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# Water column distribution of zooplanktonic size classes derived from in-situ plankton profilers: Potential use to contextualize contaminant loads in plankton

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## ABSTRACT

Pollution is one of the main anthropogenic threats to marine ecosystems. Studies analysing the accumulation and transfer of contaminants in planktonic food webs tend to rely on samples collected in discrete water bodies. Here, we assessed the representativeness of measurements at the chlorophyll-a maximum layer during the MERITE-HIPPOCAMPE cruise for the entire water column by investigating the vertical distribution of particles and plankton obtained by in-situ optical profilers at nine stations across the Mediterranean Sea. We identified specific conditions where the interpretation of results from contaminant analyses can be improved by detailing plankton size structure and vertical distributions. First, the presence of higher than usual plankton concentrations can result in sampling issues that will affect biomass estimation within each size class and therefore bias our understanding of the contaminant dynamics. Secondly, the presence of an unsampled water layer with high zooplankton biomass might imply non-resolved contaminant pathways along the trophic structure. This study lays the basis for optimizing sampling strategy in contaminant studies.

## 1. Introduction

Pollution is one of the main anthropogenic threats to marine ecosystem where persistent contaminants that are bioaccumulated in lower trophic levels can be biomagnified across the marine food web (Borgå et al., 2012). Trophodynamics at the base of the food web play a key role in defining bioaccumulation of contaminants (Castro-Jiménez et al., 2021; Tiano et al., 2014) with subsequent effect on the so-called biological pump of contaminants (Galbán-Malagón et al., 2012; González-Gaya et al., 2019). The lower trophic levels are generally characterized by a high diversity in terms of species, functional groups and morphological features (Carlotti and Poggiale, 2010). Many species have short generation times and complex life cycles that can involve a shift from one functional group to another (e.g., meroplankton). These intricate dynamics make it considerably more difficult to identify trophic structures and interactions. One common procedure in contaminant studies and other marine ecological studies has been to size-fractionate samples. This practice is rooted in the notion that the distribution of plankton biomass across size classes can indicate trophodynamics, including trophic structure and efficiency in transferring the biomass

from lower to higher trophic levels (Basedow et al., 2016; Espinasse et al., 2014b; Hunt et al., 2015).

For size-fractionation and subsequent contaminant analysis it is necessary to collect large volumes of water to get sufficient plankton biomass. During scientific cruises, sampling is usually focussed on one depth layer, the chlorophyll maximum layer (CML), to reach these high plankton biomasses in the available time. This procedure induces two potential biases: first, at high concentrations of phytoplankton and detrital material, net filtration can alter the size structure of the sample due to net clogging, and potential avoidance of larger organisms; second, net sampling and mechanical size fractionation can cause destruction or even disappearance of more fragile organisms and aggregates (i.e., marine snow) (Aldredge and Silver, 1988). Furthermore, net sampling with a particular mesh size is tailored to quantitatively sample only a given fraction of the entire plankton community. Non-destructive sensors have the capability to overcome and control these potential biases and can thus ensure the validity of contaminant analyses. A vertical deployment of those sensors throughout the water column will also reveal how representative contaminant analyses in the CML are for the entire water column.

Optical sensors to observe vertical particle distributions in the plankton size range are useful tools for observing distribution patterns as a function of water column physical and biogeochemical characteristics (Berline et al., 2021; Espinasse et al., 2014a; Schultes et al., 2013). They provide high resolution profiles of plankton abundance and biomass with unaltered size structure within a specific size range. During the MERITE HIPPOCAMPE cruise a combination of in-situ optical data and net samples were collected along a North to South transect across the Mediterranean Sea, covering various environments. Using this dataset as a case study, we aim to (1) compare the size distribution obtained from net-derived vs in situ-derived optical measurements in the CML, (2) describe the vertical distribution of zooplankton size classes across the whole water column and thus to check the representativeness of samples collected in the CML, and (3) improve the interpretation of contaminant measurements obtained in zooplankton size classes in the CML in the context of contaminant transfer in the pelagic food web.

