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Biogeosciences Discussions

Interactive comment on "Dynamics of microphytoplankton abundance and diversity in NW Mediterranean Sea during late summer condition (DYNAPROC 2 cruise; September–October 2004)" *by* S. Lasternas et al.

S. Lasternas et al.

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Review 1 This manuscript deals .... a special issue containing the results of the DYNAPROC 2 cruise.

Authors' comment 1: No comment

Nevertheless, the ms needs some improvements before publication. Specifically, emphasis is put on the almost nil effect of environmental disturbances to infer that the system was close to the steady sate and, therefore, concludes that the change observed in regularity was due to competitive exclusion. Nonetheless, plankton distribu-

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tion in the sea is essentially heterogeneous and observations at a fixed point may be strongly affected by currents, which habitually transport plankton patches with different composition and trophic status. Surface currents, in turn, are influenced by wind, and at least 3 wind events with velocities higher than 30 knots occurred during the period of observations. Consequently, it is difficult to accept, without information on surface currents, that transport did not influence the observed plankton evolution.

Authors' comment 2: This aspect of the influence of current was considered in a early version of the paper but was not fully developed. As noticed by Reviewer#1, the presence of Scrippsiella sp. was mentioned in order to highlight the intrusion of a low-salinity water mass. Emphasis was put on the wind effects and consequently a paragraph dealing with current features will be added. Also the current impact will be investigated through the relationship between phytoplankton composition and stratification index.

Specific comments Microphytoplankton should be replaced by microplankton in the title.... Check the spelling.

Authors' comment 3: Will be corrected

In the last sentence of the abstract it is mentioned that the value of taxonomic studies .....of modifications in the circulation.

Authors' comment 4: The aim of this study was to assess the short-term temporal variations in microplankton and the effect of disturbances. As our results highlighted the presence of some species characteristic from warm waters, the possible impact of global warming was mentioned in the discussion, but it is not the main purpose of our work. The global change implications thus will be removed from the abstract.

Although meteorological and hydrological results are given in other companion papers (Andersen et al.; Raybaud et al.), the results are presented, and later discussed, within the environmental context: maximum abundance under or below thermocline, diatoms

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located in or at around the deep chlorophyll maximum, Scrippsiella in the intrusions of low salinity water. Therefore, it would be of great value the inclusion of few figures showing these main features, which could facilitate go directly throughout the paper without reading other papers.

Authors' comment 5: Agreed.

I find the discussion too long and somewhat unnecessary in some cases.....Taxonomic determinations are not frequently used because of the great effort and long time needed to get the information, which can be obtained with less accuracy but more rapidly using other techniques.

Authors' comment 6: As mentioned by reviewer, taxonomic determinations are rarely used because of time and effort needed. The ms aimed, in part, to argue in favor of expending effort in taxonomic determination while relating for example the role of Dinoflagellates within the foodweb. How can we discuss the importance of the hetero-mixotrophic organisms when reductionist (e.g., methods based on pigment analysis) can not take such organisms into account? However, the discussion will be shortened following reviewer's advice.

Figures 1 to 4, but specifically figures 1 and 2 are too small, at least in the version that I have got. It is extremely difficult to see the numbers and isolines.

Authors' comment 7: Will be changed in the revised ms.

Review 2 This ms describes the abundance ..... are used to test the "Intermediate Disturbance Hypothesis".

Authors: No comment

This ms provides two tables with checklists of species....Is this relevant instead of useful data such as the maximal abundance of each species?

Authors: To give the authorities of each species found in our study is obligatory in a

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scientific paper. We will not change this. To give the maximal abundance is a good idea and we will add a column in this Table

There are not figures of hydrographical conditions, nutrients, etc. Although this ms is a part of an especial issue, data on fluorescence or the concentration of chlorophyll a are here necessary (for example for the numerous references to the deep chlorophyll maxima).

Authors: As the reviewer said, this ms is part of a special issue, and hydrological conditions as well as nutrients concentrations are then easy to found in others related papers. But we will add more graphs to describe water column to make the reading easier.

This ms remarks the originality of the daily sampling strategy.....Please be conscious of the limitations of your sampling strategy.

Authors: the reviewer did not understand. Our results do NOT correspond to samples collected from 16 different stations. The entire sentence in our paper is “In order to describe the hydrological environment, a grid of 16 stations, centered on a fixed station (“central point”), was occupied at least four times during the cruise. The main observations were done near this fixed station.” This is very clear: 16 stations, around the central point, were studied 4 times during the cruise to describe the hydrological environment. All our samples were done at or very near the central point. We will make this fact clearer.

