

ACE Basin (ACE) National Estuarine Research Reserve Nutrient Metadata
January-December 2020

Latest Update: August 8, 2024

I. Data Set and Research Descriptors

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2) Research objectives

Long-term water quality monitoring in the ACE Basin provides a unique opportunity to increase understanding of how various environmental factors influence estuarine processes. Based on discussions with local Coastal Zone Management (CZM) personnel and ACE Basin National Estuarine Research Reserve (NERR) staff knowledge of land use within the Reserve, the South Edisto River drainage basin was selected because it is well suited for studying contrasting hydrographic conditions and land use patterns.

The two major objectives of the ACE Basin monitoring program are to: 1) compare water quality conditions in shallow creeks along a salinity gradient and at different levels of development in the South Edisto River watershed and 2) track changes to the saltwater demarcation line in the South Edisto River as a result of prolonged drought, extraction of river water, and sea level rise.

The ACE NERR System Wide Monitoring Program (SWMP) began on March 3, 1995 in two tributaries of the South Edisto River, Big Bay Creek and St. Pierre Creek. In October 2002, monitoring stations were established in Fishing Creek and Mosquito Creek, tributaries of the South Edisto River. In 2014, two sites were established in the South Edisto River proper at Jehossee Island and Grove Plantation.

Four primary monitoring stations (Edisto Island [replacement for Big Bay], Fishing Creek, Mosquito Creek and St. Pierre Creek) are used to study contrasting land use patterns in the reserve. The two “treatment” sites are Edisto Island and Mosquito Creek, where boat traffic is moderate to heavy and residential and commercial development is moderate. St. Pierre Creek and Fishing Creek, where boat traffic is light and development is sparse, are designated as “control” sites. The four sites are located along a salinity gradient ranging from the polyhaline (Edisto Island & St. Pierre Creek) to mesohaline (Mosquito Creek) to oligohaline (Fishing Creek). See *Section 5 - Site Location and Character* for detailed descriptions of the sites.

The secondary stations at Jehossee Island (acejiwq) and Grove Plantation (acegpwq) are used to track changes in the saltwater demarcation line. These two stations extend our coverage of the salinity gradient in the South Edisto River. The Jehossee Island station is in the mesohaline zone, and the Grove Plantation station is in the oligohaline to freshwater zone, approximately 0.16 km (0.1 nautical miles) downstream of the legal saltwater demarcation line. See *Water Quality Metadata* for detailed descriptions of the sites.

In July 1997, the Reserve staff initiated a diel nutrient study at the Big Bay and St. Pierre monitoring stations; in 2002, St. Pierre Creek was designated as the SWMP diel site, and diel monitoring at the Big Bay site was discontinued. In February of 2002, the monthly grab nutrient-monitoring component of SWMP was initiated at the four primary monitoring stations in the ACE Basin NERR. Starting in 2016, the monthly grab samples at Edisto Island (EI) and St. Pierre (SP) were also analyzed for total nitrogen (TN) and total phosphorus (TP). In addition, grab samples were also collected from two secondary monitoring stations (Jehossee Island, JI, and Grove Plantation, GP) for analysis of TN and TP. The JI and GP data are only available from the ACE Basin NERR Research Coordinator.

a) Monthly grab sampling program

The objective of this program is to ascertain the annual and seasonal fluctuations in nutrient levels at the water quality monitoring sites. Two samples are collected from each monitoring station between mid-ebb to slack-low tide periods each month. The samples are analyzed for ammonium, nitrite + nitrate, ortho-phosphate and chlorophyll-a concentrations.

b) Diel sampling program

The objective of this program is to track the tidal fluctuations in nutrient levels during a complete diurnal tidal cycle (24 hr 48 min). Samples are collected once each month by an ISCO auto-sampler, and samples are analyzed for ammonium, nitrite + nitrate, ortho-phosphate, and chlorophyll-a concentrations.

3) Research methods

a) Monthly grab sampling program

Water samples are taken monthly at the four primary NERR water quality stations: Edisto Island (EI), St. Pierre Creek (SP), Fishing Creek (FC), and Mosquito Creek (MC) and two secondary NERR water quality stations: Jehossee Island and Grove Plantation (total nitrogen and total phosphorus only; not reported to CDMO). Two samples are collected, consecutively, at a depth of 0.3 meter below the surface, using a water-sampler or by hand. The “grab” samples are taken on the same day and between mid-ebb and slack-low tide (within 3 hours before slack-low tide). No distinction is made between neap and spring tide conditions or between morning and afternoon tides.

All samples are collected in wide-mouth, clear polypropylene bottles that are acid washed (10% HCl solution), rinsed (6x) with distilled or deionized water, and dried prior to the sampling day. At each sampling site, sample bottles are rinsed with ambient water prior to sample collection. Samples are immediately removed from natural light and placed on ice, then returned to the laboratory. Samples are filtered at the laboratory within four hours of sample retrieval. Samples are filtered through 25-mm glass fiber filters and then stored at -20°C. Chlorophyll samples are analyzed within 24 hours of collection (see Section 13 - *Analytical Methods*). Nutrient samples are shipped to the Chesapeake Biological Laboratory for analysis while chlorophyll samples are analyzed at the ACE NERR.

b) Diel sampling program

Diel monitoring occurs monthly at the St. Pierre Creek water quality station. Thirteen water samples are collected every 2 hours and 4 minutes over the complete diurnal tidal cycle (24 hr 48 min), using an ISCO auto-sampler. Sample collection begins at the predicted slack-low; and are collected at a depth of 0.5 meters below the surface. No distinction is made between neap and spring tide conditions.

All samples are collected in clear polypropylene bottles, which are acid washed (10% HCl solution), rinsed (6x) with distilled or deionized water, and dried prior to the sampling day. Due to the use of the ISCO auto-sampler, ambient water rinses prior to sample collection are not feasible, but the sampler does rinse the collection hose with the ambient water prior to collecting the sample. During the collection period, samples are kept cool by ice stored in the enclosed ISCO. When retrieved, samples are removed from the auto-sampler and placed on ice. Samples are filtered at the laboratory within four hours of sample retrieval. In the laboratory, samples are filtered through 25-mm glass fiber filters and then stored at -20°C. Chlorophyll samples are analyzed within 24 hours of collection (see Section 13 - *Analytical Methods* for more details of analyses). Nutrient samples are shipped to the Chesapeake Biological Laboratory for analysis while chlorophyll samples are analyzed at the ACE NERR.

