

**Polyunsaturated Aldehydes (PUAs):
Impacts on Microzooplankton
Grazing, Growth and Mortality due
to Predation**

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- Peter J Laventyev
- Gaya Franzè
- James Pierson

Wound-activated oxylipins, including polyunsaturated aldehydes (PUAs), produced by phytoplankton



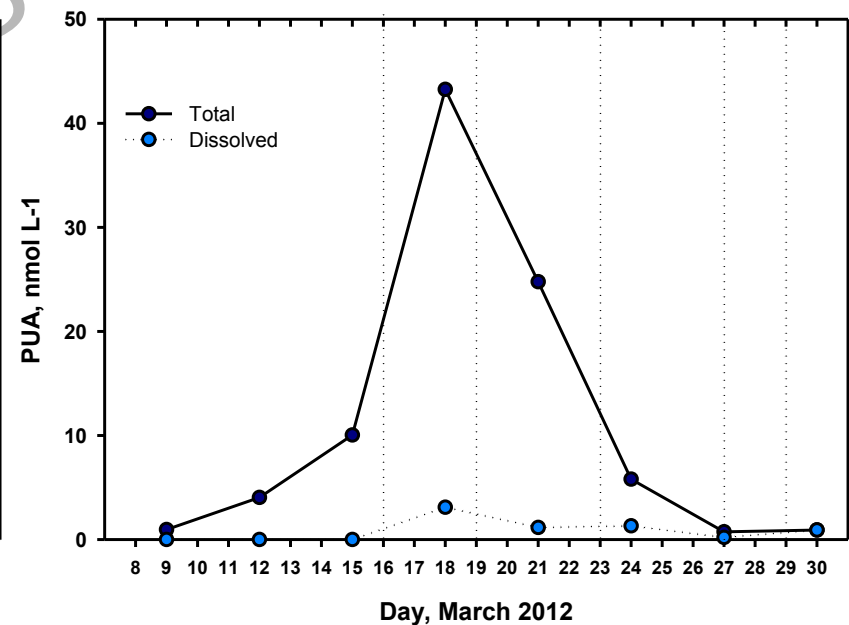
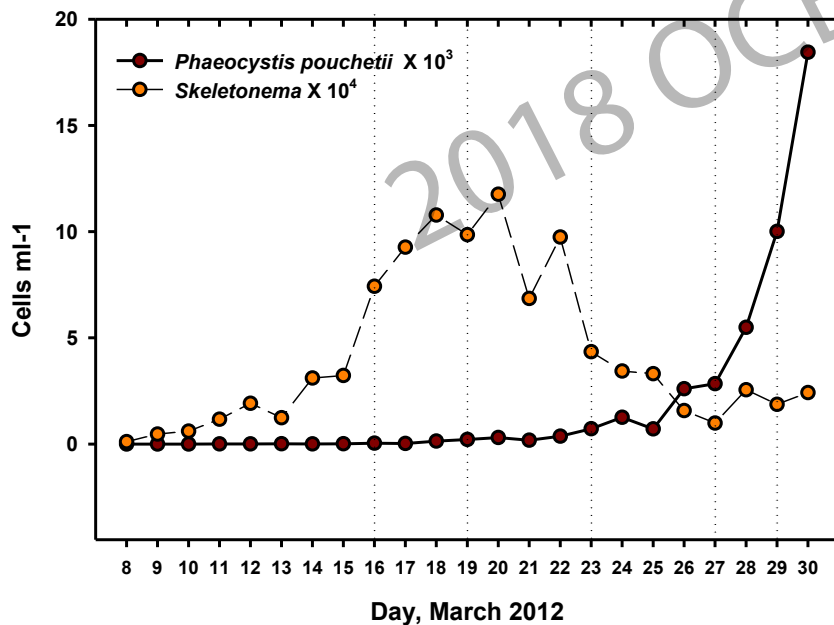
Skeletonema marinoi
(photo from Noridc phytoplankton files)

- Species and strains vary in amount and types (not just PUAs) (Watson et al. 2009)
- Nutrient stress, mechanical stress and grazing can trigger production
- Self inhibitory as well as inhibiting growth of co-occurring phytoplankton (Pohnert 2000)
- Hypothesized to deter copepods grazing (Ianora & Miralto 2010) but it is more likely they are effective against MZ grazing (Flynn & Irigoien 2009)
- *Skeletonema marinoi* produces PUAs (~1:10 , octadienal, OD : heptadienal, HD) as well as other oxylipins (Gerecht et al. 2011, Vidoudez et al. 2011). Max. 9.8 fmol/cell (Wichard et al. 2005)

Mesocosm Experiment in Norway (March 2012)



Bloom with high concentrations of *S. marinoi* (peak 10^4 cells/mL, 43 nM pPUA, ~ 3 nM dPUA).



