Appendix E

CONNECTICUT RIVER WATERSHED

2003 Chlorophyll *a* and Periphyton Technical Memorandum

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Commonwealth of Massachusetts
Executive Office of Environmental Affairs
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Introduction

Biological assessment was performed by personnel from the Massachusetts Department of Environmental Protection (MassDEP) at several stations in the Connecticut River Basin during the summer of 2003. Because the Connecticut River is a large, often deep, often slow river, it can maintain a resident population of phytoplankton. In order to learn more about the phytoplankton biomass in this river, chlorophyll *a* samples were collected to gather information on the main stem water quality and to determine if it was impacted by sources of nutrients (phosphorus and nitrogen) located along the river; in particular, agricultural runoff and discharges from wastewater treatment plants. Chlorophyll *a* is a pigment that is found in all plants and algae and provides an estimate of biomass as well as an indication of the biological production of the water body.

In the tributaries, samples were collected for the identification of periphyton, described here as including the attached microscopic and macroscopic algae. Estimates were made of the percent algal cover within the riffle of the sampling reach. Algal type and abundance were also recorded. Periphyton sampling was limited to sites chosen for macroinvertebrate/habitat investigations.

Objectives of the periphyton sampling were to provide additional information for assessment by adding another biological community to the macroinvertebrate and habitat information, and to examine temporal changes in the amount and type of algae present in the assemblage. The periphyton assessment provides information to aid in determining if the designated uses, as described in the Surface Water Quality Standards (MassDEP 1996), are being supported, threatened or lost in particular segments. Periphyton data can be used to evaluate two designated uses of the Connecticut River: Aquatic Life and Aesthetics.

Aquatic life evaluations determine if suitable habitat is available for “sustaining a native, naturally diverse, community of aquatic flora and fauna.” Natural diversity and the presence of native species may not be sustained when there are dense growths of a monoculture of a particular alga. This alteration of the community structure may indicate that the aquatic life use support is lost or threatened. Loss of parts of the food web, which is vital for aquatic life use support, may result from this alteration. In addition, the die-off and decomposition of large amounts of biomass from macroalgae can fill in the interstitial sites in the substrate and destroy this habitat for the benthic invertebrates and compromise the aquatic life use support.

The algal data are also used to determine if aesthetics have been impacted. Floating rafts of previously attached benthic mats can make a waterbody visually unappealing, as can large areas of the bottom substrates covered with long streamers of algae that can discourage waders and hinder fishermen by making the substrata slippery for walking. Fishermen can also snag their fishing lines on the filamentous algae. Nuisance amounts of algae, which can compromise aesthetics, can be determined by estimating the percent macroalgal cover in a particular habitat (e.g. riffles or pool) (Biggs 1996) (Barbour et al. 1999). Nuisance amounts of macroalgae are present, if the percent cover is greater than 40 % by filamentous green algae (Biggs 1996) (Barbour et al. 1999).

Periphyton sampling is typically done on first, second or third order streams and rivers that are small, shallow, and often fast moving. At each of the stations an estimate of the percent cover of the periphyton and benthic algae is made and samples are collected for algal identification. Periphyton samples are typically scrapes of one type of substrata in the riffle zone. The algal scrapes are used in the qualitative microscopic examination to determine the presence and relative abundance of the phyla that contributes the most to the biomass in the riffle or pool habitats. The estimate of percent cover of the filamentous algae (macroalgae) is used in conjunction with the microscopic examination to determine if uses of the river (Aquatic Life Support and Aesthetics) are lost or threatened because of excessive algal growth.

