

**The microbial community structure and nitrogen dynamics  
in a Lake Erie coastal wetland, Old Woman Creek.**

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April 22, 1998

## **Introduction**

Because large areas of the drainage basins emptying into Lake Erie are devoted to agricultural land use, non-point source inputs may have the most significant influence in providing nutrients to Lake Erie (Chapra and Robertson 1977). Such inputs predominate as surface water flows from agricultural fields into streams and rivers, eventually entering the lake. Nutrient-enriched and polluted water often first passes through coastal wetlands, which serve, therefore, as a “metabolic gate” of the limnetic ecosystems (Wetzel 1992). Although often perceived as aiding water quality by ameliorating the availability of nutrients to receiving communities (Klarer and Millie 1989), the factors involved in affecting the quality of surface water entering these coastal wetlands are not well understood.

The current paradigm in aquatic ecology places much significance on the microbial food web (Sherr et al. 1988). This consortium of bacteria, phytoplankton, and protists forms dynamic relationships with a variety of aquatic organisms, plays the leading role in consuming and regenerating inorganic nutrients (Legendre and Rassoulzadegan 1995), and may control the major biogeochemical cycles, such as nitrification (Lavrentyev et al. 1997). Microbial processes may be paramount in controlling nutrient fluxes in freshwater wetlands (Johnston 1991, Tomaszek et al. 1997). Yet relatively little work has been done in this direction. The objectives of this pilot study in a coastal wetland of Lake Erie were to explore these issues by (1) examining microbial food web structure in the water column at selected sites, and (2) determining concentrations and transformation rates of ammonium-nitrogen at these sites.

## **The study site**

Old Woman Creek (OWC) is a freshwater coastal wetland located on the south shore of Lake Erie near Huron, OH. As one of the few remaining undeveloped wetland systems in this area, OWC was set aside as State Nature Preserve and National Estuarine Research Reserve. The wetland proper is ca. 54 ha and extends about 2 km south of Lake Erie. Its predominantly agricultural watershed encompasses an area of 69.2 km<sup>2</sup>. Wetland water depth is generally less than 0.5 m, but it can exceed 1 m during periods of elevated Lake Erie water levels. OWC is characterized by a pulse-flow hydrologic regime. In the summer, intrusion of Lake Erie water and water flow through the wetland may be blocked by a barrier sand beach. Severe storms occasionally break the barrier and flow through the wetland proceeds 1 to 2 orders of magnitude more rapidly, sometimes removing much of the accumulated sediments to Lake Erie. OWC is a storm-driven ecosystem as is the case for many other Great Lakes coastal wetlands. The composition of its biotic communities is strongly influenced by fluctuating light conditions in the water column resulting from influx of particulate matter and re-suspension of bottom sediments as well as by nutrient inputs associated with surface runoff and sub-surface interflow (Klarer and Millie 1994).

## **Material and Methods**

*Sampling sites* were selected to represent different habitats within OWC. Two sites were located along the flow axis -- one in a drowned river mouth of the wetland (Mouth) and another one in the upstream portion of the wetland, near the South Rail Road (SSR). These sites were sampled on June 5, 1997 and August 27, 1997, respectively. An additional site in the mid section of OWC near a deck (Deck) represented a macrophyte-dominated habitat off the flow

axis. This site was sampled on both occasions. Samples were collected from the surface layer by forcing a clean polycarbonate carboy underwater with the mouth pointing downward, leaving the spigot at the base of the carboy open for air release. Dissolved oxygen and water temperature were measured using a YSI 50B DO-meter. Light intensity measurements were done with a LiCor LI-1000 Photometer equipped with underwater light sensors and a data logger. Subsamples for chemical and microscopic analyses were simultaneously taken from the carboys, immediately preserved as described below, and then transported to the laboratory in insulated coolers.

