Fall 11-2018

Disappearance of Phragmites in Louisiana and Nipponaclerda biwakoensis

Rachel Tugger

Disappearance of *Phragmites* in Louisiana and *Nipponaclerda biwakoensis*

by

Rachel Tuggle

Undergraduate honors thesis under the direction of

Dr. Linda Hooper-Bui

Department of Environmental Science

Submitted to the LSU Roger Hadfield Ogden Honors College in partial fulfillment of the Upper Division Honors Program.

November, 2018

Louisiana State University
& Agricultural and Mechanical College
Baton Rouge, Louisiana

Disappearance of *Phragmites* in Louisiana and *Nipponaclerda biwakoensis*

Rachel Tuggle

1. Background

Phragmites australis, commonly referred to as Roseau or common cane, is found throughout the world's temperate and tropical wetlands. Many regions see the plant as a pest because of its ability to outcompete other native wetland species and create a monoculture reed bed. *Phragmites* even secretes phytotoxins, such as gallic acid, that may harm native species trying to grow under it (Rudrappa, 2009). Unlike other areas, in Louisiana and the Gulf of Mexico, *Phragmites* is considered critical to combating the erosion of coastal wetlands.

Phragmites is fairly hardy, and it can withstand salinity up to 20 ppt and standing water up to one meter deep. It can also have extensive rhizome systems, allowing it to hold onto sediment. In regions where *Phragmites* is closely managed, like some parts of China for paper production, regular salt water tidal inundation is one of the only ways to effectively control the cane's growth (Brix et al, 2014). Despite this, there has been a rapid die-off of the plant since 2015 in Louisiana. This is considered unfavorable due to *Phragmites*' soil accretion abilities slowing the erosion of the coast and its ability to keep shipping channels intact (Ramsey, 2017).

Along with recent disappearance of the cane, the presence of an invasive Heteropteran known as Roseau cane scale (*Nipponaclerda biwakoensis*) and its invasive parasitic wasps (3) have been noted to reside in the fringes of *Phragmites* stands (Blossey, 2018). There is a native species of scale in Louisiana, but the invasive insect appears to be more populous. Despite this, the parasitic wasp that uses the scale for oviposition has been known to help control the population. The adult scale are immobile tan or brown ovals, usually in a cluster at a node underneath *Phragmites*' leaf sheaths. They tend to live, not just clustered at nodes, but together at certain positions on the plant. Juveniles (referred to as crawlers) have legs, unlike their adult forms, and they are a very light color, almost translucent. During the summer and fall months, the scale can infest the Roseau cane, and it is thought that they may contribute to the rapid decline in *Phragmites* (Zengel et al, 2018).

2. Methods

2.1 Field Sampling

Plaquemines, St. Tammany, and Cameron parishes were visited within a one month period to collect samples of *Phragmites*. In each parish, three individual populations were sampled. This was done by laying out a one meter transect at the base of each *Phragmites* stand visited. A photo was then taken of the transect to be analyzed later for vertical area coverage and also percentage of green and brown plant material. The photo had to contain the

transect measure at the base and the top of the Roseau cane stems. Ten numbers from one to one hundred centimeters were randomly generated, and the stems directly in front of those numbers on the transect were cut from the base and piece-wise to fit in labeled bags for transport back to the lab. At each site, air temperature, water temperature, dissolved oxygen (DO), salinity, conductivity, wind speed, date, and time were all recorded in field notebooks along with the stem numbers.

2.2 Lab Processing

Once back in the lab, stems were processed one by one in the order that they were collected. Because bagging required the stems to be cut piecewise, all pieces of one stem were removed from the bag together. All pieces then had their leaf sheaths peeled off as scale live under them. If some pieces had scale but others did not, those with scale were separated from those without. From the pieces with scale on them, one was randomly selected. Of the one selected, a random node was selected on the piece and cut at the node into a fifteen centimeter section.

