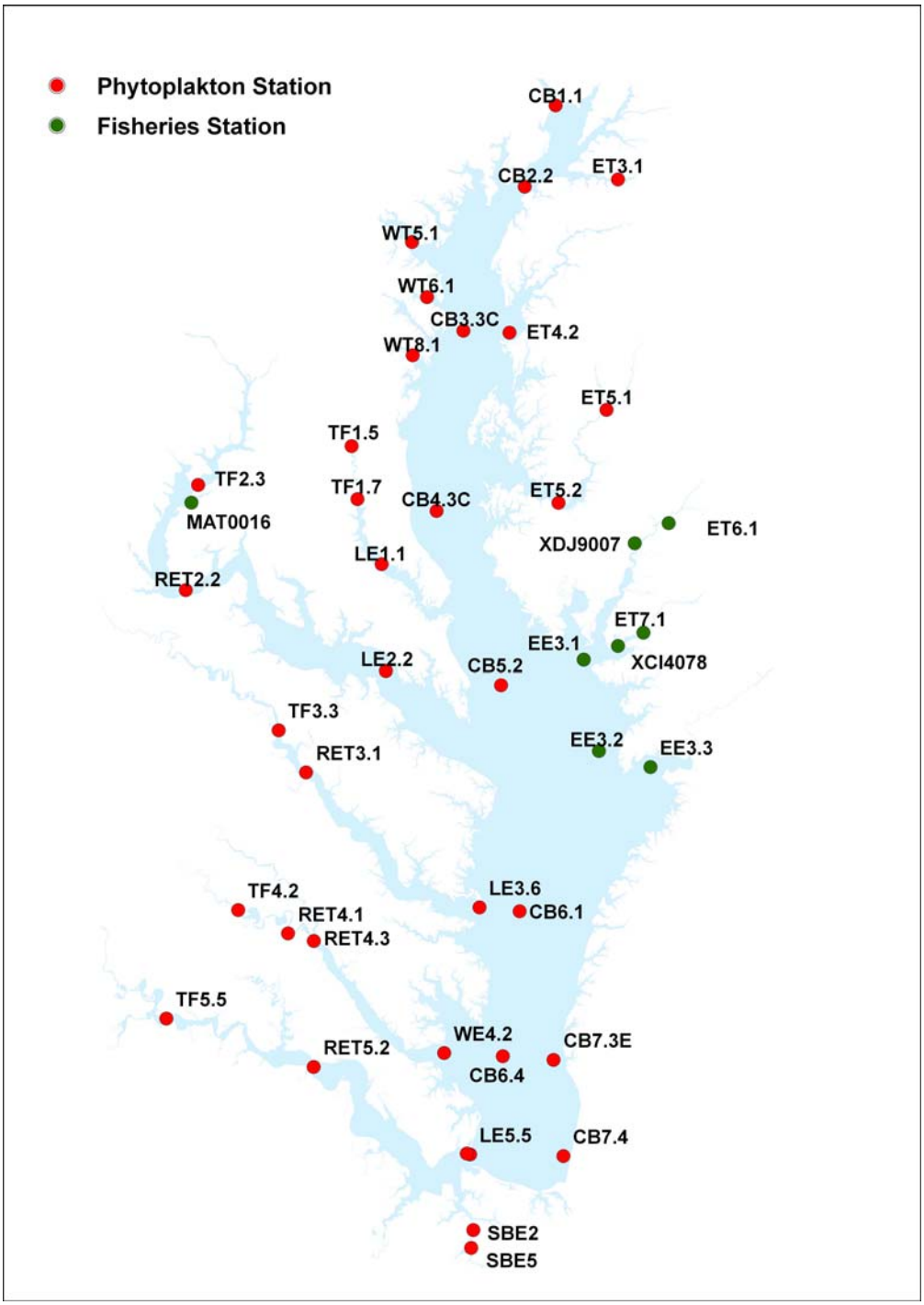
Phytoplankton Methodology

SIZE RANGE	3 - 200 µm in size
NUMBER OF STATIONS	Maryland-16 (5 Mainstem, 9 Tributary, 4 Tributary Seasonal, 8 Special Fish Study); Virginia- 14 (4 Mainstem,10 Tributary)
SAMPLING FREQUENCY	Twice Monthly-March To September, Monthly-October To February
SAMPLE COLLECTION TYPE	5 Depth Composite Sample; Above And Below Pycnocline
SAMPLING GEAR	Carboy, Hose And Water Pump
FIELD COLLECTION PROCEDURES	At each station, composite phytoplankton samples are taken from above and below the pycnocline. After the pycnocline has been determined at each station, two vertical series of five samples equidistance apart depths ate taken between the pycnocline and bottom. Water in each carboy is mixed, then a 500 milliliter sample is taken from each carboy, and is fixed with five milliliters of Lugol's solution. The pre-labeled sample bottles are transported back to the laboratory for analysis. Upon return to the lab, samples are preserved with buffered formalin (final concentration of 2% by volume)
HOLDING TIME	Cool (1-5 C), dark place for up to 3 weeks
SAMPLE ENUMERATION PREPARATION	The sample bottle is gently inverted 10-12 times and an appropriate aliquot is removed with a macropipette. This volume is generally between 1-50 mls, depending upon the density of cells and amount of sediment and detritus in the sample. The aliquot is transferred to a two-piece settling chamber which, if necessary, is topped off with distilled water. The settling chamber is capped and allowed to settle for an appropriate time (1-48 hrs). Upon settling, the upper portion of the chamber is removed and replaced with a glass plate. The sample is then transferred to a microscope for enumeration.
COUNTING CHAMBER	Utermohl Settling Chamber