Materials and Methods

Chlorophyll a

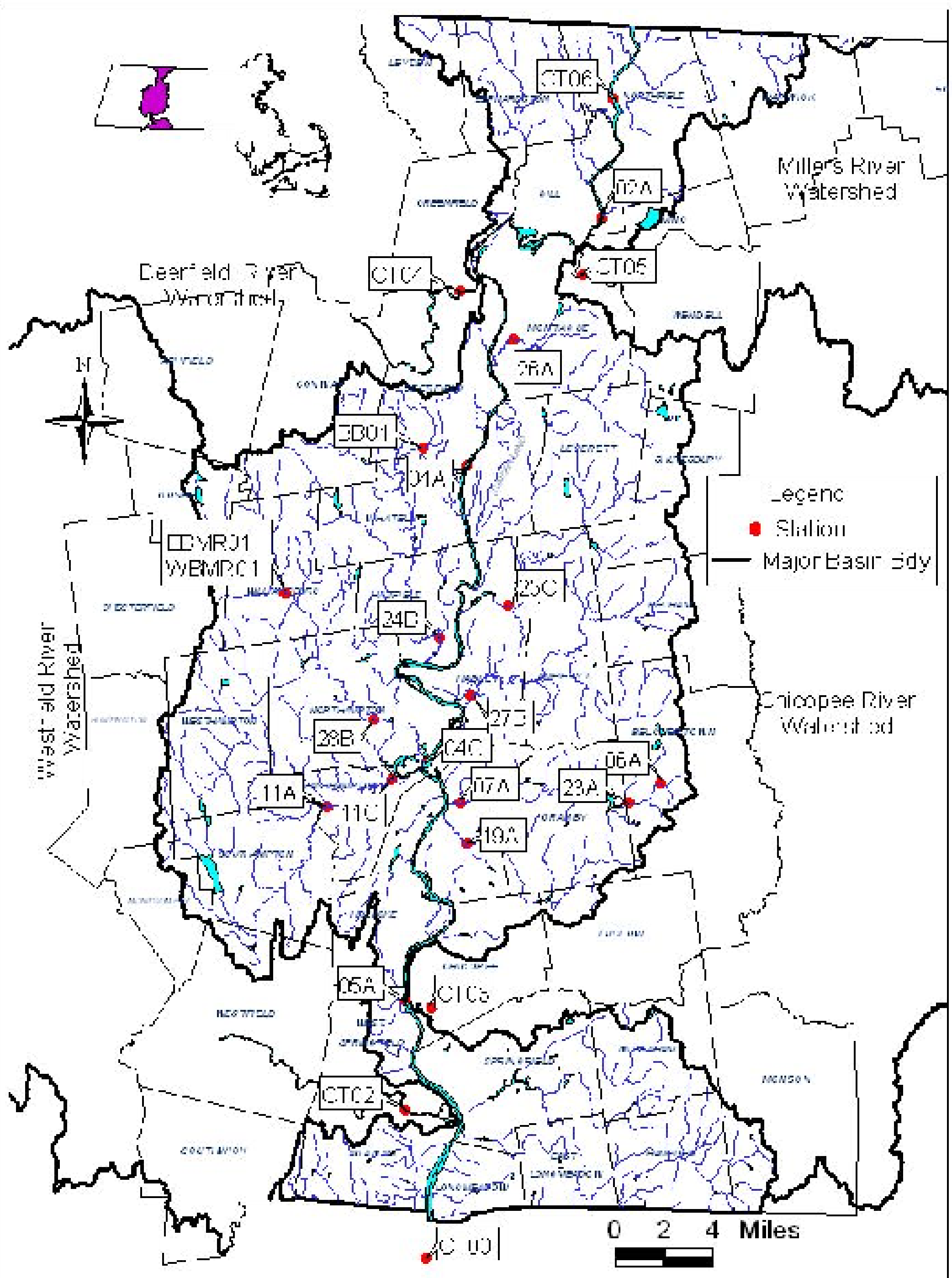
Samples for chlorophyll *a* analysis and phytoplankton identifications were collected on July 9, Aug. 6 and Sept. 10 by reaching into the main flow of the river using a pole with a sample container attached. Grab samples were collected just below the surface in plastic containers that were placed into iced coolers until they could be returned to MassDEP's laboratory in Worcester for analysis. Samples were processed within the 24-hour holding period. A list of chlorophyll *a* sampling stations is included in Table 1 and shown in Figure 1.