The fifteen centimeter section was examined under microscope to count the number of adult scale and then the number range of crawlers. Range categories for crawlers were used because crawlers can be numerous and difficult to get an exact count. The number ranges for crawlers were: 0, 1-10, 11-50, 51-100, and 100+. In a laboratory notebook, *Phragmites* stand, stem number, number of scale, crawler range, plant age, and position of scale on the stem were recorded. Plant age was estimated and categorized by juvenile, adult, and dead/old categories. Position of scale was estimated by lower, middle, and upper sections of the plant and where the scale resided.

Stems that seemingly had no scale presence at all also had a randomly selected piece and then a random fifteen centimeter section. The section was still examined under the microscope to verify that no scale or crawlers were actually present. The same data was recorded for these stems with the designation of N/A under position of scale.

2.3 Image Processing

The photos that were taken at each stand were loaded into Photoshop to be analyzed. Each photo was scaled to a 1m x 1m square by using the measure tool to see how many pixels were in an 2.54 cm (1 inch) on the transect measuring tape in the picture. That number of pixels/inch was converted to be pixels/meter. The number was then used to crop the photo to one meter high starting at the base of the *Phragmites* stand, and across starting from the end of the tape measure. If there was vegetation present or any other components

in the picture that were not *Phragmites*, the approximate areas of those components were blacked out by using the lasso and fill tool.

Then we applied the threshold function to the photo. This function converts the photo to black and white only, no grays. The threshold level was set to 38/255 pixels or about fifteen percent. This allows for areas that may include dark portions of cane to be converted to white by giving a margin of error, and that only 15% darkness and below will be converted to black. This is important due to that we want as much cane in the very front of the stand accounted for but not those in the far back. This is because scale live on fringes, and not far into the stand.

The black and white photo creates a luminosity histogram with only two signals, black and white. The white signal was selected, and the number of pixels that were white were given as well as how many pixels were present in the whole photo. This was used to calculate area coverage percentage, i.e. white pixels divided by total pixels times one hundred. The area coverage percentage was then recorded.

To find the percentage of the green and brown pixels of the area was done by taking the same cropped photo and analyzed with the color range selection tool. A range of colors were sampled of the photo with 10 percent color fuzziness until it represented the majority of the brown colors within the *Phragmites* stand. Once the colors were selected, the "Layer by Copy" function was used. This created a new layer with only the pixels that contained the selected color range. The histogram function was then used to see how many pixels were in that layer. The same was repeated for the green colors in the photo.

2.4 Statistical Analysis

All information was typed into Excel for statistical analysis. For area coverage versus number of scale, a regression was done with the area coverage of each *Phragmites* stand and the number of scale summed for the entirety of that *Phragmites* stand. The same was done for dissolved oxygen versus number of scale and salinity versus number of scale. To do a regression of wind speed versus crawler ranges, the crawler ranges were coded 1-5 with 1 being the category 0 and 5 being the category 100+. The coded crawler average for each *Phragmites* stand was then calculated and run through a regression with wind speed. Plant age and the number of scale was run through a chi-square. The brown and green pixel percentages were calculated using the pixels of the color range layer divided by the total pixels in the cropped image. A paired t-test was run between the percent of brown and green pixels, as well as a regression between the percent of brown coloration and number of scale.

3. Results and discussion

3.1 Area Coverage

There appears to be no overall trend or relation between plant area and the number of scale (Fig.1). The p-value is not significant. This graph was run both with and without the farthest right point due to odd circumstances surrounding that *Phragmites* stand. It was intentionally planted and maintained to be thinner near a properties in Plaquemines. There was no significance in either run.

