Monitoring of the Atlantic Horseshoe Crab *(Limulus polyphemus)* During the Spawning Season in the Damariscotta River Estuary

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Abstract

The purpose of this study was to summarize population surveys of the Atlantic horseshoe crab *(Limulus polyphemus)* conducted since 2002 on the Damariscotta River, Maine, by the Damariscotta River Association, and to evaluate the environmental correlates of variable abundance and spawning activity. Populations of horseshoe crab have been monitored by the Maine Department of Marine Resources (DMR) in conjunction with various volunteer groups across Maine since 2001. Concerns about the potential overharvesting of this species drove the DMR to establish rules prohibiting the harvest of horseshoe crabs. The original purpose of monitoring horseshoe crabs in Maine was to determine spatial and temporal trends in coastal populations. The objective of this study was to compile and report the results of shoreline transect surveys collected since 2002 by Damariscotta River Association volunteers at two sites in the Damariscotta River estuary: Damariscotta Mills and Days Cove. A pilot survey during 2014 at a new site, Lowes Cove, several km further downriver of the others produced no horseshoe crabs. At Damariscotta Mills and Days Cove we found that average annual counts of horseshoe crab , sex ratio, and size composition have varied without trend over the years at both sites, although Day's Cove consistently had a higher proportion of females. Males outnumbered females by a ratio of about four to one, and notably all females observed in the survey were paired with males, sometimes more than one. was Above average counts were found to occur at approximately 21° C and at within a salinity ranging of 21 to 24 ppt. Temperatures and salinities below and above these values tended to be associated with below average horseshoe crab counts. It is therefore likely that shoreline counts may be less an indicator of the abundance of horseshoe crabs in the estuary than it is of the influence of the environment on their activity and movements. Our analysis also indicates average horseshoe crab counts were strongly correlated with atmospheric temperature anomalies,and more so for the preceding winter than spring. Horseshoe crab counts were only weakly correlated with water temperatures and salinity recorded during the survey No strong association was found between horseshoe crab counts and lunar phase. While lunar phase may be a primary factor synchronizing spawning activity in other estuaries to the south, salinity and temperature appear to be important in modulating the

appearance of horseshoe crabs at our monitoring stations in this estuary.

Introduction

Horseshoe crabs are part of an ancient arthropod lineage related to spiders, the Order Xiphosura, that dates back in the fossil record 420 to 500 million years to the Cambrian period. Four species of horseshoe crabs occur worldwide and only one in North America, *Limulus polyphemus* (Schaller and Hanson, 2004). This species occurs only on the eastern coast of North America and ranges from the Yucatan Peninsula of Mexico as far north as Taunton Bay, Maine which is considered to be the northern-most extent of its range (Schaller and Thayer, 2002).

In the United States horseshoe crabs have been harvested for over a century and continue to be harvested in select locations, mostly in Delaware Bay. One of the largest and oldest uses of harvested horseshoe crabs has been in agriculture, as fertilizer or feed for live-stock. The diet of *L. polyphemus* primarily consists of large polychaete and nemertean worms and various bivalve mollusks. Because their diet included highly prized commercial clams, various shellfish organizations label the horseshoe crab a pest that needed to be controlled to maintain commercial production. Horseshoe crabs were once even viewed as “beach litter” that needed to be cleaned up so as not to deter recreational activity (Born, 1982). Use of horseshoe crabs as bait by the American eel and conch fisheries is thought to be the most significant threat to Maine's crab population. Personal accounts of this mass removal of horseshoe crabs for bait noted that “truckloads” of individuals were taken from multiple shorelines as often as every tide. A 1996 estimate of the fishing mortality accounted for at least two million individuals throughout the Atlantic Coast. Lastly, and most importantly, the pharmaceutical and medical device industries use blood extracts from horseshoe crabs as an indicator of bacterial contamination because their blood cells are ultra-sensitive to endotoxin, a toxin that is present inside a bacterial cell and is released when the cell disintegrates. This industry bleeds individuals and then releases them back into the wild, however, it is estimated that 10-15 percent of these bled individuals do not survive. This percentage accounts for an estimated mortality of 20,000 to 38,000 individuals per year (Schaller and Hanson, 2004).

From an ecological standpoint *L. polyphemus* plays a large role in the food web. Their foraging activity for large worms and bivalves involves overturning the sediment, an activity that releases particles and nutrients into the water column which serve as food for micro-organisms. Although large horseshoe crabs probably escape most predators, their eggs and young are consumed by various organisms. Shorebirds and small fish depend on the eggs and larvae as a large food source and declining egg production may have a large negative impact on horseshoe crab survivorship. Endangered sea turtles depend on adolescent horseshoe crabs as a food source and could also be negatively affected by large scale depletion of this food source (Schaller and Hanson 2004). As with many other estuarine organisms pollution, shore development, and habitat degradation pose a large threat to the remaining populations of horseshoe crabs.

The unregulated over-harvesting of *L. polyphemus* dramatically reduced the number of individuals in major breeding sites all across Maine's coastline. Although exact historical numbers are not known today it would be difficult to imagine catching enough horseshoe crabs to fill truckloads referred to in previous anecdotal accounts. Limitations on harvesting in 2001 and the eventual closure in 2002 prevented further depletion of remaining populations of *L. polyphemus*. In a long living species such as this that takes nine to 10 years to reach sexual maturity, it would seem that recovery of these populations to near historic values would take a considerable amount of time even with protection measures (Schaller and Hanson 2004).

The Maine DMR horseshoe crab surveys began in 2001 and have been conducted annually ever since. Surveys began at Damariscotta Mills or Day's Cove in the Damariscotta River in 2002. The purpose of these surveys is to establish quantitative baseline population data, and determine whether horseshoe crab populations are stable or declining. Sites were selected on the basis of a report by Born (1982), prepared for the Maine State Planning Office. Counts are conducted at sites from Casco Bay to Frenchman's Bay during the spring spawning season when horseshoe crabs become conspicuous in shallow water near the shore (Schaller et. al., 2005).

This study was sponsored by the Damariscotta River Association. The DRA is a nonprofit conservation land trust organization that strives to preserve and promote the natural, cultural, and historical heritage of the Damariscotta River and adjacent areas for the benefit of all. The DRA is an active participating group with the Maine DMR horseshoe crab surveys, although the Maine DMR no longer conducts these surveys. The DRA is one of the few organizations that conducts the surveys independently. The DRA specifically wanted to know whether horseshoe crab populations have changed, how environmental factors affect their populations, and if any additional protection measures need to be implemented to preserve these populations.

Therefore, the objective of this study was to compile horseshoe crab survey data collected on the Damariscotta River from 2001 through 2014 in order to evaluate the environmental correlates of distribution and abundance during the spawning season. We attempted to determine if the populations, sex ratios, spawning activity, and size of mating individuals have changed over time. We analyzed data collected from the same two sites in the Damariscotta River that have been monitored since 2002 (Damariscotta Mills, Day's Cove), as well as a new site (Lowes Cove) situated further down river.

Methods

Horseshoe crab surveys in the Damariscotta River began in 2002 at Damariscotta Mills and Day's Cove. An additional site, Lowes Cove was added in 2014 to assess the occurrence of crabs further down river (Figure 1). Starting in early May each year volunteers conducted daily surveys beginning approximately 30 min before high tide. At each site the survey was conducted along a fixed transect staked off along the shore.. Each survey transect was measured in 10-m segments spanning 100-m of the shoreline for a total of 10 segments. During the 2014 survey each location was remeasured and extended to 12-m segments spanning 144 m of the shoreline.. Also during the 2014 survey data were taken every three days instead of every day to minimize human disturbance to each location. Counts of horseshoe crabs were restricted to the immediate shoreline. The volunteers flipped a coin to determine the starting end of the survey. If the animal was within 1-m of the water's edge it was designated as being “IN” and if it was outside that boundary it was “OUT.” The Maine DMR only used horseshoe crabs designated is “IN” for their annual surveys. In the present analysis, however, we included horseshoe crab classified as both “IN” and “OUT” as our index of abundance. We also recorded whether males and females were coupled in amplexus. During the spawning season it is not uncommon for several males to form a train behind the female. Daily counts were averaged over the number of days surveyed to standardize horseshoe crab counts to numbers per 100-m transect per day.

Water temperatures were recorded at each site with a mercury thermometer; salinity measurements were taken using a refractometer. Horseshoe crab size was measured inconsistently between 2002 and 2014 (Table 1). During years when they were measured, all crabs were measured on the survey, except on occasions when they were extremely abundant. On those occasions the first individual encountered in each 10-m segment of the transect was measured. Carapace width was measured with a ruler placed on the ventral side across the widest part of the carapace.

We evaluated the relationship between environmental conditions and the number of horseshoe crabs in our counts in three ways. First, we graphically depicted the association between horseshoe crab counts and environmental conditions by plotting the frequency of above average horseshoe crab counts for the year over a range of environmental conditions. We examined the frequency distribution of above average daily counts across a range of temperature and salinity values, as well as across the lunar cycle. In this analysis, a daily count was considered above average if it exceeded the mean of all the daily counts for that year.

