# A Rapid Assessment Protocol for the Identification of Invasive Species in the Albemarle-Pamlico National Estuary

by

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## Abstract

The Albemarle-Pamlico Estuarine System (APES) is the second largest estuary in the continental U.S. comprising 3,000 square miles of open water and a wide variety of physical and chemical characteristics. These characteristics allow for a highly diverse community composition, but also make APES a favorable host for the settlement and propagation of invasive species. In an effort to gain information regarding the invasive species already existing in APES, the Albemarle-Pamlico National Estuary Partnership would like to conduct an annual rapid assessment survey of the estuary. This rapid assessment protocol outlines suggested sampling sites within brackish and saline areas of the estuary for fouling, intertidal and benthic habitats. In addition, a directory of potential samplers, field forms, a sample database, and a trip budget were developed as part of this protocol.

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#### Introduction

The Albemarle-Pamlico Estuarine System (APES) is the second largest estuary in the continental United States after the Chesapeake Bay and spans the northern coast of North Carolina into southern Virginia (Paerl, Rossignol et al. 2009). This estuarine system is made up of six river basins: The Pasquotank, Chowan, and Roanoke River basins flow into the Albemarle Sound; the Tar-Pamlico and Neuse river basins flow into the Pamlico Sound; and the White Oak river basin flows into the Bogue, Back and Core Sounds, as seen in Figure 1. The sounds of the system include the Albemarle, Pamlico, Back Bay, Bogue, Core, Croatan, Currituck, and Roanoke sounds. This estuarine system comprises 3,000 square miles of open water and supports a number of aquatic and terrestrial species. The salinity of the estuary ranges from freshwater inland, to marine at the intersection with the Atlantic. The large range in salinities allows for niche differentiation and a potential for a highly diverse community composition of aquatic organisms (Cognetti and Maltagliati 2000). This highly diverse ecosystem supports an array of ecological and economic functions of high importance, such as forestry, agriculture, commercial and recreational fishing, tourism, mining, energy development as well as a high variation in land use, as seen in figure 2 (Johnston, McGarvey et al. 2011). Additionally, APES acts as a nursery and fishing ground for about 80% of the entire Southeastern Atlantic U.S. fishery (Paerl, Rossignol et al. 2009). Due to the vast ecosystem services that the APES provides, the system received a Congressional designation as an Estuary of National Significance in 1987.

The North Carolina Department of Environment and Natural Resources' (NCDENR) Albemarle-Pamlico National Estuary Partnership (APNEP) has, at its core, a mission to identify, protect, and restore the significant resources of the APES. Since APNEP's inception, the program has been following components of ecosystem-based management (EBM). However, an EBM approach for the estuary has recently been in the process implementation and has been formalized in APNEP's 2012 Ecosystem Assessment Report 2012 (Carpenter and Lindsay Dubbs 2012). In addition to outlining the required actions for this new management plan, the report calls for high quality scientific data, which would be used to assess the success the EBM implementation as well as for decision makers to rely on.





**Figure 2.** Land use in the Albemarle-Pamlico Estuarine System. Image from Johnston et al., 2011.

## **Invasive Species**

All the characteristics that make APES a great host for species diversity also make it a favorable host for the settlement and propagation of invasive species. Invasive species are non-indigenous organisms that have a competitive advantage over native species and include macrophytes, invertebrates, and vertebrates. Their presence often results in adverse effects to the economy, environment and/or ecology of the ecosystem (Alonso, Deichmann et al. 2011). Major vectors for the propagation of invasive species into aquatic systems include international shipping vessels, intentional releases of aquaria, and recreational boating (Diaz, Smith et al. 2012). A few invasive species that have already been detected in the brackish and saline areas of APNEP by the National Park Service include the Asian shore crab (*Hemigrapsus sanguineus*), striped barnacle (*Balanus amphitrite*), and Suminoe oyster (*Crassostrea asiakensis*). Table A1 in the appendix contains a more comprehensive list of potential species by salinity tolerance.

Through the National Invasive Species Act of 1996, the U.S. Coast Guard has required shipping vessels arriving at any U.S. port to release their ballast water mid ocean prior to arrival in order to control the introduction of non-native species. Failure of vessels to submit Ballast Water reports can result in a civil penalty or Class C Felony charge for non-submittal (USCG 2012). Aquarium trade is a major vector for the introduction of invasive species into freshwater systems, especially in regards to algae (Diaz, Smith et al. 2012). However, if these species have high salinity tolerances they may also be able to inhabit brackish habitats. In addition, there is increasing evidence that aquarium trade is contributing to the introduction to many marine species (Courtenay and Stauffer 1990). Introduction of aquarium species, whether intentional or unintentional, has been attributed to the release of unwanted pets into public waterways (Diaz, Smith et al. 2012). Once an area has been invaded, recreational boating can be a major secondary vector for the spread of an invasive species through the entanglement or entrainment in fishing gear, boat hulls, and or trailers during the transport of standing water (Johnson, Ricciardi et al. 2001; Rothlisberger, Chadderton et al. 2010; Stasko, Patenaude et al. 2012). Kelly et al. (Kelly, Wantola et al. 2013) found poor boat hygiene to be correlated with the number of zooplankton transported within the vessel's standing water. Therefore, it is important to increase boater education regarding the importance of boat hygiene by supplying boaters with information and rinsing stations at boat access locations. Several states have boating regulations in place that require boaters to drain the hulls and rinse their vessels after entering a waterway in order to prevent the spread of invasive species. Such regulation would not be out of the question for APNEP, but would be difficult to regulate given that the estuary spans two states.

