Fall 1983

Ecological studies of the surface microlayer of small ponds at the UWM Field Station

Charles C. Remsen
University of Wisconsin - Milwaukee

James S. Maki
University of Wisconsin-Milwaukee

Savas C. Danos
University of Wisconsin-Milwaukee

Kenneth E. Estep
University of Wisconsin-Milwaukee

ECOLOGICAL STUDIES OF THE SURFACE MICROLAYER OF SMALL PONDS AT THE UWM FIELD STATION

CHARLES C. REMSEN, JAMES S. MAKI[1], SAVAS C. DANOS[2], AND KENNETH W. ESTEP[3]

Center for Great Lakes Studies, University of Wisconsin–Milwaukee
Milwaukee, Wisconsin 53201

ABSTRACT

The seasonal variation and enrichment of nutrients, pigments, bacteria, fungi and algae in the surface microlayer and subsurface waters were investigated in three ponds at the University of Wisconsin-Milwaukee Field Station, Saukville, Ozaukee County, Wisconsin. Samples were collected intermittently from June, 1978 through October, 1981.

Microlayer samples were collected using a glass plate and a screen sampler. All ponds showed dramatic seasonal variations in nutrients, microorganisms and algae in both surface and subsurface waters. The data indicate that physical factors such as adsubble processes, antirain and atmospheric deposition, along with biological factors such as heterotrophic mineralization and autotrophic uptake, play significant roles in causing the enrichment or lack of enrichment of materials within the microlayer. Furthermore, this study suggests that surface microlayers, particularly in shallow environments where algal species are adapted to high light conditions, can be sites of high biological activity.

INTRODUCTION

This paper summarizes ecological studies that began in 1977 and are continuing through the present time on the surface microlayer (neuston) of three small ponds at the UWM Field Station. Studies in marine and freshwater environments over the last 30 years have demonstrated that surface microlayers possess different physical, chemical and biological characteristics than the underlying subsurface waters. Visible slicks are often present in these environments due to the concentration of hydrophobic material in a surface film of 0.1-1.0 um (Sieburth 1965). These slicks may contain high concentrations of both inorganic and organic compounds creating a surface layer that is chemically distinct from the subsurface waters. The surface microlayer has attracted considerable attention recently because it is the entry point of many atmospherically delivered toxicants into the aquatic ecosystem. Chlorinated hydrocarbons, such as PCB's and various heavy metals are among the pollutants that are found in the surface microlayer, usually in relatively high concentrations.

[1] Present address: Laboratory of Microbial Ecology, Harvard University, Cambridge, MA 02138

[2] Present address: Littleton Water Department, Littleton, MA 01460

[3] Present address: Graduate School of Oceanography, University of Rhode Island, Kingston, RI 02881

Our interest in the surface microlayer (or surface film) is basically two-fold: (a) To examine the distribution and activities of microorganisms inhabiting this distinct environment, and in particular, how they are able to cope with the stress (in the form of UV radiation and high concentration of pollutants) to which they are constantly exposed; and (b) to determine whether the activities of these surface film microorganisms contribute to the "enrichment" of nutrients in subsurface waters.

METHODS

Three ponds have been studied; two of them designated pond #1 and pond #3 have surface areas of 51 m^2 and 60 m^2 and maximum depths of 1.3 m and 0.7 m, respectively. A third pond, pond #A, and some distance from the other two, has a surface area of approximately 192 m^2 and a fairly uniform depth of about 1 m. The position of these ponds in relation to the UWM Field Station and Cedarburg Bog is shown in Figure 1.

Surface microlayer samples were collected using sterile/acid-washed glass plate (GP) samplers (Harvey and Burzell 1972) (sample thickness 50 $\pm$ 20 um) and a sterile/acid-washed stainless steel screen (SC) sampler (Garrett 1965; Sieburth 1965) (sample thickness 320 $\pm$ 55 um).

Water samples (both surface microlayer and subsurface) were collected every 2-3 weeks for seasonal studies, and on occasion, every 4-6 hours to examine diel fluctuations. Samples were subdivided and analyses were made for nutrient chemistry (Danos 1980; Danos et al. 1983), pigments, bacteria, fungi (Maki 1982; Maki and Remsen 1983) and algae (Estep 1982). The usual limnological data were also collected (temperature, oxygen, pH, etc.) as well as local climatological data. Enrichment factors (E_f) were calculated for nutrients, pigments, algae and bacteria using the equation of Piotrowicz et al. (1972):

$$E_f = \frac{\text{Conc. (x) surface microlayer}}{\text{Conc. (x) subsurface}} - 1$$

E_f values greater than zero indicate enrichment within the microlayer fraction, as compared to subsurface waters, while values less than zero indicate a lack of microlayer enrichment.

Association among parameters was compared seasonally using non-parametric rank correlation analysis. All correlations are Spearman rank and reported as r values (Zar 1974).

RESULTS

Seasonal variation

Seasonal integrated values for various parameters are from experimental pond #1 from June 1978 through August 1979. Data included here are chlorophyll *a* and phaeophytin *a* (Figure 2), total phosphorous and total organic nitrogen (Figure 3), and integrated bacterial population estimates from both direct counts and colony-forming units (CFU) on agar (Figure 4).