Mesocosm Experiment in Norway (March 2012)

- Included dilution experiments to measure MZ grazing
- Previous research in Bering Sea suggested that during blooms, dilution water might inhibit phytoplankton growth, violating assumptions of technique (Stoecker et al. 2014)
- Added treatment of dilution water passed through a carbon cellulose (CC) cartridge to remove organics, including PUA

Rise of Bloom:

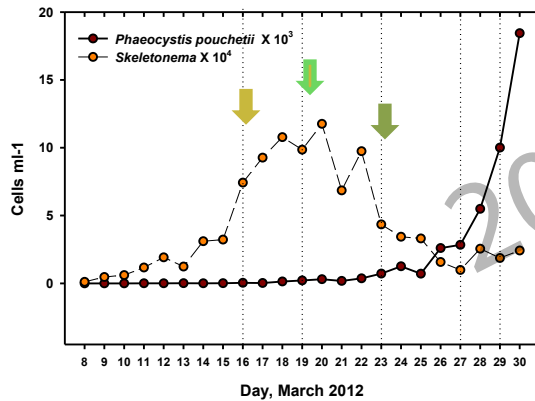
Net growth of phytoplankton positive & similar in WSW and diluted SW, but with Carbon Cellulose treatment net growth rate increases

Peak of Bloom:

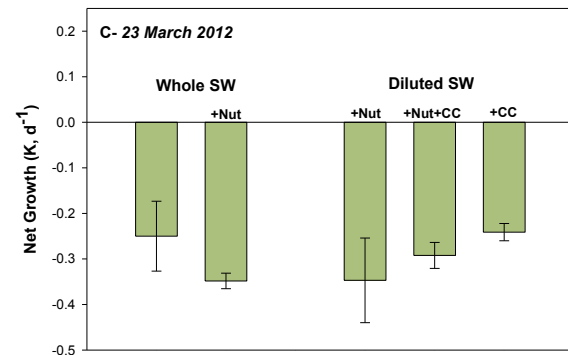
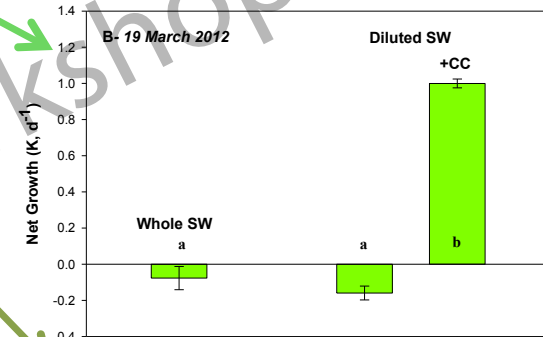
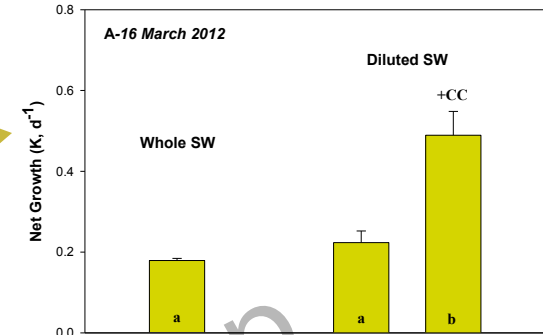
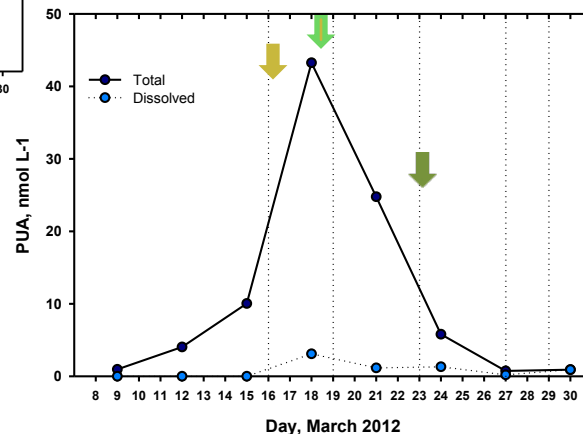
Net growth rate of phytoplankton negative in WSW & diluted SW, but with Carbon Cellulose treatment net growth rate positive and high

Bloom Crash:

Net growth of phytoplankton negative in all treatments



Organics released during preparation of FSW can be inhibitory



Stoecker et al. 2015

PUA and *Skeletonema marinoi* Addition Experiments in Chesapeake Bay and Coastal Atlantic



Dilution & Copepod Grazing Experiments with 2 types of added treatments & natural assemblages

1. Addition of dissolved PUA (1:10 OD:HD) to mimic light, moderate and dense blooms of *S. marinoi* based on PUA production of 7.5 fmol/cell and assumption 10% cells lyse & produce dPUA

Low +2.2 nM total PUA

Medium +5.5nM total PUA

High +22 nM total PUA

2. Addition of high PUA producing strain of *S. marinoi* (Skel 2092, ~1-2 fmol/cell) or low PUA strain
2000-4000 cells/mL
(Light to moderate bloom densities)

