

REPLIES TO REVIEWERS

We would like to thank the reviewers for their constructive comments that helped improve our manuscript. Further we give our response to the comments of both reviewers point by point:

RC: Reviewer Comments, AC: Authors Comments and reply

R1 refers to Reviewer 1 and R2 to Reviewer 2

Replies to Reviewer 1

RC General Comments:

This manuscript presents a DEB-bioaccumulation model for microplastics. The model was calibrated and corroborated with field data available in the North Sea and Northern Ionian Sea, showing some skill in reproducing the (few) available observations. The topic is of interest to the readership of this Journal. The manuscript is very well written and clear. The model, the simulations and the analyses are robust and discussed thoroughly. I have a number of comments that I reported in the pdf version of the manuscript that I am attaching to this review. Here I will mention just two moderate concerns of mine regarding this work. 1) The authors used an ocean-colour chlorophyll product as input of the DEB model. However, this product might be biased in optically complex coastal waters, such as the Southern North Sea considered in this manuscript. The issue is relevant, because the authors pointed out the impact of the high chlorophyll concentration on the results they obtained in the North Sea. I recommend that the author discuss the reliability of the chlorophyll product they used. For example, they could compare the ocean colour product with in situ chlorophyll data from the ICES database (<https://www.ices.dk/marine-data/data-portals/Pages/default.aspx>), or with the NSBC climatology (<https://icdc.cen.uni-hamburg.de/1/daten/ocean/knsc-hydrographic0/>) 2) The authors should point out and discuss a bit more extensively some flaws in the results of their simulations and analysis (e.g. the overestimation of the observed MCs in Figure 6, and the mismatch between the regression results and the data at two sites in Figure 13). I appreciated that these flaws were clearly mentioned in the conclusions. I don't think that these issues compromised the value of the work. More minor to moderate issues are mentioned in the attached pdf of the manuscript.

AC Reply

We would like to thank R1 for carefully reading the manuscript and the very useful comments. With regard to the two moderate concerns:

1) The satellite dataset that we used presented a much better spatial and temporal coverage in our study area, as compared to other available datasets, such as the regional CHL-a satellite product, recommended by the reviewer (CMEMS,

OCEANCOLOUR_ATL_CHL_L3_REP_OBSERVATIONS_009_067). As demonstrated in several previous studies (i.e. Sara et al., 2011, 2012, Monaco & McQuaid, 2018) it is crucial to include daily CHL-a data to force the DEB model, in order to properly simulate the daily fluctuations of the environmental forcing data and thus the MPs accumulation.

In order to assess the reliability of the chlorophyll product that we used, we have followed the reviewer's recommendation and compared the ocean color product as an input of the DEB model, with in situ data available from the ICES database (<https://www.ices.dk/marine-data/data-portals/Pages/default.aspx>). Specifically, we used the in situ chlorophyll-a (CHL-a) data from the ICES database, that is derived from the surface layer (0-3m depth), and covers our study period of 2007-2011. A mean value, regarding depth, was computed, as representative of the upper surface layer. The in situ CHL-a data, which are included within our study area (Sec. 2.4, Southern North Sea, 51.08°-51.44° N, 2.19°-3.45° E) were averaged spatially in order to fit the "box model" of our study. Apparently some days were missing during this time period (2007-2011), while some days there were more than one measurement and then mean daily values were computed. The results from the comparison are presented also in Fig. 1 of the revised manuscript, along with the used satellite data (CMEMS-Globcolour, OCEANCOLOUR_ATL_CHL_L4_REP_OBSERVATIONS_009_098). The used satellite data are in quite good agreement with the available in situ data, presenting relatively small error (RMSE and correlation coefficient computed). The result of this comparison is shown also at the attached Fig.1 here.

The same comparison was conducted also with the regional CHL-a satellite product, suggested by the reviewer (CMEMS, OCEANCOLOUR_ATL_CHL_L3_REP_OBSERVATIONS_009_067), against the in situ data described above. We attached the Fig. 2 (only here) that shows the result of this comparison. The statistical analysis resulted in relatively higher RMSE and lower correlation coefficient, as compared to the one computed with the other (Globcolour) dataset.

