

Net heterotrophy in productive prairie wetlands with high DOC concentrations

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ABSTRACT: Millions of productive wetlands dot the North American Great Plains. Although these wetlands are key ecological features of the Canadian prairies, their microbial food webs remain relatively unstudied. Over 3 yr, pelagic primary (PP) and bacterial production (BP) and biomass were monitored in 2 wetlands in Central Saskatchewan, Canada. PP ranged from 8.8 to 3911.0 mg C m⁻³ d⁻¹ (\bar{x} = 612.0 mg C m⁻³ d⁻¹), while rates of BP ranged from 8.2 to 678.0 mg C m⁻³ d⁻¹ (\bar{x} = 140.0 mg C m⁻³ d⁻¹). Nutrients, light and temperature did not appear as major factors influencing these rates. Seasonal mean ratios of PP:BP revealed that both wetlands were net autotrophic (\bar{x} PP:BP = 7.4; range 0.08 to 42.6), which was not surprising given their eutrophic status. On a smaller temporal scale, these wetlands were, on average, net heterotrophic (PP:BP < 1) on 33% of the sampling dates. High pelagic bacterial carbon demand (greater than twice the phytoplankton carbon production) indicated that bacterial metabolism was not dependent on autochthonous carbon sources. Biological availability of dissolved organic carbon (DOC) was low in both wetlands (\bar{x} = 3.8%). But this percentage of high ambient concentrations was enough to satisfy bacterial carbon demand. Based on seasonal averages, BP and bacterial numbers decreased across increasing trophic gradients (as chl *a* or total phosphorus). This phenomenon could also be observed across a seasonal gradient of changing chl *a* concentrations. Prairie wetlands not only experience periods of net heterotrophy, but bacterial carbon demand and production per unit biomass are high, suggesting an important role for bacteria in the metabolism of these eutrophic ecosystems.

KEY WORDS: Heterotrophy · DOC · Autotrophy · Primary production · Bacterial production · Prairie wetlands

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INTRODUCTION

Prairie wetlands are key ecological components of the North American prairie region. Highly productive and shallow (<1.0 m), these water bodies support 50 to 80% of the continent's waterfowl population in any given year (LaBaugh et al. 1996). Typically, they have dissolved organic carbon (DOC) concentrations >10 mg l⁻¹ and sometimes >100 mg l⁻¹ (Arts et al. 2000). They are also characterized by high concentrations of nitrogen and phosphorus (Waiser 2001a). Despite their ecological importance, we know relatively little about overall ecosystem metabolism in these wetlands.

In aquatic ecosystems, photosynthesis (primary production; PP) and respiration are the major anabolic and catabolic pathways, respectively (Cole et al. 2000). The

predominance of one or the other determines whether an aquatic system will store, export or break down organic matter. When gross PP (GPP) exceeds respiration (R), the system is net autotrophic, and when R exceeds GPP, net heterotrophy prevails (Cole et al. 2000). Aquatic systems with high DOC concentrations and low total phosphorus (TP) concentrations tend to be net heterotrophic, while those exhibiting low DOC and high TP tend towards net autotrophy (Cole et al. 2000, Hanson et al. 2003). Recent studies have also indicated that DOC concentrations below 10 mg l⁻¹ favour net autotrophy (Prairie et al. 2002, Hanson et al. 2003). However, there are systems, like prairie wetlands, which fall outside the boundaries of either the high DOC–low nutrient or low DOC–high nutrient scenarios. Unfortunately, our ability to evaluate which

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processes dominate in these wetlands has been hampered by the lack of estimates of either respiration or bacterial and algal production.

In freshwater and marine environments, bacterial numbers and production (BP) do not increase as rapidly as algal biomass across a trophic gradient of increasing nutrient concentrations (Bird & Kalff 1984, Sommaruga & Robarts 1997, Biddanda et al. 2001). Factors contributing to this observed reduction in bacterial numbers include increasing concentrations of particulate organic carbon (POC), elevated particle sinking rates, increased bacterivory and decreased excretion of organic carbon (Cotner & Biddanda 2002). As a result, the importance of bacteria, relative to phytoplankton, is thought to recede across an ascending trophic gradient. In one cross system study, however, BP per unit biomass increased across an increasing trophic gradient (Sommaruga & Robarts 1997). This observation lent support to the hypothesis, originally set forth by Bird & Kalff (1984), that bacteria are important in the metabolism of eutrophic ecosystems. But relatively few studies have examined the quantitative importance of bacteria in highly productive aquatic ecosystems, like shallow prairie wetlands. Consequently, our knowledge of the microbial ecology of such eutrophic systems has advanced only minimally (Sommaruga & Robarts 1997).

This study had 2 major objectives. The first was to ascertain whether prairie wetlands were net auto- or heterotrophic based on seasonal means of PP:BP (Jansson et al. 2000) and to assess, on shorter time scales, whether there was any evidence for periods of net heterotrophy. In order to do this, we monitored seasonal variations in pelagic algal and bacterial production rates and biomass in 2 prairie wetlands over a 3 yr period. Secondly, we wanted to establish the importance of bacterial processes in these highly productive ecosystems. Consequently, bacterial numbers, BP, and BP per unit biomass were examined across an increasing trophic gradient expressed as either chl *a* or TP. The information generated would allow some insight into the significance of bacterial processes to the metabolism of these highly productive aquatic ecosystems, and add to our sparse knowledge of their microbial ecology.

MATERIALS AND METHODS

Study sites. The St. Denis National Wildlife Area, 40 km east of Saskatoon, Saskatchewan, Canada (106° 06' W, 52° 02' N), is located within the mixed-grass prairie-parkland ecotone, covering 385 ha in the rolling morainic topography of the Minichinas Hills (Driver & Peden 1977). This area is unique in that it contains a high proportion of untilled land, in contrast to the sur-

rounding cultivated land. It is underlain by approximately 100 m of low permeability, clay-rich glacial tills from which dark brown orthic soil (containing 10 to 40% of B horizon material) has developed (van der Kamp et al. 1999). There are more than 100 wetlands within the St. Denis Wildlife Area, ranging from ephemeral to permanent, and freshwater to saline.

Two wetlands, one highland and one lowland, were chosen as research sites for this study. Pond 50 at 31 000 m² in area, is a centrally drained, semi-permanent lowland wetland encircled by marsh. Water levels fluctuate widely on a yearly basis and are driven by evaporative processes. Although filled with water most years, the pond may completely disappear depending on spring water inputs from snowmelt and runoff (Woo & Rowsell 1993). During the course of this study (1998 to 2000) there was always water in Pond 50, although levels declined from May through October in all years. Pond 50 is alkaline (\bar{x} pH = 8.7 ± 0.5; ± values given after mean are SD unless otherwise noted), saline (total dissolved solids, TDS, \bar{x} = 4.0 g l⁻¹ data from 1993 to 1996) and the seasonal mean value for chl *a* is 8.3 µg l⁻¹ (Waiser 2001b). Major cations, Ca, Mg and Na, average 162 (±52.7), 1208 (±569), and 800 (±357) mg l⁻¹, respectively, while major anions, K, Cl and SO₄ average 158 (±71.6), 161 (±1.2) and 6321 (±3004.7) mg l⁻¹, respectively (M.J.W. unpubl. data). Two sampling sites were chosen at Pond 50; one centrally located in the open water (sampled from early May to mid-October in 1998, 1999 and 2000) and the other at a shallow reed-bed site adjacent to the shore (sampled in 2000 from late May until the end of July when the site dried up).

