Microbial Ecology

Estuarine Microbial Food Web Patterns in a Lake Erie Coastal Wetland

P.J. Lavrentyev[1], M.J. McCarthy[2], D.M. Klarer[3], F. Jochem[4] and W.S. Gardner[2]

(1) Department of Biology, The University of Akron, Akron, OH 44325, USA
(2) Marine Science Institute, The University of Texas, Port Aransas, TX 78373, USA
(3) The Old Woman Creek National Estuarine Research Reserve, Huron, OH 44839, USA
(4) Marine Biology Program, Florida International University, Miami, FL 33181, USA

Received: 18 November 2003 / Accepted: 13 May 2004 / Online publication: 9 November 2004

Abstract

Composition and distribution of planktonic protists were examined relative to microbial food web dynamics (growth, grazing, and nitrogen cycling rates) at the Old Woman Creek (OWC) National Estuarine Research Reserve during an episodic storm event in July 2003. More than 150 protistan taxa were identified based on morphology. Species richness and microbial biomass measured via microscopy and flow cytometry increased along a stream–lake (Lake Erie) transect and peaked at the confluence. Water column ammonium (NH_4^+) uptake (0.06 to 1.82 μM N h^{-1}) and regeneration (0.04 to 0.55 μM N h^{-1}) rates, measured using $^{15}NH_4^+$ isotope dilution, followed the same pattern. Large light/dark NH_4^+ uptake differences were observed in the hypereutrophic OWC interior, but not at the phosphorus-limited Lake Erie site, reflecting the microbial community structural shift from net autotrophic to net heterotrophic. Despite this shift, microbial grazers (mostly choreotrich ciliates, taxon-specific growth rates up to 2.9 d^{-1}) controlled nanophytoplankton and bacteria at all sites by consuming 76 to 110% and 56 to 97% of their daily production, respectively, in dilution experiments. Overall, distribution patterns and dynamics of microbial communities in OWC resemble those in marine estuaries, where plankton productivity increases along the river–sea gradient and reaches its maximum at the confluence.

Introduction

Aquatic microbial communities encompass a wide array of heterotrophic, autotrophic, and mixotrophic (i.e., capable of combining autotrophic and heterotrophic nutrition) prokaryotes and eukaryotes, which together comprise the microbial food web [62]. In the Laurentian Great Lakes, abundant assemblages of ciliates and flagellates [8, 14, 66] exhibit short turnover times [10, 39], exert grazing pressure on picoplankton [26, 40, 53], and form direct trophic links to crustacean mesozooplankton [9, 43]. However, despite more than a century of microbiological studies in this region ([36] and references therein), the knowledge of major microbial components is incomplete. Specifically, little is known about the structure and dynamics of microbial communities in coastal wetlands, which play the critical role of ''metabolic gates'' to the Great Lakes [71].

Abiotic gradients in natural waters provide a tool to examine relationships between taxonomic composition and dynamics of microbial communities [41, 68]. Estuaries, defined as semi-isolated coastal areas that are diluted by freshwater discharge [35], present a dynamic physical environment characterized by sharp gradients in salinity, nutrient concentrations, turbidity, and temperature. Interactions among these factors typically result in enhanced plankton productivity [27, 55, 72] and nitrogen cycling rates [3, 44] in the mixing zone as compared to offshore waters. Estuarine protists, specifically ciliates, form abundant and diverse assemblages in the mesohaline zone [13, 24] and consume a large proportion of bacterial and phytoplankton production [4, 18, 50]. In Lake Erie, which exhibits physical processes similar to the coastal ocean, coastal wetlands and marshes play the role of estuaries. Although salinity is not a factor, lake waters and streams combine in freshwater ''estuaries'' to form a third type of water, which is chemically different from either the streams or the lake. Specifically, phosphorus-rich storm runoff water mixes with P-deficient lake water. In addition, storm-driven standing waves (seiches) play the role of tides.

Correspondence to: P.J. Lavrentyev; E-mail: peter3@uakron.edu

The objectives of the present study in Old Woman Creek (OWC), a Lake Erie coastal wetland, were to (a) estimate morphological diversity of planktonic protists; (b) determine abundance and relative biomass of microbial food web constituents (bacteria, phytoplankton, phagotrophic protests, and rotifers) along the stream–lake gradient; and (c) measure growth and grazing rates (herbivory and bacterivory) of microbial plankton in conjunction with nitrogen (N) cycling rate measurements. We expected that OWC would act as a typical estuary, where biological communities are regulated by the shifting turbidity–nutrient gradient. This expectation led to the following testable hypotheses: (I) maximum protist species richness and microbial biomass occur at the OWC mouth, where lake water mixes with stream water; (II) microbial grazers exert tight top-down control on bacteria and nanophytoplankton at the confluence by consuming at least 50% of their daily production; (III) this zone is associated with elevated nutrient cycling rates as compared to the upstream and lake sections.

Material and Methods

The Study Site. The study was conducted at the Old Woman Creek National Estuarine Research Reserve and Nature State Preserve, one of the few undisturbed wetland systems remaining along the Ohio shoreline of Lake Erie. OWC is a hypereutrophic ecosystem characterized by a pulse-flow hydrologic regime and diverse aquatic biota [30–32]. In summer, Lake Erie water intrusion and water flow through the wetland may be blocked by a barrier sand beach. Occasionally, storms break the barrier, and downstream flow proceeds through the wetland one to two orders of magnitude more rapidly than normal, sometimes removing large amounts of accumulated sediments to Lake Erie. Seiche activities can flood the wetland with lake water, which drains over the course of 14 h or less.

Sampling. Four sites were sampled along the 2.5 km stream–lake transect (Fig. 1). These sites represented contrasting parts of OWC: the upper channel (the stream site in the text), the broad, shallow, inner basin (the wetland site), the confluence zone (the mouth site), and nearshore Lake Erie (the lake site). Collectively, the former three sites are referred to as the OWC interior. At the time of sampling, the water column depths were ~1.0 m, 0.5 m, 1.5 m, and 2.0 m at the above four sites, respectively. The creek mouth remained open to the lake during the study. Comparative sampling was conducted at all four sites on 1 July 2003. Experiments were conducted on 2 July 2003 (lake and wetland sites) and 4 July 2003 (mouth and stream sites).

Water Column Profiles and Hydrochemistry. At each site, water temperature and specific conductivity were measured using a Hydrolab Datasonde/Surveyor IV multiprobe. Additionally, pH, dissolved oxygen, and turbidity were measured as a part of NOAA biweekly monitoring effort. *In situ* nutrient concentrations were measured in filtered (0.2 μm syringe filters; Osmonics) water samples via high-performance liquid chromatography (HPLC; NH_4^+; [20]) and automated flow injection analysis (NO_3^-, NO_2^-, and o-PO_4^{3-}; Lachat Quikchem 8000 FIA). Chlorophyll *a* (Chl) was collected from 50–100 mL triplicate samples onto 0.2 μm polycarbonate filters, immediately frozen, extracted overnight in 90% ice-cold acetone, and measured by a nonacidic method [70] using a Turner Designs TD700 fluorometer.

Microbial Composition and Abundance. To address hypothesis I, triplicate samples were collected from the subsurface layer (~30 cm) for counts of phytoplankton, microzooplankton, heterotrophic nanoflagellates (HNF), and bacteria at each site. Formaldehyde-fixed (1% final concentration) nanoflagellates were counted by epifluorescence microscopy (EFM) following staining with DAPI [60]. A dual-band filter set was used to visualize this fluorochrome and Chl simultaneously. Microplankton (including ciliates, algae, cyanobacteria, and rotifers) were preserved with acid Lugol's iodine (1% final concentration) and counted using the settling technique. At least 30 protist cells were sized for abundant taxa (fewer for less abundant taxa) from each site, corrected for shrinkage [48], and converted to carbon [47, 54]. Rotifer biomass was determined according to Fahnenstiel et al. [14]. Microbial species diversity was estimated using the Shannon–Weiner function [34] based on plankton biomass (excluding bacteria).