## **Rapid Assessment Protocol**

In order to prevent and eradicate the spread of exotic and invasive species within the estuary, it is important for APNEP to know what species of concern are already present in the estuary. However, since a large census of the whole estuary would be both time and cost prohibitive, APNEP has decided to use a Rapid Assessment Survey (RAS) as a means to gain information regarding exotic and invasive species inhabiting the estuary including aquatic vegetation, invertebrates and vertebrates. A RAS is a conservation effort geared at obtaining a large amount of high quality biological information in a short period of time that can be shared widely (EPA 2009). A RAS brings together a team of local experts in their fields as collaborators from universities, museums, government agencies, etc. along with a sampling lead to take care of all the logistics regarding sampling, equipment, and accommodations in order to maximize sampling efforts (Alonso, Deichmann et al. 2011).

RAS is a popular method of surveying areas for invasive species and have been successfully executed by various National Estuary Programs (NEPs) including the Massachusetts Bays Program as well as the San Francisco Estuary Partnership in California and the Puget Sound Partnership in Washington State (Partnership 2010; Massachusetts 2013; Partnership 2013).

The objective of this project is to develop a Rapid Assessment Protocol (RAP) for APNEP to conduct a RAS in the saline and brackish areas within APES with a focus on the Pamlico Sound. This survey will be conducted biannually, or intermittently, and will take place during late summer when invertebrates are at their peak of maturity and thus easiest to identify. This RAP will provide APNEP with 1) suggested sampling sites by habitat and salinity type; 2) a directory of suggested local experts with contact information; 3) a trip budget; 4) an Access database loaded with sampling sites, sampler directory, suspected species, and data entry forms for

subsequent sampling trips; 5) field forms; and 6) a project proposal for the development of a mobile application by students at the Duke Computer Science Department.

#### **Sampling Sites**

Sampling site selection is one of the most important aspects in the preparation of a RAP, as an appropriate site selection will maximize the number of species found. Therefore, in order to maximize the diversity of species encountered during each trip it is necessary for the sampling sites to be as diverse as possible (Alonso, Deichmann et al. 2011). Sampling sites throughout the estuarine system were selected first by salinity and then by habitat type using spatial analysis (ArcGIS). Saline (> 30 ppt) and brackish (0.5-30 ppt) areas were identified within the estuary and marinas, intertidal, and subtidal/pelagic habitats were selected for each of the two salinity categories. As per APNEP's request, efforts were focused on the Pamlico estuary since higher invasion is suspected in this region. However, areas of the Bogue Sound were also included since these have greater salinities and are in close proximity to areas of high population as well as the Port of Morehead City.

A total of 26 sites were selected for the sampling trip under the assumption that each site will take an average of 1 hour to survey with some variability in sampling time between sites. This results in a total of about 5 hours of sampling per day plus transportation between sites and breaks. A greater number of sites were selected from the marinas category as well as all brackish areas due to suspicion of higher invasion potential. As seen in Table 1, 14 sites were selected from the marinas category, which includes 8 brackish and 6 saline sites. Intertidal and subtidal/pelagic categories each have 6 total sites with 4 brackish and 2 saline sites.

	Marinas	Intertidal	Subtidal/Pelagic
Brackish	8	4	4
Saline	6	2	2
Total number of sites	14	б	б

**Table 1.** Overview of number of sites by habitat salinity

#### **Marinas**

Marinas were chosen as sampling sites for the identification of fouling organisms since these organisms readily settle on wooden dock pilings and other objects that may be submerged including ropes and tires. Therefore, marinas make for good sampling locations since they are highly prone to invasion due to high boat traffic and are easily accessible for sampling. These sites were selected using the 'Sampling by Location' tool in ArcGIS 10.1 and were queried by selecting those marinas within 100 meters of either saline or brackish water. From there, each marina was searched online and those that seemed more exclusive such as yacht clubs were avoided where possible due to better control of fouling communities.

During the summer of 2000, the state of North Carolina began a voluntary program called Green Marina in which NC marinas that meet certain criteria set by the state can become clean certified. Criteria set by the program include the implementation of best management practices (BMPs) for issues regarding hazardous waste, sewage, boat maintenance activities, education, training, and fish waste (Management 2010). Although a few of the sites that were chosen during the site selection process were NC Certified Clean Marinas, they were not chosen based on this certification. It may be possible that if these establishments are willing to go through the trouble of implementing BMPs, they may also be more meticulous in the removal of fouling organisms from pilings and boats. However, if this is not the case and the implementation of BMPs allows for more successful invasion, it would be appropriate to reconsider NC Certified Clean sites for subsequent sampling events. Figure 3 and Table 2 show all Fouling sampling sites.

Site name	City	Lat	Long	Salinity	NC Clean Certified
Duke Marine Lab/ NOAA	Beaufort	34.71886	-76.67225	Saline	YES
Fort Macon Marina	Atlantic Beach	34.69926	-76.73096	Saline	
Harker's Island Fishing Center	Harkers Island	34.69375	-76.55957	Saline	YES
Island Harbor Marina and Marine Center	Emerald Isle	34.66881	-77.04512	Saline	
Morehead Gulf Docks	Morehead City	34.72002	-76.70934	Saline	
Radio Island Marina	Beaufort	34.72060	-76.68435	Saline	
Columbia Marina	Columbia	35.91778	-76.25407	Brackish	
Cypress Landing Marina	Chocowinity	35.50187	-77.04612	Brackish	YES
Dowry Creek Marina	Belhaven	35.53365	-76.53560	Brackish	
Manteo Waterfront Marina	Manteo	35.90998	-75.66900	Brackish	YES
Mariner's Wharf	Elizabeth City	35.29801	-76.21884	Brackish	
Morehead City Yacht Basin	Morehead City	34.72110	-76.70386	Brackish	
Oriental Harbor Village Center and Marina	Oriental	35.02417	-76.69546	Brackish	
Tidewater Marine Co., Inc.	New Bern	35.09112	-77.04654	Brackish	

Table 2. Fouling Community Sampling S	bites



Figure 3. Map of selected marinas in both, brackish and saline habitats

#### Intertidal

Intertidal areas are those located in zones that area exposed to the atmosphere during the low tide. As such, intertidal communities are distinct from sub-tidal communities because they can withstand the stresses associated with exposure to the atmosphere, changes in temperature and possible desiccation (Pechenik 2005).

