Submerged Aquatic Vegetation (SAV) SONAR Mapping Surveys in low-salinity habitats: Neuse River

Final Report to Coastal Recreational Fishing License Fund
May 17, 2018

Task Order # 6795

Grant Award Period: July 1, 2016 – Dec 31, 2017

Performance Reporting Period: July 1, 2016 – Dec 31, 2017

Project Costs:

Expenditures for the Period:

Category	Expenditures
Personnel	$ 35,140
Fringe	$ 3,204
Travel	$ 2,245
Equipment	$ 2,500
Supplies	$ 3,687
Construction	$
Contractual	$ 4,481
Other	$ 5,146
Total Direct	$ 56,403
Indirect	$ 8,461
TOTAL	$ 64,864

Total Cumulative Expenditures: $ 64,864
Total Remaining Balance: $ 12,239

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Abstract

Submerged Aquatic Vegetation is essential fish habitat, supporting a diversity of species of marine and estuarine fishes and invertebrates that are commercially and recreationally important. Species such as spotted seatrout, red drum, southern flounder, American eels, pinfish, penaeid shrimp and blue crabs live in seagrass beds and SAV areas in the estuaries of North Carolina. In this Coastal Recreational Fisheries License Fund project, the Neuse River Estuary was surveyed for the presence of Submerged Aquatic Vegetation (SAV) using shore-parallel single-beam SONAR methodology with underwater video verification at regularly spaced (330 m) points. This shore-parallel Rapid Assessment (RA) survey at 1-m bathymetry was used to establish ten Sentinel Sites (each nominally 50 ha, or 123 acres), which were based on the criteria of having previously had SAV, either during our RA survey or historically, for anticipated long-term monitoring. Sentinel site surveys were done using 40 SONAR transects spaced at 25 m, oriented along a shore-normal onshore-offshore axis, in depths from 0.5 m to 2.5 m. At each sentinel site, 100 points were selected at random along the 40 transects in water less than 2 m depth for underwater video verification (comparison with SONAR imagery). Sentinel site transects were extended into shallow water (10 of 40 shore-normal transects selected at random) and were sampled with quadrats and core samplers in depths less than 0.5, using a 1 m^2 quadrat methods and snorkeling surveys done at 0.25, 0.5, 0.75, and 1.0 m depth. SONAR data was processed using the Lowrance and Navico-CMap (http://ciBiobase.com) kriging algorithm for estimating biovolume.

During this survey, SAV was discovered and mapped on the RA survey and within two of the sentinel site surveys. Where SAV was confirmed, it was in the low salinity tributaries (NR-SS-07 in the Trent River, NR-SS-06 in the Neuse River near New Bern). The accuracy of the SONAR method was good, averaging 67% agreement with video verification points on RA surveys, although there were some very low accuracies. Accuracy was better for the intensively sampled sentinel site surveys (average agreement 79%). Most of the agreement error was due to false positives (the SONAR shows SAV as present, but underwater video inspection did not show SAV); false positives occurred in both types of surveys. We recommend that these sentinel sites be monitored by NCDMF staff using modified APNEP SAV boat-based monitoring SONAR and underwater video protocols (Kenworthy et al. 2012) in the future.

Introduction

The submerged aquatic vegetation (SAV) of an estuary provides essential fish habitat. Numerous species of recreationally important fishes use SAV as a protective cover when they are attacked by predatory invertebrates, fishes, and mammals (Jackson et al. 2001; Heck et al. 2003; Barbier et al. 2011). Clams, bay scallops, penaeid shrimp and blue crabs are more abundant in SAV areas than nearby unvegetated areas. Seagrasses also provide significant benefits in terms of carbon sequestration, sediment stabilization, erosion control, and primary productivity that supports estuarine and coastal food webs (Christian and Luczkovich 1999; De Boer 2007; Barbier et al. 2011; Fourqurean et al. 2012; Lamb et al. 2017). However, seagrass and SAV habitat have been found to be declining in many estuaries worldwide (Orth et al. 2006; Waycott et al. 2009; Orth et al. 2010). In North Carolina, SAV losses are apparent from historical observations but the extent to which SAV has been lost remains undocumented. North Carolina has had as the primary objective of the Coastal Habitat Protection Plan (Hart et al. 2016) a goal to develop statistically valid monitoring and mapping program for SAV and seagrass resources in the state in order to protect and conserve this important fishery habitat.

As part of establishing a baseline for the monitoring of SAV in North Carolina estuarine areas, a survey of the submerged aquatic vegetation (SAV) was conducted in the Neuse River Estuary in spring, summer, and fall months during 2016-2017. We used underwater video and single-beam SONAR methods in conjunction with shore-based quadrat and core samples along these transects to survey the SAV along Neuse River shorelines. The work was done to achieve the following objectives:

1. To collect SAV Rapid Assessment surveys along 10-km transects spanning the entire Neuse River shorelines and tributaries.
2. To use the SAV data collected during Rapid Assessment surveys on the Neuse River estuary to select ten Sentinel Sites in the Neuse River estuary and establish a baseline survey for long-term monitoring using the APNEP SAV monitoring protocols using SONAR and underwater video survey of 40 shore-normal transects spaced at 25m in a 1000 x 500 m area (Kenworthy et al. 2012).
3. To estimate the area covered by SAV in the ten Sentinel Sites in 2017 as a baseline for future observations.

Previous Work to Establish Sentinel Sites in North Carolina Estuaries

We began surveys along the North Carolina shorelines for SAV in 2010 as a part of an original CRFL-funded project (Kenworthy et al. 2012) to develop Albemarle-Pamlico Natioanl Estuarine Partnership (APNEP) SAV monitoring protocols. Initially, we worked with small areas of the coast in areas historically with SAV coverage (Newport River near Beaufort, Blount's Bay in the Pamlico River, Sandy Point in the Albemarle, Currituck Sound) to develop these SAV protocols using in this report.

We have previously used the APNEP SAV monitoring protocols in Albemarle Sound (J. J. Luczkovich and Zenil 2016b) and the Pamlico River (J. J. Luczkovich and Zenil 2016a). This Rapid Assessment methodology led to the selection of ten Sentinel Sites in Albemarle Sound. Here, we surveyed 300 kilometers in the Neuse River in 2016 and 2017 using the same Rapid Assessment protocols and 10 Sentinel Sites were selected for SAV monitoring (Figure 1).

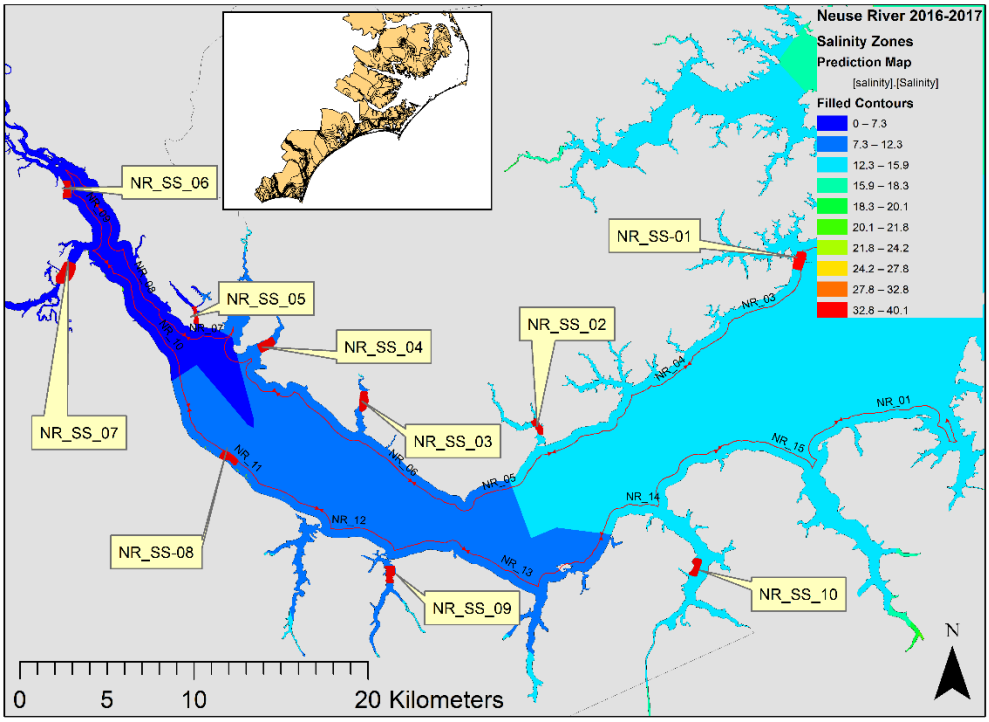

Figure 1 Neuse River Rapid Assessment transects (NR_RA) and Sentinel Sites (NR_SS).

METHODS

Rapid Assessment Transects

The Rapid Assessment Protocol was described in Kenworthy et al. (2012). In a brief recap of this protocol, a small vessel was operated at ~3 knots (1.54 m•s^{-1}) with SONAR transducers mounted on a bracket amidships (Lowrance HDS7 echosounder, 200-kHz single-beam and side-scan transducers, and BioSonics DTX echosounder with either 400-kHz or 200-kHz transducers). The vessel was piloted along a 10-km transect at 1 m depth parallel to the shoreline. This depth was based on previously studied onshore-offshore transects along a depth gradient that showed the peak SAV percent cover occurred 0.7 – 1.0 m depths, and if SAV was present in an area, it would be observed on SONAR at 1 m. Surveying was done along the entire shoreline of the Neuse River Estuary and tributaries as indicated in Figure 1. At intervals of 330 m from the start of each transect, we dropped a video camera (Sartek, model #SDC-MSS) mounted 13-cm (the focal length of the camera) above the end of a pole to the bottom and obtained 30 video comparison points along the transect. The area of the bottom that is in view

with this camera system is approximately 0.06 m^2 (Figure 2)

Figure 2. The bottom view of the video drop camera as measured under laboratory conditions with the camera mounted 13-cm above the bottom. The grid squares are 10 cm on a side, or 100 cm^2.

A GPS (latitude, longitude in decimal degrees) waypoint was set on the Lowrance HDS7 at each comparison point. The presence or absence of SAV was noted on field data sheets along with the waypoints and later these data from video inspection of the bottom and SONAR log files at each point were compared and assessed for agreement. Once the data files were obtained, they were transferred to be processed in the laboratory by methods described below.

Our methods differed in that we used a wider spacing of video verification points than the Kenworthy et al. (2012) protocol. The Rapid Assessment protocol developed by Kenworthy et al. (2012) used a spacing of 100 m between video points. While that was desirable for the development of the method, which only examined nine 10-km transects (90 km total) in great detail, it became impractical to apply when applied over a greater distance (thirty 10-km transects or 300 km). The wider spacing of 330 m between video points was used in the present study to cover the very large areas in the Neuse River Estuary (NRE). This was also used in the previous RA transects done in Albemarle Sound (J. J. Luczkovich and Zenil 2016b) and the Pamlico River Estuary (J. J. Luczkovich and Zenil 2016a). The patch length of SAV along the shore was used to determine the distance to use between video verification points. Kenworthy et al. (2012) determined the patch length of SAV was large in certain areas like Albemarle Sound (at Sandy Point, 1730 m; mean maximum patch length = 424 m), but the average patch length was relatively small (4.9 m). The best spacing between video verification points might be ~5 m, but this would represent a huge sampling effort at the scale of the entire NRE (60,000 video verification points). Smaller spacing could be done in the future at targeted sites once the largest areas of SAV are mapped and monitored across the entire Neuse River in this preliminary large-scale effort. As a result, our method used here confirmed the presence of SAV large beds (at maximum patch lengths), but missed very small patches or average-sized SAV patches. We relied on SONAR methods to uncover these smaller areas of SAV.

Sentinel Sites

Sentinel Sites were established from a set of possible sentinel sites, each 1-km long x 0.5 km from shore covering the entire shoreline of the estuaries under investigation, and selections were made according to the following criteria:

1) Presence of SAV in a historical GIS map layer provided by the NC Division of Water Quality, NC DMF, and NOAA;
2) The presence of SAV in the rapid assessment (RA) surveys conducted in fall of 2016 and spring of 2017;
3) Presence of SAV in video verification points in the RA surveys.

Bins that met those criteria were included in the final list of potential sentinel sites. The sites were selected utilized the "select by location" tool in ArcMap GIS. The bin layer was used as the "target layer" and the historical layer was used as the first "source layer." The "select features from" selection mode were used and the "intersect" was selected as the spatial query. To that selection, we further selected from "current selection" those that intersect with "SAV Video present" and "SAV SONAR present." Each of the bins has an individual identification number and 10 random bins were selected from the pool. The original proposal stated that we would sample 20 sentinel sites, but after discussion with the NC DENR staff (Anne Deaton), it was decided that it would be better to include the Neuse tributaries in the Spring 2017 work period, deviating from the original plan, and monitor 10 sites. Therefore, ten sentinel sites were established (Figure 1).

Sentinel sites transect delineation: Each of these sentinel sites is nominally defined by an area of 1000 m along the shore by 500 m offshore (50 ha or 123.6 acres). The sentinel sites were monitored for SAV using sonar and underwater video as explained in the alongshore study above, but sentinel sites had 40 shore-normal transect lines spaced 25 m apart. The transect lines were selected a priori using Digital Shoreline Analysis System (DSAS), which is a free ArcMap GIS tool commonly used to generate transect lines (Thieler et al. 2003). In addition, 100 random video verification points were generated in ArcMap GIS to compare the SONAR accuracy to the video data.

SONAR data analysis:
SONAR log files (*.sl2) recorded from the Lowrance echosounder were uploaded to the ciBiobase SAV mapping cloud-based data analysis and long-term storage website (www.cibiobase.com). The ciBioBase analysis on this website uses an SAV classification algorithm that is similar to the SAVEWS (SAV Early Warning System) developed by the US Army Corps of Engineers (Sabol et al. 2009). Once SAV has been classified as present at a DGPS location, the ciBiobase analysis software applies a geostatistical kriging algorithm to estimate the percentage biovolume of SAV along the transects. After processing, the files of vegetation percentage biovolume (BV) were exported from the ciBiobase account and imported into ArcMap GIS as *.csv files. As each of the RA transects and sentinel sites surveys had 30-40 individual SONAR log files, individual SONAR log files were merged within each RA transect and sentinel site area utilizing the "merge trips" function in the ciBiobase interface. Once the original SONAR log files were merged within the RA transect or sentinel site, they were reprocessed by ciBiobase for depth, SAV presence, bottom composition and percentage vegetation biovolume by the ciBiobase algorithm analysis. The ciBiobase cloud-based analysis software generated "vegetation" layer data that was exported as either "point data" (georeferenced SONAR data averaged at recorded GPS positions) or "grid data" (kriging interpolation output layer with points at regular intervals, ~5 m apart) into a .csv file. A single

"point" in the point file output represents a summary of sonar pings and the derived bottom and canopy depths for a GPS location. Individual point data thus represent an irregularly spaced dataset that may have overlaps or gaps in the points due to vessel speed variation during acquisition. On the other hand, the "grid" data exported from ciBiobase are actually geostatistical interpolated grids: SONAR data were interpolated by ciBiobase kriging algorithms and evenly spaced (~5 m) values representing the kriging output of aggregated data points were exported. Both of these exported files included an individual record id number, latitude and longitude (WGS 1984 datum), depth to plant canopy (in feet), plant height (in feet), percentage biovolume, and the date the original SONAR file was created. Percentage biovolume (BV) is defined as (from the www.ciBiobase.com website):

$$BV = \frac{Plant\ height\ (m)}{depth\ (m)} \times 100$$

with BV measured from the average of all "good" SONAR signals received at each DGPS location (obtained at 1-s intervals). When the echosounder data were processed using the ciBiobase algorithm a filter was applied so that only "good" signals were used to process the vegetation data by ciBiobase. The quality-assurance screening algorithm was used during data processing by ciBioBase to clean the data so that lost-bottom and noisy signals were not included in estimates of SAV presence and BV, and thus 10-20 SONAR records were averaged every 1-3 m (depending on vessel speed). GPS location fixes are reported by the Lowrance HDS7 echosounder along the transect every 1 s, GPS uses WAAS real-time differential correction (if available), and the GPS receiver will track up to 32 satellites, so given the vessel speed we used (~3-5 knots), the data collection points were spaced between 1 and 3 m apart. Because the GPS location fixes were obtained 10 times per s (10 Hz) and SONAR pings were obtained 20 times per second (20 Hz) by the Lowrance HDS7 echosounder, this combination produced a position accuracy of less than 1.0 m. Bottom depth and plant height were given in feet, so after exporting the data new columns with the information in meters was generated for each of the files. Once transformed, the .csv files were imported into ArcMap 10.5.1. The data were imported as tables and displayed using the echosounder GPS default WGS 1986 projection, but then re-projected on a map using NAD 1983 HARN UTM Zone 18 N coordinate system so that distances represented meters. Maps with the percentage biovolume information and percent coverage for the area were generated utilizing Arc Map GIS. See Appendix I for step-by-step procedures that were used to import ciBiobase data into Arc Map 10.5.1.

