

Referee #1

Dear Reviewer,

We would like to thank you for your constructive comments and advices. Here you can find the discussion on your specific comments. We hope to address to all these comments and your technical corrections. Following your comments, we provide the new version of the paper, which was deeply revised in most of the sections, and in some case rewritten.

Received and published: 5 March 2015

M. Sammartino and co-authors investigate the seasonal and interannual variability of three phytoplankton size classes (pico-, nano and micro-plankton) in the Mediterranean Sea, using SeaWiFS observations and an empirical model that relates total chlorophyll to the three phytoplankton size classes. The authors start by evaluating the performance of two models that estimate phytoplankton size classes from total chlorophyll, using in situ observations of phytoplankton pigments in the Mediterranean Sea. Based on this evaluation, the better performing model is chosen and used to estimate phytoplankton size classes (fraction of each size class to total chlorophyll) from SeaWiFS estimates (entire mission 1997-2010) of total chlorophyll in the Mediterranean Sea, estimated from a regional bio-optical model developed to account for both case 1 and 2 waters, and tailored for the Mediterranean Sea. The paper then describes regional, seasonal and inter-annual variations in phytoplankton size classes and total chlorophyll in the Mediterranean Sea over the 1997-2010 periods. These changes are discussed in the context of changes in physical forcing and current oceanographic understanding in the Mediterranean Sea. There is increasing effort use ocean-colour remote sensing for deriving additional information about the phytoplankton, other than chlorophyll biomass, such as size structure. This manuscript addresses an important issue of interest to ocean-colour scientists and to the wider marine community. Information on phytoplankton community structure is important to biogeochemical modellers, for ecosystem studies and for understanding the marine carbon cycle. Whereas, recent studies have looked at phytoplankton community structure in the Mediterranean Sea, such as that of Uitz et al. (2012, Global Biogeochem. Cy., 26, GB2024) and Navarro et al. (2014, Remote Sens. Environ., 152, 557–575), to my knowledge, the abundance-based approaches for estimating size-fractionated chlorophyll from satellite have not been tested (directly) in the Mediterranean Sea. Therefore, there is potential in this work. However, I feel that the paper requires major work to strengthen aspects of the analysis and to improve the grammar of the paper. My comments are partitioned into general comments, which focus on particular aspects of the paper which I feel require addressing, and specific comments aimed mainly to improve the grammar of the paper and address some more minor issues. I hope you find my comments useful.

General Comments

A) The comparison between the Brewin et al. (2010) and the Hirata et al. (2011) algorithms is not a fair comparison for a number of reasons:

(1) the parameters of the Brewin et al. (2010) algorithm were developed from data collected solely in the Atlantic Ocean (assuming you are using the parameters from the Brewin et al. (2010) model? Are you?), whereas the Hirata et al. (2011) algorithm was tuned to a global dataset inclusive of measurements collected in the Mediterranean Sea (see their Fig 1). The Mediterranean data used by Hirata et al. (2011) is from SeaBASS and, considering your dataset is also from SeaBASS, your evaluation of this algorithm is not independent!

First of all, it is important to underline that the Brewin model used in the paper is the Brewin et al. (2011) in which the model coefficients were retrieved by fitting the PSCs and TChl-*a* relationship with NOMAD global dataset. We thanks the reviewer for noticing that, since, citing both Brewin papers, one could think that we used the Brewin et al. (2010) model, developed from data collected solely in the Atlantic Ocean. In the new version of the paper, we clearly states that we used the Brewin et al. 2011 model.

The review is right to underline that SeaBASS (but also the NOMAD) dataset used by Hirata (or Brewin) to develop the model includes the Mediterranean data, therefore our evaluation cannot be considered fully independent. Nevertheless, our comparison provides information about the distribution of the Mediterranean measures respect the best-fit curve based on a largest, global SeaBASS/NOMAD datasets. Note that only the 15% of SeaBASS dataset are acquired in the Mediterranean Sea, while in the case of NOMAD, if we apply all the filters described in Brewin et al. (2011), no data falls in the Mediterranean basin, thus making the dataset used by Brewin et al. (2011) fully independent from our dataset. Since the global model regression is obtained by minimizing the error between the model estimates and in situ measurements on the overall range of the chlorophyll *a* values, it make sense to evaluate the performance of the model over a specific subset of data. This is particularly important for the Mediterranean Sea where optical characteristics deviate from the global ocean.

In the revised version of section 3 we clearly motivate why we performed a validation of the global models on the Mediterranean Basin.

*(2) The method used to estimate size fractions from pigments follows that of Brewin et al. (2010) and not Hirata et al. (2011). A fairer assessment would be to use the HPLC method to determine each size class developed for each approach. Note for instance, Hirata et al. (2011) uses a fucoxanthin adjustment, which probably favours the Brewin et al. (2010) for the fraction of microplankton (e.g. note the better fit to the fraction of microplankton at low chl-*a* in your Fig. 1 a). The reason the Hirata et al. (2011) algorithm produces higher estimates of nanoplankton is that this fucoxanthin is attributed to nanoplankton at low TChl-*a* (not done by Brewin et al. 2010) and also that chl-*b* is attributed to nanoplankton (attributed to picoplankton by Brewin et al. 2010). The fact that the Hirata et al. (2011) fits the data better than the Brewin et al. (2010) model, despite using the Brewin et al. (2010) HPLC method is strange, and if anything, tells you how different these relationships may be in the Mediterranean Sea.*

The review is right in affirming that a fairer assessment would be to use the HPLC method to determine each classes developed for each model approach. Therefore, to prepare the review of the paper, we estimate each size fraction from pigments following the two methods, that is Hirata et al. (2011) and Brewin et al. (2011) respectively, as suggested by the reviewer. Unfortunately, when we applied the fucoxanthin adjustment and the Hirata in situ PSCs classification to Mediterranean dataset, it results that the Hirata model is not suitable for the basin. In fact, the nanoplankton is strongly underestimated by the Hirata model and vice-versa the picoplankton is overestimated. Therefore, we decided to use the Brewin et al. (2011) in situ PSCs classification and model approach for the Mediterranean PSCs analysis.

B) *A fairer way of inter-comparing these size-class models would be to partition your Mediterranean data in two datasets: one for algorithm training (say 80% of the data) and one for model validation (say 20% of your data, judging by Fig 1 it looks like you have lots of data?). Then re-tune these algorithms (simple least-square fits) to the training datasets, and evaluate their performance on the validation dataset. This is what Hirata et al. (2011) did? To me it seems a little naïve to assume these*

algorithms would work automatically in the Mediterranean Sea, when considering they have been developed using datasets from many different regions of the ocean, and given the unique bio-optical characteristics of the Mediterranean Sea? This would also:

1) likely result in more realistic satellite products of size-fractionated chl-a in the Mediterranean Sea (Figs. 3-9)

2) provide an additional novelty to your paper, as to my knowledge, the Brewin et al. (2010) and Hirata et al. (2011) algorithms have not been tuned to the Mediterranean Sea before.

If you do this (and also with respect to your initial comparison in the paper), be careful when comparing the Brewin et al. (2010) and Hirata et al. (2011) algorithms directly, as one should also consider the number of model parameters used in the approaches. The Hirata et al. (2011) model uses 8 parameters to compute the three size fraction (3 for microplankton and 5 for picoplankton (8 for nano as it is computed as the difference between the two)) whereas Brewin et al. (2010) only uses 4 parameters (2 for pico, 2 for micro (4 for nano)). This can be done using the Akaike Information Criterion, which evaluates if there is a significant improvement in model performance when using additional parameters.

We agree with the reviewer that we could re-tune the Hirata and Brewin algorithms to the specific case of the Mediterranean Sea, but this is not the goal of this paper. Our aim is to study the spatial and temporal variability of the PSCs in the basin from satellite data. It is obvious that the assumption that global algorithms can automatically work in a regional context is naïve, but it is also naïve to proceed to the regional re-tuning before verifying the accuracy of existing models. This is the reason why we made the evaluation of the selected algorithms over the Mediterranean Sea before any further analysis. Furthermore, since the Brewin model is based on chlorophyll *a*, the specific bio-optical characteristics of the Mediterranean waters are taken, at least partially, into account by the use of regional chlorophyll estimates based on MedOC4 that was developed using a Mediterranean bio-optical dataset.

Nevertheless, since the results, that we obtained applying the Hirata HPLC-based in situ PSCs classification to the Mediterranean dataset, highlight how important is the definition of the relative contribution of diagnostic pigments to each PSCs classes, we decided instead to re-tuning the Brewin coefficients, to use a new Mediterranean relationship between diagnostic pigments and chlorophyll *a*. The Mediterranean HPLC dataset has been used to re-defining the coefficients of the Uitz et al. (2006) relationship in order to take into account the Mediterranean pigment characteristics.

When the new Mediterranean DP relationships are applied to the SeaBASS dataset the percent mean bias error between in situ and Brewin model become 21%, -5% and -7% for micro, nano and pico respectively. These values are of the same order of magnitude of the errors found by Brewin et al. (2010) for the AMT dataset (MBE pico= 10%; MBE nano=11%; MBE micro =12%, see Figure 5 in Brewin et al. 2010) and close to those obtained when he used the NOMAD dataset (ranging 11-13%). Instead, if we use the original Uitz et al. (2006) DP coefficients into the Brewin model we obtained larger %bias especially for nanoplankton, which reaches values of -34% (see Table2 in the revised paper).

This indicates that the definition of contribution of each diagnostic pigment to nano, micro and pico fraction is a key factor. These new results are presented and discussed in the revised version of the paper.

Finally, we agree with the reviewer that the regional re-tuning of the algorithms it could be of interest and it could result into improved PSCs satellite products but this will be the goal of our future paper.

C) Certain sections are poorly written (e.g. Introduction and Section 7), while others are better (e.g. abstract and discussion). The writing needs to be improved in certain areas. I have listed many cases

in my specific comments below, but there are likely to be examples I have missed. Please go carefully over each sentence of the paper and make sure it is grammatically sound. There are many cases throughout the manuscript where there are one line paragraphs? Was this intentional? If so it is not good grammatically? Or was this a formatting issue?

Thank you for noticing this, in the new version of the paper we deeply revised the text in order to improve the quality of the writing. We have tried to change and correct some sentences following your advices. The text was also corrected by proofreading service.

D) There may be seasonal and inter-annual changes in the relationships between size fraction and total chlorophyll presented by Hirata et al. (2011) (and Brewin et al. (2010)). This is something that needs to be discussed, considering the application of the models to the entire SeaWiFS time-series (1997-2010). If there are shown to changes with season or with years, this may cause biases in your results.

We agreed with the reviewer that seasonal and interannual changes in the relationship between size fractions and chlorophyll *a* may occur in the ocean. The models do not consider this variability since they are tuned using the entire global in situ dataset and therefore assuming that the contribution of each PSCs to Chlorophyll is independent from the time and location measurements. This is a real crude approach, mainly justified by the limited number of HPLC in situ observations. In the conclusion, we now highlight and discuss the limit of this approach.

Specific comments

Page 163: Line 20: *Racault et al. (2012) paper focuses on phenology (derived from total chl-a) and not phytoplankton community composition.*

Thank you for the correction, we have changed the period and the reference.

Page 164: Line 18: *suggest changing “the climate change” to “climate change”, i.e. remove “the”.*
Done

Page 164: Line 23: *suggest changing “the flow cytometry” to “flow cytometry”, i.e. remove “the”.*
Done

Page 164: Line 27-28: *suggest changing “it is possible to collect a considerable dataset of” to “there exists considerable data on”. Also be careful here, none of these datasets provide dimensions of the phytoplankton. In all cases dimensions are inferred from the in situ data (flow cytometry (scattering and fluorescence signatures); HPLC (pigments); spectrophotometry (light absorption)). There are also other methods, for instance, filtration of water through filter-pads of a known size together with in vitro fluorometric chlorophyll-a extraction is one of the oldest methods of measuring size fractionated chlorophyll.*

We know that “none of these datasets provide dimensions of the phytoplankton” so the sentence has been modified to avoid misleading interpretation.

Page 165: Line 6: *after the word “backscattering”, I would suggest adding “, derived from remote-sensing reflectance,”.*

Done

Page 165: Line 9: *suggest changing “the recent years” to “recent years”, i.e. remove “the”.*

Done

Page 165: Line 10: change “the optical variable” to “optical variables”.

Done

Page 165: Line 18: “radiance measure the chlorophyll a effect” does not make sense, please clarify.

Done

Page 165: Line 19-20: Absorption and backscattering are not single variables, these coefficients vary with wavelength!

We have changed it

Page 165: Line 23: Suggest changing the word “sunders” to “partitions”

Done

Page 165: Line 26: These models were not all applied at global scale (Fujiwara et al. 2011, for instance, was based in the Chukchi and Bering Sea)!

Yes, it is right we have modified the sentence

Page 166: Lines 4-5: This sentence does not make sense? Please re-write.

Ok, thank you, we have revised it

Page 166: Lines 13-14: I don't like the use of the word “shows singular” here, I would suggest changing to “has unique” and add “when compared with other regions” after the words “water column”.

Done

Page 168: Line 11: the word “through” is spelt wrong.

Ok, thank you

Page 168: Line 12: “well performs with” does not make sense, I suggest changing it to “performs well when compared with”.

We have changed it, thank you

Page 168: Line 13: Suggest changing “the most of the” to “most”.

Done

Page 168: Line 14: What units are RMS and bias in? Are they log10 units? Please clarify?

In the new version of the manuscript, we clarified that the Volpe et al. (2012a) evaluation was made using log 10 units. The RMS and bias referred to the Table 4 of Volpe et al. (2012a), cited in the text, are expressed in mgm^{-3} .

Page 168: Lines 17-23: It would be useful to have additional information on the in situ data here? How many measurements are they? What is the distribution of the in situ data? A table detailing this would be useful?

A new table with information on the in situ dataset is now added (see Table 1).

Page 169: Line 19: This is not true, the Brewin et al. (2010) model does not compute each size class using a separate exponential expression. The model only is fitted to two size classes (pico- and pico+nano-).

Yes, it is true, thank you for highlight the error, we have adjusted it.

Page 170: Lines 2-5: You have already described the datasets in section 2.2?

Corrected.

Page 170: Lines 12-14: *Not surprising considering the Hirata et al. (2011) model was parameterised using a fucoxanthin adjustment on the HPLC data for micro, that was not used on the in situ data here?*

The sentence has been removed.

Page 170: Line 15-18: *Worth considering the reason why the Hirata et al. (2011) model has a different shape to the Brewin et al. (2010) model for nanoplankton, irrespective of a comparison with the Med data. This is because in the Hirata et al. (2011) approach fucoxanthin is added to nanoplankton at low chl-a, and chl-b is included in nanoplankton, not done in the Brewin et al. (2010) model.*

We removed the use of the Hirata model in the present version of paper (see reply of point B).

Page 170: Lines 19-21: *Worth considering differences in the number of parameters in the two models used to compute picoplankton fractions (2 for Brewin et al. (2010) model whereas 5 for the Hirata et al. (2011) model).*

We removed the use of the Hirata model in the present version of paper (see reply of point B).