The Albemarle-Pamlico Estuary is sheltered from the effect of lunar tides by a group of barrier islands called the 'Outer Banks' (Haase, Eggleston et al. 2012). Intermittent breaks in connectivity between islands allow for the flow of saline water through inlets (Lin, Xie et al.

2007). Consequently, tides within the estuary are predominately driven by wind currents, which are highly variable in velocity, yet seasonally predicable. Southwesterly winds dominate in the late-spring/summer while northeasterly winds dominate in the late-summer/fall (Xie and Eggleston 1999). These characteristics create a 'micro tidal coast', which is defined by Hayes (Hayes 1975) as having a tidal range of approximately 1 meter in height. The few areas on the coast that have a lunar tidal influence are located near inlets from the Atlantic. However, high water velocities coming through the inlets result in low nutrient contents and coarse sediment particles, making these areas unfavorable to most organisms (Peterson and Peterson 1979).

Intertidal habitats within the Albemarle-Pamlico Estuary are highly variable and include beaches, salt marshes, mudflats, sand flats, and eel grass beds (Dover and Kirby-Smith 1979). For the purpose of the RAS, four sites were selected from the brackish area of the estuary and two from the saline areas as seen in figure 4. Brackish areas are more variable in substrate, thus potentially rendering a higher number of exotic and invasive species as the site diversity is maximized. A GIS layer of substrate types within the estuary was obtained from the North Carolina Division of Marine Fisheries, which divides areas of the estuary into polygons and categorizes them under the following classifications:

'Intertidal Firm Vegetated Shell'
'Intertidal Firm Vegetated w/o Shell'
'Intertidal Hard Non-vegetated Shell'
'Intertidal Hard Non-vegetated w/o Shell'
'Intertidal Hard Vegetated Shell'
'Intertidal Hard Vegetated w/o Shell'
'Intertidal Soft Non-vegetated Shell'
'Intertidal Soft Non-vegetated w/o Shell'
'Intertidal Soft Vegetated Shell'
'Intertidal Soft Vegetated w/o Shell'

'Not Mapped'

Selecting intertidal sites based on substrate type proved to be very challenging due to the large selection of sites in each category, given the vast area of the estuary. Instead, a probability of invasion by pathways was taken in which sites were selected based on their proximity to pathways of invasion. Such pathways were determined by assessing proximity to cities with populations greater than 2,000 as well as proximity to ports and boat ramps.

Site name	City	Lat	Long	Salinity	Substrate Type
					'Intertidal Hard
Pea Island National Refuge	Manteo, NC	35.682	-75.488	Brackish	Vegetated Shell'
					'Intertidal Firm
Oyster Point Campground	Morehead				Vegetated w/o
	City	34.752	-76.765	Brackish	Shell'
					'Intertidal Hard
Rudolph					Non-Vegetated
-	Beaufort	34.759	-76.665	Brackish	Shell'
					'Intertidal Hard
					Non-Vegetated
	Northeast of	25.014	76 204	D 1'1	w/o Shell' and
Cedar Island	Beaufort	35.014	-76.304	Brackish	'Intertidal Firm
					Vegetated w/o
					Shell'
Rachel Carson National					Middle Marsh-
Estuarine Sanctuary	Beaufort	34.715	-76.742	Saline	'seagrass bed'
					'Intertidal Hard
					non-vegetated
Emerald Isle	Emerald Isle	34.68	-77.004	Saline	Shell'

 Table 3. Intertidal community sampling sites



Figure 4. Map of selected intertidal sites in both, brackish and saline habitats

## Subtidal/Pelagic

The subtidal zone the shallow part of the littoral zone that is permanently covered with water. As such, it can host a wide variety of species based on substrate. The pelagic zone is any water that is neither close to the benthos nor near the shore. It is therefore the water column in open-water areas.

The Pamlico Estuary is a bar built type of estuary and is characterized by having shallow depths of generally 2 to 3 meters with a mean depth of 4 meters (Reed, Dickey et al. 2008). Due to the shallow depth of the estuary and the fact that estuarine benthos are low in diversity, the benthic

and pelagic zones were grouped into a single category (Little 2000). As seen in figure 5, four sites were selected from brackish areas and two from saline areas. Sites were selected based on potential of invisibility, access, and site diversity. Areas adjacent to inlets were avoided because high flow velocities around inlets result in low biodiversity. Sampling method will be variable by site, but will include beach seine, coarse netting for vertebrates, fine netting for invertebrate larvae, and hand sampling from grass beds and mudflats.

