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Evaluating The Success Of Two Monitoring Techniques Of Newly Stocked *Lampsilis fasciola* In The Upper French Broad

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Abstract

About one third of the world's freshwater mussels (order Unionida, families Margaritiferidae and Unionidae) reside in North America, with the majority ranging in the southeast United States. Unfortunately, due to anthropogenic disturbance, Unionid mussels are considered to be one of the most imperiled groups of animals in the world. North Carolina is home to over 65 Unionid species, over 50% of which are federally listed as Threatened or Endangered. In order to combat this decline, stocking and relocation techniques are commonly used. In this study, we reintroduce historically-occurring Unionid mussels into the French Broad River (FBR) and compare post-stocking monitoring techniques. *Lampsilis fasciola* mussels were reared at the Marion fish hatchery, Passive Integrated Transponder (PIT) tagged and stocked in the upper FBR. The release site was surveyed seven times over 3 months using radio frequency identification (RFID) and visual encounter surveys (VES) to identify released mussels. RFID provided a higher level of encounter, with an average of 83.4% detection, compared to an average 3.21% detection using VES. The information gathered from these efforts provide data for conservation measures in the future to further help stabilize threatened and endangered species, including *Alasmidonta raveneliana* populations.

1. Introduction

North America is home to 298 species of freshwater mussels (Bivalvia, Unionida), which makes up about 1/3 of mussels species worldwide[21]. North Carolina inhabits over 65 of these species[11]. Unfortunately, over 50% of North Carolina's mussels are state listed and over 25% are additionally federally listed[18]. Like many rivers near developed cities, the French Broad River (FBR) has been critically impacted by sedimentation, agriculture activity, sewage line breaks, and the destruction of riparian buffers[15]. These pollutants, along with the exploitation of Unionid nacre, have contributed to the decline in mussel diversity over time in the FBR. Unfortunately, mussel populations continue to decline over their geographical range. Many populations are at peril today due to damming, exotic mussel species introduction, and sedimentation from construction[17]. Damming creates distribution barriers for host fish, making it impossible for mussel glochidia to reach portions of the river[20]. Furthermore, the introduction of exotics directly compete with mussels for resources, where Unionid filtration rates are significantly reduced in areas where exotics coinhabit[3]. Additionally, pollutants such as sedimentation smother Unionid gills and decrease oxygen levels within the reach.

Unionid species richness has declined dramatically in the FBR basin since the first comprehensive species list conducted by Ortmann in 1918. Ortmann's species list includes 25 historically known freshwater mussel species occurring in the FBR basin[13] (Table 1). Today, there are currently 7 known naturally occurring and established species found in the FBR basin[9,11,18] (Table 2). Of the naturally occurring populations existing today, 4 are listed vulnerable, as their populations are as risk of extinction[6]. This includes the federally listed[18] Appalachian elktoe mussel, *Alasmidonta raveneliana*. It is critical to address this decline, because freshwater mussels play an important role within the ecosystem. Mussels provide native fishes and wildlife with food, they aid in the stabilization of river bedload[19] and they filter out bacteria from within our rivers and streams. Numerous studies have been conducted which support

that mussels aid in filtering unwanted viruses and bacterias like *Staphylococcus* and *Escherichia coli* from the water column. In a study conducted in 2016, poliovirus counts were significantly reduced and *E. coli* counts were reduced almost completely after 24h in the presence of mussels[10]. Further, the restoration of native mussels could act as bioremediation within the FBR and help aid in the restoration of its water quality.

Although there is more work to be done, the French Broad River seems to be getting cleaner over the past couple years, as conservationists and local environmental stewards devote efforts to cleanups and outreach. As a result, the FBR is becoming more stable for suitable habitat for Unidionids, as it once was reflected by the data in Ortmann's 1918 survey. In April 2018, biologist Jason Mays[9] conducted a survey in which the intolerant and endangered Appalachian elktoe was sited making this occurrence the furthest downstream the mussel has been recorded since Ortmann's survey[13]. In June 2019 we found *A. raveneliana* the furthest upstream on the FBR it has ever been found on record[11,13,18]. Thus, the FBR seems to be currently supporting small numbers of *A. raveneliana* today better than it has in many years which is a great sign for future conservation efforts.

