Weeks Bay (WKB) NERR Nutrient Metadata
January – December 2005
Latest Update: July 22, 2025

I. Data Set and Research Descriptors

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2.) Research objectives:

a. Monthly Grab

Monthly grab samples were collected to quantify the spatial variability of important nutrients in the water column between sites representing freshwater and estuarine fractions of the local salinity gradient.

b. Diel Sampling Program

Once per month, duplicate samples were collected every 130 minutes through a tidal cycle to quantify the temporal variability of important nutrients and sediment loading in the water column as a function of tidal forcing.

3.) Research methods:

a. Monthly Grab Sampling Program

Monthly grab samples were taken at four stations within the Weeks Bay estuary. Samples were taken at four WBNERR datatsonde stations, site Weeks Bay (WB), Middle Bay (MB), Magnolia River (MR), and Fish River (FR). Attempts were made to collect grab samples within one hour prior to slack low tide. Discrepancies between actual tide conditions, as determined via in-situ datalogger data, to those predicted often resulted in collections being made outside of the intended tidal range. Examination of the corresponding datasonde file for each site and time will give the user actual tidal conditions. No distinction was made between neap and spring tide conditions. Rainfall conditions prior to grab sampling were not considered. When possible, Grab samples were obtained in conjunction with the deployment of an ISCO 3700 portable sampler. (See 3b) Duplicate samples were collected using a FieldMaster™ sample collection device lowered to 0.5 meters from the bottom.
At site Fish River samples were collected both at depth and at surface, the surface duplicate samples were taken first and are noted with an earlier time in the dataset.

All samples were collected in opaque, 500 mL, Nalgene sample bottles that were previously acid washed and then rinsed with distilled water. Samples were immediately placed on ice and stored in a dark cooler then returned to the laboratory. Once in the laboratory samples were processed for nutrient, solid, and Chlą analysis.

b. Diel Sampling Program:
Twelve duplicate samples are collected each month using an ISCO 3700 portable auto sampler. Sampler was programmed to make two 500 mL collections every 130 minutes throughout a complete tidal cycle. No distinction between neap and spring tide was considered. Samples are stored on ice in 500 mL semi-transparent ISCO containers within the body of the sampler. Sample containers for nutrient determinations are acid washed and rinsed with distilled water prior to sampler deployment. Sampler was programmed to flush collection line 3 times prior to sample uptake. As soon as possible after the final collection time the samples were returned to the laboratory for nutrient, solids, and Chlą analysis.

4.) Site Location and Character

Weeks Bay (30 23' N, 87 50' W) is a small, shallow, microtidal sub-estuary, located on the eastern shore of Mobile Bay in the northeren Gulf of Mexico. It is nearly diamond shaped, and its longitudinal axis (3.4 km long) runs nearly north-south from the head, where the Fish River flows in, to the mouth, where water is exchanged with Mobile Bay. Its widest point (3.1 km) is located near the center of the estuary, where the Magnolia River discharges into eastern side of Weeks Bay. Average depth is 1.4m, although there are two areas where depths are significantly greater. The first is in the mouth of the bay where the average depth is 6m; the second is about 100m upstream of the mouth of the Fish River, where the average depth is 3.5 m. Tides are principally diurnal, and have a mean range of 0.4 m at all 4 sampling sites in the estuary. The Fish River drainage basin encompasses 14300 hectares and contributes approximately 73% to the total incoming freshwater flow with the Magnolia River supplying the rest. Mean combined discharge is 9 cubic meters per second; although freshets up to 4 times larger occur throughout the year. These characteristics result in a freshwater residence time of 13 days under average discharge conditions, with a range from 0.5 to 100 days. Salinity in Weeks Bay varies substantially both temporally and spatially. During periods of high flow in the river, salinity in the bay may be fresh from the head to the mouth, except in the deeper holes of the estuary that are not as easily flushed. However, during periods of low flow in the river, wind velocity and tidal stage are strong factors influencing salinity structure. Salinity greater than 25 ppt is infrequently observed in Weeks Bay and is usually restricted to the southern portion of the estuary near the mouth. There are no known pollutants in the estuary.

Site FR (Fish River; 30 24.97'N, 87 49.37'W) is located near the mouth of Fish River at a depth of about 2 m. Sediment type is sandy-silt, and there are small patches of *Vallisneria* sp. Growing near (but not directly under) the Data logger. Land use in the watershed is agricultural, forested and residential with the residential portion rapidly increasing. Directly surrounding the site, land use is residential and forested. Nutrient concentrations ranging from approximately 4-100 μM and <0.5 to 7.5 μM, respectively, over the previous 10 years

(Alabama Department of Environmental Management, Geological Survey of Alabama.) Over the past year, salinity ranged from 0 to 25 ppt at this site.

Site WB (Weeks Bay; 30 22.85'N, 87 49.92' W) is located near the southeast shore of Weeks Bay, about 0.5 km from the mouth of the estuary. Mean water depth at this site is about 0.9 m; sediment type is sandy-silt. Land use around this site is almost exclusively residential, with the agriculture occurring inland. Nutrient concentrations are much lower at this site than at site Fish River. Over the previous 10 years, nitrate and phosphate concentrations here ranged from approximately 0.1 to 50 µM and 0.04 to 0.45 µM, respectively. Salinity ranged from approximately 5 to 27 ppt over the past year.

Site MB (Middle Bay; 30 23.768N, 87 50.010 W) is located near the middle of Weeks Bay, approximately 1.1 kilometers from the southeastern shoreline. Mean water depth at this site is 1.5 m. Bottom sediments are a soft silty-clay with no sub-aquatic vegetation present. Salinity ranges from 0 to 25 ppt.

Site MR (Magnolia River; 30 23.398 N, 87 49.059 W) is located near the mouth of the Magnolia River. Mean water depth at this site is 1.1m; bottom sediments are silty-clay. Salinity ranges from 0 to 24 ppt. The site is approximately 20m from the southern shoreline. A bottomland hardwood forest interspersed with patches of woody shrubs dominates the southern shoreline. Approximately 40 m north of the site is a needle rush dominated marsh with extends approximately 200 m along the Magnolia River and along the north and east fringes of the bay.