We also used linear least squares regression to quantify the correlation between seasonal average atmospheric temperature and the year's average daily crab counts. In this case, we acquired atmospheric temperature data from NASA's Giovanni database (NASA 2014). Data comprised monthly temperature averages from January 2001 to December 2014 for an area encompassing latitude 44.1 - 43.9 N and longitude 69.5 - 69.6 W, centered on the upper Damariscotta River. We examined correlations of horseshoe crab counts with mean atmospheric temperatures for winter (Jan – Mar) prior to and during the spring (Apr-Jun) concurrent with the surveys. Yearly anomalies for both temperature and crab counts were calculated by subtracting yearly values from multiyear average between 2002 and 2014. Similarly, we used a regression approach to assess the correlation between the seasonal average water temperature and salinity measured during the surveys with the year's average of the daily crab counts.

Table 1. Extent of survey effort at the three study sites between 2002-2014. (n = number of days counts were done; NS: No Survey)

	Damariscotta Mills	Day's Cove	Lowes Cove
2002	Count and Sizes (*n* = 31)	Count and Sizes (*n* = 11)	NS
2003	Count (*n* = 9)	Count (*n* = 17)	NS
2004	Count (*n* = 14)	Count (*n* = 17)	NS
2005	NS	NS	NS
2006	Count and Sizes (*n* = 13)	Count and Sizes (*n* = 20)	NS
2007	Count (*n* = 17)	NS	NS
2008	Count and Sizes (*n* = 27)	NS	NS
2009	NS	NS	NS
2010	Count (*n* = 36)	NS	NS
2011	Count (*n* = 30)	NS	NS
2012	NS	NS	NS
2013	NS	NS	NS
2014	Count and Sizes (*n* = 10)	Count and Sizes (*n* = 11)	Count and Sizes (*n* = 11)

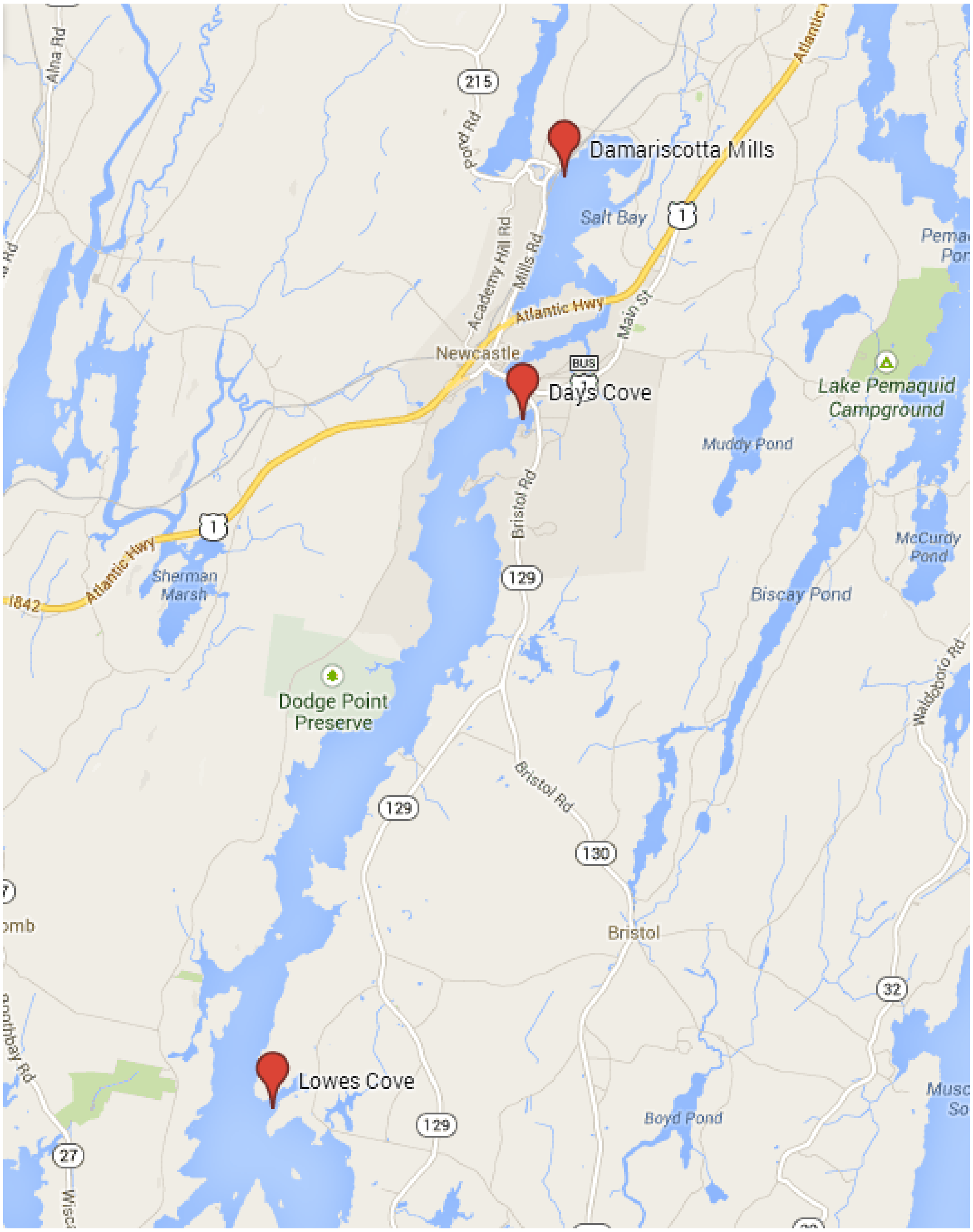

Figure 1. Location of horseshoe crab survey sites in the Damariscotta River estuary. Damariscotta Mills (44.061106, -69.522373), Day's Cove (44.027698, -69.530012), and Lowes Cove (43.933862, -69.577435).

Results

Counts of *L polyphemus* at Days Cove and Damariscotta Mills varied from a few to a few hundred over the time series (Fig. 2 A - B). No horseshoe crabs were found at Lowes Cove in 2014 and the site was therefore excluded from further analysis. Yearly composite figures of the raw data for each location can be found in the appendix.

At both Days Cove and Damariscotta Mills male horseshoe crabs outnumbered females by a ratio of about four to one. Males comprised 82% of the population at the Mills and 68% at Days Cove (Fig. 2 C - D). Notably, all females recorded were found in amplexus (Fig. 2 E – F). The number of males coupled with a female ranged from one to as many as 10, but most frequently there were one or two. At Damariscotta Mills the number of males in amplexus averaged 1.5 (SD = 1.1), and at Day's Cove, 1.2 (SD = 0.4) (Fig. 2 E - F).

The size of both males and females remained relatively constant at both sites. Over the time series, horseshoe crab carapace width at Damariscotta Mills averaged 147 mm (SD = 12 mm) for males and 184 mm (SD = 13 mm) for females; and at Day's Cove males averaged 149 mm (SD = 11 mm) and females 180 mm (SD = 18 mm) (Fig. 2 G - H).

At Damariscotta Mills and Days Cove above average horseshoe crab counts occurred most frequently when temperature was approximately 21°C, and salinity 21-24 ppt (Fig. 3 A - D). Above average counts were not associated with any particular lunar phase (Fig. 3 E - F).

Horseshoe crab counts were positively related to atmospheric temperature anomalies at both sites and for both the preceding winter and spring temperature anomalies (Fig. 4 A - D). However, only the winter temperature anomalies produced a statistically significant correlation at both sites ($p < 0.05$; Fig. 4 A - B).

Horseshoe crab counts were generally positively related to water temperature anomalies and negatively related to salinity anomalies at both sites, but none of these relationships was statistically significant (Fig. 5 A – D).

