Authors's reply to Anonymous Referee #2 comments on on egusphere-2022-1008 Tzortzis et al.

Dear referee,

Thank you very much for your constructive comments and suggestions, as well as your corrections of the grammar and spelling in order to improve the quality of the manuscript. According to the Biogeosciences guidelines, hereinafter we address the main points you raised. We are confident that following your remarks and suggestions, as well as the ones from the other anonymous referee, we can improve our manuscript for publication in Biogeosciences. Your comments are in black, our replies in blue.

General Comments

Fine-scale physical processes affect the community structures and productivity of marine plankton at various time scales, but the study to explore them are relatively limited due to technical difficulties. This study aims at untangling this problem by applying the combination of a semi-Lagrangian survey, semi-continuous sampling, and biomathematical models. This approach is novel, and the results obtained from the field survey in the Mediterranean Sea seem to be reasonable. I believe that their approach may open the door to the elucidation of complex physical processes that affect marine microbial ecology, though there are still some problems to be answered.

We thank you for this encouraging comment on our scientific approach.

The first problem is that the objective of the present study (this article) is ambiguous. I understand the final goal of their study, but the results obtained this time are too primitive for that. Based on the results obtained, the authors should reconstruct the objective(s) of the "present" study. The authors should be clear about whether this manuscript concentrated on the development of a new method or aimed to elucidate the effects of the frontal structure observed in the South Mediterranean Sea on phytoplankton structures to some degree. In addition to Introduction and Abstract, the title of the article possibly should be changed in association with that.

We acknowledge that we did not sufficiently detail our scientific questions. Our present study is closely related to our previous work (Tzortzis et al., 2021) on the description of the physical characteristics of a **frontal finescale structure** and its effects on the distribution of phytoplankton abundances, in the south part of the Balearic Islands (Mediterranean Sea). The importance of the frontal area studied in the present article is due to the fact that most of the in-situ studies related to the physical-biological coupling at finescale have focused on extreme situations occurring in boundary currents, where intense fronts and dramatic contrasts in water properties are met but are not representative of the global ocean. Indeed, on the contrary, vast oceanic regions are dominated by weak fronts continuously created, moved

and dissipated, and which separate different water masses with similar properties. Very few studies exist in these regions due to the difficulty of performing in situ experiments over these short-lived and small features. In our previous work Tzortzis et al. (2021) we showed that the fine-scale front observed during our cruise in 2018 in an oligotrophic region as the SW Mediterranean maintains the driving role on phytoplankton diversity also in these moderately energetic regions. Nevertheless, this first study did not explain this particular distribution of phytoplankton, and open questions remain: **Is it exclusively driven by the dynamics of the front ? Or, do biological processes also play a role ?**
In the present study, we attempt to explain the patterns of phytoplankton abundances by focusing on the cellular dynamic of these organisms using the size-structured population model developed by Sosik et al. (2003). This is possible thanks to the analysis of phytoplanktonic cells at the single cell level.

We agree that we did not develop enough the potential effect of the frontal dynamics on the structure of the phytoplankton community, especially in the Discussion. We will rework this part, detailing more in depth the potential effect of vertical velocities and water masses properties (temperature, salinity, nutrients) on the phytoplankton communities.

Furthermore, we did not sufficiently discuss the novelty of our methodology, and its implication for future oceanographic cruises. Although some studies have already used the size-structured population model (Sosik et al., 2003 ; Ribalet et al., 2010, Dugenne et al., 2014 ; Marrec et al., 2018) or other models (Geider et al., 1997 ; MacIntyre et al., 2000) to compute phytoplankton growth rates, the novelty of our study is its application in a context of a Lagrangian sampling strategy. Moreover, we applied this model on several phytoplankton groups (not only *Synechococcus* or *Prochlorococcus*, like most studies). To our knowledge, this has not been done before (except the study of Dugenne et al. (2014) which applied it to specific diatoms). We think our methodology applied here, paves the way for future studies.

The second problem is about the robustness and significance of the estimates of growth and loss rates. When we compare two or more values, the intervals of confidence or possible standard errors are indispensable. However, in the present manuscript, there are no remarks on that. If possible, please add the statistical information.

We agree that it is important to add intervals of confidence; in the previous literature this is not done, probably due to the difficulties in calculating them. In any case, as suggested also by the other referee, we have completely reworked the section concerning the methodology of the size-structured population model (section 2.3 in the manuscript). We provide this part further in the present document, in the case that you cannot consult our answer for the other referee. We hope that this new version clarifies the principle of this model. Indeed, statistics are already included in the model: the growth rates were estimated using the maximum likelihood function and 200 iterations were run to estimate the standard deviation of group-specific growth rates using a Markov Chain Monte Carlo (Geyer, 1992 ; Neal, 1993).

English grammatical errors are relatively frequent in this manuscript. The authors should

have it checked by a native speaker or some editorial service. For example, “Numerical simulation have shown” (L3), “Since several years” (L5), and “a precious information” (L7).

Thank you, we will rework the manuscrit with the help of a new co-author English speaker.

These are general comments on this manuscript. The followings are minor specific comments.

Specific Comments

L71 “satellite SWOT will be launched” is correct.

Sorry for the mistake. The SWOT satellite is now in orbit ! It was launched on December 16th 2022. We will modify this section following also the suggestions of the other referee.

L74 What do the authors mean by “moderate energy”? Which energy? And in which way is it important in the selection of the present study site?