In addition to conventional microscopic counts, nanophytoplankton (here photosynthetic eukaryotes 3–20 μm) from triplicate experimental samples (see below) were counted via an imaging-in-flow system (FlowCAM; [63]). This system represents a combination flow cytometer/progressive scan digital video camera. We analyzed cells within continuous flows of 0.2 to 1 ml min^{-1}, depending on phytoplankton abundance. Every cell passing through the flow chamber was measured by the FlowCAM and data were obtained on cell linear dimensions and chlorophyll content. We used a custom redesigned FlowCAM, which in addition to the features described above was equipped with an argon gas blue laser (Melles Griot, 20 mW, 488 nm) and apochromatic optics (0.85 numerical aperture). This combination improved fluorescence detection and resolution up to 0.5 μm per pixel. In our test runs, we were able to detect most phytoplankton cells >3 μm. The flow-cytometry data were verified using conventional microscopic techniques described above.

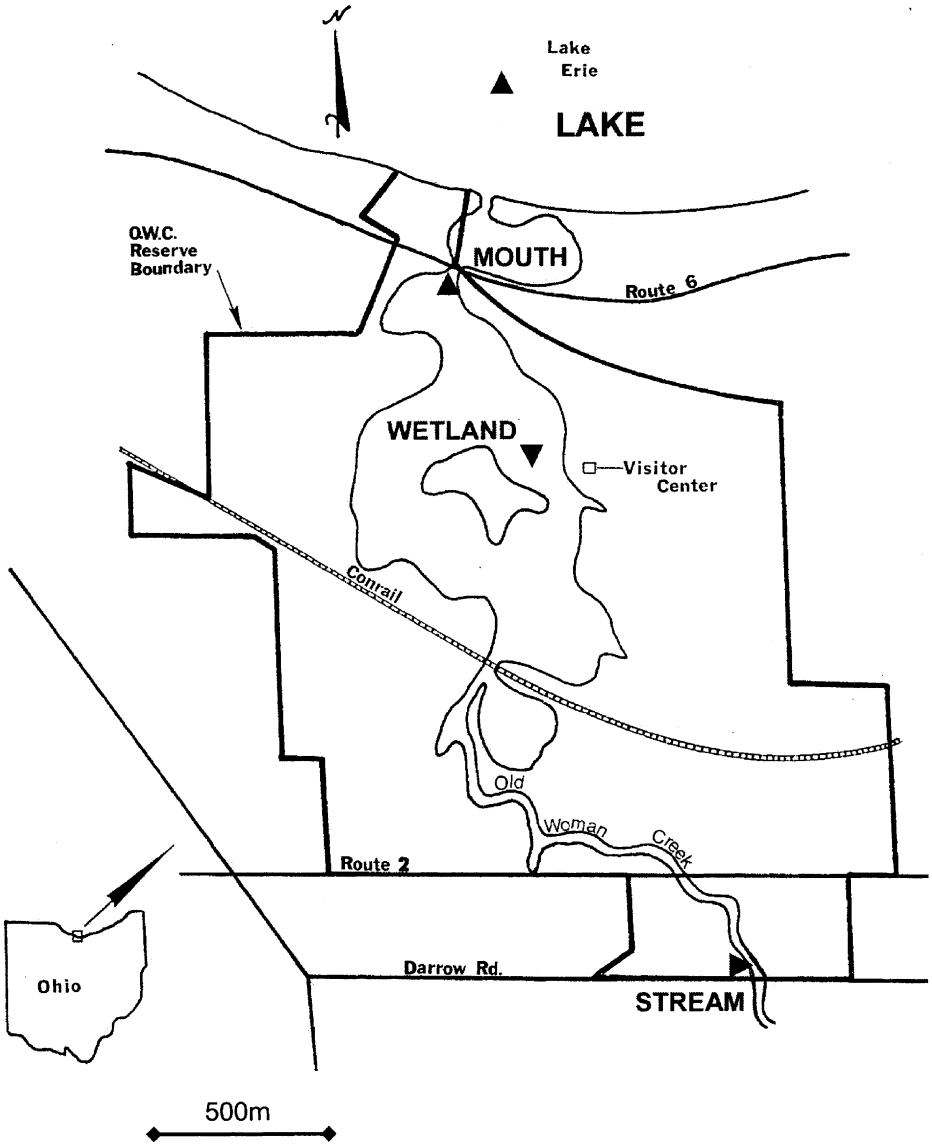

Figure 1. The sampling sites within the Old Woman Creek National Estuarine Research Reserve (OWC). The insert shows OWC location in Ohio.

Protist identification included *in vivo* observations and examination of fixed material from integrated samples obtained from each site under an Olympus IX-70 microscope equipped with differential interference contrast, apochromatic optics, a SPOT-2 digital camera, and a video attachment (Olympus-750). Morphological characteristics of protists were confirmed using silver staining [64] and scanning electron microscopy [42].

Bacteria stained with SYBR Green after RNAse treatment [28] were counted via a Becton-Dickinson FACSort flow cytometer from triplicate 1% (final concentration) formalin-fixed samples stored in liquid N. Bacterial biovolumes (at least 1500 cells per sample) were determined from DAPI-stained filters using a SPOT-2 digital camera, EFM, and Image Pro 4.5 software and converted to carbon using DAPI-corrected conversion factor [46].

Microbial Trophodynamics. To address hypothesis II, growth and grazing mortality of planktonic protists and bacteria were examined via dilution [38]. The advantages and disadvantages of this approach have been discussed [19, 37, 65]. Water from each site was prescreened through a 153 μm mesh-size Nitex nylon screen using reverse gravity flow filtration to remove crustacean zooplankton. Preliminary counts and Chl measurements indicated no significant plankton loss during the screening step. The resulting samples were diluted at different proportions (percentage of the whole water—100%, 60%, 30%, 10%) with filtered (in-line 3.0 μm and 0.2 μm Pall Science capsule filters) water from each site. The use of gravity filtration and large-capacity capsules, rather than membrane filters, for dilution preparation in this study should have lessened physical damage to plankton and resulting organic contamination during

Table 1. Physical and chemical characteristics of the study sites

Site	*Stream*	*Wetland*	*Mouth*	*Lake*
Specific conductivity (μohm)	710	680	640	270
Turbidity (NTU)	14.0	85.2	62.3	3.8
Temperature (°C)	22.4	25.1	25.8	22.2
Dissolved oxygen (μg L^{-1})	5.04	3.30	3.62	7.34
pH	7.55	7.42	7.41	7.92
o-PO_4 (μM)	0.27 ± 0.02	0.21 ± 0.07	0.21 ± 0.05	0.01 ± 0.01
NO_3–N (μM)	74.5 ± 0.38	18.5 ± 0.10	9.81 ± 0.44	54.1 ± 3.47
NO_2–N (μM)	0.50 ± 0.05	1.03 ± 0.07	1.62 ± 0.24	0.32 ± 0.11
NH_4–N (μM)	5.31 ± 0.63	25.2 ± 0.22	34.3 ± 2.67	3.10 ± 0.22
$N0_3$:NH_4	14.0	0.73	0.29	17.4
DIN (μM)	80.3	44.7	45.7	57.5
DIN:o-PO_4	297	213	218	5,748
Chlorophyll *a* (μg L^{-1})	1.45 ± 0.02	10.7 ± 0.60	29.1 ± 0.06	0.83 ± 0.04

