

Human disturbance on marine life

The effect of noise pollution on hatching and settlement in the sea squirt *Ciona intestinalis*

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Background and Aim

Sound waves travel efficiently in water and are therefore an important sensory cue for marine animals and organisms, as they use sound for communication and orientation (1). The oceans are filled with human-made disturbances, leading to an increase in anthropogenic (human-made) noise. This can be considered as noise pollution and may affect the behavior and development of marine organisms (2).

The ascidian *Ciona intestinalis*, a filter-feeding tunicate and a model chordate organism provides a simple and informative system to study the effect of acoustic stress (3).

The aim of this project was to investigate how anthropogenic noise affects hatching rate and settlement rate of *Ciona intestinalis* larvae.

Why *Ciona intestinalis*?

- Easy access
- Free-swimming larva with notochord and dorsal nerve cord (primitive chordate feature)
- Compact genome (~2600 cells)
- Predictable developmental program
- Nearest invertebrate relative to humans (vertebrates) (3).

Methods

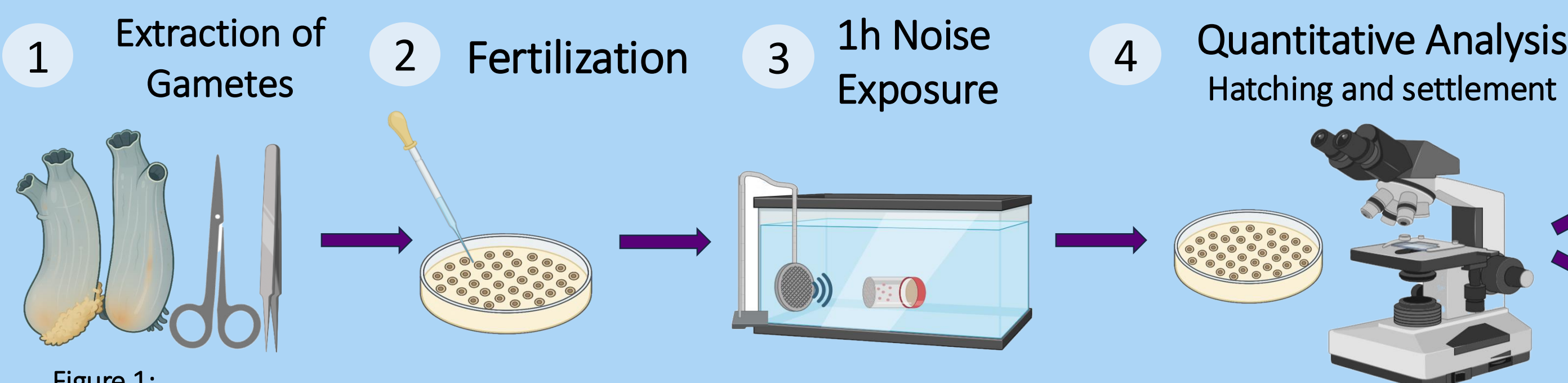


Figure 1:

Adult *Ciona intestinalis* were dissected for gamete extraction and used for fertilization. Fertilized eggs were exposed to sound for 1h at different noise levels during either the gastrula or tailbud stage. The samples were then incubated for hatching and settlement to occur. Following incubation, hatching and settlement rates were quantified.

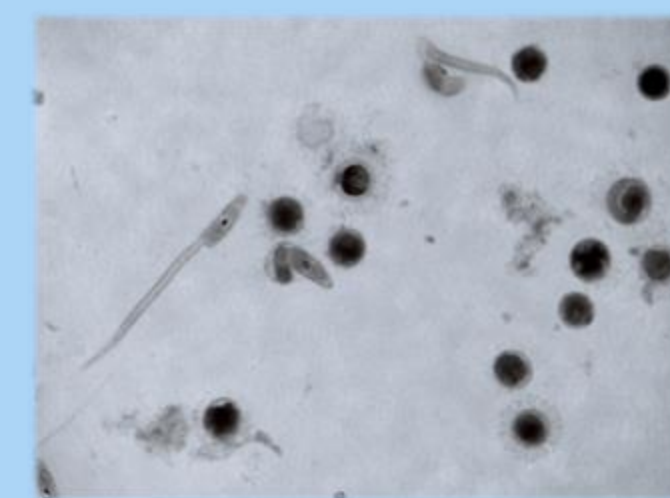


Figure 2: Micrograph picture of hatching exposure group for volume 20.