Here, we mean that Mediterranean frontal structures are often less intense than those found in boundary currents such as the Kuroshio, that are able to generate vertical velocities in order of 30 m day^{-1} (Clayton et al., 2014). By contrast, vertical velocities in the Mediterranean sea are in the order of 8 m day^{-1} (Barceló-Llull et al., 2021, Tzortzis et al., 2021).

Below is a map of the surface eddy kinetic energy by Pascual et al. (2006), where the contrast between the Mediterranean sea and the western boundary currents is evident.
We will add this reference and explanation in the new version of the Methods section.

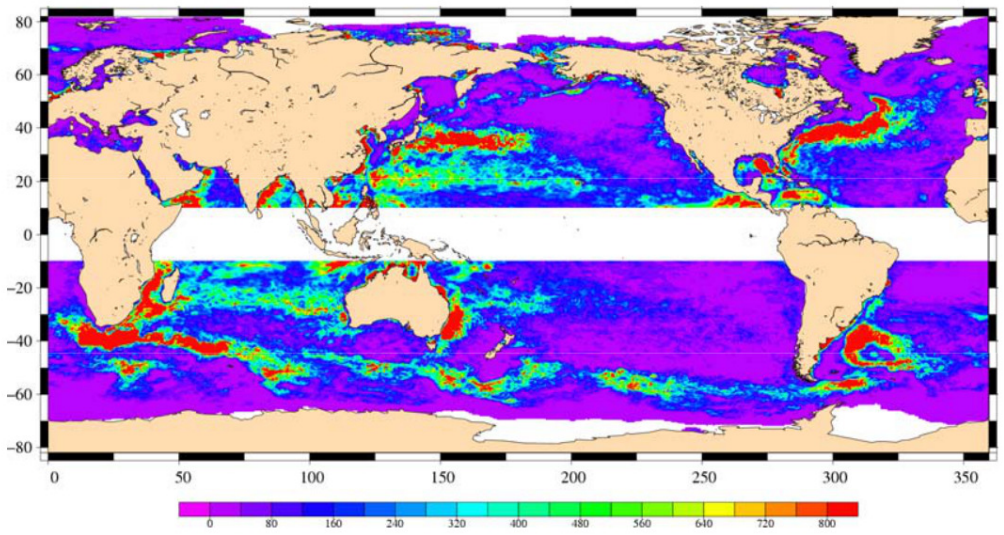

Figure: Eddy kinetic energy (EKE) estimated with 4 altimetric missions (Jason-1 + T/Pinterlaced + ERS-2/ENVISAT + GFO). Units are $cm^2\ s^{-2}$. Figure extracted from Pascual et al. (2006).

L91 “have been measured” should be “were measured”.

Thank you, we will modify it when we rework the manuscrit.

L109 Was the categorization of phytoplankton populations (functional groups) on cytograms made manually on a somewhat arbitrary criterion or semi-automatedly using something like machine learning? How do the authors guarantee the validity and consistency of the categorization?

We have identified several groups of phytoplankton by flow cytometry. This categorization of phytoplankton groups on 2 dimensional plots (cytograms) was made **manually using the conventional criterion determined by flow cytometrists**. Phytoplankton groups were resolved on the basis of their light scatter (namely forward scatter FWS and sideward scatter SWS) and fluorescence (red FLR and orange FLO fluorescence ranges) properties. For instance, *Synechococcus* was unambiguously put in evidence thanks to its higher FLO intensity induced by the presence of phycoerythrin pigments. The optical characteristics of each phytoplankton cluster (or group) are provided in the literature (Dubelaar and Jonker, 2000 ; Reynolds, 2006 ; Thyssen et al., 2008 ; Edwards et al., 2015). We based our categorization on these previous studies. We identified typical phytoplankton groups of the Mediterranean Sea already found by previous works using flow cytometry (Thyssen et al., 2014 ; Marrec et al., 2018). Most of the publications using flow cytometry data to study planktonic cells perform the same way, and rely entirely on the literature and the expertise of the flow cytometrists.

Application of Artificial Intelligence as machine learning to cytometry data is currently under development in our laboratory and the recent work of Fuchs et al. (2022) provided promising results. Unfortunately, this approach is not yet mature enough, which is why we do not use it here.

L114 Show us the time and space (cruise length) ranges that a single sample covers.

We will provide a detailed information modifying the sentence as follow:
"The flow cytometer was connected to sea surface continuous flow, through the system of the thermosalinograph (TSG), (depth: 3 m). The flow cytometer sampled the seawater in a dedicated small container called "subsampler", that isolates the seawater during its analysis. Between two consecutive samples the subsampler was flushed continuously by the seawater circuit of the ship in order to clean and renew the seawater. The subsampler isolated the seawater every 30 min, and two distinct protocols (FLR6 and FLR25) were run sequentially: for FLR6 about 1.3 mL were analyzed in 420 s and for FLR25 about 4 mL were analyzed in 600 s. The use of the subsampler to isolate the volume of seawater subsampled by the flow cytometer allowed us to ignore the movement of the ship, while the flow cytometer performed its analysis. This way the volume analyzed corresponds to a point location rather than a volume spread on the ~2 km covered by the ship in 30 min".

L183 What do the authors mean by "put in evidence"?

We will change it by "identify": "Four eukaryotic picophytoplankton groups **were identified**."

L197 "A similar distribution is observed" should be "A similar distribution was observed". Most of the sentences in this paragraph should be rewritten to past tense.

