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RAINFALL DRIVES SYNCHRONOUS SPAWNING EPISODES IN THE SCALY PEARL OYSTER, *PINCTADA LONGISQUAMOSA* ON SAN SALVADOR ISLAND, BAHAMAS

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ABSTRACT

Using growth data compiled over the past four years, we determined "birthdates" for a population of scaly pearl oysters collected in January, 2013 from Six Pack Pond on San Salvador Island, Bahamas. We plotted birthdates with rainfall data collected by Dr. R. Laurence Davis and found a correlation between periods of heavy rainfall and periods of intense oyster spawning. We dubbed these episodes "storm-spawn" events. We took advantage of being on island June 11, 2013, approximately 2-and-a-half weeks after a six-inch rain, to assess oyster-spawning activity directly by using a plankton tow in three different ponds. "D"-form veliger larvae were found swimming in Six Pack Pond and Mermaid Pond, but not in Oyster Pond. This is consistent with a model suggesting that ponds with high-volume conduit activity are buffered against changes in temperature and salinity brought on by storm events. Hence, Six Pack Pond (with no measurable tidal activity suggesting low conduit exchange) is the most susceptible to storm-driven changes in salinity/ temperature. The 6-inch May rainfall event clearly induced spawning here. Oyster Pond, with significant tidal activity indicating high conduit exchange, showed no evidence of spawning. In Mermaid Pond (a pond with intermediate conduit activity as evidenced by the delay in tidal fluxes), oysters also showed storm-spawn activity. These findings suggest that life history characteristics can be dramatically affected by differences in physical characteristics of the habitats that they occupy highlighting the uniqueness of San Salvador's anchialine ponds as a collection of natural experiments in community structuring and setting the stage for rapid evolutionary adaptation.

INTRODUCTION

Since 2001 we have followed life history characteristics in the scaly pearl oyster (*Pinctada longisquamosa*) from a series of inland, anchialine ponds located on San Salvador Island in the Bahamas. As a control group, we have also sampled *P. longisquamosa* from the Florida Keys (specifically from the gulf side of Key Largo).

For marine bivalves, temperature is the principle factor regulating gametogenesis (Barber and Blake, 2006), though food availability contributes as well (Dutertre et al., 2009). In general, reproduction, development, growth and survival of bivalves are all affected by fluctuations in both temperature and salinity (Wang, et al., 2012). In one collection made on San Salvador at bimonthly intervals throughout the year, we learned that *P. longisquamosa* on San Salvador become gravid twice a year (during the spring and late fall months). This is consistent with a report by Lubet and Aloui (1987) suggesting that bivalves exhibit an optimum "temperature window" for gamete formation, an event that should occur twice, once during spring and once during the fall.

Nevertheless, the interval of peak egg and sperm production appears quite broad, spread over months. Being gravid for a number of months during the year raises the question: how do broadcast spawners such as marine bivalves, synchronize spawning activities to maximize breeding success? Clearly, for a lone male to spawn when no females are shedding their gametes would be a futile strategy. If oysters could utilize environmental cues to trigger breeding, this would permit synchronized spawning, and greatly increase the likelihood of successful fertilization.

Three lines of evidence point to storm activity as a potential trigger for synchronized spawning in one of our inland populations of pearl oysters: 1) the remarkable (synchronous) recovery of the Six Pack pond population following hurricane (or severe deluge) decimations in 1999, 2004, 2007 and 2011; 2) the analysis of growth data indicating spawning events coincident with major storms; and 3) direct observation of the emergence of larvae in the weeks following a storm event.

METHODS

In January, 2013, Oysters were collected from Six Pack Pond along randomly oriented transect lines within 25 meters of shore. Hinge length for each oyster was measured using hand-held calipers and measuring along the straight edge of the oyster's hinge. A von Bertalanffy growth plot generated previously (Figure 1, see Carlson, et al., 2010) was used to convert hinge-length data into age data, and a table of "birthdates" was generated and plotted.

San Salvador rainfall data was obtained from Dr. R. Laurence Davis for the year of 2011. An especially intense rainfall was noted for the period from Sept. 17- October 12 (five feet of rain in four consecutive downpours!) Rainfall data and calculated oyster spawning periods were plotted on the same graph to evaluate correlation.

