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Assessing Blackmouth Shiner (Notropis melanostomus) habitat in the Pascagoula River using a habitat inundation index based on time series Landsat data

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Abstract:

The Blackmouth Shiner (*Notropis melanostomus*) is a small North American cyprinid that is listed as a Species of Concern due its relatively small range, occurring at only a few sites in Mississippi, Alabama, and Florida. Due to limited data and the small number of actual samples of *N. melanostomus*, the true characteristics of viable habitat and distribution remain unknown. The objective of my research was to utilize remote sensing data to gain a better understanding of the habitat characteristics where the *N. melanostomus* has been collected and use this information to identify other areas were populations are likely to occur during future sampling efforts. In particular, Landsat data were used to map the spatial and temporal extent of water inundation over a 20-year time-series within floodplain water bodies surrounding the Pascagoula River to determine the effects on the presence or absence of Blackmouth Shiners at historic collection sites. These characteristics could be used to inform future site selections within the Pascagoula River drainage as well as identify other river systems that have similar inundation patterns and morphology within and proximal to the known range.

Keywords: *Notropis melanostomus,* Blackmouth Shiner, Landsat, Pascagoula, inundation

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LITERATURE REVIEW

Blackmouth Shiner *(Notropis melanostomus)*

Description

The Blackmouth Shiner, (*Notropis melanostomus*), is a small American cyprinid that has been declared threatened by the American Fisheries Society (Williams et al. 1989), due to its relatively rare appearance in just a few locations in Florida, Alabama, and Mississippi (O'Connell et al., 1998). It has also been given an imperiled status globally due to the scarcity of the species worldwide (Litt et al., 2000). Given this status, the study and conservation of the habitat and range are vital to the understanding and preservation of the species.

N. melanostomus is a short-lived (less than 2 years) and small cyprinid, measuring from 21 mm to 38 mm standard length at maturity. The early descriptions of Bortone (1989) expanded by Suttkus and Bailey (1990) and Bortone (1993) provide most of the biological and phenological data known about *N. melanostomus* (O'Connell et al., 1998; Suttkus & Bailey, 1990). One of the distinguishing characteristics is the size, which is notably smaller than other cyprinid species. Another important feature is the long length and number of gill rakers (15-18), which is considerably more than other *Notropis* that typically have 4-11 or 11-13 gill rakers. *N. melanostomus* also has a distinctive large, upturned mouth with black pigmentation on the inside of the lower jaw (Suttkus & Bailey, 1990).

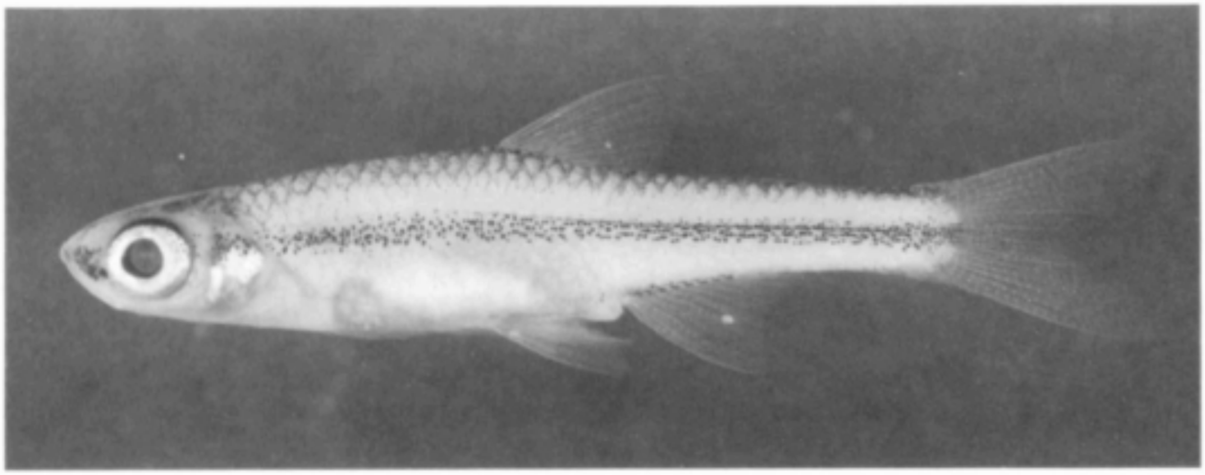

Figure 1: Notropis melanostomus. Photo by J. Suttkus. (Suttkus & Bailey, 1990)

History

First discovered in 1939 in Pond Creek in Santa Rosa County, Florida, another encounter with the *N. melanostomus* was not recorded again until 1976, when a large collection was made at the same location. Subsequently in 1977, *N. melanostomus* was collected in flood pools of the Shoal River in Walton County, Florida. (Suttkus & Bailey, 1990)

The Pond Creek and the Shoal River watersheds remain the only known locations within the State of Florida, that *N. melanostomus* maintain populations. Since the discovery of the species only two other disparate populations were identified. One population was encountered in 1986 in Mississippi, in the Black Creek tributary of the Pascagoula River (Suttkus & Bailey, 1990). This rare fish species was officially described and named in 1989 by S.A. Bortone (Bortone 1989). This led to the identification of three locations in the floodplain ponds of the Pascagoula River Drainage. The sampling focused on the main channels and did not extensively survey the many backwater areas found in abundance in the area.

After the suggestion from Suttkus and Bailey (1989) that there were "other undiscovered disjunct populations" of *N. melanostomus* in the Pascagoula River which contains a large network of floodplain ponds and oxbow lakes, O'Connell et al. (1998) conducted more extensive surveys for *N. melanostomus* at 35 sites in the Pascagoula floodplain from April to June, 1995 (O'Connell et al., 1998). Sites were selected based on the habitat descriptions of Bortone (1993) who indicated a perceived preference for backwater areas with aquatic vegetation. The goals were to find new populations and gain a better understanding of the habitat preferences of *N. melanostomus*. This effort led to the discovery of eight new sites, which are located in the ephemeral ponds and oxbow lakes that are isolated from the main channel of the Pascagoula River except during high water events (O'Connell et al., 1998).

Morphometric statistics, such as standard length, scale and ray counts, were taken on all of the 439 specimens collected during this study. It is notable that most (414 of 439) of the individuals collected were juveniles, based on a smaller size class. (O'Connell et al., 1998)

After unsuccessful surveys in 1998, 1999, 2001, and 2002, a study conducted by O'Connell et al. in 2005, attempted to determine if the sampling efforts to date provided enough information to describe with confidence the actual range of *N. melanostomus.* Using a method developed by Ponder et al. (2001) to analyze the spatial distribution and test recent sampling to six background populations of associated species to identify sampling "gaps" in *N. melanostomus* data. This study provided several key conclusions; there is not sufficient sampling effort, increased understanding of habitat and focused sampling can lead to greater confidence in distributions, and this statistical method could be used to quantify the effectiveness of future sampling efforts. The main conclusion was that there is not sufficient information to confine the actual range of *N. melanostomus*. This was confirmed by an isolated discovery of *N. melanostomus* in the Mobile River drainage in Alabama in 2003 (O'Connell et al., 2005).

In an attempt to test the effects of Hurricane Katrina on the habitat of *N. melanostomus*, O'Connell et al. returned in 2007-2008 to the sites that were surveyed in 1995 in the Pascagoula floodplain. *N. melanostomus* were collected at only two of the eight 1995 sites. Some of these sites were found to have dried up and therefore were no longer suitable habitats. One new location, at Luther Lake was discovered during this sampling period. This site is adjacent to a historic site and was not sampled in 1995. There was a notable decline in sites and numbers of *N. melanostomus* since the successful 1995 collections. The reason for the decline is unknown but could reflect changes in habitat conditions such as low water level at the time of sampling. The effect of Hurricane Katrina could not be determined but generally the sites appeared to be relatively unchanged since the storm and the 1995 conditions. Water level was noted as the main difference and the water would likely return to higher levels during the next flood cycle of the river. The discovery of the new population at Luther Lake suggests that there are still undiscovered populations throughout the floodplain (O'Connell et al. 2013).