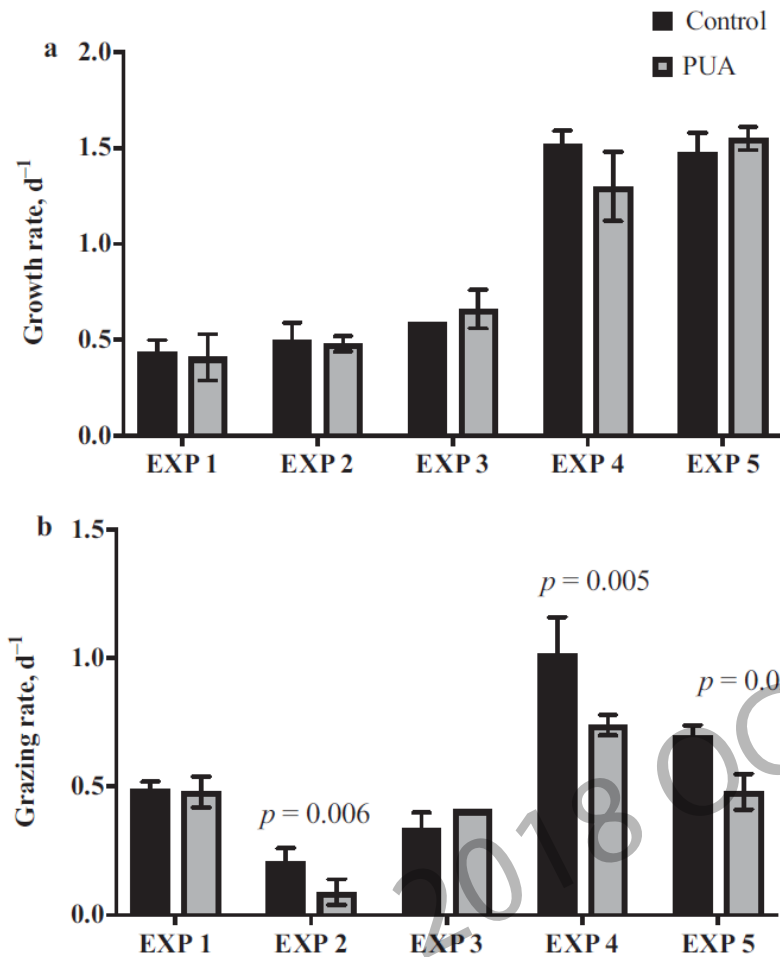


Fig. 3. Phytoplankton growth (a) and microzooplankton herbivory (b) in dilution experiments. Bars = ± 1 standard deviation; numbers = *p*-values of Student *t*-test.

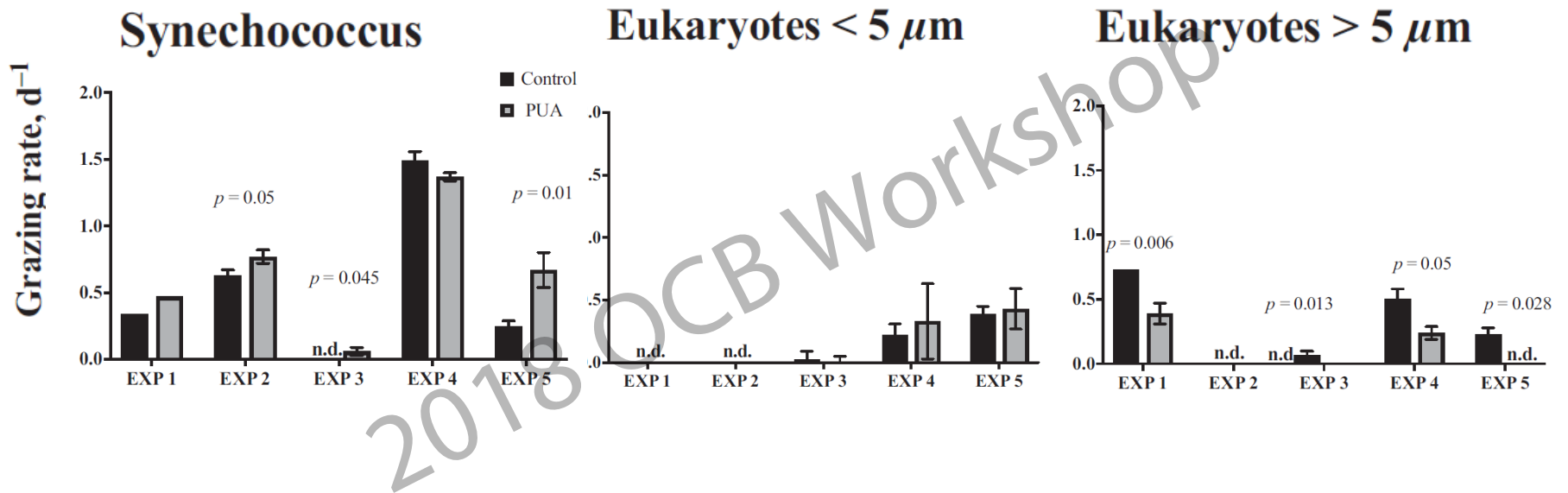
Exps 1-3 CB, 4 & 5 Coastal Atlantic;
Exps 1,4,5 : 5.5 nM, Exps 2 & 3: 2.2 nM.

