Changes to the *Pseudo-nitzschia* Lipidome After Exposure to Copepodamides Using UHPLC Coupled with High Resolution Multireflecting TOF Mass Spectrometry

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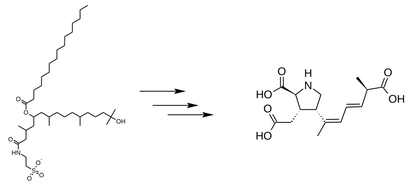
INTRODUCTION

Copepods are a widespread and abundant group of zooplankton in the ocean. They feed on marine phytoplankton including diatoms such as *Pseudo-nitzschia*. *Pseudo-nitzschia* cells sense chemical cues from the environment, including copepodamides, a class of bioactive taurine conjugated lipids that are excreted by copepods.[1] Copepodamides are known to induce defensive traits in multiple taxa of marine phytoplankton. *Pseudo-nitzschia* respond to copepodamides by regulating the production of domoic acid, a potent neurotoxin.[1]



Copepodamide Exposure

Domoic Acid Production

Domoic acid can then accumulate in filter feeding organisms that consume the phytoplankton. Cascading effects through the marine food web lead to amnesiac shellfish poisoning in humans, as well as brain and organ damage to other marine mammals and birds.[2,3]

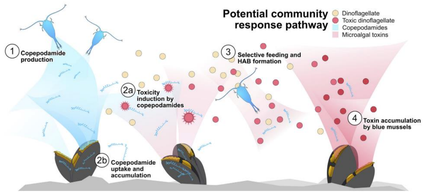


Figure 1. Hypothesized community response pathway from copepodamide production to the accumulation of biotoxins in mussels. (1) Copepods exude copepodamides to the surrounding water (2a) Copepodamides trigger increased toxin production in some harmful taxa, and (2b) accumulate in filter feeding bivalves. (3) Copepods feed selectively on less toxic prey items, thereby increasing the relative abundance of the harmful taxa. (4) Mussels accumulate toxins while feeding on a community with larger proportion of harmful taxa and grazer-induced cell-specific toxin content. Contributions from copepods and toxic dinoflagellates are indicated by blue and red, respectively. *(Reproduced from Limnol. Oceanogr.* ***66,*** *2021.)*

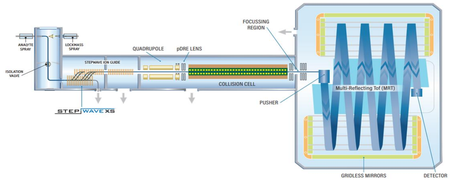


Figure 2. Schematic for Water Xevo ™ MRT Multireflecting TOF Mass Spectrometer. The effective flight path length is 4 meters with a resolution of greater than 100,000 (FWHM) and sub part per million mass accuracy.

METHODS

Cell Culture

Pseudo-nitzschia multiseries (EBL 27) cells were exposed to 0, 1, or 10 nM copepodamide for 72 hours in f/2 media (n=4). Cultures were incubated on a horizontal shaker (120 rpm) at 15°C with a 12 hr light/dark cycle.

Extraction

Cell pellet was extracted in MTBE:MeOH:H_2O (10:3:2.5) and dried down under N_2 and reconstituted in 100 µL 30:70 H_2O:ACN prior to analysis

Waters ACQUITY™ Premier UHPLC System

MP A: Water with1 mM Ammonium Formate, 0.1% Formic Acid

MP B: 95% Acetonitrile: 5% Water with 1 mm Ammonium Formate, 0.1% Formic Acid

Column: ACQUITY™ Premier CSH™ Phenyl Hexyl, 2.1x150

1.7 µm *dp*, operated at 50 C° and flow rate of 400 µL/min

Gradient Elution

Time	%A	% B	Curve
0	30	70	
10	1	99	6
13.4	1	99	6
13.5	30	70	1

Waters Xevo MRT MS

MS Tuned to 100,000 resolution and operated in either ES+ or ES– mode with Leucine Enkephalin lock mass

Data Acquisition 10 Hz in MS^E mode

CE Ramped from 20 to 50 V in High Energy Function

Data Acquisition 50 Hz in DDA mode

CE Ramped from 20 to 50 V in MS/MS Functions

DETECTION AND CHARACTERIZATION OF LIPIDS USING DIA AND DDA TECHNIQUES

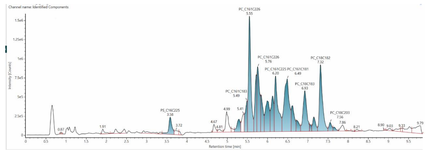


Figure 3. Positive Ion LC/MSE chromatogram of lipids identified in Pseudo-nitzschia. Candidates were searched against lipid libraries with ±1.5 ppm mass measurement tolerance.