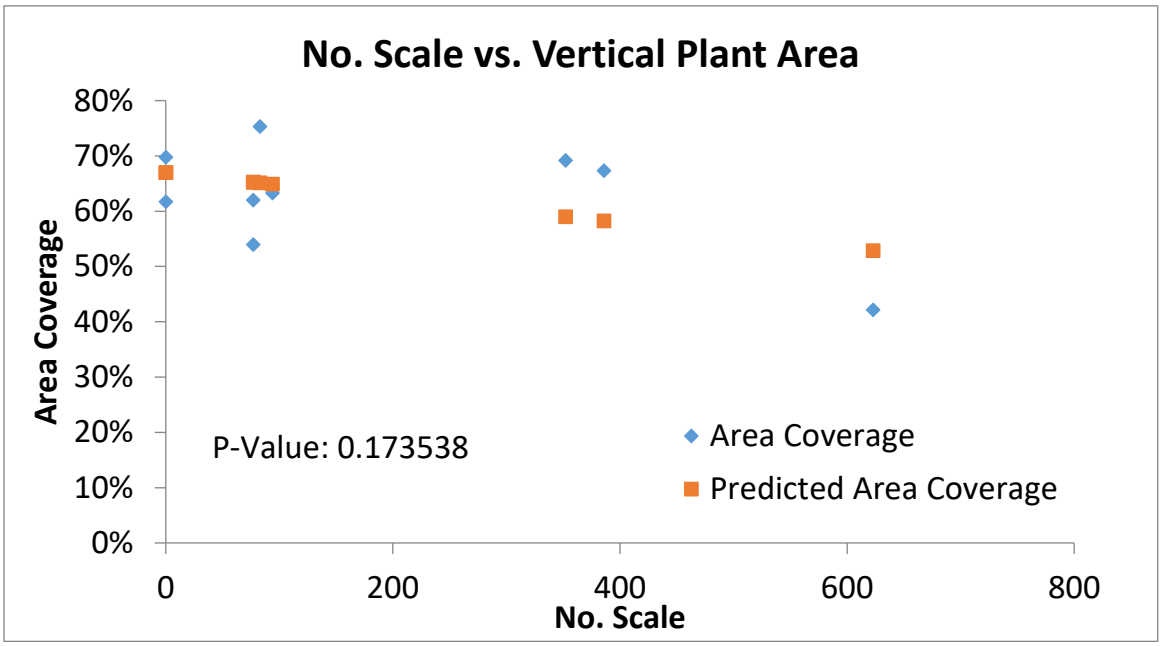

Fig. 1. Regression between the number of scale and the percent of vertical area coverage. The number of scale were compiled per *Phragmites* stand (10 stems per stand) and then run against the percentage of vertical area coverage of a 1m x 1m area of the reed stand.

3.2 Salinity

There also appears to be no relationship, trend-wise or significance-wise, between salinity and the number of scale in a *Phragmites* stand (Fig. 2). The p-value is particularly high (0.820475).

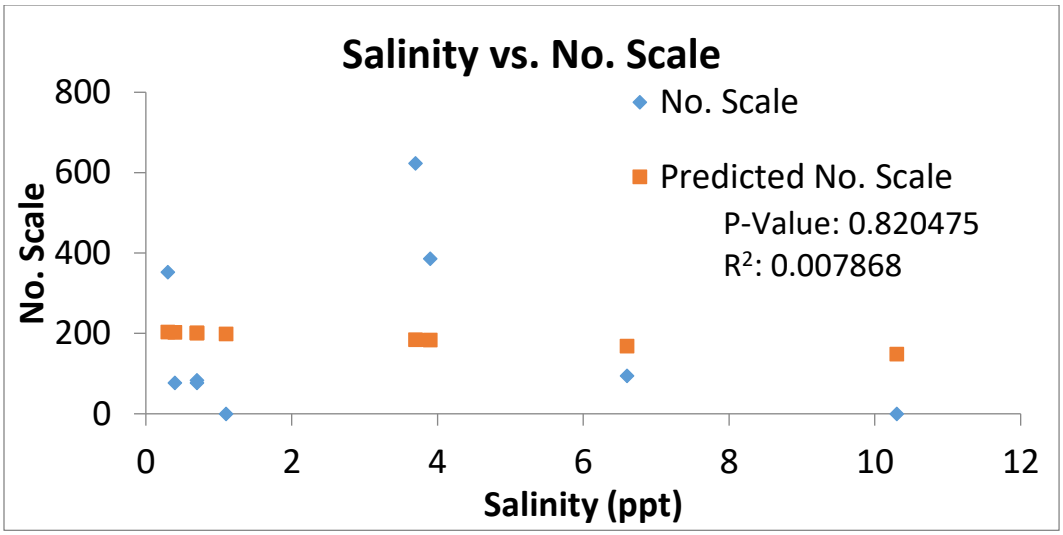

Fig. 2. Regression between salinity and number of scale. Salinity was taken at each site with a sensor. If there was no water directly under the reed stand, the salinity of the closest source of water was taken.