Thank you, we will carefully check verb tenses in the reworked manuscript.

L204 "In addition to the cell abundances measured along the route of the ship, the phytoplankton diurnal cycle in the two water masses was also reconstructed" This sentence means that the cell abundances were reconstructed first. But, of course, they were not "reconstructed". Rewrite.

We will rework this sentence as follows: "The phytoplankton diurnal cycle in the two water masses was reconstructed [...]."

L205 "each water mass" should be "either water mass"?

Thank you, we will modify it when we rework the manuscrit.

L207 "This adaptive Lagrangian approach allows sampling of the different functional groups of phytoplankton in each water mass" Different functional groups of phytoplankton in

different water masses can be sampled using another approach. I think that this is not the benefit of the adaptive Lagrangian approach. Explain it more appropriately.

We are sorry for the lack of clarity. Following the suggestions of the other referee, in the reworked manuscript we will move this part from the Discussion to the Methods section.
We also will modify the sentences as follow:

"The PROTEVSMED-SWOT cruise followed an adaptive Lagrangian strategy to measure at **high spatial and temporal resolution** several physical and biological variables with both in situ sensors and analysis of the sea surface water intake. The vessel route was designed ad-hoc on the basis of daily remote sensing dataset provided by the Software Package for an Adaptive Satellite-based Sampling for Oceanographic cruises (SPASSO, https://spasso.mio.osupytheas.fr, last access: February 6, 2023). SPASSO used altimetry-derived currents **from AVISO** ("Archiving, Validation and Interpretation of Satellite Oceanographic", https://www.aviso.altimetry.fr, last access: January 18, 2023) and ocean color observations. Chlorophyll a concentrations ([chl*a*], level 3, 1 km resolution) were provided by **CMEMS**, "Copernicus Marine Environment Monitoring Service", https://marine.copernicus.eu, last access: January 18, 2023. In addition, ocean color composite maps were provided by **CLS with the support of the CNES**). They were constructed using a simple weighted average over the previous 5 d of data gathered by the Suomi/NPP/VIIRS sensor. SPASSO generated maps of dynamical and biogeochemical structures in both near real time (NRT) and delayed time (DT).
Maps of [chl*a*] allowed us to identify two water masses, characterized by distinct [chl*a*] values and separated by a zonal front at around 38° 30' N. This front was also detected using the in situ horizontal velocities, temperature and salinity, as described in our previous study. These two water masses were sampled along a designated route of the ship, represented in black in Fig. 1. Special attention was paid to adapting the temporal sampling in order to measure the phytoplankton diel cycle in each water mass. This was achieved by continuously sampling across both water masses along transects. While the ship did not remain in each water mass for 24h, day-to-day variability remained low and measurements from several days were combined into one diel cycle (Fig. 1). The shape depicted by the ship's track led us to call these area north–south (NS) hippodrome (bold black line in Fig. 1) performed between 11 May at 02:00 and 13 May at 08:30 UTC."

L217 "Furthermore, the comparison between the biovolume observed in situ and the biovolume predicted by the model is sound and confirms that the model-predicted cell size distributions well recapitulated the diurnal cycle reflecting either growth or cell division."
Could the authors show any data or figure to support this?

We apologize for the lack of clarity. This sentence refers to Figure 4 in the manuscript. In order to also take into account the suggestion of the other referee, we will modify the text as follows:
"For *Synechococcus*, in the older AW the observed size distribution (i.e., observed biovolume) is similar to the prediction of the model (i.e., predicted biovolume). Both display

a day-long large size-class distribution centered approximately on 0.3 µm^3. In the younger AW (Fig. 4a, 4c) the distributions of biovolume observed and predicted are narrower than in the older AW and centered approximately on 0.2 µm^3 (Fig. 4b, 4d)."

L223 As mentioned in my General Comments, I request the authors to show the interval of confidence or something that can evaluate the robustness of the estimates presented by the present method. This will enable us to compare the values of different phytoplankton groups and water masses on a statistical basis. I can find something like that in Table 2, but I fail to see what it means. When the authors consider the interval, is it significant to discuss the "difference" between the two water masses?

In the manuscript, the standard deviation of the growth rates is indicated in Table 2, but in the reworked version we will also include it in the text. As previously mentioned, we will completely rework the methodological section concerning the size-structured population model, following the comments of the other referee.

The size-structured population model

We used the size-structured population model described by Sosik et al. (2003) and adapted by Dugenne et al. (2014) and Marrec et al. (2018), to estimate phytoplankton in situ growth rates.

The model uses as input the phytoplankton cell volume (biovolume) derived from cell light scatter intensities (FWS). Biovolumes were estimated using coefficients previously obtained by measuring a set of silica beads with the flow cytometer following the same settings used for phytoplankton analysis. The coefficients β_0 and β_1 used to convert FWS (arbitrary units, a.u.) to biovolume v (µm^3) were derived from a log-log regression between FSW and silica bead volumes. These methods come from the studies of Koch et al. (1996) and Foladori et al. (2008).

$v = exp(\beta_0) \times FWS^{\beta 1}$ with in our case $\beta_1 = 0.9228$ and $\beta_0 = -5.8702$

In the size-structured population model, cells are classified into several size classes according to their dimensions at time t. Δv is chosen in order to have enough number of classes m to cover the entire observed biovolume v, from v_1 to v_m (cf Figure A). Classes are logarithmically spaced as follows:

for i in 1,2,…,m $v_i = v_1 \, 2^{(i-1)\Delta v}$ with Δv constant

For *Synechococcus*, $\Delta v = \frac{1}{6}$ and $m = 40$, so that the model size classes encompassed our full measured size distributions.