Page 170 and Table 1: *Is the improvement in the Hirata et al. (2011) model significant? When considering also the number of parameters in the models?*

We removed the use of the Hirata model in the present version of paper (see reply of point B).

Page 171: Line 5: *“10-log” should be “log-10”*

Done

Page 171: Line 10: *“a zoom”????*

Corrected

Page 173: Line 5: *Suggest changing the word “rising” to “transfer”.*

Done

Page 173: Line 29: *“A widespread of micro component” does not make sense?*

Corrected

Page 174: Line 3: *Suggest changing the words “...which is at west of the..” to “...which is west of...”*

Done

Page 174: Line 5-6: *“..due to a new mixing of the water afterwards the break of the thermocline..” please improve English (e.g. “due to the water column becoming mixed after the breakdown of the thermocline”)*

Thank you; we have changed the sentence using your suggestion

Page 176: Line 3: *Not surprising really, considering the model uses Chl-a only as input?*

Yes, you are right, but this sentence was useful to introduce the subsequent analysis on phytoplankton size classes, we have revised it.

Page 177: Line 23: *Remove the words “that is” and change “bioregions” to “regions”.*

Done

Page 179: Line 1-3: *Please re-write these sentences.*

Done

Page 179: Line 28: *“In Fig. 9d and a constant increase..” does not make sense please re-write.*

Done

Page 180: Line 8: Suggest adding the word “unique” in front of the word “optical” in this sentence.
Ok, thank you

Page 180: Line 16-18: I am not sure that your analysis “revealed that the Hirata model, even if developed for the global ocean, can be used also in the Mediterranean Sea without tuning its empirical coefficients.” It is very likely that the model will improve in the Mediterranean if tuned to data in the Mediterranean!

As we discussed above the major issue is the definition of the relationship between chlorophyll and diagnostic pigments (see Section 3).

Page 183: lines 20-21: Change “river run off grows up” to river run off increases”
Done

Page 184: lines 13-15: I am not convinced that you “clearly demonstrated that pico, nano and micro classes often coexist and their relative contribution to TChl-a cannot be neglected”, these are relationship fixed in the Hirata et al. (2011) model?

The sentence was removed.

Table 1: I am not sure how useful the RMSE and MBE are in linear space. Is your dataset not log-normally distributed with respect to total chlorophyll? For instance, in oligotrophic waters a RMSE of greater than 0.15 mg m⁻³ is very large when considering the concentrations of pico-, nano- and micro- are likely much lower? I would suggest providing statistical results in log-space, if your dataset is log-normally distributed? These statistical tests assume normal distribution, and I strongly doubt your dataset is normally distributed in linear (normal) space.

We decided to maintain the statistics in the linear space in order to obtain values comparable with the results presented by Brewin for the global ocean (see Fig. 6 in Brewin et al., 2010). In addition, we added the RMSE%, the MBE% and the Pearson relation coefficients in log10 scale, so we can have an evaluation of % of errors which takes into account that the chlorophyll *a* is not normal distributed covering a large range of values.

Figure 1: Requires units on the x-axis.

Ok, thank you

Figure 2: Requires units on the x-axis (assuming this is degrees?).

Yes, they are degrees. We have specified them.

Figures 5 and 6 are very difficult to see? Also I can barely read the units *unless I zoom right in on the PDF).

Thank you for noticing it. The original figure was an A4 size. The generation of the PDF made by Ocean Science reduced the resolution of the images. We hope that in the final version of the paper when the postscript images will be used the problem will be solved.

Spatio-temporal variability of micro-, nano- and pico-phytoplankton in the Mediterranean Sea from satellite ocean colour data of SeaWiFS

M. Sammartino¹, A. Di Cicco¹, S. Marullo², and R. Santoleri¹

¹CNR – Istituto di Scienze dell’Atmosfera e del Clima, Rome, Italy

²ENEA, Agenzia nazionale per le nuove tecnologie, l’energia e lo sviluppo economico sostenibile, Centro Ricerche Frascati, Frascati, Italy

Keywords: Phytoplankton Size Classes; Ocean Colour; Mediterranean Sea.

Correspondence e-mail: michela.sammartino@artov.isac.cnr.it

Abstract

The seasonal and year-to-year variability of the [Phytoplankton Size Classes \(PSCs\)](#) spatial distribution has been examined in the Mediterranean Sea [by](#) using the entire time series of Sea-viewing Wide Field-of-view Sensor (SeaWiFS) space observations (1998 to 2010). PSCs daily maps have been determined using an empirical model based on a synoptic relationship between surface chlorophyll *a* and diagnostic pigments referred to different taxonomic groups. [The analysis of micro-, nano- and pico-phytoplankton satellite time series \(1998–2010\) describes, quantitatively, the algal assemblage structure over the basin and reveals that the main contribution to the chlorophyll *a* in most of the Mediterranean Sea comes from the pico-phytoplankton component, above all in poor nutrient environments.](#) Regions with different and peculiar features are the northwestern Mediterranean Sea, the Alborán Sea and several coastal areas, such as the north Adriatic Sea. In these areas, local interactions between physical and biological components modulate the competition between the three phytoplankton size classes. It results that, during the spring bloom season, micro-phytoplankton dominates in areas of intense vertical winter mixing and deep/intermediate water formation while, in coastal areas, micro-phytoplankton dominates in all seasons, [because of the nutrient](#) supply from the terrestrial inputs. In the Alborán Sea, [where](#) the Atlantic inflow modulates the nutrient availability, any predominance of one class over the other two has been observed. Nano-phytoplankton component instead remains widespread over the entire basin along the year, and its contribution to the TChl *a* is of the order of [30–40%](#). [The largest inter-annual signal occurs in the northwestern Mediterranean Sea, driven by the year-to-year variation in intensity and extension of the spring bloom, followed by the Alborán Sea, in which the inter-annual variability is strongly modulated by the Atlantic inflow.](#)

In absence of sufficient in situ data of community composition, the satellite-based analysis demonstrated that pico, nano and micro classes often coexist. The predominance of one group over the other ones is

strongly dependent on the physical-biological processes occurring at mesoscale. These processes directly influence the nutrient and light availability, which are the principal forcing for the algae growth.

1 Introduction

Phytoplankton represents an important element for the survival and comprehension of the marine ecosystem. Its scientific importance is owing to its ecological role in the global carbon cycle and greenhouse effect (Park et al., 2015). Phytoplankton plays a key role in the biological carbon pump not only for its consumption of inorganic carbon during photosynthesis but also for the transport of organic carbon from the surface to deep layers of ocean. Moreover, phytoplankton contributes to the primary production, due to its rapid turnover and to the great extension of the ocean on the earth's surface (Falkowski et al., 1998).

Phytoplankton biomass bio-geographic distribution, on global and regional scales, is directly influenced by biological, chemical and physical factors such as light, nutrients availability, presence of competitors, predators, as well as temperature and pH, which are all connected to the local dynamic of water masses. These biotic and abiotic factors create a complex system in which the phytoplankton, in being a primary producer, plays a relevant role (Reynolds, 1989) and represents the first step of the ecological pyramid as well as the food web (Klausches et al., 2012).

The availability of light and nutrients strongly influences the phytoplankton biomass and community structure; when nutrients are reduced, the smaller component of algal biomass predominates on the bigger one, but when the system shift to inverse bio-geochemical condition, the community tends to change its structure, in being predominated by large cells. These types of changes could have a strong impact on the marine system and on the stoichiometry, carbon storage and biogeochemistry (Marinov et al., 2010). Any change of marine ecosystem state is also reflected in new morphological and physiological adjustments, just like the change of size for each specific trophic stadium (Thingstad and Rassoulzadegan, 1999).

Thanks to the relationship between dimensions and pigmentary content, different taxa or stages of growth in the same taxon, photosynthetic efficiency and bio-optical phytoplankton properties (Chisholm, 1992; Organelli et al., 2007; Raven, 1998), "cell size" becomes an important descriptor of the community structure. Indeed, phytoplankton cell size and pigment content are some of the physiological traits that influence the rate of acquiring and processing energy and materials from the environment (Brown et al., 2004). Size and biodiversity of phytoplankton community can modulate the amount of carbon fixed and exported into the deep sea with respect to the nutrient availability (Finkel et al., 2010).

A shift in the phytoplankton size structure from a dominance of picoplankton to predominance of larger nano- and micro-phytoplankton is associated with a shift in the pelagic food web (Finkel et al., 2010). The dimension of cells and consequently the structure of the algal community can influence the trophic organization of the marine ecosystem and the ability to produce more organic matter to be transferred across the successive trophic stages (Marañón et al., 2012).

69 Given the importance of cell size in understanding the relationship between phytoplankton assemblage and
70 marine ecosystem dynamic, it is common to classify the algal community in micro-, nano- and pico-
71 phytoplankton. One of the most common Phytoplankton Size Classes (PSCs) definition identify the size ranges
72 of the phytoplankton cells as follow: micro $> 20 \mu\text{m}$, the nano from 2 to $20 \mu\text{m}$ and pico $< 2 \mu\text{m}$ (Sieburth et
73 al., 1978). In oligotrophic waters the pico-phytoplankton provides a relevant contribution to the total content
74 of chlorophyll *a* (Agawin et al., 2000), being the latter defined as the sum of chlorophyll *a*, its allomers and
75 epimers, divinyl-chlorophyll *a*, chlorophyllid *a* (Hooker et al., 2005) and called TChl *a* or chlorophyll *a* in
76 follow. On the contrary, in eutrophic water where cells have the opportunity to grow due to the availability
77 of nutrients and light, the larger cells prevail (Irwin et al., 2006).

78 In terms of biogeochemical function and role, size structure of phytoplankton communities provides
79 important information such as the knowledge of the community composition itself (Vidussi et al., 2001;
80 Chisholm, 1992; Raven, 1998). Indeed, in some cases, several biogeochemical functions correspond to a
81 particular taxon or size class; for instance, cyanobacteria often represents a large group of
82 picophytoplanktonic nitrogen-fixers. They are able to fix and use the forms of atmospheric nitrogen, thereby
83 having a direct impact on climate change. Yet, the principal components of the micro-phytoplankton, diatoms
84 and dinoflagellates, play a dominant role in the Carbon flux into deeper waters (Nair et al., 2008;
85 Sathyendranath, 2014). In these cases a PFTs (Phytoplankton Functional Types) classification is adopted, in
86 which each type defines a group of different species with a common ecological function.

87 Information about the composition of phytoplankton community structure can be obtained from the analysis
88 of in situ samples using different laboratory techniques such as: flow-cytometry, which provides information
89 about the number and the dimensions of the fluorescent cells in a specific water sample volume; HPLC (High
90 Pressure Liquid Chromatography), which is used to retrieve the composition and concentration of the
91 pigments content of the cells; spectrophotometry, which provides the pigment light absorption in the visible
92 spectrum, and filtration of water through filter-pads of a known size together with in vitro fluorometric
93 chlorophyll *a* extraction. As a result, there exist considerable data on in situ dimensional classes measures
94 that could be useful, also, for other applications like calibration and validation of satellite PSCs algorithms.

95 From space, the composition of the community is detected by exploiting the signature of the different species
96 and classes on the optical properties in the water column. Light absorption of a cell is affected by its pigment
97 “package effect” (Morel and Bricaud, 1981; Bricaud et al., 2004), which, in describing the chlorophyll *a*
98 efficiency in the light harvesting, is a direct function of the pigment cellular concentration and therefore of
99 the “cell size” (Chisholm, 1992; Raven, 1998; Basset et al., 2009).

100 Concentration of chlorophyll *a*, light absorption and backscattering signals, derived from remote-sensing
101 reflectance, are the main ocean colour variables that provide synoptic and multi temporal information about
102 phytoplankton distribution. Several satellite models have been developed in recent years to classify the algal
103 cells on the basis of optical variable measured from space. These are usually divided into two main classes:

104 direct models, which [exploit](#) the optical properties directly captured by the sensor; indirect models, as those
105 based on the strong relationship between the chlorophyll *a* concentration and the functional groups or taxa
106 [and PSCs](#) (Moisan et al., 2012).

107 Another classification of these methods is based on the spectral-response and abundance-based approaches
108 (Brewin et al. 2011a). The spectral-response models analyze the differences in the shape of the light
109 reflectance/absorption spectrum to provide information about different phytoplankton classes; an example
110 of this model is the Alvain's et al. (2005, 2008) one, in which different phytoplankton groups are identified
111 from the normalized *water leaving radiance* data. These authors exploit the anomalies in the spectral
112 signature of a specific taxon or a specific type of community after removing the chlorophyll *a* signal from the
113 radiance measure. The abundance-based models, instead, exploit the information coming from the
114 magnitude of chlorophyll *a* biomass or light absorption to separate one group from another (Devred et al.
115 2006; Uitz et al. 2006; Hirata et al. 2008; Brewin et al. 2010; Brewin et al, 2011b; Hirata et al. 2011). Most of
116 the satellite PSCs models are based on a [specific](#) variable: e.g. the absorption coefficient [at different](#)
117 [wavelength of the cells](#) (Sathyendranath et al., 2001), or the backscattering coefficient (Kostadinov et al.,
118 2009). Others are mixed [models](#), just as in the case of Fujiwara et al. (2011), in which the algorithm [partitions](#)
119 [between](#) the pico + nano-phytoplankton community [and](#) the micro one, involving the absorption and
120 backscattering coefficients.

121 Most of the models described above, were developed for the global ocean and applied to infer phytoplankton
122 composition or classes from space allowing to study their seasonal and inter-annual variability at global scales
123 (Brewin et al., 2010, 2011b; Hirata et al., 2008, 2011; Uitz et al., 2006; Mouw and Youder, 2010). In this paper,
124 [instead](#), we used [a](#) chlorophyll *a* based model to estimate phytoplankton composition in the Mediterranean
125 Sea with the aim of studying the spatial and temporal variability of phytoplankton assemblage dynamics. [The](#)
126 [choice to test a chlorophyll *a*-based model rather than spectral-based ones was inspired by the possibility to](#)
127 [check the global model performances at regional scale by using available in situ observations. Indeed, in](#)
128 [Mediterranean Sea, the number of in situ data related to Diagnostic Pigments \(sum of seven marker pigments](#)
129 [intended as size taxonomic pigments, DP, Vidussi et al., 2001\) is much greater than the optical](#)
130 [measurements, which are very limited and not always freely available.](#)

131 Presently, chlorophyll *a* estimates from ocean colour data were widely used to study the Mediterranean
132 phytoplankton biomass variability at basin and sub-basin scales (e.g. Antoine and Morel, 1996a, 1996b;
133 Santoleri et al., 2003; Bosc et al., 2004; Volpe et al., 2012b). Only recently, Navarro et al. (2014), adapted the
134 PHYSAT method of Alvain et al. (2005) to the Mediterranean Basin bio-optical characteristics, thereby
135 providing a regional algorithm to estimate dominant phytoplankton groups (Nanoeukaryotes,
136 *Prochlorococcus*, *Synechococcus*, diatoms and coccolithophores) from MODIS water leaving radiance
137 measures.