3.3 Dissolved Oxygen

Likewise, for dissolved oxygen and the number of scale, there appears to be no significant relationship or trend between the two (Fig. 3). The p-value in this, like the one before, is particularly high (0.991466).

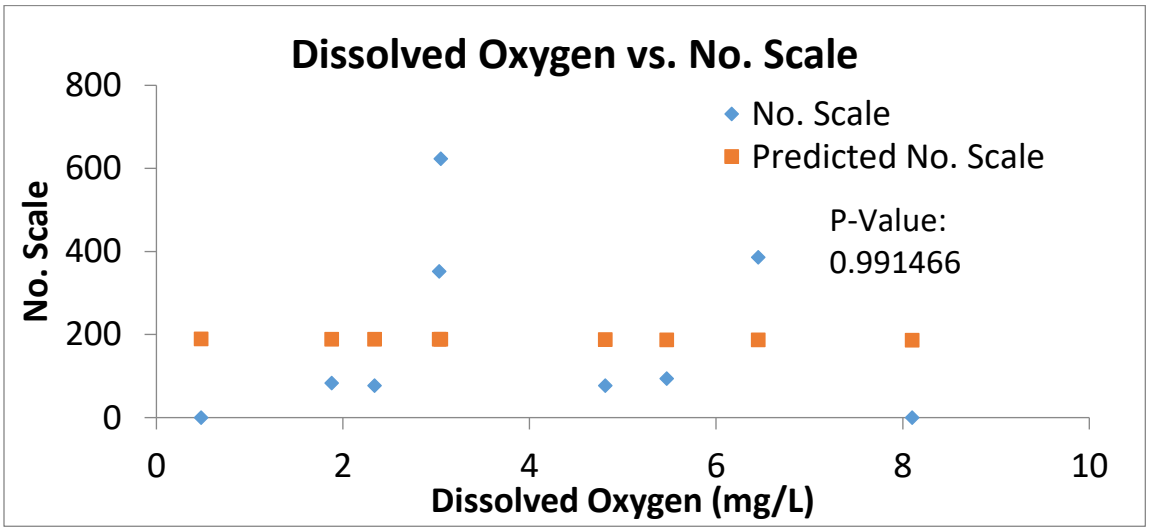

Fig. 3. Regression between dissolved oxygen and the number of scale. Dissolved oxygen was taken in nearest source of water to the *Phragmites* stand. The number of scale was compiled from ten sample stems from each reed stand.

3.4 Wind Speed and Crawlers

Like the others, there is no significant relationship, but a general trend can be seen between wind speed and the average crawler mean (Fig. 4). This could possibly be because a few crawlers may get blown away by high winds.