Habitat

N. melanostomus is known to inhabit the backwaters of streams and rivers that are partly or completely separated from the main channel (Suttkus & Bailey, 1990). Although no specimens have been collected in the main channel, it is understood from the intermittent and varying distributions in the floodplains that *N. melanostomus* survives in the main channel, and is deposited in these backwater areas during high river stages. O'Connell et al. (2013) describe this as a 'lottery' system of dispersal where it is a matter of chance where the species ends up after a flood event (O'Connell et al., 2013). It is possible that when *N. melanostomus* land in a water body that contains the right criteria, they can breed and sustain a population. At some locations populations have been quite numerous, particularly in Florida where Bortone (1993) described "several thousand individuals" and a couple of locations along the Pascagoula O'Connell et al. (1998) noted schools numbering in the hundreds (Bortone, 1993; O'Connell et al., 1998).

Many of the locations where *N. melanostomus* have been collected are ephemeral water-bodies. Typically, these backwater ponds have clear water and submersed aquatic vegetation, including *Potamogeton, Nymphaea, and Najas* (Suttkus & Bailey, 1990; O'Connell et al., 1998). O'Connell et al. (1998) performed statistical analysis on the five environmental variables that were measured at all of the sampling sites including pH, turbidity, capture depth, water, and air temperature. Upon comparison of the environmental factors between the presence or absence of *N. melanostomus* revealed a correlation with submersed vegetation, sand, and detritus. Among these the presence of vegetation had the most critical value. Canonical variate analysis suggested that water clarity had a positive relationship where *N. melanostomus* sites were significantly less turbid. (O'Connell et al., 1998)

Of all of the habitat characteristics that determine the location of *N. melanostomus,* generally the floodplain itself maybe the most important. This species has only been collected in backwater areas that are semi-connected to a larger river or stream.

Pascagoula River

The Pascagoula River is a unique watershed because it is the largest river in the lower 48 states that has not been majorly altered by human activity. Attempts to dam the river have been stopped by the efforts of conservationists and environmentalists. Much of this area has even avoided the widespread logging that decimated many of the bottomland hardwood forests throughout the Southeastern United States. The river is able to overflow into the floodplain unimpeded by the series of levees and dams found along many other river systems that were constructed to protect farmland and infrastructure.

Through the confluence of many factors the Pascagoula River and much of the surrounding swamps and forests were spared from the development and industrialization much of America experienced in the second half of the twentieth century. Mississippi was one of the states least affected by the post-war boom in growth seen in much of the United States. Mississippi was slow to develop partially due to the remnants of the reputation from some of the more disturbing chapters of the civil rights movement. Much of the land around the Pascagoula was in the holdings of a large lumber company that was slow to develop the tract (Schueler 1980).

The area became officially protected starting in the 1970s after the work of Graham Wisner and The Nature Conservancy's Dave Morine to partner with the state of Mississippi to buy 35,000 acres of pristine hardwood swamp forest surrounding the Pascagoula River from the Pascagoula Hardwood Company (Schueler 1980). This was a historic agreement which was the largest private loan for the purpose of conservation. This resulted in the creation of the Mississippi Natural Heritage Program which represents a formal partnership between The

Nature Conservancy (TNC) and state legislators and agencies. The state of Mississippi now owns the land and created the Pascagoula River State Wildlife Management Area (PRSWMA). The state and TNC continues to acquire land for conservation and now there is almost 70,000 acres of buffer around an 80-mile stretch the main channel of the river between TNC and state managed land (Stowe & Tirpak). TNC continues to build partnerships through the formation of the Pascagoula River Basin Alliance that brings together other conservation groups, state agencies, private businesses and the public to share information and raise awareness about this unique area (Stowe & Tirpak).

Through luck and the persistent efforts of a diverse group of people with a common goal, the Pascagoula River has been preserved and serves as a refuge to many species. Since its main channel has remained un-dammed the natural ebbs and flows of the river into the floodplain have been able to continue in the area. This unique habitat has allowed rare species such as *N. melanostomus* to survive.

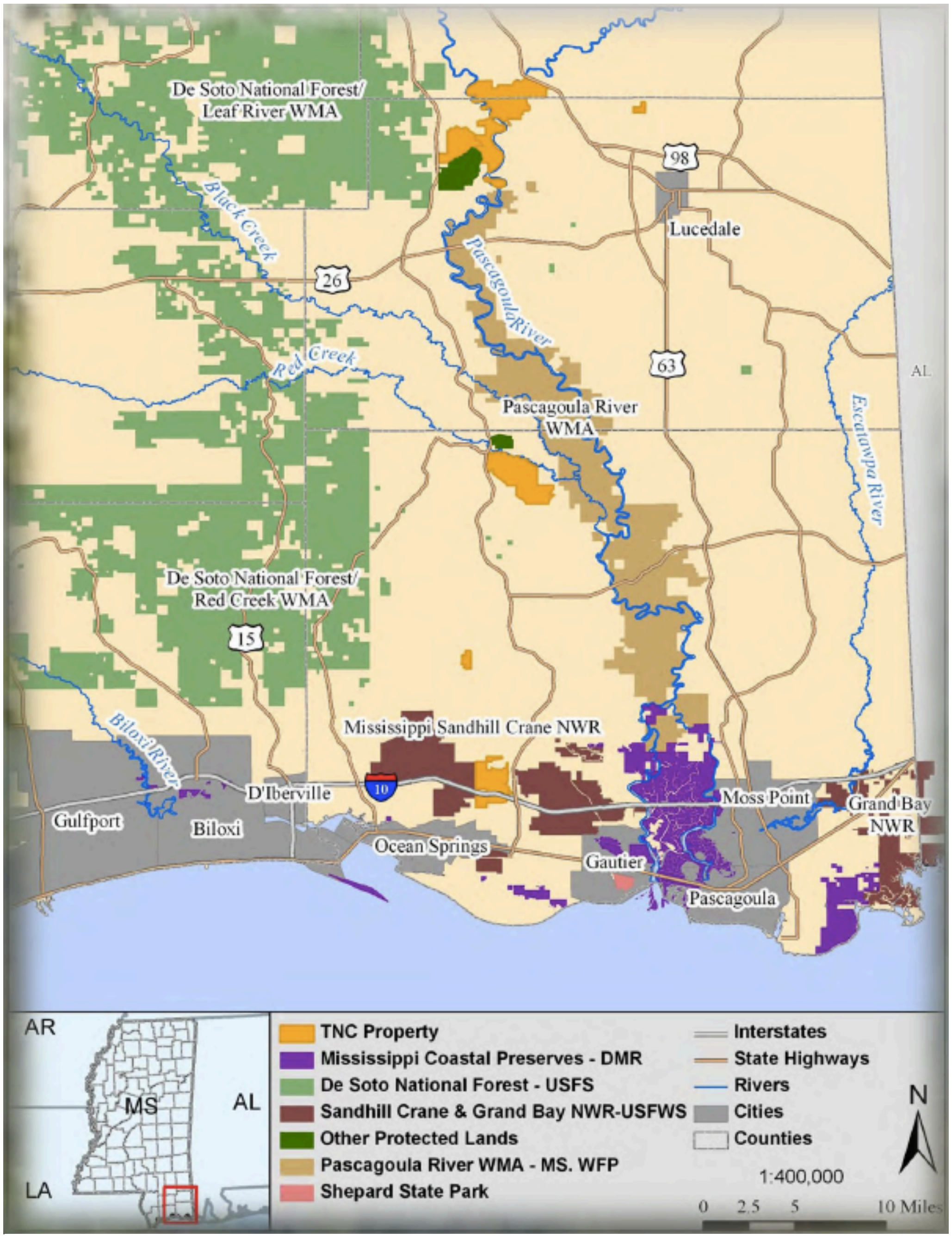

Figure 2: Map of the Pascagoula River protected areas. (adapted from: Stowe and Tirpak, TNC poster)

INTRODUCTION

Objectives

The objective of this research was to utilize remote sensing data to gain a better understanding of the habitat characteristics where *N. melanostomus* has been collected in the Pascagoula River and use this information to identify other areas where populations are likely to be found during future sampling efforts. Previous studies have made an attempt to identify key characteristics of the preferred habitat to improve sampling effectiveness and have found links between the presence of submersed aquatic vegetation and less turbid water (O'Connell et al., 1998). In Mississippi, *N. melanostomus* has only been found in the backwater oxbow lakes and ephemeral ponds in the floodplain of the Pascagoula drainage. Since the Pascagoula has been protected from levees and dams, it is able to experience the natural flooding cycles making the floodplain a dynamic environment where the ponds have a variety of patterns of inundation depending on topography, depth and distance from the river. This research attempts to investigate the correlation between the differences in inundation frequency and the presence of *N. melanostomus*.