Site name	Lat	Long	Salinity	Substrate Type	Sampling Method
Mill Creek	34.785	-76.722	Brackish	'Subtidal Soft Non- vegetated w/o Shell'	Beach Seine
Pea Island National Wildlife Refuge	35.673	-75.524	Brackish	'Subtidal Hard Vegetated w/o Shell'	Netting
Swan Quarter National Refuge	35.369	-76.334	Brackish	Mixed	Netting
Cedar Island Bay	34.992	-76.302	Brackish	'Subtidal Soft Vegetated w/o Shell'	Seining/Push netting
Back Sound Subtidal	34.662	-76.505	Saline	'Subtidal Hard Vegetated Shell'	Shovel and box sieve
Rachel Carson National Estuarine Sanctuary (South Marsh)	34.712	-76.74	Saline	'Subtidal Soft Non-Vegetated w/o Shell'	Hand (protected area)

 Table 4. Subtidal/pelagic community sampling sites



Figure 5. Map of selected Benthic/Pelagic sites in both, brackish and saline habitats

## **Sampling Team**

The sampling team will be made up of taxonomic experts in their field, students, and a support team. Ideally, the team will include experts in macrophytes, invertebrates, and vertebrates as well as one or two people handling logistics. The sampling team is expected to commit to the whole duration of the sampling trip; identify species in the field and verify them to the species level (if possible) at a later time in the laboratory; maintain a list of species identified and verified; preserve and archive voucher specimens; and properly document species at each location at the end of the survey. Sampling team will not be monetarily compensated, but will be provided with proper food and accommodations.

## **Plan of Action**

Prior to each sampling trip, sampling coordinators will be responsible for logistics regarding contacting samplers, accommodations, equipment, and instrument calibration. In addition, it will be imperative for samplers to have an itinerary for each sampling day. It is important for the needs and concerns of the samplers to be met since they will not be receiving monetary compensation for their efforts.

Once in the field, the data manager can be a student or APNEP employee who is in charge of basic data recording at each sampling location as well as measurements of water quality parameters while samplers make observations. Electronic equipment such as a tablet can be useful in entering data directly into worksheets or MS database. However, collection of data on waterproof paper and subsequent electronic entry is also an option.

Upon arrival to each of the sampling sites, site and water quality information should be collected and recorded in the sampling forms (Appendix Figures A1 and A2). Site information includes location, site type, sampler names, date, and time of collection. Water quality information will include temperature, pH, salinity, conductivity, turbidity, and dissolved oxygen. Individual sample information will include organism type, salinity where it was found, phylum, genus, and species. In the event that an organism cannot be identified, the specimen will be collected in a jar with ethanol solution and properly labeled for later identification.

## **Equipment Needed**

A variety of tools and equipment will be needed during each sampling event. Site information will require the use of a GPS unit. Water quality measurements require the use of probes for the measurement of the aforementioned water quality parameters as well as buffer solutions for instrument calibration. Fortunately, APNEP already conducts water quality sampling throughout the estuary and thus has access to these instruments. Sampling for organisms will require the use of a variety of scrapers, nets, jars, and dissecting tools as seen in Table 5.

General	Water Quality	Sampling
GPS Units	Calibration solutions	Scrapers
Waterproof paper	Temperature probe	Seining net
Clipboards	pH probe	Larval net
Pencils	Refractometer	Coarse pelagic net
Tablet (optional)	Turbidity probe	Jars
Boat	Dissolved oxygen probe	Trays/pans
Kayak		Sample labels
Таре		Solvent
		Dissecting tools
		Buckets
		Box sieve
		Shovels

 Table 5. Tools needed

The cost of each sampling trip will vary depending on the equipment that has already been purchased for previous sampling trips, thus making the first trip the most costly. A trip budget was developed (Table A2) working under the assumption that APNEP has access to water quality instruments and vehicles. It is estimated that the cost of the first trip will be about \$3,351.00 with each subsequent trip ranging from \$1,268.00 to \$1,924.00 as seen in Table 6. It is important to note that these figures are approximations since the price of fuel, distance traveled, housing costs and some of the equipment costs are subject to vary widely.

Table 6. Estimated cost p	er trip
Trip	Cost
First trip	\$3,351.00
Subsequent trip min	\$1,268.00
Subsequent trip max	\$1,924.00

#### Database

A database was developed for the purpose of storing all data related to this project. Within the database, information regarding sampling sites, organisms collected with taxonomy, and samplers for each trip can be located. Having a database facilitates data manipulation by allowing the querying of desired information. In addition, the fact that all samples are geocoded through relationships with sampling sites allows for this to be used as a geodatabase in conjunction with ArcGIS.

In order to facilitate data entry for those who are not familiar with MS Access, user-friendly forms were developed. A number of organisms that have already been sighted in the estuary by other agencies have been loaded onto database under the 'Organisms' table for ease of data entry. When beginning data entry, it is most useful to add any new samplers whose information is not already in the database as well as new sampling sites. After this information has been entered, users should proceed to entering water quality parameters under the "Sampling Site Info" form then enter information on each sample. Figure 4 shows the tables and relationships between these within the database.



Figure 4. MS Access database tables and relationships

## **Public Participation**

In addition to data acquired through the annual sampling trips, the project would greatly benefit from the implementation of a reporting procedure for visitors to the estuary who have the ability to upload pictures of suspected invasive species with a location, date and time. Presently the Massachusetts Bays Program has a page on their website where visitors can upload pictures of suspected exotics within the bays. However, given the popularity and versatility of mobile applications, the design of such an application for public participation would increase the quality of the data collected while at the same time educating and engaging the public. Many such applications already exist, each with a different goal. For example, the application "What's Invasive!" helps visitors to report invasive species found in national parks by providing the application with the common name of the organism, quantity observed (one, few, or many), coordinates, a picture, and additional notes (UCLA). This application is made to engage the greater public while contributing information to the park service. On the other hand, there are also public engagement applications that require more information on each organism in order for the data collected to be utilized by the scientific community. One example of one such application is Calflora, which in addition to location asks more specific information regarding the plant in addition such as scientific name (genus and species), life form (fern, grass, annual, or perennial), whether the plant is native, rarity, elevation, category (monocot, dicot, etc.), and community (Calflora 2012).