4) Site location and character

ACE Basin National Estuarine Research Reserve is one of the largest undeveloped estuaries on the East Coast. The study area encompasses the Ashepoo, Combahee and South Edisto River basins, which empty into St. Helena Sound. The NERR consists of approximately 60,702 ha (150,000 acres) of diverse estuarine wetlands providing preserved habitats for fish and wildlife.

The South Edisto River has a drainage area of approximately 394,176 ha (974,030 ac), encompassing the area between Four Holes Swamp and St. Helena Sound. The river receives considerable input of freshwater (average annual streamflow is 74 m³/s, 2613.29 ft³/sec). The official saltwater-freshwater demarcation line on the river lies at river mile 20 (32.19 km); however, during periods of very low flow, the saltwater interface can intrude to river mile 32 (51.5 km), which is approximately 12 river miles (19.31 km) from the inland boundary of the reserve. Salt marshes of smooth cordgrass (*Spartina alterniflora*) dominate the wetlands in the euhaline, polyhaline, and mesohaline, while waterfowl impoundments are the dominant land cover in the oligohaline and fresh waters.

The average tidal range in the South Edisto River is approximately 2.0 m (6.6 ft), with a maximum of 2.8 m (9.2 ft) and a minimum of 1.4 m (4.6 ft). The bottom habitat at all stations consists of mud which is intermixed with dead shell hash at the saltwater sites.

All ACE NERR historical nutrient/pigment monitoring stations:

Station Code	SWMP Status	Station Name	Location	Active Dates	Reason Decommissioned	Notes
aceeinut	P	Edisto Island	32.5040 N -80.3247 W	01/01/2015 - current	NA	Replaced Big Bay
acefcnut	P	Fishing Creek	32.63593 N -80.36556 W	10/01/2002 - current	NA	NA
acemcnut	P	Mosquito Creek	32.5558 N -80.4380 W	10/01/2002 - current	NA	NA
acespnut	P	St. Pierre	32.52800 N -80.36144 W	03/01/1995 - current	NA	NA
acebbnut	P	Big Bay	32.4941 N -80.3241 W	03/01/1995 - 12/31/2014	see Big Bay description below	Replaced by Edisto Island

Primary Monitoring Stations

Three of the four primary stations (Edisto Island, Fishing Creek, and St. Pierre Creek) are in tributaries of the South Edisto River and one station (Mosquito Creek) is in a tributary of both the South Edisto and Ashepoo rivers. The descriptions of the sites are as follow:

Edisto Island (EI) – GPS coordinates: 32.5040 N and -80.3247 W

On January 1, 2015, Edisto Island water quality station replaced the Big Bay station as a primary station. The Edisto Island station is approximately 1.27 km (0.68 nautical miles) upstream of the previous site (Big Bay) and is located on a dock at the Edisto Beach State Park. Water quality data was collected at both stations for 8 months and the overall results were very similar. Edisto Island station is also designated as a "treatment" site because of its proximity to developed areas. In 2020, the mean depth at the station was 2.66 m (8.9 ft), and the mean salinity was 27.50 practical salinity units (psu).

The eastern bank of the Big Bay Creek, at the new station is bordered by *Spartina alterniflora* and *Salicornia virginica*. The high ground is dominated by maritime forest, characterized by live oak (*Quercus virginiana*), slash pine (*Pinus taeda*); and cabbage palmetto (*Sabal palmetto*). A marsh island with no high ground borders the western bank while American oyster (*Crassostrea virginica*) forms a reef along both creek banks. Boat traffic is heavy, especially during the warmer months, and the creek is closed to shellfish harvesting because of the surrounding human activities. Nonpoint sources of pollution, including fertilizers, pesticides, herbicides and PAHs, to the monitoring station are surface runoff from lawns, golf courses, and paved ramps. Docks and bulkheads are constructed of concrete or wood treated with creosote, CCA-treated, or Wolmanized process.

Fishing Creek (FC) – GPS coordinates: 32.63593 N and -80.36556 W

This monitoring station is in a tributary of Fishing Creek, approximately 2 km (1.08 nautical miles) from the mouth of the creek and is located approximately 5 m (16.41 ft) from the northern bank of the creek. The tributary flows through the eastern half of Jehossee Island, a Wildlife Management Area (WMA) protected by the USFWS, and Fishing Creek forms the northeast border of the island. The station is surrounded by extensive *Spartina cynosuroides* marsh and vast mud flats. The upland area is characterized by slash pine, live oak, and cabbage palmetto. In 2020, the mean depth at the station was 2.45 m (8.0 ft), and the mean salinity was 6.3 psu.

The Fishing Creek monitoring station is designated as a "control" site because there is no development in the immediate area, and boat traffic is relatively light in the creek. The WMA contains impoundments (formerly rice fields) that are managed as wildlife habitat for endangered fauna and migratory waterfowl. No pesticides or herbicides are applied to the wetlands. Water level in the wetland is regulated by rice trunks that control the flow of water between the impoundment and the South Edisto River.

Mosquito Creek (MC) – GPS coordinates: 32.5558 N and -80.4380 W

This monitoring station is in Mosquito Creek (a tributary of both the South Edisto and Ashepoo rivers), approximately 2.51 km (1.36 nautical miles) from the Ashepoo River and 12 km (6.48 nautical miles) from the South Edisto River, and it is approximately 5 m (16.41 ft) from the southern bank of the creek. In 2020, the mean depth at the station was 4.04 m (13.3 ft), and the mean salinity was 13.3 psu.

The Mosquito Creek station is designated as a "treatment" site because of the land use practices in the surrounding area. Agriculture fields and impounded wetlands are found upstream of the monitoring station. Approximately fifteen docks constructed of creosote, concrete, Wolmanized or CCA treated wood; a public boat landing; a commercial seafood business with commercial shrimp boats and a fueling dock are located approximately 1.00 km (0.54 nautical miles) downstream of the monitoring station. The major contributor of nonpoint source pollution to the monitoring station is surface runoff from the impoundments and agricultural lands that contain high levels of nutrients and, at times, herbicides and pesticides. Impoundment trunks open and drain into the creek increasing the nutrient load and possibly introducing herbicides and pesticides. Vegetation in the area includes salt marsh dominated by *Spartina alterniflora* and *Juncus roemerianus*. Upland fringe areas consist of cabbage palmetto, live oaks and pine trees.

