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Biochemical Responses of Blue Mussel (*Mytilus* spp.) to Trophic PFAS Exposure

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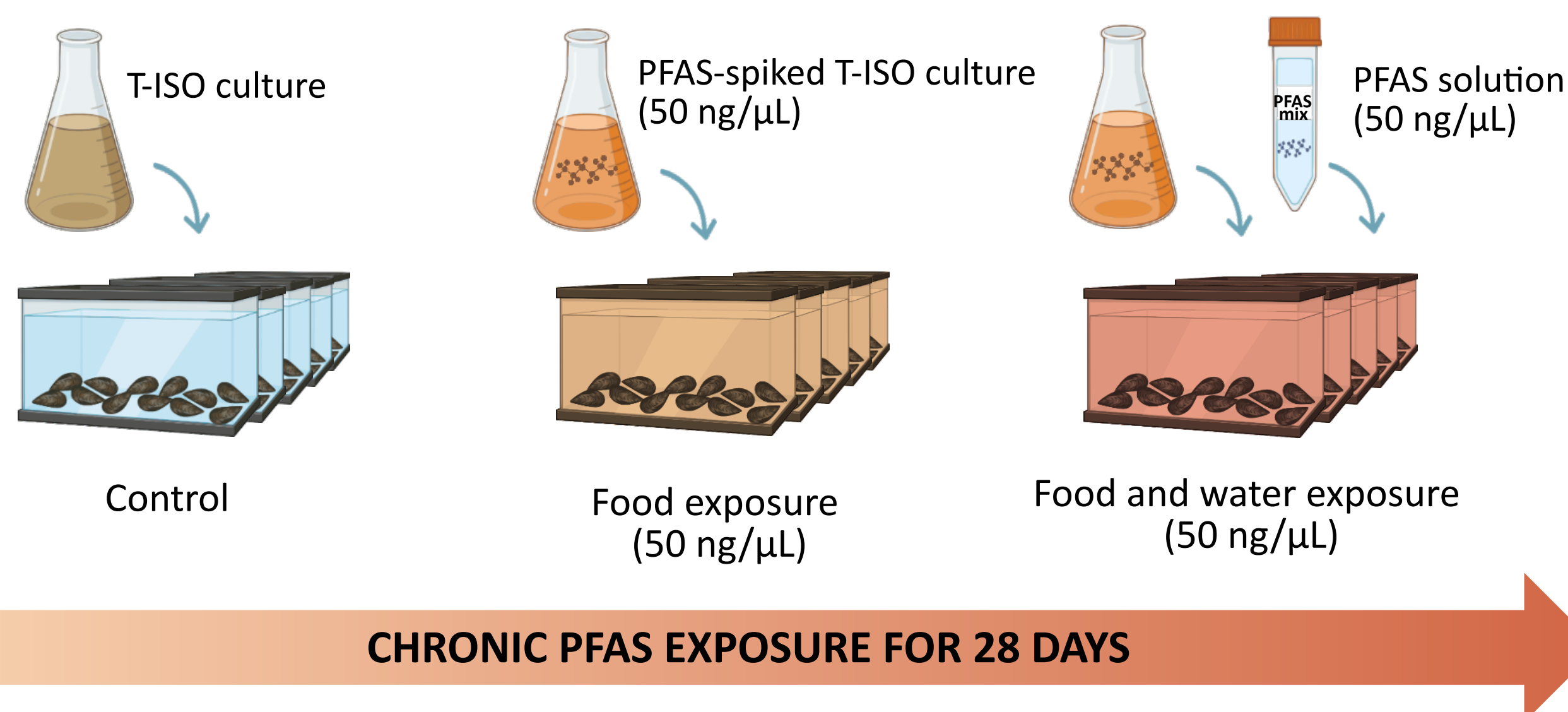
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INTRODUCTION