In particular, Landsat data were used to map the spatial and temporal extent of water inundation over a 20-year time-series to track patterns within floodplain water bodies surrounding the Pascagoula River to determine the effects on the presence or absence of *N. melanostomus* at historic collection sites. These characteristics could be used to inform future site selections within the Pascagoula River drainage as well as identify other river systems that have similar inundation patterns and morphology within and proximal to the known range.

Study Area

The study covers the main channel and floodplains of the Pascagoula River in George and Jackson counties in southeastern Mississippi. Beginning in the north near the town of Merrill, in George County, where the Leaf and Chickasawhay rivers converge to form the Pascagoula River and extending south along the river into Jackson County with the southern most site near Wade Vancleave Rd. This area covers a path 2 miles wide and 28 miles along the length of the river. Most of the areas in this study are within the managed areas of the TNC and the PSWMA.

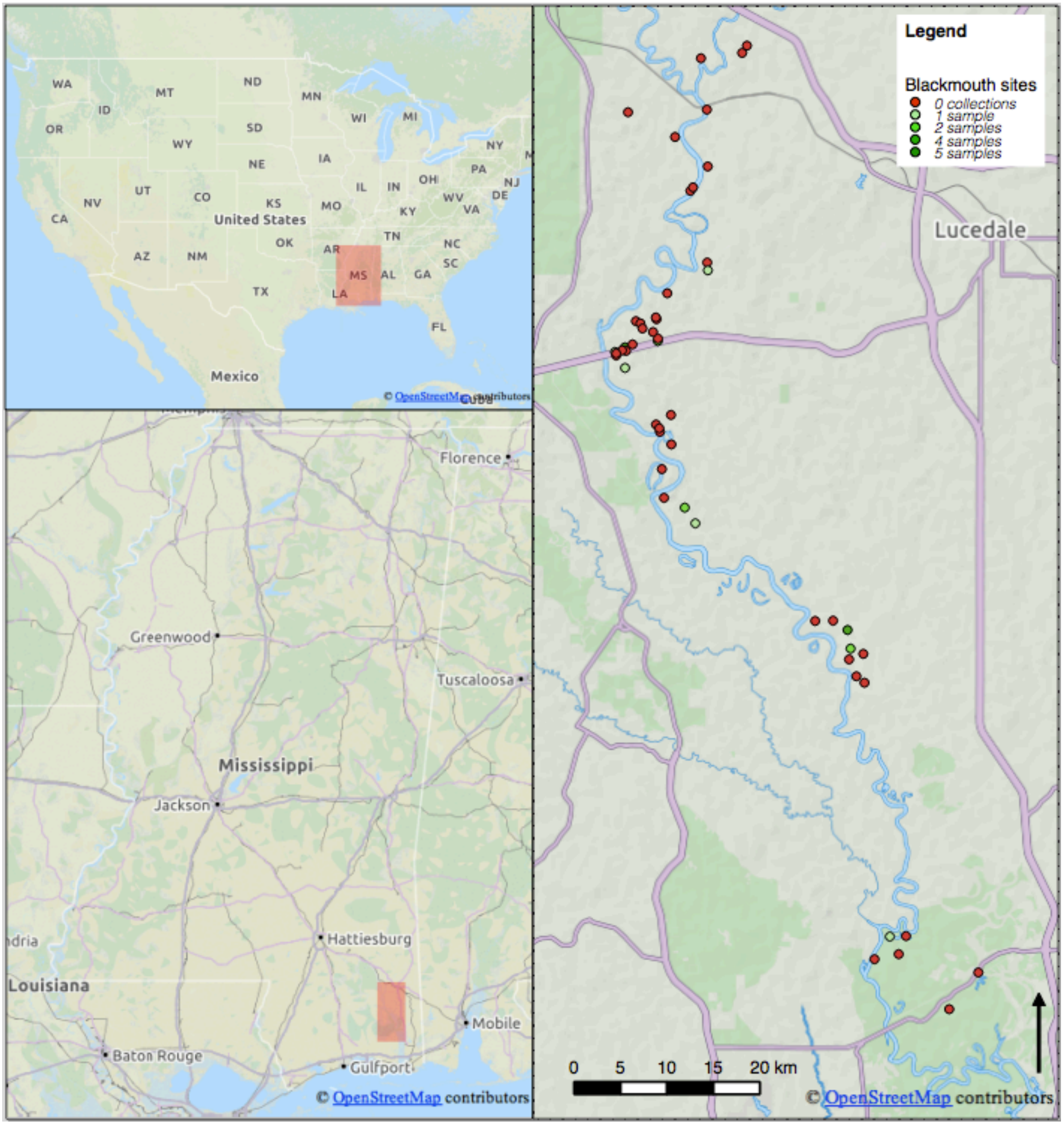

Figure 3: Study area covers the main channel of the Pascagoula River in southeastern Mississippi. Green dots depict locations where Blackmouth Shiner (*N. melanostomus*) were collected and red dots depict unsuccessful sampling sites.

METHODS

Landsat

Landsat Thematic Mapper (TM) was selected for this application because of the balance of spatial and temporal resolution. Since the goals of this research were to characterize the inundation patterns of small ponds in the floodplain, the highest spatial resolution (greatest detail in each scene) and highest temporal resolution (most scenes per time period) are preferred. There are satellite data with finer spatial resolution others with more frequent repeat frequencies. Most sensors with finer spatial resolution must sacrifice the temporal resolution. The sensors that have higher temporal resolution or more frequent repeat frequencies have much courser spatial resolution.

The spatial resolution is the area of land surface that is represented by each pixel contained in each scene. For Landsat data each pixel represents $30m^2$ of land surface. Due to the small amount of land area covered by some of the ponds in the study area, the large spatial resolution of other sensors, such as $250m^2$ resolution of Moderate Resolution Imaging Spectroradiometer (MODIS) would not have been able to detect changes in the smaller ponds.

The temporal resolution is the pass rate or the frequency that the satellite passes the same area to take images. For Landsat data any location the pass rate is every 16 days. MODIS provides daily data but was limited due to the spatial resolution.

In order to capture the fluctuations in water inundation in the small ponds and sloughs of the Pascagoula River, Landsat data provided the best combination of spatial and temporal resolution available.

The other advantage to Landsat data is the continuity of the Landsat mission. Landsat is the longest running Earth observation satellite system and has been continuously collecting multispectral data of the Earth since the 1970s. This allows for applications such as this one to have a data set that covers the entire time period of the study.

Image Selection

Landsat Thematic Mapper (TM) surface reflectance data were collected from the United States Geological Survey (USGS) Earth Explorer website for the years 1990 to 2011 for path 22 row 39, which covers the entire lower Pascagoula River study area. Dates were selected based on less than 10% cloud cover with an effort to acquire a summer and winter image for each year and to cover as much of the entire time period as possible. By selecting scenes from different seasons in each year, a variety of conditions over the 20 years would be represented.