Dissolved PUA additions (1:10 OD:HD, 2.2 or 5.5 nM) had little effect on phytoplankton (chl a) growth but decreased MZ grazing in 3/5 experiments

Franzè et al. 2017

Effects of dPUA addition on MZ grazing on different phytoplankton categories

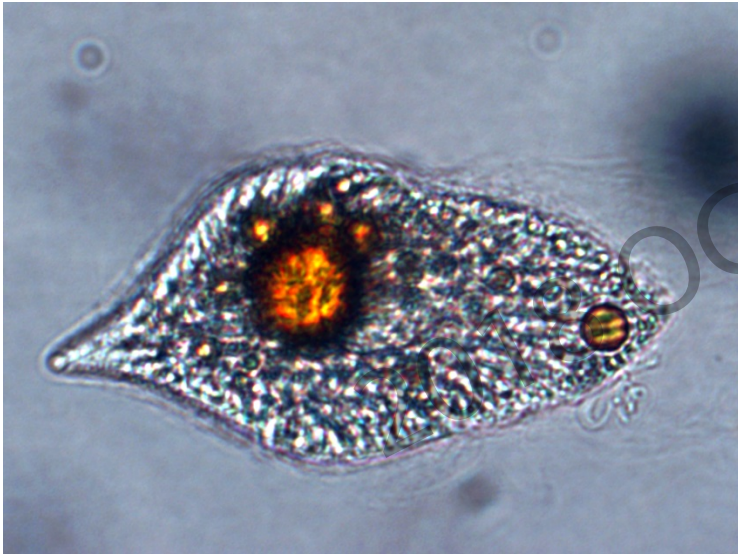
(Franzè et al. 2017)



+ dPUA increased MZ grazing on Synechococcus

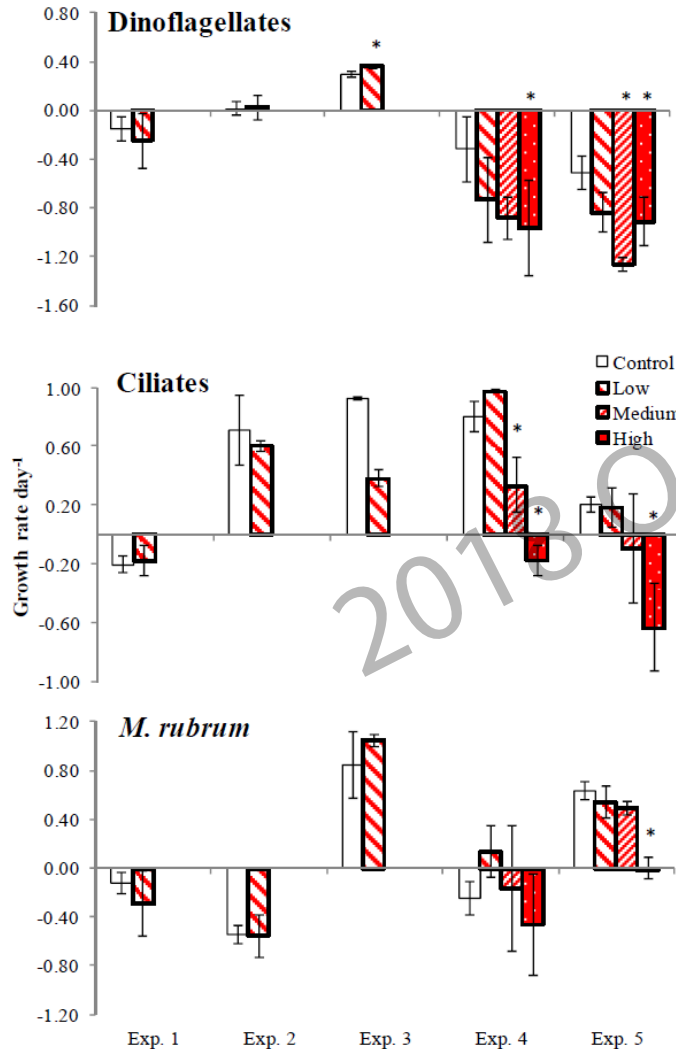
+dPUA decreased MZ grazing on > 5 μM eukaryotes

Effects of PUA Microzooplankton



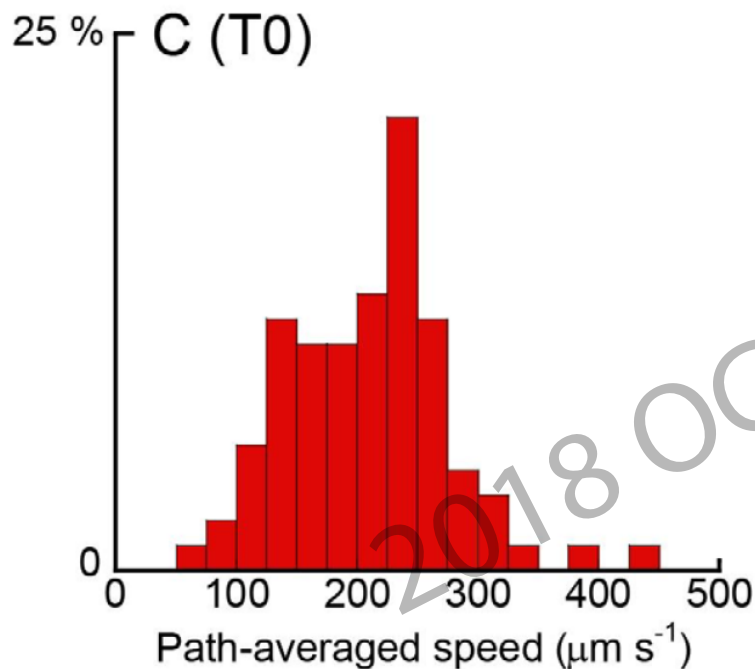
Effects of addition of dPUA (Low=2.2, Medium=5.5, High =22 nM) on MZ growth in natural assemblages