5.) Coded variable definitions

a. Station codes (column "A" of nutrient data report):
   wkbwbnut = Weeks Bay NERR, site Weeks bay nutrient data
   wkbfrnut = Weeks Bay NERR, site Fish River nutrient data
   wkbmrnut = Weeks Bay NERR, site Magnolia River nutrient data
   wkbmbnut = Weeks Bay NERR, site Middle Bay nutrient data

b. Monitoring program (column "D" of nutrient data report) :
   1 = Monthly grab sample
   2 = Diel grab sample

6.) Data collection period

The first water samples collected for 2005 SWMP nutrient monitoring program occurred on January 5 at 2152 and the last was collected on December 21 at 1129. Individual collection dates and times for both the monthly grab program and diel program are reported below.

Diel Sample Collection

Station code	Begin Date	Begin Time	End Date	End Time

wkbfrnut	1/5/2005	21:52	1/7/2005	0:27
wkbfrnut	2/8/2005	8:41	2/9/2005	8:31
wkbfrnut	3/29/2005	12:26	3/30/2005	11:16
wkbfrnut	4/27/2005	11:58	4/28/2005	11:48
wkbfrnut	5/24/2005	10:05	5/25/2005	10:00
wkbfrnut	6/20/2005	8:08	6/21/2005	7:48
wkbfrnut	7/17/2005	5:54	7/18/2005	4:44
wkbfrnut	8/16/2005	6:38	8/17/2005	6:28
wkbfrnut	9/22/2005	0:30	9/22/2005	22:20
wkbfrnut	10/17/2005	6:43	10/18/2005	6:33
wkbfrnut	11/8/2005	14:21	11/9/2005	15:11
wkbfrnut	12/20/2005	11:39	12/21/2005	11:29

Monthly Grab Sample Collection:

Station Code	Date	0.5 m above Bottom Duplicates	Surface Duplicates
wkbfrnut	1/5/2005	21:35	21:30
wkbfrnut	2/8/2005	8:35	8:30
wkbfrnut	3/30/2005	11:15	11:10
wkbfrnut	4/28/2005	11:45	11:40
wkbfrnut	5/25/2005	10:00	9:55
wkbfrnut	6/21/2005	7:50	7:45
wkbfrnut	7/18/2005	4:40	4:35
wkbfrnut	8/17/2005	6:15	6:10
wkbfrnut	9/22/2005	8:35	8:30
wkbfrnut	10/18/2005	6:20	6:15
wkbfrnut	11/9/2005	15:05	15:00
wkbfrnut	12/21/2005	11:35	11:30
wkbmbnut	1/5/2005	21:14	
wkbmbnut	2/8/2005	8:10	
wkbmbnut	3/30/2005	10:55	
wkbmbnut	4/28/2005	11:20	
wkbmbnut	5/25/2005	9:30	
wkbmbnut	6/21/2005	7:15	
wkbmbnut	7/18/2005	4:10	
wkbmbnut	8/17/2005	5:45	
wkbmbnut	9/22/2005	8:10	
wkbmbnut	10/18/2005	5:55	
wkbmbnut	11/9/2005	14:40	
wkbmbnut	12/21/2005	11:07	
wkbmrnut	1/5/2005	21:20	
wkbmrnut	2/8/2005	8:20	
wkbmrnut	3/30/2005	11:02	
wkbmrnut	4/28/2005	11:30	
wkbmrnut	5/25/2005	9:45	
wkbmrnut	6/21/2005	7:30	

wkbmrnut	7/18/2005	4:20
wkbmrnut	8/17/2005	5:55
wkbmrnut	9/22/2005	8:20
wkbmrnut	10/18/2005	6:05
wkbmrnut	11/9/2005	14:50
wkbmrnut	12/21/2005	11:18
wkbwbnut	1/5/2005	21:00
wkbwbnut	2/8/2005	8:00
wkbwbnut	3/30/2005	10:45
wkbwbnut	4/28/2005	11:05
wkbwbnut	5/25/2005	9:15
wkbwbnut	6/21/2005	7:00
wkbwbnut	7/18/2005	4:00
wkbwbnut	8/17/2005	5:30
wkbwbnut	9/22/2005	8:00
wkbwbnut	10/18/2005	5:45
wkbwbnut	11/9/2005	14:30
wkbwbnut	12/21/2005	11:00

7.) Associated researcher and projects

It is possible for interested researchers to correlate weather and water quality parameters with diel and grab sampling times by referencing the 2005 Weeks Bay NERR weather data and water quality data available via the Central Data Management Office or contacting the Weeks Bay NERR Research Coordinator.

8.) Distribution

NOAA/ERD retains the right to analyze, synthesize and publish summaries of the NERRS System-wide Monitoring Program data. The PI retains the right to be fully credited for having collected and processed the data. Following academic courtesy standards, the PI and NERR site where the data were collected will be contacted and fully acknowledged in any subsequent publications in which any part of the data are used. Manuscripts resulting from this NOAA/OCRM supported research that are produced for publication in open literature, including referred scientific journals, will acknowledge that the research was conducted under an award from the Estuarine Reserves Division, Office of Ocean and atmospheric administration. 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.

NERR water quality and nutrient data metadata can be obtained from the Research Coordinator at the individual NERR site (please see Section 1 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 http://cdmo.baruch.sc.edu. Data are available in text tab-delimited format, Microsoft Excel spreadsheet format and comma-delimited format.

II. Physical Structure Descriptors

9.) Entry verification

Samples were collected and analysis performed in accordance with Weeks Bay National Estuarine Research Reserve's Standard Operating Procedures for Water Chemistry (appendix A). Analysis data were recorded in both a laboratory log book and electronically in spreadsheet form. These data were then transferred in general formatting into the comprehensive Excel form employed by the NERR system for yearly reporting purposes. Data were checked twice for transfer accuracy. After accuracy checks were completed the Central Data Management Office derived Nutrient Rounding Macro was used to round data values to the appropriate number of decimal places.