A Turner Designs, Inc. TD-700 fluorometer was used in the chlorophyll *a* analysis (MassDEP 2000). Fifty milliliters of sample water were filtered through a glass fiber filter. The filter was ground using a motor driven grinder and a glass pestle. The ground material was transferred to plastic centrifuge tubes that were kept in the dark and refrigerated for 24 hours while the chlorophyll *a* extraction continued in 90% acetone. The plastic centrifuge tubes were kept in the dark, brought to room temperature, and then decanted into borosilicate disposable cuvettes that were placed in the TD-700 fluorometer for analysis. Results are reported in mg chlorophyll *a* per m^3 water.

Table 1. 2003 Connecticut River Chlorophyll *a* Sampling Locations

Station ID	Location	Mile Point
CT06	**Connecticut River**-Route 10 Bridge, Northfield	64.4
02A	**Connecticut River**-Downstream of Fourmile Brook confluence, Northfield and east of Pisgah Mountain Rd., Gill	58.7
04A	**Connecticut River**-Route 116, Deerfield/Sunderland	40.2
04C	**Connecticut River**-Upstream of the confluence of the Mill River, near the Oxbow, Northampton/Hadley	22.4
05A	**Connecticut River**-Route 90 boat launch, West Springfield/Chicopee	9.9
CT00	**Connecticut River**-At the USGS flow gage #01184000 downstream of Route 190, Suffield/Enfield, Connecticut	-2.9
07A	**Bachelor Brook**-At Route 47 (Hadley St.), South Hadley	0.9
11A	**Manhan River**-Loudville Rd., Easthampton	5.6
11C	**Manhan River**-Fort Hill Rd., Easthampton	0.8
27B	**Fort River**-At Route 47, Hadley	0.6
24B	**Mill River**-Maple St., Hatfield	2.1
BB01	**Bloody Brook**-Whately Rd., Deerfield	1.6
25C	**Mill River**-Mill River Lane, Hadley	0.9

Figure 1- Sampling Locations in the Connecticut River Watershed



Map is from Mitchell (2005)

Periphyton Identifications and Relative Abundance

Periphyton samples were gathered along with the macroinvertebrate samples and habitat information using methods described in Barbour et al (1999). Sampling was done by the macroinvertebrate sampling crew and consisted of randomly scraping rocks and cobble substrates, typically within the riffle area, but other habitats were occasionally sampled. Material was removed with a knife or by hand from rock substrata and then added to labeled glass vials containing sample water. Table 3 contains descriptions of the station locations where periphyton was collected. The samples were transported to the lab at MassDEP-Worcester in one liter plastic jars containing stream water to keep them cool. Once at the lab, they were refrigerated until identifications were completed. Samples held longer than a week were preserved using M^3 with a dose rate of 2 ml of preservative per 100 ml of sample (Reinke 1984).

Vials were shaken to get uniform samples before subsampling. Filamentous algae were removed first, identified separately and then the remainder of the sample was examined. An Olympus BH2 compound microscope with Nomarski optics was used for the identifications (Appendix B contains the references used for identifications). Slides were typically examined under 200 power. A modified method for periphyton analysis developed by Bahls (1993) was used. The scheme developed by Bahls for determining abundance on a slide is as follows:

R (rare)	fewer than one cell per field of view at 200x, on the average;
C (common)	at least one, but fewer than five cells per field of view;
VC (very common)	between 5 and 25 cells per field;
A (abundant)	more than 25 cells per field, but countable;
VA (very abundant)	number of cells per field too numerous to count.

A visual determination was also made of whether or not the algal covering was composed of micro or macroalgae, in particular, the green filamentous algae. The microalgae typically appear as a thin film, often green or blue-green, or as a brown floc. Macroalgal (green filamentous algae) cover over greater than 40% of the substrata in the riffle/run is considered to be indicative of organic enrichment (Barbour et al 1999) to the extent that the aesthetic quality of the stream may be compromised.