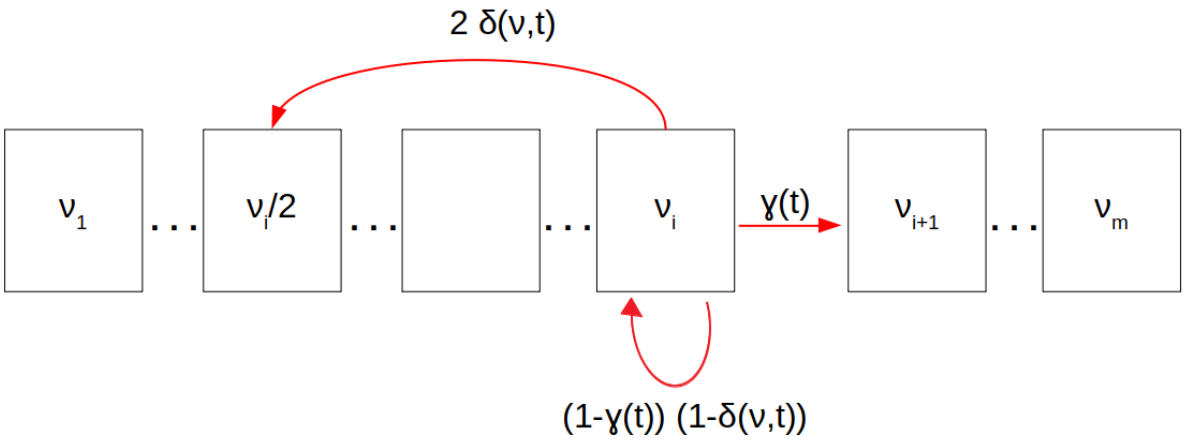

Figure A: Cell cycle stages in the size-structured population model. Cells may grow to the next size class (γ) or be at equilibrium ($1-\gamma(t)$) ($1-\delta(\nu,t)$). Above a particular size, cells are large enough to divide in two daughter cells with probability (δ). Figure adapted from Sosik et al. (2003).

At any time t, the number of cells in size classes $\vec{N}$ (and $\vec{w}$ its corresponding normalized distribution), was projected to $t + dt$ via matrix multiplication:

$$\vec{N}(t + dt) = A(t)\,\vec{N}(t) \qquad \text{and} \qquad \vec{w}(t + dt) = \frac{A(t)\,\vec{N}(t)}{\sum A(t)\,\vec{N}(t)}$$

We chose dt = 10 min (i.e., $\frac{10}{60}$ h) as Sosik et al. (2003) and Dugenne et al. (2014), because for this time step, cells are unlikely to grow more than one size class.

$A(t)$ is a tridiagonal transition matrix that contains (cf Figure B):

1) γ: the probability of cellular growth
2) δ: the probability of cells entering mitosis
3) the cells stasis, i.e., the probability for cells to maintain their state (i.e size) in equilibrium during the temporal projection.

$$\begin{array}{c} \\ v_1(t+dt) \\ \vdots \\ v_j(t+dt) \\ \vdots \\ v_m(t+dt) \end{array} \begin{array}{c} \begin{array}{ccccc} v_1(t) & \dots & v_j(t) & \dots & v_m(t) \end{array} \\ \begin{pmatrix} 1-\gamma(t) & \dots & 2.\delta(v_j,t) & \dots & 0 \\ & & & & \\ 0 & \ddots & (1-\gamma(t)).(1-\delta(v_j,t)) & \ddots & 0 \\ & & & & \\ 0 & \ddots & 0 & \ddots & 1-\delta(v_m,t) \end{pmatrix} \end{array}$$

Figure B: Matrix transition A(t). (Figure extracted from Dugenne, 2017 (thesis)).

The temporal projection

$$N_{|v=v1}\,(t+dt) = (1 - \gamma(t)).N_{v=v1}(t) + 2\delta(v_j, t).N_{v=vj}(t)$$

$$N_{|v=vm}\,(t+dt) = (1 - \delta(t)).N_{v=vm}(t) + \gamma(t).N_{v=vm-1}(t)$$

Probability of cellular growth

The probability of cells growing (γ) to the next size class depends only on the light intensity (irradiance) necessary for photosynthesis, expressed as:

$$\gamma(t) = \gamma_{max}\,[\,1 - exp(-\frac{E(t)}{E^*})]$$

γ_{max}: maximum proportion of cells growing (dimensionless quantity)
E: irradiance (µE m^{-2} s^{-1})
E*: irradiance normalizing constant (µE m^{-2} s^{-1})

Probability of cells entering mitosis

According to Dugenne et al. (2014), δ expresses a proportion (between 0 and 1) modeled by the combination of two Normal distributions ($\mathcal{N}$). One is linked to the cell size, the other is linked to the time of cell division. Both imply an optimum, reached at $\bar{v}$ and $\bar{t}$ respectively, for cell division above which the cell size and the timing of division is suboptimal.

$$\delta(t, v) = \delta_{max}\,\mathcal{N}(\bar{v},\sigma_v^2)\,\mathcal{N}(\bar{t},\sigma_t^2)$$