St. Pierre Creek (SP) – GPS coordinates: 32.52800 N and -80.36144 W

This monitoring station is in a small tributary of St. Pierre Creek, approximately 0.25 km (0.13 nautical miles) from the mouth of the creek, and it is approximately 5 m (16.41 ft) from the northern bank of the creek. The tributary flows through the southern portion of Bailey Island, and the creek forms the eastern border of the island. The monitoring station is surrounded by a wide expanse of *Spartina alterniflora* marsh. Extensive mud flats and oyster reefs fringe the banks. Maritime forest communities comprised of species such as wax myrtles (*Morella cerifera*), live oaks (*Quercus virginiana*), and palmettos dominate the upland areas. In 2020, the mean depth at the station was 1.95 m (6.5 ft), and the mean salinity was 26.4 psu.

The St. Pierre Creek station is designated as a “control” site because development in the immediate area was sparse when the station was established on March 3, 1995, and the tributary is subject to relatively light boat traffic. In 1996, the 695-acre (281.26 ha) island was sold, and the owners partnered with The Nature Conservancy to design a conservation-based development. Four hundred and three acres in the center of Bailey Island were set aside as a nature preserve that is managed by The Nature Conservancy, and the number of residential lots on the remaining 292 acres (118.17 ha) is limited to 67 (27.11 ha). Access to the island is limited to one bridge and all roads on the island are single lane and made of crushed seashells. In addition, a conservation manual was developed for the property owners that provide specific lot designs and construction guidelines as well as landscaping guidelines to protect the maritime and estuarine habitats.

Inactive Monitoring Stations

Big Bay (BB) - GPS coordinates: 32.4941 N and -80.3241 W

This monitoring station was in Big Bay Creek proper, approximately 2 km (1.24 mi) from the mouth of the creek and was located about 5 m (16.41 ft) from the southern bank of the creek. It was a "treatment" site because it was subject to nonpoint source pollution and was surrounded by moderate level of development. The southern bank of the Big Bay Creek near this station was bordered by residential and commercial development, with little setback from the bordering *Spartina alterniflora* marsh. For instance, there are over forty private docks, two commercial seafood docks and a marina with 75 slips, three paved boat ramps, and two fueling areas along the southern bank. Docks and bulkheads are constructed of concrete, or creosote, CCA-treated or Wolmanized material. Boat traffic was heavy, especially during the warmer months, and the creek is closed to shellfish harvesting because of the surrounding human activities. The major sources of nonpoint source pollution were surface runoff from lawns, golf courses, and paved ramps that contain fertilizers, pesticides, herbicides and PAHs. All of the high ground along the southern bank was developed (i.e., residential homes, condominiums and restaurants); and maritime plant communities have been replaced by golf courses, lawns and ornamental gardens. Small patches of a few maritime species (i.e. live oak (*Quercus virginiana*), cabbage palmetto (*Sabal palmetto*), and Southern red cedar (*Juniperus silicicola*)) are found along the roads. In contrast, the northern bank was bordered by a wide expanse of *Spartina alterniflora* marsh, and no high ground is present. American oyster (*Crassostrea virginica*) forms a reef along the creek banks, especially the northern side, and on intertidal mud flats within the creek. The site was moved to Edisto Island due to the dock upon which it was located was owned by a private individual that was not maintaining the structure. Water quality data was collected at both stations for 8 months and the overall results were very similar.

5) Coded variable definitions

Each individual sample is given a 3-part name code in addition to the date of grab sample collection or start date of diurnal sample collection. The name code gives the Reserve 3-letter code, station 2-letter code and SWMP program 2-letter code.

Sampling Station:	Site code:
Edisto Island	EI
Fishing Creek	FC
Mosquito Creek	MC
St. Pierre	SP

3 Part Name Codes:

acecinut = ACE Basin Edisto Island nutrient samples
acefcnut = ACE Basin Fishing Creek nutrient samples
acemcnut = ACE Basin Mosquito Creek nutrient samples
acespnut = ACE Basin St. Pierre Creek nutrient samples

Monitoring Programs:

Monthly grab sample program = 1
Diel grab sample program = 2

6) Data collection period

a) Grab sampling (sample collection time listed in Eastern Standard Time)

The SWMP grab sample program began in 2002 for St. Pierre Creek, Big Bay Creek, Fishing Creek, and Mosquito Creek with Edisto Island replacing Big Bay Creek in 2015.

Site	Sample Date	Rep 1 Time	Sample Date	Rep 2 Time
EI	01/07/2020	08:49	01/07/2020	08:50
EI	02/05/2020	10:29	02/05/2020	10:30
EI	03/18/2020	09:01	03/18/2020	09:02
EI	04/2020 No samples collected due to COVID work from home orders			
EI	05/2020 No samples collected due to COVID work from home orders			
EI	06/02/2020	10:30	06/02/2020	10:32
EI	07/01/2020	09:50	07/01/2020	09:51
EI	08/27/2020	08:40	08/27/2020	08:42
EI	09/30/2020	11:37	09/30/2020	11:38
EI	10/13/2020	10:02	10/13/2020	10:03
EI	11/13/2020	11:30	11/13/2020	11:31
EI	12/10/2020	07:16	12/10/2020	07:17

Site	Sample Date	Rep 1 Time	Sample Date	Rep 2 Time
FC	01/07/2020	10:04	01/07/2020	10:05
FC	02/05/2020	10:16	02/05/2020	10:17
FC	03/18/2020	09:40	03/18/2020	09:43
FC	04/2020 No samples collected due to COVID work from home orders			
FC	05/2020 No samples collected due to COVID work from home orders			
FC	06/02/2020	10:31	06/02/2020	10:32
FC	07/01/2020	09:41	07/01/2020	09:42
FC	08/27/2020	09:29	08/27/2020	09:30
FC	09/30/2020	12:05	09/30/2020	12:06
FC	10/13/2020	10:01	10/13/2020	10:02
FC	11/13/2020	11:53	11/13/2020	11:54
FC	12/10/2020	09:10	12/10/2020	09:11

