Year-round patterns in bacterial production and biomass in Lake Simcoe, Ontario, Canada: are heterotrophic bacteria a significant contributor to low hypolimnetic oxygen?

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Abstract

Bacteria serve an important function in aquatic environments and are associated with nutrient regeneration, carbon (C) metabolism, and secondary production. This is the first study to measure heterotrophic bacterial production, abundance, biomass, and biovolume in Lake Simcoe, Ontario. Excessive phosphorus loading resulting in low hypolimnetic oxygen concentrations has impacted the cold-water fishery in Lake Simcoe. We tested the hypothesis that bacteria contributed to dissolved oxygen declines in Lake Simcoe and examined the environmental factors impacting bacterial activity. Spatial and temporal variations in the microbial community were measured from June 2010 to July 2011. A dual-isotope method (³²H-TdR and ¹⁴C-leu) was used, resulting in mean (± standard deviation) annual epilimnetic bacterial production estimates of 0.130 ± 0.173 and 0.268 ± 0.304 µg C L⁻¹ h⁻¹, respectively. The mean annual bacterial abundance was 1.49 ± 1.53 cells × 10⁹ L⁻¹, with a mean biomass of 1.21 ± 1.34 µg C L⁻¹ and a mean biovolume of 0.0043 ± 0.0030 µm³ cell⁻¹. These estimates had distinct seasonal patterns, with consistently lower bacterial activity in the winter relative to the spring, summer, and fall. Differences between epilimnetic and hypolimnetic bacterial activity were inconsistent for the 4 bacterial parameters measured. Lake temperature, chlorophyll a, and dissolved organic C concentrations were the most significant factors influencing the annual epilimnetic patterns in the examined bacterial parameters. Annual bacterial production was low in Lake Simcoe and does not seem to be a major contributor to the low hypolimnetic oxygen concentrations in the lake.

Key words: ³²H-thymidine, ¹⁴C-leucine, bacterial activity, dual isotope, eutrophication, hypolimnetic oxygen, microbial, phosphorus

Introduction

Bacterial production, which is the rate of synthesis of biomass by heterotrophic bacterioplankton (Fuhrman and Azam 1980, 1982), is an important parameter that provides information on food web dynamics in lakes. For example, the bacterial biomass is potentially available to grazers (Chin-Leo and Kirchman 1988), thus contributing to carbon (C) flow through the aquatic food web (Smith and Azam 1992). Degradation of organic matter by bacteria is an oxygen demanding process, however, and high bacterial activity can contribute significantly to low hypolimnetic oxygen conditions (Goosen et al. 1995). Bacterial production rates have also been used as an indicator of bacterial response to changing environmental conditions (Chin-Leo and Kirchman 1988, Cole and Pace 1995). Many factors can limit bacterial production and abundance in lakes, such as nutrient availability (e.g., organic C), temperature, viral lysis (Weinbauer and Peduzzi 1995), and predation and grazing by zooplankton...
(Gardner et al. 1986, Pace and Cole 1996) and dreissenid mussels (Frischer et al. 2000). Competition for resources between bacteria and phytoplankton can also limit bacterial production and abundance (Caron 1994, Kirchman 1994).

In oligotrophic lakes, bacteria are the major competitor for dissolved nutrients such as phosphorus (P) and nitrogen (N; Cotner and Wetzel 1992). Although bacteria are superior competitors for P, they also depend on dissolved organic carbon (DOC) from primary production (autochthonous DOC; Olsen et al. 2002) and terrestrial sources (allochthonous DOC; Smith and Prairie 2004). Chrost et al. (2000) found that autochthonous C was much more microbiologically labile than allochthonous C. Labile DOC released by phytoplankton (extracellular organic carbon [EOC]; Meyer et al. 1987, Moran and Hodson 1990, Chrost et al. 2000), and aquatic macrophytes (Murray and Hodson 1985), and allochthonous DOC released from sources such as agriculture and sewage are the primary energy sources for bacterial production in lakes (Meyer et al. 1987, Moran and Hodson 1990). Dependence on DOC by bacteria may explain why bacteria and phytoplankton can coexist (Currie and Kalff 1984). Bacteria differ from most other heterotrophic organisms in that they have the ability to assimilate dissolved organic matter (DOM; Cole and Pace 1995), which can be utilized by bacterivores and grazers (Cole and Pace 1995, Chrost et al. 2000), and thus are a significant link to higher consumers (Ducklow et al. 1986).

Lake Simcoe is under the threat of eutrophication due to high total P (TP) loading from the watershed, which has resulted in the excess growth of phytoplankton and submerged aquatic vegetation (Young et al. 2011). Decomposition of this plant and algal material by bacteria can contribute to declines in hypolimnetic oxygen (O₂) concentrations (Goosen et al. 1995), but their relative contribution to the process is poorly understood. The saturation concentration of dissolved O₂ in a lake is inversely related to water temperature; however, photosynthesis and respiration can significantly alter lake O₂ levels (Horne and Goldman 1994). In Lake Simcoe, end-of-summer hypolimnetic dissolved O₂ concentrations have increased from approximately 2 mg L⁻¹ in the 1980s to 5 mg L⁻¹ in 2008 (Young et al. 2011); however, these values are still well below the target concentration of 7 mg L⁻¹ required for natural cold-water fish reproduction (Young et al. 2011). Models developed for Lake Simcoe (Young et al. 2011) suggest that chlorophyll a (Chl-a) concentrations do not solely account for the low hypolimnetic O₂ concentrations.

Although there have been numerous bacterial studies in aquatic systems (e.g., Jonas et al. 1988, Kirchman 2001, Sturluson et al. 2008), few have been conducted over a sufficient length of time to allow seasonal variation to be clearly identified. In general, bacterial studies have focused on the spring, coinciding with the spring phytoplankton bloom, and the summer, when primary and secondary production rates are generally maximal (Hwang and Heath 1997, DeBruyn et al. 2004, Sturluson et al. 2008). Here, we provide the first assessment of heterotrophic bacterial activity in Lake Simcoe and investigate whether bacteria contributed to hypolimnetic O₂ declines. This study also enhances our understanding of the importance of bacteria in the aquatic food web of temperate lakes on a year-round basis. We performed spatially extensive sampling over an entire year to assess seasonal and spatial variation in bacteria and to identify the environmental factors that impact bacterial activity.

**Study site**

Lake Simcoe is the largest inland lake in Southern Ontario, excluding the Laurentian Great Lakes. Located at 44º25ʹN; 79º20ʹW, it has a volume of 1.03 × 10⁸ m³, a surface area of 722 km², and a mean depth of 14.2 m (Nicholls 1995). With average open-water TP concentrations of about 14 µg L⁻¹ in 2010 (North et al. 2013), Lake Simcoe is considered oligo-mesotrophic. Its major inflows from the south and east drain heavily urbanized and agricultural areas, and it outflows into Lake Couchiching to the north (Young et al. 2010). The lake freezes over completely most winters from late December to early April (Hawryshyn et al. 2012). It is a dimictic lake that is typically stratified from June to October; however, the length of stratification has increased since the 1980s, possibly due to climate change (Stainsby et al. 2011).