Lavrentyev et al. 2015

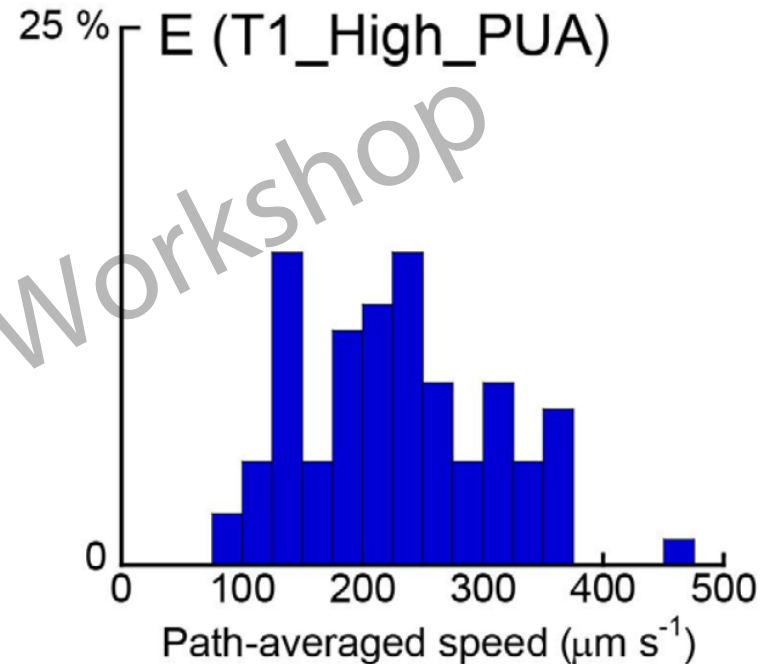


- Dinoflagellates and most ciliates were inhibited by 5.5-22 nM doses.
- *Mesodinium rubrum* appeared to be less sensitive.

Preliminary Results: PUA addition effects swimming of heterotrophic dinoflagellate *Protoperidium* sp. at 20 nM dissolved PUA (1:10 OD:HD) (Jiang et al., unpubl. data).



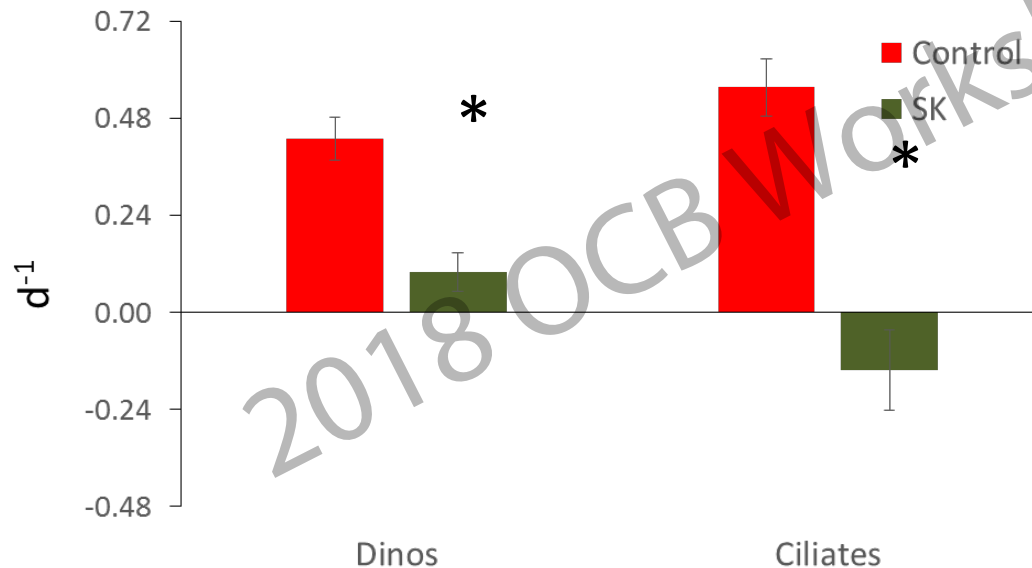
Path-averaged speed are close to normally distributed; the cells swim along helical paths of similar geometry.



Distributions deviates from normal; the cells swim either slower or faster.



