Multi-year investigation of *Pseudo-nitzschia* species assemblages in eastern Maine

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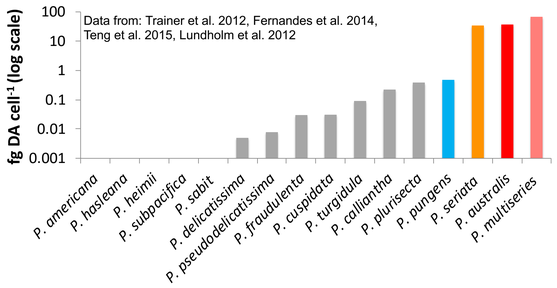
I. Domoic acid and *Pseudo-nitzschia* spp. in the Gulf of Maine (GOM)

- Roughly 26 *Pseudo-nitzschia* species produce the neurotoxin domoic acid (DA) and toxin production is not constitutive and varies across species.
- DA may result in Domoic Acid Poisoning in wildlife and can accumulate in filter–feeding shellfish and finfish, causing Amnesic Shellfish Poisoning (ASP) if consumed by humans.
- The Gulf of Maine (GOM) experienced its first ASP closure in eastern Maine in September 2016, coincident with first observations of *P. australis* there.

Fourteen species described in the Gulf of Maine based on published reports:
P. americana, P. calliantha, P. cuspidata, P. delicatissima, P. fraudulenta, P. hasleana, P. heimii, P. multiseries, P. pseudodelicatissima, P. pungens, P. seriata, P. subpacifica, P. turgidula, and *P. plurisecta* (+ *P. sabit, P. caciantha, P. australis,* and an unclassified *P.* sp.) → **18-26 spp. suspected**



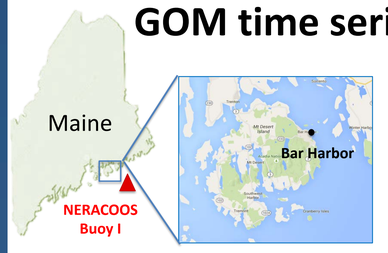
Given the emerging threat of *P. australis* and ASP toxins in the GOM, we hypothesize that:
- Interannual differences in the severity of DA events from 2013-2019 is linked to species composition.
- Early detection of toxic species can provide advance warning of toxic events.

To address these hypotheses, we have focused on the following:
AIM 1: Develop enhanced monitoring program for toxic *Pseudo-nitzschia* species within existing state shellfish management frameworks.

AIM 2: Examine trends in species composition, cellular abundance, environmental conditions, and toxin levels in the GOM during 2013-2019 to inform ongoing observation and forecasting efforts.

Bates et al., 2018 and references therein

II. Genetic analysis integrated into GOM time series sampling

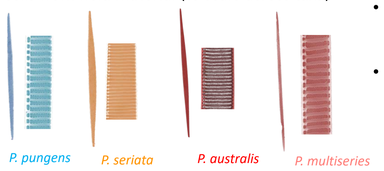


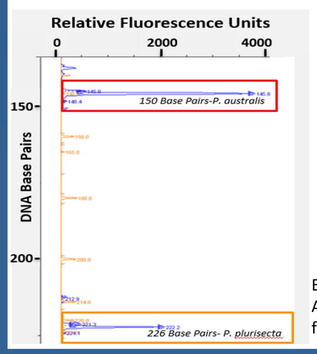
2013-2019 Time Series Data
Mount Desert Island, Maine

- ~Weekly collaborative sampling at Bar Harbor, ME from 2013 to 2019 augmented existing state sampling (cell abundance, temperature, and salinity) to include pDA, DNA, and dissolved nutrients.
- Surface temperature and salinity data were obtained from NERACOOS Buoy I

Year	Sampling Months	# of ARISA Samples
2013	July-October	20
2014	May-October	26
2015	February-September	21
2016	June-October	15
2017	May-December	33
2018	May-September	19
2019	March-November	35

SEM images of Gulf of Maine *Pseudo-nitzschia* cells from field material (to scale across taxa)