**Methods**

**Field sampling**

Monthly sampling occurred from June to December 2010 and February to July 2011 at 10 lake stations (Fig. 1). To have a continuous year-round dataset, we collected additional biweekly to monthly samples between January and June 2011 from the Beaverton Water Treatment Plant (WTP) unchlorinated intake pipe (Fig. 1, Table 1). The intake of the pipe was located at a 7.6 m deep site approximately 986 m from the shoreline, while the pipe itself was suspended 3.05 m above the lake bottom. Sampling seasons were defined as winter (under ice: Jan–Mar), spring (Apr–May), summer (Jun–Oct), and fall (Nov–Dec). In this year-round study, thermal stratification was observed during the summer at stations >20 m deep. The entire water column was mixed from top to bottom the rest of the year with the exception of slight winter stratification under ice.
Monthly lake water samples were collected during 2-day cruises from approximately 07:00 to 19:00 h; however, the order the stations were sampled and the approximate time of water collection varied month to month, depending on weather conditions. Epilimnetic integrated water samples were collected from the surface to 1 m above the thermocline (defined by a change in water temperature gradient >1 °C m\(^{-1}\); Wetzel 2001), and hypolimnetic samples were collected from a discrete depth 2 m above the lake bottom. Water samples were collected in acid-washed 20 L carboys that were protected from exposure to direct sunlight and temperature fluctuations until they were processed the same evening. Under-ice sampling was conducted in a similar manner at the same 10 lake stations, although ice conditions dictated the frequency each station was sampled throughout the winter. Winter water samples were collected with a Van Dorn sampler at discrete depths ranging from 0 to 7 m through a 30 cm sampling hole. For sampling at the Beaverton WTP intake pipe, 20 L of raw, unchlorinated water was collected directly from the intake pipe between 09:00 and 11:00 h.

**Water chemistry**

Depth-profiled temperature and \(O_2\) concentrations were recorded at each lake station throughout the year using a YSI meter (model 6600 V2; Yellow Springs Instruments, Yellow Springs, Ohio, USA) with an ROX optical \(O_2\) probe that was calibrated daily. Chl-\(a\) concentrations were determined using lake water prefiltered through 200 µm nylon (Nitex) filters. Size-fractionated subsamples were filtered through a series of polycarbonate membranes (20 and 2 µm). In each step, the filtrate was filtered onto glass fiber filters (GFF, nominal pore-size 0.7 µm) and stored in the dark at −20 °C. Each filter was passively extracted with 90% acetone in the freezer. A fluorometer (Turner Designs 10-AU; Turner Designs, Sunnyvale, California, USA) that was calibrated yearly with pure Chl-\(a\) was used to determine the pheophytin-corrected Chl-\(a\) concentrations (Smith et al. 1999).

Standard colourimetric methods were used to analyze whole water (prescreened through a 200 µm nylon filter) for TP and filtered (0.2 µm) total dissolved P (TDP; OMOE 2007). Soluble reactive P (SRP) samples were filtered (0.2 µm) and analyzed according to Stainton et al. (1977) with a detection limit of 0.372 µg L\(^{-1}\). P. Samples were also filtered (0.45 µm) and analyzed for DOC using a Shimadzu TOC-VMCPH analyzer (Chiyoda-ku, Tokyo, Japan).
Bacterial production

Bacterial production was measured using simultaneous incorporation of methyl-\(^3\)H thymidine and \(^{14}\)C-leucine (Chin-Leo and Kirchman 1988). Water samples (5 mL) were dispensed into ethanol-rinsed glass scintillation vials along with 20 nM of methyl-\(^3\)H thymidine (80 Curies mmol\(^{-1}\), PerkinElmer) and 50 nM of \(^{14}\)C-leucine (300 milliCuries mmol\(^{-1}\), PerkinElmer).

Saturation experiments were performed each year to determine the saturating concentrations of leucine and thymidine in the same manner as described in the paragraph below, with the exception of the amount of each isotope added into each vial. To validate the choice of a saturating concentration of leucine and thymidine, final concentrations of 0, 20, 40, 50, 80, 150, 200, and 250 nM; and 0, 10, 20, 40, 80, 100, and 120 nM, respectively, were added to individual vials that contained 5 mL prefiltered (200 μm Nitex) lake water collected from the epilimnion of stations E50 and K42 (Fig. 1) on 24 July 2010 and 14 July 2011, respectively. No thymidine was added to leucine saturating experiment vials, and no leucine was added to vials for thymidine saturating experiments. Final concentrations of 50 nM \(^{14}\)C-leucine and 20 nM of \(^3\)H-thymidine were found to be saturating and were used for all subsequent assays.

Fresh (<24 h) working solutions of thymidine and leucine were sterilized by filtration (0.2 μm) before addition to subsamples within an hour; 5 mL of 10% (weight/weight) trichloroacetic acid (TCA, Sigma; St. Louis, Missouri, USA), was added to one of the 4 subsamples for immediate termination after both isotopes were added. Terminated “time-zero” subsamples served as blanks to control for abiotic adsorption of the working solution. Time-zero subsamples were carried through the incubation procedure in parallel with the live subsamples. Samples from each station contained one time-zero subsample and 3 live subsamples. All subsamples were incubated in the dark for 1 h at in situ collection temperature, then terminated by placing them on ice for 5 min followed by the addition of 5 mL of ice-cold 10% (wt/wt) TCA and left to sit for 5 min before filtering. Subsamples were filtered through a cellulose nitrate membrane filter (25 mm diameter, 0.2 μm, VWR; Radnor, Pennsylvania, USA) followed by a 2-fold rinse with 3 mL of ice-cold 10% TCA and 10 mL of prefiltered lake water from the respective stations, followed by 3 mL of 95% ethanol. Cold TCA-insoluble material was retained on the filters and was measured with a Packard Tri-Carb 1600CA liquid scintillation counter (Perkin Elmer; Waltham, Massachusetts, USA). Thymidine incorporation was converted to cell production (cells L\(^{-1}\) h\(^{-1}\)) by the calibration factors 2.0 × 10⁸ cells mmol\(^{-1}\), \(^3\)H incorporated, and 0.04 μm\(^3\) cell\(^{-1}\) and 1 × 10⁶ conversion to C production (Bell 1993). Leucine incorporation was converted to protein production using the fractions 100/7.3 mole percent of leucine in protein, 131.2 g molecular weight of leucine, and 0.86 g of C per protein (Kirchman 2001). On a few occasions, bacterial production rates were measured to be below zero; these were reported as zero values.

Bacterial abundance, biomass, and biovolume

At each station, a 20 mL subsample of prescreened lake water (200 μm) was collected for bacterial abundance and biomass determination. Subsamples were preserved in glass scintillation vials with 5% paraformaldehyde solution (Sigma 95% powder paraformaldehyde) and kept at 4 °C until subsamples could be processed (within 6 months). Bacterial abundance was determined using an epifluorescence microscope (Zeiss Axioscope; Thornwood, New York, USA). Aliquots (1 mL) were stained with DNA-DAPI (4’6’-diamidino-2-phenylindole) then filtered onto 0.2 μm pore-size black polycarbonate membrane filter backed with a 0.45 μm white filter. For each measurement of bacterial abundance, at least 200 cells or 40 fields of view, whichever came first, were counted. Northern Eclipse Imaging System (Missisauaga, Ontario, Canada) was used to measure at least 200 cells per sample to determine the biomass of the sample. Using the measurements from Northern Eclipse, biovolume (μm\(^3\) cell\(^{-1}\)) estimates were calculated using Bratbak’s (1993) formula. Total bacterial biomass (mg C L\(^{-1}\)) was calculated by multiplying biovolume with a carbon conversion factor of 200 fg C μm\(^{-3}\) (Bratbak 1993) followed by the multiplication with bacterial abundance.

Statistical analyses

SYSTAT 13 (Chicago, Illinois, USA) was used for all data analysis. Transformations (log\(_{10}\) for all parameters) were applied to ensure that the data met the assumptions of normality. One-way analysis of variance (ANOVA) tests were performed followed by Tukey-Kramer post hoc tests with a significance value of \(p < 0.05\) to determine the differences among seasons and to examine spatial differences for both epilimnetic and hypolimnetic values separately for all bacterial parameters measured. Pearson correlations were performed using log\(_{10}\) transformed means, calculated by averaging the 3 replicates for each bacterial parameter within each site, to examine the relationships among bacterial parameters and with associated water quality data. Pearson correlation coefficients (\(r\)) and Bonferroni probabilities (significance value of \(p < 0.05\)) are reported. Based on an ANOVA test, samples from the WTP were not significantly different from epilimnetic.
samples from nearby stations E50 and E51 (TdR incorporation $F_{[2,33]} = 1.692, p = 0.20$ and leucine incorporation $F_{[2,48]} = 0.517, p = 0.60$; Fig. 1); therefore, the WTP was treated as an 11th epilimnetic lake station in all analyses.