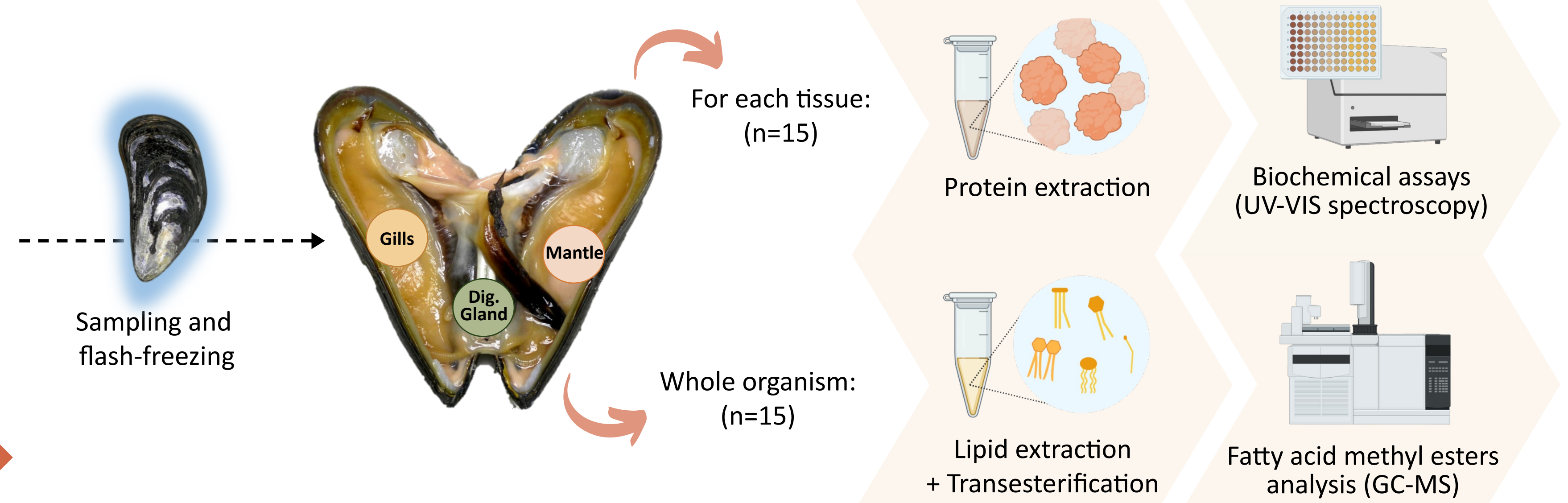
Per- and polyfluoroalkyl substances (PFAS) represent a class of **emerging contaminants** with over 4,700 variants, raising concerns for marine ecosystems due to their **pervasive presence and unknown impacts**. Despite regulatory efforts, the use of PFAS alternatives persists, necessitating a deeper understanding of their effects on marine organisms. Our aim was to assess the **biochemical effects** of chronic exposure of blue mussels (*Mytilus* spp.) to a **mixture of PFAS at environmentally relevant doses via water and food routes**.

MATERIAL & METHODS

1. Experimental Design



2. Sample Preparation and Analysis



FATTY ACID PROFILES

1. Identification of Key Fatty Acids Influenced by PFAS Exposure

Fatty acids with a proportion greater than 0.05% of the total fatty acids were considered for the analysis.