Pond 109 varies in area from 1000 to 5000 m² and is located in undulating upland underlain by glacial till of the Battleford and Floral formations (Hayashi 1996). Wetland margin vegetation consists of silver willow *Salix alba argentea*, trembling aspen *Populus tremuloides*, and balsam poplar *Populus balsamifera*, while sedge *Carex* spp. and spike rush *Eleocharis* spp. dominate the wetland proper. Agricultural land totally surrounds this small wetland. Pond 109 is ephemeral, usually disappearing by late August or earlier, depending on the extent of snowmelt and summer precipitation. The period of inundation (defined as the number of months for which water is present at the center) varied between 0 and 8 mo for the interval 1980 to 1996 with an average of 3.6 mo (Hayashi 1996). During this study, Pond 109 disappeared at the end of July in 1998 and 1999. In 2000, as a result of low snowfall and runoff, Pond 109 filled only briefly in the spring. The majority of seasonal water loss in this pond is due to infiltration (Hayashi 1996). In contrast to Pond 50, Pond 109 is a freshwater (TDS 0.23 g l⁻¹), slightly alkaline (\bar{x} pH = 7.4 ± 0.4) wetland with higher seasonal mean concentrations of chl *a* (44.3 µg l⁻¹) (Waiser

2001b). The major cations Ca, Mg and Na average 14.6 (± 5.0), 11.3 (± 1.3), and 17.7 (± 5.1) mg l⁻¹, respectively, while the major anions K, Cl and SO₄ average 26.4 (± 5.8), 3.5 (± 1.2) and 10.8 (± 3.4) mg l⁻¹, respectively (Waiser 2001b). Fish were not present in either pond.

Water collection. Water for all variables measured was collected from the open water of each pond using a clean plastic bucket. This water was subsequently screened through 153 μ m Nitex mesh to remove large zooplankters.

Chemical measurements. Every 2 wk, water samples were collected (as above) for nutrient analyses including TP, soluble reactive phosphorus (SRP), ammonium nitrogen (NH₄), nitrate-nitrite nitrogen (NO₂+NO₃), DOC, and particulate organic carbon and nitrogen (POC, PON). Samples were prepared and filtered according to methods in Environment Canada (1992), placed on ice, and transported to the lab. Inorganic nutrients were analysed using Technicon AutoAnalyzers, while a CHN analyzer was used for organic nutrients (Environment Canada 1992).

Temperature. Surface water temperature was measured between 10:00 and 11:00 h on each sampling date using a Cole-Parmer portable thermistor (Cole Parmer). The maximum time between temperature measurement on each day at each pond was 30 min. In 2000, 3 Onset Optic Stowaway temperature loggers (temperature range -4 to +37°C; Onset Computer Corp.) were placed in Pond 50 in order to monitor temperatures at various depths throughout the ice-free season. Loggers were mounted on a stake which had been driven into the sediments near the center of the pond. Pond depth at this location was approximately 25 cm and loggers were positioned just under the surface and then at 10 and 20 cm (surface, middle and just above the bottom of the pond). Temperature data were logged at half hour intervals.

Partial pressure CO₂ (PCO₂). PCO₂ was calculated using the United States Geological Survey software program NETPATH (an interactive program for calculating net geochemical reactions; available at <http://water.usgs.gov/software/netpath.html>). Temperature, alkalinity, and pH data collected for this study and major ion data from Waiser (2001b) were used as the input variables.

Chl a. Aliquots of water from both ponds were drawn through 47 mm GF/C filters (Whatman; nominal pore-size 1.2 μ m) for subsequent chl *a* analyses. Filters were placed in plastic Petri dishes, wrapped in foil and transported on ice back to the lab. Chl *a* was extracted using 90% boiling ethanol and analysed fluorometrically using a Turner Designs Model 10-AU digital fluorometer (Waiser & Robarts 1997).

PP. Pelagic PP was measured *in situ* every week during the ice-free season for 3 yr (1998 to 2000) in Pond

50 and 2 yr in Pond 109 (1998 to 1999). pH and temperature were measured using a Cole-Parmer portable meter, and total alkalinity determined by titration with 0.1 N HCl to an end point of pH 4.5 (Robarts et al. 1992). For PP measurements, aliquots of screened water were dispensed into 1 dark and 2 light bottles and approximately 400 μ l of NaH¹⁴CO₃ (0.26 MBq) added to each bottle. Bottles were clipped to a plexiglas disk and then incubated *in situ* at approximately 10 cm depth for 4 h (10:00 to 14:00 h). Following injection of the bottles, aliquots of the ¹⁴C solution were pipetted into a CO₂ trapping agent to verify ¹⁴C concentration added to the bottles. At the end of incubation, bottles were placed in a light-tight case until filtration. Samples were filtered through 0.45 μ m pore-size Sartorius cellulose nitrate filters under gentle vacuum. Filters were then arranged in scintillation vials containing 500 μ l of 1 N HCl and placed, uncapped, in a fume hood overnight to remove unincorporated ¹⁴C (Robarts et al. 1992). The next day, filters were dissolved in 10 ml of Filter Count and counted on a Canberra Packard 1900 CA scintillation counter. Volumetric rates of PP (mg C m⁻³ h⁻¹) were calculated from ¹⁴C incorporation rates and ¹²C concentrations (from temperature, pH and alkalinity data) available to phytoplankton (Robarts et al. 1992). Daily rates were estimated by multiplying hourly rates by 10 (Cole et al. 1988).

Bacterial numbers (BN). At each sampling time, 2 \times 10 ml aliquots of screened water were placed into sterile 20 ml Vacutainer tubes and preserved with 200 μ l of Lugol's solution. Subsequently, bacteria were stained using DAPI and counted using an epifluorescent microscope (Waiser 2001b).

BP. Measurements of BP were conducted at the same time as PP. For these estimates, 15 nM [methyl]-³H thymidine (TdR) was added to each of 3, 10 ml samples (2 live and 1 control). Controls were prepared by addition of 500 μ l of formaldehyde followed by 500 μ l of 5 N NaOH (Waiser 2001b). Tubes containing the samples were fixed onto a Plexiglas disk and incubated *in situ* for 30 min. Previous experiments showed that 15 nM TdR saturated, but did not inhibit, bacterial uptake in the 2 ponds and that uptake was linear over the 30 min incubation period (data not shown). At the end of 30 min, live samples were killed in the same manner as controls. All were placed on ice for transport to the lab. DNA was extracted according to the method of Robarts & Wicks (1989). The amount of TdR incorporated into DNA was counted using a Canberra Packard 1900 CA liquid scintillation spectrometer.