MICROSCOPE TYPE	Inverted Phase contrast or Normanski interference-contrast preferred
ENUMERATION PROCEDURE	At 600X magnification, twenty random fields will be counted for taxa >=3 and <=5 microns in diameter. No colonies, trichomes or filaments counted. All cells indentified using the following categories: CENTRIC DIATOM < 10UM, CRYPTOMONAS <10UM, PENNATE DIATOM < 10UM, UNIDENTIFIED GREEN CELLS 3-5UM UNIDENTIFIED MICRO-PHYTOFLAGELLATES <10UM At 300X magnification, a minimum of twenty random fields and 200 algal units > 5 microns in largest dimension will be counted. If 200 units are not tallied in 10 fields, cells in additional fields will be enumerated until 200 units have been enumerated. All colonies, trichomes, & filaments are counted at this magnification. Each colonies, trichome or filament is considered to be a single algal unit. Very large (>60 Microns) or rare species (less than 1 cell in less than 10 Grids) not counted in this scan. At 125X magnification, the entire chamber will be scanned for taxa which were not enumerated at the other two magnifications.
ARCHIVE SAMPLES	The remainder of the sample is permitted to settle for at least 72 hours before concentration to a volume of 20-25 milliliters for archiving. Archive samples are required to be held for 5 years.
PRESERVATIVES	Acid Lugol's and 37% Buffered Formalin
INDICATOR PRODUCTS	Phytoplankton Index of Biotic Integrity

Phytoplankton Methodology

QA COUNTS	Quality assurance counts are enumerated for 5% of the total number of samples. A complete new sample aliquot is settled and completely enumerated. The QA sample must have a total density and dominant taxa densities within 80% of the original count (80% is dictated by the precision calculated for the counting protocol).