Therefore, given also the better spatial and temporal coverage, we concluded that the satellite dataset that we used is optimal in our study area and time period.

2) Changes were made according to the reviewer's comment at the corresponding parts throughout the manuscript. Specifically, we have pointed out and discussed more extensively some flaws in the results of our simulations and analysis, namely the overestimation of the observed MPs in Figure 6, and the mismatch between the regression results and the data at two sites in Figure 13 of the manuscript. These points are listed in detail below, based on the corresponding comments of R1 in the manuscript.

All the line numbers written in RC are the line numbers of the R1's attached pdf manuscript, while the line numbers in AC correspond to the revised manuscript.

RC: line 15 replace "by" with "in"

AC: line 16 done.

RC: line 19 add "mussel"

AC: line 20 done.

RC: line 22 How long was the time window?

AC: line 24-25 the information was added “after 4 years and 1 year simulation”.

RC: line 35 "those": is it referred to MPs or sources?

AC: line 37 it is referred to MPs and was replaced with “those particles”.

RC: line 56 replace “from” with “at”

AC: line 58 done.

RC: line 194-195 please clarify what you mean with "represented", in this context

AC: line 196-197 the clarification was done by adding additional information in this sentence “assuming that all parameters referred to silt (or inedible particles) are applicable also to MPs particles”

RC: line 240 this implies that the last term in eq. 18 has the dimension of "particles" (C), rather than C/time. Please check the units

AC: line 242-252 The Reviewer is right. We reconstructed the Eq. 18 adding a parameter k_f (d^{-1}) in the third term that represents the post-ingestive selection mechanism utilized by the mussel to incorporate indigestible material (i.e. MPs) into faeces. This parameter is calibrated at a constant value for both study areas and illustrates the mussel's mechanism to discriminate between particles in the gut based on physicochemical criteria according to Ward et al. (2019) review study. This parameter also compensates the units of the third term of Eq. 18 to C/time.

The whole paragraph was reconstructed to adjust the new information (line 242-254) of the revised manuscript.

RC: line 244 This should be defined just before eq. 18, to help the reader understanding the equation

AC: line 238-240 done.

RC: line 250 chlorophyll-a concentration includes large to pico functional groups of phytoplankton. Are all the groups suitable to feed mussels? If not, please clarify the limitation of this assumption in the context of your work.

AC: line 264-268 The information was added about the size limitations of the suspended matter that a mussel is able to filter.

RC: line 251 Has this result of Hatzonikolakis have general validity, or was it referred to a particular location/experiment/condition? Please discuss the extent to which this result can be reliable in the context of your application

AC: line 269-276 Although the result of Hatzonikolakis et al. (2017) was referred to a particular location/condition, the same result has been demonstrated also in other studies (i.e. Troost et al., 2010)

referred to different locations and conditions. Considering that POC contributes to the mussel diet when the CHL-a concentration is low enough (Troost et al., 2010) and that our study areas (North Sea is a more eutrophic environment and N. Ionian Sea refers to mussel farm and thus suitable conditions regarding CHL-a) do not present low Chl-a values, CHL-a can be assumed to be the dominant food source of mussels. The relevant information was added in these lines of the revised manuscript.

RC: line 258-260 Why did you not use the regional chlorophyll product available for the North West Shelf-Seas in the CMEMS catalogue? I think the regional product would have been preferable, because it takes account of the complexity of the optical waters in the coastal North Sea.

AC: line 287-296 This question has been answered in our first comment under the general R1's comment. In this part we added the result of the comparison between the used satellite dataset and the available in situ data from the ICES database. The in situ data were also added in Fig. 1 of the revised manuscript. For more details about the comparison between the recommended regional chlorophyll product and the in situ data, the reader may see the first AC reply.

RC: line 266 Similar to my previous question: why did you not use the CMEMS product for the Mediterranean Sea?