Thymidine incorporation was converted into bacterial cell production using a conversion factor of 2.0 \times 10¹⁸ bacterial cells produced per mole TdR (Coveney & Wetzel 1988). Conversion factors for freshwater and marine systems vary from 1.0 \times 10¹⁷ to 60 \times 10¹⁸ cells

produced per mole TdR (Robarts & Zohary 1993). Our choice of this particular conversion factor is therefore a conservative one. A factor of 20 fg C per bacterial cell was then used to convert cell numbers to an estimate of carbon produced (Lee & Fuhrman 1987, Reitner et al. 1999). Because the carbon content of most bacterial cells is in the 10 to 20 fg C cell⁻¹ (Cotner & Biddanda 2002), we chose the upper number as more representative of carbon content of bacterial cells in eutrophic ecosystems. Daily volumetric rates were estimated by multiplying hourly rates by 24 (Cole et al. 1988).

Ratios of PP:BP were calculated on the basis of estimated daily volumetric rates (Jansson et al. 2000). For calculation of bacterial carbon demand, BP measurements were corrected using a 35% growth efficiency factor. Bacterial growth efficiency (BGE) increases across a trophic gradient from 10% in oligotrophic to 40% in the most productive systems (del Giorgio et al. 1997). The choice

Table 1. Means, SDs and ranges in concentrations of chl *a*, total P (TP), soluble reactive P (SRP), nitrate plus nitrite N (NO₂+NO₃), ammonium N, total dissolved N (TDN), particulate organic N and C (PON and POC respectively), dissolved organic C (DOC), total dissolved solids (TDS) and values for alkalinity and pH in Ponds 50 and 109. Data presented are means from 3 yr in Pond 50 (1998–2000) and 1 yr in Pond 109 (1999). All concentrations are in µg l⁻¹, except for DOC (mg l⁻¹) and TDS (g l⁻¹). Alkalinity is expressed as mg l⁻¹ CaCO₃

	Pond 50		Pond 109	
	Mean ± SD	Range	Mean ± SD	Range
TP	116.4 ± 37.8	81–277	3285 ± 78.7	2260–3950
SRP	11.6 ± 8.3	3–40	3030 ± 642	2220–3670
NO ₂ +NO ₃	10.4 ± 1.83	10–19	12.7 ± 4.2	10–19
NH ₄	163.3 ± 78.9	50–390	107 ± 39.9	65–160
TDN	4819 ± 2018	525–9570	2223 ± 179	1920–2450
POC	1198 ± 1177	322–5320	2312 ± 1606	400–7000
PON	176 ± 197	110–838	522 ± 491	110–1950
DOC	61.8 ± 21.9	21–120	25.0 ± 2.2	23.8–27.9
pH	8.7 ± 0.5	8.1–9.7	7.4 ± 0.4	6.9–8.2
Alkalinity	590 ± 147	405–830	181 ± 51.5	70–240
Chl <i>a</i>	8.3 ± 7.7	1.2–34.8	44.3 ± 59.8	3.2–268
TDS	3.7 ± 1.7	0.8–7.7	0.2 ± 0.1	0.2–0.7

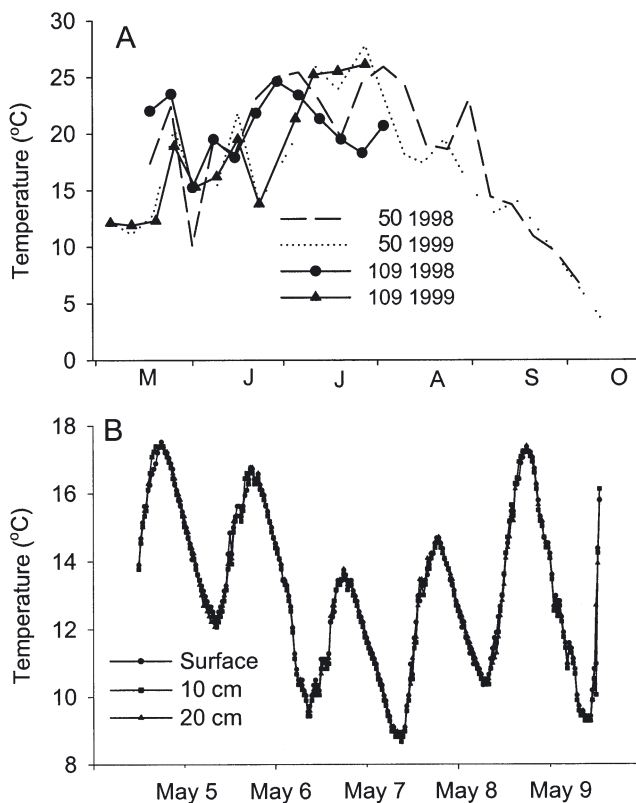


Fig. 1. (A) Seasonal variation in temperature at Ponds 50 and 109 for 1998 to 1999 and (B) diurnal variation in temperature at 3 depths in Pond 50 in May 2000

of a 35% BGE appears to be warranted for our wetlands, but the possibility remains that it could be lower. A lower BGE would have the effect of increasing our estimates for bacterial carbon demand. Bacterial numbers and production were normalized to chl *a* concentration for each sample date. Normalized numbers were then plotted against either chl *a* or TP in order to establish whether numbers and production decreased across an increasing trophic gradient (cf. Biddanda et al. 2001). BP was also normalized to bacterial biomass (BN were converted to biomass using a factor of 20 fg C per cell; see above). Means were then calculated and plotted against chl *a* concentration. This was done in an effort to establish if BP per unit biomass increased or decreased across an increasing trophic gradient (cf. Sommaruga & Robarts 1997).

RESULTS

Temperature and inorganic nutrients

Both ponds warmed up quickly following ice-out in the spring (Fig. 1A). For example, temperatures in both ponds by mid-May averaged 12.5°C over the study period. Warmest water temperatures were experienced between mid-June and the end of August and averaged 21.2°C (±3.4). Fall temperatures in Pond 50 (September to October) averaged 11.9°C (±5.0) over the 3 yr study period. Temperature data collected at 3 depths in Pond 50 indicated that this pond did not thermally stratify (Fig. 1B).

Nutrient data were pooled and averaged over a 3 yr period for Pond 50 (1998 to 2000), but only over 1 yr (1999) for Pond 109. Generally, concentrations of all major nutrients, except $\text{NH}_4\text{-N}$, TDN and DOC were higher in Pond 109 than Pond 50 (Table 1). Greatest differences were seen in values for TP and SRP which were 28 and 26 times higher, respectively, in Pond 109, while DOC was approximately 2.5 times higher in Pond 50. Also higher in Pond 50 were concentrations of $\text{NH}_4\text{-N}$ and TDN (Table 1).

Partial pressure of CO_2

Seasonal averages for CO_2 partial pressure in Pond 50 in 1998, 1999 and 2000 were 2790 ± 430 , 1094 ± 419 , and 355 ± 466 μATM , respectively. The value for the reed-bed site at Pond 50 in 2000 was 1265 ± 1140 μATM . In 1999, mean CO_2 partial pressure at Pond 109 was 1136 ± 178 μATM .