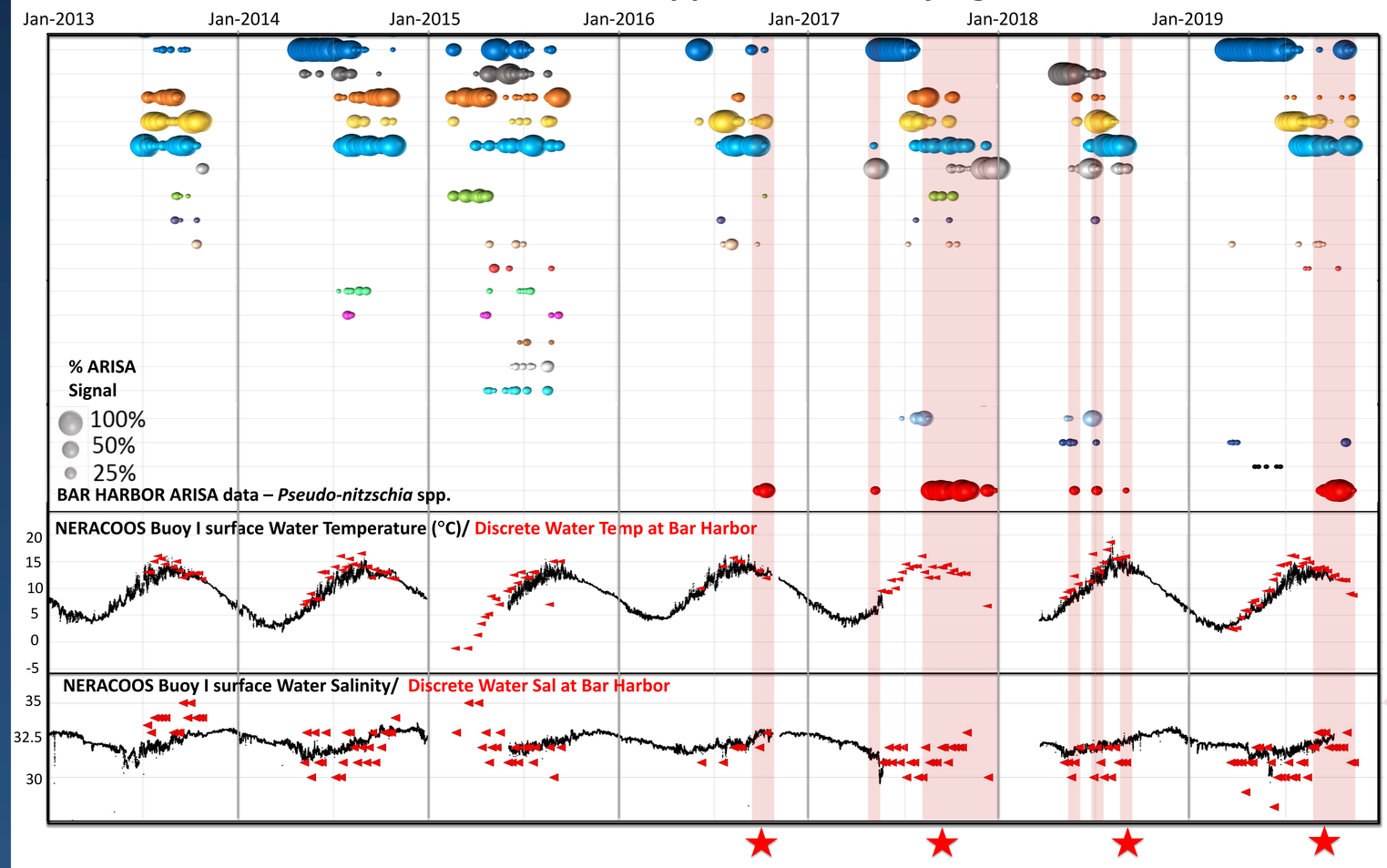


Example ARISA fingerprint

- Species share similar features and cannot be identified via light microscopy.
- Seawater for DNA samples was filtered and then frozen at -80°C or preserved in DNA lysis buffer until extraction, depending on capabilities of the sampler.
- DNA fingerprinting known as ARISA was used to identify *Pseudo-nitzschia* species assemblages.
- Genus-specific PCR primers were used to amplify a length variable region of the internal transcribed spacer 1 (ITS1). Species were assigned to ARISA fragments based on GOM *Pseudo-nitzschia* sequences.
- Scanning electron microscopy (SEM) and targeted ITS1 sequencing was conducted on a subset of samples.