**Results**

**Spatial distribution of bacteria in Lake Simcoe**

The epilimnetic data were used to examine the spatial distribution of bacteria across the lake. There were few differences in annual bacterial production, abundance, biomass, and biovolume among the 11 stations sampled. Annually, the nearshore station T2 had significantly higher bacterial production (for both leucine and thymidine incorporated) and abundance than the other 10 stations (Tukey-Kramer test $p < 0.05$). Overall, there were no significant differences among stations for annual bacterial biomass or biovolume (Tukey-Kramer test $p > 0.05$) with the exception of station T2, where biomass was 4.1 times higher than at the WTP ($p < 0.05$). Due to lack of spatial differences across the 11 stations, seasonal analyses were performed by combining all stations.

**Seasonality of bacterial production in Lake Simcoe**

The seasonal patterns in bacterial production were measured from the incorporation rates of $^3$H-thymidine and $^{14}$C-leucine. The mean annual epilimnetic bacterial production rates for $^3$H-TdR and $^{14}$C-leucine incorporation were $0.130 \pm 0.173$ ($n = 241$) and $0.268 \pm 0.304$ mg C L$^{-1}$ h$^{-1}$ ($n = 292$), respectively (Table 2). Estimates from leucine incorporation for annual epilimnetic samples were significantly higher than the thymidine estimates ($F_{[1,500]} = 21.962, p < 0.0005$); however, both methods illustrated similar temporal trends (Fig. 2a and b). Significant differences were also observed in bacterial production measured from the incorporation rates of $^3$H-thymidine compared with $^{14}$C-leucine incorporation in the winter ($F_{[1,93]} = 6.567, p = 0.012$), summer ($F_{[1,225]} = 48.549, p < 0.0005$), and fall ($F_{[1,88]} = 13.186, p < 0.0005$). No significant difference was observed between the 2 methods in the spring ($F_{[1,109]} = 0.874, p = 0.352$).

In this year-round study, thermal stratification was observed at deep stations (>20 m) during the summer, and slight inverse stratification was observed under ice in the winter. During stratification, the lakewide epilimnetic bacterial production rates were significantly higher than the hypolimnetic rates (TdR: incorporation, $F_{[1,114]} = 38.146, p < 0.0005$; leucine incorporation, $F_{[1,205]} = 38.312, p < 0.0005$; Fig. 2a,b). Bacterial production exhibited a distinct temporal pattern; maximum production values were reached during the stratified summer (Jun: $0.486 \pm 0.242$ and Jul: $0.614 \pm 0.441$ mg C L$^{-1}$ h$^{-1}$) with the leucine method, while the lowest rates were measured during the winter, under ice ($0.035 \pm 0.042$ mg C L$^{-1}$ h$^{-1}$; Fig. 2b; Table 2 and 3). With the thymidine method, maximum production was observed in the isothermal spring (May: $0.441 \pm 0.256$ mg C L$^{-1}$ h$^{-1}$; Fig. 2a; Table 2 and 3), likely because June samples were not measured. After peaking in June (May for TdR), production declined and remained relatively low until the following April, with the exception of a second smaller peak in March ($0.049 \pm 0.053$ mg C L$^{-1}$ h$^{-1}$), just before ice break-up (Fig. 2a and b).

All annual and seasonal correlation analyses were conducted using the leucine bacterial data only due to its larger sample size. When bacterial parameters were summarized as annual means, there were no significant correlations between bacterial production and the other bacterial activity parameters (Table 4); however, bacterial production was positively correlated with biomass in the fall ($r = 0.537, p = 0.039$). No significant correlations were observed in winter, spring, or summer (Table 4).

Annual, epilimnetic bacterial production was positively correlated with surface water temperature ($r = 0.517, p = <0.0005$) and DOC concentration ($r = 0.207, p = 0.049$), but negatively correlated with Chl-a concentrations (20–200 and 2–20 μm; $r = -0.238, p = 0.022$, and $r = -0.202, p = 0.050$, respectively) and TDP concentrations ($r = -0.278, p = 0.008$; Table 5). In the stratified summer, bacterial production was negatively correlated with Chl-a concentrations (20–200 μm; $r = -0.304, p = 0.028$; Table 5). There were no significant correlations of bacterial production with any of the water quality or environmental parameters in the winter (Table 5).

At the beginning of summer, water temperatures reached approximately 17 °C, and the relationship between bacterial production and temperature started to uncouple and was not observed again until the end of summer when temperatures began to decline. Further analysis of these relationships showed that log$_{10}$-transformed bacterial production rates were correlated with water temperature, but only at temperatures below 17 °C (Fig. 3a). During the period of time when water temperature was above 17 °C, however, log$_{10}$-transformed bacterial production rates showed a strong relationship with TDP concentrations (Fig. 3b).

**Seasonality of bacterial abundance, biomass, and biovolume**

At the annual scale, there were no significant differences in bacterial abundance ($F_{[1,133]} = 0.094, p = 0.760$; Fig. 2c) or biomass ($F_{[1,133]} = 0.274, p = 0.602$; Fig. 2d) between the epilimnion and the hypolimnion; however, hypolim-
Table 2. Mean (±SD; with n value in parentheses) measurements of bacterial activity (bacterial production [BP], bacterial abundance [BA], bacterial biovolume [BV], and bacterial biomass [BB]) and environmental factors (temperature, O₂, Chl-a, phosphorus, and DOC concentrations) for 11 sampling stations in Lake Simcoe from June 2010 to July 2011; hypolimnetic samples were collected only when stratification was present.

