

An Ecological Study of Hunting Creek

2013

FINAL REPORT

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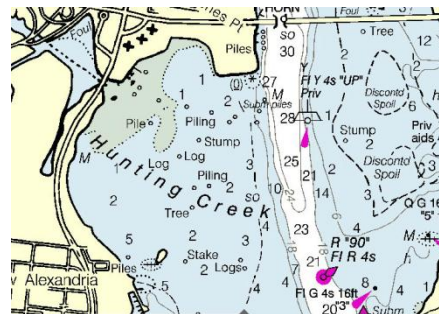


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Executive Summary

Hunting Creek is an embayment of the tidal Potomac River located just downstream of the City of Alexandria and the I-95/I-495 Woodrow Wilson bridge. This embayment receives treated wastewater from the Alexandria Renew Enterprises wastewater treatment plant and inflow from Cameron Run which drains most of the Cities of Alexandria and Falls Church and much of eastern Fairfax County. Hunting Creek is bordered on the north by the City of Alexandria and on the west and south by the George Washington Memorial Parkway. Due to its tidal nature and shallowness, the embayment does not seasonally stratify vertically, and its water is flushed by rainstorms and mixes readily with the adjacent tidal Potomac River mainstem. Beginning in 2013 the Potomac Environmental Research and Education in collaboration with Alexandria Renew Enterprises initiated a program to monitor water quality and biological communities in the Hunting Creek area including stations in the embayment itself and the adjacent river mainstem. This document presents study findings from 2013 in the context of a longer record from other tidal Potomac sites.

The Chesapeake Bay, of which the tidal Potomac River is a major subestuary, is the largest and most productive coastal system in the United States. The use of the Bay as a fisheries and recreational resource has been threatened by overenrichment with nutrients which can cause nuisance algal blooms, hypoxia in stratified areas, and declining fisheries. As a major discharger of treated wastewater into Hunting Creek, AlexRenew has been proactive in decreasing nutrient loading since the late 1970's.

The ecological study reported here provides documentation of the current state of water quality and biological resources in Hunting Creek. The year 2013 was characterized by above normal temperatures from April through July with highest monthly average of 27.3°C in July. Monthly precipitation was somewhat below normal for spring, but well above normal in June and July with 36.6 cm in those two months compared with an average of 17.3 cm. Rainfall was again above normal in both August and September. Mean monthly discharge of the mainstem Potomac at Little Falls was near normal during most of the study period, but dropped to below normal levels in September. Local tributary inflow into Hunting Creek from Cameron Run was generally somewhat below normal except in June when it was very high, three times normal. August and September were below normal. High flows again occurred October.

Water temperature tracked air temperature on a seasonal basis with little difference among the three sites. Water quality mapping revealed that the shallow areas responded more quickly to short-term temperature fluctuations than the deeper waters. Specific conductance was generally in the 200-500 $\mu\text{S}/\text{cm}$ range at all sites, typical of freshwater. ARE 1, nearest the AlexRenew discharge, was consistently highest. Specific conductance and chloride did not show a consistent seasonal pattern except at Station 1 with highs in spring and fall. Water quality mapping showed highest values near wastewater discharge plumes either from AlexRenew or Blue Plains. Dissolved oxygen did not show a consistent seasonal pattern or variation among sites, most always in the 80-120 % saturation range. Water quality mapping showed highest DO levels in the shoreline part

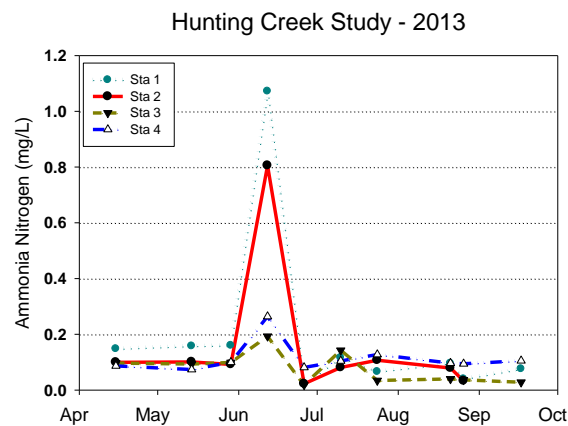
of Hunting Creek in June and the river channel area in August. pH was generally in the 7-8 range at all sites except in the early June aftermath of a major local rainfall even when values dropped lower. Water quality mapping suggested highest values generally near the river channel. Total alkalinity was generally 60-90 mg/L as CaCO_3 with somewhat lower values at ARE1 and in the aftermath of the June flow event.

Light penetration was generally slightly higher in the river than in Hunting Creek as indicated by Secchi disk depth and light attenuation coefficient. Secchi depth was higher in the spring and reached a minimum in the wake of the June high flows. Light attenuation took a very strong drop in June, but otherwise was fairly constant. Another measure of light penetration is turbidity which is negatively related to light penetration. Turbidity was highest in the wake of the June high flow event. Turbidity mapping indicated greatest values in Hunting Creek.

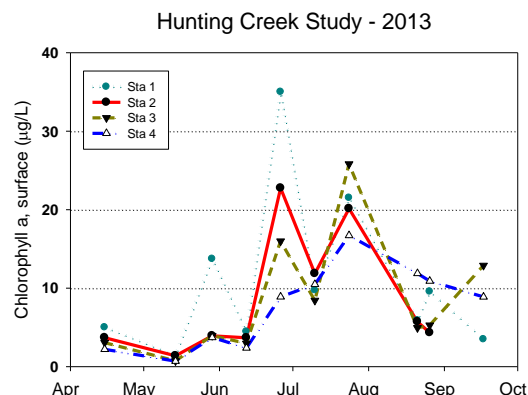
Ammonia nitrogen was very low (<0.2 mg/L) on most dates in at all stations except at ARE1 and ARE2 in the wake of the June storms. Nitrate was found at moderate levels at most sites in the spring and decreased steadily in the river and at Station 3. Values were elevated in June and July at ARE1 and ARE2. Nitrite nitrogen was much lower being less than 0.02 mg/L in almost all of the samples. Organic nitrogen at ARE3 and ARE4 increased steadily from May through

August, but was generally higher and more variable at Stations 1 and 2. Total phosphorus was generally in the range 0.05 – 0.10 mg/L, but was generally quite variable. SRP values were generally somewhat lower being mostly below 0.05 mg/L except in May and early June at Stations 1 and 2. N to P ratio (by weight) was quite variable, but was always within the range of P limitation. BOD showed much fluctuation between dates, but was consistently higher at ARE1 and ARE2. Total suspended solids and volatile suspended solids values peaked in late May and June.

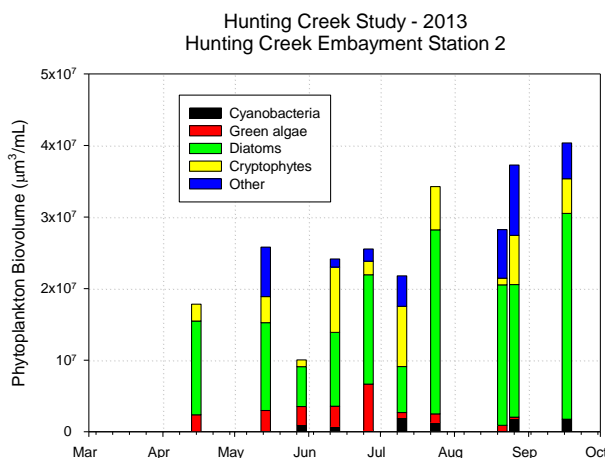
Since only one year of data was available for Hunting Creek, comparisons were made with the Gunston Cove database to gain a contextual understanding of our findings. We compared the median of HC values in 2013 with the interquartile range of values for Gunston Cove from 2005-2012. Hunting Creek results seemed to fall right in the same range as those for Gunston Cove for many parameters like temperature, dissolved oxygen, specific conductance, pH, and TSS. For other parameters like VSS and chlorophyll a, Hunting Creek was lower. This may be explained by the wet year which flushed algae from the system and perhaps Hunting Creek has generally lower residence time and decreased phytoplankton. Studies during a lower flow year will help to resolve this.



Algal populations as measured by chlorophyll a were generally very low (<5 ug/L) through mid-June, but increased to the 10-25 ug/L range from late June through July. Values declined again in August and September. Chlorophyll a in the river showed a more consistent rise and fall, while Hunting Creek and particularly ARE1 were much more variable. Chlorophyll mapping showed higher concentrations in the embayment in June, but in August concentrations were higher in the river, matching semimonthly



data. Other phytoplankton parameters were measured at only two sites (ARE2 and ARE4). Phytoplankton density followed similar seasonal patterns at both sites. Peak values were in July at ARE2, but slightly higher levels were seen in the river in August. Phytoplankton cell density at both sites was dominated by cyanobacteria and diatoms, but cryptophytes and green algae were occasionally important. *Oscillatoria* was the most persistently abundant contributor to cyanobacterial cell density with *Merismopedia* and *Microcystis* occasionally dominant. The most important diatom contributors to cell density were discoid centrics, *Melosira*, and some pinnate diatoms. *Chroomonas* and *Cryptomonas* were also numerous. Diatoms overwhelmingly dominated phytoplankton biovolume for the year. By this measure cyanobacteria were insignificant due to their smaller size. *Oscillatoria* was only cyanobacterium of any consequence. *Melosira* was clearly the most important diatom with discoid centrics being abundant at times at the



embayment site. *Cryptomonas* made an important contribution to phytoplankton biovolume in many samples and in some *Euglena* and/or *Trachelomonas* were important due to their large size. In general these patterns are similar to observations in the Gunston Cove study except that the differences between embayment and river sites are much generally much greater in Gunston Cove mainly due to lower values in the Hunting Creek embayment.

Rotifers were the most numerous zooplankton in the study area with similar abundance patterns in the embayment and river. Rotifer density was low for most of the years, but attained substantial numbers in midsummer at both sites. *Brachionus* was the overwhelming dominant on most dates with *Synchaeta* being important in late June in the embayment. The small cladoceran *Bosmina* was fairly abundant in the river in late July, but was more limited in the embayment. The larger cladoceran *Diaphanosoma*, which often attained values of over 10,000/m³ in Gunston Cove was found in only very low

numbers at both sites in the Hunting Creek study. Values for other cladocera such as *Daphnia*, *Ceriodaphnia*, and *Moina* were also much reduced in Hunting Creek relative to Gunston Cove. *Leptodora*, the predaceous cladoceran, was also very low in the embayment, but was found at values more typical of the Gunston Cove study in the river. The greatly reduced densities of these cladocera, especially *Diaphanosoma*, may be related to higher flushing rates in Hunting Creek, particularly in June that strongly disrupted the populations' development. The seasonal pattern of nauplii (immature copepods) was almost identical at the two study sites. Nauplii densities increased slowly but steadily through late July and stayed relatively high in August. *Eurytemora*, a calanoid copepod, attained only very low values in the cove, apparently being another victim of short residence times. But found a large peak in late June that carried over into early July. Other calanoids were also at peak abundance at that time. Cyclopoid copepods were not very abundant at either site.

Triplicate petite ponar samples were collected at ARE2, ARE3, and ARE4 monthly from May through August. Oligochaetes were the most common invertebrates collected in these samples. Chironomid (midge) larvae made up the most of the remaining organisms at most stations. At ARE 2 there was a substantial contribution from gastropods (snails), amphipods (scuds), and bivalves.

We collected a total of 1524 fish larvae (ichthyoplankton) in Hunting Creek. The ichthyoplankton was dominated by the species *Alosa pseudoharengus* (Alewife). The Family Clupeidae (to which alewife belongs) was overall the dominant taxon and represented close to 75% of the total catch. The density of these clupeid larvae was at its peak in late May with a density of 40 specimens per 10m³. Of the larvae collected outside the Family Clupeidae, members of the genus *Morone* (striped bass or white perch) were dominant. Their production peak was in early May with a density of 25 specimens per 10m³.

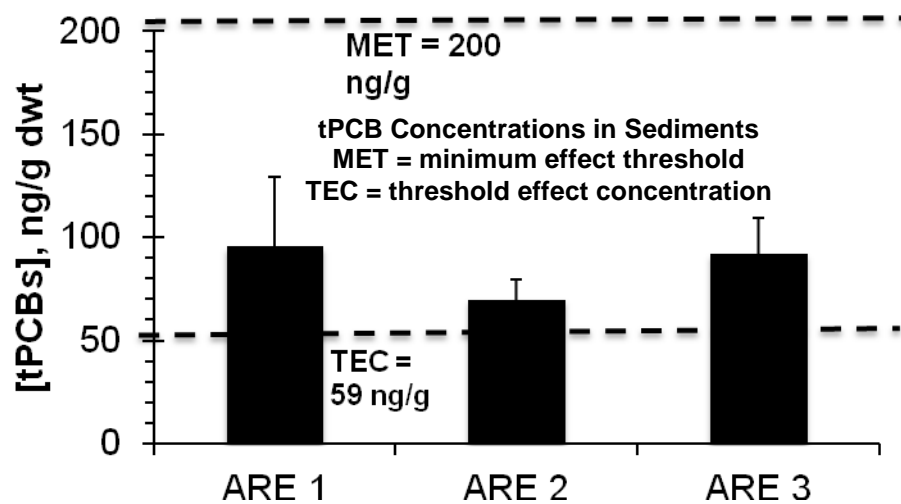
We collected a total of 995 adult and juvenile fish specimens comprising 16 species representing 10 families by trawling. White perch (*Morone americana*) was most abundant in these collections, with spottail shiner (*Notropis hudsonius*) and tessellated darter (*Etheostoma olmstedii*) as close second and third most abundant respectively. The abundance of the other species collected with trawls was one to two orders of magnitude lower. The abundance of blue catfish was an order of magnitude higher than brown bullhead, which is an indication of the newly established dominance of the invasive blue catfish over similar species in Potomac River tributaries (both the invasive blue catfish and the native brown bullhead belong to the family Ictaluridae). Blue catfish was only found in the Potomac mainstem, while brown bullhead was collected in Hunting Creek, which indicates that Hunting Creek may provide important habitat for native species. To put the invasion of blue catfish in perspective; they still only constitute 1.2 % of the total abundance found in trawls.

Seine collections yielded 2669 adult and juvenile fish specimens comprising 26 species from 13 families. In seines, the most abundant species by far was banded killifish (*Fundulus diaphonus*), followed by herrings (*Alosa* sp.). Other species that occurred at high abundance were white perch, spottail shiner, and mummichog (*Fundulus heteroclitus*). The highest abundance was found in early May, where high numbers of

banded killifish and herring constituted half of the total catch of the season. In the case of the herrings, these were juveniles that were spawned earlier in the season in Potomac River tributaries and spend time in shallow habitats such as Hunting Creek before swimming out to the Chesapeake Bay and the Atlantic.

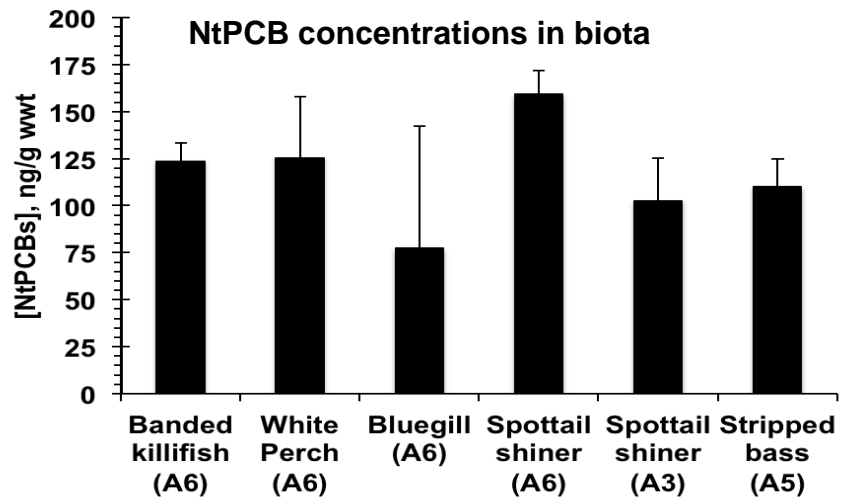
The spawning of these anadromous fishes was monitored in Cameron Run during river herring spawning season from mid-March to mid-May. Anadromous fishes such as river herring (collective name for alewife and blueback herring) migrate from the sea into freshwater to spawn. Cameron Run has several water control weirs blocking upstream access for anadromous fishes, but our reasoning was that the section of Cameron Run before the first weir could already provide river herring spawning habitat. No river herring spawning had been confirmed yet in Cameron Run by the Virginia Department of Game and Inland Fisheries (Alan Weaver, pers. comm.). Our finding of one adult alewife and several river herring larvae in the anadromous survey has now confirmed the use of Cameron Run as spawning habitat for river herring, which is currently a species of concern. This information yields fisheries management implications. Future adjustments to our sampling location within Cameron Run may provide a better estimate of the size of the spawning population in Cameron Run.

The ecological survey of Hunting Creek surficial sediments and biota revealed several important observations. The first was that total-PCBs (tPCBs) were widely detected

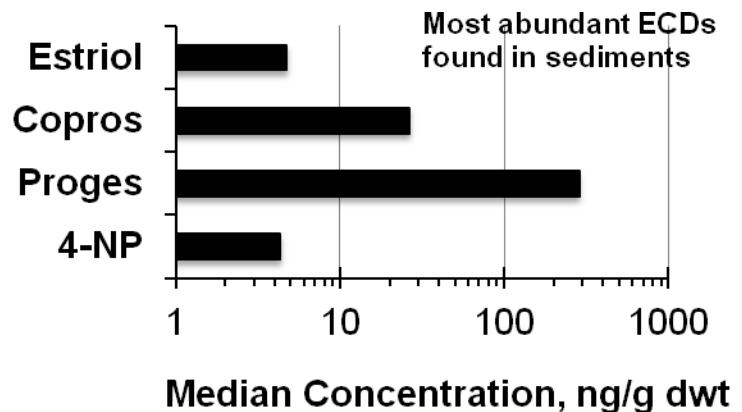


in all sediments and biota in the region (100% detection frequency). The concentrations of PCBs in sediments ranged from clean to lightly polluted, but none of the samples suggested that toxic effects are highly probable based on regulatory criteria (sediment quality guidelines). All PCB concentrations in surficial sediments were below minimum effect threshold criteria (i.e., MET). A lack of observed gradients and geospatial correlation existed for PCBs in sediments, as PCBs appeared to be widely dispersed and at relatively consistent concentrations within the region.

PCBs were detected in all biota and at all locations in Hunting Creek (100% detection frequency). The concentrations of surrogate normalized total-PCB concentrations (NtPCBs) ranged from 70 to 155 ng/g and were similar to slightly greater than PCB concentrations detected in fish previously

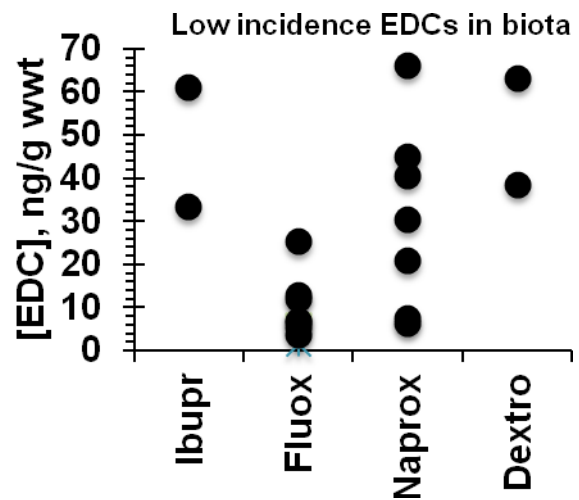


collected nearby from Dyke Marsh, VA, in 2000, which were 50 ng/g. PCBs observed in fish from Hunting Creek showed no significant differences among species, indicating any ecologically stratified partitioning of PCBs was not evident. Factors such as species, size, age, feeding habits or other factors were not identified as important ecological processes regulating PCBs at this location. EDCs were detected in sediments and biota from Hunting Creek, but stand in contrast to the observations of PCB concentrations. The EDCs were highly variable in terms of both incidence (detection frequency) and concentrations. The most frequently detected EDCs in sediments included 4-nonylphenol (non-ionic surfactant), progesterone (steroid), coprostanol (sewage sterol marker) and estriol (steroid), ranging from 4 to 280 ng/g dry weight, while detection frequencies observed were 100%, 100%, 100% and 75%, respectively. The concentrations of these four EDCs were highly variable, such that gradients or near-field geospatial differences could not be resolved. The second group of EDCs in sediments were those with low detection frequencies (<50%), and included atrazine (herbicide), diphenylhydramine (over the counter drug, OTCD), fluoxetine (SSRI antidepressant), naproxen (NSAID), dextromethorphan (OTCD), 17 β -estradiol (steroid) and norgestrel (steroid). Many of these EDCs were detected too infrequently to establish average concentrations, but represent chemicals present in the environment but at trace levels.



The incidence and concentrations of EDCs detected in biota samples resembled those in sediments with respect to variability. The most frequently detected EDCs in biota included triclosan (antibacterial agent) and bisphenol A (plastics), with DFs of 55% and 64%, respectively. The median concentrations of triclosan and bisphenol A in biota from Hunting Creek ranged from 4 to 26 ng/g wet weight (wwt), respectively.

The EDCs with DFs <50% in biota included, ibuprofen (NSAID), fluoxetine (SSRI), naproxen (NSAID) and dextromethorphan (OTCD), which did correspond with the same minor EDCs detected in surficial sediments. Concentrations of the low detection frequency EDCs ranged from 1 to 68 ng/g wwt. The EDC concentrations in biota were sparse and variable such that no differences between species, ARE sampling location or time series could be resolved. The most frequently detected EDCs in biota did not correspond to the EDCs detected in sediments.



We recommend that:

1. The basic ecosystem monitoring should continue. The year 2013 was unusually wet especially in early summer. A range of climatic conditions is needed to effectively establish baseline conditions in Hunting Creek. Several years of study will be needed to effectively bracket conditions in the embayment.
2. Water quality mapping should be continued and efforts should be made to more fully cover the study area on mapping days. This will provide much needed spatial resolution of water quality patterns.
3. Anadromous fish sampling is an important part of this monitoring program and has gained interest now that the stock of river herring has collapsed, and a moratorium on these taxa has been established in 2012. The discovery of river herring spawning in Cameron Run increases the importance of continuing studies of anadromous fish in the study area. We also intend to investigate a slight relocation of the sampling site to try to be more effective in collections.
4. We recommend that EDC sampling and analysis extend to inputs to and outputs from the AlexRenew WRRF to better understand the source of residues observed in the Hunting Creek area.
5. We recommend continuing ongoing methods development for the GCMS analysis of EDCs in biota. The current EDC method for biota samples shows low recoveries of some EDC analytes. An MS student in Chemistry is completing an MS research project on method development targeting this issue.
6. We recommend studies of *E. coli* abundance and distribution by adding it to standard sampling protocol and sediment studies related to CSO outfalls.

List of Abbreviations

BOD	Biochemical oxygen demand
cfs	cubic feet per second
DO	Dissolved oxygen
ha	hectare
l	liter
LOWESS	locally weighted sum of squares trend line
m	meter
mg	milligram
MGD	Million gallons per day
NS	not statistically significant
NTU	Nephelometric turbidity units
SAV	Submersed aquatic vegetation
SRP	Soluble reactive phosphorus
TP	Total phosphorus
TSS	Total suspended solids
um	micrometer
VSS	Volatile suspended solids
#	number

THE AQUATIC MONITORING PROGRAM
FOR THE HUNTING CREEK AREA
OF THE TIDAL FRESHWATER POTOMAC RIVER

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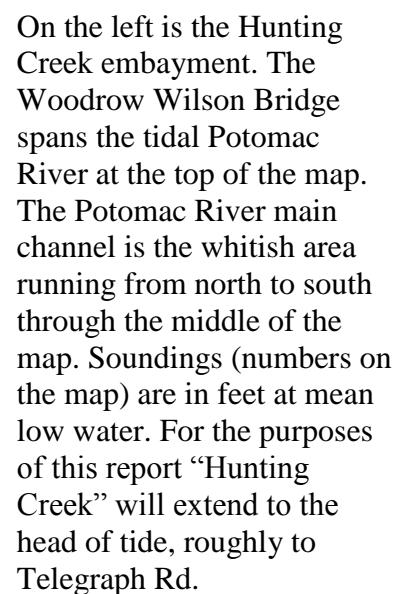
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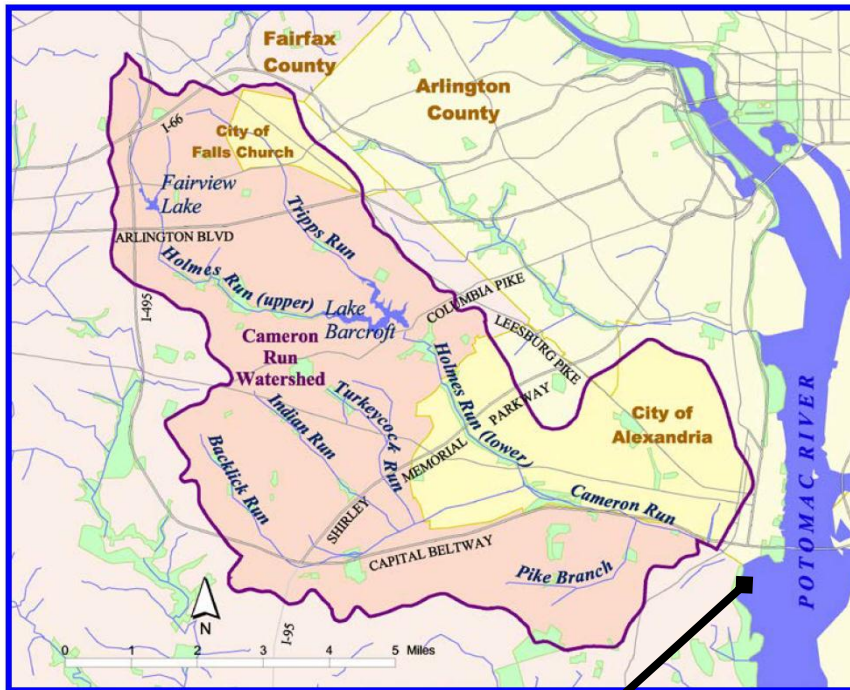
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This work was in response to a request from Karen Pallansch, Chief Executive Officer of Alexandria Renew Enterprises, operator of the wastewater reclamation facility which served about 350,000 in the City of Alexandria and County of Fairfax in northern Virginia. The study is patterned on the long-running Gunston Cove Study which the Potomac Environmental Research and Education Center has been conducting in partnership with the County of Fairfax Department of Public Works and Environmental Services since 1984. The goal of these studies is to provide baseline and on-going trend analysis of the ecosystems receiving reclaimed water from these facilities with the objective of adaptive management of these valuable freshwater resources. This will facilitate the formulation of well-grounded management strategies for maintenance and improvement of water quality and biotic resources in the tidal Potomac. A secondary, but important educational goal, is to provide training for Mason graduate and undergraduate students in water quality and biological monitoring and assessment.

Hunting Creek is an embayment of the tidal Potomac River located just downstream of the City of Alexandria and the Woodrow Wilson Bridge. Waters are shallow with the entire embayment having a depth of 2 m or less at mean tide. According to the “Environmental Atlas of the Potomac Estuary” (Lippson et al. 1981), the mean depth of Hunting Creek is 1.0m, a surface area is 2.26 km², and a volume of 2.1 x 10⁶ m³.

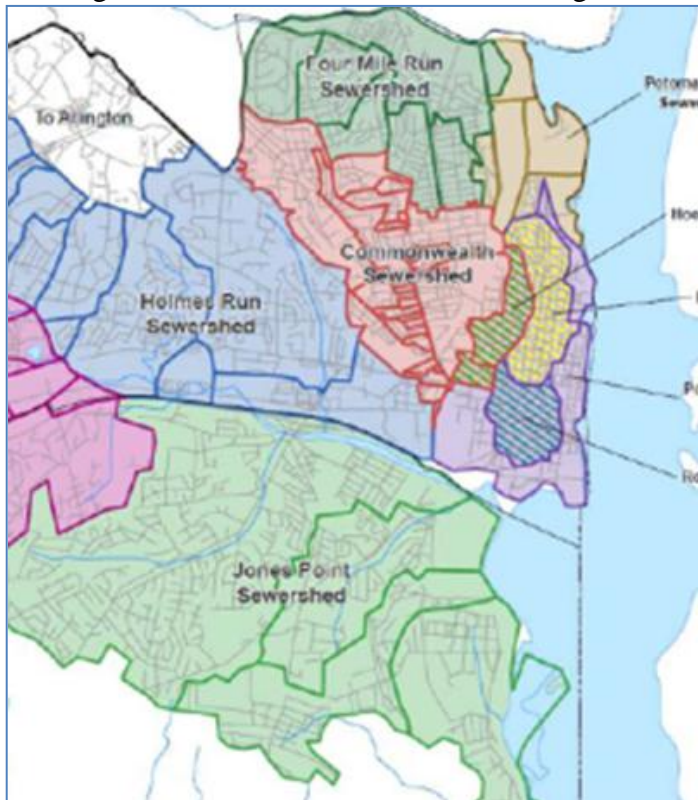




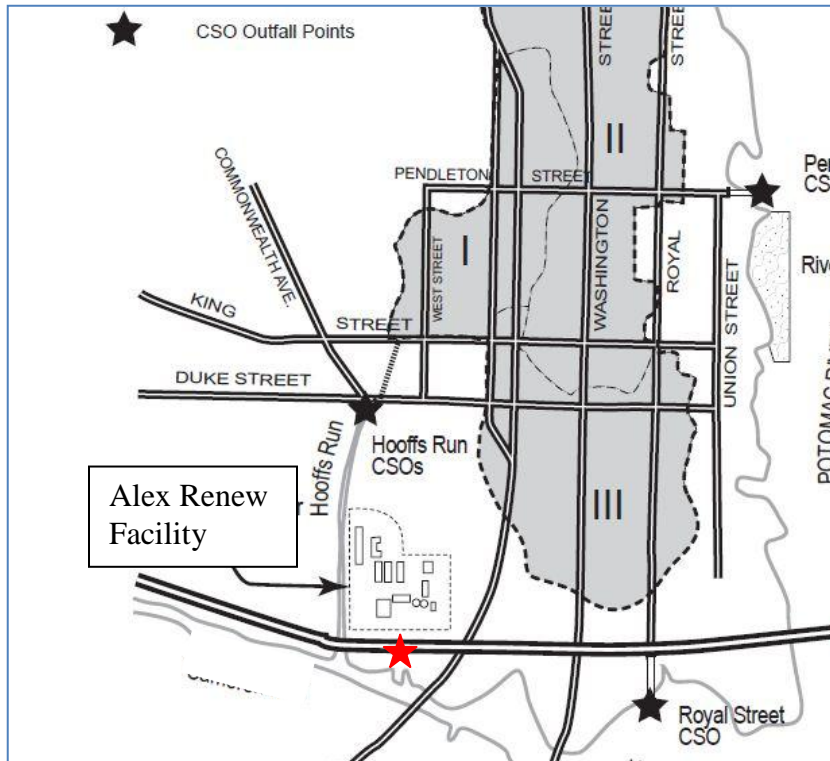
Hunting Creek embayment

On the left is a map of the Hunting Creek watershed. Cameron Run is the freshwater stream which drains the vast majority of the watershed of Hunting Creek. The watershed is predominantly suburban in nature with areas of higher density commercial and residential development. The watershed has an area of 44 square miles and drains most of the Cities of Alexandria and Falls Church and much of east central Fairfax County. A major aquatic feature of the watershed is Lake Barcroft. The suburban land uses in the watershed are a source of nonpoint pollution to Hunting Creek.

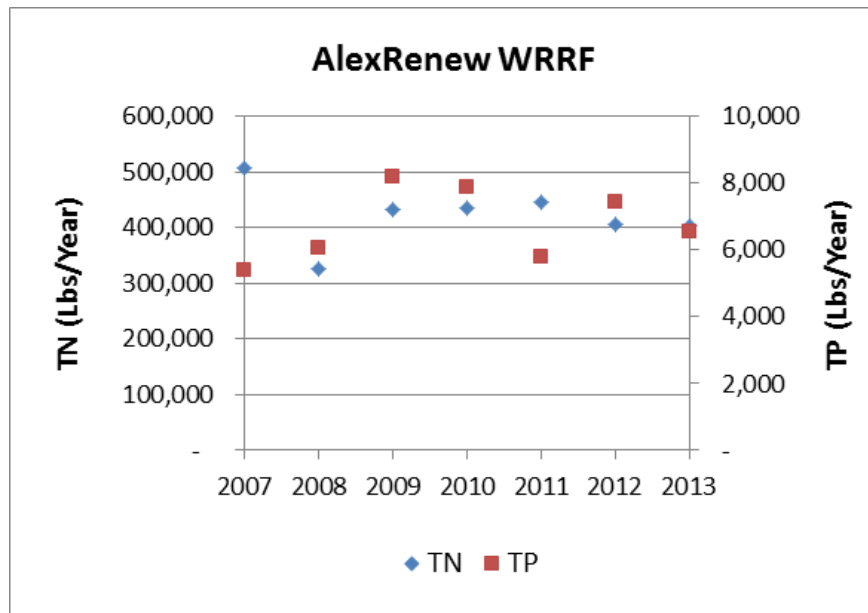
The AlexRenew WRRF serves an area similar in extent to the Cameron Run watershed with the addition of some areas along the Potomac shoreline from Four Mile Run to Dyke Marsh. The effluent of the Alexandria Renew Enterprises plant enters the upper tidal reach of Hunting Creek under the Rt 1/I-95 interchange.



The map at the left shows the sewersheds which contribute to the AlexRenew WRRF. Of particular note are the shaded areas within the City of Alexandria. These sewersheds (Hoofs Run, Pendleton, and Royal St.) all contain combined sewers meaning that domestic wastewater is co-mingled with street runoff. Under most conditions, all of this water is directed to the AlexRenew WRRF for treatment. But in extreme runoff conditions (like torrential rains), some may be diverted directly into the tidal Potomac via a Combined Sewer outfall (CSO).



The map at the left is an enlargement of the area where the Alex Renew WRRF is found and where the discharge sites of the CSO's are located. Note the close proximity of two of the CSO's to the Alex Renew WRRF discharge (shown as red star).



The graph at the left shows the loading of nitrogen and phosphorus from the Alexandria Renew WRRF for the last seven years. Loadings of both nutrient elements have remained fairly constant at about 400,000 lb/yr (181,000 kg/yr) for nitrogen and 7,000 lb/yr (3175 kg/yr) for phosphorus.

Ecology of the Freshwater Tidal Potomac

The tidal Potomac River is an integral part of the Chesapeake Bay tidal system and at its mouth the Potomac is contiguous to the bay proper. The tidal Potomac is often called a subestuary of the Chesapeake Bay and as such it is the largest subestuary of the bay in terms of size and amount of freshwater input. The mixing of freshwater with saltwater is the hallmark of an estuary. While the water elevation in an estuary is “sea level”, the water contained in an estuary is not pure sea water such as found in the ocean. Pure ocean sea water has a salt concentration of about 35 parts per thousand (ppt). Water in Chesapeake Bay ranges from about 30 ppt near its mouth to 0 ppt in the upper reaches where there is substantial freshwater inflow such as in the upper tidal Potomac River. Salinity at a given location is determined by the balance between freshwater input and salt water mixing in from the ocean. It generally varies with season being lower in spring when freshwater inflows are greater and higher in summer when there is less freshwater inflow.

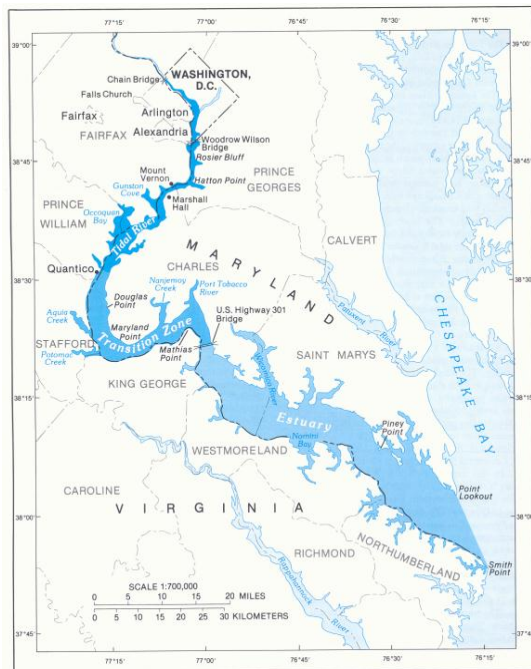


Figure 2. The tidal Potomac River and Estuary.

(map courtesy USGS)

The tidal Potomac is generally divided into three salinity zones as indicated by the map to the left:

- Estuarine or Mesohaline zone (6-14 ppt)
- Transition or Oligohaline zone (0.5-6 ppt)
- Tidal River or Tidal Fresh zone (<0.5 ppt)

Hunting Creek is in the upper part of the Tidal River/Tidal Fresh zone and as such it never experiences detectable salinity

Within the tidal freshwater zone, the flora and fauna are generally characterized by the same species that would occur in a freshwater lake in this area and the food web is similar. Primary producers are freshwater species of submersed aquatic vegetation (SAV) such as native taxa *Vallisneria americana* (water celery), *Potamogeton* spp. (pondweeds), and *Ceratophyllum* (coontail) as well as introduced species such as *Hydrilla verticillata* (hydrilla) and *Myriophyllum spicatum* (water milfoil). Historical accounts indicate that most of the shallow area of the tidal freshwater Potomac were colonized by SAV around 1900 (Carter et al. 1985).

The other group of important primary producers are phytoplankton, a mixed assemblage of algae and cyanobacteria which may turn over rapidly on a seasonal basis. The dominant groups of phytoplankton in the tidal freshwater Potomac are diatoms (considered a good food source for aquatic consumers) and cyanobacteria (considered a less desirable food source for aquatic consumers). For the latter part of the 20th century, the high nutrient loadings into the river favored cyanobacteria over both diatoms and SAV resulting large production of undesirable food for consumers. In the last decade or so, as nutrient reductions have become manifest, cyanobacteria have decreased and diatoms and SAV have increased.

The biomass contained in the cells of phytoplankton nourishes the growth of zooplankton and benthic macroinvertebrates which provide an essential food supply for the juvenile and smaller fish. These in turn provide food for the larger fish like striped bass and largemouth bass. The species of zooplankton and benthos found in the tidal fresh zone are similar to those found in lakes in the area, but the fish fauna is augmented by species that migrate in and out from the open interface with the estuary.

Resident fish species include typical lake species such as sunfish (*Lepomis* spp.), bass (*Micropterus* spp.), crappie (*Pomoxis* spp.) as well as estuarine species such as white perch (*Morone americana*) and killifish (*Fundulus* spp.). Species which spend part of their year in the area include striped bass (*Morone saxatilis*) and river herrings and shad (*Alosa* spp.).

Larval fishes are transitional stages in the development of juvenile fishes. They range in development from newly hatched, embryonic fish to juvenile fish with morphological features similar to those of an adult. Many fishes such as clupeids (herring family), white perch, striped bass, and yellow perch disperse their eggs and sperm into the open water. The larvae of these species are carried with the current and termed “ichthyoplankton”. Other fish species such as sunfish and bass lay their eggs in “nests” on the bottom and their larvae are rare in the plankton.

After hatching from the egg, the larva draws nutrition from a yolk sack for a few days time. When the yolk sack diminishes to nothing, the fish begins a life of feeding on other organisms. This post yolk sack larva feeds on small planktonic organisms (mostly small zooplankton) for a period of several days. It continues to be a fragile, almost transparent, larva and suffers high mortality to predatory zooplankton and juvenile and adult fishes of many species, including its own. When it has fed enough, it changes into an opaque juvenile, with greatly enhanced swimming ability. It can no longer be caught with a slow-moving plankton net, but is soon susceptible to capture with the seine or trawl net.

METHODS

A. Profiles and Plankton: Sampling Day

Sampling was conducted on a semimonthly basis at stations representing both Hunting Creek and the Potomac mainstem (Figure 1). One station (ARE 1) was located near the mouth of Cameron Run in the small bay located just west of the George Washington Parkway bridge. Sampling was generally conducted at ARE 1 from shore by wading out 5-7 m from shore. Two stations (ARE 2 & 3) were located in the Hunting Creek embayment proper. A fourth station was located in the river channel about 100 m upstream from Buoy 90. Dates for sampling as well as weather conditions on sampling dates and immediately preceding days are shown in Table 1.



Figure 1. Hunting Creek area of the Tidal Potomac River showing sampling stations. ARE 1, 2, 3, and 4 represent water quality stations, ARE 2 and 4 are the phytoplankton and zooplankton and ARE 5 and 6 are the fish seine stations.

Table 1
Hunting Creek Study: Sampling Dates and Weather Data for 2013

Date	Type of Sampling					Avg Daily Temp (°C)		Precipitation (cm)	
	WP	B	D	T	S	1-Day	3-Day	1-Day	3-Day
April 10				T	S	25.0	22.4	0	0
April 15	WP					15.6	15.4	T	T
May 8				T	S	18.9	17.0	0.10	1.51
May 14	WP	B234				12.8	13.7	0	T
May 22				T	S	25.6	24.3	0.05	0.06
May 29	WP	B1				26.7	22.4	0	3.40
June 5				T	S	21.1	22.2	0	1.37
June 11	WP	B234				25.0	24.4	0	7.06
June 14			D			22.8	24.1	T	T
June 19				T	S	24.1	24.4	0	0.60
June 26	WP	B1				27.8	28.0	0.03	0.09
July 10	WP					28.3	27.4	1.45	1.56
July 17				T	S	31.7	30.9	0	0
July 24	WP	B234				26.7	27.8	0	0.27
July 26				T	S	24.4	24.8	0	0
August 7			D	T	S	26.1	23.7	T	0.19
August 21	WP	B4		T	S	27.2	24.8	0.18	0.18
August 26	WP	B23				25.0	24.1	0	0
Sept 10				T	S	28.3	25.9	0	0.01
Sept 17	WP					17.2	19.1	0	0.13

Type of Sampling: WP: Water quality (samples to AlexRenew Lab), profiles and plankton, B: benthos (station numbers indicated), D: dataflow (water quality mapping), T: fish collected by trawling, S: fish collected by seining.
T under Precipitation equals “trace”.

Sampling was initiated at 10:15 am. Four types of measurements or samples were obtained at each station: (1) depth profiles of temperature, conductivity, dissolved oxygen, pH, and irradiance (photosynthetically active radiation) measured directly in the field; (2) water samples for GMU lab determination of chlorophyll *a* and phytoplankton species composition and abundance; (3) water samples for determination of N and P forms, BOD, COD alkalinity, hardness, suspended solids, chloride, and pH by the Alexandria Renew Enterprises lab; (4) net sampling of zooplankton and ichthyoplankton.

Profiles of temperature, conductivity, and dissolved oxygen were conducted at each station using a YSI 6600 datasonde with temperature, conductivity, dissolved oxygen and pH probes. Measurements were taken at 0.3 m increments to the bottom at the embayment stations. In the river measurements were made with the sonde at depths of 0.3 m and 2.0 m increments to the bottom. Meters were checked for calibration before and after sampling. Profiles of irradiance (photosynthetically active radiation, PAR) were collected with a LI-COR underwater flat scalar PAR probe. PAR measurements were taken at 10 cm intervals to a depth of 1.0 m. Simultaneous measurements were made with a terrestrial probe in air during each profile to correct for changes in ambient light if needed. Secchi depth was also determined. The readings of at least two crew members were averaged due to variability in eye sensitivity among individuals.

A 1-liter depth-composited sample was constructed from equal volumes of water collected at each of three depths (0.3 m below the surface, middepth, and 0.3 m off of the bottom) using a submersible bilge pump. A 100-mL aliquot of this sample was preserved immediately with acid Lugol's iodine for later identification and enumeration of phytoplankton at stations ARE2 and ARE4. The remainder of the sample was placed in an insulated cooler with ice. A separate 1-liter sample was collected from 0.3 m using the submersible bilge pump and placed in the insulated cooler with ice for lab analysis of surface chlorophyll *a*. These samples were analyzed by Mason.

Separate 2-liter samples were collected monthly at each station from just below the surface (0.3 m) and near the bottom (0.3 m off bottom) at each station using the submersible pump. This water was promptly delivered to the nearby Alexandria Renew Laboratory for determination of nitrogen, phosphorus, BOD, TSS, VSS, pH, total alkalinity, and chloride.

At stations ARE2 and ARE4, microzooplankton was collected by pumping 32 liters from each of three depths (0.3 m, middepth, and 0.3 m off the bottom) through a 44 μm mesh sieve. The sieve consisted of a 12-inch long cylinder of 6-inch diameter PVC pipe with a piece of 44 μm nitex net glued to one end. The 44 μm cloth was backed by a larger mesh cloth to protect it. The pumped water was passed through this sieve from each depth and then the collected microzooplankton was backflushed into the sample bottle. The resulting sample was treated with about 50 mL of club soda and then preserved with formalin containing a small amount of rose bengal to a concentration of 5-10%.

At stations ARE2 and ARE4, macrozooplankton was collected by towing a 202 μm net (0.3 m opening, 2 m long) for 1 minute at each of three depths (near surface, middepth, and near

bottom). Ichthyoplankton (larval fish) was sampled by towing a 333 μm net (0.5 m opening, 2 m long) for 2 minutes at each of the same depths at Stations ARE2 and ARE4. In the embayment, the boat made a large arc during the tow while in the river the net was towed in a more linear fashion along the channel. Macrozooplankton tows were about 300 m and ichthyoplankton tows about 600 m. Actual distance depended on specific wind conditions and tidal current intensity and direction, but an attempt was made to maintain a constant slow forward speed (approximately 2 miles per hour) through the water during the tow. The net was not towed directly in the wake of the engine. A General Oceanics flowmeter, fitted into the mouth of each net, was used to establish the exact towing distance. During towing the three depths were attained by playing out rope equivalent to about 1.5-2 times the desired depth. Samples which had obviously scraped bottom were discarded and the tow was repeated. Flowmeter readings taken before and after towing allowed precise determination of the distance towed and when multiplied by the area of the opening produced the total volume of water filtered.

Macrozooplankton and ichthyoplankton were preserved immediately with formalin to a concentration of 5-10%. Rose bengal formalin with club soda pretreatment was used for macrozooplankton, but for ichthyoplankton only clear formalin was used. Macrozooplankton was collected on each sampling trip; ichthyoplankton collections ended after July because larval fish were normally not found after this time.

Benthic macroinvertebrate samples were collected monthly at stations ARE 2, ARE3, and ARE4. Due to access issues benthic samples were collected only twice at ARE1 when a boat was able to access the area upstream of the GW Parkway bridge. Three samples were collected at each station using a petite ponar grab. The bottom material was sieved through a 0.5 mm stainless steel sieve and resulting organisms were preserved in rose bengal formalin for lab analysis.

Samples for water quality determination were maintained on ice delivered to the Alexandria Renew Enterprises (AlexRenew) Laboratory by 2 pm on sampling day and returned to GMU by 3 pm. At GMU 10-15 mL aliquots of both depth-integrated and surface samples were filtered through 0.45 μm membrane filters (Gelman GN-6 and Millipore MF HAWP) at a vacuum of less than 10 lbs/in² for chlorophyll *a* and pheopigment determination. During the final phases of filtration, 0.1 mL of MgCO₃ suspension (1 g/100 mL water) was added to the filter to prevent premature acidification. Filters were stored in 20 mL plastic scintillation vials in the lab freezer for later analysis. Seston dry weight and seston organic weight were measured by filtering 200-400 mL of depth-integrated sample through a pretared glass fiber filter (Whatman 984AH).

Sampling day activities were normally completed by 5:30 pm.

B. Profiles and Plankton: Follow-up Analyses

Chlorophyll *a* samples were extracted in a ground glass tissue grinder to which 4 mL of dimethyl sulfoxide (DMSO) was added. The filter disintegrated in the DMSO and was ground for about 1 minute by rotating the grinder under moderate hand pressure. The ground suspension was transferred back to its scintillation vial by rinsing with 90% acetone. Ground samples were

stored in the refrigerator overnight. Samples were removed from the refrigerator and centrifuged for 5 minutes to remove residual particulates.

Chlorophyll *a* concentration in the extracts was determined flurometrically using a Turner Designs Model 10 field fluorometer configured for chlorophyll analysis as specified by the manufacturer. The instrument was calibrated using standards obtained from Turner Designs. Fluorescence was determined before and after acidification with 2 drops of 10% HCl. Chlorophyll *a* was calculated from the following equation which corrects for pheophytin interference:

$$\text{Chlorophyll } a \text{ (}\mu\text{g/L)} = F_s R_s (R_b - R_a) / (R_s - 1)$$

where F_s = concentration per unit fluorescence for pure chlorophyll *a*

R_s = fluorescence before acid / fluorescence after acid for pure chlorophyll *a*

R_b = fluorescence of sample before acid

R_a = fluorescence of sample after acid

All chlorophyll analyses were completed within one month of sample collection.

Phytoplankton species composition and abundance was determined using the inverted microscope-settling chamber technique (Lund et al. 1958). Ten milliliters of well-mixed algal sample were added to a settling chamber and allowed to stand for several hours. The chamber was then placed on an inverted microscope and random fields were enumerated. At least two hundred cells were identified to species and enumerated on each slide. Counts were converted to number per mL by dividing number counted by the volume counted. Biovolume of individual cells of each species was determined by measuring dimensions microscopically and applying volume formulae for appropriate solid shapes.