Standard errors were calculated from three random water samples collected on 1 July 2003.

filtration steps [16]. Each sample was amended with 50 μg P L^{-1} (final concentration, KH_2PO_4) and incubated in duplicate, glass 1-L containers *in situ* on a submersed plankton wheel (75 cm diameter, 0.25 rpm.) for 24 h. An additional set of undiluted samples was incubated without nutrient additions to estimate *in situ* growth rates [6]. At the beginning of incubations and after 24 h, microbial grazers (ciliates, HNF, and rotifers) were collected from whole-water bottles, and bacteria and nanophytoplankton were collected from all bottles. Apparent growth rates of microorganisms (μ) were calculated assuming exponential growth (ln [N_t/N_0], where N_0 and N_t were initial and final abundances, respectively). Growth (μ) and grazing mortality rates (g) of bacteria and phytonanoplankton were estimated from linear regression of the apparent growth rates versus dilution. Growth (μ) rates of HNF and ciliates were estimated based on their initial and final concentrations in whole-water bottles. Grazing mortality estimates were corrected for the growth of grazers in undiluted controls following the methods described by Gallegos et al. [19]. The growth rates of microorganisms in experimental (whole water) and control bottles were compared via single factor ANOVA.

Nitrogen Cycling Rates. To address hypothesis III, water column N dynamics were evaluated in a separate set of bottles simultaneously with dilution experiments. Water column NH_4^+ regeneration and potential uptake rates were measured using isotope dilution experiments with $^{15}NH_4^+$ (99.8 atom % ^{15} N; Isotec). Water was spiked with 8 (lake) and 16 μM (other sites; final concentration) $^{15}NH_4^+$, partitioned into triplicate light and dark bottles (70 mL Corning tissue culture bottles; dark bottles wrapped with aluminum foil), and incubated in a mesh bag floated in OWC near site 3. Initial (0 h) and final (24 h) samples were collected in 8 mL Wheaton vials and frozen for transport to the laboratory. Samples were thawed and analyzed for total NH_4^+ concentration and atom % ^{15} N using HPLC [20]. Ammonium regeneration and potential uptake rates were calculated using the Blackburn/Caperon model [2, 5]. Since higher than ambient additions were used in this study, NH_4^+ uptake rates have been qualified as "potential" NH_4^+ uptake rates.

Results

Water Column Profiles and Hydrochemistry. Specific conductivity decreased along the stream-lake transect (Table 1). Turbidity and temperature were higher at the wetland and mouth sites than at the stream and lake sites, whereas dissolved oxygen concentration was slightly lower at the two former sites. Soluble reactive phosphorus (SRP, o-PO_4^{3-}) concentrations were <0.3 μM at all stations. Dissolved inorganic N (DIN) concentrations were highest at the stream site (93% NO_3^-) followed by the lake (94% NO_3^-), mouth (75% NH_4^+), and wetland (56% NH_4^+) sites.

Microbial Composition and Abundance. More than 150 protist species and forms were identified (Table 2). Alveolata (mostly ciliates) were the most represented group in our samples, followed by Stramenopila (mostly diatoms and chrysophytes), and Chlorophyta. Other protists included Eglenophyta, Cryptophyta, Zoomastigophora (kinetoplastids and choanoflagellates), and Sarcodina (testacea, heliozoa, and euamoeba). The mouth site was by far the most species rich followed by the wetland site, whereas the lake and stream sites had fewer species. The taxonomic distribution of chlorophytes was the most contrasting; 34 species were found at the mouth site and five or fewer at other sites. Additionally, eight species of cyanobacteria also were observed in the collected samples. In contrast to the species richness, the biomass-based Shannon diversity index (H′, bit)

Table 2. Taxonomic list of planktonic protists found in the Old Woman Creek NERR

	St.1	*St.2*	*St.3*	*St.4*
ALVEOLATA (54)	15	27	34	25
Askenasia sp.			+	+
Askenasia volvox		+		
Aspidisca sp.		+		
Balanion planktonicum		+	+	+
Ceratium herudinella		+	+	
Ceratium sp.		+		+
Codonella cratera			+	+
Cyclidium sp.	+	+	+	+
Didinium nasutum		+	+	
Enchelydon sp.			+	
Euplotes sp.		+		
Halteria chlorelligra		+	+	
Halteria cirrifera	+	+	+	+
Holophrya nigricans			+	
Holophrya simplex	+	+	+	
Holophrya sp.		+		+
Hypotrich unidentified	+			
Limnostrombidium pelagicum	+	+	+	+
Litonotus sp.		+		
Mesodinium acarus				+
Mesodinium pulex			+	
Monodinium sp.	+	+	+	
Oxytricha sp.	+	+	+	
Paradileptus elephantinus		+	+	
Paramecium sp.			+	
Pelagodileptus trachelioides			+	
Pelagostrombidium fallax		+	+	
Pelagostrombidium mirabile				+
Pelagostrombidium sp.			+	
Pelagovorticella mayeri				+
Peridinium sp.		+	+	
Pleuronema sp.	+			
Rhabdoaskenasia minima				+
Rimostrombidium brachykinetum		+		
Rimostrombidium humile	+		+	+
Rimostrombidium lacustris		+	+	+
Stentor sp.		+		
Strobilidium sp.2	+		+	
Strombidium sp.				+
Strombidium viride			+	+
Stylonichia sp.			+	
Tinitnnidium sp.	+	+	+	+
Tintinnidium fluviatile			+	+
Tintinnopsis sp.			+	
Uronema sp.	+			
Urotricha farcta	+	+	+	+
Urotricha furcata	+	+	+	+
Urotricha globosa	+	+	+	
Urotricha pelgica				+
Vorticella anastatica				+
Vorticella aquadulcis (complex)			+	+
Vorticella sp.			+	
Woloszynskia sp.		+		
CHLOROPHYTA (35)	3	5	34	3
Actinastrum hantzschii		+	+	
Actinastrum sp.		+		
Chlamydomonas sp.	+	+	+	
Chlamydomonas sp. 1	+		+	+
Chlamydomonos sp.2			+	+
Chlorogonium minimum			+	
Closterium sp.			+	

(Continued)

Table 2. Continued

	St.1	*St.2*	*St.3*	*St.4*
Colonial green (coccoid)			+	
Didymocystis sp.	+		+	
Didymogenes palatine			+	
Eudorina unicocca			+	
Franceia ovalis			+	
Golenkinia radiate			+	
Kirchneriella subsolitaria			+	
Lagerheimia balatonica		+	+	+
Lagerheimia genevensis			+	
Monoraphidium griffithii			+	
Monoraphidium tortile			+	
Nephrochlamys subsoliteria			+	
Oocystic lacustris			+	
Oocystis parva			+	
Oocystis pusilla			+	
Oocystis sp.			+	
Oocystis submarina			+	
Pediastrum duplex var. *duplex*			+	
Scenedesmus bicaudatus			+	
Scenedesmus dimorphus			+	
S. intermedias var. *indicus*			+	
Scenedesmus opoliensis		+	+	
Scenedesmus quadricauda			+	
S. quadricauda var. *longispina*			+	
Scenedesmus sp.			+	
Schroederia setigera			+	
Sphaerocystis sp.			+	
Tetraedron regulare			+	
STRAMENOPILA (36)	11	17	18	12
Achnanthes lanceolata			+	
Achnanthes sp.		+		
Actinocyclus normanii				+
Asterionella formosa				+
Aulacoseira italica			+	
A. italica var. *tenuissima*			+	
Aulocoseira alpegina		+	+	
Cyclotella atomus			+	
Cyclotella meneghiniana	+	+	+	
Cyclotella radiosa			+	
Cymatopleura solea	+			
Dinobryon sp.				+
Fragilaria vaucheriae			+	
Fragilaria sp.		+		
Gomphonema parvulum		+		
Mallomonas sp.		+	+	
Melosira distans var. *alpegina*			+	
Navicula capitata var. *hungarica*	+	+		
Navicula pupula		+		
Navicula sp.	+	+	+	
Nitzschia acicularis	+	+	+	
N. communis var. *abbreviata*				+
Nitzschia frustulum	+	+	+	+
Nitzschia sp.	+	+	+	+
Ochromonas nana	+	+	+	
Ochromonas sp.	+			+
Pennate diatom		+		
Rhoicosphenia abbreviata	+			+
Spumella sp.		+	+	
Stephanodiscus rotula			+	+
Stephanodiscus sp.			+	
Stichochrysis sp.		+		+
Surirella minuta		+		+