<table>
<thead>
<tr>
<th>Units</th>
<th>Annual</th>
<th>Winter</th>
<th>Epilimnetic Spring</th>
<th>Summer</th>
<th>Fall</th>
<th>Hypolimnetic Winter</th>
<th>Summer</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP (TdR)</td>
<td>0.130 ± 0.173 (241)</td>
<td>0.015 ± 0.030 (48)</td>
<td>0.362 ± 0.259 (45)</td>
<td>0.116 ± 0.090 (103)</td>
<td>0.051 ± 0.028 (45)</td>
<td>0.004 ± 0.008 (21)</td>
<td>0.034 ± 0.050 (53)</td>
</tr>
<tr>
<td>BP (Leu)</td>
<td>0.268 ± 0.304 (292)</td>
<td>0.035 ± 0.042 (48)</td>
<td>0.368 ± 0.220 (45)</td>
<td>0.365 ± 0.347 (154)</td>
<td>0.081 ± 0.048 (45)</td>
<td>0.031 ± 0.029 (21)</td>
<td>0.067 ± 0.083 (53)</td>
</tr>
<tr>
<td>BA (cells L⁻¹)</td>
<td>± 1.53 × 10⁹ (105)</td>
<td>± 2.44 × 10⁶ (16)</td>
<td>± 1.33 × 10⁹ (15)</td>
<td>± 1.31 × 10⁹ (59)</td>
<td>± 2.54 × 10⁹ (15)</td>
<td>± 3.71 × 10⁹ (7)</td>
<td>± 9.68 × 10⁸ (23)</td>
</tr>
<tr>
<td>BV (μm³ cell⁻¹)</td>
<td>0.0043 ± 0.0030 (105)</td>
<td>0.0032 ± 0.0022 (16)</td>
<td>0.0033 ± 0.0007 (15)</td>
<td>0.0050 ± 0.0037 (59)</td>
<td>0.0037 ± 0.0009 (15)</td>
<td>0.0039 ± 0.0023 (7)</td>
<td>0.0052 ± 0.0025 (23)</td>
</tr>
<tr>
<td>BB (μg C L⁻¹)</td>
<td>1.21 ± 1.34 (105)</td>
<td>0.23 ± 0.15 (16)</td>
<td>0.93 ± 0.69 (15)</td>
<td>1.41 ± 1.25 (59)</td>
<td>1.72 ± 2.15 (15)</td>
<td>0.28 ± 0.17 (7)</td>
<td>1.49 ± 1.01 (23)</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>13.9 ± 8.4 (106)</td>
<td>0.7 ± 0.9 (16)</td>
<td>7.2 ± 2.4 (15)</td>
<td>20.6 ± 2.9 (60)</td>
<td>7.9 ± 1.8 (15)</td>
<td>1.8 ± 1.1 (7)</td>
<td>10.6 ± 2.1 (18)</td>
</tr>
<tr>
<td>O₂ (mg L⁻¹)</td>
<td>9.6 ± 2.7 (97)</td>
<td>10.4 ± 3.7 (12)</td>
<td>12.6 ± 0.6 (12)</td>
<td>8.4 ± 2.3 (58)</td>
<td>11.2 ± 0.7 (15)</td>
<td>8.9 ± 4.2 (7)</td>
<td>7.6 ± 2.8 (22)</td>
</tr>
<tr>
<td>Chl-a (20–200 μm)</td>
<td>1.9 ± 2.1 (106)</td>
<td>3.0 ± 4.6 (16)</td>
<td>1.6 ± 1.5 (15)</td>
<td>1.5 ± 1.0 (60)</td>
<td>2.6 ± 1.4 (15)</td>
<td>NA</td>
<td>0.6 ± 0.5 (24)</td>
</tr>
<tr>
<td>Chl-a (2–20 μm)</td>
<td>1.4 ± 1.7 (106)</td>
<td>2.5 ± 3.8 (16)</td>
<td>1.3 ± 1.3 (15)</td>
<td>1.1 ± 0.8 (60)</td>
<td>1.4 ± 0.7 (15)</td>
<td>NA</td>
<td>0.4 ± 0.4 (14)</td>
</tr>
<tr>
<td>Chl-a (0.7–2 μm)</td>
<td>0.6 ± 0.4 (105)</td>
<td>0.3 ± 0.2 (16)</td>
<td>0.4 ± 0.2 (15)</td>
<td>0.7 ± 0.4 (59)</td>
<td>0.9 ± 0.4 (15)</td>
<td>NA</td>
<td>0.2 ± 0.2 (14)</td>
</tr>
<tr>
<td>DOC (μM)</td>
<td>339.5 ± 210.0 (105)</td>
<td>325.4 ± 9.5 (16)</td>
<td>332.0 ± 13.6 (15)</td>
<td>343.5 ± 24.4 (59)</td>
<td>346.1 ± 11.3 (15)</td>
<td>NA</td>
<td>314.3 ± 15.6 (24)</td>
</tr>
<tr>
<td>TP (μM)</td>
<td>0.27 ± 0.06 (106)</td>
<td>0.27 ± 0.03 (16)</td>
<td>0.27 ± 0.07 (15)</td>
<td>0.27 ± 0.07 (60)</td>
<td>0.30 ± 0.07 (15)</td>
<td>NA</td>
<td>0.30 ± 0.13 (24)</td>
</tr>
<tr>
<td>TDP (μM)</td>
<td>0.15 ± 0.05 (104)</td>
<td>0.19 ± 0.04 (16)</td>
<td>0.14 ± 0.02 (14)</td>
<td>0.13 ± 0.04 (59)</td>
<td>0.18 ± 0.04 (15)</td>
<td>NA</td>
<td>0.20 ± 0.13 (24)</td>
</tr>
<tr>
<td>SRP (μM)</td>
<td>0.06 ± 0.04 (104)</td>
<td>0.07 ± 0.03 (16)</td>
<td>0.06 ± 0.03 (15)</td>
<td>0.05 ± 0.04 (58)</td>
<td>0.08 ± 0.05 (15)</td>
<td>NA</td>
<td>0.09 ± 0.11 (24)</td>
</tr>
</tbody>
</table>
netic bacterial biovolume was significantly higher than epilimnetic biovolume ($F_{[1,133]} = 3.391, p = 0.049$; Fig. 2c). Annual epilimnetic mean abundance and biomass were $1.49 \times 10^9 \pm 1.53 \times 10^9$ ($n = 105$; Table 2) and $1.10 \pm 1.25$ μg C L$^{-1}$ ($n = 105$; Table 2), respectively. Distinct temporal patterns were evident in bacterial biomass, with biomass increasing during the spring to peak in June ($2.02 \pm 1.66$ μg C L$^{-1}$) and again in November ($2.34 \pm 2.42$ μg C L$^{-1}$), followed by a decrease in December ($0.459 \pm 0.307$ μg C L$^{-1}$; Fig. 2d). Bacterial abundance peaked in November ($3.12 \times 10^9 \pm 2.77 \times 10^9$ cells L$^{-1}$) and then decreased in December ($6.43 \times 10^8 \pm 4.30 \times 10^8$ cells L$^{-1}$; Fig. 2c). There were no significant differences among spring, summer, and fall for abundance and biomass (Table 3); however, bacterial abundance and biomass were significantly lower in the winter relative to the other 3 seasons (Table 3). Epilimnetic annual mean bacterial biovolume was $0.0043 \pm 0.0032$ μm$^3$ cell$^{-1}$ ($n = 105$; Fig. 2c; Table 2).
Fig. 2. Temporal variation in bacterial production in the epilimnion (solid line) and hypolimnion (dashed line) for (a) $^3$H-thymidine incorporated ($n = 315$) and (b) $^{14}$C-leucine incorporated ($n = 366$) samples for the period of July 2010 to July 2011, inclusive. Temporal variation in epilimnetic and hypolimnetic estimates of (c) bacterial abundance ($n = 135$), (d) bacterial biomass ($n = 135$), and (e) bacterial biovolume ($n = 135$) for the period of June 2010 to July 2011, inclusive. Each data point represents the overall mean of all stations sampled calculated by averaging the means of the 3 replicates for each station sampled during that week. Error bars represent the standard deviation of the mean. Sample size ($n$) refers to entire dataset on figure; 11 stations, 3 replicates, and 2 depths.
The lowest biovolume was measured in the winter, under ice (0.0032 ± 0.0022 μm³ cell⁻¹; Table 3). Similar to bacterial production, the maximum biovolume was observed in June (0.0081 ± 0.0060 μm³ cell⁻¹). During the spring, bacterial abundance and biovolume were negatively correlated (r = -0.664, p = 0.007; Table 4).

Due to autocorrelation resulting from bacterial biomass calculations based on measured values of bacterial abundance and biovolume, significant positive relationships were observed between bacterial abundance and biovolume with biomass (r = 0.907, p < 0.001, and r = 0.438, p < 0.001, respectively; Table 4).

