Effects of Chemical Inducers on Larval Oyster Settlement in Suboptimal Conditions

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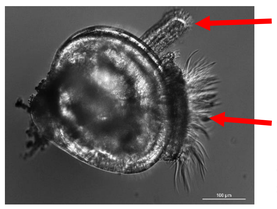
Abstract

Oysters provide important economic value for coastal communities, and ecological benefits like shoreline protection, water-quality control, and habitat for other species. In the Gulf of Mexico, oyster populations have significantly decreased due to disasters like Hurricane Katrina and openings of the Bonnet Carré Spillway. Although many resources are being put into oyster reef restoration, the vulnerable larval stages before settlement/metamorphosis can limit restoration success. The pediveliger stage is particularly important because the transition of this larval stage to juveniles determines the number of oysters that can survive and develop into adults. Previous studies have found positive effects of chemical inducers on oyster settlement/metamorphosis, but inducers are currently not used in oyster aquaculture or restoration in the Gulf of Mexico. In this study, we assessed the potential of three different neurotransmitters to induce settlement/metamorphosis in the Eastern oyster (*Crassostrea virginica*). We first conducted 1-hour behavioral assays to observe *C. virginica* pediveligers exposed to epinephrine, L-DOPA, and GABA at 10^{-5} M in low (5 ppt) and control (15 ppt) salinity and at different concentrations (10^{-6} M, 10^{-5} M, 10^{-4} M) in control salinity. Overall, GABA had little effect on settlement of metamorphosis at any concentration tested. Epinephrine caused a higher proportion of inactivity, which is indicative of metamorphosis behavior, that increased at higher concentrations. L-DOPA caused a higher proportion of foot extension, indicative of settlement behavior, that increased with higher concentrations. We also conducted a 48-hour assay on *C. virginica* pediveligers exposed to the same chemical inducers (10^{-5} M) at the same salinities and at two dissolved oxygen (DO) levels (hypoxic or ambient) to determine whether pediveligers settled, metamorphosed, or died. There was no significant increase in metamorphosis with exposure to any of the chemical inducers. There was, however, a significant negative impact of low salinity and low DO exposure on metamorphosis. Further research is needed to determine which inducer, and at what concentration, can significantly increase settlement/metamorphosis. If accomplished, this would be a cost effective way to increase oyster production to help with restoration efforts of oyster reefs in the future.

Behavior Background

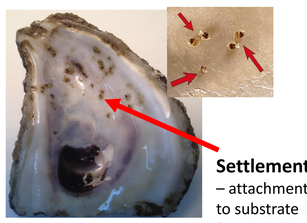
Open shell Pediveliger (14+ days)



Foot (movement indicative of searching settlement behavior)

Velum cilia (used to swim or filter water, indicative of activity other than settlement/metamorphosis)

Juveniles on shell

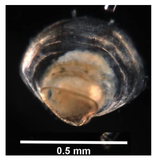


Settlement – attachment to substrate (e.g., adult oyster shell)

Metamorphosis – irreversible transition from larval stage to juvenile (attached/unattached)

Free swimming larval stage

Pediveliger → **Juvenile (spat)**

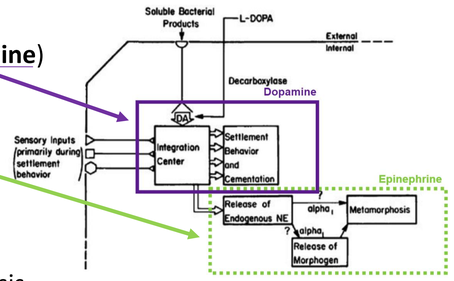


Sessile juvenile stage

Chemical Inducer Background

Chemical Inducers used:

- L-DOPA (precursor to **dopamine**)
- GABA
- Epinephrine



Expected results:

- L-DOPA – trigger settlement behavior
- GABA – trigger metamorphosis
- Epinephrine – trigger metamorphosis

Figure 1: Previous literature has shown that different neurotransmitters can trigger different larval behaviors in molluscs (settlement behavior and metamorphosis). Figure adapted from Coon et al. (1990) *Marine Biology.*

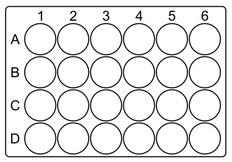
References

1. Coon, S.L., Fitt, W.K. & Bonar, D.B. Competence and delay of metamorphosis in the Pacific oyster *Crassostrea gigas* . *Mar. Biol.* **106**, 379–387 (1990). https://doi.org/10.1007/BF01344316
2. Baker, S. M., & Mann, R. (1994). Description of metamorphic phases in the oyster Crassostrea virginica and effects of hypoxia on metamorphosis. Marine Ecology Progress Series, 104, 91.

