Rhythmic larval release in the estuarine crab *Dyspanopeus sayi*: entrainment by temperature cycles

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INTRODUCTION

- Marine and estuarine crabs brood attached eggs and release larvae according to a circadian rhythm controlled by diel and tidal cycles[1].
- Normally, larval release occurs within a 4 hour interval after sunset[3].
- *Dyspanopeus sayi* is a subtidal crab that reproduces in the spring, summer, and fall.
- The circadian rhythm in *D. sayi* can be entrained by light:dark cycles[2].
- *D. sayi* is also exposed to diel temperature cycles, however, temperature has not yet been tested as an entrainment cue.

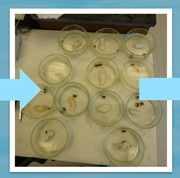
Figure 1: Female *D.sayi*

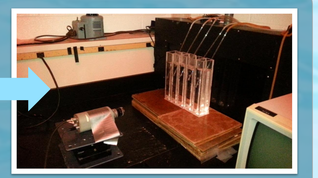
Figure 2: Ovigerous *D.sayi*

OBJECTIVES

1) Determine whether a circadian rhythm for larval release can be entrained by temperature cycles
2) Determine which environmental cycle is a stronger entrainment cue, the light:dark or temperature cycles

METHODOLOGY





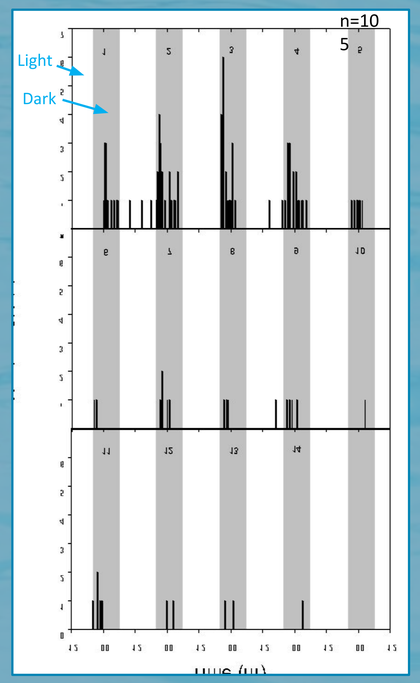
Demonstration of an endogenous rhythm
- Times of larval release were recorded
- An autocorrelation analysis and MESA test were run in MATLAB to determine the free running period of larval release times

Entrainment by reverse temperature cycles
- Environmental chamber created a reverse diel temperature cycle
- Δ10°C trial: 18°C from 6:00hr-20:00 hr & 28°C from 20:00hr-6:00hr
- Δ5°C trial: 18°C from 6:00hr-20:00 hr & 23°C from 20:00hr-6:00hr
- Placed in chamber for 4 days and then placed in constant conditions (CC)

Evaluation of temperature cycle strength
- Environmental chamber created a reverse diel temperature cycle and a light:dark cycle
- Δ10°C cycle: 18°C from 6:00hr-20:00 hr & 28°C from 20:00hr-6:00hr
- Ambient light:dark cycle: light from 6:00hr-20:00 hr & darkness from 20:00hr-6:00hr
- Placed in chamber for least 3 days and then to CC on day of predicted release.

RESULTS

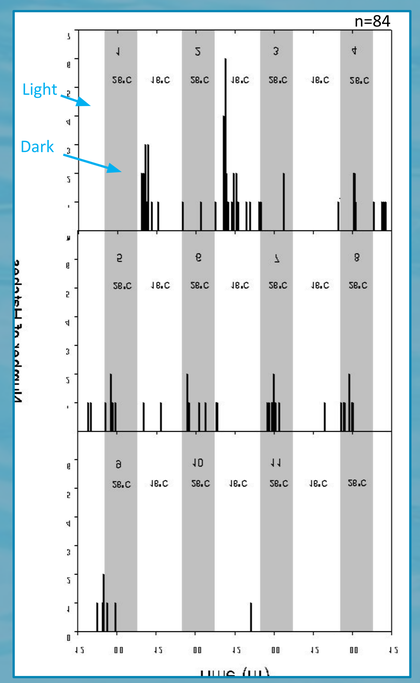
1. Demonstration of Endogenous Rhythm: Control