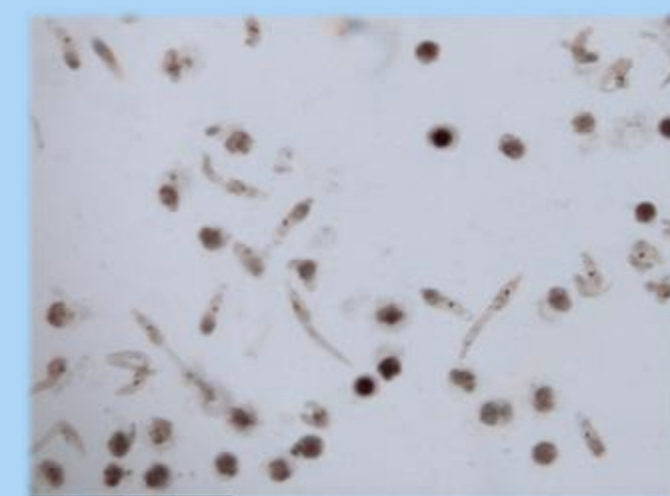
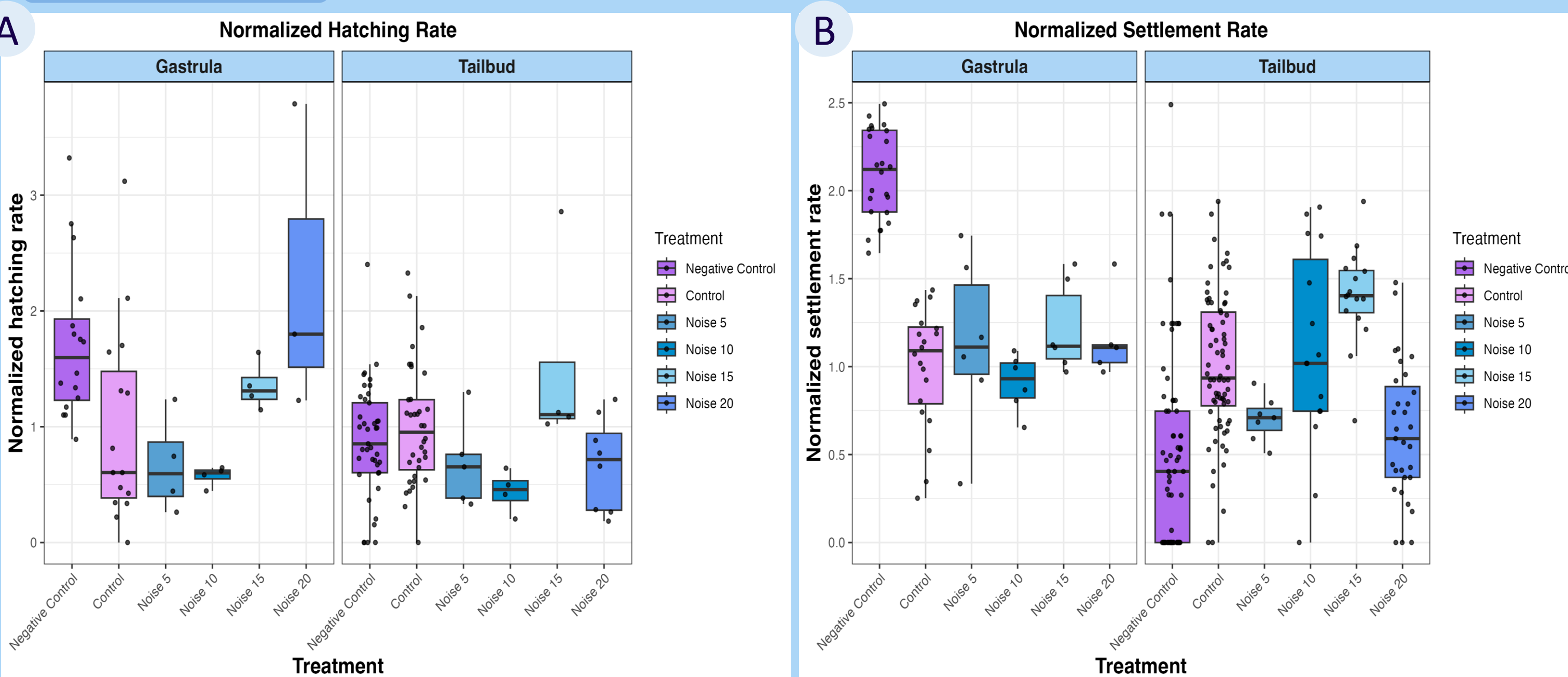


