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Introduction

- Some diatoms within the genus *Pseudo-nitzschia* produce harmful algae blooms (HABs) in coastal waters.
- HABs can produce domoic acid (DA) which can bioaccumulate in the tissues of filter-feeders.
- DA is a potent neurotoxin responsible for causing amnesic shellfish poisoning (ASP) in humans.
- Currently, there are 52 total number of known *Pseudo-nitzschia* species. Of these 52 species, 26 were found to be toxigenic DA producers.
- As *Pseudo-nitzschia* is the primary microalgal source of DA, and HABs are increasing globally, the importance of monitoring and researching *Pseudo-nitzschia* populations becomes necessary.



Figure 1. Microscopic image of *Pseudo-nitzschia* spp. (<https://hab.who.edu/species/species-by-name/pseudo-nitzschia/>)

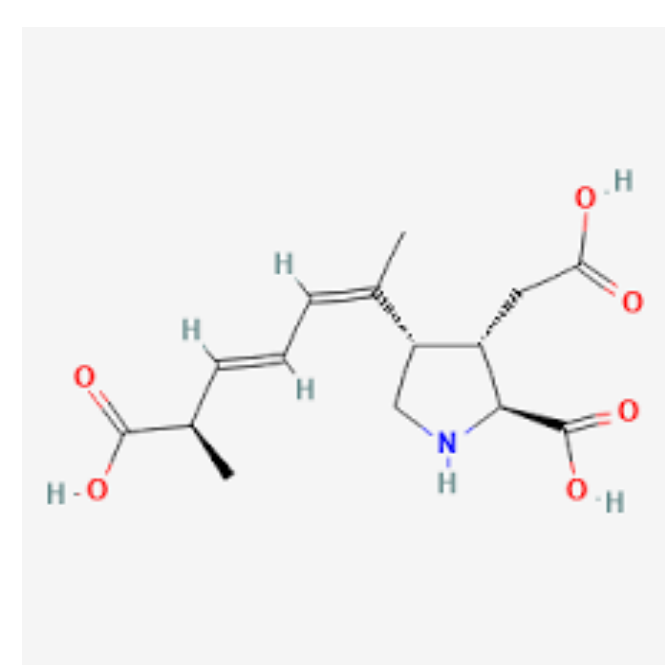


Figure 2. Domoic Acid chemical structure (<https://hab.who.edu/species/species-by-name/pseudo-nitzschia/>)



Figure 3. Typical HAB public safety poster (<https://summitcountyhealth.org/public-announcements/algae-bloom-oct17/>)

Objectives

- To collect eDNA from water samples oyster landing at at **Huntington Beach State Park** and screen for the presence of *Pseudo-nitzschia* using molecular sequence data
- To determine the specificity and applicability of **HRMF/HRMR primers** in developing a molecular assay to identify *Pseudo-nitzschia* within public shellfish harvesting areas along the Grand Strand (SC).



Figure 4. A) Map of Oyster Landing sample collection at Huntington Beach State Park, SC (southcarolinaparks.com). B) Aerial photograph of shellfish collection grounds at HBSP (www.youtube.com/watch?LM2oZMnli)

