

W Nanoemulsion Delivery of Ecologically Safe Agents for Burrowing Ghost Shrimp Control in Willapa Bay

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INTRODUCTION & SITE VISIT

Native burrowing ghost shrimp in Willapa Bay pose a large threat to the oyster industry by destabilizing oyster beds, endangering workers in unstable soil, and damaging crop yields. Previously used harsh chemical pesticides, such as carbaryl and imidacloprid, were effective but banned due to harmful effects on non-target estuarine species. **Other non-chemical control approaches, such as mechanical and electrical methods are less scalable** than deploying pesticides [1]. This project will test three organic agents: **Eugenol, PyGanic, and Chitosan**, and their characteristics within a **nanoemulsion delivery system**.



"Man-made systems create man-made issues" notes Kathleen Nesbit, co-owner of Goose Point Oysters.

Figure 1. Capstone team on work barge.

"The estuary is a 'built environment' and its responsible management is our priority."

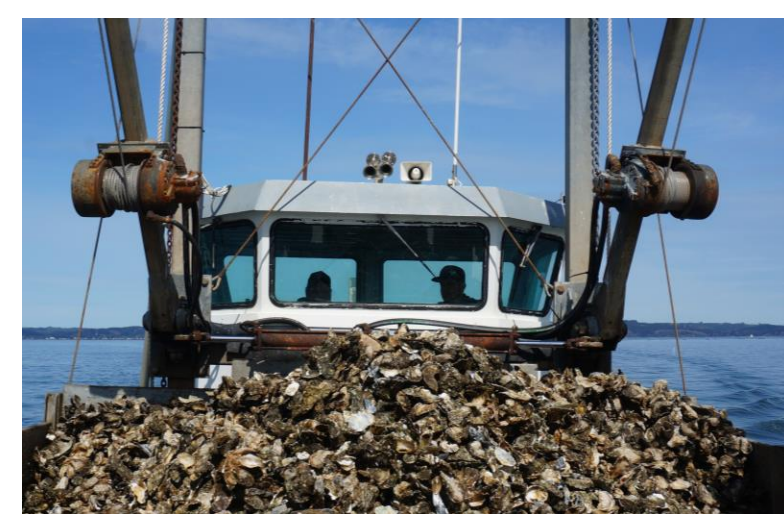


Figure 2. Barge loaded with oysters.

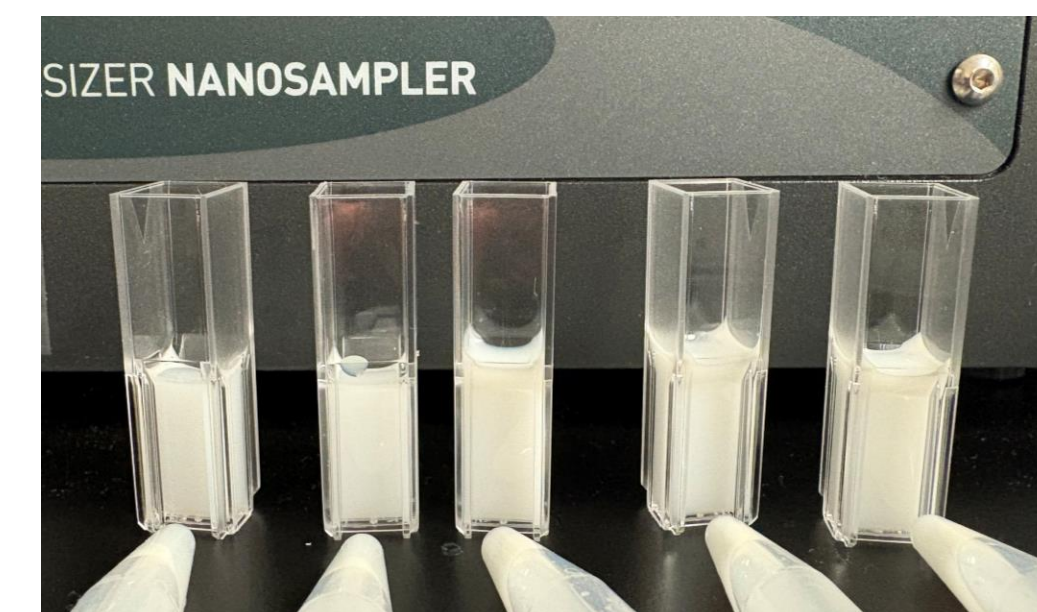


"Rural business investments increase the resilience of rural communities."