Microzooplankton and macrozooplankton samples were rinsed by sieving a well-mixed subsample of known volume and resuspending it in tap water. This allowed subsample volume to be adjusted to obtain an appropriate number of organisms for counting and for formalin preservative to be purged to avoid fume inhalation during counting. One mL subsamples were placed in a Sedgewick-Rafter counting cell and whole slides were analyzed until at least 200 animals had been identified and enumerated. A minimum of two slides was examined for each sample. References for identification were: Ward and Whipple (1959), Pennak (1978), and Rutner-Kolisko (1974). Zooplankton counts were converted to number per liter (microzooplankton) or per cubic meter (macrozooplankton) with the following formula:

$$\text{Zooplankton (\#/L or \#/m}^3\text{)} = NV_s / (V_c V_f)$$

where N = number of individuals counted

V_s = volume of reconstituted sample, (mL)

V_c = volume of reconstituted sample counted, (mL)

V_f = volume of water sieved, (L or m^3)

Ichthyoplankton samples were sieved through a 333 μm sieve to remove formalin and then reconstituted in ethanol. Larval fish were picked from the reconstituted sample with the aid

of a stereo dissecting microscope. Identification of ichthyoplankton was made to family and further to genus and species where possible. If the number of animals in the sample exceeded several hundred, then the sample was split with a plankton splitter and the resulting counts were multiplied by the subsampling factor. The works Hogue et al. (1976), Jones et al. (1978), Lippson and Moran (1974), and Mansueti and Hardy (1967) were used for identification. The number of ichthyoplankton in each sample was expressed as number per 10 m³ using the following formula:

$$\text{Ichthyoplankton (\#/10m}^3\text{)} = 10N/V$$

where N = number ichthyoplankton in the sample
V = volume of water filtered, (m³)

C. Adult and Juvenile Fish

Fishes were sampled by trawling at stations ARE 3 and 4, and seining at stations ARE 5 and ARE 6 (Figure 1). For trawling, a try-net bottom trawl with a 15-foot horizontal opening, a ¾ inch square body mesh and a ¼ inch square cod end mesh was used. The otter boards were 12 inches by 24 inches. Towing speed was 2-3 miles per hour and tow length was 5 minutes. The trawls were towed upriver parallel to the channel at ARE 4, and following the curve of the 'cove' away from the channel at ARE 3. The direction of tow should not be crucial. Dates of sampling and weather conditions are found in Table 1.

Seining was performed with a bag seine that was 50 feet long, 3 feet high, and made of knotted nylon with a ¼ inch square mesh. The bag is located in the middle of the net and measures 3 ft³. The seining procedure was standardized as much as possible. The net was stretched out perpendicular to the shore with the shore end right at the water line. The net was then pulled parallel to the shore for a distance of 100 feet by a worker at each end moving at a slow walk. Actual distance was recorded if in any circumstance it was lower than 100 feet. At the end of the prescribed distance, the offshore end of the net was swung in an arc to the shore and the net pulled up on the beach to trap the fish. Dates for seine sampling were the same as those for trawl sampling (Table 1).

After the catch from these two gear types was hauled in, the fishes were measured for standard length and total length to the nearest 0.5 cm. Standard length is the distance from the front tip of the snout to the end of the vertebral column and base of the caudal fin. This is evident in a crease perpendicular to the axis of the body when the caudal fin is pulled to the side. Total length is the distance from the tip of the snout to the tip of the longer lobe of the caudal fin, measured by straightening the longer lobe toward the midline.

If the identification of the fish was not certain in the field, the specimen was preserved in 70% ethanol and identified later in the lab. Fishes kept for chemical analysis were kept on ice wrapped in aluminum foil until frozen in the lab. All fishes retained for laboratory analysis or identification were first euthanized by submerging them in an ice sludge conform AICUC protocol. Identification was based on characteristics in dichotomous keys found in several books and articles, including Jenkins and Burkhead (1983), Hildebrand and Schroeder (1928), Loos et

al (1972), Dahlberg (1975), Scott and Crossman (1973), Bigelow and Schroeder (1953), Eddy and Underhill (1978), Page and Burr (1998), and Douglass (1999).

D. Submersed Aquatic Vegetation

Data on coverage and composition of submersed aquatic vegetation (SAV) were obtained from the SAV webpage of the Virginia Institute of Marine Science (<http://www.vims.edu/bio/sav>). Information on this web site was obtained from aerial photographs near the time of peak SAV abundance as well as ground surveys which were used to determine species composition.

E. Benthic Macroinvertebrates

Benthic macroinvertebrates were sampled using a petite ponar sampler at all stations. Triplicate samples were collected at each station monthly except at ARE1 where access by boat was more limited. Bottom samples were sieved on-site through a 0.5 mm stainless steel sieve and preserved with rose bengal formalin. In the laboratory benthic samples were rinsed with tap water through a 0.5 mm sieve to remove formalin preservative and resuspended in tap water. All organisms were picked, sorted, identified and enumerated.

F. Water Quality Mapping (Dataflow)

On two additional dates *in situ* water quality mapping was conducted by slowly transiting up to 10 transects within the Hunting Creek study area as water was pumped through a chamber containing a YSI 6600 sonde equipped with temperature, specific conductance, dissolved oxygen, pH, turbidity, and chlorophyll probes. Readings were recorded at 15 second intervals along with simultaneous GPS position readings. Every 2 minutes water samples were collected for chlorophyll calibration. These surveys allowed a much better understanding of spatial patterns in water quality within the Hunting Creek area which facilitated interpretation of data from the fixed stations. This approach is in wide use in the Chesapeake Bay region by both Virginia and Maryland under the name “dataflow”. 2013 Hunting Creek data was useful but more information is needed under a range of conditions.

G. Data Analysis

Data for each parameter were entered into spreadsheets (Excel or SigmaPlot) for graphing of temporal and spatial patterns. SYSTAT was used for statistical calculations and to create illustrations of the water quality mapping cruises. JMP v8.0.1 was used for fish graphs. Other data analysis approaches are explained in the text.

RESULTS

A. Climatic and Hydrologic Factors

In 2013 air temperature was substantially above average from April through July. August and September were about normal (Table 2). July was the warmest month and was 0.9°C above normal. There were 27 days with maximum temperature above 32.2°C (90°F) during 2013 compared with 4 in 2004, 18 in 2005, 29 in 2006, 33 in 2007, 31 in 2008, 16 days in 2009, 62 in 2010, 42 in 2011, and 42 in 2012. Precipitation was below normal from March through May, but well above normal in June and somewhat above normal in July. In August and September rainfall was very low. The largest daily rainfall totals were both in June: 7.26 cm (2.86 in) on June 28 and 7.04 cm (2.77 in) on June 10. The period June 6 to June 10 totaled 13.44 cm (5.29 in), an amount which would be expected to generate runoff flushing the study area.

Table 2. Meteorological Data for 2013. National Airport. Monthly Summary.

MONTH	Air Temp (°C)		Precipitation (cm)	
March	6.6	(8.1)	7.1	(9.1)
April	14.9	(13.4)	7.0	(7.0)
May	19.3	(18.7)	7.2	(9.7)
June	24.7	(23.6)	25.3	(8.0)
July	27.3	(26.2)	11.3	(9.3)
August	25.1	(25.2)	3.5	(8.7)
September	21.8	(21.4)	3.1	(9.6)
October	16.9	(14.9)	15.9	(8.2)
November		(9.3)		(7.7)
December		(4.2)		(7.8)

Note: 2013 monthly averages or totals are shown accompanied by long-term monthly averages (1971-2000).

Source: Local Climatological Data. National Climatic Data Center, National Oceanic and Atmospheric Administration.

Table 3. Monthly mean discharge at USGS Stations representing freshwater flow into the study area. (+) 2013 month > 2x Long Term Avg. (-) 2013 month < ½ Long Term Avg.

	Potomac River at Little Falls (cfs)		Cameron Run at Wheeler Ave (cfs)	
	2013	Long Term Average	2013	Long Term Average
January	16757	13700	43.6	41
February	19983	16600	31.2	45
March	19526	23600	40.7	55
April	14364	20400	26.3	42
May	19048	15000	28.2	41
June	10539	9030	137.9 (+)	38
July	6095	4820	52.7	31
August	3399	4550	9.5 (-)	28
September	2534 (-)	5040	10.0 (-)	38
October		5930	107.7 (+)	33

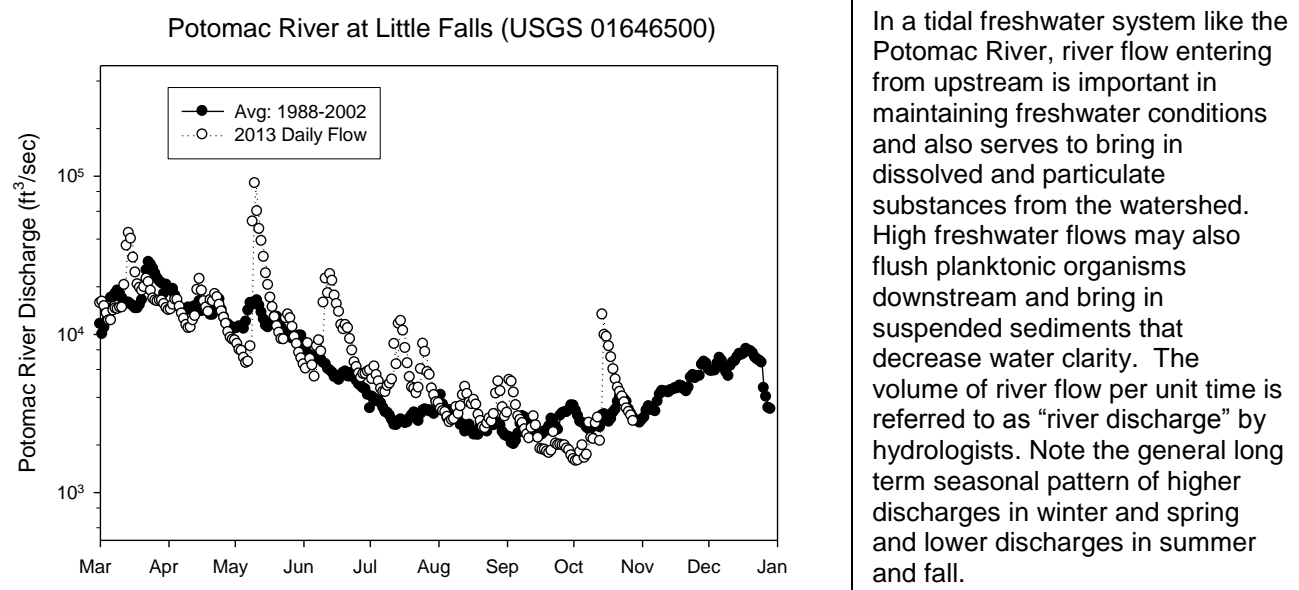


Figure 2. Mean Daily Discharge: Potomac River at Little Falls (USGS Data). Month tick is at the beginning of the month.

Potomac River discharge during 2013 was generally about average from February through September (Table 3, Figure 2). Looking more closely discharge was a bit below normal in March and April, but clearly above average in May. August and September were clearly below average. Cameron Run flows were slightly below average from January through May. However, in June average flow for 2013 was over 3 times the normal average in keeping with the high rainfall total presented on the previous page. In August and September Cameron Run flows were well below normal.

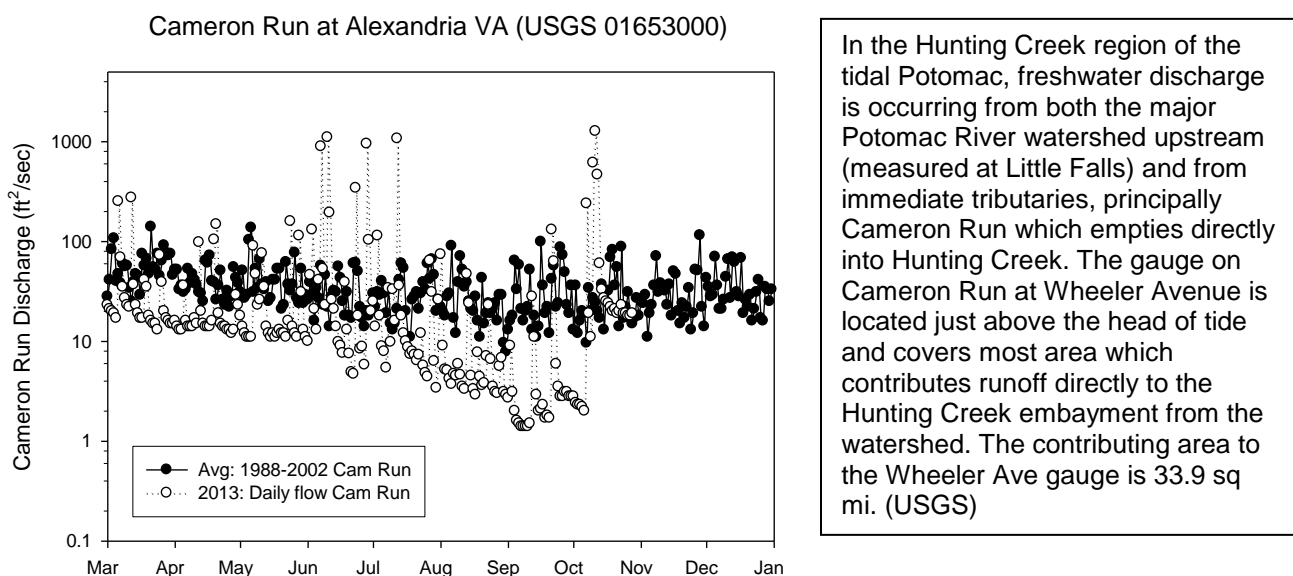
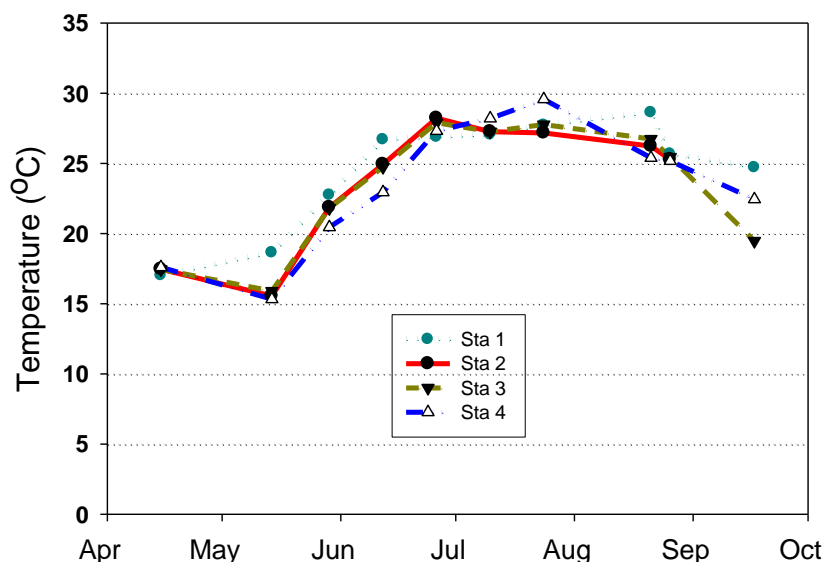


Figure 3. Mean Daily Discharge: Cameron Run at Alexandria (Wheeler Ave) (USGS Data).

B. Physico-chemical Parameters – 2013

Hunting Creek Study - 2013

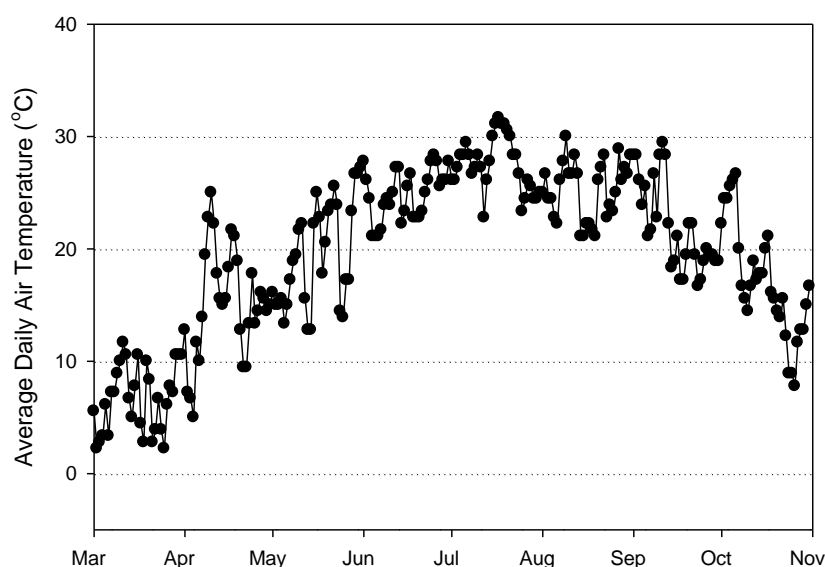


Water temperature is an important factor affecting both water quality and aquatic life. In a well-mixed system like the tidal Potomac, water temperatures are generally fairly uniform with depth. In a shallow mixed system such as the tidal Potomac, water temperature often closely tracks daily changes in air temperature.

Figure 4. Water Temperature ($^{\circ}\text{C}$). GMU Field Data. Month tick is at first day of month.

In 2013, water temperature followed the typical seasonal pattern at all stations (Figure 4). Most stations showed a slight decline from April to early May followed by a steady increase during the spring and early summer with both stations reaching values between 25°C and 30°C by late June. For most of the summer, all stations showed similar water temperatures between 25°C and 30°C . Water temperature declined in late August and September. Average daily air temperature exhibited the same general features: decline between mid April and early May, fairly uniform summer temperatures, and decline in September.

National Airport Temperature - 2013



Mean daily air temperature (Figure 5) was a good predictor of water temperature (Figure 4).

Figure 5. Average Daily Air Temperature ($^{\circ}\text{C}$) at Reagan National Airport.

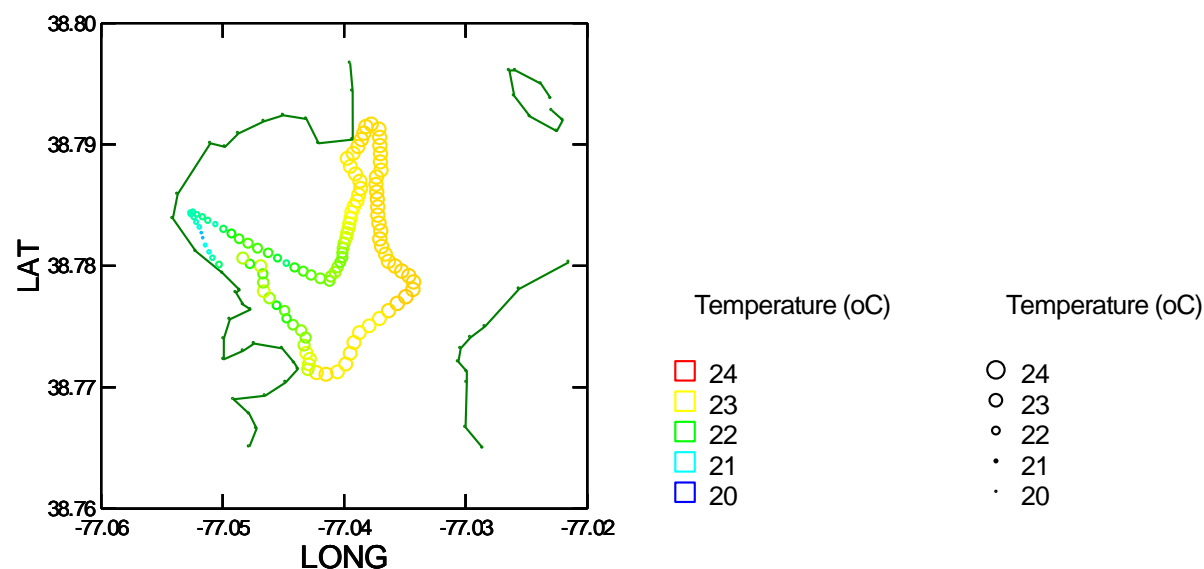


Figure 6a. Water Quality Mapping. June 14, 2013. Temperature (°C).

Mapping of water temperature was conducted on two dates in 2013: June 14 and August 7. Temperatures were 20-24°C in June and 23-26°C in August (Figure 6a & 6b). In June mapping was entirely within Hunting Creek and the adjacent river channel area. Temperatures were uniformly the lowest in Hunting Creek near the shore and increased were higher in and near the river channel. On June 14 air temperatures dropped markedly after several days of warmer weather. This was reflected in the lower water temperatures observed in the shallow Hunting Creek area, whereas temperatures remained somewhat higher in the deeper river areas. A similar phenomenon was observed on August 7. Mean air temperature on August 7 was 23.7°C while it was over 25°C a week earlier.

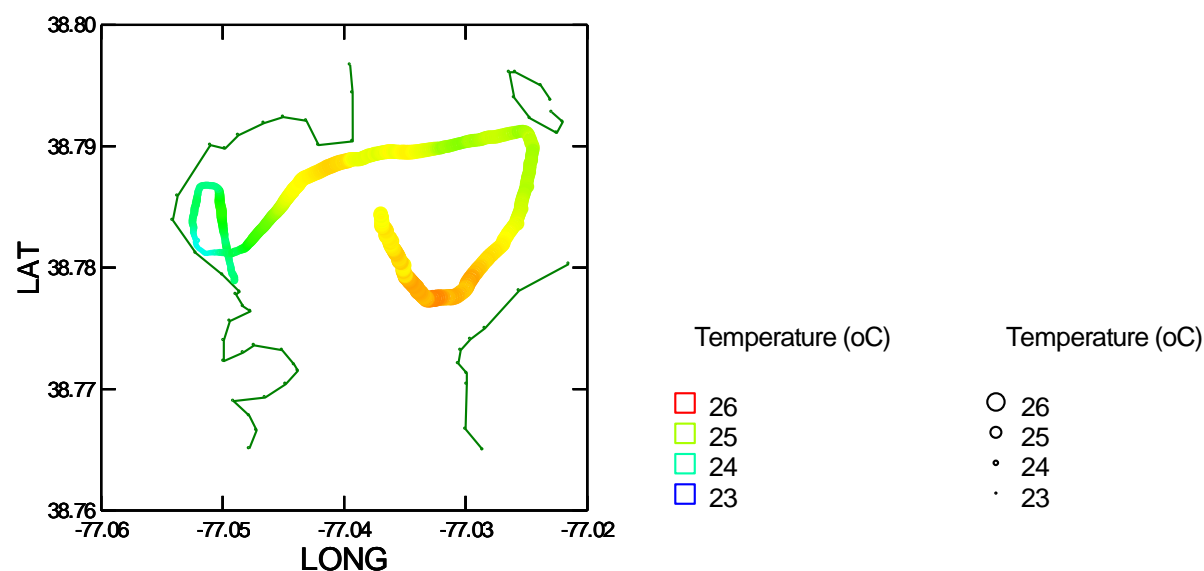


Figure 6b. Water Quality Mapping. August 7, 2013. Temperature (°C).

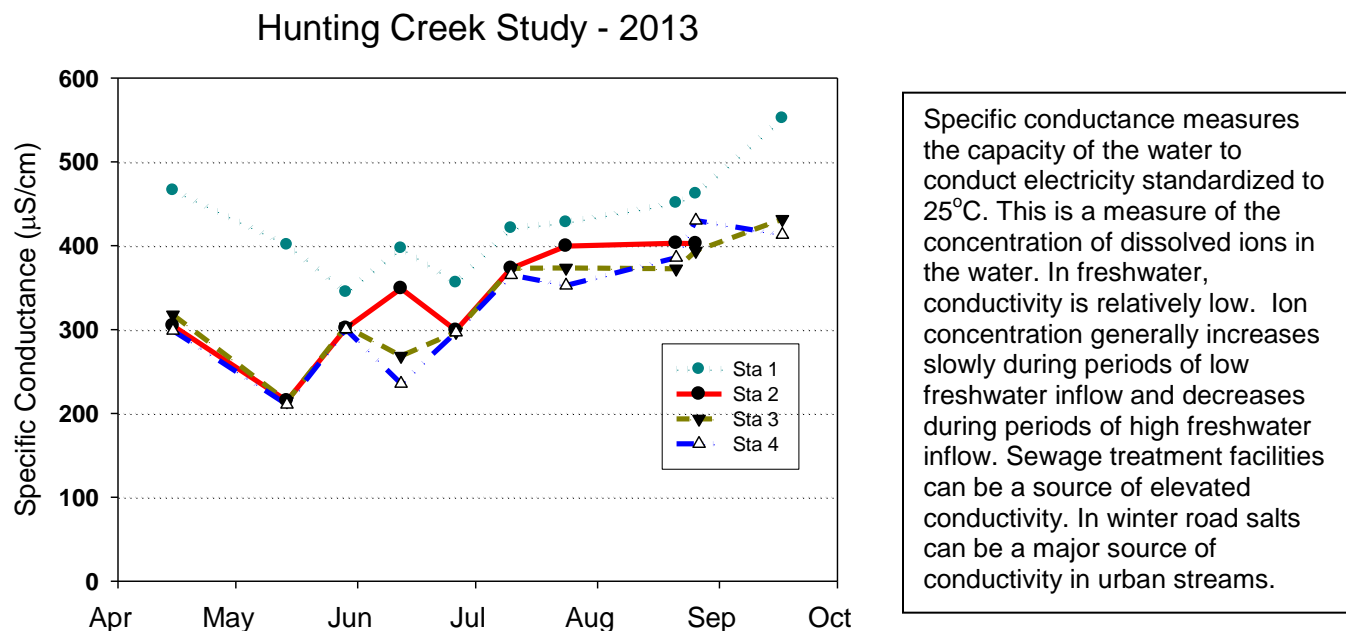


Figure 7. Specific Conductance ($\mu\text{S}/\text{cm}$). GMU Field Data. Month tick is at first day of month.

During most of 2013, specific conductance (Figure 7) exhibited similar seasonal patterns over the entire study area. Conductance decreased at Sta 1 and remained rather stable at the other stations during the period from April through late June. This was at least partially due to the high flows in June. July through September found a steady increase. Values were always higher at Sta 1 in keeping with its proximity to the AlexRenew outfall. Chloride exhibited a similar pattern (Figure 8), with higher values at Sta 1.

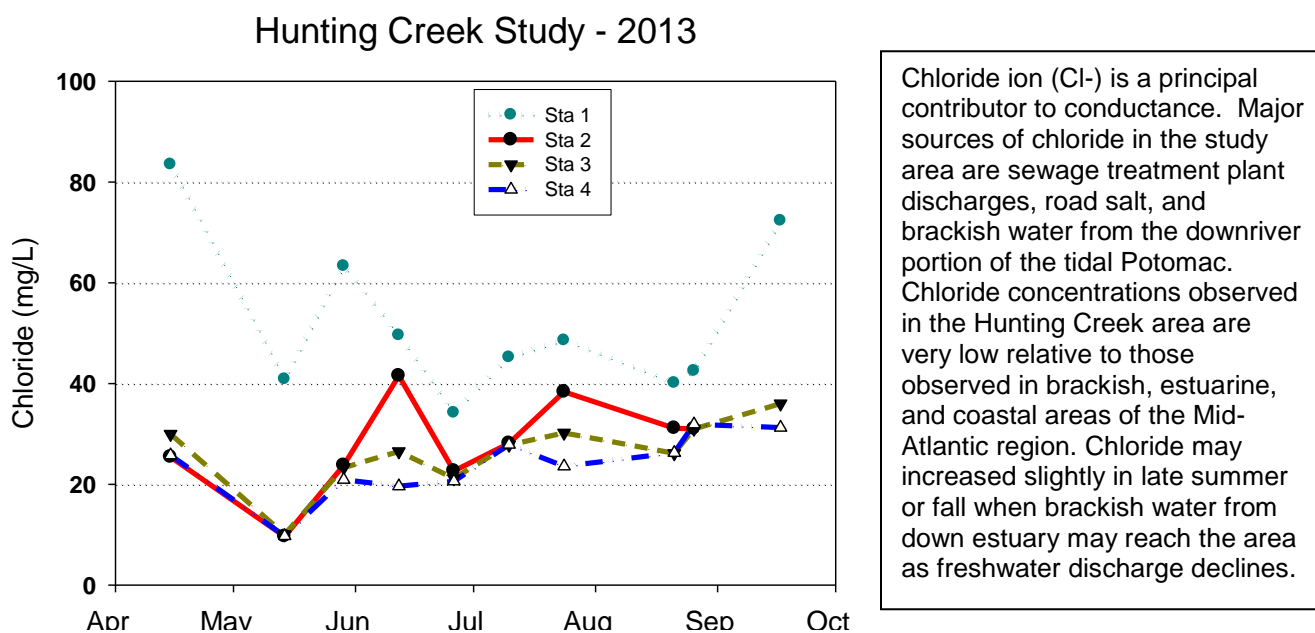


Figure 8. Chloride (mg/L). Alexandria Renew Lab Data. Month tick is at first day of month.

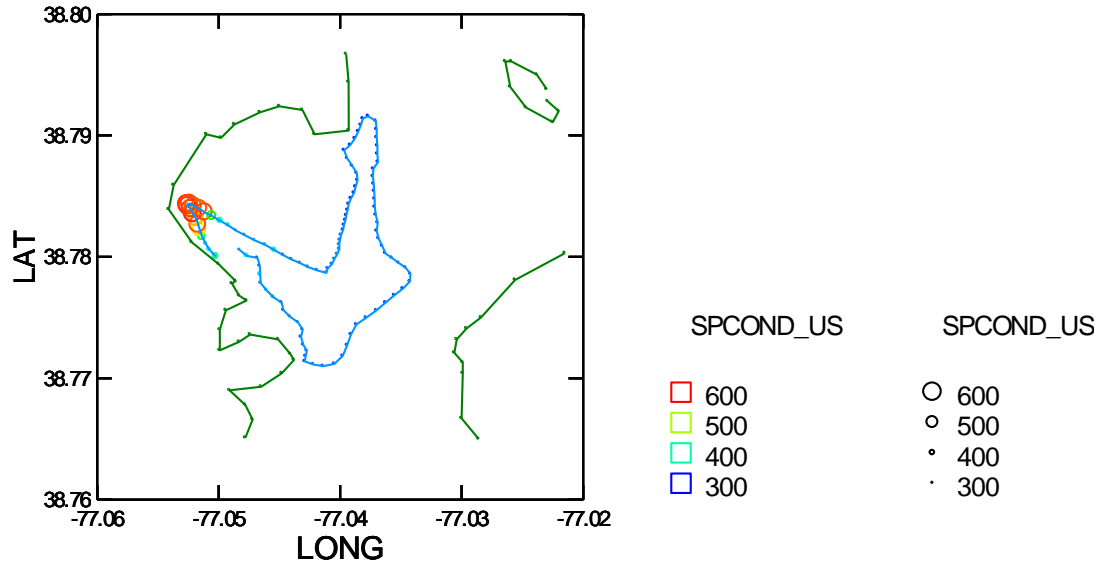


Figure 9a. Water Quality Mapping. June 14, 2013. Specific conductance (μS).

Mapping of specific conductance identified two gradients. In the June sampling there was a clear increase as the monitoring vessel moved from the Belhaven marina north along the Hunting Creek shoreline toward the area where Cameron Run enters the embayment (Figure 9a). This was probably related to elevated levels of ions in Cameron Run from the Alexandria Renew effluent that comes in just upstream. Conductivity levels went from the ambient river values of about 300 μS to near 600 μS . On August 7 this area of elevated conductance was again observed, but values were only slightly elevated (Figure 9b). An additional area of elevated values was found on the Maryland side of the channel with values nearing 420 μS . This elevated area was probably due to Blue Plains effluent concentrating along the Maryland shoreline. None of the elevated levels observed in this study were large enough to negatively affect the biota.

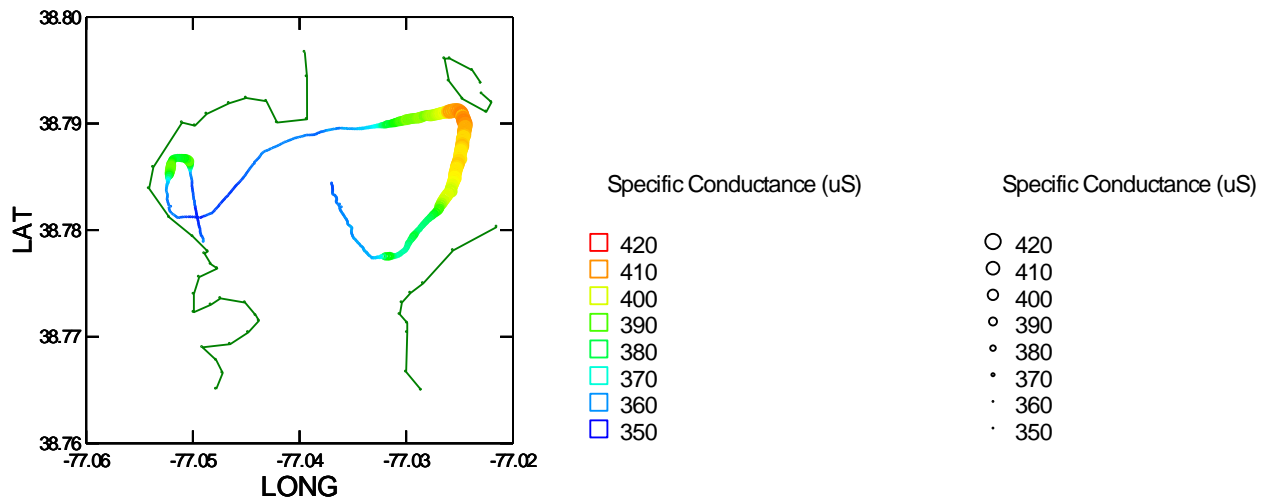


Figure 9b. Water Quality Mapping. August 7, 2013. Specific conductance (μS).

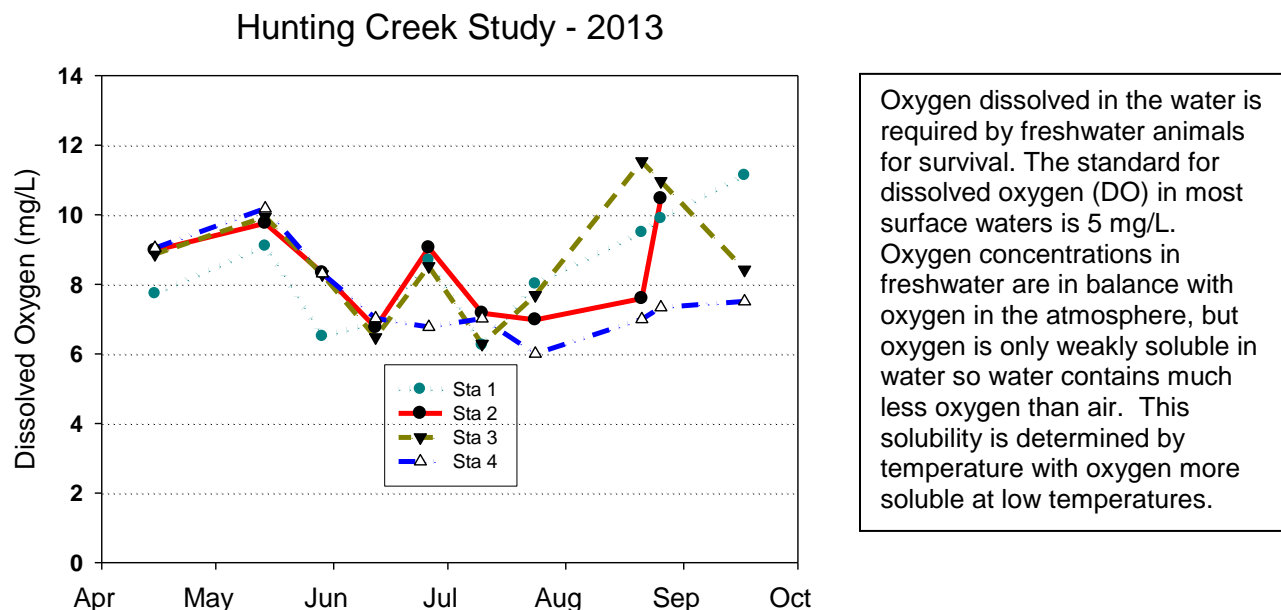


Figure 10. Dissolved Oxygen (mg/L). GMU Field Data. Month tick is at first day of month.

From April through early June dissolved oxygen values followed similar patterns at all stations (Figure 10). Beginning in late June and continuing through the rest of the year, there was a lot more divergence in values. At the river station (Sta. 4) values were fairly constant at about 7 mg/L. Station 3 exhibited the highest values and all three Hunting Cr stations were high in August. Looking at DO as percent saturation (Figure 11) revealed that for the period from April through June, values were at or slightly below saturation. However in August percent saturation was substantially higher suggesting that photosynthesis by algae and SAV was strongly active. All values reported here were instantaneous values at the time of collection.

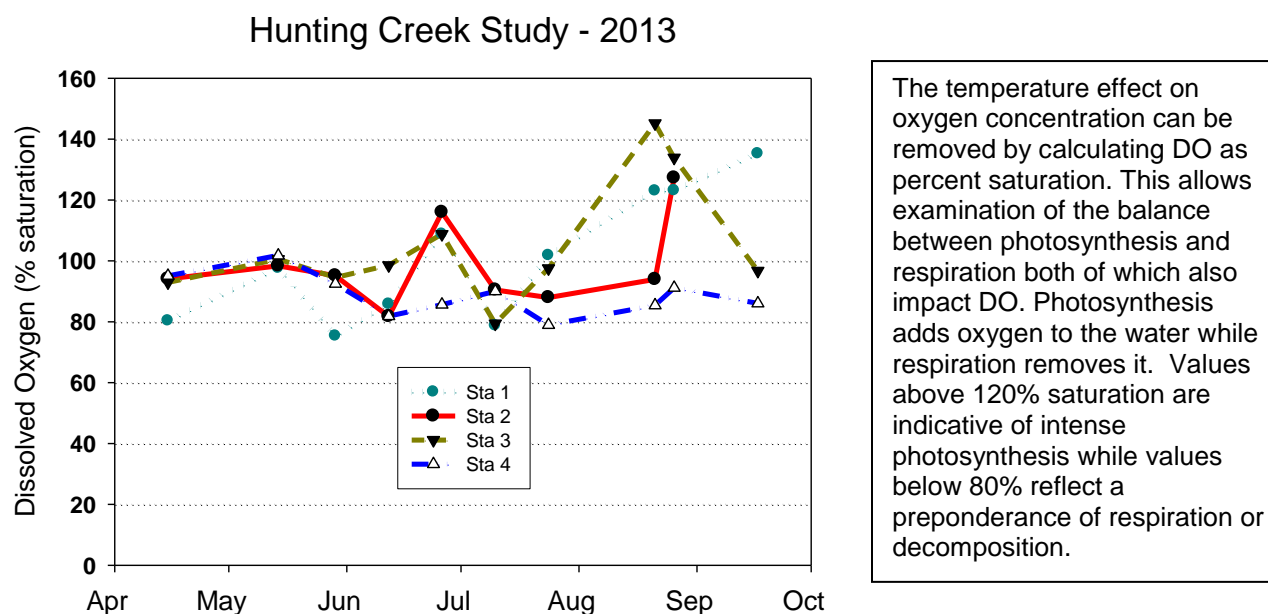


Figure 11. Dissolved Oxygen (% saturation). GMU Field Data. Month tick is at first day.

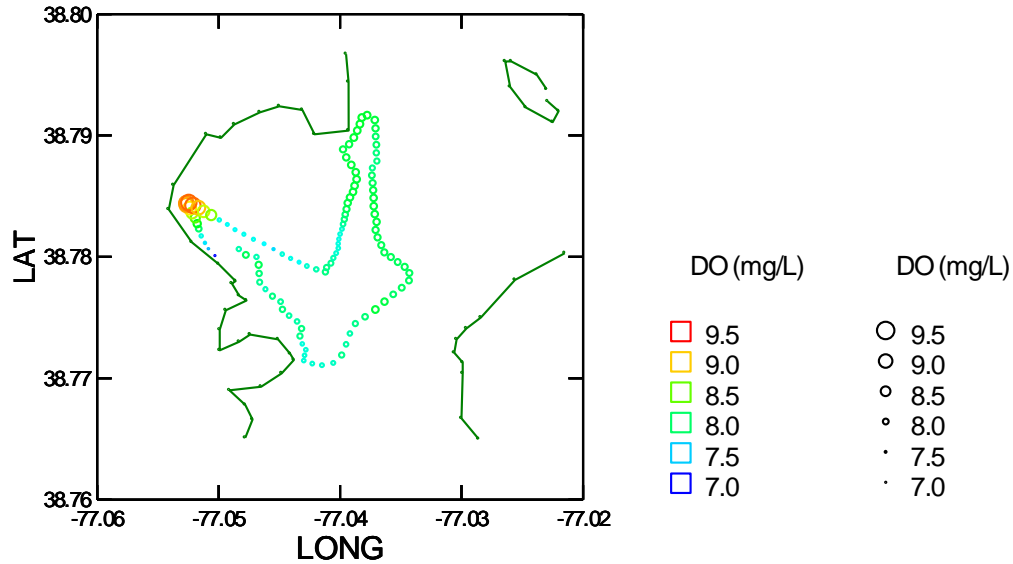


Figure 12a. Water Quality Mapping. June 14, 2013. Dissolved oxygen (mg/L).

On the June mapping cruise, dissolved oxygen values were in the 7.0-9.5 mg/L range which translates into 70-110 percent saturation (Figures 12a & 12b). The highest values were clearly observed at the northern end of the Hunting Creek nearshore transect indicating significant photosynthetic activity from phytoplankton or SAV. Due to low water it was not possible to get any closer to Cameron Run inlet, but these higher readings could have persisted or intensified further north toward the inlet. In the outer part of Hunting Creek and out into the river values were fairly uniform in the 7.5-8.5 mg/L range (80-90% saturation).

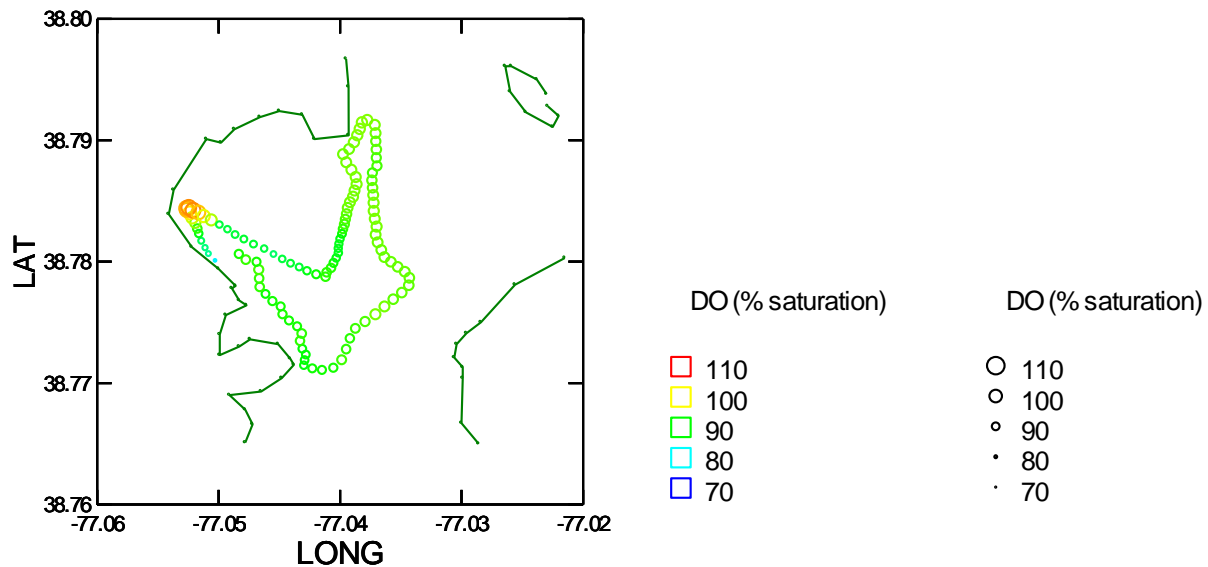


Figure 12b. Water Quality Mapping. June 14, 2013. Dissolved oxygen (percent saturation).

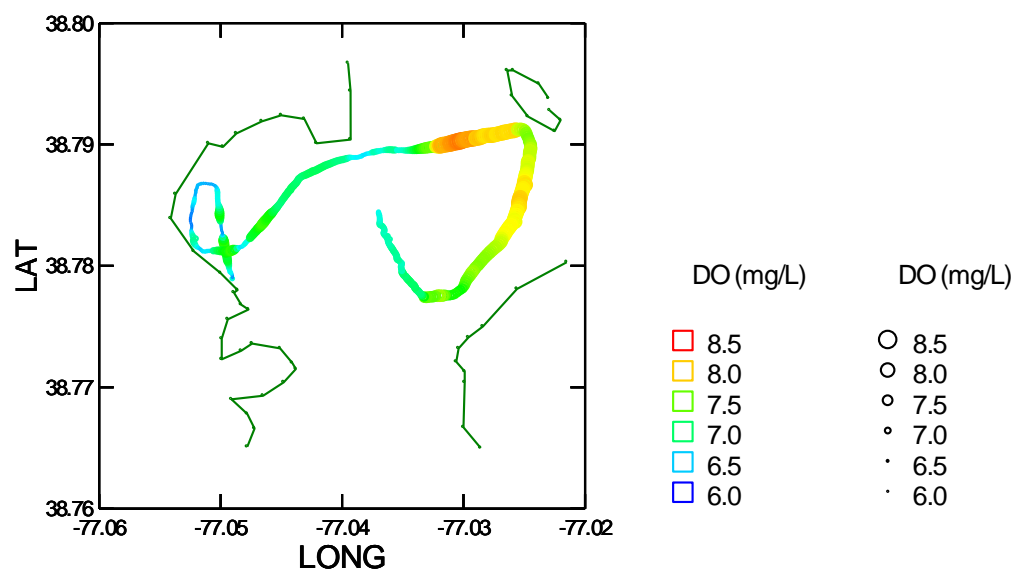


Figure 13a. Water Quality Mapping. August 7, 2013. Dissolved oxygen (mg/L).

On August 7 the mapping cruise found moderate values of dissolved oxygen in the Hunting Creek embayment and higher values in the river channel (Figures 13a & 13b). Values in the channel area were at or very near saturation whereas they were substantially below saturation in the embayment. While there was clearly a difference in dissolved oxygen between the two areas, part of the difference in percent saturation was due to the cooler water in the embayment. Cooler water can hold more oxygen, increasing the value in the denominator of the percentage equation.

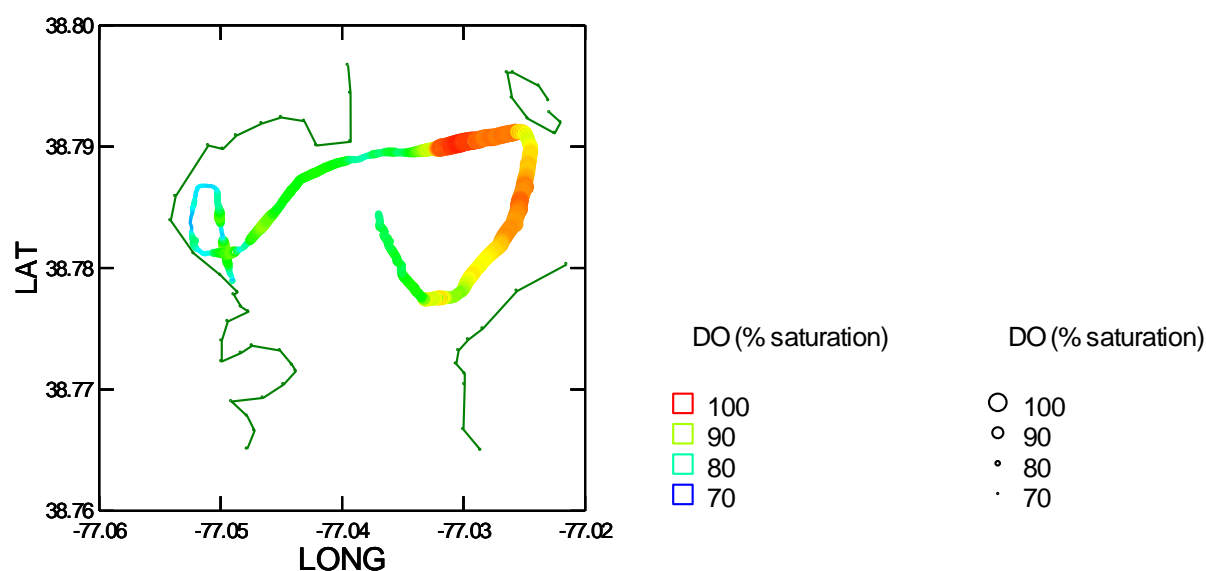


Figure 13b. Water Quality Mapping. August 7, 2013. Dissolved oxygen (percent saturation).