## 2. Methods

### 2.1. Introduction of the cruise

The MERITE-HIPPOCAMPE cruise took place in April–May 2019 aboard the RV *Antea* in the Western Mediterranean Sea. An extensive description of the cruise objectives and sampling strategies along with a summary of basic environmental conditions observed at each station can be found in Tedetti et al. (2023). A brief description including information relevant to the present study will be presented here. The main objective of the research project was to give a better understanding on the role played by plankton in transferring contaminants toward the higher components of the trophic chain. As a semi-enclosed sea, highly impacted by anthropogenic activities, the Mediterranean Sea represents a case study. Nine stations were sampled along a latitudinal transect

from the French coast in the North to the Gulf of Gabès (Tunisia) in the South, covering various types of environments (e.g., distance to the coast, hydrology, level of exposure to anthropic stressors) shaping different ecosystems (Fig. 1). At each station, measurements were conducted on a wide range of organic and inorganic contaminants including biotic metals and metalloids, organic contaminants (PAHs, PCBs, PBDEs, PFASs), radionuclides ( $^{137}\text{Cs}$ ) and microplastics, to estimate their concentrations per unity of biomass ( $\text{g g}^{-1}$  dry weight).

### 2.2. Net sampling for contaminant analysis

A Multiple Plankton Sampler (Midi type, Hydro-Bios) was deployed horizontally to sample plankton in a layer centered around the CML. The sampling device consisted of five nets with a mesh size of  $60\ \mu\text{m}$  and a section area of  $0.25\ \text{m}^2$ , which are successively opened and closed. Each net sampled a water volume of about  $50$  to  $80\ \text{m}^3$ . The sampled biomass was retrieved from the six cod-ends and pooled together to reach the amount of biomass required for contaminant analysis. The total biomass was size-fractionated using a column of sieves with decreasing mesh sizes splitting plankton into five size classes ( $60$ – $200$ ,  $200$ – $500$ ,  $500$ – $1000$ ,  $1000$ – $2000$  and  $>2000\ \mu\text{m}$ ). The wet weight was measured for each of the size classes and converted into biomass concentration ( $\text{mg m}^{-3}$ ) using estimations of volume of water filtered. In addition, vertical distributions of temperature, salinity and chlorophyll *a* concentration (chl*a*) were recorded using a fluorometer (Aqua Tracka, Chelsea ctg) coupled with a CTD Seabird SBE 911plus.

### 2.3. In-situ profilers

Two optical sensors collected vertical profiles at each station: a Laser In-Situ Scattering and Transmissometry instrument (LISST-Holo, Sequoia Scientific, Inc., WA, USA), and a Laser Optical Plankton Counter