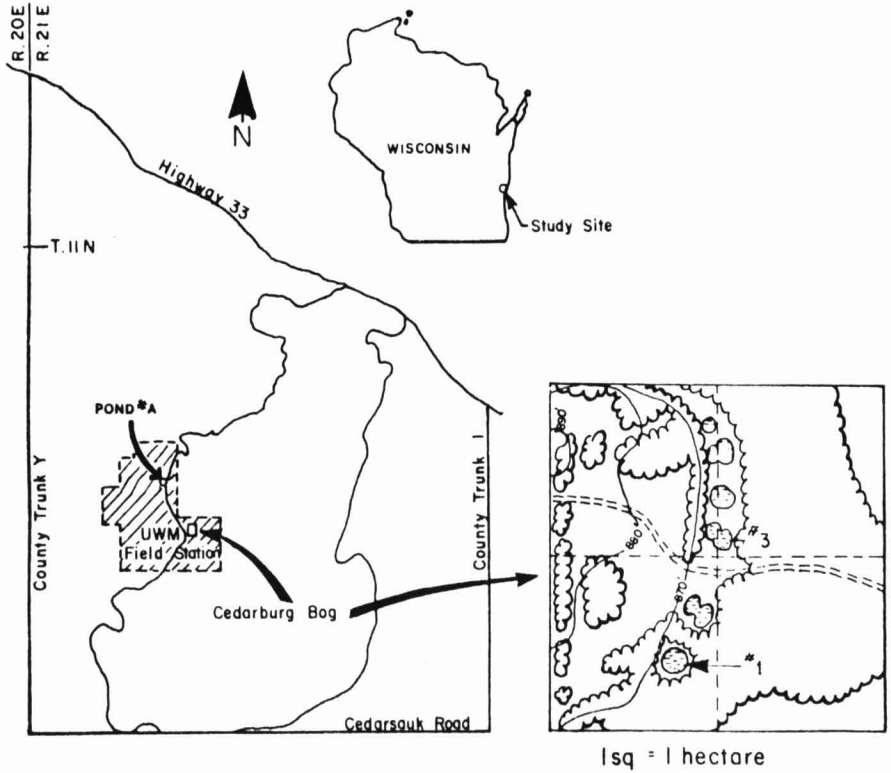

Figure 1. The locations of the experimental ponds.

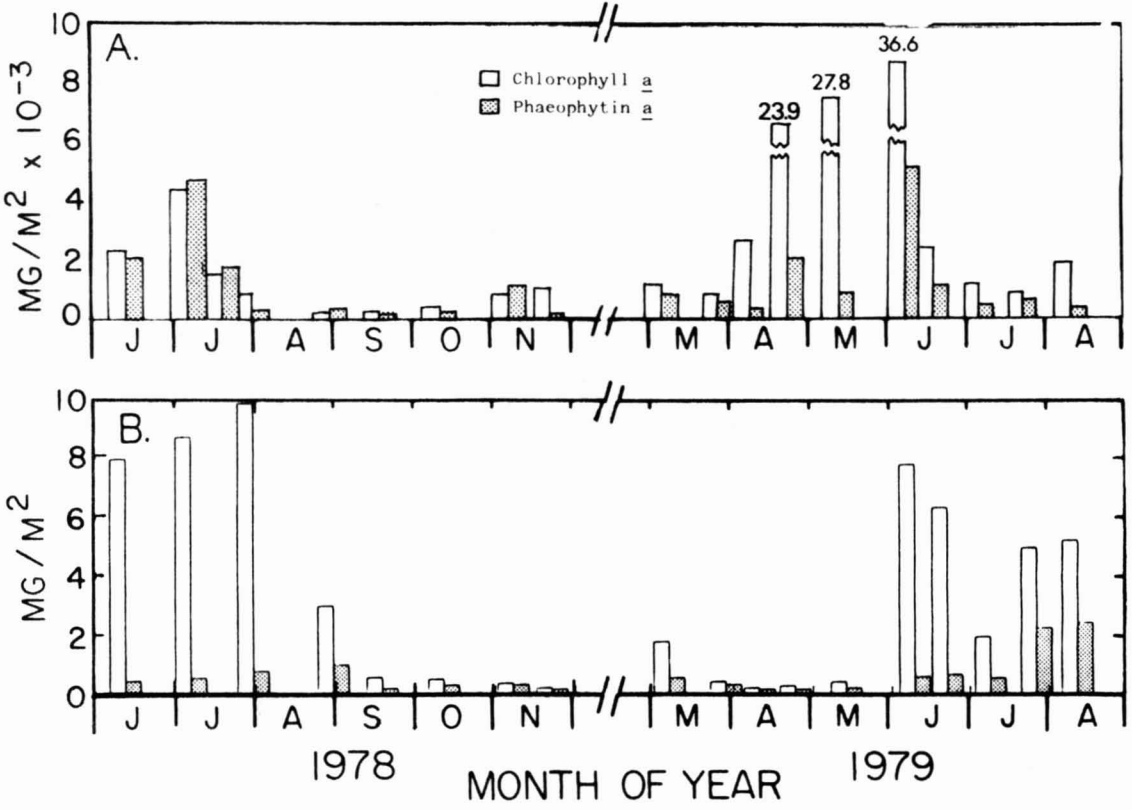

Figure 2. Integrated values of chlorophyll a and phaeophytin a for Pond #1. (A) Microlayer integration from 0 to 320 um. (B) Subsurface integration from 5 to 50 cm.