Chl *a*

Chl *a* concentrations were statistically higher in Pond 109 than 50 in 1998 and 1999 (Mann-Whitney rank sum test for pooled data: $p < 0.001$; $T = 1124$) (Fig. 2A,B,E,F, Table 1). In Pond 50 in 2000, mean chl *a* concentrations in the reed bed (26.6 ± 27.7 $\mu\text{g l}^{-1}$; range 3.9 to 119.9; $n = 14$) were higher than in the open water (11.9 ± 11.1 $\mu\text{g l}^{-1}$; range 1.2 to 34.8; $n = 21$) (Mann-Whitney rank sum test: $p = 0.029$; $T = 346$) (Fig. 2C,D).

In Pond 50, the highest peaks in chl *a* always occurred after the middle of July, except in 1999 when a smaller peak in phytoplankton biomass was observed in spring (May 6; Fig. 2B). Pronounced depressions in algal biomass during the first part of the year mirrored patterns in PP seen in this pond (see next section). In Pond 109, highest chl *a* concentrations always occurred just before the pond dried up: August 4 in 1998 (Fig. 2E) and July 29 in 1999 (Fig. 2F).

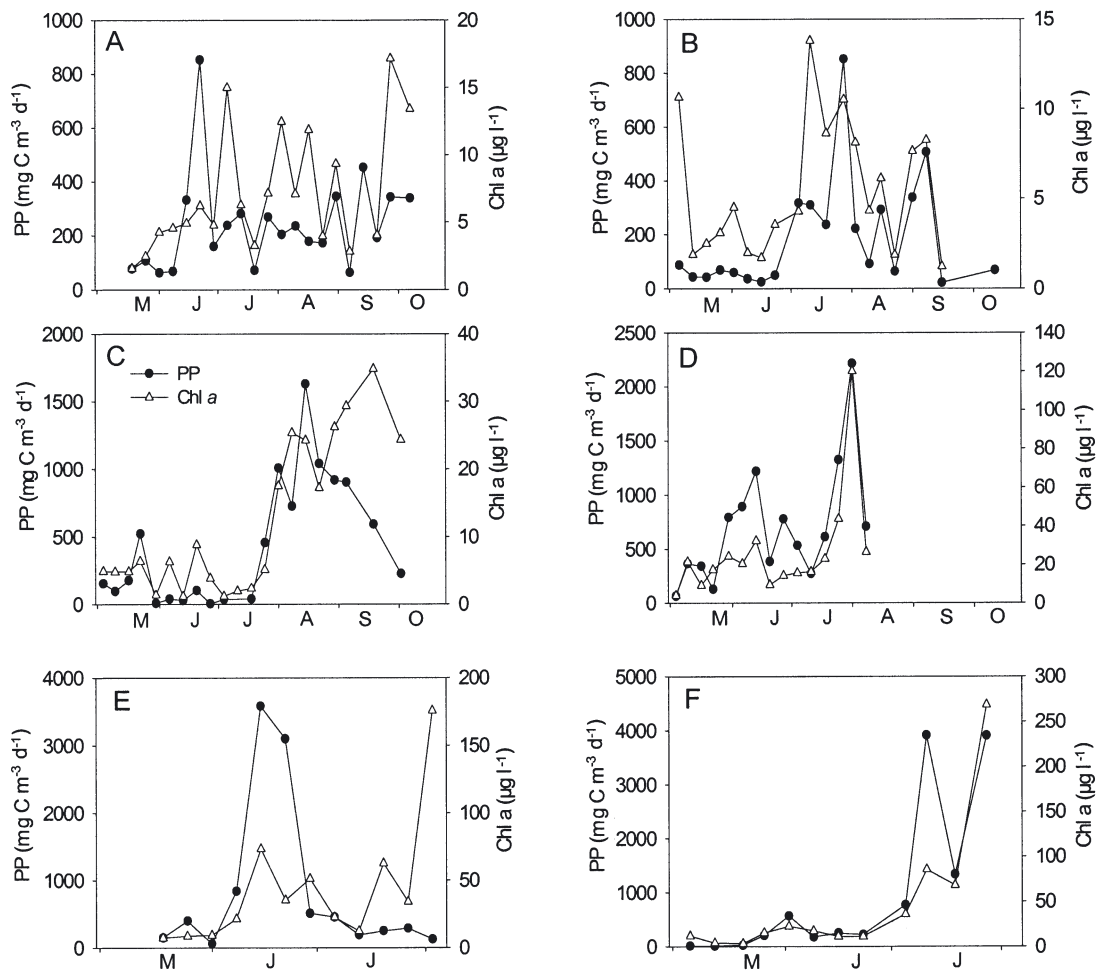


Fig. 2. Seasonal variation in primary production (PP) and chl *a* at Pond 50 in (A) 1998, (B) 1999, (C) 2000, at the reed-bed site in (D) Pond 50 in 2000, and (E) Pond 109 in 1998 and (F) in 1999

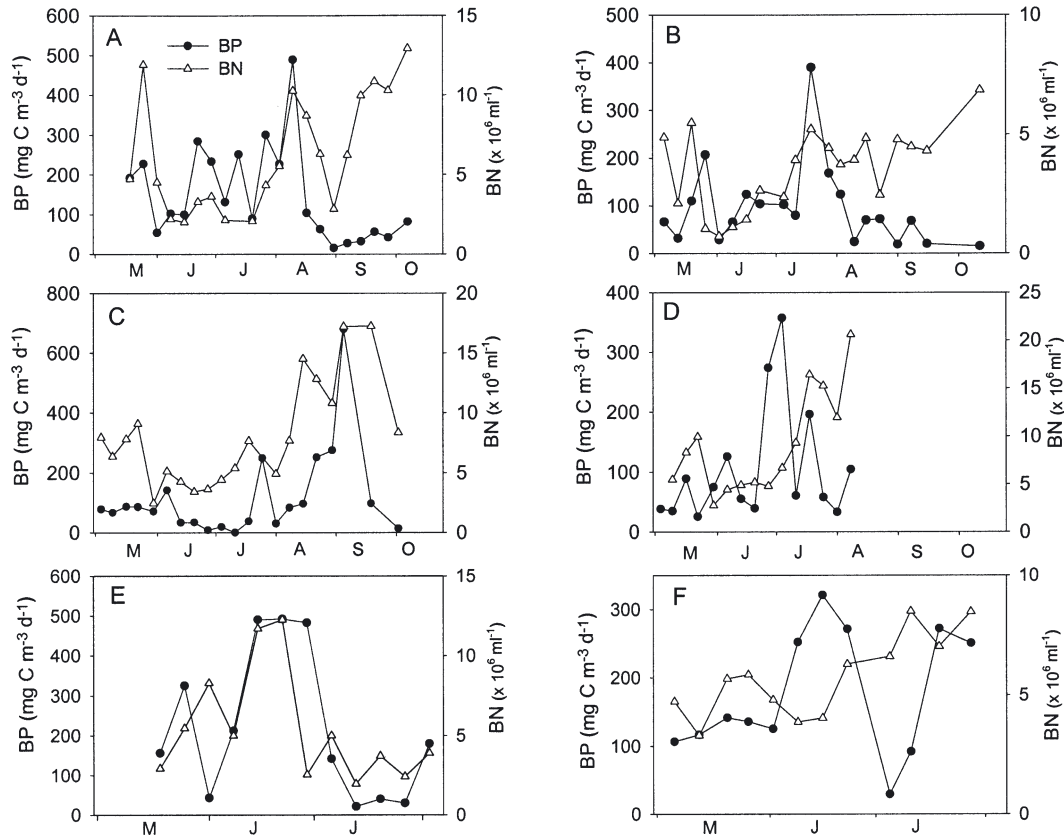


Fig. 3. Seasonal variation in bacterial production (BP) and bacterial numbers (BN) at Pond 50 in (A) 1998, (B) 1999, (C) 2000, at the reed-bed site in (D) Pond 50 in 2000, and (E) Pond 109 in 1998 and (F) in 1999

