Experimental Methodology for the Remote and Autonomous Monitoring of Oyster Reef Habitats and Oyster Reef Restoration

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Summary

The development and execution of this project was made possible through varied professional and academic experiences from previous work in monitoring juvenile fish populations and water quality in the Chesapeake Bay to training in Geographical Information Systems, Spatial Statistics, Ecology, Hydrology and Water Resources, and Strategies in Watershed Management at Johns Hopkins as well as my partnership with Dr. Jim Blanchard of the Unmanned Autonomous Systems Academy (UASA). The assemblage of technical information and creation of procedural documents in this document represents the first attempt at monitoring of oyster reefs in this manner. As such, this is a working document and will attempt to accurately represent the experimental nature of it's establishment.

This project details the creation of an experimental habitat assessment protocol utilizing small Unmanned Aircraft Systems (sUAS) and Remotely Operated Vehicles (ROVs) for the monitoring of oyster reef habitats and oyster reef restoration projects. With as little as 43% of oyster restoration projects in the Chesapeake having monitoring data on record (Kramer and Sellner 2009) (Kennedy et al. 2011), this paper explores the feasibility of increasing the amount of monitored reefs through the utilization of this experimental autonomous method in place of traditional monitoring methods. The goal of this paper is to provide the procedural framework and background information needed to recreate this experimental design. Specifically, this paper is intended for organizations that are vested in the success of oyster reef restoration projects. Oyster reefs play an important role in the health of tidal habitats by providing ecosystem services such as water column filtration, increased substrate complexity, and shoreline erosion protection. The importance of developing this novel method of habitat assessment is to reduce the impact of observation in the system which is being studied and to increase the feasibility of oyster monitoring for the many restoration projects around the world.

The many stages of this project provided valuable experience in project development, research design, field operations, geographical information systems (GIS), sUAS and ROV operations, as well as design and execution of sampling protocols.

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1.0 Introduction and Background

1.1 Background of Oysters Declines in Chesapeake

The experimental oyster reef monitoring methodology proposed by this capstone project is an attempt to create an efficient and accurate monitoring protocol for oyster reefs and oyster reef restoration projects and, additionally, to establish procedures for an eDNA based community composition survey in a highly replicable and minimally invasive manner. This project will propose, for the first time, a minimally invasive method for community composition surveying through the application of eDNA sampling.

Oyster reefs play an important role in the health of tidal habitats by providing ecosystem services such as water column filtration, increased substrate complexity, and shoreline erosion protection. Due to commercial and environmental pressures throughout the early to mid-20th century, Chesapeake Bay eastern oyster (Crassostrea *virginica*) populations and the ecosystem services they provide were decimated. Recent decades have seen increased efforts to restore eastern oyster populations via reef reconstruction projects throughout the Chesapeake. Meta-analyses of restoration projects in the Chesapeake Bay from 1990-2007(Kramer and Sellner 2009, Kennedy et al. 2011) indicate that few have included monitoring in their restoration goals.

Current monitoring techniques can involve high levels of human contact with the reef (i.e. walking along the perimeter of the reef for project footprint, and deploying a level and rod or transit pole with self-leveling laser on reef crest for reef height). These methods can cause undue damage and disturbance to the reef and may also be time consuming and costly depending upon the frequency at which they are employed.

Additionally, traditional oyster reef monitoring methods involve high degrees of interaction between the sampler and area being sampled. This, theoretically, could lead to potential influences upon the data variables being collect. For example, if environmental DNA (eDNA) is required for a reef habitat community composition analysis (species level), then human DNA that might be introduced into the environment (as well as the sample) when being collected. Furthermore, larger vessels or scuba surveys may cause disturbance in silt deposits on the reef which will impact water chemistry variables, imagery, as well as eDNA. Unmanned Aircraft Systems (UAS) and surface water Remote Vehicles (RVs) could provide an efficient and accurate replacement or supplement to traditional monitoring methods. The purpose of this project is to develop and demonstrate a replicable, novel, and noninvasive oyster reef habitat quality assessment and environmental DNA (eDNA) based community composition surveys in order to assist restoration decision making and habitat monitoring in the Chesapeake Bay watershed.

1.2 Oyster Restoration and Monitoring Efforts

Meta-analyses from 2009 (Kramer and Sellner 2009) and 2011 (Kennedy et al. 2011) found that despite the focus on restoration of oyster reef in recent decades, most restoration projects have not been monitored “to an extent that allows for comparison” (NOAA). These studies analyzed 78,0000 records from 1035 sites and found that “few” sites were continuously monitored, restoration goals were not well defined, and that “only 43 % of the datasets included both a restoration and monitoring component.” (NOAA) Kennedy et al. (2011) stressed the need for restoration projects to include clearly stated goals as well as quantitative sampling with adequate replication and sample sizes, and emphasized the importance of pre- and post-restoration monitoring (NOAA). Habitat assessment of oyster reefs is critical for monitoring their development as well as their ability to provide ecosystem services (such as water column filtration) within their immediate habitats. Traditional monitoring techniques (i.e. walking along the perimeter or through the reef for necessary data points) requires much more human interaction with the reef habitat. The importance of developing this novel method of habitat assessment is to reduce the impact of observation in the system which is being studied.

UASs have been shown to be viable options for marine ecosystem restoration monitoring, and specifically oyster reef monitoring. One study in 2019 (Windle 2019) compared the efficacy of UAS based surveys to ground based surveys when looking at reef-scale measures of area and morphology and found that "…UAS can be used to accurately monitor intertidal oyster reefs over time and that proper ground control techniques will improve measurements of reef morphology." Compared to ground based monitoring, the study found that aerial imagery analysis allows for greater point generation when delineating reef edges with "minimal increase in effort." (Windle 2019). The study utilized UASs with "real-time kinematic global positioning system (RTK-GPS), images were processed using structure from motion (SfM) stereo photogrammetry techniques with and without the use of ground control point (GCP) correction." (Windle 2019).

Previous studies exploring the feasibility of UASs in reef monitoring stated that some drawbacks of the method are "…related to image acquisition methods. For example, image quality of submerged landscapes from UAS is highly dependent on a number of environmental conditions including (but not limited to) sun angle, cloud cover, surface disturbance by wind, and turbidity (Joyce et al., 2018; Nahirnick et al., 2019)." (Ridge 2020). In order to minimize these drawbacks, the authors stated that, "…specific mission planning insights, like adjusting UAS altitude, orientation of acquired imagery (away from sun), or understanding the limitations of the data collected for a certain habitat. Additional drawbacks of this proposed remote sensing method include the inability to perform sex ratio determinations and gonad development status check on collected oyster specimens. Future studies and RVs may include the technology necessary to collect those samples remotely for later laboratory analysis.

1.3 Site Selection and Details

The site currently under consideration for the establishment of the sampling protocol is the Dobbins Island Reef site on the Magothy River, located north and west of Annapolis, Maryland (Figure 7). This site was selected due to prior knowledge of it's location, recent monitoring activity, and the recent installation of "reef balls" (Figure 3). Reef Balls are domelike concrete structures containing that provide a growing structure for juvenile oysters (spat). They are designed to elevate oyster reef off of the benthic substrate so that they filer the water column. Reef balls are perforated with large holes in order to allow for increased water flow. According to the Maryland Department of Natural Resources' 2021 Anne Arundel Complex Oyster Restoration Plan, this site is 4 acres, has a mud and dredged shell substrate, and contains 60 reef balls with a reef ball installation as recent as October 2021. Oyster densities (number of oysters per square meter) were measured at this site in 2018 through the Magothy River Association Dive

Survey and showed average densities of 6 oysters per meter squared and shell an average shell volume of 17.1 liters.

2.0 Literature Review

2.1 Small Unmanned Aerial Systems (sUAS)

sUASs have been shown to be viable options for marine ecosystem restoration monitoring, and specifically oyster reef monitoring. One study in 2019 (Windle 2019) compared the efficacy of UAS based surveys to ground based surveys when looking at reef-scale measures of area and morphology and found that "…UAS can be used to accurately monitor intertidal oyster reefs over time and that proper ground control techniques will improve measurements of reef morphology." Compared to ground based monitoring, the study found that aerial imagery analysis allows for greater point generation when delineating reef edges with "minimal increase in effort." (Windle 2019). The study utilized UASs with "real-time kinematic global positioning system (RTK-GPS), images were processed using structure from motion (SfM) stereo photogrammetry techniques with and without the use of ground control point (GCP) correction." (Windle 2019)

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The remotely operated vehicle (ROV) in this study presents an opportunity to gather data in new ways that expands on the benefits UASs bring to the environmental sciences. This investigation represents a potential leap forward in remote environmental scienceBy adding water quality monitoring, biodiversity monitoring through eDNA metabarcoding analysis, benthic imaging, and sonar data to the list of things ROVs/UASs are capable of performing. This capstone presents these methods as a valuable addition in the assessment spatial-temporal change, impact assessment, and restoration success of oyster reef habitats where traditional remote sensing methods and monitoring techniques are either impractical or potentially damaging to the sensitive reef structures being studied.

2.2 Metrics of Interest

National Oceanic and Atmospheric Administration in partnership with others defined a list of “Universal Metrics” (physical reef characteristics) and Universal Environmental Metrics (water quality variables) necessary for the successful monitoring of oyster restoration projects. The “Universal Metrics” were identified as: 1) reef volume dimension; (2) reef height; (3) oyster density; and (4) oyster size-frequency distributions. Oyster reefs increase the spatial heterogeneity on the substrate of the estuarine environment which in turn creates an array of discrete habitat patches (Huffaker 1963). Structurally and biologically complex oyster reefs (comprising both oysters and other invertebrates) are beneficial in providing fish and other species with shelter, food, and nursery services. Reef height, or elevation, has implications for proximal shoreline habitats and is a key metric for success of the oyster reef as it represents the habitat's vulnerability to – and ability to keep pace with – sea level rise. (Ridge 2020).

The "Universal Environmental Variables" were identified as: (1) water temperature; (2) salinity; and (3) dissolved oxygen (for subtidal reefs). Water temperature influences oyster reproduction, survival, growth, and exposure to disease. C. *Virginica* has an optimum salinity range of 14-34 psu (practical salinity units) and deviations from this range can have an impact on growth and mortality as well as diseases such as Perkinsus *marinus* (Dermo) and Haplosporidium *nelsoni* (MSX). Dissolved oxygen levels below 2 mg/L are known to have negative effects on the settlement, growth, and survival of oysters (e.g., Baker and Mann 1992; Johnson et al. 2009).

One metric of interest that sets this capstone aside is the environmental DNA (eDNA). Relatively new to biodiversity and community composition analyses, eDNA, which is defined by the "presence of complex mixtures of genomic DNA from multi-species dispersed within biological and biological substrates (e.g., seawater and sediment)." (Kozial). eDNA can will be recovered from water samples and through DNA extraction, it will be sequenced and assigned to taxa (or species) and offers a powerful way to survey biodiversity (Kozial). This study will implement a DNA analysis procedure called metabarcoding in which the eDNA sample containing DNA from multiple species, can be sequenced in parallel. The DNA is then identified by it's binding to reference DNA strands, or "barcodes", and in this way species are identified (Kozial). This technique serves one of the goals of this capstone which is to initiate the collection of a community composition or biodiversity analysis. Studies employing DNA sampling protocols to test for the presence or absence of a single species have provided valuable data in the past, but for this study, metabarcoding is a perfect fit.