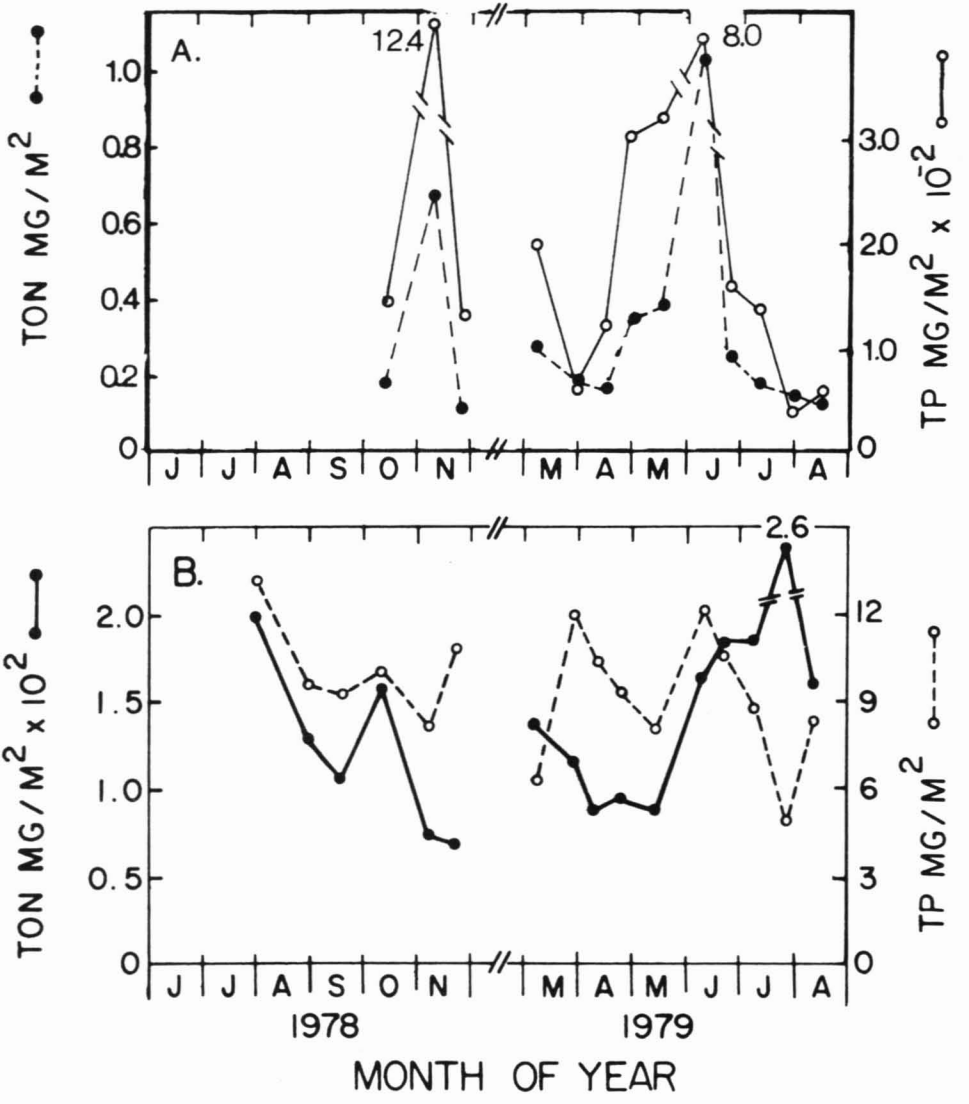

Figure 3. Pond #1 integrated values for total organic nitrogen (TON, p) and for total phosphorous (TP, 0). (A) Microlayer integration from 0 to 320 um. (B) Subsurface integration from 5 to 320 um.

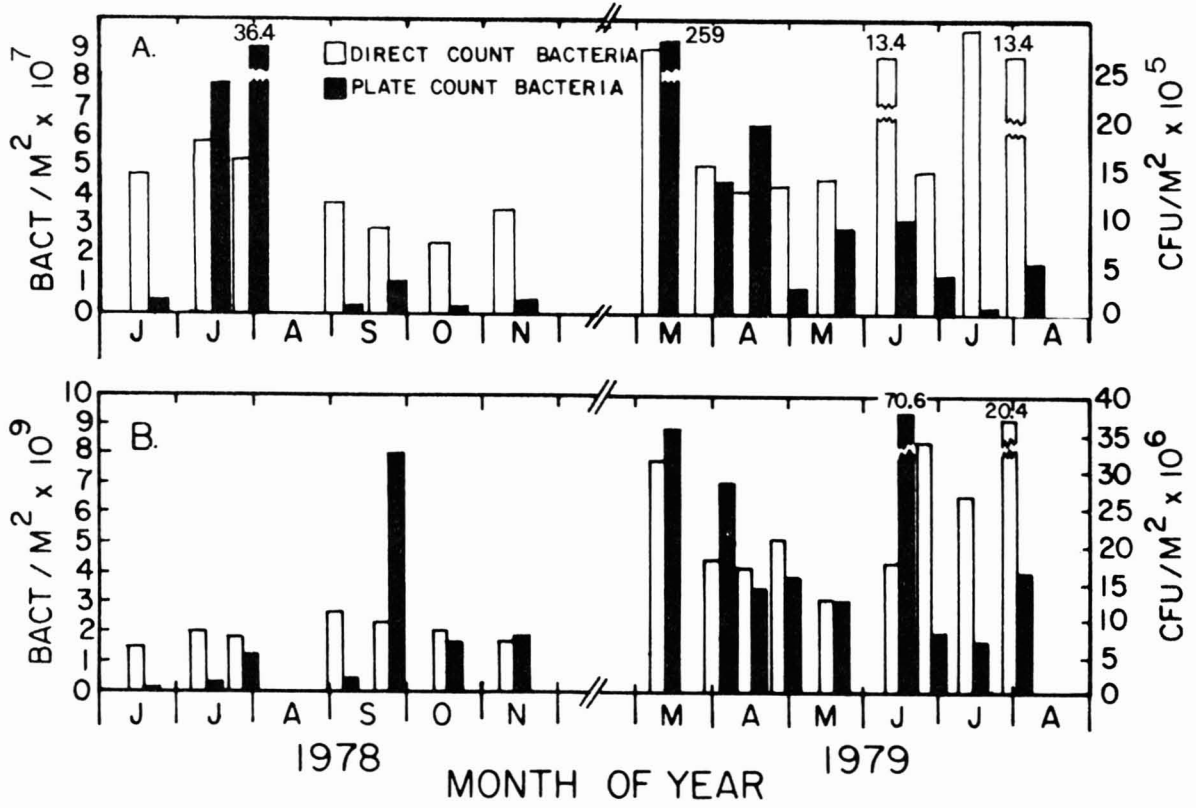

Figure 4. Pond #1 integrated values for direct count bacteria and plate count bacteria (CFU). (A) Microlayer integration from 0 to 320 um. (B) Subsurface integration from 5 to 50 cm.