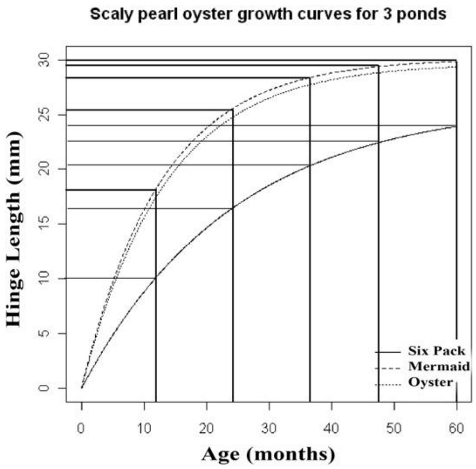

Figure 1. A von Bertalanffy growth curve generated for oysters in three anchialine ponds. Hinge length is plotted against age in months. This tool allows one to estimate age (and hence spawning dates) for oysters based on their shell-dimensions.

Veliger larvae were collected from Oyster Pond, Six-Pack Pond and Mermaid Pond using a 20 micron mesh plankton tow net. Approximately 100 meters of surface water were sampled from each site by towing the net behind us as we swam across the pond (Figure 2). Veligers were photographed and identified using an AO light microscope and a hand-held cell-phone camera.

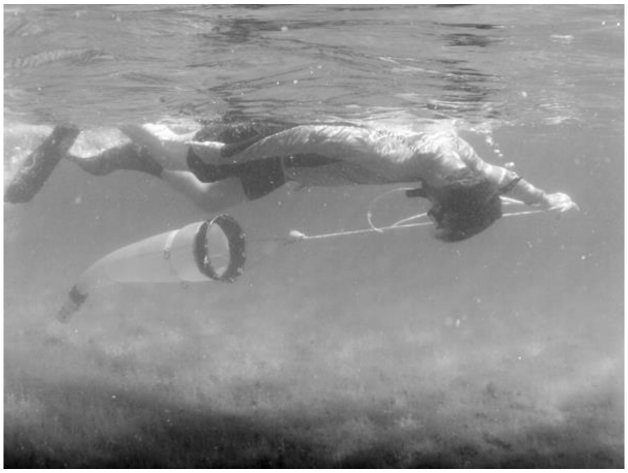

Figure 2. Hauling a plankton net through surface waters of Oyster Pond.

RESULTS

A History of Periodic Oyster Decimation in Six-Pack Pond, Coupled with Rapid Population Recovery.

We have documented four episodes of catastrophic oyster decimation in Six Pack Pond, followed by dramatic periods of repopulation (Figure 3). In 2001, we observed a community of uniformly small (juvenile) oysters densely populating the algal floor of Six Pack Pond (mean hinge length: 8 mm, or size class "B"). Over the next two years these oysters grew (mean hinge length 15.06 in 2003). We interpret this to be a population rebound 16 months after Hurricane Floyd (Sept. 13, 1999). Two things are notable: there were no large, mature oysters in the near-shore population. This argues for the elimination of the larger, adult population during the storm event. Second, by June, the population was thoroughly re-established! This argues that despite annihilation of the adult population, a subsequent renewal population was established. We hypothesized that this storm event triggered mass spawning even as the adult population was being exterminated.

In 2005 we were able to test this hypothesis. Hurricane Frances struck the island on Sept 1, 2004. Four months later we found the adult population devastated (only six live adult oysters were found after 2 hrs of searching). Curiously, the oysters in Oyster Pond (a more protected inland pond with active conduits) were relatively unaffected. Returning in June, we found the Six Pack population fully restored! These observations reinforced our impression that this population is uniquely vulnerable to storm impact, and that this population can be re-established within 9-10 months. Again, we suspected that recovery was driven by a "storm-spawn" phenomenon hypothesizing that the planktonic "veliger" larvae found refuge in the deeper (saltier) waters of the pond.

The third event was Hurricane Noel (Nov. 1, 2007). Curiously, Noel did not strike the island directly, and winds did not develop significantly over San Salvador. What did impact the island was rainfall. Nearly two feet of rain fell on San Salvador. We surveyed Six Pack Pond five months later and found the most severe population decimation yet (there were no oysters visible and salinity had dropped to 22 ppt). This caused us to refine our hypothesis. It was not the high winds and subsequent wave action that killed off the oysters in Six Pack Pond, it was more likely a drop in salinity. Again, the population reasserted itself by January 2009.

Finally, we were able to survey oyster populations following a strictly rainfall driven event, a torrential (five foot) rain that fell during the last weeks of September and into October 2011. Five months after this event, no adult oysters could be found in Six Pack Pond, yet by January 2013, the population had once again recovered.