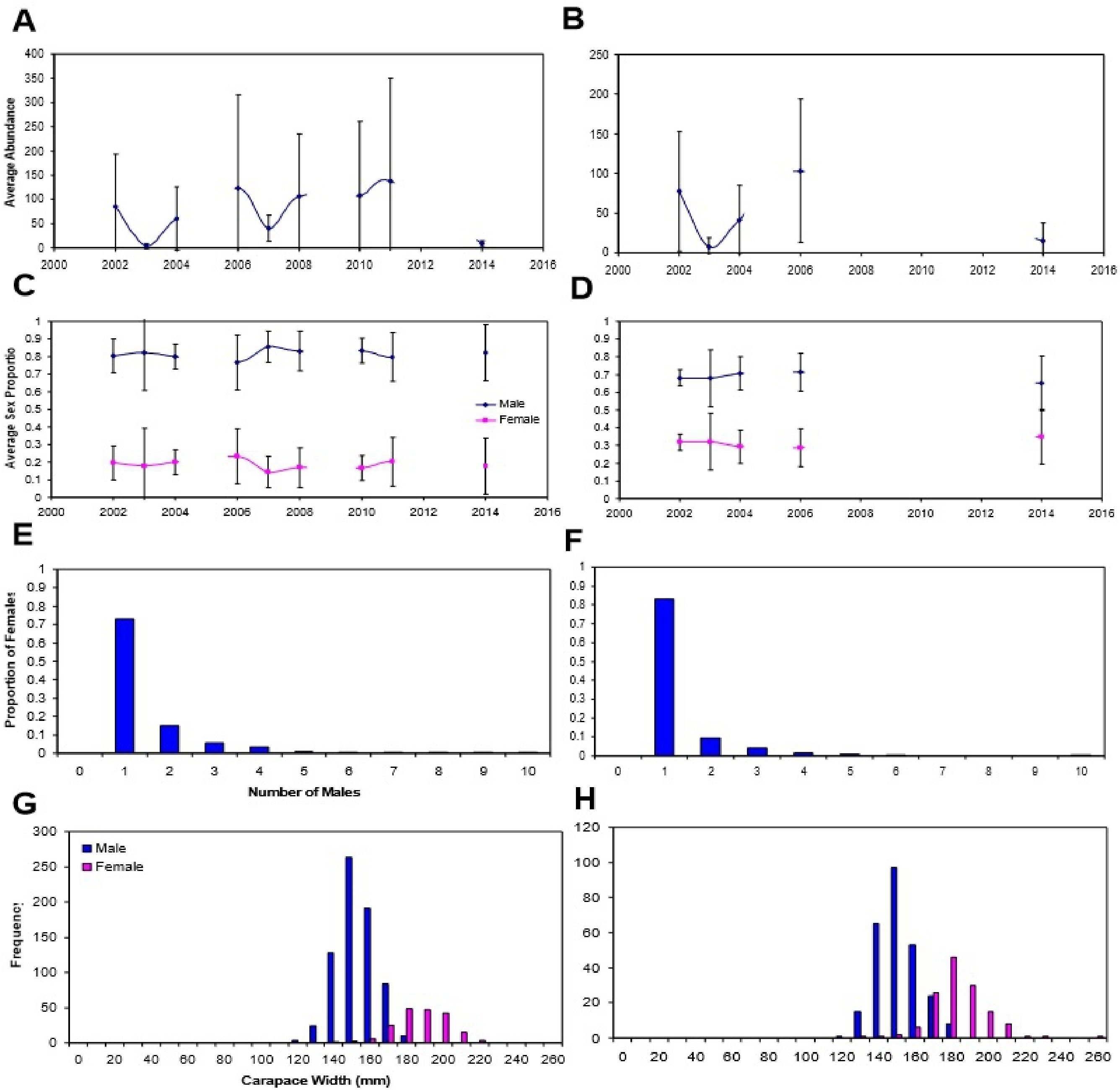

Figure 2. Raw data from Damariscotta Mills (Left) and Day's Cove (Right); 2002-2014. A and B: Average daily number of horseshoe crabs per 100-m transect, error bars are standard deviation. C and D: Average sex proportion, error bars are standard deviation. E and F: Proportional frequency of females with the number of attached males. G and H: Horseshoe crab size composition for each sex.

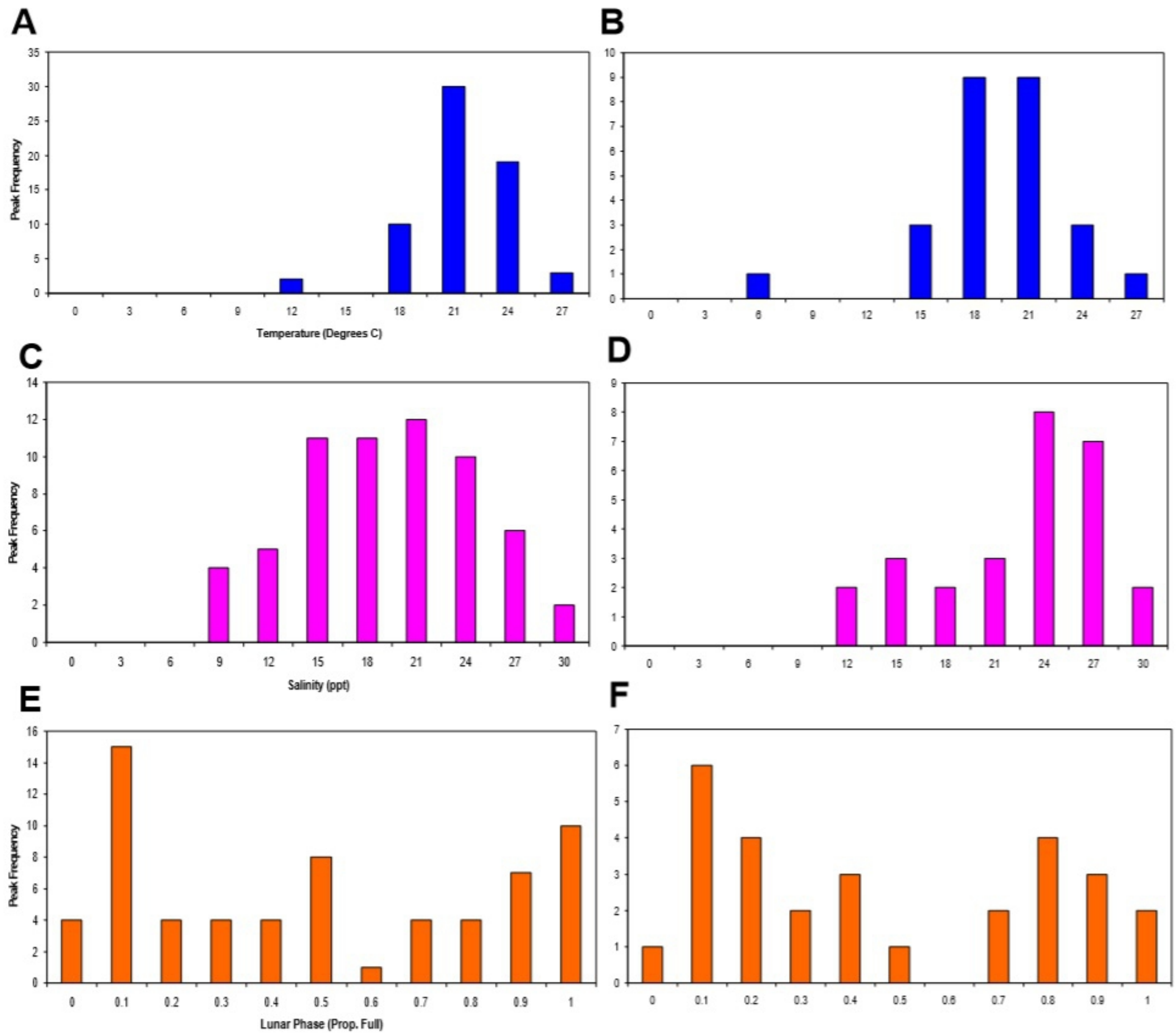

Figure 3. Frequency distributions of above average horseshoe crab counts at Damariscotta Mills (Left) and Day's Cove (Right) plotted against ranges of temperature (A – B), salinity (C-D), and lunar phase (E – F). For lunar phase, 1 = full moon, 0 = new moon