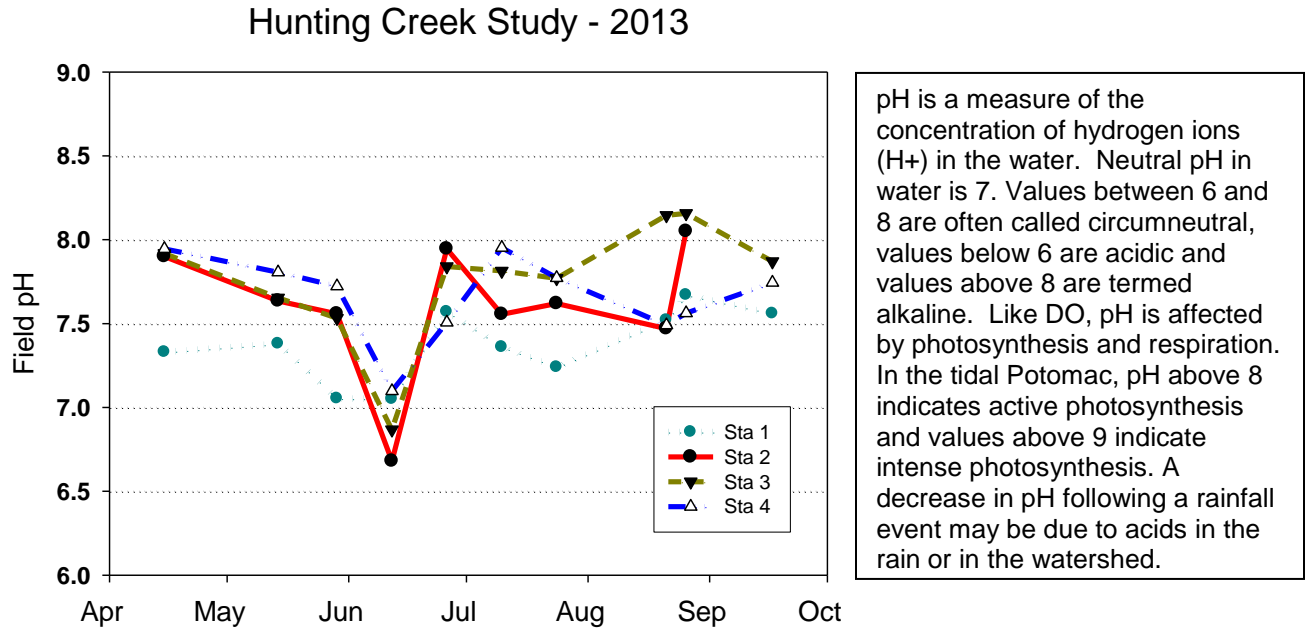


Figure 14. pH. GMU Field Data. Month tick is at first day of month.

Field pH at Sta. 1 basically did not change much during the whole study period being 7.0-7.6. At the other stations pH was generally similar from April through late June exhibiting a major decline in early June in samples collected immediately after a period of large runoff volumes (Figure 14). By the following date, pH had rebounded at these stations and peaked above 8 at Sta. 2 and Sta. 3 in late August. Lab pH was more uniform among the stations (Figure 15). The minimum was still found in early June and Sta. 3 still exceeded 8 in late August.

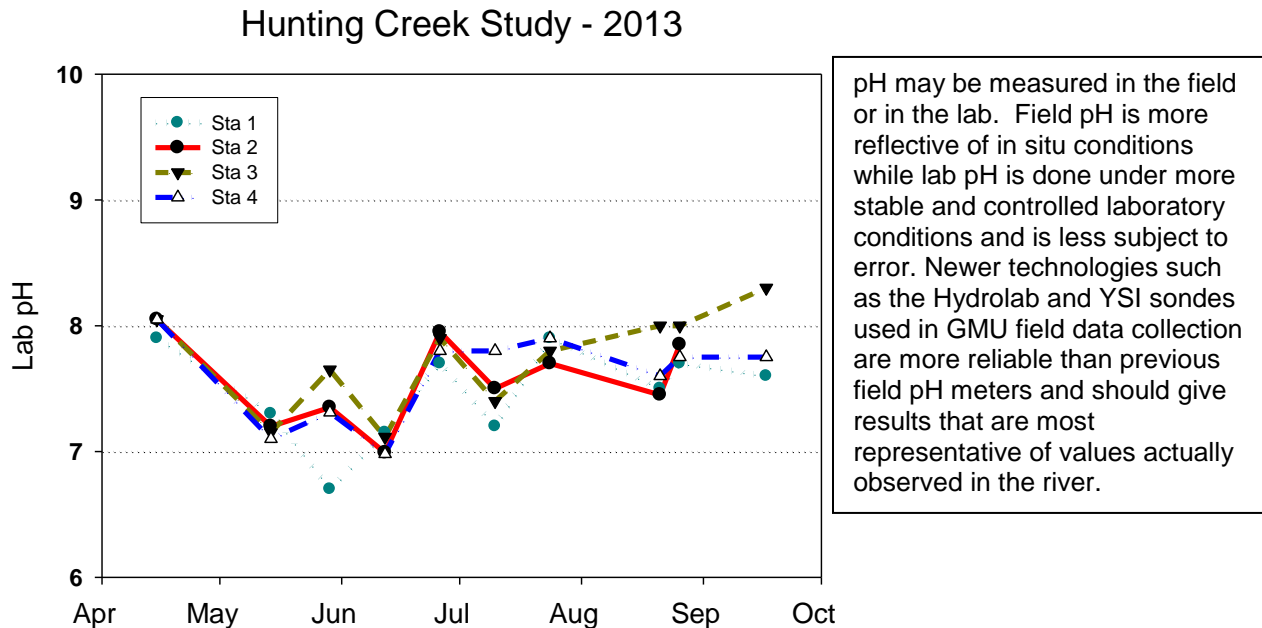


Figure 15. pH. AlexRenew Lab Data. Month tick is at first day of month.

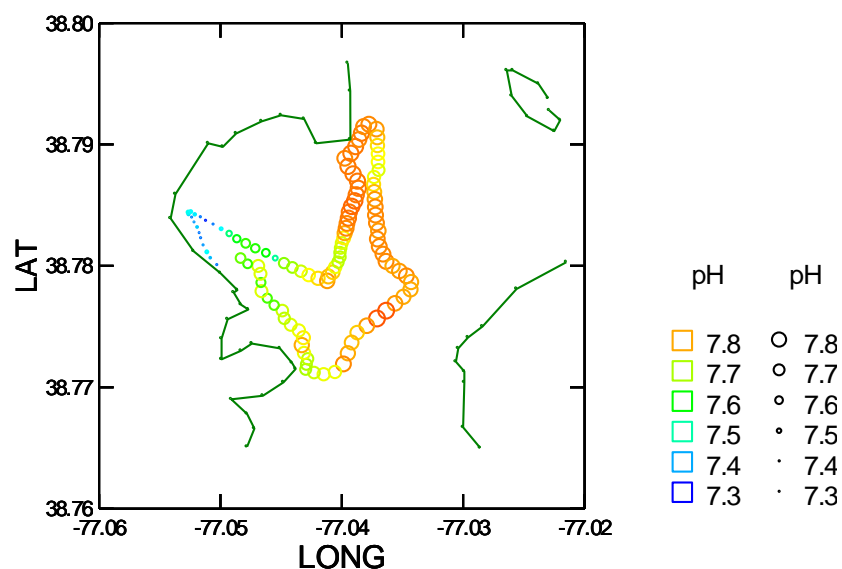


Figure 16a. Water Quality Mapping. June 14, 2013. pH.

In the June cruise pH was lowest in upper Hunting Creek at about 7.3 and gradually increased moving out into the river channel area where values were up to 7.8 (Figure 16a). This was a little surprising given the elevated dissolved oxygen and chlorophyll found in upper Hunting Creek on this date, both of which point to higher photosynthetic activity which should have increased pH. In the August cruise pH was more variable in Hunting Creek with values ranging from 7.4 to nearly 7.8 at various locations (Figure 16b). In outer Hunting Creek pH was fairly constant at around 7.6, but then ramped up noticeably in mid channel to 7.8 before declining markedly on the Maryland side. Interestingly, the southern transect across the river did not show a strong increase. Of course all of the values here are in a relatively narrow range.

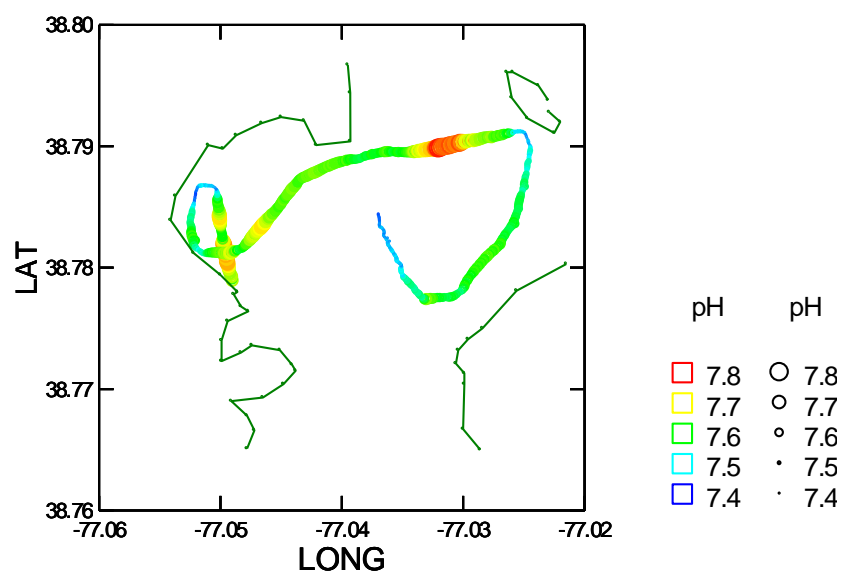


Figure 16b. Water Quality Mapping. August 7, 2013. pH.

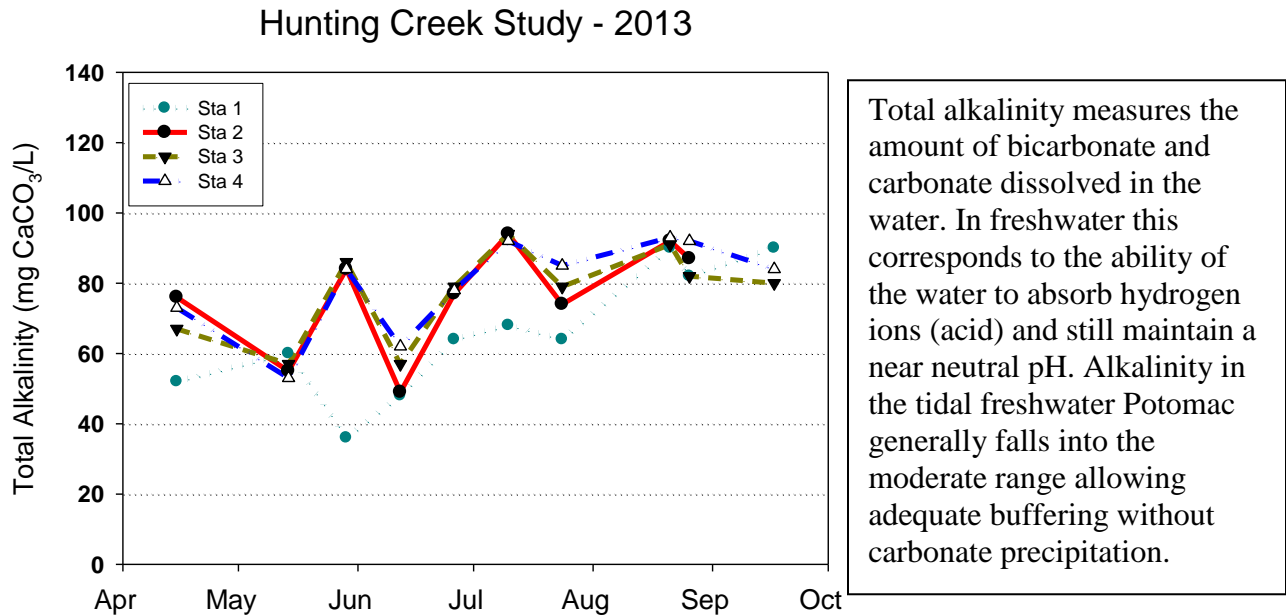


Figure 17. Total Alkalinity (mg/L as CaCO_3). AlexRenew Lab data. Month tick is at first day.

Total alkalinity was generally slightly lower at Sta. 1 than at the other stations which were very similar in their seasonal patterns (Figure 17). The effects of the early June flow event are clear in the decline in alkalinity (dilution). Water clarity as reflected by Secchi disk depth was generally higher in the river although values at all stations were very similar in the late May through July period (Figure 18). Water clarity improved in August and September, but actual Secchi Disk measurements were impeded by SAV and by having the disk reach bottom and still be visible. The values observed here are typical of those found in other part of the tidal Potomac River.

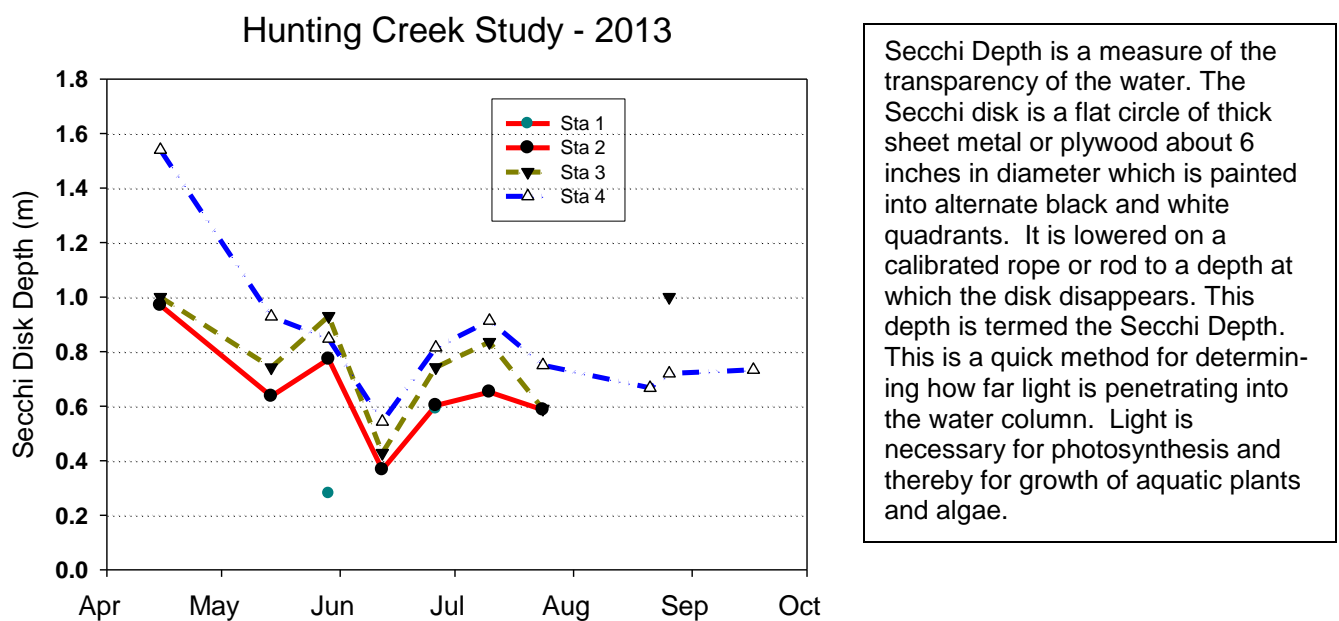


Figure 18. Secchi Disk Depth (m). GMU Field Data. Month tick is at first day of month.

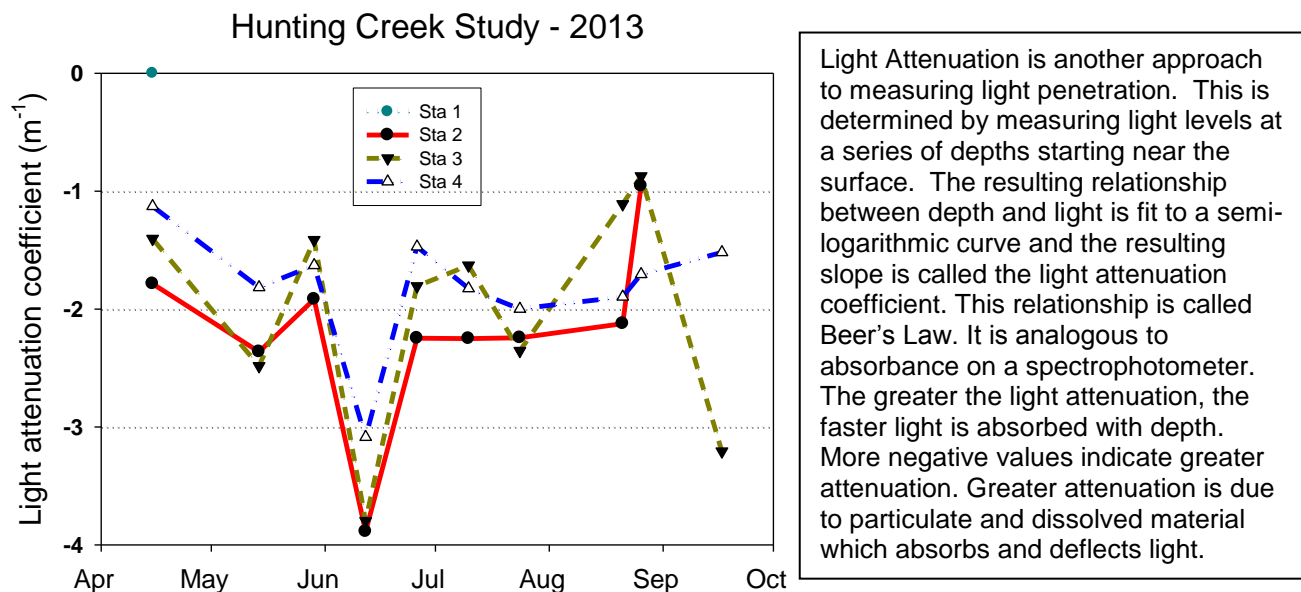


Figure 19. Light Attenuation Coefficient (m^{-1}). GMU Field Data. Month tick is at first day of month.

Light attenuation coefficient data generally fell in the range -1.0 to -3.0 m^{-1} (Figure 19). A major exception to this occurred in early June when values plunged due to the particulate matter brought in by the strong rains at this time. Another drop was found in September at Sta. 3 perhaps due to the low tide that day. Turbidity followed similar patterns with a clear increase in mid June corresponding to the strong rainfall and subsequent runoff events. Turbidity also showed an increase in September (Figure 20).

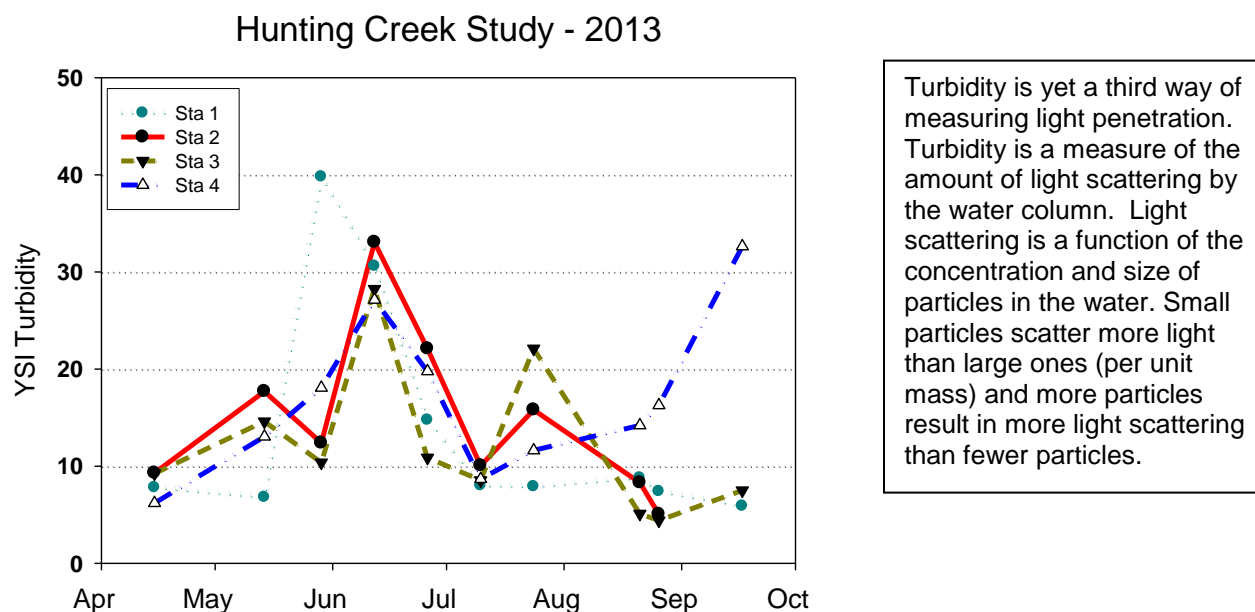


Figure 20. Turbidity (NTU). GMU Lab Data. Month tick is at first day of month.

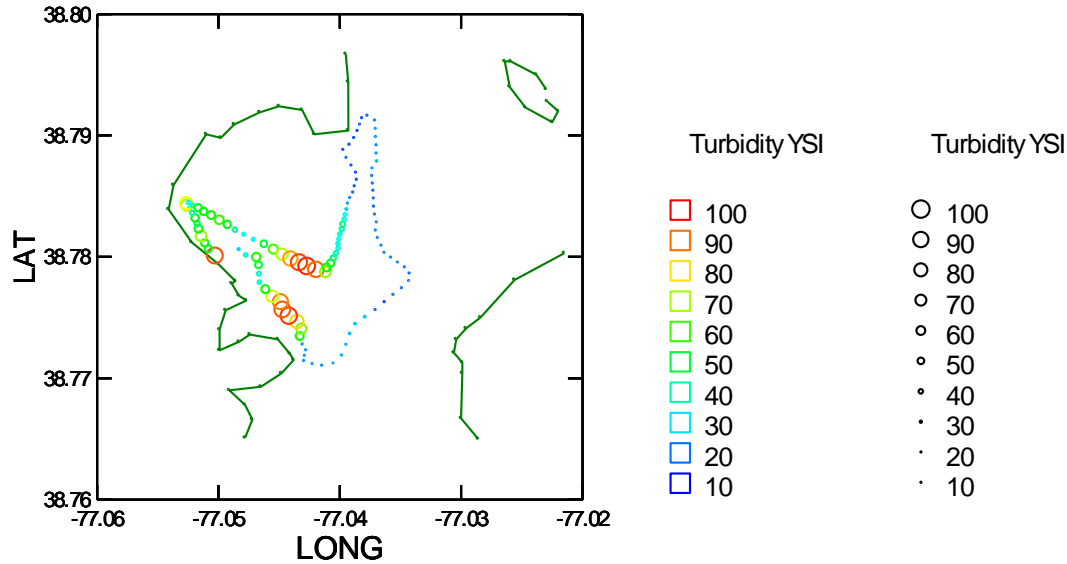


Figure 21a. Water Quality Mapping. June 14, 2013. Turbidity YSI.

On the June mapping cruise there was a marked difference in turbidity levels and variability between Hunting Creek and the Potomac mainstem (Figure 21a). Within Hunting Creek values fluctuated between 40 and 100 NTU. There was an isolated hot spot near the beginning of the transects and then there appeared to be a north-south band of high turbidity that intersected two transects near the mouth of Hunting Creek. Moving out of Hunting Creek into the river channel, turbidity dropped off rapidly to around 10 NTU. In August turbidity was lower over the whole area generally less than 15 NTU (Figure 21b). The lowest values were actually observed in Hunting Creek. Isolated values at or just above 15 NTU were found in a number of areas, but no substantial hot spots were apparent.

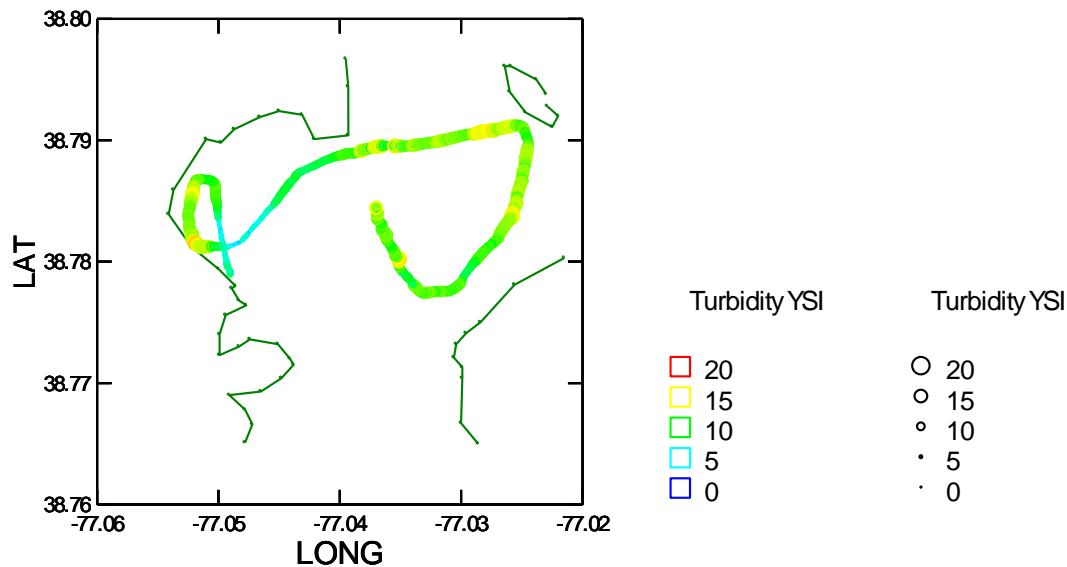


Figure 21b. Water Quality Mapping. August 7, 2013. Turbidity YSI.

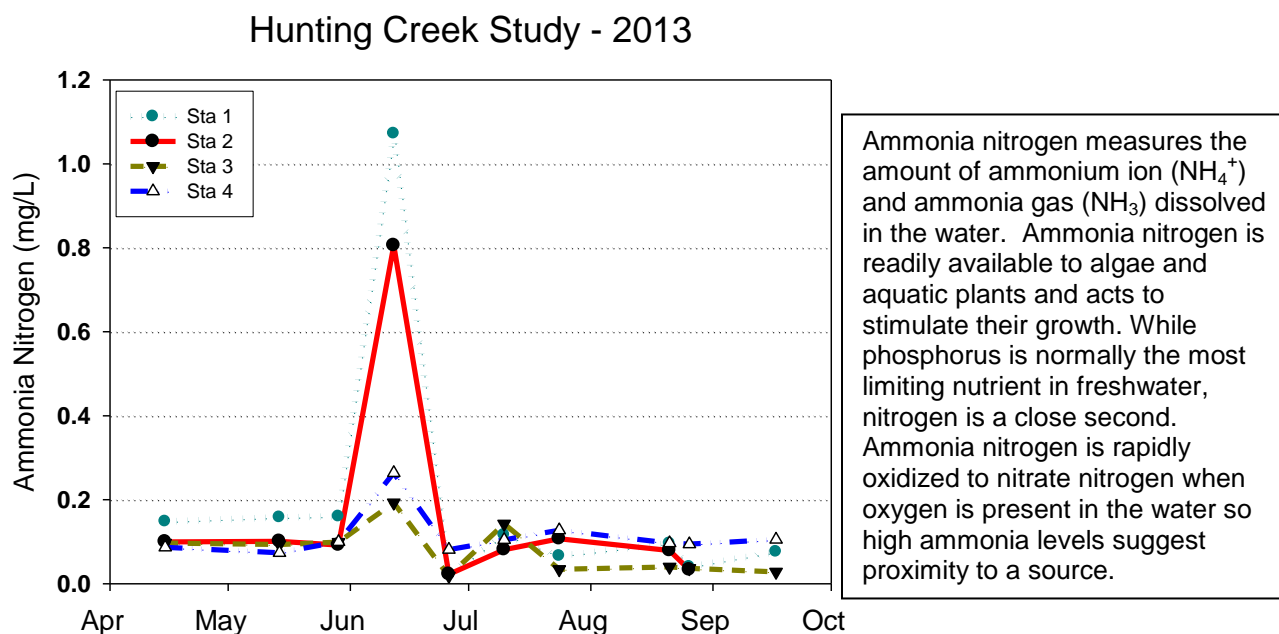


Figure 22. Ammonia Nitrogen (mg/L). AlexRenew Lab Data. Month tick is at first day of month.

Ammonia nitrogen was consistently low (<0.2 mg/L) at all stations for most of the study period (Figure 22). The exception were at Sta 1 and Sta 2 during the June flow event suggesting a source of ammonia mobilized by the rain event and entering upper Hunting Creek. Nitrate nitrogen levels were generally rather consistent at Sta. 3 and Sta. 4 at about 0.5-1.0 mg/L through the study period (Figure 23). Values were much more variable at Sta. 1 and Sta. 2 presumably related to varying inputs entering upper Hunting Creek.

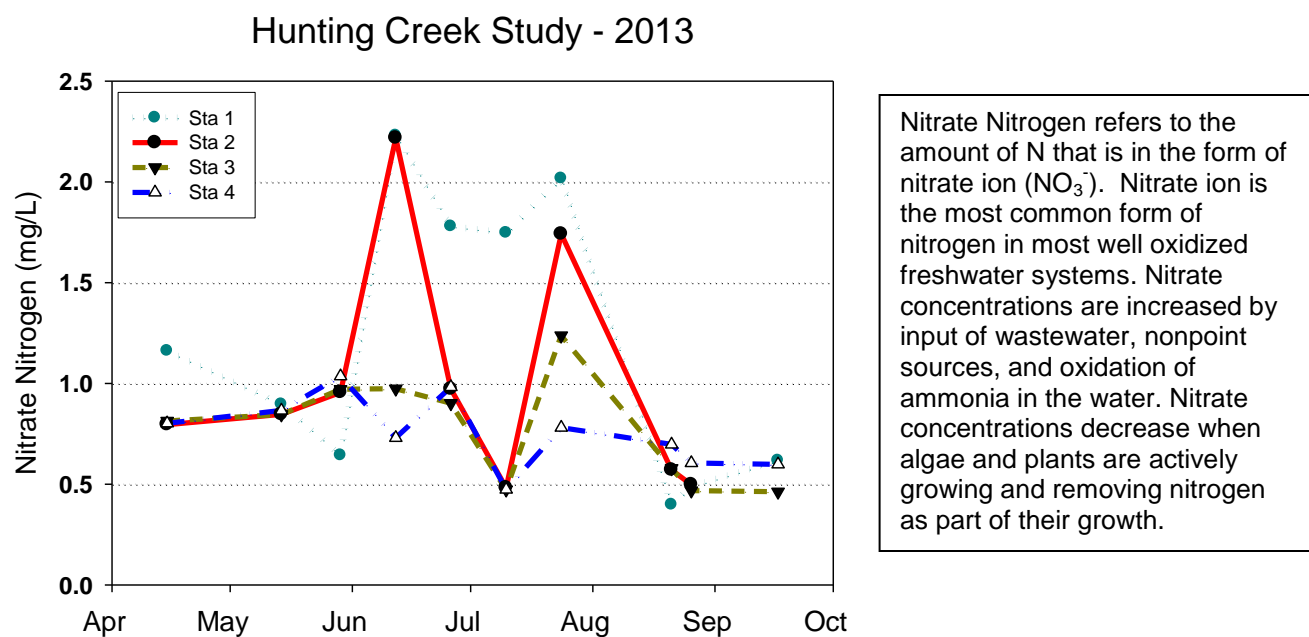


Figure 23. Nitrate Nitrogen (mg/L). AlexRenew Lab Data. Month tick is at first day of month.

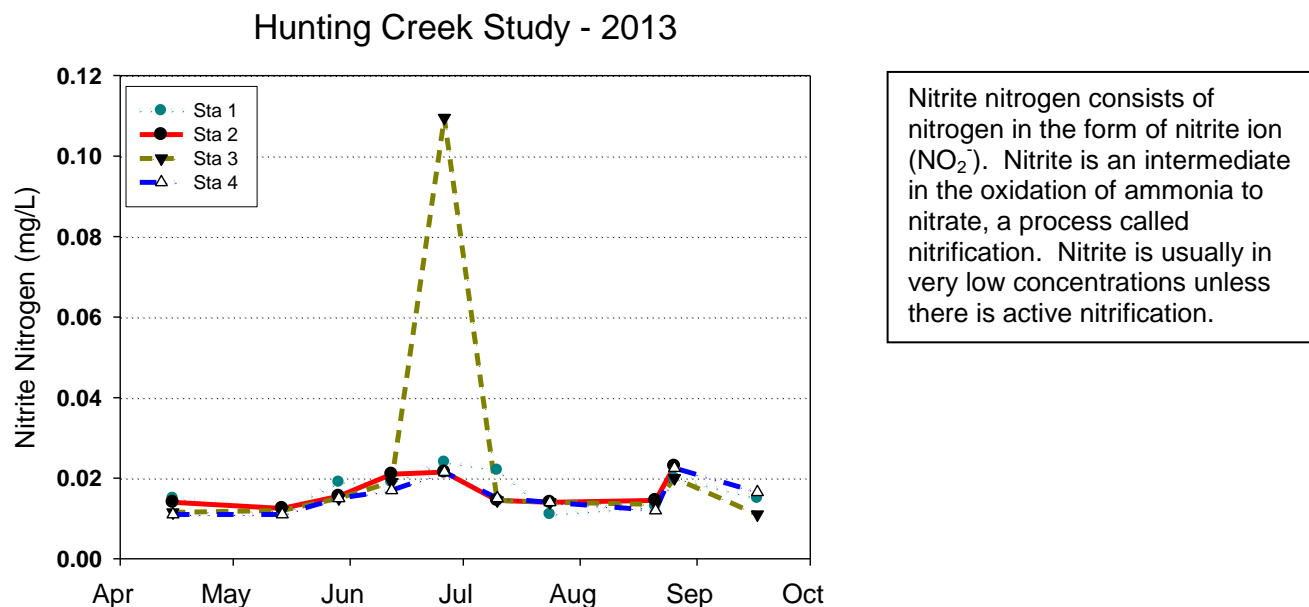


Figure 24. Nitrite Nitrogen (mg/L). AlexRenew Lab Data. Month tick is at first day of month.

In particular summer values were quite high at these two stations. Nitrite nitrogen remained low throughout the year with on exception in late June at Sta. 3 (Figure 24). Organic nitrogen exhibited a gradual increase over the study period at all stations reaching a maximum in late July or August (Figure 25). The highest values were observed at Sta. 1. As with many parameters, changes at Sta 4 (river) were much more gradual than at the embayment stations.

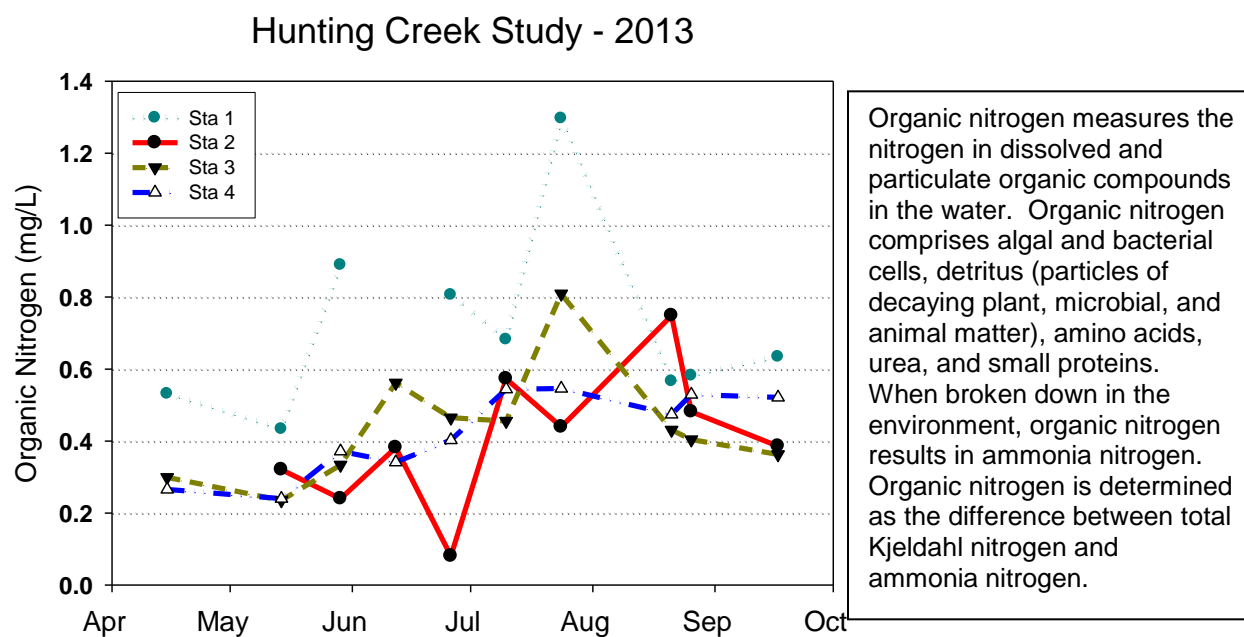
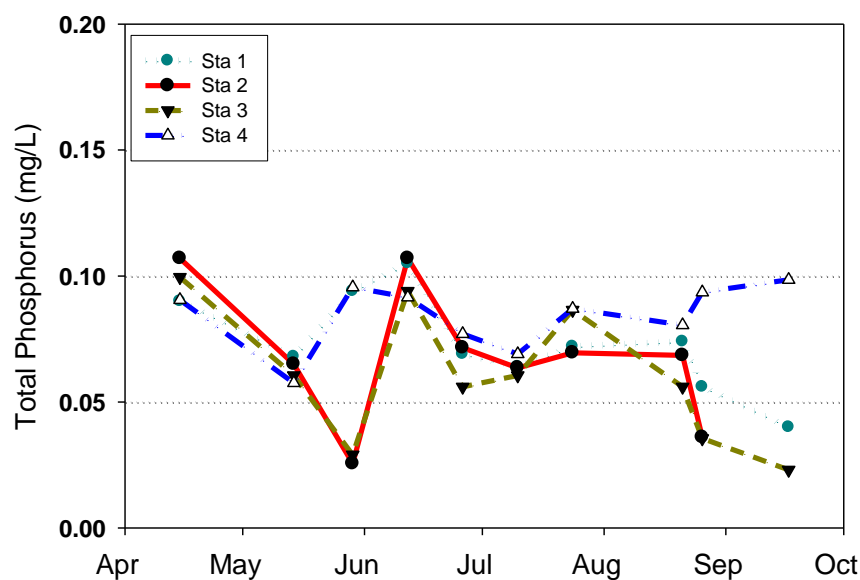


Figure 25. Organic Nitrogen (mg/L). AlexRenew Lab Data. Month tick is at first day of month.

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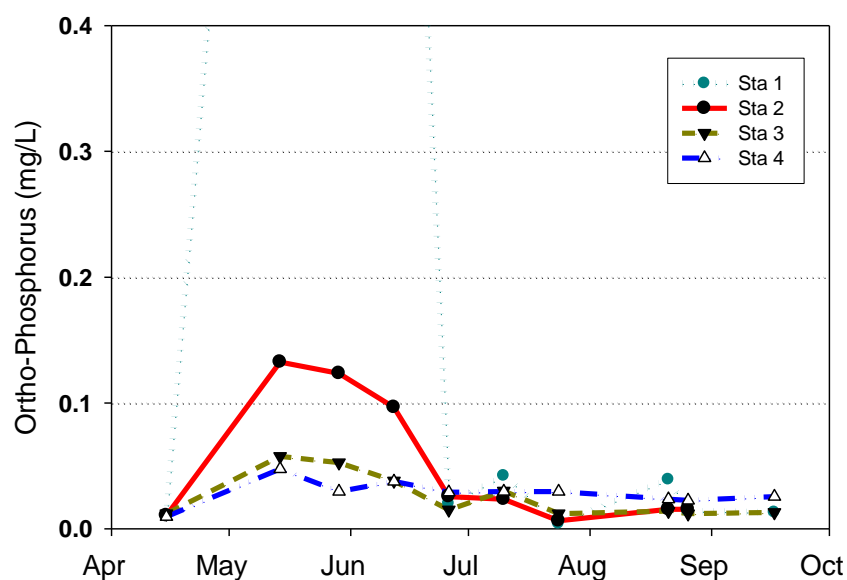


Phosphorus (P) is often the limiting nutrient in freshwater ecosystems. As such the concentration of P can set the upper limit for algal growth. Total phosphorus is the best measure of P availability in freshwater since much of the P is tied up in biological tissue such as algal cells. Total P includes phosphate ion (PO_4^{3-}) as well as phosphate inside cells and phosphate bound to inorganic particles such as clays.

Figure 26. Total Phosphorus (mg/L). AlexRenew Lab Data. Month tick is at first day of month.

Total phosphorus quite variable was generally in the range 0.05 to 0.10 mg/L (Figure 26). A marked dip was seen in late May at Sta. 2 and Sta. 3. Soluble reactive phosphorus was consistently higher at Sta. 1 and Sta. 2 during the spring and early summer, but was similar at all stations from July on (Figure 27).

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Soluble reactive phosphorus (SRP) is a measure of phosphate ion (PO_4^{3-}). Phosphate ion is the form in which P is most available to primary producers such as algae and aquatic plants in freshwater. However, SRP is often inversely related to the activity of primary producers because they tend to take it up so rapidly. So, higher levels of SRP indicate either a local source of SRP to the waterbody or limitation by a factor other than P.

Figure 27. Soluble Reactive Phosphorus (mg/L). AlexRenew Lab Data. Month tick is at first day of month.

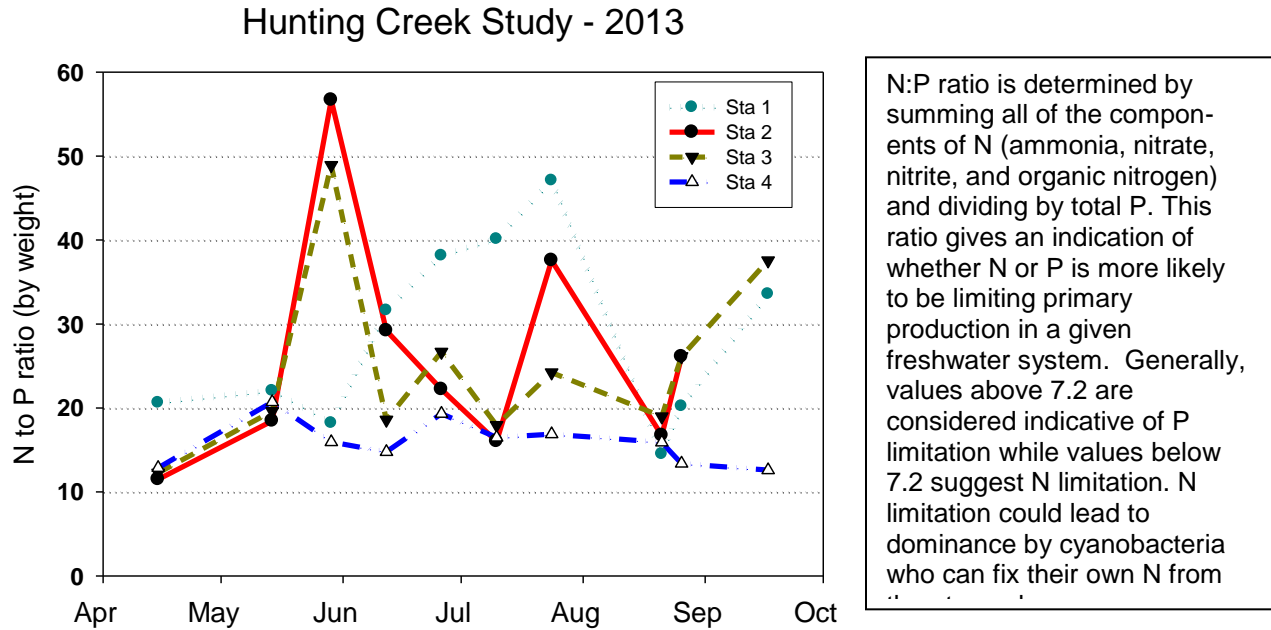


Figure 28. N/P Ratio (by mass). AlexRenew Lab Data. Month tick is at first day of month.

N/P ratio consistently pointed to P limitation. At Sta. 2 and Sta. 3 a similar seasonal pattern was observed at both stations with an increase to high values by late May and a decline through mid summer (Figure 28). The increase at Sta. 1 was more gradual and peaked in late July. Values at the river Sta. 4 were fairly constant through the year. Biochemical oxygen demand (BOD) often 1 or 2 mg/L at all stations (Figure 29). Exceptions to this were most common at Sta. 1 and to a lesser extent at Sta. 2.

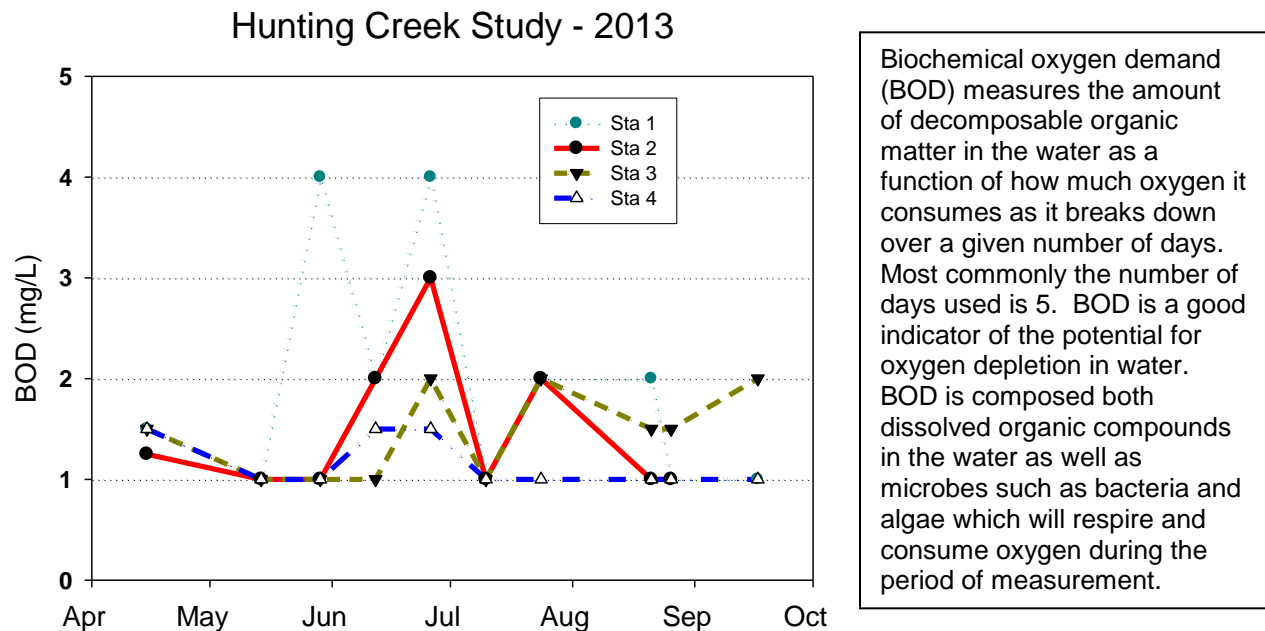


Figure 29. Biochemical Oxygen Demand (mg/L). AlexRenew Lab Data. Month tick is at first day of month.

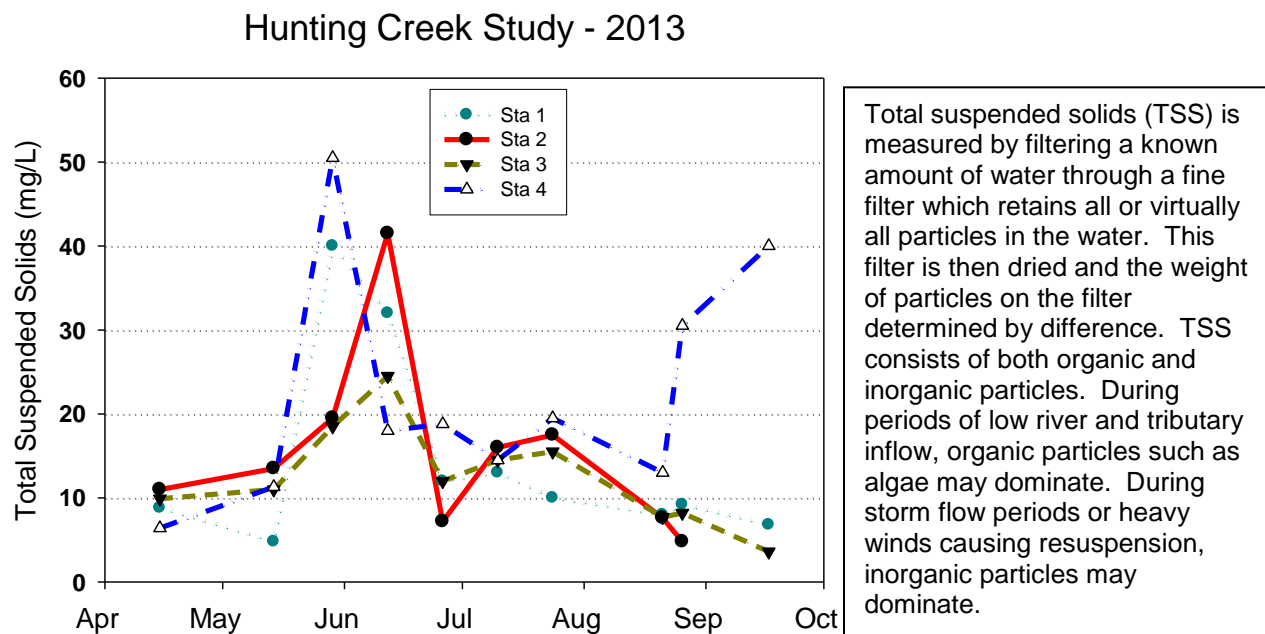


Figure 30. Total Suspended Solids (mg/L). AlexRenew Lab Data. Month tick is at first day of month.