Two meteorological events modified the environmental ....See a description of the method in Dolan et al. (2002) Microzooplankton diversity...; Deep-Sea Res I 49 1217-1232.

Authors: This is a very important point, but we believe reviewer 2 has confused bias linked to H’, J’ or S and counting error of abundance. We have considered these problems previously in some detail (see Tunin-Ley et al., 2007 and 2009,

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respectively in Journal of Phycology and in MEPS). Please see below one part of the ms Tunin-Ley et al. (2007), dealing with study of Ceratium diversity and bias related to each diversity index.

“Calculation and estimation of biodiversity indices. Biodiversity was estimated for three replicates as taxonomic richness (S = number of taxa), Shannon’s diversity index (H’ = –Ppi log2 pi„ where pi = ni ⁄N, ni = number of individuals of one taxon, and N = total number of individuals), and Pielou’s regularity index (J ‘ = H’ ⁄ log2 S). Richness and Shannon’s index are respectively biased by the sampling effort and by the sample size (Dallot 1998). In many cases, values of these indices are underestimated; especially richness (Carpentier and Lepêtre 1999), so we used the nonparametric jackknife 1 method (Manly 1991) to obtain an estimated value that partially corrects this bias for each indicator. For richness, jackknife 1 = SO+{r1(n–1) ⁄ n}, where SO is the observed taxonomic richness, n the number of replicates, and r1 the number of taxa occurring in one single replicate. For diversity and regularity, jackknife 1 = SFi ⁄ n, with Fi = nSt–(n–1)Sti–1, where St is the estimation of the indicator for the n replicates, and Sti–1 the estimation of the indicator for the n–1 replicates. This method appears to be a good intermediate choice in terms of bias and accuracy, according to Carpentier and Lepêtre (1999). Moreover, these estimations were calculated for a cumulated abundance of 100 cells per replicate (i.e., a total of 300 cells per sample). Magurran (2004) recommended abundance values between 200 and 500 individuals for diversity calculation.”

Jackknife 1 method could not be used in our study during DYNAPROC 2 cruise because no replicates were available. However, we counted more than 200 samples, which represents a very important counting effort and is very time consuming. Based on data given in Fig. 1a, most samples had more than 20 000 ind./l. This means that more than 200 cells were counted in 100 ml. With this number of cells, we have a good estimation of H’ and J’; the bias will probably be more important

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for S, but we always count Richness in the whole 100 ml. This means that S evolution really makes sense. Another important point is that for each value of a sample (this mean each black point in Fig. 1 and 2), values of surrounding samples are coherent, even for abundances (Fig. 2). Besides, rare species can not be accurately enumerated in countings when using the Üthermohl method, even by analysing greater volumes such as 2000 ml (see the discussion paragraph on sampling strategy in Tunin-ley et al. 2007). For numerous species with low abundances, the analysis of several dozens of liters, which is only feasible from a net sampling, is necessary. For this reason we choose to employ both hydrological bottles sampling and net sampling, in order to obtain the best and more accurate representation of the microplanktonic assemblage.

Studies on the phytoplankton abundance and diversity have been numerous. Margalef has extensively used the diversity indexes in Mediterranean Sea. However, it is unusual the absence of any citation to Margalef’s ecological papers.

Authors: as said by reviewer 2, “Margalef has extensively used the diversity indexes in Mediterranean Sea” and he is always cited. Even if the work of Margalef is a reference in the topic we choose to use references to more modern authors.

The calculation of the diversity index requires a representative number of specimens in each sample and taxonomical expertise to differentiate the species. This ms is presented as a detailed diversity study, including very rare species (Page 5165, line 27: it is necessary to take into account rare species).

Authors: Yes, it is important to consider rare species. For instance, even if you have 1 ind. of sp A in 100 ml, this will not really change total abundance, but it has an impact on Richness.

I have doubts on the accurate identification of common species.....of warming in the Mediterranean Sea.

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Authors: Quite obviously, the reviewer is correct in saying that “cryo” is related to “cold”. The genus Corethron has been considered as monospecific for a very long time and C. cryophilum was described as a cosmopolitan species in our references. Even european website on algae (Algae base) still consider C. cryophilum as the current name and C. hystrix as its synonym. The phytoplankton nomenclature is continuously subject to modifications as phylogenetic studies validate or invalidate taxonomic names. The validity of a taxon is often closely debated by taxonomists and it takes time before a new name is agreed by consensus and updated in taxonomic books of reference. Therefore, our identification of C. cryophilum can not be considered as a mistake in species determination. Taking into account the possibility of taxonomic confusion, we will replace C. criophylum with C. hystrix, with reference to Crawford et al., 1998.