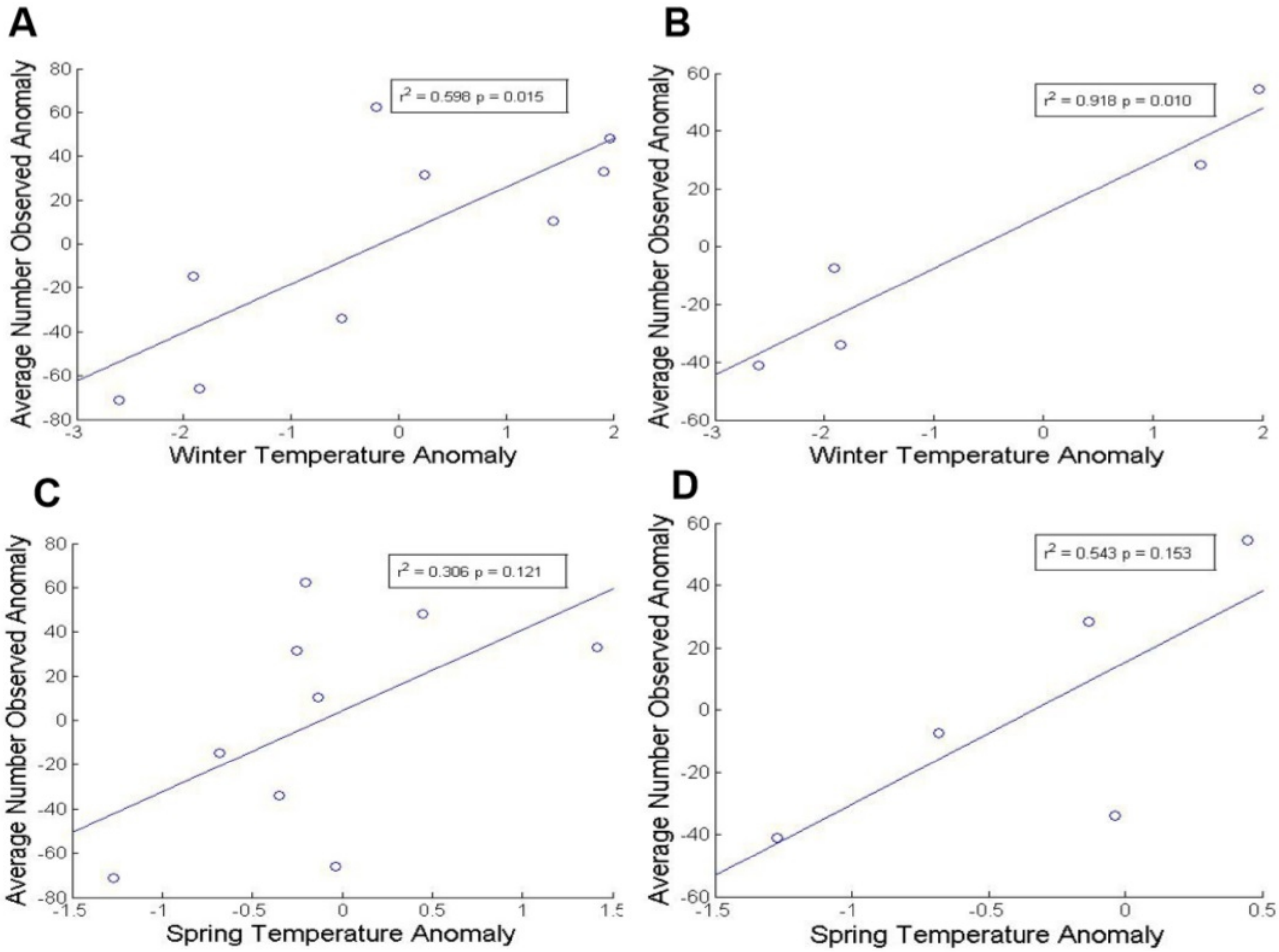

Figure 4. Correlation between variability in yearly mean horseshoe crab counts, and seasonal atmospheric temperature anomalies for Damariscotta Mills (left) and Day's Cove (right) for winter (January-March; A and B): and spring (April-June; C and D) from 2002-2014.

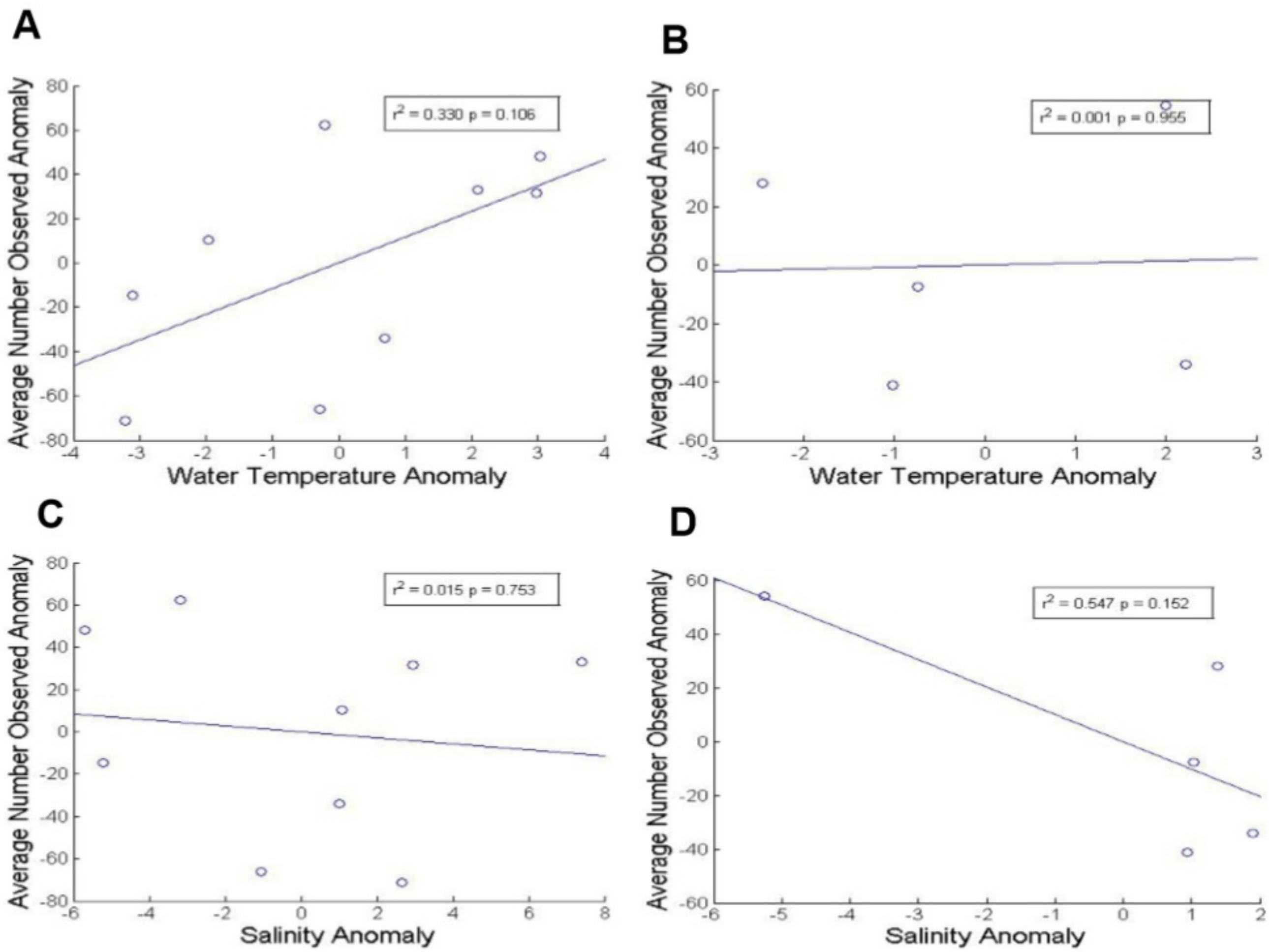

Figure 5. Correlation between variability in yearly mean horseshoe crab counts and seasonal atmospheric temperature anomalies for Damariscotta Mills (left) and Day's Cove (right) for winter (January-March; A and B): and spring (April-June; C and D) from 2002-2014.

Discussion

One of the major goals of this analysis was to quantify spatial and temporal patterns in the horseshoe crab population at Damariscotta Mills and Day's Cove, and to assess environmental correlates of temporal variability in the horseshoe crab counts at these sites. Our results suggest that horseshoe crab populations in the upper Damariscotta River have varied without trend since 2002, notwithstanding gaps in the time series.

One of our most notable observations was that 100 percent of the females observed were in amplexus and that there was consistently an excess of males available during the spawning season. This suggests strong competition among males for mates and that sperm limitation is not likely to be a concern in this population. During the spawning season unattached males will try to displace an attached male (Schaller and Thayer, 2002). The number of males attached to a female can be an indication of the level of competition among males for females. An increase or decrease in this level of competition could be indicative of changing population dynamics or species survivorship. Stable abundance, size distribution and sex ratios over the time series may be taken as a sign that recruitment and mortality rates are at a relative equilibrium. Declines in any of these indicators might be reason for concern (Stearns and Koella 1986). Maine DMR's protection measures for horseshoe crabs were fully implemented by 2002 so any changes in survey counts are unlikely to be attributed to harvesting, leaving the environment as the most likely driver of change. Furthermore, we caution that horseshoe crab counts are likely to be strongly influenced by environmental conditions.

Our results indicate that temperature is a strong correlate of variability in horseshoe crab counts at our study sites during the spawning season. Year-to-year differences in horseshoe crab counts were more strongly correlated with seasonal average atmospheric temperature than with the average water temperature measured during the spawning season. Crabs were most abundant during years with the warmest atmospheric temperature, especially during the preceding winter. Surprisingly, atmospheric temperatures of the preceding winter were a better predictor of variability in the occurrence of horseshoe crabs at our study sites. If the frequency distribution of above average crab counts across a range of observed water temperatures is any indication, the optimum water temperature may be estimated to be approximately 21°C. Much cooler temperatures may inhibit movement, and much higher temperatures may be physiologically stressful and force horseshoe crabs into deeper water and away from detection. It is therefore important to recognize that counts taken at the edge of the shore, as has been done in this study, may be less a reflection of abundance than of activity and movements.

Our results also indicate that salinity variability may also affect the horseshoe crab counts at our study sites during the spawning season. Our results suggest the optimum salinity may be estimated to be between 21 and 24 ppt. However, yearly variability in average horseshoe crab counts was not linearly correlated with salinity measured during the surveys. Our results are consistent with those of Schaller et. al. (2005) who demonstrated that periods of dry weather coincided with increases of horseshoe crab abundance while periods of wet weather coincided with decreases in abundance. Salinity fluctuations in this case may be a proxy for variable precipitation.

This study does not suggest a strong role of lunar periodicity in the variability of horseshoe crab counts at our sites, although studies conducted further south suggest that lunar phase is the greatest predictor of peak spawning activity. Schaller et. al. (2005) claimed that lunar phase is more useful in predicting the seasonal onset of spawning, than of peak spawning activity. Barlow et. al. (1984) reported that larger masses of horseshoe crabs migrated ashore during the new and full moon. In our analysis we found that peak spawning aggregations did not localize around the new and full moon.

