

# MERCURY CONCENTRATIONS AND TRANSFERS IN PHYTO- AND ZOOPLANKTON COMMUNITIES IN A COASTAL MEDITERRANEAN ECOSYSTEM (BAY OF TOULON, FRANCE)

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**Abstract** – Planktonic organisms, at the basis of marine trophic networks, play an essential role in the transfer of energy and matter, including contaminants, from the environment to marine organisms. However, only few studies focus on the planktonic compartment as a whole and on the influence of its structure and composition on trophic networks functioning and transfers of contaminants. The accumulation of mercury causes organisms health problems. So, fishes' mercury contamination has widely been studied but the role of the first trophic levels in its intake and transfers is yet to be investigated. The Bay of Toulon is known for its high concentrations of mercury in sediments, thus it is a perfect site to focus on mercury contamination of the planktonic compartment as a continuum. The aim of this study was to characterize the whole plankton continuum (from pico-, nano- phytoplankton to meso-zooplankton) from a taxonomic point of view, its trophic organization and mercury contamination. Monthly samples were collected in the Bay of Toulon, from September 2020 to September 2021, by horizontal plankton net tows, sieved through different mesh size to collect 4 size classes (>500, 500-200, 200-100 and 100-20 µm). The last fraction (<20 µm) was collected by concentrating the smallest fraction by centrifugation. Taxonomic analysis were carried out for each separated fraction. Mercury analysis were performed with an AMA 254 mercury analyzer. Results showed a higher abundance of zooplankton, pico-, nano-phytoplankton and bacteria in the Little Bay, and micro- phytoplankton in the Large Bay. Overall, mercury concentrations were higher in the LiB. Contrarily to the standard mercury biomagnification pattern commonly observed in higher trophic levels, in this study, the smaller size classes (<20 and 100-20 µm) presented higher mercury concentrations decreasing while size increased (until 200-100 µm), followed by an inversion of the trend between the two largest size classes (500-200 and >500 µm).

## Introduction

Planktonic organisms, at the interface between the biotic and abiotic compartments, occupy a key position in the organic matter, energy and contaminants transfers in marine biota [1]. The structure, composition and dynamics of planktonic communities may be highly impacted by variations of climatic and hydrological conditions or concentrations in

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contaminants [18]. These variations may significantly affect the functioning of marine ecosystems and induce modifications in marine trophic networks [10].

Zooplanktonic organisms and fishes have widely been studied as models of metal transfers, contrary to smaller organisms such as phytoplankton [2]. The Mediterranean Sea is known for its history of mercury inputs from natural and anthropic origins. The accumulation of mercury, more specifically methylmercury, causes organisms numerous health problems [3]. So, fishes' mercury contamination has widely been studied [7] but the role of the first trophic levels in its intake and transfers is yet to be investigated [4]. The Bay of Toulon is known for its high concentrations of mercury in sediments, thus it is a perfect site to focus on mercury contamination of the planktonic compartment as a continuum.

In a global context of questioning on the influence of the structure and organization of planktonic communities on the integration of contaminants and their transfers in marine trophic networks, the objectives of this study were to: *i*) collect, separate and identify planktonic matter in size classes representative of the trophic organization of the planktonic communities; *ii*) characterize the levels of mercury contamination in planktonic trophic networks in a Mediterranean coastal bay. To achieve this, samples of the planktonic compartment were fractionated in different size classes, each one taxonomically identified and their total mercury levels measured.

## Materials and methods

The Bay of Toulon is divided in the Little Bay (LiB), semi-closed and characterized by high levels of anthropic activities and the Large Bay (LaB) open on the sea and less impacted by anthropic activities [15]. Samples were collected monthly, from September 2020 to September 2021, in the LiB (start Lat.: 43°06'30"N, Long.: 05°55'00"E) and the LaB (start Lat.: 43°05'45"N, Long.: 05°56'30"E). They were collected with horizontal plankton net traits (mesh size 80 µm, 0,5 m diameter and 2,5 m long) hauled outside the wake of the boat (1,8 knots, 45 minutes). Samples were sieved through different mesh sizes (500, 200, 100 and 20 µm) to collect 4 size classes (fraction 1: >500 µm; fraction 2: 500-200 µm; fraction 3: 200-100 µm and fraction 4: 100-20 µm). The remaining fraction was centrifuged (15 min, 3000 rpm) to concentrate and retrieve fraction 5 (<20 µm). For fractions 1 to 4, aliquots were retrieved to taxonomically identify the phyto- and zooplanktonic communities, respectively preserved using a 0.3 % lugol and 4 % buffered formaldehyde solutions. For fraction 5, aliquots were fixed with a 0.25 % glutaraldehyde and 0.01 % pluronic acid solution. Samples were frozen, freeze dried and homogenized by grinding before analysis. Zooplankton taxonomic identifications were accomplished according to Folsom's method [11] using a binocular magnifier Leica® M125C and the phytoplankton ones as per Utermöhl's method [17] using an inverted epifluorescence microscope Olympus® IMT2. For fraction 5, flow cytometry was used to identify organisms with an Accuri C6 BD Bioscience® [9]. Mercury levels analysis were carried out using a semi-automatic mercury analyzer ALTEC® AMA 254 [7].