Underwater video analysis: the underwater water video data was brought to the lab, and each video was analyzed for the presence/absence of SAV. In addition, the type of substrate at each video point was classified (i.e. SAV, sand, stump, and detritus). The presence or absence of SAV, substrate type, and latitude and longitude data was stored in an excel file for each of the transects. The information was imported into ArcGIS and projected in the same way as the SONAR data.

Video-verification points: At the alongshore Rapid Assessment (RA) and shore-normal transects within Sentinel Sites (SS), we performed video verification of SONAR. Due to the vessel drifting during the video examination of the bottom, some of the video points may not co-occur on the SONAR transects but are within 3 m of each video waypoint. Along the RA transects,

video verification waypoints were selected at regular intervals in the field every 330 m using the GPS to determine the distance from the start of the RA transect or the last video verification point visited. Within each of the sentinel sites, we selected 100 random points in advance for video verification along the transect lines (with a criterion that they have a minimum distance of 1 m separation). We created the random points along a transect line using the Arc Map 10.5.1 "create random waypoints" tool (under Toolbox/Data Management/Sampling), which were exported to the Lowrance GPS as a GPX file.

A procedure was used to import the data to ArcMap 10.5 from ciBiobase vegetation layer (kriging layer of SAV biovolume) as an exported grid file (~4-5 m cell spacing) within a 25-m width along the vessel track. This procedure results in a map display in ArcMap that is the same as the default display in ciBiobase, and it is a bit of an extrapolation beyond the smaller swath collected with the echosounder (just 20-cm wide below the vessel), but this was necessary for visualization of the SONAR layer at the scale of the whole Neuse River Estuary (NRE). Because of interpolation of multiple SONAR pings in an area as the vessel moved, these interpolated outputs are what are being evaluated versus the video, which allows inspection of a very small area (0.25 m^{-2}) of the bottom. After importing, the grid layer output of the ciBiobase kriging algorithm (with 4-5 m cell size between grid points) was projected in NAD 1983 HARN UTM Zone 18N, and a spline interpolation procedure with a grid cell size of 0.8 m was applied. This spline procedure further smoothed the grid file as a raster image and made the SAV SONAR layer a continuous band 25-m wide along the vessel track. A color ramp developed for this report was applied to the classified image, with biovolume < 0.05 shown as tan (SAV absent), > 0.05 shown as light green (SAV present in low density), and > 0.50 shown as dark green (SAV high density). After over-plotting the data from the video point comparisons file in ArcMap and the SAV biovolume from the SONAR, it was possible to calculate the accuracy measure:

$$Accuracy\ \% = \frac{True\ Positive\ Points + True\ Negative\ Points}{Video\ Points} \times 100$$

Where:
True Positive Points are those in agreement that SAV is present on both SONAR and video at a given point.
True Negative Points are those in agreement that SAV is absent on both SONAR and video at a given point.

The procedure for this video and SONAR comparison is given step-by-step in Appendix II. ArcMap ESRI Shapefiles with the video data latitude and longitude with SAV presence (SAV = 1) or absence (SAV = 0) have been placed on the Open Science Framework archive for this project: osf.io/af354. (J. Luczkovich et al. 2018). From these shapefiles, *.csv data can be exported.

In order to understand species composition of the SAV discovered at the sentinel sites and to cover areas unreachable by boat with SONAR and video, quadrat and core samples were taken along randomly selected transects that were extended into shallow water. Ten of the 40 transects were selected at random within each sentinel site. A snorkeler and note taker observed the

percentage cover of the SAV with three 1 m^2 quadrats placed at four depth strata: 0.25m, 0.5m, 0.75m, and 1.0m depths placed along each transect, using a marked PVC pole to establish each depth. GPS positions were taken at each depth stratum along each transect sampled. Random placement of the quadrats was done on a 25-m cross transect at each of the depth strata listed (Duarte and Kirkman 2001). Cores (15.24 cm diameter) were taken at a depth stratum if SAV was discovered within the quadrat samples. Core samples were identified to species and SAV dry biomass determined. Dry biomass was measured after drying in an oven at 60°C for 48 h to ensure constant dry mass was obtained.

RESULTS

The salinity of the Neuse River estuary is largely fresh water on the western side, rising to 14 ppt or more at transects and sites located at the mouth of the estuary (Figure 1). As a result of this salinity gradient, most of the SAV (most species present and observed in this survey in the Neuse do not survive in salinity greater than 10 ppt) was in the western end of the estuary.

Rapid Assessment Survey: Rapid Assessment surveys were conducted in Fall of 2016 (NR-RA-01 through NR-RA-15, in the main part of Neuse River estuary, completed 13 September through 2 November 2016) and Spring 2017 (NR-RA-17 through NR-RA-30, tributaries from 15 May and 2 June 2017). Interpretation of the SONAR and video comparison points agree that the major areas with SAV were identified in very low-salinity regions and in some of the tributaries of the Neuse River. Extensive SAV areas were discovered and mapped in Beard Creek (NR-RA-19); the western upstream end of the Neuse River estuary, in the Trent River, close to the junction with the Neuse River near New Bern, NC (NR-RA-22); and in the Neuse River by New Bern, across from and near Bridgeton, NC (NR-RA-); other smaller areas of SAV were discovered during the RA surveys in tributaries farther downstream on the Neuse River, such as in Fairfield Harbor (NR-RA-21), in Upper Broad Creek (NR-RA-21), in Goose Creek (NR-RA-20), in Beard Creek (NR-RA-19), in Dawson's Creek (NR-RA-18), and Broad Creek (NR-RA-17), all or which are on the North side of the river, and in Hancock Creek (NR-RA-24) and Adams Creek (NR-RA- 27 and NR-RA-28) on the south side of the river (Figure 2). A good example of the RA transects that revealed high amounts of SAV was in Beard Creek Overall accuracy (agreement of SONAR and video) of the rapid assessment method was 66.5% (Table 1); there was a high false positive rate (32.1%) and a low false negative rate (1.4%). In the Trent River, where SAV was dense in places, this RA survey yielded higher accuracy than in a previous study (Kenworthy et al. 2012) using similar methods. The accuracy of the SONAR as compared with underwater video in the Trent River was 88.9% (24 agreements in 27 video point comparisons), with two false positives (SONAR indicated SAV, but without video confirmation), and one false negative (SONAR indicated no SAV, but video confirmed SAV; Figure 3). A lower than average accuracy was observed in transects NR-RA-01 (e.g., Figure 4), NR-RA-08, NR-RA-09, NR-RA-14, NR-RA-16, NR-RA-20, NR-RA-23, NR-RA-26, NR-RA-27, and NR-RA-28. These transects had high levels of false positives, in which the SONAR indicated SAV was present but video inspection did not confirm the presence of SAV. The lower accuracy due to false positives occurring in these transects, as compared with others, could have been due to vessel GPS position inaccuracy (± 3 m), improper transducer alignment (this condition was noticed by the ciBiobase reviewer for NR-RA-28, which had 0% accuracy), very sparse SAV, small SAV beds, patchy SAV distribution (the video camera area was small relative to the SONAR coverage, and the video points are widely spaced, so that these factors make SAV

rare and unlikely to occur in both the video and SONAR), or the presence of other material (detritus or algae) on the bottom that was not SAV.

Plant height is a factor of error in any SONAR method, as short plants will be missed due to a plant height threshold criterion that is applied in the SAV detection algorithm. In ciBiobase, only SAV plants taller than 5% of the water depth (at 1 m, this would be a 5-cm plant height threshold) are declared present in the analysis and kriging output. Plant height is incorporated in the percentage biovolume estimate, and only values > 0.05 BV are included in the kriging analysis. Plants that are shorter than this plant height criterion are often visible at video verification points, but because they are excluded from kriging of SONAR data, these plants result in a false negative SAV video points. This happened occasionally (9 points of 629 along RA transects, or 1.4% false negatives in Table 1; 86 of 1002 points in the sentinel site surveys, or 8.5% false negatives in Table 3).

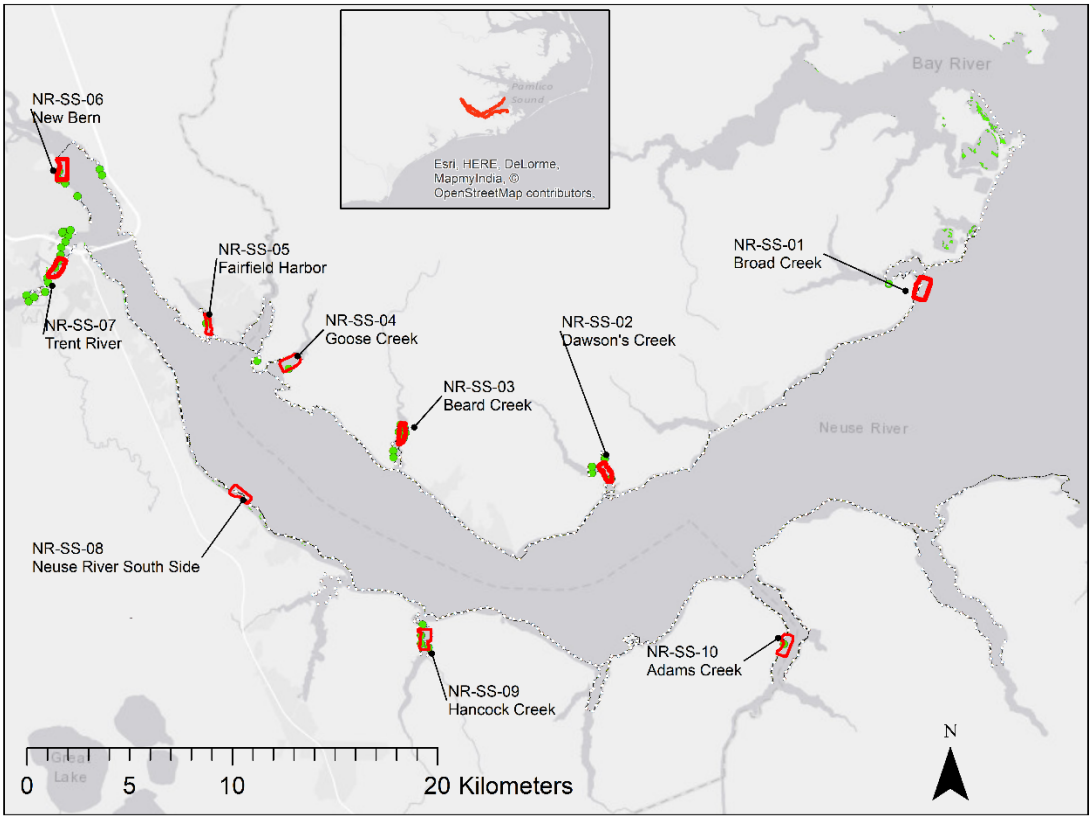

Figure 3. Sentinel Site locations as identified by the criteria listed in the text. Green symbols indicate SAV-positive video comparison points in the RA survey. The video comparison points indicate the presence of SAV (large green symbols) and SAV-negative video points (small open white symbols along SONAR track). Green polygons (e.g., NR-RA-10, NR-RA-11) show the historical SAV map layer obtained from previous work (Kenworthy et al. 2012).

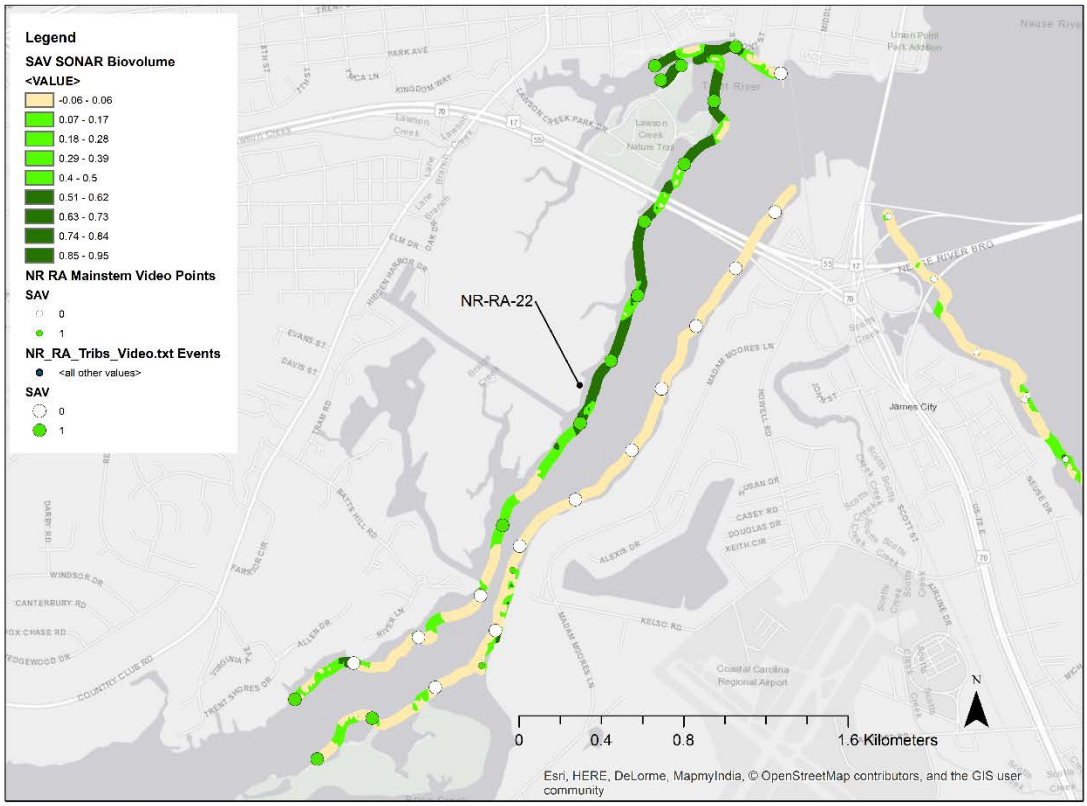

Figure 4. Rapid assessment transects in the Trent River surveyed 16 May 2017. Comparison of the SONAR track alongshore (NR-RA-22) and underwater video comparison points (SAV-positive is shown as green points, SAV-negative as white points). The colors show the biovolume of SAV as measured along the transect. Accuracy was 88.9%.