Total suspended solids was generally in the range 5-20 mg/L. (Figure 30). Major exceptions to this were observed at all stations in late May and early June. A further exception was observed at Sta. 4 in September. Volatile suspended solids showed a similar seasonal pattern at lower values (Figure 31).

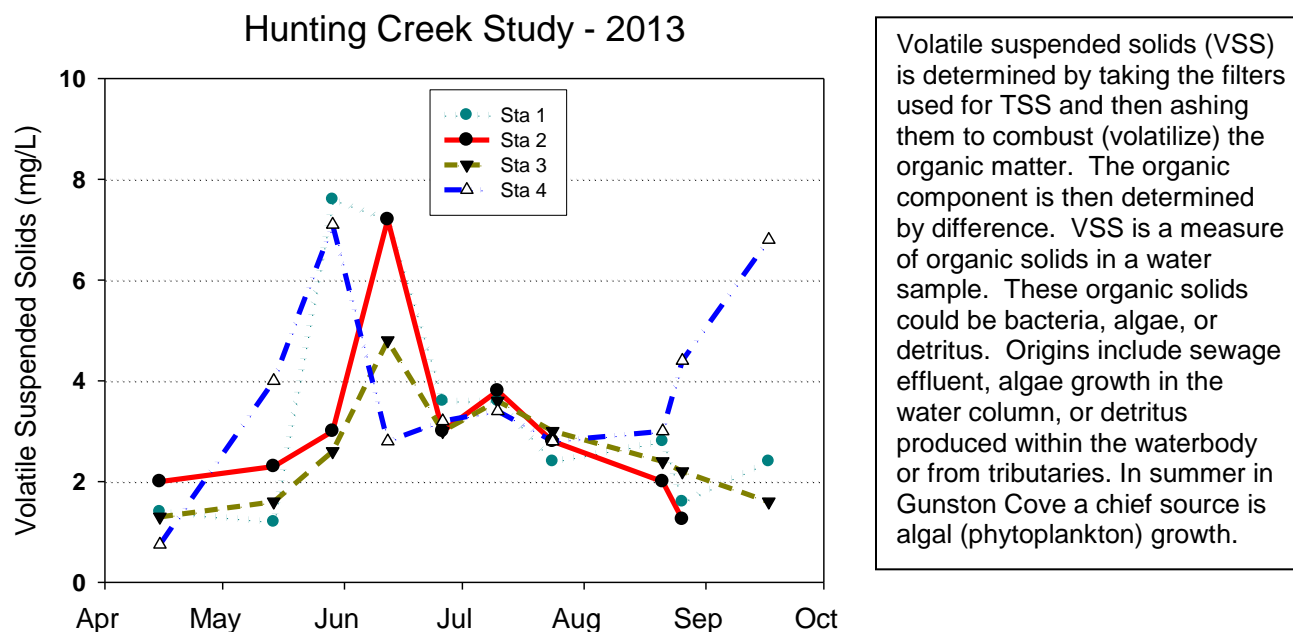
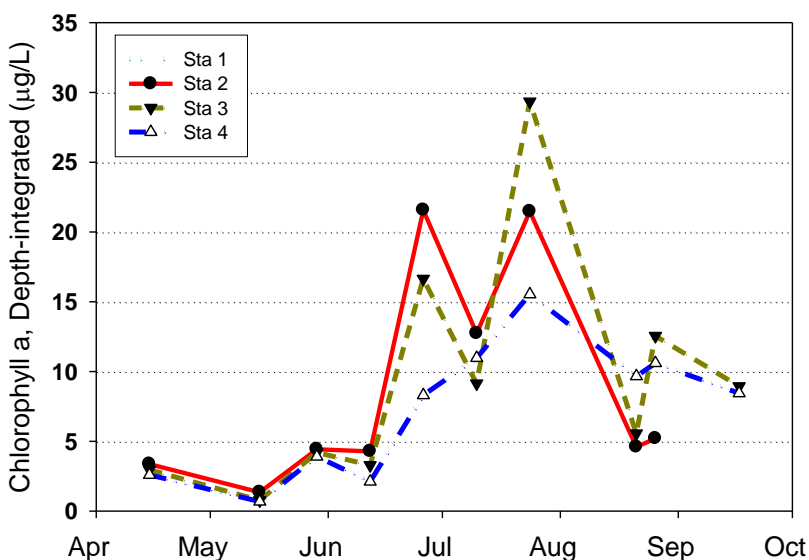


Figure 31. Volatile Suspended Solids (mg/L). AlexRenew Lab Data. Month tick is at first day of month.

C. Phytoplankton -2013

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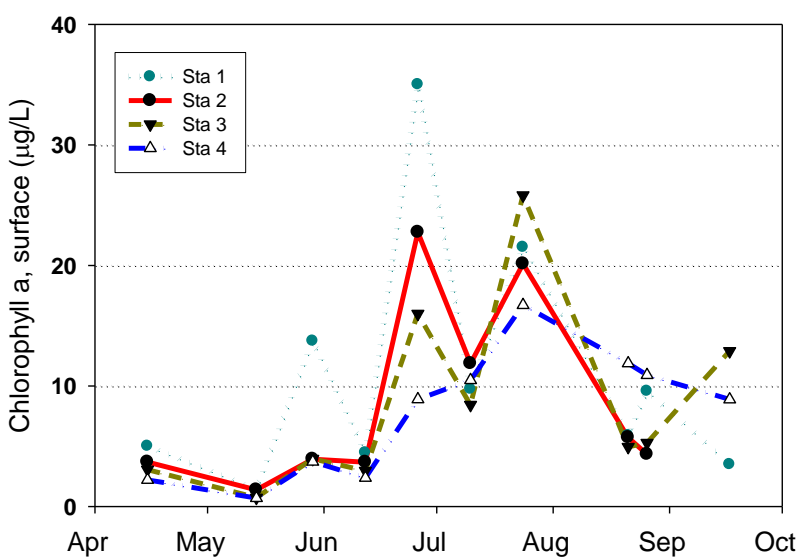


Chlorophyll a is a measure of the amount of algae growing in the water column. These suspended algae are called phytoplankton, meaning "plant wanderers". In addition to the true algae (greens, diatoms, cryptophytes, etc.) the term phytoplankton includes cyanobacteria (sometimes known as "blue-green" algae). Both depth-integrated and surface chlorophyll values are measured due to the capacity of phytoplankton to aggregate near the surface under certain conditions.

Figure 32. Chlorophyll *a* (µg/L). Depth-integrated. GMU Lab Data. Month tick is at the first day of month.

Chlorophyll *a* exhibited a clear seasonal pattern at all stations with values increasing in June and July and decreasing through September (Figure 32). Values increased from about 5 µg/L in the spring to 20-30 µg/L for the summer months at the embayment stations. At the river station (Sta. 4) values peaked at about 15 µg/L. Greater variability was observed at the embayment stations. Sta. 1 values were actually higher on some dates than at other stations (Figure 33). Surface chlorophyll showed similar spatial and temporal patterns, but values were generally slightly lower.

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In the tidal freshwater Potomac generally, there is very little difference in surface and depth-integrated chlorophyll levels because tidal action keeps the water well-mixed which overcomes any potential surface aggregation by the phytoplankton. Summer chlorophyll concentrations above 30 µg/L are generally considered characteristic or eutrophic conditions.

Figure 33. Chlorophyll *a* (µg/L). Surface. GMU Lab Data. Month tick is at first day of month.

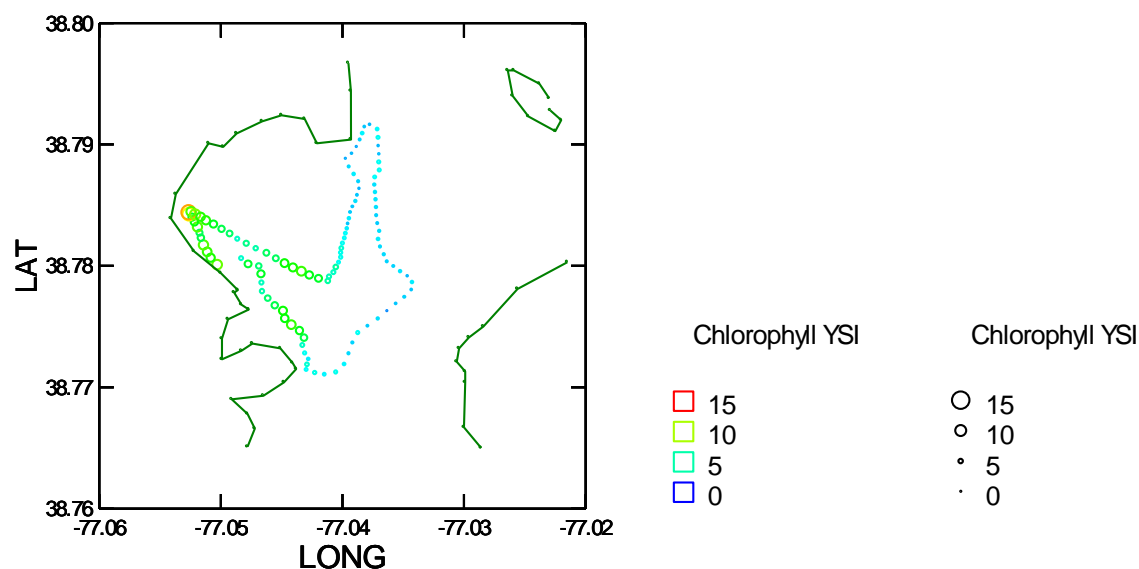


Figure 34a. Water Quality Mapping. June 14, 2013. Chlorophyll YSI (mg/L).

On the June cruise chlorophyll levels were elevated in Hunting Creek relative to the river mainstem (Figure 34a). Values increased steadily from the outer part of Hunting Creek to the most northwesterly point in the cruise path attaining a level of 15 $\mu\text{g/L}$. Values in the river were about 5 $\mu\text{g/L}$. On the August cruise the range of values was similar, but the situation was reversed in that values were generally around 5 $\mu\text{g/L}$ in Hunting Creek and near 15 $\mu\text{g/L}$ in the river channel area (Figure 34b). It should be noted that these values are not directly comparable to those in Figures 32 and 33 above due to methodological differences.

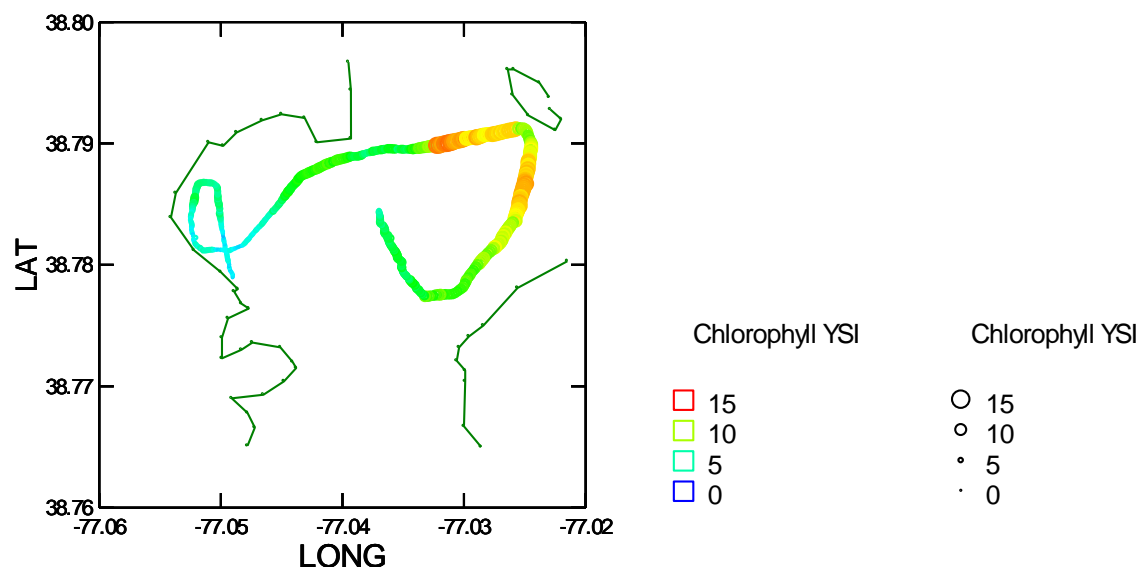
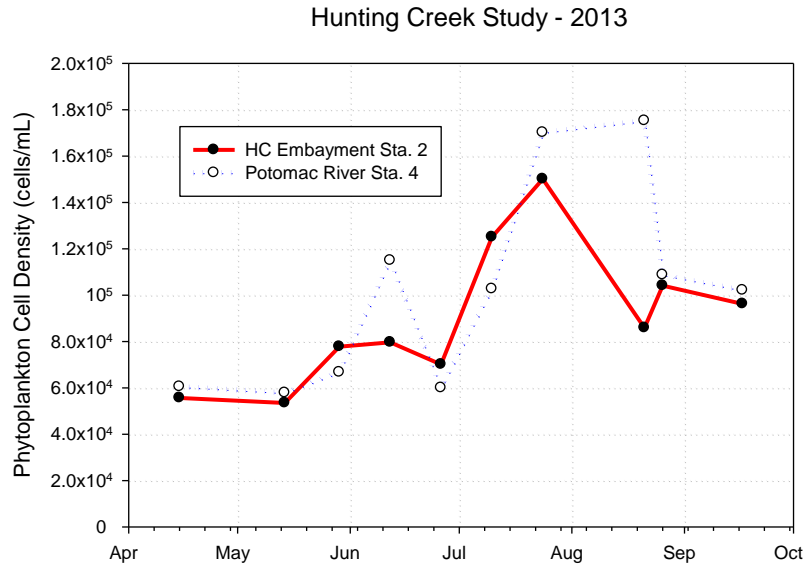


Figure 34b. Water Quality Mapping. August 7, 2013. Chlorophyll YSI (mg/L).



Phytoplankton cell density provides a measure of the number of algal cells per unit volume. This is a rough measure of the abundance of phytoplankton, but does not discriminate between large and small cells. Therefore, a large number of small cells may actually represent less biomass (weight of living tissue) than a smaller number of large cells. However, small cells are typically more active than larger ones so cell density is probably a better indicator of activity than of biomass. The smaller cells are mostly cyanobacteria.

Figure 35. Phytoplankton Density (cells/mL).

Phytoplankton density was generally low from April through May in both embayment and river (Figure 35). At both stations, a clear rise was observed in cell density during July. This rise was sustained into August in the river, but dropped back in the embayment. Total biovolume indicated two maxima at both stations (Figure 36). Cove biovolume was highest in the April sample from the river station. Aside from this unusually high value, there was little seasonal trend in the data. The embayment station was generally somewhat higher and exhibited a net increase over the study period. In the river there were a lot of ups and downs, but no clear seasonal trend.

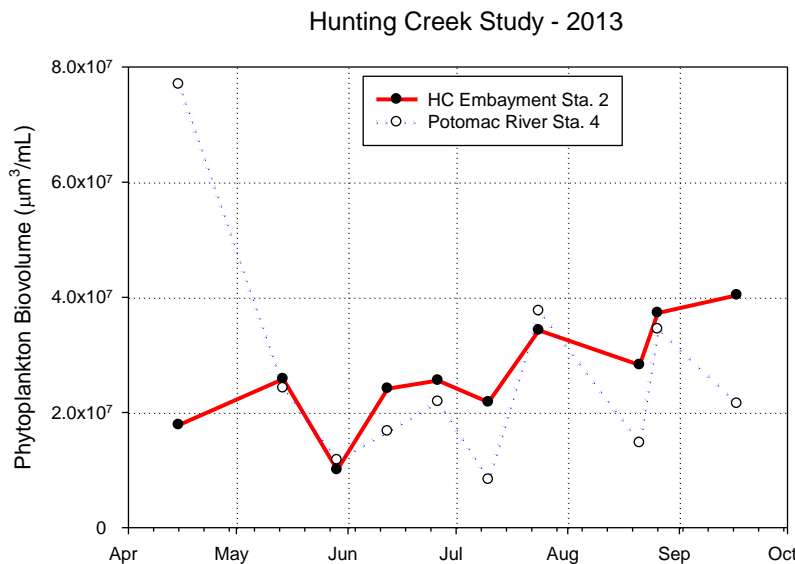


Figure 36. Phytoplankton Biovolume (um³/mL).

The volume of individual cells of each species is determined by approximating the cells of each species to an appropriate geometric shape (e.g. sphere, cylinder, cone, cube, etc.) and then making the measurements of the appropriate dimensions under the microscope. Total phytoplankton biovolume (shown here) is determined by multiplying the cell density of each species by the biovolume of each cell of that species. Biovolume accounts for the differing size of various phytoplankton cells and is probably a better measure of biomass. However, it does not account for the varying amount of water and other nonliving constituents in cells.

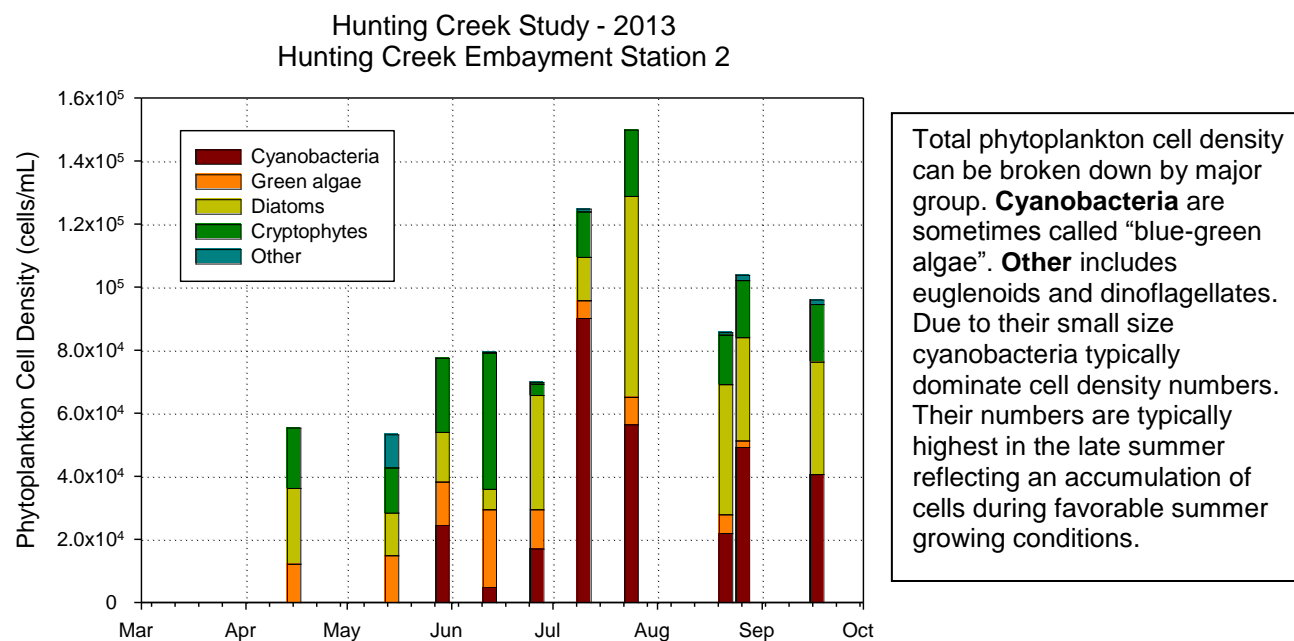


Figure 37. Phytoplankton Density by Major Group (cells/mL). Hunting Creek.

Phytoplankton density in Hunting Creek was fairly evenly divided among the major groups in spring and early summer, but in early July cyanobacteria were clearly dominant. In August and September cyanobacteria and diatoms were co-dominant (Figure 37). At the river station, patterns and densities were roughly similar with cyanobacteria dominance being somewhat more persistent in the fall (Figure 38).

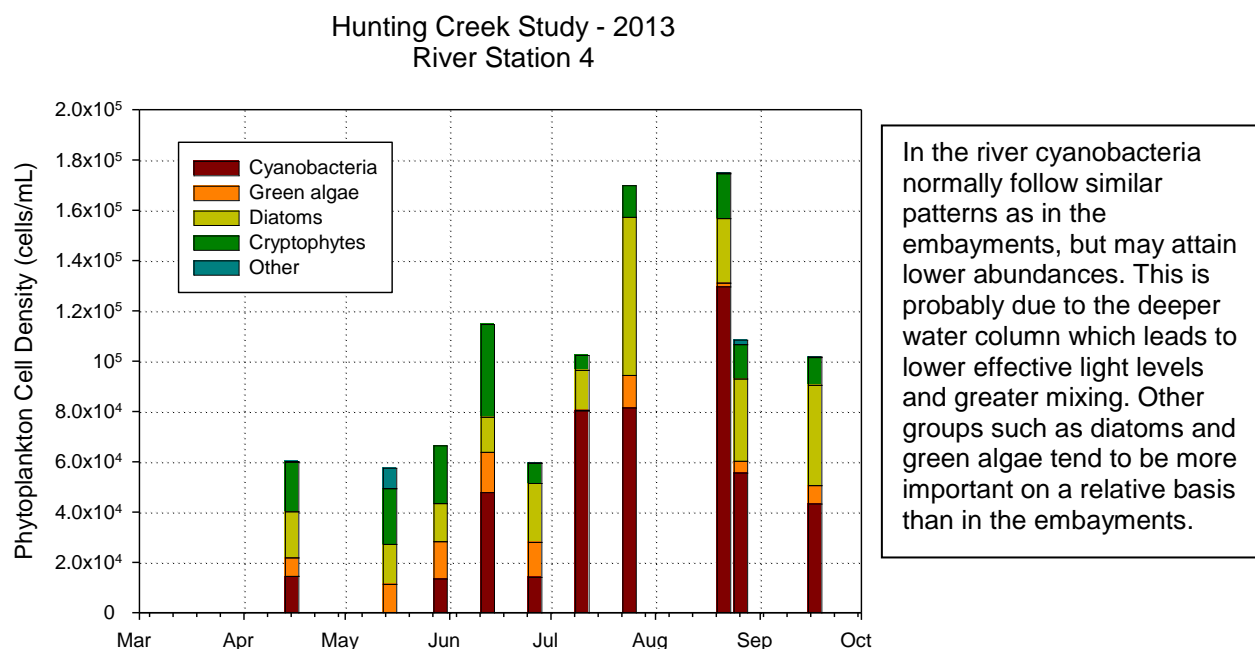


Figure 38. Phytoplankton Density by Major Group (cells/mL). River.

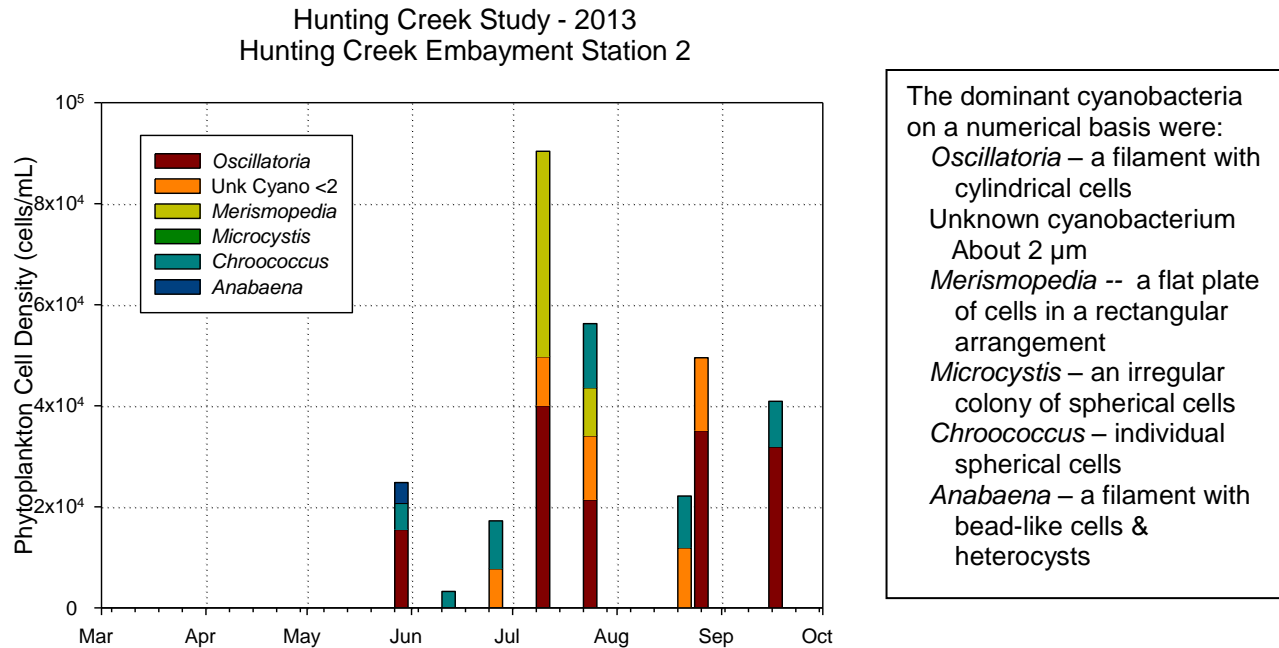


Figure 39. Phytoplankton Density by Dominant Cyanobacteria (cells/mL). Gunston Cove.

Oscillatoria was common in Hunting Creek phytoplankton samples and together with *Merismopedia* and UnkCyano<2 (unknown cyanobacterium less than 2 μ m) made up most of the cyanobacterium cell density. *Microcystis* and *Anabaena*, two taxa that can be problematic, were at low levels in all samples (Figure 39). In the river cyanobacteria were more numerous than in the cove and while *Oscillatoria*, *Merismopedia*, and UnkCyano<2 were still present, *Microcystis* and *Anabaena* were somewhat prominent in selected samples (Figure 40).

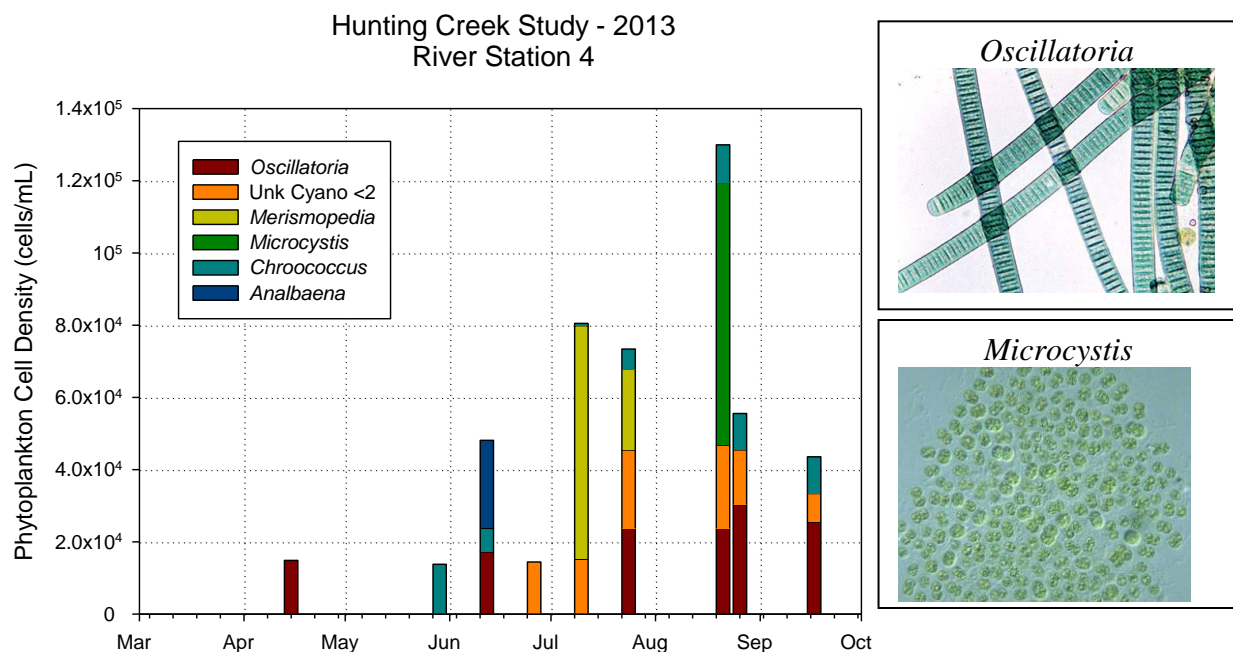


Figure 40. Phytoplankton Density by Dominant Cyanobacteria (cells/mL). River.

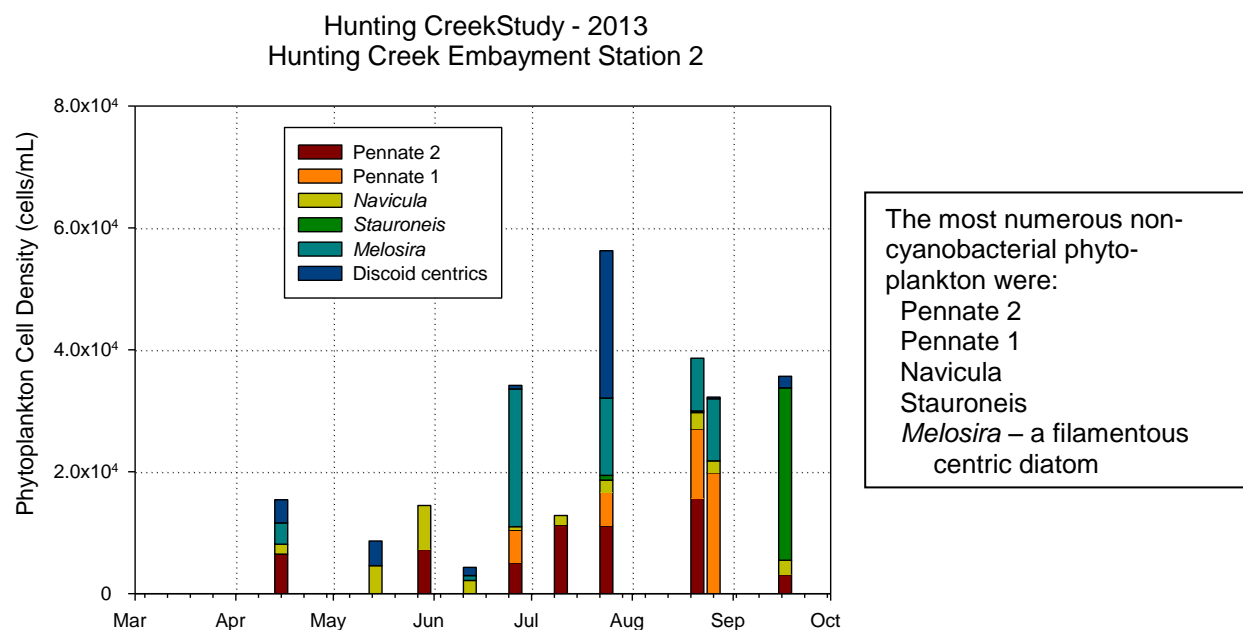


Figure 41. Phytoplankton Density (#/mL) by Dominant Diatom Taxa. Hunting Creek.

In terms of cell density, a variety of diatoms shared dominance at the Hunting Creek station (Figure 41). *Melosira* and discoid centrics were major contributors to the higher values recorded in July and August. Pennate 2 was found in almost all samples and Pennate 1 was most important August. In the river, Pennate 2 was a constant contributor and *Melosira* and discoid centrics were dominant in the late July maximum (Figure 42).

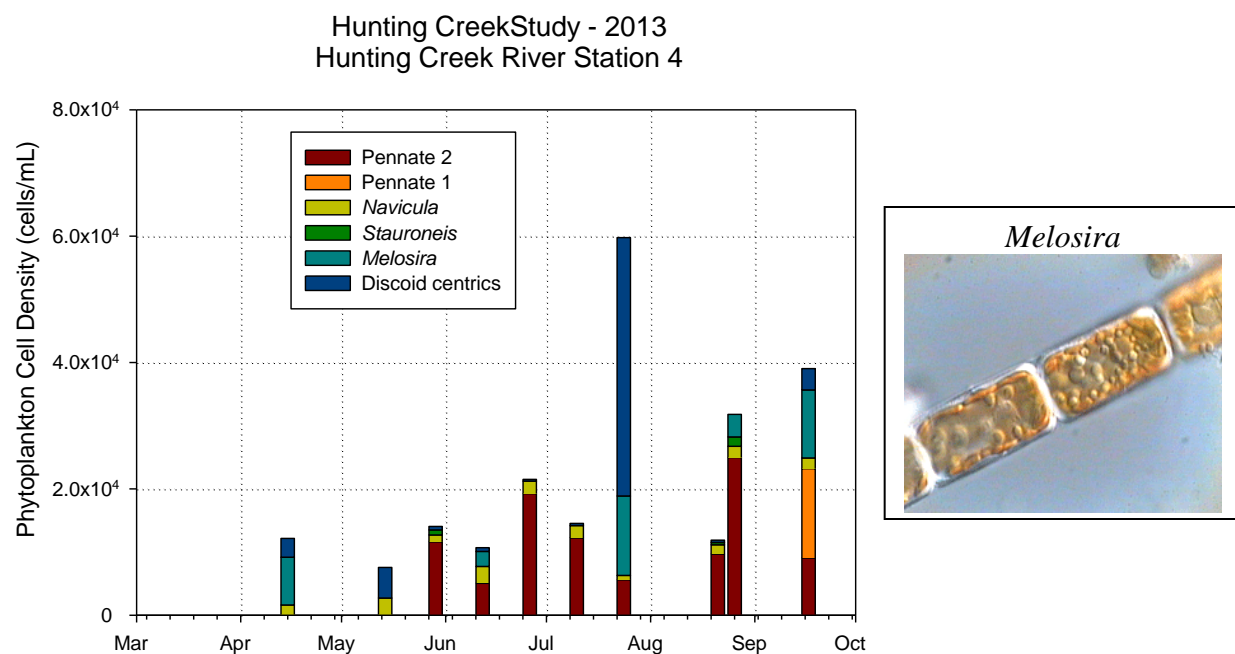


Figure 42. Phytoplankton Density (#/mL) by Dominant Diatom Taxa. River.

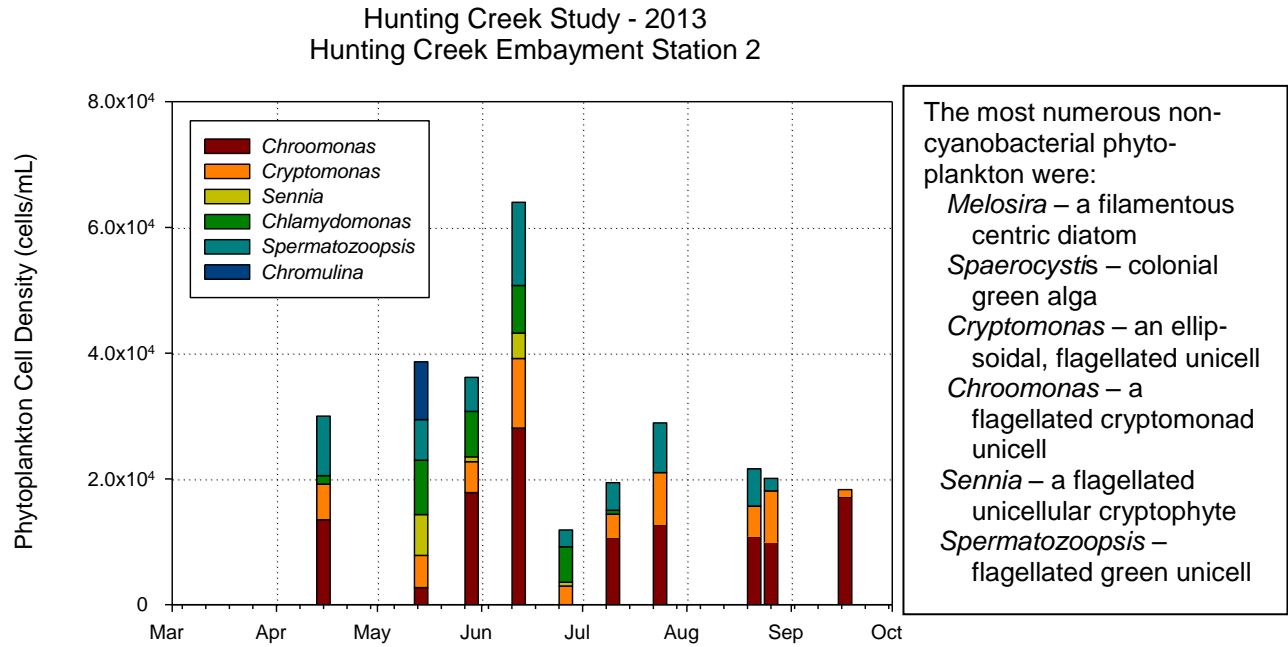


Figure 43. Phytoplankton Density (#/mL) by Dominant Other Taxa. Hunting Creek.

Phytoplankton species that were neither cyanobacteria nor diatoms were grouped together as “other” for these graphs. In the cove *Chroomonas* was the most common dominant (Figure 43). In the river *Chroomonas* was still important, but *Cryptomonas* took on a larger role (Figure 44).

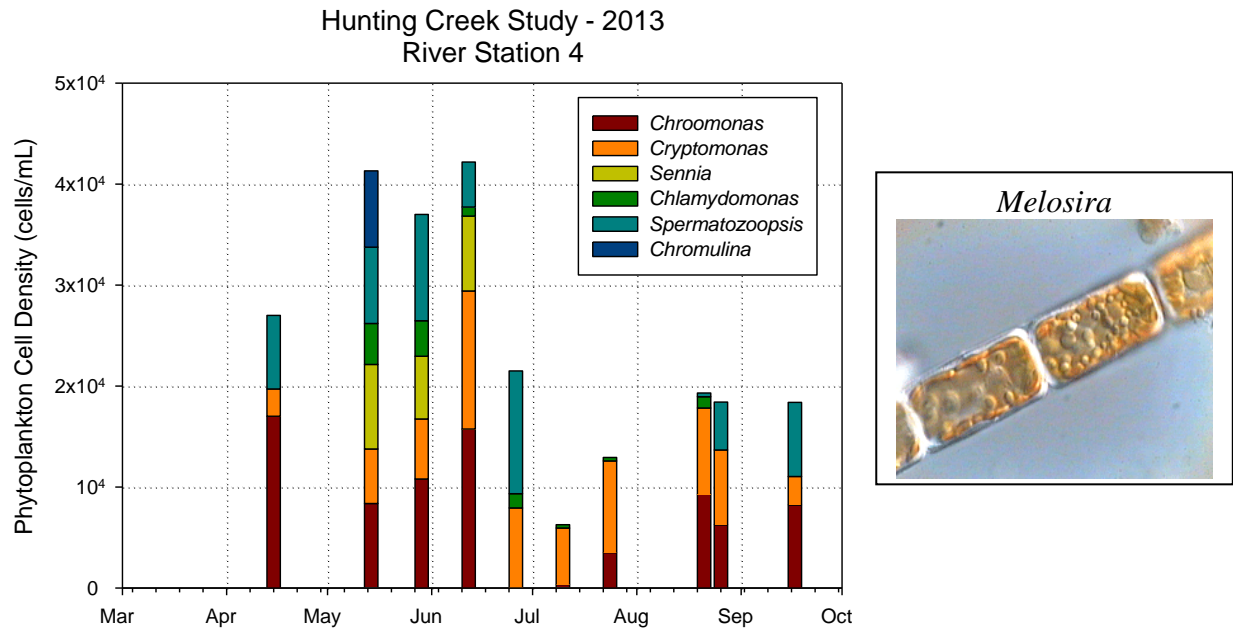


Figure 44. Phytoplankton Density (#/mL) by Dominant Taxa. River.

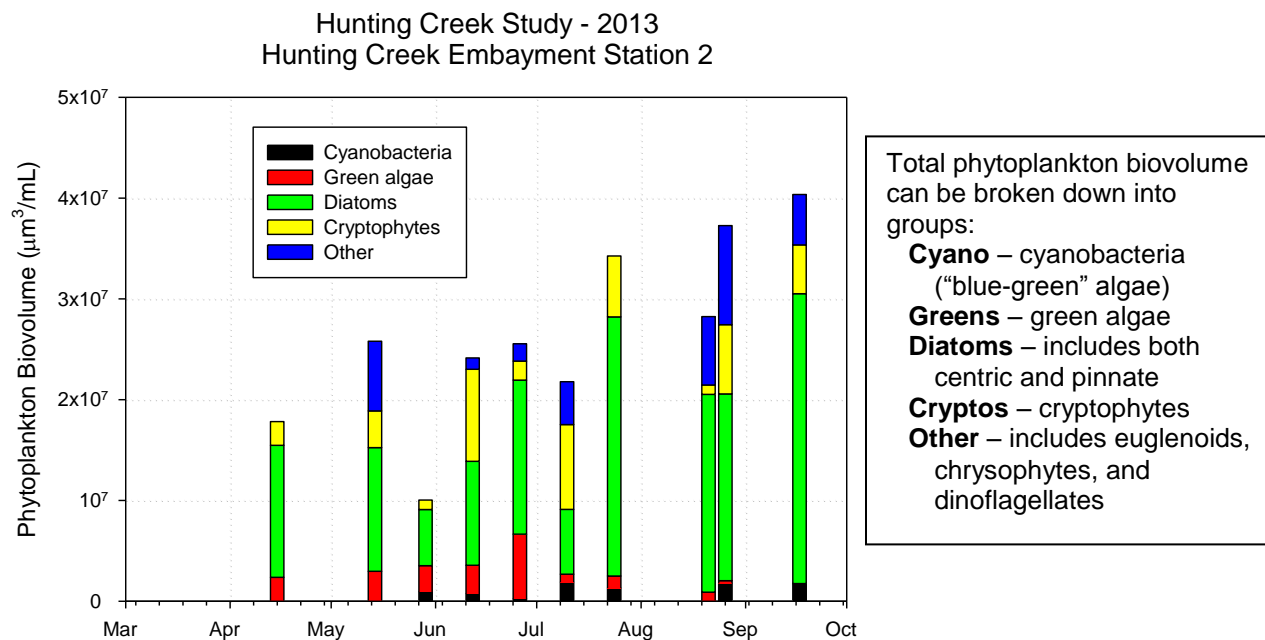


Figure 45. Phytoplankton Biovolume ($\mu\text{m}^3/\text{mL}$) by Major Groups. Hunting Creek.

In the cove diatoms were dominant in biovolume in most samples (Figure 45). Cryptophytes were codominant in a couple of samples. In the river, diatoms were even more overwhelming in their dominance (Figure 46).

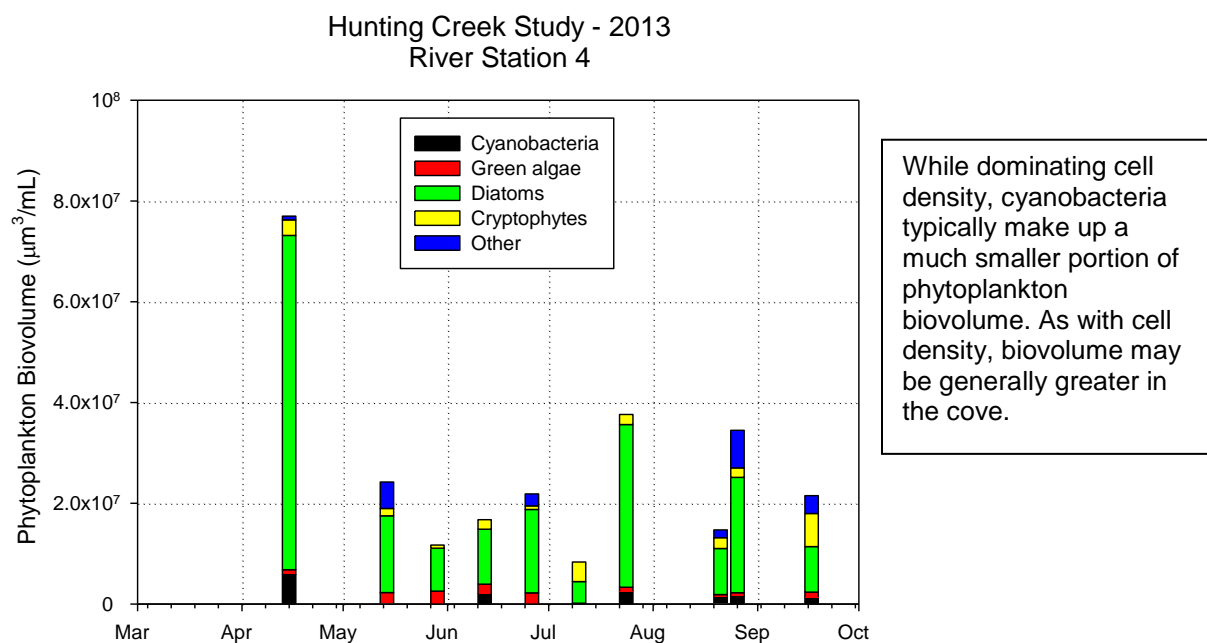


Figure 46. Phytoplankton Biovolume ($\mu\text{m}^3/\text{mL}$) by Major Groups. River.

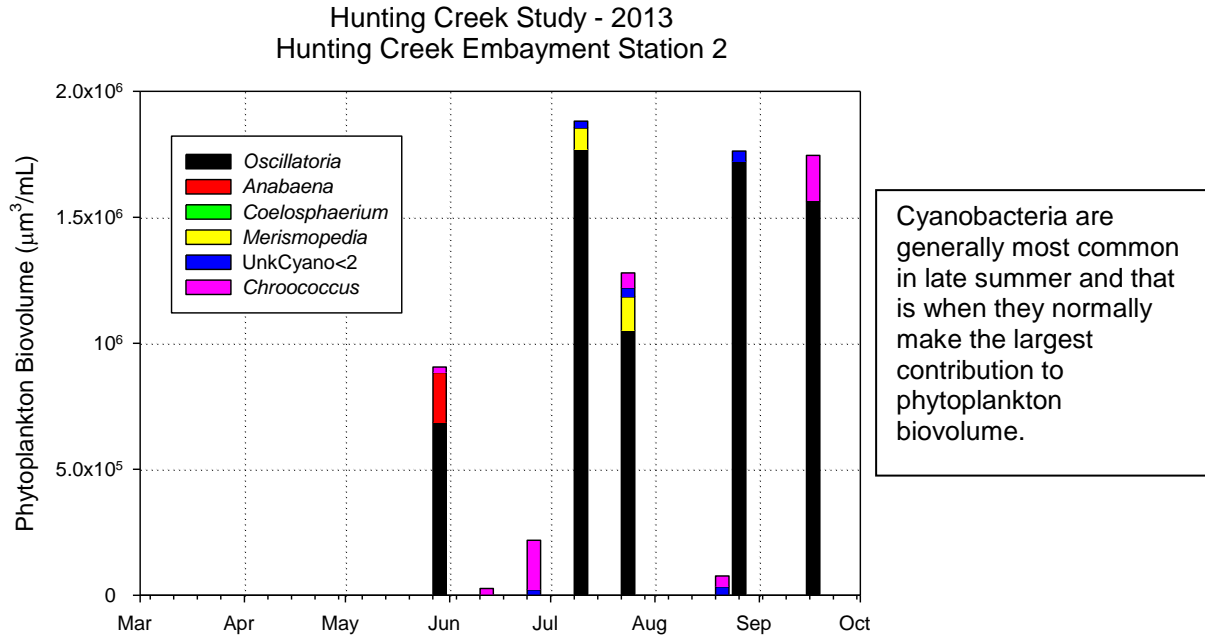


Figure 47. Phytoplankton Biovolume ($\mu\text{m}^3/\text{mL}$) by Cyanobacteria Taxa. Hunting Creek.

In Hunting Creek *Oscillatoria* was the overwhelmingly dominant cyanobacterium in terms of biovolume for most of the year (Figure 47). *Anabaena* made a showing in late May and *Chroococcus* was actually dominant in the two June samples, but at low levels. In the river, a similar pattern in was observed with *Oscillatoria* normally dominant (Figure 48). The *Anabaena* pulse was in early June and *Coelosphaerium* was co-dominant in late July.

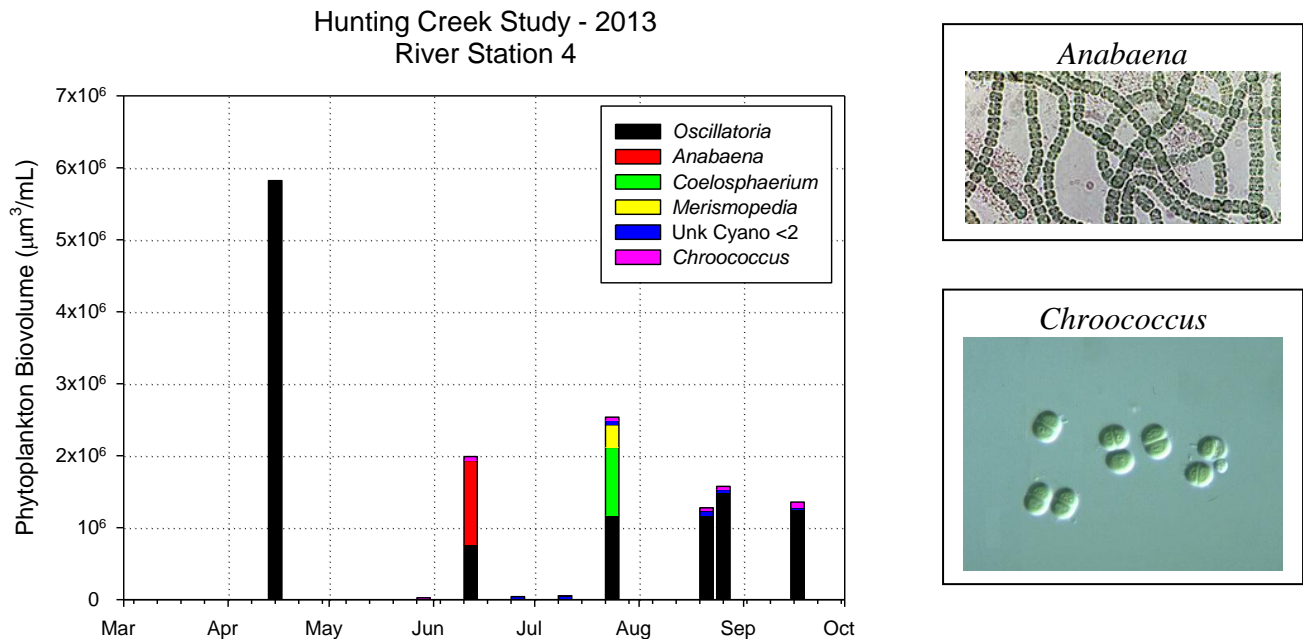


Figure 48. Phytoplankton Biovolume ($\mu\text{m}^3/\text{mL}$) by Cyanobacterial Taxa. River.