AC: line 305-311 The chosen CHL-a dataset was found preferable, as compared with other available remote sensing datasets (i.e. CMEMS chlorophyll product for Mediterranean Sea), since it presented a better spatial and temporal coverage (Hourany et al., 2019, Garnesson et al., 2019). Unfortunately, available in situ data are very scarce in the study area and therefore an extended comparison between remote and in situ data could not be conducted. However, we have compared the used satellite data (Globcolour) and the proposed product (CMEMS for Mediterranean Sea) with very few available (unpublished) in situ data, which were obtained in the framework of WFD (Water Framework Directive data kindly provided by Georgia Assimakopoulou) (Fig. 3 attached here). The few available CHL-a data in our study area (N 39.49°-39.65°, E 20.09°-20.23°) - specifically "Station Igoumenitsa" (N 39.5°, E 20.2281°) and "Station Kalamas" (N 39.6°, E 20.1439°)- were sampled at 2 m depth, covering the time period of our study (November 2014 to December 2015). The sampling dates were the same in both stations (8-Dec-14, 8-Mar-15, 12-Dec-15), allowing us to compute the mean CHL-a dataset (final 3 values) representative of our "box model". The comparison between the used satellite dataset, the proposed (regional) satellite product and the few in situ data showed the better temporal coverage and a slightly lower error for our satellite dataset (Globcolour) than the regional CMEMS product (Fig. 3 here).

RC line 272 Worth mentioning that PFT satellite products for your study regions were provided by Di Cicco et al., 2017 and Brewin et al 2017 and are now an operational product of CMEMS

AC line 315 the references were added.

RC line 281 Are you sure? Looking at your data I would say the peak is in Spring, isn't it? See also, e.g., Widdicombe et al. (2010), Journal of Plankton Research (although that paper refers to the English Channel)

AC line 322-325 the whole sentence has been clarified. We referred to the rivers discharge peaking at winter period (Van Beusekom et al., 2009), and not to the CHL-a concentration and/or productivity, which indeed peaks at spring season in the North Sea study area (shown also at Fig. 1 of the manuscript).

RC line 407 can you assume that the behavior of the model is close to linear within this range of parameter variation? In fact, SI is meaningful if the linear approximation is adequate

AC line 450-451 The R1's concern is justifiable, since we are aware of the general nonlinear effect of some parameters (i.e. temperature) on the DEB model; however within this range of parameter variation, we assumed that the model approximates the linear behavior. Our intention was to examine if the perturbed variables/parameters have an effect (or not) on the simulated MPs accumulation, in order to proceed with the development of the regression model, relating directly the environmental MPs concentration with the variables/parameters that had high effect on model's result (CHL-a, temperature and the mussel's weight and MPs load) (Eq. 20 of the manuscript). Nonetheless, the same method (sensitivity index, SI) has been also applied in other studies, which intended to examine the model's sensitivity on specific variables/parameters regarding the mussel growth (Casas and Bacher, 2006, Rosland et al., 2009, Béjaoui-Omri et al., 2014, Hatzonikolakis et al., 2017).

RC line 430 the format could be better

AC line 477 the format was changed.

RC line 430 Please define C. Does it represent OBSERVATIONS of C in the environment?

AC line 480 done. C represents the simulated MPs accumulation in the mussel.

RC line 446 Seems to me that Fig 4 is referred to before Figure 3. Please revise the order of the figures

AC line 493 done.

RC line 447 can you provide an estimate of the error, please?

AC line 494 A standard deviation was estimated by tuning the model with various X_k values and comparing the model's simulation with the available field data. The model's result with the estimated value range ($X_k = 8 \pm 1.5 \text{ mg m}^{-3}$) was in agreement with the field data and within their standard deviation.

RC line 453 Was the French site a clean one?

AC line 499-505 The sentence was revised to better justify the high value of X_k , regarding the food quality of the mussel's diet. Apart from the fact that the French site presented lower CHL-a concentrations compared to our study area (North Sea), the DEB model applied at the French site, did not include inedible particles in the mussel's food (so it was assumed clean of inedible particles). On the other hand, in our study the inedible particles (i.e. MPs) have been incorporated in the mussel's diet through the modified relation of the functional response f (Eq. 5, Table 1 in the manuscript), which regulates the assimilation rate and thus the mussel's growth (see also lines 202-208 in the manuscript).