***Experimental manipulations:*** In N dynamics experiments, potential uptake rates and regeneration rates were examined by spiking and incubating water samples with 10  $\mu\text{M}$  (final concentration) of  $^{15}\text{NH}_4^+$  and sampling them intervals to measure changes in the concentrations and isotope ratios (atom %  $^{15}\text{N}$ ) over time. Sixty-ml plastic syringes served as incubation vessels. The syringes were covered with one layer of black nylon window screen and duct tape for light and dark treatments, respectively. The light treatment blocked about 53% of the incident light (Gardner et al. In press). Incubation intervals were at 0.33, 3, and 8h, and 0.33, 2, 6, and 21 h in June and August, respectively. Samples for determination of concentrations and isotope ratios of ammonium were collected by attaching a syringe filter (Rainin® Nylon-66 0.2  $\mu\text{m}$  pore-size 25 mm diameter) to an incubation syringe and pushing ca 10 ml of sample through the filter. The first 3-ml of sample rinsed the filter and the filtrate was discarded; the remaining 7-ml of filtrate was collected in a clean glass vial (Wheaton® #224884) and immediately frozen for subsequent analysis.

***Chemical analyses:*** Ambient concentrations of ammonium were measured using a UNICAM UV-2 uv-vis spectrum fluorometer (Zadorojny et al. 1973). High performance liquid

chromatography (HPLC) was used to analyze concentration and atom %  $^{15}\text{N}$  of the ammonium in N dynamic experiments (Gardner et al. 1995). With this technique, concentration of ammonium is determined by peak area and atom %  $^{15}\text{N}$  is determined by the shift in retention time of the ammonium peak caused by the presence of  $^{15}\text{NH}_4^+$ . Both measurements were quantified by comparison to results from an internal standard of ammonium injected 7.0 min before the sample was injected. Potential uptake and regeneration rates were calculated from the linear regressions calculated using these measurements and the Blackburn/Caperon model (Blackburn 1979; Caperon et al. 1979).

**Microscopic analyses:** Phytoplankton was preserved with Lugol's iodine (Prescott 1979), concentrated from 1 l to 10 ml through several sedimentation steps, and counted in 0.4 ml aliquots under a Zeiss 16 microscope. The number of fields depended upon density of algal populations. In addition, live samples were examined to aid identification of flagellate species. Bacteria, preserved with 1% (final concentration) formaldehyde and stained with DAPI (Porter and Feig 1980), were counted on 0.2- $\mu\text{m}$  pore size black polycarbonate membrane filters under a Zeiss epifluorescence microscope. Nanoplankton preserved with 1% (final concentration) formaldehyde were counted using the dual-staining (DAPI/FITC) protocol (Sherr et al. 1993) on 0.8- $\mu\text{m}$  black filters. The presence or absence of chlorophyll autofluorescence distinguished photosynthetic and heterotrophic flagellates from each other. All preparations for epifluorescence microscopy were made shortly after sampling and stored frozen at  $-20^\circ\text{C}$ . Ciliates and other microzooplankton were counted in 10 to 50 ml settling chambers using a Wild phase contrast inverted microscope (magnification 125 – 625 x) after fixation of whole water samples with 1% (final concentration) freshly prepared acid Lugol's iodine. Phytoplankton and protists were identified to the species level whenever possible. Biomass of phytoplankton and protozoa was estimated from linear dimensions of individual cells by

approximating geometric solids and converting their biovolume to carbon according to Montagnes et al. (1994) and Putt and Stoecker (1989). Bacterial biomass was determined with allometric equations (Norland 1993) and then an average was calculated for each size category.

## Results and Discussion

Severe storms had preceded the experiments and as a result the wetland was open to Lake Erie on both sampling occasions. This hydrologic situation corresponded to high levels of turbidity (> 800 NTU) and ammonium (20  $\mu\text{M}$ ). Light intensity measured in the surface water varied between 200 and > 1000  $\mu\text{M s}^{-1} \text{m}^{-2}$ , and reflected light intensity was between 8.6 and 45.8  $\mu\text{M s}^{-1} \text{m}^{-2}$ . Water temperature varied from 15.8 to 20.4°C and from 19.1 to 25.9 °C during the experimental incubations in June and August, respectively. Dissolved oxygen concentrations were ca. 6 and 8  $\text{mg l}^{-1}$  on the first and second sampling dates, respectively.