(Continued)

Table 2. Continued

	St.1	St.2	St.3	St.4
Surirella ovata	+			
Synedra acus				+
Synedra sp.	+			
OTHER PROTISTS (27)	11	17	18	12
Bodo saltans		+	+	
Campylomonas marssoni			+	+
Carteria globasa	+			
Carteria sp.	+			
Codosiga botrytis			+	+
Cryptomonas erosa	+	+	+	+
Diploeca sp.		+	+	
Euglena acus			+	
Euglena deses			+	
Euglena proxima		+		
Euglena sp.	+	+	+	
Haptophyte unknown				+
Kathablepharis ovalis			+	+
Phacotaceae sp.			+	
Phacus sp.			+	
Phacus stokosii			+	
Rhodomonas sp.	+	+		
R. minuta var. *nannoplanctonica*			+	+
Rhynchomomas sp.			+	
Strombomonas acuminata			+	
Strombomonas sp.		+		
Trachelomonas abrupta var. *minor*			+	
Trachelomonas sp.		+	+	
Trachelomonas sp. I		+		
Difflugia sp.			+	
Actinophrys sol				+
Euamoeba sp.	+	+	+	
TOTAL PROTISTS (152)	36	59	104	44

+: species present. Numbers indicate the number of species found at each site and in within each taxonomic group.

remained at approximately the same level in the OWC interior (1.92 to 2.10) and declined at the lake site (1.77).

Maximum Chl concentration (Table 1) and phytoplankton abundance (Fig. 2) and biomass (Fig. 3) were found at the mouth site, with minima at the lake site. Phytoplankton abundance and biomass were due primarily to diatoms (*Cyclotella menenghiana* and *Navicula* sp.) and the cryptophyte *Cryptomonas erosa* at the stream site (Fig. 4A,B). These diatoms also were abundant at the wetland site, but most biomass at this site was formed by large euglenophytes, *Euglena* sp. and *E. proxima*. The mouth site phytoplankton were dominated numerically by nanodiatoms and green flagellates (*Chlamydomonas* sp.), whereas most phytoplankton biomass was due to large green algae (*Closterium* sp. and *Sphaerocystis* sp.), the cryptophyte *Cryptomonas erosa*, and euglenophytes (*Trachelomonas* sp., *Euglena acus*). At the lake site, the nanocryptophyte *Rhodomonas minima* var. *nannoplanctonica* dominated phytoplankton. Diatoms (*Actinocyclus normanii* var. *subsalsa*, *Asterionella formosa*, *Stephanodiscus rotula*) and chrysophyte flagellates co-dominated in terms of biomass and abundance, respectively. Phytoplankton size structure varied along the transect: nanophytoplankton made up about half of the total phytoplankton abundance in the OWC interior and >90% at the lake site (almost all due to flagellates). Nanophytoplankton relative contribution to total phytoplankton biomass declined from the stream site to the wetland site, and then increased sharply at the lake site (Table 3).

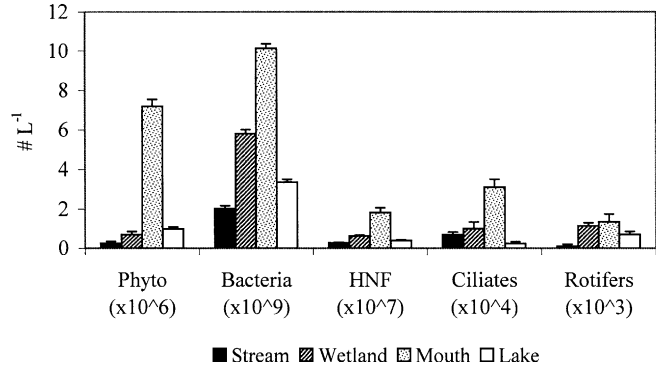

Figure 2. Abundance of phytoplankton (Phyto), bacteria, heterotrophic flagellates (HNF), ciliates, and rotifers along the stream-lake transect on 1 July 2004. Standard errors were calculated from three random samples.

The abundance (Fig. 2) and biomass (Fig. 3) of heterotrophic plankton (bacteria, HNF, ciliates, and rotifers) also peaked at the mouth site. HNF assemblage was formed primarily by the colorless chrysophytes *Spumella* sp., *Ochromonas* sp., and *Chromulina nana* at all four sites. The kinetoplastids *Bodo saltans* and *Rhycomonas* sp. were common at the wetland and mouth sites and the choanoflagellate *Codosiga botritis* at the lake site. Among ciliates, the choreotrich *Rimostrombidium humile* dominated numerically at all sites (Fig. 5A). Together with loricated choreotrichs (tintinnids *Tintinnidium fluviatile* and *Tintinnopsis* sp.), this species made up >90%

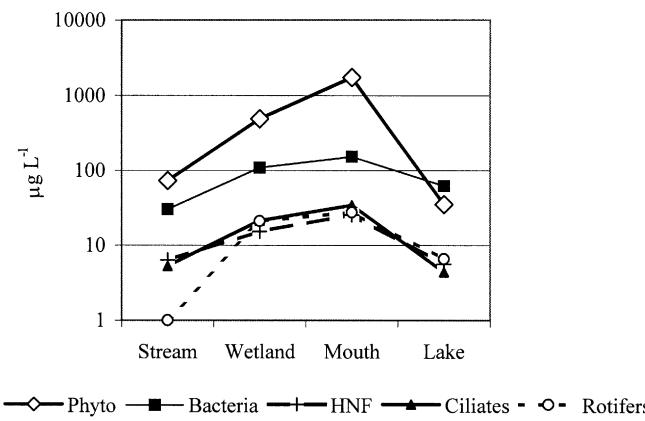

Figure 3. Biomass of phytoplankton (Phyto), bacteria, heterotrophic flagellates (HNF), ciliates, and rotifers along the stream–lake transect on 1 July 2004.