The purpose of this study was to begin restoring native fauna and facilitate different survey techniques to provide information for conservation measures in the future to further help stabilize threatened and endangered species, including *Alasmidonta raveneliana* populations. We accomplished this by 1.) propagating and stocking the Waveyrayed Lampmussel (*Lampsilis fasciola*), whose populations are currently stable,[6] into the upper FBR and monitoring this effort and 2.) attempting to achieve a better understanding of what happens to individuals that go undetected. Additionally, the reintroduction of *L. fasciola* marks this species return into the FBR after over a 100 year absence[13,14]. We hypothesize that Passive Integrated Transponder (PIT) scanning efforts will generate a more accurate representation of population density over snorkeling efforts. Additionally, we hypothesize that individuals that were undetected were present but undetected.

Table 1. Native freshwater mussel species (n=25) indicated by Ortmann's 1918[13] survey of the French Broad River Basin, NC and TN (Sevier County), including the currently accepted Latin and common names respectively.

Ortmann 1918	Accepted Name (Synonym)	Common Name
Nephronaias ligamentina	*Actinonaias ligamentina* (Lamarck, 1819)	Mucket Mussel
Alasmidonta raveneliana	*Alasmidonta raveneliana* (Lea, 1834)	Appalachian Elktoe
Alasmidonta viridis	*Alasmidonta viridis* (Rafinesque, 1820)	Slippershell
Amblema plicata	*Amblema plicata* (Say, 1817)	Threeridge
Quadrula pustulosa	*Cyclonaias pustulosa* (Lea, 1831)	Pimpleback
Rotundaria tuberculata	*Cyclonaias tuberculata* (Rafinesque, 1820)	Purple Wartyback
Elliptio niger	*Elliptio crassidens* (Lamarck, 1819)	Elephant Ear
E. dilatatus	*Elliptio dilatata* (Rafinesque, 1820)	Spike
Truncilla arcformis	*Epioblasma arcaeformis* (Lea, 1831)	Arc-form Pearly Mussel
Truncilla capsaeformis	*Epioblasma capsaeformis* (Lea, 1834)	Oyster Mussel
Fusconaia barnesiana	*Fusconaia barnesiana* (Lea, 1838)	Tennessee Pigtoe
Fusconaia pilaris	*Fusconaia subrotunda* (Lea, 1831)	Longsolid
Lampsilis fasciola	*Lampsilis fasciola* (Rafinesque, 1820)	Wavey-rayed Lampmussel
Eurynia recta	*Leptodea fragilis* (Rafinesque, 1820)	Fragile Papershell
Medionidus plateolus	*Medionidus conradicus* (I. Lea, 1834)	Cumberland Moccasinshell
Plethobasus cyphyus	*Plethobasus cyphyus* (Rafinesque, 1820)	Sheepnose
Plethobasus cooperianus	*Plethobasus cooperianus* (Lea, 1834)	Orange-footed Pimpleback
Pleurobema obliquum	*Pleurobema cordatum* (Rafinesque, 1820)	Ohio Pigtoe
Pleurobema oviforme	*Pleurobema oviforme* (Conrad, 1834)	Tennessee Clubshell
Lexingtonia dolabelloides	*Pleuronaia dolabelloides* (Lea, 1840)	Slabside pearlymussel
Proptera alata	*Potamilus alatus* (Say, 1817)	Pink Heelsplitter
Ellipsaria fasciolaris	*Ptychobranchus fasciolaris* (Rafinesque,1820)	Kidneyshell

Strophitus edentulus	*Strophitus undulatus* (Say, 1817)	Creeper
Taxolasma lividum	*Toxolasma lividum* (Rafinesque, 1831)	Purple Lillyput
Eurynia nebulosa	*Villosa iris* (Lea, 1829)	Rainbow Mussel

Table 2. Current Unionid species list (n=7) for the French Broad River Basin, NC and TN (Sevier County)[9,11,18].

Accepted Name	Common Name
Alasmidonta raveneliana	Appalachian Elktoe
Alasmidonta viridis	Slippershell
Fusconaia subrotunda	Longsolid
Pleurobema oviforme[*1]	Tennessee Clubshell
Potamilus alatus	Pink Heelsplitter
Strophitus undulatus	Creeper
Villosa iris	Rainbow Mussel

2. **Methods**

2.1 Site Selection and Plot Set-Up

Lampsilis fasciola were stocked in a 10 x 30 m stretch of the upper French Broad River (FBR), located in Rosman, NC. Site was selected based on substrate suitability and future conservation efforts planned for this reach of the river. The riverbed was stable, with preferred sand to rock ratios. Additionally, the FBR has historical records of mussel populations, including *L. fasciola*[13].

