EXHIBIT F

APPENDIX D – 1998 DEP DWM BIOLOGICAL MONITORING TECHNICAL MEMORANDUM: Chlorophyll a, Phytoplankton and Periphyton

Prepared by: **Joan Beskenis, Division of Watershed Management**

Date: **March 22, 2000**

Subject: **Nashua River 1998 Chlorophyll a, Phytoplankton and Periphyton Sampling**

Introduction

During the summer of 1998, representatives from the Division of Watershed Management and DEP-CERO participated in various aspects of biological and water quality sampling as part of the larger "Year 2" information and data gathering effort in the Nashua River watershed. Four sites along the main stem Nashua River were selected for a comprehensive analysis of factors affecting the dissolved oxygen dynamics in the water column. This information was to be used for the proper calibration and verification of a water quality simulation model. During two separate survey weeks, personnel from the USEPA New England Regional Laboratory (NERL) deployed continuous read-out oxygen sensors and data loggers for a period of three days, thus providing information pertaining to the diel oxygen patterns resulting from algal community photosynthesis and respiration, benthic demand, and other factors. At the same time, DEP mobilized a special sampling crew to obtain water samples from those same four sites for chlorophyll analysis and phytoplankton counts, and to measure *in situ* the dissolved oxygen concentration at several depths throughout the water column. In addition to the impoundment studies described above, periphyton sampling was performed in the wadable segments of the mainstem Nashua River and selected tributaries in conjunction with macroinvertebrate sampling and habitat assessment.

Phytoplankton/Chlorophyll a Results

General comments can be made regarding the qualitative assessment of the algae present in the reach of the Nashua River extending from the Ice House Dam Impoundment in Shirley to the outlet of Pepperell Pond in Pepperell. Phytoplankton samples were collected on July 21 and 22, 1998 at four sites: Ice House Dam Impoundment, Nashua River at Groton School, Groton, the inlet to Pepperell Pond, Pepperell and the outlet from Pepperell Pond. This sampling was repeated on August 11 and 12. Two types of algal samples were collected: 1) a grab sample which was collected at approximately 0.5 m below the surface of the water, and 2) integrated algal samples which were collected at most locations and on most dates. These samples were collected by lowering a weighted ½ -inch inner diameter plastic tube down to just off the bottom, pinching the top closed, and then opening the tube and emptying the contents into a clean, plastic jar. This was repeated until the desired volume was obtained. Care was taken to avoid collecting sediment in the sample. This depth-integrated sample, collected from the entire photic zone, is representative of the entire phytoplankton community; whereas the grab sample is selective for those organisms that are found close to the surface. This may or may not be representative of the algal community species composition.

Both the grab and integrated (IA) chlorophyll sample analyses from the July and August surveys are presented in Table D1. The IA samples always exhibited slightly higher chlorophyll values than did the grab samples. Chlorophyll a values ranged from a low of 1.12 mg/m^3 measured on July 22, 1998 at the Groton School to a high of 10.14 mg/m^3 at the outlet from Pepperell Pond, measured on that same date. This pattern was repeated in the August sampling. At that time Groton School again had the lowest value (2.38 $mg/m^{3)}$ while the outlet from Pepperell Pond was 19.6 mg/m^3.

In addition to the chlorophyll analysis, samples were examined to provide information on the algal community composition, which could provide evidence of excessive nutrient enrichment or other water quality problems. Nevertheless, at the time of the sampling in July and August there were no algal blooms evident, and little or no blue-green algae were present at any of the sampling sites (Table D2). This suggests that nutrients, in combination with other environmental factors, were not causing excessive algal growth. The green algae were represented by several more planktonic genera in August than in July. The dominance of the green algae in the outlet from Pepperell Pond, along with the elevated chlorophyll values, would contribute to the classification of this reach of the river as mesotrophic.

Table D1. Nashua River Chlorophyll a (mg/m^3)-1998.

lab #	sample #	date	location	chlorophyll a (mg/m^3)
10	81-0045IA	7/22/98	Outlet Pepperrell Pond	10.14
11	81-0046A	7/22/98	Inlet Pepperrell Pond	2.66
12	81-0047IA	7/22/98	Inlet Pepperrell Pond	3.5
13	81-0048A	7/22/98	Groton School	1.12
14	81-0049IA	7/22/98	Groton School	1.54
15	81-0050A	7/22/98	Groton School-duplicate	1.12
16	81-0052A	7/22/98	Ice House Dam	5.79
26	81-0077A	8/12/98	Outlet Pepperell Pond	19.6
28	81-0080A	8/12/98	Inlet Pepperrell Pond	3.22
30	81-0081	8/12/98	Groton School	2.38
32	81-0082A	8/12/98	Ice House Dam	3.22
33	81-0082IA	8/12/98	Ice House Dam	3.64
33D	81-0082CA	8/12/98	Lab duplicate	3.78

