CONNECTICUT DEPARTMENT OF ENVIRONMENTAL PROTECTION
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Monitoring Phytoplankton Community Composition in Long Island Sound With HPLC Photopigment Profiles

FACT SHEET March 2005 Gina McCarthy, Commissioner

Why are phytoplankton important?

Phytoplankton are microscopic sized plants living in the water. While most of them are single-cell organisms, they are a diverse group varying in size, shape and pigment content (Figure 1). Phytoplankton are the major primary producers in most estuaries and open coastal waters. They fix carbon dioxide and produce organic materials that larger organisms in the food chain consume. However, too much phytoplankton in the water column is usually an indication of eutrophication, the process caused by nutrient over-enrichment.

The amount, or biomass, of phytoplankton present is important to understanding their function in a system, and is often estimated by measuring the amount of chlorophyll. However, a measure of biomass alone tells us little about the composition of the phytoplankton – what groups or species are present, in what proportions, and with what characteristics – and it is these details of the phytoplankton community that can tell us the most about the overall function, and health, of the system. Each phytoplankton species or class has its own unique nutrient and light requirements and adaptive strategies, such as those that affect sinking rate and nutrient uptake kinetics. Phytoplankton also vary in their nutritional value to grazers. Consequently, phytoplankton community structure affects other trophic levels (e.g., zooplankton and fish) and the overall health of ecosystems.

In Long Island Sound (LIS), one of the most pressing environmental problems is hypoxia, a low oxygen condition that develops each summer in the bottom water of the western Sound. These low summer oxygen concentrations are inadequate to support healthy fish populations. Hypoxia is a symptom of nutrient over-enrichment (eutrophy) fueled by phytoplankton growth. When phytoplankton die or cycle through the food chain and sink to the bottom, the microbial decomposition of the organic material consumes oxygen. Coupled with the seasonal water column stratification that prevents mixing of oxygenated surface waters to the bottom, a hypoxic condition results.

Continuous monitoring of phytoplankton will allow us to see a change (if there is any) in phytoplankton community structure over time. This is particularly meaningful as we continue to implement the nitrogen management plan (decreasing the nitrogen loading to the Sound) and expect a decrease in phytoplankton biomass in the water column and an increase in bottom oxygen concentration. Availability of nitrogen could affect the phytoplankton composition in time and space which in turn affect other trophic levels in the water.

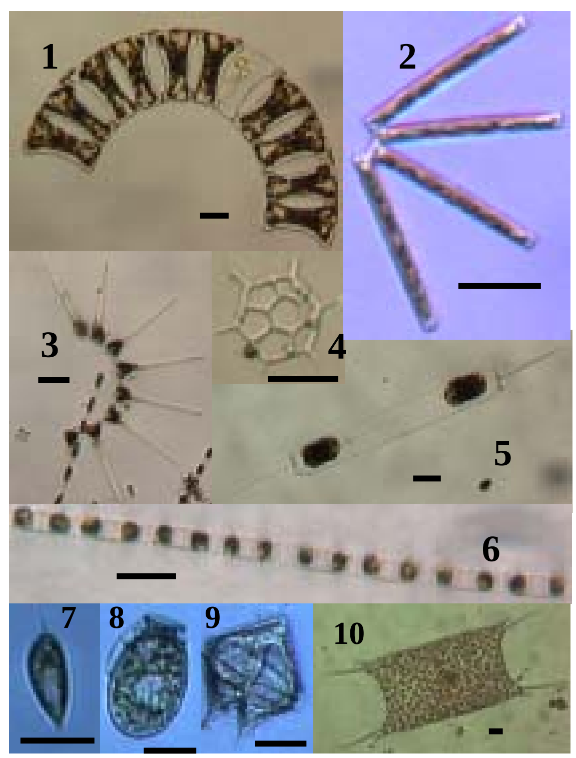

Figure 1. Examples of phytoplankton from Long Island Sound (CT DEP sample file photos; Judy Li). Scale bar = 20 µm. 1. *Eucampia zodiacus*; 2. *Thalassionema nitzschioides*; 3. *Asterionella japonica*; 4. *Distephanus speculum*; 5. *Ditylum brightwellii*; 6. *Skeletonema costatum.* 7. *Prorocentrum sp.*; 8. *Dinophysis sp.*; 9. *Protoperidinium sp.*; 10. *Odontella sinensis.* Species 1, 2, 3, 5, 6 and 10 are diatoms. Species 7 to 9 are dinoflagellates and species 4 is a silicoflagellate.