Given these results, we speculate that temperature may be a more important determinant of spawning activity near the northern edged of the geographic range of *L. polyphemus* than it is in the south. In a previous report of horseshoe crab surveys conducted in Maine, Schaller et. al. (2005) observed that high spawning activity corresponded to periods of warm weather while decreases in spawning activity corresponded to periods of wet weather. They hypothesized that temperature could be important to horseshoe crab spawning activity. Our results are consistent with Schaller et. al. (2005). The spawning season for *L. polyphemus* is during May and June, which in Maine has highly fluctuating weather conditions ranging from well below freezing with heavy snow and rain to sunny and warm. Since horseshoe crab spawning activity is greatly affected by temperature and salinity fluctuations, we can claim that these two factors are of greater importance than lunar phase at these two locations. Although lunar phase and tide height are biologically important to the synchronization of spawning activity in southern parts of the species' range studies of *L. polyphemus* at the northern extent of its range suggest spawning activity is more strongly linked to favorable temperature conditions.

The horseshoe crab is an important member of the Damariscotta River's ecological community and understanding how it reacts to the environment is vital to understanding how this species responds to environmental stimuli. We have determined that temperature and salinity may be of greater importance than lunar phase to horseshoe crab activity, and will likely influence the numbers counted in shoreline surveys in May and June. Thus counts taken at the water's edge may be more indicative of horseshoe crab activity levels than their abundance. All females were in amplexus with males. This suggests that the females in the populations at these two sites are not limited by opportunities to mate, but there is likely a high level of competition among males for females. At both sites males outnumbered females by a ratio of about four to one. Females tend to have a carapace width 30 mm larger than males. Additional research on the behavioral changes of horseshoe crabs in response to fluctuating temperature and salinity would be beneficial in understanding how and why horseshoe crab abundances fluctuate dramatically from year to year. The only published research on horseshoe crab abundance and environmental variables are the yearly horseshoe crab reports conducted by the Maine DMR. Their analysis however does not include a correlation analysis between horseshoe crab abundance and environmental factors. We recommend that the DRA continue the monitoring of horseshoe crabs. However, we suggest that transect length should be reduced back to 100-m and that surveys should be conducted at least every other day.

Acknowledgments

Funding for this study was provided by the Damariscotta River Association. The University of Maine's School of Marine Sciences and the Darling Marine Center in Walpole, ME provided the facilities and knowledgeable staff that contributed this data analysis. Peter Thayer of the Maine Department of Marine Resources provided the historic data set without which this study would not be possible. Andrew Thomas at UMaine's School of Marine Sciences assisted with the analysis of NASA atmospheric temperature data. Lastly we thank all the DRA volunteers who put in the time and effort to monitor this species from 2002 to 2014. Without the tremendous effort from all of these individuals there would be little known about horseshoe crabs in the Damariscotta River.

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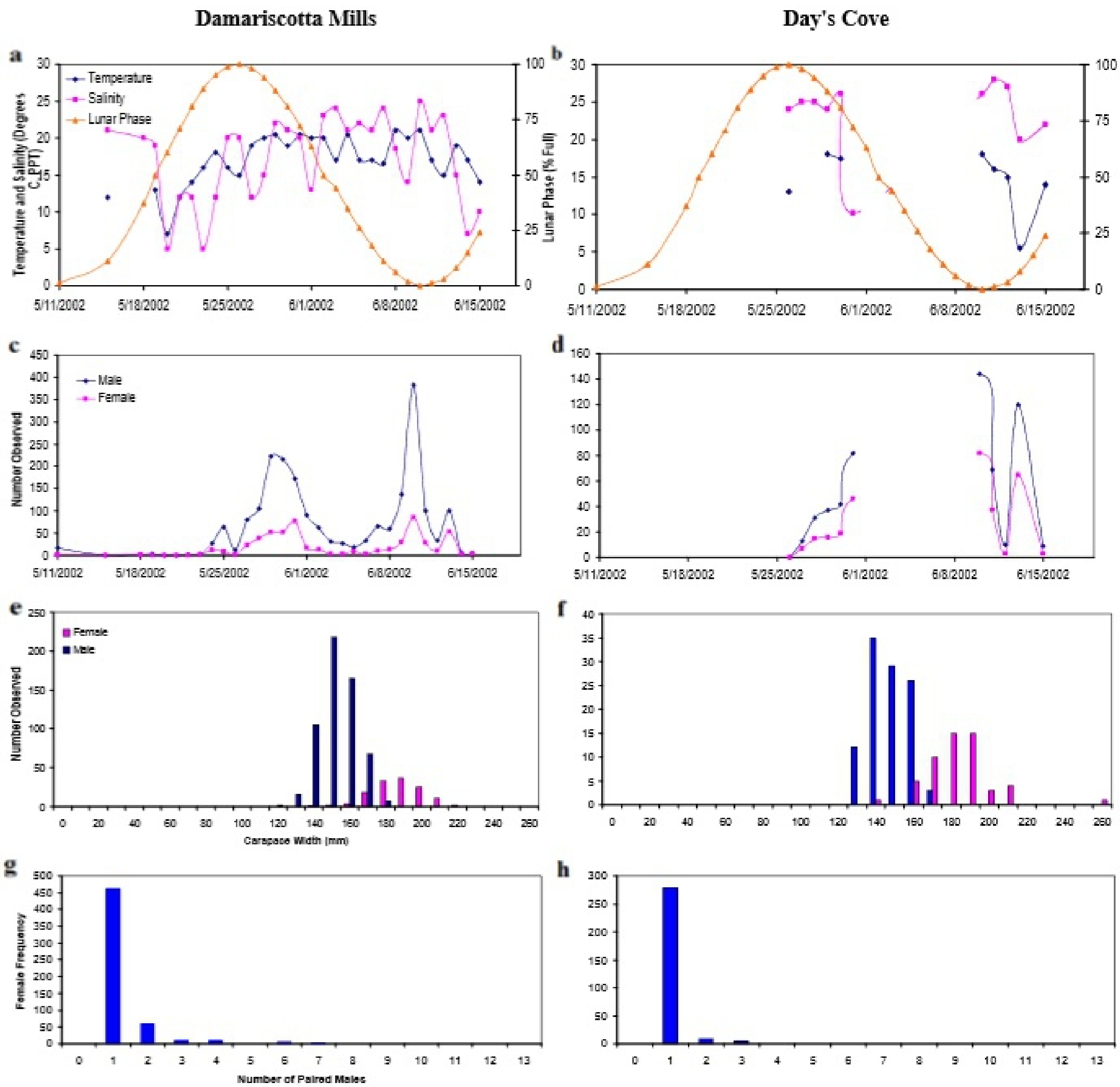
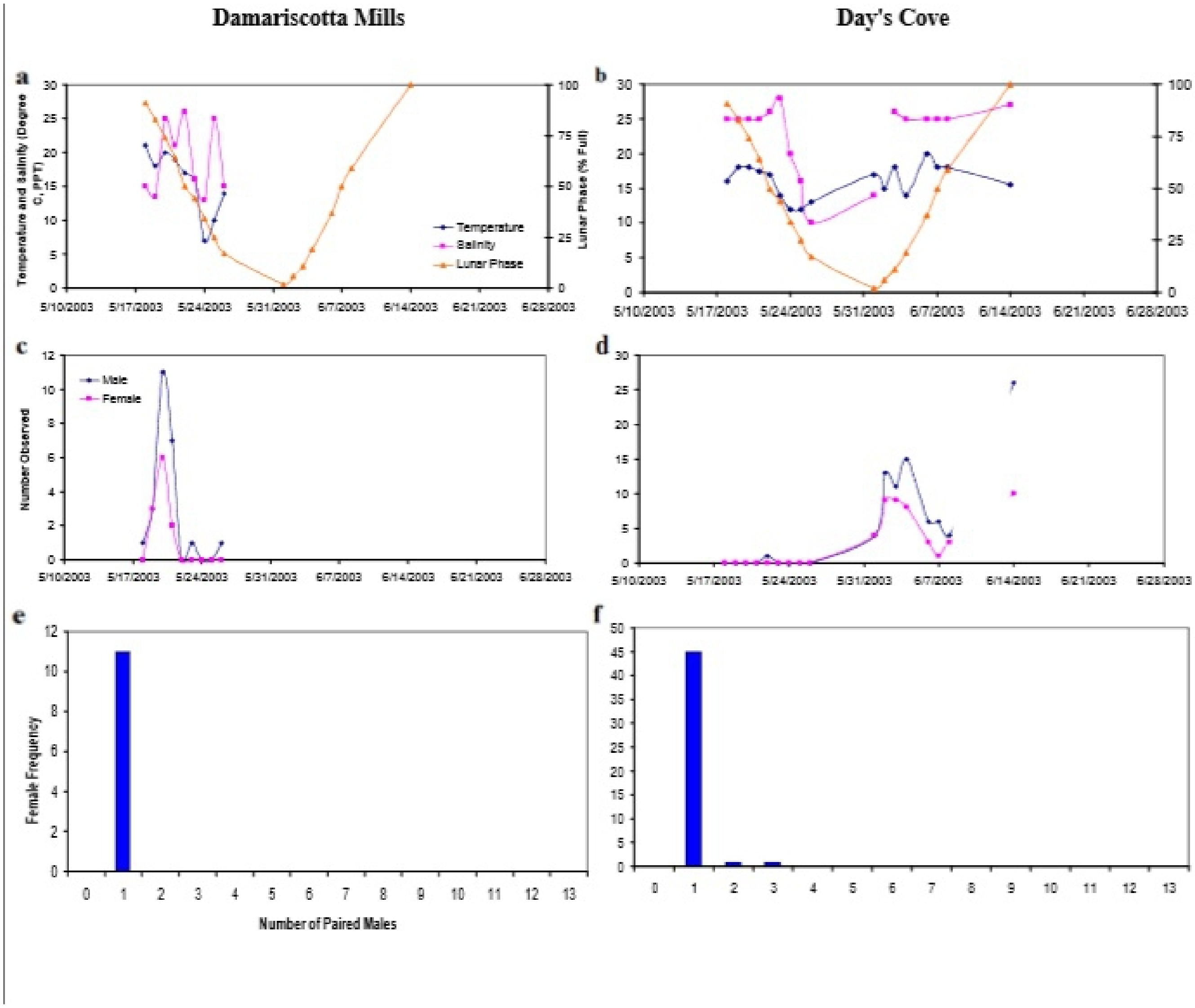
Appendix