Figure 3. Oyster farmers moving oyster crop to new bed.

APPROACH & METHODS

Nanoemulsions are nano-sized colloidal suspensions composed of oil (active agent), water, and surfactants.



Nanoemulsions offer:
- higher stability
- controlled release
- targeted delivery [2]

Figure 4. PyGanic nanoemulsion samples in size measurement cuvettes for DLS analysis.

- Form nanoemulsions with eugenol and PyGanic and measure droplet size, polydispersity index (PDI), and zeta potential using Dynamic Light Scattering technology.
- Investigate effects of bay conditions (temperature and salinity) on nanoemulsion characteristics.
- Optimize droplet size and zeta potential for stability and targeted release of agent with chitosan/surfactants.

AGENT SELECTION

- Eugenol** is the active component of clove oil, a biodegradable, plant-based essential oil. Eugenol disrupts neurophysiological systems essential for feeding and maintaining burrows, leading to death at high enough concentrations. [3]
- PyGanic** is an "emulsifiable concentrate", organic pesticide used commercially in agriculture with an active component of pyrethrin. Pyrethrins affect the sodium channel opening in the shrimp, leading to neurotoxic effects like paralysis and death. Although acutely toxic to off-target species [4], it breaks down rapidly in water and with sunlight exposure [5].
- Chitosan** is an environmentally-benign biopolymer derived from crustacean shells, and acts both as a bioactive agent and nanoemulsion stabilizer. Chitosan disrupts membranes and gills of shrimp, leading to reduced respiration and death [6]. It is a positively charged polymer in aquatic environments.

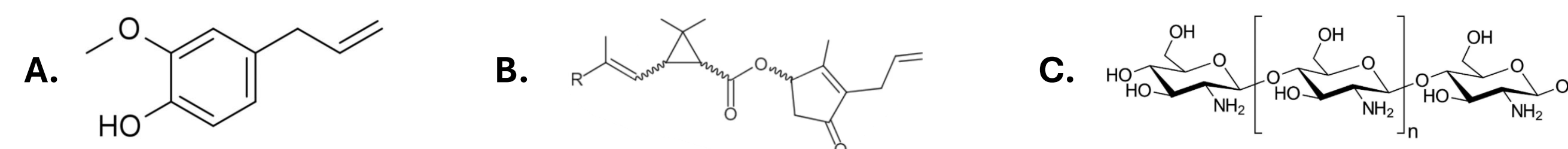


Figure 5. Chemical structures of (A) eugenol, (B) pyrethroid, and (C) chitosan. These compounds feature aromatic rings and/or long hydrocarbon chains, resulting in hydrophobic behavior which enables encapsulation into nano-sized droplets.

CONCLUSIONS

- Surfactant (Chitosan) flipping negative zeta potential to positive suggests **targeted delivery to shrimp burrows due to (+) attraction to (-) negatively charged bay sediment**.
- Reduction of nanoemulsion size with increased synthesis temperature suggests **summer nanoemulsion application for faster diffusion of smaller particles in bay sediment**.
- No droplet change in size with varied salinity**.
- High PDI of **Eugenol nanoemulsions indicate stable nanoemulsions were not formed, addition of surfactant is required**.

