## CREEK Project’s Microzooplankton Seasonal Monitoring Database

### Dataset Title:
CREEK Project’s Microzooplankton Seasonal Monitoring Database for Eight Creeks in the North Inlet Estuary, South Carolina: 1997-1999

### Investigator Information:
<table>
<thead>
<tr>
<th>Investigator 1</th>
<th>Investigator 2</th>
<th>Investigator 3</th>
<th>Data Manager</th>
</tr>
</thead>
<tbody>
<tr>
<td>Michael</td>
<td>Dennis</td>
<td>Richard</td>
<td>Franklin</td>
</tr>
<tr>
<td>Wetz</td>
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</tr>
</tbody>
</table>

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### Data Set Credit
Supported by the National Science Foundation’s Research Experience for Undergraduates (REU) Program, grant DEB-9509057, the US ECOHAB Program, sponsored by NOAA/NSF/NEA/ONR, grant NABIOPO943, NOAA grant NABSAA-D-SG672, and EPA grant R826944-01-0.

### Data File Name
CREEK_Projects_Microzooplankton_Seasonal_Monitoring_Database.zip

### Beginning Date
01-Nov-1996

### End Date
01-May-2000

### Number of Data Records
653

### Research Location
North Inlet Estuary

**Geographic Description:**
Eight creeks reside in North Inlet Estuary, four off of Clambank Creek, and four off of Town Creek. The North Inlet Estuary (33.30N, 79.10W) lies east of the uplands of Hobcaw Barony (also known as the Belle W. Baruch Property). The Estuary is located in Georgetown County, South Carolina.

**Sampling Site Location Map:**
Map of the eight creek sites (Link):
[Map Link](http://links.baruch.sc.edu/Data/CREEK/CreekMicroZPK/Metadata/CREEK.MICROZPK.FINAL.note.pdf)

**Location Bounding Box:**
- West Bounding Coordinate: 79.192
- East Bounding Coordinate: 79.167
- North Bounding Coordinate: 33.35
- South Bounding Coordinate: 33.327

**Or if single point location:**
- Latitude: 33.336
- Longitude: 79.19
- Elevation: 0

### Taxonomic Coverage:
**Organisms studied:**
- Eastern oyster (Crassostrea virginica), Microzooplankton

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This condensed metadata is from the original, more extensive metadata created on 2/24/2005 by Ginger Ogburn-Matthews. If needed, the original may be accessed at:
[Original Metadata Link](http://links.baruch.sc.edu/Data/CREEK/CreekMicroZPK/Metadata/CREEK.MICROZPK.FINAL.note.pdf)

Links and email addresses in the original have not been updated as those locations and people may no longer be available.

The data manager identified on this page should be contacted for any questions about the data.

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Tracking of the status of this project is available:

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If you have any questions or need more information, please contact the data manager identified on this page.
Abstract:
A group of eight tidal creeks dominated by oysters, Crassostrea virginica, in North Inlet, South Carolina, USA were studied using a replicated BACI (Before-After Control-Impact) design in which all creeks are sampled simultaneously. Before the pre-manipulation year, oyster biomass in each creek was manipulated so that all eight creeks had an equal oyster biomass to water volume ratio of 8 grams dry body weight of oysters per cubic meter. Detailed geomorphological observations were made on each creek as the study began. Nutrients and chlorophyll a were measured weekly in each creek and exhibited seasonal and inter-annual influences. Phytoplankton pigment levels were measured with high performance liquid chromatography (HPLC). Intensive planktonic - microbial loop and nekton samplings were conducted seasonally. Oyster growth was measured monthly. In the second or manipulation year, the role of oysters was tested by removing them from four creeks. Planktonic abundance and dispersal of the oyster parasite, Perkinsus marinus, nekton abundance and biomass, oyster biomass, growth and survival, changes in water chemistry, and phytoplankton pigment levels were also investigated in the tidal creeks during this manipulation year.

Purpose:
The CREEK Project was initiated in order to investigate the hypothesis that oyster reefs control the structure and function of intertidal creeks. In order to verify the outcome of the project, other variables (i.e. water chemistry, chlorophyll a, suspended sediment, nekton, and phytoplankton) were monitored to provide ecosystem level information and understanding about the role of oyster reefs.