Site	Sample Date	Rep 1 Time	Sample Date	Rep 2 Time
MC	01/07/2020	10:58	01/07/2020	10:59
MC	02/05/2020	09:00	02/05/2020	09:01
MC	03/18/2020	08:46	03/18/2020	08:47
MC	04/2020 No samples collected due to COVID work from home orders			
MC	05/2020 No samples collected due to COVID work from home orders			
MC	06/02/2020	09:21	06/02/2020	09:22
MC	07/01/2020	08:37	07/01/2020	08:38
MC	08/27/2020	07:25	08/27/2020	07:26
MC	09/30/2020	11:11	09/30/2020	11:12

MC	10/13/2020	09:01	10/13/2020	09:02
MC	11/13/2020	11:01	11/13/2020	11:02
MC	12/10/2020	08:16	12/10/2020	08:17

Site	Sample Date	Rep 1 Time	Sample Date	Rep 2 Time
SP	01/07/2020	09:15	01/07/2020	09:16
SP	02/05/2020	08:12	02/05/2020	08:13
SP	03/18/2020	08:07	03/18/2020	08:08
SP	04/2020 No samples collected due to COVID work from home orders			
SP	05/2020 No samples collected due to COVID work from home orders			
SP	06/02/2020	08:26	06/02/2020	08:27
SP	07/01/2020	07:46	07/01/2020	07:47
SP	08/27/2020	06:54	08/27/2020	06:55
SP	09/30/2020	10:24	09/30/2020	10:25
SP	10/13/2020	08:01	10/13/2020	08:02
SP	11/13/2020	10:12	11/13/2020	10:13
SP	12/10/2020	07:45	12/10/2020	07:46

b) Diel sampling (sample collection time listed in Eastern Standard Time)

The SWMP diel sample program began in 2002 for the St. Pierre Creek site.

Site	Start Date	Start Time	End Date	End Time
SP	01/06/2020	10:52	01/07/2020	11:40
SP	02/05/2020	11:11	02/06/2020	11:59
SP	03/18/2020	10:12	03/19/2020	11:00
SP	04/2020 No samples collected due to COVID work from home orders			
SP	05/2020 No samples collected due to COVID work from home orders			
SP	06/16/2020	10:34	06/17/2020	11:22
SP	07/01/2020	10:45	07/02/2020	11:33
SP	08/12/2020	08:11	08/13/2020	08:59
SP	09/2020 No samples collected due to severe weather week of deployment			
SP	10/13/2020	11:01	10/14/2020	11:49
SP	11/23/2020	08:59	11/24/2020	09:48
SP	12/10/2020	10:21	12/11/2020	11:09

7) Associated researchers and projects

As part of the System-wide Monitoring Program (SWMP), and weather and water quality data are obtained with a Campbell Scientific CR1000 data logger and YSI 6600-EDS or YSI EXO2 data loggers, respectively. Diel nutrient samples are gathered once per month at the St. Pierre water quality monitoring station, and grab samples are obtained at each of the primary stations once per month. The concentrations of the following parameters are measured and recorded for the nutrient monitoring program: ammonium (NH₄), nitrite + nitrate (NO₂ + NO₃), ortho-phosphate (PO₄), and chlorophyll-a (Chl-a). Historic water quality, nutrient, and weather data can be obtained at <http://cdmo.baruch.sc.edu>. Information about other studies conducted in the ACE Basin may be obtained from the ACE NERR Research Coordinator.

As part of the System-wide Monitoring Program (SWMP), ACE NERR also monitors 15-minute meteorological and water quality data which may be correlated with this nutrient/pigment dataset. The meteorological (Bennett's Point) and water quality data from the stations (GP and SP) are telemetered to the CDMO and HADS. These data are available at <http://cdmo.baruch.sc.edu>. Information about other studies conducted in the ACE Basin may be obtained from the ACE Basin NERR Research Coordinator.

8) Distribution

NOAA retains the right to analyze, synthesize and publish summaries of the NERRS System-wide Monitoring Program data. The NERRS retains the right to be fully credited for having collected and processed the data. Following academic courtesy standards, the NERR site where the data were collected should be contacted and fully acknowledged in any subsequent publications in which any part of the data are used. The data set enclosed within this package/transmission is only as good as the quality assurance and quality control procedures outlined by the enclosed metadata reporting statement. The user bears all responsibility for its subsequent use/misuse in any further analyses or comparisons. The Federal government does not assume liability to the Recipient or third persons, nor will the Federal government reimburse or indemnify the Recipient for its liability due to any losses resulting in any way from the use of this data.

Requested citation format:

NOAA National Estuarine Research Reserve System (NERRS). System-wide Monitoring Program. Data accessed from the NOAA NERRS Centralized Data Management Office website: www.nerrsdata.org; accessed 12 October 2020.

NERR nutrient data and metadata can be obtained from the Research Coordinator at the individual NERR site (please see Principal investigators and contact persons), from the Data Manager at the Centralized Data Management Office (please see personnel directory under the general information link on the CDMO home page) and online at the CDMO home page www.nerrsdata.org. Data are available in comma separated version format.

II. Physical Structure Descriptors

9) Entry verification

Nutrient data are entered into a Microsoft Excel worksheet and processed using the NutrientQAQC Excel macro. The NutrientQAQC macro sets up the data worksheet, metadata worksheets, and MDL worksheet; adds chosen parameters and facilitates data entry; allows the user to set the number of significant figures to be reported for each parameter and rounds using banker's rounding rules; allows the user to input MDL values and then automatically flags/codes measured values below MDL and inserts the MDL; calculates parameters chosen by the user and automatically flags/codes for component values below MDL, negative calculated values, and missing data; allows the user to apply QAQC flags and codes to the data; produces summary statistics; graphs selected parameters for review; and exports the resulting data file to the CDMO for tertiary QAQC and assimilation into the CDMO's authoritative online database.

Chesapeake Biological Laboratory calculates and reports results in mg/L.