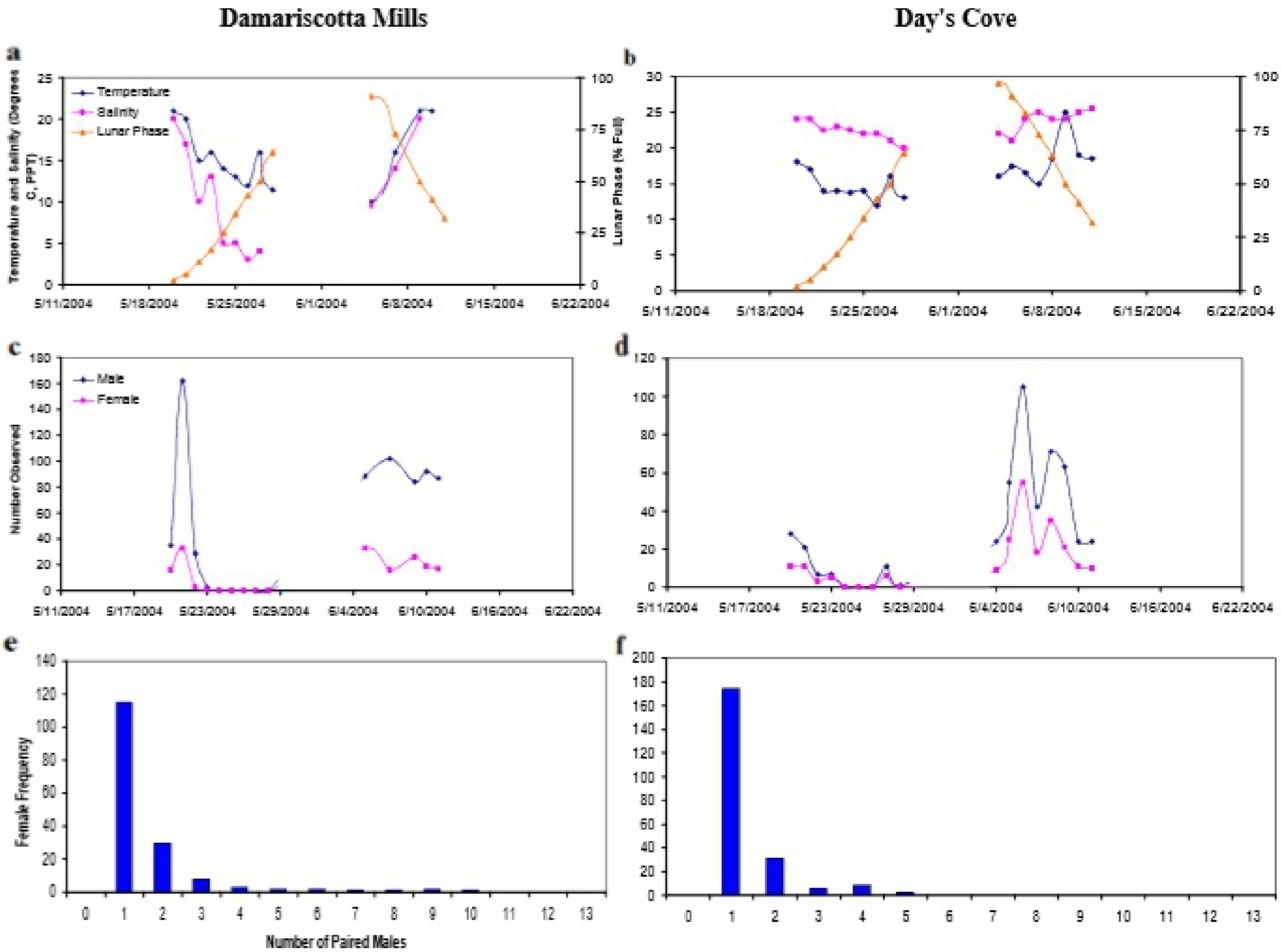
Damariscotta River horseshoe crab survey summary by year from 2002 – 2014.



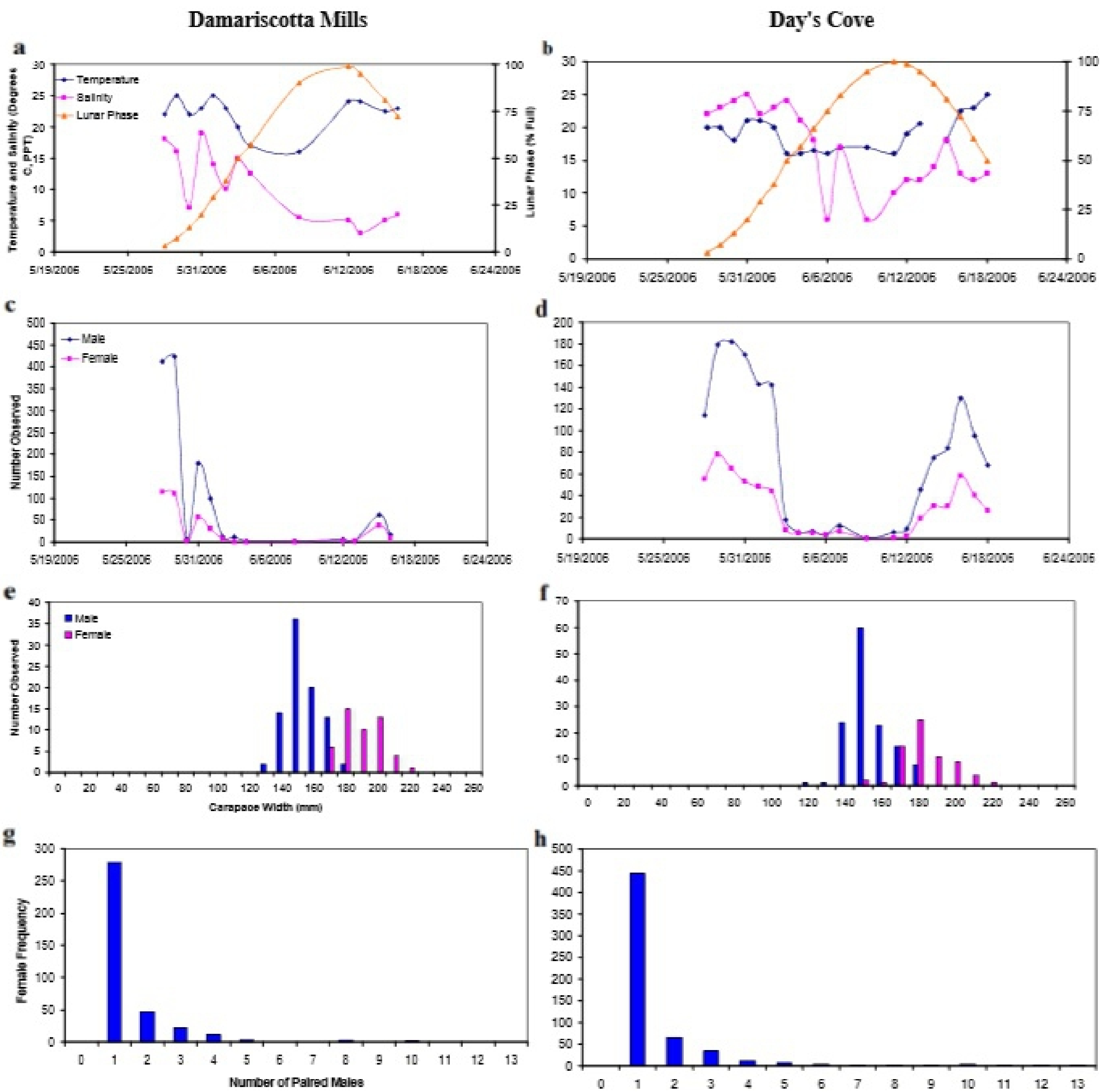
Appendix Figure 1. Horseshoe crab survey data for Damariscotta Mills and Day's Cove; 2002. A and B: Temperature, Salinity, and Lunar Phase variation during survey. C and D: Horseshoe crab abundance. E and F: Size frequency distribution for each sex. G and H: Frequency distribution for females paired with *n* males.