δ_{max}: maximum proportion of cells entering mitosis (dimensionless quantity)
$\bar{v}$: mean of the size Normal distribution (µm^3)
σ_v: standard deviation of the size Normal distribution (µm^3)
$\bar{t}$: mean of the time Normal distribution (h)
σ_t: standard deviation of the time Normal distribution (h)

Cells stasis

A third functional proportion is included in the transition matrix $A(t)$, to represent cell stasis. Since this function illustrates a non-transition, it is modeled by the proportion of cells that neither divided nor grew between t and $t + dt$.

$$[1 - \gamma(t)]\,[1 - \delta(t, v)]$$

Optimal parameters

The set of parameters, θ is estimated by maximum likelihood function, assuming errors between observed $\vec{w}$ and predicted $\widehat{w}$ normalized size distributions. Their standard deviations are estimated by a Markov Chain Monte Carlo approach (Geyer, 1992 ; Neal, 1993) that sample θ from their prior density distribution, obtained after running 200 optimizations on bootstrapped residuals to approximate the parameter posterior distribution using the normal likelihood. (The likelihood function represents the probability of random variable realizations conditional on particular values of the statistical parameters).

$$\theta = [\gamma_{max},\ E^*,\ \delta_{max},\ \bar{v},\ \sigma_v,\ \bar{t},\ \sigma_t] = argmin(\sum(\theta))$$

$$\sum(\theta) = \sum_{t}^{t+dt} \sum_{i=1}^{m} (w(t) - \widehat{w}(t,\theta))^2$$

$$\widehat{w}(t,\theta) = A(t\text{-}dt,\ \theta)\ \vec{w}(t\text{-}dt) \quad \text{and} \quad \widehat{N}(t,\theta) = A(t\text{-}dt,\ \theta)\ \vec{N}(t\text{-}dt)$$

Table: Model parameters being optimized.

Parameters	Definition	Interval	Units
γ_{max}	Maximum proportions of cells in growing phase	[0, 1]	∅
E^*	Irradiance normalizing constant	[0,∞[	µE m^{-2} s^{-1}
δ_{max}	Maximum proportion of cells entering mitosis	[0,1]	∅
$\bar{v}$	Mean of the size Normal distribution	[v_{min} , v_{max}]	μm^3

σ_v	Standard deviation of the size Normal distribution	$[10^{-06},\infty[$	µm^3
$\bar{t}$	Mean of the time Normal distribution	$[1, 24\frac{1}{dt}+1]$	hours
σ_t	Standard deviation of the time Normal distribution	$[10^{-06},\infty[$	hours

Growth rate and loss rate

Once optimal parameters are identified, the growth rate is calculated using the time projection of the initial size distribution $\vec{N}$. 200 iterations were run to estimate the standard deviation of group-specific growth rates.

$$\mu_{size} = \frac{1}{t+dt} ln\left(\frac{\sum_{i=1}^{m} \widehat{N}_i(t+dt)}{\sum_{i=1}^{m} \vec{N}_i(t)}\right)$$

$\vec{N}$: observed size distribution at $t = 0$ (cells cm^{-3})
i: ith size class
$\widehat{N}$: predicted size distribution (cells cm^{-3})
m: number of size classes
dt: time step (h)
$t + dt$: temporal integration of the distribution projection (h), $t + dt = 24$ h
μ_{size}: growth rates (day^{-1})

The model estimates a population intrinsic growth rate μ_{size} , and a specific loss rate, over a 24 h period.
The daily average population loss rate $\bar{l}$ is obtained by the difference between the intrinsic growth rates μ_{size} and the hourly logarithmic of observed size distribution $\vec{N}$.

$$\bar{l} = \int^{dt} \mu_{size}(dt) - \frac{1}{dt} ln\left(\frac{\vec{N}(t+dt)}{\vec{N}(t)}\right)$$

L223 What do the authors mean by a negative loss rate? I think that it should be shown as a positive value if the loss term is significant.

The model estimates a population intrinsic growth rate, μ_{size} , and a specific loss rate, *l(t)*, over a 24 h period. The loss term includes both biological losses (grazing or death, always negative) but also physical losses (e.g., advection, which can be positive or negative, see Sosik et al., 2003).

L224 "a low division rate" should be "a low loss rate"?

Indeed it is "a low loss rate".

L225 We are not able to judge whether the difference is "significant", without an appropriate statistical figure. Did the authors conduct a statistical test? In which way? What was the level of significance?

We did not conduct a statistical test, but in the reworked manuscript we plan to measure the "fit" of the model, i.e. compare observed vs predicted cell distributions (e.g. Fig. 4a vs 4c). The idea is to recover the error used to define the "best fit" and the best parameters (i.e. error of the selected model).

L244 "largest cells of *Synechococcus* are dominant" This sounds unnatural. "large cells" or "larger cells" may sound more natural.

Thank you for your English corrections.

L245 "This is due to the fact that the older AW is composed of *Synechococcus* cells transiting in all the cell cycle stages all day long". That the older AW is composed of *Synechococcus* cells transiting in all the cell cycle stages all day long is not a "fact", but a suggestion or speculation derived from the present observation. The authors should be more careful about it.

The sentence will be reformulated as follows: "The model results suggest that in the older AW *Synechococcus* cells transit in all the cell cycle stages all day long."

L250 "The patchiness of a distribution" laterally means how frequently "patches" are observed in that distribution. It does not mean how dispersed it is over a wide range. This misunderstanding may be critical in this discussion.

