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# Metabolomics approach reveals disruption of metabolic pathways in the marine bivalve *Mytilus galloprovincialis* exposed to a WWTP effluent extract

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## 1. Introduction

Conventional wastewater treatment plants (WWTP) discharge a highly diverse range of organic contaminants in aquatic environments [1][2]. Because of the high demographic pressure, coastlines are affected by contaminant discharges through the development of WWTP sea outfalls, but also by contributions from catchment areas. The health of those important ecosystems could be threatened by contaminant release. In this context, new approaches can be helpful to assess the effects of multi-contamination on marine organisms without *a priori* knowledge. Environmental metabolomics could be one of those approaches since it is able to provide meaningful information on the biochemical response of organisms to a stress[3]. The aim of the present study was to develop a metabolomics approach to highlight key metabolites disrupted by WWTP effluent exposure and then elucidate the biological effects of such exposure on Mediterranean mussels (*Mytilus galloprovincialis*).

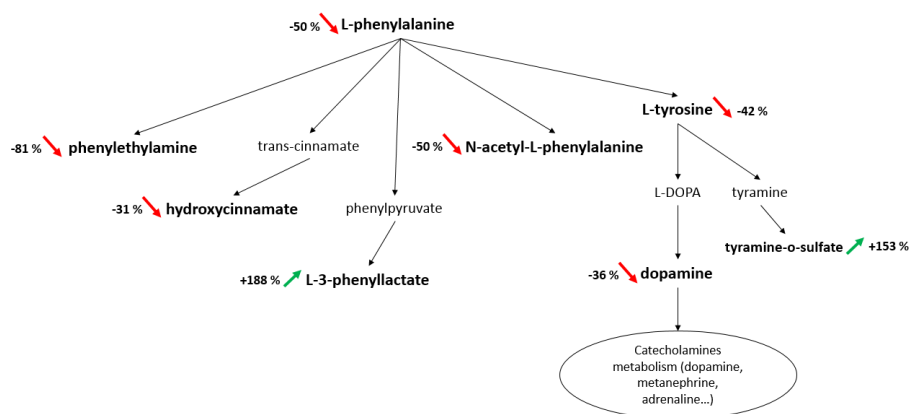
## 2. Materials and methods

An exposure has been performed in controlled laboratory conditions. Mussels (n=50, 5 mussels per becher of 2L) were acclimatised for seven days. Then, 25 mussels were exposed to a WWTP effluent extract corresponding to an environmental dilution of 5% while 25 mussels were exposed to solvent (100µL MeOH into 2L of seawater). During the acclimatisation and exposure periods, filtered seawater was continuously aerated and renewed daily (static renewal) and mussels were fed once daily with the marine green alga *Tetraselmis suecica* at constant density (10,000 cells/mL). Seven days following the exposure, mussels were dissected and their sex determined. No mortality was observed during the exposure period. Metabolic fingerprints were generated from digestive gland by liquid chromatography-high resolution mass spectrometry (control group: n=9 males, n=10 females and n=6 undifferentiated; exposed group: n=10 males, n=10 females and n=5 undifferentiated). Afterwards, univariate and multivariate tests were applied to highlight disrupted metabolites in males and females following exposure. A gender specific response was also assessed quantitatively and qualitatively by an ANOVA Multiblock OPLS (AMOPLS). This method takes advantage from the structured design of multifactorial experiments to decompose and quantify the sources of variability, and investigate main effects (exposure, gender) or interaction(s) (exposure×gender) using dedicated components [4]. Moreover, to better understand these observed effects, the WWTP effluent extract was characterised based on a suspect screening approach of 80 contaminants in an attempt to provide a first understanding of the relationship between observed effects and contaminants.

## 3. Results and discussion

### 3.1. WWTP effluent effects on male mussels

Treated male mussels showed numerous metabolites altered in response to WWTP effluent exposure. The highlighted metabolites belong mainly to amino acids metabolism (e.g. tyrosine, phenylalanine, leucine, proline, etc.), neurohormones (dopamine and a serotonin metabolite), purine and pyrimidine metabolism (e.g. adenosine, adenine, guanine, uracil etc.), citric acid cycle intermediates (e.g. malate, fumarate), and a component involved in oxidative stress defence (oxidised glutathione). Figure 1 shows an example of altered metabolic pathways, *i.e.* phenylalanine and tyrosine metabolism. Modulation of these metabolites could have effects on several biological processes, which may lead to a negative impact of organism fitness, such as energy metabolism, DNA, RNA and protein synthesis, osmoregulation, byssus formation and reproduction. The characterisation of the WWTP effluent extract has allowed the detection of 38 PhACs and their metabolites from various therapeutic classes, and 4 pesticides, occurring in the WWTP effluent extract. Based on previously published literature, some of them can be related to the observed effects, which supports our hypotheses about the impacted biological processes.



**Figure 1: Partial map of phenylalanine and tyrosine metabolism in the response of male mussels to WWTP effluent exposure. Metabolites modulated by exposure are shown in bold. Red arrows correspond to down-modulations and green arrows to up-modulations (exposed compared to control). Direct connections between metabolites are indicated with full arrows.**

### 3.2. Do males and females respond identically?

Female mussels also showed many impacted metabolites. Some of them were common to male, while many others seemed to be specific of female response. Thanks to the AMOPLS analysis, we were able to decompose the variability: the exposure effect (independently of gender) represented 7% ( $p < 0.01$ ) of the total variability, and the exposure × gender interaction, i.e. the gender specific response to WWTP effluent exposure, represented 4% ( $p < 0.05$ ) of the total variability. Meaningful biochemical information was obtained from the components related to each effect. The former highlighted metabolites modulated in response to exposure whatever the gender. The latter highlighted key metabolites involved in gender specific response (for example dopamine modulation was only observed in male in response to the WWTP exposure). This may provide a better understanding of response variabilities to the exposure.

## 4. Conclusions

In addition to enriching knowledge about the impact of WWTP effluents in marine environment, our work demonstrates the relevance of a metabolomics approach to highlight key molecular events triggered by chemical exposure, which could lead to adverse outcomes at the individual level. In addition, it underlines that gender has to be taken into account for further research on response of organisms to stress. AMOPLS is a promising approach to better characterise multiple factors of variability in response to a stress.

## 5. References

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