Figure 3: Micrograph picture of settlement control group for volume 15.

Results



Noise pollution sound

Artificial underwater noise amplified at 63 Hz and 125 Hz 1/8 octave band
Noise 5 – 134.9 dB re 1μPa
Noise 10 – 141.8 dB re 1μPa
Noise 15 – 149.9 dB re 1μPa
Noise 20 – 157.6 dB re 1μPa

For both hatching and settlement, the interaction between developmental stage and treatment was statistically significant (Hatching: $p=0.00036$ and Settlement: $p=2 \times 10^{-16}$), demonstrating that the effect of noise exposure differed between gastrula and tailbud embryos.

A) Hatching was significantly lower after tailbud exposure compared to gastrula exposure ($p < 0.00019$).

Within each stage, noise exposure showed no significant effect on the hatching when compared to control. A weak trend was observed, showing higher hatching at Noise 20 in gastrula ($p=0.089$). Pairwise comparison between exposed groups suggests reduced hatching at noise 10, indicating a possible sensitivity window.

B) Settlement was significantly lower after tailbud exposure compared to gastrula exposure ($p < 2 \times 10^{-16}$).

For gastrula, noise exposure showed no significant effect on the settlement when compared to control.

For tailbud, settlement was higher at noise 15 ($p=0.022$) and lower at noise 20 (0.0025) relative to control, suggesting a possible response to different noise intensities.

Conclusion

Treatment of embryos appeared to affect both hatching and settlement depending on the developmental stage of exposure, although other experimental factors, e.g., applied mechanical stress, parental batch and season, may also contribute.

Larvae exposed at tailbud appeared more affected than those exposed at gastrula.

Hatching showed no significant effect of noise exposure within each developmental stage.

For settlement, tailbud embryos showed significantly higher settlement at Noise 15 and lower settlement at Noise 20 compared to the control.

Developmental stages could seem to affect the response, possibly due to the differences in structural development between gastrula and tailbud embryos. At tailbud, chordate features such as the notochord, simple nervous system, developing musculature and sensory structures are present. In contrast, the gastrula undergoes early patterning, with tissue yet to be differentiated. This may suggest that tailbud is more prone to be affected by applied stress.



Figure 5: *Ciona intestinalis*
Photo: Alexandre Jan (4)

References:

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2. Peng C, Zhao X, Liu G. Noise in the Sea and Its Impacts on Marine Organisms. *Int J Environ Res Public Health*. 2015 Sep 30;12(10):12304-23. doi: 10.3390/ijerph121012304. PMID: 26437424; PMCID: PMC4626970.
3. Matthew J Kourakis, William C Smith (2015) The Natural History of Model Organisms: An organismal perspective on *C. intestinalis* development, origins and diversification *eLife* 4:e06024
4. Alexandre Jan