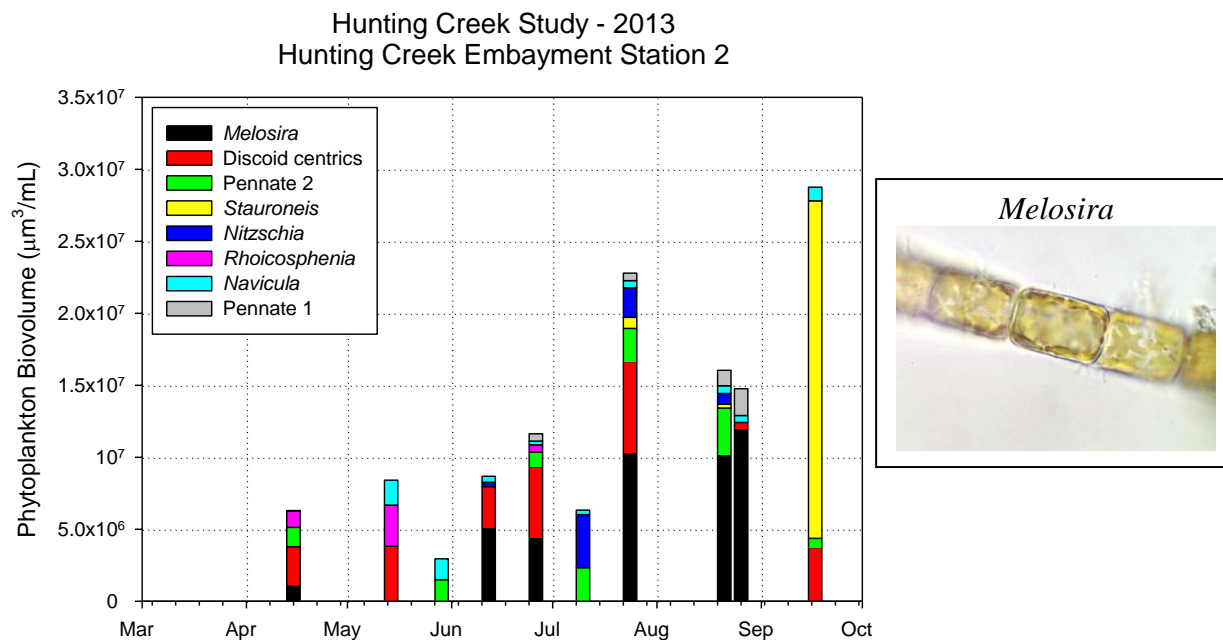


Figure 49. Phytoplankton Biovolume ($\mu\text{m}^3/\text{mL}$) by Dominant Diatom Taxa. Hunting Creek.

In biovolume, there were more taxa of importance than will cell density. Either *Melosira* or discoid centrics or both were generally dominant. A number of other taxa were sporadic in their occurrence, but could be important on those dates. Principal of these was *Stauroneis* in September (Figure 49). In the river, *Melosira* was less consistently dominant, but did have a big role in the April peak (Figure 50).

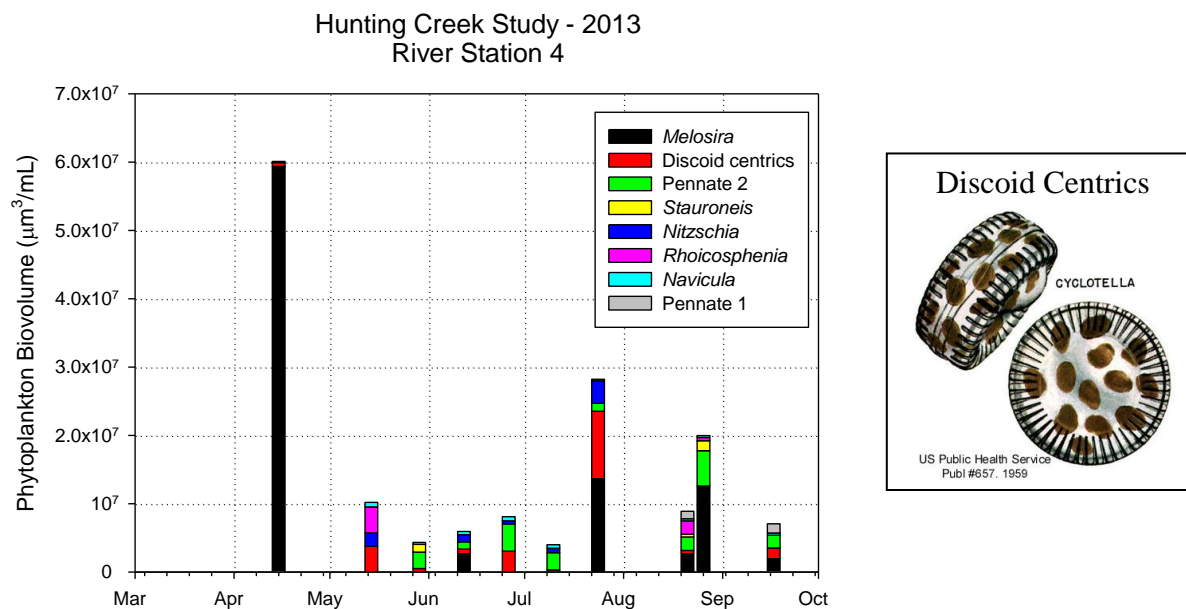


Figure 50. Phytoplankton Biovolume ($\mu\text{m}^3/\text{mL}$) by Dominant Diatom Taxon. River.

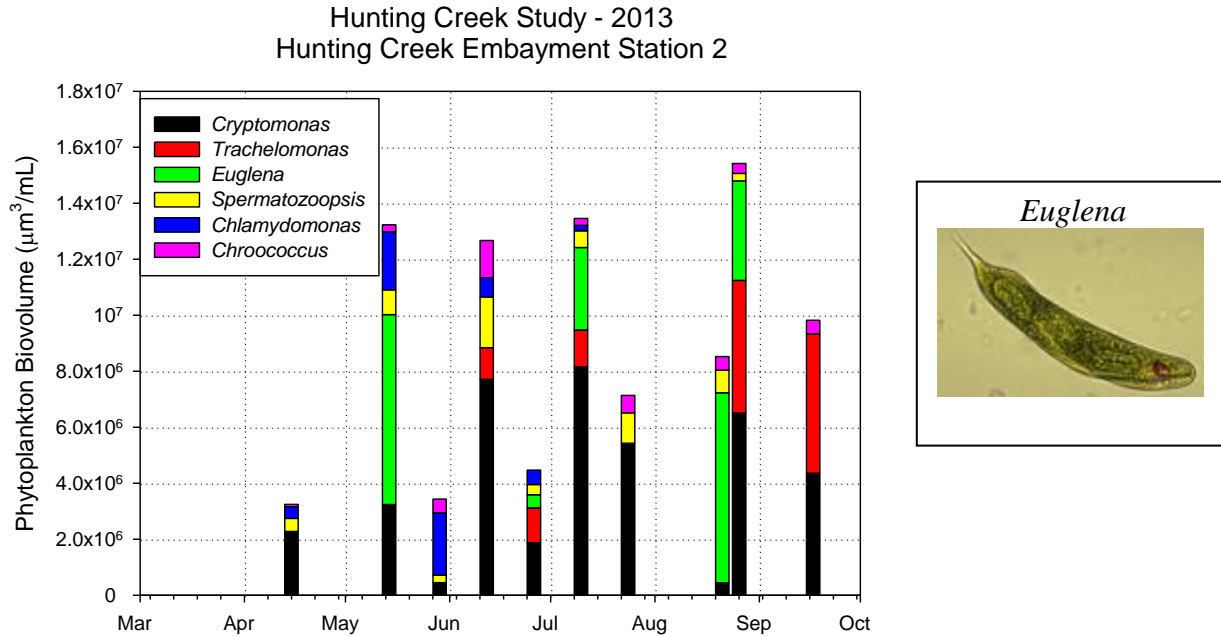


Figure 51. Phytoplankton Biovolume ($\mu\text{m}^3/\text{mL}$) by Dominant Other Taxa. Hunting Creek.

Cryptomonas was the most important component of noncyanobacterial biovolume in Hunting Creek for much of the year followed by *Euglena* and *Trachelomonas* (Figure 51). In the river the same taxa were most prominent (Figure 52). *Spermatozoopsis* made a strong showing in some spring samples.

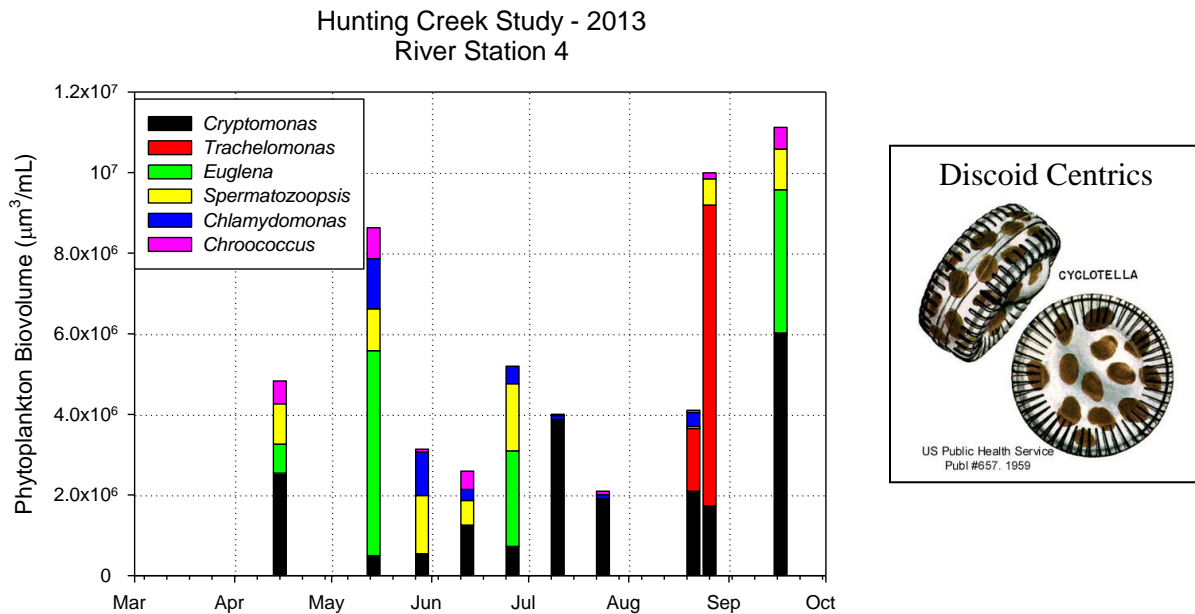


Figure 52. Phytoplankton Biovolume ($\mu\text{m}^3/\text{mL}$) by Dominant Other Taxon. River.

D. Zooplankton – 2013

Hunting Creek Study - 2013 - Station 2

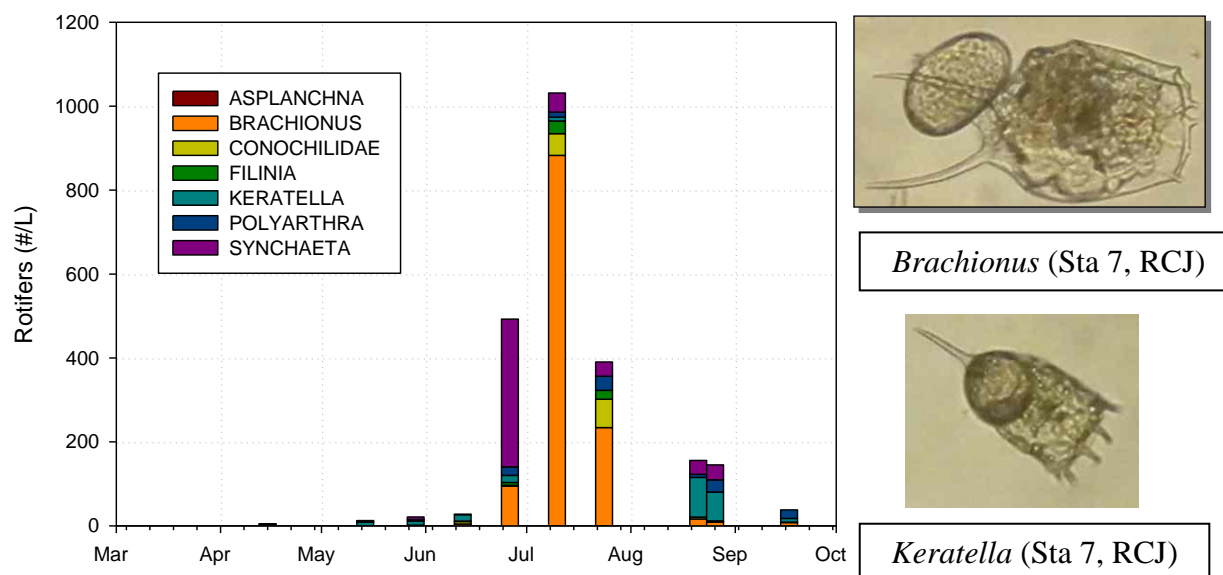


Figure 53. Rotifer Density by Dominant Taxa (#/L). Hunting Creek.

In Hunting Creek, rotifers increased from low values during April through early June to just over 1000/L in early August (Figure 53). *Synchaeta* was dominant in late June followed by *Brachionus* in July. *Keratella* was dominant at the reduced levels found in August. In the river rotifers demonstrated a similar seasonal pattern with slight differences in dominance patterns (Figure 54). *Brachionus* alone was dominant in the late June and July samples with the most abundance. *Keratella* was again most important in August.

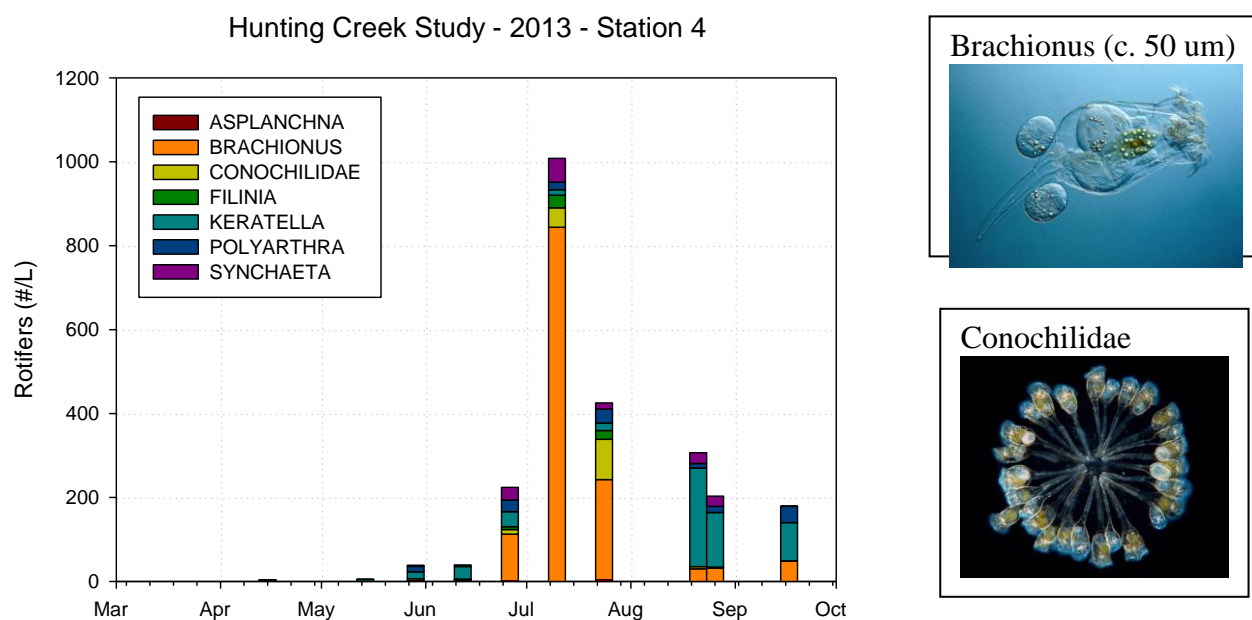


Figure 54. Rotifer Density by Dominant Taxa (#/L). River.

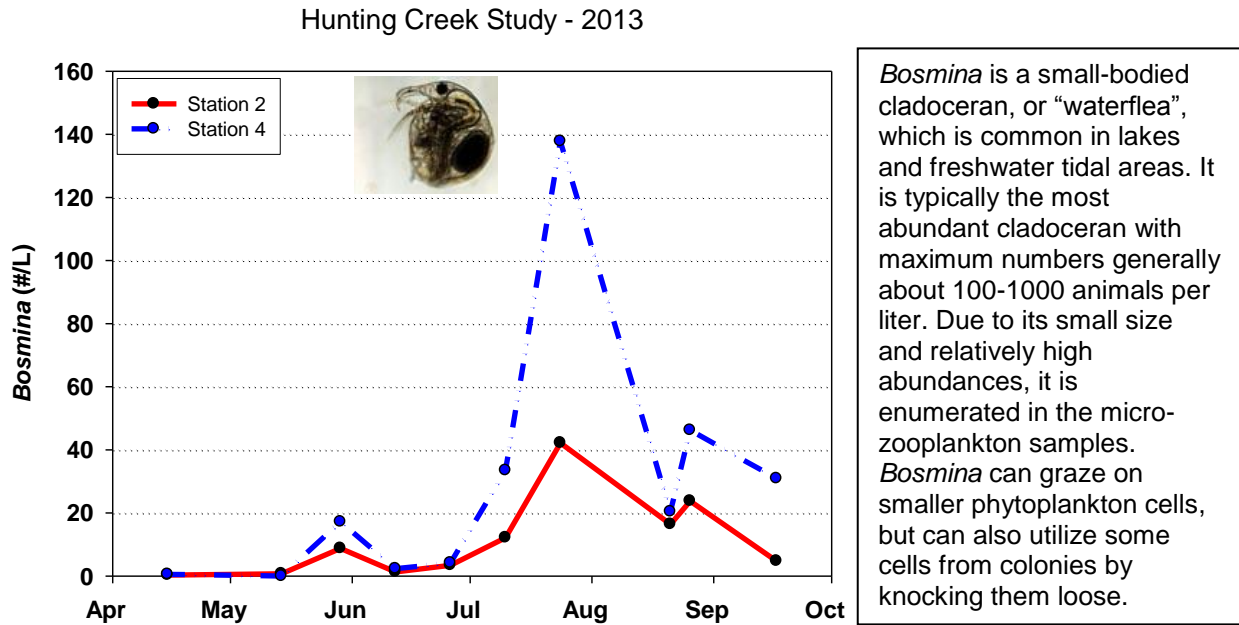


Figure 55. *Bosmina* Density by Station (#/L).

In 2013 the small cladoceran *Bosmina* followed similar patterns in both Hunting Creek and the river. *Bosmina* exhibited a slight increase in late May, but dropped in June perhaps due to flushing from the rain events. In July a major increase was observed with values reaching 140/L at the river station and 40/L in Hunting Creek (Figure 55). *Diaphanosoma*, typically the most abundant larger cladoceran in Gunston Cove, exhibited two peaks in Hunting Creek, each attaining about 500/m³ (Figure 56). In the river, there was a smoother pattern, but lower maximum of about 160/m³.

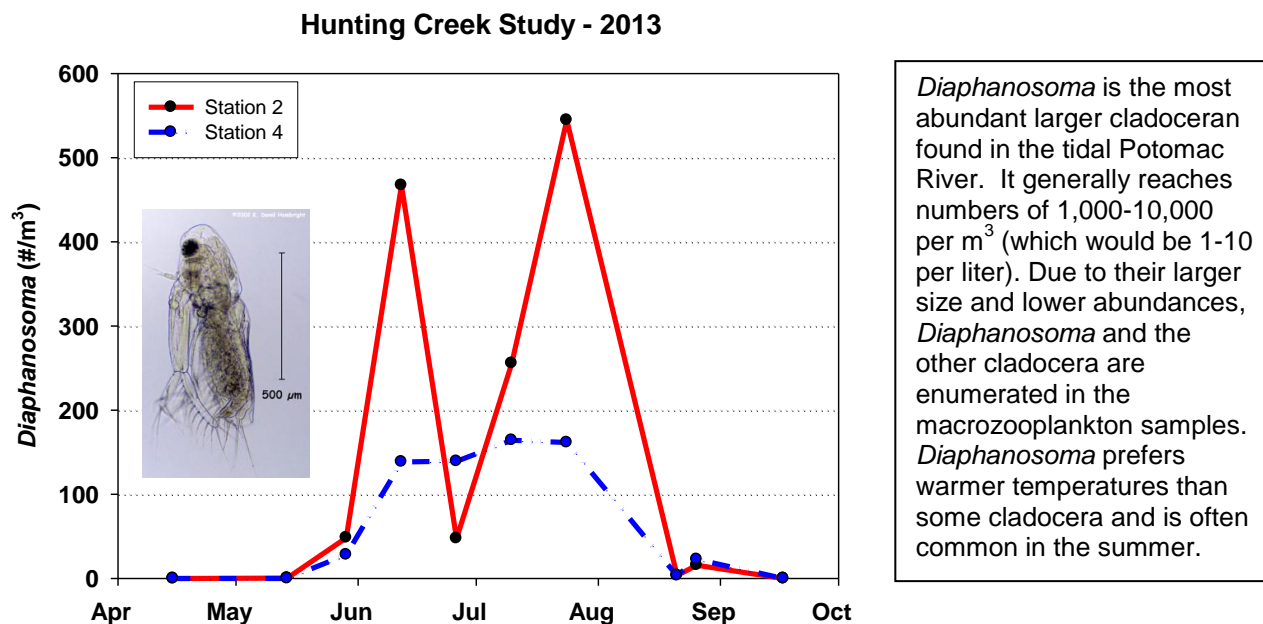


Figure 56. *Diaphanosoma* Density by Station (#/m³).

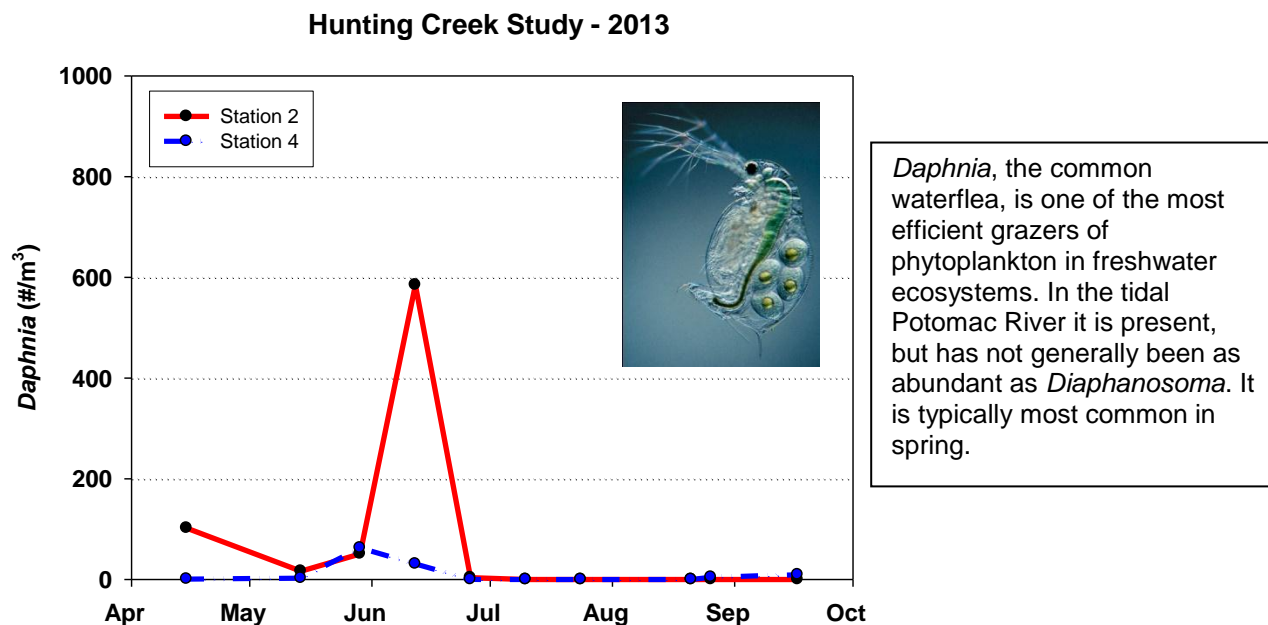


Figure 57. *Daphnia* Density by Station (#/m³).

Daphnia was found at rather low levels that peaked in early June in Hunting Creek at about 600/m³ (Figure 57). *Daphnia* was uncommon in the river. *Ceriodaphnia* was also present mainly in June, but at even lower levels in Hunting Creek (Figure 58).

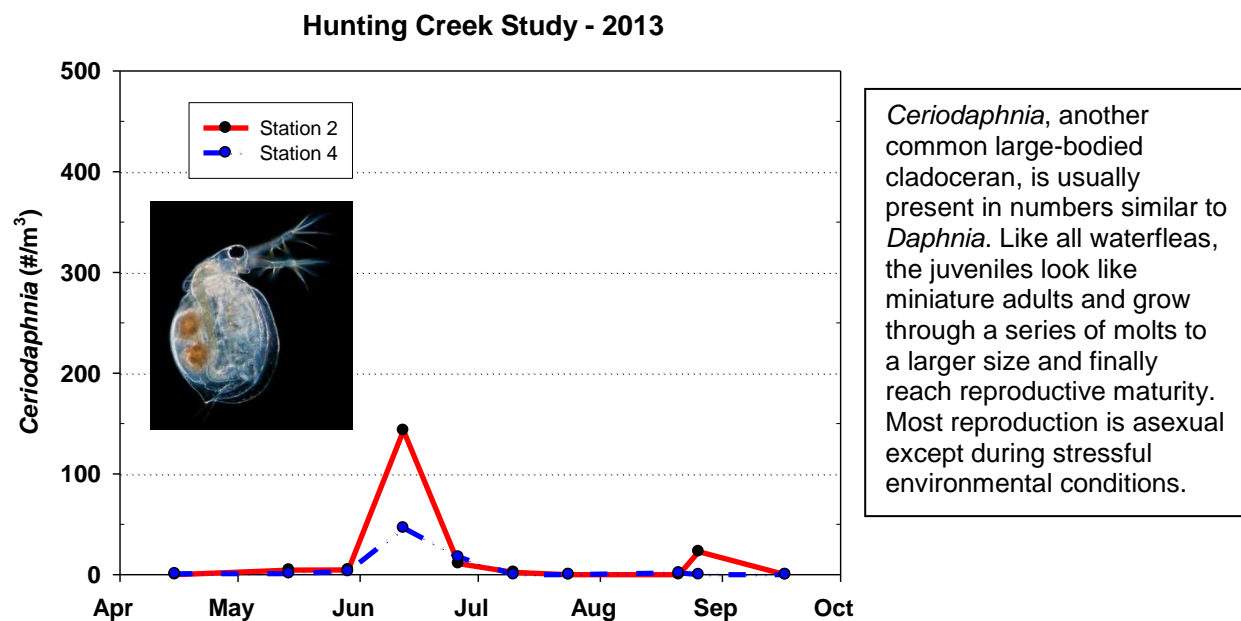


Figure 58. *Ceriodaphnia* Density by Station (#/m³).

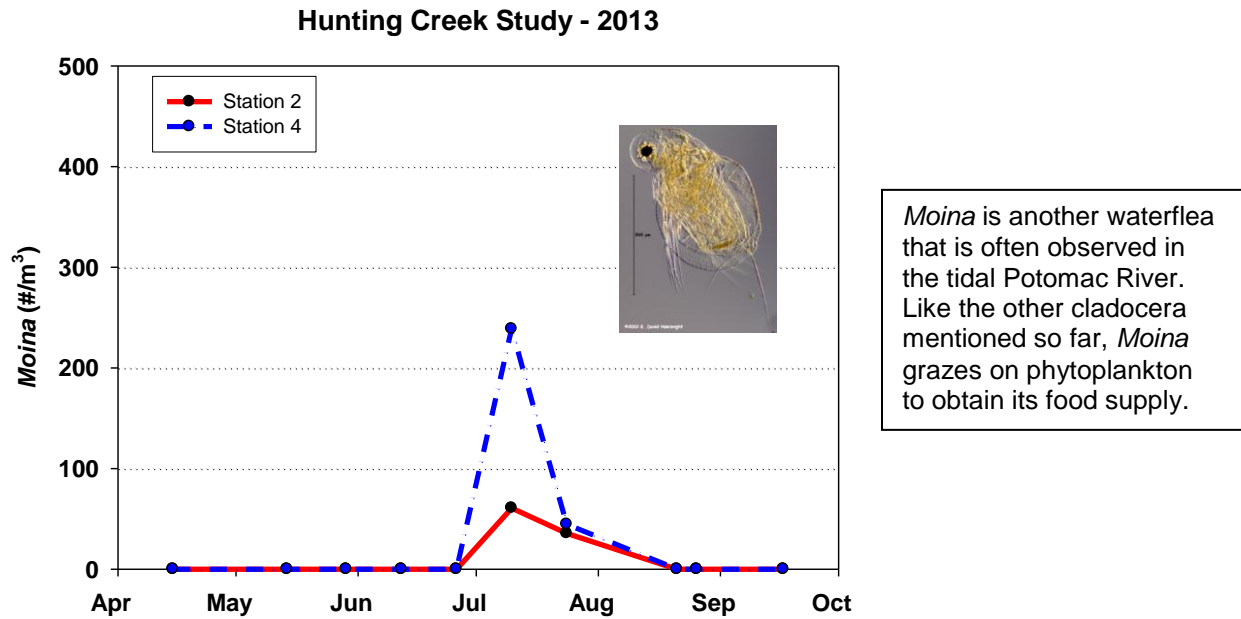


Figure 59. *Moina* Density by Station (#/m³).

Moina was actually more common in the river, but was restricted to the month of July (Figure 59). *Leptodora*, the large cladoceran predator, was consistently present in Hunting Creek from late May through July, but reached higher levels at the river station in late June and early July before dropping off markedly (Figure 60).

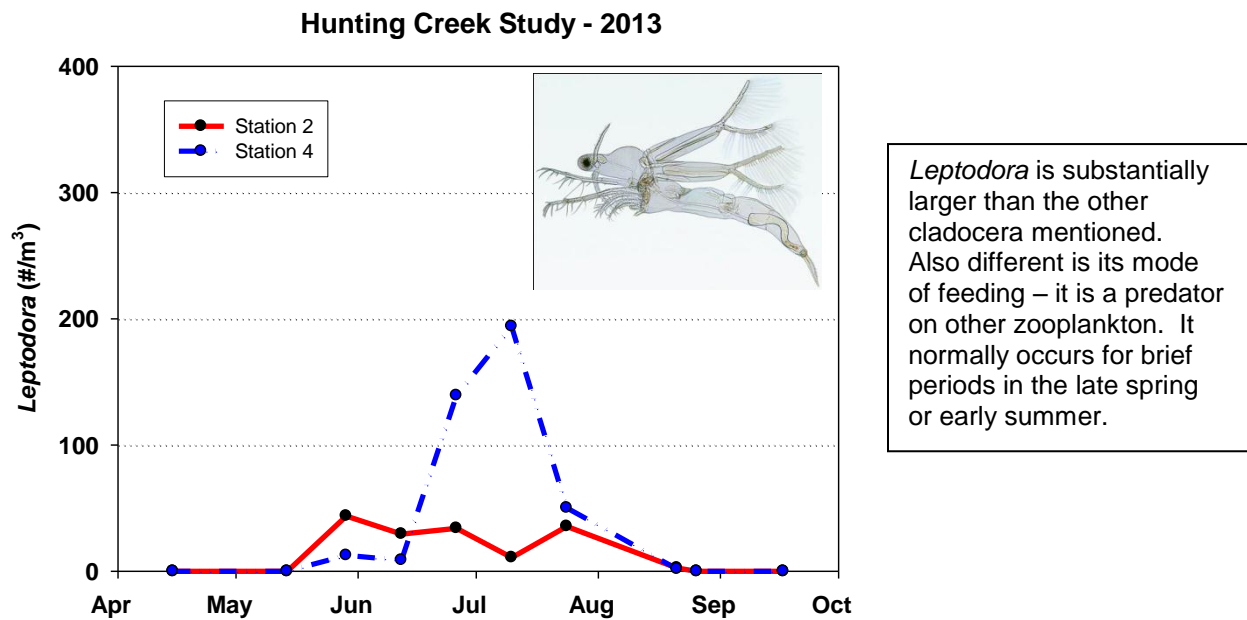


Figure 60. *Leptodora* Density by Station (#/m³).

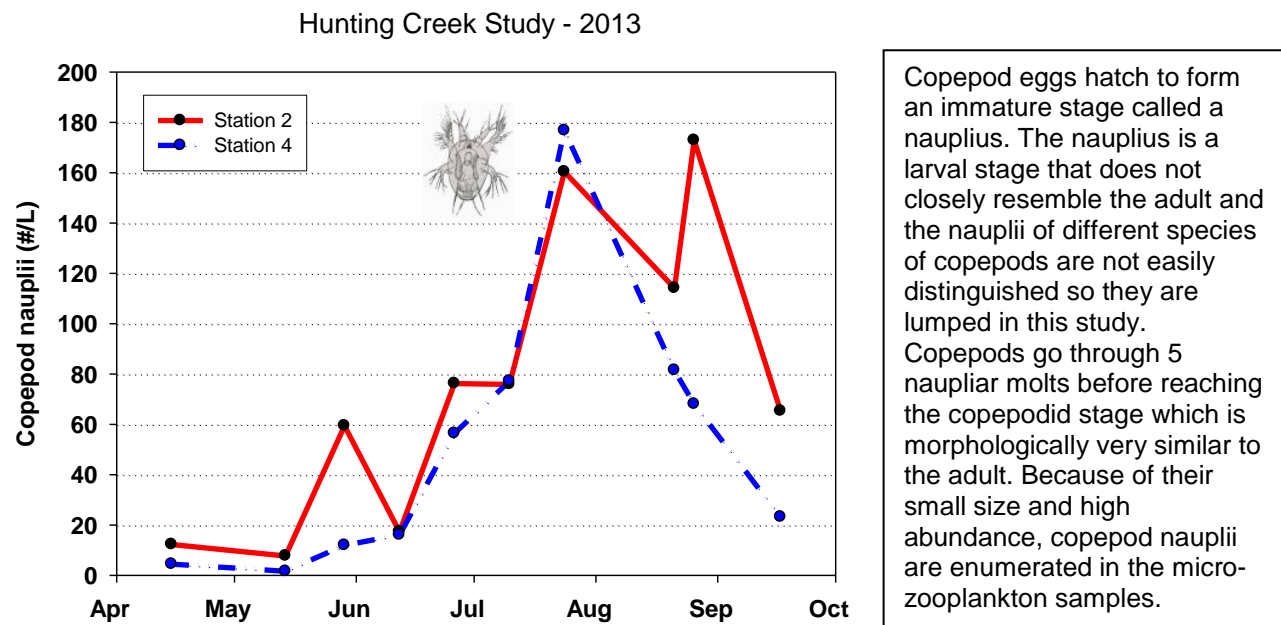


Figure 61. Copepod Nauplii Density by Station (#/L).

Copepod nauplii were present at low levels in April and early May (Figure 61). An increase in Hunting Creek in late May was reversed in early June, but recovered to increase to higher values during the remainder of the study period. The June – July increase followed a very similar pattern and values at the two stations reached 160-180/L. *Eurytemora* exhibited muted values in Hunting Creek, but attained very high densities of over 6500/m³ in late June in the river (Figure 62)..

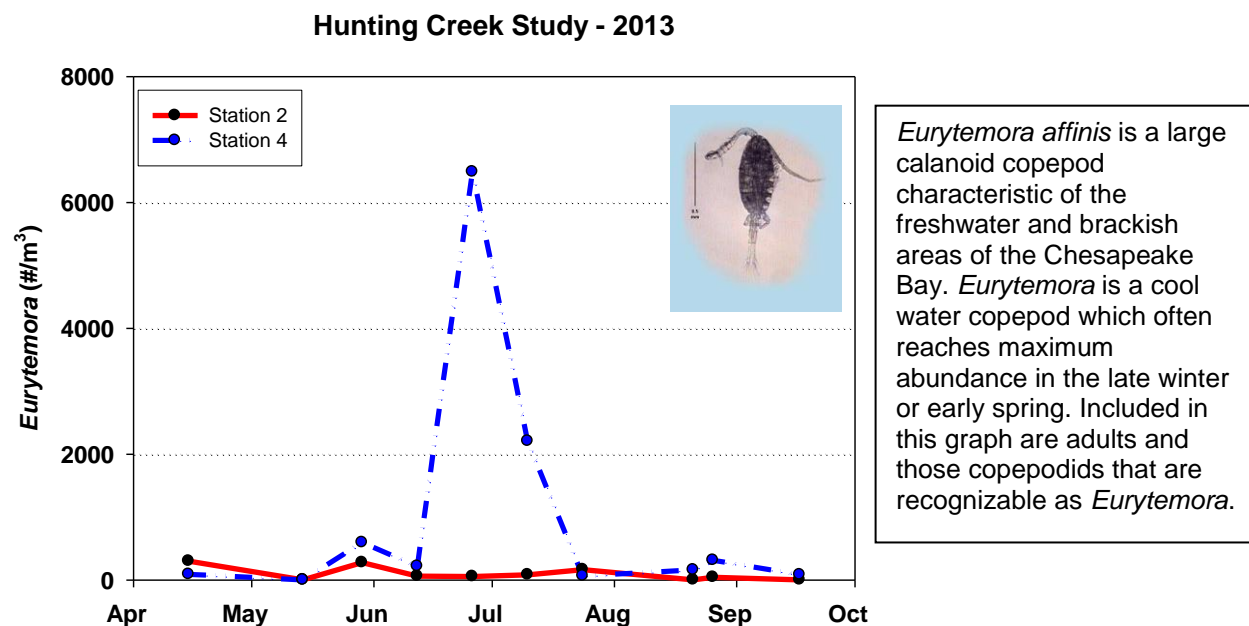


Figure 62. *Eurytemora* Density by Station (#/m³).

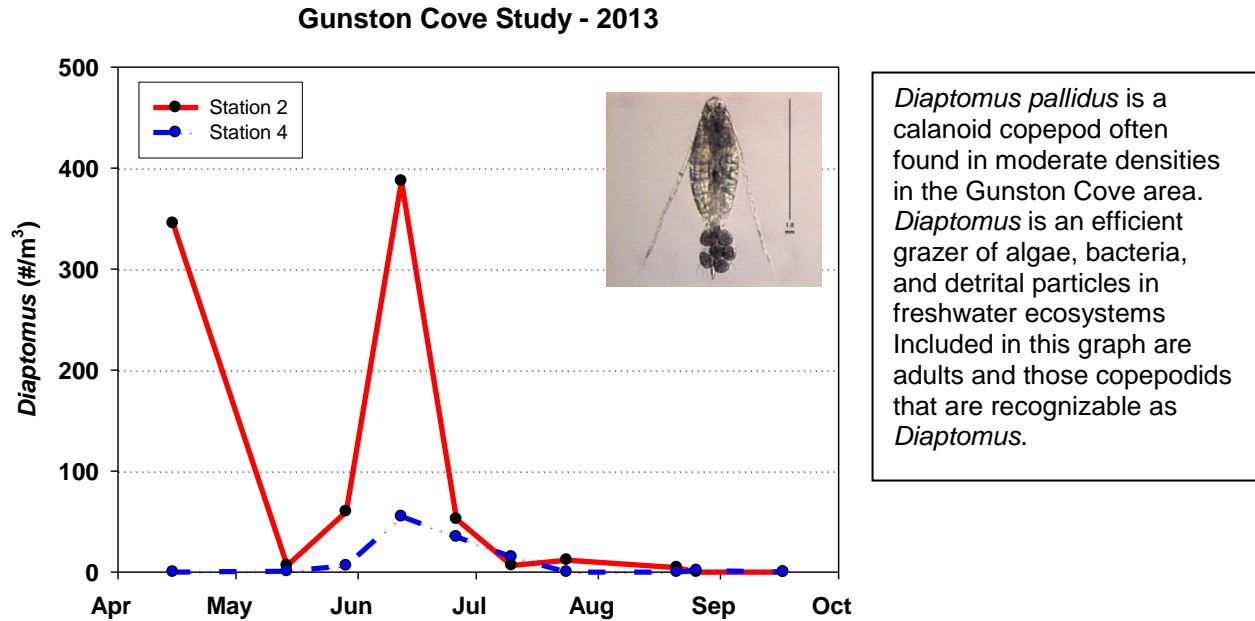


Figure 63. *Diaptomus* Density by Station (#/m³).

Diaptomus was more common in Hunting Creek than in the river reaching a maximum of 400/m³ in early June (Figure 63). Other calanoid copepods were not common in Hunting Creek, but attained a value of about 1500/m³ in the river in late June (Figure 64).

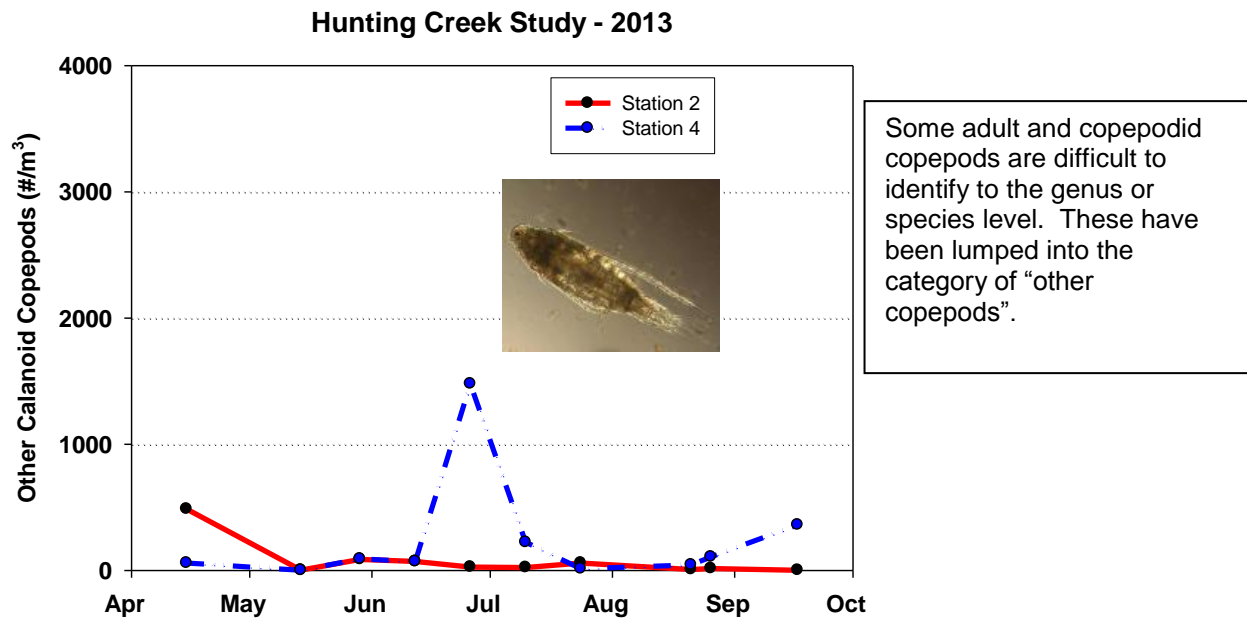


Figure 64. Other Calanoids Density by Station (#/m³).

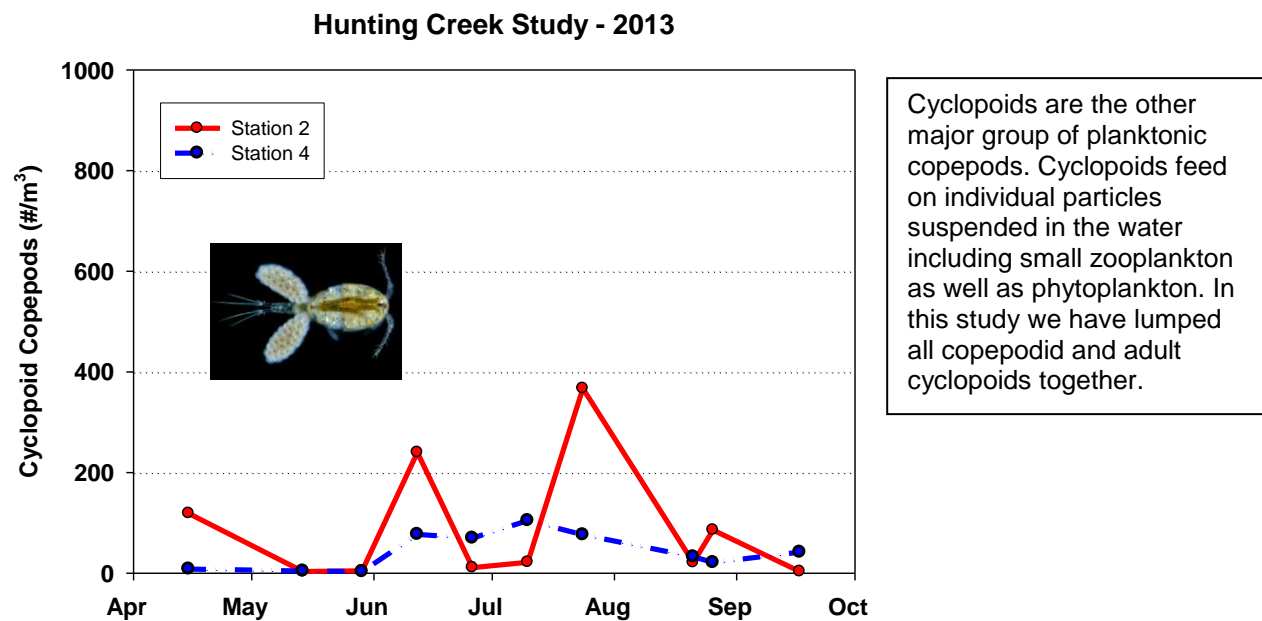


Figure 65. Cyclopoid Copepods by Station (#/m³).

Cyclopoid copepods exhibited a seasonal increase attaining highest values in early July at both stations (Figure 65). As with other zooplankton densities were much more variable in Hunting Creek than in the river.

E. Ichthyoplankton – 2013

We collected 14 samples (7 at Station ARE 2 and 7 at Station ARE 4) during the months April through July and obtained a total of 1524 larvae (Table 4). The fish larvae are often difficult to distinguish at the species level, thus some of the counts are only to the genus or even family level. The dominant taxon category was comprised of larvae identified as the family Clupeidae with 29.5% of the catch, but this taxa represents all clupeid larvae (all *Alosa* sp. and *Dorosoma* sp.) that could not be identified to a lower taxonomic level. *Alosa pseudoharengus* (Alewife) were second in rank (19.8 %). *Morone* sp., *Dorosoma* sp., and *Alosa aestivalis* were common too, comprising 18.8%, 12% and 10.3% of total collections respectively. *Dorosoma* sp. are likely all gizzard shad; the alternative (threadfin shad) is rarely found this high up the Potomac River, while *Morone* sp. is likely dominated by white perch but contains striped bass as well. Other species were collected in much lower numbers (Table 4).