The 10 x 30 meter plot ran alongside the southeast bank of the river, the bank acting as one side of the 30 m stretch of the plot. Bank pins were set up 30 meters apart using flagging to indicate where the plot boundaries lay. A meter tape was used to measure out 10 m from each bank pin into the river and a rebar stake was hammered into the riverbed to plot out the corners of the research area. During sampling, a meter tape was laid out onto the river bed between rebar stakes to better indicate the edge of the plot area. This process was repeated each time when visiting the survey site.

2.2 PIT tagging and stocking

Juvenile *Lampsilis fasciola* mussels (n=300) were propagated and obtained from the North Carolina Wildlife Resources Commission fish hatchery located in Marion, NC. Each mussel was from the same 2016 Pigeon River broodstock. Each mussel was tagged using 8 mm x 1.4 mm PIT tags at about age 3. One PIT tag was glued centrally onto the left value of each mussel using *Loctite Ultragel* superglue (Henkel Adhesives, Düsseldorf, Germany). Once the superglue was dry (~30-40 seconds) each tag was then further secured onto the exterior of the shells by completely encapsulating the PIT tag with *WaterWeld* epoxy putty (J-B Weld, Atlanta, Georgia)[7]. Only ~5 individuals were worked on at a time to reduce stress on the animals. Mussels were limited to 2-3 minutes out of the water. About 25 mussels per hour were tagged using this method.

Mussels were stocked on June 3, 2019, in a 300 m^2 area. All 300 of the tagged mussels were placed in a blind fashion, where individuals who placed the animals were not later conducting surveys. Animals were placed about 1 m^2 apart.

2.3 Surveying techniques

2.3.1 visual encounter survey

Two different surveying techniques were used and compared; visual encounter surveys (VES) and radio frequency identification (RFID) using tag scanning. VES were conducted using mask and snorkel. A single surveyor (Surveyor 1), who was not involved in placing the animals, conducted all the surveys. Surveyor 1 conducted surveys weekly for 3 weeks, then every 2 weeks for 3 weeks then monthly until the surveys were complete. Surveys were conducted on June 6, June 13, June 24, July 11, July 25, August 9, and August 23 of 2019. VES were conducted one additional day when RFID surveys were not, on 2019 September 6. On some occasions, there was a second surveyor (Surveyor 2) with more experience included in the surveys with whom to compare encounter rates. Surveyor 2 conducted surveys on June 13, July 11, August 8 and September 6 of 2019. Surveyor 1 had average skill level, with about 2 years experience conducting mussel surveys. Surveyor 2 had very experienced skill level with 12+ years of experience. Each time the surveys were conducted the surveyor would snorkel between 60 and 70 minutes, or until the entire plot had been searched. Surveyors worked though the plot in a zig zag fashion, moving from downstream to upstream and vice versa, covering about a half meter stretch at a time. Dead mussels were included in counts, then removed from the plot and recorded. Time spent conducting VES varied based on water clarity (secchi reading), weather conditions, etc. After completing the survey, total mussel catch per unit effort (CPUE) was calculated by dividing number of mussels (n_{visual}) visually detected each survey by the total time spent searching (n_{visual} /minute)[16]. Linear regression was used to see if CPUE was associated with water clarity using R Studios. Water clarity was measured using secchi disk readings and data from CPUE from Surveyor 1 were used.

2.3.2 radio frequency identification

The other technique used was radio frequency identification (RFID). This was implemented by scanning for PIT tags using a *Biomark* HPR Plus Reader with *Biomark* BP Lite Portable Antenna. The HPR reader and scanner are used simultaneously and the reader picks up unique PIT tag numbers when the wand hovers over the tagged animal. The wand was used to scan the bottom of the river using a sweeping motion. The sweeping motions are typically ~1 meter wide. The user of the HPR reader always conducted their survey after the visual, to eliminate the possibility of mussel agitation, causing the mussels to close their valves and making them hard to detect visually. Scanning surveys lasted the same amount of time as the visual surveys. Scanning surveys were conducted in the same zigzag fashion as the visual surveys, however, moving perpendicular to water flow, making sure to overlap portions to ensure no animals went undetected.