10) Parameter titles and variable names by category

Data Category	Parameter	Variable Name	Units of Measure
Phosphorus and Nitrogen:			
	*Orthophosphate	PO4F	mg/L as P
	*Ammonium, Filtered	NH4F	mg/L as N
	*Nitrite + Nitrate, Filtered	NO23F	mg/L as N
	Dissolved Inorganic Nitrogen	DIN	mg/L as N
	Total Nitrogen	TN	mg/L as N
	Total Phosphorus	TP	mg/L as P
Plant Pigments:			
	*Chlorophyll a	CHLA_N	µg/L

Notes:

1. Time is coded based on a 2400 clock and is referenced to Standard Time.

2. Reserves have the option of measuring either NO₂ and NO₃ or they may substitute NO₃ for individual analyses if they can show that NO₂ is a minor component relative to NO₃. ACE NERR staff and the Analytical Laboratory staff have determined that separate NO₂ and NO₃ data were not necessary because the concentration of NO₂ is negligible when compared to the concentration of NO₃.

11) Measured or calculated laboratory parameters

a) Parameters measured directly

Nitrogen species:	NH ₄ F, NO ₂ 3F, TN
Phosphorus species:	PO ₄ F, TP
Other:	CHLA_N

b) Calculated parameters

DIN	NO ₂ 3F+NH ₄ F
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12) Limits of detection

Method Detection Limits (MDL), the lowest concentration of a parameter that an analytical procedure can reliably detect, have been established by Chesapeake Biological Laboratory. The MDL is determined as 3 times the standard deviation of a minimum of 7 replicates of a single low concentration sample. These values are reviewed and revised periodically.

Parameter	Start Date	End Date	MDL	Revisited
PO ₄ F	01/01/2020	12/31/2020	0.0034	05/01/2020
NH ₄ F	01/01/2020	05/01/2020	0.013	
NH ₄ F	05/01/2020	12/31/2020	0.009	05/01/2020
NO ₂ 3F	01/01/2020	08/01/2020	0.0007	
NO ₂ 3F	08/01/2020	12/31/2020	0.0015	08/01/2020
NO ₂ 3F (ASTM)	01/01/2020	12/31/2020	0.0057	07/23/2020
TN	01/01/2020	12/31/2020	0.05	05/01/2020
TP	01/01/2020	12/31/2020	0.0015	05/01/2020
CHL-A	01/01/2020	02/01/2020	0.9	
CHL-A	02/01/2020	12/31/2020	0.7	02/01/2020

NO₂3F samples analyzed with the ASTM 4500 Method (See Section 13 b.) are listed below. It should be noted that samples analyzed using this method have a MDL of 0.0057.

Date Filtered	Sample
Grab Samples	
07/01/2020	FC, MC
ISCO Samples	
07/02/2020	ISCO 3, 8, 12

13) Laboratory methods

The supplies used by the ACE Basin NERR to collect and process water samples are: 1) 1-L wide-mouth clear polypropylene bottles; 2) 1-L clear polypropylene ISCO bottles; 3) 60-ml scintillation vials and caps; 4) 15-ml polypropylene centrifuge tubes and caps; 5) borosilicate culture vials; 6) 60-ml filtering apparatus [polypropylene syringe, syringe plunger and syringe filter holder with 25 mm filter size]; 7) 25 mm glass microfiber filter paper; 8) pump pipette (variable volume: 0.5-5 ml) and polypropylene pipette tips; 8) 90% acetone solution; and 9) 5% hydrochloric acid.

Prior to sample collection, the sample bottles, scintillation vials and caps, and filtering apparatus are acid-washed with a 10% hydrochloric acid bath, rinsed (6x) with distilled or deionized water, and dried. The dried bottles are capped, and the filtering apparatus is covered and stored.

b) Filtering Process

The samples are filtered by ACE NERR staff within four hours of collection. While wearing gloves one 25 mm filter is placed in a filter holder using clean forceps to prevent contamination. The sample bottle is gently agitated by 10 inversions to suspend the particulates. The filter syringe is “seeded” with the sample water by rinsing it with sample water. The filter holder is attached to the syringe and 50 ml of agitated sample water is added to the filter apparatus (syringe with attached filter holder). The filter apparatus is positioned over a scintillation vial and filter plunger inserted and slowly pushed upon. Both scintillation vials are seeded with the sample water by filling them with filtered water and then discarded. After filtering the 50 ml, the filter holder is removed from the syringe, covered, and set aside. If sediments and other solids in the sample prevented the filtration of the entire 50 ml, the volume is filtered on a laboratory sheet and the unfiltered portion discarded.

Using a clean filtering apparatus, the filtering process is repeated as above, with the filtered water retained as the sample collected in the scintillation vials. Samples are placed in a -20°C freezer after filtering. Nutrient samples are shipped to the CBL on dry ice via overnight courier. Upon reaching CBL they are placed in a -20°C freezer.

The two filters to be analyzed for chlorophyll are examined for similar color and coverage of particulates. If similar, then each filter is placed in a labeled centrifuge tube filled with 10 ml of 90% acetone. The tube is capped and covered with foil. If the density and coverage of particulates on one filter is noticeably different than the duplicate filter, both filters are discarded and the filtering process is repeated until two filters of similar color and coverage are obtained. After filtering all of the samples, the centrifuge tubes are stored in a freezer at -20°C until analysis the following day by the ACE NERR.

a) Parameter: NH4F

CBL Method: 19

Method Reference: 4500-NH3 G-1997

Method Descriptor: This method is dependent upon the Berthelot Reaction, during which a blue colored compound, closely related to indophenol, forms when an ammonium salt solution is added to sodium phenoxide. Filtered samples are complexed with sodium potassium tartrate and sodium citrate. The complexed sample reacts with alkaline phenol and hypochlorite, catalyzed by sodium nitroprusside, yielding an intense blue color suitable for photometric measurement. The method is suitable for NH₄ concentrations 0.001 to 1.68 mg NH₄-N/L.

Preservation Method: Water collected for ammonium should be filtered through a Whatman GF/F glass fiber filter (nominal pore size 0.7 µm) or equivalent, and measured for salinity. Water should be acidified to a pH of <2 and cooled to 4°C. The AutoAnalyzer vial container (sample cups) should be clean and sample rinsed. Acidified ammonium samples may be stored up to 28 days at 4°C. Non acidified ammonium samples may be refrigerated at 4°C for no longer than one day. Prior to analysis, check samples and adjust pH accordingly. Sample pH should be between 5 and 9.

b) Parameter: NO3F

CBL Method: 17

Method Reference: EPA 353.2

Method Descriptor: Filtered samples are passed through a granulated copper-cadmium column to reduce nitrate to nitrite. The nitrite, both that which was reduced from nitrate and nitrite that was originally present, is then determined by diazotizing with sulfanilamide and coupling with N-1-naphthylethylenediamine dihydrochloride to form a colored azo dye.