3.0 Materials and Technology

3.1 TriYak and Nautical Surveys

The surface water ROV system (TriYak) is a custom built remote control vehicle from the Unmanned Autonomous Systems Academy's (UASA) Land.Air.Sea Robots (™) Program (Figure 1a). The design is a miniature trimaran with a child's size ocean kayak in the center position with miniature pontoons attached to the side for stabilization. On either side of the center pontoon/kayak are two small electric outboard motors that can perform forward, backward, and side to side maneuvers. Mounted to the center of the TriYak is a metal frame that houses the equipment needed for data collection as well as a battery based power source with the associated remote control radio hardware. Additional equipment mounted to the TriYak consists of a Lowrance HSD7 Sonar, a 120-V AC motor with peristaltic pump head, water sample collection bottle, HoboDisk temperature and turbidity sensors, and a debris filter.

The TriYak will be launched from the vessel in a location proximal to the oyster reef and be remotely piloted to the location of the reef. Once the TriYak has arrived at the center of, or point of interest, on the reef, water samples will be collected by the radio controlled pump system. Once the samples are collected, the pilot will maneuver the TriYak back to the vessel for sample processing. Once sample processing is complete, the pilot will maneuver the TriYak back to the oyster reef and along a rectangular or square area, pilot the TriYak in transects that covers the areal footprint of the reef.

3.2 Sonar

The ROV will gather coordinates for all variables of interest utilizing the Global Positioning System (GPS) technology on the Lowrance HSD7 Sonar system. SONAR systems produce pulses of acoustic energy and records the reflected sound from the river-bottom into an image based off of the relative hardness of the benthic substrate (Guillen & Mokrech). According to Guillen & Mokrech, “The resolution of sonar imagery is affected by the speed of boat that is deploying the side-scan sonar system with higher resolution images are produced at lower boat speeds.”. The sonar system on the TriYak is equipped for side scan and down scan active imaging. This will allow for a side profile and a “top-down” view of the oyster reef. With the down-scan images including depth measurements and the side-scan images including width measurement, a 2 dimensional calculation of reef ball extent can be calculated. These calculations are a proxy measurement for potential oyster reef growth. Utilizing a sonar system attached to our “TriYak” ROV will allow for accurate recording of the location and areal extent of the oyster reef, the location of water quality measurements, the location of eDNA and water sample collection, as well as other variables. “Dense objects such as oyster shells reflect more sonar energy, whereas soft objects such as mud absorb energy and reflect weaker signals.” (Guillen & Mokrech). The main objects of interest necessitating the use of sonar in this project are the reef balls on the river bottom which would produce very strong and characteristic feedback on sonar.

3.21 Reef Volumetric Calculation Methods

Volumes of reef balls will be calculated as a proxy measurement for reef growth. he Magothy River Association indicated to the survey team that Dobbins Hill has recently seen an installation of reef balls, and for that reason (along with technical specifications from the manufacturers of the reef balls molds) a blank or non-oyster containing standard should be located proximal to the previously installed reef balls. This will allow for a standardized control structure to measured and compared against. Volumetric calculations of the reef balls structures at Dobbins Hill reef will be performed in ImageJ, an open-source image analysis and processing software that was developed by the National Institutes of Health. ImageJ has been shown to be a successful tool for many applications including the image based analysis of fish otoliths (or ear bones) for estimated age data (Goncalves et. al). The radius of randomly selected reef balls will be sourced from the sonar images with the depth readings serving as reference length used in the calculations. The known depth readings from the sonar data will allow the program and the user to define distance or length based upon the amount of pixels in an established standard (Section 7.2).

An assumption made for the calculation of the reef ball volumes is that the shape of the reef balls is a hemisphere (or half of a sphere). Therefore the volume of the reef balls will be calculated with the following equation:

Volume of Hemisphere: $V = \frac{2}{3}\pi r^3$

3.3 Water Quality Sampling

The water quality and sample collection suite consists of the peristaltic pump, debris filter, and sample bottle, which allows for the remote collection of eDNA for community composition analyses and water for in-situ water chemistry data. Water quality data (DO, Turbidity, Temperature, Salinity) will be collected remotely and brought back to the vessel to be measured manually with the Vernier Labquest equipment. There are three HoboDisk sensors (temperature and turbidity) that are attached to the TriYak. One on top of the TriYak facing upwards, one on the bottom of the debris filter, and one on top of the debris filter (Figure 1b). The placement of these sensors will allow for a calculation of turbidity (via lumens) in the water column between the surface and the depth of water collection.

3.4 eDNA Sampling

Environmental DNA (eDNA) samples were collected in the same manner and at the same time as the water quality samples. In order to avoid contamination of the sample the following steps were taken. the sample collection bottle was rinsed with purified tap water prior to sampling. Furthermore, the eDNA sample was collected before any water quality measurements were taken in order to avoid contamination of the sample post collection. 250 mL of water was collected for each eDNA sample. The filter sizes attach to the end of the sampling pump is 5 microns. The eDNA sampling kit take advantage of a 1 micron paper filter to trap DNA. 60 mL of water is the initial amount of water required to be filtered per directions from the analytical lab. That being said, filtration of the water sample should use as much water as in order to ensure a representative sample Once the samples are filtered, they are set with a preservative and then shipped to be analyzed and processed by Jonah Ventures in Boulder

Colorado. This study takes advantage of DNA metabarcoding which allows for many species to be identified in each sample.

3.5 Submersible ROV

For the acquisition of photographic and video-graphic data of the benthic habitat and oyster reef structure, this study utilized a commercially available solution, the Powervision PowerRay tethered ROVs. The PowerRay is a submersible ROV that is equipped with a forward facing high resolution camera with lighting as well as sonar for bottom scanning and depth control. The maximum distance that the submersible can operate is 300m as it is limited by the cabled tether to the vessel or location occupied by the pilot. The ROV is manually piloted via remote control and provides the pilot a live view of the video, sonar, and depth reading. The goal of the submersible is to collect video and photographic data of the reef structure as well as substrate type and quality. This data will be classified qualitatively in a similar manner to Heggie and Ogburn (2021), where reef structure, health, and coexisting species count were classified based on a 4 point scale. With the ability to observe reef texture and potential substrate intrusion, the images collected from the submersible will provide high quality data that compliments the sonar scans from the TriYak.

3.6 Small Unmanned Aircraft Systems and Aerial Surveys

Aerial photography taken from traditional aircraft and geospatial imaging taken from satellites, such as the LANSAT project, are well established methods for mapping features at broad spatial scales (Guillen and Mokrech). These traditional methods have provided the backbone of modern environmental monitoring but lack spatial resolution (level of image detail), have very high costs, and are not always in service, or available, when data is needed (Guillen and Mokrech). On the other hand a UAS with GPS, digital cameras, and autopilot can be used to monitor wildlife and features of interest such as oyster reefs (Anderson and Gaston 2013). For the acquisition of aerial photography and videography, this study will utilize a commercially available sUAS in the DJI Phantom 4 quadcopter. The DJI Phantom 4 was equipped with a Sentera and 4K resolution camera that will collect RGB and near infrared (NIR) images that will serve the purpose of providing high quality geo-referenced images that properly position the reef for later analysis. The combination of both UAS and ROV data provides the opportunity to verify the relative accuracy and precision of each method in mapping the oyster reef habitat.

Flight planning and clearance logistics were performed by and in coordination with Dr. Jim Blanchard and the pilots under his command. All operations were conducted with clearance from the Federal Aviation Administration through the filing of a "Notice to Airmen" (NOTAM) (Figure 5). Filing of a NOTAM with the FAA consists of reporting the sUAS operational location, flight range, altitude, time length of operation, operator ID, and sponsoring organization. Once a NOTAM is successfully filed, airspace will be reserved for your mission and will appear on live aeronautical navigational charts viewable to all pilots operating near the airspace. On the day of the flight, the Flight Team coordinated with local air traffic control services and manned aircraft in the area to ensure no risk was posed to other aircraft operating in the area. During the flight operation, preflight and postflight checklists were deployed to ensure the airworthiness of the sUAS, the safety of the operation, and the safety of the researchers on the vessel (Appendix 10.3). During the operation of the sUAS, one individual was, at all times, responsible for observing the airspace to prevent risk of interference with the airspace and mission.

Aerial surveys are key for establishing the areal extent of certain reefs that are above a certain depth and are exposed to the surface and air conditions. For this study, the reefs of interest were not tidally influenced, therefore the aerial surveys were used to plot the location of the reefs in relation to the current shoreline. Additionally, the sUAS was utilized to monitor the movement of the TriYak in order to accurately collect the required parameters. The sUAS was piloted manually to a set location above the reef with a buoy acting as a reference point (Figure 8). This procedure allowed for the TriYak to be piloted along a grid pattern over the footprint of the oyster reef.

3.7

Sitescan

Georeferenced images from the DJI Phantom 4 sUAS were collected through an the automated flight programing software Sitescan. Sitescan allows for a flight path to be preprogrammed and executed automatically by the sUAS. The GPS coordinates for the oyster reef are required to be input into the software for the center point of the area of interest. Once the reef location is entered, an areal polygon can be created that includes your oyster reef and other points of interest (Figure 8). The size of the polygon will determine the time of the flight, the amount of batteries required during the flight, and the number of images taken during the flight.

3.8 Software

ReefMaster is a software application that maps bathymetric data from sonar equipment. ReefMaster is available to the public for $200.00 USD and a free trial of 14 days is available. For the purposes of this study, the free trial version was utilized. Analyses and tools available in the free trial include underwater mapping, sidescan sonar mosaic, waypoint management, multi-and channel sonar viewer. Tracks from the sonar collections will appear as “layers” upon a basemap in the ReefMaster software and contain spatially and temporally explicit data as well as sonar images, depth, and vessel speed. Sonar tracks from multiple runs of the TriYak will be aggregated in order to provide a complete representation of the reef and the surrounding environment. Shapefiles of this data will be exported to ArcGIS Pro for visualization and presentation purposes. ArcGIS is a widely used program in the environmental sciences and is powerful tool for presenting geographic and environmental data. Water quality and UAS imaging will be embedded into the sonar data.

3.9 eDNA Kits

Environmental DNA kits are from Jonah Ventures, which provides a citizen science friendly approach to DNA sampling and processing. Aquatic eDNA kits are $89 USD and provide sequencing for fishes and phytoplankton. Instructions for using these kits are detailed in the procedural results section as well as the appendix.

Sampling instructions (Section 4.0 and Appendix 10.2) are included on their website as well as inside the packaging for each sample. In order to submit and review results from sampling, each user must register on their website. Then, through their their mobile app, JonahDNA, information about the samples collected need to be entered (Figure 4). The required information for each sample includes the preassigned Kit ID number, your site name, volume of water filtered, latitude, longitude, date, time, an option to make the data public or not, as well as any notes you may want to add for the sample. Results for the metabarcoding analysis take approximately 2 months.