Statistical Design/Statistical Field Design of Creeks
A replicated BACI (Before-After Control-Impact) design with eight similar tidal creeks as replicates was used for this Creek project. Creeks were additionally assigned to one of four blocks based on their physical locations within the estuary and suspected or known spatial differences at this scale. Blocking was deemed important because Clambank Creek creeks drain an upland area whereas Town Creek creeks do not border any uplands, and because there is a salinity gradient from north to south with those creeks further south more likely to experience low salinity spillover from Winyah Bay during "flushes". The "Before Manipulation Year" began in March 1997 and ended in February 1998. The "After Manipulation Year" began in March 1998 following the removal of oysters from 4 randomly selected creeks, two each in Clambank and Town Creeks. Thus, the CREEK study satisfies a number of concerns raised by Harbottle (1984): (1) there are control creeks; (2) the creeks are replicated; and (3) the creeks are sampled repeatedly, both before and after the intervention. In addition, the design heeds the recommendation of Stewart-Oaten et al.'s (1986) by sampling all creeks simultaneously. The statistical analysis after the intervention year is an adaptation of Stewart-Oaten et al.'s (1986) proposed analysis.

Field Collection Procedures and Protocols (Tidal Creek Morphology)
A detailed topographic/bathymetric survey of each creek and its basin was conducted utilizing a Topcon total station. All elevations were referenced to a common datum that are in turn referenced to eight USGS permanent benchmarks. These data generated estimates of creek length, width, cross-sectional area at mouth, surface area, and water volume.

Field Collection Procedures and Protocols (Planktonic and Microbial Loop Sampling)
The planktonic food web in the experimental system was examined using a series of bioassay experiments. Replicate samples were collected at a morning mid-ebb tide at each of the eight experimental creeks and dispensed into 1-liter acid-cleaned polycarbonate bottles.

Laboratory Collection Procedures and Protocols (Planktonic and Microbial Loop)
Samples were incubated under various treatments designed to examine the effect of substrate enrichment or reduced grazing pressure on phytoplankton community biomass (chlorophyll a). The treatments included 4 µM NH4 addition, 20 µM glycine addition, and a 20:1 dilution treatment used to reduce grazing pressure on phytoplankton by decreasing encounter rates between microzooplankton and phytoplankton prey (Larwood and Hassett 1982). Lewisii et al. (1998) have found, from experiments involving serial dilution of North Inlet water, that a 20:1 dilution fell within the range where grazer reduction over 72 hours was saturated. Bottles were incubated in raceways containing flowing estuarine water to simulate tidal creek temperatures. Overhead fluorescent cool white bulbs provided uniform irradiance adjusted to a light/dark cycle simulating natural conditions. Water samples were mechanically stirred (gently) at uniform rates between bottles. Chlorophyll a was measured daily at mid-day over the 72-hour time course.
Field Collection Procedures and Protocols (Nekton Abundance and Biomass)
Nekton seasonal abundance and biomass were determined for each creek. Simultaneous collections of nekton were made with block nets set at early morning slack high tide at all eight creek mouths. Catches were removed from the block nets, and pools within each creek bed were seined at low tide to provide a complete assessment of fish and motile macroinvertebrate use of the creeks. To investigate the role of oyster reefs on nekton, live oysters were removed from creeks 1, 4, 5, and 8. In 1998, all eight creeks were again sampled for nekton, following the same procedures established in 1997. All samples were frozen.

Laboratory Collection Procedures and Protocols (Nekton Abundance and Biomass)
To determine species biomass and abundance, two-person teams collected all nekton species at sampling stations with respect to distance from mouth and depth of reef at collection site.

Field Collection Procedures and Protocols (Oyster Biomass)
Pre-manipulation refers to the period of time that data were collected from the intertidal creeks after adjusting all eight creek oyster biomass to 8 grams dry body weight per cubic meter and before removal of oysters from the four manipulated creeks. Post-manipulation is that period following removal of oysters from the four manipulated creeks. Survey maps were used to determine the contours of the creeks, the volume of water in the creeks, and the location of oyster reefs within the creeks. Before the pre-manipulation year observations began, the area of each creek covered by oyster reef was measured from air surveys. Oysters from 10 quadrats (0.25 m²) distributed at different elevations along the length of each creek were collected and total dry body weight per quadrat averaged for each creek. Oyster biomass for each creek was then estimated by multiplying the average biomass per quadrat by the area of the reef within the creek. Oysters were then redistributed among creeks by hand to yield an oyster biomass of 8 grams dry body weight per cubic meter of water volume in each creek. Oyster biomass estimates were made from converting length measurements to dry body weight using the allometric relationship published in Dame 1972. The grams dry body per cubic meter relationship was used because it more realistically describes the benthic-pelagic coupling of the oysters to the water column (Dame 1993).

