Report CNR Short Term Mobility 2018

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STM Goals

The goals of this STM project was:

1. Provide new understanding of how plankton food web structure is affected by the presence of polyunsaturated aldehydes that are produced by diatoms during blooms
2. Establish new and enhance existing collaborations between US and Italian plankton ecologists.

Background

Diatoms autotrophic microplankton that dominate phytoplankton biomass during blooms and in nutrient replete coastal and estuarine regions around the world. Many diatom species produce polyunsaturated aldehydes or PUA, which are allelopathic and cytotoxic chemicals that can induce reproductive failure in a variety of animal zooplankton, including copepods. Much less attention has been paid to the effect of these chemicals on protistan microzooplankton such as dinoflagellates and ciliates, which are the dominant grazers on diatoms under most conditions. Previous work suggests that the presence of PUA can reduce the grazing pressure on diatoms and increase grazing on microzooplankton by copepods (Lavrentyev et al. 2015; Franzé et al. 2017;

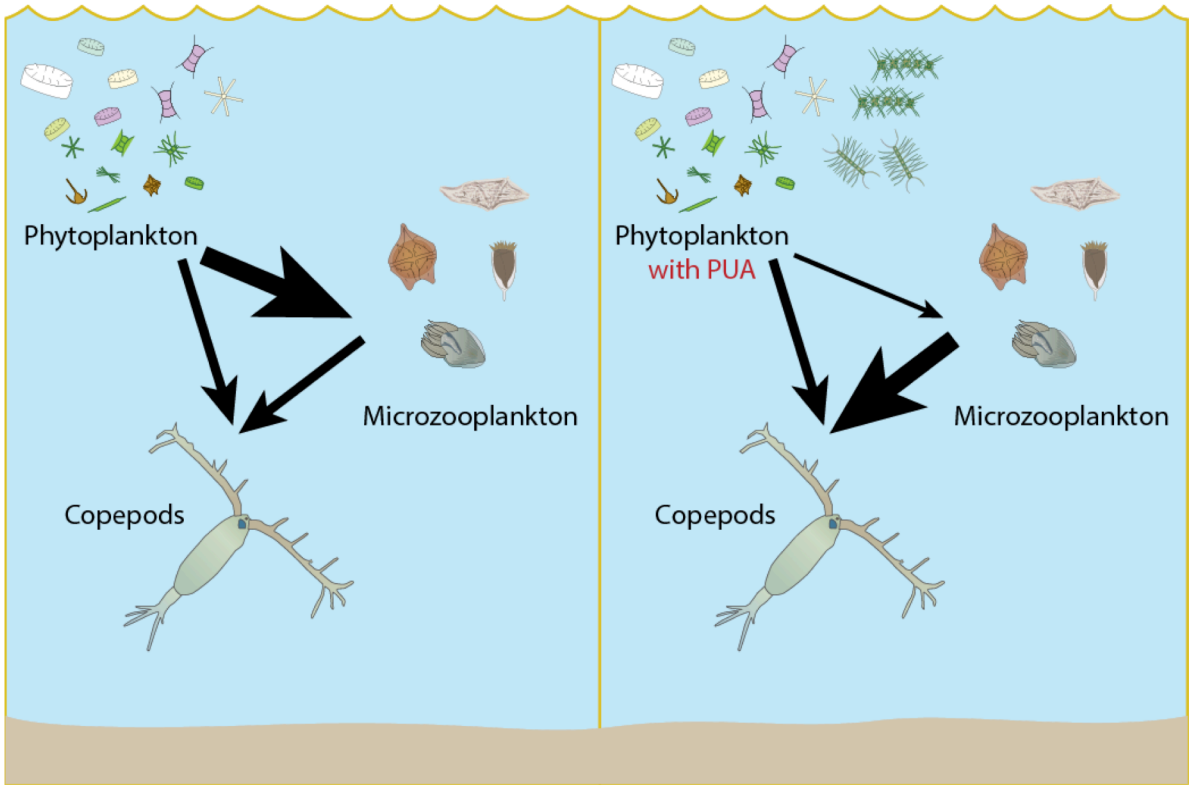

Figure 1. Conceptual diagram of hypothesized PUA impacts on planktonic food webs. Left panel shows food web without the presence of PUA, right panel shows the food web in the presence of PUA.

Fig. 1). These effects are likely to induce changes throughout the planktonic food web that may eventually affect both export flux of carbon to the deep ocean and higher trophic level production as they modify the flow and fate of primary production.