Results

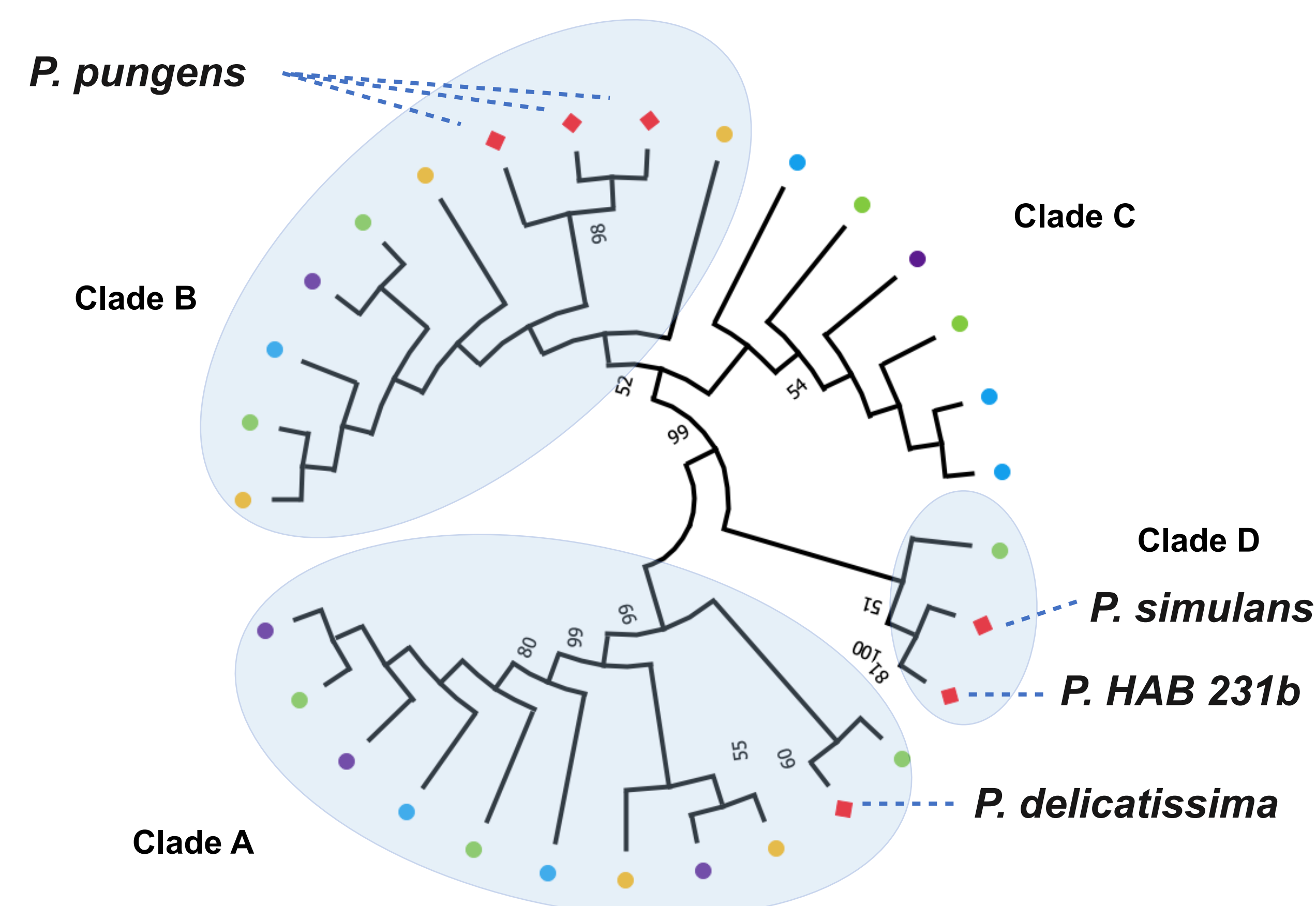


Figure 5. Maximum likelihood Phylogenetic Tree indicating eDNA sequences relatedness to known domoic acid producing *Pseudo-nitzschia* species. (using General Time Reversible model with a discreet Gamma distribution). Bootstrap values (1000 replicates) over 50% shown.

Collection Dates	Average DNA Concentration (ng/mL)	Location Within Tree (clades)	Likely Domoic Acid Production
June 22, 2021	1.67x10 ⁴	A, B, C, D	+++
June 23, 2021	2.16x10 ⁴	A, B, C	+
July 7, 2021	1.64x10 ⁴	A, B, C	+
July 26, 2021	4.9x10 ³	A, B, C	++
August 2, 2021	1.42x10 ³	N/A	N/A