RFID surveys were conducted the same dates as VES, except for 6 September 2019, when only VES were conducted. After all surveys were conducted, the data were uploaded using *BioTerm* (Biomark) software, which transferred all PIT tag numbers detected that event, including their respective GPS coordinates each event. The data were then further analyzed using Excel to remove duplicates, identifying the unique number of individuals detected.

To account for any movement of the mussels, the GPS coordinates for 27 individuals were chosen at random and analyzed. The GPS coordinates from the 27 individuals on each survey day was mapped to see if any movements were occurring. Unfortunately, there is a 3m error on *Biomark* GPS coordinates. We attempted to account for the 3m error by adding a 3m buffer to coordinates in ArcMap (version 10.6.1). We then considered points with buffers that did not touch or overlap other buffers as a movement occurrence.

After completing the survey, total mussel catch per unit effort (CPUE) was calculated by dividing number of mussels (n_{RFID}) detected by the PIT tag reader by the total time spent searching (n_{RFID}/minute)[16]. After surveys were conducted, VES were compared to RFID surveys using t-tests. Other information collected during each survey included water pH, conductivity, temperate and secchi readings. Additionally, brief weather descriptions were collected on site. Data were recorded into excel and analyzed using R studios.

3. Results

3.1 Visual detections

VES detected significantly less individuals than the PIT tag reader each effort ($t=1.76$, $df=14$, $p < 0.0001$). The lowest number of mussels detected visually were 1 and the highest 29 (Fig. 1). The highest number of mussels detected was on study day 5. On average, 9.6 individuals were visually encountered by Surveyor 1 and 20.25 by Surveyor 2. Surveyor 2 visual detections ranged between 13 and 25 individuals. Although fewer *L. fasciola* were detected on days with lower secchi readings, there was no significant correlation between catch per unit effort (CPUE) and water clarity ($R^2=0.2991$, $F=1,6$ $p=0.09$, Fig.2). VES had a significantly lower CPUE rate than RFID ($t=1.94$, $df=6$, $p=5.68^{-07}$, Fig.

3). CPUE ranged between 0.02 (n_{visual}/ min.) and 0.45 (n_{visual}/ min.)(Fig.3). Secchi readings ranged from 0.9 m to 3.79 m, with an average of 2.19 m.

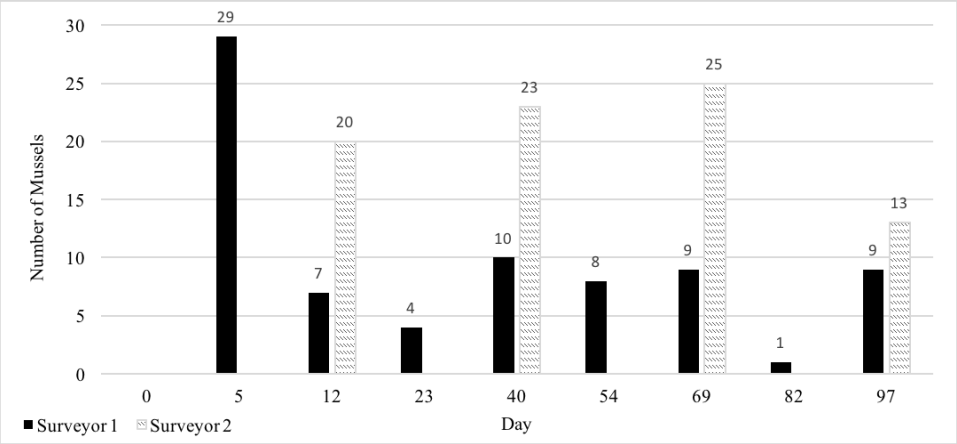

Figure 1. Number of mussels visually detected while conducting snorkel- surveys on study day n. *Lampsilis fasciola* (n=300) were stocked on 3 June 2019, study day 0.