Therefore, the objective of this work is dual: i) to understand how well a simple empirical model solely based on chlorophyll *a* data, as Brewin et al. (2011b) referred as BR henceforth, can describe the phytoplankton biomass distribution in the Mediterranean Sea; ii) to study the spatial–temporal variability of the three phytoplankton size classes (micro, nano and pico) in this basin, by applying the selected model to the ocean colour products. This paper will be the first attempt to describe the seasonal and inter-annual evolution of the phytoplankton size classes assemblage during the entire SeaWiFS era. In Section 2, we presented the satellite and in situ data we use. In the same section we briefly describe the selected PSCs model is. In Section 3 we describe BR model validation over the Mediterranean Sea, using HPLC observation. Finally, the variability and distribution of PSCs is analyzed at different scales of time and space (Sections 4, 5 and 6). Conclusions (Section 7) summarizes the results and presents the future perspectives.

148

1.1 The study area

The Mediterranean Sea (Fig. 1), although relatively small, is characterized by a circulation that can be compared to that of a large-scale ocean. It is among all, the most interesting of the semi-enclosed seas because of the great range of processes and interactions that occur within it (Robinson and Golnaraghi, 1994). Most of the physical and biological processes that characterize the global ocean, many of which are not well known or understood, occur analogously in the Mediterranean Sea (Siokou-Frangou et al., 2010; Lacombe et al., 1981; Robinson and Golnaraghi, 1994). These biological and physical processes interact with each other and influence, directly, the distribution of the phytoplankton and zooplankton community and the optical properties of the seawater. Unlike the other seas and oceans, the Mediterranean Sea has unique optical properties of the water column, when compared with other regions, with “oligotrophic waters less blue (30 %) and greener (15%) than the global ocean” (Volpe et al., 2007). Many hypotheses were developed in the past to understand and justify the reason why the Mediterranean Sea shows these properties. One of them is relative to the high yellow substance content, which can be responsible of an enhancement of absorbing material (Claustre and Maritorena, 2003; Morel and Gentili, 2009). Another hypothesis attributes this effect to the presence of coccoliths (D’Ortenzio et al. 2002, Gitelson et al., 1996), while a third hypothesis is related to the presence of submicron Saharan dust in suspension in the surface layer (Claustre et al., 2002). Finally, Volpe et al. (2007) suggest that the different phytoplankton community structure, typical of the basin, could alter the spectral signature and therefore be responsible of peculiar colour of the Mediterranean Sea. Nowadays, it does not exist a univocal response, which can optimally justify the Mediterranean “greener” than other oceans. Therefore, this peculiarity has made it necessary to develop regional bio-optical algorithms in order to estimate chlorophyll *a* concentration from in situ optical measurements and satellite data (D’Ortenzio et al., 2002, Volpe et al., 2007; Santoleri et al. 2008). Finally, the optical properties of the Mediterranean Sea suggest verifying whether a PSCs model designed for global ocean applications can perform similarly in the Mediterranean Sea.

173

174 **2 Data & Methods**

175

176 **2.1 Satellite data and processing**

177 The satellite data used in this work comes from Sea-viewing Wide Field-of-view Sensor (SeaWiFS). They are
178 daily chlorophyll *a* Level 3 (L3) data (resolution 1.1 km), from 1998 to 2010, produced by the Satellite
179 Oceanography Group (GOS) of the Institute of Atmospheric Sciences and Climate (ISAC) of the Italian National
180 Research Council (CNR), and made available to scientific community. We used the so called Mediterranean
181 Case1Case2 merged chlorophyll *a* product (GOS Chl_1-2). These daily chlorophyll *a* fields are derived from L1
182 SeaWiFS passes applying two different bio-optical regional algorithms for open and coastal waters (see Volpe
183 et al., 2012a, for the details of processing). The MedOC4 algorithm is used to retrieve chlorophyll *a* in the
184 Case 1 waters (Volpe et al., 2007) and while the Ad4, is applied for the Case 2 waters (Berthon and Zibordi,
185 2004). The identification of the optical properties of each pixel is based on the D'Alimonte's method
186 (D'Alimonte et al., 2003), which takes into account the entire spectrum from the blue band to NIR, for both
187 Case1 and Case2 waters types. For intermediate waters, a weighted average of the two algorithms based on
188 the distance between the actual reflectance spectra and the reference one for the Case 1 and 2 waters is
189 applied.

190 The choice of applying an algorithm born specifically for the Mediterranean Case 1 waters, as MedOC4,
191 provides a more realistic value of TChl *a*, as demonstrated by Volpe et al. (2007), who showed that NASA
192 SeaWiFS chlorophyll *a* fields are affected by an uncertainty of the order of 100% (Volpe et al., 2007) and
193 confirmed by several authors. The MedOC4 algorithm was developed from a readjustment of the NASA
194 algorithm OC4 (O'Reilly et al., 1998), in which the coefficients were obtained from a fourth power polynomial
195 regression fit between log-transformed in situ Mediterranean chlorophyll *a* concentration and maximum
196 band ratios at a specific wavelength obtained by in situ optical profiles (Volpe et al., 2007). Similarly, the Ad4
197 has been tuned by using the bio-optical dataset acquired by JRC in the Venice Tower located in the North
198 Adriatic Sea.

199 Besides the use of a regional algorithm, all the data distributed by GOS and those distributed by MyOcean
200 OCTAC to the end-users are quality checked. The daily TChl *a* fields, used as input in this work, were subjected
201 to quality assessment through classical matchup analysis (called offline validation in Volpe et al., 2012a).
202 Volpe et al. (2012a) demonstrates that the SeaWiFS Mediterranean regional products match up well to the
203 corresponding in situ data showing the following statistical results: the correlation coefficient (r^2) 0.815,
204 Root Mean Square Error (RMS) 0.253 mgm^{-3} , bias -0.019 mgm^{-3} , relative (RPD) 15% and absolute (ADP) 51%
205 percentage differences (see Table 4 in Volpe et al., 2012a). Given the log-normal chlorophyll *a* distribution,
206 r^2 , RMS and bias are calculated over log-transformed quantities, while RPD and APD over untransformed
207 pairs of values.

208 Here, daily chlorophyll a maps, at 4 km of resolution, were used to compute monthly maps covering the
209 SeaWiFS era (1998–2010), then the monthly means were averaged to compute monthly climatology.
210 Moreover, TChl a fields at monthly and climatological scales were then used to support the analysis of
211 phytoplankton biomass variability. In these maps, the chlorophyll a concentration is expressed as base log-
212 10 transformed considering the log-normal distribution of this pigment.
213 The BR method was then applied to compute the PSCs daily fields over the Mediterranean Sea for entire
214 SeaWiFS time series. This model expresses the TChl a concentration as the sum of pico, nano and micro-
215 phytoplankton chlorophyll a fraction, and each class is computed by using a simple function of the
216 chlorophyll a . For more details about the algorithm, see Brewin et al. (2011b). The daily PSCs fields are then
217 used to produce monthly climatological fields.

218

219 **2.2 In situ data and processing**

220 The in situ dataset used in this paper is the SeaBASS HPLC-based diagnostic pigments dataset (Werdell and
221 Bailey, 2005). All the data acquired in the Mediterranean Sea were extracted from this global dataset and
222 used for model validation purpose. The Mediterranean SeaBASS dataset (referred as “MED in situ”,
223 hereafter) consists of 1454 samples acquired in the basin since 1999 and represents the 15% of the global
224 SeaBASS data. The MED in situ data were acquired during two trans-Mediterranean cruises (Prosop99, and
225 Boum08) covering the basin from Gibraltar to eastern Mediterranean, and near the Boussole mooring where
226 periodic measurements are carried out from 2001 to 2006. The details of the in situ observation in terms of
227 location, period of sampling, TChl a value ranges and sampling depth are reported in Table 1. Even if most of
228 the data were acquired at the Bussole sampling site, the measurements still covers the entire range values
229 of the Mediterranean chlorophyll a variability, with values ranging from less than 0.05 to more than 5 mgm^{-3} .
230

231 The Med in situ pigment dataset was quality checked and filtered by applying the same procedure used by
232 Brewin et al. (2011b). Following Aiken et al. (2009), outliers were determined from the regression of
233 accessory pigments against TChl a excluding values behind the 95% of confidence interval of the regression.
234 This reduces the number of samples from 1454 to 1085.

235 This dataset was then used to compute the in situ quantification of PSCs following the methods described in
236 the Brewin et al. (2011b), based on the previous works of Vidussi et al. (2001) and Uitz et al. (2006).

237 We point out that NOMAD dataset used by Brewin et al. (2011b) to develop their PSCs model, after filtering,
238 does not include any Mediterranean data point; therefore, our Mediterranean dataset can be considered
239 fully independent.

240

3 Brewin model performances over the Mediterranean Sea

The MED in situ is used to evaluate, for the first time, the BR model accuracy over the Mediterranean Sea (Table 2 and Fig. 2). Figure 2 (left panels, a-c) shows the micro, nano and pico-phytoplankton fractions, obtained by applying the Uitz et al. (2006) DP coefficients, as a function of the TChl a . A rather large scatter of the data around the model curves suggests that, in the real world, the relative abundance of micro, nano and pico-phytoplankton cannot be a simple function of the chlorophyll a concentration alone. In particular, the BR model strongly underestimates nano plankton fraction measured in the Mediterranean basin in the entire range of TChl a values, while overestimates the pico fraction for TChl a concentrations less than 0.8 mg/m³; only for micro plankton the curve falls in middle of the observed cloud of data points. These results are quantitatively confirmed by the statistical analysis, which shows a log10 bias error of -4%, -26% and 67% for micro, pico and nano fractions respectively.

The poor performance of the model can be due to the particular optical properties of Mediterranean waters, which makes this basin unique with respect to the other oceans (see section 1). For this reason, before to start performing any new adjustment of the BR coefficients, we first investigate whether if a different relation between DP and chlorophyll a in the Mediterranean basin can be responsible for the observed biases. This allows us also to verify the Volpe et al. (2007) hypothesis, which considers the different assemblage of the phytoplankton community structure as one of the possible causes responsible of the greener colour of the Mediterranean Sea. Recently, Di Cicco (2014) provided a regional DP and chlorophyll a relationship, which is entirely based on Mediterranean data. She, by applying the Gieskes et al. (1988) approach to the MED in situ data, performed a new multiple regression analysis to evaluate whether a different pigment ratios of the phytoplankton community can occur in the basin and showed that the use of Uitz DP-TChl a relationship results in an underestimation of the Mediterranean TChl a estimate overall its range values; namely the Utiz line fit has a slope coefficient less than 1. The new MED DP-TChl a relationship found by Di Cicco (2014) is:

$$TChla = 1.999[Zea] + 1.624[TChlb] + 2.088[Allo] + 0.861[19' Hex-fuco] + 0.405[19' But-Fuco] + 1.74[Fuco] + 1.172[Peri] \quad \text{eq. (1)}$$

in which each PSCs fraction is computed as follows:

$$f_{pico} = (1.999[Zea] + 1.624[TChlb])/TChla \quad \text{eq. (2)}$$

$$f_{nano} = (2.088[Allo] + 0.861[19' Hex-fuco] + 0.405[19' But-fuco])/TChla \quad \text{eq. (3)}$$

$$f_{micro} = (1.74[Fuco] + 1.172[Peri])/TChla \quad \text{eq. (4)}$$

For more details about the new coefficient retrieval, see Di Cicco (2014). Consequently, we applied the new Di Cicco (2014) coefficients to obtain the new in situ PSCs classification to be compared with the BR model. Effectively, the improved performances of the model with respect to the in situ PSCs fractions (shown in Fig. 2, d-f) highlights how important the relation between the diagnostic pigments and TChl a content is. Figure 2 summarizes the comparison between the BR satellite model and the in situ PSCs fractions as obtained by using, respectively, the Uitz et al. (2006) DP coefficients (left panel of Fig. 2, a-c) and Di Cicco (2014) ones (right panel of Fig. 2, d-f), while the statistical results are shown in Table 2. Figure 2 shows that the in situ Uitz PSCs classification is not suitable for the Mediterranean Sea and a regional classification is therefore necessary. This is evident, in particular, for the nano-phytoplankton case (Fig. 2 e), in which the use of MED DP relationship shifts down the cloud points and results in a better performance of BR model with the log10 %mean bias error falling from 67% to only 8%. By observing the pico scatter plot (Fig. 2f), the dots are now distributed around the model curve for the all range of chlorophyll a values, and the % log10 bias decreases from -26% to 15%. Micro component represents a similar behavior, both applying the global coefficients and the Mediterranean ones, as confirmed by the statistical results. The statistics in Table 2, computed both in linear scale and in log-transformed scale using the reference equation of Table 3, confirms that the use of Di Cicco DP relationship is a key factor to improve the in situ PSCs classification. When equation (1) is used, the errors we found applying the BR model result in a MBE% range from -5% to 21% which is of same order that is found by Brewin et al. (2010) by using an independent dataset (from 11% to 13.3%). Consequently, we conclude that an adaptation of the BR model coefficients for the Mediterranean case is not a priority considering the limited margin for improvement left after the tuning of the Uitz DP-TChl a coefficients.

297

298 **4 Seasonal variability of spatial distribution of the PSCs in the Mediterranean Sea**

The seasonal evolution of the chlorophyll a distribution in the Mediterranean Sea is driven by the life cycle of the phytoplanktonic organisms that follows the typical succession of temperate areas, with a high biomass increase in late winter/early spring and a decrease in summertime, and a second smaller bloom in autumn. PSCs variability follows this oscillation mostly driven by the evolution of the chlorophyll a concentration and its West-East gradient (see Fig 1S (a-c) in additional material). This spatial gradient is one of the dominant features of the chlorophyll a distribution in the Mediterranean Sea and reinforces the paradigm of an extremely oligotrophic Eastern basin and a more productive Western side (D'Ortenzio et al., 2009). We investigated the seasonal variability of this spatial gradient by computing the variation of monthly chlorophyll a climatology moving from West to East along the basin (Fig. 3). In this figure, each colored line represents a climatological month and the chlorophyll a value at a given longitude is obtained by averaging all the sea pixels from north to south, excluding those closer than 20 km from the coast to restrict the calculus to open ocean waters.

311 A decreasing trend of this surface chlorophyll *a* mean concentration, moving from West to East, is observed
312 in all the months of the year (Fig. 3). The curves highlight the occurrence of an enhanced seasonal cycle in
313 the western Mediterranean with respect to the eastern Mediterranean Sea, generally characterized by
314 oligotrophic conditions in all the months of the year. Oligotrophic conditions dominate in the western
315 Mediterranean Sea during summer, while during spring, the occurrence of the blooms is marked by two
316 distinct peaks at 4° and 9° E associated to the Gulf of Lions and Ligurian Sea respectively. The peak at 13° E,
317 instead, is the signature of the rich chlorophyll *a* area of the North Adriatic Sea.

318 The observed West to East decreasing trend is consistent with a similar trend observed in the nutrient
319 concentrations by Siokou-Frangou et al. (2010) and by Santinelli et al. (2012). These concentrations are
320 generally very low, according with the general oligotrophy of the basin, mainly linked to the lack of
321 phosphorous, which represents a limiting factor for phytoplankton community's growth (Zohary and
322 Robarts, 1998; Ribera D'Alcalà et al., 2003; Krom et al., 2004).