The seasonal changes in the number of fungal CFU in the surface microlayer of experimental pond #3 are presented in Figure 5. Fungal CFU exhibited a peak in numbers in early-to-mid-summer.

Algal succession and seasonal changes in experimental pond #A during 1981 are clearly demonstrated by a diagram of the dominant and sub-dominant species. Figure 6 is a plot of the cube root of the relative abundance of these species for the season. The succession of flagellate (Rhodomonas, Cryptomonas and Chlamydomonas) and diatom (Synedra and Fragilaria sp.) species can be seen along with their relationship to water temperature. Diatoms and flagellates underwent succession somewhat independently of one another, populations of the former being more stable and long lasting.

Microlayer enrichment

Table 1 presents calculated mean enrichment factors (E_f) for nutrients, chlorophyll a, and bacteria within the surface microlayer of experimental pond #1 and #A. With the exception of dissolved reactive silicate, all parameters sampled showed enrichment in the surface microlayer relative to subsurface waters. Heterotrophic plate count bacteria (CFU) showed the highest microlayer enrichment with an E_f of 28.5. Other organic parameters (chlorophyll a, phaeophytin a, total organic nitrogen, and total phosphorous) also showed high enrichment factors. Highest inorganic enrichment was observed for ammonia-N and dissolved reactive phosphorous, and in Pond #1, less enrichment for nitrite-N and nitrate-N.

Surface microlayer sample enrichment (E_f) and subsurface quantities of fungal CFU in ponds #1 and #3 during 1979 are presented in Tables 2 and 3. Fungal CFU were always enriched in the surface microlayers of both ponds irrespective of the microlayer sampler used, when compared to subsurface CFU.

In a separate study, enrichment factors were also determined for various algal groups from experimental pond #A in 1981 (Table 4). Enrichment factors calculated for all flagellated species were usually quite low, but were quite high during the first two weeks in June 1979. Diatoms, on the other hand, showed consistently high enrichment factors, indicating that the diatoms were almost always enriched in the surface microlayer.

Statistical results

Spearman rank correlations for the integrated microlayer data of experimental pond #1 during the sampling period are presented in Table 5. The most significant positive correlations ($p < 0.01$) are chlorophyll a and phaeophytin a ($r = 0.70$, $p < 0.01$), soluble inorganic nitrogen (SIN) and NO_3 ($r = 0.90$, $p < 0.01$), and total phosphorous and total organic nitrogen ($r = 0.85$, $p < 0.01$).

DISCUSSION

Seasonal fluctuations

Distinct temporal heterogeneity was observed in the microlayer and subsurface waters of experimental pond #1. The seasonal fluctuations of microlayer phototrophs

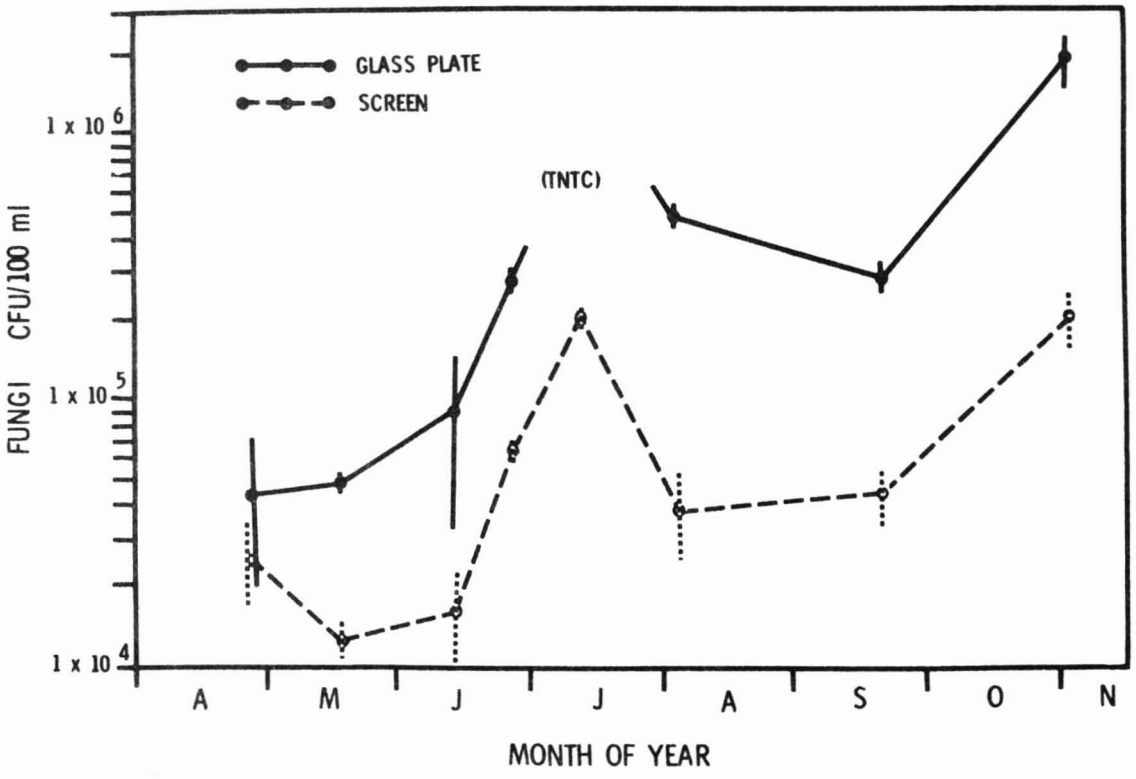

Figure 5. Fungi colony forming units (CFU) surface microlayer samples from pond #3 during 1979. Bars denote one standard deviation. TNTC = too numerous to count.