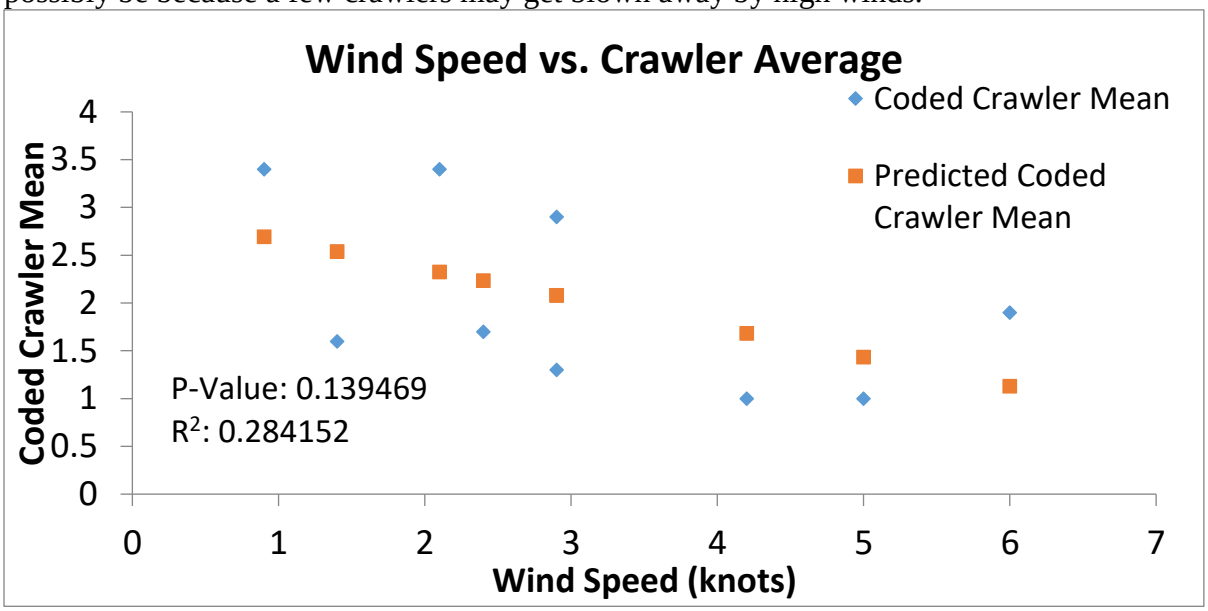

Fig. 4. Regression between wind speed and the coded mean of crawlers. Crawlers were recorded in numerical categories, thus each category was coded and for each reed stand and averaged.

3.5 Color Analysis

No significance was found in the difference between the percent of brown coloration and the percent of green coloration over the nine *Phragmites* stands (Fig. 5). Therefore, it is accepted that there was no difference between the two.

	Percent Brown	*Percent Green*
Mean	0.376455556	0.336266667
Variance	0.026226168	0.03711345
Observations	9	9
Pearson Correlation	-0.786048524	
Hypothesized Mean Difference	0	

Fig. 5. Paired t-test between the percentages of brown and green coloration. The Pearson correlation is not significant meaning that the hypothesized mean difference of 0 is accepted, and the two are not different.

3.6 Brown Coloration

With a p-value of 0.95872, the percent of brown pixels in the photos were not significant (Fig. 6). There appears to be no correlation between brown coloration and scale, thus the relative health of the plant, indicated by sick or dying plants browning, is not directly significant to the scale population of the reed stand.