Diverse assemblages of phytoplankton (Table 1) and protists (Table 2) were found in the OWC water. However, a few abundant species formed the bulk of the community biomass (Table 3). The flagellate *Cryptomonas erosa* predominated in the phytoplankton community in all samples, with the exception of the Deck site in August, where another flagellate, *Lepocinclis ovum* formed more than 30% of the phytoplankton biomass. Diatoms, mainly representing the genus *Aulocoseira*, were co-dominant, especially at the Mouth site open to Lake Erie. Small aplastidic chrysophytes were the most abundant component of heterotrophic nanoplankton in all three locations. Among microzooplankton, the choreotrichs ciliates *Rimostrombidium lacustris*, *R. humile*, and *Strobilidium* sp. formed the essential proportion of the total biomass. The Mouth site was an exception with the oligotrichs *Limnostrombidium* sp. and *Pelagostrombidium fallax*, and the peritrich *Vorticella*

*campanula* being predominant. The composition of the planktonic community reflects good water quality of OWC despite a considerable anthropogenic pressure on its ecosystem.

The absolute biomass of phytoplankton and other microorganisms was higher in August than in June, and at the sites located along the flow axes (Mouth and SSR) than at the Deck site (Table 4). The spatial difference was strongly expressed in the phytoplankton biomass. The seasonal trend was more profound in the ciliate biomass, which even exceeded that of phytoplankton at the DECK site in August. This findings suggest that a large proportion of phytoplankton production, predominated by nanoplankton-sized flagellates, may pass through ciliates in OWC. The heterotrophic-to-photosynthetic biomass ratio and bacterial-to-phytoplankton biomass ratio were higher in the latter site than in the flow axis on both sampling occasions. The consistency of this pattern is indicative that heterotrophic plankton, particularly bacteria, may be using carbon produced by macrophytes in addition to that fixed by phytoplankton at this site. The high heterotrophic nanoplankton- to-bacterial biomass ratio suggests that bacteria may also undergo a significant grazing pressure from protists. In addition, the predominant phytoplankter, *C. erosa*, may also practice bacterivory.

Due to very high concentrations of ammonium in the water column (ca. 20  $\mu\text{M}$ ) on both sampling occasions we were unable to accurately estimate ammonium cycling rates in experiments. The resolution of the  $^{15}\text{N}$  isotope dilution technique greatly depends on the background-to-signal ratio. However, we were able to estimate net ammonium uptake rates based on changes in ammonium concentrations during bottle experiments (Table 5). These rates were always higher in light incubations than in dark ones. The ratio between dark and light rates increased with the increased bacterial-to-phytoplankton biomass ratio, indicating that bacteria, possibly nitrifying ones, may be partially responsible for the dark uptake of ammonium. The differences in uptake rates

between the sampling dates sites correspond well to the difference in phytoplankton biomass, that was higher in August than in June and at the SSR site than at the Deck site.

The daily uptake-to-ambient concentration ratio increased by one order of magnitude in August, indicating that the ability of the planktonic community to absorb allochthonous nutrients brought by the run-off water increases as the season develops and plankton biomass accumulates in the water column. Inversely, these findings suggest that the wetland buffering capacity may be limited during spring and early summer, when nutrient loading from the surrounding agriculture is particularly intense.

These preliminary data on the Old Woman Creek plankton confirm the assumption that a diverse and abundant microbial assemblage inhabits this coastal wetland. The abundance and structure of the microbial food web at the studied sites implicate the potential for significant ammonium recycling rates during the periods of summer stagnation. However, the presence of storms immediately before our two sampling trips delivered high concentrations of ammonium that, in turn, precluded accurate estimates of cycling rates. Therefore, to get a more complete picture of the microbial-driven nitrogen dynamics in OWC we plan to continue these experiments through the summer of 1998.