**Fig. 3.** Temporal variation in epilimnetic bacterial production (n = 292) and (a) surface water temperature (n = 300) for the period of June 2010 to July 2011; horizontal line represents 17 ºC, above which the temperature and bacterial production relationship starts to uncouple, and (b) TDP (n = 295) for the period of June 2010 to July 2011 as well as an insert for the period of June to October 2010 (summer stratified period) when water temperatures were approximately 17 ºC. Each data point represents the mean of the epilimnetic samples calculated by averaging the means of the 3 replicates for all stations sampled during that week. Error bars represent the standard deviation of the mean. Sample size (n) refers to the epilimnetic dataset, including 3 replicates for bacterial production and one for surface temperature and TDP.
Annual epilimnetic bacterial abundance was positively correlated with temperature \((r = 0.254, p = 0.010)\), Chl-\(a\) \((0.7–2 \mu m: r = 0.305, p = 0.002)\), and DOC concentrations \((r = 0.226, p = 0.021)\;\text{Table 5}\). In the stratified summer period, epilimnetic bacterial abundance was positively correlated with Chl-\(a\) \((20–200 \mu m: r = 0.265, p = 0.043; 2–20 \mu m: r = 0.277, p = 0.034; 0.7–2 \mu m: r = 0.287, p = 0.029)\). Annual bacterial biomass was positively correlated with temperature \((r = 0.329, p = 0.0007)\) and negatively correlated with TDP \((r = -0.213, p = 0.031)\;\text{Table 5}\). Annual epilimnetic bacterial biovolumes were positively correlated with temperature \((r = 0.241, p = 0.014)\) and negatively correlated with Chl-\(a\) \((20-200 \mu m: r = -0.302, p = 0.002; 2–20 \mu m: r = -0.361, p = 0.0002; 0.7–2 \mu m: r = -0.282, p = 0.004)\), DOC \((r = -0.331, p = 0.0006), TP \((r = -0.218, p = 0.026)\), and TDP \((r = -0.228, p = 0.021)\) concentrations \(\text{Table 5}\).

At the seasonal scale, epilimnetic bacterial biovolumes were positively correlated with hypolimnetic \(O_2\) concentrations in the spring and summer \((r = 0.591, p = 0.043\) and \(r = 0.395, p = 0.002,\) respectively; \text{Table 5}\). Epilimnetic bacterial biovolume was negatively correlated with all size fractions of Chl-\(a\) \((20–200 \mu m: r = -0.419, p = 0.0009; 2–20 \mu m: r = -0.437, p = 0.0005; \text{and} 0.7–2 \mu m: r = -0.483, p = 0.0001)\), DOC \((r = -0.523, p = 0.0005)\), and TP \((r = -0.253, p = 0.050)\) concentrations during the summer \(\text{Table 5}\).

**Discussion**

We did not find any relationship between the bacterial parameters measured and hypolimnetic \(O_2\) concentrations, indicating that bacteria do not play a dominant role in \(O_2\) consuming processes in Lake Simcoe. This suggests that the low hypolimnetic \(O_2\) concentrations at the end-of-summer in Lake Simcoe are not directly related to heterotrophic bacterial production and may be more strongly related to biomass produced by autotrophs. Bacterial production accounted for 11.6 and 24\% of primary production in Lake Simcoe using thymidine and leucine incorporation, respectively \(\text{Kim 2013}\).

During the period of this study \((\text{June 2010 to July 2011})\), there was limited spatial variation in Lake Simcoe’s microbial community. Conversely, distinct seasonal patterns were observed with bacterial production, abundance, biomass, and biovolume consistently lower in the winter relative to the spring, summer, and fall. Annual differences between epilimnetic and hypolimnetic samples were inconsistent for the 4 bacterial parameters measured. Lake temperature, Chl-\(a\), and DOC concentrations were strongly correlated with annual bacterial parameters, suggesting these factors affect bacteria in the epilimnion of Lake Simcoe. Higher water temperatures were associated with greater bacterial production, biomass, and biovolume. Higher Chl-\(a\) concentrations of size fraction 20–200 \(\mu m\) were associated with decreased bacterial production, while Chl-\(a\) of size fraction 0.7–2 \(\mu m\) was negatively correlated with bacterial abundance and biovolume. In addition, higher DOC concentrations were associated with stimulated bacterial production and abundance, but decreased cell biovolume.

Both the \(^3\)H-thymidine and \(^14\)C-leucine incorporation methods illustrated similar seasonal and spatial patterns of bacterial production rates. This agreement between independent, simultaneous estimates provides confidence that the observed variations reflect real changes in the rates of bacterial production. The \(^3\)H-thymidine and \(^14\)C-leucine incorporation methods are commonly in agreement \(\text{Chin-Leo and Kirchman 1988, Jonas et al. 1988, van Looij and Riemann 1993}\). The leucine method uses the incorporation of L-leu into protein to provide a direct estimate of the growth rate of biomass and thus the flux of C into bacterial biomass.

Although the thymidine method has been used most extensively \(\text{Hollibaugh 1994, Servais 1995}\), estimating bacterial C production with the thymidine method relies on the knowledge of 3 conversion factors: cell production per mole, cell biovolume, and C content per cell volume \(\text{Jorgensen 1992}\). Discrepancies within these values have been acknowledged to contribute to differences between bacterial production from study to study and even differences between methods within a single study. Our leucine-based estimates were 1.4 times higher than the thymidine-based estimates \(\text{data from July 2010–May 2011}\), which may be attributed to unbalanced growth in bacteria that can occur when the various growth rates of macromolecular syntheses are uncoupled; this generally occurs when bacteria are shifting from one growth rate to another \(\text{Chin-Leo and Kirchman 1990}\). Changes in temperature \(\text{Shiah and Ducklow 1997}\), nutrient concentrations \(\text{Tibbles 1996}\), and bacterial growth rates \(\text{Chin-Leo and Kirchman 1988}\) have been shown to modify the ratio between leucine and thymidine; however, neither our seasonal nor monthly data exhibited a clear pattern that can account for the differences between the 2 methods. Nutrient \((\text{DOC, TP, SRP})\) and Chl-\(a\) concentrations did not differ between the winter and spring seasons \((p > 0.05)\;\text{data not shown}\), implying there were no significant changes in growth rates from the winter to spring, and that balanced growth \(\text{i.e., growth when macromolecular syntheses’ various growth rates are coupled, and thus there are no changes in growth rate}\) was observed. Changes in temperature and nutrients may have contributed to unbalanced growth during the summer, fall, and winter, however, leading to discrepancies between the 2 methods.
Table 5. Coefficients (with n value in parentheses) for the correlations between the epilimnetic bacterial parameters and the examined environmental factors (epilimnetic temperature, hypolimnetic O$_2$, Chl-$a$, phosphorus, and DOC concentrations) for 11 sampling stations in Lake Simcoe for the period of June 2010 to July 2011; correlation p-values are significant at *0.05, **0.01, and ***0.001.