Table 4. The larval fishes collected in Hunting Creek (Sta. 2) and the Potomac River (Sta. 4) in 2013

Taxon	Species	Station 2	Station 4	Total	% of Total
<i>Alosa aestivalis</i>	blueback herring	83	74	157	10.3
<i>Alosa mediocris</i>	hickory shad	26	5	31	2.0
<i>Alosa pseudoharengus</i>	alewife	178	124	302	19.8
<i>Alosa sapidissima</i>	American shad	1	0	1	<0.1
<i>Carassius auratus</i>	goldfish	1	4	5	0.3
Clupeidae	herring or shad	349	101	450	29.5
<i>Dorosoma</i> sp.	Gizzard shad	107	76	183	12.0
<i>Fundulus</i> sp.	Fundulus sp.	0	1	1	<0.1
<i>Lepomis</i> sp.	sunfish	3	1	4	0.3
<i>Menidia beryllina</i>	inland silverside	10	6	16	1.1
<i>Morone</i> sp.	perch or bass	267	19	286	18.8
<i>Notemigonus crysoleucas</i>	golden shiner	0	7	7	0.5
<i>Perca flavescens</i>	yellow perch	3	3	6	0.4
Unidentified	Unidentified	61	14	75	4.9
	Total	1068	435	1524	100

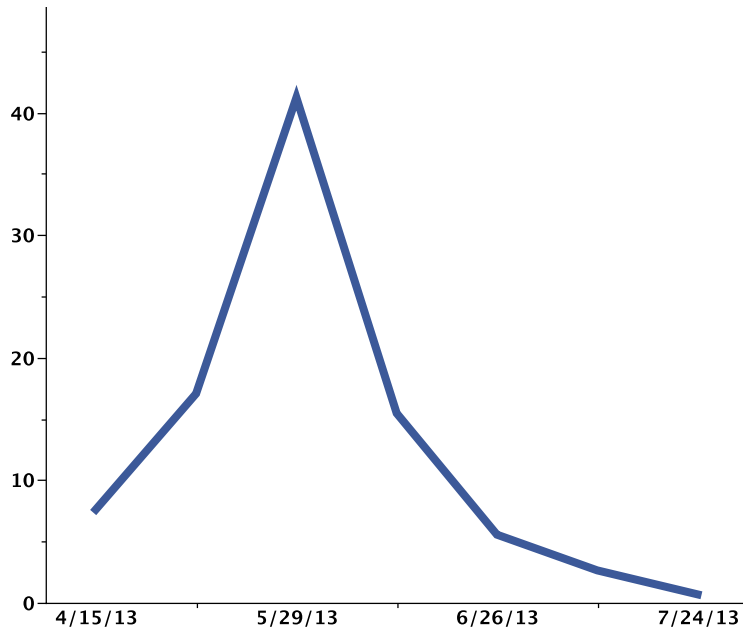


Figure 66. Density of clupeid larvae per 10m³.

Clupeid larvae in Figure 66 include blueback herring, hickory shad, alewife, American shad, gizzard shad, and threadfin shad. These have similar spawning patterns so they are lumped into one group for this analysis. Clupeids increased in the study areas in spring attaining a maximum in late May (Figure 66). White perch larvae attained maximum numbers in early May, which dominated the pattern of the other larvae combined (Figure 67).

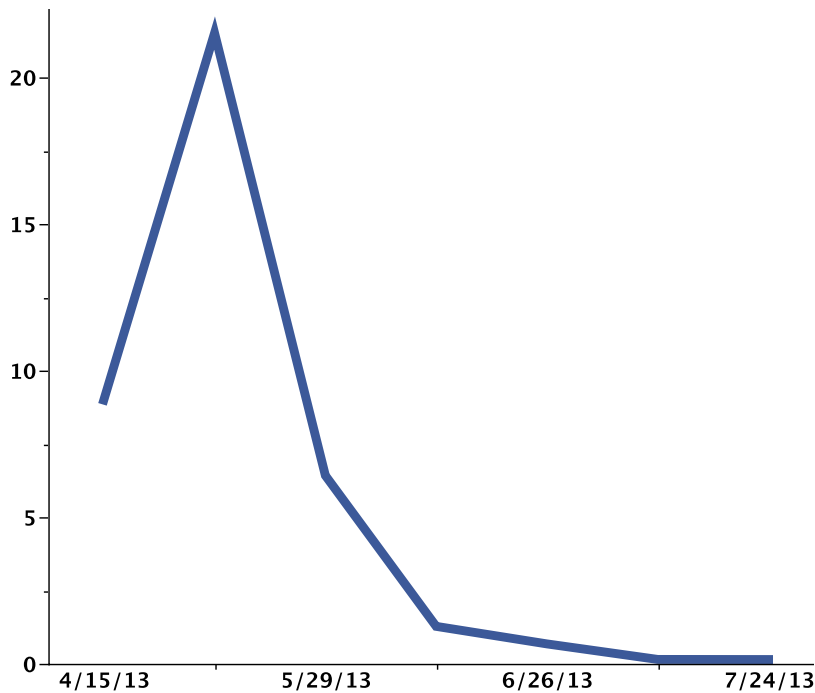


Figure 67. Density of all other larvae per 10m³.

F. Adult and juvenile fishes – 2013

Trawls

Trawl sampling was conducted between April 10 and September 10 at stations ARE 3 and ARE 4. A total of 995 fishes comprising 16 species representing 10 families were collected (Table 5). The majority (82%, numerically) of the fish collected were represented by 3 species: white perch (33.9%), spottail shiner (24.4%), and tessellated darter (23.7%). Other abundant species (annual total >1%) included: Alosas (2.71%), goldfish (2.01%), banded killifish (1.01%), bay anchovy (6.23%), blue catfish (1.21%), and yellow perch (2.11%). Other species were observed at lower abundances (Tables 5 and 6).

Table 5. Adult and juvenile fish collected by trawling. Hunting Creek - 2013

Family	Species	Common name	Abundance	% total
Atherinidae	<i>Menidia beryllina</i>	inland silverside	1	0.10
Centrarchidae	<i>Lepomis gibbosus</i>	pumpkinseed	5	0.50
	<i>Lepomis machrochirus</i>	bluegill	9	0.90
	<i>Lepomis microlophus</i>	redear sunfish	5	0.50
	<i>Lepomis sp.</i>	sunfish	3	0.30
	<i>Alosa sp.</i>	herring or shad	27	2.71
Clupeidae	<i>Dorosoma cepedianum</i>	gizzard shad	1	0.10
	<i>Carassius auratus</i>	goldfish	20	2.01
	<i>Notropis hudsonius</i>	spottail shiner	243	24.4
Cyprinodontidae	<i>Fundulus diaphanus</i>	banded killifish	10	1.01
Engraulidae	<i>Anchoa mitchilli</i>	bay anchovy	62	6.23
Ictaluridae	<i>Ameiurus nebulosus</i>	brown bullhead	2	0.20
	<i>Ictaurus furcatus</i>	blue catfish	12	1.21
Percichthyidae	<i>Morone americana</i>	white perch	337	33.9
Percidae	<i>Etheostoma olmstedii</i>	tessellated darter	236	23.7
	<i>Perca flavescens</i>	yellow perch	21	2.11
Sciaenidae	<i>Micropogonias undulatus</i>	Atlantic croaker	1	0.10
Total			995	100

Table 6. Adult and juvenile fish collected by trawling. Hunting Creek study - 2013

			4/10	5/08	5/22	6/05	6/19	7/17	7/26	8/07	8/21	9/10
Atherinidae	<i>Menidia beryllina</i>	inland silverside	0	0	0	1	0	0	0	0	0	0
Centrarchidae	<i>Lepomis gibbosus</i>	pumpkinseed	0	0	1	3	0	1	0	0	0	0
	<i>Lepomis macrochirus</i>	bluegill	0	1	0	0	5	2	0	1	0	0
	<i>Lepomis microlophus</i>	redeer sunfish	0	1	0	0	0	0	1	0	3	0
	<i>Lepomis sp.</i>	sunfish	0	2	0	0	0	0	0	1	0	0
	<i>Alosa sp.</i>	herring or shad	0	0	0	9	0	3	0	15	0	0
Clupeidae	<i>Dorosoma cepedianum</i>	gizzard shad	0	0	0	1	0	0	0	0	0	0
Cyprinidae	<i>Carassius auratus</i>	goldfish	0	0	0	0	0	0	0	1	0	19
	<i>Notropis hudsonius</i>	spottail shiner	0	4	0	29	48	152	2	8	0	0
Cyprinodontidae	<i>Fundulus diaphanus</i>	banded killifish	0	0	0	2	0	6	0	1	0	1
Engraulidae	<i>Anchoa mitchilli</i>	bay anchovy	0	0	50	0	0	1	0	8	3	0
Ictaluridae	<i>Ameiurus nebulosus</i>	brown bullhead	0	0	0	1	1	0	0	0	0	0
	<i>Ictaurus furcatus</i>	blue catfish	0	0	0	0	0	0	10	0	0	2
Percichthyidae	<i>Morone americana</i>	white perch	0	10	0	51	78	134	42	11	10	1
Percidae	<i>Etheostoma olmstedii</i>	tessellated darter	1	1	75	37	110	10	1	0	1	0
	<i>Perca flavescens</i>	yellow perch	0	0	0	2	18	1	0	0	0	0
Sciaenidae	<i>Micropogonias undulatus</i>	Atlantic croaker	0	0	1	0	0	0	0	0	0	0
Total			1	19	127	136	260	310	56	46	17	23

Most catches occurred in June and July (Tables 6). Most catches occurred at Station ARE 3, which means species actively pursue the shallower habitat in Hunting Creek from the Potomac River mainstem (Table 7). In total numbers and species richness of fish, Station ARE 3 dominated with 889 individuals from 14 species whereas Station ARE 4 had 106 individuals from 9 species. There were no centrarchids (sunfishes) in ARE 4, which are species known to be associated with submerged aquatic vegetation, while 22 individuals from at least 3 species were found in ARE 3.

Table 7. Adult and juvenile fish collected by trawling. Hunting Creek study - 2013

			3	4
Atherinidae	<i>Menidia beryllina</i>	inland silverside	0	1
Centrarchidae	<i>Lepomis gibbosus</i>	pumpkinseed	5	0
	<i>Lepomis machrochirus</i>	bluegill	9	0
	<i>Lepomis microlophus</i>	redear sunfish	5	0
	<i>Lepomis sp.</i>	sunfish	3	0
Clupeidae	<i>Alosa sp.</i>	herring or shad	4	23
	<i>Dorosoma cepedianum</i>	gizzard shad	0	1
Cyprinidae	<i>Carassius auratus</i>	goldfish	20	0
	<i>Notropis hudsonius</i>	spottail shiner	241	2
Cyprinodontidae	<i>Fundulus diaphanus</i>	banded killifish	10	0
Engraulidae	<i>Anchoa mitchilli</i>	bay anchovy	51	11
Ictaluridae	<i>Ameiurus nebulosus</i>	brown bullhead	2	0
	<i>Ictalurus furcatus</i>	blue catfish	0	12
Percichthyidae	<i>Morone americana</i>	white perch	286	51
Percidae	<i>Etheostoma olmstedii</i>	tessellated darter	232	4
	<i>Perca flavescens</i>	yellow perch	20	1
Sciaenidae	<i>Micropogonias</i>	Atlantic croaker	1	0
	<i>undulatus</i>			
Total			889	106

The six most abundant species varied in representation across the two stations (Figure 68A and B). At both stations, white perch made up a significant proportion of the total catch. Other species present at high proportions at ARE 3 were spottail shiner and tessellated darter, while the second highest abundant taxa in ARE 4 were herring or shad. Total catch of especially white perch, spottail shiner, and tessellated darter was significantly higher in ARE 3 than ARE 4. Station 3 was overall the most productive station of the two, with a total abundance more than 8 times higher than Station 4.

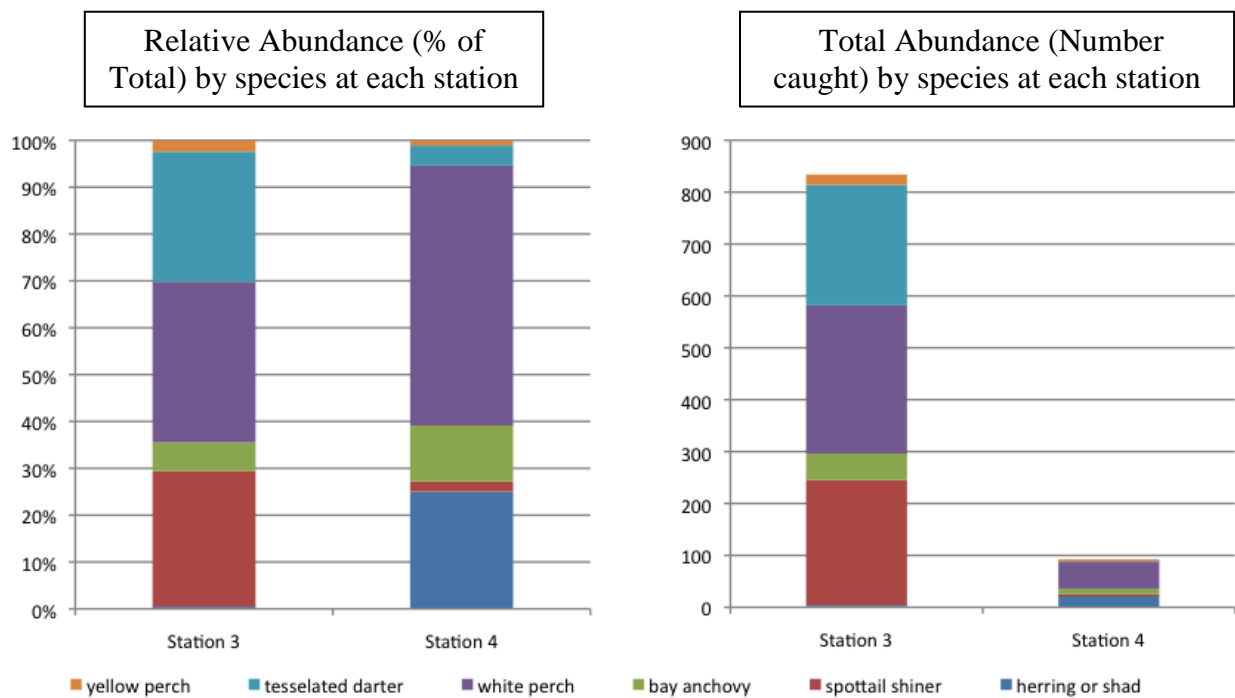


Figure 68A and B. Adult and Juvenile Fishes Collected by Trawling. Dominant Species by Station.

White perch (*Morone americana*), the most common fish in the open waters of Hunting Creek, continues to be an important commercial and popular game fish. Adults grow to over 30 cm long. Sexual maturity begins the second year at lengths greater than 9 cm. As juveniles they feed on zooplankton and macrobenthos, but as they get larger consume fish as well.

Spottail shiner (*Notropis hudsonius*), a member of the minnow family, is abundant in Hunting Creek. Spawning occurs throughout the warmer months. It reaches sexual maturity at about 5.5 cm and may attain a length of 10 cm. They feed primarily on benthic invertebrates and occasionally on algae and plants.

Trawling collects fish that are located in the open water near the bottom. Due to the shallowness of Hunting Creek, the volume collected is a substantial part of the water column. However, in the river channel, the near bottom habitat through which the trawl moves is only a small portion of the water column. Fishes tend to concentrate near the bottom or along shorelines rather than in the upper portion of the open water.

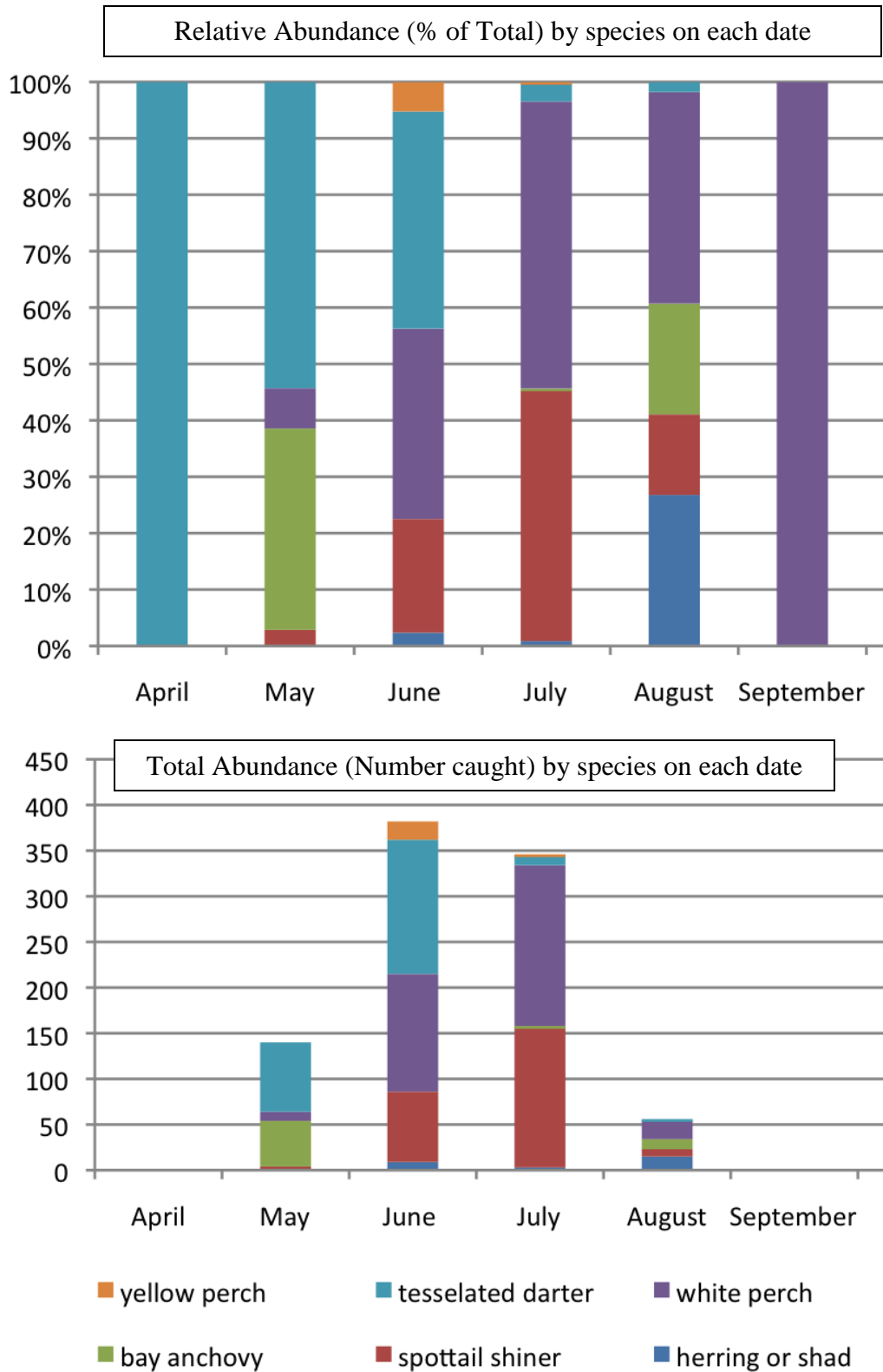


Figure 69 A&B. Adult and Juvenile Fishes Collected by Trawling. Dominant Species by Month.

Displayed as catch per month, it can be seen again that white perch was the most common species, and was present from May to September (Figure 69A and B). The relative abundance of spottail shiner had a similar distribution. Another common species were tessellated darter, which was highest in relative abundance early in the season and was slowly replaced by white perch, and was at its highest absolute abundance in June. The most productive months overall were June and July.

Seines

Seine sampling was conducted semi-monthly at 2 stations between 10 April and 10 September. As planned, only one sampling trip per month was performed in April and September.

Table 8. Adult and juvenile fish collected by seining. Hunting Creek study - 2013

Family	Species	Common name	Abundance	% total
Anguillidae	<i>Anguilla rostrata</i>	American eel	1	0.04
Atherinidae	<i>Menidia beryllina</i>	inland silverside	13	0.49
Belonidae	<i>Strongylura marina</i>	Atlantic needlefish	1	0.04
Catostomidae	<i>Carpionodes cyprinus</i>	quillback	9	0.34
Centrarchidae	<i>Lepomis gibbosus</i>	pumpkinseed	1	0.04
	<i>Lepomis macrochirus</i>	bluegill	3	0.11
	<i>Lepomis microlophus</i>	redear sunfish	1	0.04
	<i>Lepomis sp.</i>	sunfish	2	0.07
	<i>Micropterus dolomieu</i>	smallmouth bass	5	0.19
	<i>Micropterus punctulatus</i>	spotted bass	1	0.04
	<i>Micropterus salmoides</i>	large-mouth bass	3	0.11
	<i>Micropterus sp.</i>	bass species	1	0.04
	<i>Alosa sp.</i>	herring or shad	499	18.7
	<i>Dorosoma cepedianum</i>	gizzard shad	4	0.15
Cyprinidae	<i>Notemigonus crysoleucas</i>	golden shiner	2	0.07
	<i>Notropis hudsonius</i>	spottail shiner	57	2.14
	<i>Notropis sp.</i>	shiner sp.	1	0.04
Cyprinodontidae	<i>Fundulus diaphanus</i>	banded killifish	1786	66.9
	<i>Fundulus heteroclitus</i>	mummichog	53	1.99
Engraulidae	<i>Anchoa mitchilli</i>	bay anchovy	7	0.26
Ictaluridae	<i>Ameiurus nebulosus</i>	brown bullhead	1	0.04
Percichthyidae	<i>Morone americana</i>	white perch	202	7.57
	<i>Morone saxatilis</i>	striped bass	2	0.07
Percidae	<i>Etheostoma olmstedii</i>	tessellated darter	2	0.07
	<i>Perca flavescens</i>	yellow perch	1	0.04
Poeciliidae	<i>Gambusia holbrooki</i>	mosquitofish	11	0.41
Total			2669	100

The two seines stations (Stations ARE 5 and ARE 6; Figure 1) were selected as locations with shallow sloping shorelines that would enable us to tow a beach seine. The net was towed up onto the beach unless high water completely submerged the beach. In those cases, the net was towed into the boat.

A total of 10 seine samples were conducted, comprising 2669 fishes of 26 species (Table 8). The dominant species in seine catches was banded killifish (66.9%), followed by herrings (18.7%). Several other species occurred at high abundances (>50 total) including: white perch, spottail shiner, and mummichog. Other species occurred at medium or low abundances (Table 8).

Banded killifish (*Fundulus diaphanus*) is a small fish, but the most abundant species in shoreline areas. Individuals become sexually mature at about 5 cm in length and may grow to over 8 cm long. Spawning occurs throughout the warmer months over vegetation and shells. They feed on benthic invertebrates, vegetation, and very small fishes.

White perch (*Morone americana*), which was discussed earlier in the trawl section, is also a common shoreline fish as juveniles collected in seines. The juveniles of white perch are attracted to the littoral zone as habitat where their predation risk is lower and potential food intake is higher.

Seining is conducted in shallow water adjacent to the shoreline. Some fish minimize predation by congregating along the shoreline rather than disperse through the open water. The high abundance of fish in seine tows, while seines sample a smaller volume of water than trawls emphasizes the higher densities of fish along the shoreline.

Table 9. Adult and juvenile fish collected by seining. Hunting creek study - 2013

			4/10	5/08	5/22	6/05	6/19	7/17	7/26	8/07	8/21	9/10
Anguillidae	<i>Anguilla rostrata</i>	American eel	0	0	0	0	1	0	0	0	0	0
Atherinidae	<i>Menidia beryllina</i>	inland silverside	0	5	0	1	0	2	1	4	0	0
Belonidae	<i>Strongylura marina</i>	Atlantic needlefish	0	0	0	0	0	0	0	1	0	0
Catostomidae	<i>Carpionodes cyprinus</i>	quillback	0	0	0	1	8	0	0	0	0	0
Centrarchidae	<i>Lepomis gibbosus</i>	pumpkinseed	0	0	0	0	0	0	0	0	0	1
	<i>Lepomis machrochirus</i>	bluegill	1	0	1	1	0	0	0	0	0	0
	<i>Lepomis microlophus</i>	redear sunfish	1	0	0	0	0	0	0	0	0	0
	<i>Lepomis sp.</i>	sunfish	0	0	0	0	2	0	0	0	0	0
	<i>Micropterus dolomieu</i>	smallmouth bass	0	0	0	0	0	0	0	1	3	1
	<i>Micropterus punctulatus</i>	spotted bass	0	0	0	0	0	1	0	0	0	0
	<i>Micropterus salmoides</i>	large-mouth bass	0	0	0	1	0	0	0	1	0	1
	<i>Micropterus sp.</i>	bass species	0	0	0	0	1	0	0	0	0	0
Clupeidae	<i>Alosa sp.</i>	herring or shad	0	473	0	0	0	0	5	0	4	17
	<i>Dorosoma cepedianum</i>	gizzard shad	0	0	0	0	0	0	4	0	0	0
Cyprinidae	<i>Notemigonus crysoleucas</i>	golden shiner	2	0	0	0	0	0	0	0	0	0
	<i>Notropis hudsonius</i>	spottail shiner	51	2	0	0	4	0	0	0	0	0
	<i>Notropis sp.</i>	shiner sp.	0	0	0	0	0	0	1	0	0	0
Cyprinodontidae	<i>Fundulus diaphanus</i>	banded killifish	146	819	8	226	14	166	26	62	302	17
	<i>Fundulus heteroclitus</i>	mummichog	0	0	19	1	3	22	0	2	6	0
Engraulidae	<i>Anchoa mitchilli</i>	bay anchovy	0	7	0	0	0	0	0	0	0	0
Ictaluridae	<i>Ameriurus nebulosus</i>	brown bullhead	0	0	1	0	0	0	0	0	0	0
Percichthyidae	<i>Morone americana</i>	white perch	7	0	6	0	13	6	11	61	16	82
	<i>Morone saxatilis</i>	striped bass	0	0	0	0	0	0	0	0	1	1
Percidae	<i>Etheostoma olmstedii</i>	tesselated darter	0	0	0	0	2	0	0	0	0	0
	<i>Perca flavescens</i>	yellow perch	0	0	0	0	0	0	0	0	0	1
Poeciliidae	<i>Gambusia holbrooki</i>	mosquitofish	0	0	0	0	0	0	0	9	2	0
Total			208	1306	35	231	48	197	48	141	334	121

Table 10. Adult and juvenile fish collected by seining. Hunting creek study - 2013

			Station 5	Station 6
Anguillidae	<i>Anguilla rostrata</i>	American eel	1	0
Atherinidae	<i>Menidia beryllina</i>	inland silverside	1	12
Belonidae	<i>Strongylura marina</i>	Atlantic needlefish	0	1
Catostomidae	<i>Carpionodes cyprinus</i>	quillback	0	9
Centrarchidae	<i>Lepomis gibbosus</i>	pumpkinseed	1	0
	<i>Lepomis machrochirus</i>	bluegill	2	1
	<i>Lepomis microlophus</i>	redeer sunfish	0	1
	<i>Lepomis sp.</i>	sunfish	1	1
	<i>Micropterus dolomieu</i>	smallmouth bass	4	1
	<i>Micropterus punctulatus</i>	spotted bass	0	1
	<i>Micropterus salmoides</i>	large-mouth bass	1	2
	<i>Micropterus sp.</i>	bass species	1	0
Clupeidae	<i>Alosa sp.</i>	herring or shad	17	482
	<i>Dorosoma cepedianum</i>	gizzard shad	0	4
Cyprinidae	<i>Notemigonus crysoleucas</i>	golden shiner	0	2
	<i>Notropis hudsonius</i>	spottail shiner	2	55
	<i>Notropis sp.</i>	shiner sp.	0	1
Cyprinodontidae	<i>Fundulus diaphanus</i>	banded killifish	1104	682
	<i>Fundulus heteroclitus</i>	mummichog	27	26
Engraulidae	<i>Anchoa mitchilli</i>	bay anchovy	0	7
Ictaluridae	<i>Ameriurus nebulosus</i>	brown bullhead	1	0
Percichthyidae	<i>Morone americana</i>	white perch	86	116
	<i>Morone saxatilis</i>	striped bass	2	0
Percidae	<i>Etheostoma olmsted</i>	tessellated darter	0	2
	<i>Perca flavescens</i>	yellow perch	0	1
Poeciliidae	<i>Gambusia holbrooki</i>	mosquitofish	11	0
Total			1262	1407

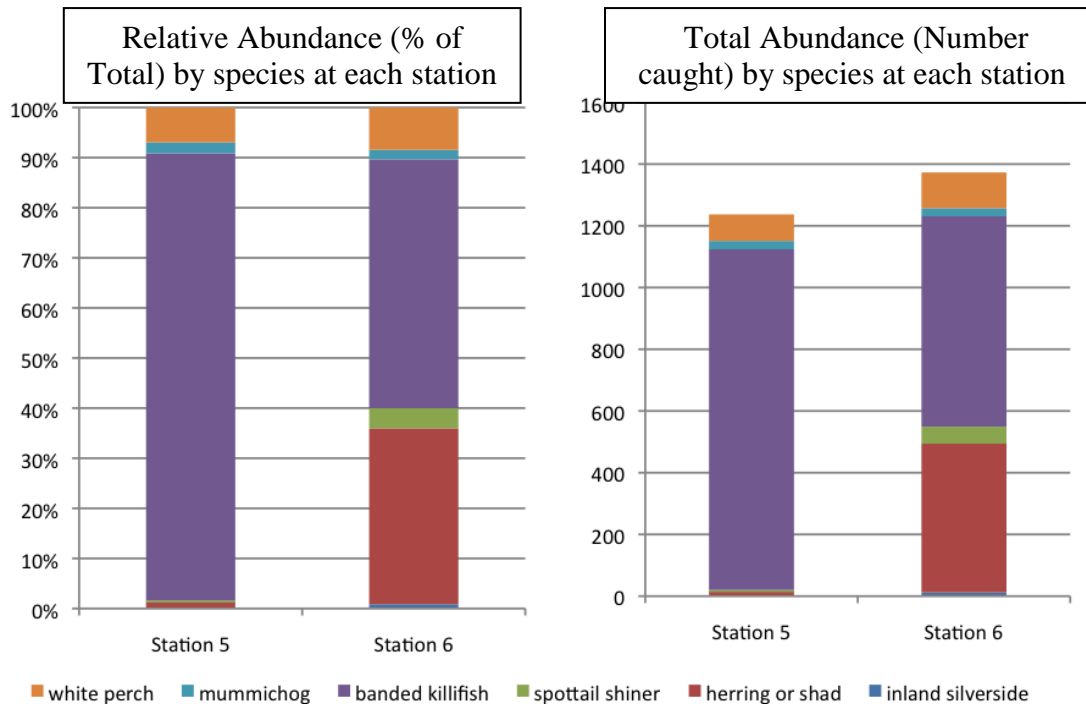


Figure 70. Adult and Juvenile Fishes Collected by Seining. Dominant Species by Station.

Banded killifish was present and dominant at both stations, in all months sampled (Figure 70 and 71). Almost all fish collected in Station 5 were banded killifish, while Station 6 saw a high abundance of herring or shad as well. The relative abundance of white perch increased slowly towards the end of the season and showed highest relative abundance in September.

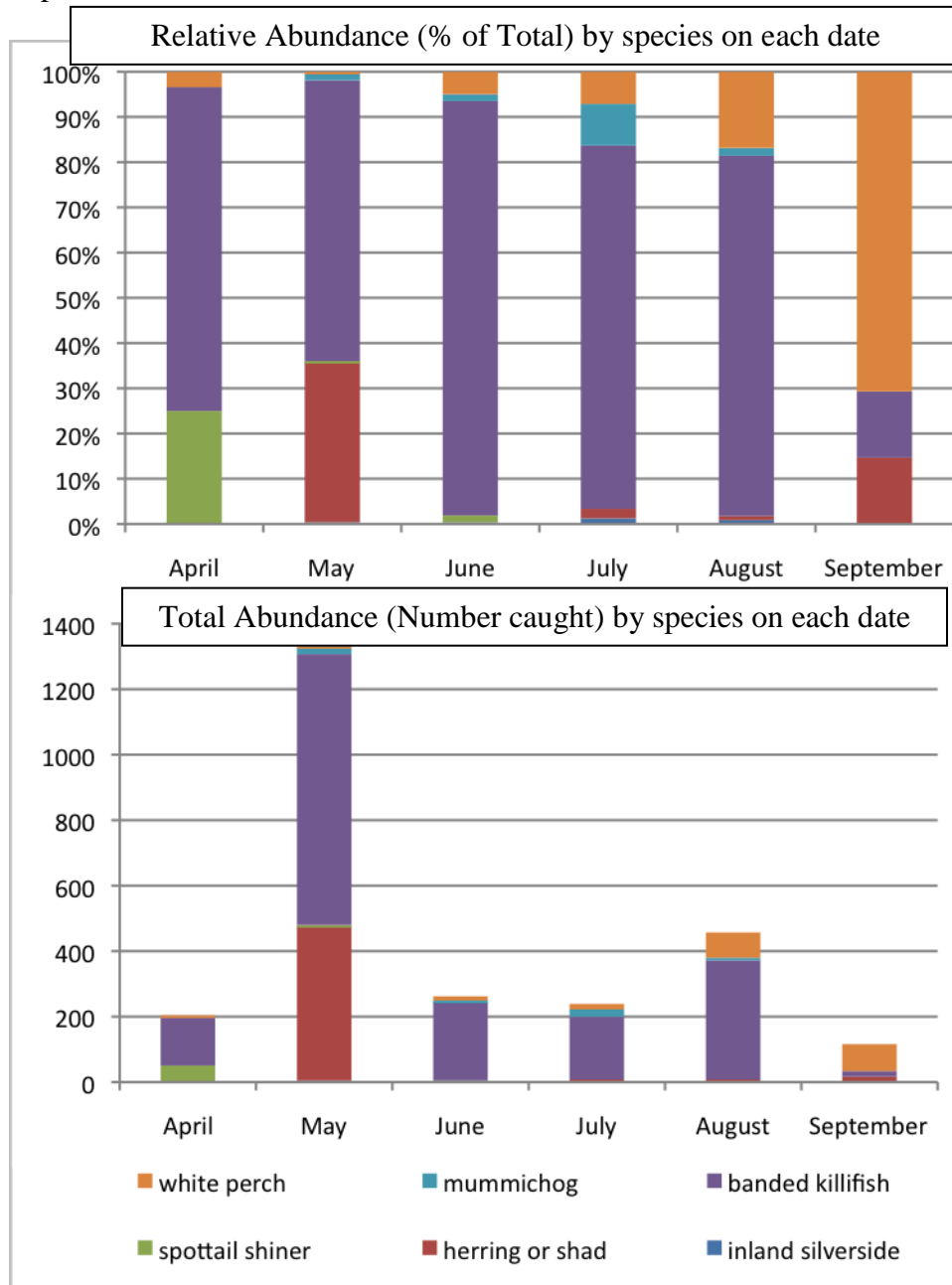


Figure 71A and B. Adult and Juvenile Fish Collected by Seining. Dominant Species by Month.

F. Submersed Aquatic Vegetation – 2013

SAV data overflights by VIMS were conducted in 2013 and Figure 72 (left) depicts the area covered by SAV that was detectable by aerial remote sensing. Note that this area was much larger than that identified in 2012 illustrating the variation that has occurred in recent years.

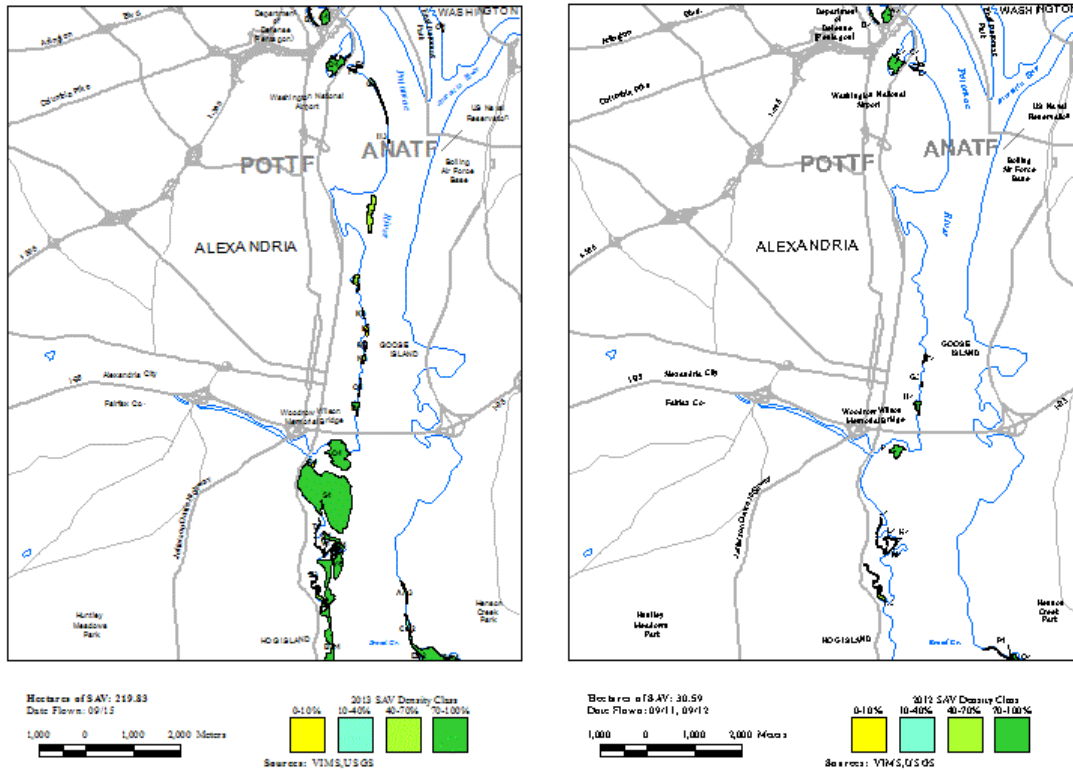


Figure 72. Distribution and density of Submersed Aquatic Vegetation (SAV) in the Hunting Creek area in 2013 (left) and 2012 (right). VIMS (<http://www.vims.edu/bio/sav/index.html>).

H. Benthic Macroinvertebrates - 2013

Triplicate petite ponar samples were collected at ARE2, ARE3, and ARE4 monthly from May through August. Due to access difficulties, only four samples were obtained at ARE1, those in May and June. Averages over all samples collected at each station are shown in Figure 73. Oligochaetes were the most common invertebrates collected in these samples ranging from 66-172 per petite ponar (Figure 73a). Chironomid (midge) larvae made up the most of the remaining organisms at most stations. At ARE 2 there was a substantial contribution from gastropods (snails), amphipods (scuds), and bivalves (Figure 73b).

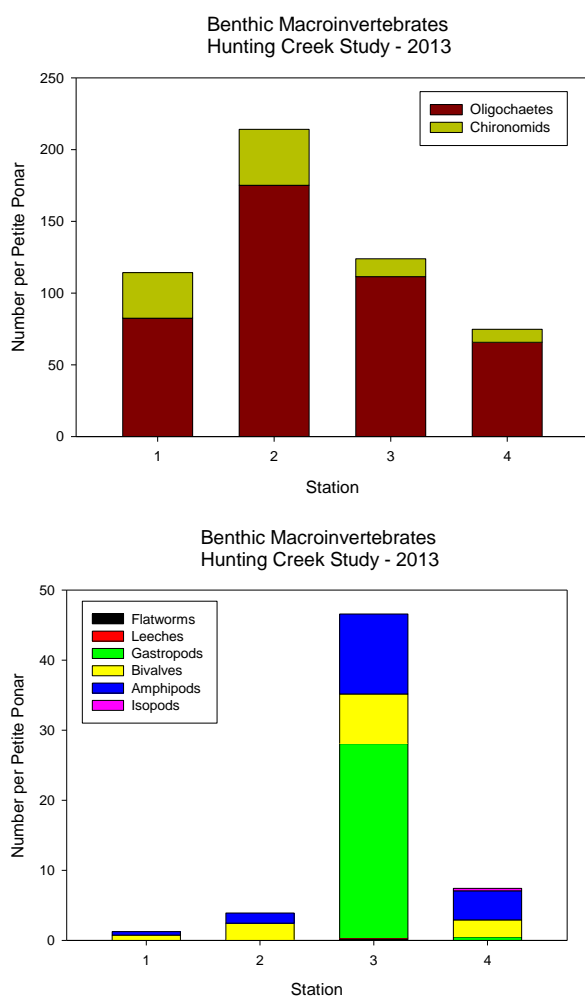


Figure 73. Average abundance of various benthic macroinvertebrate taxa in petite ponar samples collected on four dates in 2013. (a) dominant taxa. (b) “other” group from (a) broken out by taxa.

DISCUSSION

A. 2013 Synopsis

In 2013 air temperature was substantially above average from April through July. August and September were about normal; July was the warmest month. Precipitation was below normal from March through May, but well above normal in June and somewhat elevated in July. August and September were well below normal in precipitation. Potomac River discharge was about average from February through September. However, there were major flow peaks in May, June and July. In Cameron Run there were many flow spikes in June and the average flow for that month was over three times the normal average.

Water temperature followed a typical seasonal pattern at all stations. Mapping of water temperature suggested that the shallow Hunting Creek embayment responded more rapidly to changes in air temperature than the deeper river channel area. Specific conductance was elevated at ARE1 above the GW Parkway bridge and at times in upper Hunting Creek as shown by the June mapping cruise. Chloride followed a similar pattern. Dissolved oxygen was generally in the 80-120 percent saturation range indicating that neither photosynthesis nor respiration was excessive. The June mapping cruise indicated a hot spot of elevated photosynthesis in upper Hunting Creek, but this was not found in the August cruise. pH was generally in the 7-8 range at all stations, but responded negatively at all stations during June, perhaps related to the high run and river flow at that time. In the cruises, pH was generally somewhat lower in Hunting Creek than in the river mainstem. Total alkalinity was variable through time, but similar at all stations except ARE1 which was generally lower and showed a different seasonal pattern.

Secchi disk depth underwent a strong seasonal pattern at all stations with values decreasing from April through June, recovering somewhat in July. Water clarity was pretty good in August and September, but Secchi depth measurements were impeded by SAV and by reaching the bottom. The light penetration improvement in August and September was shown by decreased light attenuation and turbidity in Hunting Creek at this time. The water quality mapping cruise on June 14, which with an average wind speed of 13.3 knots (highest of summer 2013), revealed the susceptibility of Hunting Creek to wind resuspension with elevated turbidity and very high hot spots along the cruise path. And of course there was a lot of inflow in June as well that brought in suspended sediments. This was not observed on August 7, a day of lower winds and a period of low inflows.

Ammonia nitrogen was quite low (0.2 mg/L) on most dates at all stations. The exception was early June with ARE1 and ARE2 being about 1.0 mg/L on that date. Nitrate was generally about 1 mg/L in spring and showed an overall decrease through the year at ARE3 and ARE4. ARE1 and ARE2 were more variable with summer maxima of about 2 mg/L. Nitrite was generally 0.02 mg/L or less except on one date at ARE3. Organic nitrogen exhibited a gradual increase over the study period at all stations reaching a maximum in late July or August. The highest values were observed at ARE1. As with many parameters, changes at ARE4 (river) were much more gradual than at the embayment stations. Total phosphorus was mostly between 0.05 and 0.10 mg/L, but was quite variable. Soluble reactive phosphorus was quite elevated at ARE1 and ARE2 during the May and June, but was similar at all stations and quite low from July on. N to P ratio varied

markedly between stations and over time, but always remained above 10, indicative of P limitation.

BOD was generally 1 or 2 mg/L at all stations, but was sometimes elevated to 3 or 4 mg/L at ARE1 and ARE2. TSS was generally 5-20 mg/L at all station, but was elevated during the June inflows and winds at all stations. Values also increased in the river in late August and September. Volatile suspended solids followed similar seasonal and spatial patterns. Chlorophyll exhibited a general seasonal pattern of increase, but it was often punctuated at most stations by reversals from one sampling time to the next. The seasonal pattern really took off in late June and peaked in late July. The river showed a smoother seasonal progression. Interestingly, the station above GW Parkway bridge showed some of the highest chlorophyll levels. Water quality mapping showed a maximum in June in upper Hunting Creek, but not in August.

Phytoplankton cell density was fairly stable through April and May, but increased in early June only to decrease in late June before showing as substantial rise in July in both areas. Cyanobacteria and diatoms were the most important contributors to cell density in both areas. *Oscillatoria* and *Merismopedia* were the most important cyanobacteria on most dates while a variety of diatoms contributed. The cryptophytes *Chroomonas* and *Cryptomonas* were also important at both stations. Phytoplankton biovolume exhibited a progressive increase from April through September at the Hunting Creek station, but was quite variable at the river station. Biovolume was strongly dominated by diatoms on most dates. *Melosira* and discoid centrics stood out as major contributors, but there were a selection of others that were important on some dates.

Rotifers were the most numerous zooplankton and bloomed strongly in late July and August, possibly held back by the high flushing and winds in June. *Brachionus* was the most numerous genus with *Synchaeta* contributing in late July and *Keratella* in August. The small bodied cladoceran *Bosmina* reached fairly high levels in July, especially in the river. The larger bodied cldocerans like *Diaphanosoma*, *Daphnia*, *Ceriodaphnia*, and *Moina* were limited both in peak numbers and even in dates of occurrence. *Leptodora* made a strong showing in the river on two dates, but was scarcer in Hunting Creek. Copepod nauplii did well as the summer went along tracking in together at both stations with a maximum in late June. *Eurytemora* which can reach very large populations did so in the river in late June, but was always quite low in Hunting Creek. Cyclopoid copepods were not very common. Ostracods, which have been found only sporadically in Gunston Cove samples, were found at moderate densities at both stations.

B. Correlation Analysis of Hunting Creek Data

To better understand the ecological relationships in Hunting Creek and the nearby Potomac River, relationship among parameters were assessed using correlation analysis. Due to the uncertain statistical distribution of some parameters, the correlations were conducted using the Spearman rank correlation coefficient rather than the Pearson coefficient.

Since all samples were collected by PEREC personnel at the same time, it was possible to pool the data on all field and lab water quality parameters at the level of depth-averages. Three tables were constructed: PEREC field and lab parameters with each other, AlexRenew lab parameters

with each other, and PEREC field parameters against AlexRenew lab parameters.

Table 11 shows the correlations among PEREC collected water quality parameters. Temperature was highly correlated with chlorophyll measures reflecting the seasonal pattern in both. Conductivity was negatively correlated against turbidity which could be related to dilution of ions being associated with increased turbidity in the aftermath of runoff events. However, there was no relationship to TSS which should be affected in a similar way. Dissolved oxygen, measured as percent saturation, was correlated positively with pH and negatively with turbidity. Each of these could reflect a relationship to photosynthetic rate; higher photosynthetic rates would be possible with lower turbidity and produce more oxygen and higher pH. More relationships were found when dissolved oxygen was quantified as mg/L, including a positive relationship to Secchi disk and negative relationships to turbidity, chlorophyll, and suspended solids. While some of these may be meaningful, they are hard to interpret because when expressed as mg/L, DO is very closely tied to temperature. pH was positively correlated with Secchi disk and light extinction coefficient, and negatively correlated with turbidity and TSS. These correlations are consistent with pH being driven by photosynthesis such that light is enhanced by higher light transparency and all of these correlative factors are related to light levels in the water.

In addition to the relationships already mentioned, Secchi disk depth was positively correlated with light extinction coefficient and negatively correlated with turbidity and suspended solids. In addition, light extinction coefficient was negatively correlated with turbidity and TSS. And turbidity was strongly correlated with suspended solids. These correlations create a strong argument for the overall negative relationship between suspended solids and light availability in the water column. There was a strong correlation between VSS and TSS, but VSS was also strongly correlated with chlorophyll while TSS was not. The VSS-chlorophyll linkage can be explained by the fact that carbon-based algae are an important component of the volatile suspended solids while also containing chlorophyll.

The correlation coefficients among AlexRenew lab parameters are shown in Table 12. pH was strongly negatively correlated with orthophosphorus, but not total phosphorus. Negative correlations were also found between pH and ammonia nitrogen and suspended solids. Alkalinity was negatively correlated with both nitrate nitrogen and BOD. Total phosphorus and orthophosphorus were both positively correlated with ammonia nitrogen and TSS. Organic nitrogen was correlated with chloride, perhaps due to the fact that both were higher at Sta. 1, just downstream of the AlexRenew effluent. Nitrate was positively related to TSS. Nitrite was positively related to VSS and COD. TSS and VSS were highly correlated.

Table 13 contains the correlation coefficients between PEREC and AlexRenew parameters. Temperature was correlated with alkalinity, organic nitrogen, and date, all of these increasing as the months passed. Conductivity was also correlated with organic nitrogen and date, but had the strongest correlation with chloride as much of the variation in conductivity is due to changing chloride concentrations especially related to input from Cameron Run and the AlexRenew effluent. Both DO parameters showed the same correlation profile: negative relationships to total phosphorus, ammonia nitrogen and suspended solids. The negative relationships to solids could

Table 11. Spearman Rank Correlation Coefficients among PEREC collected parameters.

Spearman Correlation Matrix													
	TEMP	COND25	DOPPM	DOSAT	PH	SECCHI	EXTCOEF	YSITURB	YSICHL	TSSDIGM	VSSDIGM	CHLDI	PHEODI
TEMP	1.000												
COND25	0.202	1.000											
DOPPM	-0.263	0.010	1.000										
DOSAT	0.049	0.036	0.854	1.000									
PH	0.029	-0.118	0.516	0.419	1.000								
SECCHI	-0.160	-0.077	0.443	0.208	0.661	1.000							
EXTCOEF	0.046	0.183	0.368	0.172	0.548	0.841	1.000						
YSITURB	-0.028	-0.463	-0.511	-0.425	-0.406	-0.793	-0.534	1.000					
YSICHL	0.472	0.241	-0.422	-0.196	0.044	-0.419	-0.187	0.296	1.000				
TSSDIGM	0.150	-0.042	-0.548	-0.397	-0.570	-0.722	-0.452	0.836	0.293	1.000			
VSSDIGM	0.386	-0.013	-0.497	-0.255	-0.331	-0.737	-0.406	0.690	0.458	0.804	1.000		
CHLDI	0.697	0.577	-0.291	-0.108	0.200	-0.237	0.010	-0.053	0.867	0.141	0.462	1.000	
PHEODI	0.742	0.578	-0.483	-0.315	0.104	-0.257	-0.042	0.029	0.790	0.297	0.538	0.921	1.000

Pairwise Frequency Table													
	TEMP	COND25	DOPPM	DOSAT	PH	SECCHI	EXTCOEF	YSITURB	YSICHL	TSSDIGM	VSSDIGM	CHLDI	PHEODI
TEMP	39												
COND25	39	39											
DOPPM	39	39	39										
DOSAT	39	39	39	39									
PH	39	39	39	39	39								
SECCHI	27	27	27	27	27	27							
EXTCOEF	29	29	29	29	29	25	29						
YSITURB	39	39	39	39	39	27	29	39					
YSICHL	38	38	38	38	38	27	29	38	38				
TSSDIGM	29	29	29	29	29	25	29	29	29	29			
VSSDIGM	29	29	29	29	29	25	29	29	29	29	29		
CHLDI	29	29	29	29	29	25	29	29	29	29	29	29	
PHEODI	29	29	29	29	29	25	29	29	29	29	29	29	29

Table 12. Spearman Rank Correlation Coefficients among ARE lab parameters.