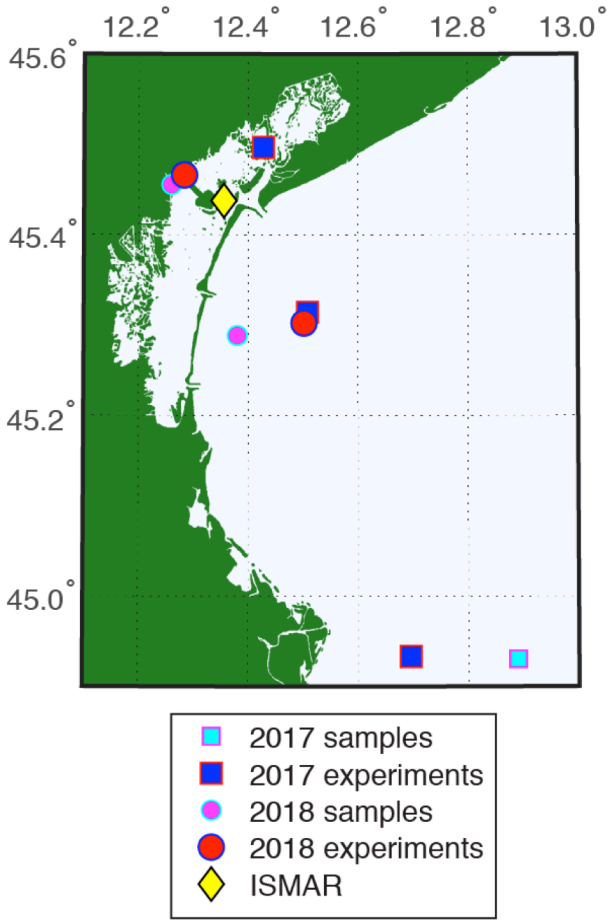

Figure 2. Station locations for this STM project (2018 stations) and from work conducted in 2017.

The Adriatic Sea, and in particular the region near the mouth of the Po River has been shown to have large diatom blooms in spring, often dominated by the species *Skeletonema marinoi* Sarno and Zingone (recently separated from the *S. costatum* complex, Sarno et al. 2005). This species is also wide spread in the northern Atlantic (Kooistra et al. 2008; Gerecht et al. 2013; Canesi and Rynearson 2016), including polar coastal waters (Zhang et al. 2010). *S. marinoi* produces several PUA, among them *trans, trans*-2,4-octadienal (Octa) and *trans, trans*-2,4-heptadienal (Hepta) (Ribalet et al 2007). The enzymatic cascade leading to PUA production in *S. marinoi* is triggered upon cell disruption and yields up to 9.8 fmol cell^{-1} (Wichard et al. 2005; Ribalet et al. 2007). During a *S. marinoi* bloom, dissolved PUA concentrations were correlated with its abundance and cell lysis rates, suggesting a potential release of these compounds into seawater without engaging the wound-activated cascade (Ribalet et al. 2014).

STM Activities

The first goal of this Short Term Mobility project was to conduct grazing experiments with microzooplankton and copepods during the period of the spring diatom bloom near the mouth of the Po River estuary and in the Venice Lagoon. These two different regions were chosen to provide contrasting physical conditions and plankton communities in order to assess how PUA changes the trophic relationships among the communities. The original goal was to conduct experiments on board a research vessel, the *G. Dalla Porta*, during a monitoring cruise near the Po River Delta. Unfortunately, the cruise was cancelled due to issues concerning the ship, but we were able to conduct the experiments with water and plankton samples collected from small boats at two locations, in

	Whole Sea Water	5% Sea Water	Copepods
Without PUA	3X	3X	3X
With PUA	3X	3X	3X

Table 1. Experimental Design

the lagoon at ISMAR station 1 (San Giuliano), and near the Acqua Alta scientific tower at station 80530 (Fig. 2). Additionally, samples were collected at two other stations, in the lagoon at ISMAR station 2 (Marghera) and at a coastal station (50530). At each station, scientists from ISMAR collected phytoplankton, zooplankton, and nutrient samples, and a CTD cast was conducted to measure the vertical profile of physical conditions at each station (Fig. 3).