<table>
<thead>
<tr>
<th>Chemical Parameter</th>
<th>Bacterial Parameter$^1$</th>
<th>Annual</th>
<th>Winter</th>
<th>Spring</th>
<th>Summer</th>
<th>Fall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epi. Temp.</td>
<td>BP</td>
<td>0.517 *** (98)</td>
<td>−0.040 (16)</td>
<td>0.116 (15)</td>
<td>0.203 (52)</td>
<td>0.210 (15)</td>
</tr>
<tr>
<td></td>
<td>BA</td>
<td>0.254 ** (105)</td>
<td>−0.361 (16)</td>
<td>0.108 (15)</td>
<td>−0.139 (59)</td>
<td>0.379 (15)</td>
</tr>
<tr>
<td></td>
<td>BB</td>
<td>0.329 *** (105)</td>
<td>−0.359 (16)</td>
<td>0.077 (15)</td>
<td>−0.223 * (59)</td>
<td>0.352 (15)</td>
</tr>
<tr>
<td></td>
<td>BV</td>
<td>0.241 ** (105)</td>
<td>−0.185 (16)</td>
<td>−0.202 (15)</td>
<td>−0.201 (59)</td>
<td>−0.126 (15)</td>
</tr>
<tr>
<td>Hypo O$_2$</td>
<td>BP</td>
<td>0.004 (89)</td>
<td>0.259 (12)</td>
<td>−0.362 (12)</td>
<td>0.273 * (50)</td>
<td>0.043 (15)</td>
</tr>
<tr>
<td></td>
<td>BA</td>
<td>−0.146 (96)</td>
<td>−0.117 (12)</td>
<td>−0.439 (12)</td>
<td>−0.254 * (57)</td>
<td>−0.249 (15)</td>
</tr>
<tr>
<td></td>
<td>BB</td>
<td>−0.065 (96)</td>
<td>−0.067 (12)</td>
<td>−0.369 (12)</td>
<td>−0.023 (57)</td>
<td>−0.245 (15)</td>
</tr>
<tr>
<td></td>
<td>BV</td>
<td>0.154 (96)</td>
<td>0.058 (12)</td>
<td>0.591 * (12)</td>
<td>0.395 *** (57)</td>
<td>0.027 (15)</td>
</tr>
<tr>
<td>Chl-$a$ (20–200 μm)</td>
<td>BP</td>
<td>−0.238 * (98)</td>
<td>−0.147 (16)</td>
<td>0.318 (15)</td>
<td>−0.304 * (52)</td>
<td>0.080 (15)</td>
</tr>
<tr>
<td></td>
<td>BA</td>
<td>0.126 (105)</td>
<td>−0.320 (16)</td>
<td>−0.022 (15)</td>
<td>0.265 * (59)</td>
<td>0.359 (15)</td>
</tr>
<tr>
<td></td>
<td>BB</td>
<td>−0.014 (105)</td>
<td>−0.293 (16)</td>
<td>−0.023 (15)</td>
<td>0.020 (59)</td>
<td>0.351 (15)</td>
</tr>
<tr>
<td></td>
<td>BV</td>
<td>−0.302 *** (105)</td>
<td>−0.021 (16)</td>
<td>0.009 (15)</td>
<td>−0.419 *** (59)</td>
<td>−0.042 (15)</td>
</tr>
<tr>
<td>Chl-$a$ (2–20 μm)</td>
<td>BP</td>
<td>−0.202 * (98)</td>
<td>−0.202 (16)</td>
<td>0.291 (15)</td>
<td>−0.264 * (52)</td>
<td>0.448 (15)</td>
</tr>
<tr>
<td></td>
<td>BA</td>
<td>0.140 (105)</td>
<td>−0.302 (16)</td>
<td>−0.022 (15)</td>
<td>0.277 * (59)</td>
<td>0.636 ** (15)</td>
</tr>
<tr>
<td></td>
<td>BB</td>
<td>−0.027 (105)</td>
<td>−0.254 (16)</td>
<td>−0.014 (15)</td>
<td>0.022 (59)</td>
<td>0.587 ** (15)</td>
</tr>
<tr>
<td></td>
<td>BV</td>
<td>−0.361 *** (105)</td>
<td>0.017 (16)</td>
<td>0.048 (15)</td>
<td>−0.437 *** (59)</td>
<td>−0.186 (15)</td>
</tr>
<tr>
<td>Chl-$a$ (0.7–2 μm)</td>
<td>BP</td>
<td>−0.058 (97)</td>
<td>−0.303 (16)</td>
<td>−0.126 (15)</td>
<td>−0.226 (51)</td>
<td>0.469 * (15)</td>
</tr>
<tr>
<td></td>
<td>BA</td>
<td>0.140 (105)</td>
<td>−0.302 (16)</td>
<td>−0.022 (15)</td>
<td>0.277 * (59)</td>
<td>0.636 ** (15)</td>
</tr>
<tr>
<td></td>
<td>BB</td>
<td>−0.027 (105)</td>
<td>−0.254 (16)</td>
<td>−0.014 (15)</td>
<td>0.022 (59)</td>
<td>0.587 ** (15)</td>
</tr>
<tr>
<td></td>
<td>BV</td>
<td>−0.302 ** (105)</td>
<td>0.017 (16)</td>
<td>0.048 (15)</td>
<td>−0.437 *** (59)</td>
<td>−0.186 (15)</td>
</tr>
<tr>
<td>DOC</td>
<td>BP</td>
<td>0.207 * (97)</td>
<td>0.416 (16)</td>
<td>0.483 * (15)</td>
<td>0.078 (51)</td>
<td>0.425 (15)</td>
</tr>
<tr>
<td></td>
<td>BA</td>
<td>0.226 * (104)</td>
<td>−0.114 (16)</td>
<td>0.031 (15)</td>
<td>0.137 (58)</td>
<td>0.331 (15)</td>
</tr>
<tr>
<td></td>
<td>BB</td>
<td>0.064 (104)</td>
<td>−0.130 (15)</td>
<td>−0.025 (15)</td>
<td>−0.143 (58)</td>
<td>0.303 (15)</td>
</tr>
<tr>
<td></td>
<td>BV</td>
<td>−0.331 *** (104)</td>
<td>−0.047 (16)</td>
<td>−0.273 (15)</td>
<td>−0.523 *** (58)</td>
<td>−0.135 (15)</td>
</tr>
<tr>
<td>TP</td>
<td>BP</td>
<td>−0.070 (98)</td>
<td>0.066 (16)</td>
<td>0.363 (15)</td>
<td>−0.010 (52)</td>
<td>−0.330 (15)</td>
</tr>
<tr>
<td></td>
<td>BA</td>
<td>0.043 (105)</td>
<td>0.036 (15)</td>
<td>−0.304 (15)</td>
<td>0.086 (59)</td>
<td>0.232 (15)</td>
</tr>
<tr>
<td></td>
<td>BB</td>
<td>−0.053 (105)</td>
<td>0.027 (16)</td>
<td>−0.275 (15)</td>
<td>−0.053 (59)</td>
<td>0.129 (15)</td>
</tr>
<tr>
<td></td>
<td>BV</td>
<td>−0.218 * (105)</td>
<td>−0.007 (16)</td>
<td>0.315 (15)</td>
<td>−0.253 * (59)</td>
<td>−0.468 * (15)</td>
</tr>
<tr>
<td>TDP</td>
<td>BP</td>
<td>−0.278 ** (96)</td>
<td>−0.512 (16)</td>
<td>0.286 (14)</td>
<td>0.179 (51)</td>
<td>−0.664 ** (15)</td>
</tr>
<tr>
<td></td>
<td>BA</td>
<td>−0.130 (103)</td>
<td>0.302 (16)</td>
<td>0.451 (14)</td>
<td>−0.048 (58)</td>
<td>−0.266 (15)</td>
</tr>
<tr>
<td></td>
<td>BB</td>
<td>−0.513 * (103)</td>
<td>0.318 (16)</td>
<td>0.425 (14)</td>
<td>−0.122 (58)</td>
<td>−0.308 (15)</td>
</tr>
<tr>
<td></td>
<td>BV</td>
<td>−0.228 * (103)</td>
<td>0.084 (16)</td>
<td>−0.388 (14)</td>
<td>−0.160 (58)</td>
<td>−0.181 (15)</td>
</tr>
<tr>
<td>SRP</td>
<td>BP</td>
<td>−0.133 (96)</td>
<td>−0.206 (16)</td>
<td>−0.008 (15)</td>
<td>0.115 (50)</td>
<td>−0.236 (15)</td>
</tr>
<tr>
<td></td>
<td>BA</td>
<td>−0.163 (103)</td>
<td>0.309 (16)</td>
<td>0.100 (15)</td>
<td>−0.228 (57)</td>
<td>−0.066 (15)</td>
</tr>
<tr>
<td></td>
<td>BB</td>
<td>−0.201 * (103)</td>
<td>0.499 * (16)</td>
<td>0.067 (15)</td>
<td>−0.241 ** (57)</td>
<td>−0.080 (15)</td>
</tr>
<tr>
<td></td>
<td>BV</td>
<td>−0.129 (103)</td>
<td>0.357 * (16)</td>
<td>−0.211 (15)</td>
<td>−0.085 (57)</td>
<td>−0.063 (15)</td>
</tr>
</tbody>
</table>

$^1$BP: Bacterial production (leucine incorporated); BA: Bacterial abundance; BB: Bacterial biomass; BV: Bacterial biovolume
Table 6. Comparison of bacterial parameters in Lake Simcoe versus published values for other temperate freshwater lakes.