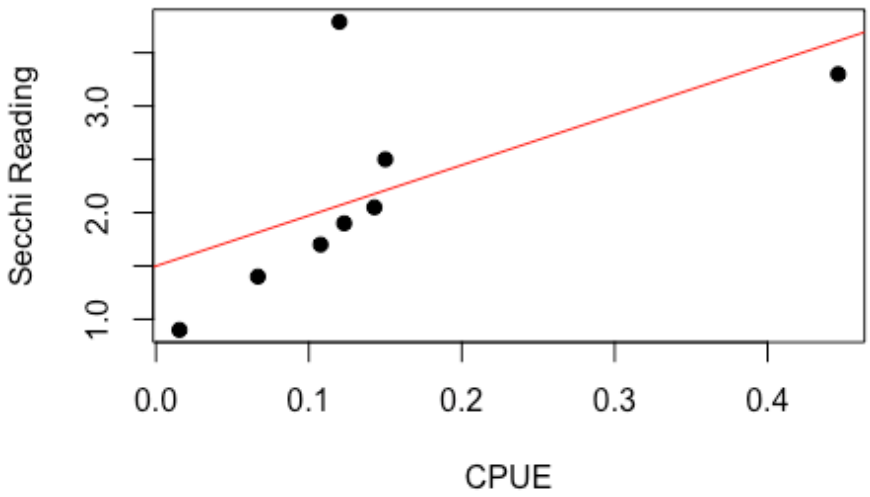

Figure 2. Relationship between CPUE (n_{visual}/minutes) of Surveyor 1 and Secchi reading (meters).

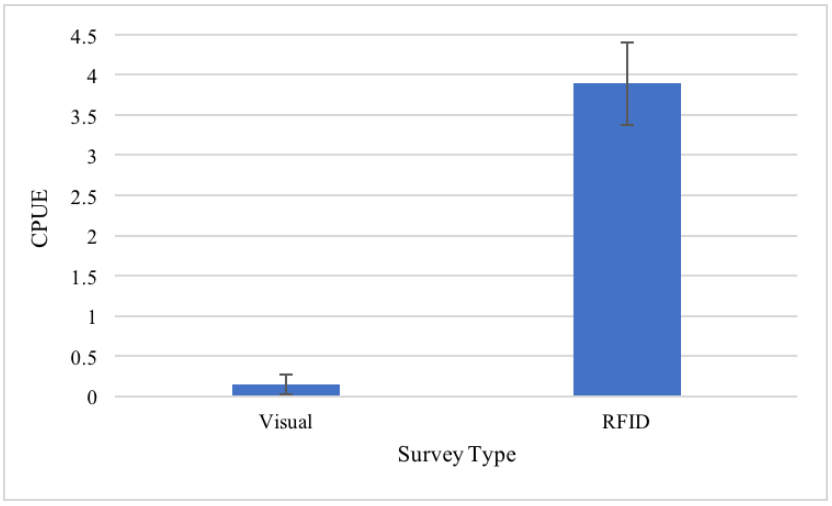

Figure 3. Mean ± SD CPUE of VES (Visual) and RFID Surveys. CPUE was measured as $n_{visual,\ RFID}$ mussels detected/ minutes spent surveying.

3.2 RFID Detections

The lowest number of mussels detected by the PIT tag reader was 198, and the highest 284 (Fig. 4). The highest number of mussels detected occurred on study day 82. On average, 250 mussels were detected, 83.4%. RFID readings had a 94.6% redetection rate (Fig. 5). There were115 individuals detected 100% of the time, while 2 individuals were never detected. Out of n=300, 298 individuals were detected at least once. Additionally, 273 mussels were detected more than half the time. CPUE ranged from 3.07 n_{RFID}/minute to 4.37 n_{RFID}/minute (Fig. 3). RFID had a significantly higher CPUE rate than VES (t=1.94, df=6, $p=5.68^{-07}$). Of the 27 analyzed individuals, 20 experienced a movement occurrence (Fig. 6).