The limit on the number of dates selected was mainly due to cloud cover. The presence of clouds in an image presents difficulty for this type of study in that the areas where clouds were present have the effect of masking out the information that is on the ground at the time the image is collected. For example, if a cloud were covering a pond that contained water at the time Landsat captured the image, the resulting classification would not indicate that water was present due to the cloud interference. There are accepted techniques for creating cloud masks in the processing of remotely sensed imagery, but for this type of classification where the classes are a binary of water or no water, the presence of masked clouds could create classifications with a bias toward less water than is actually present on the ground. For this reason, an effort was made to include images with the least amount of cloud interference as possible. This resulted in some years being under-represented or omitted in the dataset. In total, 39 separate Landsat scenes were selected for analysis.

Water Classification

Each Landsat scene was processed using QGIS (QGIS Development Team, 2015). QGIS is an open source Geographic Information System (GIS) created by Open Source Geospatial Foundation (OSGeo) that has the capabilities of geospatial image processing, analysis and map production.

The first step was to clip each scene to the study area around the river. A polygon was drawn to delineate the area of the flood plain that would be inundated when the river was at a maximum. This shape was used to cut out all images to just include the area within the polygon. This limited the amount of processing time as well focused the study just to the area that would be of interest.

Next, a land cover classification was performed using the Semi-Auto Classification plugin (Congedo et al., 2013) to identify areas with water. Other methods have been successfully used to classify water with Landsat imagery such as the establishment of thresholds in the Short Wave Infrared (SWIR) band (Allen, 2015; Frazier & Page, 2000). One of the limitations with this type of classification is the misclassification of highly turbid water (Frazier & Page, 2000). The Pascagoula River can have large quantities of suspended solids that contribute to the turbidity of the water. This results in water that could be misclassified using the SWIR band threshold method. Through experimentation with this technique, it was determined that this misclassification would be problematic for this study. For this reason, the

Semi-Auto Classification method proved to be preferable due to the ability to customize the criteria for determining what should be classified as water, accounting for the higher turbidity found in the Pascagoula River.

This process was done by selecting a series of areas identified as water in the true color composite (RGB=Bands 3, 2, 1) of a representative Landsat scene. Due to varying properties of water in different areas, such as turbidity, and the presence of submersed aquatic vegetation, a variety of locations in and proximal to the river were selected.

The spectral signatures of the selected areas were used as training classes to classify the areas with similar spectral information. This resulted in a raster image that depicted the areas that were covered in water as a value of one (blue) and all other areas were given a zero value (black) (fig. 4). This gives a spatial representation of the extent of water inundation for that date. This process was repeated for each of the 39 dates in the time series. Each raster image was quality checked against the true color composite (RGB=Bands 3, 2, 1) of that date.

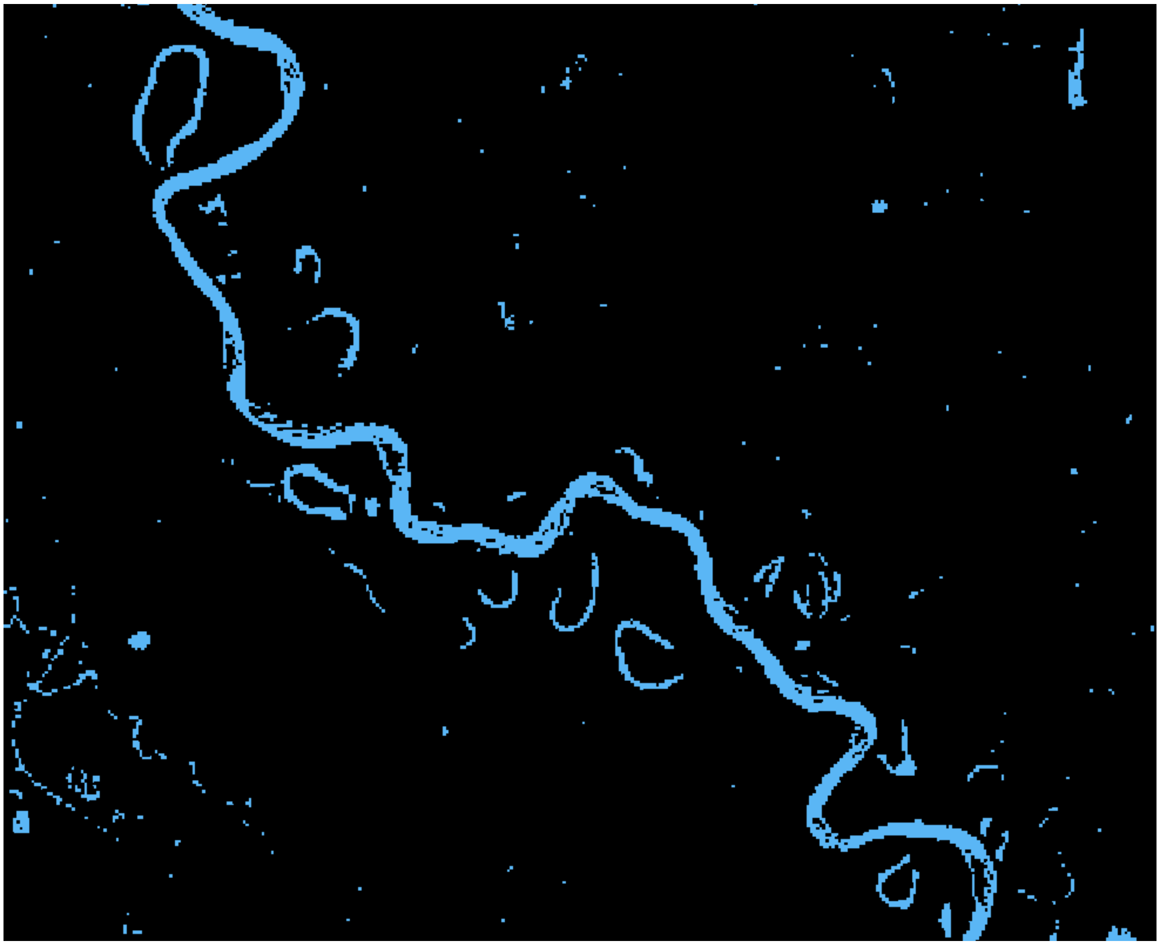

Figure 4: Example of classified Landsat raster image. Blue represents water.

Cumulative Water Index

Now that all of the Landsat scenes were classified to indicate all of the surface area that contained water for each respective date, they were combined to indicate the spatial and temporal inundation patterns. Since the water in each of the scenes was coded with a value of one, all of the separate raster layers were added together to depict the cumulative frequency value for each pixel. This resulted in a new raster layer that has values ranging from zero indicating no water for any of the dates, to thirty-nine showing water present in all of the

Landsat scenes (fig. 5). This serves as a proxy for the relative frequency of the occurrence of water per each pixel throughout the study area.

To integrate this inundation raster layer with the *N. melanostomus* sites, all historic sampling efforts were categorized by the presence or absence of *N. melanostomus* and plotted in QGIS based on the GPS coordinates from the field notes recorded during previous collections efforts (fig. 3). This included all sites sampled by O'Connell et al. in 1995, 1998, 1999, 2001, 2002, 2007, and 2008 including the nine sites where *N. melanostomus* were collected and the 31 sites where they were not found.

In order to determine the characteristics of the water bodies proximal to the sampling sites, all locations were identified and a vector layer was created with a polygon drawn around each water body that corresponded to each of the sampling sites. By using the addition raster layer and Landsat ture color composites (RGB 3, 2, 1), the full extent of the water bodies was represented in each of the polygons.

The Raster Statistics tool was used to extract the histogram values for the addition water raster layer for each of the ware body poygons. This created a count of the number of pixels classified as water for each of the addition frequency values (1-39) within the polygon water body shape. These totals were then normalized with respect to the maximum frequency value recorded, or maximum number of pixels within the water body identified as water. This method allows for the comparison of the relative frequency of water inundation within each water body and for the comparison of the various frequencies between the water bodies.