Consequently, the higher value of X_k in our study area, reflects the lower quality food and affects the half-saturation constant (X_k) according to Kooijman (2006).

RC line 464 This is not coherent with your comment of Figure 1 that CHL peaked in winter (which in fact I think was wrong)

AC See also the corresponding comment above. The comment was referred to the rivers discharge and not to the CHL-a peak. It was rephrased as mentioned, in lines 322-325 of the revised manuscript.

RC line 480 this reference comes after figure 4: please revise

AC line 532 done.

RC line 492 Please mention that the model overestimated the data range explicitly. It seems like the model reproduced a seasonal increase that was not observed.

AC line 547-548 R1's comment was considered and discussed here and also throughout the whole manuscript at the corresponding parts, as recommended.

RC line 495 Is this explanation quantifiable?

AC line 551-554 This explanation was quantified, discussed and compared with our model's result.

RC line 498-500 This sounds quite a speculation that goes too far. at the end of the day, you calibrated the model to fit the equations to the data.

AC 557-562 the whole sentence was revised, including the model's overestimation in the North Sea simulation.

RC line 500 not really in agreement in the North Sea

AC 557-562 This was commented.

RC line 554 Please mention here in the text and discuss later in the manuscript that in the North Sea the range of variability of the data and model uncertainty do not really overlap significantly at the time of the observations.

AC line 613-615 R1's comment was mentioned here and discussed in the corresponding parts of the manuscript (see also the specific parts discussed in the following comments).

RC line 594 please describe briefly the results shown in figure 13 (e.g. general overlapping of regressed and observed C, except in Hastings and Plymouth)

AC line 664-675 We described the results of Fig. 13 and discussed thoroughly about the possible explanation of the two exceptions (Hastings and Plymouth).

RC line 614-615 Please rephrase.

AC line 689-691 We rephrased the whole sentence.

RC line 623 Which approach? The seasonally variable approach? Please clarify this sentence

AC line 696-704 this was referred to our applied approach with the daily CHL-a fluctuations. In fact, it was further clarified by adding a sentence describing the evolution step that we made, by applying a daily variable approach.

RC line 627 Please remind the reader which figures presented these results.

AC line 706 done.

RC line 630 Please mention and discuss the model overestimation of MP in Figure 6.

AC line 706-712 We would like to thank R1 for the comment, as it allowed us to interpret better our results and compare further the observed field data with the model's result, regarding not only the MPs accumulation in the mussel but also the MPs elimination after 24 hours of depuration. The comment was mentioned and discussed thoroughly.

RC line 641-643 Not clear to me why this sentence is relevant here. Please clarify.

AC line 726-729 R1 is right. We also found this sentence irrelevant here, so it was deleted. The information of this sentence has been communicated to the reader earlier in the relevant part (Sec. 3.3 of the manuscript).

RC line 646-647 This sounds an over-stretched statement. please provide more evidence

AC line 731-734 We rephrased it and provided more evidence, as recommended.

RC line 662 Am I wrong, or Cenv is the dependent variable and C the predictor, in eq. 20?

AC line 751 R1 is right. This was a misprint and was corrected.

RC line 663 But in Hastings and Plymouth, why?

AC line 757-759 This was clearly stated and an explanation was suggested. The specific issue was discussed more in lines 664-675, as mentioned in a previous comment reply.

RC line 670-674 quite long sentence, please consider to split it in 2.

AC line 766-771 done.

RC line 677 Please specify what data you are talking about. MP in mussels, right?

AC line 773 This was done. Yes, we were referred to MPs in mussels.

RC line 686 overstatement. In figure 6 the data are clearly overestimated

AC line 782-784 The sentence was rephrased, including the overestimation statement.

RC line 687 replace "to be close to reality" with "to represent the natural variability"

AC line 785 done.