Field Collection Procedures and Protocols (Water Chemistry, Suspended solids, Chl a)
Water samples were taken once a week from each study creek for chemical analysis. The samples were collected approximately mid-way between the daytime high and low tide stages. Water samples were collected from the center of each creek mouth at a depth of 1 m below the surface, but not closer than about 0.1 m to the bottom. Triplicate samples were collected from each creek and all samples were collected within 45 minutes. The sample bottles were immediately placed in ice and rushed to the laboratory for analysis. Temperature was measured at each site as samples were collected.

Laboratory Procedures and Protocols (Water Chemistry, Chlorophyll a, Suspended Solids)
Salinity values were determined after the water sample was brought back into the laboratory, by placing the water from the sample onto a handheld refractometer. Seventy-five to 500 ml of the water samples were filtered through a pre-weighed pre-combusted Whatman GFF 0.7 µm (nominal pore size) glass fiber filter usually within one hour of the water sample collection at the Baruch Marine Field Laboratory’s Water Chemistry Lab to separate the particulates from the water. Samples were shaken up first before filtering began; the amount of water filtered was determined by how much sediment and other solids were in the sample. In the winter in the absence of phytoplankton blooms and when sedimentation was low, up to 400 ml were filtered. In the summer and usually after heavy rains less water was filtered: the determining factor was to get a good sample of suspended solids on the filter from the water sample in order to get beyond the minimum detection limits of the total suspended solids analysis.

A 0.7 µm (nominal pore size) glass fiber filter was used throughout the entire study to determine the cutoff between dissolved and particulate constituents in the water sample. The filtered water is then run through a Technicon Analyzer. The following water chemistry analysis used filtered aliquots (< 0.7 µm) of ammonia, nitrate, ortho phosphate, total nitrogen, total phosphorus, and dissolved organic carbon. What remained on the 0.7 µm filters were used for the Suspended solids and Chlorophyll a analysis. For more details on Water Chemistry, Chlorophyll a, and suspended solids laboratory procedures and citations, please refer to the metadata for this subproject.

Field Collection Procedures and Protocols (Phytoplankton Pigment Field Collection Protocol)
The samples were taken in triplicate (A, B, and C) approximately mid-way between the daytime high and low tide stages, from the center of each creek mouth, and at a depth of 1 m below the surface, but not closer than about 0.1 m to the bottom. Water temperatures were measured as the samples were taken (see Creek Water Chemistry data) and all samples were collected within 45 minutes. Sample bottles were immediately placed on ice and rushed to the laboratory for processing. Samples were collected on a weekly basis, however, not all samples were processed for pigments. In 1997 and 1998 duplicate samples (A and B) were processed. In 1999 only one sample (B) was used. Water samples were processed for pigments more frequently in summer months and least frequently in winter months. Processing frequency varied from every couple of months to every couple of weeks.

Laboratory Procedures and Protocols (Phytoplankton Pigment Sample Processing and HPLC Analysis)
The water samples were filtered using a 25-millimeter glass fiber grade F GF/F filter, with a vacuum of 10 inches mercury or less, within a couple hours of collection. The filter and the algae it contained were stored in a -80 degree Celsius freezer until the samples were needed. From this freezer, each sample (filter with algae) was placed into a glass vial. The HPLC technician added 2 milliliters of HPLC grade acetonitrile and agitated the mixture with a vortex mixer for about 20 seconds, or until the filter broke down. The filter and algae “sorbed” were then placed in a -20 degree Celsius Freezer overnight. The next morning, the sample was vortexed again and then syringe filtered through a 0.2 micron pore size PTFE membrane. The filtrate was stored at -20 degrees Celsius until it was analyzed, usually within a day or two. Analysis was conducted using a Beckman System Gold HPLC, Initially with the Beckman Gold software and later with the 32 Karat Gold software, following the method of Van Heukelem and Thomas (2001) specific for the Agilent IDQ C8 column.

Field Collection Procedures and Protocols (Oyster Disease Monitoring)
In August 1997, 10 oysters were collected from each of three to five discrete reefs located along the main stem of each creek. Oysters were collected during low tide and the distance from the creek mouth, creek depth, temperature and salinity were recorded. Samples were returned to the laboratory and refrigerated until processed for Perkinsus marinus infection intensity. Following the experimental removal of virtually all oysters from the experimental creeks, oysters that recruited into both experimental (removal) and control creeks were subsequently sampled using the same 1997 methodology in fall of 1999. Percent prevalence and weighted prevalences were calculated and compared among experimental and removal creeks and with respect to distance from mouth and depth of reef at collection site.