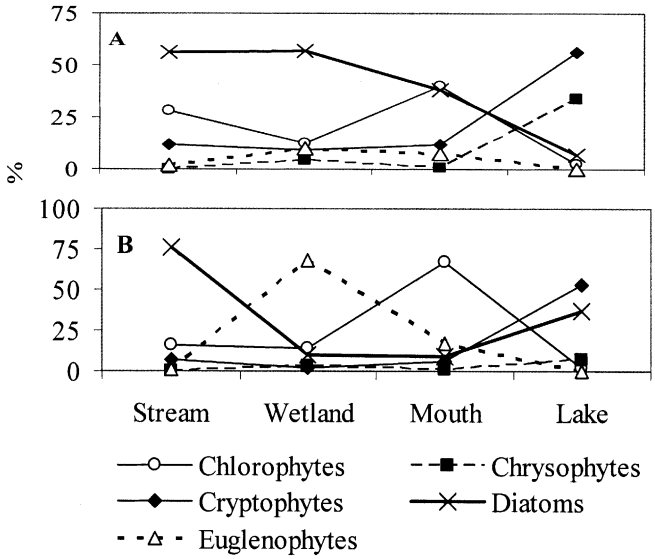

Figure 4. Relative abundance (A) and biomass (B) of phytoplankton taxonomic groups along the stream–lake gradient.

of ciliate abundance and biomass at the mouth site (Fig. 5A,B). The composition of codominant ciliates varied among the sites. The nanoplanktonic prostomate *Urotricha farcta* had maximum relative contribution at the stream site, the large chlorophyll-bearing haptorid *Didinium nasutum* and oligotrich *Strombidium viride* at the wetland site, and the oligotrich *Limnostrombidium pelagicum* at the lake site. Most of the common ciliates were planktonic with the exception of several hypotrich species (*Aspidisca* sp., *Euplotes* sp., *Stylonichia* sp.), which are meroplanktonic. Rotifers from the genera *Polyarthra*, *Testudinium*, and *Brachionus* reached their maximum biomass at the mouth site, exceeded biomass of ciliates at the lake site, and were almost absent at the stream site (Fig. 3).

Several ratios were calculated to characterize the microbial food web structure with respect to the changing nutrient environment along the stream-lake gradient (Table 3). The heterotrophic-to-autotrophic plankton biomass and heterotrophic biomass to Chl ratios decreased along the transect and then increased sharply at the net heterotrophic lake site, which was characterized by elevated values of bacterial biomass per units of NH_4^+ and SRP. The microzooplankton biomass (ciliates plus rotifers) remained >50% of nanophytoplankton biomass at all sites, including the lake site. The ratio of microzooplankton (ciliates + rotifers) to total phytoplankton biomass was minimum at the mouth site and maximum at the lake site. The protist (HNF + ciliates) to bacteria ratio remained steady in the OWC interior and declined at the lake site.

Microbial Trophodynamics. The ciliate assemblage had higher growth rates at the lake and stream sites, whereas HNF grew faster at the wetland and mouth sites (Table 4). The maximum taxon-specific growth rates of the predominant ciliates exceeded that of their assemblage at different sites (*Rimostrombidium humile* 2.65 d^{-1} at the lake site; *Tininnidium fluviatile* 2.91 d^{-1} and *Urotricha farcta* 1.67 d^{-1} at the wetland site; *Limnostrombidium pelagicum* 1.10 d^{-1} at the mouth site). No apparent population growth was detected for rotifers during the 24 h incubations.

At all four sites, dilution elicited a linear growth response in bacteria and nanophytoplankton (Table 4). Compared to the control, bacterial growth was not affected by P enrichment in dilution experiments at the stream site, increased by ~40% at the wetland ($p = 0.02$, $F = 13.5$) and mouth sites ($p < 0.01$, $F = 54.4$), and nearly tripled at the lake site ($p < 0.01$, $F = 80.3$). Maximum bacterial growth rates were measured at the stream site followed by the lake and mouth sites. The wetland site had minimum bacterial growth. Bacterial growth considerably exceeded grazing losses only at the lake site. The latter site also was the only site where P enrichment stimulated nanophytoplankton growth (~2 times that in control, $p < 0.01$, $F = 46.0$). Nanophytoplankton growth rates were maximal at the mouth site and exceeded grazing mortality only at this and the wetland sites.

Nitrogen Cycling Rates. Potential NH_4^+ uptake and regeneration rates increased along the stream–mouth transect and then declined at the lake site (Fig. 6). At all

Table 3. Microbial food web structure in OWC on 1 July 2003

	Stream	*Wetland*	*Mouth*	*Lake*
Heterotrophs: total phytoplankton (μg C μg C^{-1})	0.59	0.34	0.14	2.77
Heterotrophs: chlorophyll *a* (μg C μg Chl^{-1})	29.0	15.6	8.2	94.5
Bacteria: SRP (μg C μg P^{-1})	3.60	16.8	23.4	199.8
Bacteria: NH_4^+(μg C μg N^{-1})	0.40	0.31	0.32	1.43
Nanophytoplankton: total phytoplankton (μg C μg C^{-1})	0.11	0.10	0.07	0.61
Microzooplankton: nanophytoplankton (μg C μg C^{-1})	0.69	0.88	0.52	0.51
Microzooplankton: total phytoplankton (μg C μg C^{-1})	0.08	0.09	0.04	0.31
Protists: Bacteria (μg C μg C^{-1})	0.39	0.33	0.39	0.16

HNF: heterotrophic nanoflagellates; Microzooplankton: ciliates + rotifers; Protists: HNF + ciliates; Heterotrophs: bacteria + microzooplankton; SPR: soluble reactive phosphorus.

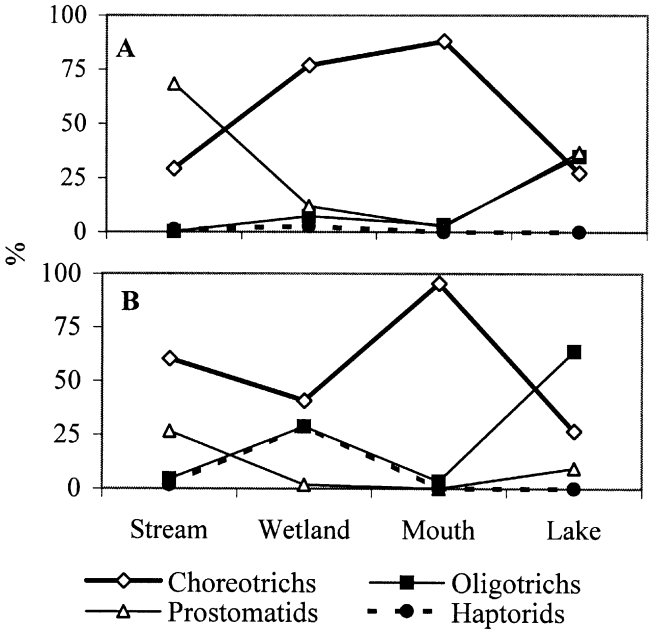

Figure 5. Relative abundance (A) and biomass (B) of ciliate taxonomic groups. Choreotrichs include aloricate and loricate (tintinnids) ciliates.

sites except the last site, light rates exceeded dark rates. These differences were more pronounced at the mouth site (dark uptake only 14% of light uptake) than at the wetland (29%) or stream (40%) sites. In contrast to uptake, no light/dark differences were observed for regeneration, except the lake site, where dark regeneration was higher than light regeneration. In light incubations, regeneration rates were ca. 70% of potential NH_4^+ uptake at the lake site and 30–50% at the other three sites. In dark incubations, regeneration exceeded uptake by a factor of 2 at the wetland and mouth sites, were about equal to uptake rates at the lake site, and decreased to 72% of uptake at the stream site.