4.0 Resulting Methods and Procedures

The results of this study, a preliminary procedural guide and methodology, are detailed in the "as-executed" stepwise field operations procedures in the section below. The footnotes of this section provide further details on the procedures and methods listed as well as lessons learned in the execution of this capstone. Due to limited sampling events and field excursions, preliminary data is solely provided for proof of concept and no conclusions or inferences as to the health of the oyster reef of interest can or should be made. These results will be detailed in the preliminary data and analysis section (Section 7.0)

A. Preparatory Procedure

1. Power on Vernier Labquest and calibrate sensor.[1]
2. Ensure Hobodisks are logging and synched (Appendix 10.5).
   I. Open HOBOware Pro software on iPad.
   II. Navigate to the devices window.
   III. To sync HOBO Pendents with the iPad, hit center button on HOBO Pendant once until it blinks blinking.[2]
   IV. Configure HOBO Pendant in order to erase previous data and create a new log file.
   V. Ensure that the times on the HOBO Pendants are accurate for recording purposes.

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[1] See appendix sections 10.8, 10.9, 10.10, 10.11, and 10.12 for device and sensor user manuals.
[2] The center button on the HOBO Pendant must be firmly pushed until the green light flashes once in order to sync with the HOBOware Pro iPad application.

3. Attach HOBO Pendants to TriYak and Debris Filter (Figures 1a and 1b).
   i. 1 on top of the TriYak.
   ii. 1 on the bottom of the debris filter cage.
   iii. 1 on the bottom of the debris filter cage.
4. Program flight pattern for sUAS in Sitescan software.
   i. Open SiteScan software.
   ii. Create a new project.
   iii. Enter latitude and longitude at the center of the point of interest.
   iv. Create new flight plan with areal survey as the flight mode.
   v. Create flight pattern polygon that incorporates the area of the reef .
   vi. In settings, select an overlap amount of 80%.
   vii. Save the created flight path.
8. Load SD cards into DJI Phantom, Sentera camera, Lowrance HDS7 Sonar, and Vernier Labquest device.
9. Ensure DJI Phantom, TriYak, iPad, remote control, and submersible ROV batteries are charged.
10. Load all materials and equipment onto the vessel.[3]
    i. 1 sUAS Case (with DJI Phantom, 4 batteries, spare propellers, and remote control)

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[3] Before departing from shore, ensure all equipment containers are closed appropriately and any access ports for wires are appropriately waterproofed. Water entering any of the equipment may cause electrical shorts or corrosion damage that may end sampling events or permanently damage the equipment. During this capstone, water entered the battery storage box due a non-waterproofed wire port and damaged a circuit board responsible for relaying power to the motors. This was remedied by relaying the power through a different circuit board. On different sampling event, water or corrosion damaged an antennae cable and prevented the signal transmitted from the remote control from reaching the TriYak ROV.

ii. 1 TriYak (with remote control, battery pack, pump motor, debris filter cage, and pontoons)
iii. 1 Powervision PowerRay ROV case (with remote control, tether cable, router box)
iv. 1 Toolbox
v. 3 HOBO Pendant data loggers
vi. 2 Apple iPads
vii. 4 eDNA kits from Jonah Ventures
viii. 4 Water sample collection bottles (250m)
ix. Notepads and pencils
x. Life vests for every survey team member

11. Launch vessel from boat ramp or slip.

B. Navigation and Preparatory Work

1. Pilot vessel at slow speed (no wake) to a location proximal to the oyster reef structure.
2. Deploy stern and bow anchors to provide a stable landing zone for
3. Launch TriYak from vessel and fasten to vessel for later deployment.

i. Ensure Lowrance Sonar is powered on, satellites locked, data is being recorded, and width of sonar scan set to 60m.

   i. Test remote control and motors with of the TriYak for power and proper orientation
   ii. Deploy Coast Guard Delta Flag on TriYak to indicate an Unmanned Vessel is being operated TriYak bow first into the water manually

iii. Ensure peristaltic pump is powered on, functioning correctly, cleared with bottled water, and secured to collection bottle.

iv. Fasten water sample collection bottle to vessel.

C. Perform sUAS airworthiness flight

1. Complete DJI pre-takeoff checklist.
2. Complete DJI takeoff checklist.
3. Test orientation of DJI Phantom.
4. Return DJI Phantom to vessel.
5. Power off, and then back on, all systems to reset Sentera Camera.
6. Replace battery in the DJI Phantom.

D. Execute Flight Plan from Sitescan with DJI Go4 app

1. Complete DJI pre-takeoff checklist.
2. Complete DJI takeoff checklist.
3. During ascent of the sUAS, verify a green light coming from the Sentera camera.
4. Record GPS coordinates of the center of the oyster reef.
5. Return DJI Phantom to vessel.
6. Remove SD cards with image data.
7. Once DJI Phantom is secured on the vessel, prepare TriYak for departure and collection of eDNA/water quality samples.

E. TriYak Water Quality Mission

1. Complete DJI pre-takeoff checklist (Appendix Section 10.3).
2. Complete DJI takeoff checklist (Appendix Section 10.3).

3. Launch DJI Phantom to monitor location of TriYak from above and follow TriYak on it's course.
4. Deploy "Delta" (Coast Guard flag for unmanned vessel) flag.
5. Pilot TriYak to location in the center of the oyster reef.
    i. Record GPS location.
6. Collect 250 mL of water for eDNA and water quality samples by flipping the right shoulder switch on the remote control, leaving the switch toggled for two minutes until the 250 mL bottle is full.
7. Pilot the TriYak back to the vessel.
8. Pilot DJI Phantom back to vessel.
9. Perform Arrival and Landing Checklist (See Appendix Section 10.3).
10. Recover the 500 mL water sample from TriYak and place it in the shade . Leave the TriYak fastened to the vessel.
11. Open sonar display box and save the data file containing the sonar transect and associated data with a file name containing the date and site location/number.
12. Process eDNA Sample (See Appendix Section .10.2)
    i. From the 2500 mL bottle, sample 60 mL of water with the provided syringe.
    ii. Screw on the syringe filter (1um) to bottom of provided syringe.
    iii. Push water through until you cannot push any more water through (refilling the syringe if needed.
    iv. Detach syringe and fill with 50 mL of air.
    v. Push 50 mL of air through the filter to dry it.
    vi. Inject 1 mL of provided preservative into the filter.

vii. Seal the sample with the 2 screw caps provided.

viii. Shake any water off the filter and replace it in the cup with the desiccant.

ix. Store sample in provided plastic bag with QR code.

x. Record sample information via pictures and notes.

11. Process Turbidity Sample.

    i. Use pipette to fill Vernier provided cuvette.

    ii. Plug in turbidity meter to Labquest device.

    iii. Set sampling procedure on Labquest device to sample every 0.2 seconds (5 samples/second)

    iv. Sample for 1 minute.

    v. Save data to Vernier Labquest device with title inclusive of date and run number.

12. Collect Dissolved Oxygen, Salinity, and pH data.

    i. Ensure SD card is inserted into Labquest device.

    ii. Program Vernier Labquest water quality data collection regiment.

    iii. Sample every 0.2 seconds (5 samples/second).

    iv. Time for sampling is 800 seconds.

    v. Insert probes into water sample

    vi. Initiate sampling run

    vii. When sampling is complete, save the file with a unique name associated to the site

    viii. Fasten *new* 250 mL bottle for additional samples, if needed.

13. Repeat steps 1-9 for each sample site.

14. Remove debris filter from TriYak after all water quality sampling events.

F. TriYak sonar mapping mission

1. Launch TriYak
2. Complete DJI Phantom pre-takeoff checklist.
3. Complete DJI Phantom takeoff checklist.
4. Launch DJI Phantom to monitor location of TriYak from above and follow TriYak on it's course.
5. Pilot the TriYak along a transect that starts furthest away from the shore and finishes closest to shore while covering the entire areal structure of the oyster reef.
6. When the area of the reef is mapped and all transects are complete, pilot the TriYak back to the vessel and attach.
7. Pilot sUAS back to vessel.
8. Perform Arrival and Landing Checklist (See Appendix Section 10.3).
9. Open sonar display box and save the data file containing the sonar transect and associated data with a file name containing the date and site location/number.
10. Turn off recording of Hobodisk data loggers via smartphone and save the data with a file name containing the date and sample location/number.
11. Repeat steps 1-9 for each sample site.
12. Retrieve TriYak from water
13. Turn off Lowrance HDS7 Sonar.
14. Disconnect TriYak motor power cables from the battery.

G. Subaquatic Reef Imaging and Habitat Classification Mission

1. Assemble and power on PowerRay systems (See Appendix Section 10.6).
2. Launch the PowerRay next to the vessel.

3. Test the orientation of the motors and remote control.[4]
4. Pilot PowerRay from vessel to sample site.
5. Submerge PowerRay to depth where benthic environment becomes visible.
6. Start video recording.
7. Execute pilot guided transect that starts furthest away from the shore and finishes closest to shore while covering the entire structure of the oyster reef.
8. When the transect is complete, turn off video recording and pilot the PowerRay back to the vessel and attach.
9. Retrieve the PowerRay from the water.[5]
10. Repeat steps 1-8 for each sample site

H. Wrap-Up Procedures

1. Tie off vessel at dock or shore.
2. Load vessel onto trailer.
3. Unload all gear from vessel on-shore.
4. Remove SD card from Labquest device.
5. Remove SD card from Lowrance HDS7 sonar.
6. Remove SD cards from DJI Phantom and Sentera camera.
7. Pack away DJI Phantom.
8. Pack away PowerRay.

---

[4] The first test of the PowerRay motors should be to move the ROV away from the vessel. Do not attempt to pilot the ROV towards the vessel as the first test.

[5] If the PowerRay ROV become stuck to the benthic environment, do not pull on the tether with full force. The tether is not load bearing and is prone to breakage and may result in loss of the ROV.

i. Rinse PowerRay with fresh water to clear out motors, clean lenses, and remove dirt and debris from surface.

ii. Drain the PowerRay by rotating it in all directions.

iii. Dry the PowerRay with microfiber towels.

iv. Dry tether port *completely* before unplugging the tether.

9. Remove HOBO Pendants from TriYak and debris filter.

10. Save data from HOBO Pendants

    i. Open HOBOware app

    ii. Select the “Run Out” button

    iii. Create a relevant file name

    iv. Export to .xlsx format

10. Pack away all remaining gear.

11. Debrief with the survey team and record any issues with maintenance, operations, safety, and training (M.O.S.T. debrief).

5.0 Discussion

The objective of this study was to establish a procedure utilizing experimental ROVs as well as UAS technology to gather environmental quality and eDNA data at a small scale oyster restoration project within the Chesapeake Bay watershed on the Magothy River in Maryland. The execution of these procedures occurred on the dates of October 23rd and October 31st of 2021. Prior to field testing, many hours of virtual planning calls, training sessions, background research were spent discussing the technology as well as the scope and goals of the project. The method and procedure established by this capstone was found have mixed results and achieved varying levels of success. The results show that reliable collection, and therefore accuracy, of the environmental data collected depends on the detail of the procedures, the capability of the technology, the training of ROV and UAS operators as well as the environmental and site characteristics, such as recent weather events, depth, type of oyster reef structure, and benthic substrate types. These procedures, established for use in future studies, were executed and logged as they were performed and represent an exploratory mission detailing the feasibility of UAS and ROV technology in the monitoring of oyster reefs and oyster reef restoration projects.