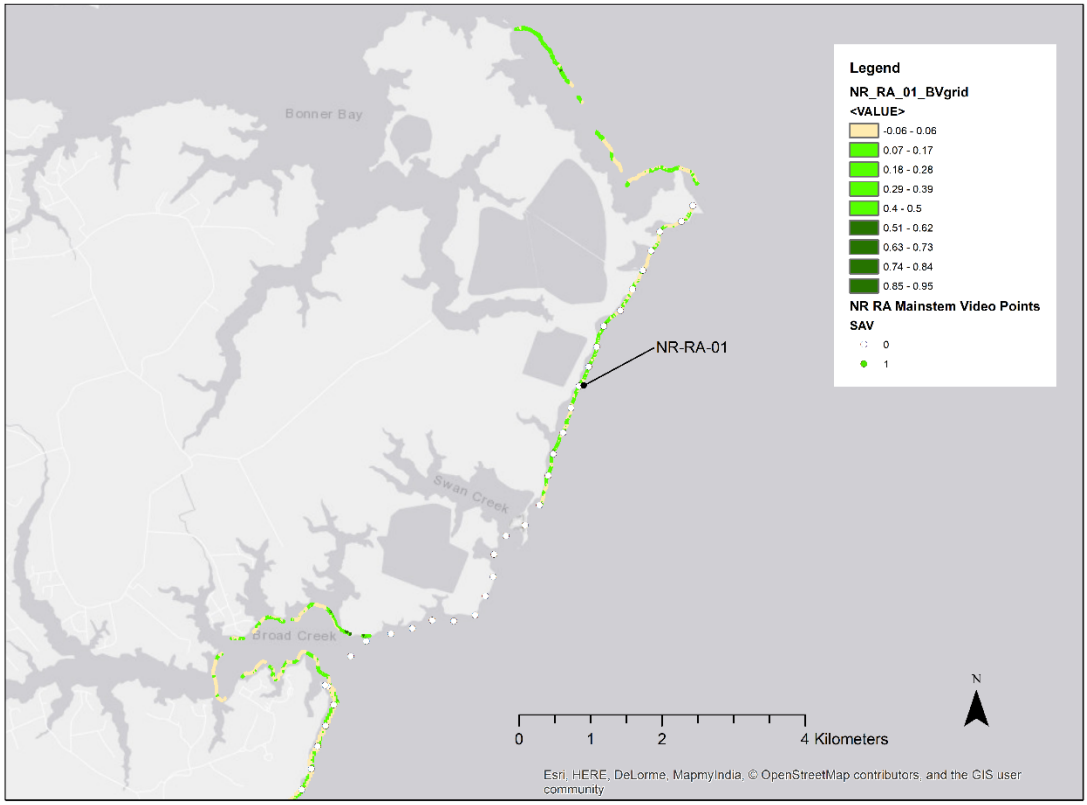

Figure 5. The SONAR track at NR-RA-01 surveyed 1 November 2016, with video comparison points. The colors show the biovolume of SAV as measured along the transect. No SAV was observed at any video comparison points; accuracy (agreement that no SAV was present) was 18.75 %, with 81.25% false positive rate (SONAR indicated SAV, but with no video confirmation).

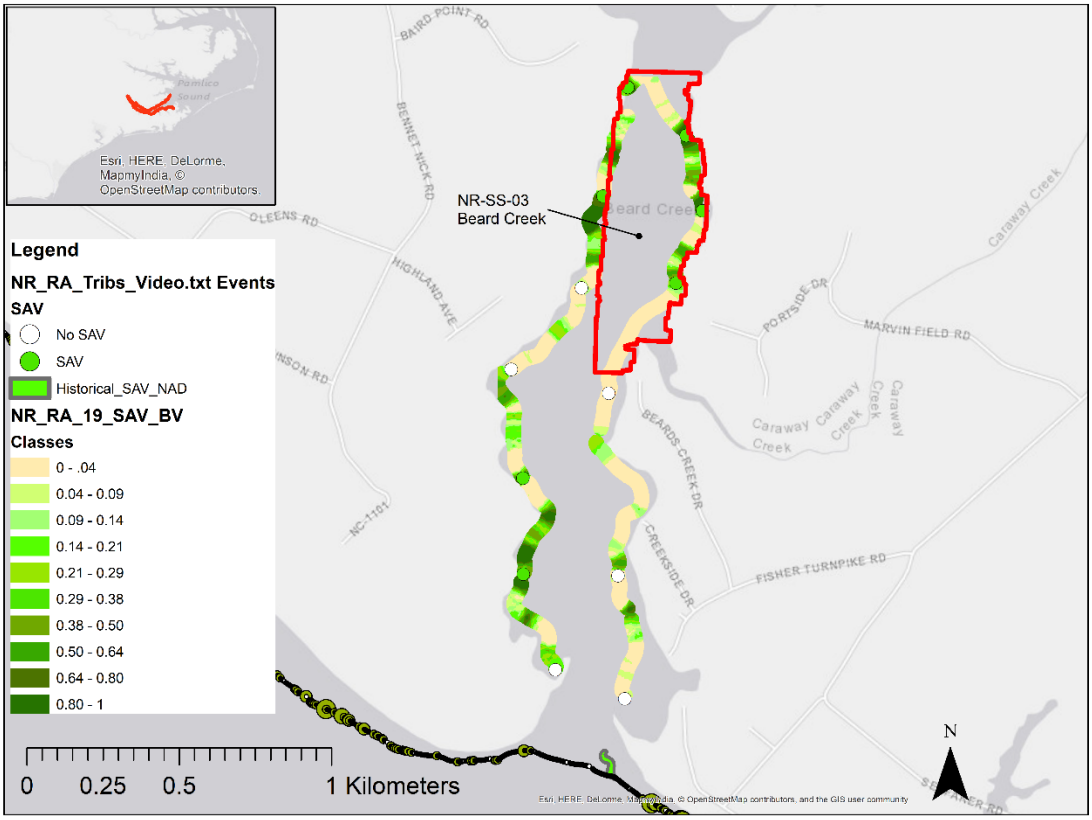

Figure 6. Rapid Assessment transect NR-RA-19 at Beard Creek (also later surveyed as part of the Sentinel Site survey, NR-SS-03 on 28 Oct 2017). Survey date for the RA transect was 31 May 2017.

Table 1. Accuracy Estimate for Rapid Assessment survey in Neuse River. Transect ID, date of survey, number of video-sonar verification points, number of agreements between SONAR and video, number of false positives (SONAR SAV positive, video SAV negative), number of false negatives (SONAR SAV negative, video SAV positive), and percentage accuracy.

Transect	Survey date	Number of Verification Points	Number of Agreements	False Positives	False Negatives	Accuracy (%)
NR-RA-01	1 Nov 2016	16	3	13	0	18.7
NR-RA-02	1 Nov 2016	30	20	10	0	66.7
NR-RA-03	26 Oct 2016	29	27	2	0	90.0
NR-RA-04	31 Oct 2016	29	15	14	0	51.7
NR-RA-05	31 Oct 2016	34	31	3	0	92.2
NR-RA-06	31 Oct 2016	24	20	4	0	83.3
NR-RA-07	13 Sep 2016	30	29	1	0	96.7
NR-RA-08	13 Sep 2016	18	8	6	4	44.4
NR-RA-09	15 Sep 2016	30	13	17	0	44.3
NR-RA-10	11 Nov 2016	21	13	8	0	61.9
NR-RA-11	28 Oct 2016	31	25	6	0	80.6
NR-RA-12	28 Oct 2016	30	25	5	0	83.3
NR-RA-13	2 Nov 2016	28	21	7	0	75.0
NR-RA-14	2 Nov 2016	28	11	17	0	39.3
NR-RA-15	2 Nov 2016	No video				
NR-RA-16	19 May 2017	12	2	10	0	16.7
NR-RA-17	19 May 2017	17	12	4	1	70.6
NR-RA-18	17 May 2017	16	12	4	0	75.0
NR-RA-19	31 May 2017	13	13	0	0	100.0
NR-RA-20	22 May 2017	9	5	3	1	55.6
NR-RA-21	22 May 2017	27	19	8	0	70.4
NR-RA-22	16 May 2017	27	24	2	1	88.9
NR-RA-23	16 May 2017	14	8	6	0	57.1
NR-RA-24	16 May 2017	15	11	3	1	73.3
NR-RA-25	1 Jun 2017	16	12	4	0	75.0
NR-RA-26	1 Jun 2017	6	4	2	0	66.7
NR-RA-27	16 May 2017	16	1	15	0	6.25
NR-RA-28	16 May 2017	19	0	19	0	0
NR-RA-29	15 May 2017	21	16	5	1	76.2
NR-RA-30	15 May 2017	24	19	4	0	79.2
Totals All Transects		**630**	**419**	**202**	**9**	**66.5**

Sentinel Site Surveys: The ten sentinel sites were visited in summer and fall of 2017. Each site was nominally 50 ha (1000 m x 500 m), but many of the sites were located in the Neuse River estuary tributaries, places where we discovered most of the SAV during the rapid assessment surveys. Thus, the actual survey area was often less than this, with the smallest area surveyed (NR-SS-05) being 26 ha (Table 2). The salinity at the sites ranged from nearly fresh water at the western end to mesohaline salinity near the eastern end of the estuary (1.1 ppt at the New Bern site NR-SS-06 to 20.6 ppt at NR-SS-10 in Adams Creek).

The biovolume of SAV from the SONAR surveys are shown for each site in the figures that follow (NR-SS-01, Figure 5; NR-SS-02, Figure 7; NR-SS-03, Figure 9; NR-SS-04, Figure 11; NR-SS-05, Figure 13; NR-SS-06, Figure 15; NR-SS-07, Figure 17; NR-SS-08, Figure 19; NR-SS-09, Figure 21; NR-SS-10 Figure 23). The color scheme shows the biovolume of SAV as varying colors of green to red, with blue representing the absence of SAV. Later, we discuss the accuracy of these SONAR maps of SAV by comparing the classifications to video inspections at random points. The amount of SAV area coverage was computed from these images by the ciBiobase software for these areas and are represented as percentage of the survey area covered by SAV in Table 2.

The areas with the most extensive SAV based on both the SONAR and underwater video were at the sites NR-SS-06 (New Bern Figure 15) and NR-SS-07 Trent River Figure 17). No SAV was observed during the sentinel site surveys at the other sites. Core sampling and quadrat surveys done at these sites showed that the SAV species present were wild celery *Vallisneria americana* (NR-SS-07) and widgeon grass *Ruppia maritima*, southern water nymph *Naja guadalupensis*, and muskgrass algae *Chara* (NR-SS-06).

There was no statistical tendency for greater coverage of SAV as measured by SONAR to occur in lower salinities (correlation test, $r = 0.001$, $p = 0.99$); however, the SAV beds that were confirmed by video and quadrat sampling in the sentinel sites were located in low salinity (NR-SS-06 and NR-SS-07, see Figure 1). Secchi depth was measured each survey date and did not show any pattern of increased SAV with greater Secchi depth (correlation test, $r = -0.009$, $p = 0.97$). However, these data are single-time-point samples taken during the SONAR survey and are not likely to be very useful in explaining long-term SAV abundance patterns. Long-term variability in salinity and Secchi depths are of greater significance for SAV occurrence and survival.

Accuracy of SONAR as compared to video was high at most sites (Table 3, mean = 79%, 72-96 % agreement; see Figure 6, Figure 8, Figure 10, Figure 14, Figure 16, Figure 18, Figure 20, Figure 22, Figure 24), but was very low (39%) at one site, NR-SS-04 (Figure 12). True positives (SAV on both SONAR and Video inspection of the sites) for SAV occurred only at sites NR-SS-06 (Figure 16) and NR-SS-07 (Figure 18) confirming the presence of SAV beds at these two sites alone. The low accuracy at NR-SS-04 was due to a very high occurrence false-positive video comparison points (SONAR indicated SAV, but video inspection of the bottom did not show SAV). These false positive points were likely due to detrital material that apparently came from adjoining salt marshes, or perhaps dead SAV from earlier growth in the season, which covered the bottom. The bottom video imagery from this site showed a good deal of flocculent

material wafting up off the bottom when the camera was placed there (Figure 25). Some emergent algae or other organisms (tunicates or hydroids) were also present on the bottom here. This flocculent layer with emergent structure on the bottom created conditions in the SONAR processing algorithm which resulting these areas being classified as having low-levels of SAV (< 0.50 Biovolume), which resulted in 61% of the comparisons being false positives (Table 3). For comparison, a video frame for NR-SS-07, Trent River, at waypoint 53 which was a true positive point (SAV present on SONAR map and in the video image) is shown in Figure 26. The dense SAV bed of wild celery *Valisneria americana* is clearly visible.

We also observed percentage cover of SAV at this sentinel site using quadrats and confirmed the species of SAV by obtaining a core sample. The map of NR-SS-07 with quadrat data plotted on the SONAR map is shown in Figure 27. The percentage cover is also provided for each quadrat shown and the color of the symbols show the presence or absence of SAV. There was very good agreement between the percent cover estimated directly by the quadrat method in the snorkeling and the SONAR survey. The SAV on the snorkeling transects was largely restricted to quadrats in depth strata 0.75 and 1.0 m; none was recorded in depths ≤ 0.5 m (Figure 28). At this site, the shore line was developed with bulkheads, and there were many docks. There was nearly 100 cover at most of the deeper quadrats, and none in the shallow waters, suggesting that the bulkheads and resulting wave action may have played a role in limiting the SAV. Light was not likely to be a limiting at these shallow depths.

Although we used the same quadrat survey technique at every sentinel site in this study, we only recorded SAV at two sites in the quadrats: NR-SS-06 (New Bern) and NR-SS-07 (Trent River). The absence of SAV at the other eight sentinel sites, the high rate of false positive video verification points and the SONAR maps showing putative SAV areas at these sites suggested that the SONAR method overestimated the SAV area of coverage. The SONAR maps thus should not be regarded as representing SAV accurately at these sites. The SONAR technique is sensitive to detritus and other material (which could represent SAV detrital material from previously growing SAV) lying on the sediment. In the future surveys that use this SONAR technique should include ground truth point for verification as we have done here, to assess accuracy.

Conclusions and Recommendations:

1) Rapid Assessment technique of the entire shoreline of the Neuse River and its tributaries revealed some areas of SAV for setting up intensive sentinel site monitoring locations. These areas were located in the lower salinity regions of the Neuse River estuary.
2) This study represents a baseline sentinel site survey for SAV in the Neuse River Estuary. Very little SAV was discovered, limited to just two sites, in the summer and fall of 2017.
3) The survey at the sentinel sites will be re-surveyed this year to see if change in SAV coverage has occurred.
4) This repeat study will be funded by APNEP through another National Fish and Wildlife Foundation Grant to examine the impacts of the coal ash spill that occurred in the Dan River (upstream in the Neuse River watershed) in 2014.

Table 2. Sentinel Site locations, latitude and longitude of the start of first transect, survey date, environmental data: temperature °C, salinity (ppt), Secchi depth (m), SAV area estimated from SONAR (ha), extent of survey Area (ha), percent of the survey area covered by SAV, mean percentage biovolume where SAV exists, and the standard deviation of the mean biovolume (BV).

Site ID	Latitude (dd)	Longitude (dd)	Date	Temper-ature °C	Salinity (ppt)	Secchi depth (m)	SAV Area (ha)	Survey Area (ha)	Percent of Area Covered by SAV	Mean Biovolume (where SAV exists)	Standard Deviation of BV
NR-SS-01	35.08818726	-76.5853773	28-Jun-17	29.5	17.01	0.8	2.7	67.2	4	11.7	10.6
NR-SS-02	35.00121689	-76.75696564	3-Jul-17	29.4	12.7	0.8	1.2	29.1	4.1	7.1	2.8
NR-SS-03	35.01338163	-76.8668379	28-Oct-17	18.3	9.6	1.1	1.7	33.0	5.2	7.2	3.7
NR-SS-04	35.05158234	-76.92350769	26-Jul-17	29.0	15.5	0.9	12.1	47.7	25.4	10.1	6.8
NR-SS-05	35.05970764	-76.97053528	19-Jul-17	29.6	6.8	1.0	3.8	26.6	14.3	9.7	6.1
NR-SS-06	35.12640478	-77.05128757	13-Oct-17	24.9	1.1	1.4	4.1	52.7	7.7	14.1	8.5
NR-SS-07	35.09107835	-77.05129658	18-Jul-17	27.4	2.7	1.3	5.7	42.3	13.4	13.6	11.1
NR-SS-08	34.98895056	-76.94648007	27-Jul-17	28.7	12.6	0.9	7.0	48.2	14.5	22.1	19.6
NR-SS-09	34.93236714	-76.85520164	1-Aug-17	26.4	15.7	1.0	2.9	52.8	5.5	13.5	10.6
NR-SS-10	34.93230802	-76.66028745	4-Aug-17	27.6	20.6	0.8	2.8	49.9	5.7	13.0	11

Table 3. Accuracy Estimate for Sentinel Site survey in Neuse River. Site ID, date of survey, number of video-sonar verification points, number of agreements between SONAR and video, number of false positives (SONAR SAV positive, video SAV negative), number of false negatives (SONAR SAV negative, video SAV positive), and percentage accuracy.