These four episodes share two things in common: storms decimate the adult oyster population of Six Pack Pond, and yet the population rebounds in less than a year. This rapid rebound argues against a mere physical displacement of adult oysters that somehow find their way back to the shallows. That view is also untenable in light of the small, uniform size of the oysters that initially repopulate the shallows. What we suggest is that environmental changes that accompany a severe storm (most notably a drop in salinity and temperature), provoke mass spawning prior to killing the adult breeding population. We further propose that the planktonic larvae can be freely distributed through the water column, finding refuge in deeper, saltier waters. Six Pack Pond is unique among the larger ponds in that it reaches depths of 4-5 meters. The reason we do not see similar storm-driven population crashes in other oyster refuges (Oyster Pond for example) is that the substantial tidal action operating through these pond's conduit system buffers the environment from wide (lethal) swings in salinity. Curiously, we have seen evidence of storm-induced spawning in Oyster Pond, without the accompanying mortality (Carlson, et al. 2010).

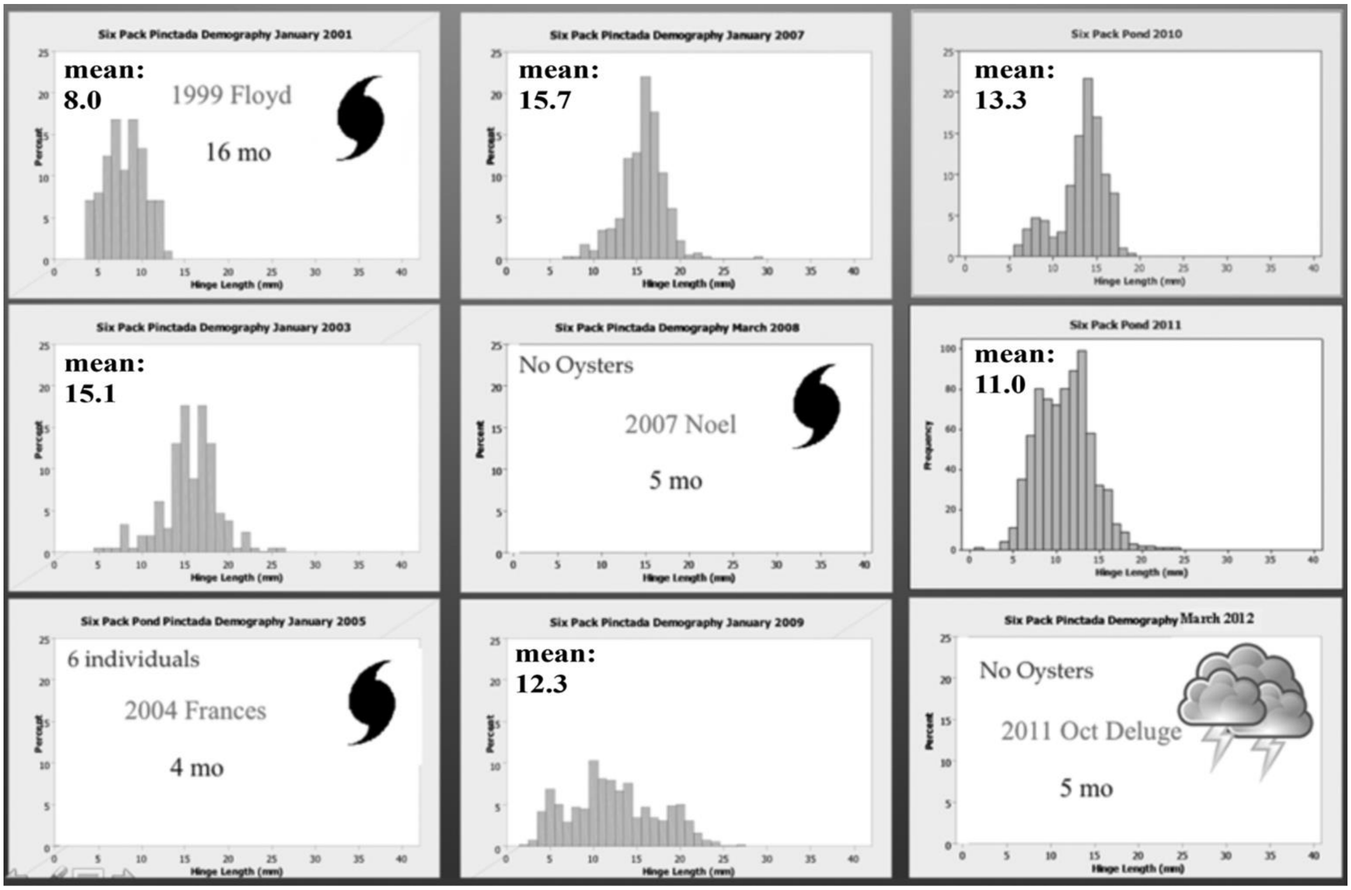

Figure 3. Oyster survey data from Six-Pack Pond from 2001 – 2012. Four catastrophic population decimations were documented associated with Hurricane Floyd (1999, absence of large adults in 2001 survey suggest population crash coupled with recovery), Hurricane Frances (2004 only 6 live oysters were found four months after storm despite two hours of