FUTURE WORK

- Replicate DLS and zeta potential tests by varying temperature, salinity, and surfactant concentration (x3) to reinforce findings
- Investigate effect of agent interaction with Willapa Bay sediment on nanodroplet size and zeta potential
- Investigation of spike injector for field scale application

RESULTS

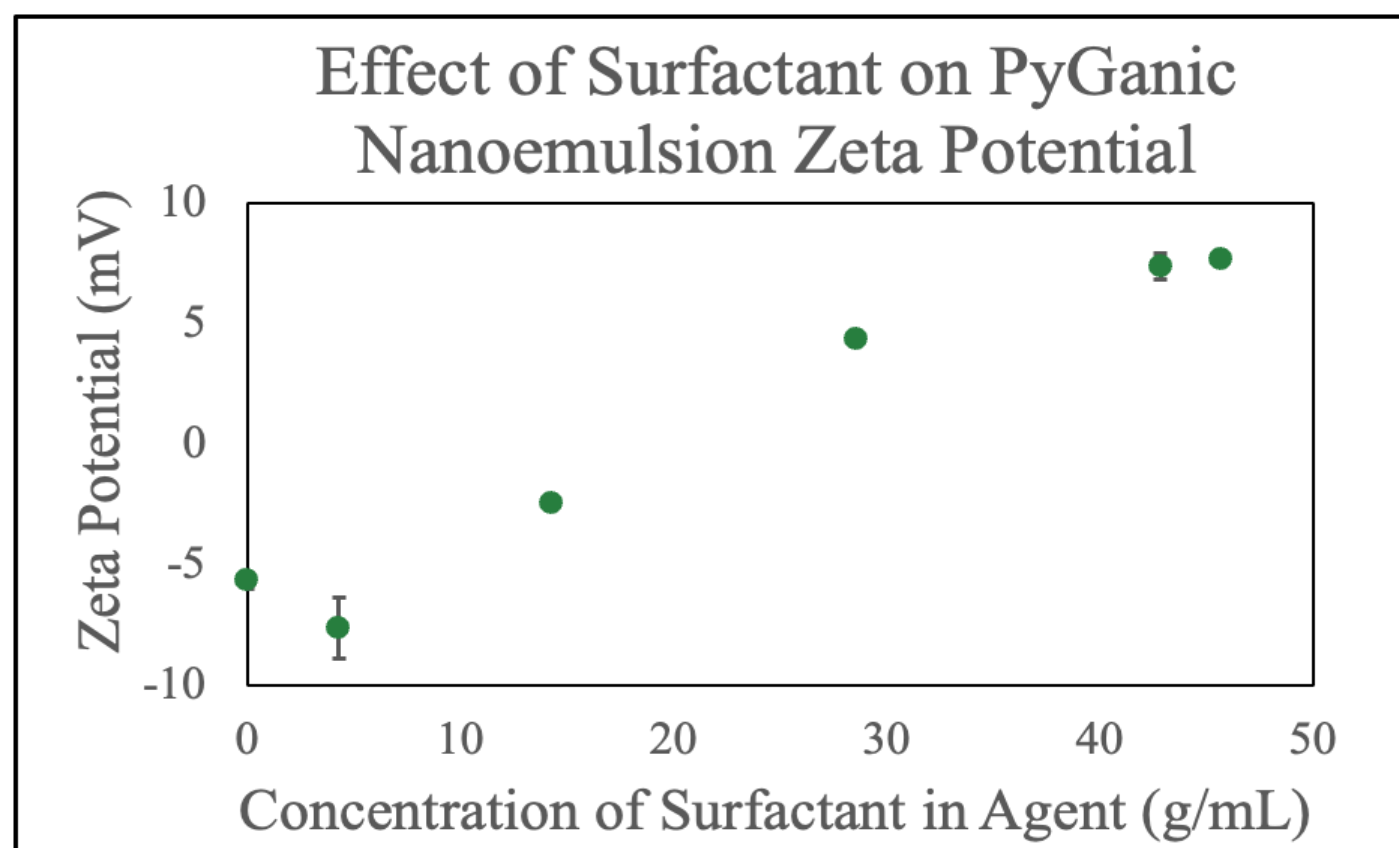


Figure 6. A steady increase in PyGanic nanoemulsion zeta potential was seen as chitosan concentration increased until eventual stalling above ~40 g/mL.