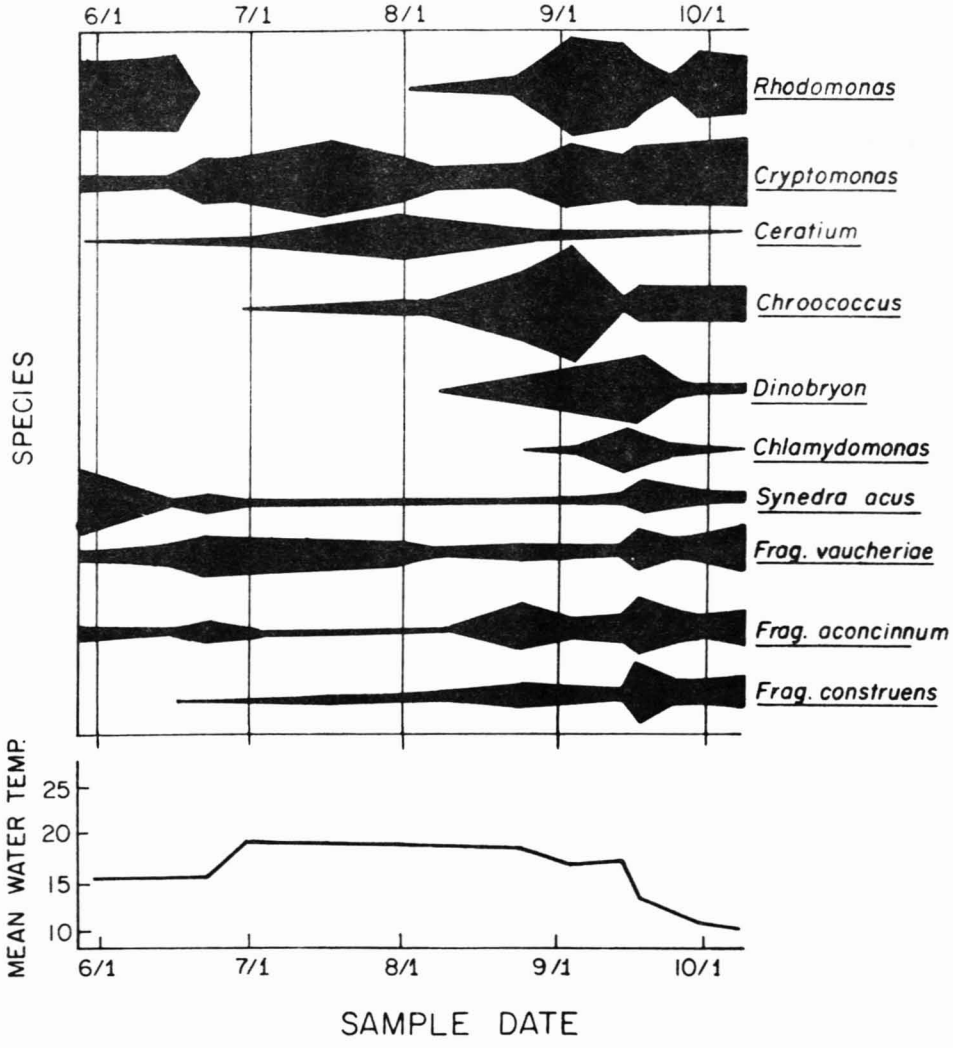

Figure 6. Algal succession and seasonal changes of algal species in pond #A as related to water temperature.

Table 1. Mean enrichment factor (E_f) for nutrients, chlorophyll a, and bacteria in the surface microlayer of experimental ponds #1 and #A.

	E_f		Range	
Parameter	Pond #1	Pond #A	Pond #1	Pond #A
NH_3-N	3.34	1.25	-0.73 to 85.1	0.16 to 3.1
NO_3-N	0.51	2.03	-0.93 to 9.09	-0.43 to 3.06
NO_2-N	1.18	9.15	-0.66 to 8.81	0.13 to 22.86
DRP	2.70	2.32	-0.62 to 17.2	1.63 to 4.04
SiO_2	-0.01	NA	-0.34 to 1.55	NA
TON	5.43	NA	-0.43 to 47.8	NA
TP	7.72	NA	-0.61 to 86.1	NA
Chlorophyll a	16.3	0.54	-0.94 to 355	-0.83 to 3.49
Phaeophytin a	6.30	2.35	-0.96 to 76.1	-0.39 to 6.91
Direct Count Bacteria	1.03	NA	-0.48 to 17.8	NA
Plate Count Bacteria	28.5	NA	-0.68 to 206	NA

NA = Data not available
DRP = Dissolved reactive phosphate
TON = Total organic nitrogen
TP = Total phosphorous

Table 2. Average enrichment (E_f) of fungi CFU in glass plate (GP) and screen (SC) samples, and subsurface fungi CFU (with ± standard deviation) x 10^3/100 ml from pond #1 during 1979.