323 Figure 3 clearly reveals that April is the month in which the maximum excursion of chlorophyll *a* across the
324 basin occurs while August shows a minimum of the longitudinal gradient. In these two months we observed
325 the two extremes of the annual chlorophyll *a* variability all the Mediterranean sub-basin, except for the
326 Adriatic Sea. Therefore, in the next sub-sessions we focus on these contrasting months for analyzing the
327 variation of the spatial distribution of micro-, nano, and pico-phytoplankton in the Mediterranean Sea
328 resulting from the application of the BR model. However, the maps of entire climatological time series can
329 be found as supplementary materials.

330

331 **4.1 Micro-phytoplankton**

332 The seasonal spring to summer excursion of micro-phytoplankton, in the first optical depth, is shown in Fig.
333 4. In August, excluding the coastal areas, the micro-phytoplankton is uniformly distributed over the entire
334 Mediterranean and its contribution to the total chlorophyll *a* is low, with values of about 12% in the Ionian-
335 Levantine Basin and 13% in the Western basin, with relative peaks of 15–25% in the Alborán Sea. These low
336 values are associated to low chlorophyll *a* concentrations. Indeed, in summertime, the water becomes
337 warmer and the stratification of the column is more marked, thereby producing a resistant thermocline that
338 limits the transfer of nutrients to surface and consequently determines a reduced photosynthetic activity
339 (Siokou-Frangou et al., 2010). This pattern persists also in June and July (see additional material). In August,
340 high values of micro-phytoplankton contribution are observed in some coastal regions characterized by a
341 high nutrients supply due to upwelling phenomena or river runoff: the Alborán Sea, the north Adriatic Sea,
342 the Gulf of Lions and the gulf of Gabes with values ranging between 35–75 %. In the Alborán Sea, the higher
343 micro-phytoplankton contribution is highlighted by water upwelled along the Spanish coasts and entrained
344 in the west Alborán gyre (Sarhan et al., 2000).

345 In April, [instead](#), the fraction of micro-phytoplankton significantly grows in the northwestern Mediterranean
346 Sea [reaching values from 30% to 57%](#). This area, included by D'Ortenzio et al. (2009) in the bloom cluster, is
347 characterized by a local dynamic in which cold winter winds can induce deep mixing extending down to
348 several hundred up to thousand meters, [a value that is large when](#) compared to the seasonal winter overturn.
349 This deep overturning process also brings up an additional supply of nutrients complementary to that
350 furnished by seasonal convection, thus modulating the spring bloom. The bloom observed in April ([Fig. 4](#)) is
351 the result of winter upwelled nutrients and phytoplankton trapped in the euphotic zone by the spring re-
352 stratification process and [by](#) the increased insolation. After this high productivity's period, micro-
353 phytoplankton contribution to the TChl *a* decreases in the whole basin, reaching its minimum in [August-](#)
354 [September](#).

355 In April, high micro-phytoplankton values are still present in the same coastal areas [where micro](#)
356 [predominates in](#) August, with the addition of the north Aegean Sea, where the signature of the Black Sea
357 outflow is now evident [in the chlorophyll map \(Fig. 4\)](#). Differently from August, the Spanish coastal water
358 reaches also the eastern Alborán Gyre, [resulting in a widespread region characterized by micro component](#).
359 In the Ionian-Levantine Basin, the contribution of the micro-phytoplankton remains low with values about of
360 [12–13%](#) and with higher values ranging from [15 to 21%](#) in the western side of the Ionian Sea and in the [area](#)
361 [west of Rhodes Island where the presence of the Rhodes Gyre facilitates the uplift of nutrients from the](#)
362 [deeper layer](#).

363 [Yet, in the Western basin, an increase of micro fraction occurs during the entire autumn/winter seasons](#) (not
364 shown) [due to water column becoming mixed after the breakdown of the thermocline](#) (Bosc et al., 2004).
365 Unlike the [spring bloom](#), [now the](#) values of chlorophyll *a* [are lower](#), in agreement with previous observations
366 (Siokou-Frangou et al., 2010). This phenomenon leads to a minor percentage of micro-phytoplankton [close](#)
367 [to 20%](#) of the TChl *a*, with some peaks in the Algerian Current [that flows along](#) the southern [boundary](#) of the
368 [western Mediterranean \(25–40%\)](#). The Eastern basin still shows low fractions of micro component during the
369 [autumn/winter months \(see November to February maps in supplement material\)](#).

370

371 **4.2 Nano-phytoplankton**

372 The amplitude of the seasonal cycle of the nano-phytoplankton component is less pronounced than the micro
373 ([Fig. 4](#)). In summer, the contribution of the nano-phytoplankton to the total chlorophyll *a* is between [18](#) and
374 [24%](#). In coastal areas, such as the North Adriatic Sea, its contribution to total chlorophyll *a* reaches [25–38 %](#),
375 with a decrease for pixels more close to the coast where micro-phytoplankton still dominates ([Fig. 5a](#)).
376 In April, the contribution of the nano-phytoplankton remains between [20](#) and [25%](#) in most of the Ionian-
377 Levantine Basin, with the exception of the Rhodes gyre, where it reaches values of about [29%](#) and the
378 western Ionian Sea, where values up to [30–36%](#) are observed approaching the coasts of Italy ([Fig. 4](#)). In the
379 western Mediterranean Sea, the values of nano contribution to total chlorophyll *a*, vary from [25](#) to [38%](#) ([Fig.](#)

380 4). Yet, in the North Adriatic Sea, the nano fraction, in April, is always between 20 and 36 %, but with a more
381 evident decrease, with respect to August, for those pixels that are closer to the coast, where the micro-
382 phytoplankton remain predominant (Fig. 5b). The variability of nano component in the remain months of the
383 year (autumn/winter) is not so high and it still shows higher values in the Western basin (28-30%) than in the
384 Eastern (20-25%), reaching peak values of 38% in gyres of the Alborán Sea and along the Algerian current.
385 In these months as in the spring, the division, in terms of oligotrophy, of the Eastern basin with respect to
386 the Western is more evident, otherwise, the months from July to September reveal, in the open ocean, an
387 invariable pattern of nano component.

388

389 4.3 Pico-phytoplankton

390 Due to the high surface/volume ratio, pico-phytoplankton seems to be more suitable to poor nutrient
391 environments often characterized by high salinity, such as those that occur in the Levantine Basin (Le Quéré
392 et al., 2005). As suggested by Uitz et al. (2012), its capacity to survive in this type of environments justifies its
393 great abundance in the Eastern basin, thus becoming the principal producer in ultra-oligotrophic waters.
394 Indeed, Figure 4 shows that in August, the pico-phytoplankton contributes to 60–70% of the TChl *a*, in the
395 offshore waters while lower values are observed in coastal waters: about 15–30% in the western Alborán
396 Gyre, 11–24% in the North Adriatic Sea and 12–34% in the Gulf of Lions. In April, in the Ionian-Levantine
397 Basin, the pico fractions values remain high, but lower than those observed in August (64–65 %), while in the
398 northwestern Mediterranean Sea a large area of low pico TChl *a* concentration occurs with values ranging
399 between 13 and 24%. Similarly, low values are observed in coastal regions, e.g. in the north Aegean Sea,
400 where the outflow of the Black Sea influences the distribution of pico class, with values ranging from 40–45%
401 in August (see also Fig. 5c). In April, the outflow of the Black Sea waters is marked by a minimum, which
402 ranges between 13 and 20 %, which now affects all the northern part of the Aegean Sea (Fig. 5d).

403 The analysis of the January to April maps (supplementary material) shows that pico component reveals a
404 contrasting variability moving from West to Eastward, with high percentages in the latter and lower in the
405 former. With the arrival of the summer season, the pico-phytoplankton seems to cover homogenously all the
406 basin with values of 70% and minima in correspondence of coastal areas. Later, the pico-phytoplankton
407 decreases in the most dynamic areas, such as along the Tunisian coast, in conjunction a micro and nano
408 fraction increase (see e.g. December maps of supplement). This is caused by the intrusion of new nutrients
409 from the deeper layer, due to the break of thermocline.

410

411 5 Inter-annual variability of chlorophyll *a* and PSCs in the Mediterranean Basin

412 The inter-annual variability of the surface chlorophyll *a* and PSCs distribution in the Mediterranean Sea is
413 shown in Figs. 6 and 7 for the two opposite months of April and August, respectively. In addition, the
414 chlorophyll *a*, micro, nano and pico fractions anomalies (respect to SeaWiFS climatology) have been

415 computed and then averaged at basin scale in order to identify potential inter-annual signals and changes
416 occurred during the SeaWiFS era. Figure 8 shows that, at basin scale, the inter-annual signal is very small (the
417 anomalies ranged from -0.04 to 0.06 $\mu\text{g L}^{-1}$) with positive anomalies peaks observed in winter 1999 and spring
418 2005 and 2006 as well as in March 2009, indicating that the inter-annual signal is essentially driven by the
419 intensity of the spring bloom. From the analysis of the anomalies it emerges also that pico oscillates between
420 reduced ranges of positive (maximum nearly to 0.02 $\mu\text{g L}^{-1}$) and negative anomalies (-0.01 $\mu\text{g L}^{-1}$), followed by
421 the nano component (maximum nearly to 0.03 $\mu\text{g L}^{-1}$ - minimum -0.02 $\mu\text{g L}^{-1}$), while micro falls in higher
422 anomaly ranges (maximum 0.04 $\mu\text{g L}^{-1}$ - minimum -0.03 $\mu\text{g L}^{-1}$).

423 The analysis of the April and August maps reveals that year-to-year variations are very small in August. In
424 April, significant variations are observed: the pico component dominates the TChl *a* concentration with
425 percentages of about 60-70%, over the entire basin, except those areas of the Western basin, characterized
426 by high and complex dynamic of the water masses. In these regions an enhanced inter-annual signal is
427 observed. In the Eastern basin these high values of pico remain constant in all years, while, in the Western
428 basin, the areas most affected by a strong decrease of pico TChl *a* contribution are located in correspondence
429 to the Gulf of Lions and in the Alborán Sea. The April time series maps (Fig. 6) reveal that the 1999, 2005 and
430 2006 are the years of highest chlorophyll *a* concentrations in the Gulf of Lions and in the coastal zones of the
431 basin. Here the contribution to TChl *a* of pico clearly decreases reaching values less than 10%, while, at the
432 same time, the micro component increases up to 60-70%, thus becoming predominant with respect to the
433 pico and also to the nano-phytoplankton fraction, which remains around to the 30-38%. This behavior results
434 into a positive peak of micro in the 2005 and 2006 anomalies time series (Fig 8). The April maps reveal that
435 nano component is not subjected to a significant year-to-year variation; however, a west to east gradient is
436 visible in all years with maximum values located offshore the Gulf of Lions, where the inter-annual variability
437 is more evident. The West to East Mediterranean oligotrophic gradient is reflected in the April micro maps
438 (Fig. 6), where the contribution to TChl *a* of the largest cells is very low, 15-19% along the entire time series,
439 highlighting the influence that poor nutrient environments, as those in the Eastern basin, have on the micro-
440 phytoplankton.

441 In August, the scenario is clearly different (Fig. 7). The chlorophyll *a* concentration is very low in most of the
442 offshore areas, although a slightly increases of TChl *a* can be observed from 2005 to 2007 in the Western
443 basin. The most evident signal of inter-annual variation is visible along the coastal zones of North Adriatic
444 Sea. The low inter-annual variability observed in August affects also the pico and nano components, but,
445 differently from micro, their contribution to TChl *a* is higher, respectively 65-70% for pico and 19-20% for
446 nano. The analysis suggests that the seasonal and inter-annual signal, observed in the TChl *a* and pico-, nano-
447 , and micro-phytoplankton time series, is driven by local processes occurring in the Mediterranean Sea, only
448 partially revealed by present basin scale analysis.

449

6 Seasonal and year-to-year variability of chlorophyll *a* and PSCs at local scale

Local processes play an important role in the ecosystem of the Mediterranean Sea interacting with the physical system that contributes to drive its evolution but *that*, in turn, is affected by it (bio-feedbacks). To investigate the year-to-year variability of processes that occur at local scale in the Mediterranean Sea, we selected four key sub-regions: the Northwestern Mediterranean Sea (NWMed), the Levantine Basin (LEV), the Alborán Sea (ALB) and the North Adriatic Sea (NADR) (see colored boxes in Fig. 1). In these regions relevant processes, such as surface currents advection, upwelling phenomena, water stratification or nutrients and river inputs occur, modulating local ecosystem variability. Results of this analysis were synthesized in Figure 9.

In NWMed Sea (Fig. 9a) the seasonal cycle of chlorophyll *a* concentration shows an increase of TChl *a* values from the initial part of the year, January–February, with maximum values in April and in March ranging from 0.4 to 1.2 $\mu\text{g L}^{-1}$. In summer, the chlorophyll *a* decreases up to 0.06 $\mu\text{g L}^{-1}$, and then, in autumn, it rises again. The analysis of the year-to-year variability reveals an absolute spring maximum in April 2005 (Fig. 9a), with a concentration of about 1.2 $\mu\text{g L}^{-1}$, followed by a decreasing trend from 2006 to 2007 and a new rising in 2008 (0.9 $\mu\text{g L}^{-1}$). From this year onwards, the lack of some months is due to the fewer number of observations recorded by SeaWiFS from 2007–2010. The accuracy of the TChl *a* variability in the NWMed sector is taken into account computing and evaluating the anomalies over the time series from 1998 to 2010 (Fig. 10a). From Figure 10a results that negative anomalies are more frequent and stronger than the positive ones, in the first part of the time series. This type of oscillations still persists up 2005, when the highest positive spring anomaly occurs (0.6 $\mu\text{g L}^{-1}$), followed by the April 2006 and 2008 positive anomalies.

The mean annual value of chlorophyll *a* concentration for NWMed and ALB (Fig. 9a, c) are quite similar, but in the latter (Fig. 9c), the seasonal cycle is less “clean” and the year-to-year variability is marked by minimal values of the spring maxima from 2001 to 2004 ranging from 0.5 to 0.7 $\mu\text{g L}^{-1}$ and relative maxima in 2000, 2005, 2006 and 2007 ranging from 1.0 to 1.1 $\mu\text{g L}^{-1}$. Intermediate values of the spring maximum are observed in the remaining years of the series. The “chaotic” pattern of the TChl *a* in the Alborán Sea is also reflected in the corresponding anomaly (Fig. 10c). In this case, the positive and negative anomalies vary among -0.4 and +0.5 $\mu\text{g L}^{-1}$. The time series anomaly reveals that the main positive peaks occur in April 2000, March 2006 and February 2007; the same peaks highlighted in the inter-annual analysis (fig. 9c). Negative anomalies prevail in 2002 and 2003.

Differently from the ALB Sea, in the LEV basin (Fig. 9b) the seasonal cycle of the chlorophyll *a* concentration is more regular. It rapidly increases from early winter months, reaching local maxima values in January–February. In summer, the chlorophyll *a* reaches minimum values and then it increases again in autumn. In Fig. 9b peak values appear in January 1999 and February 2004 reaching chlorophyll *a* concentrations of about 0.08–0.10 $\mu\text{g L}^{-1}$. Among all basins, the anomaly time series of Levantine Basin (Fig. 10b) is characterized by the smallest oscillations, ranging from -0.01 to slightly more than 0.02 $\mu\text{g L}^{-1}$. Despite these low values,

485 positive peaks occur in 2004 and 2006, while from 1998 to 2003, the times series is dominated by negative
486 values.