The method is suitable for NO₃+NO₂ concentrations 0.0007 to 0.056 mg NO₃+NO₂-N/L.

Preservation Method: Water collected for nitrite+nitrate should be filtered through a Whatman GF/F glass fiber filter (nominal pore size 0.7 μm), or equivalent and measured for salinity. Water should be acidified to a pH of <2 and cooled to 4°C. The AutoAnalyzer vial container (sample cups) should be clean and sample rinsed. Acidified nitrite+nitrate samples may be stored up to 28 days at 4°C. Non acidified nitrite+nitrate samples may be refrigerated at 4°C for no longer than one day. Prior to analysis, check samples and adjust pH accordingly. Sample pH should be between 5 and 9.

CBL Method: 21

Method Reference: EPA 353.2, Standards Methods #4500-N C, 4500-NO₃ F

Method Descriptor: Filtered samples are mixed with Nitrate Reductase (AtNaR2, commercially available, is a recombinantly produced form of eukaryotic Nitrate Reductase using a modified gene from the plant *Arabidopsis thaliana*. The enzyme AtNaR2 is produced in *Pichia pastoris* and purified from extracts of the yeast.) and NADH (β -Nicotinamide adenine dinucleotide reduced form disodium salt). The nitrite, both that which was reduced from nitrate and nitrite that was originally present, is then determined by diazotizing with sulfanilamide and coupling with N-1-naphthylethylenediamine dihydrochloride to form a colored azo dye. Filtered samples with concentrations found to be below the method detection limit are analyzed via cadmium reduction with a Technicon Bran & Luebbe AutoAnalyzer II.

The method is suitable NO₃+NO₂ concentrations 0.028 to 5.6 mg NO₃+NO₂-N/L.

Preservation Method: Water collected for NO₃+NO₂ should be frozen at $\leq -20^\circ\text{C}$. The AutoAnalyzer vial container (sample cups) should be clean and sample rinsed. Frozen NO₃+NO₂ samples may be stored up to 28 days. It has been shown that frozen QCS samples up to a year old still fall well within the control limits. NO₃+NO₂ samples may be refrigerated at 4°C for no longer than one day

c) **Parameter: PO₄F**

CBL Method: 20

Method Reference: 365.1

Method Descriptor: Filtered samples are mixed with a sulfuric acid-antimony-molybdate solution, and subsequently with an ascorbic acid solution, yielding an intense blue color suitable for photometric measurement.

The method is suitable for PO₄-P concentrations 0.0006 to 1.488 mg PO₄-P/L.

Preservation Method: Water collected for orthophosphate should be filtered through a Whatman GF/F glass fiber filter (nominal pore size 0.7 μm), or equivalent and measured for salinity. Water should be acidified to a pH of <2 and cooled to 4°C. The AutoAnalyzer vial container (sample cups) should be clean and sample rinsed. Acidified orthophosphate samples may be stored up to 28 days at 4°C. Non acidified ammonium samples may be refrigerated at 4°C for no longer than one day. Prior to analysis, check samples and adjust pH accordingly. Sample pH should be between 5 and 9.

d) **Parameter: TN**

CBL Method: 22

Method Reference: EPA 353.2

Method Descriptor: An exact amount of whole water is placed in test tubes where an exact amount of Potassium Persulfate Digestion Reagent is added. Under initially alkaline conditions and heat, nitrate is the sole nitrogen product. The now digested samples are buffered, then mixed and passed through a granulated copper-cadmium column to reduce nitrate to nitrite. The nitrite, both that which was reduced from nitrate and originally present, is then determined by diazotizing with sulfanilamide and coupling with N-1-naphthylethylenediamine dihydrochloride to form a colored azo dye.

Preservation Method: Water should be acidified to a pH of <2 and cooled to 4°C. The AutoAnalyzer vial container (sample cups) should be clean and sample rinsed. Samples may be stored for 6 months prior to analysis.

e) **Parameter: TP**

CBL Method: 24

Method Reference: 365.1

Method Descriptor:

An exact amount of filtered samples (whole water for TP) are placed in test tubes where an exact amount of Potassium Persulfate Digestion Reagent is added. Under initially alkaline conditions and heat, nitrate is the sole nitrogen product. As the potassium persulfate continues to oxidize, conditions become acidic and orthophosphate becomes the sole phosphorus product. The now digested samples are buffered, then mixed with a sulfuric acid-molybdate solution, and subsequently with an ascorbic acid solution, yielding an intense blue color suitable for photometric measurement.

Preservation Method: Water should be acidified to a pH of <2 and cooled to 4°C. The AutoAnalyzer vial container (sample cups) should be clean and sample rinsed. Samples may be stored for 6 months prior to analysis.

f) **Parameter: CHLA_N**

Method Reference: Modification of EPA Method 445.0 (EPA/600/R-92/121)

Method Descriptor: The extract is used in the procedure to determine the chlorophyll-a chemistry. Within a 24-hour period the samples are centrifuged at 3000 rpm for 10 minutes and then 5 ml are transferred to culture tubes, using a pump pipettor. The extraction is read at 440 nm wavelength, on a Turner Trilogy fluorometer. The Welschmeyer Method is used to measure chlorophyll-a without acidification.

Preservation Method: The 25-mm glass filters are placed in centrifuge tubes (one filter/tube) filled with 10 ml of 90% acetone solution and frozen for up to 24 hours. Samples have been stored for up to 72 hours without any noticeable difference in values. If samples are not to be analyzed until after a 72-hour period then the filter must be drawn dry, removed from the 10 ml of 90% acetone solution and stored in a desiccator at -20°C for up to 30 days.

14) **Field and Laboratory QAQC programs**

a) **Precision**

i) **Field variability** - Grab samples were collected monthly at each of the four primary monitoring sites. A water-sampler or hand collection was used to collect two consecutive samples at a depth of 0.3-0.5 meter below the surface. The grab samples were taken on the same day and between mid-ebb and slack-low water (~ 3 hrs before slack-low water to slack-low water).