Utilizing the experimental ROV, the TriYak, was largely successful and demonstrated the capabilities of the technology well. Water samples were collected reliably, the piloting of the ROV was intuitive, and the sonar system produced results. Navigational errors and data logging issues prevented the complete sonar imaging of the oyster reef structure with the TriYak. For this reason, Sonar tracks from the TriYak and boat, and the associated data they contain, were aggregated in order to provide data for analysis in this capstone. The ROV in this study successfully collected one 250 mL water sample which was used for both water quality data and eDNA samples. In order to improve the robustness of data and the efficiency of time and resources, the TriYak has already been slated to include a multiple sample collection system. It has also been proposed that the sampling events, as well as the piloted sonar transects, could be preprogrammed to run at specific GPS coordinates by utilizing an an autopilot system similar to SiteScan for the UAS. This would allow for more efficient water sample collection and more comprehensive sonar image acquisition, therefore more representative environmental data over the entire site. In regards to eDNA sampling, this procedural capstone took solely aquatic eDNA samples. Kozial et. al emphasize that "…no single biological substrate (or media) can capture the broad spectrum of taxa that a multi-substrate study is capable of". As the capabilities of ROV technology improve, this study should implement a multi-substrate study that includes silt depositions on the reef when present. Not including more than one substrate, or eDNA sample media, may underestimate total diversity of species recorded (Kozial).

This study required the launching of a UAS from the boat, and consequentially the anchored boat location varied slightly with wave action during the survey. For this reason, a manual launching and landing procedure, "Hand-Launch and Recovery", was executed in order to avoid the UAS from landing in it's previously assigned landing zone, which may be in the water. The desired UAS imagery for this study consisted of a live video of the TriYak transect operation, an orthomosaic of the oyster reef, and the associated images within the orthomosaic. The UAS successfully provided overhead guidance to the TriYak operator through the live video feed back to the sUAS operator.

This coordination between pilots allowed for navigational hazards to be mitigated and straight transects to be piloted. Due to the nature of the oyster reef site, the orthomosaic was an aspect of the study was not successful. The issue that cause the orthomosaic to not be completed was the lack of heterogeneity in the photos taken. In order for the images to be stitched together for the mosaic, the algorithm needs to be able to distinguish where to combine the images. The homogenous appearance of water and the lack of land features prevented the successful construction of an orthomosaic. This being said, the images that were to be compiled into the orthomosaic were still taken and are valuable pieces of information. These images have GPS coordinates associated with them and can be used to monitor changes in the environment over time. For this study, because the reef was at a depth of 4 meters and not visible from the surface, these images were used solely for reference points. Orthomosaics and the images that comprise them, are important environmental data that can be utilized for oyster reefs that are intertidal and are exposed to the surface during low tide or reef structures that are close to shore and not at too great a depth.

One part of the methodology that did not yield productive results for this study site was the use of the Powervision PowerRay submersible ROV to gather benthic photography. Although the submersible ROV theoretically provided a simple and commercially available method of image data collection, the forward facing position of the camera produced unusable data due to the inability to see the benthic environment. Other factors affecting the applicability of the submersible ROV were the combination of depth and turbidity of the water, operator variability in skill level, and the inability to track the submersible’s location while operating.

Other oyster reef studies have utilized a bottom-facing camera system and have had considerable success in classifying oyster reef structure qualitatively for reef monitoring studies. Heggie and Ogburn (2021), of the Smithsonian Ecological Research Center (SERC), utilized video from a GoPro camera attached to a “sonar frame” to develop an “assessment approach for monitoring habitat characteristics of subtidal oyster reefs differing in restoration activity”. The study was performed on 4 tributaries of the Choptank River on the Eastern Shore of Maryland. These sites have large oyster harvest areas and oyster sanctuaries with water quality that is historically better than the site on the Magothy utilized for this study. Heggie and Ogburn developed a “..categorical score ranging from 0 to 3 was compared with percent cover of oyster shell and of fouling organisms.”. For future studies, a similar drop-camera system, mounted to the TriYak with depth control, could gather benthic images of the reef quality and structure. The camera system would have a size standards within the camera frame for use in later oyster sizing studies as well as other calculations.

5.1 Future Work

Oyster reefs and the coastal environments that contain them are immensely dynamic, both spatially and temporally. Traditional monitoring efforts in the Chesapeake Bay are failing to keep up with the implementation of new oyster reef restoration projects as well as the landscape scale changes affecting the success of these projects. The monitoring method explored in this capstone could be modified in order to produce robust datasets over long time frames for reef structure, reef biodiversity, and potential oyster reef mediated water quality changes. Organizations vested in the success of oyster restoration projects may find ROV and UAS surveys that monitor similar variables as this study does, valuable for recording oyster reef propagation, survivability, as well as for potential ecological and biodiversity influences. The spatially and temporally explicit collected by the UASs and ROVs in this study is an improvement over traditional methods where space and time are not explicit.

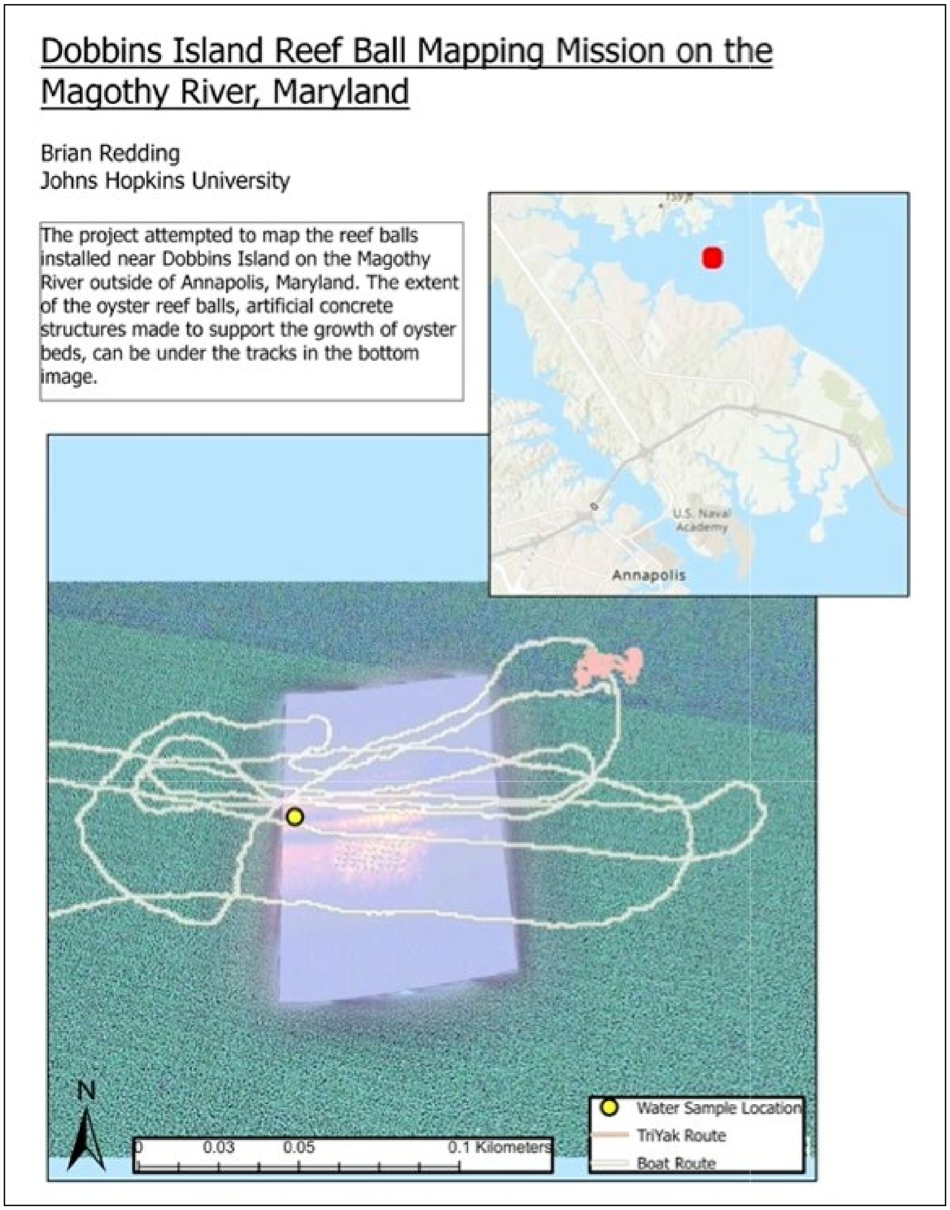
6.0 Conclusion

During the course of this capstone, background research was performed, procedures were developed, the procedures were field tested, and equipment was modified with the goal of developing and refining a novel protocol for the monitoring of oyster reefs. To this end, the goal was achieved. Due to the experimental nature of the capstone, many improvements were discovered along the way that will improve the procedures as-executed as well as the tools and technology used in future studies. This capstone demonstrated the potential for the combination of ROVs and UASs to provide robust data for many variables in a manner that is less invasive than traditional oyster monitoring efforts. With improvements to the procedures and technology presented in this capstone, the utilization of UASs and ROVs can be a time and resource efficient replacement for traditional oyster reef monitoring which would allow for the increased monitoring of oyster reef projects throughout the Chesapeake Bay and around the world.

7.0 Example Data

The section presents examples of data and results that can be produced from the methods and procedures provided in this capstone. The data and results presented are not full representation of what can be done with the technology and serve as a guide for future studies and survey crews. For each variable presented in this section there will be a brief summary of the data sourcing, analysis, and results. The example data presented below was not, and should not be, used to make conclusions about the health of the oyster reef studied.