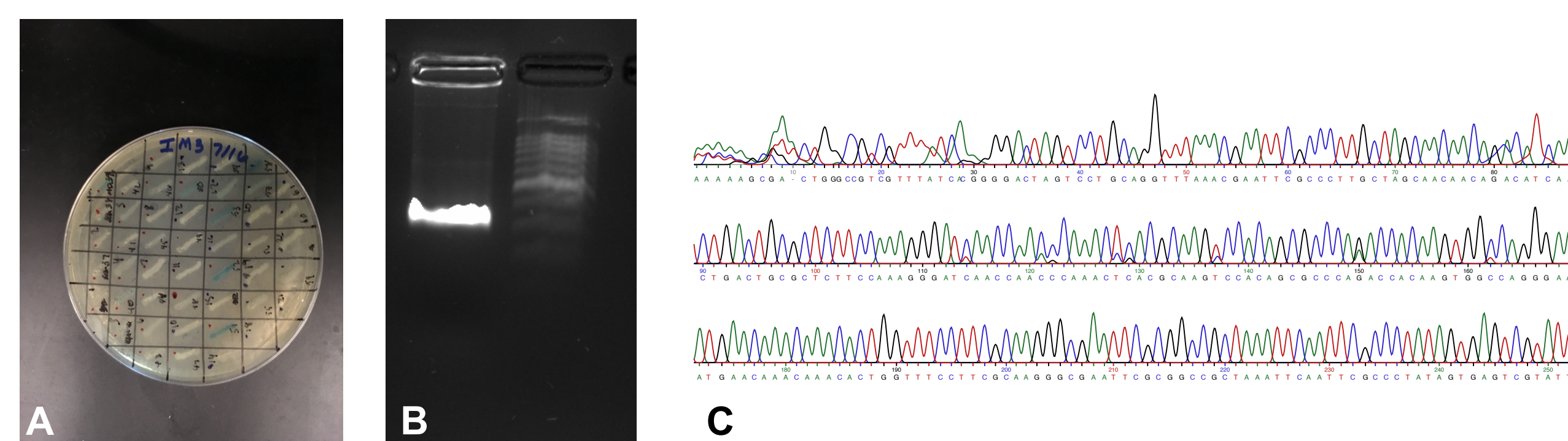


Figure 6: A) Scratch plate of picked clones of transformed *E. coli* cells (White colonies contain transformed vector with plasmid, Blue colonies contain vector only). B) Amplification product of cloned amplicon (~300bp) C) Trace file of sequenced amplicon

Related Species

Pseudo-nitzschia delicatissima

- Producer of domoic acid
- Native to Atlantic Ocean, Gulf of Maine, Gulf of Mexico, Pacific Ocean

Pseudo-nitzschia pungens

- Producer of domoic acid
- Native to Gulf of Mexico, Atlantic Ocean, Gulf of Maine, North Carolina (USA)

Pseudo-nitzschia simulans

- Producer of domoic acid
- Native to Yellow Sea, East China Sea, South China Sea

Discussion

- Found that the eDNA fell within clades of several possible domoic acid producers
- Proved that eDNA can be used to detect multiple species of this genus
- Sequence data from multiple collections of eDNA are very similar to known species of *P. nitzschia*
- If these eDNA fall within the same clade as known producers of domoic acid, it is likely they also produce DA
- DA poisoning can lead to memory loss, GI discomfort, seizures