Table 4. Microbial rates (d^{-1}) in dilution experiments on 2 July 2003 (lake and wetland sites) and 4 July 2003 (mouth and stream sites)

Group	*Rate*	*Stream*	*Wetland*	*Mouth*	*Lake*
Ciliates	μ	0.85	0.41	0.46	0.76
HNF	μ	0.28	0.48	0.36	0.23
Bacteria	μ	1.48	0.15	0.21	0.64
	g	1.43	0.11	0.19	0.37
	μ–*g*	0.05	0.04	0.02	0.27
PNAN	μ	0.82	0.78	1.00	0.79
	g	0.74	0.60	0.76	0.86
	μ–*g*	0.07	0.18	0.24	–0.07

μ: growth; *g*: mortality (adjusted); μ–*g*: net growth. Negative net growth values indicate that grazing losses exceeded growth. All rates are significant at the 95% confidence level. The rate estimation methods are described in the Methods section.

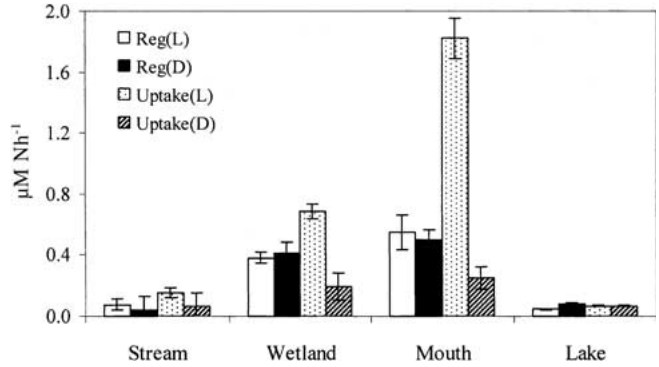

Figure 6. Ammonium regeneration (Reg) and potential uptake rates in light (L) and dark (D) bottles. Standard errors were calculated from three random water samples.

Discussion

OWC water column is inhabited by a diverse assemblage of protists. Most of them are planktonic forms, despite the shallow nature of OWC and wind-driven resuspension, which may favor meroplanktonic forms [58]. These results represent only a preliminary assessment of protist diversity in OWC. For example, our morphological analysis revealed 16 oligotrichs and choreotrichs in OWC samples, whereas their diversity based on nucleotide sequencing of small subunit (18S) ribosomal DNA was higher (23 forms based on ~100 clones; Joel Duff, University of Akron, pers. comm.). Most of these ciliates are not yet represented in GenBank, as probably will be the case with other protist groups found in OWC, particularly nanoflagellates.

The maximum microbial biomass and species richness of protists at the mouth site support hypothesis I. These findings correspond well to the data obtained from the mesohaline zone in marine estuaries [13, 24]. In contrast to species richness distribution, diversity remained at approximately the same level in the OWC interior since a few species dominated the community at each site. For example, at the mouth site, >90% of ciliate abundance was due to choreotrich ciliates, which are effective bacterivores [61]. Most of the predominant species are opportunistic, i.e., they are widely distributed [17] and exhibit rapid turnover rates, which allow them to exploit abundant recourses at OWC.

Phytoplankton composition appeared to reflect changing light–nutrient conditions along the stream–lake gradient. Chlorophytes and euglenophytes made up >80% of phytoplankton biomass at the turbid NH_4–rich wetland and mouth sites, whereas relative biomass of diatoms increased at the periphery sites (stream and lake), characterized by a better light environment and higher NO_3:NH_4 ratios. The dominance of bacteria, cryptophytes, and plastidic chrysophytes at the lake site may be associated with P-limited conditions. This observation is

supported by the elevated bacteria–SRP ratio, the results of dilution bioassays in this and other studies [25], and total phosphorus concentrations (dissolved plus particulate, 3.8 μM at the mouth site vs. 0.2 μM at the lake site; authors, unpublished data). Although picocyanobacteria not included in this study may be abundant in coastal Lake Erie (*Synechococcus* abundance up to 0.6×10^6 mL^{-1}; authors, unpublished data), the heterotrophic biomass to chlorophyll ratio confirms an increased role of heterotrophic plankton at the lake site. This observation appears to reflect a shift from algae-dominated communities in productive waters to bacteria-dominated communities in nutrient-deficient waters [11]. Bacteria can compete successfully for limiting nutrients with phytoplankton (e.g., [29]), whereas plastidic chrysophytes and other mixotrophic flagellates often resort to bacterivory to alleviate mineral deficiency [7, 56, 57].

The obtained growth and grazing mortality data indicate that, despite observed changes in microbial abundance and composition along the stream–lake gradient, microbial grazers retained their ability to control nanophytoplankton and bacteria, thus supporting hypothesis II. The accumulation of total phytoplankton biomass at the mouth site was due primarily to large green algae, which may not be consumed extensively by ciliates and rotifers. The effects of phytoplankton composition on microzooplankton herbivory rates have been described in estuarine environments [15, 45]. The growth of HNF in OWC was low, which can be attributed to ciliate top-down pressure [59, 69]. In contrast, the predominant ciliate taxa had very high population growth rates exceeding "maximum" rates predicated from allometric equations [49]. Interestingly, none of them grew that fast at their abundance maximum sites. In general, planktonic ciliate rapid growth is not surprising since these protists are adapted to take advantage of varying environments [33, 51]. Reduced predation mortality from mesozooplankton can offer only a partial explanation for the observed rates, since rotifers, some of which have been reported to feed on ciliates [1], were present. Although rotifers did not display growth in our incubations, their elevated biomass at the mouth and lake sites suggests an important trophic role and corresponds well to the data obtained in summer at the St. Joseph River mouth, Lake Michigan [21] and in the southern Chesapeake Bay [52].

Nutrient cycling rates reflect nutrient regime and MFW patterns in OWC and support hypothesis III. The highest uptake and regeneration rates corresponded to maximum MFW biomass at the mouth site. The largest difference between light and dark uptake also was observed at the mouth site, which corresponds well to its net autotrophic status. Maximum regeneration rates at this site may be associated with both grazing activities and bacterial recycling of NH_4^+, since bacteria can assimilate and release NH_4^+ at the same time [67]. At the same time, the almost balanced uptake and regeneration rates at the lake site indicate that microbial water column recycling was sufficient to satisfy community N demand at this net heterotrophic site. In contrast to the mouth site, bacteria may retain NH_4^+ (their preferred N source) at the nitrate-dominated lake site, leaving most recycling to grazers.

Ammonium cycling rates in OWC were comparable to maximum rates observed in other hypereutrophic coastal systems. For example, maximum uptake and regeneration rates of 1.2 and 1.5 μM N h^{-1}, respectively, were reported from a coastal mangrove swamp in India [12], and 4.40 and 1.10 μM N h^{-1} from the Mississippi River plume [3]. Note that these studies used tracer level $^{15}NH_4^+$ additions. Although saturating nutrient additions may alter steady-state conditions in stable isotope experiments [22], rates obtained from tracer- and high-level additions tend to converge at high ambient substrate concentrations [23].

The observed "estuarine" patterns of the microbial food web characteristics may have been influenced by a storm system that passed through OWC during the study, resulting in increased surface runoff from the watershed and physical forcing from Lake Erie. The water level fluctuated daily as much as 0.8 m at the mouth site (the authors, unpublished data). A seiche can introduce lake plankton and entrain suspended matter and nutrients at the mouth by temporarily restricting outflow from the creek [32]. These conditions are conducive to plankton growth in marine estuaries [55, 72]. In contrast, scouring may have reduced plankton numbers and species richness in the upper portion of OWC, particularly in the stream section.

Overall, distribution patterns and dynamics of the microbial food web and N cycling in OWC resemble those in "true" estuaries, despite the lack of salinity gradients. These findings stress the need for comparative studies between marine and freshwater coastal environments to provide insights into microbial diversity, dynamics, and biogeochemical processes in ecotones.