PP

Pooled data for 1998 and 1999 indicated that PP was higher in Pond 109 than Pond 50 (Mann-Whitney rank sum test: $p = 0.029$; $T = 694.5$). In Pond 50, PP at the reed bed was higher than that in the open water (Student's t -test: $p = 0.008$; $t = 2.89$) (Fig. 2C,D).

In all years, peaks in PP in Pond 50 (pelagic and reed bed) and 109 usually occurred after mid-June (Fig. 2). In 2000, however, although there was a small spring production peak in Pond 50, the highest occurred much later in the summer and lasted into early September (Fig. 2C).

BN

Over the 2 yr period, BN were higher in Pond 109 than Pond 50 (t -test: $p = 0.008$; $t = 2.75$). In 2000 there was no significant difference in mean BN between open water and reed-bed values (t -test: $p = 0.068$; $t = -1.91$) (Fig. 3C,D).

While peaks in BN in the open water of Pond 50 always occurred during the fall (Fig. 3A–C), they

occurred earlier at the reed bed site (2000; Fig. 3D). In Pond 109, the peak in BN occurred in early June 1998 (Fig. 3E) and mid-July 1999 (Fig. 3F).

BP

A rank-sum test on the pooled BP data revealed no significant difference either between the 2 ponds ($p = 0.306$; $n = 24$; $T = 638$) or between the 2 sites at Pond 50 ($p = 0.510$; $n = 16$; $T = 217$). Multiple peaks in BP were evident at all sites in all years (Fig. 3). Spring and early summer peaks in BP in both ponds were out of phase with PP peaks.

PP:BP ratios and bacterial carbon demand

Ratios of PP:BP for all years at all study sites indicated that these systems were net autotrophic (PP:BP > 1 sensu Jansson et al. 2000) (Fig. 4). Average ratios of PP:BP in Pond 50 were 3.6 ± 5.4 ($n = 21$) in 1998, 3.0 ± 4.0 ($n = 20$) in 1999, 5.6 ± 8.2 ($n = 20$) in 2000 (Fig. 4A) and 12.6 ± 17.3 ($n = 16$) in the same year in the reed bed (Fig. 4B). The same ratios in Pond 109 were $4.5 \pm$

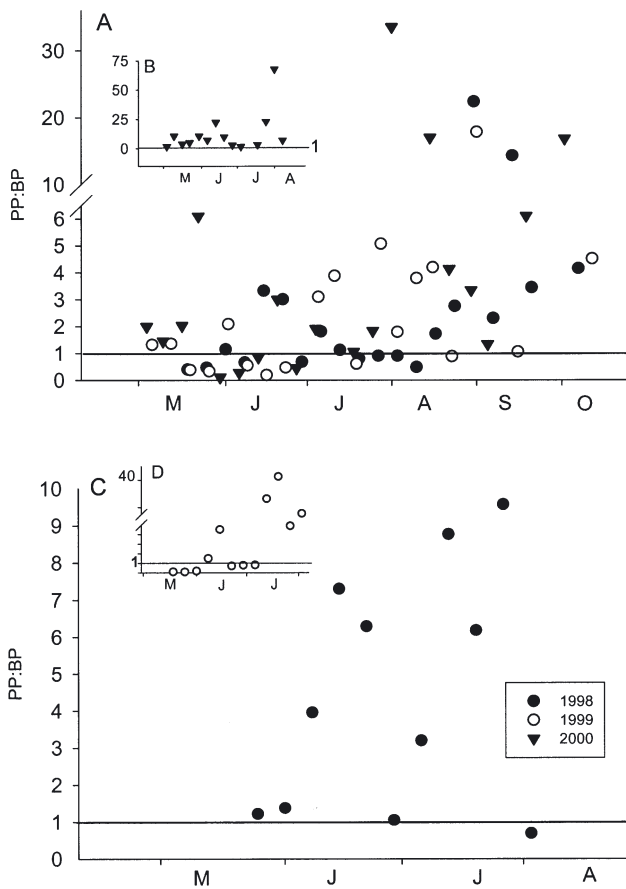


Fig. 4. Seasonal variation in the ratio of net primary production (PP) to bacterial production (BP) at (A) Pond 50 from 1998 to 2000, (B) the reed-bed site in Pond 50 in 2000 and (C) Pond 109 in 1998 and (D) 1999. Horizontal line represents the point where PP:BP equals 1.0. Ratios above this denote net autotrophy, while those below represent net heterotrophy

3.3 (1998) and 8.8 ± 13.5 in 1999 ($n = 12$ for both years) (Fig. 4C,D). Close inspection of the seasonal variation in PP:BP ratios, however, revealed that there were instances of net heterotrophy in the pelagic areas of both ponds (PP:BP < 1) (Fig. 4A,D) with the majority of these occurring before mid-July (Fig. 4A,D).

Bacterial carbon demand >100% of PP was observed in both ponds, but rarely at the reed bed site (Fig. 5A–C). Most occurrences of this phenomenon were observed before the end of July in Pond 50, and before mid-June in Pond 109 (Fig. 5A,C).

BN and BP across an increasing trophic gradient

When seasonal means were used, the ratio of BN:chl *a* decreased across an increasing trophic gradient, expressed as chl *a*, at all sites (Fig. 6A). Ratios of BN:chl *a* in Pond 50 were higher than those in Pond 109 (Mann-

Whitney rank sum: $p = 0.002$; $n = 24$; $T = 736$) and in the reed-bed site (Mann-Whitney rank sum: $p = 0.002$; $n = 13$; $T = 115$). BP and BN normalized to chl *a* decreased across the within pond seasonal trophic gradients, expressed either as chl *a* or TP (Fig. 6B–E). BP per unit biomass for all sites was high when compared to that for other aquatic ecosystems of similar trophic status (Fig. 7).