Table 1. Major phytoplankton photopigment content determined from HPLC (High-performance liquid chromatography). (from Paerl et al. 2003)

	Chlorophyll a	Div-chl a	Chlorophyll b	div-chl b	Chlorophyll c1c2	Chlorophyll c3	Alloxanththin	Antheraxanthin	β-Carotene	Canthaxanthin	Diadinoxanthin	Diatoxanthin	Echinenone	But-Fucoxanthin	Fucoxanthin	Hex-Fucoxanthin	Gyroxnthin	Lutein
Chlorophytes	x		x					x	x									x
Chrysophytes	x				x	x			x		x	x		x	x			
Cryptophytes	x				x		x		x									
Cyanobacteria (Pelagic)	x							x	x									
Cyanobacteria (benthic)	x							x	x	x			x					
Diatoms	x				x				x		x	x			x			
Dinoflagellates	x				x				x		x	x						
Euglenophytes	x		x					x	x		x	x						
Eustigmatophytes	x								x									
Prymnesiophytes (Haptophyta)	x				x				x		x	x		x	x	x		
Pelagophytes	x				x				x		x	x		x	x			
Prasinophytes	x		x						x									x
Prochlorophytes		X		x					x									
Raphidophytes	x				x				x		x	x			x			
Karenia	x				x	x					x	x		x	x	x	x	

Table 2. Comparison between the photopigment method and microscopic method for phytoplankton community assessments.

	Photopigment method	***Microscopic method***
Output	Quantitative results at class level	Quantitative results at species level plus size information
Input	Water samples and pigment ratio for known species	Water samples
Advantages	• Fast and less labor intensive • More accurate for small and hard-to-preserve species • Does not require high taxonomic skills	Can provide species-level information
Limitations	• Gives information at class level for most cases • Uncertainty associated with changes in pigment composition with environmental and physiological status	• Requires high taxonomic skills • Very labor intensive • Difficult with small and hard-to-preserve species

How are phytoplankton monitored conventionally?

Microscopic examination is the classic way of studying phytoplankton community composition. While this method gives detailed size and species information, the limitation is that nano- (2-20 µm) and pico- (0.2-2 µm) sized phytoplankton and other hard-to-preserve species may be missed or unidentifiable by the microscopic method alone. It also requires advanced taxonomic skills and is very labor intensive. An alternative way to monitor phytoplankton community structure is through photopigment analysis.

What is photopigment analysis?

Chlorophyll is a photopigment that most people are familiar with, but there are many other pigments in phytoplankton that can help "fingerprint" different algal groups. Unique pigments or pigment combinations are associated with specific phytoplankton classes or groups. While many may share the same pigment types, the relative amounts may vary (Table 1). Thus, measured pigment concentrations allows us to rapidly identify and quantify different phytoplankton classes in a mixed water sample without tedious microscopy (Table 2).

How are phytoplankton monitored through photopigment analysis?

There are 3 steps involved (Figure 2).

1. Sample collection
The first step is to collect the water sample from LIS at our selected monitoring stations.

2. Pigment analysis
Water samples are sent to a laboratory where photopigments are analyzed using a special process called HPLC (High-performance liquid chromatography).

3. Phytoplankton class analysis
The computer software, CHEMTAX was specifically developed to calculate phytoplankton class composition based on the HPLC data from the water samples (pigment data) and the known pigment ratio for target classes (reference pigment ratios). The reference pigment ratio matrix required by Chemtax was based on pigment ratios of cultured species isolated from LIS and nearby estuaries. Through the calculation from these known groups, chlorophyll or total pigment is partitioned into different phytoplankton classes in the unknown samples being analyzed.

