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# A Seasonal Study of Bacterial Community Succession in a Temperate Backwater System, Indicated by Variation in Morphotype Numbers, Biomass, and Secondary Production

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## ABSTRACT

The investigation of the bacterial community in the Kühwörter Wasser, a macrophyte-dominated arm of the River Danube backwater system near Vienna, revealed that variation in microbial densities and biomass could be related to a characteristic sequence in morphotype composition over the seasons. Maximal bacterial cell numbers and biomass occured in early summer, with values of up to $9 \times 10^9$ cells $l^{-1}$ and 122 μg C $l^{-1}$, respectively, caused by a massive increase of vibrio-shaped cells. On the other hand, in early spring, filamentous bacteria were responsible for a marked increase in bacterial biomass, making up 40% of the total bacterial biomass. Over the year, rod-shaped cells were the dominating morphotype, while the biomass of cocci was rather negligible. In winter, cell numbers and biomass showed minimal values with $2.0 \times 10^9$ cells $l^{-1}$ and 28 μg C $l^{-1}$, respectively, and bacteria were considered to be substrate and temperature limited during this period. Saturation values of the incorporation of $^3$H-thymidine into DNA, for the estimation of bacterial secondary production, varied seasonally, ranging from 5 nM to 40 nM. Thus, saturation experiments needed to be conducted on a regular basis. Also, the amount of labeled thymidine in the DNA, as a percentage of labeled thymidine in the TCA precipitate, varied over the year. Minimum values of 45% were recorded during the cold season, while maximum values of 75–80% at the beginning of June coincided with high chlorophyll *a* values and minimal $K_m$-values derived from saturation experiments. The potential role of the nitrogen-rich nucleoside thymidine as a readily utilizable substrate for bacteria during labeling experiments, under varying conditions of substrate availability, is discussed. Bacterial secondary production rates ranged from 0.3 μg C $l^{-1}$ $h^{-1}$ in winter to values of 10 μg C $l^{-1}$ $h^{-1}$ in August, where phytoplanktonic biomass reached the summer maximum, and bacterial biomass was calculated to be renewed 3 times per day. An estimation of the bacterial carbon demand showed that for the major part of the year, with the exception of early spring, the bacterioplankton community in the Kühwörter Wasser was dependent on carbon sources other than phytoplanktonic primary production.

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## Introduction

Although a large amount of information is now available from diverse aquatic ecosystems on energy-flow processes within the microbial compartment, there is a gap of information concerning the functioning of the microbial loop in the water column of backwater systems [8]. These shallow floodplain waterbodies are generally characterized by expansive submerse and emerse macrophyte beds, and usually support greater phytoplankton densities than the rivers they are derived from [7]. The investigated locality, the Kühwörter Wasser, is part of the extensive backwater system of the Danube River near Vienna, and is a nature reserve [31]. The trophic states of the different arms of the backwater system were reported to vary from meso- to severely eutrophic [16], the Kühwörter Wasser belonging to the branches at the lower end of the trophic scale, with average chlorophyll *a* values of 6 $\mu g\, l^{-1}$ over the year, and total phosphorus concentrations below 20 $\mu g\, l^{-1}$. This backwater branch has an average open water area of 0.23 $km^2$ and an average depth of 1 m. A thick sediment layer, up to 40 cm, fills the basin of the Kühwörter Wasser with submerse species such as *Myriophyllum spicatum, M. verticillatum,* and floating leaved *Nymphaeaceae* species dominating the system. A belt of emerse macrophytes, consisting mainly of *Phragmites australis, Typha latifolia,* and *Schoenoplectus lacustris,* surrounds the major part of the Kühwörter Wasser. No stratification of the water column occurs over any part of the year. In winter (January to February), a consistent ice layer covers the water column of the Kühwörter Wasser.

It has been established that heterotrophic bacteria, as an important component of the planktonic community, contribute significantly to regulating the flux of organic matter [2]. We concentrated on characterizing the heterotrophic bacterial compartment by the quantitative description of cell morphotype variations over the seasons, (i.e., cell numbers and cell volumes) to study the magnitude of energy flow in the water column of backwater systems. Seasonal bacterioplankton succession is often reflected by changes in cell volumes [40]. In this context, our aim was to determine whether the morphotype composition of a bacterioplankton community in a temperate freshwater ecosystem changes in a consistent pattern over the year, and which environmental factors could explain these variations. We also used bacterial production rates to estimate the magnitude of carbon flow through the microbial compartment. We questioned to what extent planktonic algal production supports the bacterial carbon demand in this macrophyte-dominated backwater system. The results of our study indicate that, for the major part of the year, the bacterial compartment requires a significant amount of carbon from macrophytes to support the measured production rates.