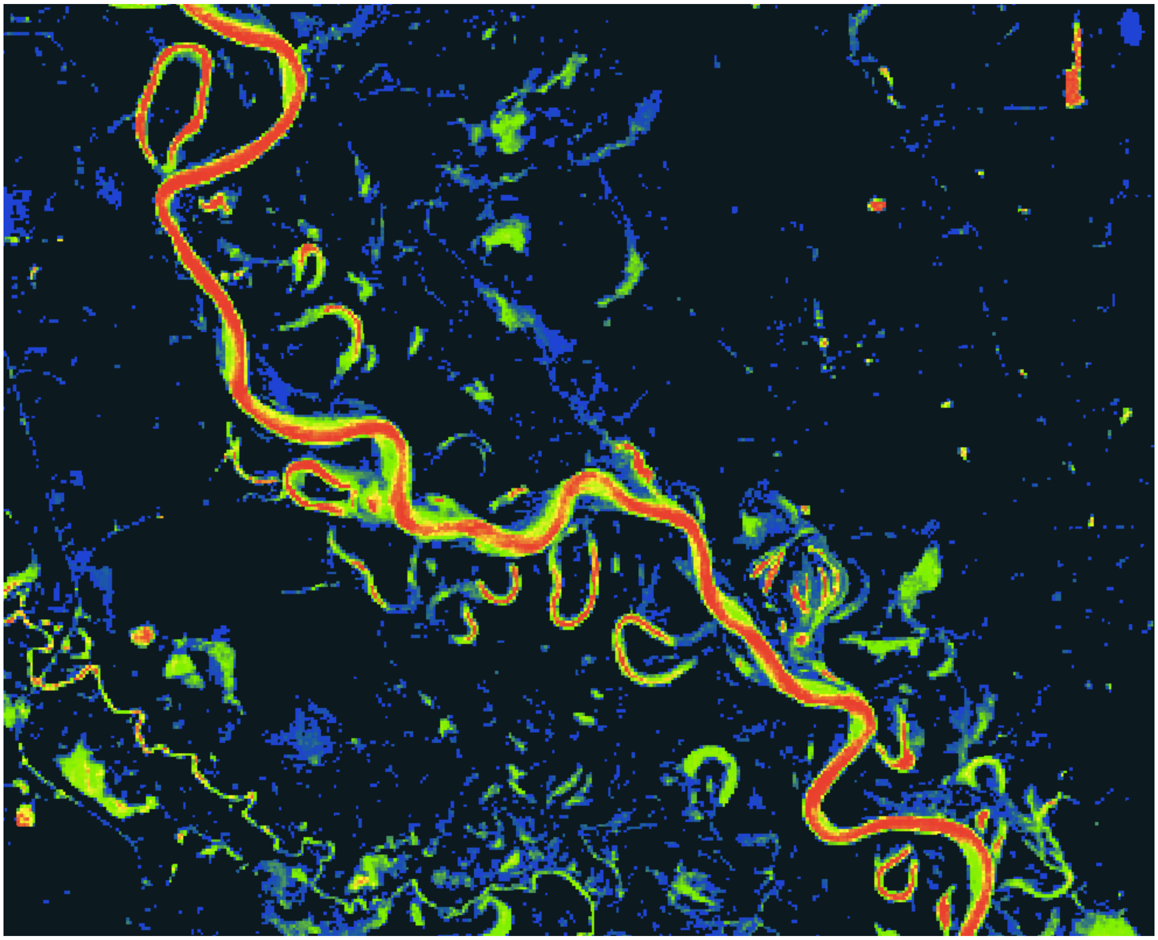

Figure 5: Example of addition water raster resulting from the summation of all single date water raster layers. Red indicates higher values, green intermediate, and blue low values.

RESULTS

Water Classification

The results of the water classification analysis show that there is a wide range of the amount of water that is in the Pascagoula floodplain within the dates that were used in this study. Some of the dates were during times where the river had overtopped the natural levees and had completely inundated much of the floodplain (fig. 6 - left) and other dates showed that there had been a drying period of no inundation and probably little rainfall and most of the backwater areas had lost much of the standing water (fig. 6 - right). These results are clear when the two figures are compared side by side.

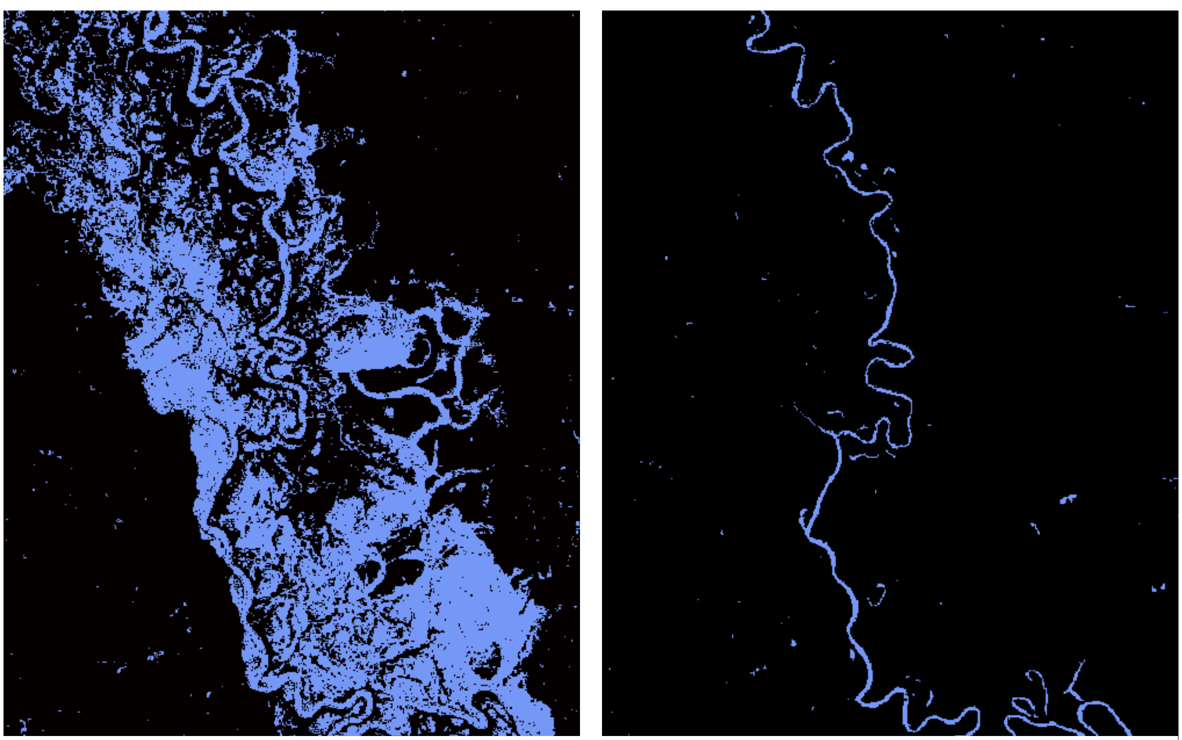

Figure 6: Example of variation in water level with the Pascagoula River drainage. Blue indicates water in each of the classified Landsat raster images. High water (left) and low water (right).

Cumulative Water Index

The results of the inundation analysis through the cumulative water index indicate a series of patterns of inundation for individual ponds within the study area. Some of the ponds are in shallow areas that are infrequently flooded and can cover a large area. Other ponds have areas that are continuously wet such as some of the oxbow lakes that are more permanent water bodies. This variation represents the fluctuation of the amount of water present in the pond through time.

By classifying each Landsat scene for water and adding all of the scenes together a relative inundation quantity can be derived. This is represented as a range of values from 1-39, where one indicates that water was present for only one of the Landsat scene dates and thirty-nine indicates that water was present for all of the dates.

The Raster Statistics Tool allowed for the extraction of the number of pixels in each water body that represented each of the addition values. Since there is a large range of sizes of ponds these numbers were normalized for each of the ponds so that range of frequency of each of the values (1-39) was comparable between ponds of various sizes. The histogram of each of these normalized values were then plotted for each of the ponds. Most of the ponds had the highest value for one. This was likely the result of one of the Landsat dates corresponding to a super-flood time when all of the ponds were at very high stage and the distal edges of the pond that become inundated on that day represent the most area of a single value within the range.