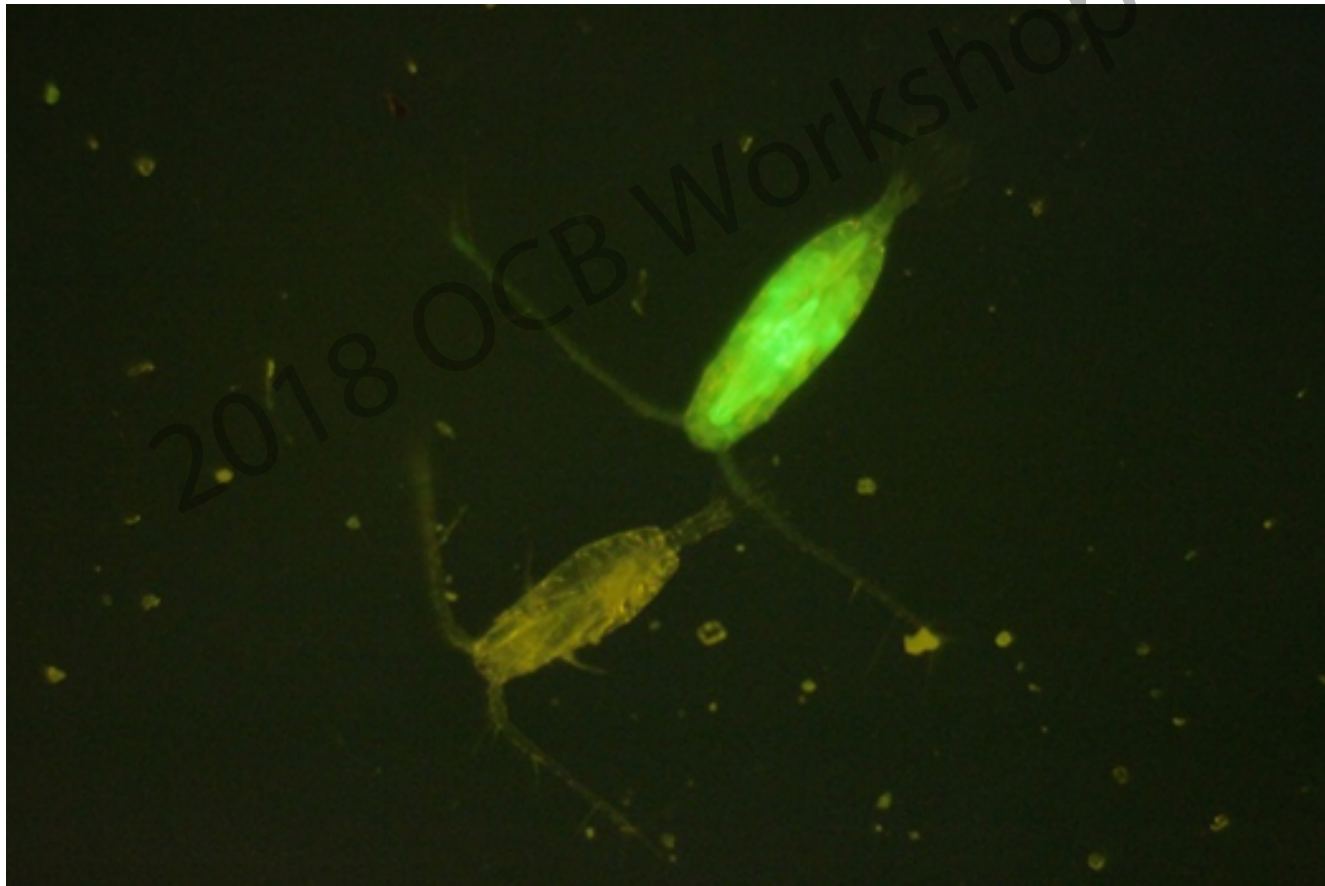
2000 cells/mL (final conc.) of *S. marinoi* (Sk 2092, high PUA strain) decreases growth (μ , d^{-1}) of dinoflagellates and ciliates in natural assemblages of plankton, Chesapeake Bay. Mean \pm SD of replicates.



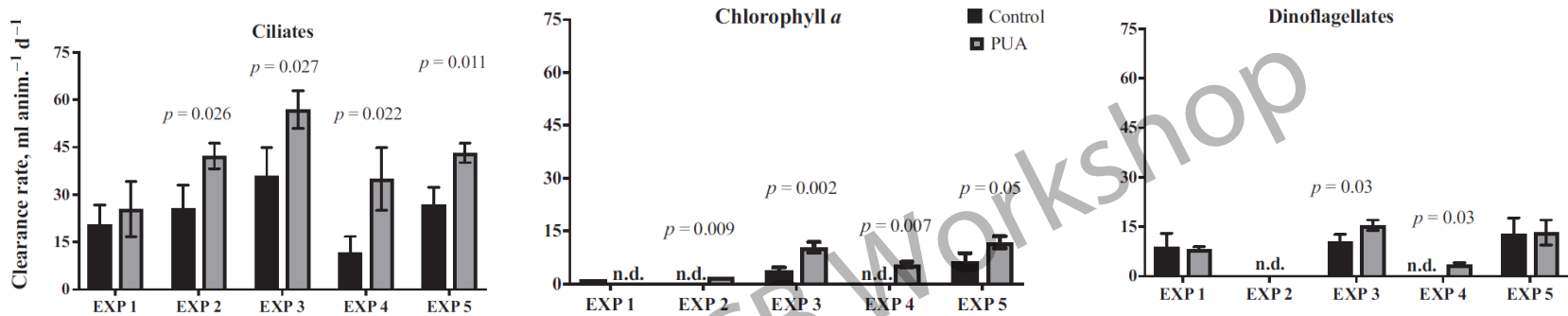
Lavrentyev, Franzè, Pierson & Stoecker (unpubl. data)