487 Differently from the LEV, where chlorophyll *a* concentrations are almost one order of magnitude lower than
488 in the other sub-basins, the NADR (Fig. 9d) exhibits the highest values of chlorophyll *a* concentration. In
489 NADR, summer minima never reach values as low as those observed in other three sub-regions contributing
490 to mask the seasonal signal. The NADR inter-annual variability of the chlorophyll *a* concentration is expressed
491 by an irregular trend from 1998 to 2002, a local minimum during 2003 and then a more weakened variability
492 from the end of 2005 to 2009. In this case, main peaks occur in 2000, 2004 and 2010 while, 2003, represent
493 the year of the lowest oscillation. North Adriatic Sea anomalies (Fig. 10d) are the most intense among the
494 four sectors reaching positive values as high as $2.0 \mu\text{gL}^{-1}$ in 2000 and negative values as low as $-0.85 \mu\text{gL}^{-1}$ in
495 2003.

496 In the NWMed basin (Fig. 9a), the contribution of pico-phytoplankton to the seasonal cycle seems to be
497 constant from year-to-year, with values not higher than $0.14 \mu\text{gL}^{-1}$ and a mean concentration of $0.08 \mu\text{gL}^{-1}$.
498 Although the differences between minima and maxima of pico in each year are low, it, however, follows a
499 seasonal variability, with higher values in late winter–early spring, and lower values in summer.

500 Nano-phytoplankton shows the same seasonal cycle of pico (Fig. 9a). Both maxima and minima occur in the
501 same months of the smallest cells, but, in this case, the excursion among them, are higher with respect to
502 those of pico. The peaks occur during the early spring season, reaching an absolute maximum of $0.38 \mu\text{gL}^{-1}$
503 in April 2005 with an annual mean of $0.08 \mu\text{gL}^{-1}$. In NWMed Sea, the largest seasonal variability is due to
504 micro-phytoplankton. Maximum values occur during the spring blooms season with the highest peak of about
505 $0.7 \mu\text{gL}^{-1}$ in April 2005. During summer, micro-phytoplankton reaches very low concentrations, below 0.02
506 μgL^{-1} .

507 In contrast with the NWMed sector, pico-phytoplankton predominates in the LEV (Fig. 9b) all year around
508 with a mean concentration of $0.03 \mu\text{gL}^{-1}$ (Fig. 9b) with a seasonal cycle nearly constant from year-to-year.

509 The nano component shows a higher variability and large seasonal differences between minima and maxima.
510 The peak values, usually, occur in January–February while low concentrations are reached in summer with a
511 mean year concentration slightly higher than $0.01 \mu\text{gL}^{-1}$.

512 Furthermore, the strong and well-known oligotrophy of this basin is reflected in the fraction of micro-
513 phytoplankton, the lowest among the three PSCs, with a mean that is very close zero.

514 Among all the four sectors, the ALB (Fig. 9c) and NADR (Fig. 9d) basins show an irregular inter-annual
515 variability with a nearly absent seasonal cycle in the NADR.

516 In the ALB basin (Fig. 9c) the pico-phytoplankton concentration are relatively low and nearly constant along
517 the entire period (mean value of $0.1 \mu\text{gL}^{-1}$), with small peaks occurring during spring months.

518 Nano-phytoplankton follows the same pattern of pico, but with a higher excursion between minima and
519 maxima. The absolute peak for nano component is in March 2005, with a concentration of 0.30 μgL^{-1} respect
520 to 1.00 μgL^{-1} of TChl α .

521 For the ALB Sea, we observe a less clean seasonal cycle and a reduced year-to-year variability, above all for
522 micro-phytoplankton fraction. Micro-phytoplankton shows a seasonal oscillation with the usual increase
523 during spring blooms and a decrease in summer, as a result of a stratification of the water column. The mean
524 contribution of micro-phytoplankton to the TChl α is about of 0.15 μgL^{-1} , while the maximum is 0.7 μgL^{-1} in
525 March 2006.

526 In the NADR basin (Fig. 9d) the seasonal signal is absent or, at least, not immediately visible. In contrast with
527 the other basins, the chlorophyll α content is very high, and the PSCs ratios show a different behavior with
528 respect to the other sectors. Pico-phytoplankton fraction is nearly constant along the entire time series with
529 low or absent seasonal variations. Pico mean fraction of the value the TChl α is 0.11 μgL^{-1} , which is still higher
530 than the mean value (0.03 μgL^{-1}) of the LEV Sea (Fig. 9b).

531 In NADR, the highest contribution to the TChl α is provided by the micro-phytoplankton (mean value 0.7 μgL^{-1}
532 ¹). It also shows inter-annual variations, but with peaks that occur in different years with respect to the other
533 three sectors. Fig. 9d reveals constant high values of micro in 2001 and 2002, two peaks in November 2000
534 (3.1 μgL^{-1}) and in May 2004 (2.5 μgL^{-1}) and the lowest values in 2003.

535

536 **7 Discussion and conclusions**

537 In this work, for the first time, we estimate the contribution of micro-, nano- and pico-phytoplankton to the
538 total chlorophyll α over the Mediterranean Sea by applying an abundance based model (Brewin et al., 2011b,
539 referred as BR) to the entire time series of the SeaWiFS mission. Since the selected model was developed by
540 using datasets from many different regions of the ocean, we started by verifying its accuracy for the
541 Mediterranean case.

542 This validation showed that the model constantly underestimates nano-phytoplankton fractions over the
543 entire range of observed TChl α concentrations while overestimates pico-phytoplankton concentration, for
544 low TChl α concentrations. These results lead to think that the specific optical properties of this basin can be
545 influenced by the phytoplankton community assemblage as suggested by Volpe et al. (2007). In fact,
546 considering that each region can be characterized by a specific pigment content, we hypothesized that the
547 different pigment ratios can represent one of the possible reason that can justify the observed deviation of
548 the model from the in situ PSCs classification. Therefore, we first investigated whether the global relation
549 between DP and chlorophyll α , used by BR model, is still valid for the Mediterranean Sea and whether the
550 use of a regionally tuned relation can contribute to reduce the observed bias between modeled and
551 measured PSCs. Our results demonstrate that the use of a regional Mediterranean DP function (Di Cicco,
552 2014) reduces the bias to values comparable with those obtained by BR at global scale and suggest that a re-

553 tuning of the empirical BR model coefficients is not a priority, with respect to the main goal of this work. We
554 concluded that, the BR model, even if developed for the global ocean, can still be used in the Mediterranean
555 Sea considering that, when applied to satellite data, the major source of uncertainty is the chlorophyll *a*
556 determination. However, the use of daily chlorophyll *a* data, reprocessed with a regional Mediterranean
557 algorithm for Case 1 and Case 2 waters, allows us to account for the unique optical properties of the
558 Mediterranean Sea, thus reducing the bias between in situ measured and satellite chlorophyll *a* estimate to
559 nearly zero (-0.02mgm^{-3}) with a relative small RMS (0.25mgm^{-3}) (see Table 4 in Volpe et al., 2012a).

560 The analysis of micro-, nano- and pico-phytoplankton satellite time series (1998–2010) allowed, for the first
561 time, to quantitatively describe the seasonal and inter-annual variability of the spatial distribution of the algal
562 assemblage structure. The results indicate that pico-phytoplankton dominates all around the year in most of
563 the Mediterranean Basin, in particular, in ultra-oligotrophic waters. Nevertheless, exceptions are: the
564 northwestern Mediterranean Sea (during the spring bloom), the Alborán Sea, and several coastal areas such
565 as the north Adriatic Sea. In the coastal areas, the contribution of micro-phytoplankton to TChl *a* is always
566 more evident, and can be explained by the high typical nutrients conditions of these regions, that favor the
567 predominance of micro-phytoplankton with respect to the other two size classes (e.g. Siokou-Frangou et al.,
568 2010). On the contrary, in the offshore waters, the contribution of nano-phytoplankton to TChl *a* is of the
569 order of 20–40% remaining mainly constant along the year (Fig. 4). This is consistent with the nano-
570 phytoplankton constant contribution to the Mediterranean primary production observed by previous
571 authors (Uitz et al., 2010, 2012).

572 In ultra-oligotrophic waters, such as those of the Levantine Basin, pico-phytoplankton prevails in the PSCs
573 climatology (section 4). This is justified by the ability of the smallest cells to exploit better the poor nutrient
574 environments, according to their high surface/volume ratio (Le Quéré et al., 2005; Timmermans et al., 2005).
575 Indeed, the summer stratification of the water column, causes a strong decrease in micro chlorophyll *a*
576 contribution, whereas, nano-phytoplankton and pico-phytoplankton survive, adapting to the warmer water
577 state (Fig. 4) (Marty and Chiaverini, 2002).

578 The typical chlorophyll *a* seasonal cycle of the temperate regions occurs in the Mediterranean Sea, with
579 maxima in spring and minima in summer. It results into a seasonal signal of the PSCs distribution,
580 characterized by an increase in the micro fraction in spring and the pico fraction in summer (Fig. 4). This mean
581 seasonal cycle can be significantly distorted in coastal regions, such as the North Adriatic Sea (Fig. 4), where
582 terrestrial inputs from rivers play an important role in modulating the nutrients supply in the upper layer of
583 the water column. In this basin, the micro class dominates all around year, in accordance with the knowledge
584 resulting from in situ measurements of the LTER (Italian Long Term Ecological Research Network) North
585 Adriatic station (Fonda Umani et al., 2005; Cataletto et al., 2012). In addition, in the Alborán Sea, in which
586 the Atlantic inflow modulates the nutrient availability, an intermediate temperate and sub-tropical seasonal
587 cycle is observed, with a chlorophyll *a* maximum in late winter–early spring (Siokou-Frangou et al., 2010). In

588 this region, our analysis reveals that, *in general*, there is not an evident predominance of one class over the
589 others all along the year (Fig. 9c). Micro, nano and pico contribution to TChl *a* is modulated by intermitted
590 processes, such as the variation of the Atlantic flow and *upwelling* events occurring along the Spanish coast,
591 which can cause a vertical uplift of nutrients, especially nitrates, to the surface water layer (Mercado et al.,
592 2005).

593 Inter-annual variability is observed in the entire basin, but the largest inter-annual signal occurs in the
594 northwestern Mediterranean Sea, driven by the year-to-year variation of the intensity and extension of the
595 spring bloom (Fig. 6). During spring relatively high values of chlorophyll *a* are observed in the whole basin
596 (Fig. 6), but above all in the Western basin and in particular in 1999, as reported in Volpe et al. (2012b). A
597 general decrease of spring chlorophyll *a* concentrations occur in 2001, affecting mostly the Eastern basin,
598 confirming Bosc et al. (2004) results. This decrease is reflected in a lower contribution of the micro fraction
599 on the TChl *a* (Fig. 6). A peak of chlorophyll *a* signal occurs in April 2005, accompanied by an increase of
600 micro-phytoplankton with respect to previous years (Figs. 6 and 8b). These anomalous high values of
601 averaged spring chlorophyll *a* field are associated with an overall increase in the concentration of chlorophyll
602 *a*, which occurs in the entire western Mediterranean Basin. They are also linked to an intensification of the
603 spring bloom in the Gulf of Lions (see Fig. 6), where an unusual and strong winter convection occurred in the
604 2005 (Volpe et al., 2012b; Font et al., 2007; Smith et al., 2008). This phenomenon results in a local increase
605 of the micro-phytoplankton fraction with respect to previous years even though the nano and pico
606 contributions to total chlorophyll *a* remain dominant at basin scale (Fig. 6). A second spring maximum is
607 observed in 2008 in both chlorophyll *a* and micro-phytoplankton (Fig. 6), related again to the enhancement
608 of the spring bloom in the Gulf of Lions.

609 The analysis of the year-to-year variability in the PSCs of the NWMed (Fig. 9a) confirms the occurrence of an
610 evident seasonal and inter-annual signal. The seasonal cycle of chlorophyll *a* and thus PSCs is the one typical
611 of temperate areas, with maxima in March and/or April (Fig. 9a). During these spring blooms, the micro-
612 phytoplankton exceeds the other classes, in light of the great amount of nutrients available in the water
613 column. Indeed, in this area, the winter deep and intermediated convection allows to bring up nutrients from
614 the deeper layer (Lévy et al., 1998a, b). This process modulates the year-to-year variability of intensity and
615 duration of spring bloom (Santoleri et al., 2003), which results into a strong inter-annual signal of the micro-
616 phytoplankton concentration, as revealed by our analysis (Fig. 9a).

617 The micro-phytoplankton dominates the inter-annual signal also in the NADR, while both nano and pico-
618 phytoplankton show slight variations (Fig. 9d). The large contribution of the biggest cells to the high values
619 of TChl *a* can be related to the presence of big rivers, such as Po, Brenta, Livenza, Adige and Isonzo. Every
620 year, their runoff causes the release of a large amount of organic particles and nutrients that support the
621 micro cell size growth and development. Our analysis shows that the peaks of micro-phytoplankton biomass,
622 usually, occur in May and November (Fig. 9d), when the river runoff increases due to the more intense rainfall

623 and snowmelt (Struglia et al., 2004; Malej et al., 1995). Anomalous events are recorded in November 2000
624 and May 2004 (Fig. 9d). In the former, the prevalence of micro-phytoplankton on the TChl α can be due to
625 the particular meteorological conditions that occurred in that year. Intense precipitations occurred in
626 November 2000 in the Po hydrographic basin (Stravisi, 2006; Russo et al., 2005), with the consequent
627 intensification of the river outflow. This intensification increased nutrients concentrations in the north
628 Adriatic Sea, contributing to the increase of micro-phytoplankton fraction, as revealed by our analysis. This
629 is also in agreement with in situ observations of the LTER station, located in the Gulf of Trieste, which shows
630 biomass peaks of micro fraction in the same year (Cataletto et al., 2012).

631 An opposite case is the Levantine Sea, where the ultra-oligotrophic regime influences the distribution and, in
632 particular, the contribution of the three PSCs to the TChl α . Fig. 9b shows that most of the TChl α is due to
633 the pico-phytoplankton class, which is predominant all along the year. This can be related to the ability of
634 the smallest cells to live and survive in extreme conditions, such as poor nutrient environments and well
635 stratified water column (Siokou-Frangou et al., 2010; Le Quéré et al., 2005). When the bloom occurs
636 (February), besides the pico class, also the nano fraction increases its contribution to TChl α .

637 In summary, in absence of sufficient in situ data of community composition, our time series analysis
638 demonstrates the potential use of ocean colour imagery for monitoring the phytoplankton assemblage in the
639 Mediterranean Basin. The possibility to identify all the components of the phytoplankton assemblage, in
640 terms of dimensional size, allowed us to provide complementary information to the present knowledge of
641 the Mediterranean phytoplankton composition, which was based so far, only on the dominant phytoplankton
642 types (Navarro et al., 2014). Our analysis demonstrated that the predominance of one group over the other
643 ones strongly depends on the physical-biological processes occurring at the mesoscale, which directly
644 influences the nutrient and light availability, i.e, the principal force for the algae growth. Our analysis
645 demonstrated that, in the evaluation of the contribution of each size class to TChl α , the ratio of diagnostic
646 pigments in relationship to chlorophyll α content is a key factor. This ratio represents one of the elements
647 that is mostly affected by the characteristics of pigment content of each specific region, which itself
648 influences all the algorithm retrieval processes. Moreover, this phenomenon can induce an eventual bias due
649 to the seasonal and inter-annual changes in the relationship between size fraction and TChl α , thus
650 representing a limit for the approaches that are based on the direct fitting of the model with in situ global or
651 regional pigment dataset.