The goal of the potential mobile application for the Albemarle-Pamlico Estuary will ultimately be determined by NCDENR and the developer. This project can be completed by upper division undergraduates at Duke University through the course COMPSCI 290: Apps, From Concept to Client, taught during Fall semester by Richard Lucic. In this course, groups of three students chose a client project and develop a mobile application for their client during the duration of the semester. A project proposal will be drafted and submitted on behalf of NCDENR as part of this project.

# Appendix

Mytilopsis leucophaeataConrad's false musselInvertebrateXXX2Cordviolphora caspiaFreshwater hydroidInvertebrateXX2Cordviolphora caspiaFreshwater hydroidInvertebrateXX2Paranais fricia tubilicid wormInvertebrateXX2Paranais fricia tubilicid wormInvertebrateXX2Ascidiella aspersaSolitary ascidianInvertebrateXX2Boccardiella figericaa spinoid wormInvertebrateXX2Bornea bisuturalisTwo-goove odostomeInvertebrateXX2Callinectes bocourtiBocourt swinning crabInvertebrateXX2Callinectes bocourtiBocourt swinning crabInvertebrateXX2Charybdis helleriian Indo-Pacific crabInvertebrateXX2Diadomene linetaOrange-striped sea amemoneInvertebrateXX2Didommane linetaConsial tunicateInvertebrateXX2Didommane linetaConsial tunicateInvertebrateXX2Didommane linetaConsial tunicateInvertebrateXX2Didommane linetaConsial tunicateInvertebrateX2Didommane linetaConsial tunicateInvertebrateX2Didommane linetaSea (Lagia exoticX2Didommane linetaC	Scientific name	Common name	Туре	Fresh	Brackish	Saline	Source
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HemgripulusInstant Sindi Sindi CalabInvertebrateXXZBoccardiella ligericaa spinoid wormInvertebrateXXZBoccardiella ligericaa spinoid wormInvertebrateXXZBotryllus schlosseriGolden star tunicateInvertebrateXXZCallinectes bocouritBocourt swimming crabInvertebrateXXZCarnicus maenasGreen crabInvertebrateXXZCuthona percaLake Merrit cuthonaInvertebrateXXZDiadumene lineataOrange-striped sea anemoneInvertebrateXXZDiadumene lineataOrange-striped sea anemoneInvertebrateXXZDiadumene lineataOrange-striped sea anemoneInvertebrateXXZDiadumene lineataOrange-striped sea anemoneInvertebrateXXZDiadumene lineataColonial tunicateInvertebrateXXZDiadumene lineataColonial tunicateInvertebrateXZDirigita exoticaWhatri roachInvertebrateXZMaeorias inexspectataBlack Sea JellyfishInvertebrateXZMoerisia lyonsia hydrozoanInvertebrateXZMoerisia lyonsihydrash snailInvertebrateXZSyela clavaAsian tunicateInvertebrateXZSyela clavaAsian tunicateInvertebrate	Paranais frici	a tubificid worm	Invertebrate	Х	Х		2
Actuality of the second seco	Hemigrapsus sanguineus	Asian Shore Crab	Invertebrate		Х	Х	2
Dectorational inferitorAXBoonea bisuturalisTwo-goove dostomeInvertebrateXXBorryllus schlosseriGolden star tunicateInvertebrateXX2Callinectes bocourtiBocourt swimming crabInvertebrateXX2Carnicus maenasGreen crabInvertebrateXX2Charybdis helleriian Indo-Pacific crabInvertebrateXX2Cuthona percaLake Merrit cuthonaInvertebrateXX2Diadumene lineataOrange-striped sea anemoneInvertebrateXX2Drymonema dalmatinumPink meanieInvertebrateXX2Pricopomatus enigmaticusAustralian tubewormInvertebrateXX2Ligia exoticaWharf roachInvertebrateXX2Maeotias inexspectataBlack Sea JellyfishInvertebrateX2Moerista lyonsia hydrozoanInvertebrateX2Mosostella myosotisMarsh snailInvertebrateX2Moerisa loyostia hydrozoanInvertebrateX2Moerisa loyostiBristlewormInvertebrateX2Moerisal ponsia hydrozoanInvertebrateX2Moerisal ponsia hydrozoanInvertebrateX2Moerisal ponsia hydrozoanInvertebrateX2Moerisal ponsia hydrozoanInvertebrateX2	Ascidiella aspersa	Solitary ascidian	Invertebrate		Х	Х	
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Carnitals materialsOrecurvitabInvertebrateXX2Charybdis helleriian Indo-Pacific crabInvertebrateXX2Diadumene lineataOrange-striped sea anemoneInvertebrateXX2Didemnum lahilleiColonial tunicateInvertebrateXX2Drymonema dalmatinumPink meanieInvertebrateXX2Ligia exoticaMustralian tubewormInvertebrateXX2Ligia exoticaWharf roachInvertebrateXX2Littorina littoreaCommon periwinkleInvertebrateXX2Maeotias inexspectataBlack Sea JellyfishInvertebrateXX2Moorisia lyonsia hydrozoanInvertebrateXX2Ostrare adulisEdible oysterInvertebrateXX2Polydora ciliataBristlewormInvertebrateXX2Synidotea laevidorsalisan isopodInvertebrateXX2Synidotea laevidorsalisan isopodInvertebrateXX2Synidotea laevidorsalisan isopodInvertebrateXX2Sphaeroma terebransWarty pilbugInvertebrateX2Crassostrea asiakensisSuminoe oysterInvertebrateX2Crassostrea asiakensisSuminoe oysterInvertebrateX2Edible oysterInvertebrateXX2<	Callinectes bocourti	Bocourt swimming crab	Invertebrate		Х	Х	