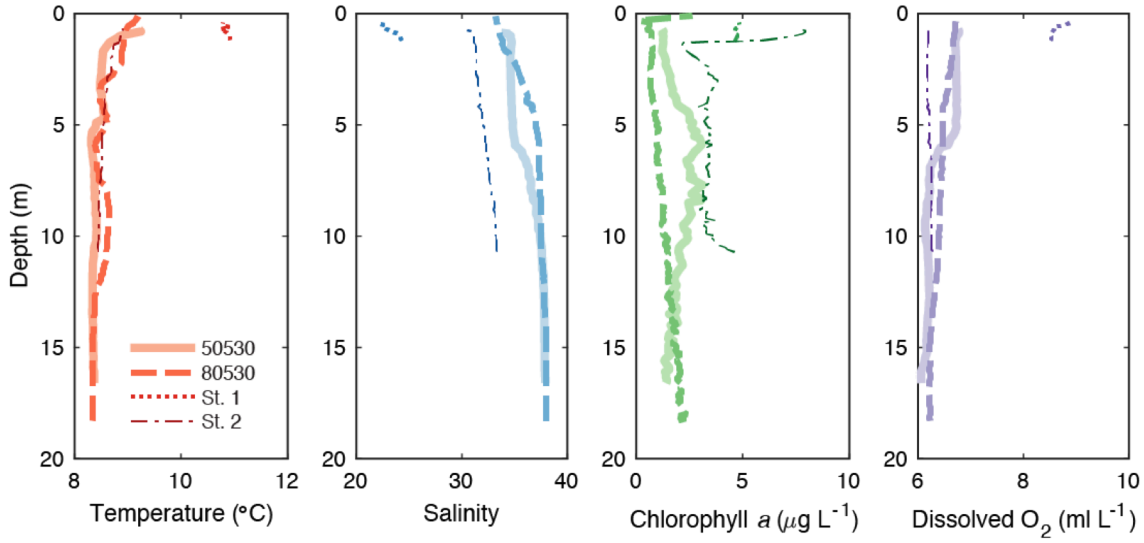

Figure 3. CTD data from each of the four stations sampled during the STM project. From left to right, panels show temperature, salinity, chlorophyll a fluorescence, and dissolved oxygen. Different stations are represented by lines of different weights and shades.

For each experiment, we measured growth of both microzooplankton and phytoplankton, as well as grazing by both microzooplankton and copepods. To do this, we incubated 18 250 ml culture flasks for 24 hours in the lagoon, consisting of triplicate bottles of six different treatments (Table 1). The whole sea water (WSW) treatments were simply whole sea water from each station that was filtered through 200 µm mesh to remove large (e.g. copepod) grazers. The 5% sea water (5%) treatments were made by mixing WSW with sea water that had been gravity filtered through a 0.2 µm cartridge filter. Copepod treatments consisted of WSW to which we added 5 *Acartia clausii* females to each bottle. For PUA treatments, we added 50 µl of a PUA stock consisting of a mixture of methanol, HEPTA and OCTA.

The copepods were collected using a 57 cm diameter ring net fitted with 200 µm mesh that was towed obliquely at each station and rinsed into 1 L plastic bottles until they were sorted at the ISMAR lab under a stereo microscope. Water and zooplankton samples used in the experiments were kept in the dark until the experiments were set up. To incubate the samples, we suspended the bottles in a plastic basket from the dock outside of the ISMAR Venezia facility. The bottles were placed in a mesh bag and covered in two layers of nylon screening to reduce photo inhibition that may occur due to high light intensity at the surface. Using the methods of Landry et al. (2008), we can calculate the microzooplankton growth and grazing rates, and the phytoplankton growth rate from the WSW and 5% treatments. Similarly, using the methods of Frost (1972) we can calculate the copepod grazing rates from the WSW and Copepod treatments.

From each treatment bottle we collected samples for chlorophyll a analysis, and from all samples bottles except the 5% treatments we collected samples for microzooplankton analysis. Additional samples were collected from each treatment bottle from the second set of experiments (with water and copepods from the Acqua Alta station, 80530) for picoplankton analysis, which will be conducted by Rafaella Casotti at the Stazione Zoologica Anton Dohrn, Napoli. These samples will be analyzed in the coming weeks.

In addition to the experimental work conducted in Venezia, quantitative analysis on experimental work that was carried out in 2017 by Drs. James Pierson and Peter Lavrentyev (from the University of Akron) during a previous visit to ISMAR Venezia was also accomplished. This work will be submitted as a peer-reviewed manuscript this year, and will be co-authored by Drs. Laverntyev, Pierson, Bastianini, and potentially other scientists from ISMAR that contributed to the work. In addition, a numerical model is being developed to quantitatively assess the impact of PUA on different aspects of the plankton community. This multiple trophic level, concentration based growth and grazing equation model was developed during the STM project.