DISCUSSION

Seasonal variation in biomass and production

PP and chl *a* in the 2 study wetlands were usually depressed through spring and into mid-summer, after which time peaks were normally seen (Fig. 2). In Pond 50, this was especially evident in 1999 and 2000. This

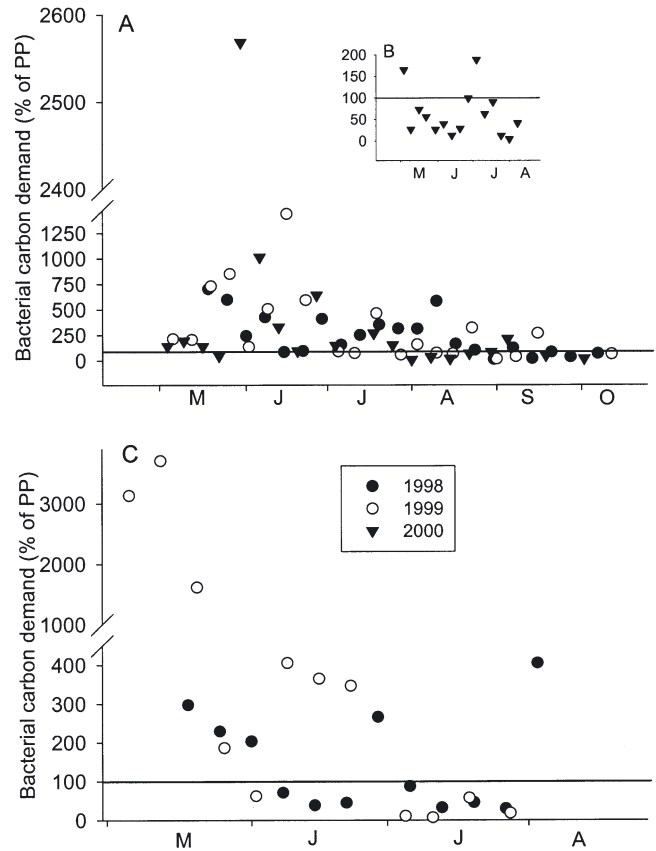


Fig. 5. Seasonal variation in bacterial carbon demand expressed as a percentage of primary production (PP) at (A) Pond 50 from 1998 to 2000, (B) the reed-bed site in Pond 50 in 2000, and (C) at Pond 109 from 1998 to 1999. Horizontal line on each graph represents the point at which bacterial carbon demand is equivalent to PP. Points above the line represent times when bacterial carbon demand exceeds PP, while points below the line denote times when PP exceeds bacterial carbon demand

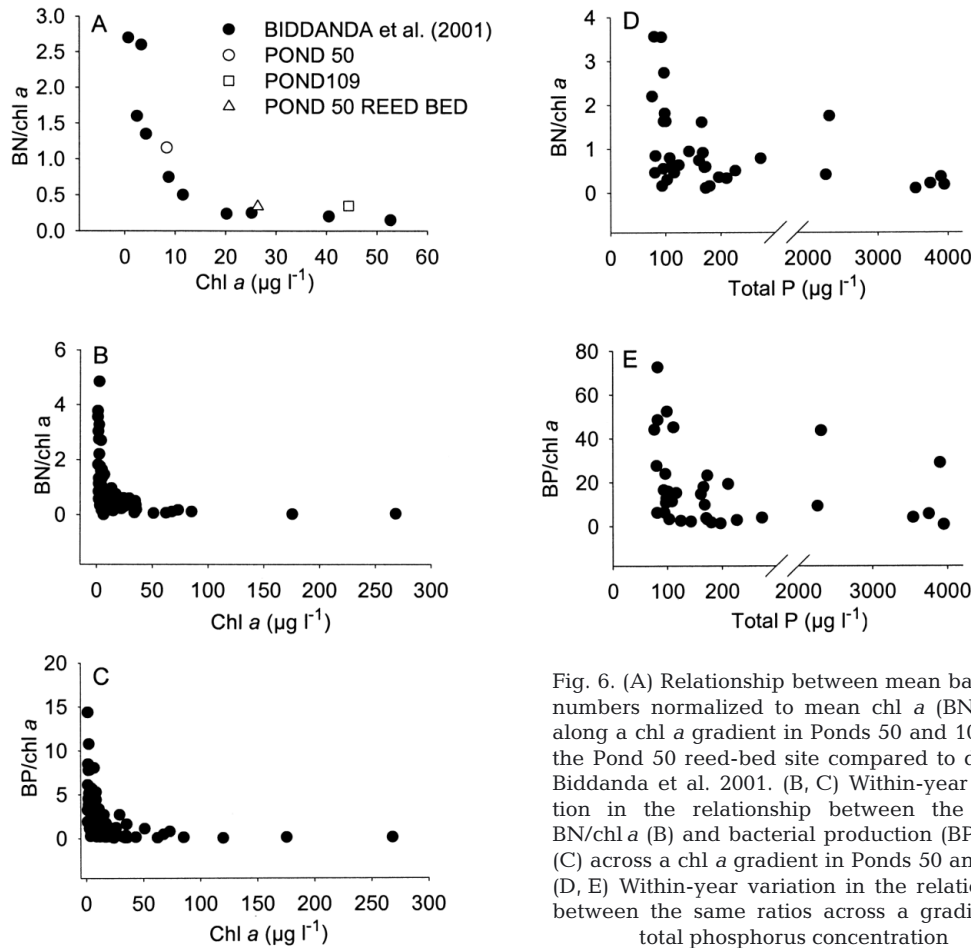


Fig. 6. (A) Relationship between mean bacterial numbers normalized to mean chl *a* (BN/chl *a*) along a chl *a* gradient in Ponds 50 and 109 and the Pond 50 reed-bed site compared to data of Biddanda et al. 2001. (B, C) Within-year variation in the relationship between the ratios BN/chl *a* (B) and bacterial production (BP)/chl *a* (C) across a chl *a* gradient in Ponds 50 and 109. (D, E) Within-year variation in the relationship between the same ratios across a gradient in total phosphorus concentration

contrasts with most lake ecosystems, which usually experience peaks in PP and chl *a* in the spring (Sommer et al. 1986). In more productive wetland systems, however, mid-summer, and even fall, maxima may occur (Crumpton 1989). In fact, the timing of biomass and production peaks reported here is similar to those recorded for productive wetlands at Delta Marsh, Manitoba (Robinson et al. 1997).

There are a number of possible explanations for this phenomenon. First, with ice-out occurring in mid- to late April, and our first sampling occurring during the first 2 wk of May, it may be that we simply missed the spring bloom in these ponds. Secondly, although this spring to mid-summer depression in chl *a* and PP might be related to nutrient limitation, there are strong indications that this is not the case. Overall concentrations of phosphorus and nitrogen are high in both ponds (Table 1) and, based on mean TP concentration, both would be considered hypertrophic (Wetzel 2001). Orthophosphate levels (most biologically available form of P) also remain measurable throughout the period of chl *a* depression. As a result of high nutrient levels, microbial communities in prairie wetlands,

including Pond 50, have been shown to be nutrient sufficient throughout the ice-free season (Waiser 2001a). It seems unlikely, therefore, that seasonal depressions in algal biomass and productivity in either pond are related to nutrient limitation.

The ability of DOC to attenuate photosynthetically active radiation (PAR) can reduce PP and algal biomass (Christensen et al. 1996, Carpenter et al. 1998). In lakes, light absorption by DOC squeezes the photic and mixing zones into a shallow layer, thus restricting autotrophic production (Cole et al. 2000). Light attenuation, consequently, might be another factor limiting algal production and biomass in these high DOC prairie systems. However, light penetration data collected as part of a larger study of these 2 wetlands indicated that the euphotic zone was deeper than actual pond depth in both (Waiser 2001b). It seems unlikely, therefore, that the spring and early summer depressions in algal biomass and production were due to light limitation.