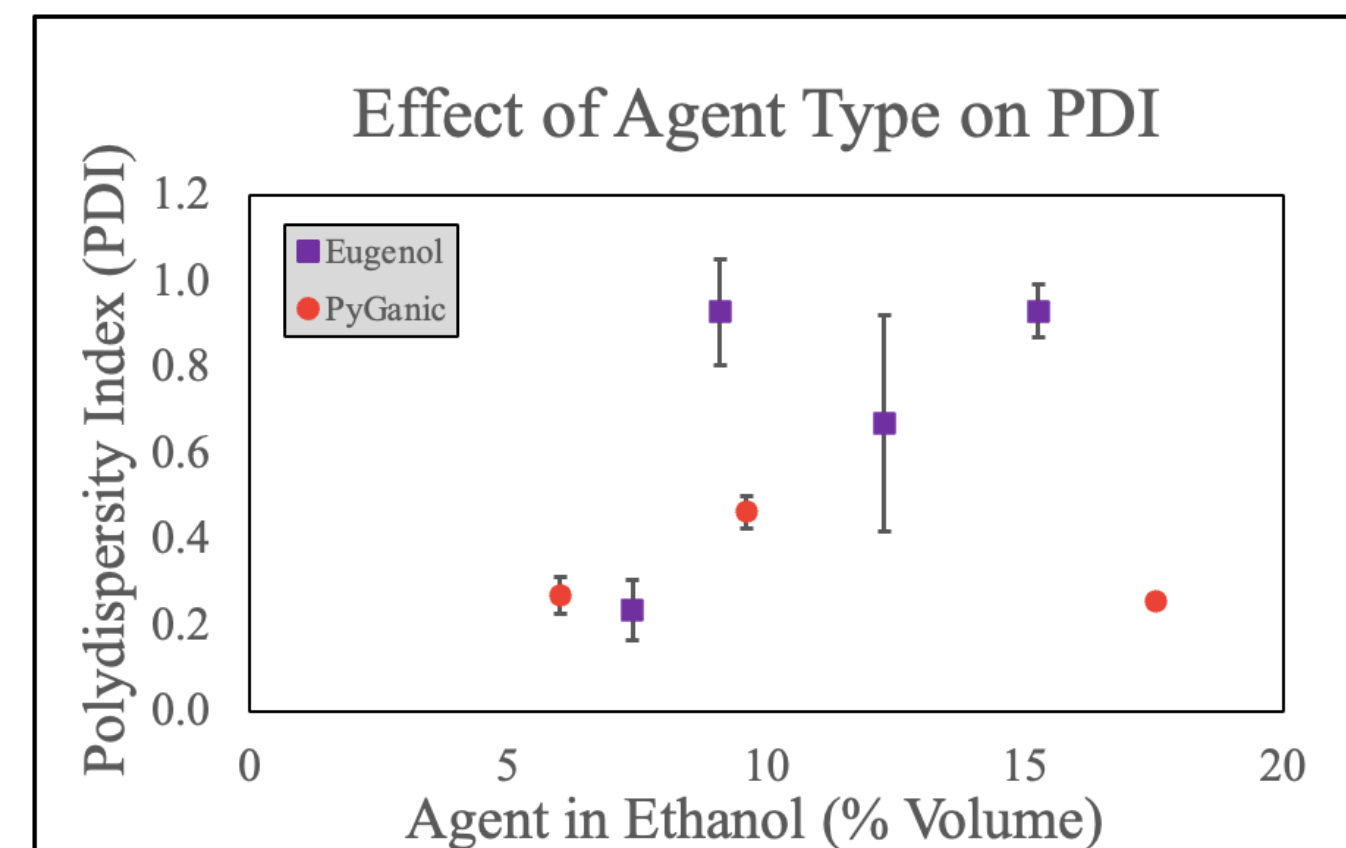


Figure 7. The PDI of eugenol increased dramatically over 10 vol. % agent/ethanol with large error bars, while PyGanic remained constant, indicating low quality eugenol data.