The ponds that showed the least frequent inundation had histogram values that showed fewer values in the upper part of the addition value range. As can be seen in the example in (fig. 7) the histogram has very few values above the 20 and most of the values represented by the lower range. This indicates that for most of the Landsat scene dates this pond contained very little or no water. The relative amount of inundation frequency can also be seen visually in the map of the pond which consists mostly of blues and greens with almost no orange or red visible. This is probably a relative flat area that only fills up during extreme flood events and does not retain water on a consistent basis.

On the opposite end of the spectrum there are ponds that showed a high level of consistent water. In the example, (fig. 8) there are higher values throughout the histogram showing that the portion of the pond that maintained water represented a large percentage of the overall pond size. This pond had a relative frequency value of 0.39 for addition value 39, meaning that there was a significant portion of the pond that contained water in every Landsat date in this study. In the map of this pond there is much less blue and green and much more orange and red than the previous example. This pond is very close to the main channel of the river and may be more likely to receive frequent additional water from the river and is probably deeper resulting in the retention of more water through time.

The third example, (fig. 9) shows a water body that exhibits some of the characteristics of both of the previous examples and has some high addition values but the histogram is still weighted toward the lower end of the range. The map shows a more even range of tones with some blue, green, orange, and red. This pond has a wide range of water inundation with a small portion that retains water consistently. There was a zero frequency for addition value 39 meaning that there was no area in the pond that contained water in every Landsat date, but there were significant levels for addition values 36, 37, and 38, indicating that the water body had some water in almost every Landsat scene date.

The results indicate that there are various types of water bodies within the Pascagoula River floodplain that have a range of relative inundation frequency. Some ponds are only wet during extreme flooding events and do not retain much water consistently. Other ponds have significant portions that retain water all of the time.

Of the three main types of inundation patterns illustrated by these examples, seven out of the eight sites where *N. melanostomus* were found were in the intermediate category. One pond was too small to accurately categorize and one fell into the more wet category. Of the 31 ponds where *N. melanostomus* was not found, 11 were in the more wet category, 12 were in the less wet category and 7 were in the intermediate category.

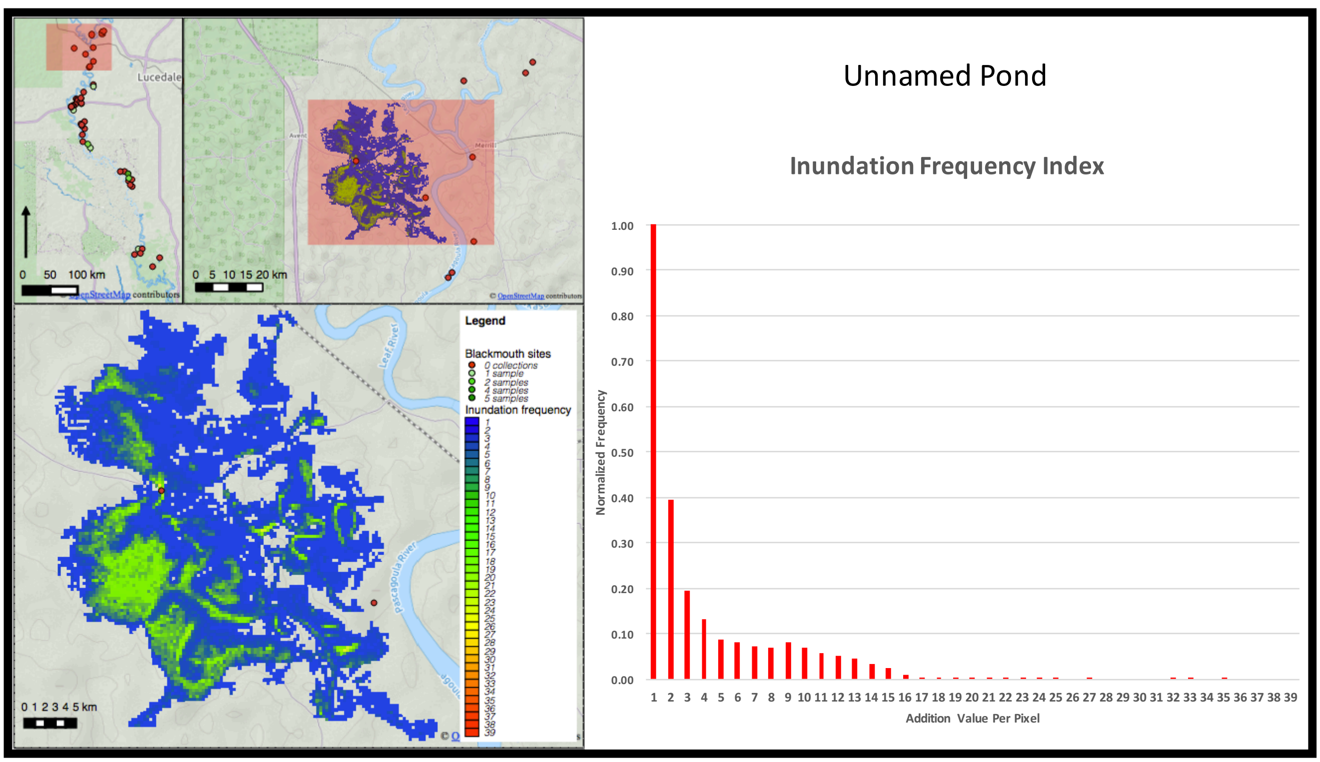

Figure 7: Map of addition raster and inundation frequency index histogram of Unnamed Pond

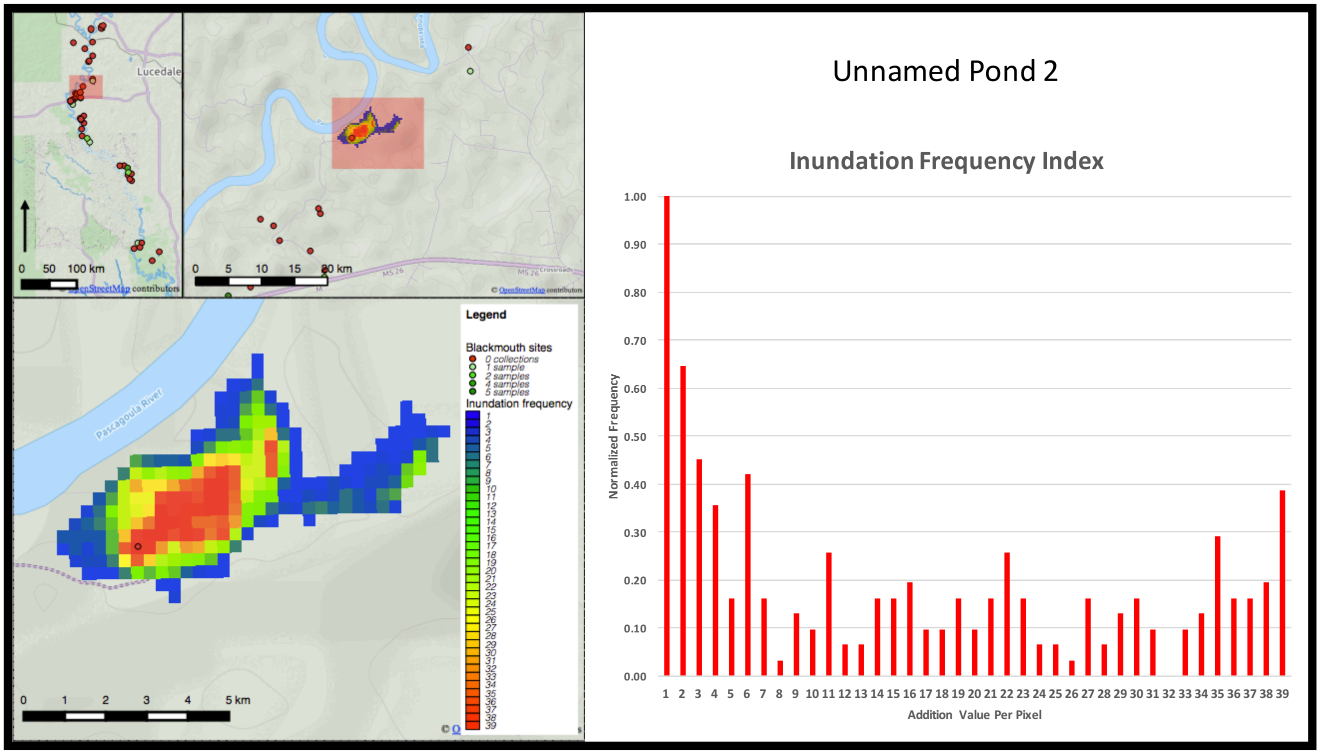

Figure 8: Map of addition raster and inundation frequency index histogram of Unnamed Pond 2