Depressions in algal biomass and production at our study sites could be linked to heavy zooplankton grazing at this time of the year, a phenomenon that has

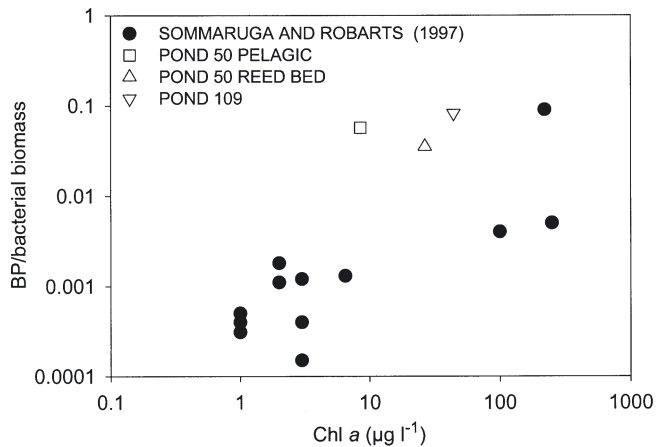


Fig. 7. Logarithmic relationship between chl *a* and bacterial production (BP; $\text{mg C m}^{-3} \text{d}^{-1}$) normalized to bacterial biomass ($\mu\text{g C l}^{-1}$) for Ponds 50 and 109 and other freshwater systems (data from Sommaruga & Robarts 1997). Bacterial numbers were converted to biomass assuming a mean cell carbon content of 20 fg cell^{-1}

been noted in other prairie wetlands (Sandilands et al. 2000). In Pond 50, although high zooplankton numbers were observed at certain times of the year, zooplankton data collection was not a part of this study, and so a link between seasonal depressions in algal biomass and production and zooplankton remains entirely speculative. Murkin & Ross (2000) aptly noted that 'A general lack of basic ecological and taxonomic information on invertebrates in prairie wetlands restricts our understanding of their role in the structure and function of these systems'.

Heterotrophic or autotrophic?

The traditional view that most aquatic ecosystems are net autotrophic ('a partial and variable truth'; Wetzel 1992) has recently been challenged. Heterotrophy is considered present if respiration exceeds PP or if net BP exceeds net PP. Although the evidence for ecosystem level net heterotrophy is preliminary (Cole 1999), reports from a variety of ecosystems tend to support its existence. Net heterotrophy in the Hudson River, for example, occurred due to inputs of allochthonous organic matter and limitation of algal production by turbidity (Findlay et al. 1991). In a series of Quebec lakes, net community respiration always exceeded net PP at chl *a* levels $<15 \mu\text{g l}^{-1}$ (del Giorgio & Peters 1994). In a cross-system overview, bacterial respiration tended to exceed net PP in aquatic ecosystems when net PP was $<100 \mu\text{g C l}^{-1} \text{d}^{-1}$ (del Giorgio et al. 1997). Variation in PCO_2 also provides one of the most sensitive indicators of biological processes associated with

the consumption and production of CO_2 (Herczeg 1987). Many lakes, especially oligotrophic ones, tend to be supersaturated with CO_2 compared to atmospheric values (Cole et al. 1994, 2000). This supersaturation has been linked to net heterotrophy (Cole 1999).

In most aquatic ecosystems, the balance between autotrophy and heterotrophy is determined by the potential for phytoplankton photosynthesis, rather than the input of external carbon. This determination was made for a series of Quebec lakes which were characterized by a much larger range in chl *a* and nutrients than DOC concentrations (del Giorgio & Peters 1994). In some aquatic ecosystems, however, changes from autotrophy to heterotrophy are due to the balance struck between the positive influence of DOC on BP and the negative influence on PP (Jansson et al. 2000). In the pelagic system of unproductive subarctic Swedish lakes, N:P ratios may be critical in determining net autotrophy or heterotrophy (Karlsson et al. 2002), while other research at Chesapeake Bay has shown that this balance may be determined by variations in the size structure of the planktonic community (White & Kemp 2001). Generally, net autotrophy is favoured and particularly evident in eutrophic systems. In these situations, larger autotrophs tend to prevail as they are better nutrient competitors at high concentrations and also have a greater capacity for nutrient storage (Cotner & Biddanda 2002). In artificially manipulated systems, nutrient additions, in the absence of herbivores, can push systems towards net autotrophy by increasing GPP (Cole et al. 2000). However, exceptions are found in high DOC systems ($\text{DOC} >20\text{--}25 \text{ mg l}^{-1}$) where the magnitude of allochthonous inputs overwhelms autochthonous production (del Giorgio & Peters 1994). Aquatic systems favouring net heterotrophy usually have high concentrations of DOC, low concentrations of nutrients, and are oligotrophic (Cole 1999, Cotner & Biddanda 2002; but also see Carignan et al. 2000, who reported net autotrophy in 12 oligotrophic lakes in the Canadian Shield). In net heterotrophic systems, respiration rates tend to be high, often in excess of PP (Pace & Cole 2000)

But what about prairie wetlands, where high DOC concentrations favour net heterotrophy, while high nutrient concentrations favour net autotrophy? Are they net heterotrophic or autotrophic? We addressed this question by determining the ratio of net PP to BP for the 2 study wetlands (following Jansson et al. 2000). Seasonal averages of PP:BP for Ponds 50 and 109 for all study years indicated that both ponds were net autotrophic (Fig. 4). This would be in line with our expectations given that these ponds are eutrophic. We did, however, have a number of concerns. The first was that PCO_2 calculations indicated supersaturation of both ponds for much of the study period, a feature

usually associated with net heterotrophy (Cole et al. 1994). It should be noted, however, that in some cases net autotrophic systems have been shown to be supersaturated with CO₂. In an oligotrophic Quebec lake (Lake Croche), for example, concomitant O₂ and CO₂ supersaturation was noted during thermal stratification (Carignan et al. 2000). Secondly was the high variation in seasonal means of PP:BP ratios (see 'Results'). A closer examination at a smaller temporal scale indicated that Pond 50 was net heterotrophic on approximately 38, 35 and 20% of the sampling dates in 1998, 1999, and 2000 (Fig. 4A). Although the majority of these dates occurred before mid-July in 1999 and 2000, in 1998 periods of net heterotrophy were evident from mid-July to early August (Fig. 4A). In Pond 109, net heterotrophy was present on 17 and 50% of the sampling dates in 1998 and 1999, respectively. In 1999, all of the incidents of net heterotrophy occurred before June 25 (Fig. 4B).