7.1 Reef Mapping Data

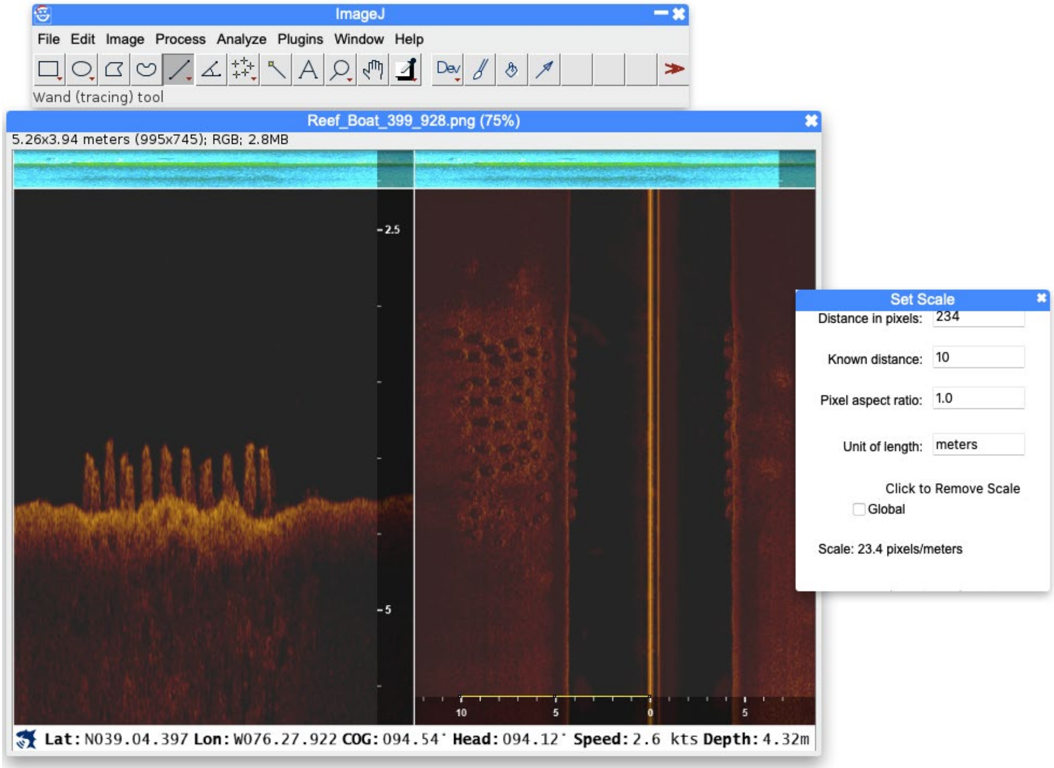


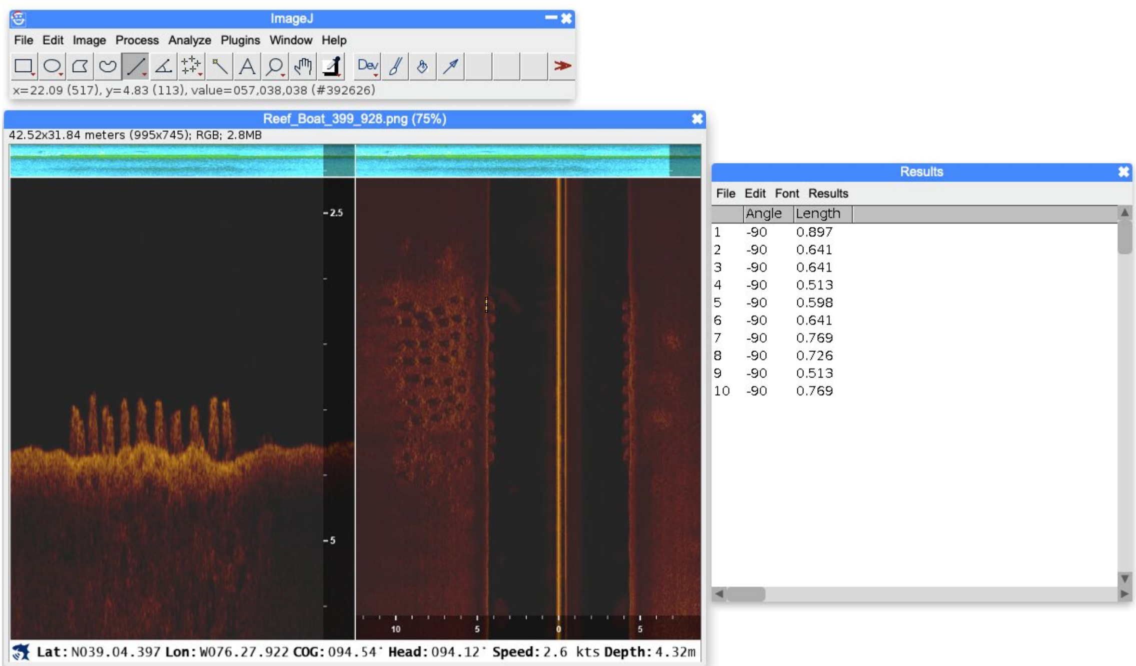
7.2 Reef Volumetric Calculations

The screenshots below display the sonar data from the Lowrance HDS7 sonar sensor. The images from the sonar sensor were exported to Reefmaster and then to ImageJ for analysis. In order to calibrate the size of objects in the image, a pixel to meter scale must be set using a known distance (in this study, the depth markings on the sonar screen). The pixel to meter scale set in the first image below is 23.4 pixels/meter. The diameters for each reef ball were measured using the top view on the right side of the screen due to the lessened impact of wave action on the smoothness of the benthic surface. The diameters measured are presented in the table of the second screenshot below. An assumption made for the calculation of the reef ball volumes is that the shape of the reef balls is a hemisphere. Therefore the volume of the reef balls will be calculated with the following equations:

Volume of Sphere: $V = \frac{4}{3}\pi r^3$

Volume of Reef Ball: $V = \frac{2}{3}\pi r^3$


ImageJ
File Edit Image Process Analyze Plugins Window Help
Wand (tracing) tool
Reef_Boat_399_928.png (75%)
5.26x3.94 meters (995x745); RGB; 2.8MB
Set Scale
Distance in pixels: 234
Known distance: 10
Pixel aspect ratio: 1.0
Unit of length: meters
Click to Remove Scale
Global
Scale: 23.4 pixels/meters
Lat: N039.04.397 Lon: W076.27.922 COG: 094.54˚ Head: 094.12˚ Speed: 2.6 kts Depth: 4.32m


ImageJ
File Edit Image Process Analyze Plugins Window Help
x=22.09 (517), y=4.83 (113), value=057,038,038 (#392626)
Reef_Boat_399_928.png (75%)
42.52x31.84 meters (995x745); RGB; 2.8MB
Results
File Edit Font Results
Angle Length
1 -90 0.897
2 -90 0.641
3 -90 0.641
4 -90 0.513
5 -90 0.598
6 -90 0.641
7 -90 0.769
8 -90 0.726
9 -90 0.513
10 -90 0.769
Lat: N039.04.397 Lon: W076.27.922 COG: 094.54˚ Head: 094.12˚ Speed: 2.6 kts Depth: 4.32m

Example Calculation

ReefBall #10 Measurements

	Diameter	r (Meters)	r (cm)	r^3	V (cm^3)	V (m^3)
10	0.769	0.385	38.450	56844.576	118935.947	0.119