Sentinel Site	Survey date	Number of Verification Points	Number of Agreements	False Positives	False Negatives	Accuracy (%)
NR-SS-01	28 Jun 2017	100	93	7	0	93.0
NR-SS-02	3 Jul 2017	100	79	21	0	79.0
NR-SS-03	28 Oct 2017	101	84	17	0	83.2
NR-SS-04	26 Jul 2017	100	39	61	0	39.8
NR-SS-05	19 Jul 2017	99	80	19	0	80.8
NR-SS-06	13 Oct 2017	100	74	16	6	78.0
NR-SS-07	18 Jul 2017	101	72	10	19	72.0
NR-SS-08	27 Jul 2017	100	96	6	0	96.0
NR-SS-09	01 Aug 2017	100	89	11	0	89.0
NR-SS-10	4 Aug 2017	101	89	12	0	88.1
Totals		**1002**	**795**	**119**	**86**	**79.3**

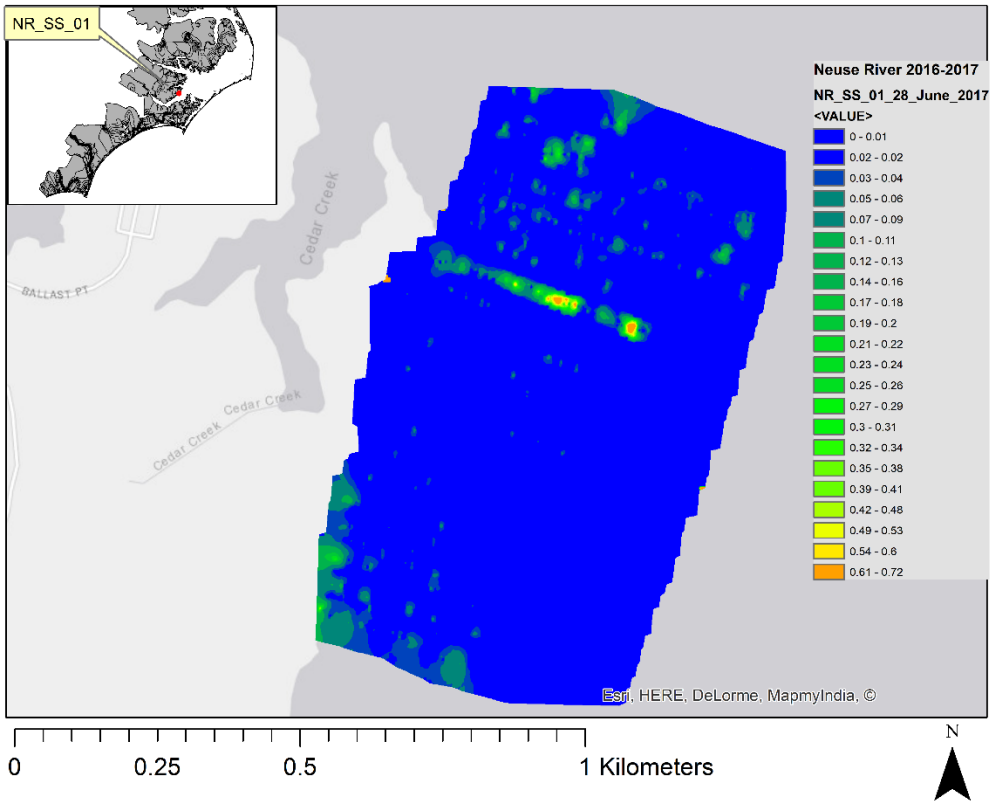

Figure 7. The Biovolume of SAV at NR_SS_01 on 28 June 2017.

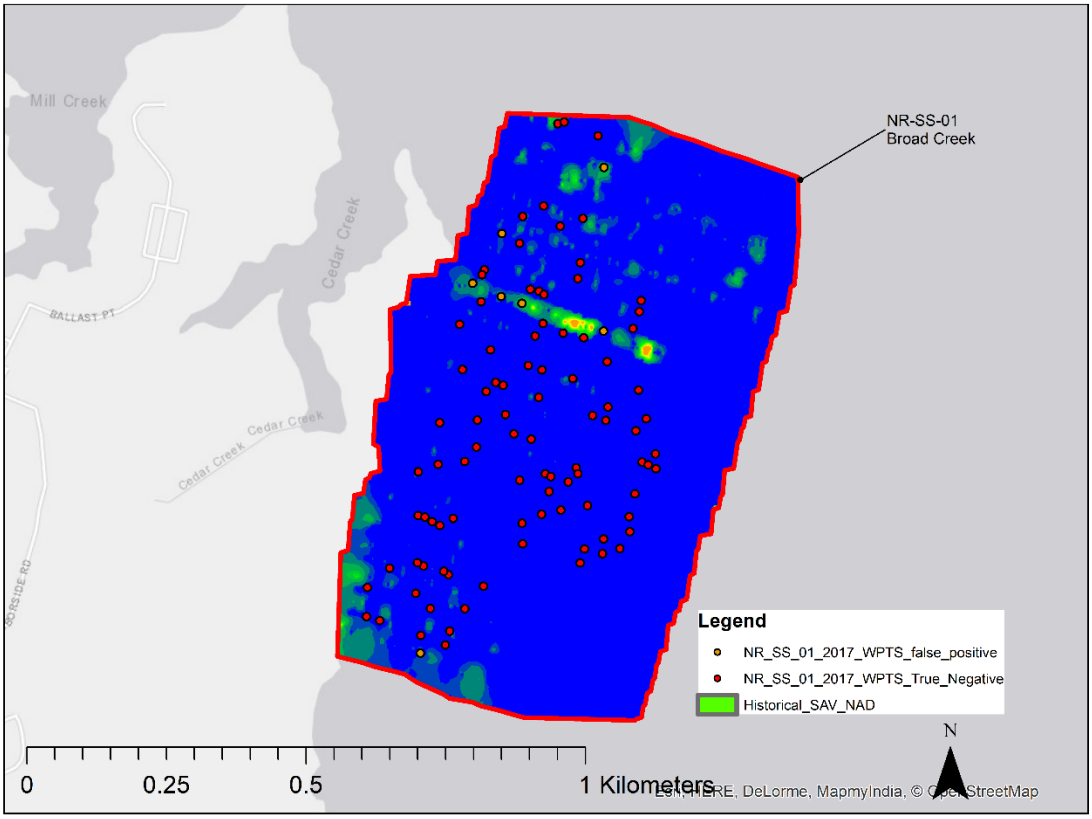

Figure 8. NR-SS-01 Video comparison points on 28 June 2017, 93% accuracy.

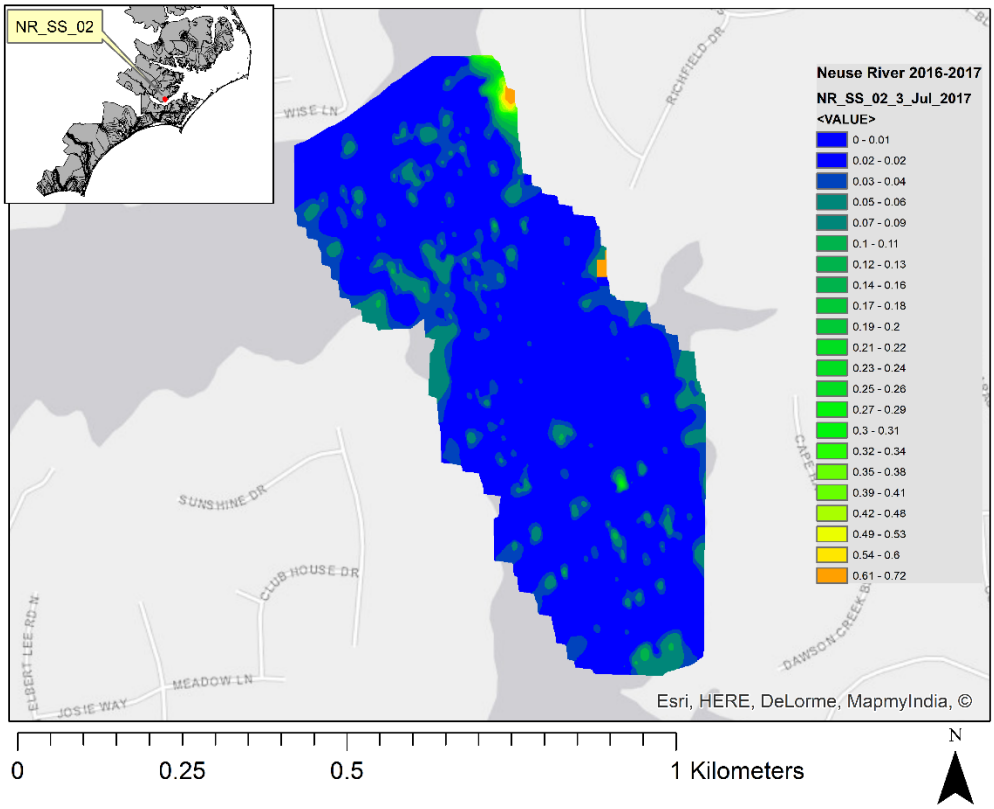

Figure 9. NR-SS-02. Biovolume of SAV in SONAR survey on 3 July 2017.

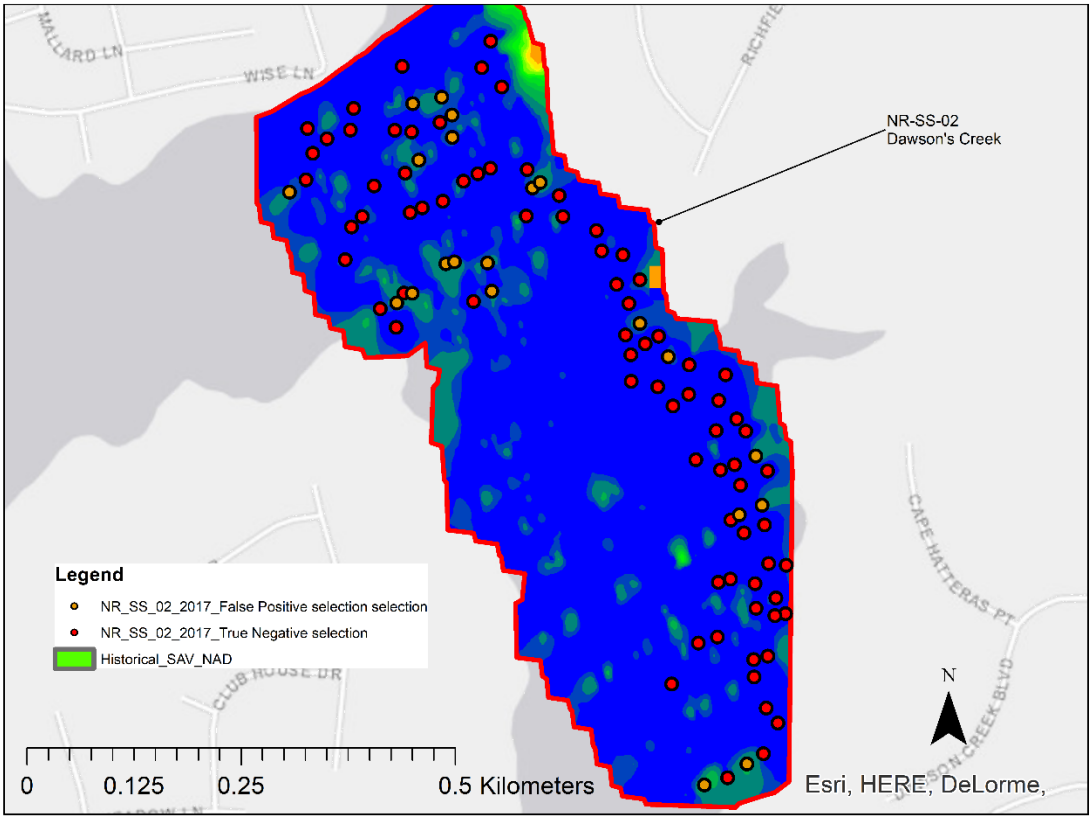

Figure 10. NR-SS-02 Video comparison points on 3 July 2017, 79% accuracy.

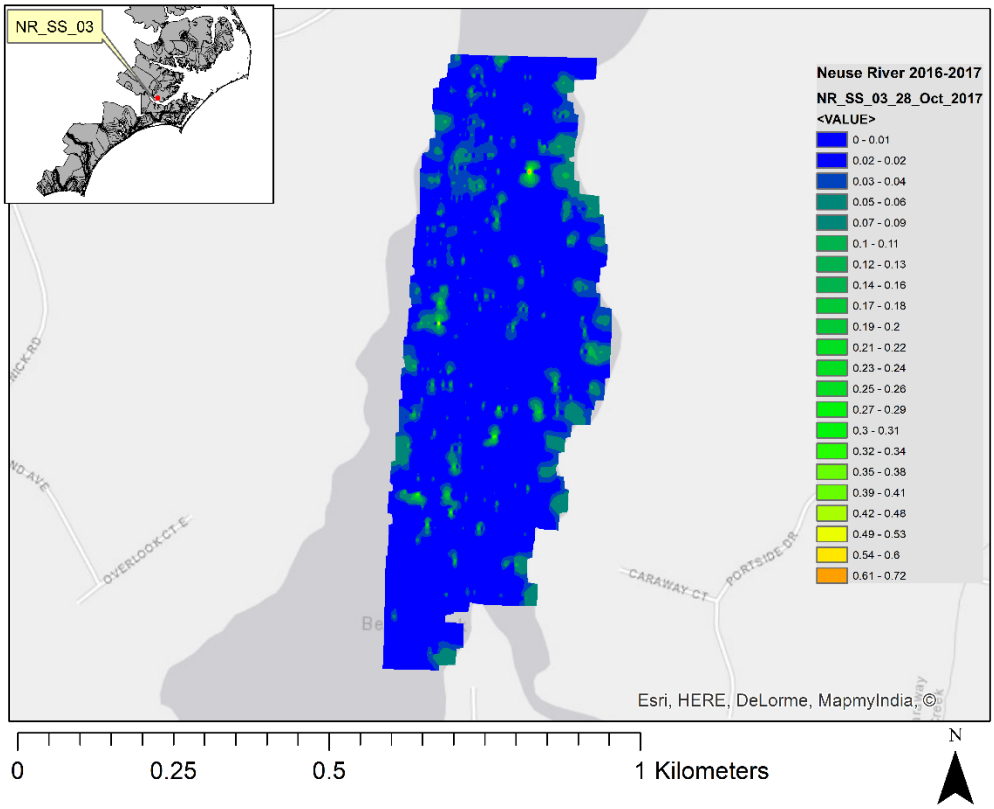

Figure 11. NR-SS-03 Biovolume of SAV in the SONAR survey on 28 Oct 2017.