10.) Parameter titles and variable names by data category

Required NOAA/NERRS System-wide Monitoring Program water quality parameters are denoted by an asterisks "*".

Data Category	Parameter	Variable	Unit of Measurement
Phosphorous and Nitrogen:			
	*Orthophosphate, filtered	PO4F	mg/l as P
	Dissolved Organic Phosphorus	DOP	mg/l as P
	Total Dissolved Phosphorous	TDP	mg/l as P
	*Nitrite + Nitrate, filtered	NO23F	mg/l as N
	* Nitrite, filtered	NO2F	mg/l as N
	*Nitrate, filtered	NO3F	mg/l as N
	*Ammonium, filtered	NH4F	mg/l as N
	Dissolved Inorganic Nitrogen	DIN	mg/l as N
Plant pigments			
	*Chlorophyll a	CHLA N	µg/l
	Phaeophytin	PHEA	µg/l
Other Lab Parameters			
	Total suspended solids	TSS	mg/l
	Total volatile solids	TVS	mg/l
	Total fixed solids	TFS	mg/l

Notes:
1. Time is coded based on a 2400 hour clock and is referenced to Standard Time.
2. Reserves have the option of measuring either NO23 or NO2 or NO3.

11.) Measured and Calculated Laboratory Parameters

a. Parameters measured directly

Nitrogen species:	NO23F, NO2F, NH4F
Phosphorus species:	PO4F, TDP
Other:	CHLA_N, PHEA, TSS, TFS

b. Calculated parameters

NO3F:	NO23F – NO2F
DIN:	NO23F + NH4F
DOP:	TDP – PO4F
TVS:	TSS – TFS

12.) Limits of Detection

13.) Method Detection Limits (MDL), the lowest concentration of a parameter that an analytical procedure can reliably detect, were determined by the Weeks Bay Lab. MDLs were determined as 3 times the standard deviation of 10 or more lab duplicate samples. MDLs are periodically reviewed. Current MDLs are listed below, please see Appendix A. Weeks Bay NERR Laboratory Standard Operating Procedures. for more information.

Parameter	Variable Method	Detection Limit	Dates in Use
Orthophosphate	PO4F	0.001 mg/L as P	2004-2005
Total Phosphorus	TDP	0.001 mg/L as P	2004-2005
Ammonium	NH4F	0.001 mg/L as N	2004-2005
Nitrite	NO2F	0.001 mg/L as N	2004-2005
Nitrite+Nitrate	NO23F	0.001 mg/L as N	2004-2005
Chlorophyll a	CHLA_N	0.1 µg/L	2004-2005
Phaeophytin	PHEA	0.1 µg/L	2004-2005
Total Suspended Solids	TSS	0.01 mg/L	2004-2005
TFS Total Fixed Solids	TFS	0.01 mg/L	2004-2005

14.) Laboratory Methods – refer to Appendix A, Weeks Bay NERR Laboratory Standard Operating Procedures.

15.) Reporting of missing data and data with concentrations lower than method detections limits.

Nutrient/Chla comment codes and definitions are provided in the following table. Missing data are denoted by a blank cell " " and commented coded with an "M". Laboratories in the NERRS System submit data that are censored at a lower detection rate limit, called the Method Detection Limit or MDL. MDL's for specific parameters are listed in the Laboratory Methods and Detection Limits Section (Section II, Part 14) of this document. Measured concentrations that are less than this limit are replaced with the minimum detection limit value and comment coded with a "B" in the variable code comment column. For example, the measured concentration of NO23F was 0.0005 mg/L as N (MDL=0.0008), the

reported value would be 0.0008 with a “B” placed in the NO23F comment code column. Calculated parameters are comment coded with a “C” and if any of the components used in the calculation are below the MDL, the calculated value is removed and also comment coded with a “B”. If a calculated value is negative, the value is removed and comment coded with an “N”.

Note: The way below MDL values are handled in the NERRS SWMP dataset was changed in November of 2011. Previously, below MDL data from 2002-2006 were also coded with a B, but replaced with -9999 place holders. Any 2002-2006 nutrient/pigment data downloaded from the CDMO prior to December November of 2011 will contain -9999s representing below MDL concentrations.

Comment Code	Definition
A	Value above upper limit of method detection
B	Value below method detection limit
C	Calculated value
D	Data deleted or calculated value could not be determined due to deleted data, see metadata for details
H	Sample held beyond specified holding time
K	Check metadata for further details
M	Data missing, sample never collected or calculated value could not be determined due to missing data
P	Significant precipitation (reserve defined, see metadata for further details)
U	Lab analysis from unpreserved sample
S	Data suspect, see metadata for further details

16.) QA/QC Programs

a) Precision:
   i) Field Variability – WKB NERR collects replicate samples at each site during grab sampling.
   ii) Laboratory Variability – One tidal cycle sample is lab duplicated and at least one chlorophyll sample is lab duplicated
   iii) Inter-organizational splits - None

b) Accuracy:
   i) Samples Spikes – For each chemical determination, at least 1 blank and 1 standard sample (or matrix spike) are tested.
   ii) Standard Reference Material Analysis – For each chemical determination, at least 1 blank and 1 standard sample (or matrix spike) are tested.
   iii) Cross Calibration Exercises - None

Refer to appendix A, Weeks Bay NERR Standard Operating Procedures for Water Chemistry. Additional QA/QC include participation in the NERRS quality assessment program as well as procedures discussed in section 8 of this document.