652 Since our pioneering work does not aim to provide a specific regional product for PSCs, we are aware that
653 more efforts need to be done on this regard. As a future perspective, we would like to extend our analysis to
654 other satellite sensors in order to enlarge the PSCs time series, but we will also consider and test other
655 models, based on different variables, with the aim to track, as much as possible, the phytoplankton
656 community evolution from space. Moreover, one of our future project will be to regionalize one of this

657 approach, as well as the BR model, in order to provide a better instrument to retrieve information about the
658 PSCs variability specifically for the Mediterranean Sea.

659

660 **Acknowledgements**

661 This work was financially supported by the EU project FP 7 PERSEUS (Policy-oriented marine Environmental
662 Research in the Southern EUropean Seas) Grant Agreement No. 287600 and the Italian RITMARE Flagship
663 Project. This work was also supported by MyOcean-2: Prototype Operational Continuity for GMES Ocean
664 Monitoring and Forecasting Service (Grant Agreement 283367). We are deeply grateful to Dr. Federico Falcini
665 for his criticism and constructive suggestions and Dr. Simone Colella for his great technical support and his
666 advices during the revision phase.

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Cruise	Date	Location	N° Samples	Depth (m)	TChl <i>a</i> range values (mg m ⁻³)	Sources
Prosop99	14/09/1999 – 03/10/1999	Transmed	255	0 – 50	0.02 – 0.89	SeaBASS
Boussole Mooring	22/07/2001 – 03/12/2006	North-Western Mediterranean Sea	1143	0 - 50	0.02 – 5.52	SeaBASS
Boum08	03/07/2008 – 18/07/2008	Transmed	33	9	0.03 – 0.15	SeaBASS
Boussole03	15/07/2008 – 19/07/2008	North-Western Mediterranean Sea	23	0 - 50	0.08 – 2.20	SeaBASS

896

897 [Table 1 Information about the in situ SeaBASS sub-dataset used for the validation of the application of BR model on the Mediterranean Sea.](#)

898

Uitz et al. (2006) coefficients					
	MBE (mg m ⁻³)	MBE%	MBE%_log10	RMSE%_log10	R_log10
Micro	0.059	14%	-4%	29%	0.6
Nano	-0.060	-34%	67%	79%	0.5
Pico	-0.002	51%	-26%	42%	0.7
Di Cicco (2014) coefficients					
	MBE (mg m ⁻³)	MBE%	MBE%_log10	RMSE%_log10	R_log10
Micro	0.066	21%	-7%	28%	0.6
Nano	-0.013	-5%	8%	22%	0.4
Pico	-0.053	-7%	15%	50%	0.8

899

900 Table 2 Statistical results from the comparison of BR model and in situ PSCs classification obtained using, in the Diagnostic Pigments Analysis, respectively the
901 Uitz et al. (2006) and the Di Cicco (2014) coefficients. Mean Bias Error (MBE) has the same dimensions of in situ observation (x in Table 3), while Mean Bias Error
902 percentages (MBE%), Root mean square error percentages (RMSE%) and linear Pearson correlation coefficients (*r*) are dimensionless and refers to a TChl *a*
903 smoothed with a 5-points running mean. Where there is “_log10” the result refers to log-transformed units, otherwise it is expressed in linear space.

Per cent Mean Bias Error	$MBE\% = \frac{1}{N} \sum_{i=1}^N \left(\frac{Model - x_i}{x_i} \right) \times 100$
Per cent Root Mean Squared Error	$RMSE\% = \frac{1}{N} \sum_{i=1}^N \left(\frac{Model - x_i}{x_i} \right)^2 \times 100$
Pearson Correlation Coefficient	$r = \frac{\sum_i (model_i - \overline{model})(x_i - \bar{x})}{\sqrt{\sum_i (model_i - \overline{model})^2} \sqrt{\sum_i (x_i - \bar{x})^2}}$
Mean Bias Error	$MBE = \frac{1}{N} \sum_{i=1}^N (Model - x_i)$

904

905 [Table 3 Basic statistical quantities used for the assessment of the comparison of the BR model applied on the Mediterranean Sea, using the two different in situ](#)
906 [PSCs classification approaches \(Uitz et al. \(2006\) and Di Cicco \(2014\) coefficients\). N is the number of observations and x is in situ measure.](#)

907

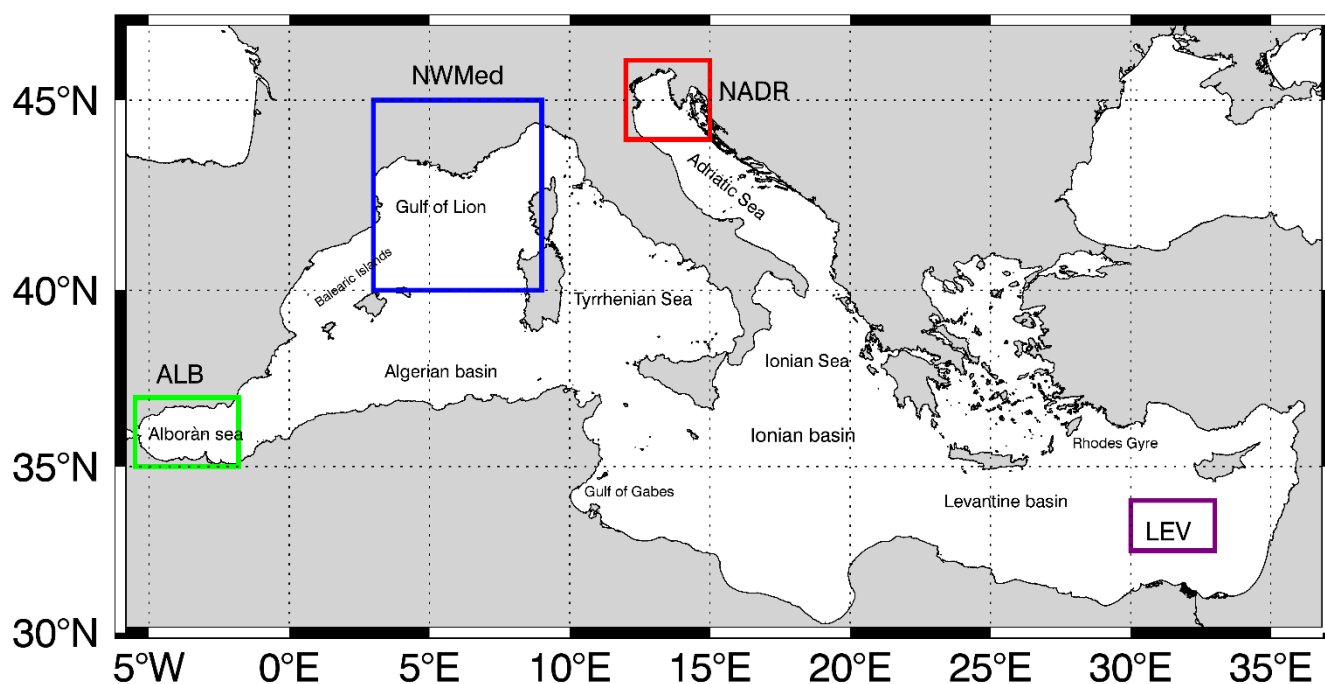
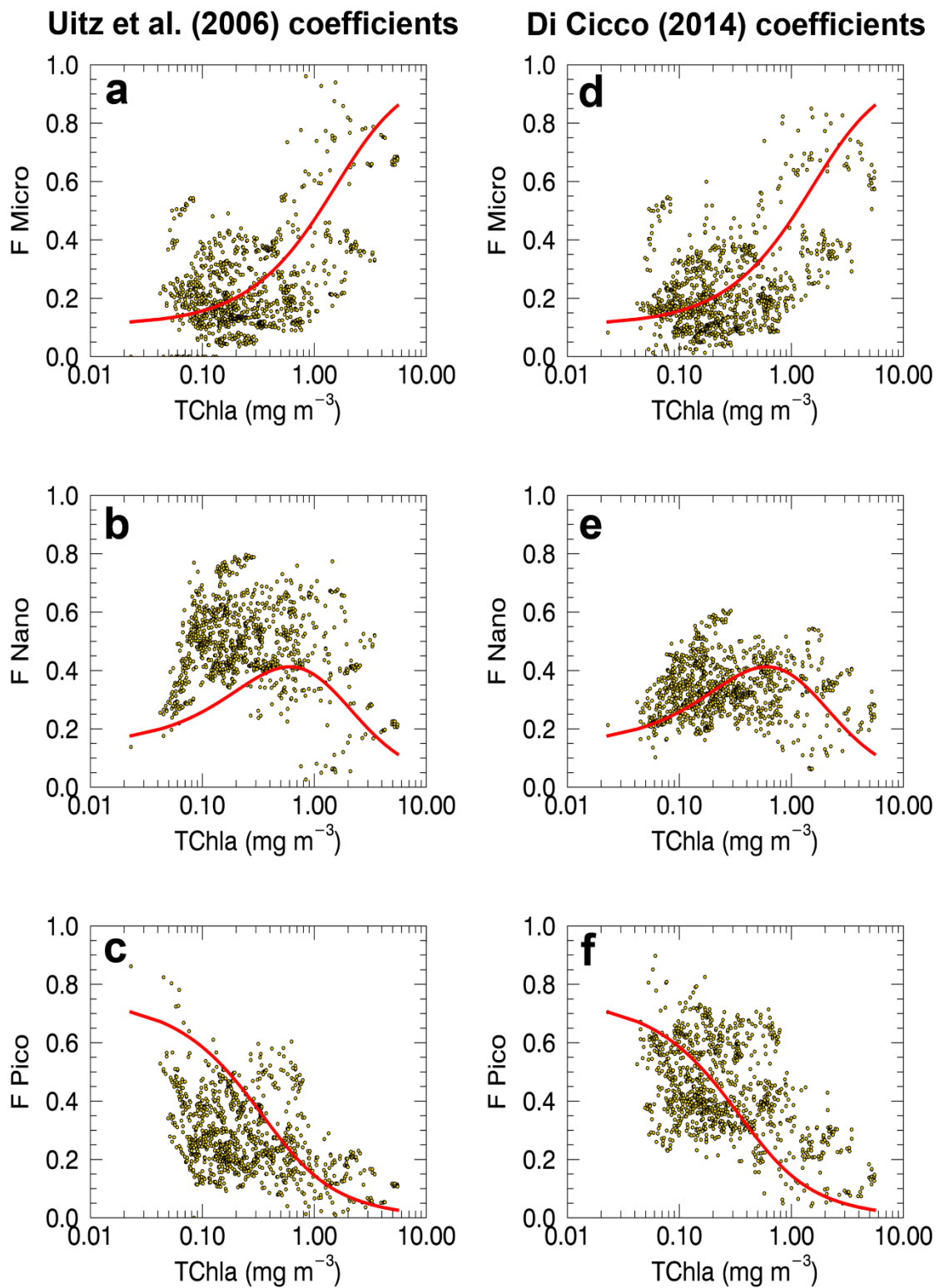


Fig. 1 Maps of the Mediterranean Sea and its most interesting basin or sub-basins. The colored box indicates the region analyzed in section 6 for the seasonal and inter-annual variability of TChl a and PSCs at local scales. The green box refers to the Alborán Sea (ALB), the blue box to the Northwestern Mediterranean Sea (NWMED), the red one indicates the North Adriatic Sea (NADR) and the purple box refers to the Levantine Sea (LEV).



914
 915 Fig. 2 BR model (red line) plotted against in situ PSCs classification (yellow dots) obtained using the Uitz et al.
 916 (2006) coefficients (a-c, on the left panel) and Di Cicco (2014) coefficients (d-f, on the right panel). The yellow
 917 dots refer to the in situ size class fractions resulting from the use of the diagnostic pigments (DP) of the
 918 SeaBASS Mediterranean subset.

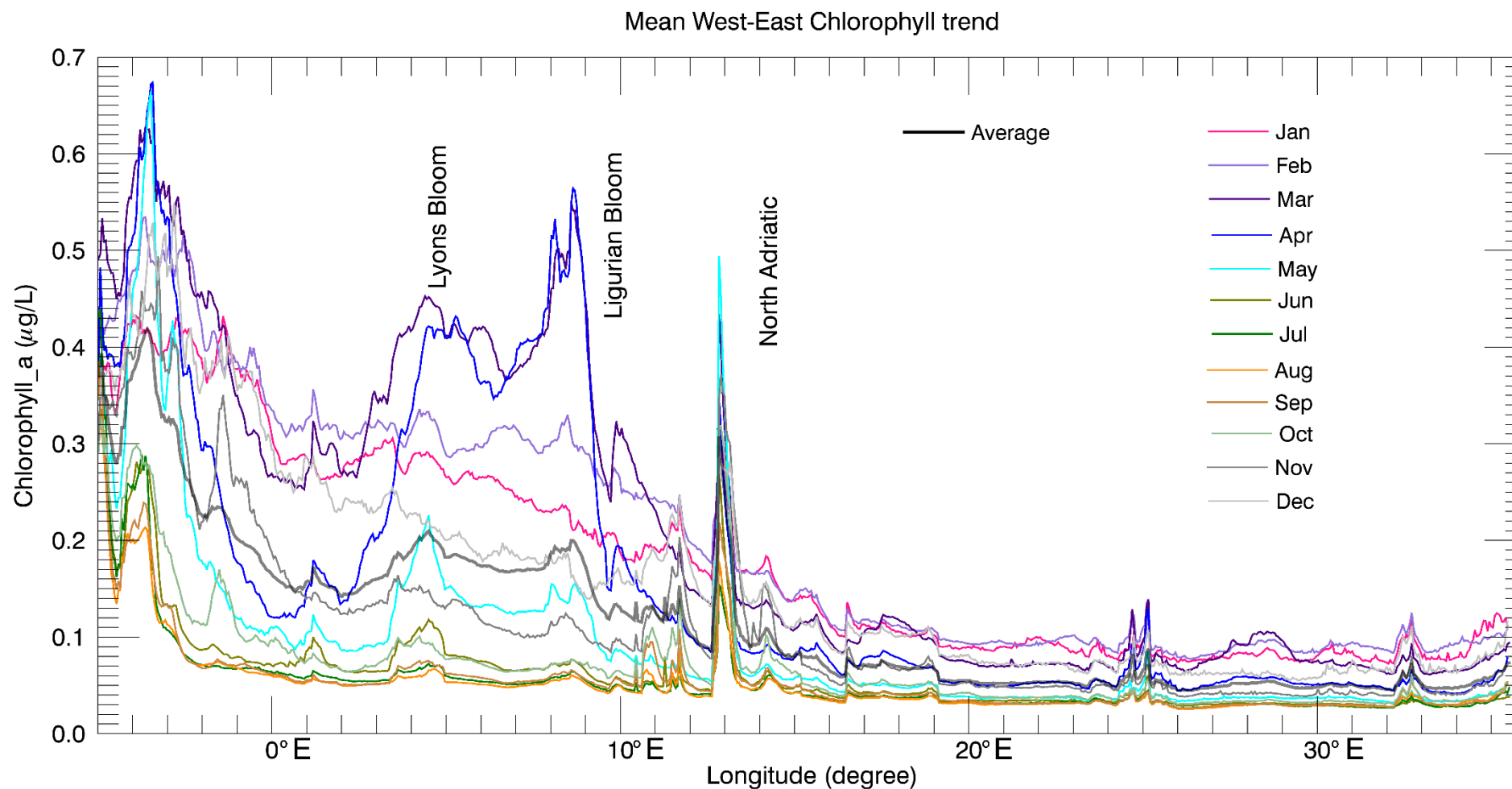


Fig.3 West-Eastward climatological monthly mean chlorophyll a concentration (mgm^{-3}) over the basin, for the time series 1998-2010. The colored lines are build up averaging all pixels from North to South for each longitude degree of the basin, moving from West to East. The high chlorophyll a values of Gulf of Lions, Ligurian Sea and North Adriatic Sea are highlighted in the figure (see also the map of Mediterranean Sea, Fig. 1).