Diel samples were collected monthly in St. Pierre Creek near the water quality station. Samples were collected every 2 hours and 4 minutes over a complete diurnal tide cycle (24 hr 48 min), using an ISCO auto-sampler. Sample collection began at the predicted slack-low, and samples were collected at a depth of 0.5 meters below the surface. Diel samples do not have replicates.

ii) **Laboratory variability** – One replication is filtered for nutrients. Typically, 10% of the samples analyzed by CBL consists of laboratory duplicates. Two replicates are filtered for each chlorophyll *a* sample.

iii) **Inter-organizational splits** – No splits: all samples were analyzed by the same laboratory

b) **Accuracy**

i) **Sample spikes** – Throughout the analytical batch, typically 10% of the total number of samples analyzed consists of laboratory spikes to assess analyte precision and analyte recovery.

ii) **Standard reference material analysis** – CBL's Standard reference materials for ammonium, nitrite+nitrate, and orthophosphate are supplied by SPEX, a US EPA certified company. The samples are analyzed at the beginning and end of every run.

iii) **Cross calibration exercises** – CBL has participated in the NERR cross calibration exercise in 2018. CBL also participates in the Chesapeake Bay Blind Audit program that was initiated in 1998.

15) **QAQC flag definitions**

QAQC flags provide documentation of the data and are applied to individual data points by insertion into the parameter's associated flag column (header preceded by an F_). QAQC flags are applied to the nutrient data during secondary QAQC to indicate data that are out of sensor range low (-4), rejected due to QAQC checks (-3), missing (-2), optional and were not collected (-1), suspect (1), and that have been corrected (5). All remaining data are flagged as having passed initial QAQC checks (0) when the data are uploaded and assimilated into the CDMO ODIS as provisional plus data. The historical data flag (4) is used to indicate data that were submitted to the CDMO prior to the initiation of secondary QAQC flags and codes (and the use of the automated primary QAQC system for WQ and MET data). This flag is only present in historical data that are exported from the CDMO ODIS.

- 4 Outside Low Sensor Range
- 3 Data Rejected due to QAQC
- 2 Missing Data
- 1 Optional SWMP Supported Parameter
- 0 Data Passed Initial QAQC Checks
- 1 Suspect Data
- 4 Historical Data: Pre-Auto QAQC
- 5 Corrected Data

16) QAQC code definitions

QAQC codes are used in conjunction with QAQC flags to provide further documentation of the data and are also applied by insertion into the associated flag column. There are three (3) different code categories, general, sensor, and comment. General errors document general problems with the sample or sample collection, sensor errors document common sensor or parameter specific problems, and comment codes are used to further document conditions or a problem with the data. Only one general or sensor error and one comment code can be applied to a particular data point. However, a record flag column (F_Record) in the nutrient data allows multiple comment codes to be applied to the entire data record.

General errors

GCM	Calculated value could not be determined due to missing data
GCR	Calculated value could not be determined due to rejected data
GDM	Data missing or sample never collected
GQD	Data rejected due to QA/QC checks
GQS	Data suspect due to QA/QC checks
GSM	See metadata

Sensor errors

SBL	Value below minimum limit of method detection
SCB	Calculated value could not be determined due to a below MDL component
SCC	Calculation with this component resulted in a negative value
SNV	Calculated value is negative
SRD	Replicate values differ substantially
SUL	Value above upper limit of method detection

Parameter Comments

CAB	Algal bloom
CDR	Sample diluted and rerun
CHB	Sample held beyond specified holding time
CIP	Ice present in sample vicinity
CIF	Flotsam present in sample vicinity
CLE	Sample collected later/earlier than scheduled

CRE Significant rain event
 CSM See metadata
 CUS Lab analysis from unpreserved sample

Record comments

CAB Algal bloom
 CHB Sample held beyond specified holding time
 CIP Ice present in sample vicinity
 CIF Flotsam present in sample vicinity
 CLE Sample collected later/earlier than scheduled
 CRE Significant rain event
 CSM See metadata
 CUS Lab analysis from unpreserved sample

Cloud cover

CCL clear (0-10%)

17) Other remarks/notes

Data may be missing due to problems with sample collection or processing. Laboratories in the NERRS System submit data that are censored at a lower detection rate limit, called the Method Detection Limit or MDL. MDLs for specific parameters are listed in the Laboratory Methods and Detection Limits Section (Section II, Part 12) of this document. Concentrations that are less than this limit are censored with the use of a QAQC flag and code, and the reported value is the method detection limit itself rather than a measured value. For example, if the measured concentration of NO23F was 0.0005 mg/l as N (MDL=0.0008), the reported value would be 0.0008 and would be flagged as out of sensor range low (-4) and coded SBL. In addition, if any of the components used to calculate a variable are below the MDL, the calculated variable is removed and flagged/coded -4 SCB. If a calculated value is negative, it is rejected and all measured components are marked suspect. If additional information on MDL's or missing, suspect, or rejected data is needed, contact the Research Coordinator at the reserve submitting the data.

Note: The way below MDL values are handled in the NERRS SWMP dataset was changed in November of 2011. Previously, below MDL data from 2007-2010 were also flagged/coded, but either reported as the measured value or a blank cell. Any 2007-2011 nutrient/pigment data downloaded from the CDMO prior to November of 2011 will reflect this difference.

Sample hold times for 2020: Nutrient samples are held at -20°C. NERRS SOP allows nutrient samples to be held for up to 28 days at -20°C, plus allows for up to 5 days for collecting, processing, and shipping samples. Chlorophyll samples may be held for 24 hours if not frozen, 30 days if frozen at -20°C, plus the 5 days for collection/processing. Samples held beyond that time period are flagged suspect and coded as <1> [GSM] (CHB).