The second goal of the STM project was to develop new collaborative efforts between US and Italian plankton ecologists. Dr. Peter Lavrentyev was present during the visit to Italy, and along with Dr. Pierson they conducted a variety of meetings with Dr. Rafaella Casotti, Dr. Cecilia Totti from Università Politecnica delle Marche, Alessandra Campanelli from ISMAR in Ancona, and Federica Grilli from ISMAR in Ancona, as well as the host Dr. Mauro Bastianini, Dr. Marco Pansera, Dr. Elisa Camatti, and Anna Schroder from ISMAR in Venezia. Novel collaborations have emerged from these meetings, and the most concrete outcome is a plan to contribute at least one chapter to an upcoming book that compares the Adriatico Mar to the Chesapeake Bay. This book is an update of a previous book (Malone et al. 1999), and will be comprised of chapters comparing and contrasting various aspects of the two ecosystems. One idea for a proposed chapter would be to examine the impact of PUA on the two systems, because there is interest and expertise from both Italian and US scientists in this question. Other ideas for chapters in this book included some assessment of the role of genetic metabarcoding in monitoring programs, a review of trends in phytoplankton and zooplankton communities, and plankton trophic dynamics. There are data available and ongoing efforts in both locations that should support chapters on at least some of these topics.

References

Canesi, K.L., and T.A. Rynearson. 2016. Temporal variation of *Skeletonema* community composition from a long-term time series in Narragansett Bay identified using high-throughput DNA sequencing. *Mar. Ecol. Prog. Ser.* 556: 1–16.

Gerecht, A. and others. 2013. Oxylipin production during a mesocosm bloom of *Skeletonema* marinoi. *J.Exp. Mar. Biol. Ecol.* 446: 159–165.

Franzè, G., Pierson, J. J., Stoecker, D. K. and Lavrentyev, P. J. (2017), Diatom-produced allelochemicals trigger trophic cascades in the planktonic food web. *Limnol. Oceanogr.* doi:10.1002/lno.10756

Kooistra, W., D. Sarno, S. Balzano, H. F. Gu, R. A. Andersen, and A. Zingone. 2008. Global diversity and biogeography of *Skeletonema* species (Bacillariophyta). *Protist* 159: 177-193.

Lavrentyev, P. J., G. Franzè, J. J. Pierson, and D. K. Stoecker. 2015. The effect of dissolved polyunsaturated aldehydes on microzooplankton growth rates in the Chesapeake Bay and Atlantic Coastal Waters. *Mar. Drugs* 13: 2834-2856.

Malone, T.C., Malej, A., Harding Jr, L.W., Smodlaka, N. and Turner, R.E., 1999. Ecosystems at the land-sea margin: Drainage Basin to Coastal Sea. American Geophysical Union. Washington, DC

Ribalet, F., T. Wichard, G. Pohnert, A. Ianora, A. Miralto, and R. Casotti. 2007. Age and nutrient limitation enhance polyunsaturated aldehyde production in marine diatoms. *Phytochemistry* 68: 2059-2067.

Ribalet, F. and others 2014. Phytoplankton cell lysis associated with polyunsaturated aldehyde release in the Northern Adriatic Sea. *PLoS One* 9: e85947 doi.org/10.1371/journal.pone.0098727.

Sarno, D., W. H. C. F. Kooistra, L. K. Medlin, I. Percopo, and A. Zingone. 2005. Diversity in the genus *Skeletonema* (Bacillariophyceae). II. An assessment of the taxonomy of *S. costatum*-like species with the description of four new species. J. Phycol. 41: 151-176.

Wichard, T., Poulet, S.A., Halsband-Lenk, C., Albaina, A., Harris, R., Liu, D., and G. Pohnert. 2005a. Survey of the chemical defence potential of diatoms: screening of fifty one species for alpha, beta, gamma, delta-unsaturated aldehydes. *J. Chem. Ecol.* 31: 949-58.

Zhang, F., He, J., Xia, L., Cai, M., Lin, L. and Y. Guang. 2010. Applying and comparing two chemometric methods in absorption spectral analysis of photopigments from Arctic microalgae. *J. Microbiol. Meth.* 83: 120-126.