The stations exhibiting higher flow velocities (i.e., Groton School and Ice House Dam Pond) had fewer green algal genera and more diatoms and flagellated genera present (Table D2). This assemblage characterizes a lotic environment that is subjected to organic enrichment. Not anticipated was the dominance of "sewage fungus" at the Groton School site in August. Sewage fungus was also found at Ice House Dam Pond along with *Euglena* sp. and *Scenedesmus* sp. These genera are commonly found in areas of organic enrichment. Some fibers that looked like paper waste were present in the sample as well.

Periphyton Results

Periphyton was collected at nine stations along the Nashua River and its tributaries during the summer of 1998. Samples were collected by personnel from DEP-DWM-Worcester in conjunction with sampling for macroinvertebrates as well as habitat assessment. In most cases, the riffle habitat was sampled in an area with an open or partially open canopy, and often in the same reach as the macroinvertebrate sampling. Periphyton samples were collected by scraping substrates into a glass vial filled with sample water. They were put on ice and transported back to the microscopy lab in Worcester (DEP-DWM) for identification. The vial was shaken to get a uniform sample before subsampling. If filamentous algae comprised most of the sample they 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 identifications. Slides were typically examined under 200 x power. Information gathered was qualitative; only the dominant algal genera were identified. Observations concerning vegetative cover were recorded on the field sheets. This information was used in conjunction with information on the predominant taxa to provide a qualitative assessment of water quality and habitat within each reach.

Current DWM field collection methods do not allow for the quantitative assessment of algal cover. Any indication of the extent of algal cover in a particular reach is based on an estimate made during the habitat assessment. Areas with extensive algal growth are certainly identified in this manner, but areas with cryptic algal genera may be overlooked. This does limit the usefulness of the data; therefore, the analysis is limited to general comments regarding a particular site. Furthermore, this analysis does not take into effect the changes that occur seasonally in the algal communities. Results of the periphyton community assessment can be found in Table D3.

Table D2. Nashua River Phytoplankton-Sampling Results for July 21-22 and August 11-12, 1998.

Class	Algal Type/Genus	Stations/Sample Type					
		Outlet Pepperrell Pond		Inlet Pepperrell Pond	Groton School	Ice House Dam Pond	
		integrated	grab	integrated	integrated	grab	integrated
Cyanophyceae	Blue-greens						
	Lyngbya		August				
Bacillariophyceae	Diatoms						
centric	*Cyclotella*		August				
	Melosira	August	August	July	August		August
	ui* Centric diatom	August			July		
pennate	*Asterionella*						
	Synedra		August				
	Fragilaria		August	July	August		
	Tabellaria						August
	Navicula						August
	Cocconeis						August
	Stauroneis			August		July	
	ui Pennate					July	August
Chlorophyceae	Greens						
	Scenedesmus	August					August
	Oocystis	July / August	August				
	Gloeocystis	July / August					
	Sphaerocystis		August	July / August	July		
	Ankistrodesmus	August			August		
	Chlamydomonas	August	August			July	August
	Dictyosphaerium		August				
	Platydorina	August					
	Pediastrum		August				August
	Pandorina	August					
	Volvox	August	August				
	Haematococcus		August				
	ui Green flagellates	August		August			
	ui Coccoid green		August	August			August
Chrysophyceae	Golden-browns						
	Uroglenopsis		August				
	Chrysococcus	August					
	ui Golden-browns	August	August			July	
Dinophyceae	Dinoflagellates						
	Ceratium	August		August			
	ui Dinoflagellates	August					August
Xanthophyceae	ui Flagellates						
Euglenophyceae	*Euglena*	August	August		August		August
	unpigmented euglenoids			July	July		
	ui Euglenoids	August		August	August		August
Cryptophyceae	*Cryptomonas*						August
"sewage fungus"					August		

* ui – unidentified

Table D3. Nashua River Periphyton from stations sampled in September 1998.