We apologize for the lack of clarity. We will remove this sentence, as we don't think this information is relevant because it is kind of repetitive with what we wrote before, and modify this part also taking into account the comments of the other referee:
"The model results suggest that in the older AW *Synechococcus* cells transit in all the cell cycle stages all day long. Furthermore, in the older AW the cells display a day-long large

size-class distribution centered approximately on 0.3 µm^3 while in the younger AW (Fig. 4a, 4c) the distribution is narrower and centered approximately on 0.2 µm^3 (Fig. 4b, 4d)."

L257 Avoid using any contraction (including "couldn't") in academic writing.

Thank you for your English correction.

L258 What is an "important biodiversity"? I believe that biodiversity is always important.

We apologize for the misuse of the adjective "important". Of course, we do agree on the importance of biodiversity! We will modify the sentence as follows:
"Picophytoplankton is often characterized by the presence of several taxa with potentially different effects on the population dynamics, whereas nanophytoplankton is mostly dominated by diatoms in the Mediterranean Sea (Siokou-Frangou et al., 2010 ; Marty et al., 2002 ; Navarro et al., 2014 ; El Hourany et al., 2019). "

L261 Does this mean that the authors should have conducted molecular analysis (e.g. metabarcoding) to elucidate which taxonomic group each flow cytometric population is composed of? Although it requires flow sorting before analysis, is it a possible future plan? Anyway, the authors mention "this hypothesis" here, but I could not find any hypothesis to be tested from this paragraph. Please reconsider the issues to be discussed here and rearrange this paragraph.

As mentioned in the methodology section, several phytoplankton groups were identified by flow cytometry. This analysis allowed us to detect various groups of eukaryotic nanophytoplankton (RNano and SNano) and eukaryotic picophytoplankton (Pico1, Pico2, Pico3, PicoHFLR). *Synechococcus* is a prokaryotic picophytoplankton, but we have made a distinction between the picophytoplankton group and *Synechococcus* group because this latter was unambiguously resolved by flow cytometry thanks to its higher FLO intensity induced by the presence of phycoerythrin pigments. Idem for Cryptophytes which also have a peculiar and unambiguous optical signature.

Unfortunately, we did not conduct molecular analysis, that is why we are not able to identify the taxa contained in pico- and nanophytoplankton groups. In our future cruise (spring 2023), we plan to use metabarcoding and metagenomic analysis to address the biodiversity of phytoplankton. We will also perform zooplankton and virus sampling to understand the effect of zooplankton grazing and viral lysis on the different phytoplankton groups.

Following the suggestions of the other referee, we will move the sentences (L 256 - 258) in section 3.3. In the reworked manuscript, we will focus on the explanation of why the size distribution of picophytoplankton is noisy whereas we obtained a clear pattern for nanophytoplankton.

L269 The authors have used the term “finescale” and the rough definition appears here for the first time. From which have the authors derived this definition? We often used the term “mesoscale” to show this spatial scale in marine processes (Dickey and Bidigare, 2005, Scientia Marina). If this term was originally defined, the authors should have shown that in Introduction.

In the manuscript, we defined the term “finescale” in the Introduction (L 20-21): “ocean structures characterized by horizontal scale of the order of 1-100 km, with a short lifetime (days-weeks)”. Following your comments, in the reworked manuscript we will develop this definition further.
Although several studies used the term “mesoscale”, in our case “finescale” seems more appropriate. Indeed, by using this term, we include a fraction of the mesoscale processes (e.g. eddies), with scales close to the first internal Rossby radius, and the submesoscale processes, with scales smaller than the first internal Rossby radius (e.g. fronts) (Capet et al., 2008a ; Capet et al., 2008b ; McWilliams, 2016 ; Lévy et al., 2018).

L272 What are “many important oceanic processes including biogeochemical cycles and biodiversity”? Unless specified, we cannot judge whether “this suggests the possibility of a close coupling between the finescale forcing and the phytoplankton distribution and growth.” Honestly, I could not understand what the authors are to discuss in this paragraph. In different water masses, phytoplankton community structures are different almost every time. We usually attribute this to different water properties that can affect phytoplankton physiology, including salinity, temperature, turbidity, and nutrient concentrations, rather than to temporal and/or spatial scales of physical processes. I am afraid that there may be a large discrepancy between the final goal of this (overall) study and possible conclusions extracted from the present results.

We agree that phytoplankton is affected by water masses properties. In this part, we were not clear enough, we propose to reformulate these sentences as follows:
“The temporal scale of finescale processes (days-weeks) is of the same magnitude as biogeochemical processes and phytoplankton cellular cycle. The rapid evolution of these finescale structures influence the phytoplankton community, suggesting the possibility of a close coupling between finescale forcing and phytoplankton distribution and growth.”

L284 How much of the two figures (Figs. 7 and A2) was extracted from the original version in Tzortzis et al. (2021)? If it is a copy of the original, the authors should not use it again but should just cite it. And the authors say “in the frontal area upwellings and downwellings occur with different intensities”, but I think that it is not reflected in Fig. 7. From this figure, I could not find any difference in the vertical velocity of the two water masses.

Figures 2 and A2 were indeed extracted from Tzortzis et al. (2021). Following your suggestion and those of the first referee, we will move these figures into supplementary information. Concerning Figure 7, we have adapted this figure from Tzortzis et al. (2021),

which is why we think that it should stay in the manuscript. Following the suggestions of the other referee, we have reworked this figure (see below) for clarity.