## Cited literature:

- Blackburn, H. T. 1979. Method for measuring rates of  $\text{NH}_4^+$  turnover in anoxic marine sediments, using a  $^{15}\text{N}$ - $\text{NH}_4^+$  dilution technique. *Appl. Environ. Microbiol.* 37: 760-765.
- Caperon, J. D. Schell, J. Hirota, and E. Laws. 1979. Ammonium excretion rates in Kaneohe Bay, Hawaii, measured by a  $^{15}\text{N}$  isotope dilution technique. *Mar. Biol.* 54:33-40.
- Chapra, S.C. and A. Robetson. 1977. Great Lakes eutrophication: the effects of point source control of phosphorus. *Science* 196: 1448-1450.
- Gardner, W. S., H. A. Bootsma, C. Evans, and P. A. St. John. 1995a. Improved chromatographic analysis of  $^{15}\text{N}$ : $^{14}\text{N}$  ratios in ammonium or nitrate for isotope addition experiments. *Mar. Chem.* 48:271-282.
- Gardner, W.S., Bootsma, H.A., Troncone, F., Lavrentyev, P.J., and Cavaletto, J.F. Nitrogen cycling rates and light effects in tropical Lake Maracaibo, Venezuela. *Limnol. Oceanogr.* In press.
- Jonhston, C.A. 1991. Sediment and nutrient retention by freshwater wetlands: Effects on surface water quality. *Crit. Rev. Environm. Control.* 21: 419-565.
- Klarer, D.M. and D.F. Millie. 1989. Amelioration of storm water quality by a freshwater estuary. *Arch. Hydrobiol.* 116: 375-389.
- Klarer, D.M. and D.F. Millie. 1994. Regulation of phytoplankton dynamics in a Laurentian Great Lakes estuary. *Hydrobiologia* 286: 97-108.
- Lavrentyev, P.J., W.S. Gardner, and J.R. Johnson. 1997. Cascading trophic effects on aquatic nitrification: Experimental evidence and potential implications. *Aquat. Microb. Ecol.* 13: 161-175.
- Legendre, L. and F. Rassoulzadegan 1995. Plankton and nutrient dynamics in marine waters. *Ophelia* 41:153-172.
- Montagnes, D.J., J.A. Bergers, P.J. Harrison, and F.J.R. Taylor. 1994. Estimating carbon, protein, and chlorophyll a from volume in marine phytoplankton. *Limnol. Oceanogr.* 39: 1044-1060.
- Norland, S. 1993. The relationship between biomass and volume of bacteria, p. 303-307. *In* P.F. Kemp, B.F. Sherr, E.B. Sherr, and J.J. Cole [eds.], *Handbook of Methods in Aquatic Microbial Ecology*. Lewis.
- Porter, K.G. and Y.G. Feig. 1980. The use of DAPI for identifying and counting aquatic microflora. *Limnol. Oceanogr.* 25:943-948.

- Prescott, G.W. 1979. How to identify the freshwater algae. William C. Brown, Co. Dubuque, 293 pp.
- Putt, M., and D.K. Stoecher. 1989. An experimentally determined carbon:volume ratio for marine oligotrichous ciliates from estuarine and coastal waters. *Limnol. Oceanogr.* 34:177-183.
- Sherr, E.B., D.A. Caron, and B.F. Sherr. 1993. Staining of heterotrophic protists for visualization via epifluorescence microscopy, p. 213-228. *In* P.F. Kemp, B.F. Sherr, E.B. Sherr, and J.J. Cole [eds.], *Handbook of Methods in Aquatic Microbial Ecology*. Lewis.
- Sherr, B.F., Sherr, E.B., and C.S. Hopkinson. 1988. Trophic interactions within pelagic microbial communities: indications of feedback regulation of carbon flow. *Hydrobiologia* 159:19-26.
- Tomaszek, J.A., Gardner, W.S., and T.H. Johengen. 1997. Denitrification in sediments of a Lake Erie coastal wetland (Old Woman Creek, Huron, Ohio, USA). *J. Great Lakes Res.* 23: 403-415.
- Wetzel, R.G. 1992. Wetlands as metabolic gates. *J. Great Lakes Res.* 18: 529-532.
- Zadorojny, C., Saxon, S., and R. Finger. 1973. Spectrophotometric determination of ammonia. *J. Water Pollution Cont.* 45: 905-912.