S. marinoi additions decreased growth of dinoflagellates and ciliates (* $p < 0.05$).

Effects of addition of dPUA or *S. marinoi* on copepod grazing

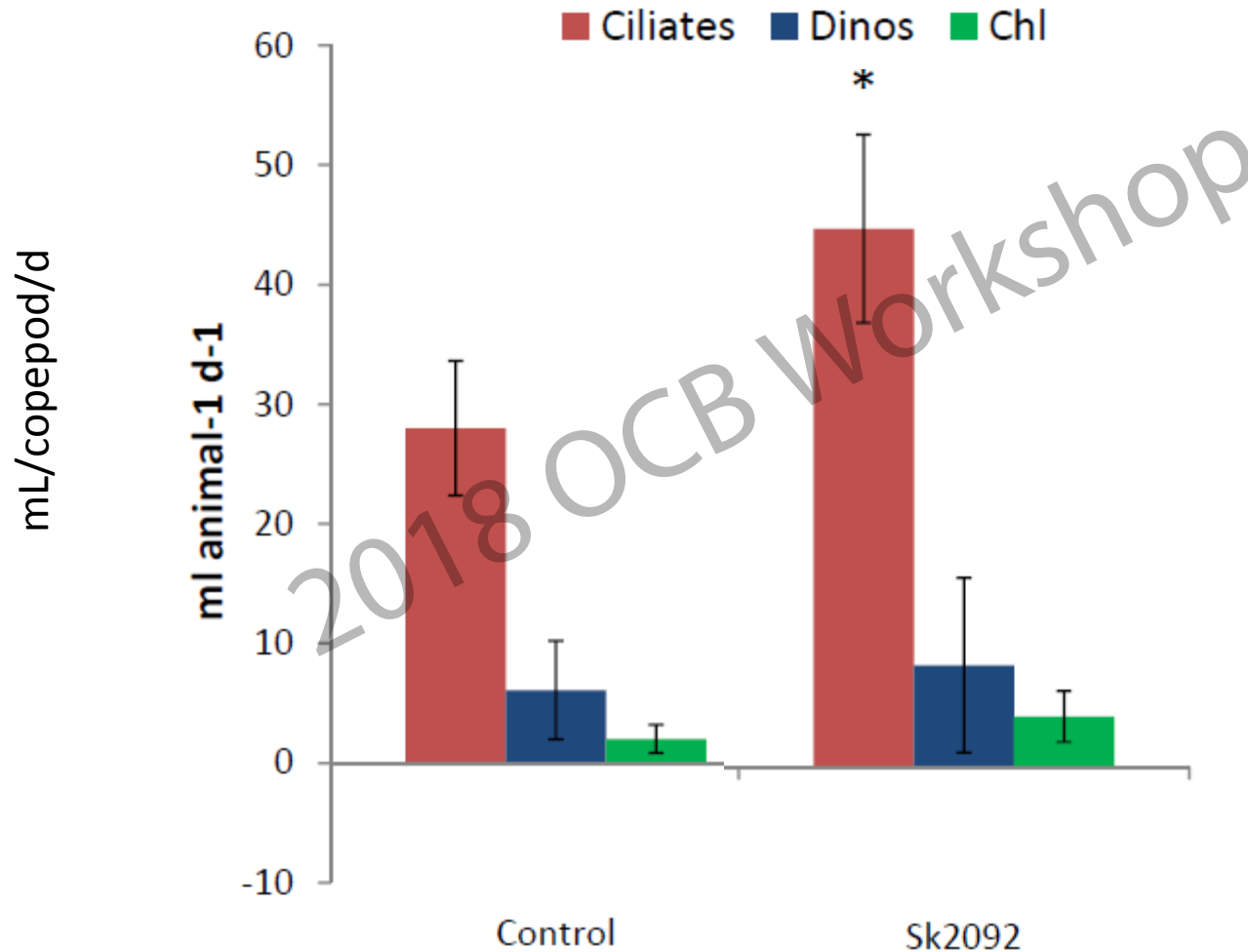


Copepod (*Acartia tonsa*) clearance stimulated by addition of dPUA



Stimulation of copepod clearance of ciliates and dinoflagellates should cause a trophic cascade, lessening top down control on phytoplankton