RC line 693 Here I do not understand: Cenv is highly variable because it is naturally variable, or because there are huge observational errors? please clarify

AC line 790-795 We clarified this by adding a sentence. We thank R1 for the comment.

RC line 729 Is this info relevant?

AC line 833 The information was deleted as we also found it irrelevant.

RC line 745 approximated? considered indirectly?

AC line 851 We replaced it with “considered indirectly”.

RC line 747-752 the logical flow of these last sentences was rather unclear to me. Please consider rephrasing this part.

AC line 851-859 We rephrased these sentences.

RC line 756-757 This is a strong critic, which I appreciate and don't consider a crucial flaw. However, such critic was not as much evident in the presentation and discussion of the results (e.g. Figure 6?). Please report the model flaw also in the presentation and discussion of the results.

AC line 864-865 This sentence was rephrased. The model flaws were reported and discussed extensively at the corresponding parts throughout the whole manuscript (i.e. presentation and discussion of the results, see also above comments reply).

RC line 772-774 I found that the sentence included redundant information, thus I suggested some changes. Please feel free to reject my suggestions

AC line 881-883 We rephrased the sentence, adjusting some changes.

RC page 40 (Figures) Can't you plot just the bottom x-axis, in black? (the upper one is redundant)

AC page 43 Fig. 8 done.

Replies to Reviewer 2

RC General Comments:

In the current manuscript, a Dynamic Energy Budget model is developed aiming to simulate the uptake and excretion rate of microplastics, by two species of mussels at two different regions (North Sea and N. Ionian Sea). The authors claim that the biophysical regime (in this case chlorophyll and sea surface temperature) influences the accumulation rates in filter feeders.

Overall, I think that the paper is well-written, without any major issues or inaccuracies. I appreciate the clear figures that allow following the manuscript. The literature is well cited and extensive. I truly enjoyed reading it; the authors have put substantial efforts in preparing their manuscript. I am, thus, recommending a few minor comments/ suggestions for their consideration. My only semi-major comment, which does not impact the overall research output, is about the overestimation of the satellite derived chlorophyll concentrations at the southern North Sea. Please see the specific comment in the next section.

AC Reply

We would like to thank R2 for his time and considerations and for carefully reading the manuscript and providing useful comments. With regard to the semi-major comment:

Since this was one of the two moderate concerns of R1, we have already examined and discussed the reliability of the satellite derived chlorophyll concentrations that were used at the southern North Sea. We compared the used satellite data (CMEMS-Globcolour), as well as, the regional satellite product (CMEMS product for the North West Shelf-Seas) with the available in situ data (ICES database) (see Fig. 1 and Fig.2 attached here). We concluded that the satellite data used, have a better spatial and temporal coverage and relatively lower error as compared to the regional CHL-a product. For more details, the reader may also see the first reply in R1's comment.

All the line numbers written in RC are the line numbers referred to the original manuscript (written in R2's review), while the line numbers in AC correspond to the revised manuscript.

Abstract

RC line 7: MavroLithari should read Mavrolithari or Mavro Lithari.

AC line 7: it should read Mavro Lithari, so we corrected it.

RC line 29-30: "... with MPs accumulation in mussel's soft tissue, temperature and chlorophyll-a." . The sentence does not flow well. Please revise.

AC line 31-32 R2 is right, so we rephrased the sentence.

Introduction

RC: It flows very well, with informative and well-referenced text. The novelty of the current study is clear.

Line 95 (and 97): DEB has been abbreviated in the abstract, please check the journal rules (if abbreviated in the abstract, is there a need to be abbreviated in the text too?).

Once you find out the rule, please apply to the species *M. edulis* and *M. galloprovincialis*

(lines 96/97).

AC We would like to thank R2.

According to the journal rules, abbreviations need to be defined in the abstract and then again at the first instance in the rest of the text. We followed this rule and abbreviated the DEB in the abstract and then at the first instance (line 97). The same was applied also for the species (abstract and line 99-100).