Date	Station/Location	Habitat	Algal class and genera
1-Sep-98	NT34 Whitman River-upstream from Rte 2A, Fitchburg	pool-run	Chlorophyceae-*Spirogyra* Bacillariophyceae-*Fragilaria*
1-Sep-98	NN09 North Branch Nashua River, downstream Fallulah Rd., Fitchburg	shallow pools	Chlorophyceae-*Mougeotia* Bacillariophyceae-*Synedra* Bacillariophyceae-ui pennate
3-Sep-98	NN13 North Branch Nashua River, downstream from Ponakin Mill, Lancaster	riffle	Chlorophyceae-flecks of compressed green algae
3-Sep-98	NS17U South Branch Nashua River-upstream from MWRA, Clinton	riffle	Rhodophyceae-*Batrachospermum* Chlorophyceae-*Ulothrix*
2-Sep-98	NT67 Nissitissit River downstream from Prescott Rd., Pepperell	riffle	Chlorophyceae-*Spirogyra*
2-Sep-98	NT68 Nissitissit River downstream from Canal St., Pepperell	riffle	Chlorophyceae-*Spirogyra* Chlorophyceae-*Rhizoclonium*
3-Sep-98	NM23B Nashua River approximately one mile downstream from Leominster Rd.and just downstream of railroad bridge, Shirley/Ayer	riffle	Chlorophyceae-*Ulothrix*
2-Sep-98	NM29 downstream from Covered Bridge, Pepperell	riffle	Bacillariophyceae-*Melosira* Moss Bacillariophyceae-*Fragilaria* Bacillariophyceae-ui pennate Bacillariophyceae-*Synedra* Chlorophyceae-*Pediastrum* Chlorophyceae-*Cosmarium* ~~Clhlorophyceae-*Scenedesmus*~~ Chlorophyceae-*Scenedesmus* Cyanophyceae-*Lyngbya* fungal mycelia
2-Sep-98	NM30 Nashua River downstream from Rte 111, Hollis, New Hampshire	riffle/pool	Chlorophyceae-*Spirogyra* Chlorophyceae-*Ulothrix*

Nashua River Periphyton Observations by Location

The South Branch Nashua River (NS17U) algal community was different from other locations. This station, located upstream from the MWRA sewage treatment plant in Clinton, receives hypolimnetic water

from the Wachusett Reservoir. The water quality characteristics and/or water temperatures apparently differ from other streams. The red alga-*Batrachospermum* sp. is found at sites exhibiting cool water temperatures, lower light levels and, often, lower nutrient regimes.

The Whitman River was sampled at station NT34, which is located upstream from Route 2A in Fitchburg. There was no evidence of nonpoint sources of pollution; however, there were vast amounts of *Spirogyra*

sp. present, which is often indicative of the presence of elevated levels of nutrients. Likewise, substrates were covered with a floc of the diatom *Fragilaria* sp., another taxon typically associated with abundant nutrients.
Station NN09 on the North Branch of the Nashua River is subjected to obvious sources of nonpoint source pollution from both commercial and industrial development. This was noted on the habitat assessment field sheet. The water column was slightly turbid and exhibited a "sewage" type smell. Comments on the habitat assessment sheet stated that there was 0% vegetative cover although a brown substance covered the cobbles (this was likely the naviculoid diatoms that were present in large numbers in the sample). Apparently, free floating drift algae (tangles of the green algae *Mougeotia* sp. and *Ulothrix* sp.) were found in the shallow pools along the left bank.

The North Branch of the Nashua River was sampled again at Ponakin Mill, Lancaster (NN13). The habitat here was different from others in that very fast riffles were present. The qualitative algal sample only had flecks of a compressed green alga. The field sheets describe a thin layer of slippery periphyton on the rocks, which was probably diatoms. Moss was also prevalent at this station although none was present in the sample collected.

Habitat assessment at the main stem Nashua River station NM23B revealed an area with obvious sources of nonpoint-source pollution. Field staff observed that there was a sewage odor and that the water was very turbid. No comments were made about the presence of surface films at this partly shaded reach, but microscopic observation revealed the presence of some *Ulothix* sp. and lots of bacteria were present. This station is also located just below the confluence with the South Branch which receives the effluent from the MWRA WWTP.

The Nashua River station (NM29), located downstream from the Covered Bridge, Pepperell, was described on the habitat field sheet as potentially receiving nonpoint source runoff from a nearby horse farm. The water column was described as turbid and opaque. Although the periphyton sample did not indicate an impacted algal community, the light transparency might have been impaired thereby reducing algal habitat. The other algae in the sample were planktonic and were basically filtered out of the water column by the abundant growth of moss on the river bottom . Genera such as *Pediastrum, Cosmarium*, and *Scenedesmus* are all found in lentic conditions and likely spill out from Pepperell Pond.

A site on the Nissitissit River (NT67) located downstream from Prescott Rd. in Pepperell, is classified as a cold water fishery and did not support a distinctive periphyton community. This partially shaded station contained shallow riffles with some *Spirogyra* present, as well as some rooted emergent macrophytes; no mention was made on the field sheet of the percent of vegetative cover, so it is assumed that nuisance amounts were not found.

