# SUPPLEMENTARY MATERIAL

## **Methods**

The use of stable isotope mixing models to identify the main feeding locations of 0-group and 1-group flounder required the discrimination between food sources throughout the Lima estuary, from the lower to the middle and upper estuaries. Carbon (δ13C) and nitrogen (δ15N) stable isotope patterns of main organic matter sources, including particulate organic matter (POM), and sediment organic matter (SOM), and main prey groups was investigated across the Lima estuary. Differences of δ13C between POM and SOM sources, and across estuarine sectors (lower, middle and upper), were tested with a two-way ANOVA coupled with a Tukey post-hoc test. Data were log-transformed in order to meet parametric assumptions. Differences of δ13C and δ15N between prey were tested with a permutational multivariate analysis of variance (PERMANOVA), using prey groups as a nested factor within estuarine sector. Pair-wise tests between levels of the estuarine sector, and prey groups within sectors were performed in a separate PERMANOVA routine. Multivariate dispersion was tested with the PERMDISP routine. The PERMANOVA and PERMDISP analyses were based on the Euclidean distance dissimilarity matrix.

## **Results**

There were no significant differences between the POM and the SOM carbon signatures (two-way ANOVA; F = 1.3, p = 0.27), across all samples. Both POM and SOM δ13C signatures varied significantly (two-way ANOVA; F = 11.1, p = 0.001) between the three estuarine sectors. The upper estuary presented a depleted carbon signature ranging from -32.66 to -25.55‰, compared to the lower (p = 0.02) and middle estuaries (Tukey post-hoc, p= 0.04), with a δ13C varying from -24.57 to -19.45 ‰. This variation was consistent with the salinity gradient, as the oligohaline upper estuary showed an average salinity of 7.2 ± 5.4, while the lower and middle estuaries had average salinities of 29.9 ± 0.1, and 29.5 ± 0.3, respectively. Stable isotope signatures of prey followed a similar pattern and varied across estuarine sectors and between prey groups within each estuarine sector (Table S1). The upper estuary prey (*Corophium* spp., Polychaeta, and Chironomidae) presented lower δ13C compared to other prey (Figure 4B). Differences between prey groups within each sector were associated to δ15N as shown by the dual isotope plot (Figure 4B). Therefore, POM, SOM and prey samples were grouped as *upstream* (upper estuarine samples), and *downstream* (lower and middle estuarine samples), and this sampled classification was used throughout the paper.

**Table S1.** Statistics of the nested PERMANOVA test for the carbon (δ13C) and nitrogen (δ15N) stable isotope variation across estuarine sectors, and between prey groups within each estuarine sector.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Source of variation | Df | SS | MS | Pseudo-F | p-value |
| Estuarine area | 2 | 466.18 | 233.09 | 12.08 | 0.03\* |
| Prey groups | 5 | 96.44 | 19.29 | 7.93 | 0.001\* |
| Res | 23 | 55.96 | 2.43 |  |  |
| Total | 30 | 1165 |  |  |  |

\*significant p-value