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Didaminent internationInvertebrateXXDidemnum lahilleiColonial tunicateInvertebrateXX2Drymonema dalmatinumPink meanieInvertebrateXX2Ficopomatus enigmaticusAustralian tubewormInvertebrateXX2Ligia exoticaWharf roachInvertebrateXX2Littorina littoreaCommon periwinkleInvertebrateXX2Maeotias inexspectataBlack Sea JellyfishInvertebrateXX2Membranipora membranaceaSea mat, Lacy crust bryazoanInvertebrateXX2Moerisia lyonsia hydrozoanInvertebrateXX2Mysostella mysostisMarsh snailInvertebrateXX2Ostrae adulisEdible oysterInvertebrateXX2Polydora ciliataBristlewormInvertebrateXX2Sygela clavaAsian tunicateInvertebrateXX2Synidotea laevidorsalisan isopodInvertebrateXX2Tereedo navalisNaval shipwormInvertebrateXX2Sphaeroma terebransWarty pillbugInvertebrateX2Hemigaspus sanquineusJapanese shore crabInvertebrateX2Crassostrea asiakensisSuminoe oysterInvertebrateX2Anguillicola crassusEel parasiteInvertebrateX2 <td>Cuthona perca</td> <td>Lake Merrit cuthona</td> <td>Invertebrate</td> <td></td> <td>Х</td> <td>Х</td> <td>2</td>	Cuthona perca	Lake Merrit cuthona	Invertebrate		Х	Х	2
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Pricopondatis enginanciasAustralian dubewormInvertebrateXXLigia exoticaWharf roachInvertebrateXX2Littorina littoreaCommon periwinkleInvertebrateXX2Maeotias inexspectataBlack Sea JellyfishInvertebrateXX2Membranipora membranaceaSea mat, Lacy crust bryazoanInvertebrateXX2Moesisia lyonsia hydrozoanInvertebrateXX2Myosotella myosotisMarsh snailInvertebrateXX2Ostrae adulisEdible oysterInvertebrateXX2Polydora ciliataBristlewormInvertebrateXX2Rapana venosaVeined Rapa whelkInvertebrateXX2Synidotea laevidorsalisan isopodInvertebrateXX2Teredo navalisNaval shipwormInvertebrateXX2Sphaeroma terebransWarty pillbugInvertebrateX2Hemigaspus sanquineusJapanese shore crabInvertebrateX2Crassostrea asiakensisSuminoe oysterInvertebrateX2Anguillicola crassusEel parasiteInvertebrateX2Balanus trigonusa barnacleInvertebrateX2Balanus trigonusa barnacleInvertebrateX2Balanus trigonusa barnacleInvertebrateX2Anguillicola cra	Drymonema dalmatinum	Pink meanie	Invertebrate		Х	Х	2
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Entonia intervaCommon performateInvertebrateXXMaeotias inexspectataBlack Sea JellyfishInvertebrateXX2Membranipora membranaceaSea mat, Lacy crust bryazoanInvertebrateXX2Moerisia lyonsia hydrozoanInvertebrateXX2Myosotella myosotisMarsh snailInvertebrateXX2Ostrae adulisEdible oysterInvertebrateXX2Polydora ciliataBristlewormInvertebrateXX2Rapana venosaVeined Rapa whelkInvertebrateXX2Synidotea laevidorsalisan isopodInvertebrateXX2Tenellia adspersaMiniature aeolisInvertebrateXX2Sphaeroma terebransWarty pillbugInvertebrateX2Hemigaspus sanquineusJapanese shore crabInvertebrateX2Anguillicola crassusEel parasiteInvertebrateX2Balanus AmphitriteStriped barnacleInvertebrateX2Balanus trigonusa barnacleInvertebrateX2	Ligia exotica	Wharf roach	Invertebrate		Х	Х	2
Mateonas messpectadaBrack Sca FerlyTishInvertebrateXXMembranipora membranaceaSea mat, Lacy crust bryazoanInvertebrateXX2Moerisia lyonsia hydrozoanInvertebrateXX2Myosotella myosotisMarsh snailInvertebrateXX2Ostrae adulisEdible oysterInvertebrateXX2Polydora ciliataBristlewormInvertebrateXX2Rapana venosaVeined Rapa whelkInvertebrateXX2Styela clavaAsian tunicateInvertebrateXX2Synidotea laevidorsalisan isopodInvertebrateXX2Tenellia adspersaMiniature aeolisInvertebrateXX2Sphaeroma terebransWarty pillbugInvertebrateX2Hemigaspus sanquineusJapanese shore crabInvertebrateX2Ralanus AmphitriteStriped barnacleInvertebrateX2Balanus AmphitriteSuminoe oysterInvertebrateX2Balanus trigonusa barnacleInvertebrateX2Balanus trigonusa barnacleInvertebrateX2	Littorina littorea	Common periwinkle	Invertebrate		Х	Х	2