RStudio and R version 4.2.0 were used to carry out the statistical analysis [12]. The Shapiro-Wilk test was used to evaluate normality. Total abundance and mercury levels were compared using the Wilcoxon signed-rank test. Non-parametric data between fractions were evaluated using ANOVA Friedman test and a post hoc Wilcoxon pairwise test with

Benjamini-Hochberg corrected p values. Parametric data between fractions were evaluated using ANOVA and a post hoc Tukey's test.

## Results

The mean abundance of the meso- zooplanktonic communities was almost 2 times higher in the LiB than in the LaB (Wilcox. test,  $p < 0,05$ ). In the LiB, there were significantly more organisms in fractions 1 ( $>500\mu\text{m}$ ), 2 ( $500\text{-}200\mu\text{m}$ ) and 3 ( $200\text{-}100\mu\text{m}$ ) than in the smallest fraction (4:  $100\text{-}20\mu\text{m}$ ). In the LaB, similar observations showed significantly more zooplankton in fractions 1, 2 and 3 than in fraction 4, but also more in fractions 1 and 2 than in fraction 3 (ANOVA, Wilcox. corr. BH,  $p < 0,05$ ; Figure 1).

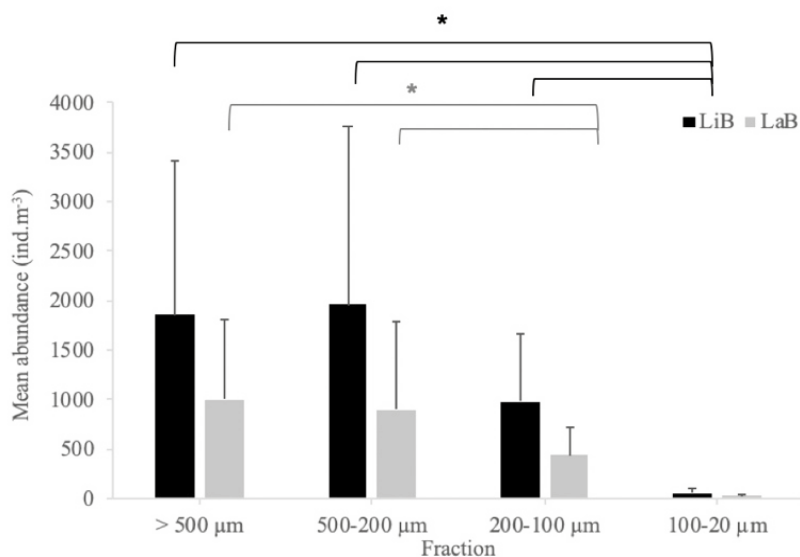


Figure 1 – Zooplankton mean abundance in fractions 1 to 4.

The abundance of Protista, Mollusca, Calanoïda and Cyclopoïda was significantly higher in the LiB (Wilcox. test,  $p < 0,05$ ), for the other taxa a similar trend was also observed. In the LiB, there were significantly more Protista and Mollusca in fraction 4 than in fractions 1, 2 and 3. Calanoïda and Cyclopoïda were more abundant in fractions 1 and 2 than in fractions 3 and 4. Finding in the LaB were comparable: Protista were dominant in fraction 4, Calanoïda and Cyclopoïda in fractions 1 and 2 (ANOVA, Wilcox. corr. BH,  $p < 0,05$ ; Figure 2). Overall, the most abundant taxa were Calanoïda and Cyclopoïda, representing more than 70 % of the communities.