16.) Other remarks

On 07/22/2025 this dataset was updated to include embedded QAQC flags and codes for anomalous/suspect, rejected, missing, and below detection limit data. System-wide monitoring data beginning in 2007 were processed to allow for QAQC flags and codes to be embedded in the data files rather than using the original single letter codes used for the nutrient and pigment dataset along with the detailed sections in the metadata document for suspect, missing, and rejected data. Please note that prior to 2007, rejected data were deleted from the dataset so they are unavailable to be used at all. Suspect, missing, rejected and below minimum detection flags and appropriate three letter codes were embedded retroactively for dataset consistency. The QAQC flag/codes corresponding to the original letter codes are detailed below.

		Historic	
Flag/code	***If also C***	**Letter Code**	Historic Code Definition
<1> [SUL]		A	Value above upper limit of method detection
<-4> [SBL]	<-4> [SCB]	B	Value below method detection limit
no need to flag/code unless combined		C	Calculated value
<-3> [GQD]	<3> [GCR]	D	Data deleted or calculated value could not be determined due to deleted data, see metadata for details
<1> (CHB)		H	Sample held beyond specified holding time
<0> (CSM) unless other flag		K	Check metadata for further details
<-2> [GDM]	<-2> [GCM]	M	Data missing, sample never collected or calculated value could not be determined due to missing data
<-3> [SNV] and <1> [SCC] for components		N	Negative calculated value
(CRE) or F_Record {CRE}		P	Significant precipitation (reserve defined, see metadata for further details)
<0> (CUS)		U	Lab analysis from unpreserved sample
<1> (CSM)		S	Data suspect, see metadata for further details

a) Significant Weather Events in 2005

Rainfall by day for 2005. These data are from the USGS rainfall gauges at Fish River (02578500) and at Magnolia River (02578300). Data from the Weeks Bay weather station can be found within WKBMET files at the CDMO website or by contacting the Research Coordinator at Weeks Bay NERR.

Month/day/year; rainfall in mm

January:
01/08/2005 15.2
01/09/2005 00.3
01/13/2005 14.7
01/14/2005 00.3
01/28/2005 03.8
01/29/2005 08.1
01/30/2005 00.3
01/31/2005 16.5

February:
02/01/2005 12.2
02/02/2005 10.9
02/09/2005 03.0
02/13/2005 18.3
02/14/2005 02.3
02/15/2005 00.3
02/23/2005 20.3
02/24/2005 06.6
02/25/2005 00.3

March:
03/03/2005 12.4
03/07/2005 10.7
03/08/2005 00.3
03/14/2005 00.3
03/15/2005 15.2
03/16/2005 11.4
03/20/2005 00.8
03/22/2005 00.3
03/25/2005 00.8
03/26/2005 20.1
03/27/2005 12.4
03/31/2005 158.8

April:
04/01/2005 145.5
04/06/2005 189.8
04/07/2005 4.3
04/11/2005 0.3
04/12/2005 36.8
04/23/2005 6.0
04/25/2005 1.0
04/26/2005 25.8
04/30/2005 149.0

May:
05/01/2005 00.3
05/10/2005 06.3
05/15/2005 05.6
05/20/2005 02.8
05/21/2005 00.3
05/27/2005 00.3
05/29/2005 09.1
05/30/2005 04.1
05/31/2005 63.5

June:
06/01/2005 26.7
06/02/2005 00.3
06/06/2005 53.3
06/07/2005 00.3
06/08/2005 10.7
06/09/2005 09.1
06/10/2005 11.2
06/11/2005 70.1
06/12/2005 00.8
06/15/2005 01.3
06/17/2005 33.7
06/18/2005 00.7
06/20/2005 17.3
06/21/2005 00.3

06/23/2005 00.5
06/24/2005 10.7
06/28/2005 00.3
06/29/2005 27.2
06/30/2005 22.6

July:
07/02/2005 37.6
07/05/2005 03.6
07/06/2005 40.1
07/08/2005 04.3
07/09/2005 00.3
07/10/2005 55.4
07/11/2005 01.8
07/14/2005 18.8
07/15/2005 29.5
07/20/2005 13.2
07/21/2005 23.4
07/24/2005 00.3
07/28/2005 01.5
07/29/2005 00.3
07/30/2005 06.9
07/31/2005 00.3

August:
08/01/2005 01.8
08/02/2005 12.9
08/03/2005 00.8
08/04/2005 16.2
08/05/2005 03.3
08/06/2005 47.7
08/07/2005 00.5
08/10/2005 04.6
08/11/2005 01.8
08/15/2005 05.3
08/16/2005 01.8
08/17/2005 02.3
08/20/2005 00.5
08/21/2005 02.5
08/22/2005 01.8
08/24/2005 08.1
08/25/2005 15.0
08/28/2005 22.1
08/29/2005 48.7
08/31/2005 28.7

September:
09/04/2005 15.0
09/05/2005 00.3
09/19/2005 01.78

09/22/2005 00.5
09/23/2005 24.9
09/24/2005 37.3
09/25/2005 17.5
09/26/2005 09.4
09/28/2005 03.0
09/30/2005 17.5

October:
10/01/2005 04.6
10/06/2005 04.1

November:
11/02/2005 23.6
11/06/2005 01.0
11/08/2005 00.3
11/12/2005 10.4
11/13/2005 00.5
11/15/2005 01.8
11/16/2005 00.8
11/21/2005 05.6
11/27/2005 27.4
11/28/2005 00.8

December:
12/05/2005 14.5
12/08/2005 00.8
12/14/2005 02.8
12/15/2005 28.2
12/17/2005 04.3
12/24/2005 22.3
12/25/2005 00.3
12/28/2005 00.3

b) Expanded explanations of deleted data for 2005

January: All fractions of Chlorophyll were deleted due to samples being held too long before determination. This caused high degree of error in repeated samples.

February through May: All phaeophytin samples were deleted due to improper concentration of the acid used in this determination.

September: 9/22/05 at 20:10, data pointy deleted from TDP column due to probable contamination of sample.

October: Several TDP samples were deleted due to probable contamination of glassware. The last 3 diel samples (10/18/05) for TFS were apparently mixed (dropped?) and were deleted.