Hubbard et al. 2008, 2014, Clark et al. 2019

III. Transitions in *Pseudo-nitzschia* spp. occur at varying time scales in GOM



ARISA taxa:
- ∗ *P. delicatissima*
- ∗ *P. obtusa*
- ✻ *P. seriata*
- ✻ *P. plurisecta*
- ✻ *P. pungens*
- *P. americana*
- ∗ *P. fraudulenta*
- Unk_223 bp
- ∗ *P. cuspidata*
- ✻ *P. multiseries*
- ∗ *P. pseudodelicatissima*
- *P. dolorosa*
- Unk_164 bp
- Unk_170 bp
- *P. caciantha*
- Unk_227 bp
- Unk_187 bp
- Unk_162 bp
- ✻ *P. australis*

✻ Five most toxic GOM species
∗ Other toxic GOM species

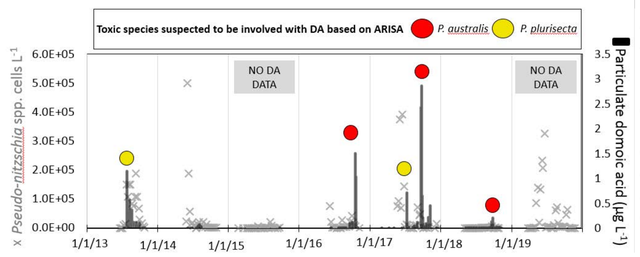
Pink inset boxes highlight when *P. australis* is present

★ **Years with DA-related shellfish harvest closures in eastern Maine**

Species assemblages dynamic across event, seasonal, and interannual time scales

- ARISA detected 19 different fragments or taxa: 13 were attributed to known GOM species, 6 were not identified based on known GOM diversity.
- In each year, seven to thirteen taxa were observed; diversity peaked in 2015, and three taxa were found only in that year.
- Seasonal species succession observed in all years: *P. delicatissima* or *P. obtusa* (a polar species) were associated with coolers waters in the spring, diversity increased in summer and into fall (e.g., *P. pungens, P. seriata, P. plurisecta* occurred most years*)*. Multiple toxic species were often observed in the same sample.
- *P. australis* was first observed in 2016, and has been observed every year since, following peak summer temperatures and with slight variations in timing and duration; no other novel species were observed in 2016 but three novel types were observed after 2016. Closures due to DA levels in shellfish occurred in association with *P. australis* from 2016-2019, with interannual differences in the severity and duration of events (ME DMR).

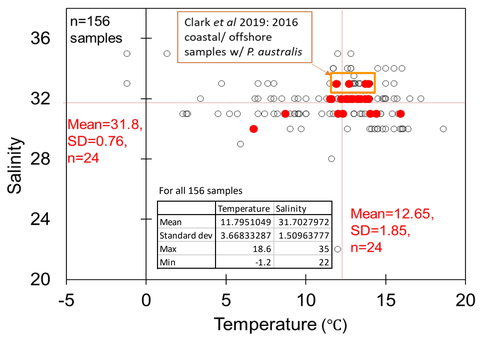
IV. *Pseudo-nitzschia* spp. and DA: 2013-2018



P. australis and *P. plurisecta* are associated with peaks in pDA, but shellfish harvest closures have only been observed during *P. australis* events.

Other toxic *P.* spp. commonly observed, and peaks in cell abundance do not always correspond with peaks in DA.

V. *Pseudo-nitzschia australis* observed annually since 2016



Temperature and salinity of Bar Harbor samples, 2013-2019 (n=156)

- Samples with *P. australis* (red; n=24) and the average temperature and salinity values of *P. australis* (dashed red lines) were compared to those from a 2016 GOM cruise (Clark et al. 2019).
- The time series data was more or less consistent with cruise observations (orange inset) and expanded our knowledge of the temperature/salinity niche of *P. australis* in the GOM.

VI. Linking field and lab studies to understand species and toxin dynamics

AIM 1:
- We have expanded our field sampling network by collaborating with other states (Massachusetts, Rhode Island, Connecticut, and New Hampshire) to sample routinely and/or during suspected DA events.
- We are also working to continue to enhance the time series in eastern Maine that was initiated in 2013 by building in situ sensors and other sampling into monitoring efforts (Project 1 and other leveraged projects).

AIM 2:
- These collective efforts also help inform statistical analyses and model development related to bloom prediction and hindcasting at event to interdecadal time scales (Project 2 and other leveraged efforts).
- Current lab studies involved cultivating *P. australis* across ecologically relevant temperature and salinity gradients to improve our understanding of growth and toxin dynamics.

Acknowledgements

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