This ms deals on the short-time variations. Phytoplankton species .... is easy for beginners in the phytoplankton identification.

Authors: The reviewer's comments with regard to our honesty do not merit response. However, we would like to point out that Ceratium species represented the major part of microphytoplankton in our net samples. With regard to other interests of studying Ceratium, please see below one part of the ms Tunin-Ley et al. (2009) in MEPS :

“Considering these challenges, we chose to focus on an armoured dinoflagellate genus, Ceratium Schrank, as a biological model to examine potential effects of global change on phytoplankton biodiversity. This cosmopolitan genus includes about 80 species (Sournia 1986), is found from polar to tropical areas and has been the focus of numerous studies and monographs since the end of the 19th century (e.g. Gourret 1883, Jørgensen 1911, 1920, Trégouboff & Rose 1957a, b, Halim 1960, Sournia 1967, Dodge 1982, Steidinger & Tangen 1997), with limited and generally traceable taxonomic changes over time. An advantage offered by this genus is that identification to species level is more feasible than for other phytoplanktonic groups, where it can be limited by the small size of the organisms or may require the use of electron

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microscopy and molecular tools. Moreover, Ceratium species are known to be sensitive to temperature in terms of biogeography (Dodge & Marshall 1994), seasonality and morphology (Sournia 1967), and have hence been proposed as biological indicators of water masses (Dodge 1993, Okolodkov 1996, Ochoa & Gómez 1997, Sanchez et al. 2000, Raine et al. 2002), current regimes (Dowidar 1973) and climate change (Dodge & Marshall 1994, Johns et al. 2003). In the northwestern Mediterranean Sea, the genus is species-rich and often dominates the armoured dinoflagellates in terms of abundance (Tunin-Ley et al. 2007).”

Concerning Ceratium identification, it is true that the size and the characteristic shape of the organisms make a priori the identification at specific level easier than for some other dinoflagellate or diatom genera. But only a considerable taxonomic expertise allows differenciation of the numerous subspecific taxa, which are often sources of misidentification for non-experts, even at the species level.

As Ceratium species have relatively long doubling time and could be mixotrophic, it was irrelevant to use them in our study? We do not think so. Our purpose was to describe and analyse the evolution of total microplankton in the context of the DYNAPROC 2 cruise. Once again, the genus Ceratium was predominant in net samples, and thus could not be ignored, with regard to its important role in its range of size and its possible interactions within the trophic web.

A species identified as Scrippsiella sp. is considered as an indicator of a coastal intrusion. The precise species identification is required because it is relevant for the results. Species such as Ceratium fusus and C. furca are also neritic bloom-forming species. However, the occurrence in open waters should not be considered intrusions of coastal waters. Taken into account that this ms is a part of a multidisciplinary oceanography study, please provide any evidence (hydrographical data, satellite image) of the coastal water intrusion.

Authors: All Scrippsiella species are considered as neretic. This is not the case of any

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Ceratium species, especially C. fusus or C. furca. Scrippsiella species identification can not be done because our samples are now old (5 years) and they are fixed in acidic lugol's. It is now impossible to see fine details.

The reviewer 2 should note that we are working on the whole microphytoplankton diversity, and that this ms is not a publication dealing with one or two species. Saying that we believe Scrippsiella sp. is sufficient, since all Scrippsiella species are neretic.

The description of the methods is excessively long. For example it is not necessary to cite all the references used for the phytoplankton identification: Tregouboff and Rose (1957a, b), Dodge (1982), Sournia (1986), Balech (1988), Hasle and Syversten (1996), Steidinger and Tangen (1997).

Authors: This suggestion is rather surprising as citation of the taxonomic authorities employed in a taxonomiç study is generally conceded to be essential.

The discussion is full of classical topics in phytoplankton ecology that are not related to the results here presented. I cannot go through all the text.

Authors: We are confused by demands to cite classical Margalef publications (see above), and to not consider classical topics in phytoplankton ecology. This seems contradictory.

I focus on the conclusions: "As DYNAPROC 2 is a multidisciplinary program, our results could easily be analysed in the view of physico-chemical and biological parameters, including zooplankton diversity and abundance as well as microbial community structure and activities. Our results highlighted the value of such data to complete and complement pigment analysis." I have read the ms and I cannot find any example of the relation between these parameters and the results. For example, what is the relation between the dominance of Ceratium and the zooplankton diversity? What is the relation to the chemo-pigments?

Authors: OK, we will discuss the relations between our results and these parameters

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in the ms.