$$V = \frac{2}{3}\pi r^3$$

$$V = \frac{2}{3}\pi 38.45^3$$

$$V = \frac{2}{3}\pi 56744.576$$

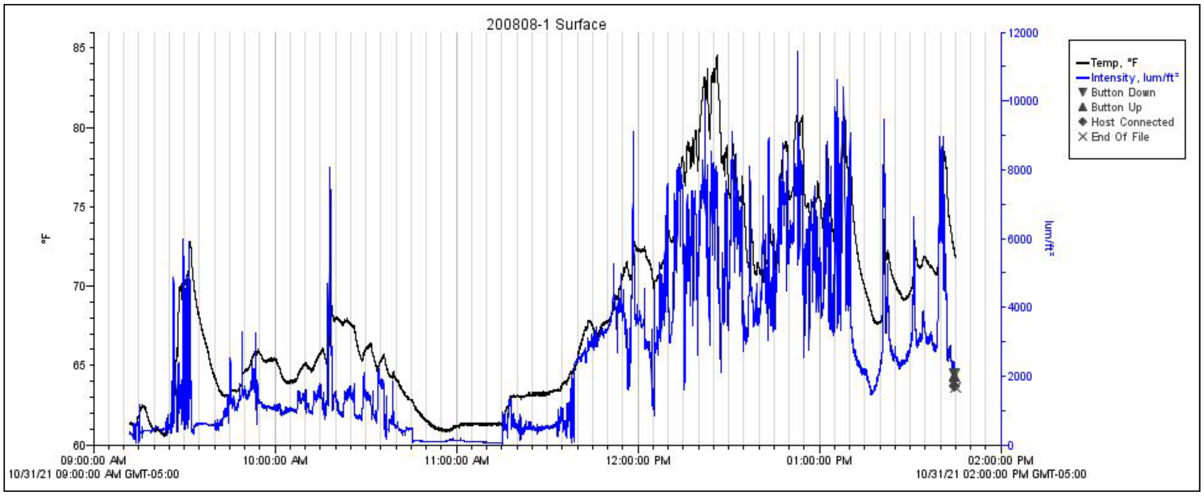
$$V = 118935.9cm3$$

$$V = 0.119m3$$

7.3 Water Quality Results

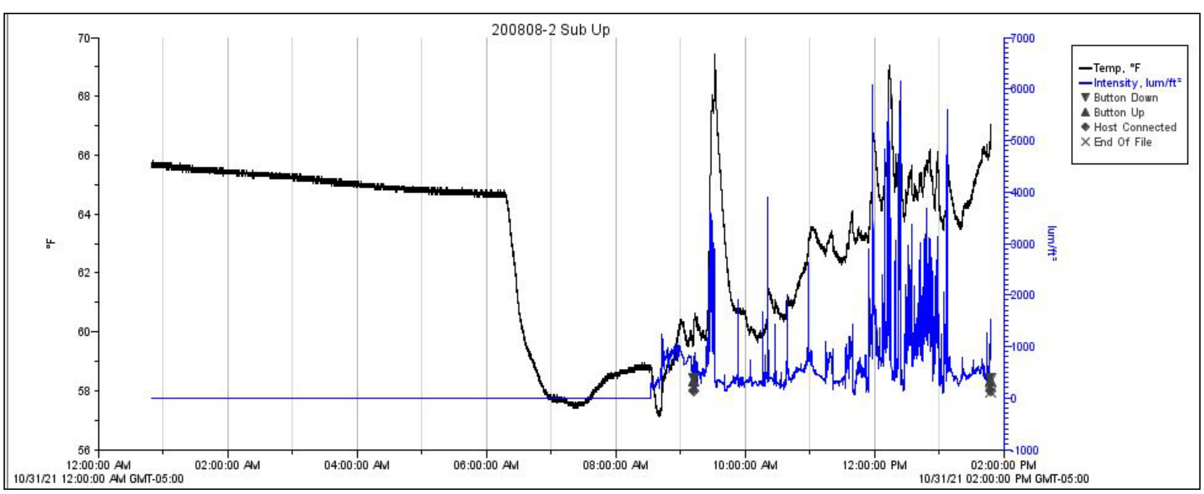
Temperature and Turbidity

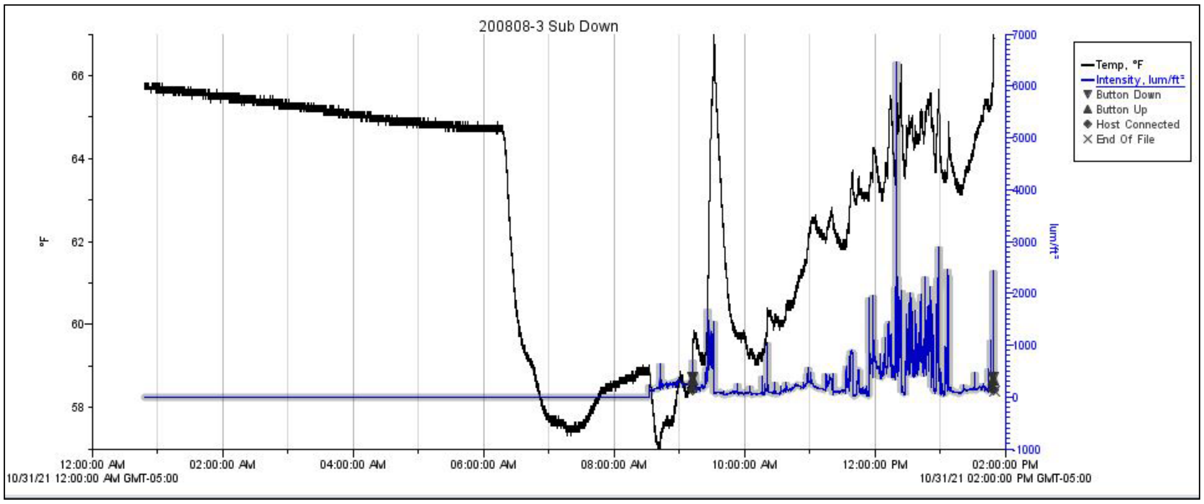
The graphs below are from the HOBO Pendant temperature and turbidity (lux) sensors and were assembled in Onset Computers' HOBOware software for desktop computer. While these graphs are for a long time period, the time interval of interest for this capstone is the time when the water quality sample was collected. This time period is between 12:45 PM and 12:47 PM on October 31st, 2021. The graphs appear in this order: Surface (on top of the TriYak), Up (on top of the debris filter facing up), and Bottom (on the bottom of the debris filter facing down). The data in these graphs are also available CSV format and it is from this format that our data will be analyzed. Due to technical constraints as well as the nature of the sampling procedure taking place over 2 minutes for a set volume of water in stasis, an average calculation will be performed for the turbidity (lux) and temperature data within this time interval. The averages calculated for the time periods for this sample were 71.99 Fahrenheit and 5283.54 lum/ft², 65.12 Fahrenheit and 1581.80 lum/ft², and 64.75 Fahrenheit and



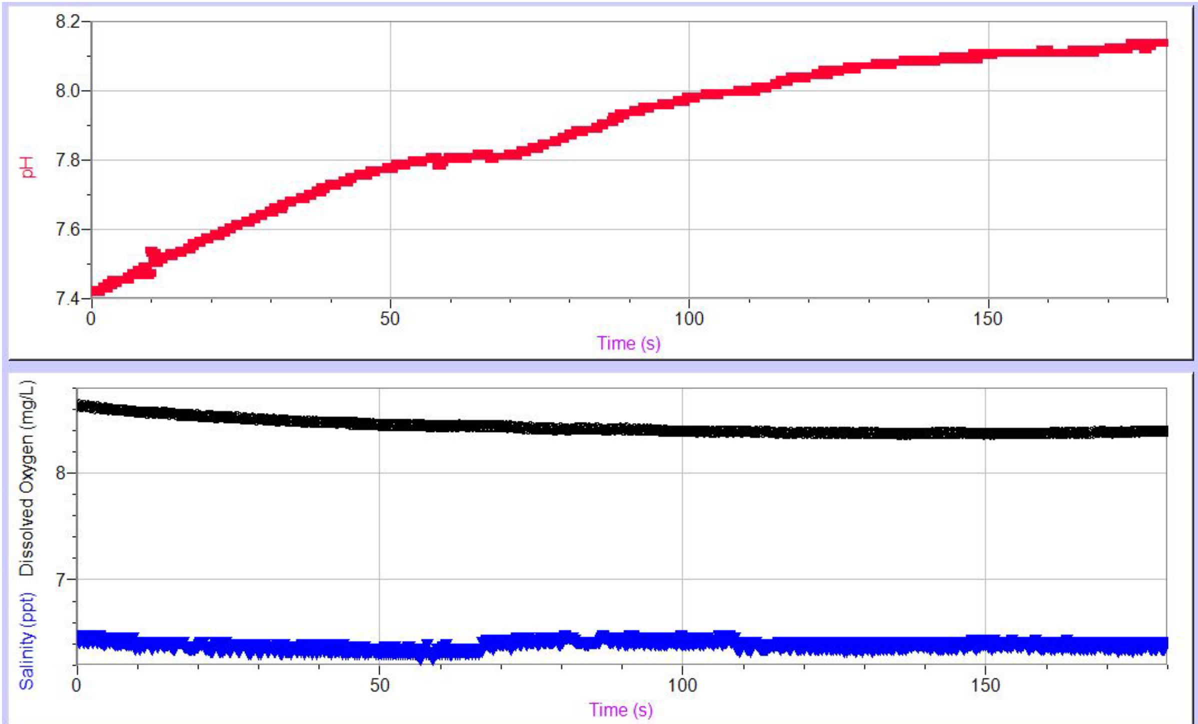
1011.89 lum/ft² for surface, up, and bottom respectively.

Salinity, Dissolved Oxygen, and pH





The graphs below are from the Vernier Labquest 2 device and the equipped dissolved oxygen (DO) in mg/L, salinity (ppt), pH, and turbidity (NTU) sensors and were assembled in Vernier Logger Lite software for desktop computer. The data in these graphs are also available CSV format and it is from this format that our data will be analyzed. Due to technical constraints as well as the nature of the sampling procedure taking place over 3 minutes for a set volume of water in stasis, an average result will be calculated for will be performed for all variables. The averages calculated for this sample are a pH of 7.88, a salinity of 6.39 ppt, dissolved oxygen of 8.43 mg/L, and turbidity (NTU) at 34.59 NTU.



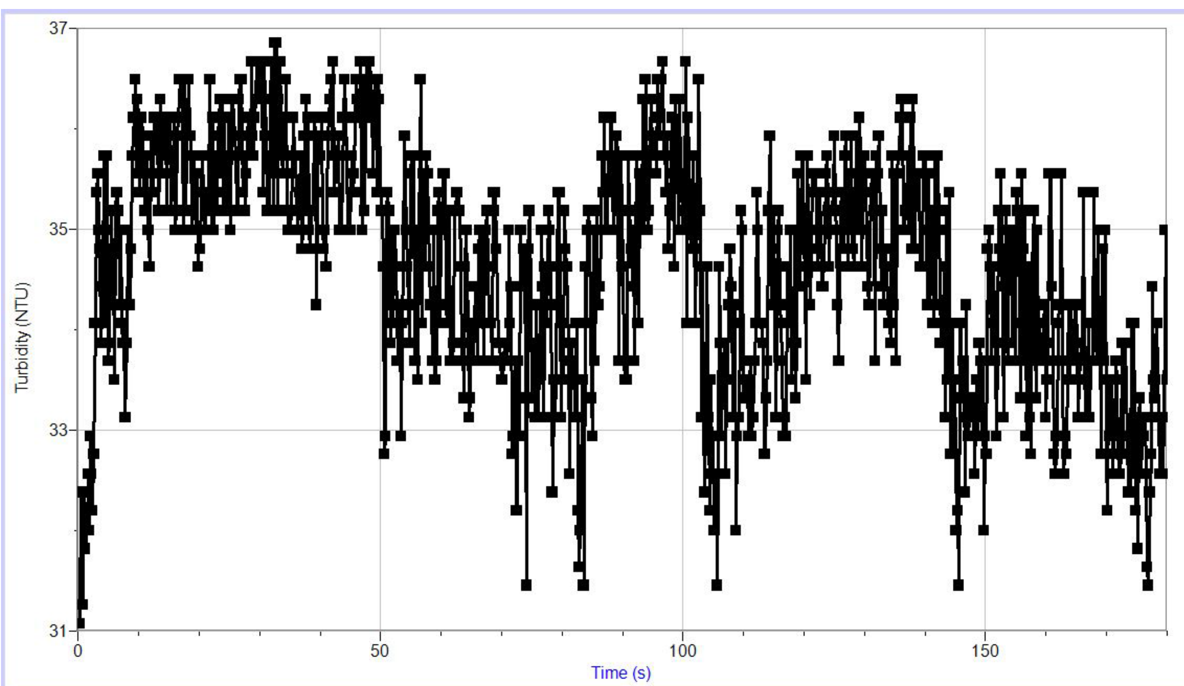
8.2
8.0
7.8
7.6
7.4
pH
0
50
100
150
Time (s)
Dissolved Oxygen (mg/L)
Salinity (ppt)
8
7
0
50
100
150
Time (s)

7.4 eDNA Based Community Composition Analysis

As of December 20th 2021, eDNA metabarcoding analysis is still being performed by Jonah Ventures in Boulder, Colorado. Samples were shipped on November 4th and received on November 5th. Delays in processing the data are expected with an estimated time frame of completion being early January 2022.

7.5 Benthic Image Classification

No images on the reef structures or benthic substrate were obtained during the limited missions for this capstone.



8.0

Figures, Photos, and Diagrams

Figure 1a. This is the custom built surface water remotely operated vehicle built by the Unmanned Autonomous Systems Academy's (UASA) Land.Air.Sea Robotics (™) (LASR) program and called the “TriYak”. The component parts are as numbered here: 1) antennae for remote controls, 2) delta flag, 3) 2 outboard electric motors, 4) waterproof battery and components housing, 5) support pontoons, 6) payload/main pontoon, 7) waterproof sonar display housing, 8) water pump antennae component housing, 9) water pump, 10) water sample collection bottle, 11) debris filter, 12) sonar sensor.

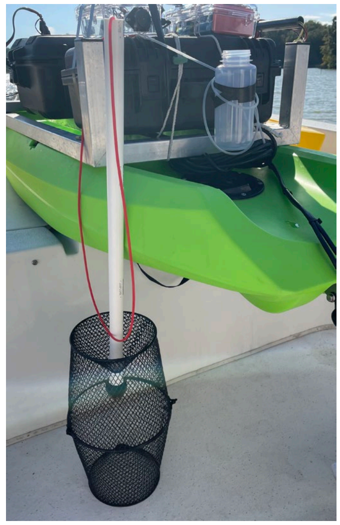


Figure 1b. This image depicts the debris filter and water pump collection system in more detail. The red pump is the in-line and white tube in the out-line.

Figure 2a. This is the topside of the Powervision PowerRay submersible remotely operated vehicle (ROV). The power light is on indicating that the tether is connected. Also on the top of the ROV is one of the depth control propellers.

Figure 2b. This is the bottom of the Powervision PowerRay submersible remotely operated vehicle (ROV).The large spherical object visible is the sonar sensor. Also on the bottom of the ROV is one of the depth control propellers.

Figure 2c. This is the remote control of the Powervision PowerRay submersible remotely operated vehicle (ROV)and user interface of the Vision+ app which displays the controls, live feed of the video camera, as well as sonar.