Acknowledgments

The National Science Foundation Microbial Observatories program (grant no. 0239997) supported this study. We thank UA students Ken Moats and Kathy Dunmire for laboratory and field assistance and Dr. Francisco Moore and the OWC NERR staff for logistical support.

References

1. Arndt, H (1993) Rotifers as predators on components of the microbial food web (bacteria, heterotrophic flagellates, ciliates): a review. Hydrobiologia 255: 231–246

2. Blackburn, TH (1979) Method for measuring rates of NH_4^+ turnover in anoxic marine sediments using a $^{15}NH_4^+$ dilution technique. Appl Environ Microbiol 37: 760–765
3. Bode, A, Dortch, Q (1996) Uptake and regeneration of inorganic nitrogen in coastal waters influenced by the Mississippi River: spatial and seasonal variations. J Plankton Res 18: 2251–2268
4. Boissonneault-Cellineri, KR, Mehta, M, Lonsdale, DJ, Caron, DA (2001) Microbial food web interactions in two Long Island embayments. Aquat Microb Ecol 26: 139–155
5. Caperon, J, Schell, D, Hirota, J, Laws, E (1979) Ammonium excretion rates in Kaneohe Bay, Hawaii, measured by a ^{15}N isotope dilution technique. Mar Biol 54: 33–40
6. Caron, DA (2001) "Protistan herbivory and bacterivory." In: Paul, JH (Ed.) *Marine Microbiology*. Methods Microbiol 30:289–315
7. Caron, DA, Sanders, RW, Lim, EL, Marrase, C, Amaral, LA, Whitney, S, Aoki, RB, Porter, KG (1993) Light-dependent phagotrophy in the freshwater mixotrophic chrysophyte. Dynobrion cylindricum 25: 93–111
8. Carrick, HJ, Fahnenstiel, GL (1990) Planktonic protozoa in Lakes Huron and Michigan: Seasonal abundance and composition of ciliates and dinoflagellates. J Great Lakes Res 16: 319–329
9. Carrick, HJ, Fahnenstiel, GL, Stoermer, EF, Wetzel, RG (1991) The importance of zooplankton–protozoan trophic couplings in Lake Michigan. Limnol Oceanogr 36: 1335–1345
10. Carrick, HJ, Fahnenstiel, GL, Taylor, WD (1992) Growth and production of planktonic protozoa in Lake Michigan: *in situ* versus in vitro comparisons and importance to food web dynamics. Limnol Oceanogr 37: 1221–1255
11. Cotner, JB, Biddanda, BA (2002) Small players, large role: microbial influence on biogeochemical processes in pelagic aquatic ecosystems. Ecosystems 5: 105–121
12. Dham, VV, Heredia, AM, Wafar, S, Wafar, M (2002) Seasonal variation in uptake and *in situ* regeneration of nitrogen in mangrove waters. Limnol Oceanogr 47: 241–254
13. Dolan, JR, Gallegos, CL (2001) Estuarine diversity of tintinnids (planktonic ciliates). J Plankton Res 23: 1009–1027
14. Fahnenstiel, GL, Krause, AE, McCormick, MJ, Carrick, HJ, Schelske, CL (1998) Structure of the planktonic food web in the St. Lawrence Great Lakes. J Great Lakes Res 23: 531–554
15. Fahnenstiel, GL, McCormick, MJ, Lang, GA, Redalje, DG, Lohrenz, SE, Markowitz, M, Wagoner, B, Carrick, HJ (1995) Taxon-specific growth and loss rates for dominant phytoplankton populations from the northern Gulf of Mexico. Mar Ecol Progr Ser 117: 229–39
16. Ferguson, RL, Buckley, EN, Palumbo, AV (1984) Response of marine bacterioplankton to differential filtration and confinement. Appl Environ Microbiol 47: 49–55
17. Foissner, W, Berger, H, Schaumburg, J (1999) Identification and ecology of limnetic plankton ciliates. Landesamtes für Wasserwirtschaft, Heft 3/99, Munich, pp 1–739
18. Froneman, PW (2002) Trophic cascading in an oligotrophic temperate estuary, South Africa. J Plankton Res 24: 807–816
19. Gallegos, CL, Vant, WN, Safi, KA (1996) Microzooplankton grazing of phytoplankton in Manukau Harbor, New Zealand. N Z J Mar Fresh Res 30: 423–434
20. Gardner, WS, Bootsma, HA, Evans, C, St. John, PA (1995) Improved chromatographic analysis of ^{15}N:^{14}N ratios in ammonium or nitrate for isotope addition experiments. Mar Chem 48: 271–282
21. Gardner, WS, Lavrentyev, PJ, Cavaletto, JF, McCarthy, MJ, Eadie, BJ, Johengen, TH, Cotner, JB (2004) Distribution and dynamics of nitrogen and microbial plankton in southern Lake Michigan during spring transition 1999-2000. J Geophys Res-Oceans 109 (C3): art. no. C03007
22. Glibert, PM (1988) Primary productivity and pelagic nitrogen cycling. In: Blackburn, TH, Sørensen, J (Eds.) Nitrogen Cycling in Coastal Marine Environments, Wiley, Chichester, UK, pp 3–31
23. Glibert, PM, Goldman, JC, Carpenter, EJ (1982) Seasonal variations in the utilization of ammonium and nitrate by phytoplankton in Vineyard Sound, Massachusetts, USA. Mar Biol 70: 237–249
24. Godhantaraman, N, Uye, S (2003) Geographical and seasonal variations in taxonomic composition, abundance and biomass of microzooplankton across a brackish-water lagoonal system of Japan. J Plankton Res 25: 465–482
25. Hernandez, I, Hwang, SJ, Heath, RT (1996) Measurement of phosphomonoesterase activity with a radiolabelled glucose-6-phosphate. Role in the phosphorus requirement of phytoplankton and bacterioplankton in a temperate mesotrophic lake. Arch Hydrobiolog 137: 265–280
26. Hwang, SJ, Heath, RT (1997) Bacterial productivity and protistan bacterivory in coastal and offshore communities of Lake Erie. Can J Fish Aquat Sci 54: 788–799
27. Iriarte, A, Madariaga, I, Revilla, M, Sarobe, A (2003) Short-term variability in microbial food web dynamics in a shallow tidal estuary. Aquat Microb Ecol 31: 145–161
28. Jochem, FJ (2001) Morphology and DNA content of bacterioplankton in the northern Gulf of Mexico: analysis by epifluorescence microscopy and flow cytometry. Aquat Microb Ecol 25: 179–194
29. Joint, I, Henriksen, P, Fonnes, GA, Bourne, D, Thingstad, TF, Riemann, B (2002) Competition for inorganic nutrients between phytoplankton and bacterioplankton in nutrient manipulated mesocosms. Aquat Microb Ecol 29: 145–159
30. Kepner, RL, Pratt, JR (1996) Characterization of surface-associated protozoan communities in a Lake Erie coastal wetlands (Old Woman Creek, Ohio). J Great Lakes Res 22: 63–76
31. Klarer, DM, Millie, DF (1992) Aquatic macrophytes and algae at Old Woman Creek estuary and other Great Lakes coastal wetlands. J Great Lakes Res 18: 622–633
32. Klarer, DM, Millie, DF (1994) Regulation of phytoplankton dynamics in a Laurentian Great Lakes estuary. Hydrobiologia 286: 97–108
33. Kopylov, AI, Moiseev, EV (1983) Reproduction rate and production of zooflagellates in the northeastern part of the Black Sea. Okeanologya 23: 640–643, [In Russian with English summary]
34. Krebs, CJ (1989) Ecological Methodology. Harper-Collins, New York
35. Lalli, CM, Parsons, TR (1997) Biological Oceanography: An Introduction. Butterworth-Heinemann, Oxford
36. Landacre, FL (1908) The protozoa of Sandusky Bay and vicinity. Proc Ohio Acad Sci 4: 1–68
37. Landry, MR (1994) Methods and controls for measuring the grazing impact of planktonic protists. Mar Microb Food Webs 8: 37–57
38. Landry, MR, Hassett, RP (1982) Estimating the grazing impact of marine micro-zooplankton. Mar Biol 67: 283–288
39. Lavrentyev, PJ, Bootsma, HA, Johengen, TH, Cavaletto, JF, Gardner, WS (1998) Microbial plankton response to recourse limitation: insights from the community structure and seston stoichiometry in Florida Bay, USA. Mar Ecol Prog Ser 165: 45–57
40. Lavrentyev, PJ, Gardner, WS, Cavaletto, JF, Beaver, JR (1995) Effects of the zebra mussel (*Dreissena polymorpha* Pallas) on protozoa and phytoplankton in Saginaw Bay, Lake Huron. J Great Lakes Res 21: 545–557
41. Lavrentyev, PJ, Gardner, WS, Johnson, JR (1997) Cascading trophic effects on aquatic nitrification: experimental evidence and potential implications. Aquat Microb Ecol 13: 161–175
42. Leadbeater , BSC (1993) Preparation of pelagic protists for electron microscopy. In: Kemp, PF, Sherr, BF, Sherr, EB, Cole, JJ (Eds.)