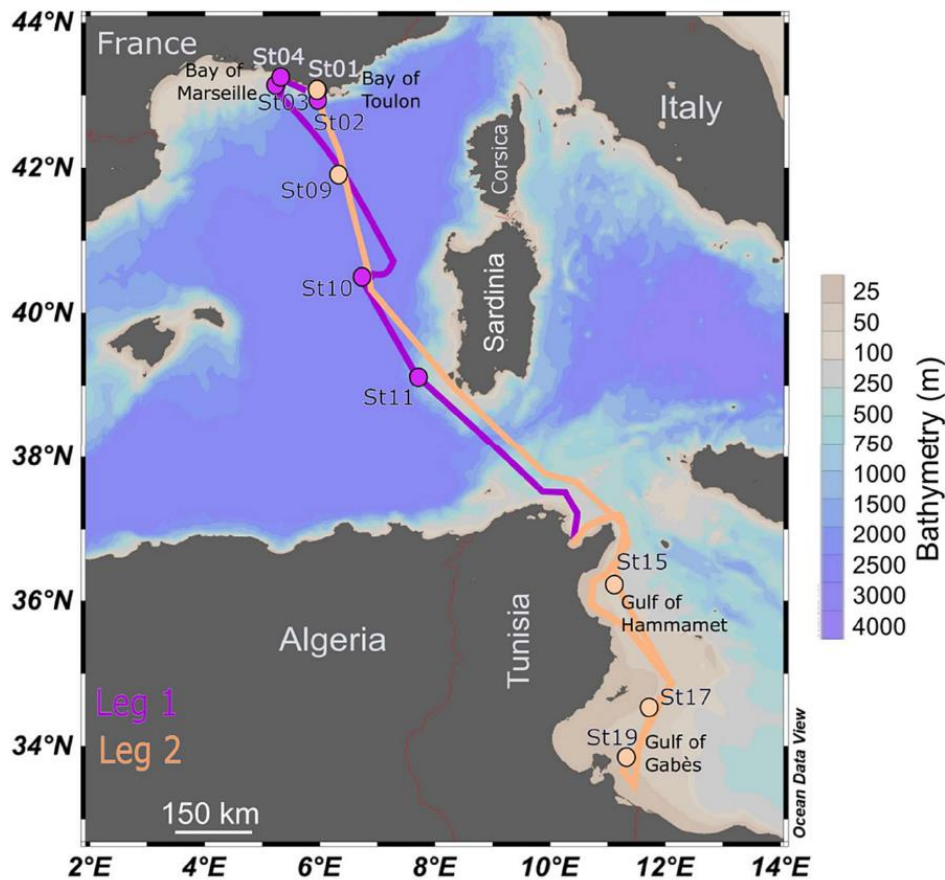


Fig. 1. Map of sampled stations during the two legs of the MERITE-HIPPOCAMPE cruise (13 April–14 May 2019) on board the RV *Antea*. Sensor data at Station 4 were not available.

(LOPC, Brooke Ocean Technology Dartmouth, Canada). The functioning of both devices roughly follows a similar concept, as they count and measure the size of particles in-situ by hitting them with a laser beam (Graham and Nimmo Smith, 2010; Herman et al., 2004). Data from both instruments were vertically binned in 10 m thick layers. Higher vertical resolution is possible but the relatively small volume of water sampled by the sensors resulted in a low number of particles measured, especially in the larger size classes. Binning data in a 10 m thick layer was found to be a good compromise ensuring relevant vertical resolution and accurate biomass estimates for all size classes. Size measurements from the sensors are provided in equivalent spherical diameter (ESD) or biovolume (length-width ratio of 3), and were converted into biomass assuming a density of  $1 \text{ mg mm}^{-3}$  (Herman and Harvey, 2006). Combined together, measurements provided by the LISST-Holo and the LOPC cover a size range from  $15 \mu\text{m}$  to a few mm ESD. More information about the merging process and the agreement between data from both sensors can be found in Fig. S1. Above  $60 \mu\text{m}$ , biomasses were summed into the same size classes as the net samples ( $60\text{--}200$ ,  $200\text{--}500$ ,  $500\text{--}1000$ ,  $1000\text{--}2000$ ,  $>2000 \mu\text{m}$ ). Only the first four size classes were kept for comparison, the larger size class ( $> 2 \text{ mm}$ ) was discarded as the volume of water analyzed by the sensors was too small to allow accurate estimations large organism concentrations.

#### 2.4. Comparison of biomass estimates from net samples and in-situ profilers

Beyond the fact that the composition of in-situ particles can differ slightly from the composition of net sampled particles (due to particle aggregations or breaking of particles during net sampling process), comparison of data from net samples and optical profilers should be done with caution. While overall both approaches usually yield similar results (Marcolin et al., 2013; Schultes and Lopes, 2009; Vandromme et al., 2014), there are a number of points to consider that can complexify direct comparison of absolute values (Espinasse et al., 2018). Because the net sampling and the in-situ profilers are most of the time carried out in two different casts, the sampled environment might not be exactly the same due to spatial heterogeneity in plankton distribution (Espinasse et al., 2014a). Estimation of the volume of water sampled can also be a source of divergence in biomass estimates but will not affect size distribution. The conversion of ESD or biovolume to biomass might also introduce small biases. Finally, because the size thresholds delimiting the size classes will apply differently depending on how the particles are split (physically with sieves vs digitally based on ESD), an offset might occur. Regarding the second and third points, a test was conducted on a separate dataset using vertical net tows done at each of the nine stations allowing to compare biomasses from nets and from full profiles of optical sensors (Fig. S2) Using sensor full profiles allows better estimation of particle density and removes potential effect of slight mismatch between sampling depths. The correlation between biomass estimates gave satisfying results although the limited number of stations does not allow to produce meaningful statistical metrics. Other points are discussed further below in the text.