Figure 3 . Pictured here (front) are reef balls installed in a tropical setting for the purpose of coral reef restoration. The reef balls in this image are of similar size (3 feet) to the ones installed at the Dobbins Hill reef. Source (**http://www.reefball.com/brochure.htm**)

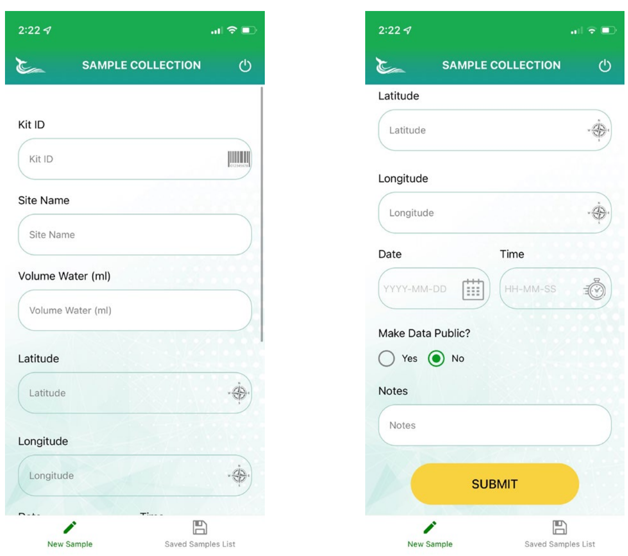
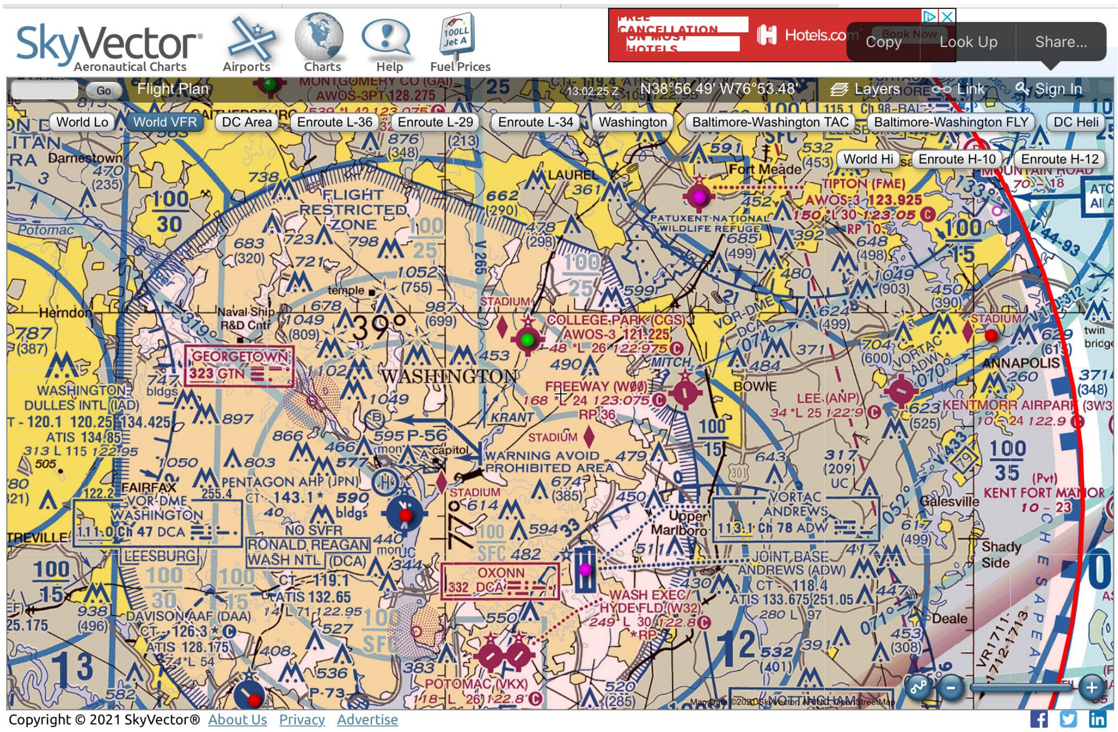
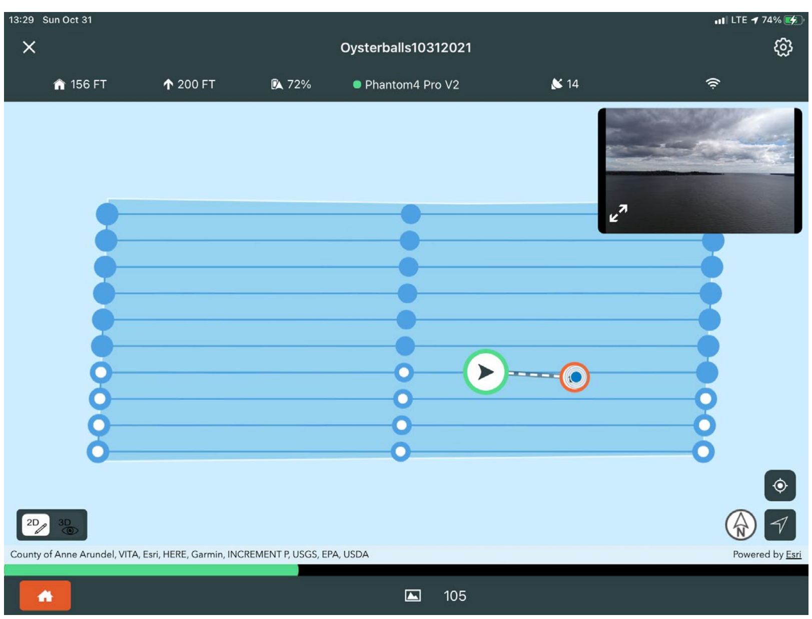


Figure 4. These screenshots from the Jonah Ventures "JonahDNA" app show the information



required when submitting samples for processing.
Figure 5. This Aeronautical chart displays the airspace for all uses in the region including the airspace utilized for the sUAS flight. The very small purple circle in the top right of the image represent the area of operation for the sUAS survey conducted for this experiment. The use of this airspace was authorized by the Federal Aviation Administration (FAA) through the filing of a



Notice to Airmen (NOTAM) by one of the FAA licensed sUAS operators on the survey team.

Figure 6. The flight pattern designed in SiteScan is displayed on the DJI Go App while the sUAS is in flight.

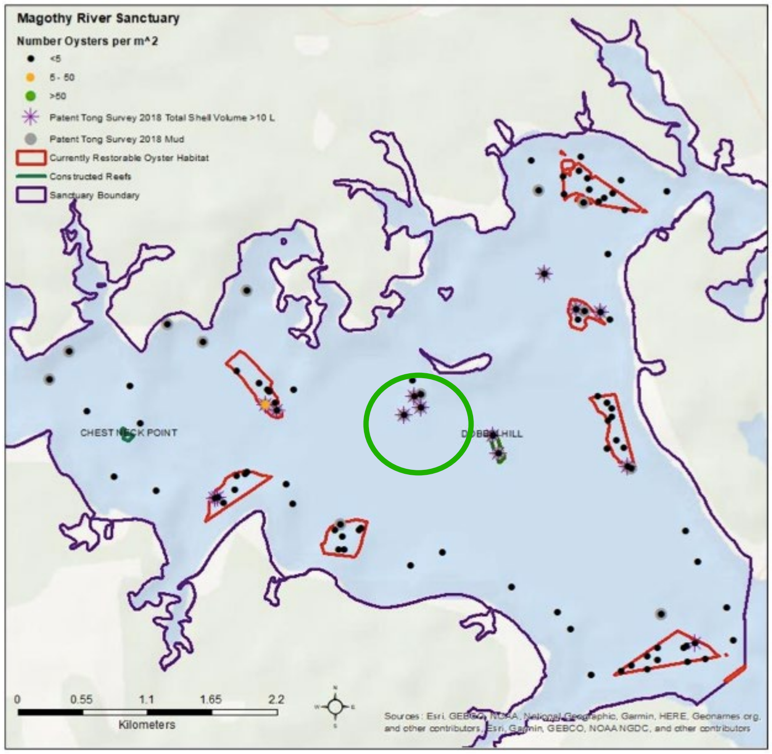
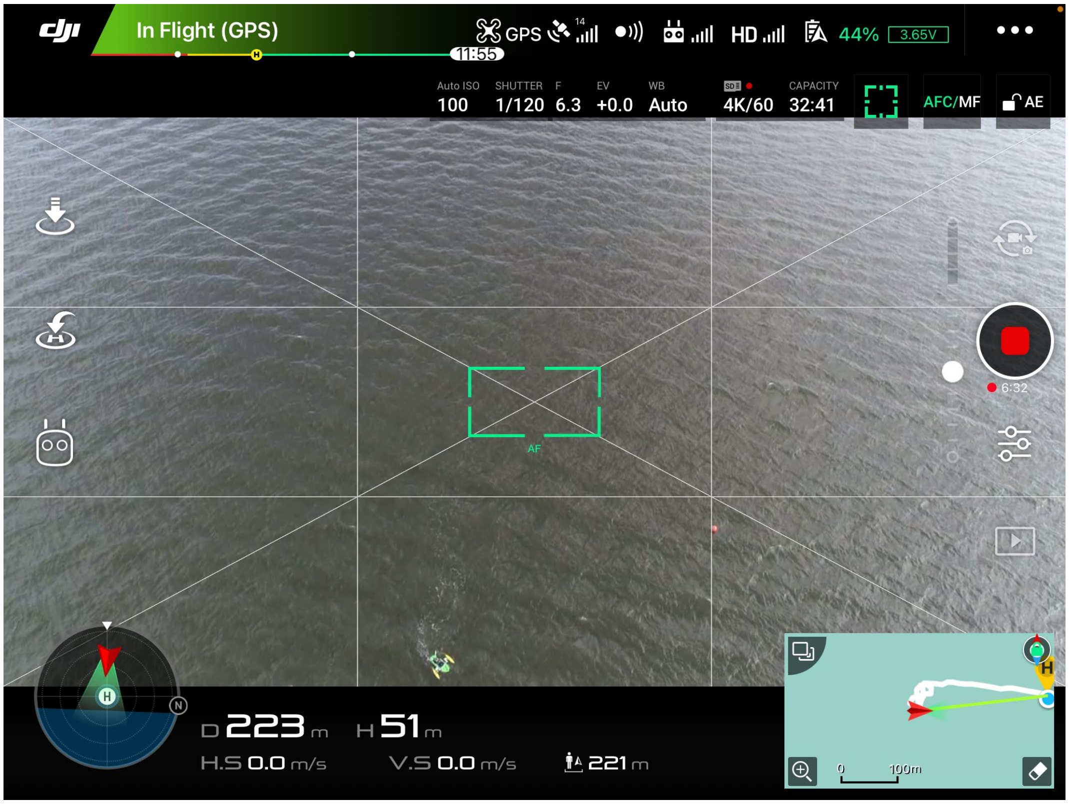


Figure 7. This map from the Maryland Department of Natural Resource's Anne Arundel Restoration plan shows the study area of interest circled in green. The data within the selection

is from the 2018 Patent tong Survey and indicates oyster densities of less than 5 oyster per



meter squared.

Figure 8. This image is a screenshot from the iPad of the sUAS (DJI Phantom Drone) operator. It shows the TriYak in operation with a point of interest (red buoy indicating the relative location of the oyster reef balls) included in the center bottom right section of the grid.