			DEPTH (cm)				
Date	GP E_f	SC E_f	5	10	20	50	100
3/31/79	35.93	17.46	1.30 (0.28)	6.00 (0.14)	0.60 (0.02)	7.50 (0.70)	ND
4/13/79	91.89	52.32	0.70 (0.42)	1.45 (0.77)	0.90 (0.14)	1.35 (0.19)	1.30 (0.27)
4/27/79	104.26	7.96	1.80 (0.27)	4.45 (0.31)	1.50 (0.08)	0.06 (0.05)	1.10 (0.28)
5/17/79	421.03	21.21	0.70 (0.24)	0.10 (0.03)	0.60 (0.06)	1.05 (0.13)	0.55 (0.06)
6/13/79	744.39	19.39	0.70 (0.40)	3.70 (0.24)	1.20 (0.28)	8.75 (0.64)	1.15 (0.12)
6/26/79	74.08	56.06	1.90 (0.56)	0.50 (0.14)	0.30 (0.14)	0.40 (0.14)	0.20 (0.14)
7/12/79	105.18	148.61	0.40 (0.28)	3.35 (0.49)	0.75 (0.35)	0.10 (0.08)	0.10 (0.05)
8/3/79	32.98	51.22	2.90 (0.15)	1.45 (0.77)	0.70 (0.28)	0.20 (0.14)	1.75 (0.18)
9/20/79	41.94	31.67	2.10 (0.13)	0.70 (0.42)	0.50 (0.14)	1.00 (0.42)	1.10 (0.14)
11/1/79	20.87	10.27	42.00 (16.97)	40.00 (42.14)	21.00 (5.65)	4.80 (0.28)	2.70 (0.98)

ND = No Data.

Table 3. Average enrichment (E_f) of fungi CFU in glass plate (GP) and screen (SC) samples, and subsurface fungi CFU (with ± standard deviation) x 10^3/100 ml from pond #3 during 1979.

			DEPTH (cm)			
Date	GP E_f	SC E_f	5	10	20	50
4/27/79	10.12	5.16	3.10 (1.55)	3.25 (1.76)	7.75 (1.61)	4.40 (0.56)
5/17/79	125.50	31.38	0.10 (0.04)	0.00 ---	3.50 (0.71)	0.00 ---
6/13/79	163.98	28.72	0.80 (0.28)	0.60 (0.14)	0.30 (0.14)	0.90 (0.42)
6/26/79	135.19	31.79	6.15 (1.20)	0.75 (0.35)	1.10 (0.28)	3.90 (0.27)
7/12/79	ND	71.40	9.50 (0.71)	2.25 (0.18)	1.05 (0.07)	4.55 (0.63)
8/3/79	59.26	3.69	2.90 (1.27)	6.30 (0.42)	40.45 (2.19)	19.85 (4.45)
9/20/79	17.91	1.90	13.25 (1.06)	15.20 (0.28)	12.50 (2.12)	19.00 (1.41)
11/1/79	64.90	5.94	34.00 (5.65)	ND	37.00 (4.24)	21.00 (1.41)

ND = No Data.

Table 4. Seasonal enrichment factors for all algal species comparing the surface microlayer with 20 cm in Pond #A.

Date	All spp.	Flagellates	Diatoms	All others
5/31/79	3.16	6.52	0.35	0.31
6/14/79	1.38	1.39	3.96	-0.12
6/22/79	-0.84	-0.96	9.35	2.46
6/30/79	0.41	0.39	2.59	0.39
7/16/79	-0.91	-0.93	3.89	0.25
7/31/79	-0.82	-0.88	2.27	0.01
8/6/79	-0.84	-0.92	13.34	4.07
8/26/79	-0.89	-0.98	1.61	-0.94
9/3/79	-0.77	-0.93	2.59	-0.68
9/14/79	-0.14	-0.15	0.42	-0.36
9/17/79	-0.37	-0.56	4.34	-0.17
9/23/79	-0.65	-0.68	-0.83	0.08
9/29/79	-0.37	-0.42	3.26	0.33
10/8/79	0.11	0.07	6.81	-0.38
Mean	-0.11	0.07	3.85	0.39

Table 5. Spearman rank correlations for pond #1 microlayer integrated data from June 1978 through August 1979.

	NO_3	NO_2	SIN	DRP	SIL	TON	TP	CHL	PHA	CFU	BDC
NH_3	-0.14	0.15	0.20	0.01	0.17	-0.23	-0.61	-0.43	0.07	-0.36	-0.25
NO_3		-0.14	0.90**	0.28	-0.01	0.07	0.32	-0.23	-0.32	-0.27	-0.37
NO_2			-0.12	0.07	-0.12	0.03	0.01	0.14	-0.04	0.20	-0.10
SIN				0.24	0.15	-0.05	0.21	-0.38	-0.35	-0.43	-0.41
DRP					-0.04	0.48	0.55	-0.04	-0.09	-0.23	-0.18
SIL						-0.64**	-0.43	-0.66**	-0.62**	-0.25	-0.45
TON							-0.85**	-0.73*	0.71*	0.36	ND
TP								0.49	0.62*	0.17	ND
CHL									0.70**	0.44	0.55*
PHA										0.28	0.37
CFU											0.48*

*Significant at p 0.05.
**Significant at p 0.01.
ND = No Data.
SIN = Soluble inorganic nitrogen.
DRP = Dissolved reactive phosphorous.
SIL = Silica.
TON = Total organic nitrogen.
TP = Total phosphorous.
CHL = Chlorophyll a.
PHA = Phaeophytin a.
CFU = Colony forming unit.
BDC = Bacterial direct count.

in this study were similar to those observed by Gallagher (1975) and Manzi et al. (1977). Low concentrations of chlorophyll a were observed in the microlayer in early spring. Chlorophyll a rose to maximum levels in early summer along with bacterial populations. These decreased in late summer and into the fall. Our data support the findings of Gallagher (1975) who also observed a strongly phototrophic neustonic community in the spring and early summer thriving on the dissolved nutrients enriched within the microlayer. However, during summer, bacterioneuston populations peaked and the phytoneuston community increased in phaeophytin, suggesting increased heterotrophic mineralization of the dying algal mat by midsummer.

The seasonal changes in fungal CFU in the surface microlayers of both ponds #1 and #3 revealed two main features: 1) an early-to-mid-summer peak and 2) an increase in late fall. Willoughby and Collins (1966) reported large numbers of fungi in the water column of Blelham Tarn after the spring thaw, but this was not observed in surface microlayer samples from either pond, although on the first sampling date for each pond the subsurface numbers of fungal CFU appeared to be much higher at some depths than on the next date. It is probable that the first sampling date was not close enough to the final thaw in either pond to make an increase more apparent.