+2000-4000 cells/mL (final conc.) of *S. marinoi* (Sk 2092, high PUA strain, ~2 fmol pPUA/cell) stimulates feeding of *Acartia tonsa* on ciliates in natural assemblages of plankton (<200 μ m), Choptank River & Coastal Atlantic. Mean +/- SD of 5 experiments



Lavrentyev, Franzè,
Pierson & Stoecker
(unpubl. data)

Summary of Experiments

- Inhibitory metabolites (PUA etc) released during filtration of SW during dense blooms can inhibit phytoplankton growth, resulting in underestimation of MZ grazing in dilution experiments.
- Dissolved PUA additions (2.2-5.5 nM) have little effect on phytoplankton growth (chl a) but can decrease MZ grazing, esp. on eukaryotes > 5 μm .
- Dissolved PUA additions (2.2-5.5 nM) decreases or inhibits growth of Dinoflagellates & Ciliates. Preliminary data indicates that PUA affects swimming of MZ.
- Dissolved PUA additions (2.2-5.5 nM) stimulate copepod clearance, esp. of Ciliates.
- Addition of high PUA *Skeletonema marinoi* (2000-4000 cells/mL) decreases or inhibits growth of Dinoflagellates & Ciliates and stimulates copepod clearance of Ciliates.

Conceptual model of effects PUA on planktonic food web



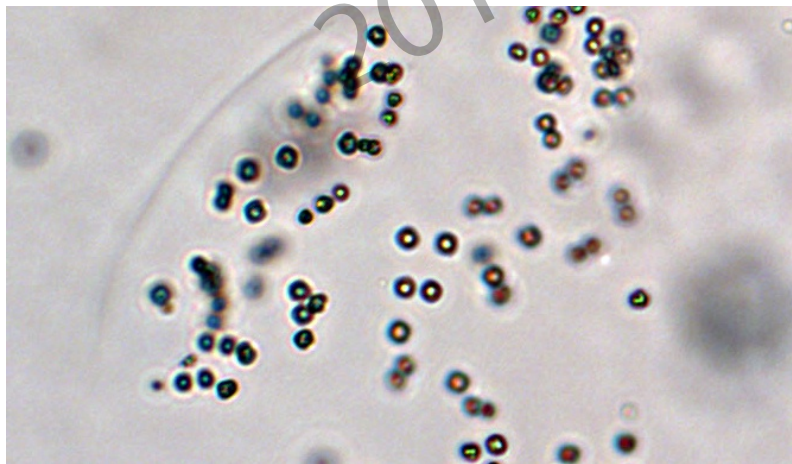
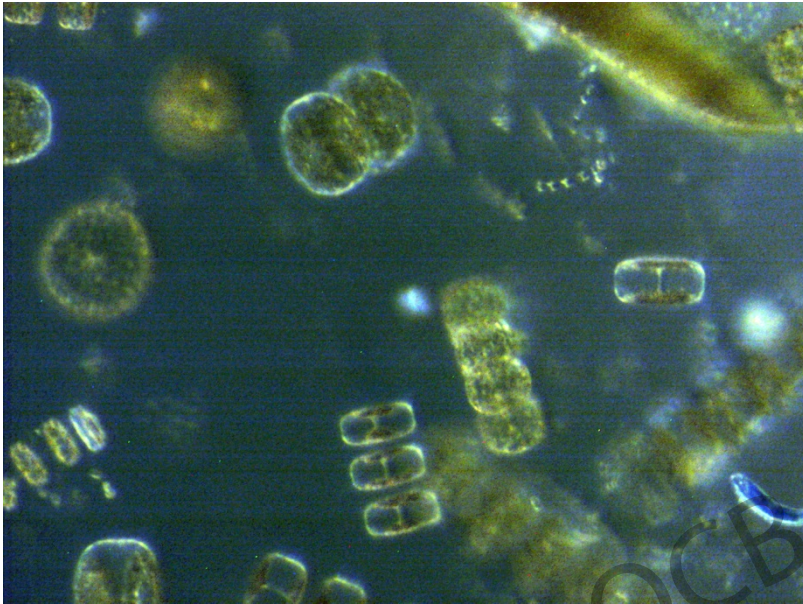
Modified from Franzè et al. 2017

Consequences for Food Web of PUA?

- Disrupt growth-grazing equilibrium.
- Opportunity for bloom development.



The Puzzle: Significant Negative Grazing Rates (g) in Dilution Experiments



- ~60 dilution experiments in Eastern Bering Sea in Summer.
- 39% of experiments g was ns ($p > 0.05$); 3% significant negative g .
- **To test if dilution itself, or metabolites released during preparation of FSW, cause $<$ in growth (μ) of phytoplankton, compared ratio of variable FL to maximum FL (F_v/F_m) in diluted (20% WSW) and WSW treatments with and without added nitrate.**
- (Fv/Fm ratio is measure of potential max quantum yield of PSII; reduction in Fv/Fm is indicator of physiological stress)

Reductions in phytoplankton “health” as indicated by reductions in variable fluorescence (F_v/F_m) in diluted seawater (20%WSW) vs. whole seawater (WSW) were associated with low estimates of MZ grazing in dilution experiments conducted in the Eastern Bering Sea in Summer (Stoecker et al. 2014).