Historical Phytoplankton and Picoplankton Monitoring Stations. Note fisheries stations were phytoplankton only stations, which were part of a special project by MDDNR in 2010

Picoplankton Methodology

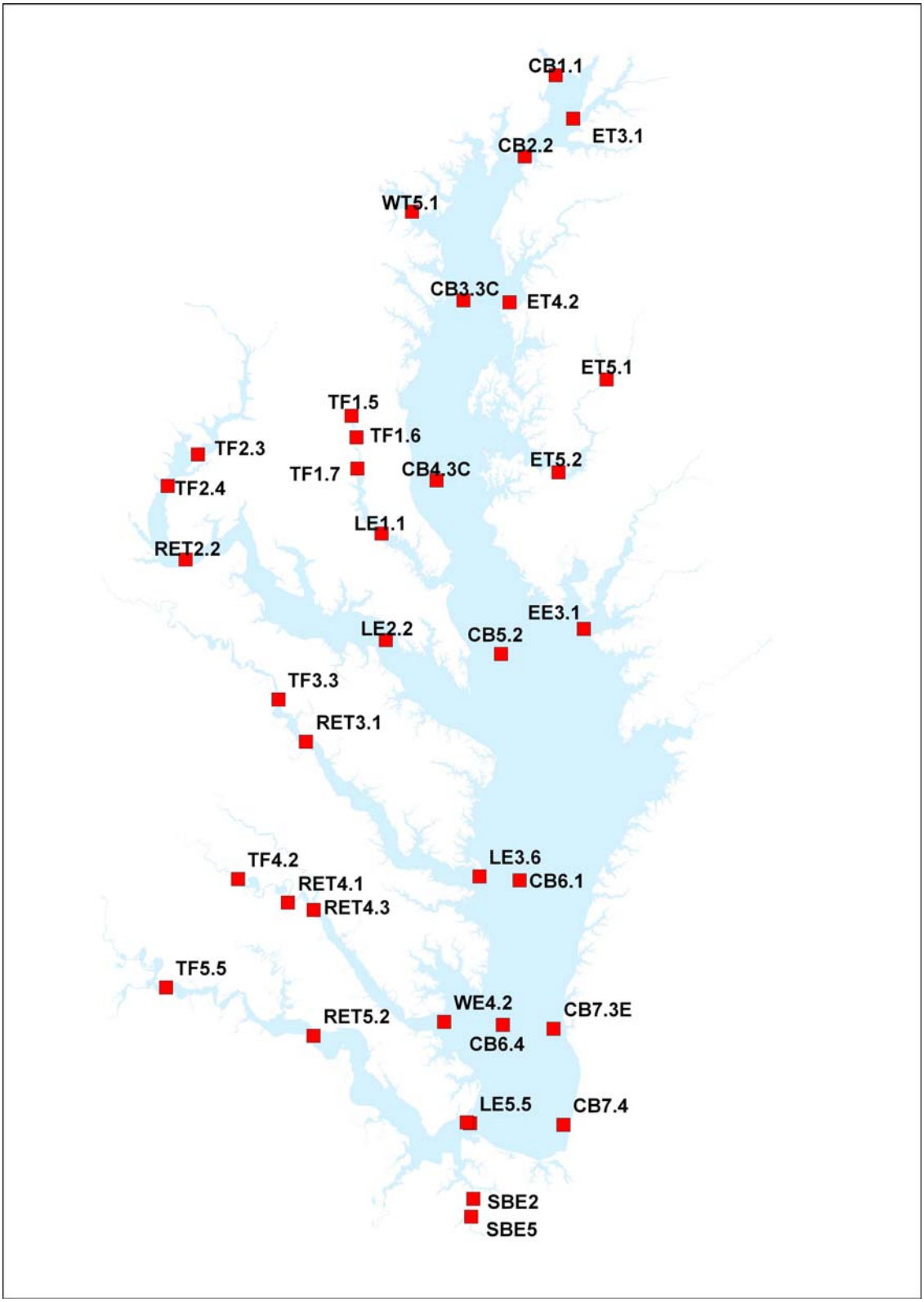
SIZE RANGE	0.2-2 µm in size
NUMBER OF STATIONS	Maryland-16 (5 Mainstem, 9 Tributary, 4 Tributary Seasonal, 8 Special Fish Study); Virginia- 14 (4 Mainstem,10 Tributary)
SAMPLING FREQUENCY	Twice Monthly-March To September, Monthly-October To February
SAMPLE COLLECTION TYPE	5 Depth Composite Sample; Above And Below Pycnocline
SAMPLING GEAR	Carboy, Hose And Water Pump
FIELD COLLECTION PROCEDURES	At each station, composite picoplankton samples are taken from above and below the pycnocline. After the pycnocline has been determined at each station, two vertical series of five samples equidistance apart depths ate taken between the pycnocline and bottom. Water in each carboy is mixed, A 125-ml subsample will be removed and placed in a Nalgene bottle containing 2-ml of gluteraldehyde. The labeled sample bottles are placed in a cooler with ice and transported back to the lab for processing.
HOLDING TIME	The field samples are returned to the lab and held in a refrigerator (4^0C) until processing begins (no longer than 7 days)