Appendix A:

Standard Operating Procedures
Water Chemistry
Weeks Bay National Estuarine Research Reserve
2005

Sample Handling

Sample Bottle and Glassware Cleaning

Samples are collected in 500 mL Nalgene® HDPE (high-density polyethelene) wide-mouthed amber bottles. Sample bottles are rinsed with 10% HCl then rinsed three (3) times with distilled water and allowed to air dry. Bottles are then rinsed 3 times with sample water before sample collection. ISCO® samples are dispensed into 1L ISCO® discreet sample bottles treated as above. After filtering, 500 mL from the ISCO bottles are transferred to wide-mouth amber Nalgene® HDPE bottles for storage.

All glass- and plastic-ware that is to be used in chemical analysis is also rinsed in 10% hydrochloric acid and then rinsed at least 3 times in distilled water.

Sampling

Site grab samples: Samples collected at the current SWMP datalogger sites at the depth of the sensors on the datalogger; at - or no more than 2 hours before - low tide. Collect 2 consecutive samples at each site. At site FR, 2 additional samples are collected at ½ m from the surface. This gives us a "surface/bottom" gradient at this deeper site. Samples are collected with a Van Dorn bottle or similar device for collecting water at a discreet depth. Collection bottle should be large enough to rinse the sample bottle three times before capturing sample. Rinse the sample bottle three times before collecting a 500 mL sample. Fill the bottle completely and cap. Store in the dark on ice until return to the lab. Temperature, dissolved oxygen, salinity, turbidity and pH are taken at the site at time of sampling with a YSI 6600 Datasonde®.

Tidal cycle samples: Samples are collected from 1 half meter above the sediments near the Fish River SWMP site. Samples are collected using an ISCO® sampler. Sampler is set to acquire a 1L sample approximately every 136 minutes to accumulate 12 single samples from low tide to low tide (Gulf of Mexico tidal cycles are 25 hours, 1 high and 1 low per day). Sampler is set to rinse the collection line 3 times before collecting the sample. Ice is placed in the sampler to cool the samples and is replaced as necessary throughout the sample collection cycle.

Laboratory Analysis

Filtration and Storage: All samples are filtered immediately upon returning to the lab and prior to storage or chemical analysis.

Samples for chlorophyll analysis: Filter and filtration apparatus are pre-rinsed with saturated magnesium carbonate solution to neutralize any acid present. A sample of known volume (for chlorophyll analysis) is filtered through a Whatman® GF/F 47 mm diameter glass fiber filter (0.7 μm). The filter is then removed, folded, and placed into 10 mL aqueous acetone (90% acetone, 10% saturated magnesium carbonate solution) in a 15 ml polypropylene disposable centrifuge tube. This tube is then stored in the freezer (=/< -20º C) in the dark for at least one week prior to analysis.

Samples for Suspended Solids analysis: A sample of known volume is drawn through a pre-weighed (to 0.01 mg) Whatman® 934/AH 47 mm diameter glass fiber filter (1.5 μm). Filters are then placed directly into the drying oven.

Samples for chemical analysis: Samples are drawn through a Whatman® GF/F 47 mm diameter glass fiber filter (0.7 μm). In the case of samples with high suspended solids, sample may first be drawn through a Whatman® GF/C 47 mm diameter glass fiber filter (1.2 μm) pre-filter. Samples are then either determined immediately, refrigerated at 4º C if they are to be determined in the next 48 hours; or, frozen at =/< -20º C. Samples are determined within a 28 day maximum hold time.

Order of Analyses: For maximum efficiency and to minimize contamination problems, the following order of analyses is followed in our lab:
1) Chlorophyll filtration
2) Suspended solids filtration
3) Filtration of all other samples
4) Ammonia determination (within 24 hours of sample collection)
5), Nitrite, Nitrate, Dissolved Reactive Phosphorus and Total Dissolved Phosphorus (in any order).

Table 1. Storage of samples and Method Detection Limits for determinations at Weeks Bay NERR.

number	determination	code	storage	units	MDL
I	Chlorophyll *a* in the presence of Pheophytin *a*	Chla Phea	filter immediately, freeze filters at <-20º C	mg/m^3 (μg/L)	0.1 mg/m^3 (μg/L)
II	Total Suspended Solids, Volatile Suspended, Fixed Suspended	TSS VSS FSS	filter immediately, store filters + residue in desiccator	mg/L	0.01 mg/L
III	Ammonia	NH4F	refrigerate (4º C), run within 24 hours	mg/L	0.01 mg/L* (0.001)
IV	Nitrite	NO2F	refrigerate (4º C) up to 48 hours, freeze (-20º C) up to 28 days	mg/L	0.01 mg/L* (0.001)

V	Nitrate + Nitrite Nitrate	NO23F NO3F	refrigerate (4º C) up to 48 hours, freeze (-20º C) up to 28 days	mg/L	0.01 mg/L* (0.001)
VI	Dissolved Reactive Phosphorus	PO4F	refrigerate up to 48 hours, freeze up to 28 days	mg/L	0.01 mg/L* (0.001)
VII	Total Dissolved Phosphorus, Dissolved Organic Phosphorus	TDP DOP	refrigerate up to 48 hours, freeze up to 28 days	mg/L	0.01 mg/L* (0.001)

*From Standard Methods, 20th Ed.; we have determined our laboratory MDL to be 0.001 mg/L.

I. Chlorophyll Analysis - Spectrophotometric Method (Standard Methods, 20th Ed. pp. 10/18 - 10/20)

Reagents:

a) Buffered Acetone Solution: Mix 90 parts acetone with 10 parts saturated magnesium carbonate solution (1.0 g magnesium carbonate in 100 mL distilled water)

b) 0.1 M HCl: Dilute 8.3 mL concentrated ACS Hydrochloric Acid with distilled water up to 1000 mL final volume.