Table 1. Species composition of phytoplankton in Old Woman Creek.

|  |                                   |
|--|-----------------------------------|
| <i>Cryptomonas</i> sp.                         | <i>Surirella ovalis</i>           |
| <i>C. erosa</i>                                | <i>Cymatopleura solea</i>         |
| <i>Rhodomonas</i> sp.                          | <i>Chrysococcus</i> sp.           |
| <i>R. minuta</i> var. <i>nannoplanktonica</i>  | <i>C. triolis</i>                 |
| <i>Aulacoseira</i> sp.                         | <i>Euglena</i> sp.                |
| <i>Aulacoseira</i> sp.                         | <i>E. acus</i>                    |
| <i>A. alpigena</i>                             | <i>E. gasterosteus</i>            |
| <i>A. islandica</i>                            | <i>Phacus pseudonordtedtii</i>    |
| <i>Melosira varians</i>                        | <i>Lepociclis</i> sp.             |
| <i>Cyclotella</i> sp.                          | <i>L. ovum</i>                    |
| <i>C. atomus</i>                               | <i>Trachelomonas</i> sp.          |
| <i>C. meneghiniana</i>                         | <i>T. volvocina</i>               |
| <i>C. pseudostelligera</i>                     | <i>Strombomonas fluviatile</i>    |
| <i>Skeletonema potamos</i>                     | <i>S. gibberosa</i>               |
| <i>Stephanodiscus</i> sp.                      | <i>Scenedesmus</i> sp.            |
| <i>S. minutulus</i>                            | <i>S. acuminatus</i>              |
| <i>S. parvus</i>                               | <i>S. deniticolatus</i>           |
| <i>Meridion circulare</i>                      | <i>S. dimorphus</i>               |
| <i>M. circulare</i> var. <i>constrictum</i>    | <i>Didymocystis planktonica</i>   |
| <i>Navicula capitata</i> var. <i>hungarica</i> | <i>Schroederia setigera</i>       |
| <i>Navicula</i> spp.                           | <i>Lagerheimia balatonica</i>     |
| <i>Fragillaria capucina</i> complex            | <i>L. genevensis</i>              |
| <i>F. construens</i> complex                   | <i>L. wratislavensis</i>          |
| <i>Achnanthes</i> sp.                          | <i>Nephrochlamys subsolitaria</i> |
| <i>A. lanceolata</i> complex                   | <i>Pediastrum duplex</i>          |
| <i>Gomphonema</i> sp.                          | <i>Cosmarium</i> sp.              |
| <i>G. parvulum</i>                             | <i>Chlamydomonas</i> sp.          |
| <i>Rhoicosheria abbreviata</i>                 | <i>Oscillatoria</i> sp.           |
| <i>Amphora pediculus</i>                       | <i>Lyngbya</i> sp.                |
| <i>Eunotia</i> sp.                             | <i>Choococcales</i> spp.          |

Table 2. Species composition of protozooplankton in Old Woman Creek.

*Arcella vulgaris*

*Chromulina nana*

*Ochromonas* sp.

*Spumella* sp.

*Bodo saltans*

*Prorodon* sp.

*Holophrya nigricans*

*Askenasia volvox*

*Urotricha furcata*

*Balanion* sp.

*Limnostrombidium* sp.

*Pelagostrombidium fallax*

*Rimostrombidium lacustris*

*R. humile*

*Strobilidium* sp.

*Tintinnidium fluviatile*

*Codonella cratera*

*Vorticella campanula*

*Euplotes* sp.