Membrandora membrandeedScandal, Eacy Crust of yazoanInvertebrateXXMoerisia lyonsia hydrozoanInvertebrateXX2Myosotella myosotisMarsh snailInvertebrateXX2Ostrae adulisEdible oysterInvertebrateXX2Polydora ciliataBristlewormInvertebrateXX2Rapana venosaVeined Rapa whelkInvertebrateXX2Styela clavaAsian tunicateInvertebrateXX2Synidotea laevidorsalisan isopodInvertebrateXX2Tenellia adspersaMiniature aeolisInvertebrateXX2Sphaeroma terebransWarty pillbugInvertebrateX2Hemigaspus sanquineusJapanese shore crabInvertebrateX2Balanus AmphitriteStriped barnacleInvertebrateX2Anguillicola crassusEel parasiteInvertebrateX2Balanus trigonusa barnacleInvertebrateX2	Maeotias inexspectata	Black Sea Jellyfish	Invertebrate		Х	Х	2
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Myosoleta myosolisMarsii sianInvertebrateXXOstrae adulisEdible oysterInvertebrateXX2Polydora ciliataBristlewormInvertebrateXX2Rapana venosaVeined Rapa whelkInvertebrateXX2Styela clavaAsian tunicateInvertebrateXX2Synidotea laevidorsalisan isopodInvertebrateXX2Tenellia adspersaMiniature aeolisInvertebrateXX2Sphaeroma terebransWarty pillbugInvertebrateX2Hemigaspus sanquineusJapanese shore crabInvertebrateX2Balanus AmphitriteStriped barnacleInvertebrateX2Anguillicola crassusEel parasiteInvertebrateX2Balanus trigonusa barnacleInvertebrateX2	Moerisia lyonsi	a hydrozoan	Invertebrate		Х	Х	2
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Polyabra childaDifferentiationInvertebrateXXRapana venosaVeined Rapa whelkInvertebrateXX2Styela clavaAsian tunicateInvertebrateXX2Synidotea laevidorsalisan isopodInvertebrateXX2Tenellia adspersaMiniature aeolisInvertebrateXX2Teredo navalisNaval shipwormInvertebrateXX2Sphaeroma terebransWarty pillbugInvertebrateX2Hemigaspus sanquineusJapanese shore crabInvertebrateX2Balanus AmphitriteStriped barnacleInvertebrateX2Anguillicola crassusEel parasiteInvertebrateX2Balanus trigonusa barnacleInvertebrateX2	Ostrae adulis	Edible oyster	Invertebrate		Х	Х	2
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Syndoled deviaorsaitsan isopodinvertebrateXXTenellia adspersaMiniature aeolisInvertebrateXX2Teredo navalisNaval shipwormInvertebrateXX2Sphaeroma terebransWarty pillbugInvertebrateX2Hemigaspus sanquineusJapanese shore crabInvertebrateX1Balanus AmphitriteStriped barnacleInvertebrateX2Crassostrea asiakensisSuminoe oysterInvertebrateX2Anguillicola crassusEel parasiteInvertebrateX2Balanus trigonusa barnacleInvertebrateX2	Styela clava	Asian tunicate	Invertebrate		Х	Х	2
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Intereation davantsNavant simp wordtInvertebrateXXSphaeroma terebransWarty pillbugInvertebrateX2Hemigaspus sanquineusJapanese shore crabInvertebrateX1Balanus AmphitriteStriped barnacleInvertebrateX2Crassostrea asiakensisSuminoe oysterInvertebrateX2Anguillicola crassusEel parasiteInvertebrateX2Balanus trigonusa barnacleInvertebrateX2	Tenellia adspersa	Miniature aeolis	Invertebrate		Х	Х	2
Sphaeronia terebransWarty pillougInvertebrateXHemigaspus sanquineusJapanese shore crabInvertebrateX1Balanus AmphitriteStriped barnacleInvertebrateX2Crassostrea asiakensisSuminoe oysterInvertebrateX2Anguillicola crassusEel parasiteInvertebrateX2Balanus trigonusa barnacleInvertebrateX2	Teredo navalis	Naval shipworm	Invertebrate		Х	Х	2
Internigaspus sanquineusSapariese since crabInvertebrateXBalanus AmphitriteStriped barnacleInvertebrateX2Crassostrea asiakensisSuminoe oysterInvertebrateX2Anguillicola crassusEel parasiteInvertebrateX2Balanus trigonusa barnacleInvertebrateX2	Sphaeroma terebrans	Warty pillbug	Invertebrate		Х		2
Datantis AmphilititeSurject ballacticInvertebrateXCrassostrea asiakensisSuminoe oysterInvertebrateX2Anguillicola crassusEel parasiteInvertebrateX2Balanus trigonusa barnacleInvertebrateX2	Hemigaspus sanquineus	Japanese shore crab	Invertebrate			Х	1
Anguillicola crassusEel parasiteInvertebrateX2Balanus trigonusa barnacleInvertebrateX2	Balanus Amphitrite	Striped barnacle	Invertebrate			X	2
Anguint cold crassisLet parasiteInvertebrateXBalanus trigonusa barnacleInvertebrateX2	Crassostrea asiakensis	Suminoe oyster	Invertebrate			X	2
	Anguillicola crassus	Eel parasite	Invertebrate			X	2
Gonionemus vertens Clinging jellyfish Invertebrate X 2	Balanus trigonus	a barnacle	Invertebrate			X	2
	Gonionemus vertens	Clinging jellyfish	Invertebrate			X	2