### 3. Results and discussion

Based on our dataset, we aimed to answer three questions which are relevant to address the main objectives of this study. The results will be presented and discussed through these questions.

#### 3.1. Comparison of in situ- vs collected-samples size distribution

- Is the size distribution of zooplankton measured from in-situ sensors comparable to that obtained by sieve fractionation of net samples?

The interpretation of the contaminant levels and associated bioaccumulation factors is based on the assumption that organisms are

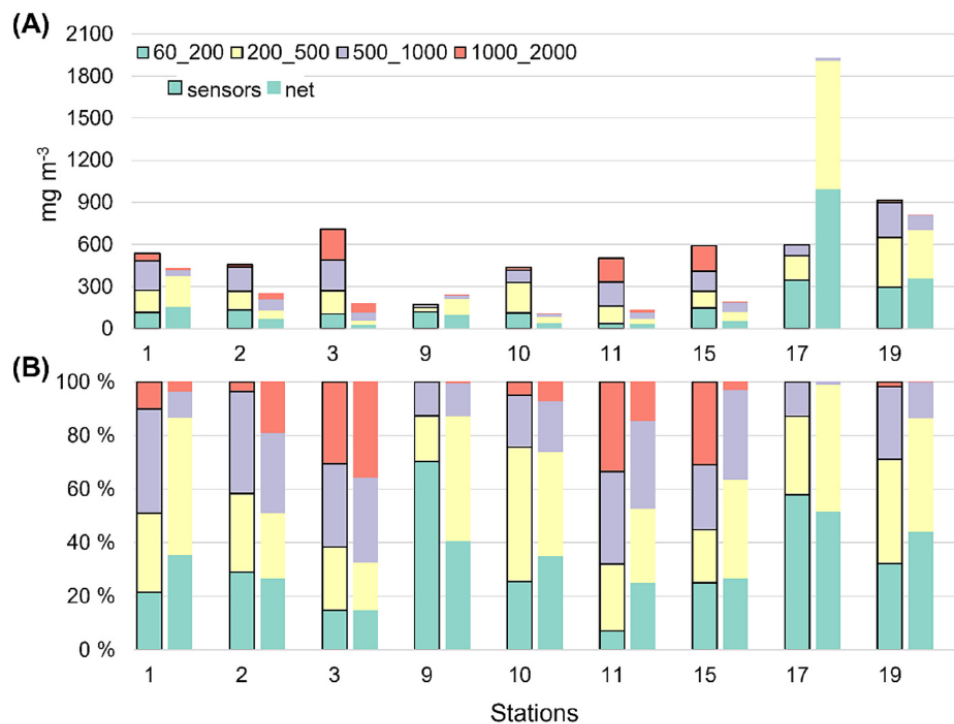
distributed in the correct size class. However, as detailed before, the process of sampling zooplankton using a net and running them through a sieve column of different meshes tends to alter size distribution. In comparison, laser-based plankton counters provide direct size measurements of particles and assign them in size-classes, although particle shape and its orientation somewhat affect optical size distributions at low particle concentrations. It is therefore important to determine if both approaches provide comparable size distributions and if not, to investigate why they differ and what cause these differences.

Among the nine stations, three showed differences in biomass size-distribution between sensors- and net-derived data: stations 1, 9 and 15 (i.e., changes in relative contribution  $>25\%$  for at least one of the size classes; Fig. 2B, Table 1). At station 1, the  $500\text{--}1000 \mu\text{m}$  size class was depleted in the net sample compared to sensor data ( $38.8\%$  vs  $9.5\%$  of total biomass). This size class was dominated by middle-size copepods, such as *Clauso* / *Paracalanus* species (Fierro-González et al., 2023). Small scale zooplankton heterogeneity is a well described phenomenon (Espinasse et al., 2014a; Trudnowska et al., 2016) and may explain this difference. However, since absolute values of biomass between both approaches did not differ significantly (Fig. 2A), it is likely that the influence of patchiness was limited. When possible, sampling replicates may smooth down this heterogeneity. At station 9, because of a phytoplankton bloom occurring, clogging of the  $200 \mu\text{m}$  sieve possibly retained particles, resulting in an artificial transfer from the  $60\text{--}200 \mu\text{m}$  to the  $200\text{--}500 \mu\text{m}$  size class. Also, copepod nauplii, which dominated the smaller size class (Fierro-González et al., 2023) might have been underestimated in net samples (Skjoldal et al., 2013). A good estimation of biomass in small size classes is particularly important for contaminant sensible to dilution processes such as PAHs (Guigue et al., 2023). At station 15, the largest size class ( $> 1 \text{ mm}$ ) had very low biomass in the net sample compared to the sensor data ( $3.2\%$  vs  $30.9\%$  of total biomass). This size class was dominated by euphausiid larvae. These organisms are physically able to escape from both laser sensors and nets (Wiebe et al., 2004). Although both types of sampling devices were towed at similar speeds and the section area is bigger for the net than for the LOPC, it is possible that escapement behavior was enhanced by the horizontal towing direction of the nets (Vereshchaka et al., 2019).