SAMPLE ENUMERATION PREPARATION	Using a Millipore apparatus, a backing 0.45 um nuclepore filter wetted with distilled water, was placed on the Millipore stem. Then a 0.20 um nuclepore filer, previously stained in an irgalan black solution was placed over the other filter. Two milliliters of the shaken water sample was added to the filter apparatus. Using a pump, and a maximum vacuum of ten centimeter for mercury, the sample are filtered until the meniscus disappears from the top of the filter. The 0.2 um nucleopore filter was removed and placed immediately on a glass slide previously moistened by breath. A drop of immersion oil was placed at the center of the filter, and then covered with a cover slip.
COUNTING CHAMBER	Glass slide
MICROSCOPE TYPE	epifluorescenct microscope equipped with a 100 watt mercury lamp
ENUMERATION PROCEDURE	Each sample was enumerated at a magnification of 1000X with an epifluorescenct microscope equipped with a 100-watt mercury lamp. The autotrophic picoplankton were counted using two filter cubes were used in order to enumerate the picoplankton - one in the excitation range of 420-490 nm and the other in the excitation range of 515-560 nm. A minimum of 200 cells and a minimum coverage of 20 fields were counted on each slide.
ARCHIVE SAMPLES	None
PRESERVATIVES	Gluteraldehyde
INDICATOR PRODUCTS	Phytoplankton Index of Biotic Integrity
QA COUNTS	Quality assurance counts are enumerated for 5% of the total number of samples. A complete new sample aliquot is settled and completely enumerated. The QA sample must have a total density within 80% of the original count (80% is dictated by the precision calculated for the counting protocol).