Spearman Correlation Matrix													
	PHLAB	ALK	TP	OP	ORGN	NO3N	NH4N	NO2N	CLD	TSS	VSS	BOD	COD
PHLAB	1.000												
ALK	0.311	1.000											
TP	-0.103	-0.359	1.000										
OP	-0.782	-0.238	0.026	1.000									
ORGN	-0.032	0.193	0.131	-0.236	1.000								
NO3N	-0.216	-0.631	0.340	0.123	-0.048	1.000							
NH4N	-0.544	-0.333	0.564	0.506	-0.007	0.205	1.000						
NO2N	-0.120	0.010	0.030	0.151	0.221	0.155	-0.032	1.000					
CLD	0.000	-0.097	0.106	-0.197	0.659	0.025	0.152	0.138	1.000				
TSS	-0.483	-0.112	0.479	0.450	0.058	0.347	0.548	0.330	-0.139	1.000			
VSS	-0.503	-0.033	0.351	0.458	0.202	0.259	0.305	0.428	0.004	0.795	1.000		
BOD	0.256	-0.419	0.249	-0.266	0.070	0.308	-0.179	0.095	0.266	-0.078	0.065	1.000	
COD	-0.414	-0.342	0.224	0.374	0.074	0.162	0.257	0.483	0.247	0.277	0.360	0.093	1.000

Pairwise Frequency Table													
	PHLAB	ALK	TP	OP	ORGN	NO3N	NH4N	NO2N	CLD	TSS	VSS	BOD	COD
PHLAB	39												
ALK	39	39											
TP	39	39	39										
OP	39	39	39	39									
ORGN	37	37	37	37	38								
NO3N	39	39	39	39	37	39							
NH4N	39	39	39	39	37	39	39						
NO2N	39	39	39	39	37	39	39	39					
CLD	39	39	39	39	37	39	39	39	39				
TSS	39	39	39	39	37	39	39	39	39	39			
VSS	39	39	39	39	37	39	39	39	39	39	39		
BOD	39	39	39	39	37	39	39	39	39	39	39	39	
COD	31	31	31	31	29	31	31	31	31	31	31	31	31

Table 13. Spearman Rank Correlation Coefficients between PEREC and ARE lab parameters.

Spearman Correlation Matrix																
	TEMP(1)	COND25	DOPPM	DOSAT	PH	SECCHI	EXTCOEF	YSITURB	YSICHL	TSSSURFGM	TSSDIGM	VSSSURFGM	VSSDIGM	CHLDI	PHEODI	CHLSF
PHLAB	0.175	0.231	0.308	0.286	0.668	0.558	0.496	-0.490	0.187	-0.341	-0.511	-0.131	-0.245	0.401	0.243	0.355
ALK	0.428	0.336	0.035	0.106	0.378	0.345	0.361	-0.372	0.263	-0.369	-0.184	-0.370	-0.144	0.523	0.606	0.296
TP	-0.058	-0.123	-0.471	-0.522	-0.327	-0.222	-0.127	0.585	-0.073	0.575	0.557	0.515	0.433	-0.141	0.029	-0.028
OP	-0.026	-0.366	-0.248	-0.264	-0.429	-0.281	-0.376	0.460	-0.118	0.265	0.353	0.053	0.140	-0.413	-0.260	-0.341
ORGN	0.501	0.598	-0.316	-0.071	-0.346	-0.417	-0.049	-0.097	0.321	0.176	0.220	0.373	0.347	0.534	0.661	0.540
NO3N	-0.117	-0.268	-0.267	-0.180	-0.440	-0.314	-0.267	0.448	0.027	0.562	0.469	0.501	0.413	-0.128	-0.202	0.017
NH4N	-0.265	-0.074	-0.610	-0.720	-0.551	-0.302	-0.262	0.389	-0.232	0.371	0.473	0.235	0.288	-0.285	-0.030	-0.331
NO2N	0.371	0.029	-0.223	-0.058	-0.140	-0.358	0.100	0.264	0.395	0.233	0.413	0.230	0.510	0.320	0.274	0.263
CLD	0.096	0.834	-0.141	-0.087	-0.377	-0.304	0.009	-0.231	0.144	-0.010	0.104	0.212	0.177	0.430	0.443	0.295
TSS	0.035	-0.308	-0.692	-0.607	-0.410	-0.438	-0.207	0.786	0.317	0.693	0.712	0.441	0.560	0.033	0.207	0.094
VSS	0.291	-0.180	-0.592	-0.411	-0.344	-0.465	-0.220	0.712	0.416	0.604	0.651	0.431	0.621	0.207	0.397	0.263
BOD	0.188	0.005	-0.018	0.063	-0.047	-0.342	-0.118	0.217	0.242	0.269	-0.007	0.394	0.237	0.318	0.111	0.421
COD	0.036	0.004	-0.084	-0.027	-0.468	-0.428	-0.161	0.263	-0.104	0.325	0.462	0.241	0.477	-0.125	-0.041	-0.158
DATE	0.485	0.653	0.010	0.227	0.163	-0.261	0.084	-0.246	0.542	-0.188	0.082	-0.115	0.135	0.667	0.653	0.519

Pairwise Frequency Table																
	TEMP(1)	COND25	DOPPM	DOSAT	PH	SECCHI	EXTCOEF	YSITURB	YSICHL	TSSSURFGM	TSSDIGM	VSSSURFGM	VSSDIGM	CHLDI	PHEODI	CHLSF
PHLAB	39	39	39	39	39	27	29	39	38	39	29	39	29	29	29	39
ALK	39	39	39	39	39	27	29	39	38	39	29	39	29	29	29	39
TP	39	39	39	39	39	27	29	39	38	39	29	39	29	29	29	39
OP	39	39	39	39	39	27	29	39	38	39	29	39	29	29	29	39
ORGN	37	37	37	37	37	26	28	37	36	37	28	37	28	28	28	37
NO3N	39	39	39	39	39	27	29	39	38	39	29	39	29	29	29	39
NH4N	39	39	39	39	39	27	29	39	38	39	29	39	29	29	29	39
NO2N	39	39	39	39	39	27	29	39	38	39	29	39	29	29	29	39
CLD	39	39	39	39	39	27	29	39	38	39	29	39	29	29	29	39
TSS	39	39	39	39	39	27	29	39	38	39	29	39	29	29	29	39
VSS	39	39	39	39	39	27	29	39	38	39	29	39	29	29	29	39
BOD	39	39	39	39	39	27	29	39	38	39	29	39	29	29	29	39
COD	31	31	31	31	31	20	23	31	30	31	23	31	23	23	23	31
DATE	39	39	39	39	39	27	29	39	38	39	29	39	29	29	29	39

be related to solids decreasing light which would inhibit photosynthesis and lower DO. The ammonia relationship may be strongly influenced by the spike in ammonia that occurred in early June which also saw a drop in DO. PEREC field pH exhibited a large number of significant relationships: positive correlations with AlexRenew lab pH and alkalinity, and negative correlations with orthophosphorus, nitrate, ammonia, TSS, and COD. The positive correlations are to be expected given the obvious tight linkage among these parameters. The negative correlations appear to be associated with the June wet period in which pH was depressed and the other parameters were accentuated.

Secchi depth and extinction coefficient had a positive relationship to AlexRenew lab pH, all declined during the early June runoff event. Secchi was also negatively related to VSS. Turbidity had a number of correlations: positive with total phosphorus, orthophosphorus, nitrate, ammonia, and suspended solids and negative with lab pH. All of these parameters showed elevated levels (except pH which dropped) early June and many had a second peak in late July. YSI chlorophyll was significantly correlated in a positive way with chloride, VSS, and date. Extracted chlorophylls (CHLDI and CHLSF) were correlated positively with alkalinity, organic nitrogen, and date. Both surface and depth-integrated TSS were positively correlated with total phosphorus, nitrate, and AlexRenew lab suspended solids. The intercorrelation of solids is to be expected and since total P is often associated with solids that is expected. The correlation with nitrate seems to be related to the elevated values in both variables in early June and late July. Two additional correlations were found with depth-integrated TSS: negative with AlexRenew lab pH and positive with ammonia nitrogen and COD.

Water quality mapping revealed some interesting spatial and temporal patterns in the study area. Correlations were done among the mapping parameters for each date. On the June cruise, all of the correlations shown in Table 14a were statistically significant at the 0.01 level. Some of the strongest correlations were with temperature which basically broke down the data into two regions: the cooler Hunting Creek embayment and the warmer river mainstem. Specific conductance, temperature, and chlorophyll were negatively correlated with temperature (higher in Hunting Creek) and pH was positively correlated with temperature (higher in the river mainstem). The lack of strong positive relationships between chlorophyll and either dissolved oxygen or pH indicates that photosynthesis was not very active in this period.

On the August cruise, temperature was not as much of a defining factor although it was still cooler in Hunting Creek than in the river mainstem (Table 14b). Specific conductance was positively correlated with quite a few variables including dissolved oxygen, turbidity and chlorophyll. This seemed to be related to the fact that all of these variables were highest in the embayments on both Virginia and Maryland side and lower in the river mainstem. There were high correlations between dissolved oxygen and chlorophyll consistent with photosynthesis effects. A positive correlation between turbidity and chlorophyll could be explained by the increase in turbidity and chlorophyll associated with elevated phytoplankton densities.

C. Comparison with Recent Gunston Cove Data

To further contextualize the observations made in the 2013 for Hunting Creek and the nearby Potomac River, we extracted summary information from the Gunston Cove study dataset.

Table 14a. Spearman Rank Correlation Coefficients between mapped parameters. June 14, 2013. (n=110). All coefficients were statistically significant given the large n. Those over 0.450 were considered strong and highlighted.

Spearman Correlation Matrix							
	TEMP	SPCOND	DOPPM	DOSAT	PH	YSITURB	YSICHL
TEMP	1.000						
SPCOND	-0.825	1.000					
DOPPM	0.272	-0.308	1.000				
DOSAT	0.391	-0.381	0.967	1.000			
PH	0.795	-0.838	0.368	0.441	1.000		
YSITURB	-0.818	0.753	-0.466	-0.549	-0.731	1.000	
YSICHL	-0.856	0.790	-0.298	-0.378	-0.832	0.872	1.000

Table 14b. Spearman Rank Correlation Coefficients between mapped parameters. August 7, 2013. (n=498). All coefficients were statistically significant given the large n. Those over 0.450 were considered strong and highlighted.

Spearman Correlation Matrix							
	TEMP	SPCOND	DOPPM	DOSAT	PH	YSITURB	YSICHL
TEMP	1.000						
SPCOND	0.187	1.000					
DOPPM	0.228	0.475	1.000				
DOSAT	0.307	0.503	0.992	1.000			
PH	-0.201	-0.327	0.377	0.336	1.000		
YSITURB	0.009	0.604	0.123	0.141	-0.395	1.000	
YSICHL	0.479	0.778	0.725	0.765	-0.014	0.467	1.000

Specifically, we took data from the period 2005-2012 for the Gunston Cove study and calculated summary statistics. We then compared those summary statistics with those derived from this 2013 Hunting Creek Study. Both datasets are similar in that they encompass both embayment and river stations, they have the same sampling period and frequency, and they use the same or nearly the same methodologies. For the current draft analysis we used depth-integrated averages for Hunting Creek and separate surface and bottom samples from Gunston Cove, but that can be aligned better in the final report.

For an initial comparison, we will examine the median values for the 2013 Hunting Creek study as compared with the lower and upper quartile values from the 2005-2012 Gunston Cove data set. If the median for Hunting Creek fell outside this range for Gunston Cove that would be noteworthy. The comparison for PEREC collected field and lab parameters can be made by comparing the median values in Table 15a with the lower and upper quartile values found in Table 15b. Based on this comparison median chlorophyll values (measured by YSI and by extraction) were lower than the lower quartile value for the pooled Gunston Cove data suggesting that phytoplankton levels in Hunting Creek in 2013 were clearly lower than the norm for the freshwater tidal Potomac as defined by Gunston Cove. For example the lower quartile for extracted depth-integrated chlorophyll (CHLDI) the 2013 Hunting Creek study was 5.6 ug/L compared with the lower quartile value for the pooled Gunston Cove data of 10.0 ug/L. Volatile suspended solids levels were also low relative to Gunston Cove. In this case the Hunting Creek 2013 value for depth-integrated VSS was 3.2 mg/L while the lower quartile for the Gunston Cove data was 5.7 mg/L. The VSS difference could be another manifestation of the differences in phytoplankton. The median value for turbidity in 2013 Hunting Creek data was almost identical with the lower quartile for the Gunston Cove data again suggesting that the Hunting Creek data was unusually low relative to the benchmark historical data for the tidal Potomac. Field pH for 2013 Hunting Creek was just above the Gunston Cove lower quartile value. All other field and lab PEREC parameters for 2013 Hunting Creek were within the Gunston Cove interquartile range.

A similar comparison was done between AlexRenew lab parameters (Table 16a) and the same suite of parameters measured by the Noman Cole Lab of Fairfax County as part of the Gunston Cove study (Table 16b). The 2013 Hunting Creek study median values of several parameters fell outside of the interquartile range as defined by the 2005-2012 Gunston Cove data. Medians for orthophosphate and ammonia nitrogen were above the upper quartile value of the reference data set while medians for organic nitrogen and volatile suspended solids were below the lower quartile value. The higher value for the two dissolved nutrient forms may be related to the nearby presence of both Blue Plains and Alexandria Renew Enterprises in the Hunting Creek area with only the Noman Cole plant close to the Gunston Cove area. The low values for organic nitrogen and volatile suspended solids are probably related to the lower phytoplankton levels in Hunting Creek suggested above. Two parameters had 2013 Hunting Creek medians that were at the Gunston Cove lower quartile value of the reference data set: Lab pH and BOD. These again could be related to the lower phytoplankton populations in Hunting Creek.

Comparison of phytoplankton count data will need to be more qualitative as data sets of Gunston Cove data have not been comprehensively compiled. Referring to Figure 112 of the 2012 Gunston Cove study report (Jones and deMutsert 2013) the range of annual average

Table 15a. Basic Statistics for PEREC collected parameters. Hunting Creek Study – 2013.

	TEMP	SP COND	DOPPM	DOSAT	PH	SECCHI	EXTCOEF	TURB YSI	CHL YSI	TSS SURF	TSS DI	VSS SURF	VSS DI	CHL DI	PHEO DI	CHL SF	PHEO SF
N of Cases	39	39	39	39	39	27	29	39	38	39	29	39	29	29	29	39	39
Minimum	0.400	210.600	6.004	75.333	6.680	0.280	-3.887	4.400	1.275	7.000	7.300	1.750	1.500	0.672	2.149	0.693	1.829
Maximum	29.562	552.000	11.535	145.150	8.157	1.540	-0.874	39.800	44.850	61.000	54.500	9.250	6.333	29.334	15.700	35.002	15.510
Median	25.170	372.500	8.287	94.525	7.620	0.742	-1.826	10.867	3.700	15.375	17.375	3.000	3.200	5.551	5.858	5.724	5.713
Mean	23.118	357.913	8.254	98.213	7.598	0.747	-1.998	14.358	6.300	18.593	20.127	3.366	3.337	8.450	6.856	8.920	6.767
Standard Error	0.901	12.128	0.236	2.728	0.054	0.047	0.139	1.437	1.311	1.799	1.668	0.240	0.198	1.302	0.726	1.242	0.614
Lower quartile	19.713	300.675	7.000	85.850	7.475	0.593	-2.277	7.925	2.300	12.750	15.269	2.425	2.693	3.344	3.491	3.676	3.423
Upper quartile	27.198	402.875	9.088	101.790	7.833	0.897	-1.508	17.970	6.100	20.951	23.875	3.616	3.600	11.363	9.760	11.864	9.378

Table 15b. Basic statistics for Gunston Cove Data 2005-2012. PEREC parameters. Depth-integrated averages.

	TEMP	COND25	DOPPM	DOSAT	PH	FLD SECCHI	EXTCOF	YSITURB	YSICHL	TSS GM	VSS GM	CHLDI	PHEDI	CHLSF	PHESF
N of Cases	168	168	160	160	164	168	154	106	104	154	154	168	168	168	168
Minimum	9.824	121.000	4.647	57.296	6.473	15.333	-8.837	-16.200	-3.980	2.857	2.143	0.851	0.727	0.769	0.215
Maximum	31.993	629.500	17.800	176.551	9.658	146.000	-1.020	244.800	29.350	180.000	26.000	75.993	30.376	81.686	31.084
Median	26.299	329.717	8.364	98.738	7.851	72.250	-2.008	13.050	8.267	15.857	7.429	16.297	8.036	15.312	7.169
Arithmetic Mean	24.596	335.741	8.764	104.481	7.952	73.874	-2.113	19.111	9.833	17.760	7.664	20.685	9.818	20.053	9.277
Standard Error	0.404	5.511	0.173	1.940	0.044	1.355	0.065	2.856	0.701	1.194	0.241	1.240	0.502	1.281	0.518
Lower quartile	21.117	286.600	6.988	84.000	7.524	64.000	-2.388	10.900	4.200	12.750	5.667	10.001	4.833	9.482	4.496
Upper quartile	28.370	384.619	10.411	121.320	8.353	81.575	-1.644	18.050	14.467	20.250	9.167	26.077	13.508	25.541	12.279

Table 16a. Basic Statistics for ARE Lab parameters. Hunting Creek Study – 2013.

	PHLAB	ALK	TP	OP	ORGN	NO3N	NH4N	NO2N	CLD	TSS	VSS	BOD	COD
N of Cases	39	39	39	39	38	39	39	39	39	39	39	39	31
Minimum	6.700	36.000	0.023	0.004	0.081	0.398	0.019	0.011	9.630	3.600	0.750	1.000	6.000
Maximum	8.300	94.000	0.107	1.081	1.296	2.230	1.072	0.110	83.480	50.500	7.600	4.000	24.400
Median	7.700	79.000	0.070	0.026	0.460	0.814	0.096	0.015	29.965	13.000	2.800	1.000	15.000
Arithmetic Mean	7.592	74.615	0.071	0.093	0.491	0.932	0.136	0.018	32.594	16.182	3.218	1.506	14.692
Standard Error	0.060	2.482	0.004	0.038	0.035	0.079	0.032	0.002	2.457	1.800	0.286	0.121	0.885
Lower quartile	7.302	62.500	0.058	0.013	0.362	0.584	0.068	0.013	23.600	8.350	2.050	1.000	11.150
Upper quartile	7.900	86.750	0.091	0.041	0.566	0.980	0.114	0.020	39.663	18.725	3.600	2.000	18.025

Table 16b. Basic statistics for Gunston Cove Data 2005-2012. Noman Cole Lab data. Surface and bottom samples separate.

	PH Lab	ALK	TP	SRP	ON	NH3N	NO3N	NO2N	NP	CLD	TSS	VSS	BOD
N of Cases	335	339	339	339	339	339	339	339	335	335	335	335	331
Minimum	6.700	26.000	0.015	0.002	0.020	0.005	0.005	0.001	3.219	4.000	1.000	0.750	1.000
Maximum	9.500	109.000	0.320	0.327	2.185	0.400	3.490	0.800	112.667	145.000	149.000	24.000	8.000
Median	7.900	72.000	0.070	0.011	0.700	0.020	0.760	0.010	21.333	30.000	17.000	4.000	2.000
Arithmetic Mean	8.007	70.720	0.074	0.015	0.730	0.041	0.744	0.022	23.342	33.881	19.354	4.618	2.243
Standard Error	0.028	0.801	0.002	0.001	0.017	0.003	0.028	0.002	0.753	1.079	0.765	0.150	0.072
Lower quartile	7.700	62.000	0.060	0.005	0.530	0.005	0.315	0.010	15.000	21.000	12.000	3.000	1.000
Upper quartile	8.400	81.000	0.080	0.020	0.849	0.060	1.017	0.030	28.475	39.750	23.000	5.400	3.000

phytoplankton cell density values since 2009 are $3\text{--}5 \times 10^5$ cells/mL for the embayment (cove) station and $1\text{--}6 \times 10^5$ cells/mL. These values are substantially higher than the 0.9×10^5 cells/mL in Hunting Creek and 1.0×10^5 in the river mainstem. This is consistent with earlier findings that suggested that phytoplankton populations were lower in the Hunting Creek area than in the Gunston Cove area. Note however that in some years the Gunston Cove stations were not much greater than the 2013 Hunting Creek values. When comparing biovolumes the numbers are closer. In 2013 biovolume in Hunting Creek ranged from $1.0\text{--}4.0 \times 10^7 \text{ um}^3/\text{mL}$ and $0.8\text{--}7.7 \times 10^7 \text{ um}^3/\text{mL}$ in the river mainstem. These compare reasonably well with the Gunston Cove study ranges of $1.1\text{--}9.7 \times 10^7 \text{ um}^3/\text{mL}$ for the cove station and $0.3\text{--}7.7 \times 10^7 \text{ um}^3/\text{mL}$ for the river station. The dominance of cyanobacteria and diatoms in phytoplankton cell density and diatoms alone in phytoplankton biovolume in the phytoplankton data is similar between the two studies as are the dominant species within each group.

Zooplankton data were available for a quantitative comparison similar to that done for water quality variables. Table 17a presents the basic statistics for microzooplankton the 2013 Hunting Creek study while those for the 2005-2012 Gunston Cove study are shown in Table 17b. Comparing medians from each table for each taxon shows that for almost all taxa (the rotifer *Synchaeta* being the only exception), Hunting Creek has lower population numbers in general. This is particularly obvious for all of the rotifers except *Synchaeta*. In fact the dominant rotifer in both areas, *Brachionus*, had a median in Hunting Creek study data that was substantially below the lower quartile of Gunston Cove data.

Examining the macrozooplankton data we find that again most of the taxa medians are lower in the 2013 Hunting Creek data than in the reference data set (Tables 18a & 18b). In particular *Diaphanosoma*, total cladocera (this excludes *Bosmina*), cyclopoid copepods, and total copepods (this excludes nauplii) have substantially lower taxa medians in the Hunting Creek data set. In fact, the medians for cyclopoid copepods and total copepods are below the lower quartile and total cladocera is very near this value. There are a variety of potential explanations for the lower zooplankton levels in the Hunting Creek area as observed in the 2013 data. Perhaps the most obvious one is the lower residence time of water in this upper tidal area as compared with further downstream at Gunston Cove. This low residence time was certainly a particular issue in 2013 when large amounts of runoff occurred in June, just when some of the zooplankton would be starting to ramp up or even going through their peak abundance periods in less wet years.

The benthic communities found at the Hunting Creek study stations compare quite well with the Gunston Cove study samples. Referring to Table 26 of the 2012 Gunston Cove report (Jones and deMutsert 2013), we see that oligochaetes and chironomids are dominant at both station with oligochaetes generally somewhat more abundant. This was certainly what we observed at the Hunting Creek study stations. A second observation about the Gunston Cove results is that other taxa such as amphipods, isopods gastropods, and bivalves were much more common at the river station than in the cove. In the Hunting Creek study, it was actually one of the embayment stations (Sta. 3) that had the greatest diversity including many of these groups. This may partially be due to the particular spot in the river which was sampled which had a rather sandy bottom.

Table 17a. Basic Statistics for Microzooplankton. Hunting Creek Study. 2013. All samples combined.

	NAUPLII	BOSMINA	ASPLANCH-NA	BRACHIONUS	CONOCHILUS	FILINIA	KERATELLA	LECANE	POLY-ARTHRA	SYN-CHAETA	TOTAL ROTIFERS
N of Cases	18	18	18	18	18	18	18	18	18	18	18
Minimum	1.589	0.000	0.000	0.095	0.000	0.000	0.000	0.000	0.190	0.000	9.055
Maximum	176.693	137.760	5.990	879.818	95.833	30.339	234.872	0.000	33.750	352.083	1058.815
Median	63.715	10.443	0.000	12.506	3.385	0.983	15.869	0.000	11.498	19.640	161.726
Arithmetic Mean	66.092	20.597	0.923	138.394	16.722	6.552	39.408	0.000	12.827	36.921	262.060
Standard Error	13.720	7.707	0.412	64.534	6.704	2.453	14.177	0.000	2.761	19.017	76.852
Lower quartile	12.282	1.311	0.000	0.417	1.215	0.053	7.910	0.000	2.094	0.952	24.583
Upper quartile	81.458	23.750	0.607	108.625	11.000	6.875	35.750	0.000	20.313	33.750	398.672

Table 17b. Basic Statistics for Microzooplankton. Gunston Cove Study 2005-2012. All samples combined.

	NAUPLII	BOSMINA	ASPLANCH-NA	BRACHIONUS	CONOCHILUS	FILINIA	KERATELLA	LECANE	POLY-ARTHRA	SYN-CHAETA	TRICHOCERCA	TOTAL ROTIFERS
N of Cases	164	164	164	164	164	164	164	164	164	164	164	164
Minimum	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.198
Maximum	876.563	585.156	837.500	4185.938	682.813	2676.609	3701.156	37.500	2445.964	1444.792	239.167	8978.125
Median	80.582	22.218	0.841	102.125	15.840	9.805	78.430	0.000	47.734	19.728	2.326	546.268
Arithmetic Mean	124.644	51.120	23.279	399.718	58.999	120.108	238.799	1.158	138.321	74.585	20.893	1095.997
Standard Error	11.139	6.343	5.922	54.617	7.258	24.302	40.582	0.369	20.935	12.759	3.192	122.324
Lower quartile	27.625	1.432	0.000	22.695	1.602	0.467	14.329	0.000	3.747	2.833	0.000	118.615
Upper quartile	185.451	60.000	16.243	409.492	77.682	71.050	222.055	0.000	156.317	81.854	22.331	1297.214

Table 18a. Basic Statistics for Macrozooplankton. Hunting Creek Study. 2013. All samples combined.

	CHYDORIDS	DAPHNIA	DIAPHANO-SOMA	LEPTODORA	TOTAL CLADOCERA	CYCLOPOID COPEPODS	CALANOID COPEPODS	HARPAC-TICIDS	TOTAL COPEPODS	OSTRACODS
N of Cases	18	18	18	18	18	18	18	18	18	18
Minimum	0.000	0.000	0.000	0.000	9.419	3.123	1.136	0.000	6.146	0.000
Maximum	59.461	584.846	544.530	193.937	2746.326	366.966	7997.805	11.317	8084.738	120.670
Median	11.333	1.680	37.772	9.811	160.188	27.000	221.369	0.000	330.812	7.219
Arithmetic Mean	12.927	47.698	113.305	31.236	385.449	70.467	832.851	1.014	907.630	18.222
Standard Error	3.362	32.297	38.198	12.454	155.479	22.459	443.317	0.654	446.085	7.364
Lower quartile	2.432	0.000	3.405	0.000	98.515	8.220	77.884	0.000	146.903	1.741
Upper quartile	16.441	30.777	161.331	35.513	295.571	85.693	521.733	0.206	696.513	11.838

Table 18b. Basic Statistics for Macrozooplankton. Gunston Cove Study. 2005-2012. All samples combined.

	CHYDORIDS	DAPHNIA	DIAPHANO-SOMA	LEPTODORA	TOTAL CLADOCERA	CYCLOPOID COPEPODS	CALANOID COPEPODS	HARPAC-TICIDS	TOTAL COPEPODS
N of Cases	164	164	164	164	164	164	164	164	164
Minimum	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	3.307
Maximum	547.043	8707.639	21422.197	2862.349	31425.226	47488.108	53286.252	410.282	54500.754
Median	0.000	0.000	239.097	8.815	614.225	327.197	767.669	0.000	1531.372
Arithmetic Mean	30.411	221.325	1745.572	158.306	2613.195	1585.648	3038.250	8.149	5604.046
Standard Error	6.063	69.926	269.872	28.824	372.211	336.410	516.702	3.066	705.138
Lower quartile	0.000	0.000	7.984	0.000	154.655	70.551	163.193	0.000	436.917
Upper quartile	19.379	71.642	1565.887	114.690	2880.738	1566.502	2571.651	0.000	7269.380

D. Fish Comparisons



The total abundance of each species at each station in 2013 reveals what the dominant fish species at each station were (Figure 74). Since the exact community structure varies with each sample taken at each station, we performed a non-parametric analysis to test if there were significant community differences between the stations. A PERMANOVA revealed that the community structure of the samples collected at each station was significantly different between stations ($Pseudo-F=3.5222$, $p=0.001$). Pairwise comparison made clear that all stations were significantly different from each other except ARE5 and ARE6. We indeed sampled a similar habitat, namely the Hunting Creek littoral zone, with the same gear (seine net) at station 5 and 6, which is reflected in similar fish communities. While ARE3 and ARE4 were sampled with the same gear (trawl), they are two different habitats: Hunting Creek and the Potomac River mainstem. We found a different community structure within Hunting Creek than in the Potomac River, which means that fish actively and selectively make use of this habitat. A SIMPER

analysis was used to determine which fish were mostly responsible for the similarities and dissimilarities between stations and samples. Even though not abundant, blue catfish was characteristic of the samples collected on ARE4. Blue catfish is an invasive species, and is apparently not actively using Hunting Creek as habitat, since 12 specimens were caught in the Potomac River mainstem and none in any of the Hunting creek samples. The species characteristic for both ARE5 and ARE6 was banded killifish, while tessellated darter was indicative of the samples from ARE3. Overall, the fish species found in Hunting Creek are pretty characteristic of Potomac River tributaries.

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Anadromous Fish Survey Cameron Run 2013

Introduction

The commercially valuable anadromous fishes in the herring family (Clupeidae) live as adults in the coastal ocean, but return to freshwater creeks and rivers to spawn. In the mid-Atlantic region, four species are present: American shad (*Alosa sapidissima*), blueback herring (*Alosa aestivalis*), alewife (*Alosa pseudoharengus*), and hickory shad (*Alosa mediocris*).

The American shad grows to be the largest and spawns in the shallow flats along the Potomac River channel. In the 1700s and early 1800s, incredibly large numbers of American shad were caught each spring as they came up the river to spawn. The records from 1814-1824 of just one fishery located at Chapman's Landing opposite Mason Neck, Virginia indicate that the annual catch varied from 27,939 to 180,755 American shad (Massmann 1961). By 1982, the numbers caught in the entire river had dwindled so much that a moratorium was placed on both commercial and sport harvest of the species. In 1995, the Interstate Commission on the Potomac River Basin began a process of capturing ripe American shad in gill nets off Dogue Creek and Fort Belvoir, stripping eggs from the females, and fertilizing the eggs with milt from males. The resulting young were raised in hatcheries for several days and then released, as fry, in the river below Great Falls (Cummins 2005). Through the 2002 season, over 15.8 million fry were released into the river, and by 2003 - the year after the restoration program ended - the population was judged strong enough to support a limited commercial fishery as bycatch in gill net fisheries. Moreover, a replacement stocking program continues (Jim Cummins, pers. comm.).

Prior to the 1900s, spawning occurred in the river as high as Great Falls (Smith and Bean 1988). In recent years spawning has occurred mostly downriver between Piscataway Creek and Mason Neck (Lippson et al. 1979). Hickory shad has similar spawning habitats and co-occurs with American shad, but is far less common than American shad or river herring, and less is known about its life history.

The alewife and blueback herring, collectively called river herring, are commercially valuable, although typically less valuable than American shad. In past centuries, their numbers were apparently even greater than those of the American shad. Massmann (1961) reported that from 1814 to 1824, the annual catch at Chapman's Landing ranged from 343,341 to 1,068,932 fish. The alewife spawns in tributary creeks of the Potomac River and travels farther into these creeks than do the other species. The blueback herring also enters creeks to spawn, but may also utilize downstream tidal embayments to spawn.

River herring were listed in 2006 by NOAA as species of concern due to widespread declining population indices. Population indices of river herring in the Potomac are available from seine surveys of juveniles conducted by MD-DNR. Juvenile catch rate indices are highly variable but have been lower in the most recent decade for both species. Since declines continued, a moratorium was established in January 2012, restricting all catches of alewife and blueback herring (4VAC 20-1260-20). Causes of river herring decline are likely a combination of long-term spawning habitat degradation and high mortalities as a result of bycatch in the menhaden fishery. The establishment of a moratorium indicates that declines are widespread, and regular

fishing regulations have not been sufficient to rebuild the stock. Using a moratorium to rebuild the stock is also an indication that the cause of the decline is largely unknown.

Identifying all areas used as spawning habitat by alewife and/or blueback herring in an important component of their conservation. There are no surveys of use of Cameron Run by these anadromous species; this study is the first to determine whether Cameron Run is currently used as a spawning habitat.

Two other herring family species are semi-anadromous and spawn in Potomac River tributaries. These are gizzard shad (*Dorosoma cepedianum*) and threadfin shad (*Dorosoma petenense*). Both are very similar morphologically and ecologically, but in our collections, threadfin shad are found downriver of Mason Neck, and gizzard shad are found upriver of Mason Neck. Neither is commercially valuable, but both are important food sources of larger predatory fishes.

Since 1988, George Mason University researchers have focused a monitoring program on the spawning of these species in other tributaries such as Pohick Creek, Accotink Creek, and, less regularly, Dogue Creek. With this study Cameron Run is added, which has not been monitored for presence of river herring or other anadromous species by either George Mason or other fisheries biologists in any previous years (Jim Cummins, pers. comm.). Use of Cameron Run by river herring upstream from where the effluent of Alexandria Renew Enterprises enters Cameron Run would signify that the ARE effluent does not deter river herring from using Cameron Run as spawning habitat.

Methods

We conducted weekly sampling trips from March 14 to May 17 in 2013. During each trip a hoop net was set blocking the complete creek to collect adults swimming upstream, and ichthyoplankton nets were set to collect larvae floating downstream. The sampling location was chosen to be upstream from the ARE effluent, and close (but downstream) of the first dam in Cameron Run (Figure 1).

Ichthyoplankton was collected by holding two conical plankton net with a mouth diameter of 0.25 m and a square mesh size of 0.333 mm in the stream current for 20 minutes. A mechanical flow meter designed for low velocity measurements was suspended in the net opening and provided estimates of water volume filtered by the net. The number of rotations of the flow meter attached to the net opening was multiplied with a factor of 0.0036 to gain volume filtered (m^3). Larval density ($\#/\text{10m}^3$) per species was calculated using the following formula:

$$\text{Larval density } (\#/\text{10m}^3) = 10N/(0.0036 * (\text{flow meter start reading} - \text{flow meter end reading}))$$

Where N is the count of the larvae of one species in one sample.

We collected 2 ichthyoplankton samples per week, and these were spaced out evenly along the stream cross-section. Coincident with plankton samples, we calculated stream discharge rate from measurements of stream cross-section area and current velocity using the following equation:

$$\text{Depth (m)} \times \text{Width (m)} \times \text{Velocity (m/s)} = \text{Discharge (m}^3/\text{s)}$$

Velocity was measured using a handheld digital flow meter that measures flow in cm/s, which had to be converted to m/s to calculate discharge. Both depth and current velocity were measured at 12 to 20 locations along the cross-section. At each sampling trip other physical parameters of the creek were recorded as well (water temperature, dissolved oxygen, pH, and conductivity).

The ichthyoplankton samples were preserved in 70% ethanol and transported to the GMU laboratory for identification and enumeration of fish larvae. Identification of larvae was accomplished with multiple taxonomic resources: primarily Lippson & Moran (1974), Jones et al. (1978), and Walsh et al. (2005). River herring (both species) have demersal eggs (tend to sink to the bottom) that are frequently adhesive. As this situation presents a significant bias, we made no attempts to quantify egg abundance in the samples. We were able to estimate total larval production (P) during the period of sampling by multiplying the larval density (m^{-3}) with total discharge (m^3) (Table 1).

The hoop net was deployed once each week in the morning and retrieved the following morning (see Figure 2). Fish in the hoop net were identified, enumerated, and measured.

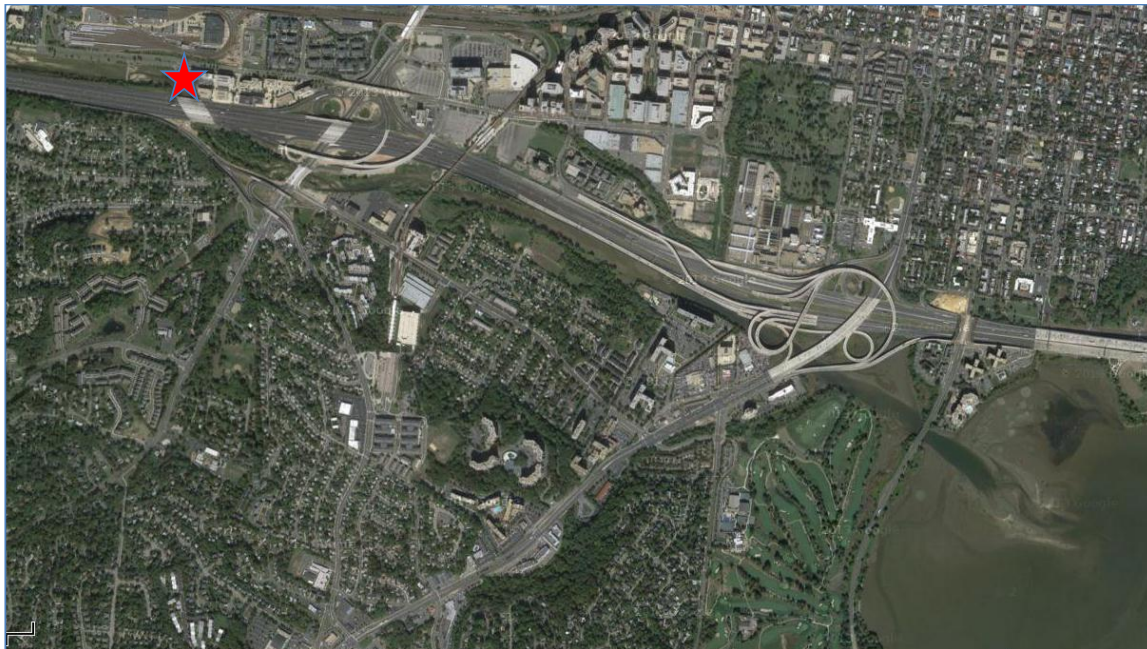


Figure 1. Sampling location Cameron Run.

Since the net were set 24 hours per week for 10 weeks, we approximated total abundance of spawning river herring during the time of collection by extrapolating the mean catch per hour per species during the time the creeks were blocked of over the total collection period as follows:

$$\text{Average catch/24 hours} * 1680 \text{ hours} = \text{total abundance of spawners}$$

Our total collection period is a good approximation of the total time of the spawning run of alewife. To determine the number of females we used a ratio of 0.5.

In response to problems with animals tearing holes in our nets in previous sampling experiences, we used a fence device in front of the mouth of the net that significantly reduces this problem. The device effectively excluded otters and similar destructive wildlife, but has slots that allowed up-running fish to be captured.



Figure 2. Hoop net deployed in Cameron Run. The top of the hoop net is exposed at both high and low tide to avoid drowning of air-breathing vertebrates. The hedging is angled downstream in order to funnel up-migrating herring into the opening of the net.

Results and Discussion

During the 10 weeks of sampling we caught one male alewife in the net on April 26, 2013. While this is of course a very low abundance, it does signify the use of Cameron Run as spawning ground for alewife, since it would have to swim upstream into Cameron Run as far as possible to be caught in the net, which is a behavior associated with spawning. The length of the alewife could not be recorded as the tail was eaten (likely by a turtle). Extrapolating over the time sampled, this could mean that the alewife spawning population is the size of 168 individuals of

which 84 are egg-bearing females. Sampling over multiple years and at a different location than this sampling season would provide us with increasingly better estimates of the spawning population of alewife in Cameron Run.

We measured creek discharge and other physical parameters at the same location and times where ichthyoplankton samples were taken, which was about 200 m downstream from the hoopnet (Table 1). Creek discharge was overall low with a peak in early April at $1.56 \text{ m}^3 \text{ s}^{-1}$. Average discharge was $0.45 \text{ m}^3 \text{ s}^{-1}$, ranging from $0.23 \text{ m}^3 \text{ s}^{-1}$ to $1.56 \text{ m}^3 \text{ s}^{-1}$. Dissolved oxygen (DO), pH, and specific conductivity (SpCond) were all in the benign range for occurrence of river herring. Water temperature (Temp) was likely too low for river herring spawning from 3/15/13 to 4/5/13, in the benign range after that.

Date	Discharge ($\text{m}^3 \text{ s}^{-1}$)	Temp ($^{\circ}\text{C}$)	SpCond ($\mu\text{S s}^{-1}$)	DO (mg l^{-1})	pH
3/15/13	0.413	5.6	531.1	12.79	7.61
3/22/13	0.259	4.5	489.2	14.33	8.05
3/29/13	0.329	7.9	651.7	12.78	7.62
4/5/13	1.555	8.9	639.7	11.95	7.99
4/9/13	0.295	19.8	535.1	11.75	8.92
4/19/13	0.382	19.1	502.3	10.08	8.1
4/26/13	0.240	16.2	480.5	11.4	8.4
5/3/13	0.239	17.4	462.8	11.11	8.26
5/7/13	0.556	16.1	506.4	10.45	7.87
5/17/13	0.228	25.5	457.5	10.4	8.43

Table 1. Discharge and other physical parameters of Cameron Run during each sampling day.

In the ichthyoplankton samples we indeed found larvae of alewife and well as blueback herring (Table 2). The numbers are low with a total of 6 alewife larvae and 1 blueback herring larva, but their sheer presence demonstrates that river herring spawning takes place in Cameron Run. Larvae of other species were present in the samples as well; these were carp (*Cyprinus carpio*), goldfish (*Carassius auratus*) and killifish (*Cyprinodontidae*) larvae.

Date	Species	Count	Volume sampled (m^3)	Larval density ($\#/\text{10m}^3$)
4/5/13	Unknown	1	11.08	0.90
4/26/13	<i>Alosa pseudoharengus</i>	6	5.62	10.68
4/26/13	<i>Carassius auratus</i>	1	5.62	1.78
4/26/13	<i>Cyprinus carpio</i>	1	5.62	1.78
4/26/13	<i>Cyprinidae</i>	1	5.62	1.78

4/26/13	<i>Cyprinodontidae</i>	1	5.62	1.78
5/3/13	<i>Carassius auratus</i>	1	7.83	1.28
5/7/13	<i>Alosa aestivalis</i>	1	7.97	1.25
5/17/13	<i>Cyprinus carpio</i>	1	6.16	1.62
5/17/13	Unknown Clupeid	1	6.16	1.62

Table 2. Larvae collected in Cameron Run. When sampling dates are not included in the table, no larvae were found in those samples (see Table 1 for all sampling dates). River herring larvae are in bold.

During the entire sampling period of 70 days, the total discharge was estimated to be on the order of 2.7 million cubic meters. Given the observed mean densities of larvae, the total production of *Alosa* larvae was estimated at approximately 139 thousand for Cameron Run (Table 3).

Statistic	Cameron Run
Mean discharge ($\text{m}^3 \text{s}^{-1}$)	0.45
Total discharge, 3/15 to 5/17 (m^3)	2,721,600.00
Total volume sampled (m^3)	138.46
Mean <i>Alosa</i> larvae density (10m^{-3})	0.51
Total river herring production (# larvae)	138,801.60
Total adult river herring (#)	168.00

Table 3. Estimation of river herring (alewife and blueback herring) larval production and spawner abundance from Cameron Run during spring 2013.

Conclusion

With no background information on spawning use of Cameron Run, it is an exiting discovery it is indeed used as spawning habitat by river herring. Both alewife and blueback herring larvae were found in Cameron Run, and one alewife adult was caught swimming upstream. Larvae do not have the ability to swim upstream; therefore they must have been spawned in Cameron Run. More productive spawning habitat than the current sampled location may be present in Cameron Run, and a suggested adjustment in coming years is to position our sampling location a few hundred meters downstream from the current location.

Although the current evidence suggests that the importance of Cameron Run may be marginal to alewife and blueback herring populations, it is important to recognize that marginal habitats may sustain fish populations during periods of declining abundance and low recruitment (Kraus and Secor 2005). Due to the recent moratorium on river herring, annual estimation of spawner abundance should be a continued priority for annual monitoring of this and other Potomac River tributaries. Anadromous fishes typically exhibit strong year-class fluctuations. Additional years of data collection (at least through 2 generation lengths ~ a decade) should provide a sufficient understanding of this variability.

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**An Ecological Study of Hunting Creek: Incidence of PCBs and Endocrine Disruptive
Chemicals in Surficial Sediments and Biota**

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Introduction

Effective assessment in aquatic ecology and water quality can be achieved from field studies that provide baseline and on-going trend analysis related to the condition of aquatic systems receiving reclaimed water or storm runoff from urban regions. The primary objective of baseline studies is to provide scientific evidence of change in ecosystems that can be directly incorporated into adaptive management strategies. The vitality of fisheries in the Potomac River is impacted by chemical emissions in the metropolitan Washington, DC region. As environmental pollution concerns arise, addressing the health of the organisms inhabiting polluted ecosystems becomes critical.

Pollutants of greatest concern in the Chesapeake Bay watershed include legacy chemicals such as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), pesticides and emerging pollutants such as pharmaceuticals and endocrine disrupting chemicals (EDCs). There is concern among water quality managers regarding health risks posed by pharmaceuticals and personal care products (PPCPs) and EDCs. The U.S. Environmental Protection Agency (EPA) has defined an EDC as “an exogenous agent that interferes with synthesis, secretion, transport, metabolism, binding action, or elimination of natural blood-borne hormones that are present in the body and are responsible for homeostasis, reproduction, and developmental process.” All endocrine disrupting chemicals can be hazardous to organisms, even at parts-per-billion concentrations, due to their ability to initiate estrogenic activity (*1*). The sources of exposure to endocrine disruptive chemicals are diverse. Introduction into the aquatic environment is through industrial waste discharge, urban stormwater runoff and wastewater treatment plants. In light of emerging evidence proving EDCs in the environment pose a serious health threat further research in EDC multi-media distribution and bioaccumulation in biota is essential. Thus, the chemical focus of the present field study is on EDCs, which include PCBs.

Fish, plankton and sediments collected from Hunting Creek (northern Virginia, USA) in 2013 (April through September) were analyzed for PCBs and selected EDCs. Hunting Creek is formed by confluence of Cameron Run and Hooff Run in Northern Virginia. This embayment receives large quantities of treated wastewater effluent. Since the 1970s Fairfax County, as a major discharger of treated wastewater into the Potomac River, has taken very proactive measures in assessing the impacts of wastewater discharge on stream ecology.

The high in-stream concentrations of anthropogenically-derived chemicals in northern Virginia are due to the dense urban development along the tributary streams and the relative frequency of wastewater treatment plant discharge compared to the freshwater flow in the stream. The relative volume of stormwater and wastewater discharges versus normal freshwater flow provides sufficient time for significant in-stream accumulation to occur. Therefore the entire aquatic food chain can be exposed to the pollutants throughout entire life cycles and across generations (4). The present study represents a first-order survey of the types and concentrations of environmental pollutants in the Potomac River that can be used to monitor and assess ecological conditions.

Study Objectives

The objectives of the present investigation included the following considerations.

1. Quantify PCBs (122 congeners) and selected contemporary EDCs in sediments, plankton and fish species collected in the Hunting Creek region of the freshwater tidal Potomac River using nets, trawls and seines. Hunting Creek receives wastewater flow from the AlexRenew facility.
2. Compare observed concentrations in the Hunting Creek environment to available water and sediment quality criteria to assess potential ecological implications.
3. Evaluate ecological factors related to the bioaccumulation of PCBs and PPCPs among biota, such as feeding habits, trophic guilds and migration where possible.

Materials and Methods

Sample Collection:

Several species of fish common to the tidal Potomac River, plankton (100 μ m) and bottom sediments (Table 1) were obtained from April through September 2013. The fish species included striped bass, smallmouth bass, white perch, banded killifish, bluegill, alewife and spottail shiner using either otter trawl, nets or 16.7 mm beach seines. Plankton were collected using 100 μ m mesh conical nets via boat tows. Benthic invertebrates were collected from bed sediments using a Ponar grab sampler (Wildco, Saginaw, MI).

Table 1. Original sampling plan for Hunting Creek ecological analysis and chemical monitoring.