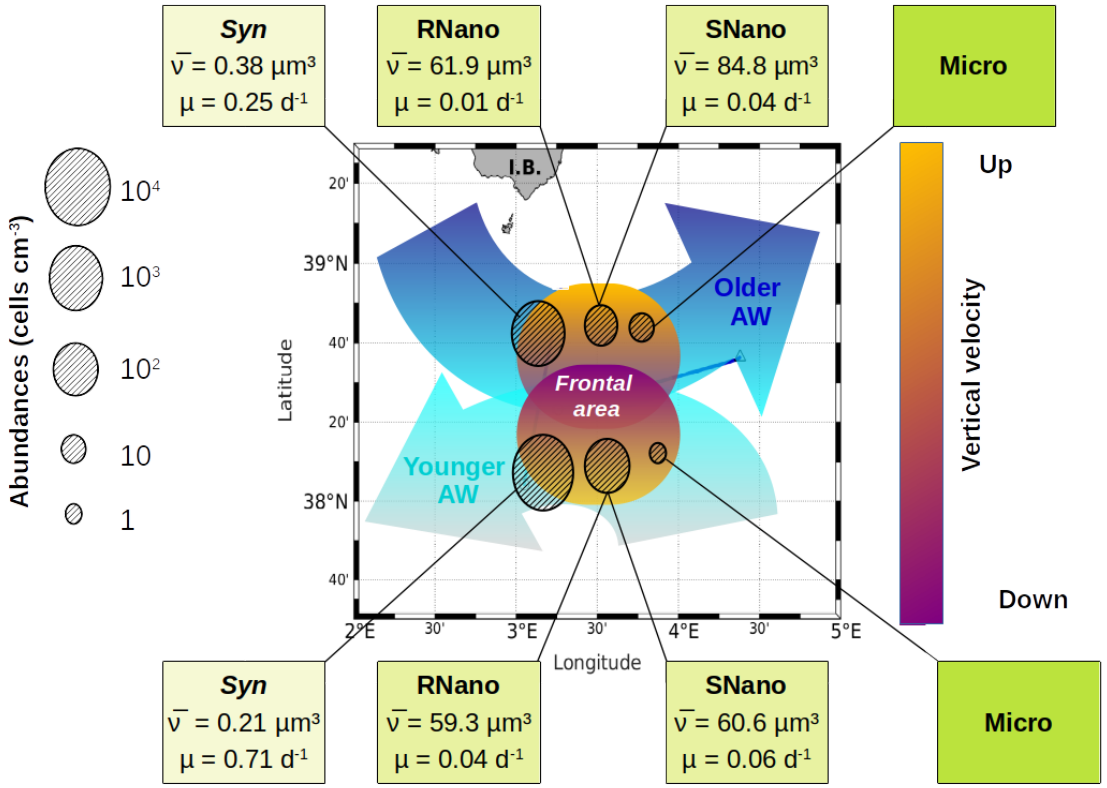

Figure 7: The contrasted distribution of phytoplankton in the frontal area. The circles represent the abundances of the several phytoplankton groups in the two water masses separated by the front. The boxes indicate the biovolume (v) and the growth rates (µ) for each phytoplankton group. Figure adapted from Tzortzis et al. (2021).

L286 The authors intended to say "spatial", not "special"? Even if so, the authors did not show "spatial" distribution in this paper. They just showed "temporal variations" in phytoplankton populations while covering two water masses.

We intended to say "special", but "particular" is more appropriate.

L289 "high phytoplankton size" is not an appropriate term.

Thank you for your corrections, maybe "the largest cells" is better.

L290 "picophytoplankton are more abundant in oligotrophic regions". This is a problematic description. First, it is true that the proportion of picophytoplankton in the total phytoplankton biomass becomes higher in the oligotrophic region compared with that in the mesotrophic or eutrophic regions. However, the absolute biomass or abundance of picophytoplankton is not always higher in the oligotrophic area. Generally speaking,

Prochlorococcus, which are adapted to ultraoligotrophic environments, are most abundant in oligotrophic waters. However, *Synechococcus* and eukaryotic picophytoplankton are more abundant in the mesotrophic region. Additionally, within the narrow trophic variation of the oligotrophic regions (typically < 0.1 µM of nitrate), a higher concentration of nutrients is sometimes related to the higher abundance of these picophytoplankton populations. Because the Mediterranean Sea is widely depleted with surface nutrients, discussion is not such a simple one as "picophytoplankton are more abundant in oligotrophic regions." I admit that this description is true for the study area, as shown in previous studies (Jacquet et al., 2010; Mena et al., 2016) as well, but it is not always related to generalization.