Free running period of 25.0 h

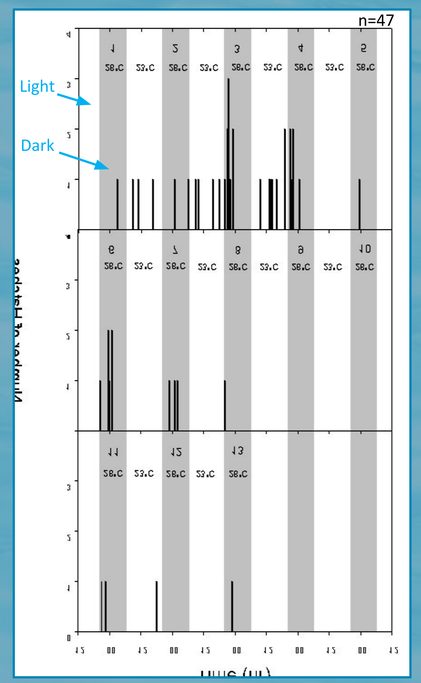
- Larval release times occurred during the dark phase (94.3%) of the ambient environment while in CC.

2. Temperature Cycle: Δ10°C



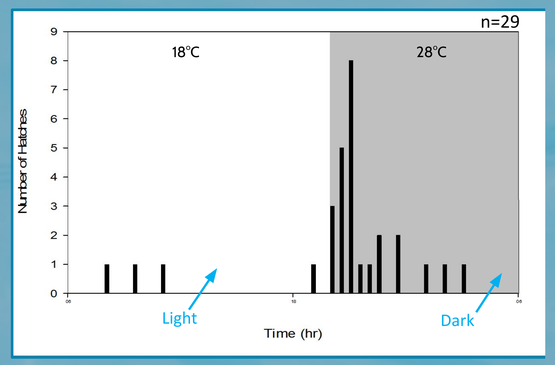
- Most larval release times (84.3%) occurred during the low temperature phase for the first **4** days in CC.
- After 4 days, the times of release reverted to the dark phase (60.6%) of the ambient light:dark cycle.

3. Temperature Cycle: Δ5°C



- Most larval release times (81.8%) occurred during the low temperature phase for the first **3** days in CC.
- After 3 days, the times of release reverted to the dark phase (95.2%) of the ambient light:dark cycle.

4. Light:dark vs. Temperature Δ10°C Cycles



- The majority (90%) of larval release times occurred during the dark phase of the light:dark cycle and the high temperature phase of the temperature cycle
- This suggests that the crabs released larvae according to a circadian rhythm entrained by the light:dark cycle.

DISCUSSION

1. The majority of larval release times occurred during the dark phase of the ambient environment. This shows that the circadian rhythm was retained even after being placed in constant conditions and thus, is an endogenous rhythm.

2. The data demonstrated entrainment to the temperature cycle during the first 4 days in CC. After 4 days, release times reverted to their original entrainment by the ambient light:dark cycle. This suggests that temperature cycles may not be a strong cue for entrainment in *D. sayi*.

3. The data showed entrainment during the first 3 days in CC, but reverted to releasing larvae during the dark phase. This suggests that the Δ 5°C was weaker at entraining the rhythm than the Δ 10°C.

4. When crabs were exposed to a temperature cycle and light:dark cycle simultaneously, crabs released larvae during the dark phase of the light:dark cycle. This proves that light:dark cycle was a stronger environmental cue for the larval release circadian rhythm in *D. sayi*.

Given that temperature cycles were successful in entraining the circadian rhythm, but were not favored when crabs were exposed to both cycles, it is possible that temperature cycles are used in conjunction with or in the absence of a light:dark cycle to entrain the larval release circadian rhythm.

References: 1) Christy, J.H. 2011. Timing of hatching and release of larvae by brachyuran crabs: patterns, adaptive significance and control. Integrative and Comparative Biology 51:62-72. 2) Forward, Jr., R.B., K.G. Sanchez, and P.P. Riley. 2016. Entrainment of the circadian rhythm in egg hatching of the crab *Dyspanopeus sayi* by chemical cues from ovigerous females. Biological Bulletin 230:15-24. 3) Ziegler, T.A., and R.B. Forward Jr. 2005. Larval release behaviors in the blue crab *Callinectes sapidus*: role of chemical cues. Journal of Experimental Marine Biology and Ecology 273:1-14.

Acknowledgements

This study was funded in part by NSF Grant No. NSF OCE-14-60180. A special thanks to Dr. Richard B. Forward Jr. for his valuable mentorship and expertise, the other REU students who assisted with crab collections, and to the Duke University Marine Laboratory for its facilities and resources.