#### 3.2. Defining the optimal sampling depth

- Does the chl<sub>a</sub> maximum layer correspond to the maximum of biomass (where most of the trophic interactions occur)?

The active transfer of contaminants along the planktonic trophic chain from autotroph synthesized biomass to zooplankton take place where zooplankton feed on newly produced material. This tends to happen slightly above the CML where primary production is usually highest and fed upon by herbivore zooplankton, or deeper in the water column where omnivorous or detritivores zooplankton feed on sinking particles (Stukel et al., 2019). If the latter situation is dominant, it is possible that the peak of zooplankton biomass and of chl<sub>a</sub> concentration will occur at different depths. In addition, trophic interactions with higher trophic level organisms such as fish larvae and juveniles sometimes occurs in the surface layer (Palomera et al., 2007). Among the nine stations, stations 1 and 10 showed different vertical positions for peaks of chl<sub>a</sub> concentration and zooplankton biomass (Figs. 3 & S3). In both cases, a zooplankton biomass peak occurred in the surface layer (top 10 m). This two-layer system may imply different trophic structures and ecosystem functioning between the surface and the water layers below. Crustacean larvae, mostly euphausiid larvae, were more abundant in the surface layer than in the rest of the column and likely contribute to this surface peak (Fierro-González et al., 2023). Euphausiid larvae have been reported to stay in the surface layer during the day (Granata et al., 2020). Their behavior is not yet fully understood, but they could access to other food sources such as copepod nauplii. As they are likely to be preyed on by fish as they grow in size, they may be an important vector



**Fig. 2.** Absolute (A) and relative (B) distribution of plankton biomasses at the chlorophyll maximum layer (CML), in four size classes, 60–200, 200–500, 500–1000 and 1000–2000  $\mu\text{m}$ , over nine stations sampled across the Mediterranean Sea from North (Marseille, France) to South (Gulf of Gabès, Tunisia). Both in-situ measurements from laser-based sensors (black frame) and net-sampled biomasses weighed in laboratory are displayed. Optical estimates are based on a 10-m layer centred around the CML depth, closely matching the layer sampled by the net.

**Table 1**

Differences between relative contribution of 60–200, 200–500, 500–1000 and 1000–2000  $\mu\text{m}$  size classes to the total pool of biomass estimated by in-situ sensors and from net samples. In bold, numbers with absolute value  $>0.25$ .

Stations	60–200 $\mu\text{m}$	200–500 $\mu\text{m}$	500–1000 $\mu\text{m}$	1000–2000 $\mu\text{m}$
St. 1	–0.14	–0.22	<b>0.29</b>	0.07
St. 2	0.02	0.05	0.08	–0.15
St. 3	0.00	0.06	–0.01	–0.05
St. 9	<b>0.30</b>	<b>–0.30</b>	0.00	–0.01
St. 10	–0.09	0.11	0.00	–0.02
St. 11	–0.18	–0.03	0.02	0.19
St. 15	–0.01	–0.17	–0.09	<b>0.28</b>
St. 17	0.06	–0.18	0.12	0.00
St. 19	–0.12	–0.03	0.14	0.02

of transferring contaminants from surface layer toward higher trophic levels. Besides, they can be mixed with the buoyant type of microplastics that tend to accumulate in the very surface layer (Chevalier et al., 2023), favoring accidental predation of these plastics by fish. However, although the biomass concentrations in the surface was the highest for these stations, it is important to note that these peaks were restricted to thin layers and therefore have a limited influence on total zooplankton biomass.