Procedure:

1) Filter a known volume of water through a 47 mm Whatman® GF/F glass fiber filter (0.7 μm)
2) Place filter into a 15 mL centrifuge tube and add 10 mL aliquot of buffered acetone.
3) Let bottle sit in freezer in the dark for a minimum of 7 days to insure complete extraction of chlorophyll. After a week, remove centrifuge tube and store in the dark at room temperature for 30 minutes.
3) Centrifuge for 5 minutes to clear acetone.
4) Take a 3 mL sample of cleared acetone-chlorophyll solution and read in 1.0 cm glass spectrophotometer cell at 750 and 664 nm. Add 0.1 mL of 0.1N HCl to 3mL sample and re-read at 750 and at 665 nm.

Note: Optical density at 664 nm should be >0.100 and <1.000. If samples are less than 0.100, use a longer path length spectrophotometer cell and correct absorbance to 1 cm BEFORE using equation.

5) Calculate chlorophyll and pheophytin using equations:

$$\text{Chlorophyll } a, \text{ mg/m}^3 = \frac{26.7 \times (od664_b - od665_a) \times V_1}{V_2 \times L}$$

$$\text{Pheophytin } a, \text{ mg/m}^3 = \frac{26.7 \times [(1.7 \times od665_a) - od664_b] \times V_1}{V_2 \times L}$$

Where: V_1= volume of extract, in liters
V_2= volume of sample, in cubic meters
L= width of cuvette in centimeters

$od664_b$, $od665_a$ = optical densities (corrected for turbidity at 750 nm) of 90% acetone extract before and after acidification, respectively

(note: 10 mL extract = 0.01 L, 250 mL sample = 0.00025 m^3)

Method Detection Limit: 0.5 mg/m^3 (µg/L)

II. Suspended Solids Analysis (Standard Methods, 20th Ed. pp. 2/57 - 2/59)

Procedure:

1) Preparation of filter disks: Insert glass fiber filter with 1.5 µm particle retention size into filtration apparatus. Apply vacuum and wash disk with 3 successive 20 mL aliquots of distilled/deionized water. Transfer filter to an inert aluminum weighing dish. Place in muffle oven and ash at 550º C for 15 minutes. Cool in a desiccator to balance temperature and weigh to 0.01 mg. Keep filter in desiccator until use. Pre-washed and Pre-ashed filters may be purchased.
2) Selection of sample size: Select sample size to yield between 2.5 and 200 mg dried residue.
3) Total Suspended Solids: Pull measured volume through filter. Place filter in drying oven at 103 - 105º C for 24 hours. Cool filter to balance temperature in a desiccator. Weigh filter + residue to 0.01 mg.

   mg total suspended solids/mL = [(A - B) X 1000]/ C
   where: A = weight of filter + dried residue, mg
   B = weight of filter, mg
   C = sample volume, mL

4) Total Volatile Solids: Place filter + dried residue in a muffle furnace and ignite at 550º C to constant weight (usually 15 to 20 minutes are required for 200 mg residue). Transfer filter to desiccator to cool to balance temperature. Weigh filter + residue to 0.01 mg.

   mg total volatile solids/mL = [(A - B) X 1000]/C
   where: A = weight of filter + residue before ignition, mg
   B = weight of filter + residue after ignition, mg
   C = sample volume, mL

5) Total Fixed Solids:

   mg total fixed solids = [(A - B) X 1000]/C
   where: A = weight of filter + residue after ignition, mg
   B = weight of filter, mg
   C = sample volume, mL

Method Detection Limit: 0.01 mg/L

Chemical Analysis

General Considerations

All chemicals used in making reagents are at least certified ACS grade or higher unless otherwise specified.

Chemical analysis methods used at Weeks Bay National Estuarine Research Reserve are from Standard Methods for the Examination of Water and Wastewater, 20th Edition, 1998.

All samples are measured using class A volumetric pipettes or class A volumetric flasks. Reagents are added to samples using repeating syringe-type pipettes.

All glass and plastic ware used in the chemical tests have been pre-cleaned with 10% HCl and then rinsed with distilled water a minimum of 3 times. Between uses, the glass and plastic ware is cleaned as outlined above and then allowed to air dry upside down in the laboratory.

III. Ammonia Analysis - Phenate Method (Standard Methods, 20th Ed. pp. 4/108 - 4/109)

Reagents:

a) Phenol-alcohol Solution: Mix 11.1 mL liquified phenol (>89%) with 95% ethanol to a final volume of 100 mL. CAUTION: TOXIC - SEE MATERIAL DATA SAFETY SHEET.
b) Sodium Nitroprusside Solution: Dissolve 0.5 g sodium nitroprusside ($Na_2Fe(CN)_5NO \cdot 2H_2O$) in 100 mL distilled/deionized water. Store in amber bottle for not more than one month. CAUTION: POISONOUS- SEE MATERIAL DATA SAFETY SHEET.
c) Alkaline Citrate Solution: Dissolve 100 g trisodium citrate and 5 g sodium hydroxide (NaOH) in distilled/deionized water. Dilute to 500 mL.
d) Sodium Hypochlorite Solution: Use a commercial solution of bleach (unscented Chlorox®- is the only brand that I have used successfully) with at least 5.25% hypochlorite. Replace every 2 months.
e) Oxidizing Solution: Mix 4 parks alkaline citrate solution to 1 part sodium hypochlorite solution. This is stable for less than 12 hours.
f) Stock Ammonium Solution: Dissolve 0.3819 g anhydrous ammonium chloride (NH_4Cl) - which has been dried at 100º C in distilled/de-ionized water and dilute to 1000 mL. 1.00 mL = 100 μg N=122 μg NH_3. It is possible to purchase this standard commercially.

Procedure:

(note: Add all reagents in a darkened room. Direct light, either natural or fluorescent will change the color of the reaction from blue to green-blue.)