1 Hour Behavioral Assay

Methods:

- Added ~10 larvae per well (1 mL)
- Added 1 µL of inducer
- 8 replicates for each assay



1st Assay Treatment:

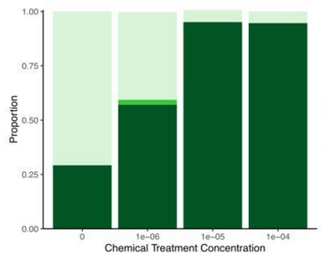
- Inducers were used at 10^{-6} M, 10^{-5} M, 10^{-4} M at ambient salinity (15 ppt)

2nd Assay Treatment:

- All inducers at 10^{-5} M
- Salinity **5 ppt** (low)/**15 ppt**

At varying concentrations-

A) Epinephrine-



B) L-DOPA-

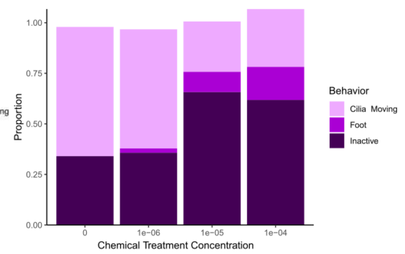
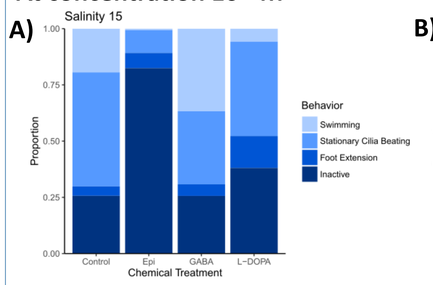


Figure 2: A)Higher proportion of inactivity as concentration increases, possibly indicative of metamorphosis. B) Higher proportion of foot extension as concentration increases. GABA is similar to control at all concentrations (not shown).

At concentration 10^{-5} M-

A)



B)

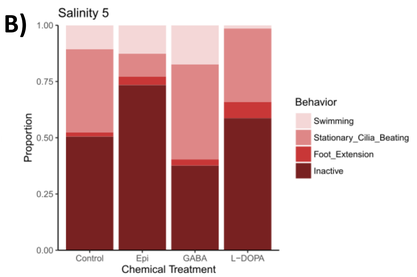


Figure 3: At both (A) 15 and (B) 5 ppt, higher proportion of inactivity in epinephrine and higher proportion of foot extension in L-DOPA, relative to control. Both behaviors were higher at 15 than 5 ppt.

48 Hour Metamorphosis Assay

- Added ~100 larvae (10 mL of stock) to each jar (473 mL)
- Added treatment water to jar containing a clean oyster shell
  - Two salinities: **5 ppt** or **15 ppt**
  - Two DO levels: Hypoxic or Ambient
- Added 0.473 mL stock chemical or DI water (10^{-5} M in jar)
- After 48 hours counted the number:
  - Attached to Jar/Lid or Shell
  - Unattached Pediveliger (**4A**)
  - Unattached Postlarvae (**4B & 4C**) or Juvenile (**4D**)
  - Dead

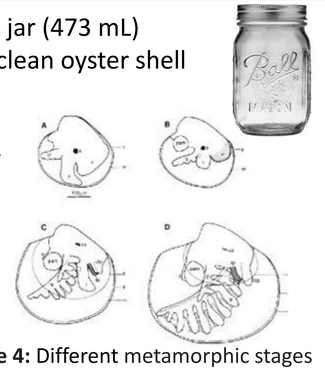
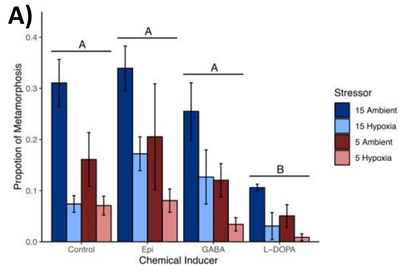
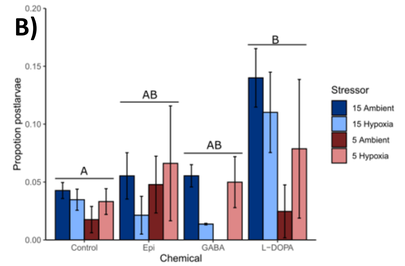


Figure 4: Different metamorphic stages from pediveliger to juvenile. Figure adapted from Baker & Mann (1994).

A)



B)



C)

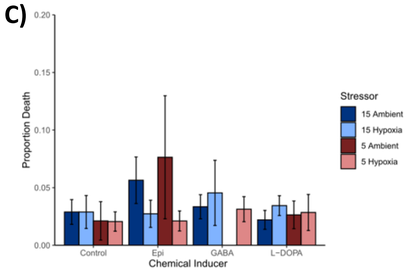


Figure 5: A) Overall, low DO and salinity had a significantly negative effect on metamorphosis. There were no significant differences in proportion of metamorphosis between any inducers/control except for L-DOPA, but B) L-DOPA had higher proportion of postlarvae stage oysters (suggesting slower growth). C) There was no significant differences in mortality among treatments.

Conclusions

- Further research is needed to identify an inducer (and concentration) that can help the survival of oysters during the transition from larvae to juvenile.
- If that can be achieved, this could significantly help increase settlement for oyster restoration efforts in a cost-effective way.
- Moving forward, L-DOPA and epinephrine will be tested at higher concentrations longer term as well as other inducers.

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