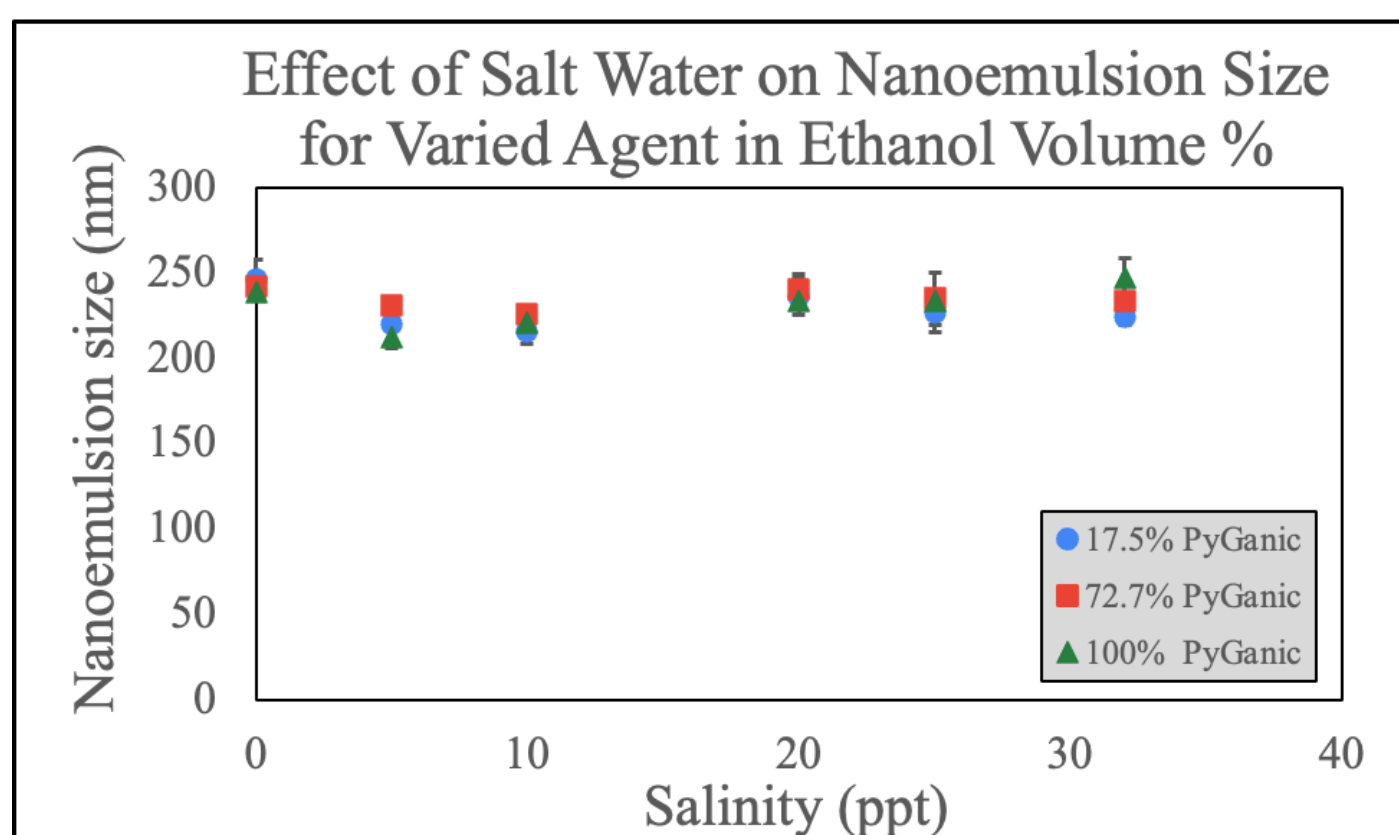


Figure 8. The size remained constant around ~250 nm for all salt concentrations between 0-32 ppt, and for varied PyGanic in ethanol volume %.