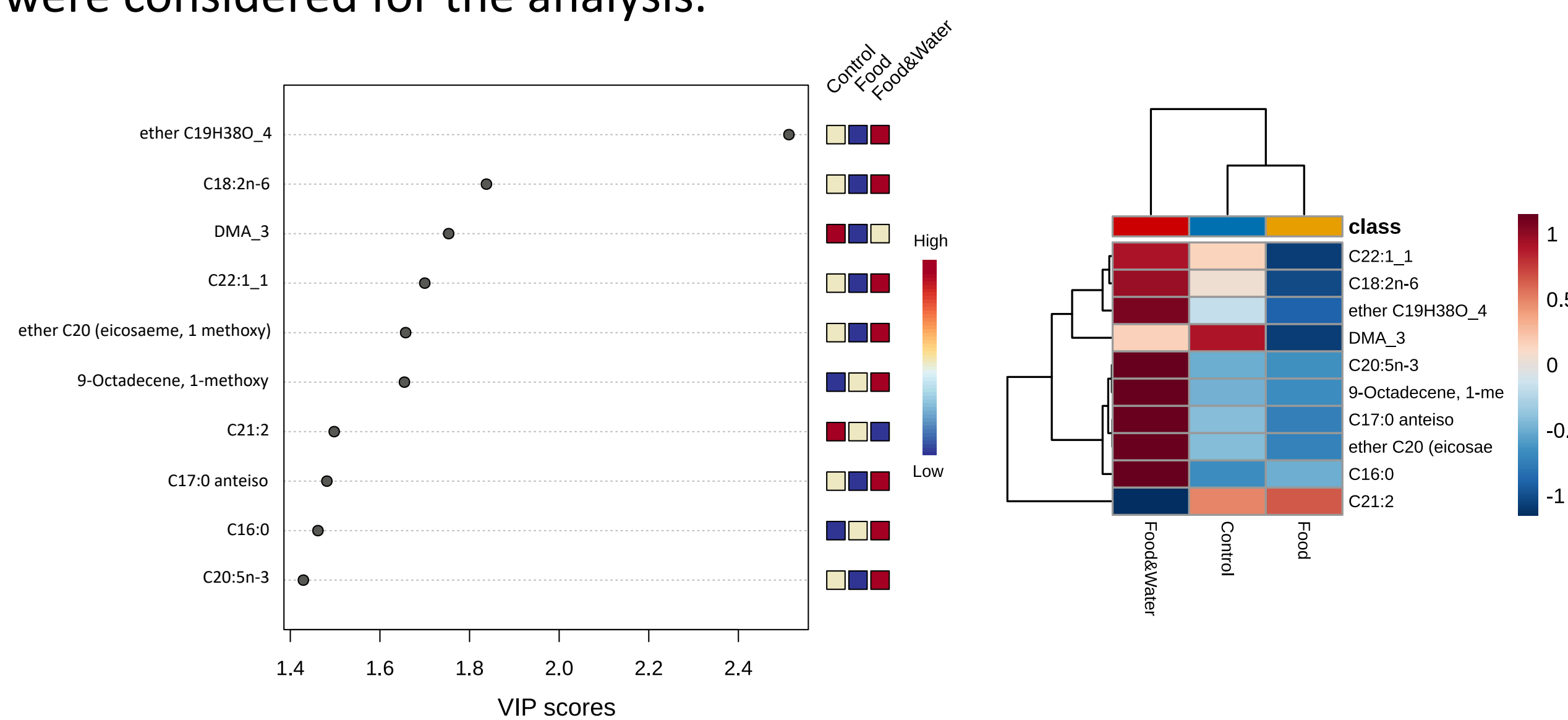


Fig. 1. VIP scores (left) and hierarchical Ward clustering heatmap (right) of the 10 most influential compounds identified by PLS-DA from normalized data.

Ether C19H38O_4 has the highest discriminant ability (VIP > 2.5), followed by several unsaturated fatty acids (C18:2n-6, C22:1, C21:2, C20:5n-3) and DMA_3, suggesting their **potential as biomarkers**.

The heatmap shows **higher fatty acid levels** in Food&Water condition, with a notable **decrease in C21:2**, indicating a specific response to PFAS exposure. Combined PFAS exposure (Food&Water) may have a **greater impact** on fatty acid composition compared to single exposure (Food).