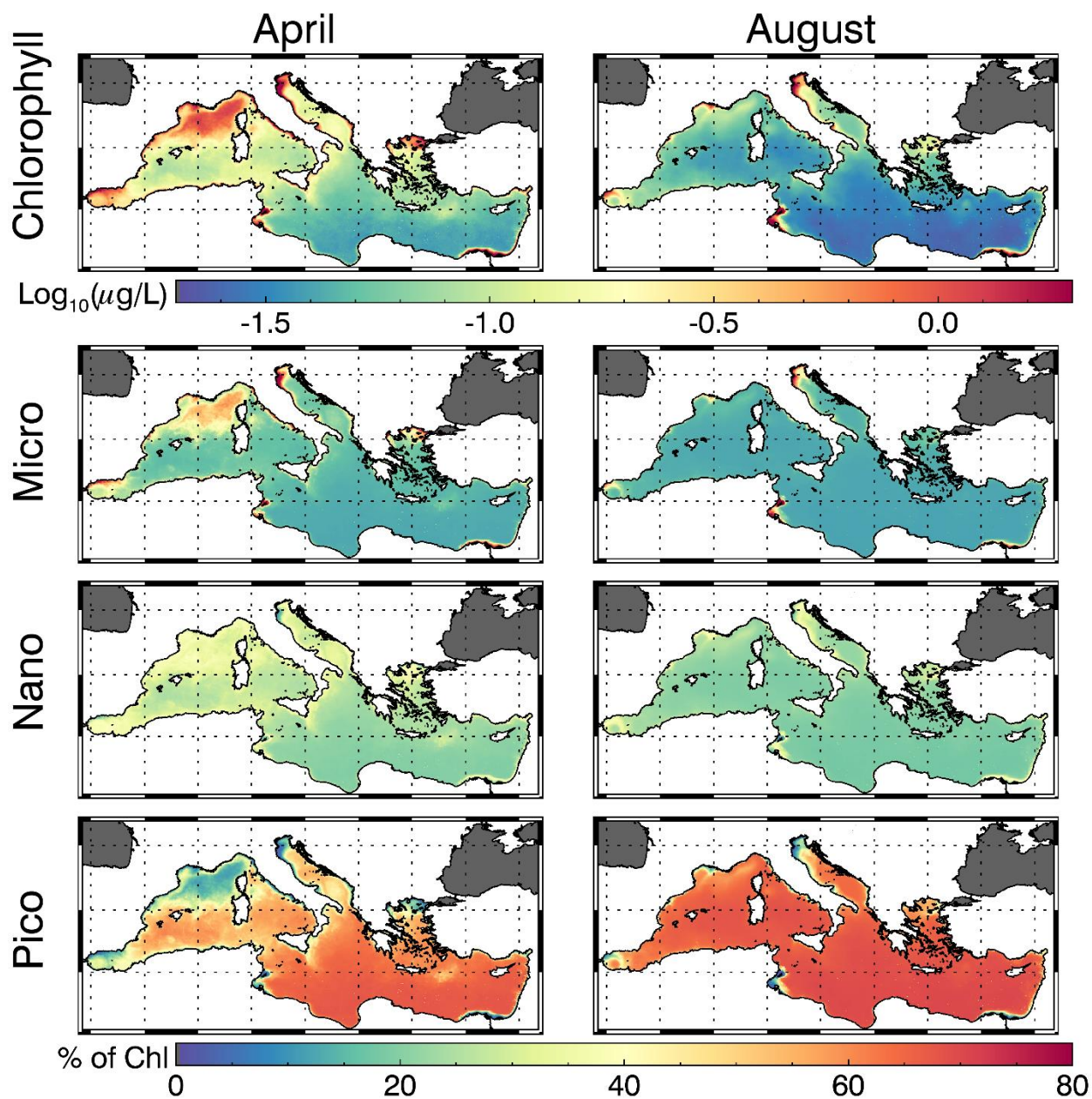


Fig. 4 Seasonal spring to summer excursion in the Mediterranean Sea of TChl *a* and PSCs. On the left panel, the April climatology (1998-2010) maps of TChl *a* (μgL^{-1}) and PSCs (%). On the right panel, the August climatology (1998-2010) maps of TChl *a* (μgL^{-1}) and PSCs (%).

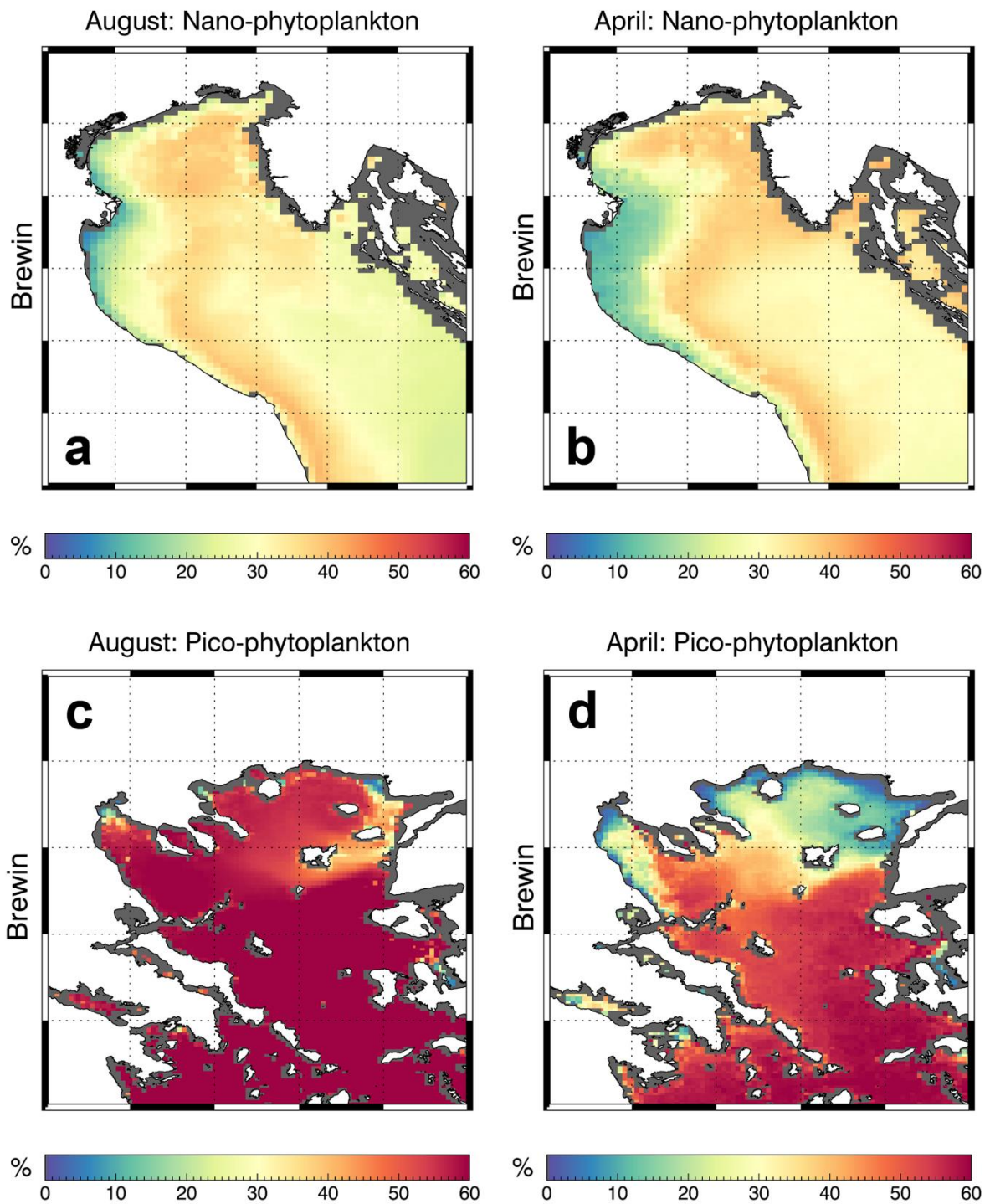


Fig. 5 Seasonal spring to summer excursion of nano- and pico-phytoplankton fractions (%) of TChl *a* in two sectors. Nano percentages (%) in the North Adriatic Sea for August (a) and April (b) climatology (1998-2010). Pico percentages (%) in the Aegean Sea for August (c) and April (d) climatology (1998-2010).

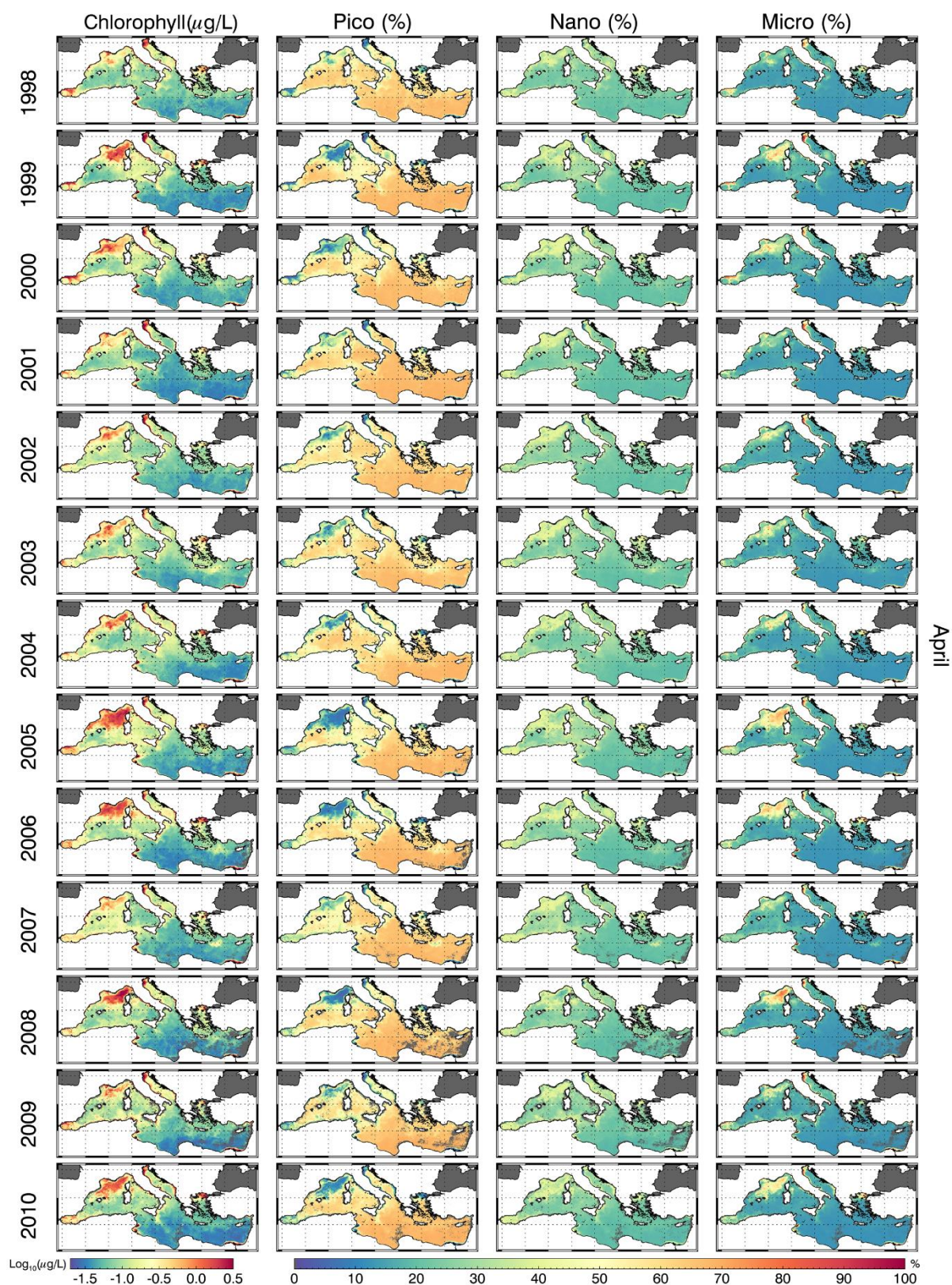


Fig. 6 Monthly maps of inter-annual variability (1998-2010) of TChl *a* and PSCs over the entire basin for April. The first panel refers to TChl *a* ($\mu\text{g/L}$), the second to Pico fraction on TChl *a* (%), the third and the fourth respectively referred to Nano and Micro fractions (%).