## Materials and Methods

### *Sampling*

From May 1991 to June 1992, triplicate samples were taken using acid-rinsed, sterilized, 1-l Schott flasks at biweekly to monthly intervals, at a depth of 40 cm between 9:30 and 10:30 a.m., at a constant station in the lower part of the Kühwörter Wasser. At each date, water temperature was determined using a mercury thermometer. Samples were transported within 1 h to the laboratory, in a cooling bag.

### *Chlorophyll* a

For chlorophyll *a* determination, 100–300 ml of the samples was filtered through Whatman GF/F filters (47 mm in diameter, England), followed by extraction for 24 h with 90% methanol at 4°C [36, 59]. The solutions were measured by fluorometry (F-2000; Hitachi, Japan) using an excitation wavelength of 435 nm and an emission wavelength of 675 nm. Standards (Spinach; C-5753) were obtained from Sigma (St. Louis, Missouri).

### *Bacterial Numbers and Biometry*

For estimation of bacterial numbers (BN) and biomass, 20-ml subsamples were transferred to acid-rinsed, sterile scintillation vials and fixed with 1 ml buffered formaldehyde (33%, pH = 7.4). The vials were stored at 4°C and counted within 14 days. For microscopic examination, the acridine orange direct count method [21] was applied. In all steps, 0.1-μm-filtered and autoclaved solutions were used. One to two milliliters of the fixed samples was mixed with an acridine orange solution to a final concentration of 0.01%. After 5 min, the mixture was filtered through a 0.1-μm pore-size filter (black; Nuclepore, California, USA), which had been mounted onto a cellulose-nitrate filter (Sartorius, 0.2 μm pore-size, Göttinger, Germany), resulting in an even distribution of the cells on the filter. Filters were observed at a magnification of 1250× with a Leitz Diaplan microscope (Germany) equipped with an HBO 50W mercury lamp (excitation wavelength 450–490 nm, cutoff filter 515 nm).

Bacteria were separated into four classes, according to their different morphology: rods; cocci; curved rods (vibrios, including spirillae); and filamentous bacteria. Cells were operationally defined as rods if their length and width differed by more than 0.1 μm [56]. Bacteria longer than 3 μm and showing a diameter smaller than or equal to 0.5 μm were defined as filamentous forms. Bacteria sizes were determined using an eyepiece micrometer. Fluorescent

latex beads with diameters of 0.11, 0.22, 0.6, and 0.88 μm were used for calibration of the sizing procedure [56]. Cell volume estimations were based on the assumption that all bacteria are spheres or cylinders with two hemispherical caps. At least 30 fields per sample were counted, and 160–200 cells were measured (> 40 per morphotype). Cellular carbon content in fg C cell$^{-1}$ ($C$) was calculated from estimated cell volumes ($V$; μm$^3$) assuming the allometric relation $C = 120\ V^{0.72}$, after Norland [35].

### *Thymidine Incorporation*

Triplicate subsamples (10 ml) of each sample were placed in acid-rinsed, sterile 20-ml polyethylene scintillation vials (Canberra Packard, Illinois, USA) in a waterbath at the measured in situ temperature (± 1°C). Concentrations of radioactive [methyl-3H]thymidine (75–85 mCi mmol$^{-1}$; NEN-Research Products, Boston) between 5 and 40 nM (final concentration) were used. Own saturation experiments indicated that these concentrations prevented isotope dilution [4, 38]. The Michaelis-Menten equation was used to describe the kinetics of the thymidine uptake into the bacterial DNA. Incubation was stopped after 30 min with 0.5 ml buffered formaldehyde (33%, pH = 7.4). In preliminary experiments, we found that incorporation of radioactive thymidine (TdR) was linear for at least one hour.