Sample Type	Name	Target Collection
Fish-pelagic	white perch (<i>Morone americana</i>)	20
Fish-pelagic	bluegill (<i>Lepomis macrochirus</i>)	10
Fish-pelagic	spottail shiner (<i>Notropis hudsonius</i>)	16
Fish-pelagic	gizzard shad (<i>Dorosoma cepedianum</i>)	16
Fish-benthopelagic	banded killifish (<i>Fundulus diaphanus</i>)	16
Fish-benthic	blue catfish (<i>Ictalurus furcatus</i>)	8
Fish-predator	largemouth bass (<i>Micropterus salmoides</i>)	8
Fish-anadromous	alewife (<i>Alosa pseudoharengus</i>)	16
Seston (plankton)	Trawls with 100 µm mesh	6
Benthic invertebrates	Ponar grab and seine	6
Alluvial Sediments	Ponar grab	18

Aquatic species were collected monthly to bi-monthly from Hunting Creek using a geospatial grid that could evaluate chemical gradients, chemical sources and species distribution (Fig. 1). Collected organisms were placed in glass jars, with Teflon lined caps (small fish, seston and invertebrates) or individually wrapped in aluminum foil (larger fish) and frozen at -20°C in laboratory storage. Fish smaller than 2 inches were pooled in a glass jar as a composite of 5. Fish from 2-4 inches were kept in a glass jar as a composite of 3. Fish larger than 4 inches were wrapped individually in aluminum foil. The maximum threshold size for fish was 10 inches. Sediment samples were obtained using a Ponar grab. Sediments were scooped with a stainless steel spatula to a depth of 5 cm (surficial layer) and placed into glass jars. Sediments were maintained frozen at -20°C in the laboratory until analysis.

Sample Preparation:

The concentrations of EDCs and PCBs in fish and sediments were quantified using microwave-assisted Extraction (MARS, CEM Corp., Matthews, NC), Florisil (60-100 mesh, J. T.

Baker, Philipsburg, NJ) clean up and gas chromatography-mass spectrometry quantification (Agilent Model 5975C GCMS series).

The frozen tissue samples were thawed and homogenized in a Die-cast 10 speed blender (Oster, Boca Raton, FL, pre-rinsed with hexane). Approximately 1 g wet-weight (Fig. 2) of the blended tissue paste was mixed with 10 g of granular, anhydrous sodium sulfate to macerate and homogenize the matrix to a dry powder using mortar and pestle. The dry powder was spiked with 50 μ L of EDC and 20 μ L of PCB surrogate standards. The saponification-extraction solution employed was 1 M KOH in methanol (1 M KOH/MeOH). About 15 mL of the extraction solvent was used to extract the analytes from the sample dry powder inside a MARS



Figure 1. Geospatial sampling grid used in the Hunting Creek study. Six sampling zones (ARE 1-6) were identified for spatial coverage and comparisons.

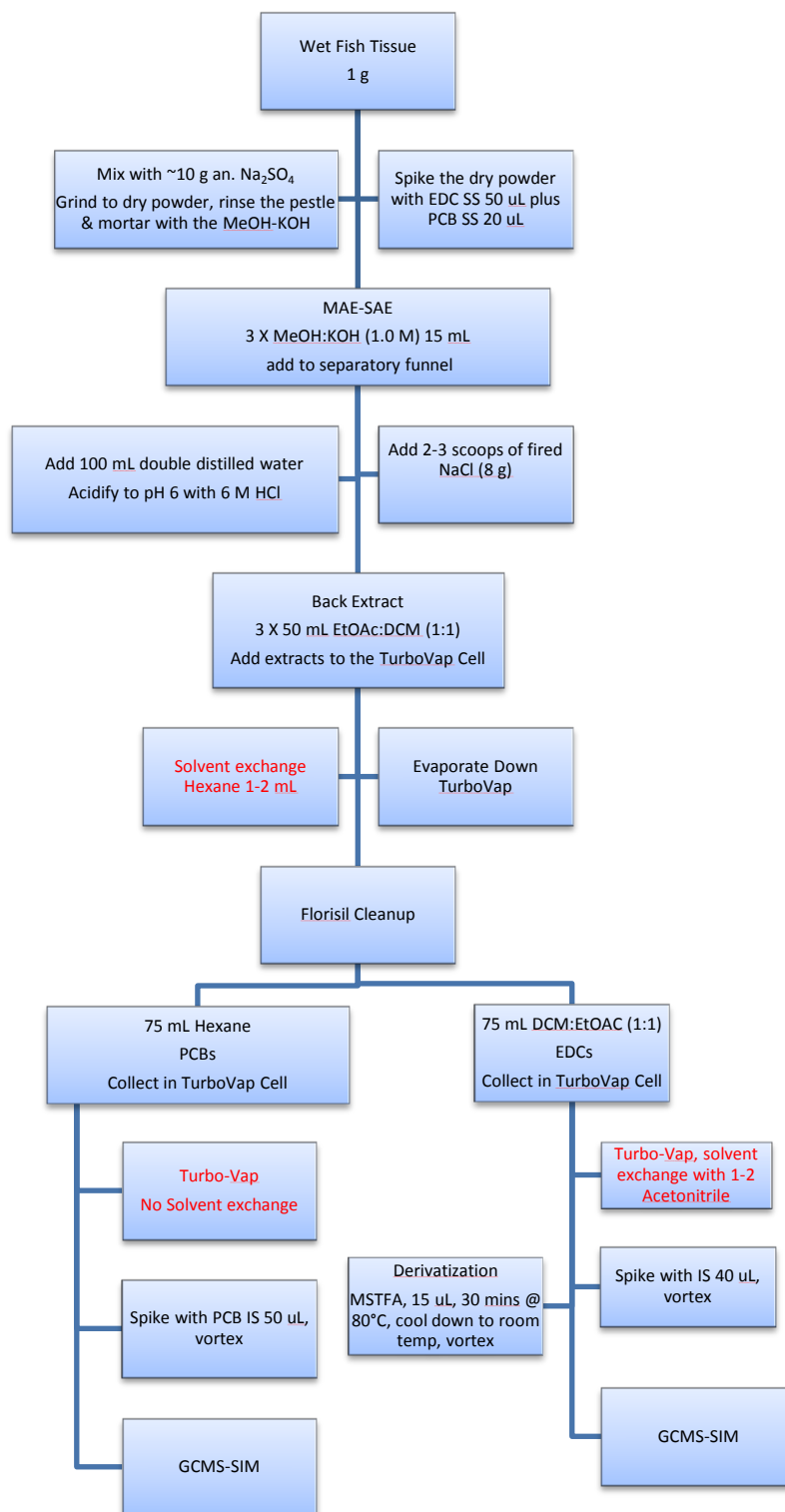


Figure 2. Tissue analysis flow diagram.

GreenChem vessel. The sample was subjected to microwave extraction-saponification at 100 °C and 600 Watts for 15 minutes. All extractions were performed in triplicate. After each sequential MAE the solvent was transferred to a 250-mL separatory funnel. The combined extracts were diluted with 100 mL of double distilled water containing ~8 g of sodium sulfate and acidified to pH 6 by adding 6 M HCl. The analytes were back extracted into 50 mL of 1:1 (v/v) dichloromethane: ethyl acetate (DCM:EtOAc) in the separatory funnel. The back extraction was repeated in triplicate and the DCM:EtOAc extracts were combined and reduced in volume to ~1 mL using a TurboVap evaporator (Zymark, Hopkinton, MA).

The concentrated tissue extracts were subjected to Florisil chromatography for clean up. The Florisil columns were comprised of 6.0 g of 5% water-deactivated Florisil sandwiched between 2.0 g of anhydrous sodium sulfate. The extracts were loaded on Florisil and eluted sequentially with 75 mL of hexane (PCBs) followed by 75 mL of DCM:EtOAc (EDCs). The separate Florisil eluents were concentrated to 0.5 mL in 300 mL TurboVap tubes. Both fractions were evaporated to a final volume of 0.5 mL. During solvent evaporation the EDC fraction was solvent-exchanged with acetonitrile.

Sediment processing (Fig. 3) was similar to the fish protocol with the exception of saponification and acidification, which is not necessary due to the lack of lipids in the sediment. The thawed wet sediment was initially centrifuged for 10 minutes at 1500 rpm (Du Pont Sorval RC-5B, New Town, CT) to dewater prior to extraction. About 1.0 g of dewatered sediment was desiccated by mixing in a mortar and pestle with 5 g of anhydrous sodium sulfate. The dry powder was with 50 µL of EDC and 20 µL of PCB surrogate standards. The dry powder was transferred to a GreenChem extraction vessel and subjected to MAE as described above for tissues using 15 mL of 1:1 DCM:EtOAc. Sediment MAE was performed in triplicate and the extracts were combined and evaporated to ~1 mL using the TurboVap. During evaporation the extracts were solvent exchanged with hexane. The concentrated extracts were subjected to Florisil clean up exactly as described above, where the hexane and DCM:EtOAc elution fractions

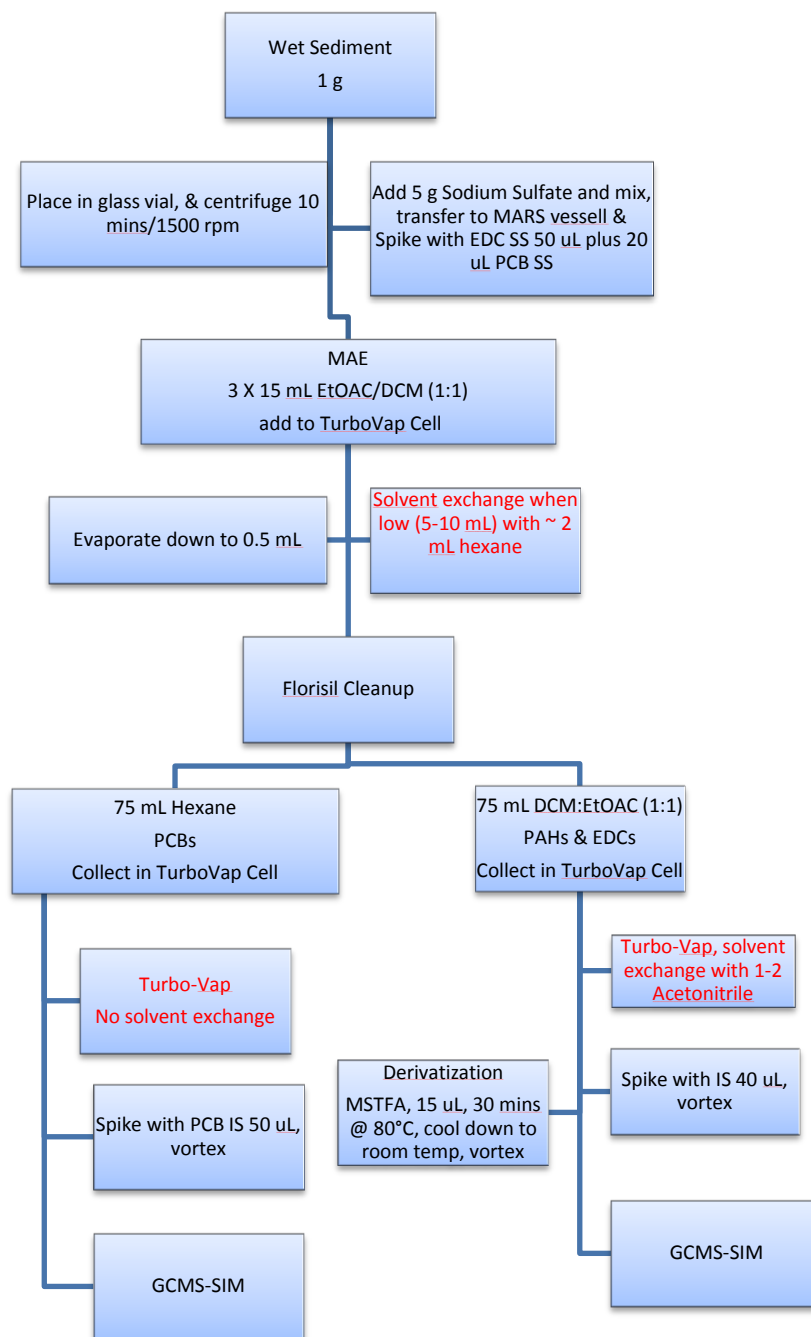


Figure 3. Sediment analysis flow diagram.

were solvent volume reduced to 0.5 mL using the TurboVap. Each fraction was transferred to a GCMS vial for analysis.

Seston were processed using the sediment protocol with the exception of vacuum filtration replacing the centrifuge step. The gelatinous plankton samples were isolated on pre-

weighted glass filter filters using vacuum filtration. The filter containing the organism was placed in a desiccator and weighted following water loss. The dried filter was homogenized with 5 g of anhydrous sodium sulfate in a mortar and pestle and subjected to MAE, solvent volume reduction and Florisil clean up to provide two elution fractions.

GCMS Analysis:

GCMS analysis was performed using an Agilent 5975 C system (fitted with a 7890A series gas chromatograph (Agilent Technologies, Santa Clara, CA). Agilent GCMS ChemStation Software was utilized in retention time locking, data acquisition, processing, and instrument control. The EDC (Table 2), PCB (Table 3) and internal/surrogate standards are listed in Tables 2-4.

PCBs and EDCs are separated and quantified using an Agilent HP-5MSi capillary column (5% biphenyl/95% dimethylsiloxane) with the following dimensions: 30 m x 0.25 mm i.d., 0.25 μ m thick film. An Agilent model 7673A automated liquid sampler (autosampler) was used to provide 10 μ L injections into the GC. An Agilent ultra-inert 2 mm dimpled inlet liner is used at the inlet for inert performance. The GC operating conditions were as follows for EDCs: the initial column temperature of 79 °C for 15 seconds, programmed to 300 °C at 710 °C/min, and kept at this temperature for 2 minutes. The helium carrier gas flow is maintained at a constant pressure of 17.3 psi. A retention time locked method, adopted from the Agilent Pesticide and Endocrine Disruptor database, using the locked retention time of chlorpyrifos methyl (16.596 min) was used as a reference. The GC operating conditions were as follows for PCBs: initial oven temperature at 70 °C for 1 minute, programmed to 150 °C at 50 °C/min, then 200 °C at 6 °C/min and finally to 280 °C at 16 °C/min; this final oven temperature is held for 17 minutes.

PCBs are evaluated as total-PCBs (tPCBs), which is derived by summing all of the 122 individual congeners shown in Table 3. Another method of PCB evaluations is through the homologue groups, which is derived from summing separately each of the congeners with similar chlorine substitution. For example, the 5Cl homologue group is obtained by summing all the pentachloro substituted PCBs (refer to Table 3). Finally, PCBs are also evaluated as the sum of the dioxin-like PCBs. The dioxin-like PCBs represent congeners that have no chlorine

substitution at the 2- and 2'- positions on the biphenyl rings. The dioxin-like congeners are considered to be the most toxic constituents in the tPCB mixture.

Table 2. List of EDCs measured in Hunting Creek samples by GCMS.

Ibuprofen	Dextromethorphan	Mestranol
Acetaminophen	Bisphenol A	19-Norethindrone
4-tert-Octylphenol	Carbamazepine	17 α -Ethinylestradiol
Atrazine	Dichlofenac	D(-)-Norgestrel
Caffeine	Diethylstilbestrol	Progesterone
Diphenhydramine	Escitalopram	Estriol
Vinclozolin	Chloramphenicol	Genistein
4-Nonylphenol	Trimethoprim	Indomethacin
Gemfibrozil	Estrone	Coprostanol
Fluoxetine	Equilin	Coumestrol
Naproxen	17 β -Estradiol	Atorvastatin
Triclosan	Testosterone	

Table 3. List of PCB congeners measured in the Hunting Creek samples by GCMS.

Number of Chlorines	CAS Structural PCB Number ^a	Number of Congeners
1	1, 2, 3	3
2	4, 5, 6, 7, 8, 9, 10, 12, 15	9
3	16, 17, 18, 19, 20, 24, 25, 27, 28, 29, 30 ^b , 31, 32, 33, 34, 37	16
4	40, 41, 42, 44, 45, 46, 47, 48, 49, 52, 56, 59, 60, 63, 64, 66, 67, 69, 70, 71, 74, (77) ^d	22
5	82, 83, 84, 85, 87, 91, 92, 93, 95, 97, 99, 101, 103 ^c , 104, (105), 109, 110, (114), 115, (118), 119, (123), 137	23
6	(128), 129, 131, 132, 134, 135, 136, 138, 140 ^c , 141, 144, 146, 147, 149, 151, 153, (156), (157), 158, 164, (167)	21
7	170, 171, 172, 173, 174, 176, 177, 178, 179, 180, 183, 185, 187, (189), 190, 191, 193	17
8	194, 195, 196, 197, 199, 203, 204 ^b , 205, 206, 207, 208	11
	Total Number of Congeners	122

^a Mills *et al.* 2007

^b IS = Internal Injection Standard

^c SS = Surrogate Standard

^d Dioxin-like congeners listed in parenthesis.

Table 4. Internal and Surrogate Standards used in GCMS analysis.

Internal Standards	Surrogate Standards
PCBs: PCB 30 & 204	PCB 103 & 140
EDCs: Acenaphthene-d ₁₀ , Phenantrene-d ₁₀ & Chrysene-d ₁₂	EDCs: Naproxen ¹³ C ₃ & Bisphenol A ¹³ C ₁₂

Electron impact (EI) mass spectra, in both full-scan mode and selected ion mode (SIM), are obtained at 70 eV with monitoring from 15 m/z to 510 m/z for full-scan mode. In SIM mode the quantifying ion and two additional qualifying ions were used for each target analyte. The quadrupole analyzer and the ion source temperatures were held constant at 150 and 230 °C respectively. All calibration and quantitation was accomplished employing ChemStation software (Agilent).

Ancillary Analysis:

The moisture content of sediment was determined gravimetrically. Sediment (~5 g) was pre-weighed, added to a porcelain thimble and heated to constant weight (~48 hrs) at 60 °C. The dried sediments were reweighed to yield moisture loss. Moisture content was used to convert the wet mass of sediment determined prior to sample extraction to dry weights.

Lipid weight (i.e., total extractable lipid) in the biota samples was determined by employing the MAE method in combination with gravimetric analysis. In this procedure, 5 g of fish was desiccated and homogenized with 50 g of anhydrous sodium sulfate similar to the extraction method described above (equivalent ratios), followed by MAE using 30 mL of 1:1 DCM:EtOAc. MAE was performed in triplicate and the combined extracts were evaporated to dryness in a pre-weighed 300 mL TurboVap cell. The residual total extracted lipid mass, reaching constant weight, was determined gravimetrically.

Sediment organic carbon and nitrogen contents were determined using a soil CN Analyzer. (Flash 2000, ThermoScientific, Waltham, MA). Approximately 1 g of sediment was oven dried and ground to a fine powder using mortar and pestle. The ground sample was transferred to a silver combustion cup and treated with 1 M HCl to degas carbon dioxide.

Degased sediment was redried and analyzed for C and N content using aspartic acid as the reference standard. All sediments were analyzed in triplicate.

Quality Assurance

1. PCBs

The surrogate standards evaluated for PCB analysis in bed sediments are shown in Fig. 4, where mean recoveries were 94% ($\pm 26\%$, 1 standard deviation) for PCB 103 and 104% ($\pm 24\%$) for PCB 140 for all the sediments analyzed (N = 12). Blanks for sediments showed below detection limits (0.01 ng/g dwt for the individual congeners) except for congeners 153, 180, 128, 118, 189 and 196, which were subtracted from the sample concentrations.

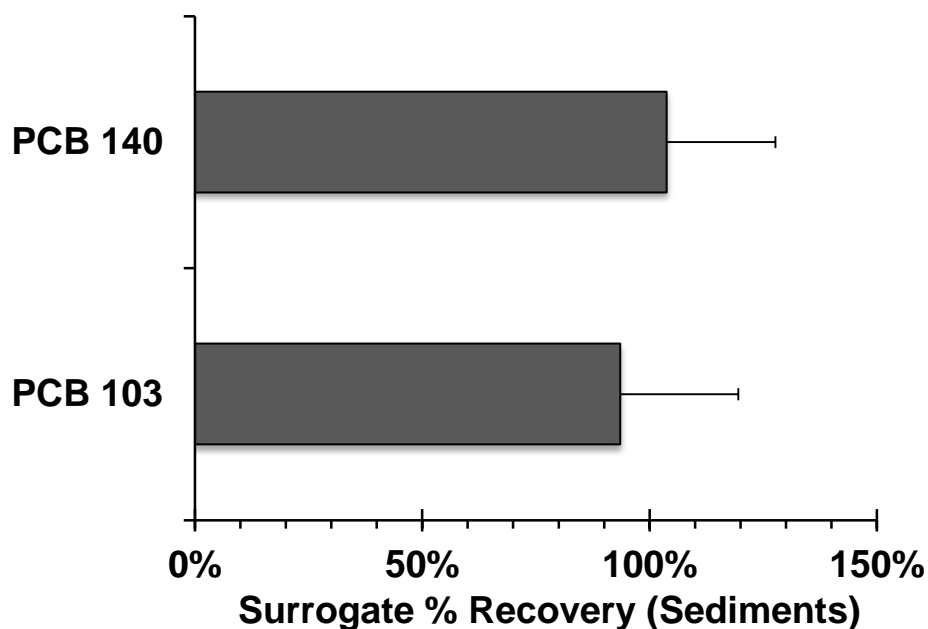


Figure 4. Overall mean (± 1 standard deviation) surrogate recoveries of PCBs (congeners 103 and 140) in Hunting Creek sediments.

The surrogate standards evaluated for PCB analysis in biota are shown in Fig. 5, where mean recoveries were 56% ($\pm 13\%$) for PCB 103 and 60% ($\pm 14\%$) for PCB 140 for all biota samples analyzed (N = 29). Surrogate recoveries were observed to be lower in biota than sediment samples because PCB analysis is yet incomplete for the DCM/EtOAc elution fraction

from Florisil clean up (see Fig. 4). PCBs showed breakthrough in the DCM/EtOAc fraction for both sediments and biota samples. Sediments samples included above include PCB analysis in both fractions, while biota samples include only the hexane fraction. Blanks for sediment showed below detection limits (0.01 ng/g dwt for the individual congeners) except for congeners 153, 180, 128, 118, 189 and 196, which were subtracted from the sample concentrations to account for the amounts detected in the blanks.

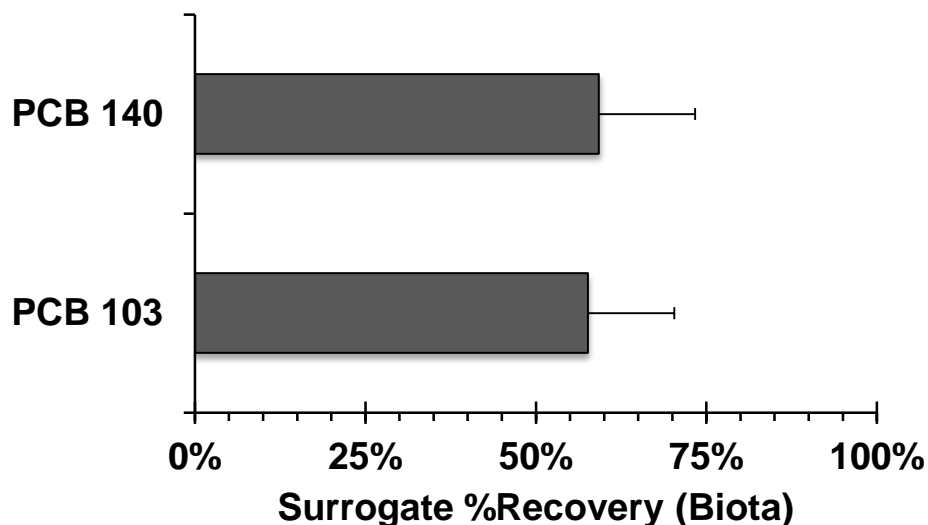


Figure 5. Overall mean (± 1 standard deviation) surrogate recoveries of PCBs (congeners 103 and 140) in Hunting Creek biota samples.

In the case of tPCBs in biota samples, a normalized concentration of tPCBs (i.e., NtPCBs) was determined to account for low performing surrogate recoveries. Any sample that showed an average surrogate recovery (103 and 140) below 1 standard deviation was normalized to the average surrogate recovery.

2. EDCs

The sources of EDCs in the aquatic environment are varied. EDCs represent a wide range of chemicals that effect the endocrine system in biota. Most of the focus in EDCs has been observed for reproductive impairment, particularly the phenomenon of intersex fish in large rivers near urban centers. The Intersex condition has been observed predominantly in black bass species captured near the vicinity of wastewater outfalls. It has been observed in the upper

Potomac River in particular by Vicki Blaser and her group at USGS, and has been correlated with pesticide runoff and runoff from animal agriculture (cattle) operations.

The sources of EDCs include legacy pollutants such as PCBs, pharmaceuticals, particularly human therapeutic birth control agents such as estriol and progesterone, plasticizers, pesticides, and household chemicals. There is no single chemical class of EDCs. As such, the sources of EDCs are industrial discharge, agricultural runoff, stormwater runoff, particularly from urban areas, and wastewater discharge. We have no way of apportioning sources at this time.

The surrogate recoveries for the EDCs in biota are shown in Fig. 6. The overall mean surrogate recovery for bisphenol A C-13 across all biota samples was 74% ($\pm 50\%$). The overall mean surrogate recovery for naproxen was 22% ($\pm 20\%$) reflecting the variable performance and difficulty of analyzing contemporary EDCs by GCMS. The naproxen surrogate recoveries varied between 4-69% in biota reflecting the difficulty in quantifying all EDC analytes efficiently. Alternative methods are under development in our laboratory to enhance the performance of underperforming acidic EDC analytes, which are particularly problematic in tissue matrices. Method detection limits for the EDCs in biota and sediments varied from 0.2 to 25 ng/g. Blanks showed some detections of bisphenol A only, that were subtracted from the sample concentrations when present to correct for background contributions to measured concentrations. Surrogate recoveries for the EDCs in sediments (bisphenol A C13 and naproxen C13,d3) are currently unavailable and will be provided in the final report.

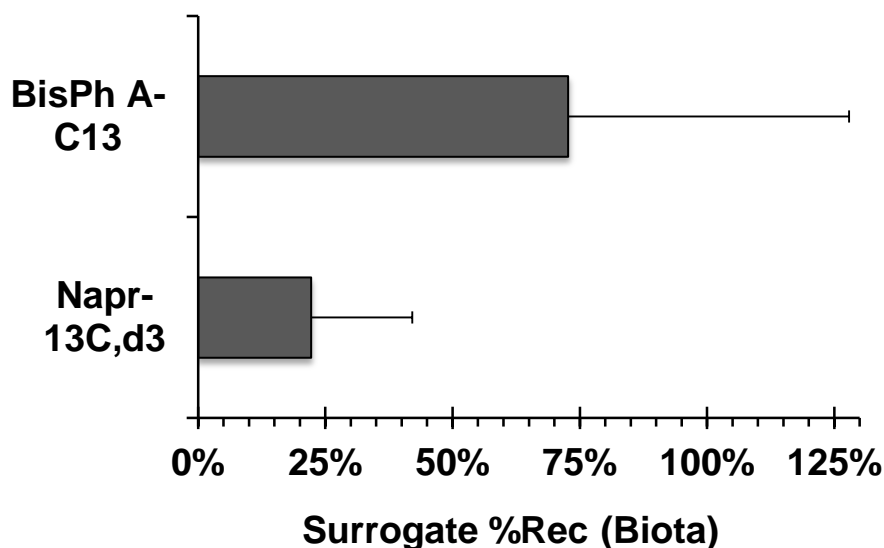


Figure 6. Overall mean (\pm 1 standard deviation) surrogate recoveries for EDCs in biota. BisPh A C13 = bisphenol A C-13; Napr 13C,d3 = naproxen C-13,d₃.

Results

1. PCBs in Sediments

The mean concentrations of tPCBs in Hunting Creek sediments ranged from 70 to 95 ng/g dw (Fig. 7). There were no significant differences (95% confidence level) in tPCB concentrations between the sampled ARE sites ($p > 0.05$, ANOVA), establishing the absence of any downstream gradient of PCBs within Hunting Creek. The measured tPCB concentrations were all above the consensus threshold effect concentration (TEC) of 59 ng/g (McDonald 2000) for establishing toxic effects in benthic organisms. Concentrations of toxic substances above the TEC may be expected to show some low level of toxicity. However, all measured tPCB concentrations were below the minimum effect threshold (MET) of 200 ng/g dw (McDonald et al. 2000), a concentration that is considered to be clean to lightly polluted but showing little no toxicity to the majority of sediment dwelling organisms.

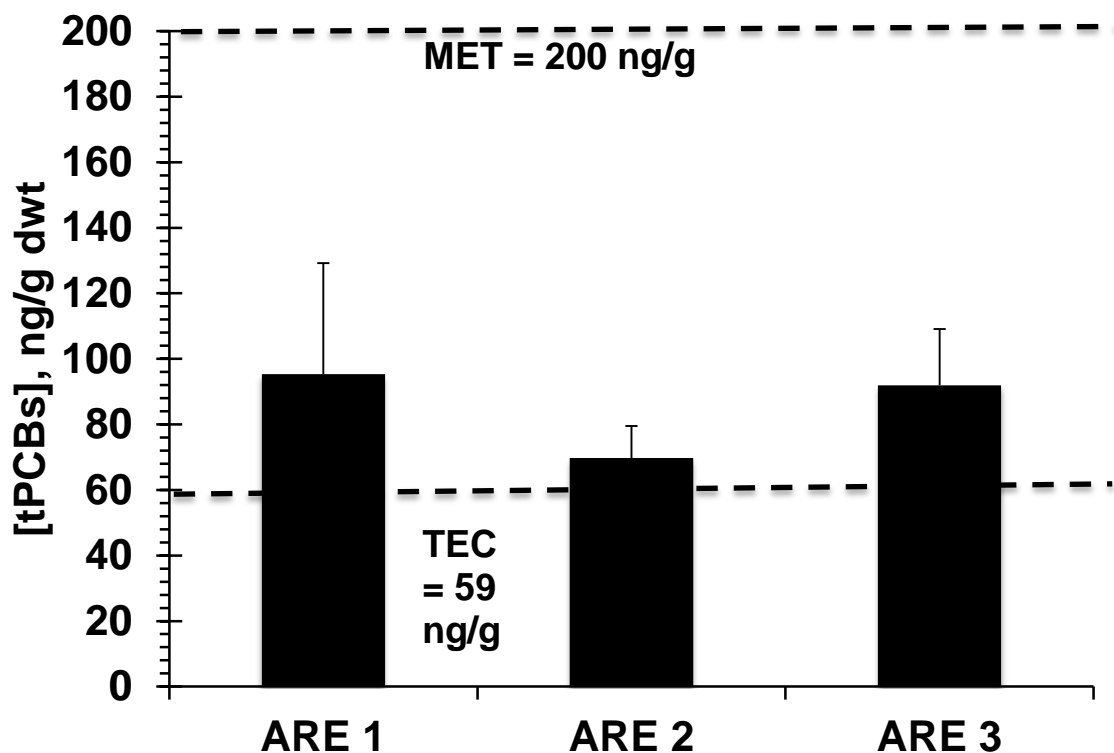


Figure 7. Mean (± 1 standard deviation) concentrations (ng/g dry weight) of total-PCBs in Hunting Creek sediments. The Area designations correspond to Fig. 1. TEC = Threshold Effect Concentration.

The PCB homologue patterns observed in sediments are illustrated in Fig. 8, where the penta- and hexa-chlorine substituted congeners dominate in the PCB mixture. The PCB homologue distribution was similar among all the sediment samples indicating a common source. The relative abundance of the PCB mixture in Hunting Creek indicated Aroclor 1254 (pentaCl maximum) and 1260 (hexaCl maximum) were the primary sources based on the homologue distribution patterns.

2. PCBs in Biota

The mean tPCB concentrations in fish species (Fig.9) ranged from 77 (bluegill) to 159 ng/g ww (spottail shiner at ARE 6). No statistically significant differences in mean tPCB concentrations were observed between fish species, collection date or geospatial distribution in sampling (ANOVA, $p > 0.05$). Fish showed higher tPCB concentrations (overall mean of 116

ng/g ww) than did sediments (86 ng/g dwt), indicating a greater PCB enrichment in fish tissues relative to bottom sediments.

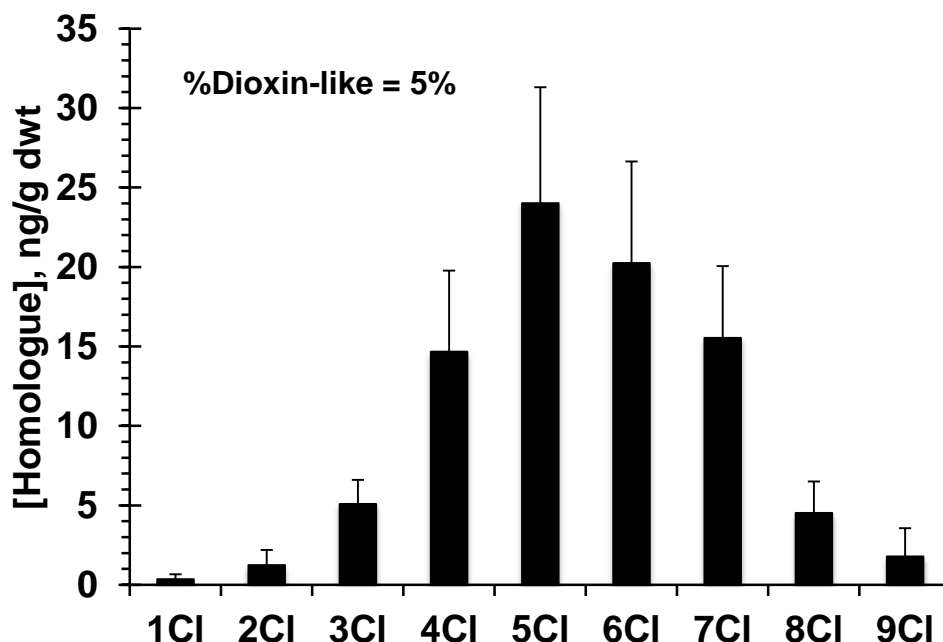


Figure 8. Mean PCB homologue concentrations (ng/g dry weight) for all sediment samples. Numbers represent the sum of similar chlorine-substituted congeners within the homologue groups.

The fish species analyzed included banded killifish (N=3), white perch (N=7), bluegill (N=3), spottail shiner (N=4 for ARE 6 and N=3 for ARE 3) and striped bass (N=3). Fish species not analyzed for PCBs included blue catfish and largemouth bass (no individuals obtained for analysis) and alewife.

The distribution pattern of PCB homologue concentrations observed in fish resembled that seen in sediments (Fig. 10), with the penta- and hexachloroPCBs being the dominant homologue classes in the tPCB mixture. Although no difference in homologue distribution profiles was observed between sediments and fish, fish showed a greater enrichment of the dioxin-like congeners (19% of tPCBs) relative to sediments (5% of tPCBs) in tPCBs. This observation can be derived from the lower metabolic rates of degradation in fish of the dioxin-congeners.

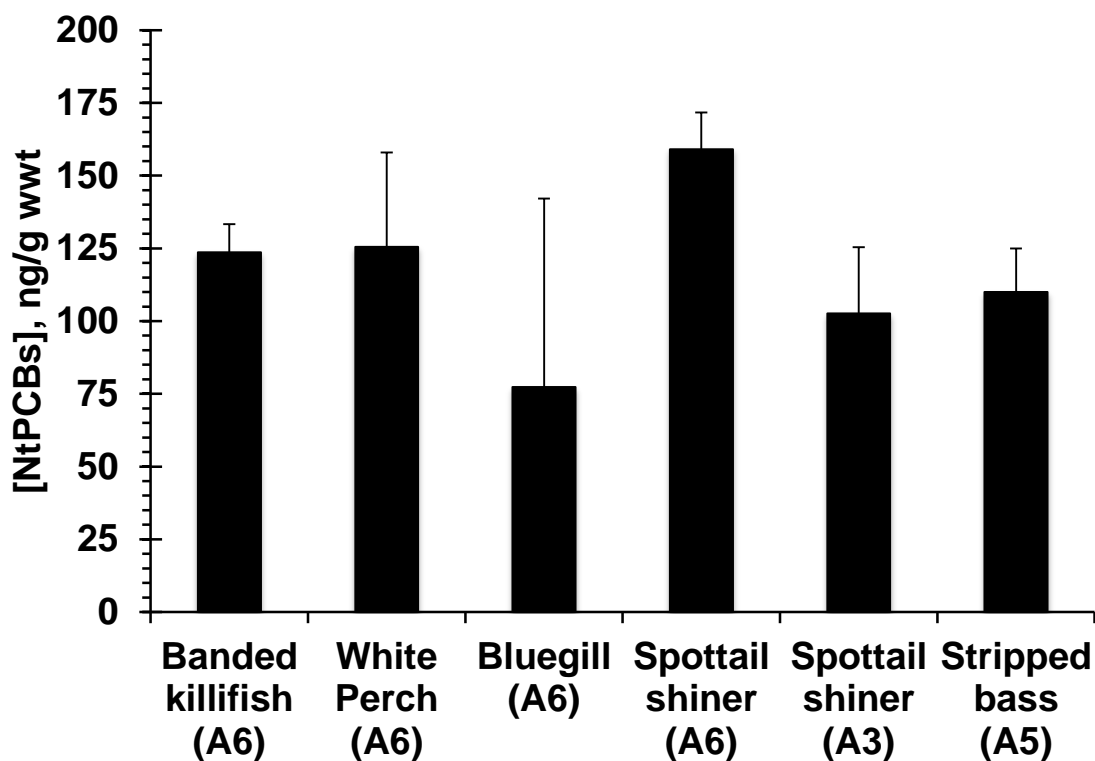


Figure 9. Total-PCB (tPCB) concentrations in biota collected from Hunting Creek. Species are identified location of collection (A#) according to Fig. 1.

3. EDCs in Sediments

The incidence and concentrations of the EDCs were sporadic and variable, although some trends were evident. Unlike the PCBs in surficial sediments, the EDCs do not show consistent occurrence and distribution. The EDCs can be grouped into two general categories, the first being those with the higher detection frequencies (DFs) >70% and the second being those found with DFs <40% in the sediments analyzed. The most frequently detected EDCs in sediments included 4-nonylphenol (non-ionic surfactant), progesterone (steroid), coprostanol (sewage sterol marker) and estriol (steroid), with DFs of 100%, 100%, 100% and 75%, respectively. The median concentrations of the most frequently detected EDCs are shown in Fig. 11, ranging from 4 to 280 ng/g dwt. The concentrations of these four EDCs were highly variable, such that gradients or geospatial differences could not be resolved.

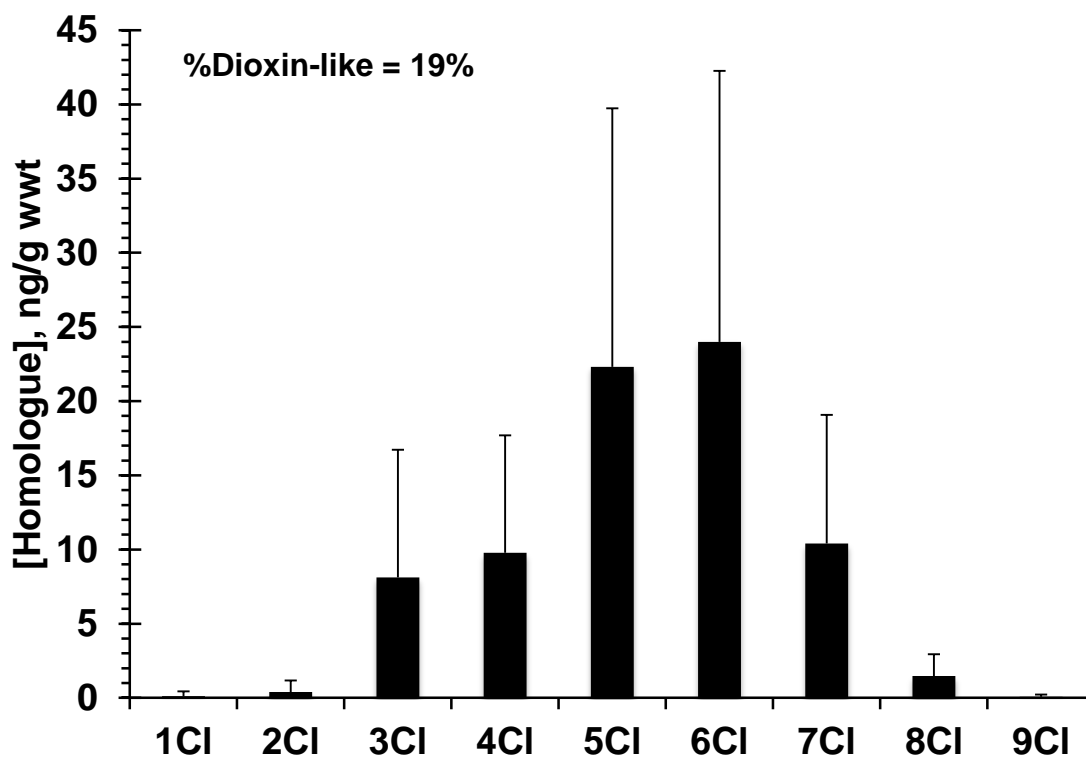


Figure 10. Mean PCB homologue concentrations in all fish samples. Numbers represent the sum of similar numbers of chlorine-substituted congener within the homologue groups.

The second group of EDCs in sediments were those with low detection frequencies (<50%), and included atrazine (herbicide), diphenylhydramine (over the counter drug, OTCD), fluoxetine (SSRI antidepressant), naproxen (NSAID), dextromethorphan (OTCD), 17 β -estradiol (steroid) and norgestrel (steroid). Many of these EDCs were detected too infrequently to establish average concentrations, but represent chemicals present in the environment but at trace levels.

4. EDCs in Biota

The incidence and concentrations of EDCs detected in biota samples resembled those in sediments with respect to variability. The most frequently detected EDCs in biota included triclosan (antibacterial agent) and bisphenol A (plastics), with DFs of 55% and 64%, respectively. The median concentrations of triclosan and bisphenol A in biota from Hunting Creek are shown in Fig. 12, ranging from 4 to 26 ng/g wwt.

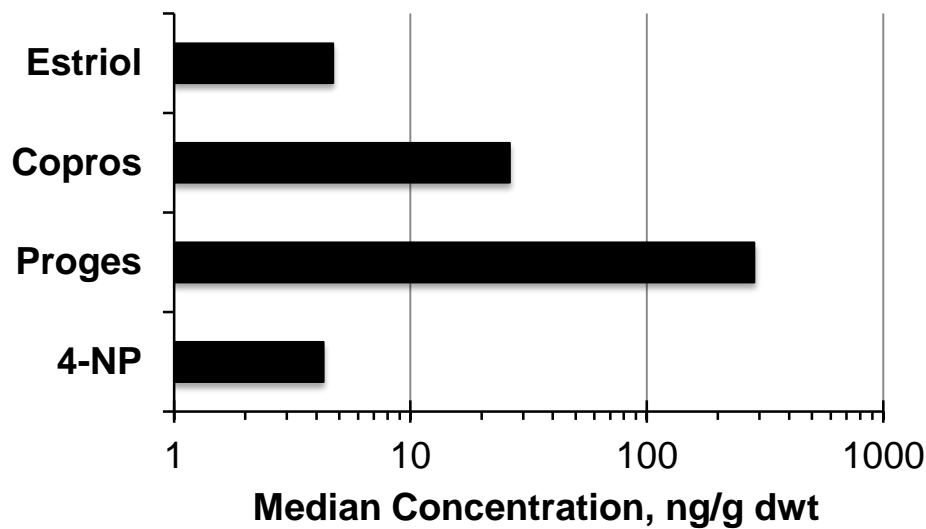


Figure 11. Median concentrations of EDCs detected >75% frequency in surficial sediments. 4-NP = 4-nonylphenol, proges = progesterone, copros = coprostanol.

The EDCs with DFs <50% in biota included, ibuprofen (NSAID), fluoxetine (SSRI), naproxen (NSAID) and dextromethoraphan (OTCD), which did correspond with the same minor EDCs detected in surficial sediments. Individual concentrations of these EDCs in biota are shown in Fig. 13. Concentrations of the low detection frequency EDCs ranged from 1 to 68 ng/g wwt.

The EDC concentrations in biota were sparse and variable such that no differences between species, ARE sampling location or time series could be resolved. The most frequently detected EDCs in biota did not correspond to the EDCs detected in sediments.

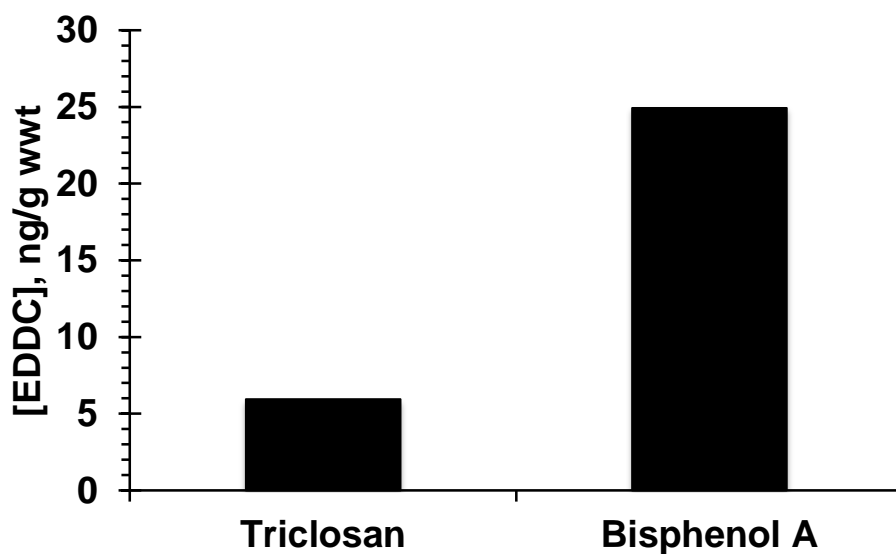


Figure 12. Median concentrations of triclosan and bisphenol A detected in all biota samples collected from Hunting Creek.

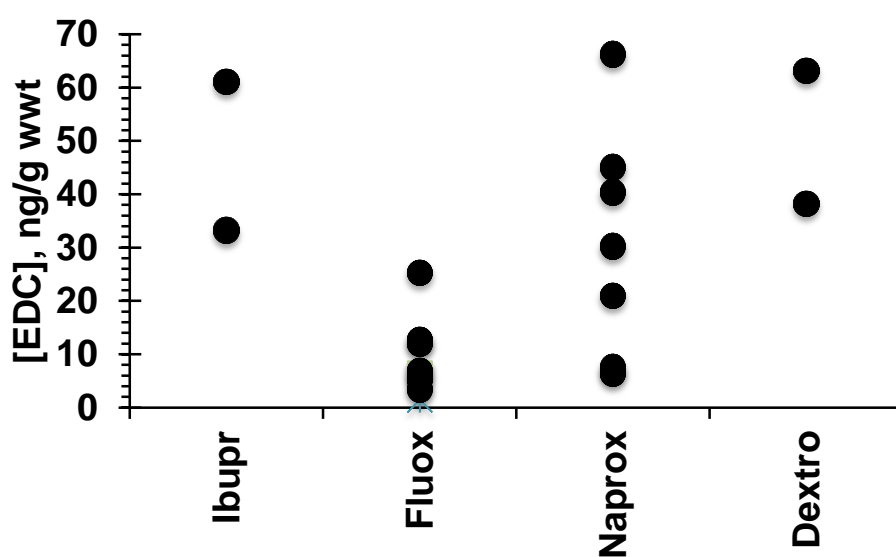


Figure 13. Individual biota concentrations of low detection frequency EDCs in Hunting Creek. Ibupr = ibuprofen, Fluox = fluoxetine, Naprox = naproxen and Dextro = dextromethorphan.

Discussion

The ecological survey of Hunting Creek surficial sediments and biota revealed several important observations. The first is that PCBs are widely detected in all sediments and biota in the local area. The concentrations of PCBs in sediments ranged from clean to lightly polluted, but no samples suggested that toxic effects are highly probable based on regulatory criteria (sediment quality guidelines). All PCB concentrations in surficial sediments were below minimum effect threshold criteria. A lack of observed gradients and geospatial correlation existed for PCBs in sediments, as they appear to be widely dispersed and uniform within the region.

PCBs were detected in all biota and at all locations in Hunting Creek. The concentrations observed at Hunting Creek were similar to slightly greater than PCB concentrations detected in fish collected from Dyke Marsh, VA (Crimmins et al. 2000). PCBs observed in fish from Hunting Creek showed no significant differences among species, indicating any ecologically stratified partitioning of PCBs was not evident. Factors such as species, size, age, feeding habits or other factors were not identified as important ecological processes regulating PCBs at this location.

EDCs were detected in sediments and biota from Hunting Creek, but stand in contrast to the observations of PCB concentrations. The EDCs were highly variable in terms of both incidence (detection frequency) and concentrations. The greatest divergence of EDCs from PCBs is that surficial sediments and biota showed a difference in chemicals that predominated in each matrix. Sediments show a predominance of steroids, while biota bioaccumulated primarily triclosan and bisphenol A in their tissues. Since there exist no regulatory criteria for EDCs in sediments or tissues, the influence of these chemicals in toxicity cannot be evaluated.

Work in Progress

Current work in progress is to complete the evaluation of all analytical QA and measured sample concentrations to provide the final dataset for the year 1 study. Priorities include the following list:

- Reanalyze 6 sediment samples for EDCs with the proper addition of surrogate standards

- Assess the flagged PCB concentrations (low surrogate recoveries) for PCBs in biota (3 white perch samples from ARE 5)
- Pursue ongoing methods development for the GCMS analysis of EDCs in biota. The current EDC method for biota samples shows low recoveries of some EDC analytes. An MS student in Chemistry is completing an MS research project on method development targeting this issue.
- Propose a new sampling plan and strategy for the next year of the study. For example, it is desirable to sample fewer species with a greater number of replicate analyses

Acknowledgments

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