1) Measure a 25 mL sample and pour it into a numbered 50 mL Erlenmeyer flask.
2) Prepare a set of 5 standards appropriate to the concentration of the samples - treat standards the same as the samples.
3) Add 1 mL phenol-alcohol solution and mix
4) Add 1 mL sodium nitroprusside solution and mix.
5) Add 2.5 mL oxidizing solution and mix.
6) Cover samples with parafilm
7) Let stand in dark at room temperature for at least one hour. (color is stable for 24 hours)

8) Read color absorption at 640 nm (with a 1 cm or greater path length cuvette) and determine concentration in mg/L from a graph of absorbance vs concentration.

Method Detection Limit: 0.001 mg/L

IV. Nitrite - Colorimetric Method (Standard Methods, 20th Ed. pp. 4/112 - 4/114)

Reagents:

a) Color Reagent: To 800 mL distilled water add 100 mL 85% phosphoric acid and 10 g sulfanilamide. After dissolving sulfanilamide completely, add 1 g N-(1-naphthyl)-ethylenediamine dihydrochloride (N.E.D.). Mix to dissolve and then dilute to 1000 mL with distilled/deionized water. Solution is stable for about a month when stored in a dark bottle in the refrigerator.
b) Standard Nitrite Solution: Purchase this standard commercially. Problems with standardization of a "home-made" standard far outweigh the cost of a commercial standard.

Procedure:

1) Measure out 50 mL sample or smaller portion diluted to 50 mL and pour into a 125 mL Erlenmeyer flask.
2) Neutralize sample to between pH 5 and pH 9 with either 1N HCl or 1N NH_4OH.
3) Add 2 mL Color Reagent and mix.
4) Let the color develop for more than 10 minutes and less than 2 hours before measuring absorbance at 543 nm against a distilled water-reagent blank.
5) Prepare at least 5 standards in the appropriate range from the standard solution. Treat standards as samples. Determine concentration in mg/L from a calibration curve comparing concentration with absorbance of standards.

Method Detection Limit: 0.001 mg/L

V. Nitrate-Cadmium Reduction, Colorimetric Method (Standard Methods, 20th Ed. pp. 4/117 - 4/118)

Reagents:

a) Copper-cadmium granules: Wash 25 g 20- to 100-mesh Cd granules with 6N HCl and rinse with distilled water (at least 3 rinses). Swirl Cd with 100 ml 2% $CuSO_4$ solution for 5 minutes or until blue color partially fades. Decant and repeat with 100 mL fresh $CuSO_4$ until a brown colloidal precipitate develops. Wash Cu-Cd granules copiously (at least 10 times) with distilled water to remove all precipitated Cu.
b) Color Reagent: To 800 mL distilled/deionized water add 100 mL 85% phosphoric acid and 10 g sulfanilamide. After dissolving sulfanilamide completely, add 1 g N-(1-naphthyl)-ethylenediamine dihydrochloride (N.E.D.). Mix to dissolve and then dilute to 1000 mL with distilled/deionized water. Solution is stable for about a month when stored in a dark bottle in the refrigerator.
c) Ammonium chloride-EDTA Solution: Dissolve 13 g ammonium chloride (NH_4Cl) and 1.7 g disodium EDTA in 900 mL distilled/deionized water. Adjust pH to 8.5 with concentrated NH_4OH and then dilute to 1000 mL.

d) Dilute Ammonium Chloride-EDTA Solution: Dilute 300 mL of ammonium chloride/EDTA solution to 500 mL with distilled/deionized water.
e) Hydrochloric Acid, 6N: Mix equal amounts of concentrated HCl and distilled/deionized water.
f) Copper Sulfate Solution, 2%: Dissolve 20 gm $CuSO_4 \cdot 5H_2O$ in 500 mL distilled/deionized water and dilute to 1 L.
g) Stock Nitrate Solution: Dry potassium nitrate (KNO_3) in an oven at 105º C for 24 hours. Dissolve 0.7218 g in distilled/deionized water and dilute to 1000 mL. Preserve with 2 mL $CHCl_3$. 1.00 mL = 100 μg NO_3-N. It is possible to purchase this standard commercially.
h) Standard Nitrate Solution: Dilute 50.0 mL stock nitrate solution to 500 mL with distilled/deionized water. 1.00 mL = 10 μg NO_3-N.
i) Standard Nitrite Solution: Purchase this standard commercially. Problems with standardization of a "home-made" nitrite standard far outweigh the cost of a commercial standard.

Procedure:

1) Preparation of reduction column: Insert a glass wool plug into bottom of reduction column and fill with distilled water. Add sufficient Cu-Cd granules to make a column of granules approximately 18.5 cm long in reduction column. Ensure that water levels remain above granules at all times to prevent entrapment of air. Wash column with 200 mL dilute ammonium chloride-EDTA Solution. Activate column by passing through it, at 7 to 10 mL/minute, 100 mL of a solution composed of 25 mL of a 1.0 mg nitrate/L standard and 75 mL of the dilute ammonium chloride-EDTA Solution. This only has to be done once when the columns are made. It is not necessary to wash the column between samples. Do not allow column to go dry. Store column in dilute ammonium chloride-EDTA solution.
2) Adjust pH of sample water to between 5 and 9 with dilute HCl or dilute NaOH.
3) To a 25.0 mL sample or a portion diluted to 25.0 mL, add 75.0 mL dilute ammonium chloride-EDTA solution and mix. Pass this through the column at a rate of 7 to 11 mL/ minute. Discard the first 50 mL collected. Use 50 mL of the remainder and treat as a nitrite sample.
4) As soon as possible and absolutely no later than 15 minutes after collection, add 2 mL color reagent and mix.
5) Let the samples sit for more than 10 minutes and less than 2 hours before measuring absorbance at 543 nm against a distilled water-reagent blank.
6) Prepare at least 5 standards in the appropriate range from the standard solution. Treat standards as samples. Determine concentration in mg/L from a calibration curve comparing concentration with absorbance of standards.
7) To check column efficiency, compare nitrate standard passed through column with a nitrite standard of equal concentration (NO_3/NO_2 - N mg/L). Column should be re-activated when efficiency falls below 75%.