DNA extraction was performed according to Wicks and Robarts [60]. Briefly, after the addition of formaldehyde, the solutions were subsequently chilled and acidified with ice-cold TCA (5% final conc.). After 20 min, precipitates were collected on 0.1-μm pore-size cellulose nitrate 25-mm-diameter filters (Sartorius). For the extraction of proteins the filters were treated with 5 ml of phenol-chloroform (50% w/v) followed by 5 ml of ice-cold 80% v/v ethanol. Filters were transferred into 7-ml scintillation vials (Canberra Packard) and, after drying overnight, dissolved in 0.75 ml of cellosolve (2-ethoxyethanol) for 2 h. Scintillation cocktail (4.5 ml, Filter Count; Canberra Packard) was added, and, after 24 h, radioactivity was measured with a Canberra Packard scintillation counter (1900 TR). Counts were automatically corrected for quenching using a stored standard curve and a machine counting efficiency program. To determine the initial radioactivity added, 100-μl portions were withdrawn from the samples at the beginning of the assay and counted after the addition of 4.5 ml of Ultima Gold scintillation cocktail (24 h of storage) from Packard.

Bacterial cell production was calculated using an empirically derived conversion factor of $2.8 \times 10^{18}$ cells produced mol$^{-1}$ TdR incoporated into DNA [31]. The obtained values of cell production were converted into bacterial carbon production by multiplying this value with the estimated mean cellular carbon content. TdR incorporation into the cold TCA precipitate (considered usually as total macromolecules) was measured seasonally.

### *Turnover Time*

The turnover time of the bacterial population was estimated by dividing bacterial biomass (BBM, μg C l$^{-1}$) by the bacterial secondary production (BSP, μg C l$^{-1}$ h$^{-1}$).

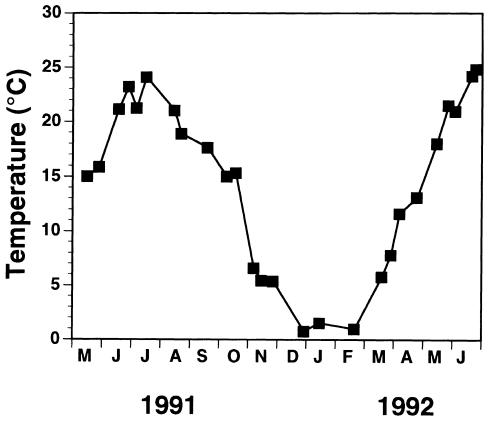


Fig. 1. Variation in the water temperature in the backwater Kühwörter Wasser during the investigation period.

## Results

### *Temperature*

Water temperature varied between 25°C in summer and a few degrees above 0°C in the winter, when the creek was covered with ice (Fig. 1).

### *Chlorophyll* a

Figure 2 shows that the magnitude of the chlorophyll *a* concentration pattern differed markedly between spring 1991 and 1992. In 1991, a distinct peak was found in May, reaching values of 20 μg C l$^{-1}$, while during June 1991 there was a marked decrease in concentration. After the summer phytoplankton bloom in August, values dropped to 4 μg l$^{-1}$ and remained at a constant level until November. From December 1991 until February 1992 the concentrations were low. During spring 1992 two small peaks in concentrations were observed, one occurred in March and the other at the end of April. No chlorophyll *a* peak was recorded for May 1992, indicating the decrease in pigments after the spring peak occurred earlier than in 1991. Values during June 1992 were at least 5 times lower than during June 1991.

### *Bacterial Numbers*

Total bacterial numbers averaged $4.0 \times 10^9$ cells l$^{-1}$ (SD: $1.5 \times 10^9$), showing highest values in summer with densities of $6.8 \times 10^9$ (1991) and $9.0 \times 10^9$ cells l$^{-1}$ (1992), and lowest values of about $2.0 \times 10^9$ cells l$^{-1}$ during the cold period (Fig. 3). In addition to the summer peak, a maximum of $5.8 \times 10^9$ cells l$^{-1}$ could be observed in spring 1991. During

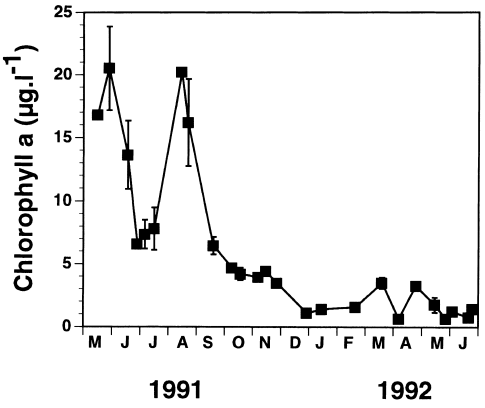


Fig. 2. Variation in chlorophyll *a* in the backwater Kühwörter Wasser during the investigation period; *bars* represent 2 standard deviations of the three replicates.