Results

Chlorophyll a

Channel characteristics of the Connecticut River, such as depth and retention time, favor the establishment of an indigenous phytoplankton population. The biomass of the phytoplankton was estimated by determining the chlorophyll *a* concentration in a water column sample. The chlorophyll results remained fairly constant over the sampling period (Table 2) as most stations exhibited the same value or less than a 1.0 mg/m^3 change from July to September. Exceptions to this were station 11 C on the Manhan River which had its highest algal production in August (5.1 mg/m^3 chlorophyll a) but then dropped in September to 1.8 mg/m^3. Bloody Brook (BB01) peaked in July at 8.8 mg/m^3, but then decreased in August and September.

Table 2. 2003 Connecticut River Water-column Chlorophyll *a* Data (mg/m^3)				
Station ID	**Water Column color/transparency**	**Sampling Dates**		
		July 9	***August 6***	***September 10***
CT06	Water typically colored brown	<1.0	1.0	<1.0
02A	Water column was usually clear	<1.0 (1.1)*	1.3 (1.1)	1.6 (1.7)
04A	Water column slightly turbid and brown	<1.0 (<1.0)	--	<1.0
04C	Slightly turbid, brown	<1.0	1.3	1.1
05A	Water was typically slightly turbid, and brown	1.4	1.0	1.7
CT00	Water column clear	--	1.7 (1.6)	2.3
07A	Water always colored tan or brown and turbid	--	1.3	<1.0
11A	Water usually clear, yet low gradient and pasture land	--	2.1	<1.0
11C	Water brown colored	--	5.1	1.8
27B	Water was brown and turbid	--	3.1	<1.0
24B	Slightly turbid, brown	1.3	--	--
BB01	Water usually brown	8.8 (7.9)	3.2 (5.7)	3.4
25C	Water was usually slightly turbid and brown	1.5 (1.3)	--	--
* Values for duplicate samples appear in parentheses				

Periphyton

The three periphyton sampling locations, their percent canopy cover and percent algal cover are described in Table 3. Appendix A lists algal genera that were identified at these sites.

Table 3. 2003 Periphyton samples from selected Connecticut River Tributaries				
Unique ID	**Location**	**% Canopy Cover**	**% Algal Cover**	**Dominant Algae in riffle**
B0510	Mill River (Hatfield), ~100-meters upstream of Mountain Drive, below the confluence of West Brook, Hatfield, MA	50	65	Filamentous cyanobacteria *Phormidium* VA.
B0507	Stony Brook, ~30-meters upstream of powerlines, downstream from Route 116, South Hadley, MA	90	2	Filamentous green *Cladophora glomerata* and diatom *Cocconeis* sp.
B0515	Sawmill River, upstream at South Ferry Road, Montague, MA	70	30	Diatom chain (*Melosira brevigulata*)- planktonic, lake organisms

The Stony Brook station (B0507) had only 2% algal cover, and a high percentage of the river bottom was shaded by the canopy (90%) (Table 3). Isolated clumps of the green filamentous alga *Cladophora glomerata* were recovered in the algal scrapes (Table 3, Appendix A).

At the Mill River location (B0510) the percent algal cover was high at 65% with filamentous cover in the riffle dominated by the cyanobacteria-*Phormidium* sp. Although *Phormidium* sp. covered a large part of the substrata, the short microscopic filaments do not have the same nuisance factor as macroscopic algae. Canopy cover here was the lowest of the three stations at 50%.

According to field sheets, non-point source pollution was evident at the Sawmill River in Montague (B0515). Cows had access to the river at this station and their droppings were found in the riparian zone. The water column was slightly turbid and had a grayish color. The diatom chain *Melosira brevigulata* was a major constituent of the periphyton that covered 30% of the substrata in the riffle.

Discussion

Algal production, as indicated by the chlorophyll *a* values, was low at the stations included for sampling at the Connecticut River. As indicated in Table 3, many of the stations had highly colored and often turbid water. Agricultural land-use is prevalent throughout this watershed. In the technical memorandum presenting the 2003 water quality data for the Connecticut River Mitchell (2005) mentions possible sources for the turbidity present in the water column. The turbidity may have resulted from the sandy soil types that formed the banks of the river in several areas like CT06, 11A, 11C, where slumping or erosion of sandy/muddy banks was noted (Mitchell 2005). This common phenomenon along the Connecticut River could be caused by erosion of lake-bottom deposits (Typically clay, silt and sand) that are prevalent along both sides of the river-remnants of glacial Lake Hitchcock, which extended up to the Massachusetts border with Vermont.