<table>
<thead>
<tr>
<th>Location</th>
<th>Production TdR method μg C L$^{-1}$ h$^{-1}$</th>
<th>Production Leu method μg C L$^{-1}$ h$^{-1}$</th>
<th>Abundance cells L$^{-1}$</th>
<th>Biovolume μm$^3$ cell$^{-1}$</th>
<th>Biomass μg C L$^{-1}$</th>
<th>Sampling period</th>
<th>Methods</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lake Simcoe</td>
<td>0.116</td>
<td>0.365</td>
<td>1.56 E × 10$^9$</td>
<td>0.0050</td>
<td>1.41</td>
<td>Jun–Oct</td>
<td>DAPI method, Epifluorescence microscope, 3$^3$H-TdR (20 nM), 14$^C$-Leucine (50 nM), Filtration method</td>
<td>Current study</td>
</tr>
<tr>
<td>Lake Erie</td>
<td>0.05–0.91</td>
<td>5.2–13.1 × 10$^9$</td>
<td></td>
<td></td>
<td></td>
<td>Jul–Aug</td>
<td>3$^3$H-TdR, Epifluorescence microscope</td>
<td>Hwang and Heath 1997</td>
</tr>
<tr>
<td></td>
<td>1.74</td>
<td>1.260</td>
<td>1.8–4.6 × 10$^9$</td>
<td></td>
<td></td>
<td></td>
<td>Acridine Orange Method, Dark uptake, 20 nM TdR, 40 nM Leu, Filtration method</td>
<td>Wilhelm and Smith 2000</td>
</tr>
<tr>
<td>Lake Erie (eastern basin)</td>
<td>0.088</td>
<td>3.90 × 10$^9$</td>
<td></td>
<td></td>
<td></td>
<td>Jul</td>
<td>3$^3$H-Leucine (20 nM), Microcentrifuge method</td>
<td>DeBruyn et al. 2004</td>
</tr>
<tr>
<td>Lake Michigan</td>
<td>0.115</td>
<td>1.13 × 10$^9$</td>
<td></td>
<td></td>
<td></td>
<td>May–Jun</td>
<td>Acridine Orange Method, Epifluorescence microscope, 3$^3$H-Leucine (20 nM), Filtration method</td>
<td>Biddanda and Cotner 2002</td>
</tr>
<tr>
<td></td>
<td>0.005–0.044</td>
<td>5.67–9.98 × 10$^8$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>DAPI method</td>
<td>Heath and Munawar 2004</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>70.7</td>
<td></td>
<td></td>
<td></td>
<td>Aug</td>
<td>3$^3$H-Leucine incorporated</td>
<td>Munawar et al. 2009</td>
</tr>
<tr>
<td>Lake Baikal</td>
<td>0.6–1.5 × 10$^9$</td>
<td>0.03–0.104</td>
<td>5.88–21.15</td>
<td></td>
<td></td>
<td></td>
<td>Used fresh weight to convert to biomass</td>
<td>Straškrábová et al. 2005</td>
</tr>
</tbody>
</table>
Do summer heterotrophic bacterial parameters represent the maximum seasonal values in temperate large lakes?

Because comparable bacterial studies are lacking lakes of a similar size to Lake Simcoe, we compared our bacterial data to those from larger temperate lakes. Our bacterial production rates and abundances were within the ranges presented in the literature for heterotrophic bacteria in temperate freshwater ecosystems (Table 6). In contrast, bacterial biovolume and biomass in Lake Simcoe were lower than those reported for other large freshwater systems such as Lake Superior (Munawar et al. 2009) and Lake Baikal (Straškrábová et al. 2005; Table 6).

The lowest bacterial production rates in Lake Simcoe were measured under ice during the winter while the maximum values consistently occurred in the summer, although a smaller second peak was observed in March under ice. At low temperatures, bacteria require increased substrate concentrations to grow and respire at rates comparable to those under high temperatures (Pomeroy and Wiebe 2001). The March bacterial production peak coincided with a temporary increase in Chl-α concentrations, suggesting enhanced substrate levels may have compensated for the bacterial metabolism temperature suppression (Biddanda and Cotner 2002). Further research is required to determine if this bacterial production pulse was an annual event or an anomaly. Although there were no significant differences found among bacterial abundances in the spring, summer, and fall, we did observe a November peak in abundance. These results indicate that sampling in the summer will capture peak bacterial production rates but will not be representative of the fall to spring period when production is reduced and will miss the fall abundance peak.

In the Mediterranean Sea, bacterial abundance was found to be lowest during the well-mixed periods, which Trabelsi and Rassoulzadegan (2011) partly attributed to high viral abundance during lake mixing. To date, no viral work has been conducted in Lake Simcoe. Grazing by protozoans also plays a role in controlling bacteria (Sherr et al. 1983, Chrost et al. 2000, Kalff 2002) and can be an important channel for transferring bacterial biomass to upper trophic levels. In the Laurentian Great Lakes (lakes Huron and Michigan), average protozoan abundance is 4–15 cells mL⁻¹, with the lowest abundance occurring in the winter and the highest abundances measured from May–June and October–November (Carrick and Fahrenstiel 1990).

Protozoan abundance has not been reported for Lake Simcoe, but seasonal patterns in bacterial abundance reflect the protozoan seasonal dynamics reported for lakes Huron and Michigan, with low abundances in the winter and high abundances in the spring to early summer and late summer to early fall. In July, when both bacterial abundance and biomass decreased, bacterial production was at its maximum and biovolume exhibited a small peak. This indicates fewer cells were being produced but their mean size had increased. Bacterial biovolume in Lake Simcoe was lower than that reported in other freshwater studies (Table 6). Novitsky and Morita (1976) reported a decrease in bacterial biovolume of starved cells, which may have been the case during the winter in Lake Simcoe when the smallest biovolumes were measured. In Lake Erie, maximum bacterial biovolumes were observed in May and July, while minimum biovolumes occurred in June and August (Hwang and Heath 1997). In contrast, maximum bacterial biovolume for Lake Simcoe was observed in June, when bacterial production was at a maximum. Increased temperature and C availability during this time may have allowed production of larger bacterial cells.

Factors controlling bacterial production in Lake Simcoe

Bacterial production rates are affected by both abiotic factors (e.g., temperature, substrate availability, and nutrient concentrations; Jonas et al. 1988, Kirchman 1990, Pomeroy and Wiebe 1993), and biotic factors (e.g., grazing; Button 1994, Sherr and Sherr 2002). Numerous studies have shown that temperature and bacterial production are positively correlated (Servais 1989, Findlay et al. 1991, Coveney and Wetzel 1995), as was seen in Lake Simcoe. In some temperate lakes, bacterial production and temperature were correlated below a certain critical level (10 °C for Lake Michigan, Scavia and Laird 1987; 14 °C for Mirror Lake, Ochs et al. 1995). At temperatures above this critical level, bacterial production may be limited by nutrient concentrations (Morris and Lewis 1992) and substrate quality (Kirchman 1990), as well as bacterial grazing and viral lysis. Thus, temperature may also regulate nutrient competition between bacteria and phytoplankton (Pomeroy and Wiebe 1988, Ameryk et al. 2005). In Lake Simcoe, this critical temperature seems to be 17 °C, above which bacterial production and water temperatures were uncoupled. Grazing by protozoa could be responsible for the decoupling between bacterial production and water temperature seen here at temperatures above 17 °C. At the same time that protozoa peaked in lakes Huron and Michigan, bacterial production in Lake Simcoe was tightly coupled with TDP.
Annual bacterial production was significantly correlated with Chl-\(a\) (20–200 \(\mu\)m) and DOC concentrations. DOC and Chl-\(a\) concentrations are useful variables for estimating the supply of organic matter (Chrost et al. 2000) to heterotrophic bacteria. As Chl-\(a\) concentrations increased in Lake Simcoe, bacterial production decreased, likely a result of decreased P (TDP and SRP) availability for bacteria due to uptake by phytoplankton. One source of labile DOC is through the release by phytoplankton (Chrost et al. 2000). Similar to the findings of Biddanda and Cotner (2002), our DOC concentrations were positively correlated with Chl-\(a\) concentrations, particularly the 0.7–2 \(\mu\)m size fraction. Annual bacterial abundance and biovolume were also significantly correlated with both Chl-\(a\) and DOC concentrations, implying that organic matter from phytoplankton seem to provide an important substrate for not only bacterial production, but abundance and biovolume as well.