searching, followed by population recovery in June 2005, data not shown), Hurricane Noel (2007, zero live oysters were found in March 2008, 5 months after storm, population recovered by Jan. 2009), non-hurricane related decimation documented 5 months after Oct. deluge in 2011 (population recovered by Jan. 2013, data not shown).

Correlating Rainfall Events with Spawning Dates.

In 2010 we developed a growth model for the oysters of San Salvador's inland ponds (Figure 1). This allowed us to measure an oyster (hinge length data) and use that number to determine its age and hence, calculate its date of birth (spawning date). In January 2013, we measured a sample of oysters collected from Six Pack Pond. By converting hinge length data to age data, we were able to plot their effective spawning dates. This data appears in Figure 4. There was some uncertainty about which growth curve to use. Our von Bertalanffy Growth curves were calculated from oysters during a non-storm year in which the salinity of Six-Pack Pond was rather high (>40ppt). In January 2013, Six Pack Pond salinity was significantly lower, (26.4 ppt) as it was still recovering from the October 2011 deluge. In normal years, in which Six Pack Pond is somewhat hypersaline, we have seen slower growth of oysters. We could use the hypersaline Six-Pack growth rates, or imagine that, with near normal salinity, growth rates are more like those of Oyster Pond.

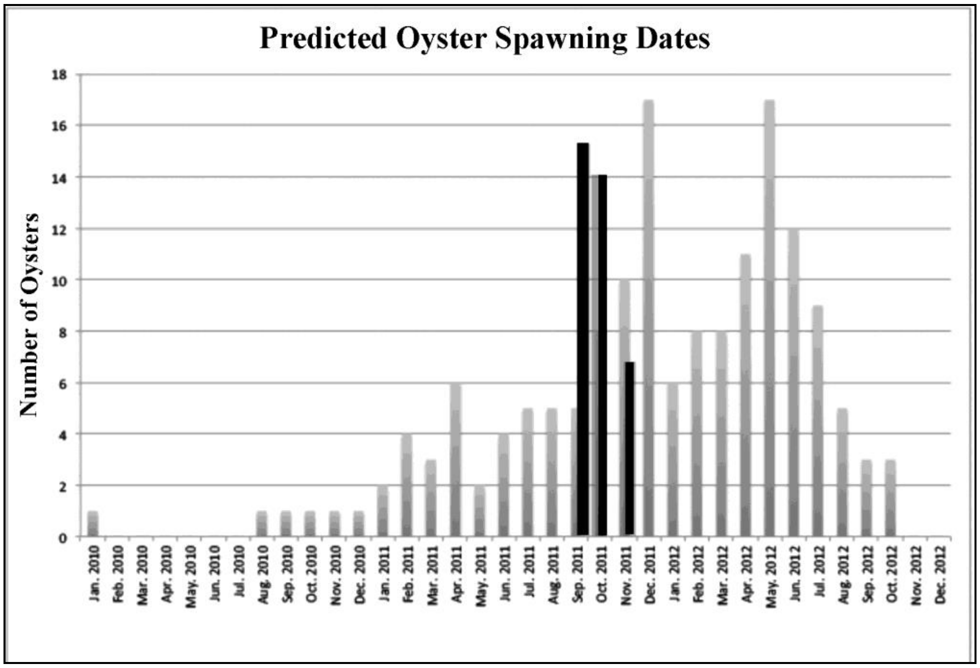

Figure 4. Five foot rainfall event (black) plotted against estimates of oyster spawning dates (gray). Oysters were sampled from Six-Pack Pond, January 2013.