Handbook of Methods in Aquatic Microbial Ecology, Boca Raton, Lewis, pp 241–252
43. LeBlanc, JS, Taylor, WD, Johannsson, OE (1997) The feeding ecology of the cyclopoid copepod *Diacyclops thomasi* in Lake Ontario. J Great Lakes Res 23: 369–381
44. Lehrter, JC, Pennock, JR, McManus, GB (1999) Microzooplankton grazing and nitrogen excretion across a surface estuarine–coastal interface. Estuaries 22: 113–125
45. Lewitus, AJ, Koepfler, ET, Morris, JT (1998) Seasonal variation in the regulation of phytoplankton by nitrogen and grazing in a salt-marsh estuary. Limnol Oceanogr 43: 636–646
46. Loferer-Krößbacher, M, Klima, J, Psenner, R (1998) Determination of bacterial cell dry mass by transmission electron microscopy and densitometric image analysis. Appl Environ Microbiol 64: 688–694
47. Menden-Deuer, S, Lessard, EJ (2000) Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. Limnol Oceangor 54: 569–579
48. Montagnes, DJS, Berges, JA, Harrison, PJ, Taylor, JFR (1994) Estimating carbon, protein, and chlorophyll a from volume in marine phytoplankton. Limnol Oceanogr 39: 1044–1060
49. Müller, H, Geller, W (1993) Maximum growth rates of ciliated protozoa: the dependence on body size and temperature reconsidered. Arch Hydrobiol 126: 315–327
50. Murrel, MC, Hollibaugh, JT (1998) Microzooplankton grazing in northern San Francisco Bay measured by the dilution method. Aquat Microb Ecol 15: 53–63
51. Nielsen, TG, Kiørbe, T (1991) Effects of a storm event on the structure of the pelagic food web with special emphasis on planktonic ciliates. J Plankton Res 13: 35–51
52. Park, GS, Marshall, HG (2000) The trophic contributions of rotifers in tidal freshwater and estuarine habitats. Estuar Coast Shelf Sci 51: 729–742
53. Pernie, GL, Scavia, D, Pace, ML, Carrick, HJ (1990) Micrograzer impact and substrate limitation of bacterioplankton in Lake Michigan. Can J Fish Aquat Sci 47: 1836–1841
54. Putt, M, Stoecker, DK (1989) An experimentally determined carbon: volume ratio for marine oligotrichous ciliates from estuarine and coastal waters. Limnol Oceanogr 34: 177–183
55. Roman, MR, Holliday, DV, Sanford, LP (2001) Temporal and spatial patterns of zooplankton in the Chesapeake Bay turbidity maximum. Mar Ecol Prog Ser 213: 215–227
56. Rothhaupt, KO (1997) Nutrient turnover by freshwater bacterivorous flagellates: differences between a heterotrophic and a mixotrophic chrysophyte. Aquat Microb Ecol 12: 65–70
57. Sanders, RW, Caron, DA, Davidson, JM, Dennett, MR, Moran, DM (2001) Nutrient acquisition and population growth of a mixotrophic alga in axenic and bacterized cultures. Microb Ecol 42: 513–523
58. Schelske, CL, Carrick, HJ, Aldridge, FJ (1995) Can wind-induced resuspension of meroplankton affect phytoplankton dynamics? J N Am Benthol Soc 14: 616–630
59. Sheldon, RW, Nival, P, Rassoulzadegan, F (1986) An experimental investigation of fiagellate-ciliate-copepod food chain with some observations relevant to the linear biomass hypothesis. Limnol Oceanogr 31: 184–188
60. Sherr, EB, Caron, DA, Sherr, BF (1993) Staining of heterotrophic protists for visualization via epifluorescence microscopy. In: Kemp, PF, Sherr, BF, Sherr, EB, Cole, JJ (Eds.) Handbook of Methods in Aquatic Microbial Ecology, Lewis, Boca Raton, FL, pp 213–228
61. Sherr, EB, Rassoulzadegan, F, Sherr, BF (1989) Bacterivory by pelagic choreotrichous ciliates in coastal waters of the Mediterranean Sea. Mar Ecol Progr Ser 55: 235–240
62. Sherr, EB, Sherr, BF (2000) Marine microbes: an overview. In: Kirchman, DL (Ed.) Microbial Ecology of the Oceans, Wiley, New York, pp 13–46
63. Sieracki, CK, Sieracki, ME, Yentch, CS (1998) An imaging-in-flow system for automated analysis of marine microplankton. Mar Ecol Prog Ser 168: 285–296
64. Skibbe, O (1994) An improved quantitative protargol stain for ciliates and other planktonic protists. Arch Hydrobiol 130: 339–347
65. Strom, S (2000) Bacterivory: interactions between bacteria and their grazers. In: Kirchman, DL (Ed.) Microbial Ecology of the Oceans, Wiley, New York, pp 351–386
66. Taylor, WD, Heynen, ML (1987) Seasonal and vertical distribution of Ciliophora in Lake Ontario. Can J Fish Aquat Sci 44: 2185–2191
67. Tupas, L, Koike, I (1991) Simultaneous uptake and regeneration of ammonium by mixed assemblages of heterotrophic marine bacteria. Mar Ecol Prog Ser 70: 273–282
68. Turner, JT, Roff, JC (1993) Trophic levels and trophospecies in marine plankton: lessons from the microbial food web. Mar Microb Food Web 7: 225–248
69. Weisse, T (1991) The annual cycle of heterotrophic freshwater nanoflagellates: role of bottom-up versus top-down control. J Plankton Res 13: 167–185
70. Welschmeyer, NA (1994) Fluorometric analysis of chlorophyll-a in the presence of chlorophyll-b and pheopigments. Limnol Oceanogr 39: 1985–1992
71. Wetzel, RG (1992) Wetlands as metabolic gates. J Great Lakes Res 18: 529–532
72. Yin, KD, Goldblatt, RH, Harrison, PJ, St John, MA, Clifford, PJ, Beamish, RJ (1997) Importance of wind and river discharge in influencing nutrient dynamics and phytoplankton production in summer in the central Strait of Georgia. Mar Ecol Prog Ser 161: 173–183