*Samples held longer than allowed by NERRS protocols

Date Filtered	Date Analyzed			
All	PO4F	NH4F	NO23F	CHLA_N
Grab Samples				
01/07/2020	01/16/2020	01/16/2020	01/24/2020 (FC, EI) 01/27/2020 (SP1, MC) *02/11/2020 (SP2)	01/08/2020
02/05/2020	02/19/2020	02/19/2020	02/24/2020	02/06/2020

03/18/2020	WFH	WFH	WFH, COVID	03/19/2020
06/02/2020	*07/17/2020	*07/17/2020	*07/06/2020	06/03/2020
07/01/2020	07/21/2020	07/21/2020	*08/06/2020	07/02/2020
08/27/2020	09/21/2020	09/21/2020	09/11/2020	08/28/2020
09/30/2020	10/14/2020	10/14/2020	10/15/2020	10/01/2020
10/13/2020	11/03/2020	11/03/2020	10/28/2020	10/14/2020
11/13/2020	12/07/2020	12/07/2020	11/23/2020	11/14/2020
12/10/2020	12/18/2020	12/18/2020	12/17/2020	12/11/2020
ISCO Samples				
01/07/2020	01/16/2020	01/16/2020	*02/11/2020	01/08/2020
02/06/2019	02/19/2020	02/19/2020	02/11/2020	02/07/2020
03/19/2020	WFH	WFH	WFH, COVID	03/20/2020
06/17/2020	07/17/2020	07/17/2020	07/06/2020	06/18/2020
07/02/2020	07/21/2020	07/21/2020	*08/06/2020	07/03/2020
08/13/2020	08/24/2020	08/24/2020	08/26/2020	08/14/2020
10/14/2020	11/03/2020	11/03/2020	10/28/2020	10/15/2020
11/24/2020	12/15/2020	12/15/2020	12/09/2020	11/25/2020
12/11/2020	12/18/2020	12/18/2020	12/17/2020	12/12/2020
Date Collected		Date Analyzed		
		TN		TP
Grab Samples				
01/07/2020		01/17/2020		01/17/2020
02/05/2020		03/03/2020		03/03/2020
06/02/2020		08/13/2020		08/13/2020
07/01/2020		08/17/2020		08/17/2020
08/27/2020		09/15/2020		09/15/2020
09/30/2020		10/13/2020		10/13/2020
10/13/2020		12/03/2020		12/03/2020
11/03/2020		12/14/2020		12/14/2020
12/10/2020		02/04/2021		02/04/2021

Precipitation Blanket Statement

The Reserve is over 72,846 ha, and it encompasses most of the estuarine portion of the 320,000-ha ACE Basin watershed, extending 27.14 km northward from the mouth of St. Helena Sound to its inland boundary at the defunct *SCCL* railroad. Due to the immense size of the Reserve it is not uncommon to observe heavy rainfall in one area of the Reserve but not in another area.

Data Editing/Flagging Notes: Organized by Sample type, and Code

Grab Samples: (Monitoring Program 1)

All Sites

Rejected Data (Flag <-3>)

Missing Data (Flag <-2>)

The 03/18/2020 grab sampling event was successful and samples were processed for chlorophyll *a*; however, COVID work from home orders precluded processing of nutrient samples in a reasonable time frame (e.g., several months). The values are flagged as <-2> [GDM] (CSM) or <-2> [GCM] (CSM) for DIN.

Suspect Data (Flag <1>)

Passed Initial QAQC Checks (Flag <0>)

Edisto Island

Rejected Data (Flag <-3>)

Missing Data (Flag <-2>)

A grab sample was collected on 01/07/2020 08:49; however, both chlorophyll *a* centrifuge tubes holding the filters cracked and no samples was available for analysis. The value is flagged as <-2> [GDM] (CSM).

Suspect Data (Flag <1>)

A grab sample was collected on 01/07/2020 08:50; however, one of the two chlorophyll *a* centrifuge tubes holding the filters cracked and no sample from this laboratory replicate was available for processing. Therefore, the value is based on only one laboratory replicate instead of the two normally used for chlorophyll *a*. The value is flagged as <1> [GDM] (CSM).

The differences in field replicates for the identified parameter during the following sampling events were suspect. The percent difference between field replicates was greater than 50. The values are flagged as <1> [SRD] (CSM) or <1> [SRD] (CHB) if replicates were also held past the holding times.

Nitrite+Nitrate	07/01/2020 (0.0116 and 0.0444 mg/L)
DIN	07/01/2020 (0.026 and 0.057 mg/L)

The differences in field replicates for the identified parameter during the following sampling events were suspect. The percent difference between field replicates was greater than 50%. The values are flagged as <1>[SRD] (CSM) if replicates were also held past the holding times.

Nitrite+Nitrate	08/27/2020 (0.0935 and 0.0520 mg/L)
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Passed Initial QAQC Checks (Flag <0>)

Fishing Creek

Rejected Data (Flag <-3>)

Missing Data (Flag <-2>)

Suspect Data (Flag <1>)

Passed Initial QAQC Checks (Flag <0>)

Mosquito Creek

Rejected Data (Flag <-3>)

Missing Data (Flag <-2>)

Suspect Data (Flag <1>)

Passed Initial QAQC Checks (Flag <0>)

St. Pierre Creek

Rejected Data (Flag <-3>)

Missing Data (Flag <-2>)

Suspect Data (Flag <1>)

Passed Initial QAQC Checks (Flag <0>)

Diel Samples: St. Pierre Creek (Monitoring Program 2)

Rejected Data (Flag <-3>)

Missing Data (Flag <-2>)

The 03/18/2020 ISCO sampling event was successful and the chlorophyll *a* samples were processed; however, COVID work from home orders precluded processing of nutrient samples in a reasonable time frame (e.g., several months). The values are flagged as <-2> [GDM] (CSM) or <-2> [GCM] (CSM) for DIN.

Suspect Data (Flag <1>)

Passed Initial QAQC Checks (Flag <0>)

Nutrient Equipment History

Turner 10-AU-005-CE Fluorometer

1994 - Fluorometer (S/N: 0057 LRXX) was purchased in **April**.

2004 - Fluorometer was sent to Turner designs for a standard repair in **November**.

2012 - Fluorometer was calibrated in **July**.

2013 - Fluorometer was calibrated in **May**.

2014 - Fluorometer was sent in Turner designs for a standard repair in **October**.

2014 - Calibration during **December** but was not successful, so unit was re-set to default settings.

2016 - Fluorometer was calibrated in **July**.

2016 - Solid standard was replaced in **August**.

2018 - Fluorometer was calibrated in **February**.

2019 - Fluorometer was calibrated in **February**.

Turner Trilogy Fluorometer

2020 - Fluorometer was calibrated in **October**.