2. Comparison of Key Fatty Acids Proportions Between Conditions

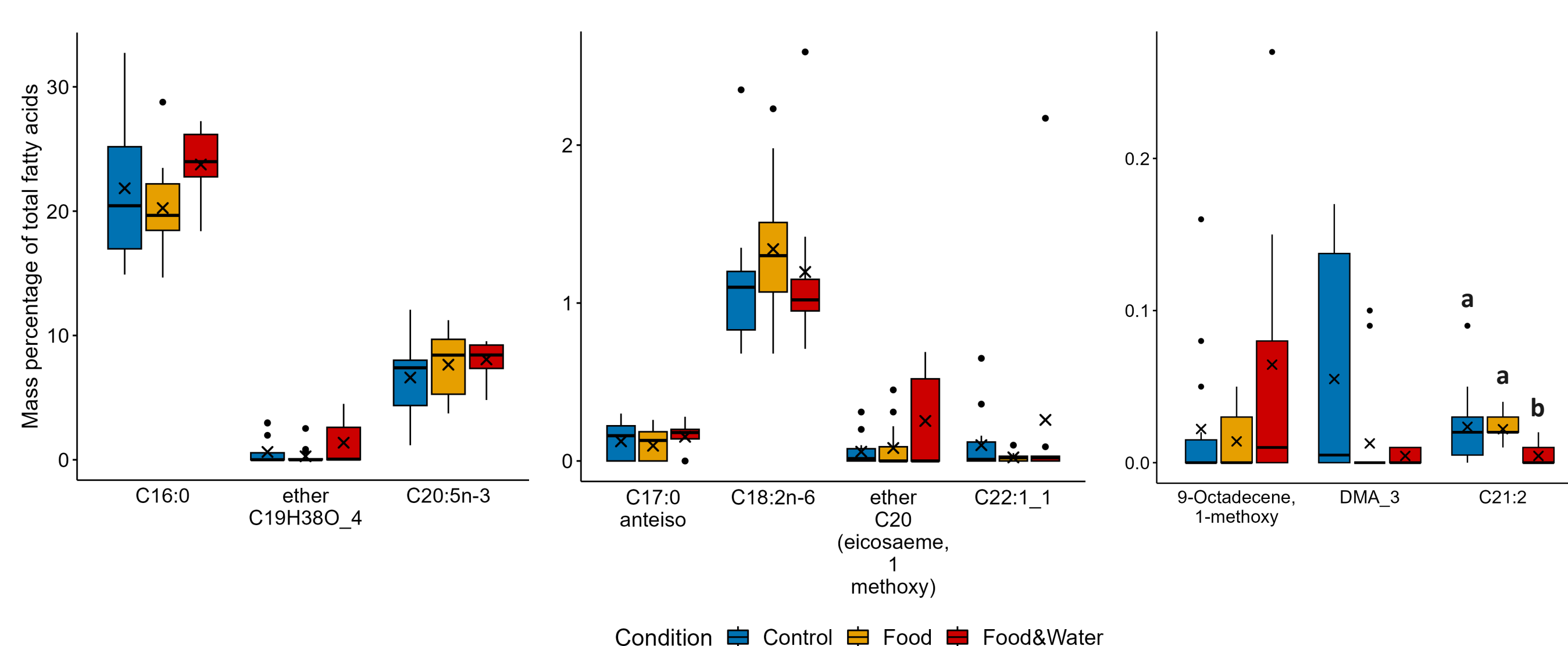


Fig. 2. Proportions (expressed as % total fatty acids) of the 10 most influential compounds identified by PLS-DA. Letters indicates significant differences between conditions (Kruskal-Wallis, $p < 0.05$).

PFAS exposure has **minimal impact** on most fatty acid proportions, except for **C21:2** which is significantly reduced in Food&Water condition, indicating a **specific response to PFAS**.

Ether C19H38O_4 shows no significant difference between conditions, **questioning VIP scores' predictability of actual differences**.