Table A1. Potential invasive species

Teredo bartschi	Bartchi shipworm	Invertebrate			Х	2
Thecacera pennigera	a nudibranch	Invertebrate			Х	2
Tritonia plebeia	European nudibranch	Invertebrate			X	2
Megabalanus coccopoma	Titan acorn barnacle	Invertebrate			X	2
Perna vidris	Green mussel	Invertebrate			Х	2
Panicum repens	Torpedograss	Vegetation	Х	Х		1
Antithamnion pectinatum	a red algae	Vegetation			Х	2
Bonnemaisonia hamifera	Bonnemaison's hook weed	Vegetation			Х	2
Cladophora sericea	Green algae	Vegetation			Х	2
Codium fragile fragile	Dead man's fingers	Vegetation			Х	2, 3
Grateloupia turuturu	a red algae	Vegetation			Х	2
Neosiphonia harveyi	Red algae, a rhodophyta	Vegetation			Х	2, 3
Antithamnion hubbsii	NA	Vegetation			Х	3
Gracilaria vermiculophylla	Red alga/Ohmi	Vegetation			Х	3
Lomentaria orcadensis	Red alga	Vegetation			Х	3
Odontella sinensis	Chinese diatom	Vegetation				3
Polysiphonia breviarticulata	Polysiphonia breviarticulata	Vegetation				3
Porphyra suborbiculata	Red Laver	Vegetation				3
Striaria attenuata	Stried branched weed	Vegetation				3
Dorosoma petenense	threadfin shad	Vertebrate	Х	Х	Х	1
Oncorhynchus mykiss	rainbow trout	Vertebrate	Х	Х	Х	1
Oncorhynchus nerka	kokanee	Vertebrate	Х	Х	Х	1
Salmo salar	Atlantic salmon	Vertebrate	Х	Х	Х	1
Osmerus mordax	Rainbow smelt	Vertebrate	Х	Х	X	2
Oreochromis aureus	blue tilapia	Vertebrate	Х	X		1
Oreochromis mossambicus	Mozambique tilapia	Vertebrate	Х	Х		1
Tilapia zillii	redbelly tilapia	Vertebrate	Х	Х		1, 2
Tinca tinca	tench	Vertebrate	Х	X		1
Pterois volitans	Red Lionfish	Vertebrate			X	2

<sup>1</sup>Summary Report of Nonindigenous Aquatic Species in U.S. Fish and Wildlife Service Region 4

 $^{2}http://www.nature.nps.gov/water/marine invasives/search.cfm$ 

 $^{3}http://invasions.si.edu/nemesis/browseDB/searchBioregions.jsp$ 



Rapid Assessment Survey Sampling Form Albemarle-Pamlico National Estuary Partnership NCDENR

#### Site Information

Site Name			
Latitude		Longitude	
Site Type	Marina 🗆	Intertidal	Subtidal/Pelagic 🗆
Sampler Names			
Date		Time	
Water Quality			
Temperature		Salinity	
pH		Conductivity	
Turbidity		Dissolved oxygen	
ndividual Sampl	le #1		
Туре	Primary Producer	Invertebrate	Vertebrate
Habitat	Fresh Water 🗆	Brackish	Marine 🗆
Phylum			
Genus			
Species			
Unknown	Provide sample number		
ndividual Sampl	le #2		
Туре	Primary Producer 🗆	Invertebrate	Vertebrate
Habitat	Fresh Water 🗆	Brackish	Marine 🗆
Phylum			
Genus			
Species			
Unknown	Provide sample number		
ndividual Sampl	le #3		
Туре	Primary Producer	Invertebrate	Vertebrate
Habitat	Fresh Water 🗆	Brackish	Marine 🗆
Phylum			
C			
Genus			
Genus Species			

Figure A1. Sampling Field Form

	Albemarle-Pamlico National Est NCDENR	uary Partnership	
Additional San	nles		
Sampling Site			
Date			
Individual Sam	ple #		
Туре	Primary Producer	Invertebrate 🗆	Vertebrate
Habitat	Fresh Water 🗆	Brackish	Marine 🗏
Phylum			
Genus			
Species			
Unknown 🗆	Provide sample number		
Habitat	Fresh Water	Brackish	Marine
Phylum			
1			
Genus			
Genus Species			
	Provide sample number		
Species	Provide sample number		
Species Unknown			
Species Unknown		Invertebrate	Vertebrate
Species Unknown Individual Sam Type Habitat	ple #	Invertebrate Brackish	Vertebrate
Species Unknown	ple # Primary Producer		
Species Unknown	ple # Primary Producer		
Species Unknown	ple # Primary Producer		
Species Unknown Individual Sam Type Habitat Phylum Genus	ple # Primary Producer		

Figure A2. Sampling field form for additional samples

Purchase	Number of units	Price per unit	Total cost	Single purchase	Intermittent Purchase
Buckets	2	3.00	6.00	X	
Таре	3	5.00	15.00	X	
Scraper	4	6.00	24.00	X	
Clipboards with storage	5	15.00	75.00	X	
Shovels	2	15.00	30.00	X	
Trays/pans	5	20.00	100.00	X	
Box sieve	2	20.00	40.00	X	
sample coolers	2	25.00	50.00	X	
Food cooler	1	25.00	25.00	X	
Dissecting tools (blades, foreceps, and dissecting scissors)	3	40.00	120.00	х	
Refractometer	1	100.00	100.00	X	
Seining net	1	142.00	142.00	X	
GPS Units	1	150.00	150.00	X	
Larval net	1	150.00	150.00	X	
Coarse pelagic net	1	150.00	150.00	X	
Tablet (optional)	1	250.00	250.00	X	
Sample labels (pack)	1	15.00	15.00		X
Waterproof copy paper (200 pack)	1	50.00	50.00		Х
250 ml Polyethylene bottles (pack of 24)	2	54.00	108.00		X
pH 4 Calibration solution (6 pack)	1	85.00	85.00		X
pH 7 Calibration solution (6 pack)	1	85.00	85.00		Х
1 oz Glass Vials (case of 400)	1	100.00	100.00		Х
Turbidity calibration solution (6pack)	1	100.00	100.00		Х
Conductivity solution (6 pack)	1	113.00	113.00		Х
Fuel (gallons)	80	3.20	256.00		
70% Isopropyl Alcohol (6 pack)	1	12.00	12.00		
Foood	1	300.00	300.00		
Housing Rental (5 bedroom weekly)	1	700.00	700.00		

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