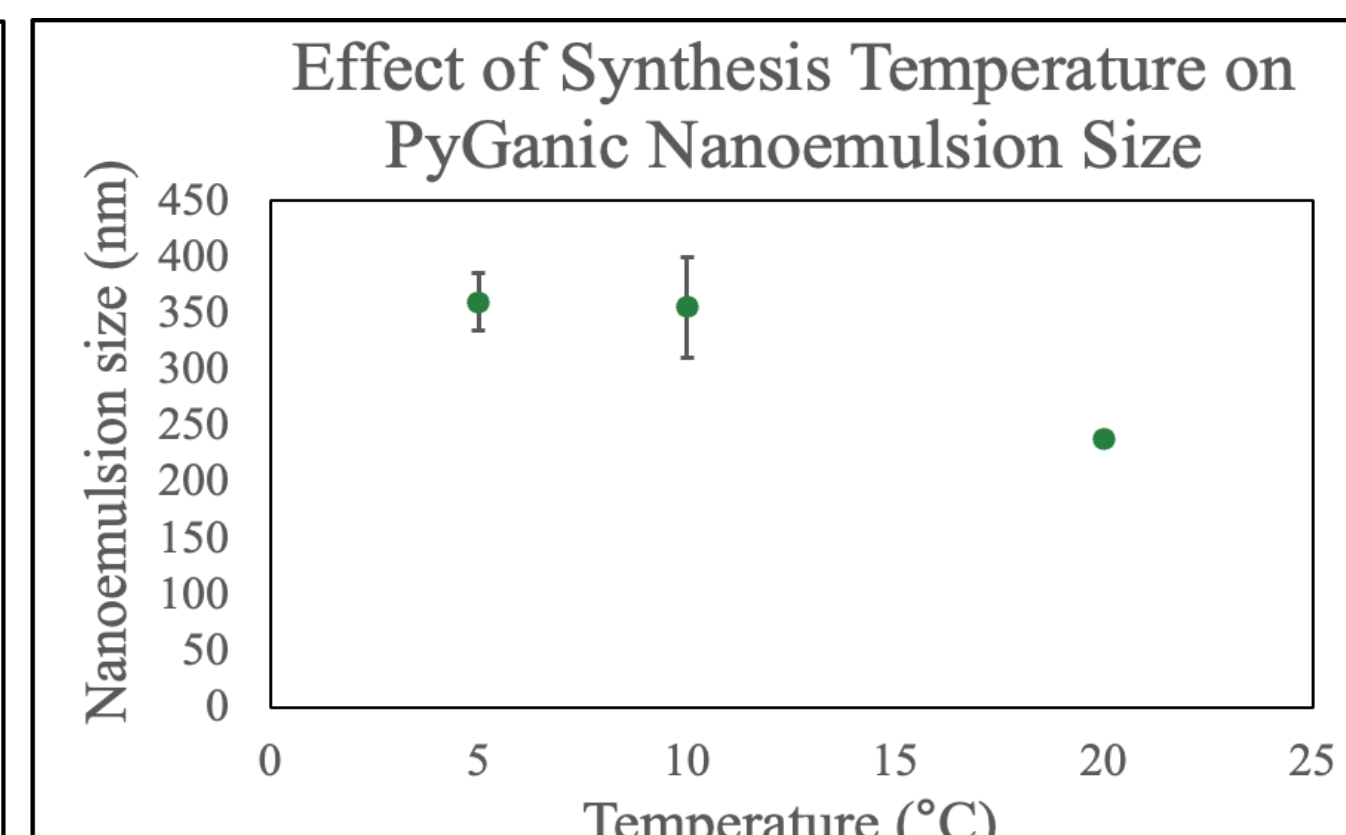


Figure 9. A decrease in nanoemulsion size is seen as the synthesis temperature increased.

Note: Error bars on all figures indicate multiple measurements of the same sample.