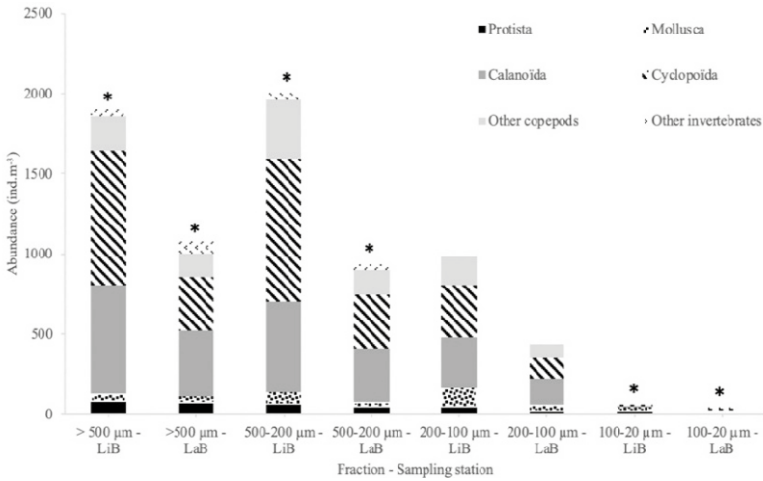


Figure 2 – Proportion of zooplanktonic taxa in fractions 1 to 4.

The mean abundance of the phytoplanktonic communities was almost 2 times higher in the LaB (Wilcox. test,  $p < 0,05$ ). In the LiB, there was drastically more phytoplankton in the smaller fractions (3 and 4) than in the biggest fractions (1 and 2). In the LaB, corresponding findings showed more phytoplankton in fractions 3 and 4 than in fractions 1 and 2, but also in fraction 4 than in fraction 3 (ANOVA, Wilcox. corr. BH,  $p < 0,05$ ; Figure 3).

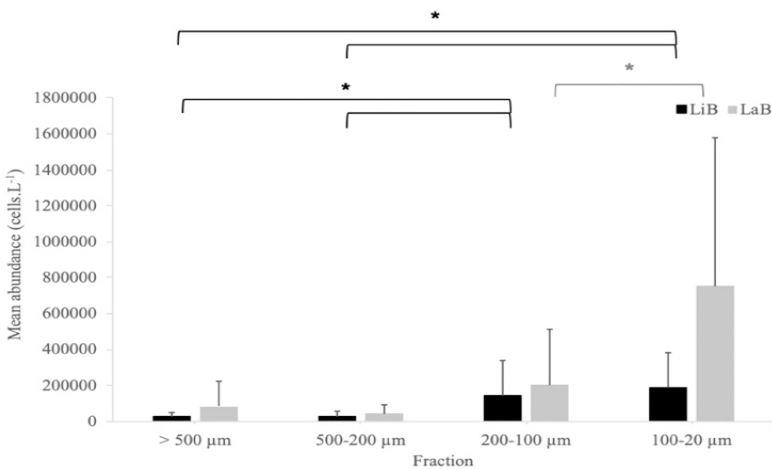


Figure 3 – Phytoplankton mean abundance in fractions 1 to 4.

The abundance of Bacillariophyceae was significantly higher in the LaB (Wilcox. test,  $p < 0,05$ ), for the other taxa an analogous trend was also observed. Bacillariophyceae were

drastically more abundant in fractions 3 and 4 than in fractions 1 and 2 in the LiB, whereas in the LaB they were only predominant in the fourth fraction (ANOVA, Wilcox. corr. BH,  $p < 0,05$ ; Figure 4). A trend of greater Dinophyceae and other flagellates abundance in the smaller size classes was also identified in both bays.

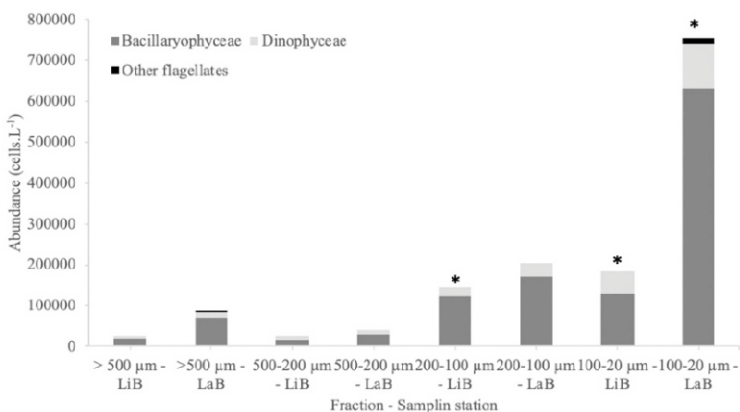


Figure 4 – Proportion of each phytoplankton taxa in fractions 1 to 4 in the LiB and the LaB.