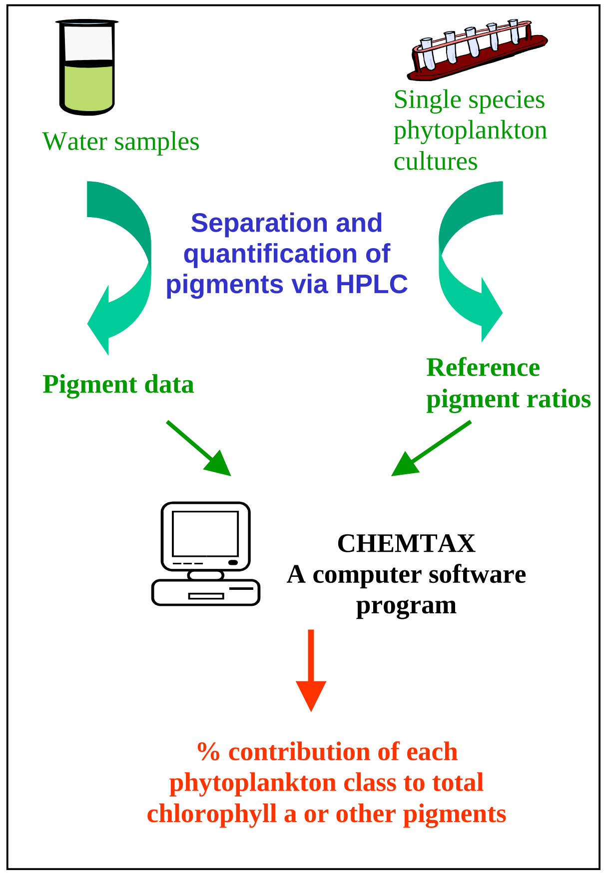

Figure 2. Flow chart for phytoplankton analysis through photopigments.

Table 3. Contribution of each phytoplankton group/class to total pigment. Data is based on Sound-wide average of all samples, April 2002-June 2004. CTDEP LIS Monitoring Program.

Group / class	% contribution
Diatoms	51.03
Cryptophyceae	14.01
Dinoflagellates	9.14
Prymnesiophyceae	7.50
Euglenophyceae	6.85
Cyanobacteria	4.24
Prasinophyceae	3.94
Raphidophyceae	1.98
Chrysophyceae	0.79
Chlorophyceae	0.28

Phytoplankton community composition in Long Island Sound

CTDEP's Long Island Sound Water Quality Monitoring Program began to collect samples for HPLC photopigment analysis in April 2002. The following discussion is based on samples taken April 2002 through June 2004.

Chemical taxonomic analysis using HPLC pigment profiles revealed that diatoms are the most abundant phytoplankton group in the Sound (Table 3), contributing over half of the total phytoplankton biomass. The second most abundant group is cryptophytes followed by dinoflagellates.

There is a distinct seasonal pattern in phytoplankton community composition in Long Island Sound. Diversity is low during the winter, and diatoms dominate, accounting for 70-90% of the total phytoplankton biomass. Diversity is higher in the summer. Although diatoms continue to be the dominant group during the summer months, they account for much less of the total (about 30% of total pigments), with a much more diverse phytoplankton community present. From June to August, for example, dinoflagellates and prasinophytes exhibit annual highs in their percent contribution relative to total phytoplankton.

Although there was a clear spatial gradient in phytoplankton biomass in the Sound, with higher biomass in the western Sound, which is generally richer in nutrients, and lower biomass in the eastern Sound, the community composition did not exhibit such a gradient. The distributions of diatoms, cryptophytes and dinoflagellates were extremely similar across the Sound.

Photopigment method - advantages, limitations and ways to improve the results

Photopigment method has proven to be an efficient way of monitoring phytoplankton. It is less time consuming and does not require advanced taxonomic skills. It shows great advantages over the microscopic method particularly with small-sized and hard to preserve species. However, with few exceptions, it only provides information at class level since few species can be identified and quantified. Another limitation is the uncertainty in the results due mainly to the change in phytoplankton's pigment concentrations, as affected by environmental conditions such as light intensity, nutrient availability, and physiological status.

To improve the estimation by photopigment method, the reference pigment ratio matrix should be constructed with pigment data from as many locally isolated species as possible. Verification of results by microscopic examination on a regular basis is also recommended. With these efforts, photopigment analysis techniques in LIS will continue to improve.

Further readings:

1. Mackey, M. D., Higgins, H. W., Mackey, D. J. and Wright, S. W. 1997. CHEMTAX user's manual: a program for estimating class abundances from chemical markers – application to HPLC measurements of phytoplankton pigments. CSIRO Marine Laboratories Report 229, Hobart, Australia, ISBN 0 643 06040 5, 47 pp.

2. Paerl, H.W., Valdes, L.M., Pinckney, J. L., Piehler, M. F., Dyble, J. and Moisaander, P. H. 2003. Phytoplankton photopigments as indicators of estuarine and coastal eutrophication. *BioScience* 53: 1-12.

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