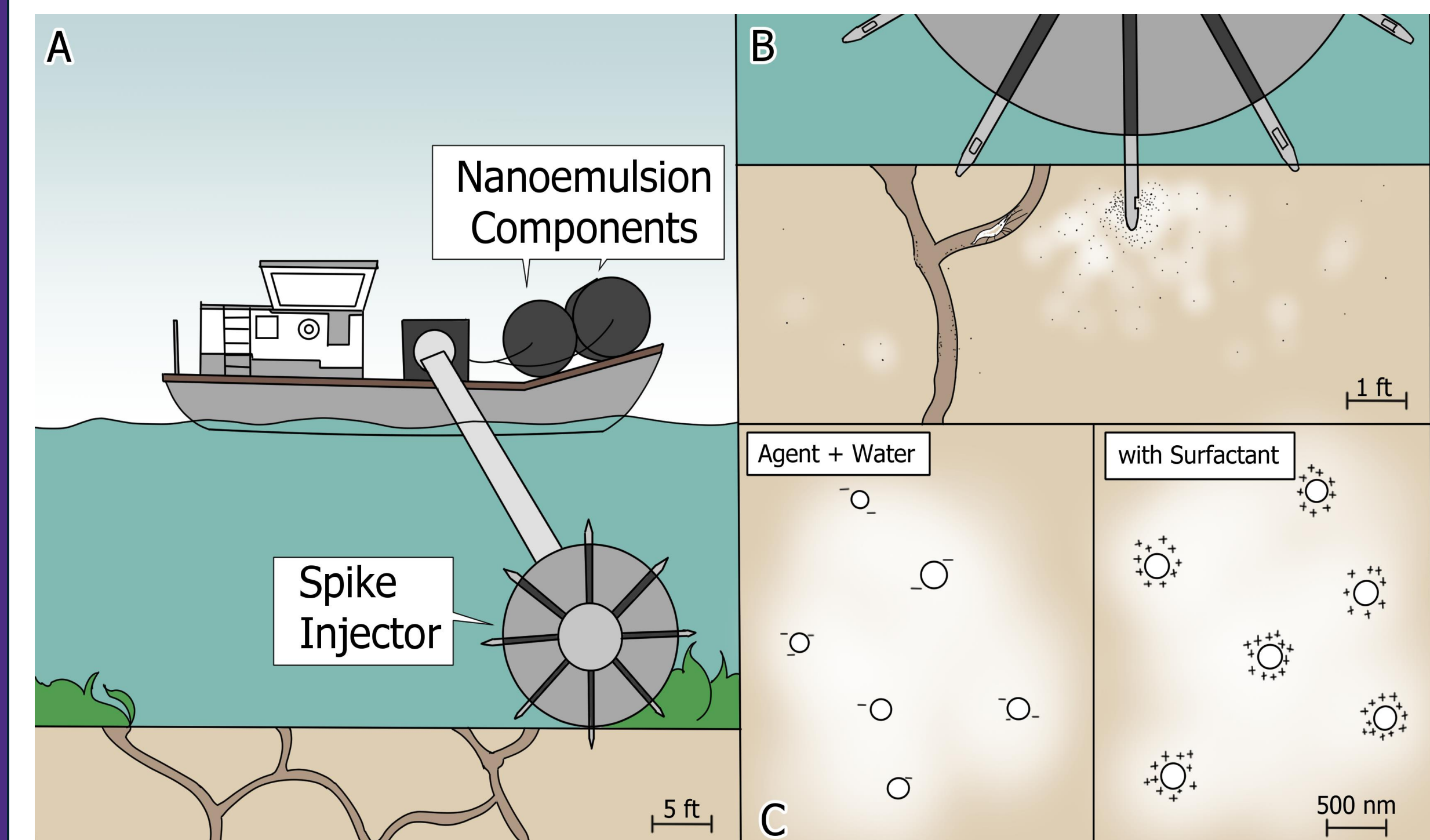


Figure 10. Proposed delivery system of nanoemulsion with spike injector. Nanoemulsion components: agent, surfactant (Chitosan), and bay water (diluent), are mixed onboard the work barge and pumped through spike injector. The injector is rolled across the bay floor and is connected to barge with adjustable anchors to allow for changes in bay depth. Pressurized delivery with high pressure pump system ensures targeted and localized containment of nanoemulsion within bay sediment.

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