Laboratory Procedures and Protocols (Oyster Disease Monitoring)
Samples were returned to the laboratory and refrigerated until processed for Perkinsus marinus infection intensity. P. marinus Infection intensity was determined using Ray’s fluid thioglycollate medium (RTG-M) assay (Ray, SM 1966).

Field Collection Procedures and Protocols (Internal and External Creek Habitat Survey)
The internal habitats of all eight intertidal creeks (4 in Clambank Creek, 4 in Town Creek) were surveyed manually (on foot) using tape measures during low tide. One tape was positioned along the central axis of the creek and a shorter tape was used to determine width of the creek bottom (from the lowest points of adjacent creek banks) perpendicular to the axis. The widths were determined every one meter along the lengths of the main creek and each tributary.
<table>
<thead>
<tr>
<th>Variable Name</th>
<th>Variable Description</th>
<th>Units</th>
<th>Measurement Scale</th>
<th>Code Information</th>
<th>Number Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creek</td>
<td>numbering identification of each tidal creek within North Inlet Estuary where samples were collected; creeks 1-4 were creeklets running into Clam Bank Creek; creeks 5-8 were creeklets running into Town Creek. See map for creek numbering and location within North Inlet Estuary.</td>
<td>1,2,3,4,5,6,7,8</td>
<td>nominal</td>
<td>1-4(Creeklets running into Clam Bank Creek)</td>
<td>integer</td>
</tr>
<tr>
<td>Date</td>
<td>month/day/year (mm/dd/yyyy) that the sample was collected (not necessarily processed or analyzed)</td>
<td>1,2,3,4,5,6,7,8</td>
<td>datetime</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bag</td>
<td>the letter identification of each of the four bags deployed in the creeks. Bag A was closest to the creek mouth; B was the next deployed bag, and D was the bag deployed furthest up into the creek.</td>
<td>A,B,C,D</td>
<td>nominal</td>
<td></td>
<td>integer</td>
</tr>
<tr>
<td>Oyster#</td>
<td>the numbering identification of each of the 25 oysters in each bag</td>
<td>1,2,3,4,5,6,7,8</td>
<td>nominal</td>
<td></td>
<td>integer</td>
</tr>
<tr>
<td>Initial</td>
<td>the shell height measurement at the deployment date.</td>
<td>millimeters</td>
<td>ratio</td>
<td></td>
<td>real</td>
</tr>
<tr>
<td>Final</td>
<td>the shell height measurement after each deployment.</td>
<td>millimeters</td>
<td>ratio</td>
<td></td>
<td>real</td>
</tr>
<tr>
<td>Growth</td>
<td>Final Oyster Shell Height – Initial Oyster Shell Height. (This calculation was done for all of the oysters on each date.)</td>
<td>millimeters</td>
<td>ratio</td>
<td></td>
<td>real</td>
</tr>
<tr>
<td>Days</td>
<td>the number of days between the deployment date and the recovery date.</td>
<td>day</td>
<td>datetime</td>
<td></td>
<td>integer</td>
</tr>
<tr>
<td>Daily Growth</td>
<td>Growth divided by the number of elapsed days, i.e. 8/18/97 – 10/21/97 = 64 days.</td>
<td>millimeters</td>
<td>ratio</td>
<td></td>
<td>real</td>
</tr>
<tr>
<td>Pigment Concentrations</td>
<td>Pigment concentrations (reported in nanograms per milliliter) were calculated by the HPLC technician using the following formula: (Peak Area * Retention Time / Volume). See the Process Section for more information on this equation. The HPLC technician felt that the resulting concentration values were accurate to the second decimal place and that the third place was a valid estimate.</td>
<td>nanograms per milliliter</td>
<td>ratio</td>
<td></td>
<td>real</td>
</tr>
<tr>
<td>Creek/Rep</td>
<td>The creek number (1-8) and replicate designation (A-B) are integers and have no decimal places assigned to them.</td>
<td></td>
<td>nominal</td>
<td></td>
<td>integer</td>
</tr>
<tr>
<td>Total Nitrogen Filtered (TNF)</td>
<td>The Technicians used for these analyses report values to three decimal places, but due to variations and slight contamination of the oxidizing reagent, handling, and processing of the water samples, the accuracy of the final values is only to ± 1 micromoles per liter. However, the measurements (reported in micromoles per liter) are reported with one decimal place. Negative calculated values for total nitrogen and phosphorus can occur and negative values are the result of mathematical manipulation of the raw values.</td>
<td>micromoles per liter</td>
<td>ratio</td>
<td>± 1 micromoles per liter</td>
<td>real</td>
</tr>
<tr>
<td>Parameter</td>
<td>Description</td>
<td>Accuracy</td>
<td>Unit</td>
<td></td>
<td></td>
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<tr>
<td>-----------------------------------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>-----------</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Total Phosphorus Filtered (TPF)</td>