A number of factors, including temperature, may influence development of net heterotrophic and autotrophic periods in these 2 prairie wetlands. In some systems, P:R ratios are generally higher in the spring, an attribute related to cooler temperatures which had a smaller effect on phytoplankton than on bacterial respiration (del Giorgio & Peters 1994). But prairie wetlands, like the ones in this study, are shallow and warm up quickly following ice-out (Fig. 1A). Moreover, there was no significant relationship when BP in both Ponds 50 and 109 was regressed versus temperature (data not shown). In the 2 study wetlands, there does not appear to be a strong relationship between BP and spring temperature and peaks can and do occur in May and June (Fig. 3). Another contributory factor could be zooplankton grazing. In Lake Søbygård, the ratio of BP:PP increased (PP:BP decreased) with increasing grazing pressure (Jeppesen et al. 1992). In this lake, BP was stimulated either by zooplankton feeding on potential bacterial predators or by fueling bacteria with labile carbon substrates released during grazing (Jeppesen et al. 1997). Grazing pressure in spring and early summer in prairie wetlands may contribute to net heterotrophic periods by stimulating BP and keeping algal biomass and rates of production low. When zooplankton crash, the combination of release from predation pressure and surfeit of nutrients may create the right conditions for strong periods of net autotrophy seen after mid-summer in these 2 ponds. But further research is certainly required to confirm this speculative link between zooplankton grazing and the development of net heterotrophic periods.

During periods of net heterotrophy, bacterial carbon demand was high, far in excess of what phytoplankton could provide. In 1998, 1999 and 2000, for example, bacterial carbon demand in the pelagic area in Pond 50

during these periods was 3.5, 4.8 and 5.1 times greater, respectively, than PP (Fig. 5A). In Pond 109, bacterial carbon demand was in excess by 1.6 and 13.5 times in 1998 and 1999, respectively (Fig. 5C). In shallow Lake Neusiedl, Austria, bacterial carbon demand exceeded phytoplankton production by an order of magnitude (Reitner et al. 1999). The authors hypothesized that most of the carbon required by bacteria was supplied by large standing crops of *Phragmites* spp. and their associated periphytes. Clearly, bacteria in prairie wetlands must be relying on carbon sources not associated with pelagic phytoplankton to satisfy this high carbon demand. Although these sources have not been identified, it seems likely that bacteria are tapping into the vast stores of DOC present in these wetland systems. This DOC is likely a mix of carbon terrestrially derived and that produced within the basin, either through decomposition or exudation by zooplankton and periphyton. Rates of periphytic algal production are high in these ponds (Waiser 2001b), but it is not known to what extent they may supply excreted organic carbon suitable for bacterial use. Although the biological availability of DOC in both study wetlands is low, only a small percentage of the high ambient concentrations would be required to meet the bacterial carbon demand. Based on our estimates, only 0.2% of the DOC in Pond 50 and 0.8% in Pond 109 would be required on a daily basis to fulfill bacterial requirements. Biologically available carbon experiments conducted on these 2 ponds revealed that, on average, 1.7% (± 1.2) of the DOC in Pond 50, and 5.9% (± 2.2) in Pond 109, is available to bacteria (Waiser 2001b). Also of interest is the fact that wetland DOC is photoreactive and made more available to bacteria after exposure to solar radiation (Waiser 2001b). It seems likely, therefore, that DOC could theoretically fulfill the bacterial carbon demand in these 2 ponds.

Does the importance of bacteria decrease across a trophic gradient?

Although heterotrophic organisms tend to dominate the biomass in oligotrophic systems, their abundance does not keep track with increasing algal biomass across a trophic gradient (Bird & Kalff 1984, Sommaruga & Robarts 1997). This phenomenon has been linked to a number of factors including the ability of bacteria to acquire nutrients at low ambient concentrations in oligotrophic systems and the fact that bacteria are grazed or subject to viral attack in eutrophic systems. In 10 Minnesota kettle lakes and Lake Superior, bacterial numbers per unit chl *a* (BN:chl *a*) decreased across a gradient from oligotrophy to eutrophy (Biddanda et al. 2001). When seasonal averages of BN:chl *a*

from our study wetlands were added to the plot of Biddanda et al. (2001) there was a good fit of our data with that from his study lakes (Fig 6A). In prairie wetlands, bacterial numbers appear to decrease along a trophic gradient when seasonal averages are used.

In the study of Biddanda et al. (2001), chl *a* concentrations ranged from 0.57 $\mu\text{g l}^{-1}$ in the most oligotrophic to 52.7 $\mu\text{g l}^{-1}$ in the eutrophic system. Chl *a* concentrations in each study wetland, however, varied across a trophic gradient spanning oligotrophy to eutrophy within one season, as they do in many aquatic ecosystems (Fig. 2). Our data clearly show that over shorter time scales, bacterial numbers and production tend to increase when concentrations of chl *a* and TP are low and vice versa (Fig. 6B–E). To our knowledge, this is the first time that this type of seasonal observation has been made in any aquatic ecosystem. Data presented here highlight the shorter time scales over which this phenomenon can be observed and underline the importance of looking at seasonal trends as well as seasonal averages. While studying the spring bloom in Lake Constance, Weisse et al. (1990) noted that the bloom could conceivably be subdivided into several stages, each exhibiting different features. The ecological importance, moreover, could not be fully evaluated when only average annual biomass or cumulative production was considered.

It is unclear at this time why bacterial numbers and production do not keep pace with increasing algal biomass in these ponds. But bacterial growth can be limited by bacterivory (Biddanda et al. 2001) and viral numbers and activity are extremely high in these ponds (Robarts & Bird 1998). Because bacterial numbers and production per unit chl *a* decrease across a trophic gradient, it is also believed that the relative importance of bacteria to material and energy flux also decreases (Cotner & Biddanda 2002). In eutrophic aquatic systems, lower excretion of organic carbon and/or increased particle sinking fluxes may contribute to this decreased importance (Cotner & Biddanda 2002). However, our data seem to suggest that the influence of bacteria does not decline in highly productive systems (cf. Sommaruga & Robarts 1997). First, our BP data clearly show that even these eutrophic prairie systems experience periods of net heterotrophy (Fig. 4A,C). During these times, pelagic BP exceeds PP. Secondly, bacterial carbon demand in these systems is high and exceeds pelagic phytoplankton production on 57% of the sampling dates in both ponds (Fig. 5A,C). Finally, in many hypertrophic ecosystems, like prairie wetlands, although bacterial numbers do not increase as rapidly as algal biomass with increasing trophity, BP per unit biomass increases (Fig. 7). Because of the high production per cell, Sommaruga & Robarts (1997) stated that bacteria probably do not

play a lesser role in lake metabolism in highly productive systems, thereby supporting the hypothesis originally set out by Bird & Kalff (1984). Our data support these observations and point to the need for more detailed studies of the importance of bacteria to material and energy flux in eutrophic aquatic ecosystems.

Acknowledgements. The authors would like to thank Garth van der Kamp for useful discussions. We also thank Bopiah Biddanda and 2 anonymous reviewers whose comments improved the manuscript. Vijay Tumber, Jennifer Holm and Randy Schmidt provided valuable technical and laboratory assistance. This research was made possible by funding through the Institute of Wetland and Waterfowl Research and Environment Canada's National Water Research Institute to R.D.R. and M.J.W.

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Editorial responsibility: Robert Sanders, Philadelphia, Pennsylvania, USA

*Submitted: May 20, 2003; Accepted: September 26, 2003
Proofs received from author(s): January 20, 2004*