BIOCHEMICAL MARKERS

1. Data Distribution Overview

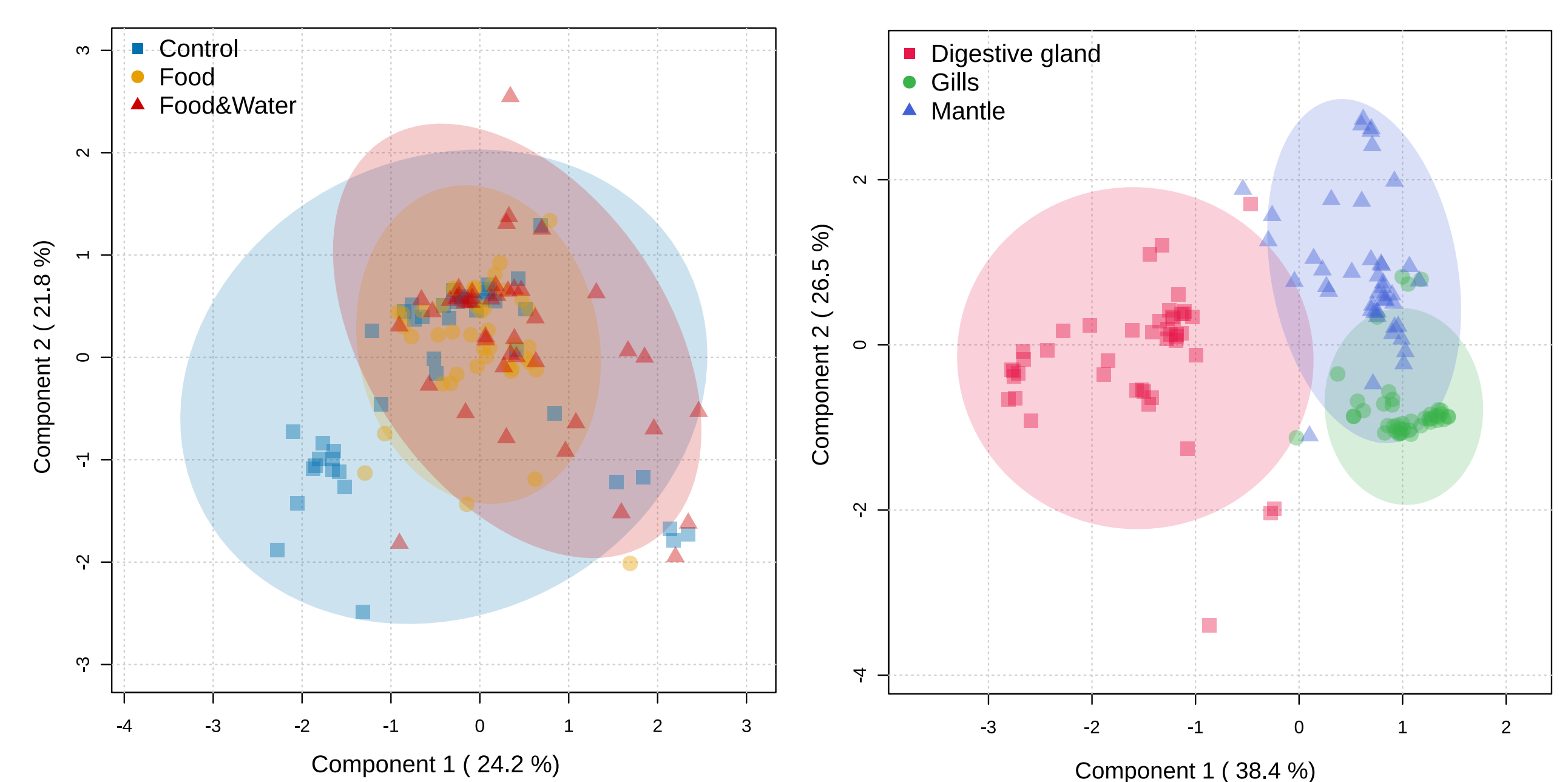


Fig. 3. Score plots of samples by tissue type (left) and experimental condition (right).