<td>The Technicon Autoanalyzer used for these analyses reports values to three decimal places, but due to variations and slight contamination of the oxidizing reagent, handling, and processing of the water samples, the accuracy of the final values is only to ± 1 micromoles per liter. However, the measurements (reported in micromoles per liter) are reported with one decimal place. Negative calculated values for total nitrogen and phosphorus can occur and negative values are the result of mathematical manipulation of the raw values.</td>
<td>± 1 micromoles per liter</td>
<td>real</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ortho phosphate (PO4)</td>
<td>The handling and processing of these nutrients is more accurate, and there is less room for error. The measurements (reported in micromoles per liter) are given with two decimal places.</td>
<td>± 0.08 micromoles per liter</td>
<td>ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ammonia (NH4)</td>
<td>The handling and processing of these nutrients is more accurate, and there is less room for error. The measurements (reported in micromoles per liter) are given with two decimal places.</td>
<td>± 0.2 micromoles per liter</td>
<td>real</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrate (NO3)</td>
<td>The handling and processing of these nutrients is more accurate, and there is less room for error. The measurements (reported in micromoles per liter) are given with two decimal places.</td>
<td>± 0.1 micromoles per liter</td>
<td>real</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dissolved Organic Carbon (DOC)</td>
<td>A Shimadzu Carbon analyzer was used that reads to the nearest one hundredth, but because the final value is an average of the three values, the nearest tenth of a milligram per liter is used (one decimal place).</td>
<td>± 0.1 milligrams per liter</td>
<td>real</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Suspended Solids (SusSol)</td>
<td>The balance reads to the fourth decimal place of a gram (ten thousands of a gram), but humidity in the air can influence the filters, so the 3rd decimal place is read and assumed accurate (±0.001 g = ± 1 milligram). Measurements are reported in grams/liter with three decimal places in the database.</td>
<td>± 0.001 grams per liter (± 1 milligram per liter)</td>
<td>ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organic Suspended Solids (OSS)</td>
<td>The balance reads to the fourth decimal place of a gram (ten thousands of a gram), but humidity in the air can influence the filters, so the 3rd decimal place is read and assumed accurate (±0.001 g = ± 1 milligram). Measurements are reported in grams/liter with three decimal places in the database.</td>
<td>± 0.001 grams per liter (± 1 milligram per liter)</td>
<td>ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inorganic Suspended Solids (ISS)</td>
<td>The balance reads to the fourth decimal place of a gram (ten thousands of a gram), but humidity in the air can influence the filters, so the 3rd decimal place is read and assumed accurate (±0.001 g = ± 1 milligram). Measurements are reported in grams/liter with three decimal places in the database.</td>
<td>± 0.001 grams per liter (± 1 milligram per liter)</td>
<td>ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorophyll a (Chla)</td>
<td>Strickland and Parsons (1972) say that detection limits depend upon the volume filtered and the sensitivity of the fluorometer. Using a Turner fluorometer, the accuracy limit documentation states a limit of 0.01 μg/L, when 2 L was filtered. Because we filter 10-20 ml, the accuracy would be much less. We are estimating that our chlorophyll accuracy is 0.1 μg/L at best. No tests have been done to verify this. Measurements are reported in micrograms/liter with two decimal places in the database.</td>
<td>± 0.1 micrograms per liter (μg/l)</td>
<td>ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salinity</td>
<td>Refractometer has lines that represent every two part per thousand and can be read to the nearest part per thousand but the instrument is only accurate ± 2 parts per thousand. The measurements are reported in parts per thousand with no decimal places in the database.</td>
<td>± 2 parts per thousand</td>
<td>real</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water Temperature</td>
<td>Thermometers have lines for each degree C. Could only read to the nearest 1°C with no decimal places in the database.</td>
<td>± 1 degrees Celsius</td>
<td>ratio</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>