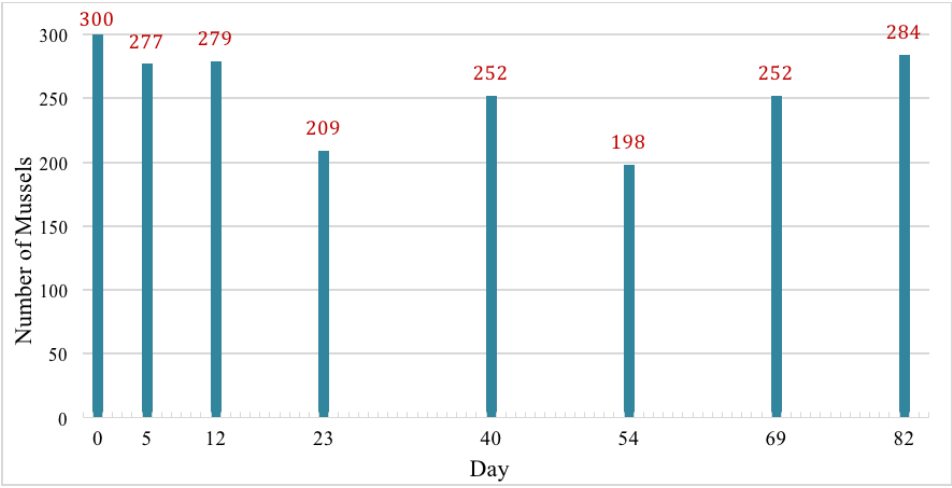

Figure 4. Number of PIT tags detected by *Biomark* PIT-tag reader on study day n. Days are represented by one tick mark. *Lampsilis fasciola* (n=300) were stocked on 3 June 2019, study day 0.

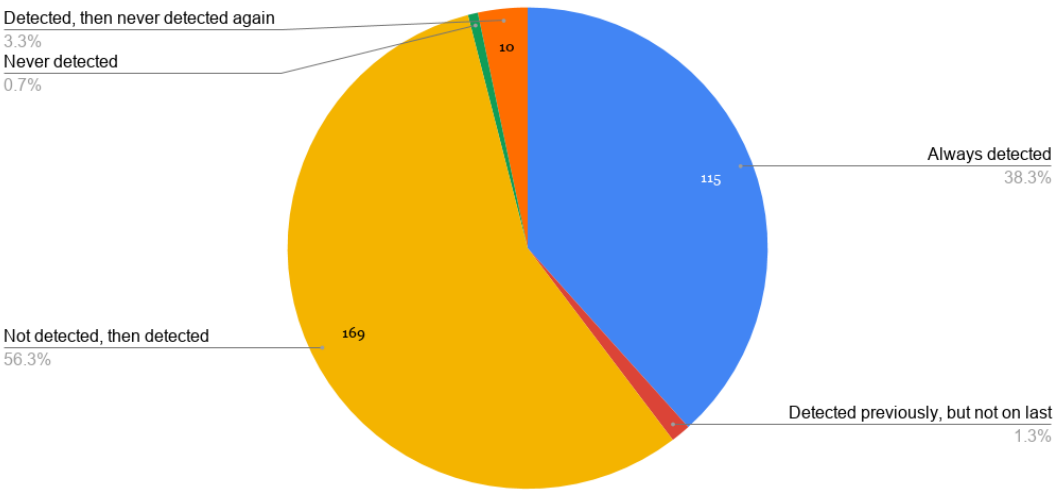

Figure 5. Breakdown of RFID data percentages in 5 categories: 1.) Detected at least once, then never detected again n=10; 2.) Never detected n=2; 3.) Not detected, then detected n=169; 4.) Always detected n=115; 5.) Detected previously, but not on the last survey, n=4; . No individual was included in >1 category.