The mean abundance of the pico-, nano- phytoplanktonic and bacterial communities was almost 4 times greater in the LiB than in the LaB (Wilcox. test,  $p < 0,05$ ; Figure 5).

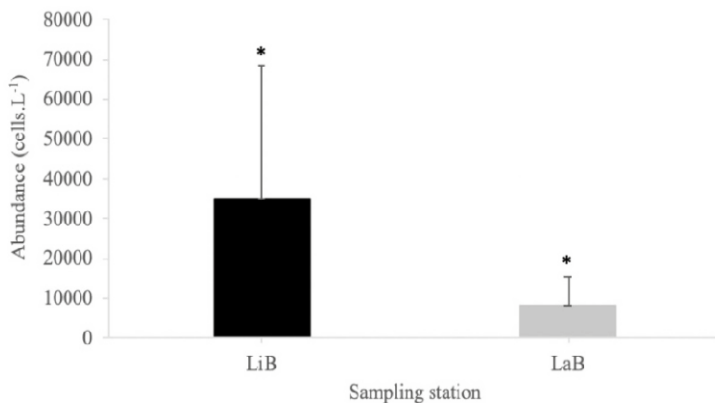


Figure 5 – Pico-, nano- phytoplankton and bacterial mean abundance in fraction 5 in the LiB and the LaB.

Overall, *Synechococcus* and Bacteria were the most abundant taxa in both bays. The abundance of *Prochlorococcus*, Picoeukaryots, Nanoeukaryots and Cryptophyceae was

significantly more important in the LiB, and the same trend was observed for *Synechococcus* and Bacteria (Wilcox. test,  $p < 0,05$ , Figure 6).

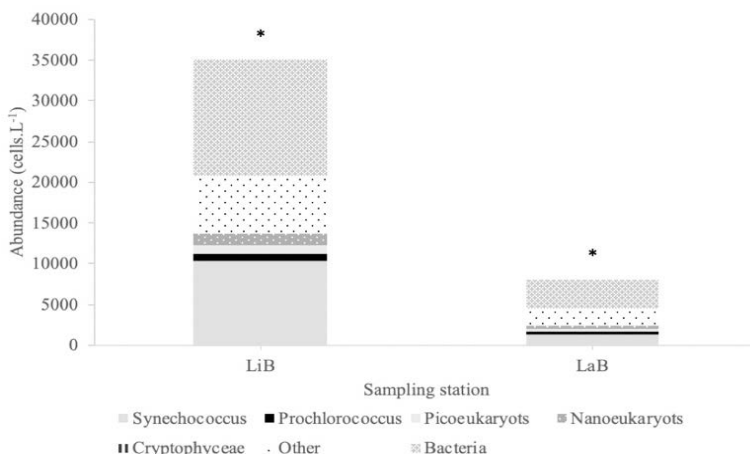


Figure 6 – Proportion of each pico- nano- phytoplanktonic and bacterial taxa in fraction 5 in the LiB and the LaB.

Mercury concentrations in each fraction were almost twice as important in the LiB than in the LaB (Wilcox. test,  $p < 0,05$ ). In the LiB, they were critically more important in fractions 5 and 4 than in fractions 1, 2 and 3 (ANOVA, Tukey,  $p < 0,05$ , Figure 7). The smallest size classes were the most contaminated, a trend of biodilution was remarked from fraction 5 through 2 followed by an inversion of the trend with an onset of biomagnification between fractions 1 and 2.

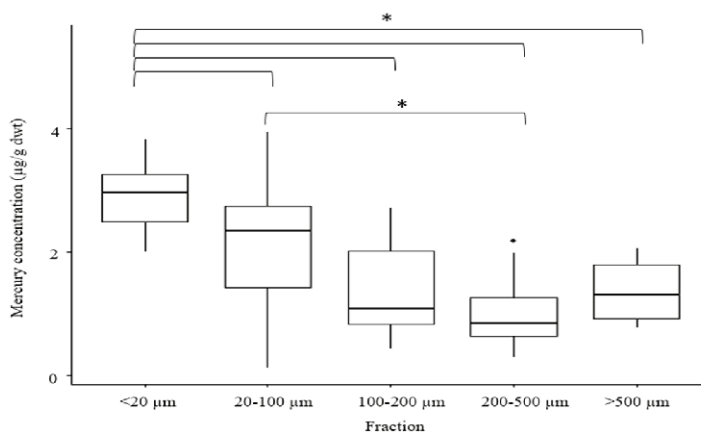


Figure 7 – Mercury concentrations in each fraction in the LiB.