The majority of algal seasonal biomass in experimental pond #A was composed of flagellated species. Dominant species rapidly succeeded one another, often resulting in a change in the dominant species from one sample date to another. This pattern of rapid succession is to be expected in shallow, eutrophic systems with rapidly fluctuating environmental conditions.

Subdominance was equally divided between flagellate and diatom species. Diatoms were most abundant in the fall when water temperatures began to drop, a phenomenon observed in many freshwater systems (Hutchinson 1967). Diatom populations showed greater stability than the flagellates, as evidenced by the less rapid succession and longer persistence of individual species. For example, during the period from 5/31/81 to 9/14/81, diatoms exhibited a succession of Synedra acus to Fragilaria vaucheriae to F. aconcinnum (Estep and Remsen 1983) at a time when six flagellates replaced one another in rapid succession.

Surface microlayer enrichment

In a small freshwater pond the surface microlayer differs substantially in its biological and chemical composition from subsurface waters. With the exception of dissolved reactive silicate, all chemical parameters were enriched in the surface microlayer of our experimental ponds. Liss (1975) speculated that dissolved silicon, unlike dissolved and particulate organic matter, is not transported to the surface microlayer by rising bubbles. In addition, the strong negative correlations between dissolved silicate and chlorophyll a within the microlayer suggests the possibility of active uptake of silicate during periods of phototrophic

growth (e.g. flagellates and diatoms). Previous studies of marine and freshwater environments (Goering and Menzel 1965; Williams 1967; Nishizawa 1971; Hatcher and Parker 1974a, b) have not reported concurrent enrichment of all three dissolved inorganic nitrogen species and dissolved reactive phosphorous within the microlayer, as was observed in our studies and by Saijo et al. (1974). This may have been a result of infrequent sampling in previous studies.

The presence of large numbers of fungi in the surface microlayer could be important in the cycling of nutrients and dissolved organic carbon (DOC) in this zone, particularly since proteins (Baier 1970), lipids (Larsson et al. 1974) and carbohydrates (Seiburth et al. 1976) have been found to be important organic components of surface films. Previous investigators have reported fungi in concentrations from 102 to 105 ml^{-1} in both marine and freshwater surface microlayers (Hatcher and Parker 1974a; Crow et al. 1975; Ahearn et al. 1977; Dutka and Kwan 1978; Kjelleberg et al. 1979). The number of fungal CFU that we found in surface microlayer samples of ponds #1 and #3 generally fall within the above range. The average enrichment factor for fungi CFU was always positive, indicating that the highest populations were in the surface microlayer of the ponds.

The high numbers of microorganisms in the microlayer may be concentrated there by mechanisms similar to those which concentrate organic material (Liss 1975). Bacterial concentration by adsorption to rising bubbles is well documented (Bezdek and Carlucci 1974; Blanchard and Syzdek 1978). Furthermore, Tsyban and Polishchuck (1969), Hatcher and Parker (1974a, b) and Lion et al. (1979) postulated that microorganisms may associate with organic detritus rising to the water's surface.

Significant and striking differences occurred between surface microlayer and subsurface algal population abundances on a majority of sample dates in pond #A. Surface microlayer algal enrichment was a transient phenomenon; on some days the surface microlayer abundance was higher than the subsurface, while on other days the opposite was true. This variation in enrichment was similar to the results of Parker and Hatcher (1974a) in a study of three freshwater environments in Virginia. The variation in stratification pattern and the lack of species unique to either the microlayer or the subsurface waters suggests that the surface microlayer assemblage was the result of a constant transfer of algae between the microlayer and the underlying waters and the benthos, rather than an independently reproducing, wholly isolated community. However, the data suggest that algal species can be divided into four functional groups based on their relationship to the surface microlayer:

Group 1. Flagellates that were always in low abundance in the microlayer relative to subsurface waters. This group was represented by Dinobryon and Ceratium.

Group 2. Flagellates that were sometimes enriched and sometimes scarce in the surface microlayer. This group was composed of Chlamydomonas, Cryptomonas and Rhodomonoas.

Group 3. Non-motile algae, excluding diatoms, which exhibited a neutral reaction to the surface microlayer.

Group 4. The diatoms, which showed a specific affinity for the surface microlayer.

In conclusion, our study of small freshwater ponds indicates that the surface microlayer differs substantially in its biological and chemical composition with respect to subsurface waters. Living and dead biogenic materials, as well as dissolved nutrients (excluding silicates) are highly enriched in the surface microlayer and show definite seasonal fluctuations. Chlorophyll a and bacterial populations show a unimodal annual cycle with maximum concentrations observed in the early summer. Dissolved nutrients, on the other hand, show a bimodal cycle with maximum values observed in the spring and fall. Physical mechanisms such as antirain (organic debris filled with gas rising from the sediments) and adsubble processes (rising gas bubbles) appear to play major roles in the enrichment of the surface microlayer. However, in the spring and early summer, biological processes may be more important and in situ growth of the phytoneuston and bacterioneuston occurs. Once in situ growth occurs, the biological community structure itself has profound effects on the enrichment or depletion of microlayer materials. Phototrophic dominance may cause nutrient depletion, while heterotrophic dominance may cause nutrient enrichment.

Financial and technical support for this research was provided in part by The Center for Great Lakes Studies, The University of Wisconsin-Milwaukee, and in part by a grant from The Joyce Foundation. The technical assistance of R. Ristic, D. Welch and J. Flores is greatly appreciated.

LITERATURE CITED

Ahearn, D. G., S. A. Crow, and W. L. Cook. 1977. Microbial interactions with pesticides in estuarine surface slicks. U. S. Environmental Protection Agency, Gulf Breeze, Florida. EPA-R-8033141.