Method Detection Limit: 0.001 mg/L

VI. Dissolved Reactive Phosphorus- Ascorbic Acid Method (Standard Methods 20th Ed. pp. 4/146 - 4/147)

Reagents:

a) 5N Sulfuric Acid Solution: Dilute 70 mL ACS certified concentrated H_2SO_4 with distilled water to 500 mL.
b) Potassium Antimony1 Tartrate solution (P.A.T.): Dissolve 1.3715 g potassium antimonyl tartrate ($K(SbO)C_4H_4O_6 \cdot \frac{1}{2}H_2O$) in 400 mL distilled/deionized water and dilute to 500 mL. Store in glass-stoppered bottle.
c) Ammonium Molybdate Solution: Dissolve 20 g ammonium molybdate ($(NH_4)_6Mo_7O_{24} \cdot 4H_2O$) in 500 mL distilled/deionized water. Store in plastic bottle at 4º C.
d) Ascorbic Acid: 0.1M: Dissolve 1.76 gm ascorbic acid in 100 mL distilled/deionized water. Solution is stable for about 1 week at 4°C.
e) Combined Reagent: Mix the above reagents in the following proportions for 100 mL of combined reagent: 50 mL 5N H_2SO_4, 5 mL P.A.T., 15 mL ammonium molybdate, and 30 mL ascorbic acid. Mix after the addition of each reagent. Let all reagents reach room temperature before mixing and mix in the order given. The reagent is stable for 4 hours.
f) Phenolphthalein Indicator Solution: Use prepared indicator solution.
g) Stock Phosphate Solution: Dissolve 219.5 mg anhydrous potassium dihydrogen phosphate (KH_2PO_4) in distilled/deionized water and dilute to 1000 mL. 1.00 mL = 50.0:g PO_4–P. It is possible to purchase this standard commercially.
h) Standard Phosphate Solution: Dilute 50.0 mL standard solution to 1000 ml. with distilled water. 1.00 ml.= 2.50:g P

Procedures:

1) Measure a 50.0 mL sample into a 125 mL Erlenmeyer flask.
2) Add 1drop pheno1phtalein indicator. If a pink color develops, add 5N H_2SO_4 solution drop by drop until color disappears.
3) Add 8.0 mL combined reagent and mix thoroughly.
4) After 10 minutes and before 30 minutes read color absorbance at 880 nm.
5) Prepare calibration curve from a series of 6 standards. Use a distilled/deionized water + combined reagent blank to zero the spectrophotometer. Plot absorbance vs. concentration to give formula for determination of samples.

Note: In case of highly colored water or turbid water, prepare blank from sample water by adding all reagents but ascorbic acid and potassium antimony1 tartrate.

Method Detection Limit: 0.001 mg/L

VII. Total Dissolved Phosphorus-Persulfate Digestion Method (Standard Methods, 20th Ed. pp. 4/142 - 4/144)

Reagents:

a) Sulfuric Acid Solution: Carefully add 300 mL concentrated H_2SO_4 to approximately 600 mL distilled/deionized water. Cool and dilute to 1000 mL.
b) Sodium Hydroxide, 1N: Add 40 g Certified ACS NaOH to distilled/deionized water and dilute to 1 L.
c) Persulfate: Use certified ACS solid ammonium persulfate $(NH_4)_2S_2O_8$ or certified ACS solid potassium persulfate ($K_2S_2O_8$).
d) Phenolphthalein Indicator Solution: Use prepared indicator solution.

Procedure:

1) Measure 50 mL of sample to a 250 mL Erlenmeyer flask. Add 1drop phenolphthalein indicator and discharge color with H_2SO_4 added one drop at a time.
2) Add 1 mL H_2SO_4 solution and 0.4 g persulfate (using a volumetric ceramic spoon).
3) Boil on hotplate until only 5-10 mL remains. DO NOT BOIL TO DRYNESS.
4) Cool and dilute with 30 mL distilled/deionized water. Add 1drop phenolphthalein indicator and add NaOH solution until you get pink color.
5) Discharge color by adding H_2SO_4 drop by drop. Make up to 50 mL with distilled/deionized water.
6) Take this 50 mL sample and treat as soluble reactive phosphorus sample. (See above test)

Note: Carry a set of standards through the digestion procedure.
Method Detection Limit: 0.001 mg/L

Quality Control Program

To ensure the accuracy of the chemical data, a program of quality control procedures has been implemented.

Definitions

Blank - a quality control measurement that checks for contamination in reagents or in the distilled/deionized water utilized in the chemical test. A sample of distilled/deionized water is analyzed as an unknown sample.

Bottle Blank- a quality control measurement that checks for contamination carried over from sample bottle cleaning. Fill sample bottle with distilled/deionized water and analyze as an unknown sample.

Field Replicate Samples- separate samples collected sequentially in the field at the same time and place. They are analyzed in the lab as separate samples to assess the precision in field sampling

Laboratory Duplicate Samples- separate sub-samples taken from the same sample bottle in the laboratory to assess the variability in the testing procedures

(Quality Control) Standard Sample- a known concentration of standard is made up and diluted to sample volume using distilled water and analyzed as an unknown sample. Serves as quality control on the sample preparation process and verification of calibration curve

(Matrix) Spike- a known amount of standard is added to a sample to determine percent recovery of the specific chemical being tested. This is used to determine if there are any interferences in the chemical analysis process.

Calibration Curve- a graph of absorption measured by the instrument against known standards. The range of standards should bracket the expected range in concentrations of the unknown samples. Most often produces a straight line.

Method Detection Limit (MDL) – A laboratory replication (greater than 7) is made of a single sample and the standard deviation is determined for this replication. The MDL is 3XSD for the replication.

Quality Control at Weeks Bay National Estuarine Research Reserve

For each chemical determination, at least 1 blank and 1 standard sample (or matrix spike) are tested. Site grab samples are all triplicated (field replication) and one tidal cycle sample is lab duplicated. At least 1 Chlorophyll sample is lab duplicated.