In the LaB similar observation showed higher mercury concentrations in fractions 5 and 4, but in fraction 1 than in fraction 2 (ANOVA, Wilcox. corr. BH,  $p < 0,05$ ; Figure 8). In the LaB too, the smallest size classes were the most contaminated in mercury and a trend of biodilution could be observed between fraction 5 and fraction 3 followed by an inversion of the trend with an onset of biomagnification between fractions 1 and 2.

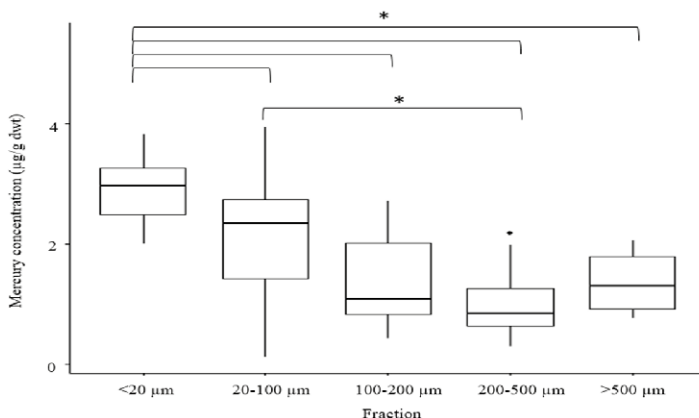


Figure 8 – Mercury concentrations in each fraction in the LaB.

## Discussion

Firstly, observations showed that zooplankton, pico-, nano- phytoplankton and bacteria were more abundant in the LiB but micro-, phytoplankton were more abundant in the LaB. These results are coherent with previous observations for every community except the micro- phytoplanktonic community. Indeed, between 2006 and 2007, Rossi and Jamet [14] described a more abundant phytoplanktonic community in the LiB. This dissimilarity could be explained by the distinct composition and size structure of communities in both bays. Secondly, the results indicated that Copepods, Bacillariophyceae and Synechococcus were the dominant taxa in their respective communities and were consistent with previous observations at the study site by Serranito *et al.* [15], Rossi and Jamet [13] and Cocelet *et al.* [5], respectively.

The mercury concentration in this study were greater than those in other studies conducted in the Gulf of Lion and in the Bay of Biscay [6, 7]. The higher levels of mercury recorded in all the fractions in the LiB could be attributed to the historical contamination of mercury in the LiB [16]. These differences could also be attributed to the specific trophic status in each bay and their impacts on the functioning of the ecosystems resulting in the distinct patterns of mercury integration in planktonic organisms observed during this study. The results also showed that the smallest fractions are the most contaminated by mercury. The smaller size classes could be the place of the beginning of integration of mercury in the planktonic trophic networks. The first part of the mercury levels quantification shows an absence of mercury biomagnification, or more likely, an apparent biodilution in the smaller size classes (< 20 µm to 200-100 µm), these results are in stark contrast to the traditionally

accepted mercury transfer scheme in marine ecosystem, where mercury biomagnifies with the increase of trophic levels [8]. But, the second part of the results (between 200-100  $\mu\text{m}$  and  $> 500 \mu\text{m}$ ), were congruent with conventional patterns of mercury biomagnification between higher trophic levels. The results raise the hypothesis of distinct mercury integration schemes in phyto- and zooplanktonic organisms and highlights the importance of the trophic functioning of the studied ecosystems.

## Conclusion

This study allowed to characterize the composition and structure of the planktonic compartment in the Bay of Toulon (LiB and LaB). The zooplanktonic, pico-, nano-phytoplanktonic and bacterial communities were more abundant in the LiB but the micro-, phytoplanktonic community was most present in the LaB. The zooplanktonic community was dominated by Copepods, Bacillariophyceae and Synechococcus were the most abundant micro- and pico- nano- phytoplanktonic taxa, respectively. Mercury concentration were greater in the LiB than in the LaB. Contrarily to the standard mercury biomagnification pattern commonly observed in higher trophic levels, in this study, the smaller size classes ( $<20$  and  $100-20 \mu\text{m}$ ) presented higher mercury concentrations decreasing while size increased (until  $200-100\mu\text{m}$ ), followed by an inversion of the trend between the two largest size classes ( $500-200$  and  $>500 \mu\text{m}$ ). This raises the hypothesis of distinct mercury integration schemes in phyto- and zooplanktonic organisms and highlights the importance of the trophic functioning of ecosystems.

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