Other sources of turbidity could be from non-point source run-off. Stations 02A-Northfield, 27 B-Amherst and 25C-University of Massachusetts all receive run-off from towns. Station 11C is located 0.75 miles below the Easthampton Wastewater Treatment Plant, another source of solids and nutrients to the river. Agricultural run-off may impact stations 02A, 05A, 11A, 27B and 25 C (Mitchell 2005).

The turbid and colored waters may have limited algal productivity by reducing available light penetration. Chlorophyll *a* values (an indicator of algal production) were often 1 mg/m^3 or less from stations that stretched from mile point 64.4 down to mile point –2.9 at CT00 in Enfield, Connecticut.

A closed canopy appeared to affect periphyton production at tributary sites including B0507 and B0515. A significant inverse relationship (r^2 equal to .9959 (F=0.040783) was found in a regression using % algal cover (y) and % canopy cover (x).

In areas with elevated nutrients and open canopy the green filamentous alga *Cladophora glomerata* is often found in abundance. The growth of this alga at B0507 might be more luxuriant if the canopy was more open.

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Appendix A: 2003 Connecticut River Tributary Periphyton - Algal Taxonomic Identifications and Abundance Data					
Unique ID	**Location**	**Date**	**Family**	**Genus/Species**	**Abundance***
B0515	Sawmill River, upstream at South Ferry Road, Montague, MA	22-July	Bacillariophyceae	*Fragilaria* sp.	C
			Bacillariophyceae	***Melosira brevigulata***	**VA**
			Bacillariophyceae	*Synedra* sp.	C
			Bacillariophyceae	*Ui** pennate diatoms*	R
			Chlorophyceae	*Chlamydomonas* sp.	C
			Chlorophyceae	*Closterium* sp.	C
			Chlorophyceae	*Scenedesmus* sp.	C
			Chlorophyceae	*Spirogyra* sp.	C
			Chlorophyceae	*Ui** desmids*	C
			Cyanophyceae	*Oscillatoria* sp.	R
B0507	Stony Brook, ~30-meters upstream of powerlines, downstream from Route 116, South Hadley, MA	22-July	Bacillariophyceae	***Cocconeis* sp.**	**VA**
			Chlorophyceae	***Cladophora glomerata***	**VA**
B0510 *Sample 1*	Mill River (Hatfield), ~100-meters upstream of Mountain Drive, below the confluence of West Brook, Hatfield, MA	23-July	Bacillariophyceae	*Cymbella* sp.	R
			Bacillariophyceae	*Cyclotella* sp.	R
			Bacillariophyceae	*Navicula* sp.	A
			Bacillariophyceae	*Pinnularia* sp.	R
			Bacillariophyceae	*Surirella* sp.	R
			Chlorophyceae	*Scenedesmus* sp.	R
			Chlorophyceae	*Staurastrum* sp.	R
			Cyanophyceae	***Phormidium* sp.**	**VA**
			Euglenophyceae	*Euglena* sp.	R
B0510 *Sample 2*	Mill River (Hatfield), ~100-meters upstream of Mountain Drive, below the confluence of West Brook, Hatfield, MA	23-July	Bacillariophyceae	***Cocconeis* sp.**	**VA**
			Bacillariophyceae	*Cyclotella* sp.	VC
			Chlorophyceae	*Closterium* sp.	R
			Chlorophyceae	*Microspora* sp.	R
			Chlorophyceae	*Ulothrix* sp.	A
			Chlorophyceae	ui** filament	VC
			Cyanophyceae	*Cylindrocapsa* sp.	A

* R (rare)
C (common)
VC (very common)
A (abundant)
VA (very abundant**)**

** unidentified

Appendix B: References Used for Taxonomic Identifications of the Algae

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