See Phytoplankton Methodology for Station Locations.

Mesozooplankton Methodology

SIZE RANGE	0.2 mm-2 mm
NUMBER OF STATIONS	Maryland-16 (5 Mainstem, 9 Tributary, 4 Tributary Seasonal); Virginia- 14 (4 Mainstem,12 Tributary)
SAMPLING FREQUENCY	Twice Monthly-March To September, Monthly-October To February
SAMPLE COLLECTION TYPE	Composite Whole Water Column Tow
SAMPLING GEAR	20-cm Bongo Net (202 µm Mesh Net) Equipped With Flow Meters
FIELD COLLECTION PROCEDURES	Samples are obtained by towing a 20-cm bongo net (202 µm mesh net) equipped with flow meters in a stepped oblique fashion. Two complete tows are made and combined for zooplankton. The entire water column was sampled by first deploying the gear just above the bottom and raising the net in timed progressive steps, usually 0.5 to 1.5 minutes/step. Flow meter readings were taken before and after each tow to determine the volume of water filtered. Replicate samples collections are composited into one sample for laboratory analysis. The count sample is preserved a 10% formalin solution.
HOLDING TIME	Samples are usually processed within 1 or 2 months after collection.
SAMPLE ENUMERATION PREPARATION	Sample are prepared for enumeration by pouring each sample into a 63 um sieve under the hood and rinse off all formalin. Each sample is then sieved through a 500 um sieve which is suspended over a 63 um sieve, to remove grasses, large fish and other detritus are present in the sample. Samples having exceptionally large volumes of zooplankton are first split with the Folsom Plankton Splitter. Following washing, plankton are transferred to a calibrated beaker and diluted to a fixed volume. The ideal dilution volume/subsample volume combination is approximately one hundred organisms in a one or two ml subsample. Next, the samples are mixed thoroughly by constant aeration.
COUNTING CHAMBER	Circular chamber for dissecting Microscope
MICROSCOPE TYPE	Dissecting Microscope
ENUMERATION PROCEDURE	a hierarchical counting technique is employed to obtain density estimates. A 1-ml subsample is immediately removed with a Henson-Stempel Pipette (H-S pipette) and a first counting at least 60 individuals of the most dominant forms (e.g. Acartia tonsa) in a small sub sample (usually 1 - 2 milliliters). This is followed by 5- and 10- milliliter sub samples from which all species that had counts less than 60 in the previous sub sample are counted. Macro zooplankton (amphipods, shrimp, etc.) are identified when observed in sub samples. In addition, all samples, after the standard hierarchical counting technique, were filtered through an 850-micrometer sieve. Mesozooplankton that were retained in the 850-micrometer sieve that were not previously identified in the sub samples and/or macro zooplankton were counted and identified. .
ARCHIVE SAMPLES	After processing, samples are archived for at least five years after the final report.
PRESERVATIVES	10% Formalin
INDICATOR PRODUCTS	Publish Polyhaline Zooplankton IBI, Larval Food Availability Index
QA COUNTS	Standard quality control for laboratory processing of zooplankton samples consists of recounting at least 10% of the samples. Replicate samples must have less than a error is 10%.

Mesozooplankton Methodology



Historical Mesozooplankton Monitoring Stations. Note: No monitoring has occurred since September 2002.

Microzooplankton Methodology

SIZE RANGE	20-200 in size
NUMBER OF STATIONS	Maryland-16 (5 Mainstem, 9 Tributary, 4 Tributary Seasonal); Virginia- 14 (4 Mainstem,12 Tributary)
SAMPLING FREQUENCY	Twice Monthly-March To September, Monthly-October To February
SAMPLE COLLECTION TYPE	5 Depth Composite Sample; Above And Below Pycnocline
SAMPLING GEAR	Carboy, Hose, Water Pump and 44 µm Mesh Net
FIELD COLLECTION PROCEDURES	Two composite samples (30-l) will be collected using a small diaphragm pump and hose connected to a sampling tube (missile) that will be lowered to ten depths spread evenly through the water column (depths will include 0.5 m below the surface, at the pycnocline and 1 meter above the bottom). The whole water microzooplankton samples will be decanted from the carboy and then placed into a 500-ml sample bottle. The samples will be preserved with acid Lugol's solution to a final concentration of 2 %. Net microzooplankton will be collected by pumping water from each of 10 depths spread evenly over the entire water column into a 44 ?m-mesh net held on deck. The samples collected in the 44 um-mesh net will be rinsed down and decanted into a jar containing buffered formaldehyde (final concentration of 2%) and transferred to the laboratory.
HOLDING TIME	Samples are usually processed within 1 or 2 months after collection.
SAMPLE ENUMERATION PREPARATION	For the enumeration of whole water microzooplankton, a 5-25 ml subsample will be removed from the sample jar for settling. This amount depends on how much detritus and plankton are in the sample. The appropriate aliquot will be pipetted into a Sedgewick-Rafter cell settling chamber and allowed to settle. In the enumeration of net microzooplankton, each sample will be gently mixed and a 1-ml aliquot removed with a Stempel pipet and put into a Sedgwick Rafter cell with a drop of Rose Bengal for enumeration with a compound microscope at 100X magnification. The sample will be allowed to set for 10 minutes before counting.
COUNTING CHAMBER	Sedgewick-Rafter Cell
MICROSCOPE TYPE	Compound Microscope.
ENUMERATION PROCEDURE	For Whole water sample enumeration, The entire Sedgewick-Rafter cell chamber will be examined at 250X with an inverted microscope to obtain a minimum count of 100 organisms. If 100 organisms are not counted, another subsample will be settled. Any organism that was abundant in the first aliquot (more than 60) will not be counted in the second aliquot. In the enumeration of net microzooplankton, each sample will be gently mixed and a 1-ml aliquot removed with a Stempel pipet and put into a Sedgwick-Rafter cell with a drop of Rose Bengal for enumeration with a compound microscope at 100X magnification. The sample will be allowed to set for 10 minutes before counting. At least one chamber (1 ml) will be counted for each sample and if the total count does not reach 250 organisms, subsequent 1 ml aliquots will be enumerated until a count of 250 or more organisms is obtained or a maximum of 3 ml examined. If a certain organism is abundant (more than 60 per chamber), it will not be counted in the subsequent 1 ml aliquots for a given sample.
ARCHIVE SAMPLES	After processing, samples are archived for at least five years after the final report.