Sample clustering is primarily driven by tissue type, with minimal influence from experimental conditions, suggesting **strong tissue-specific biomarker responses**.

2. Tissue-Specific Variations in Biomarker Levels

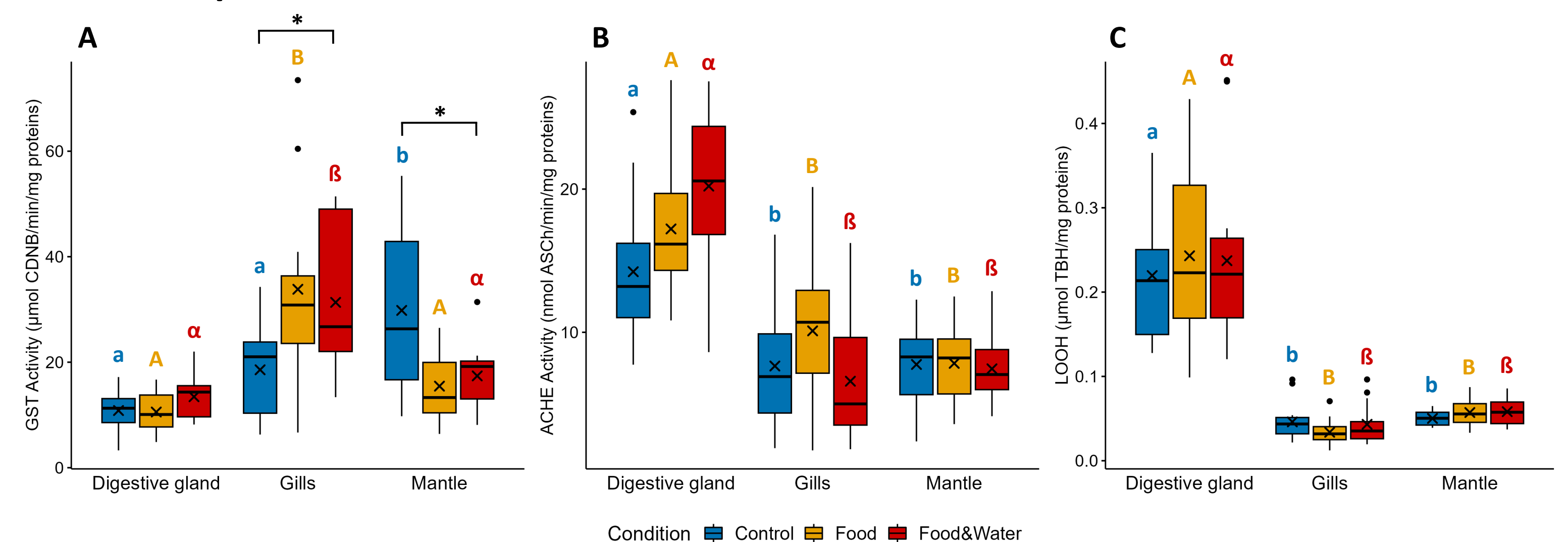


Fig. 4. GST activity (A), AChE activity (B) and LOOH concentrations (C) in *Mytilus* spp. under three experimental conditions (Control, Food, Food&Water). Letters indicate significant differences between tissues, and asterisks indicate significant differences between conditions (Two-way ANOVA, $p < 0.05$).

Significant differences in biomarker levels across tissues for each condition, with few significant variations between conditions within a specific tissue, except for GST activity, which is higher in gills and lower in mantle in Food&Water compared to Control. This confirms that **tissue type is the dominant factor influencing biomarker responses**.

CONCLUSION

Combined PFAS exposure (via food and water) has a greater impact on fatty acid composition and GST activity in *Mytilus* spp. compared to single exposure routes. Specifically, the fatty acid C21:2 is significantly reduced under combined exposure, indicating a specific biochemical response to PFAS at environmental concentrations.