This also raises questions about how the impact of diel vertical migration (DVM) could be accounting for, as active contaminant transfer occurs through feeding, and organisms of different sizes follow different patterns in terms of DVM depth range and feeding timing. For example, among copepod species, small size copepods are known to undergo DVM of narrower amplitude than copepods of larger size (Ohman and Romagnan, 2016).

### 3.3. Impact of changes in size distribution through the water column

- Does the zooplankton size distribution in the CML remain constant over the water column? If not, what could be the consequences for

estimates of contaminant concentration integrated over the water column?

Changes in zooplankton size structure is likely to influence the efficiency of contaminant bioaccumulation and modify the pathways taken by the contaminants to transfer higher up in the trophic chain. To be able to extrapolate the results found in the CML to the entire water column, it is therefore important to assess if the size distribution changed with depth. If the CML is constrained to a thin layer, where most of the biomass is accumulated, then changes in distribution size with depth should no affect significantly integrated estimates. On the other hand, if the biomass distribution is rather uniform along the water column or if another biomass peak takes place above or below the sampling depth, then extrapolate results from the CML can lead to biased estimates.

Among the nine stations, stations 17 and 19 profiles showed a drastic change in size distribution with depth, characterized by an increase in biomass in the larger size class, occurring in the surface layer (Figs. 3 & S4). At these stations, the bottom layer was characterized by large amounts of detrital materials and the quasi absence of large ( $> 1 \text{ mm}$ ) copepods and gelatinous organisms (Fierro-González et al., 2023). Turbid waters are not a favourable environment for gelatinous filter-feeders (Briseño-Avena et al., 2020; Paffenhöfer et al., 1991). Particularly for appendicularians, for which in addition to lowering the food quality (López-Urrutia et al., 2003), accumulation of detritus can result in house clogging (Tiselius et al., 2003). In the surface, large size copepods such as *Centropages* spp., and chaetognaths dominated the biomass in the size class  $>1 \text{ mm}$  (Fierro-González et al., 2023). These two stations were also characterized by high densities of small pelagic fish (Table 2) (Ben Abdallah et al., 2018), which usually feed in the surface layer at night (Palomera et al., 2007), implying active trophic transfer. Active trophic transfer can result in biomagnification of contaminants along the food web. Methylmercury and PCBs for example, are some of the toxic contaminants that have been shown to accumulate toward higher trophic levels (Tiano et al., 2014, Tésan et al. this issue).