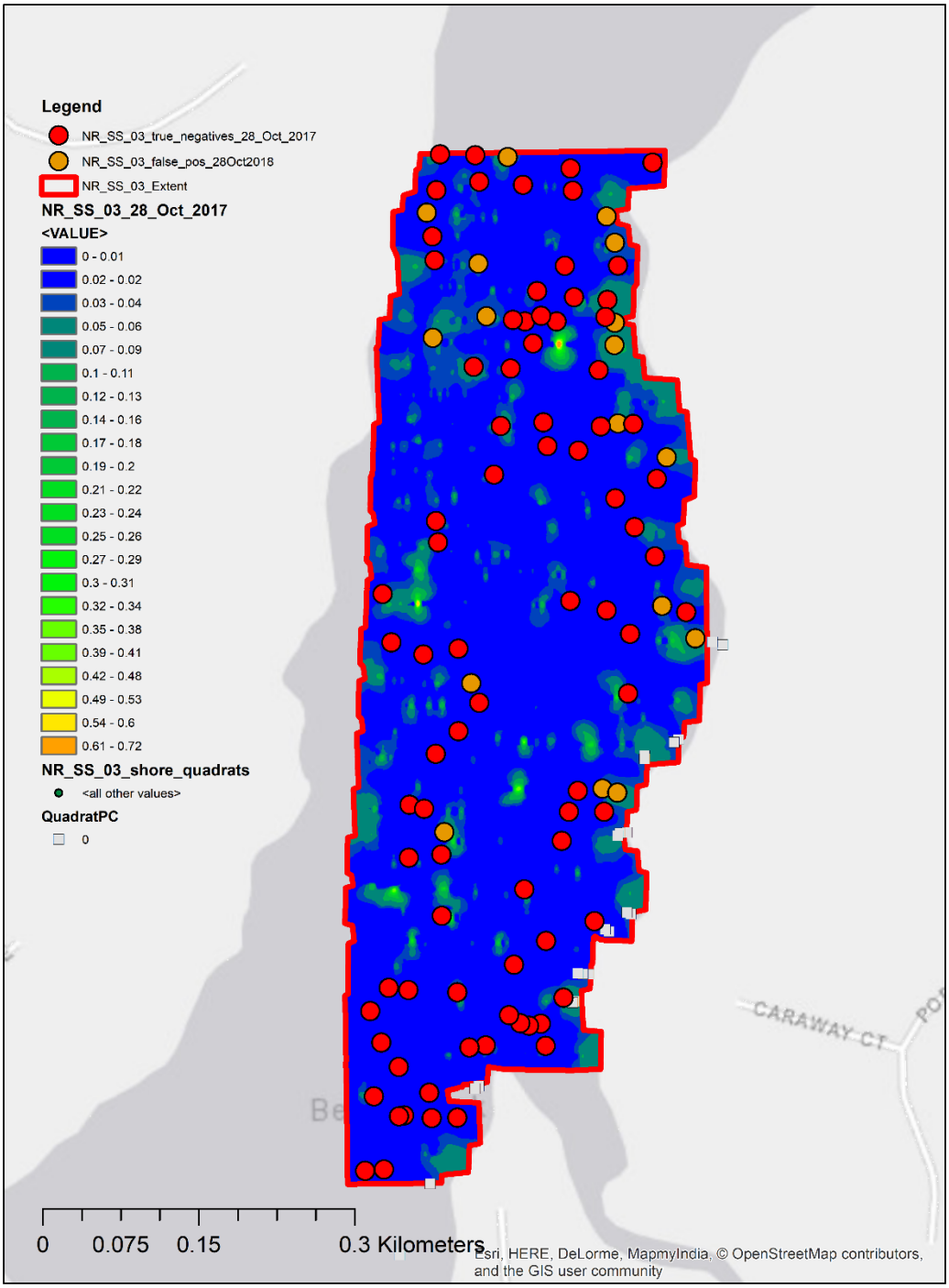

Figure 12. NR-SS-03 Video comparison points on 28 Oct 2017, showing true negatives (red points, SONAR and video agree), false positives (ochre points, SONAR indicated SAV, with none observed on video). Video and SONAR produced 83% accuracy. Gray boxes show where quadrat samples were taken, but no SAV was observed on this date.

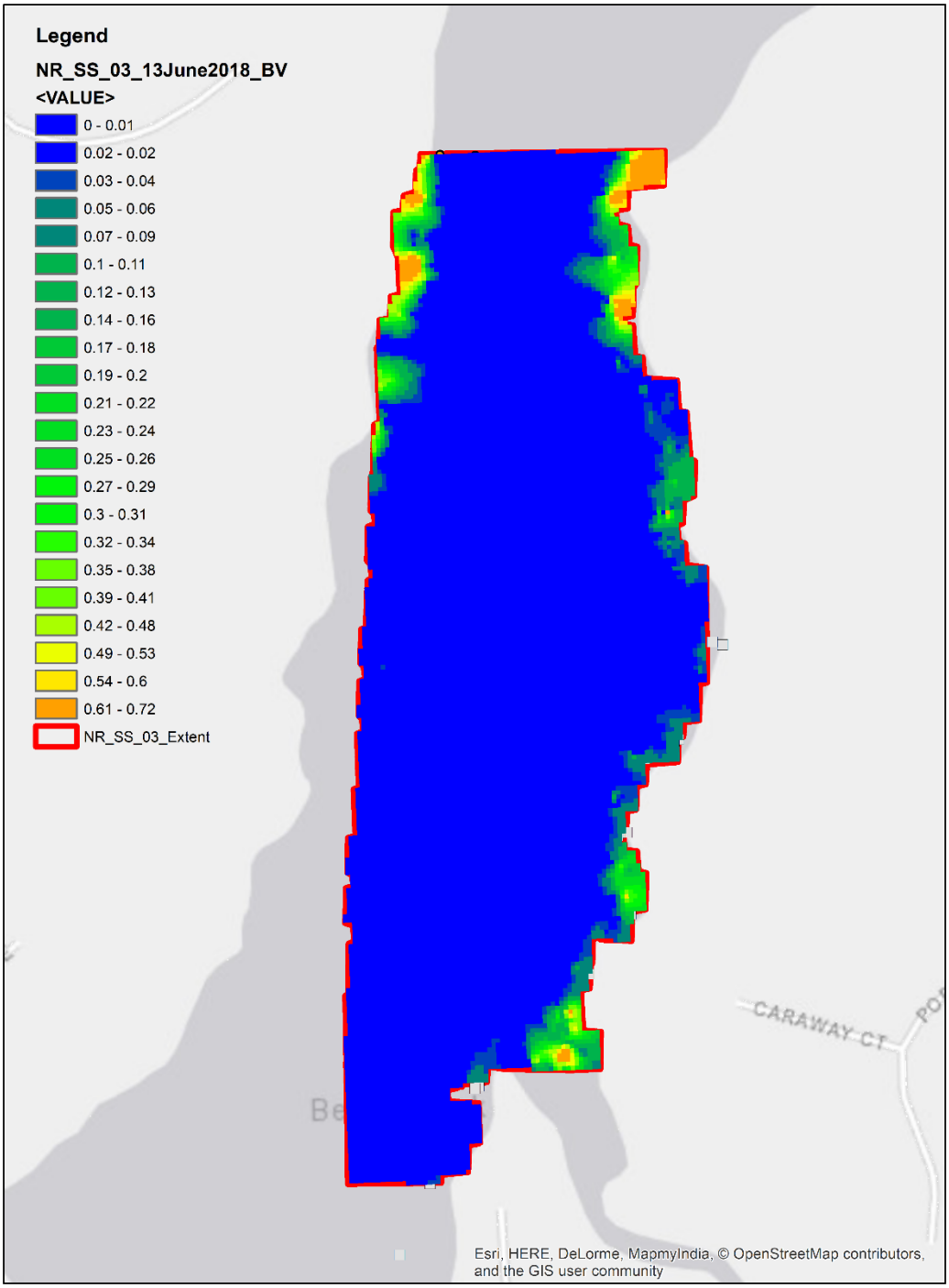

Figure 13. NR-SS-03 Biovolume of SAV in the SONAR survey on 213 Jun 2018 (for comparison with 28 Oct 2017). Underwater video and quadrat samples are still being collected and processed now, but there was SAV present at this sentinel site.

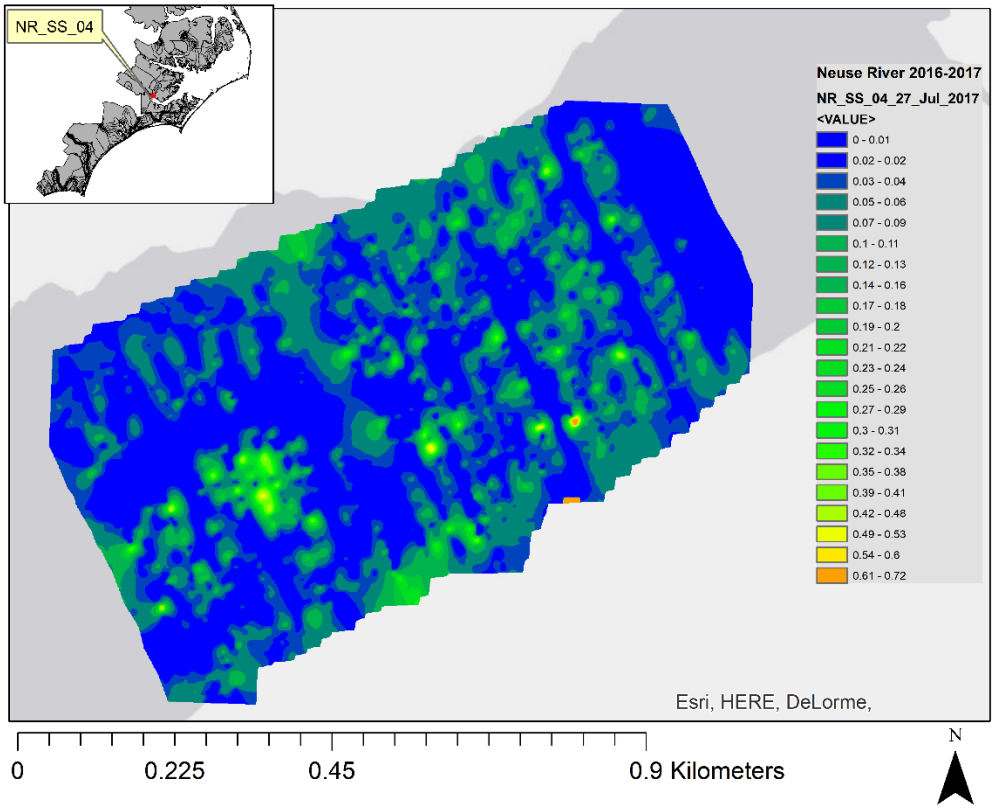

Figure 14. NR-SS-04 Biovolume of SAV in SONAR survey.

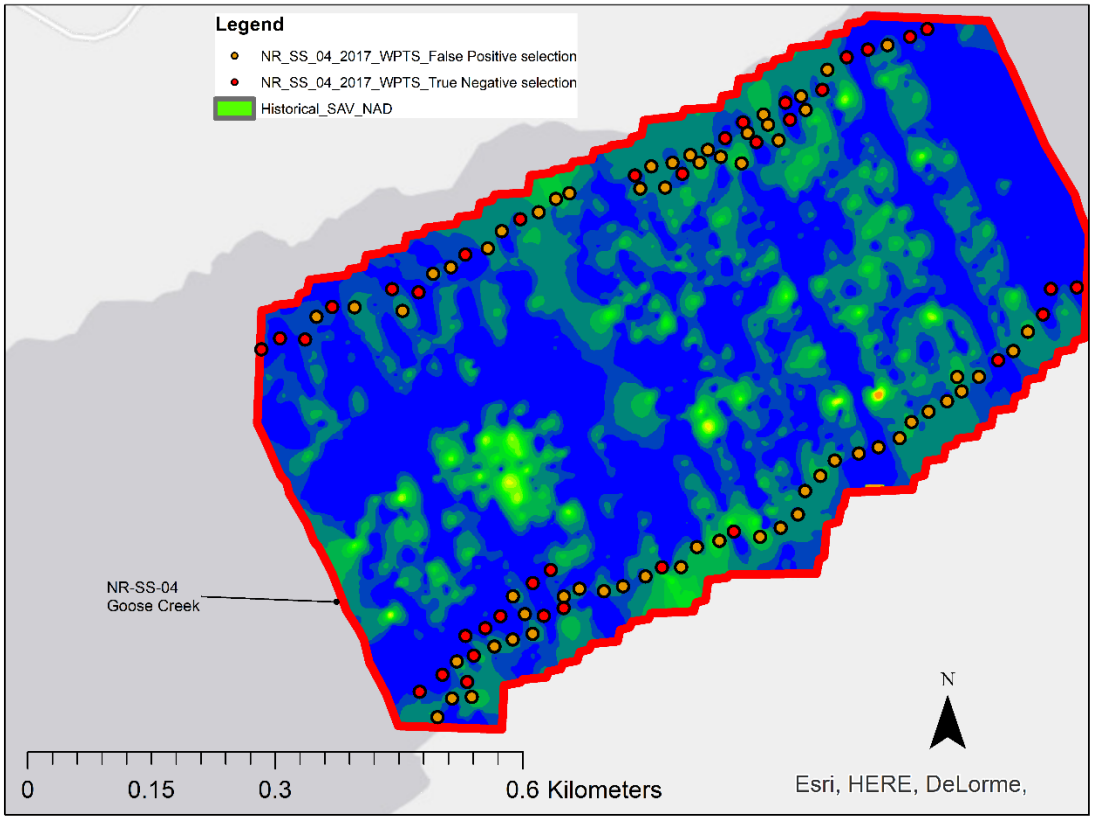

Figure 15. Video comparison points for NR-SS-04, 39 % accuracy. No SAV was observed on video, but there was a good deal of peat and other organic matter from nearby marshes on the underwater video. Some algae was observed on the bottom as well.

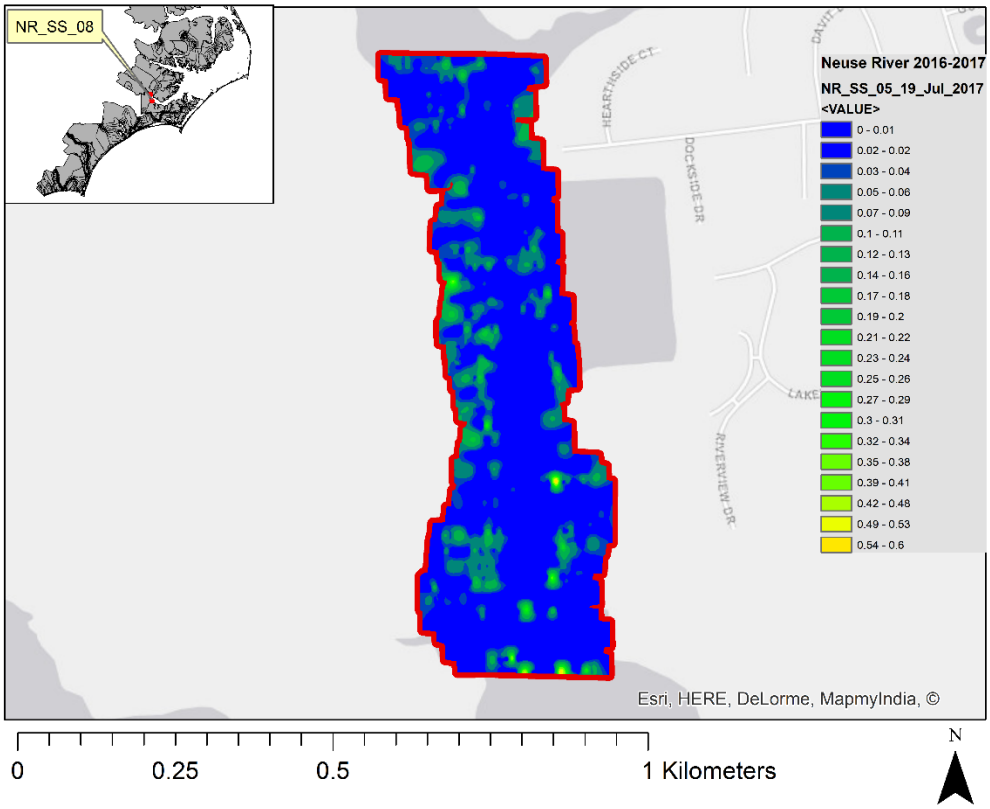

Figure 16. NR-SS-05 Biovolume of SAV in SONAR survey.