Microzooplankton Methodology

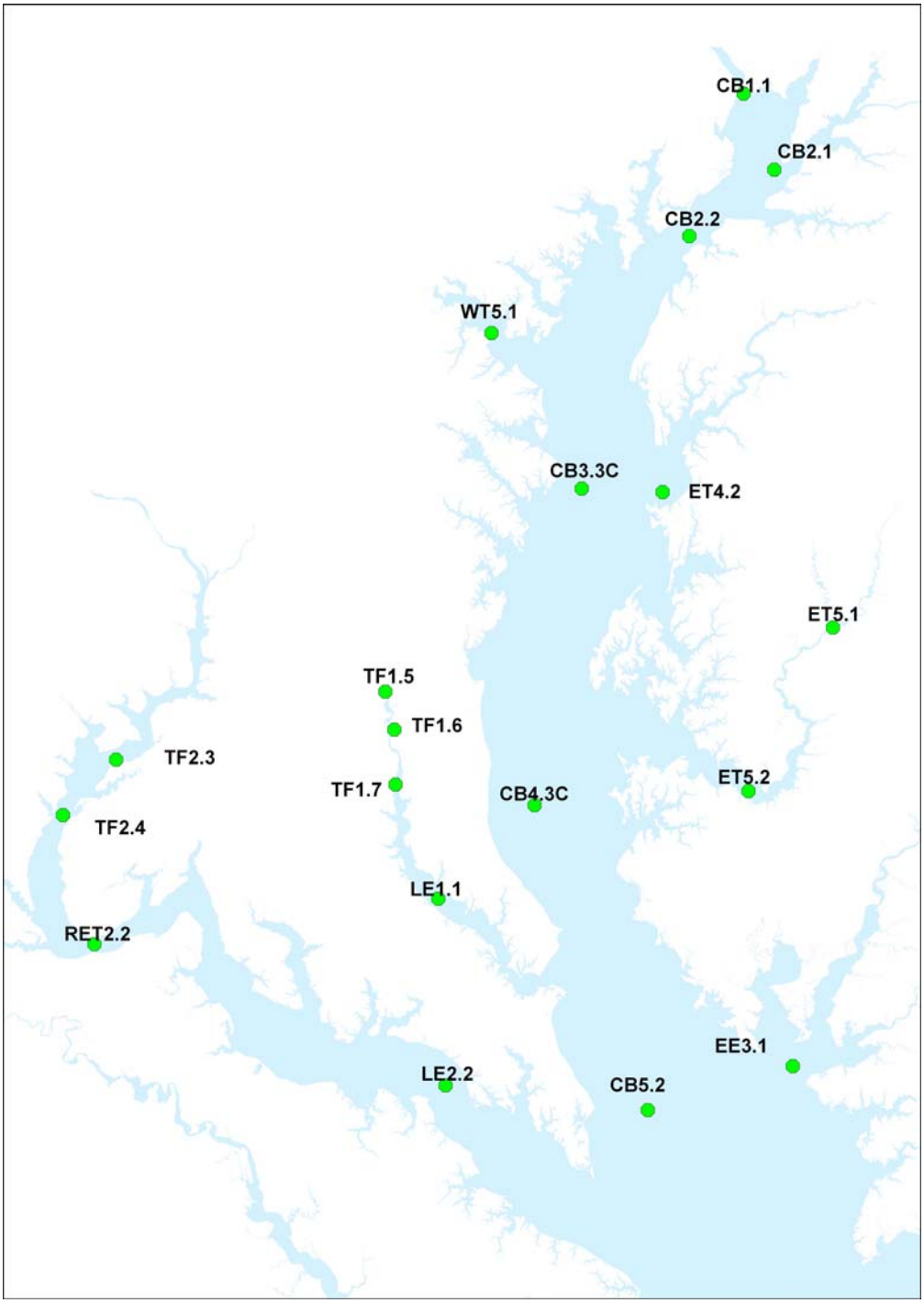
PRESERVATIVES	Whole water-acid Lugol's solution to a final concentration of 2 %; Net Sample-buffered formaldehyde (final concentration of 2%)
INDICATOR PRODUCTS	None Currently
QA COUNTS	Random sample recounts of previously counted microzooplankton samples are undertaken in order to determine counting error. One sample/20 samples is blindly selected and recounted. The recount total cell density must fall within 10 % of the total for the original count or the sample is counted again until 2 samples' total densities are within 10 % of one another.