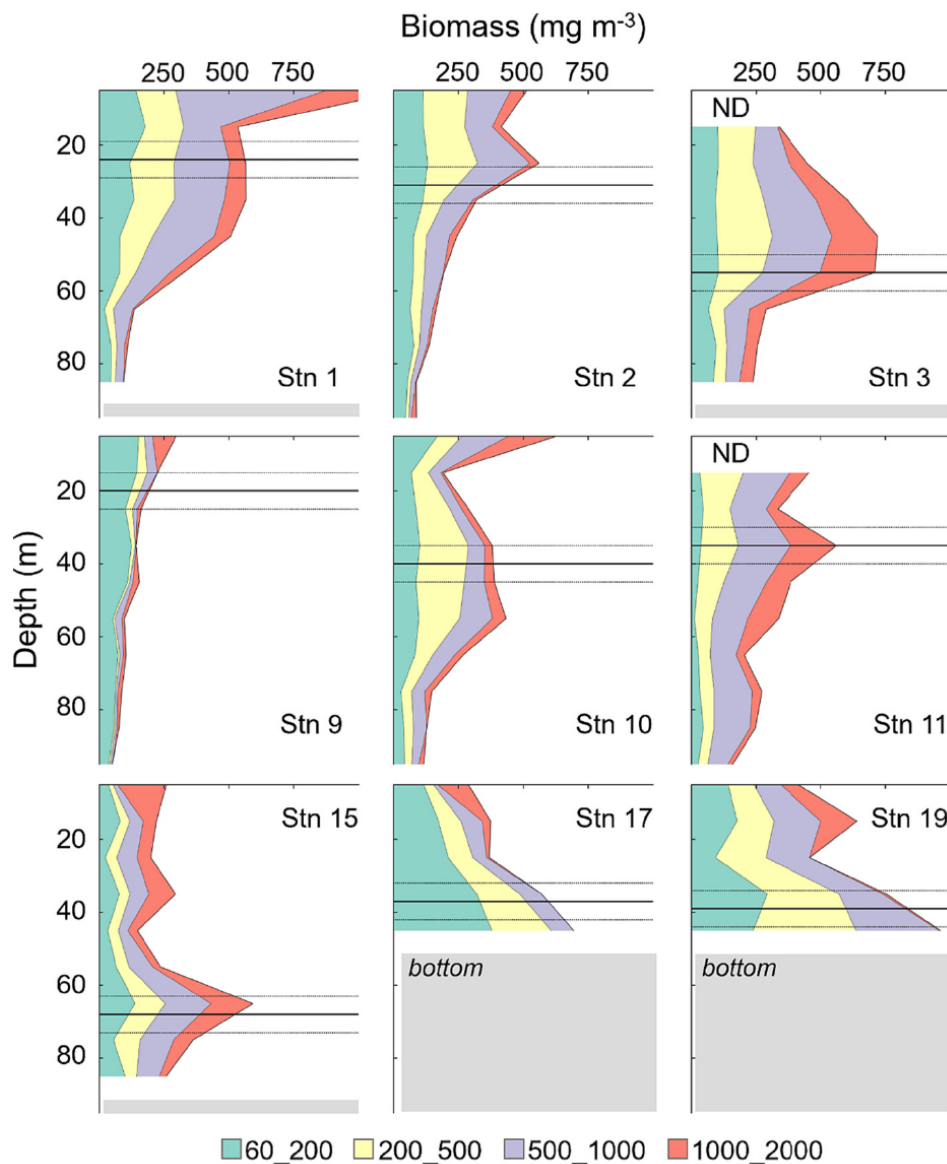


Fig. 3. Vertical distributions of plankton biomass in four size classes as measured by in-situ laser sensors. The chl<sub>a</sub> maximum layer where the samples were collected for contaminant analyses (see Fig. 2) is shown (black lines). The upper and lower bounds (dotted lines) define the water layer in which the sensor data were integrated. ND: no data available.

Chifflet et al. (2023) also showed strong biomagnification of some of the metals and metalloids, and specifically in the southern coastal area (station 15, 17 and 19).

### 3.4. Identifying environmental factors affecting contaminant estimates

To be able to anticipate the situations in which one or several of the conditions discussed above are not met, and therefore for which the quantification and interpretation of the bioaccumulation and transfer of organic and metallic contaminants within planktonic food web might be biased, we investigated for leading patterns in environmental conditions. This could be used as a template for designing further sampling strategy, and define when results should be taken with caution and whether the use of additional information might be needed for better interpretation. Although we only have a limited number of stations, and acknowledge that this effort should be further extended, the environmental conditions observed during the cruise covered a large range of configuration in terms of production, stratification, temperature and bathymetry. Table 2 summarizes the environmental conditions observed at each station (see also Fig. S5 for profiles) along with information related to the three questions discussed previously. The situations where

the interpretation of the contaminant dynamics should be done with caution can be grouped into three scenarios: (i) CML close to the bottom (shallow coastal stations 17 and 19) due to sediment resuspension resulting in different plankton communities in the surface layer and at the CML depth; (ii) high phytoplankton bloom enhancing problems linked with sampling procedure (clogging, larger organisms escaping nets) (stations 9 and 15); (iii) presence of a zooplankton peak in the surface layer not being related to a chl<sub>a</sub> peak (stations 1 and 10). The latter situation is difficult to anticipate based on environmental data alone, and in this case the use of in-situ sensors is required.