Appendix Figure 2. Horseshoe crab survey data for Damariscotta Mills and Day's Cove; 2003. A and B: Temperature, Salinity, and Lunar Phase variation during survey. C and D: Horseshoe crab abundance. E and F: Frequency distribution for females paired with *n* males.



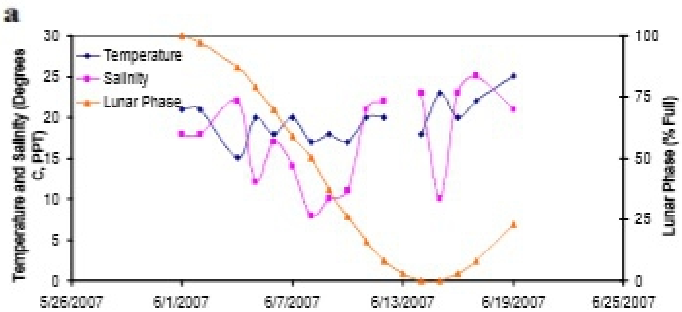
Appendix Figure 3. Horseshoe crab survey data for Damariscotta Mills and Day's Cove; 2004. A and B: Temperature, Salinity, and Lunar Phase variation during survey. C and D: Horseshoe crab abundance. E and F: Frequency distribution for females paired with *n* males.



Appendix Figure 4. Horseshoe crab survey data for Damariscotta Mills and Day's Cove; 2006. A and B: Temperature, Salinity, and Lunar Phase variation during survey. C and D: Horseshoe crab abundance. E and F: Size frequency distribution for each sex. G and H: Frequency distribution for females paired with *n* males.

Damariscotta Mills

Day's Cove



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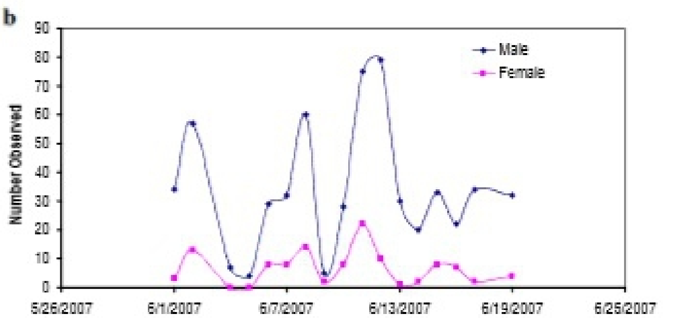

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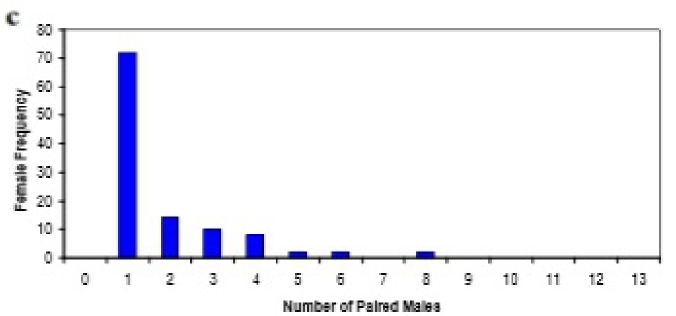

No
Data
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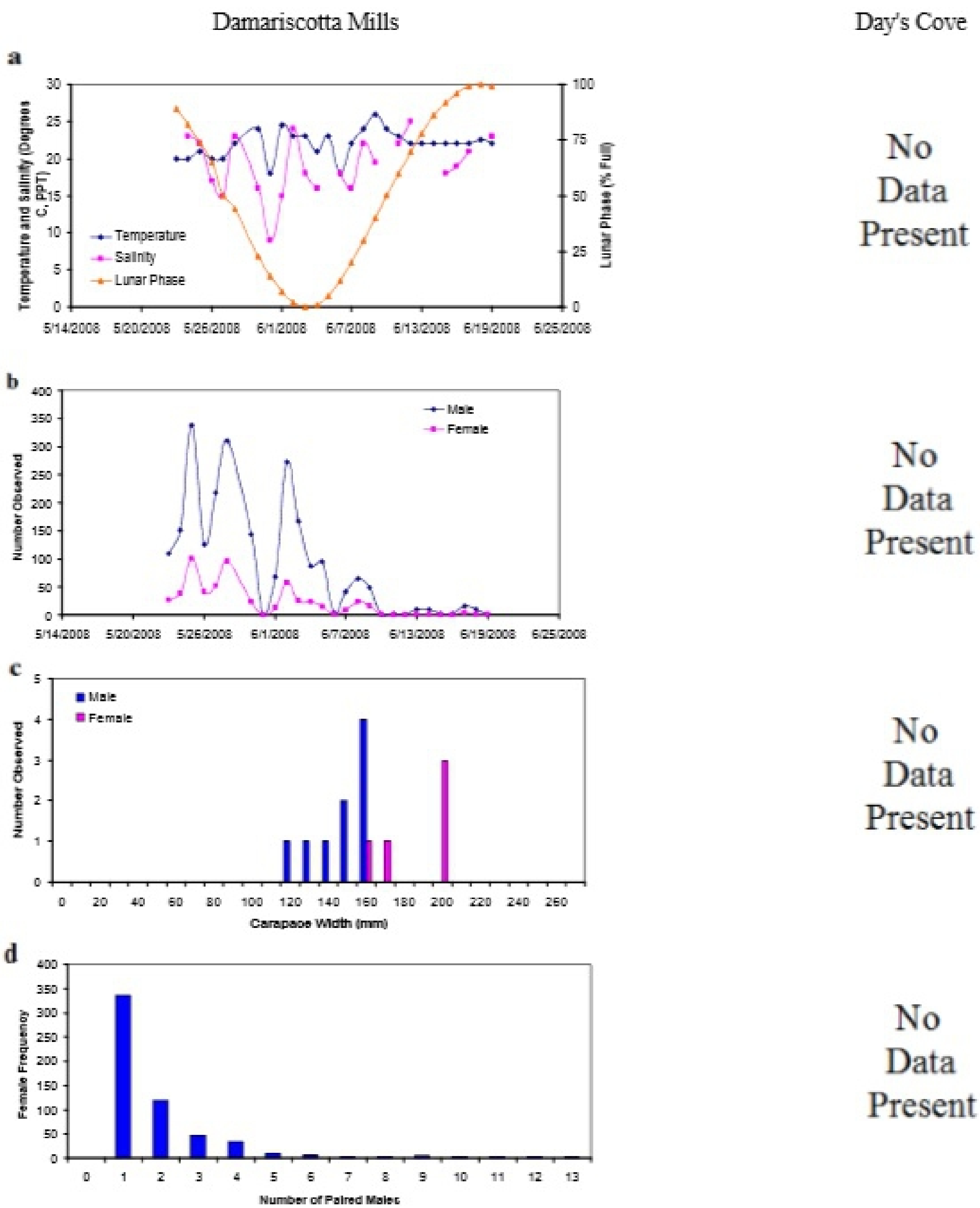
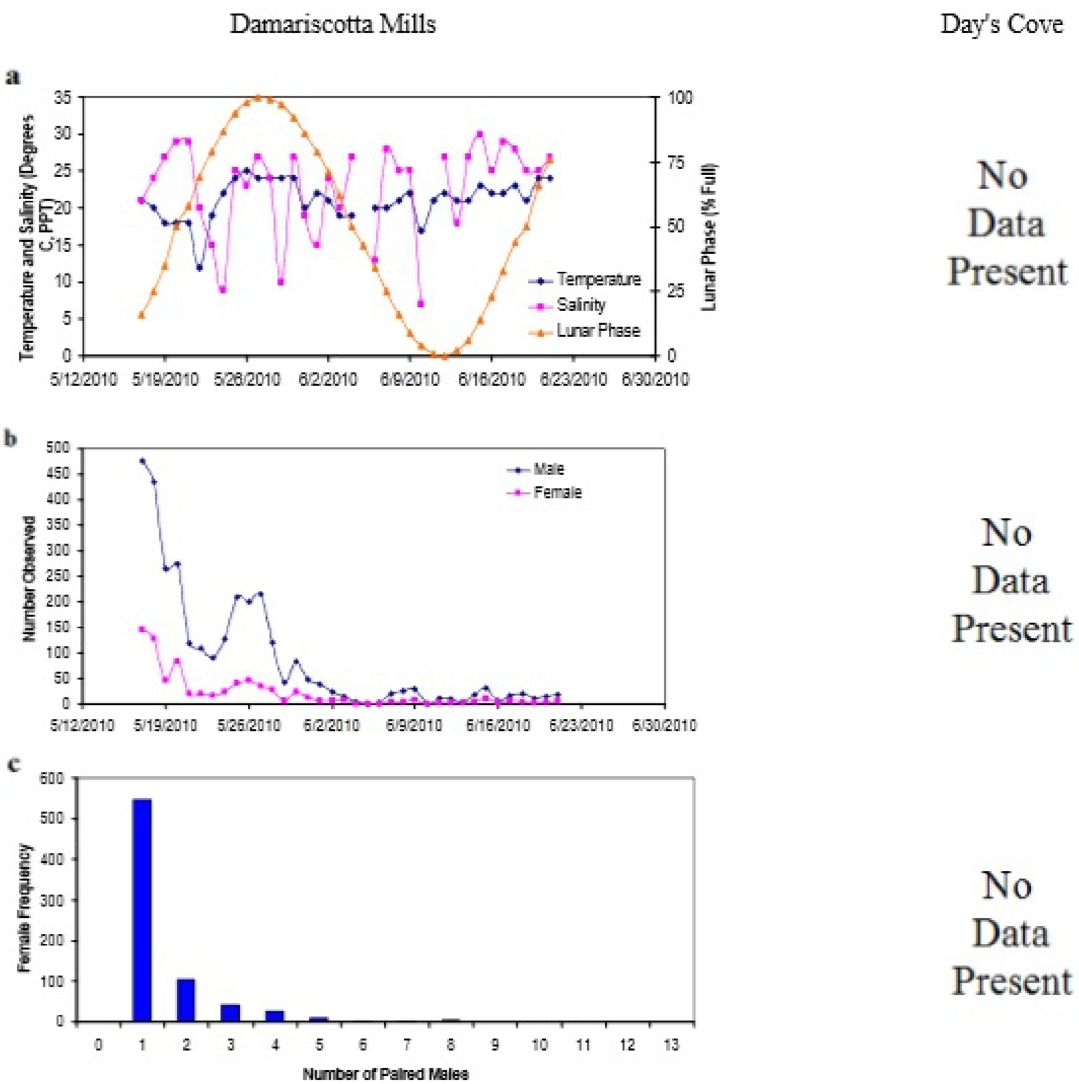
Appendix Figure 5. Horseshoe crab survey data for Damariscotta Mills and Day's Cove; 2007. A: Temperature, Salinity, and Lunar Phase variation during survey. B: Horseshoe crab abundance. C: Frequency distribution for females paired with *n* males.