"Moreover the theoretical ecology feature, the knowledge of microphytoplankton short term abundance and diversity evolution supplied complementary information of biogeochemical, biological and ecological interests. It allowed a better understanding of the interactions between autotrophs and nutrients as well as trophic relations with zooplankton". Please provide examples of the relation of the results and the biogeochemical fluxes. This is relevant for the readers of Biogeosciences.

Authors: Relations of our results and biogeochemical fluxes have already been provided in discussion part (importance of silicoflagellates, N2 fixation, etc…)

"Species indicators confirmed the arrival of coastal water and the possible long term warming of NW Mediterranean. We also found some very rare dinoflagellates species, which need genetic analysis to clarify their phylogeny." In a paper focused on the short time variations, the conclusion deals on aspects such as long term warming.

Authors: why not, as we found species that could indicate a warming of Mediterranean Sea?

The last sentence of the conclusion in a paper to be published in Biogeosciences remarks the importance of genetic analysis of very rare species. I disagree; very rare species (only observed each 40 years) have not a significant influence in the pelagic food webs. I do not consider the genetic analysis of rare dinoflagellates as a priority for future research in biological oceanography.

Authors: We do not say that very rare species have a significant influence on pelagic food web. As those species were present in our study, we think it is important to cite them, in order to contribute to a better understanding of their distribution and to complete available records which are very useful to synthesis on phytoplankton geographical distribution. Furthermore, as those species are very rare, we need more information about description, validity of name, as well as biology and ecology. With

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regard to these aspects, molecular biology is a powerful tool that can provide valuable information.

This paper should be presented as a description of the phytoplankton composition and abundance and the relationship to the environmental variables (temperature, salinity), nutrients and pigments.

Authors: OK.

Phytoplankton identification is a laborious task carried out by a single individual. There is an excess of co-authors that does not contribute with any data.

Authors: The reviewer's comment does not merit a response.

The authors should avoid originalities such as testing classical hypothesis in ecology and a discussion in topics that are not related to the dataset.

Authors: As revealing commented above, reviewer 2 did not read all of the discussion (“The discussion is full of classical topics in phytoplankton ecology ….. I cannot go through all the text.”). Then how can it be said that our discussion is not related to our dataset?

Author’s conclusion: Even if this implies that the ms might not be published in BG, authors can not take into account several comments of reviewers 2, which are neither constructive nor scientifically motivated, and which discredit more than help to improve the ms. Authors will take into account the comments of ref 1 and 3.

Review 3 General comments

The manuscript (MS) contains an impressive taxonomic work. In my opinion the intrinsic value of this effort and information might be suitable for publication. However, after reading several times the MS, my impression is that the data treatment and discussion of the results does not correspond with the big effort under taken to obtain the taxonomic information. Furthermore, the title and abstract generate high expectation

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in the reader interested in global change, biogeochemical cycles and trophic interactions, but results and discussion derives in a charged taxonomic description and lacks in physical-biological coupling. This might be due, to the fact that the authors did not found the expected change from stratiﬁcation to mixing conditions they were looking for to test the IDH hypothesis. However, if the test of the IDH hypothesis was the aim of the research carried out, it is surprising that the authors mention difficulties to determine and measure disturbance. I think that a stratiﬁcation index, turbulence measurements or even nutrient input might serve as estimation of a disturbance that affects plankton composition.

Authors’ comment 1: Index stratification deserves to be investigated as turbulence measurements. We will add and analyse those parameters in our revised paper.

The authors mention that the DYNAPROC2 is a multidisciplinary program and that the results could easily be analyzed in the view of physical-chemical and biological parameters (Page, 5185, line 13). But no physical and chemical data were shown in the MS. .....to show the association of different species to distinct water masses.

Authors’ comment 2: We agree with the reviewer and changes advised will be included in the corrected version.

The authors mention taxonomic singularities and link them to global changes, but for these statements long-time observations are necessary. However, as the authors indicate taxonomic approaches are rare and without the implementation of regular monitor programs the detection of a rear species at a certain date does not mean that the species just arrived because of global warming, but that finally the taxonomist encountered the species. Most of the results and conclusion are not new and trivial. Of course, as diatoms, also the presence of silicioﬂagellates inﬂuence the carbon and silicate cycle, and the presence of nitrogen ﬁxing organisms sustain primary production. But something more should be said about the amount of carbon, silicate or nitrogen in the species and, more important, about the canalization

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of these compounds through the pelagic ecosystem. For biogeochemical cycles it does not matter the name of the particle (cell) but the composition and the possible faith of the particle.

Authors’ comment 3: Accepted, we understand the point of view of Reviewer, however, the detection of rare species that may have specific fate and implications within biogeochemical cycles was pointed out in our ms. The proper purpose was to highlight the importance of taxonomic approach that are often ignored in generalist analysis.