Choosing the latter rationale, we plotted the birthdates seen in Figure 4 (in gray).

There were clearly two prominent breeding episodes, one extending from October-December, 2011, and a second peak, five months later, extending from April-June, 2012. It should be noted that the pond-salinity was doubtless undergoing a dynamic shift from hypo-saline conditions (following the five foot rainfall of 2011) to the more saline conditions of spring and summer. Growth rates were likely affected.

During 2011, Dr. R. Laurence Davis collected rainfall data, which he generously shared with us. The most significant rainfall event was the October deluge (plotted in black, Figure 4). It is noteworthy that this storm correlates with the onset of the earlier spawning episode. It is also noteworthy that the second predicted spawning event (spring 2012) fell five months after the proposed storm-spawn event. From earlier studies we know that it takes oysters approximately five months for our oysters to reach sexual. Hence the second spawning event most likely represents the first spawning episode from the October "storm-spawn" progeny.

Direct Evidence of Storm-induced Spawning.

In June 2013, we arrived on island two and a half weeks following a six-inch rainfall event. We sampled suspended plankton in three different oyster habitats: Oyster Pond (with strong conduit activity that buffers pond salinity against all but the most dramatic rainfall events), Mermaid Pond (with sluggish conduit activity and salinity levels that fluctuate modestly in response to storms), and Six Pack Pond (with no measurable tidal flux suggesting minimal conduit activity and dramatic swings in storm-driven salinity). Plankton tows yielded viable D-form veliger larvae from both Mermaid Pond and Six Pack pond, but not from Oyster Pond (Figure 5).

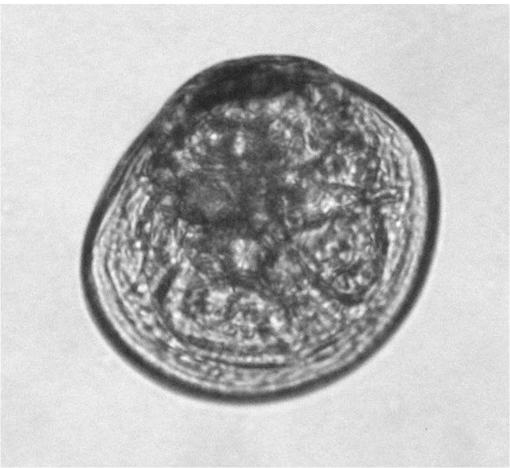

Figure 5. D-stage veliger larvae collected from plankton tow in Six Pack Pond.

Pearl oyster veligers typically remain suspended in the water column for 2-4 weeks before settling and becoming attached to the substrate by byssal threads (Southgate and Lucas, 2008). The fact that we observed free-swimming larvae in pond-water plankton, argues that they had been spawned during this recent storm event, probably in response to a sudden drop in temperature and salinity.

The absence of larvae in Oyster Pond argues that the May 2013 storm, while sufficient to induce spawning in the less-protected pond environments, was insufficient to trigger a synchronous spawning event in the well-purged Oyster Pond system. There also was no evidence of mortality in the Six Pack population. This suggests that a six-inch rainfall, while sufficient to trigger spawning in both Six Pack Pond and Mermaid Pond, was not sufficient to drop salinity to lethal levels (a fact confirmed by salinity measurements: Six Pack Pond = 29.3 ppt up from January 26.4 ppt).

DISCUSSION

The anchialine ponds of San Salvador Island offer a unique assemblage of habitats, allowing researchers to exploit a rich gradient of environmental conditions (Halvorson et al., 2012). Ponds range from hyper-saline to brackish, from zero measurable turnover in water chemistry to dramatic twice-daily turnovers driven by extensive subterranean conduits winding through miles of chambers to the sea. Each pond also hosts a unique assemblage of flora and fauna. We have taken advantage of these natural experiments to explore the interplay between habitat and natural history.

This year we focused our efforts on determining the natural trigger(s) for spawning in the scaly pearl oyster, *Pinctada longisquamosa*. We assembled three lines of evidence all suggesting that this marine bivalve is keenly attuned to dramatic weather events common to the West Indies and Caribbean. Indeed, it seems likely that these delicate pearl oysters (and perhaps many marine invertebrates) have evolved life-history characteristics in an evolutionary dance with severe storms, even depending on them to drive and synchronize their seasonal breeding episodes.