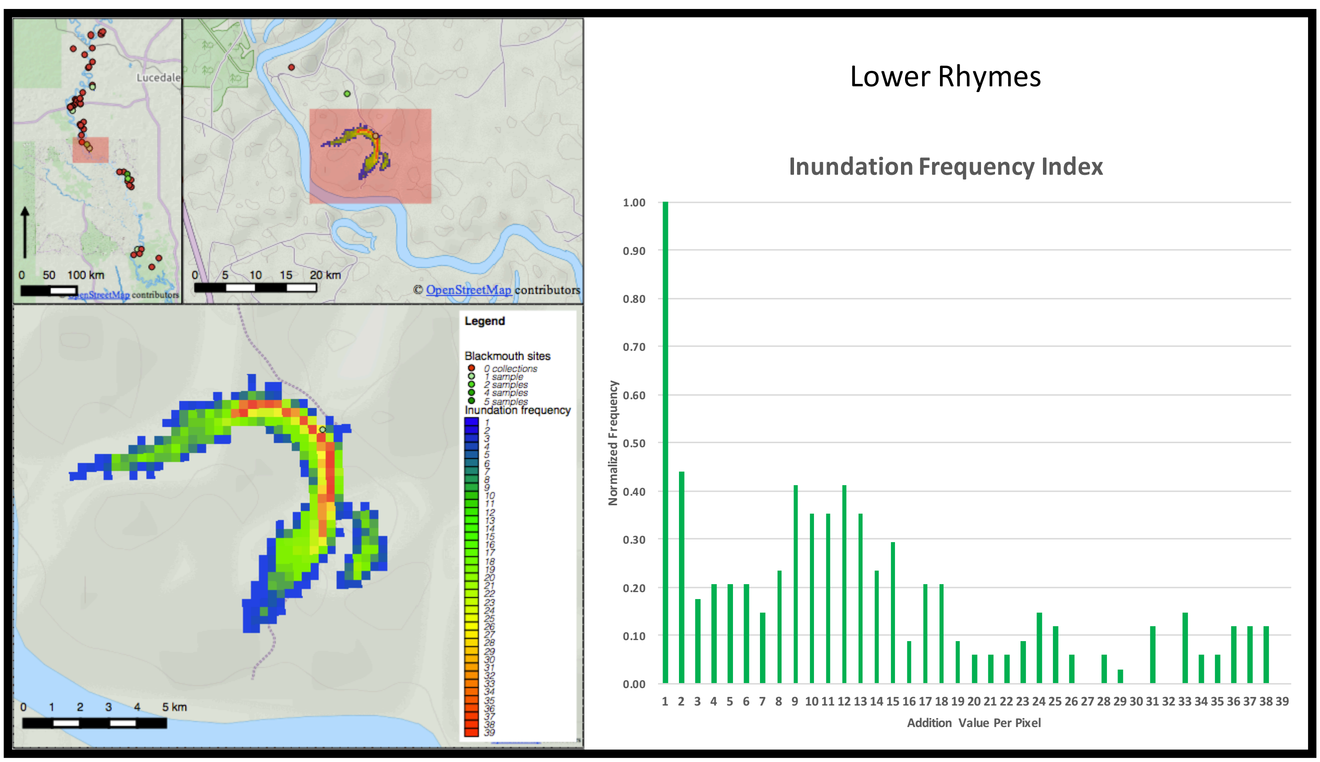

Figure 9: Map of addition raster and inundation frequency index histogram of Lower Rhymes

DISCUSSION

Upon review of the histograms of the addition layers of each of the water-bodies a pattern emerged. Although each location exhibits different characteristics, there is a trend in inundation frequency among the locations where *N. melanostomus* were encountered that differed from the locations where *N. melanostomus* were not found. The examination of the relationship between the portion of the pond that maintains a continuous water level (higher numbers in the histogram) and areas that are more infrequently inundated (lower numbers in the histogram), suggests that *N. melanostomus* survives and reproduces in ponds that maintain a balance between how much of the pond stays wet. By normalizing the values in each pond and taking the average of the inundation frequency of all of the ponds where *N. melanostomus* was found shows the pattern of this relationship for the dates used in this study (fig. 10). The same process was used for the ponds where *N. melanostomus* were not found.

The plots for the averages of all of the *N. melanostomus* ponds versus the average of all of the non *N. melanostomus* ponds does not reveal much of a distinction between the two types, but there are a variety of different inundation patterns. The ponds where *N. melanostomus* were not found can be separated into three different classes; ponds that are less wet (fig. 7), more wet (fig. 8), and intermediate (fig. 9). The more wet type represents more established ponds that have a larger portion of the pond that consistently maintains water. The less wet type represents ponds that are only intermittently inundated and do not have a significant portion of the pond that constantly retains water. Both of these types of scenarios could be problematic for the survival of *N. melanostomus.*

The ponds that are categorized as “more wet” could limit *N. melanostomus* populations through the ability of larger species to survive and become predators. In these floodplain habitats the water levels fluctuate through time. Even if *N. melanostomus* can become established in the distillate edges where they can avoid predation during times of higher water, when the water recedes they would be forced into areas that the predators are inhabiting. This would create a situation where smaller species, such as *N. melanostomus*, are not able to sustain populations, due to the presence of larger predator species.

The ponds that are categorized as “less wet” could limit *N. melanostomus* populations because of the lack of sustained water for longer periods of time. Most species of fishes, with a few notable exceptions, require a minimum amount of water to survive. Some of these locations experience a wide range from wet to dry and are shallow flat areas even when wet. A breeding pair of *N. melanostomus* may be deposited into one of these types of ponds and may reproduce but the population would be killed off as soon as the water recedes and the pond dries up.

The third type of pond shows similar characteristics to the suitable ponds where *N. melanostomus* were encountered. The fact that *N. melanostomus* were not found in these locations does not indicate definitively that these areas are not suitable. This may only be a function of the “lottery” effect as described by O’Connell et. al (1998) in which there is likely a random factor in the possibility that a new population would be established in a pond (O’Connell et al. 1998). First, a breeding pair of fish would have to be washed into the area and survive long enough to reproduce. Due to the relative infrequent events of total inundation of the floodplain, these possible migration events are limited. It is assumed that *N. melanostomus* do not exist in the main river channel in high density since none have been collected from the main channel of the river (though one historic collection was in a backwater portion of the river). The possibility that a particular pond would get the breeding pair during flood stage would be minimal.

Another distinct possibility is that *N. melanostomus* relies on these ephemeral and backwater areas as breeding and rearing areas. Rather than a habitat necessary for a sustained population, these habitats are the locations in which the most successful breeding can take place due to the protection provided by the inability of predators to survive. This hypothesis was suggested by O'Connell et al. (1998) as "the possible result of a temporally and spatially unique spawning event" and is supported by the disproportionate number of juveniles (414 out of 439) collected in 1995 (O'Connell et al., 1998). If this spawning behavior is part of the life cycle of *N. melanostomus* then much more study of the reproduction of this rare species needs to be conducted to understand the importance of these areas to the long-term survival of the species.