Green filamentous algae, in this case *Rhizoclonium* and *Spiroyra* predominated downstream at station NT68 on the Nissitissit River. Field notes state that this location had only a small amount of filamentous algae as well as sparse growth of aquatic vascular plants. Although there was a potential source of pollution from an adjacent oil company, there was no evidence of problems in the stream.

The main stem Nashua River was also sampled at station NM30, located in Hollis, New Hampshire at Route 111. *Spirogyra* sp. and *Ulothrix* sp. dominated the periphyton. This main stem station has bedrock outcrops, deep riffles and pools. When it was sampled in 1985, the dominant drift algae in the pools was a different green, *Hydrodictyon* sp. Fortunately, this nuisance alga was not found during the 1998 sampling. An estimate of the percentage of the reach with vegetative cover (60%) was included on the field sheet. This was composed of *Elodea* and *Myriophyllum* sp. (rooted submergents), and free-floating *Lemna* and *Wolfia*. In addition to algae, moss covered the rocks.

Discussion

Physical and biological disturbances of periphyton and other aquatic communties may be indicated by changes in both biomass and species composition. Biggs (1996) describes three temporal patterns of biomass distribution that can be distinguished among streams: 1) relatively constant disturbance, low biomass, 2) cycles of accrual and sloughing; and 3) seasonal cycles. The relatively constant, low biomass can occur as a result of frequent disturbance. In the summer of 1998, flooding was not a frequent phenomenon so this type of disturbance was minimal. During periods of extended flow stability (i.e., 4-10 weeks), the accumulation of biomass can occur (Douglas,1958). This "accrual and sloughing" pattern was a likely phenomenon at the Nashua River sites visited in 1998. During periods of relative flow stability, populations of filamentous algae, such as *Spirogyra*, which are otherwise vulnerable to flooding, can increase. This may have been the situation exhibited at one or two Nashua River stations, in particular NM30, on the main stem, and NT34 on the Whitman River. Station NT 34 contained both stable (50 % cobble) and unstable (50% sand and gravel) substrates. This station scored very poorly in the habitat assessment for embeddedness (6 out of 20 points). During higher flow periods it is likely that scouring of the surfaces would occur at this site; however, during the period sampled, long filamentous strands of algae were able to build up. Also of consideration at these sites is the level of nutrients available for algal growth. Periphyton communities are often dominated by erect stalked diatoms and/or filamentous green algae when the level of disturbance is low to medium and nutrients are medium to plentiful as found at station NN09 (Biggs, 1996). Biomass also builds up under these conditions. It is conjectured that grazing by fish and macroinvertebrates cannot keep up with algal growth. In these situations long filamentous growths of algae can occur on stable substrates and/or flocs of diatoms can cover all substrates.

Conclusions and Recommendations

There is some indication of excess nutrients and low/medium hydrologic disturbance in this basin. Drought conditions for the past several years have certainly affected adversely many of our rivers, allowing the concentration of many pollutants in depositional zones and reducing scouring events. Many stations that were evaluated in the habitat assessment indicated that nonpoint sources of pollution were in evidence. An effort should be made to remove these sources and to monitor improvements through the evaluation of changes in the algal community, biomass and percent cover.

Literature Cited

Biggs, B. J. F..1996. Patterns in benthic algae of streams. IN: Algal Ecology*: Freshwater Benthic Ecosystems.* edit. R. J. Stevenson, M. L. Bothwell, R. L. Lowe. Academic Press. San Diego.

Biggs, B. J. F. and Gerbeaux, P. J. 1996. Epilithic periphyton in temperate rivers: community composition in relation to differences in disturbance regimes. NIWA-Christchurch Misc. Rep. 177, 1-33. IN: Algal Ecology: *Freshwater Benthic Ecosystems.* edit. R. J. Stevenson, M. L. Bothwell, R. L. Lowe. Academic Press. San Diego.

Biggs, B. J. F. and Price, G. M. 1987. A survey of filamentous algal proliferation's in New Zealand rivers. *N. Z. J. Mar. Freshwater Res.* 21:175-191.

Douglas, B. 1958. The ecology of the attached diatoms and other algae in a stony stream. J. Ecol. 46:295-322.

Peterson, C. G. and Stevenson, R. J. 1990. Post-spate development of epilithic algal communities in different current environments. *Can. J. Bot.* 68: 2092-2102.

Technical Services Branch. 1990. *A Biological Assessment of Water Quality Conditions in the Nashua River and Selected Tributaries: Results of the 1985 Survey.* Mass. Dept. of Environmental Protection, Westborough, Massachusetts .