Thank you for your analysis. We will rework this part taking into account your comments, as follows:

"In our study, the older AW is characterized by larger cells of *Synechococcus* and nanophytoplankton with low abundances, whereas the younger AW is dominated by small cells with high abundances. Furthermore, microphytoplankton (i.e largest type of phytoplankton) is more abundant in older AW than in the younger AW. A possible explanation is that these two water masses do not have the same nutrient concentration, thus favoring certain phytoplankton groups.
Bethoux (1989) and Schroeder et al. (2010) have observed that the older AW is slightly more enriched with nutrients than the younger AW because during its circulation across the Mediterranean basin, the older AW receives nutrient inputs from the continent (river discharges, rain, wind). While in our study we do not have nutrient data, we can suppose that the nutrient distribution across the two water masses should be similar to the one measured during the previous studies of Bethoux (1989) and Schroeder et al. (2010).
We propose that the enhancement in nutrient in the older AW explains the corresponding phytoplankton cell size and abundances distributions. Our hypothesis is supported by the fact that our results are in agreements with those of Jacquet et al. (2010) and Mena et al. (2016) which also found the highest abundances of the small phytoplankton (*Synechococcus* and picophytoplankton) in the most oligotrophic waters, i.e., the younger AW. Furthermore, previous studies have shown that the proportion of picophytoplankton in the total phytoplankton biomass is higher in the oligotrophic region compared with that in the mesotrophic or eutrophic regions (Zhang et al., 2008 ; Cerino et al., 2012). Indeed, their better surface:size ratio due to their small size confers them a better capacity to inhabit areas with very low nutrient concentration compared to larger phytoplankton (Kiørboe, 1993 ; Marañón, 2015). Since our study area is always oligotrophic (Moutin et al., 2012), a small variation of the nutrient concentration (typically < 0.1 µM of nitrate) is sufficient to generate higher abundance of picophytoplankton."

L295 "If in our study we do not have nutrient data" I do not understand the intention. Are the authors unclear whether they have nutrient data themselves?

We will modify the sentence as follows:

"Unfortunately, it was not possible for both technical and funding reasons to perform nutrient measurements during the 2018 cruise, which is why we cannot provide nutrient concentrations of both water masses. We acknowledge the importance of this information and these measurements are planned for our future cruise this year."

L305 Here the authors abandoned the trial to estimate the effects of physical processes on irradiance received by phytoplankton, but is it impossible to compare them from the results of vertical velocity in the two water masses?

Following also the suggestion of the other referee, we will modify the beginning of this section as follows:
"Previous studies have well established that vertical motions impact biogeochemistry (Mahadevan & Tandon, 2006 ; Mahadevan, 2016 ; McGillicuddy, 2016). Upward vertical velocities drive deep nutrients into the euphotic layer and also move the phytoplankton cells along the water column resulting in changing light conditions."

L309 Although the authors succeeded in estimating intrinsic growth rates of various phytoplankton populations in the two different water masses using novel methodologies, the conclusion remarks here seem too superficial and primitive. The authors did not discuss the validity or robustness of the methodology or did not discuss the interactive connections among physical fields, chemical environments, and phytoplankton growth with quantitative comparisons.

We will completely rework the Conclusion taking into account your comments and those of the other reviewer:

"Phytoplankton structure and dynamics are a complex result of many interacting biological and physical phenomena. Finescale structures, and in particular fronts, generate vertical velocities which displace phytoplankton cells and nutrients in the water column, thus influencing phytoplankton communities. These mechanisms are only partially understood because the spatial scale of these structures and their ephemeral nature make them particularly difficult to study in situ; as a consequence only a few studies have been performed in finescale frontal regions. The estimates of specific growth rates for the various phytoplankton groups is one of the keys to better understand how environmental conditions affect phytoplankton dynamics. In this study, we followed the dynamics of several phytoplankton groups in two distinct water masses both in terms of hydrology and phytoplankton abundances, in order to explain their particular distribution.

The originality of our work resides in the fact that we used a size-structured population model applied in two water masses identified using a Lagrangian sampling strategy. To our knowledge that has never been done before. This strategy allowed us to reconstruct the diurnal cycle for several phytoplankton groups and to identify contrasted dynamics in the two water masses. For *Synechococcus* and nanophytoplankton, we found the higher cell size in the older AW located to the north of the front, associated with the lower abundances. A possible explanation is that the older AW is more enriched in nutrients than the younger AW,

thus favoring the largest cells. This remains a hypothesis because of a lack of nutrient data. Furthermore, in the oligotrophic region such as the Mediterranean Sea, a narrow trophic variation of the nutrient concentration is sufficient to generate higher abundance of phytoplankton. Another novelty of our study is that we applied this model on several phytoplankton groups identified by flow cytometry, whereas previous studies only applied it to *Synechococcus* and *Prochlorococcus*. We obtained good results for *Synechococcus* and nanophytoplankton. However, our results were noisy for picophytoplankton groups probably because these latter contain several taxa with differing dynamics.

Our work paves the way for many research perspectives. Direct integration of growth rates in biogeochemical models (Cullen et al., 1993) should be taken into account for a better assessment of the biogeochemical contribution of phytoplankton in oligotrophic ecosystems and to better forecast its evolution in the context of global change. Furthermore, we plan future experiments again in the South Western Mediterranean in spring 2023, after the launch of the SWOT satellite which will provide high resolution altimetry-derived current. Involving high-resolution nutrient measurements (and also high-precision ones, considering the oligotrophy of the Mediterranean Sea), coupled with metabarcoding (to address the biodiversity of phytoplankton), zooplankton and virus sampling, we will improve the understanding of zooplankton grazing and viral lysis on the different phytoplankton groups. Furthermore, we aim to explore how biogeochemical and ecological role of the finescales in regions of weak circulation are different from the ones more documented in highly energetic regions like boundary currents. In the Mediterranean sea, the low nutrient content is indeed the perfect condition when addressing this question, because even weak horizontal or vertical nutrient redistributions associated with the finescale circulation are likely to result in a biological response (Talmy et al., 2014 ; Hashihama et al., 2021).”

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