Speciﬁc comments The title, mention microphytoplankton, and the reader get the impression that a two-month sampling was carried out. However, the real sampling includes autotrophic and heterotrophic organisms and the two sampling methods (hydrographic bottles and net sampling) do not coincide with the recognized limits of microplankton (20-200 micrometer). In the first method the authors analyze also part of nanoplankton, while the net sampling only is representative for plankton bigger than 53 mm. The sampling does not correspond to a nearly daily sampling from October-September as inferred in the abstract, but to four 5-days sampling intervals in one month (between 17 of September to 17 of October). This should be clarified from the very beginning.

Authors’ comment 4: Ok, we will clarify the title and the abstract since it appears confusing. We should also clarify limits given by Sieburth et al (1978) about size range of phytoplankton. Indeed our ms includes phytoplankton organisms lower than 20μm but which belong to Dinoflagellates, that are commonly defined as microplanktonic organisms. Within a same genus cells may belong either to nano- or microplankton, but they often correspond to the same functional group. Thus, the classification should be established by function group or size group. Here we referred to the theoretical size range of microplankton to define groups of interest and the most appropriate sampling strategy. Thus, hydrological bottle sampling allowed to collect cells that are abundant, relatively small or delicate, and included nanoplanktonic cells. Net

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sampling allowed to collect the larger fraction of microplankton and the rarest taxa. The combination of the two sampling methods provides the best representation of the microplanktonic assemblage. We focussed our study on microphytoplankton, but all the taxa having a potential importance, including microzooplankton, were enumerated. Regarding to the study duration, we will bring clarification from the very beginning as required by Reviewer3.

Following the structure of the sampling strategy, the authors refer always to Leg 1 and Leg 2, but it would be more precise to refer always to the sampling cycle (1, 2, 3, and 4). Accordingly, in spite of describing slight decreases and increases of the calculated indexes (diversity, species richness, regularity) during a disrupted sampling, it could be of help to verify if their exist a statistical difference among the four sampling cycles. It does not need a lot of figures, description and discussion, to check if the perturbation during the cruise affects the taxonomic composition of the planktonic community. But it seems that there are no statistical differences and also no signiﬁcant perturbations, and the manuscript focus on a spatio-temporal description, resumed in the Correspondence Analysis.

Authors’ comment 5: Advice provided by Reviewer 3 will be investigated during the ms correction process.

In the absence of clear temporal patterns, and a persistence of stratified conditions it might be more interesting look for vertical changes in the diversity indices, than looking for temporal changes giving high importance to the last point of almost 18 sampling days (ﬁgure 4); especially if during the last sampling cycle only two samples of net sampling could be taken. Considering the elevate sampling resolution in the vertical (sampling in 10 meter interval between surface and 90m depth, page 5167, line 10) there is a lot of information suitable for publication. By the way, I could not find to which depth correspond the diversity indexes corresponding to the bottle sampling shown in ﬁgure 3.

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Authors’ comment 6: The vertical variation in diversity indexes for bottle samples will be plotted. The diversity indexes in figure 3 corresponds to integrated indexes, to allow comparison with diversity indexes describing net samples.

Overall, results and discussion is too long. In spite of long descriptions, some correlations could be calculated .....Looking for all possible links between taxonomy and the encountered salinity minimum, authors makes contradictions. At one hand, the low salinity is due to advection of coastal water as indicated by Scrippsiella (and the associated community, page 5181, line 2), on the other hand they use the same event to explain a change in the taxonomic composition due to competition, suggesting that the low salinity is a stress factor for diatoms which are substituted by silicioﬂagellates.

Authors’ comment 7: The presence of Scrippsiella confirmed the advection of coastal water with less salinity. But this lower salinity may also affect the composition in species due to the different response of the organisms.

Some technical corrections Concerning the diversity indexes I would change the term of regularity for evenness, in fact this is the term used by Raybaud et al (same issue) for the same indices.

Authors’ comment 8: OK

Page 5171 Line 23: I suggest substituting “salinity isoclines” by halocline. If there is more than one fluorescence, chlorophyll or diatom peak, I suggest a numeration from surface to depth. Page 5172, Line16: Practical Salinity Units (PSU), is not in use Line 20: I suppose that the second peak of diatoms correspond to the 50m peak of fluorescence mentioned in the last line of page 5171. Page 5166 The Lines 1 -15 should be included in material and method.

Authors’ comment 9: OK

Interactive comment on Biogeosciences Discuss., 5, 5163, 2008.