Abrupt changes in either temperature, (De la Roche, et al. 2002, Velasco, et al. 2007, Pronker, et al. 2008, Matias et al. 2009, Nava and Garcı´a de Severeyn 2010, Soria, et al. 2010) or salinity (Southgate and Lee 1998, Nava and Garcı´a de Severeyn 2010) have been shown to trigger spawning in cultivated bivalve cultures. The presence of mature gametes can also trigger spawning (Ellis 1998). The three inland ponds under investigation exhibit an environmental "Goldilocks Effect" when it comes to oyster life histories. Six Pack Pond exhibits wild fluctuations in temperature and salinity in response to severe storm activity. The oyster population inhabiting Six Pack Pond exhibits a precarious existence swinging from annihilation of the adult population, through mass storm-induced spawning, to rapid repopulation in the aftermath. It is only by virtue of its unusual depth, (we suspect) that oysters have not been exterminated once and for all. Oyster Pond, on the other hand, is protected from storm-induced changes by having a twice-daily flushing of its surface waters through a massive system of underground conduits. Indeed, we suspect that the underground waters represent a type of reservoir against which the pond waters are flushed and exchanged. The result, we propose, is that mild to moderate storms are unable to bring about the changes in temperature or salinity necessary to trigger or synchronize spawning in these oysters. Consequently, the Oyster Pond population grows progressively senescent as mature oysters age, and juveniles are not recruited. It takes a severe storm (more than 6 inches of rain for example) to trigger spawning in this population. This pond could be said to suffer from an overly-protected habitat, and is only rescued from senescence by periodic hurricanes or deluge. Finally, we have Mermaid Pond. This habitat appears to find itself in the middle. It has conduits that actively turn over the surface waters, but these conduits appear sluggish. We have recorded strong drops in salinity that persist for weeks following a severe storm. Oysters in this habitat appear to benefit from regular, storm-induced spawning events, without the periodic decimations seen in Six Pack Pond.

Spawning events are triggered against a backdrop of annual fluctuation in gamete "readiness". Egg and sperm production do not appear to be constant throughout the year. Rather, there appear to be two broad "waves" of gamete maturation, one in spring and the other in fall. In 2008 and 2009, temperature data was collected from several of the inland ponds on a monthly basis (Figure 6). As mentioned by Lubet and Aloui (1987), bivalves might have an optimum "temperature window" for gametogenesis. If this is so, then we may be close to identifying environmental parameters that drive both gamete maturation and synchronous spawning in this particular marine invertebrate.

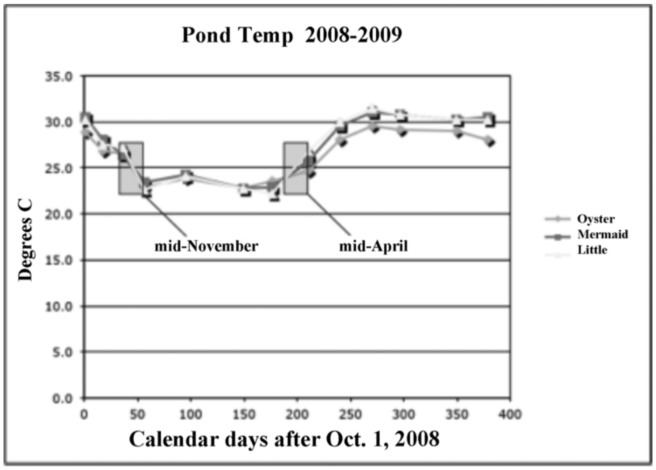

Figure 6. Pond temperature data showing an annual cycle consistent across all three pond habitats. Boxes highlight periods when temperatures fall between $22^{o}C$ and $27^{o}C$, a hypothetical "optimum" for oyster gametogenesis.

Together, these habitats afford us a remarkable opportunity to investigate the dynamic interplay between organismal life histories and environmental conditions. This has the potential to allow inquiry into both physiological adaptation (how breeding behavior is driven by environmental cues) and evolutionary adaptation (how genetic programs underlying mechanisms of sex-determination can be "tuned" to natural cycles of environmental change). To this point, it is interesting to learn that in a different bivalve, *Crassostrea gigas*, sex determination may be influenced by ambient temperature (Santerre, et al., 2013). This study in natural history continues to play itself out against the backdrop of climate change, and may help us to appreciate some of the complex and unexpected consequences of our changing times.

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