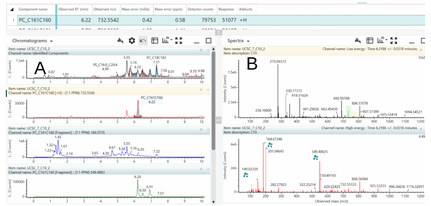


Figure 4. Representative data for palmitoleoly palmitoyl phosphatidylcholine PC 16:1 16:0 from ESI+ve LC/MS^E analysis a lipid extract from pseudo-nitzschia cells exposed to 10 nM copepodamide. Panel A Extracted ion traces for fragment ions for the PC headgroup and Panel B. High and low energy MSE spectra showing mass measurement error (0.58 ppm) on the precursor ion and the PC headgroup fragment ion.

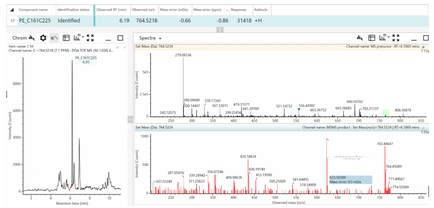


Figure 5. Representative MS and MS/MS ESI+ve spectra of palmitoleoly docosapentaenoyl phosphatidylethanolamine obtained from DDA analysis of a lipid extract from pseudo-nitzschia cells exposed to 10 nm copepodamide. Fragmentation of the PE precursor results in the loss of headgroup.

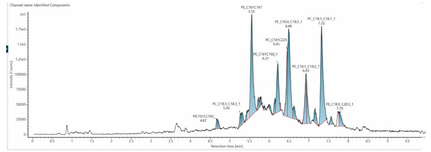


Figure 6. Negative Ion LC/MSE chromatogram of lipids identified in Pseudo-nitzschia. Candidates were searched against lipid libraries with ±1.5 ppm mass measurement tolerance.

Figure 7. Representative data for palmitoleoly palmitoyl phosphatidyethanolamine PC 16:1 16:0 from ESI-ve LC/MS^E analysis a lipid extract from pseudo-nitzschia cells exposed to 10 nM copepodamide. Panel A Extracted ion traces for fragment ions for the palmitoleyl fragment ion. High and low energy MSE spectra showing mass measurement error (0.3 ppm) on the precursor ion and the palmitoleoly fragment ion.

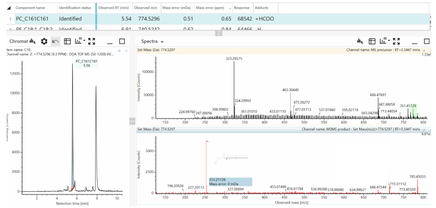


Figure 8. Representative MS and MS/MS ESI-ve spectra of palmitoleoly phosphatidylcholine obtained from DDA analysis of a lipid extract from pseudo-nitzschia cells exposed to 10 nm copepodamide. Major fragments include the palmitoleic acyl anion.

STATISTICAL ANALYSIS

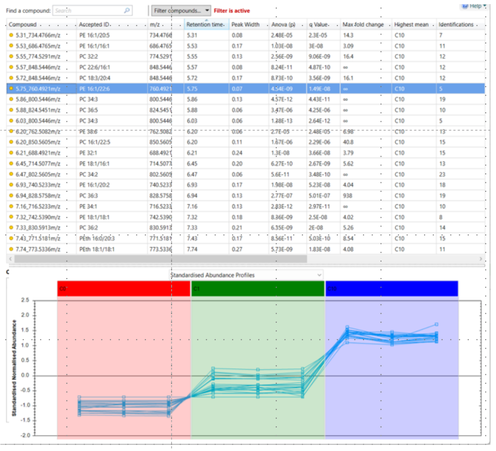


Table 1. Phospholipids observed in pseudo-nitzschia that are significantly elevated (ANOVA p>0.0005) in a dose dependent fashion with increasing copepodamide exposure. Data from triplicate LC/MS^E injections was processed in Progenesis QI™ software for time alignment, normalization and statisitical testing. Compounds were then searched against the LipidMaps database (downloaded 052025) with 1.5 ppm mass measurement tolerance on precursor ions and 3 ppm on fragment ions.

CONCLUSIONS

- **Rapid, high sensitivity detection and characterization of *pseudo-nitzschi*a lipidome using DIA and DDA techniques.**
- **Use of 1.5 ppm m/z error cutoff for increased confidence of lipid identification.**
- **Significant remodeling of *Pseudo-nitzschia* lipidome after exposure to copepodamide.**
- **Dose dependent elevation of palmitoleic acid (C16:1) containing phospholipids, especially phosphatidylethanolamines.**

References

1. Selander, E. *et al., Sci. Adv.* ***2,*** *2019, 3-9.*
2. Trapp, A., *et al., Limnol. Oceanogr.* **66,** 2021, 3455-3471.
3. Bargu, S, *et al., Mar. Ecol. Prog. Ser.* **418,** 213-222.

The authors declare no competing interests.

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