If inundation frequency and water retention are the key factors in the ability of *N. melanostomus* to establish populations or reproduce, then there is a specific signature to pattern of the most suitable habitats. They are locations that experience enough water level fluctuation to create the conditions for the initial deposition of the founding pair during high water connection to the river but only retain a small amount of water over longer time scales so that larger predator species cannot also survive within the same pond. This requires a specific set of requirements that could be mapped throughout the watershed and to identify other possible locations that show similar conditions.

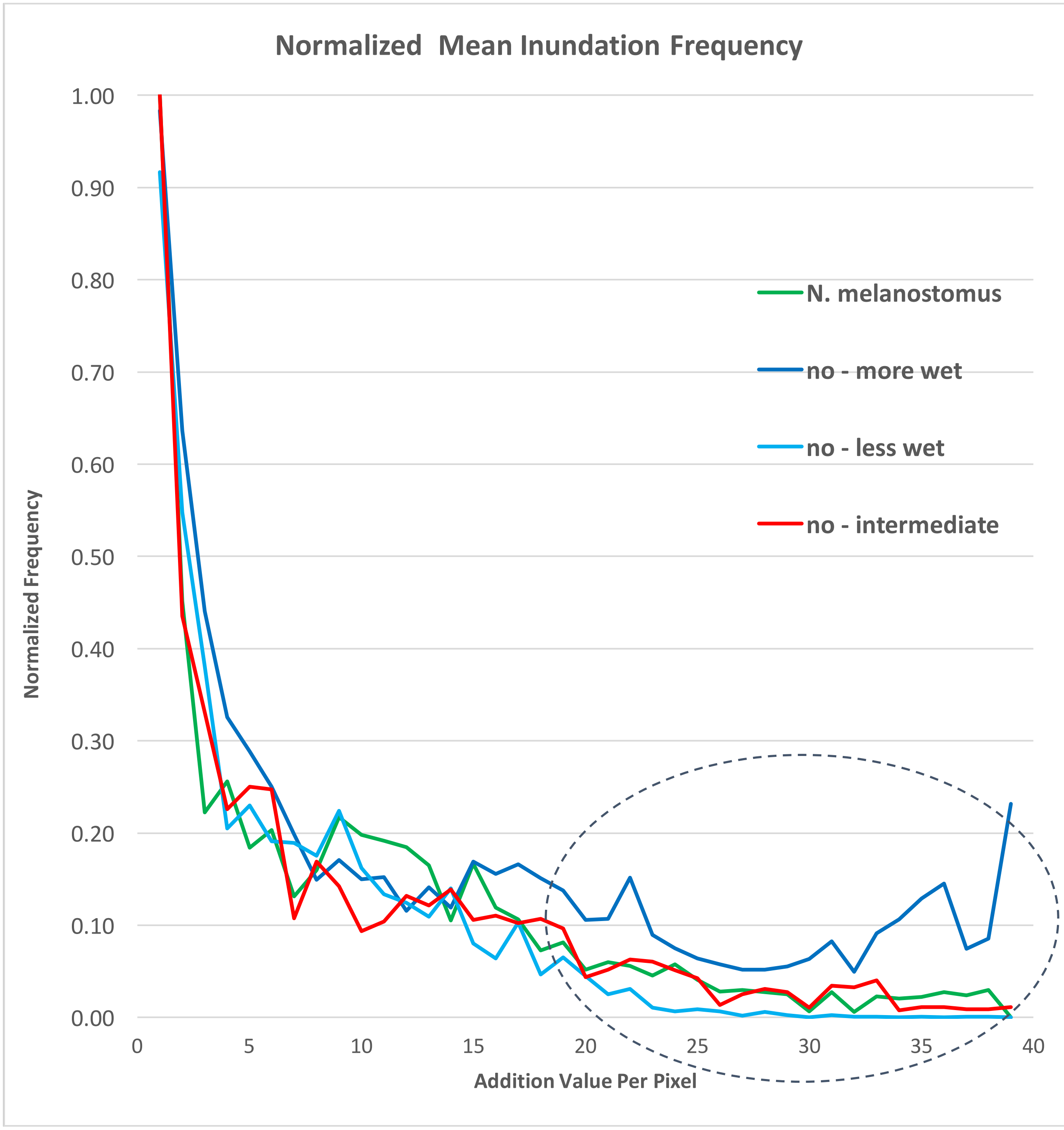

Figure 10: Normalized Mean Inundation Frequency. Plot of the mean of the normalized frequencies of the addition value per pixel histogram values (calculated from the addition of the water classification of all Landsat scenes) for four groups of water bodies. *N. melanostomus* sites (green) = water bodies where *N. melanostomus* were collected. Water bodies where no *N. melanostomus* were found separated into three groups: No – more wet (dark blue) = water bodies that are more consistently wet, no – less wet (light blue) = water bodies that are infrequently inundated, no – intermediate (red) = water bodies that are moderately inundated. Circled area indicates most significant differences between pond types, wetter ponds (dark blue) have higher values showing more consistent water retention, and less wet ponds (light blue) have low or zero values showing no consistent water retention. Intermediate ponds show a similar trend showing a small portion of the pond that hold consistent water.

CONCLUSIONS

The methods used in this study show promise for the study of the habitat characteristics of *N. melanostomus* and other rare riverine fishes. The importance of the frequency of inundation and the ability of a pond to hold water, play a vital role in the survival and proliferation of some species such as *N. melanostomus*.

There are several possible sources of error and difficulty in the interpretation of the results in this study. Due to the relatively small sample size and sites to examine these relationships, there may not be sufficient information to characterize the relative importance of some of these factors. The size of some of the water bodies was a limiting factor due to the 30 m spatial resolution of Landsat data. The Semi-Automatic classification method can lead to the over or under estimation of water due to the many possible variations in the characteristics of water.

Future work could improve on the methods used in this study that would lead to a better understanding of the inundation patterns and the character of the floodplain ponds that are *N. melanostomus* habitats. The first would be to link each pixel from the classified water images to the river gages in the Pascagoula River similar to the method described by Y. Allen (Allen, 2015). By correlating the presence or absence of water to the stage of the river, a more complete picture of the patterns of inundation could be created based on the greater temporal resolution provided by the daily measurement of the river gage height measurements.

River gage data could also be used to plan the timing of future sampling to follow inundation events after the turbidity has settled, to identify new locations and monitor the population success.

Another method that would improve the understanding of the area would be to interpret elevation data for the floodplain. Lidar data for the Pascagoula watershed are available and would provide a detailed elevation dataset. These data could be used to determine the depth of each of the ponds. The depth could be a key factor on the ability of *N. melanostomus* survival because deeper ponds could provide refuge for large predators in the deeper portions of the pond. Even if *N. melanostomus* are able to avoid predation in the shallow edges during times of high water levels, they would be forced into the deeper areas in periods of lower water levels. There could also be a threshold on the shallowness of ponds where *N. melanostomus* could survive but only for short periods, due to the pond drying out.

Despite the limitations of this study, valuable information about the subtle variations of inundation patterns suggest that there are two limiting factors on either end of the inundation frequency range for suitable habitat. On the more wet side, the ponds that have too large a portion that stays consistently wet may not be suitable habitats, possibly due to the ability of larger predators to survive in these areas. On the less wet side, the ponds that have too small a portion that stay consistently wet are not suitable habitats due to the inability of populations to survive when the pond dries up too frequently.

The greatest possible addition to the understanding of the habitats of this species would be more field sampling. Due to the rarity and limited range of *N. melanostomus*, statistical correlations between the presence of the species and suitable habitat characteristics are difficult (O'Connell et al., 2005). Ultimately the conclusions and hypotheses explored in this study can only be supported by more field sampling to increase the number of known sites. The method of inundation analysis through the classification of Landsat imagery, can provide the data necessary to determine the most likely locations to yield *N. melanostomus* in future sampling efforts. Once the sites are established, repeated sampling on a seasonal basis would reveal important information of how populations fluctuate through time and at various water levels.

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