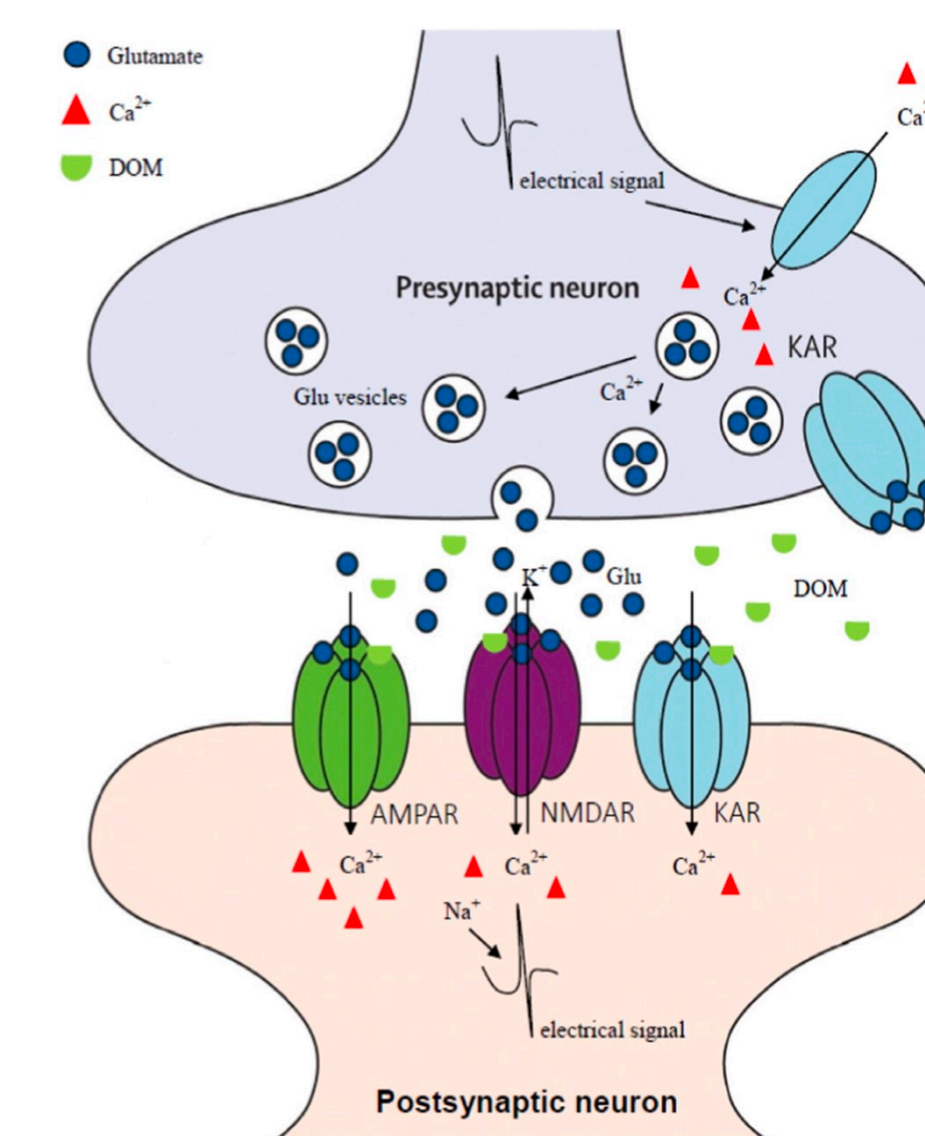


Figure 7: Synaptic action of Domoic Acid (DOM) from Saeed, A. et al. (2017). Domoic acid: Attributes, exposure risks, innovative detection techniques and therapeutics. *Algal Research*, 24, 97–110. <https://doi.org/10.1016/j.algal.2017.02.007>

Future Work

- In the upcoming future, the goal is to optimize qPCR conditions to determine specific taxa of *Pseudo-nitzschia*.
- The ultimate goal is to calculate concentrations of domoic acid within the waters and living oysters of Huntington Beach State Park.

Methodology

Sample Collection and Filtration

- > Oyster Landing Murrells Inlet, SC
- > Vacuum Filtration

DNA Extraction

- > Qiagen RNeasy® Micro Plant Kit

DNA Quantification

- > Qubit 2.0 Fluorometer

DNA Purification

- > Agencourt AMPure XP

DNA Amplification and Detection

- > PCR
- > HRMF/HRMR Primers
- > Gel Electrophoresis

Cloning of PCR Amplicons

- > Topo TA Cloning
- > Vector: pCR®2.1

DNA Sequencing

- > Sequencing primer: M13 F/R

DNA Sequence Analysis

- > Alignment MUSCLE 3.0
- > Tree Construction RAxML 8

Acknowledgments

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