Given the importance of P as a limiting nutrient to phytoplankton, combined with the strong role it plays in the management of Lake Simcoe (Young et al. 2011), we compared trends in bacterial parameters with the different forms of P. With increasing TP and TDP concentrations, bacterial cells were smaller, which may be attributed to an increase in phytoplankton biomass. Stronger competition between bacteria and phytoplankton for P may occur during periods of increased P concentrations; thus, P may be limiting for both phytoplankton and bacteria in Lake Simcoe. Although bacterial production was occurring, bacterial cell size may have been limited by availability of P during the summer.

With increasing P concentrations there could be an increase in predator populations, which could also contribute to smaller bacterial cell size. Bacteria are selectively grazed by protozoan bacterivores, which tend to select the largest, fastest growing cells (Sherr et al. 1992). In eutrophic systems there should be a positive relationship between frequency of larger, rapidly growing cells and protozoan bacterivory (Sherr et al. 1992). Zooplankton and dreissenid mussels can also selectively graze larger bacterial cells, thus eliminating larger cells at a faster rate than smaller ones in the water column. When protozoans are the dominant grazers, however, zooplankton and dreissenids feed on smaller bacterial cells (Kalff 2002). In addition, viral lysis is known to account for a significant fraction of bacterial mortality in eutrophic systems (Weinbauer and Peduzzi 1995). Overall, in Lake Simcoe the relationship between P concentrations and small-sized bacteria may be attributed to increased competition for nutrients by phytoplankton or changes in grazing dynamics protozoans, zooplankton, and dreissenids.

### Relationship between bacterial activity and lake trophic status

Within Lake Simcoe, a range in trophic status can be observed from eutrophic conditions in Cook’s Bay (21.8 mg L\(^{-1}\) annual ice-free mean TP concentration in 2010), to the oligotrophic main basin and Kempenfelt Bay (12.0 and 12.5 mg L\(^{-1}\), respectively, annual ice-free mean TP concentrations in 2010; Wetzel 2001, North et al. 2013). Trophic status of a lake can influence bacterial activity measurements; however, the lack of spatial variation in bacterial activity from our sampling sites may be due to the limited range and overall low bacterial activity in Lake Simcoe. Chrost et al. (2000) found that autochthonous C was much more microbiologically labile than allochthonous C. They also observed that the highest proportion of labile DOC was found in enriched lakes. Labile DOC that is released by phytoplankton (EOC; Meyer et al. 1987, Moran and Hodson 1990, Chrost et al. 2000), aquatic macrophytes (Murray and Hodson 1985), and allochthonous DOC from sources such as agriculture and sewage are the most significant energy sources for bacterial production (Meyer et al. 1987, Moran and Hodson 1990).

Due to the range in trophic status across the lake, variation in bacterial activity was expected, but was not observed. The lack of spatial differences may be due to competition with phytoplankton, increased grazing pressure, and free versus attached bacteria. Eutrophic Cook’s Bay has high biomass of both phytoplankton (North et al. 2013) and submerged aquatic vegetation (SAV; Depew et al. 2011, Ginn 2011). SAV has high nutrient demands and also serves as habitat for other organisms including bacteria. This may result in numerous bacteria attached to the vegetation while free-floating bacteria dominate at other sites; the former would not be included in measurements made in this study. Perhaps as a result of such high primary producer biomass, higher zooplankton abundances have been reported in Cook’s Bay relative to other areas of the lake (North et al. 2013). This increased grazing pressure may be contributing to the low bacterial abundance. An additional, but untested, contributing factor to bacterial mortality could be high viral lysis rates, typical of eutrophic systems (Weinbauer and Peduzzi 1995).

### Why was there no relationship between bacterial production and measures of abundance?

In contrast to previous studies (Biddanda and Cotner 2002, DeBruyn et al. 2004), epilimnetic bacterial production in Lake Simcoe was not significantly correlated to bacterial abundance, biomass, or biovolume.
The lack of relationships may be attributed to a change in the proportion of metabolically active bacteria (DeBruyn et al. 2004). The DAPI stain used in this study was a DNA-specific, fluorescing stain commonly used to stain bacteria; however, it does not differentiate between living and dead cells (Madigan et al. 2000), whereas bacterial production only measures viable cells.

Other researchers have found that a significant proportion of aquatic heterotrophic bacterial populations can be in an inactive state, especially under starvation conditions (Cole 1982, Goulder 1991, Smith and del Giorgio 2003), which could explain why bacterial production, abundance, biomass, and biovolume were significantly lower in winter than in other seasons. The addition of limiting nutrients may stimulate inactive bacteria to become active, thus increasing production rates while maintaining the same bacterial abundance (DeBruyn et al. 2004). There may be evidence of this occurring in Lake Simcoe; during fall mixis, with increased P concentrations in the water column, bacterial production was strongly correlated to both bacterial abundance and biomass.

Additionally, the lack of relationship between bacterial production and the other bacterial parameters could be due to grazing pressure on bacteria by protozoans and viral lysis (Chin-Leo and Kirchman 1990). Grazing by protozoans plays an important role in controlling bacterial numbers (Sherr et al. 1983, Chrost et al. 2000, Kalff, 2002), therefore, abundance is less responsive to environmental conditions (nutrients and temperature) and more responsive to grazing than bacterial production. In addition, viral lysis can affect bacterial production and abundance relationships because viral lysis redistributes nutrients to bacteria, which can be associated with an increase in bacterial production but a decrease in bacterial abundance.

Our study has provided insights on bacterial activity in Lake Simcoe, but additional work is needed to more fully describe bacteria in Lake Simcoe; in particular, multivariate analysis may help to understand the environmental factors affecting bacterial activity and seasonal dynamics. Future bacterial studies should also investigate heterotrophic respiration rates in the system on a seasonal and spatial basis, and characterize the influence of viral abundances and activity on bacteria. It is also unknown what influence protozoan and dreissenid grazing have on bacterial populations in the lake.

**Conclusions**

This study consisted of extensive temporal and spatial measurements of bacterial activity in Lake Simcoe to determine if bacteria contribute to the low hypolimnetic O$_2$ concentrations in the lake, as well as to investigate the factors influencing annual epilimnetic patterns in the 4 bacterial parameters measured. Annual bacterial production was low in Lake Simcoe relative to other systems, and we found no evidence for heterotrophic bacterial activity contributing to low hypolimnetic O$_2$ conditions. Although thymidine and leucine incorporation methods resulted in significantly different bacterial production estimates, the resultant temporal and spatial patterns were similar between the 2 methods. We found that bacterial production rates were lowest under the ice during the winter, while the maximum values consistently occurred during the open-water season. Maximum bacterial abundance occurred in November in this study, thus sampling exclusively in the spring and summer may result in an underestimation of bacterial abundance.

In addition, by not sampling winter abundances, researchers may be over-estimating annual abundance rates. Annual differences between the mean epilimnetic and hypolimnetic samples for the bacterial parameters were inconsistent as one was not consistently higher than the other. Due to the lack of spatial differences in bacterial activity across the length of the lake, future work may not require such extensive spatial sampling. In Lake Simcoe, bacterial activity was positively correlated with high lake temperature and high DOC concentrations with the exception of bacterial biovolume, which was negatively correlated with DOC, most likely due to the abundance of smaller cells being produced. To our knowledge, ours is the first investigation of heterotrophic bacterial activity in Lake Simcoe, and we hope our work will initiate further research on this important component of the Lake Simcoe ecosystem.

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