See Mesozooplankton Methodology for Station Locations.

Gelatinous Zooplankton Methodology

SIZE RANGE	20+ mm
NUMBER OF STATIONS	13 Bay wide
SAMPLING FREQUENCY	Twice-Monthly July-September
SAMPLE COLLECTION TYPE	Composite Whole Water Column Tow
SAMPLING GEAR	50-cm Bongo Net (505 µm Mesh Net) Equipped With Flow Meters
FIELD COLLECTION PROCEDURES	Samples are obtained by towing a 50-cm bongo net (505 µm mesh net) equipped with flow meters in a stepped oblique fashion. Two complete tows are made and combined for zooplankton. The entire water column was sampled by first deploying the gear just above the bottom and raising the net in timed progressive steps, usually 0.5 to 1.5 minutes/step. Flow meter readings were taken before and after each tow to determine the volume of water filtered.
HOLDING TIME	Not Applicable, samples enumerated in field.
SAMPLE ENUMERATION PREPARATION	Beroe, Hydrozoans, Mnemiopsis, and true Jellyfish are removed from samples and sorted in the field after and their numbers and settled volumes were recorded from the nets.
COUNTING CHAMBER	Not Applicable-All samples will be processed live in field.
MICROSCOPE TYPE	Plastic beakers and graduated cylinders
ENUMERATION PROCEDURE	All individuals > 1 cm for medusae and length for ctenophores will be counted in samples in which a species is represented by < 100 individuals. If > 100 individuals of a species are present, total number of individuals > 1 cm will be estimated from the sample volume and number of individuals in a subsample containing at least 50 individuals. Digital photographs will be used to verify identification when necessary. All gelatinous zooplankton were reported as count and volumes in the four classes -Beroe, Hydrozoans, Mnemiopsis, and true Jellyfish. All gelatinous zooplankton were removed from samples and sorted in the field after sample preservation, their numbers and settled volumes were recorded from the net that was used as the count sample.
ARCHIVE SAMPLES	Not Applicable
PRESERVATIVES	Not Applicable
INDICATOR PRODUCTS	None Currently
QA COUNTS	None clearly established

Gelatinous Zooplankton Methodology



Historical Gelatinous Zooplankton Monitoring Stations. Note: No monitoring has occurred since September 2002. Data from Virginia was irregular and did not meet CBP data and QA standard.