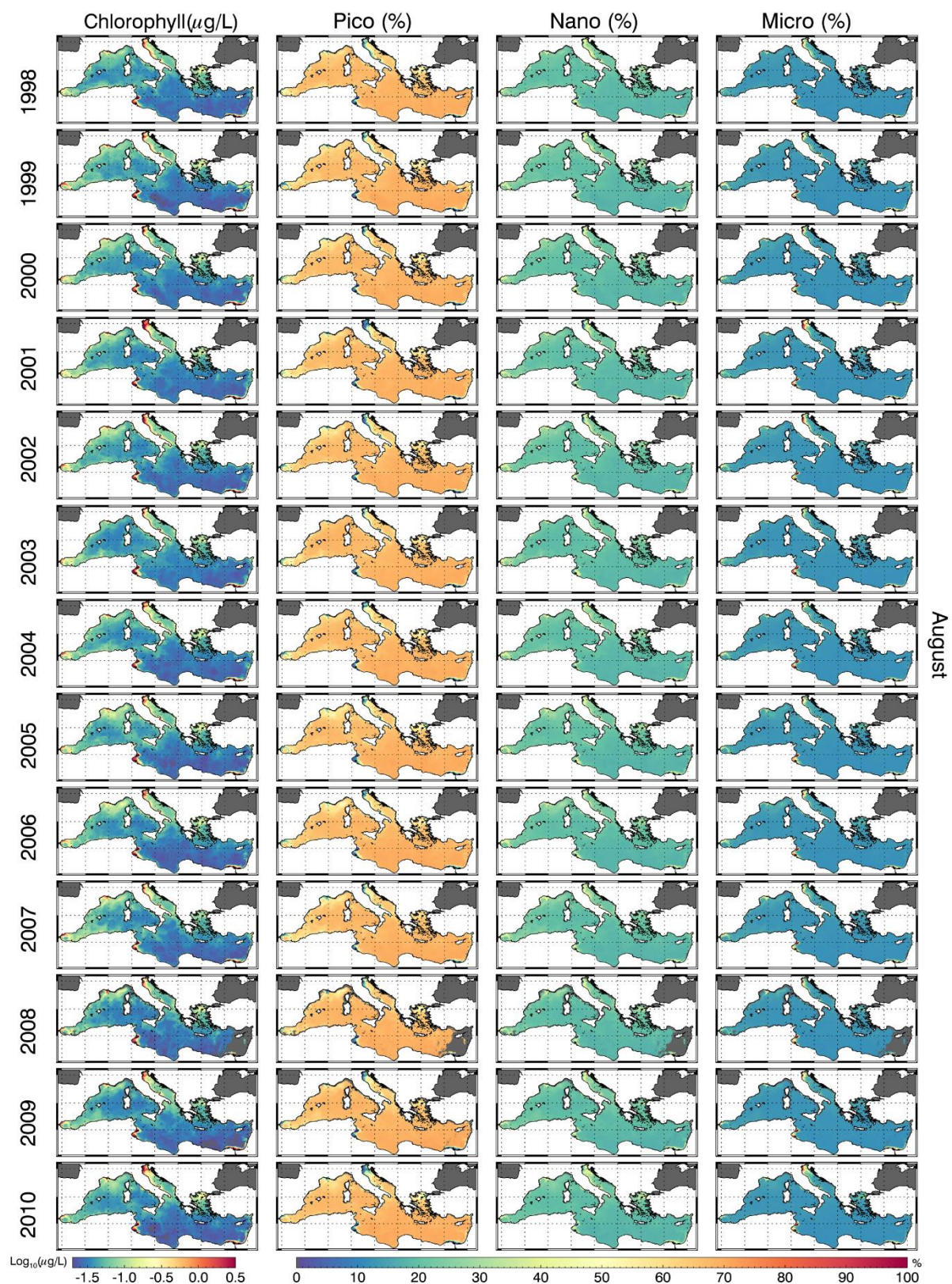


Fig. 7 Monthly maps of inter-annual variability (1998-2010) of TChl *a* and PSCs over the entire basin for August. The first panel refers to TChl *a* ($\mu\text{g/L}^{-1}$), the second to Pico fraction on TChl *a* (%), the third and the fourth respectively referred to Nano and Micro fraction (%).

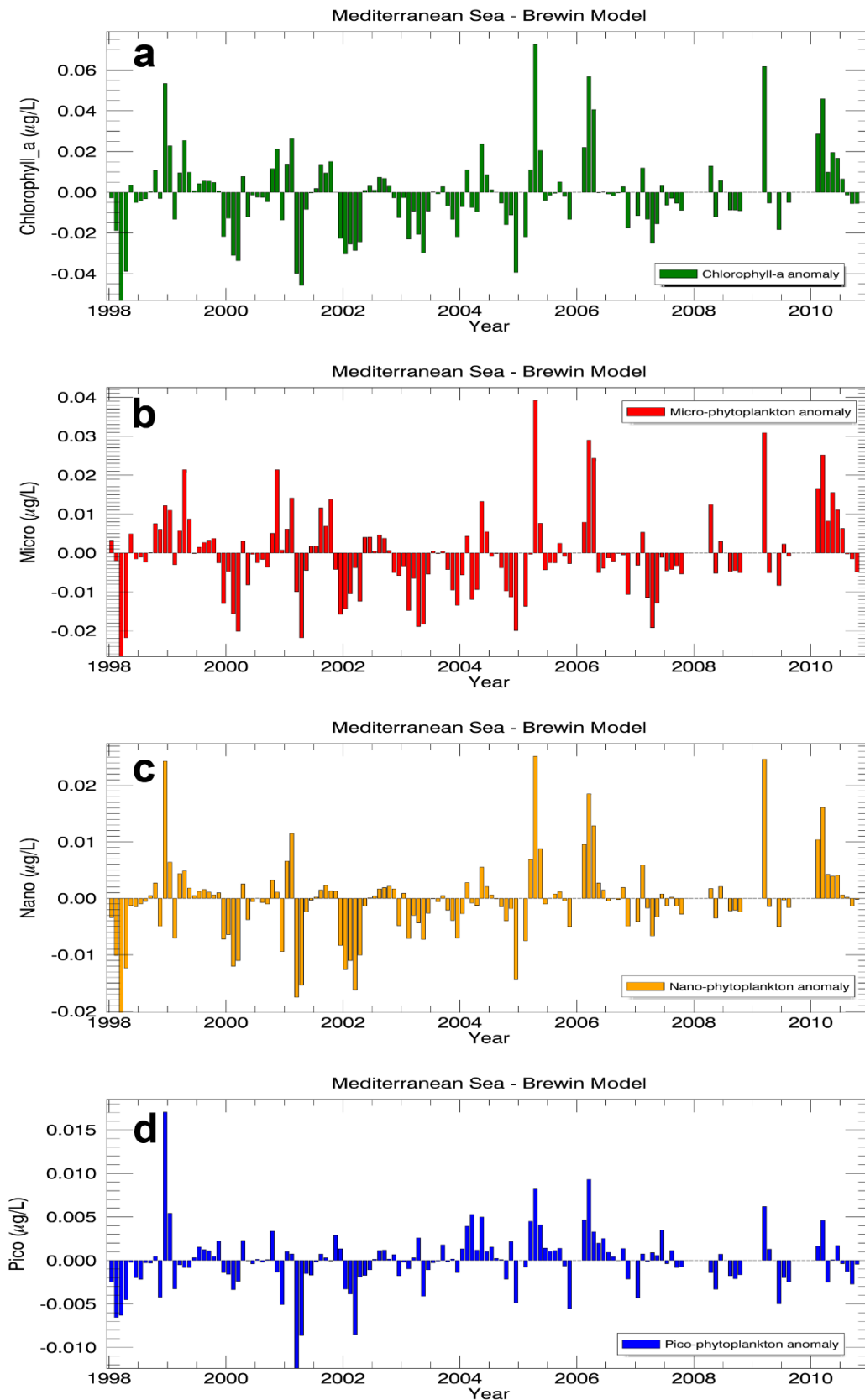
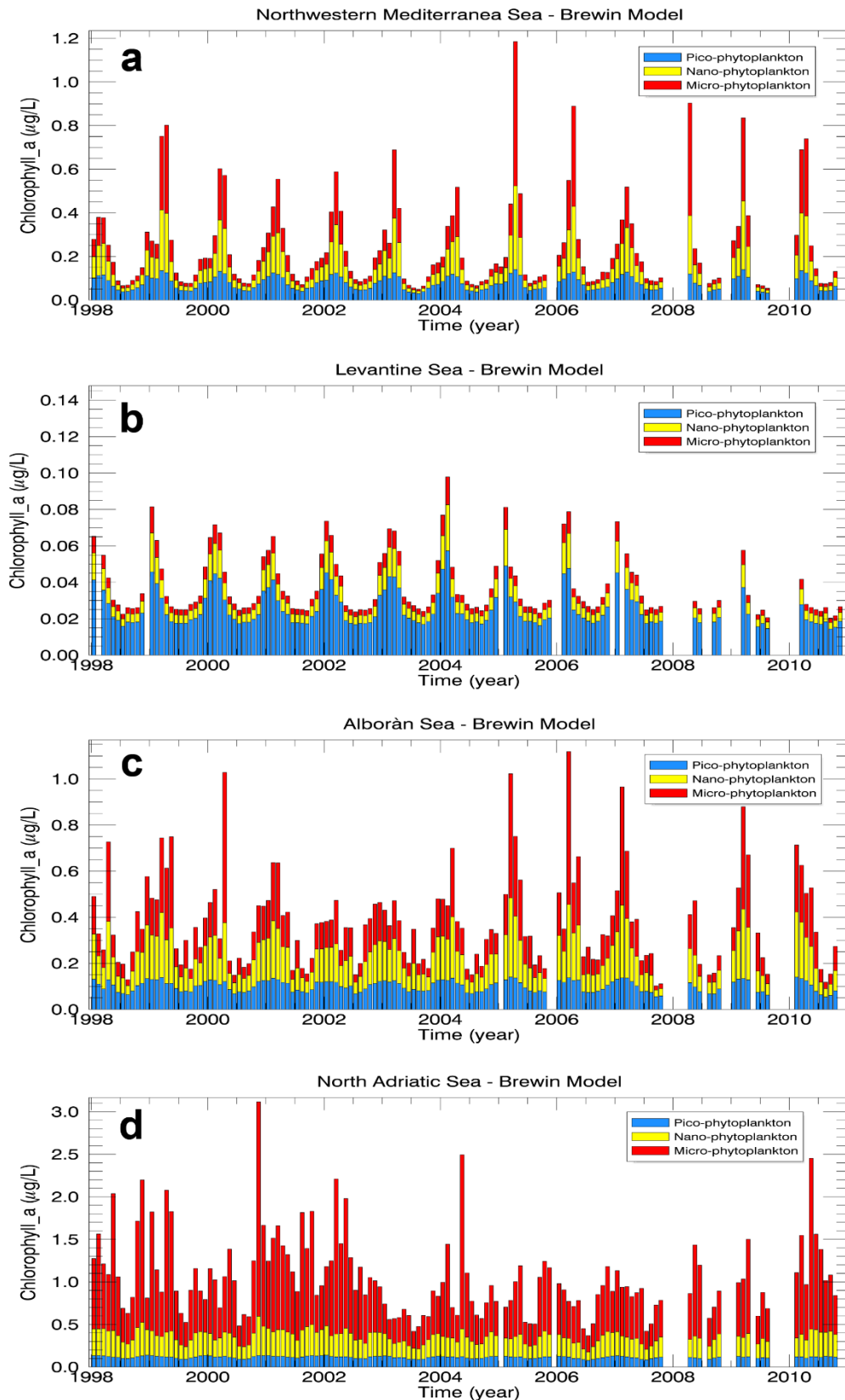


Fig. 8 Monthly anomalies computed for the entire time series (1998-2010) over the Mediterranean basin. Gaps in the time series correspond to months where less than 90% of observations were recorded in the basin. From top to bottom, there are the anomalies of TChl a , micro contribution to TChl a , nano contribution to TChl a , pico contribution to TChl a .



948

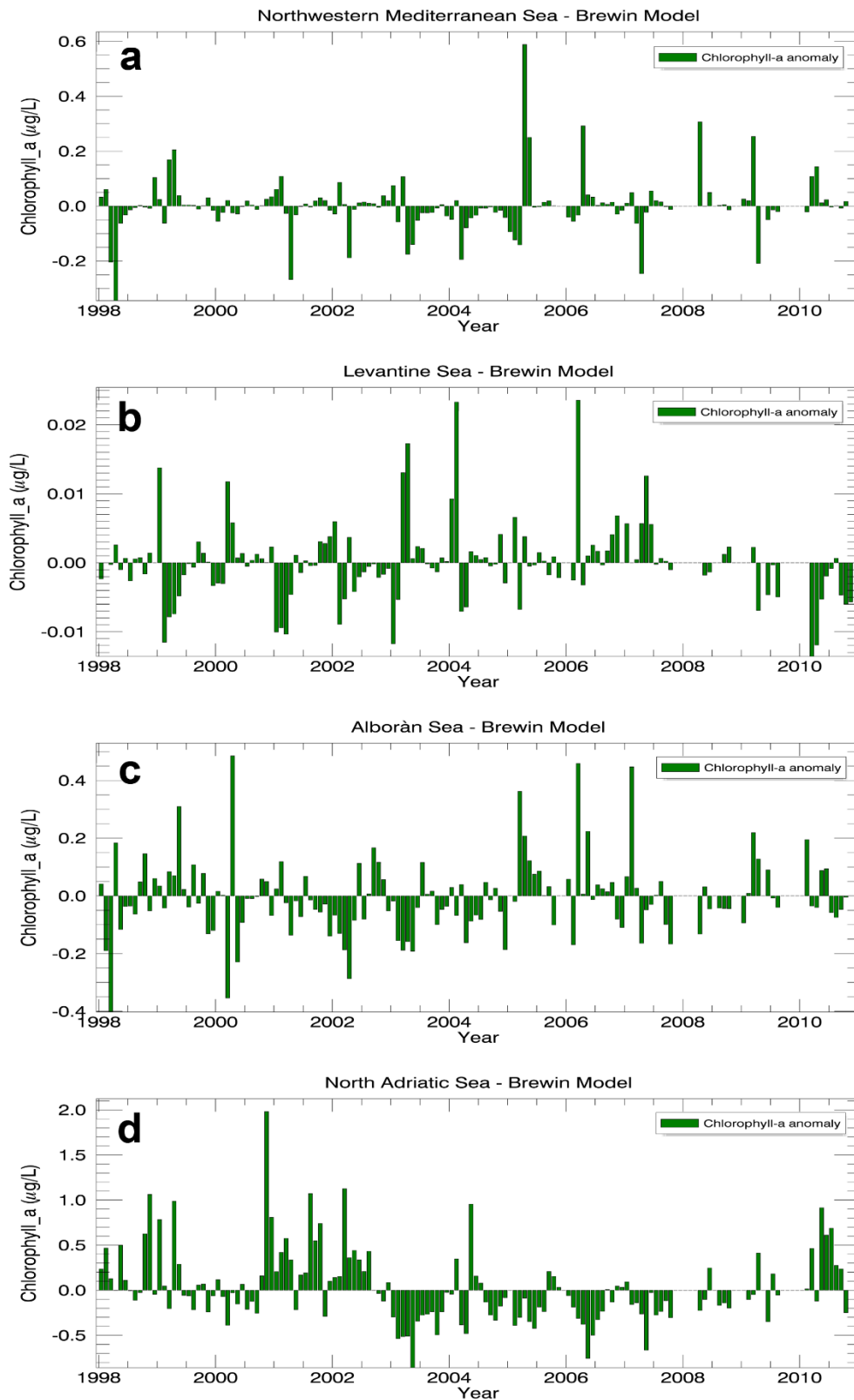
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Fig. 9 Inter-annual variability of the contribution of micro-, nano- and pico-phytoplankton to the TChl a (μgL^{-1}) from 1998 to 2010 in the four sectors: Northwestern Mediterranean Sea (a), Levantine Sea (b), Alborán Sea (c), North Adriatic Sea (d). Gaps in the time series correspond to months where less than 90% of observations were recorded in the region.



953

954 Fig. 10 Monthly anomalies of TChl a computed for the entire time series (1998-2010) over each of the four
 955 sectors. Gaps in the time series correspond to months where less than 90% of observations were recorded
 956 in the region. From top to bottom there are the anomalies of TChl a in the Northwestern Mediterranean Sea
 957 (NWMed), Levantine Sea (LEV), Alborán Sea (ALB) and North Adriatic Sea (NADR).