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10.0 Appendix

10.1 - Image J Software

- https://imagej.nih.gov/ij/index.html

10.2 - Jonah Ventures eDNA Sampling Protocol

- https://jonahventures.com/wp-content/uploads/instructions.water_.horizontal.11panel.05252021-01.png

10.3 - sUAS UASA Flight Checklist

READY ROOM BRIEFINGS		
PLANNING		
Pilot Flying	**Mission Planning**	**Pilot Not Flying**
PIC/VO/PF/PNF to Aircraft	☐ **Aircrew Requirements / 10-10 VO Assessment**	**PIC/VO/PF/PNF to Aircraft**
EVALUATION COMPLETE	☐ Map-Based Site Evaluation	EVALUATION COMPLETE
PRINTED & SCREEN SHOT	☐ Airspace (Class, Restrictions, TFRs, NOTAMs, Airports)	PRINTED & SCREEN SHOT
NOT REQUIRED or SCREEN SHOT	☐ LAANC Airspace Authorization Code	NOT REQUIRED or SCREEN SHOT
NOT REQUIRED or ***TalkMarker Enabled***	☐ DJI Airspace Authorization Code	NOT REQUIRED or ***TalkMarker*** Enabled
FAA SOURCE PRINTED & SCREEN SHOT	☐ Weather (Ceiling, Visibility, Winds, Precip, Daylight)	FAA SOURCE PRINTED & SCREEN SHOT
CHECKED	☐ Cell Phone Coverage	CHECKED
PRINTED	☐ Waivers Required	PRINTED
GROUND STATIONS	☐ Cache Mission Area Maps	GROUND STATIONS
PACKED PER IMAGE CHECKLIST	☐ Field Safety Go Kit	PACKED PER IMAGE CHECKLIST
PREFLIGHT		
Pilot Flying	**Crew Brief**	**Pilot Not Flying**
PIC ***TalkMarker*** Then Data	☐ Date/Time/Location	PIC ***TalkMarker*** Then DATA
VFR LOS	☐ Mission	VFR LOS
AGL PLUS 500' & 3SM	☐ Weather	AGL PLUS 500' & 3SM
DATA AGL BEARING DISTANCE	☐ Obstacles; MOCA Meters Above LZ; Quadrant	DATA AGL BEARING DISTANCE
120M or ACTUAL or LAANC then value	☐ Max Altitude Set	**120M or ACTUAL or LAANC then value**
MOCA + 10M or LAANC then value	☐ **RTH Altitude Set**	**MOCA + 10M or LAANC then value**
500M or ACTUAL or LAANC then value	☐ Max Distance Set	**500M or ACTUAL or LAANC then value**
RETURN TO HOME / HOVER / LAND	☐ RC Signal Lost Set	**RETURN TO HOME / HOVER / LAND**
DO NOT STOP IN AIR	☐ Stop Motors In Air	DO NOT STOP IN AIR
SET OFF	☐ **Auto Landing Gear**	**SET OFF**
SET ON Reg #	☐ **Remote ID**	**SET ON Reg #**
SET ON or BWDVR	☐ **Record Audio in DJI App or BWDVR**	**SET ON or BWDVR**
SET ON	☐ **DJI Cache Auto Manage/Clear**	**SET ON**
TalkMarker Then LOCATION & TIME	☐ Debrief Location/Time	***TalkMarker*** Then LOCATION & TIME
POSTFLIGHT		
Pilot Flying	**After Action Review:** Quick Cycles Around Crew Until All Pass	**Pilot Not Flying**
BWDVR/iPad/AC Connected to MOBAARS	☐ Connect sUAS System to MOBAARS	BWDVR/iPad/AC Connected to MOBAARS
PIC ***TalkMarker*** Then Data	☐ Date/Time/Location	PIC ***TalkMarker*** Then Data
PIC Mandatory	☐ All Crew Names	VO Mandatory if Used
Good/Bad List First PASS; Then AAR	☐ Maintenance	Good/Bad List First PASS; Then AAR
Good/Bad List First PASS; Then AAR	☐ Operations	Good/Bad List First PASS; Then AAR
Good/Bad List First PASS; Then AAR	☐ Safety	Good/Bad List First PASS; Then AAR
Good/Bad List First PASS; Then AAR	☐ Training	Good/Bad List First PASS; Then AAR

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FLIGHT DECK PREP CHECKLIST

PREFLIGHT - EVOLUTION		
Pilot Flying	**Flight Station Prep Checklist**	**Pilot Not Flying**
READY GPS	☐ MOBAARS Cameras	READY GPS
IN PLACE	☐ Signs, Cones, Flags	IN PLACE
ON	☐ Personal Protective Equipment	ON
COMPLETE	☐ Flight Area Inspection	COMPLETE
AIRPLANE MODE	☐ Cell Phones	AIRPLANE MODE
WIFI OFF	☐ iPads WiFi Off	WIFI OFF
FLIGHT DECK - HOPS		
Pilot Flying	**Aircraft Prep Checklist**	**Pilot Not Flying**
COMPLETE	☐ Aircraft Inspection	COMPLETE
INSPECTED & SECURE	☐ Propellers	**VERIFIED**
COMPLETE	☐ Camera/Gimbal/ND Filter Inspection	**VERIFIED**
COMPLETE	☐ Micro SD Card Installed w/ Sufficient Free Space	COMPLETE
CHECKED/RECORDED	☐ Aircraft Battery	CHECKED/RECORDED
SETUP/CHARGED	☐ Remote Controller w/iPAD/Glare Shield	SETUP/CHARGED
MAPS CACHED & WIFI OFF	☐ iPAD	MAPS CACHED & WIFI OFF
ALPHA DATA BRAVO DATA	☐ Handheld Radios Tuned & Checked Intra & Inter	ALPHA DATA BRAVO DATA

FLIGHT DECK SECURE CHECKLIST

POSTFLIGHT - EVOLUTION		
Pilot Flying	**Shutdown Checklist**	**Pilot Not Flying**
OFF	☐ Aircraft Battery	OFF
OFF	☐ iPad(s)	OFF
OFF	☐ Remote Controller(s)	OFF
PIC ***TalkMarker***	☐ PIC Hot Wash for MOST Immediate Corrections	PIC ***TalkMarker***
OPERATION | MISSION | EVOLUTION TERMINATION & sUAS COMPONENT AIRWORTHINESS ASSESSMENT		
Pilot Flying	**Recover Aircraft Checklist**	**Pilot Not Flying**
INSPECTED & REMOVED	☐ Propellers	INSPECTED & REMOVED
INSPECTED & REMOVED	☐ iPad Cable(s)	INSPECTED & REMOVED
INSPECTED & REMOVED	☐ iPad/Glare Shield	INSPECTED & REMOVED
INSPECTED & REMOVED	☐ Remote Controller(s)	INSPECTED & REMOVED
INSPECTED & REMOVED	☐ Aircraft	INSPECTED & REMOVED
DATA	☐ Battery Status Each	DATA

CONFIRMED or VISIBLE MODE	☐ **Confirm Satellite Lock (minimum of 11)**	**CONFIRMED or VISIBLE MODE**
CLEAR	☐ Clear Aircraft Ground Area	CLEAR
CAMERA ON HORIZON	☐ Camera C1 to Horizon	CAMERA ON HORIZON
ARMED	☐ Start Engines	ARMED
LOCATION CORRECT	☐ Verify Home Point Set Correct Location	LOCATION CORRECT
DATA	☐ Fuel Percentage	DATA
TalkMarker VISIBLE	☐ Low Fuel Dot	***TalkMarker*** VISIBLE
DEPARTING	☐ Announce Takeoff	DEPARTING
CALL MSA AGL	☐ Climb Above LZ Minimum Safe Altitude (Min 10M)	CALL MSA AGL
COMPLETE ABOVE 10M	☐ Control Check	COMPLETE ABOVE 10M
GEAR UP	☐ Landing Gear	GEAR UP
CORRECT	☐ Orientation Lights	CORRECT
TalkMarker VISIBLE	☐ Head Home Icon	***TalkMarker*** VISIBLE

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ARRIVAL & LANDING CHECKLIST

ARRIVAL		
Pilot Flying	**Landing Checklist**	**Pilot Not Flying**
RADIO INTENTIONS	☐ Coordinate Multiple AC Landing	RADIO INTENTIONS
TalkMarker ARRIVING	☐ Announce Landing 70M Out	***TalkMarker*** ARRIVING
LANDING		
Pilot Flying	**Landing Checklist**	**Pilot Not Flying**
TalkMarker CALL WHEN at MSA ALT	☐ Hold Above LZ Minimum Safe Altitude	***TalkMarker*** Call ALT when at MSA
GEAR DOWN	☐ Landing Gear Down at 10M	GEAR DOWN
LANDING	☐ Visually Clear Landing Area	LANDING
CALL 2M	☐ 2M Final Approach Fix	CALL 2M
CAMERA UP	☐ Roll Camera Up (unless blind landing)	CAMERA UP
CALL ON DECK	☐ Land Aircraft	CALL ON DECK
CALL DISARMED	☐ Shutdown Engines	CALL DISARMED
POWER REMAINING	☐ Fuel Percentage	POWER REMAINING
POWER REMAINING PF then PNF	☐ Remote Controller(s)	POWER REMAINING PF then PNF
POWER REMAINING PF then PNF	☐ iPad(s)	POWER REMAINING PF then PNF
GRANTED LEFT HAND OFF/DENIED	☐ Battery Replacement	PERMISSION TO APPROACH
TalkMarker RECORDING STOPPED	☐ Stop Video Recording	***TalkMarker*** RECORDING STOPPED

10.4 - Lowrance HDS-5, HDS-7, HDS-5m & HDS-7m Operation Manual
- https://softwaredownloads.navico.com/Lowrance/FTP/Lowrance_Software%20-%20Copy/Documents/HDS-5-7-5M-7M_OM_EN_988-10041-001_w.pdf

10.5 - Onset Computers HOBO Pendant User Manual
- https://www.onsetcomp.com/files/manual_pdfs/21536-M%20MX2201%20and%20MX2202%20Manual.pdf

10.6 - PowerVision PowerRay User Manual
- https://www.powervision.me/pv/manual/PowerRay_App_quick_start_guide_en_210806.pdf

10.7 - ReefMaster Software
- https://reefmaster.com.au/index.php/downloads/try-reefmaster

10.8 - Vernier Labquest 2 User Manual
- http://www2.vernier.com/manuals/labquest2_user_manual.pdf

10.9 - Vernier Dissolved Oxygen Probe User Manual
- https://www.vernier.com/files/manuals/do-bta/do-bta.pdf

10.10 - Vernier pH Probe User Manual

- https://www.vernier.com/files/manuals/ph-bta/ph-bta.pdf

10.11 - Vernier Salinity Sensor User Manual
- https://www.vernier.com/files/manuals/sal-bta/sal-bta.pdf

10.12 - Vernier Turbidity Sensor User Manual
- https://www.vernier.com/files/manuals/trb-bta/trb-bta.pdf