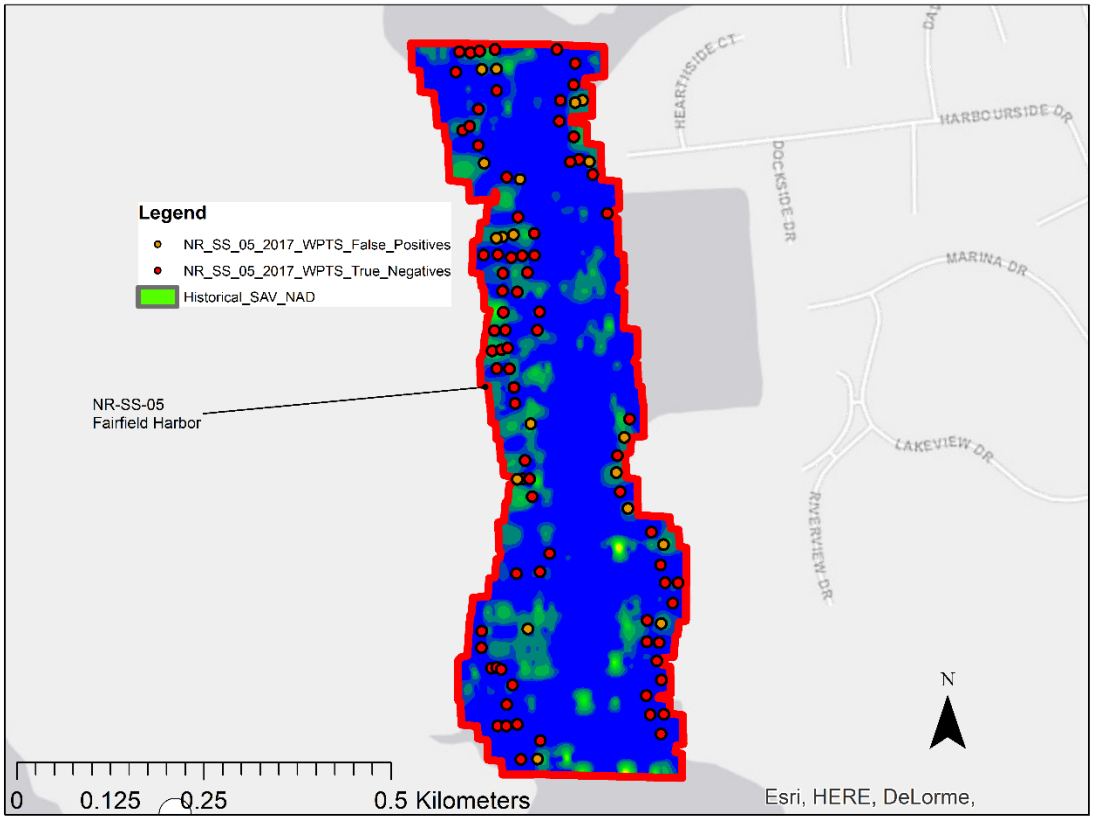

Figure 17. NR-SS-05 Biovolume of SAV in SONAR survey, 80 % accuracy.

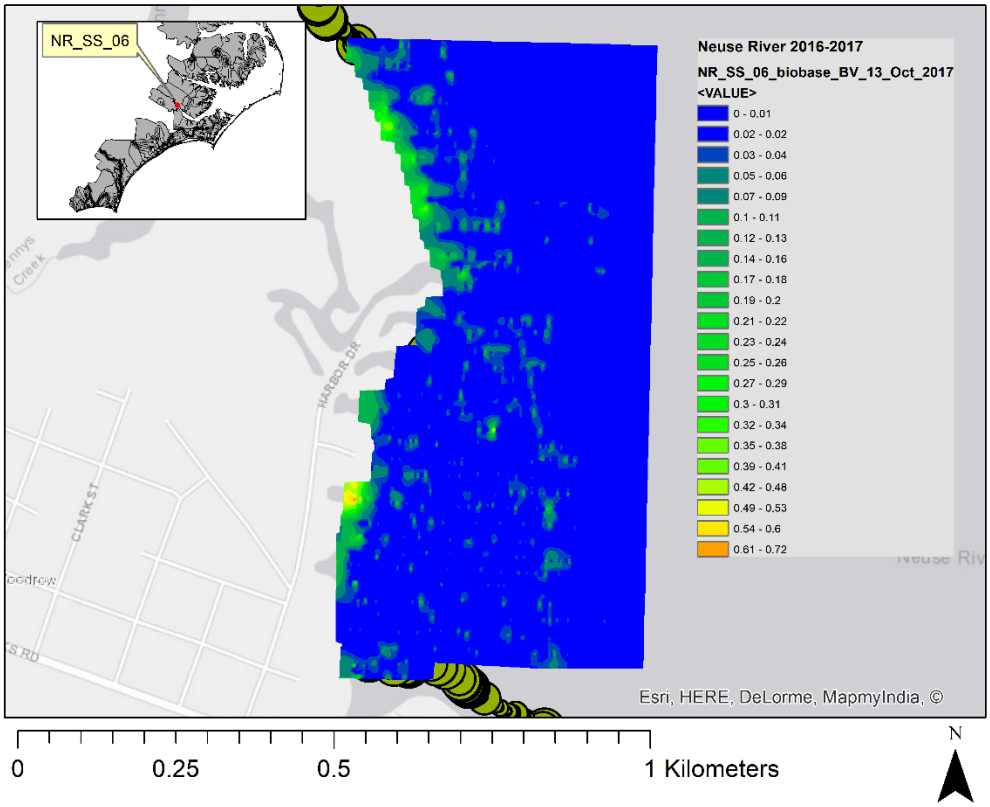

Figure 18. NR-SS-06 biovolume of SAV SONAR.

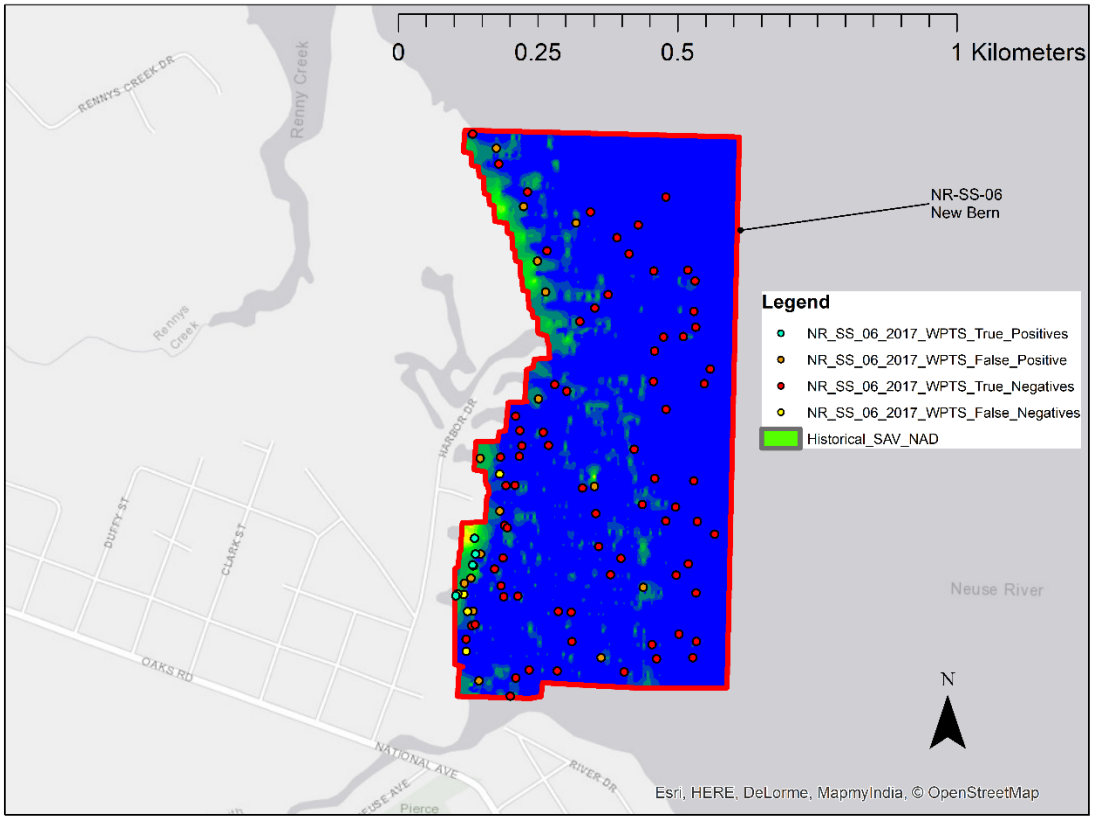

Figure 19. NR-SS-06 video comparison points, 74% accuracy.

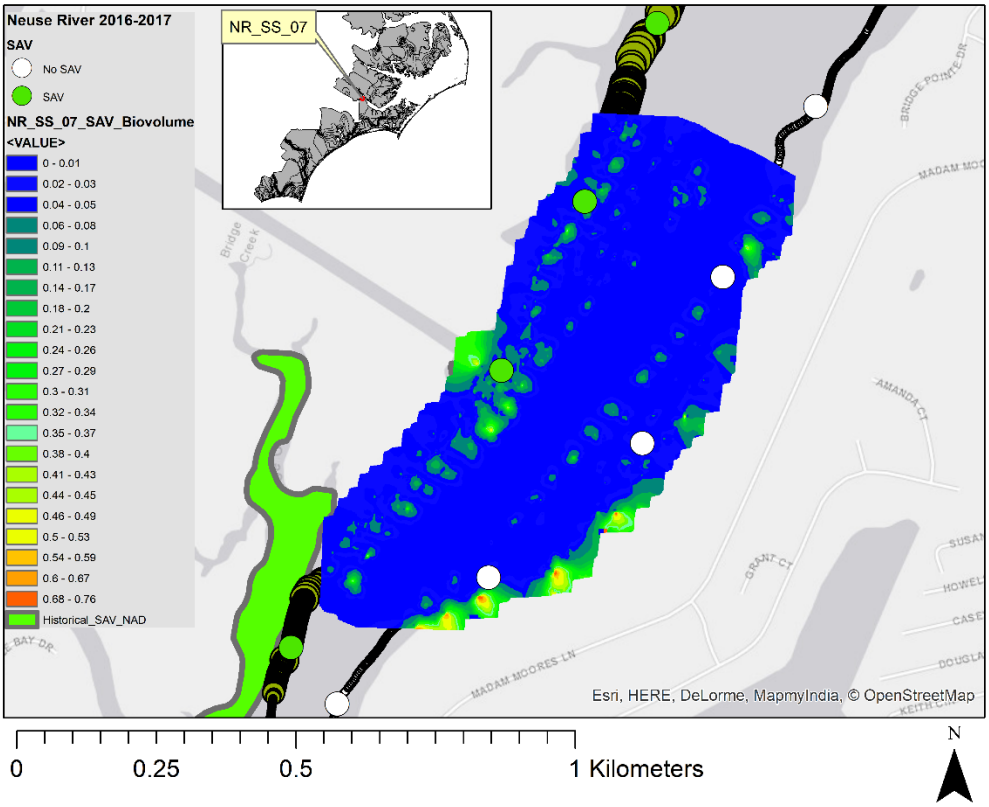

Figure 20. NR-SS-07. Biovolume of SAV on SONAR. NR-RA-22 sonar track is also shown with video verification points (SAV present, green symbols, SAV absent white symbols).

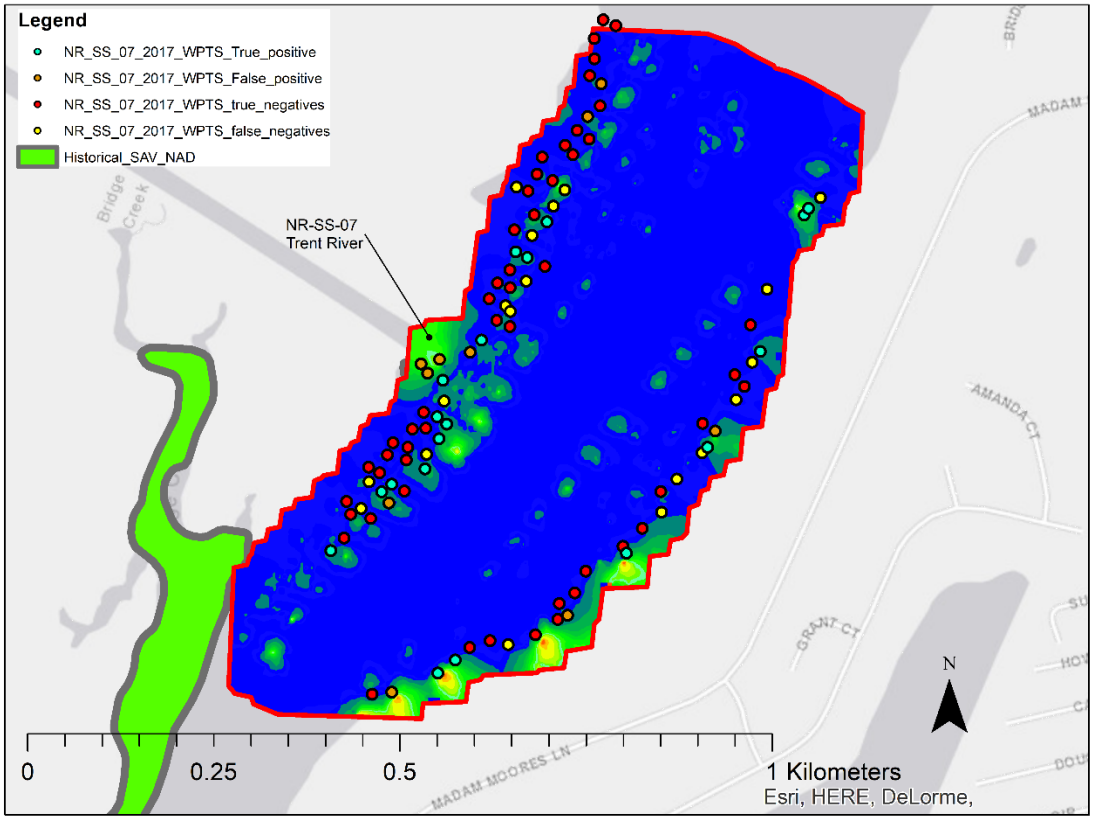

Figure 21. NR-SS-07 video comparison points, 71.3% accuracy.

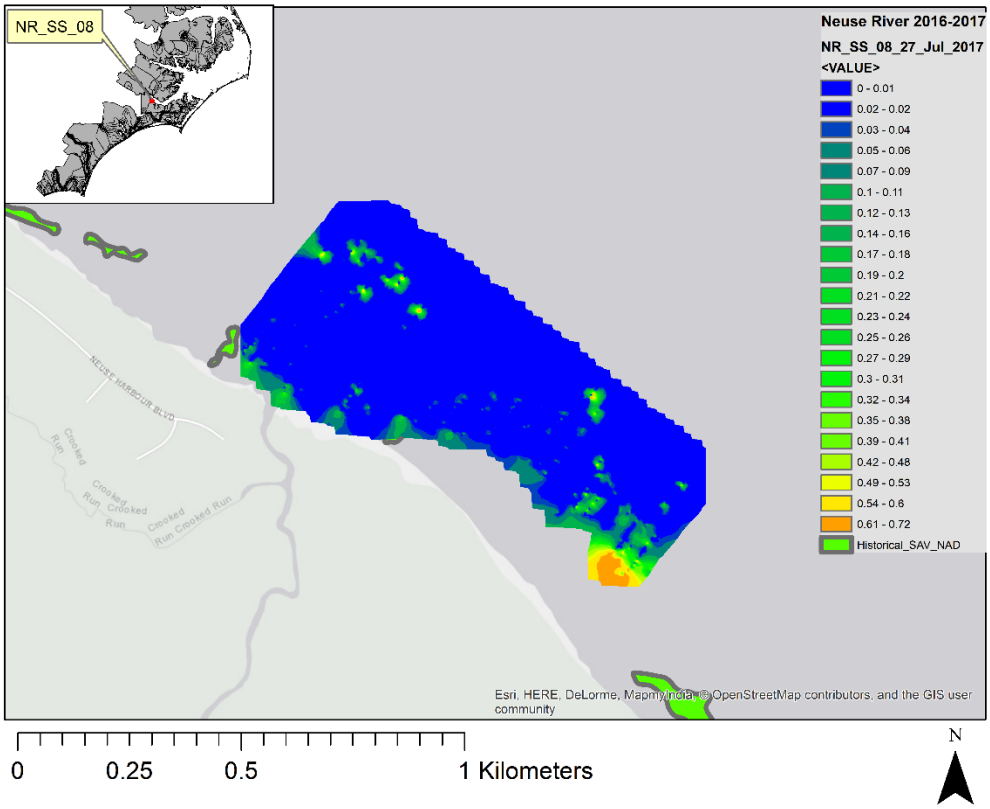

Figure 22. NR-SS-08. Biovolume of SAV on SONAR.