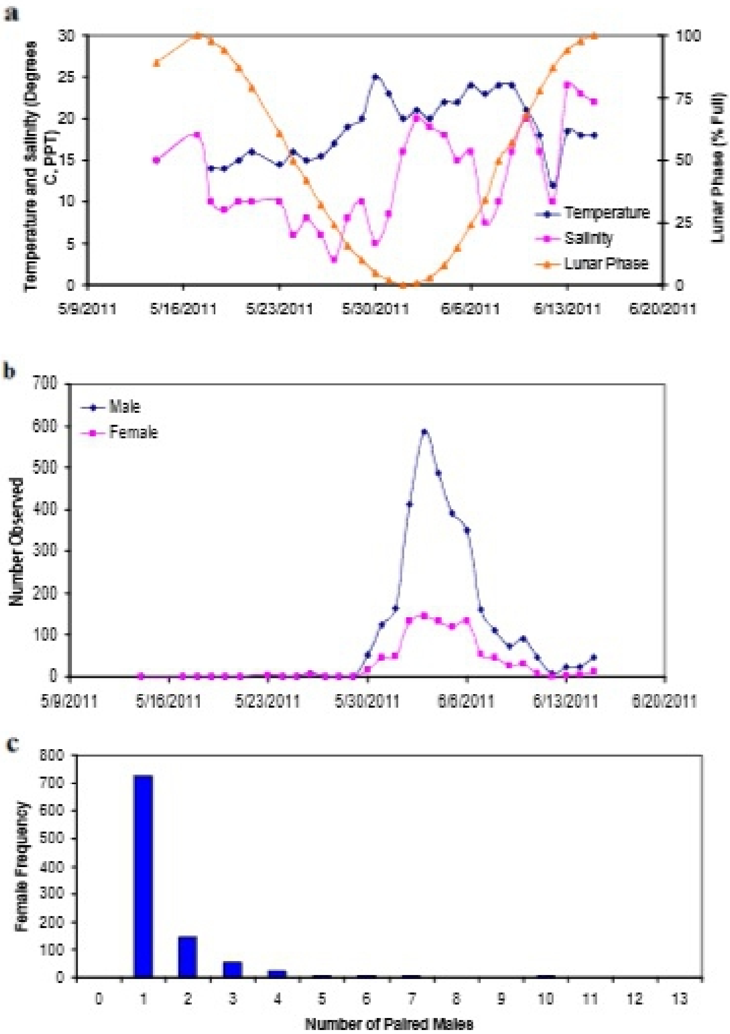


Appendix Figure 6. Horseshoe crab survey data for Damariscotta Mills and Day's Cove; 2008. A: Temperature, Salinity, and Lunar Phase variation during survey. B: Horseshoe crab abundance. C: Size frequency distribution for each sex. D: Frequency distribution for females paired with *n* males.



Appendix Figure 7. Horseshoe crab survey data for Damariscotta Mills and Day's Cove; 2010. A: Temperature, Salinity, and Lunar Phase variation during survey. B: Horseshoe crab abundance. C: Frequency distribution for females paired with *n* males.

Damariscotta Mills



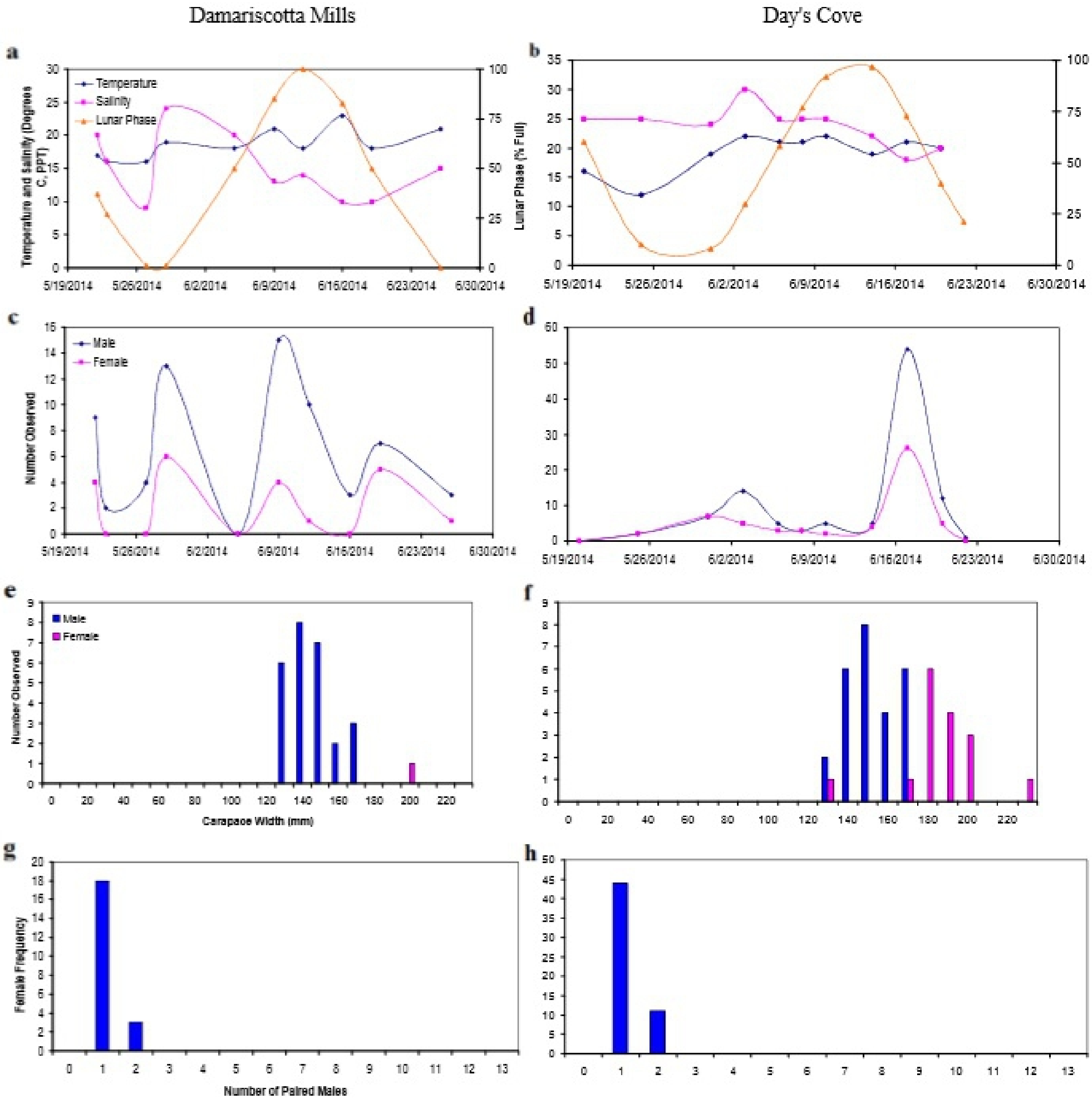
Day's Cove

No Data Present

No Data Present

No Data Present

Appendix Figure 8. Horseshoe crab survey data for Damariscotta Mills and Day's Cove; 2011. A: Temperature, Salinity, and Lunar Phase variation during survey. B: Horseshoe crab abundance. C: Frequency distribution for females paired with *n* males.



Appendix Figure 9. Horseshoe crab survey data for Damariscotta Mills and Day's Cove; 2014. A and B: Temperature, Salinity, and Lunar Phase variation during survey. C and D: Horseshoe crab abundance. E and F: Size frequency distribution for each sex. G and H: Frequency distribution for females paired with *n* males.