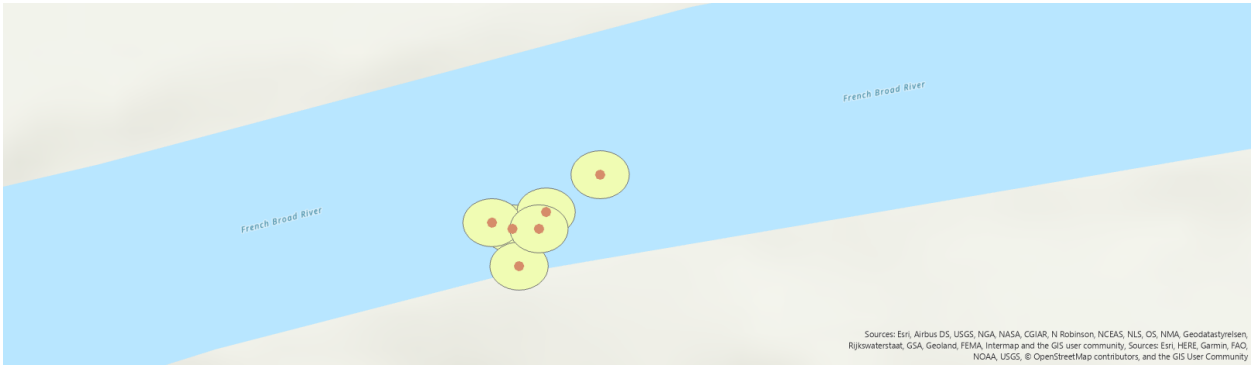

Figure 6. Individual 384.0A03029CC1 with 3m buffer added to GPS points captured each RFID survey. Map created using ArcMap 2018.

4. Discussion

The data collected supports our hypothesis and RFID surveying proved much more effective than VES, detecting 83.4 % of individuals on average. Additionally, RFID CPUE rates were significantly higher than VES rates (Fig.3). On average, Surveyor 2 visually encountered more mussels than Surveyor 1, owing to more experience (Fig. 1). Although VES detection rates (CPUE) seemed to increase with water clarity (secchi reading), there was no significant correlation between CPUE and water clarity (Fig. 2). Perhaps this could be explained by sample size error.

Although PIT tagging can be costly and time consuming[8]. RFID has a much more accurate return on occurring stocked individuals. These results are also shown in a similar study conducted by Zydlewski et al. who found about 40% more individuals using RFID than visual surveys[22]. These findings are important because they give conservationist a better understanding of how stocked individuals are stabilizing in their new habitat. Additionally, RFID leads to more encounter opportunity, thus having the flexibility to collect data on a higher sample size than visually encountered individuals. RFID gives a much higher CPUE rate (Fig. 3), meaning there is a higher return rate given the same amount of effort. Aquatics biologists often survey multiple sites in one day[5], thus a higher CPUE allows for maximization of site surveys within a certain number of survey time allotted per site, allowing for more sites to be surveyed in one day[4].

Because there is variation when looking at the breakdown of detection per individual, further investigation is warranted. The breakdown is as follows: 1.) 10 were detected at least once, then never detected again; 2.) 2 were never detected; 3.) 169 were not detected, then detected; 4.) 115 were always detected and 5.) 4 were previously detected, but not detected on the last survey (Fig. 5). Detection of individuals in scenario 1 could be explained by mussels potentially losing their tags; or perhaps were washed downstream from fluctuating water volume, flow intensity or stress[4]. The lack of detection can certainly be explained in scenario 2 by death, as shells for these individuals were found and identified using RFID.

There were overall more individuals 'not detected, then later detected' (scenario 3). Perhaps the area in which these mussels were inhabiting was missed by the wand. The wand must be directly over the tag to trigger detection, thus tags that were not directly hovered over would conclude undetected. This could be explained by different experience levels by the reader operator. Another reasoning for scenario 3 could be that the mussels were moving in and out of the plot boundaries. Although Unionids are typically very sedentary animals, it is possible they exhibit movement, especially when acclimating to a new environment[5]. Of the 27 individuals that were studied and mapped to analyze any possible movements of the mussels, 20 of them had instances of a movement occurrence, therefore movement could be a possibility. However, there is a lot of room for error in this reasoning, but because there is enough variation looking at our data, we consider further research on movement being a factor for scenario 3.

Missed detection for scenario 3 is unlikely to occur from mussels burying too deep because Unionids bury, on average, up to 20 cm depths[1] and the *Biomark* BP Lite Portable Antenna has a maximum detection distance ranging from 30.5 cm to 43.2 cm, depending on tag orientation, length, and electromagnetic interference[12]. Individuals in scenario 4 were always detected. This could be explained by tag retention and the ability of individuals to acclimate appropriately to their new environment.

Future research should continue to test questions that arose with this study. The literature shows that little research has been studied of mussel movements. Obtaining a better understanding of Unionid home-rage could perhaps generate a more thorough understanding for missed detections. Additionally, further data should be collected and analyzed to see if the experience levels of the tag reader operator correlates with RFID detection.

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7. Endnotes

1. 1. Occurrence record in basin may be result of misidentification.[11]