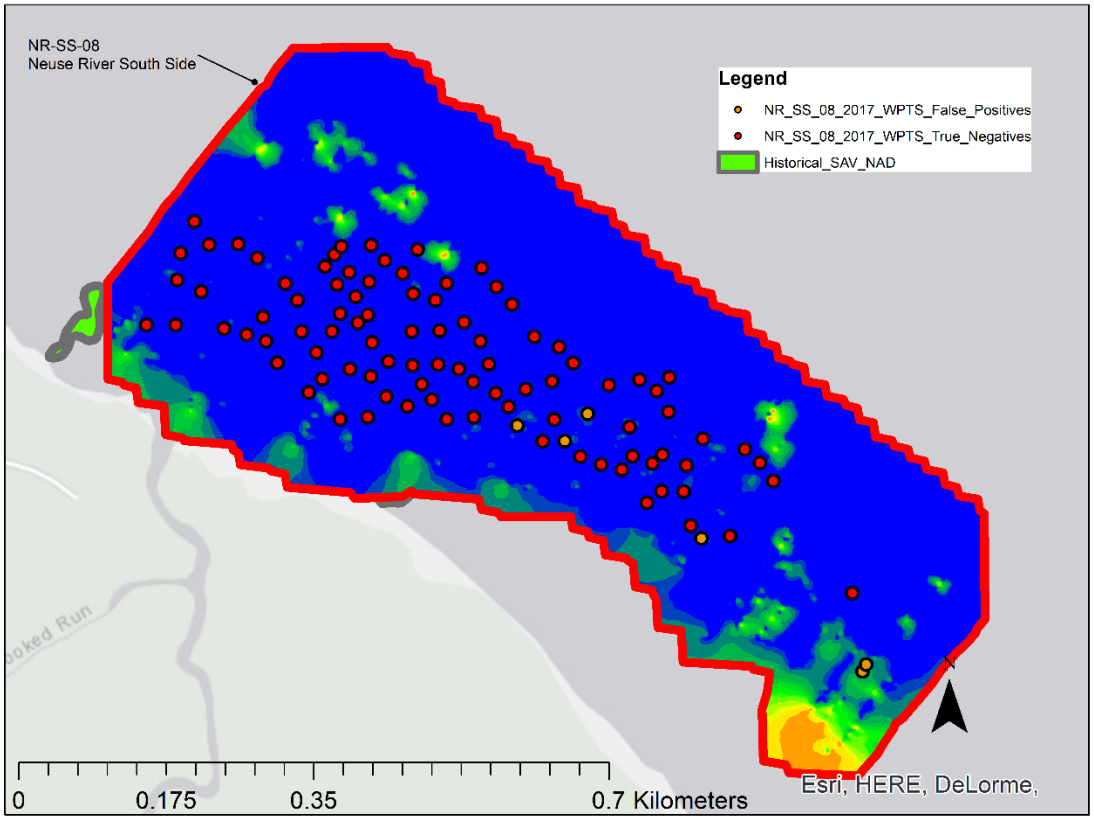

Figure 23. NR-SS-08. Video comparison points, 96% accuracy.

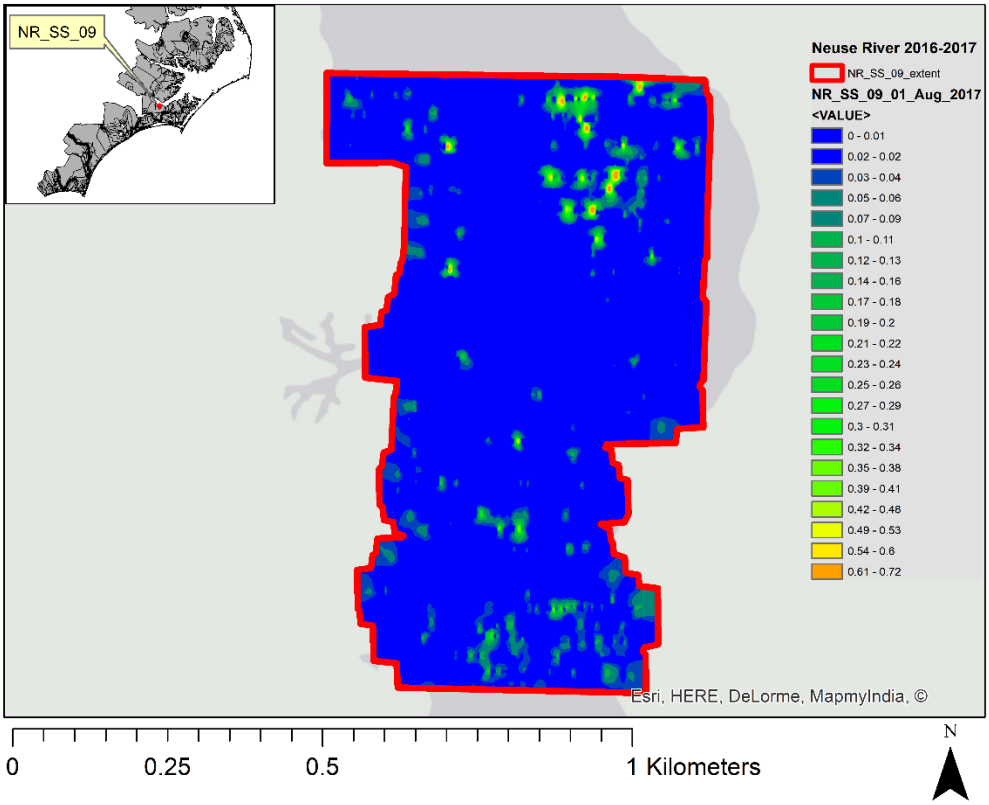

Figure 24. NR-SS-09. Biovolume of SAV on SONAR.

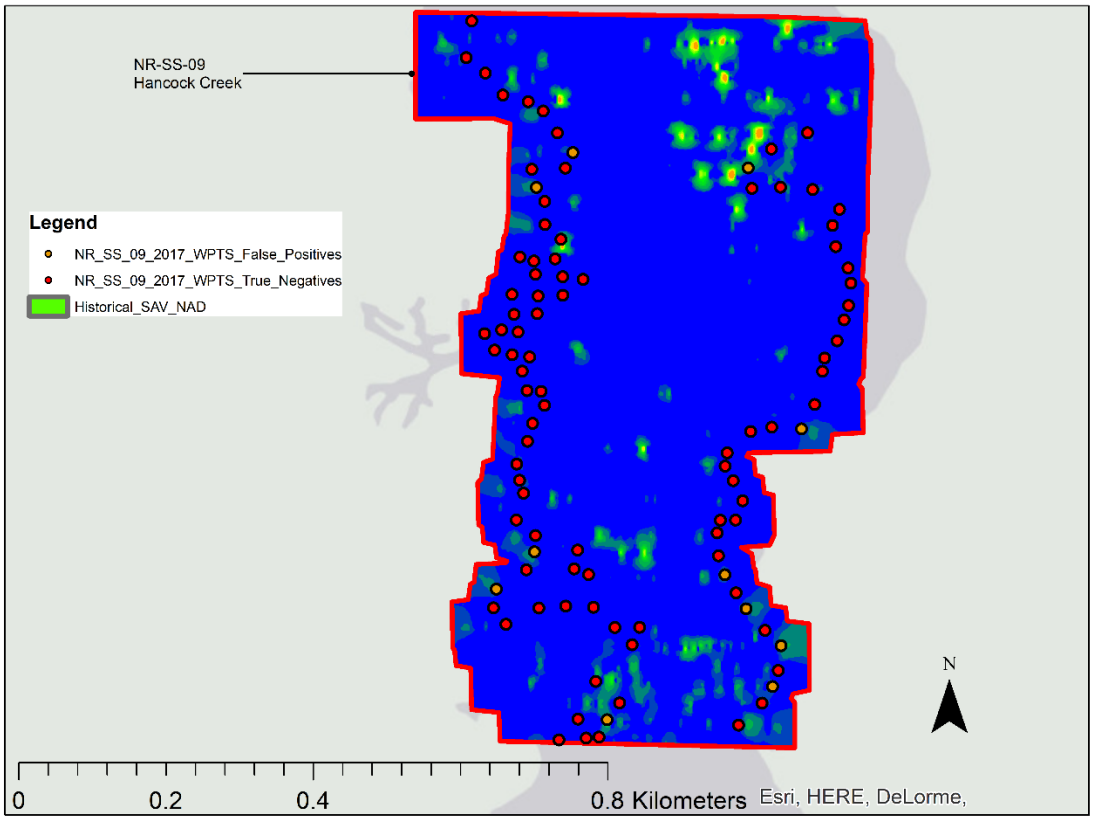

Figure 25. NR-SS-09. Video comparison points, 89% accuracy.

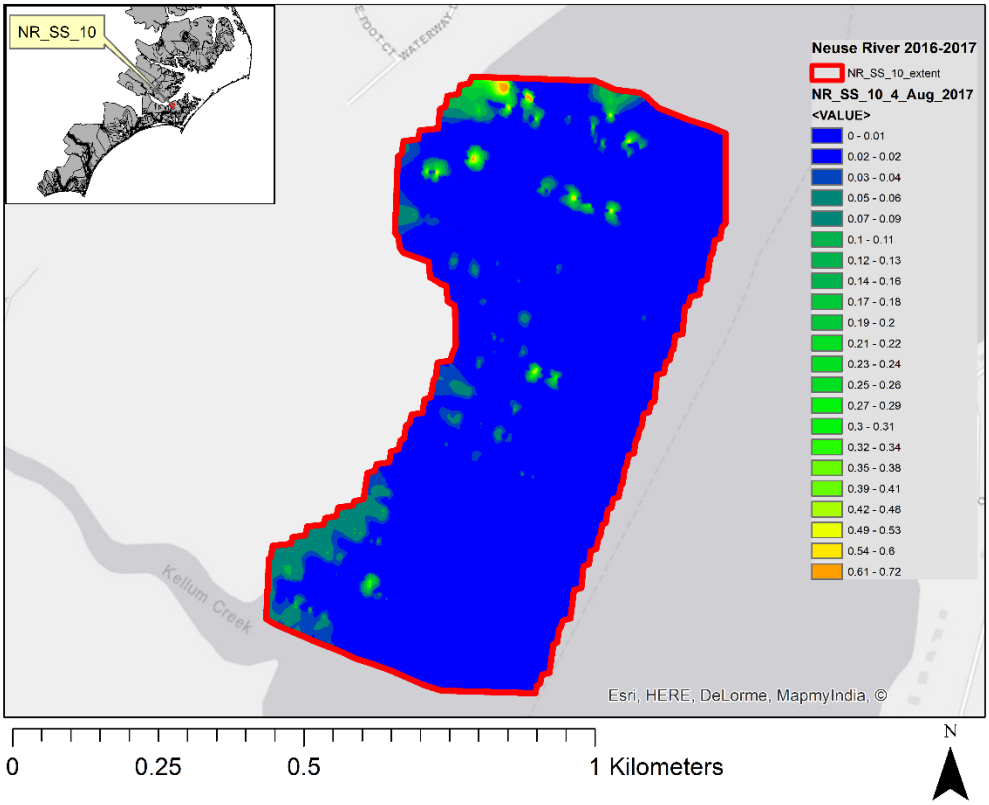

Figure 26. NR-SS-10. Biovolume of SAV on SONAR.

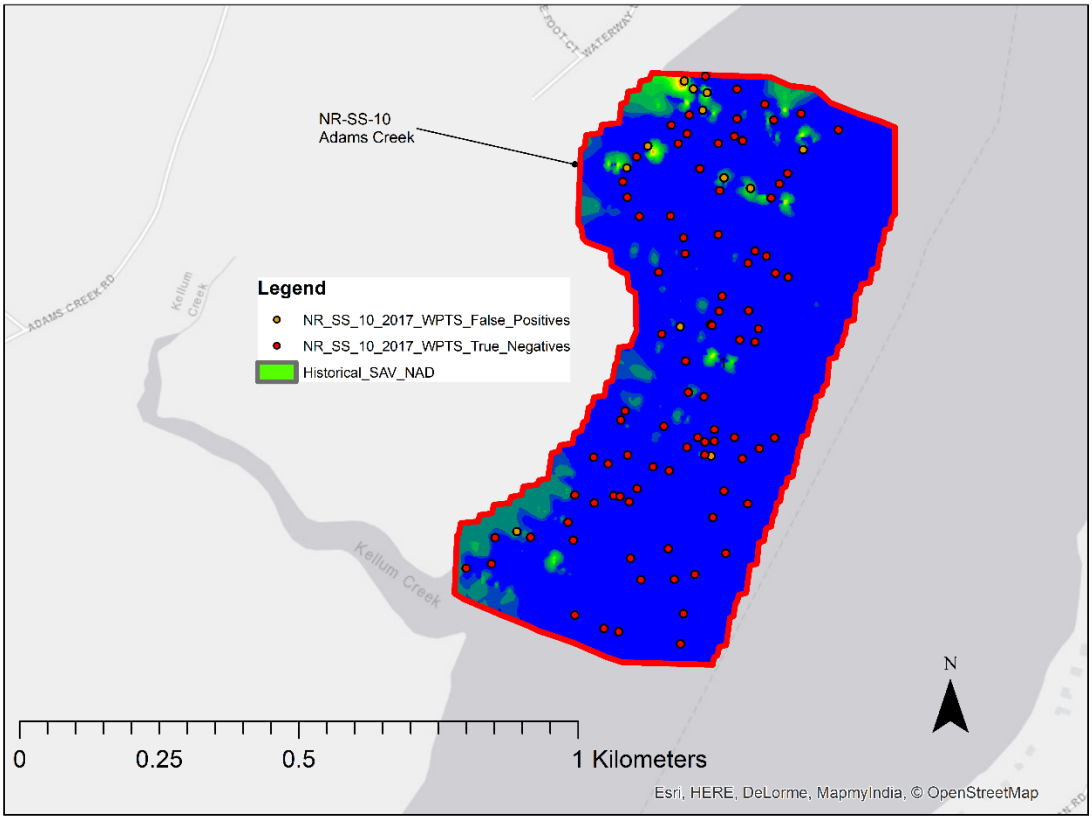

Figure 27. NR-SS-10, video comparison points, 88 % accuracy.