*Hypotrichidium conicum*

*Cyclidium* sp.

*Cinetochilium margaritaceum*

Table 3. Relative contribution of the predominant ( $\geq 10\%$ ) algal and protist species to the total phytoplankton and microzooplankton biomass, respectively.

| Species  | Mouth 6-5-97 | Deck 6-6-97 | Deck 8-26-97 | SSR 8-27-97 |
|--|--------------|-------------|--------------|-------------|
| PHYTOPLANKTON                                  | %            | %           | %            | %           |
| <i>Cryptomonas erosa</i>                       | 27.7         | 46.1        | 14.4         | 45.1        |
| <i>Aulacoseira distans</i>                     | 2.0          | 10.3        | 7.7          | 9.1         |
| <i>Melosira varians</i>                        | 17.2         | 0.0         | 0.0          | 4.7         |
| <i>Cyclotella</i> spp.                         | 10.5         | 10.2        | 7.6          | 6.3         |
| <i>Navicula capitata</i> var. <i>hungarica</i> | 2.1          | 11.0        | 0.7          | 1.5         |
| <i>Lepocinclis ovum</i>                        | 0.0          | 0.0         | 30.6         | 9.1         |
| CILIATES                                       |              |             |              |             |
| <i>Rimostrombidium lacustris</i>               | -            | 47.0        | 30.3         | 27.1        |
| <i>R. humile</i>                               | 1.1          | 12.1        | 6.8          | 7.2         |
| <i>Strobilidium</i> sp.                        | -            | 28.1        | 49.2         | 43.2        |
| <i>Limnostrombidium</i> sp.                    | 21.7         | -           | -            | -           |
| <i>Pelagostrombidium fallax</i>                | 48.4         | -           | -            | 7.9         |
| <i>Vorticella campanula</i>                    | 22.0         | -           | -            | -           |

Table 4. The biomass ( $\mu\text{g C l}^{-1}$ ) and structure of the microbial food web in Old Woman Creek.

PHYT – phytoplankton, BAC – bacteria, HNAN – heterotrophic nanoplankton, MICRO – microzooplankton and ciliates. HET:PHOT - the ratios of heterophic-to photosynthetic plankton biomass, HNAN:BAC – the ratio of heterotrophic nanoplankton-to-bacteria biomass, MICRO:PHYT – the microzooplankton-to phytoplankton biomass ratio, and BAC:PHYT – the bacterial-to-phytoplankton biomass ratio.

|            | Mouth 6-5-97 | Deck 6-6-97 | Deck 8-26-97 | SRR 8-27-97 |
|------------|--------------|-------------|--------------|-------------|
| PHYTO      | 180.3        | 74.4        | 86.7         | 332.1       |
| BAC        | 69.2         | 38.8        | 65.5         | 57.8        |
| HNAN       | 16.9         | 12.1        | 16.6         | 22.6        |
| MICRO      | 27.3         | 19.6        | 104.4        | 130.9       |
| HET:PHOT   | 0.63         | 0.95        | 2.15         | 0.64        |
| HNAN:BAC   | 0.24         | 0.31        | 0.25         | 0.39        |
| MICRO:PHYT | 0.15         | 0.26        | 1.20         | 0.39        |
| BAC:PHYT   | 0.38         | 0.52        | 0.76         | 0.17        |

Table 5. Net ammonium uptake rates ( $\mu\text{M h}^{-1}$ ) in light and dark incubations, the ratio of dark to light uptake rates, and the ratio of daily light uptake rate to ambient  $\text{NH}_4^+$  concentration.

|   | Deck 6-6-97 | Deck 8-26-97 | SRR 8-27-97 |
|---|-------------|--------------|-------------|
| Light   | 0.13        | 0.46         | 2.75        |
| Dark  | 0.07        | 0.29         | 0.77        |
| Dark / light  | 0.54        | 0.63         | 0.28        |
| Daily Light Uptake/ Ambient $\text{NH}_4^+$ Concentration | 0.16        | 0.55         | 3.30        |