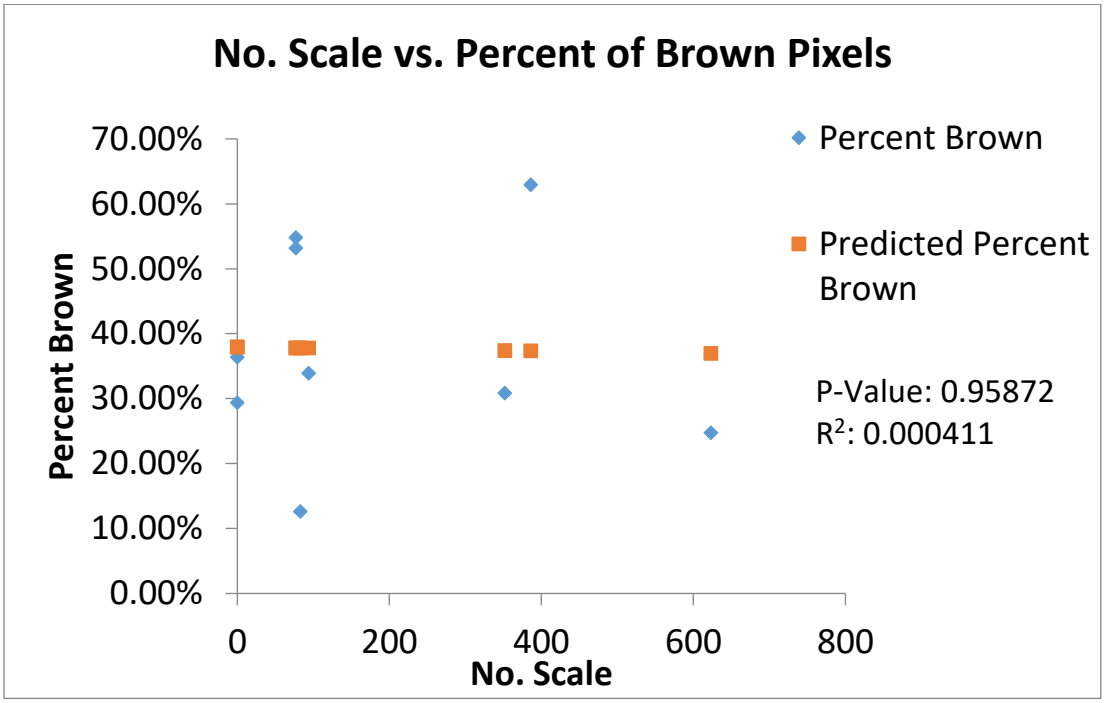

Fig. 6. Regression between percentage of brown coloration of *Phragmites* in a 1m x 1m photo and the number of scale in each *Phragmites* stand.

3.7 Plant Age

As expected, there is a significant relationship between the age of a plant and the number of scale inhabiting it (Fig. 7). The p-value is very low (3.74151E-11), lending it a strong significance.

Plant Age	No. Scale	No. Stems	Mean Scale	Expected	Chi^2 P-Value
Juvenile	95	13	7.31	14.56	3.74151E-11
Live Adult	1,578	44	35.80	14.56	
Old/Dead	19	33	0.58	14.56	

Fig. 7. Chi-squared analysis between age groups of *Phragmites* and the scale.

3.8 Discussion

In many papers concerning *Phragmites*, much of the relationship between scale and *Phragmites* is unknown, and it is mostly conjecture that the scale has a major role in the die-off of the reed as the direct relationship has not been deeply studied. This paper points to the unlikeliness that the scale are the cause of the Roseau cane's disappearance. Other studies have found that in places of high growth Roseau cane, tidal inundation is one of the only effective methods that keep the cane from spreading. These statistics, therefore, indicated that the general conjecture may not be accurate. The scale may still cause damage to the plant because they are parasitic in nature, but it is increasingly unlikely that they are a major cause of Roseau cane death. They may a symptom of the problem rather than the actual driver.

4. Summary and conclusion

Phragmites area coverage on the fringes of stands, where scale live, cannot be directly linked to the number of scale inhabiting them. No significant relationship between the salinity, dissolved oxygen, wind speed, or area coverage of *Phragmites* to the number of scale appears to exist in this experiment. The relative health of the plant, indicated by accelerated browning associated with sick or dying cane, also does not seem to have a significant relationship with the number of scale in a *Phragmites* stand. This may indicate that other factors other than *Nipponaclerda biwakoensis* are responsible for the rapid decline of *Phragmites* in Louisiana and the Gulf. Further research may include more samples of *Phragmites* and measurements for toxins, like sulfides, in the soil, water table levels, or plant pathogens.

5. References

Rudrappa, T., Choi, Y. S., Levia, D. F., Legates, D. R., Lee, K. H., & Bais, H. P. ,2009, *Phragmites* australis root secreted phytotoxin undergoes photo-degradation to execute severe phytotoxicity: *Plant signaling & behavior*, *4*(6), 506-13.

Bernd Blossey, Patrick Häfliger, Lisa Tewksbury, Andrea Dávalos, Richard Casagrande, 2018, Complete host specificity test plant list and associated data to assess host specificity of *Archanara geminipuncta* and *Archanara neurica*, two potential biocontrol agents for invasive *Phragmites australis* in North America: Data in Brief, Volume 19, August 2018, Pages 1755-1764

Brix et al, 2014, Large-scale management of common reed, *Phragmites australis*, for paper production: Ecological Engineering 73 (2014) 760-769

Ramsey, E.W., III, and Rangoonwala, Amina, 2017, Mapping the change of *Phragmites* australis live biomass in the lower Mississippi River Delta marshes: U.S. Geological Survey Open-File Report 2017–1098, https://doi.org/10.3133/ofr20171098.

Zengel et al, 2018, Vegetation recovery in an oil-impacted and burned *Phragmites australis* tidal freshwater marsh: Science of the Total Environment 612 (2018) 231-237