Baier, R. E. 1970. Surface quality assessment of natural bodies of water. Proc. 13th Conf. Great Lakes Res. 1970: 114-127.

Bezdek, H. H., and A. F. Carlucci. 1974. Surface concentration of marine bacteria. Limnol. Oceanogr. 17: 566-569.

Blanchard, D. C., and B. C. Parker. 1977. The freshwater to air transfer of microorganisms and organic matter. In Aquatic microbial communities. J. Cairns (ed.), Garland Pub. Co. New York. p. 625-658.

Crow, S. A., D. G. Ahearn, W. L. Cook, and A. W. Bourquin. 1975. Densities of bacteria and fungi in coastal surface films as determined by a membrane-adsorption procedure. Limnol. Oceanogr. 20: 644-646.

Danos, S. C., J. S. Maki, and C. C. Remsen. 1983. Stratification of microorganisms and nutrients in the surface microlayer of small freshwater ponds. Hydrobiologia 98: 193-202.

Dutka, B. J., and K. K. Kwan. 1978. Health-indicator bacteria in water-surface microlayers. Can. J. Microbiol. 24: 187-188.

Estep, K. W. 1982. Interactions between algal populations and the surface microlayer in a small Wisconsin pond. M. S. Thesis, University of Wisconsin-Milwaukee.

Estep, K. W., and C. C. Remsen. 1983. A new species of Fragilaria from the midwestern United States. Nova Hedwigia 37: (in press).

Gallagher, J. L. 1975. The significance of the surface film in salt marsh plankton metabolism. Limnol. Oceanogr. 20: 120-123.

Garrett, W. D. 1965. Collection of slick-forming materials from the sea surface. Limnol. Oceanogr. 10: 602-605.

Goering, J. J., and D. W. Menzel. 1965. The nutrient chemistry of the sea surface. Deep Sea Res. 12: 839-843.

Hatcher, R. F. and B. C. Parker. 1974a. Investigations of freshwater surface microlayers. Virginia Polytechnic Institute and State University, Water Resources Center, Bull. 64, 84 p.

Hatcher, R. F., and B. C. Parker. 1974b. Microbiological and chemical enrichment of freshwater-surface microlayers relative to the bulk-subsurface water. Can. J. Microbiol. 20: 1051-1057.

Harvey, G. W., and L. A. Burzell. 1972. A simple microlayer method for small samples. Limnol. Oceanogr. 17: 156-157.

Hutchinson, G. E. 1967. A Treatise on Limnology, Vol. 2, J. Wiley and Sons, Somerset, N.J., 1115 p.

Kjelleberg, S., T. A. Stenstrom, and G. Odham. 1979. Comparative study of different hydrophobic devices for sampling lipid surface films and adherent microorganisms. Mar. Biol. 53: 21-25.

Larsson, K., G. Odham, and A. Sodergren. 1974. On lipid surface films on the sea. I. A simple method for sampling and studies of composition. Mar. Chem. 2: 49-57.

Lion, L. W., R. W. Harvey, L. Y. Young, and J. O. Lackie. 1979. Particulate matter: its association with microorganisms and trace metals in an estuarine salt marsh microlayer. Environ. Sci. Technol. 13: 1522-1525.

Liss, P. S. 1975. Chemistry of the sea surface microlayer. In Chemical Oceanography. 2nd ed. Vol. 2. Edited by J. P. Riley and G. Skirrow. Academic Press, New York. 193-243.

Maki, J. S. 1982. Investigations of microbial populations and their activity in freshwater surface microlayers. Ph.D. Dissertation, University of Wisconsin-Milwaukee.

Maki, J. S. and C. C. Remsen. 1983. A membrane adsorption-SEM technique for observing neuston organisms. Microb. Ecol. (in press).

Manzi, J. J., P. E. Stofan, and J. L. Dupuy. 1977. Spatial heterogeneity of phytoplankton populations in estuarine surface microlayers. Mar. Biol. 41: 29-38.

Nishizawa, S. 1971. Concentration of organic and inorganic materials in the surface skin at the equator, 155° W. Bull. Plank. Soc. Japan 18: 42-44.

Piotrowicz, S. R., B. J. Ray, G. L. Hoffman, and R. A. Duce. 1972. Trace metal enrichment in the sea-surface microlayer. J. Geophys. Res. 77: 5243-5254.

Saijo, Y., O. Mitramura, and K. Ogiyama. 1974. Chemical composition of surface film in the coastal sea area and in freshwater lakes. Jap. J. Limnol. 35: 110-116.

Sieburth, J. McN. 1965. Bacteriological samplers for air-water and water-sediment interfaces. Ocean Sci. Ocean Eng., Trans. Jt. Conf. Mar. Tech. Soc. and ASLO, Washington, D. C., 1064-1068.

Sieburth, J. McN., P. J. Williams, K. M. Johnson, C. M. Burney, D. M. Lavoie, K. R. Hinga, D. A. Carson, F. W. French, P. W. Johnson, and P. G. Davis. 1976. Dissolved organic matter and heterotrophic microneuston in the surface microlayer of the North Atlantic. Science 194: 1415-1418.

Tsyban, A. V., and L. N. Polishchuck. 1969. The interrelationship of bacterioneuston and zooneuston. Hydrobiol. J. 5: 37-44.

Willoughby, L. G., and V. G. Collins. 1966. A study of the distribution of fungal spores and bacteria in Blelham Tarn and its associated streams. Nova Hedwigia 12: 150-171.

Williams, P. M. 1967. Sea surface chemistry: organic and inorganic nitrogen and phosphorous in surface films and subsurface waters. Deep Sea Res. 14: 791-800.

Zar, J. H. 1974. Biostatistical analysis. Prentice-Hall Inc., Englewood Cliffs, N. J.