Materials and Methods

RC line 112: The North Sea is a marginal sea rather a semi-enclosed environment (clear openings and influence from the Atlantic from both sides).

AC line 116 The suggestion was done. “semi-enclosed” was replaced with “marginal”.

RC line 180: DEB is abbreviated again – please revise.

AC line 184 R2 is right, this was corrected.

RC line 250: chlorophyll-a concentrations (CHL-a, an index of phytoplankton biomass).

AC line 266: done.

RC line 258: in the future, please consider using the OC-CCI Chl-a product, which is a better product for coastal regions. Also available in Copernicus.

AC line 289-298 We have already discussed the reliability of the satellite product that we used in our study area for the specific time period above (in our first reply to R1 and R2 comments) and concluded that the used satellite CHL-a data were suitable for the specific place and time period. However, we thank R2 for the kind recommendation and we will consider this in a future relevant study.

RC line 265: it should read (i.e. SeaWiFS, MERIS, MODIS, VIIRS and OLCI-a). All these are abbreviations and not just names.

AC line 301 R2 is right and we corrected it.

RC: Please provide a simple reasoning why two different products of Chl-a were used for the two different study areas.

AC: This question has been answered also earlier in R1’s comment (“why did you not use the CMEMS product for the Mediterranean Sea?”). We provide the same answer here for the reader’s convenience:

“AC: line 305-311 The answer was included to the added part of the revised manuscript. Moreover, to justify our reply to R1’s question we compared the used satellite data (Globcolour) and the proposed product (CMEMS for Mediterranean Sea) with very few available in situ data, which were obtained in the framework of WFD (Water Framework Directive data kindly provided by

Georgia Assimakopoulou). Unfortunately, there are not enough available in situ data for the specific area (Fig. 3 attached here). The available few CHL-a data are included in our study area (N 39.49°-39.65°, E 20.09°-20.23°) - specifically “Station Igoumenitsa” (N 39.5°, E 20.2281°) and “Station Kalamas” (N 39.6°, E 20.1439°) - and sampled at 2 m depth, covering the time period of our study (November 2014 to December 2015). The sampling dates were the same in both stations (8-Dec-14, 8-Mar-15, 12-Dec-15), allowing us to compute the mean CHL-a dataset (final 3 values) representative of our “box model”. The comparison between our used satellite data, the proposed satellite product and the few in situ data showed a better temporal coverage and a slightly lower error for our satellite dataset (Globcolour) than the proposed CMEMS product (Fig. 4 here)”

RC line 275: $_{0.88 \text{ mg chl-a m}^{-3}}$ = should read $_{0.88 \text{ mg m}^{-3}}$. Please change throughout.

AC This was changed throughout the manuscript.

RC line 282: sea surface temperature has been already abbreviated, please use the abbreviation (i.e. SST)

AC line 327 done.

RC line 325: “...Pouvreau et al., 2006).In order to...” insert a space after the punctuation mark (full stop).

AC line 370 done.

RC line 278 and Figure 1: it is a bit worrying to observe such high chlorophyll values in the coastal North Sea region. In reality, this environment is eutrophic (no doubt) and certainly very high concentrations are expected. However, your North Sea region belongs to CASE II waters, where algorithms tend to overestimate chlorophyll concentrations. In optically-complex Case II waters, Chl-a can not readily be distinguished from particulate matter and/or yellow substances (dissolved organic matter) and so global chlorophyll algorithms are less reliable. This has to be communicated to the readers.

Please add a few sentences to acknowledge the issue. To facilitate your revision, you will find this reference very useful:

International Ocean-Colour Coordinating Group – IOCCG (2000), Remote sensing of ocean colour in coastal, and other optically-complex waters, Rep. Int. Ocean-Colour Coord. Group 3, edited by S. Sathyendranath, Dartmouth, N. S., Canada. The IOCCG reports are freely available and Open-Access.

AC line 289-298 We justified the satellite product that we used in previous comments and added a few sentences to acknowledge the issue as requested. Also the above suggested reference was added.