## 4. Conclusion

Our results confirm that in most cases sampling at the DCM is the best strategy to ensure both high amounts of biomass and representativeness of the contaminant situation over the entire water column. However, we showed that in some specific cases the interpretation of the results can be more complex. There are two main points that emerged from this study. First, some of the issues are linked with the difficulty to sample zooplankton quantitatively across the full size spectrum. Copepod nauplii tends to go through net mesh while larger organisms have the

**Table 2**

General description of the sampling sites and environmental conditions at the time of sampling for the nine stations distributed along a North-South transect in the Mediterranean Sea. General features were taken from (Tedetti et al., 2023). strat: = stratification.

Station	General features	Environmental conditions	Net vs sensors-sampled particles	CML vs biomass peak	Consistency of size structure
St 1. Bay of Toulon 91 m	Nearly closed urbanised bay; Intermittently bloom area or bloom area	Mixed waters; low, homogeneous chl a	< 500–1000 $\mu\text{m}$	Biomass peak in surface	Uniform
St 2. Offshore Toulon 1770 m	Limit of the continental shelf; Intermittently bloom area or bloom area	Weak strat. ca 30 m; medium chl a peak at 30 m	Good agreement	Matching peaks	Uniform
St 3. Offshore Marseille 95 m	Southeast entrance to the Gulf of Lion; Intrusions of the Ligurian-Provençal current; Intermittently bloom area or bloom area	Mixed waters; low, peak chl a at 55 m	Good agreement	Matching peaks	Uniform
St 9. Offshore 2575 m	North of the North Balearic Front; Winter convection area; Bloom area	Weak strat. ca 80 m: high peak chl a at 10 m	> 200–500 $\mu\text{m}$	Matching peaks	Uniform
St 10. Offshore 2791 m	Slightly North of the North Balearic Front; Intermittently bloom area	Gradual strat; low peak chl a at 40 m	Good agreement	Biomass peak in surface	Uniform
St 11. Offshore 1378 m	South of the North Balearic Front; Presence of mesoscale eddies; No bloom area	Strong strat. ca 100 m; low homogeneous chl a	Good agreement	Matching peaks	Uniform
St 15. Gulf of Hammamet 100 m	Close to the Sicily Channel; Possible entrance of Atlantic Tunisian Current branch; No bloom area; High density of small pelagic fishes	Gradual strat; low, peak chl a at 70 m	< 1–2 mm	Matching peaks	Uniform
St 17. North of Gulf of Gabès 50 m	Shallow area, influence of tides and Atlantic Tunisian Current; Coastal bloom area; High density of small pelagic fishes	Mixed waters; low homogeneous chl a; sed. Resus.	Good agreement	Matching peaks	Larger plankton in surface layer
St 19. South of Gulf of Gabès 50 m	Shallow area, influence of tides and Atlantic Tunisian Current; Coastal bloom area; High density of small pelagic fishes	Weak strat. ca 25 m; medium chl a peak at 45 m; sed. Resus.	Good agreement	Matching peaks	Larger plankton in surface layer

ability to avoid nets resulting in underestimation for the smallest and largest zooplankton size classes, respectively. Second, a two-layer system, with differing peaks in the vertical profiles of phytoplankton and zooplankton biomass or a second zooplankton peak, was observed at some stations, sometimes linked with sediment resuspension in the bottom layer. In such case, the trophic interactions are likely to be important in the surface layer and therefore contaminant transfer toward higher trophic levels could be non negligible. This could be particularly significant since most of contaminants usually exhibit highest concentrations in the surface layer due to various inputs including atmospheric deposition.

#### CRedit authorship contribution statement

BE, MP and FC conceptualized the study. MP, FC, DM and CC collected the data. BE processed and analyzed the data, produced the figures and made the first draft. All co-authors contributed to the final drafting of the manuscript and approved the submitted version.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data availability

All data from the MERITE-HIPPOCAMPE cruise (<https://doi.org/10.17600/18000900>); Tedetti and Tronczynski, 2019) is stored in the MISTRALS-SEDOO database (<https://mistrals.sedoo.fr/MERITE/>) and will be made publicly accessible once all the articles related to the cruise are published in the present special issue. In the meantime, data can be obtained upon request from the corresponding author. In addition, navigation data and CTD profiles from the MERITE-HIPPOCAMPE cruise are available via the IFREMER/SISMER database (<https://data.ifremer.fr/SISMER>).

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online

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