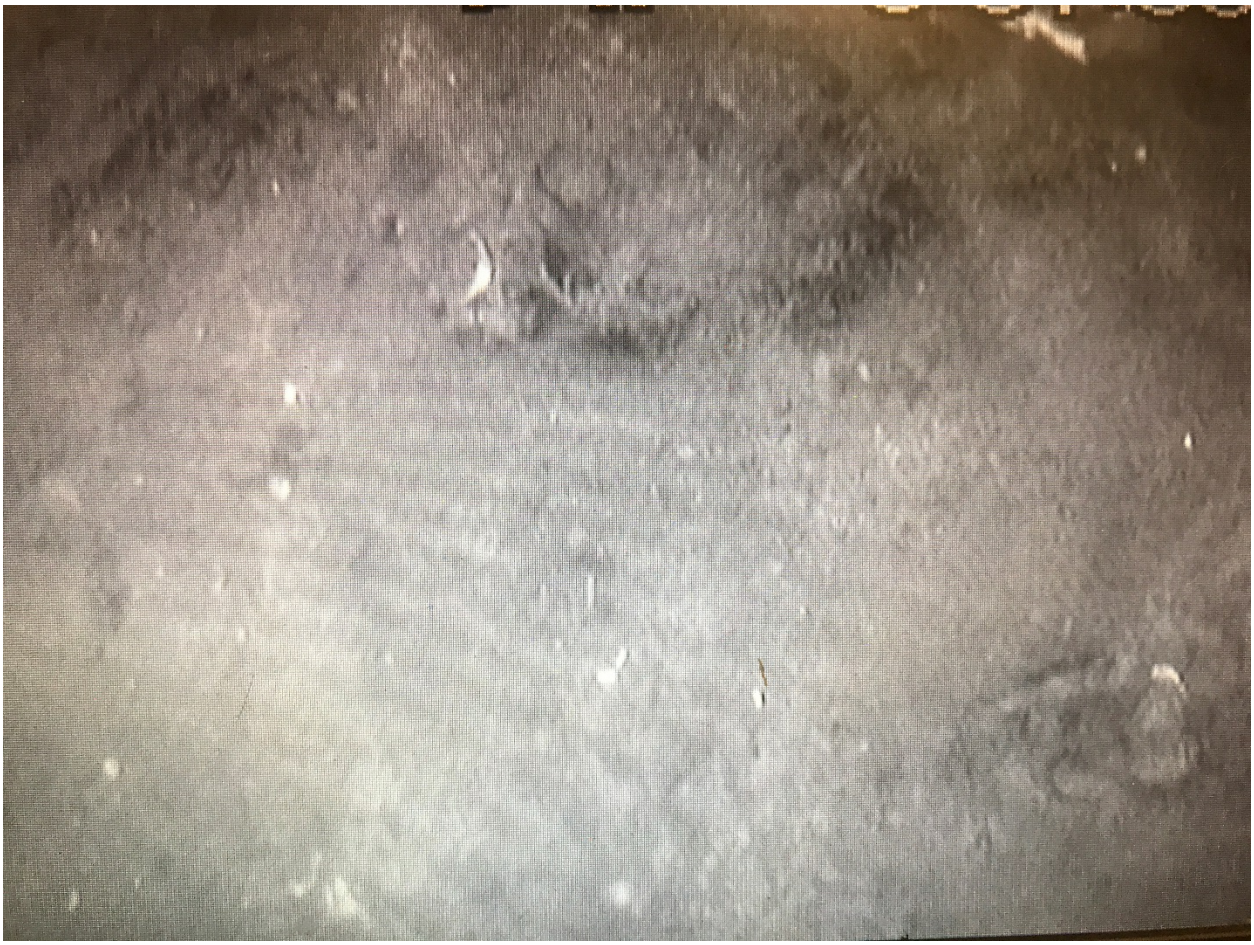

Figure 28. Bottom image of NR-SS-04, waypoint 18, a false-positive video comparison point. In the upper part of the image, flocculent detrital material is visible, which would waft up at the camera pole touched bottom. The layer of detrital material was thick enough to be picked up by SONAR and appears as SAV on the biovolume SONAR imagery (Figure 14).

*Figure 29. Bottom image from video camera at NR-SS-07, Trent River Waypoint 53. A true-positive SAV video comparison point (SAV on SONAR and underwater video). This is wild celery (*Valisneria americana*).*

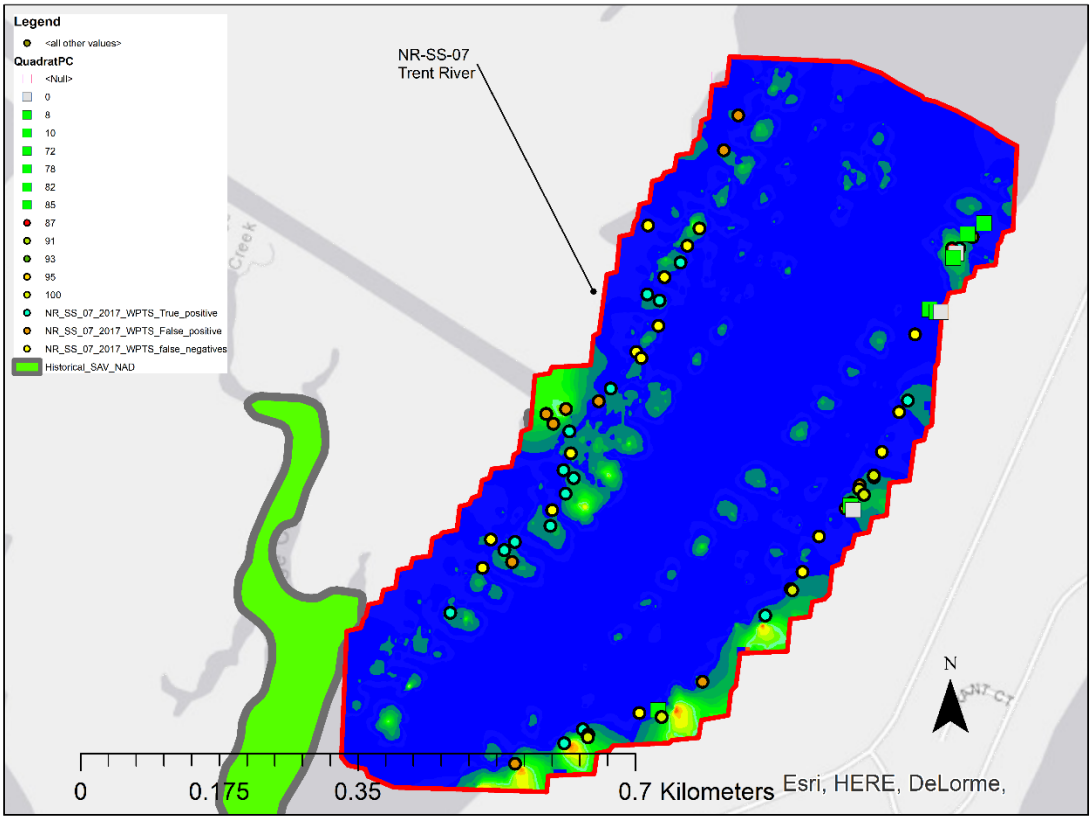

Figure 30. Quadrat samples (1 m^2) taken by snorkelers at depths of 0.25, 0.5, 0.75, and 1.0 m depths along transects at NR-SS-07 on 18 July 2017. SAV was present in quadrats (green squares) at depths 0.75 and 1.0 m; at depths < 0.5 m, SAV was absent (gray squares).

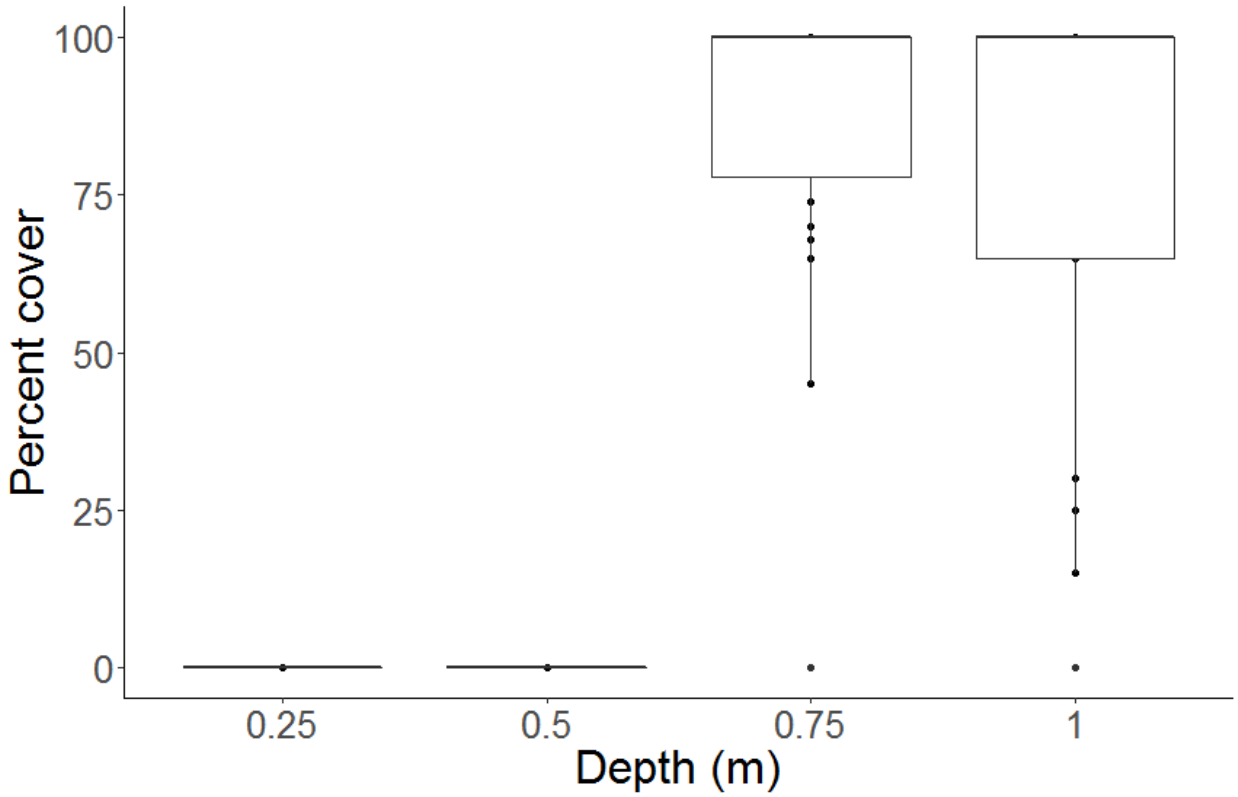

Figure 31. The depth distribution of SAV along snorkeling transects at NR-SS-07 (Trent River). No SAV occurred at depths <0.7m. The SAV was dense at deeper sites along the transect (0.75 and 1.0 m). The SAV at this site was wild celery Valisneria americana*. In most cases, bulkheads prevented the snorkelers from sampling at depths = 0.25 m. This site was almost completely bulk-headed for the entire length of the shore with many piers and docks.*

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Appendix:

I. **Procedures used to create SAV biovolume maps from ciBiobase export files. This procedure follows one proscribed by the folks at ciBiobase (part of CMap and Navico).**

For greater details, please see:
https://www.cibiobase.com/Downloads/T1ConvertingciBioBaseGridstoRaster.pdf and
https://www.cibiobase.com/Downloads/T5CreatingPubQualityImagery.pdf

1. Locate the appropriate SONAR logs on the ciBiobase system.
2. Individual SONAR log files were first merged within ciBiobase to combine the individual raw data transects into appropriate Sentinel Site (NR-SS-XX) and Rapid Assessment (NR-RA-XX) areas; note that the RA files normally have a SAV prediction swath along the vessel track that is 25 m wide. The actual swath width of the down-looking SONAR transducer along the vessel track is less than 1 m wide.
3. Select one Sentinel Site or Rapid Assessment merge file for export. On the "Export" tab, choose "Vegetation" data and select "Grid" data for export as csv file.
4. Import the csv file into ArcMap 10.5 using "Add Data"
5. Choose display X Y data. Use WGS 1984 as the geographic coordinate system.
6. Using ARC toolbox Project into UTM Zone 18 N
7. Check distance between grid points using ruler (in m). Most grid points are 4.17 m apart
8. Using Arc toolbox, choose "Spline" to convert the grid points. Use a cell size 5 x smaller than the grid points to interpolate, with grid size now = 4.17/5 = 0.835 m.
9. Choose Feature to Raster for the spline output. Output is a raster file, but it is very much larger that the survey area. So, in the next steps, we will clip it to a polygon delimited from the actual survey.
10. Convert the original grid points (feature) to raster (Feature to Raster in Arc Toolbox Conversion).
11. Use the Con tool with true = 1 and false = 0, input is the raster just created.
12. Raster to Polygon of Con tool layer output. The polygon will now delimit the actual area of the original survey.
13. Use Raster Clip tool to clip the spline output from Step 9 to the survey area polygon from Step 12.
14. Set symbology of the clipped raster layer obtained in Step 13. The symbology used in this report for the sentinel sites is the one used by ciBiobase for their default maps and SONAR imagery. Once edited, the symbology can be saved as a color ramp and reused on different images. The classified raster symbology was edited manually in Arc Map and is given in the table below:

Biovolume	Red	Green	Blue	Color
< 0 - 0	0	0	255	Dark Blue
0 - 0.05	0	0	255	Dark Blue
0.05 - 0.10	0	134	121	Dark green
0.10 - 0.15	0	179	76	Green
0.15 - 0.20	0	201	54	Green
0.20 - 0.25	0	223	32	Green
0.25 - 0.30	0	245	10	Green
0.30 - 0.35	37	255	0	Light green
0.35 - 0.40	103	255	0	Light green
0.40 - 0.45	169	255	0	Light green
0.45 - 0.50	235	255	0	Light green
0.50 - 0.55	255	231	0	Yellow
0.55 - 0.60	255	195	0	Yellow-Orange
0.60 - 0.65	255	160	0	Orange
0.65 - 0.70	255	124	0	Orange
0.70 - 0.75	255	94	0	Orange
0.75 - 0.80	255	70	0	Light red
0.80 - 0.85	255	47	0	Light red
0.85 - 0.90	255	24	0	Red
0.90 - 0.95	255	1	0	Red
0.95 - 1	255	0	0	Red
> 1		255	0	Red

II. Procedures for verifying the accuracy of SONAR imagery against the underwater video inspection of the bottom at randomly selected comparison points.

1. A text (*.csv) file of underwater video comparison points was downloaded from the Lowrance GPS as waypoints, with columns labeled: waypoint ID, latitude, longitude. For each waypoint, SAV was scored presence (SAV=1) or absence (SAV=0) on video by reviewing the video recording taken at each waypoint. This scoring was done in a spreadsheet editor (Excel) then exported to comma separated values (csv) text files.
2. After importing the csv file into ARC Map, and displaying this as XY data in the WGS 84 geographic coordinate system and then was projected into UTM Zone 18N. The resulting layer of video waypoints and attribute table was over-plotted on the SONAR raster files created above.
3. Green symbols were used to indicate SAV positive points from video inspection and white symbols were used for SAV negative points.
4. Labels were displayed onscreen to show the waypoint ID for each comparison point to be scored.
5. Next, the video waypoints in the attribute table was edited interactively to include presence/absence SONAR data in a new "SONAR" column.
6. First, a field was added called "SONAR", to score the presence or absence of SAV as displayed by the SONAR kriging raster files imported from ciBiobase.
7. The editing tool was used to edit the video waypoints attribute table, while examining the SONAR kriging raster file and over-plotted video waypoints on the screen.
8. A SONAR SAV presence or absence score was obtained for each waypoint by examining the SONAR maps imported from ciBiobase while editing the imported video waypoints file in Arc Map. The four categories for the accuracy analysis are as follows:

SONAR and video Agree no SAV present "True negatives"		SONAR and video Agree SAV present "True positives"		SAV on SONAR No SAV on video "False positives"		SAV on video No SAV on SONAR "False negatives"	
SAV	SONAR	SAV	SONAR	SAV	SONAR	SAV	SONAR
0	0	1	1	0	1	1	0

Different color symbols were used to display these four categories. Accuracy was computed by summing the true positive and true negative and dividing by the total number of comparison points.

III. Abbreviations used in this report:

ArcMap – a GIS software package from ESRI (Environmental Science Research Institute)
APNEP – Albemarle Pamlico National Estuarine Partnership
BV – percentage biovolume of SAV; the percentage of the water column occupied by SAV where it occurs
ciBiobase – A software package available on the internet to analyze SONAR data for SAV presence
CRFL – Coastal Recreational Fisheries License
DGPS - differential GPS; it augments those GPS signals with signals from ground radio stations that have improved the accuracy of position locations to less than 1 m.
DSAS – Digital Shoreline Analysis System, an extension for Arc Map 10.5.1 used by our research team to develop transects perpendicular to the coast
GIS – Geographic Information System
GPS – Global Positioning System; standard GPS uses NAVSTAR satellites to broadcast signals received by GPS receivers;
kHz – kilohertz (1,000 Hertz)
NAD 1983 HARN UTM Zone 18 N – a geographic map projection for the Universal Transverse Mercator 18N zone of the earth, using a 1983 datum, which includes North Carolina. The distance units of this system on the ground are given in meters.
Navico CMap – The company that makes Lowrance echosounder and ciBiobase
NCDENR- North Carolina Department of Environment and Natural Resources
NCDMF – North Carolina Division of Marine Fisheries
NOAA - National Oceanic and Atmospheric Administration
RA – Rapid Assessment transect or method
SAV - Submerged Aquatic Vegetation
SAVEWS - SAV Early Warning System algorithm that was used to determine SAV presence by United States Army Corps of Engineers
SONAR – Sound Navigation and Ranging
WAAS – Wide Area Augmentation System, GPS ground-reference radio stations that provide improved GPS accuracy (< 1.0 m)
WGS 84 – World Geographic System coordinate system using an earth-centered datum in 1984; used in the Department of Defense's United States GPS systems; default coordinate system for the GPS receivers used in this study.