RESULTS

RC line 448: please use the same units (they are the same after all $\text{mg m}^{-3} = \text{g l}^{-1}$)

AC line 497 this was corrected.

END.

Figures

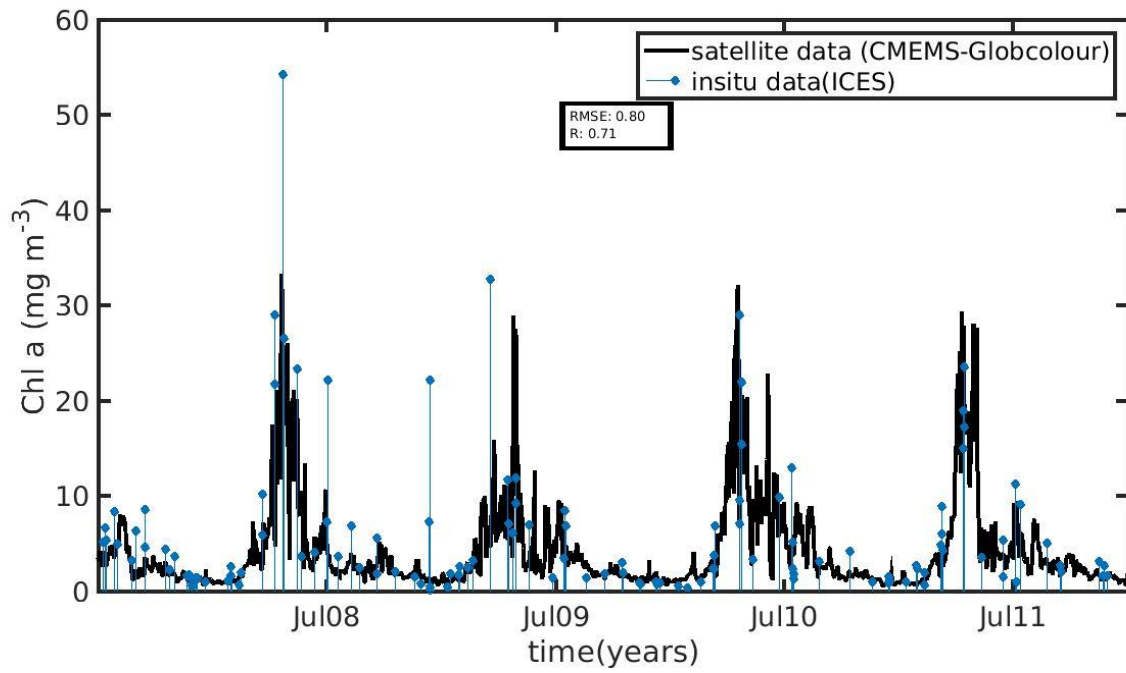


Fig.1. Comparison between the satellite data (CMEMS-Globcolour) with the available in situ data (ICES database)

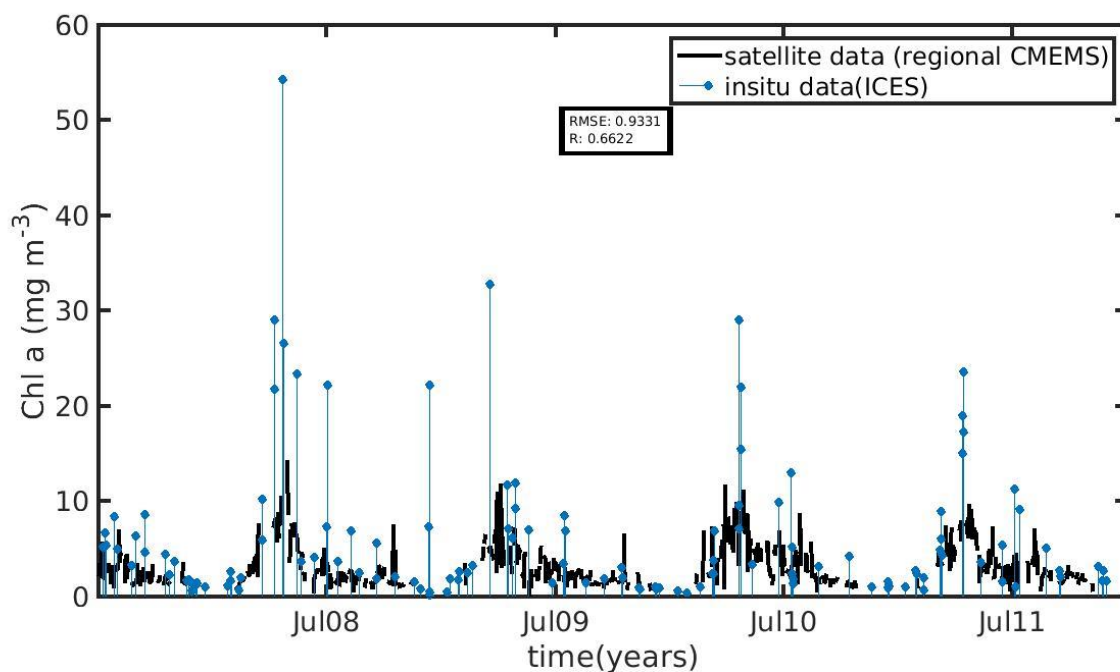


Fig. 2. Comparison between the satellite data (regional CMEMS) with the available in situ data (ICES database).

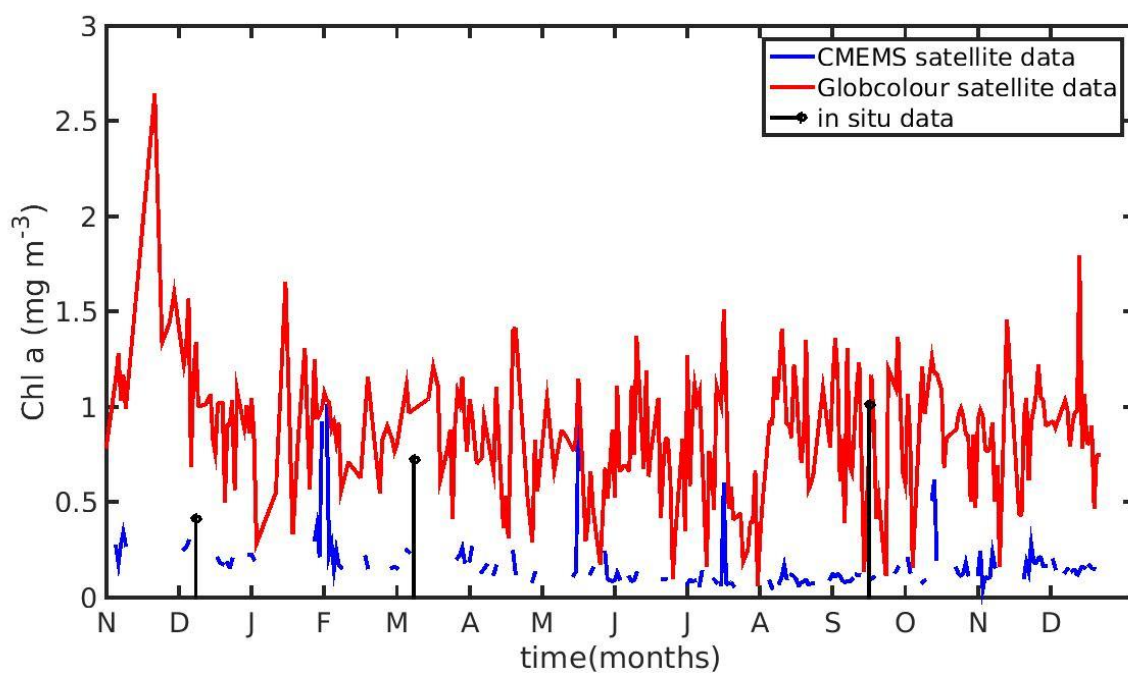


Fig. 3. Comparison between the Globcolour satellite data, the CMEMS satellite data for the Mediterranean Sea and the (few) available in situ data.