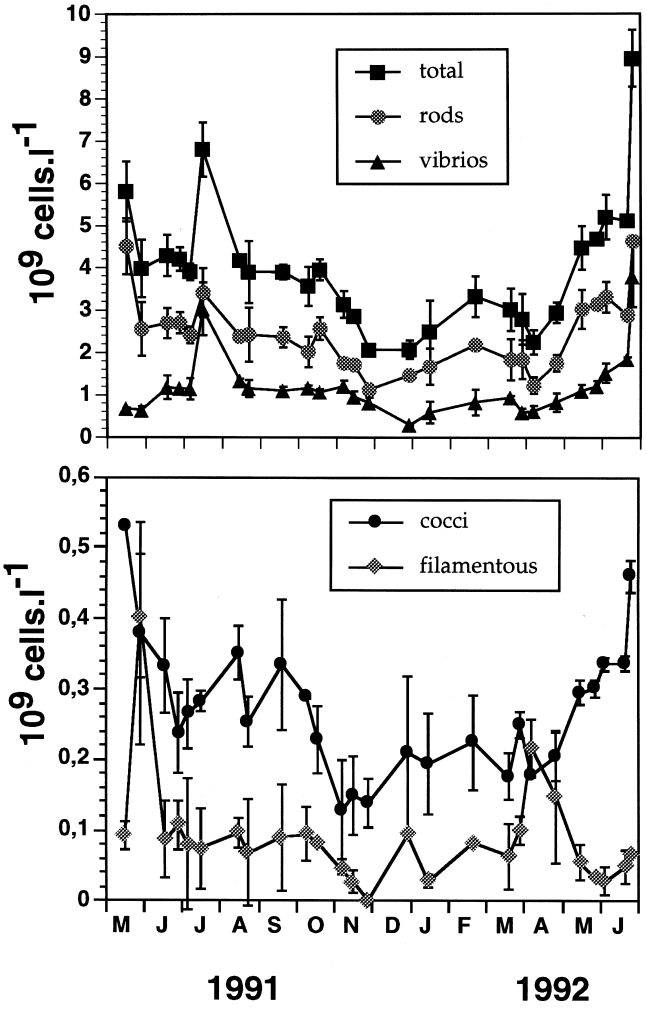


Fig. 3. Total bacterial numbers and numbers of rods, vibrios, cocci, and filamentous bacteria in the Kühwörter Wasser; *bars* represent 2 standard deviations of the three replicates.

both summer maxima, a large increase in numbers of vibrio-shaped cells was responsible for the high amount of bacterial cells, whereas during the spring maximum, the rod-shaped morphotype was the dominating form.

The amount of rod-shaped cells ranged from $1.1 \times 10^9$ in November to $4.5 \times 10^9$ and $4.6 \times 10^9$ cells $l^{-1}$ in May 1991 and June 1992, respectively, with a mean value of $2.5 \times 10^9$ cells $l^{-1}$ (SD: $0.9 \times 10^9$) over the seasons. Densities of vibrio-shaped cells showed a mean value of $1.2 \times 10^9$ cells $l^{-1}$ (SD: $0.7 \times 10^9$) and exhibited two marked peaks, one at the end of July 1991, with $3.0 \times 10^9$ cells $l^{-1}$, and one at the end of June 1992, with $3.8 \times 10^9$ cells $l^{-1}$. The lowest value during the year ($0.3 \times 10^9$ cells $l^{-1}$) was observed in December.

Cocci and filamentous forms were only minor components of total bacterial numbers (Fig. 3). Densities of cocci varied from between $0.1 \times 10^9$ cells $l^{-1}$ in November, and $0.5 \times 10^9$ cells $l^{-1}$ in spring; densities of filamentous bacteria averaged $0.09 \times 10^9$ cells $l^{-1}$ (SD: $0.08 \times 10^9$) over the seasons with two marked peaks in spring. At the end of May 1991, $0.4 \times 10^9$ filamentous cells $\times l^{-1}$ were counted, while in 1992, populations of $0.22 \times 10^9$ cells $l^{-1}$ were reached in the middle of April.

Expressed as a percentage of total bacterial numbers, rods were the dominating morphology with 62% (SD: 6%) over all seasons. Vibrios comprised, on average, 29% (SD: 8%) to total BN with maximal values of 45 and 42% in July 1991 and June 1992, respectively. The proportion of coccal forms was rather constant at 7% (SD: 1.5%), over the seasons, while filamentous forms showed strong seasonal variation. Densities ranged from below detection in November to 10% and 9% of total BN in May 1991 and April 1992, respectively.

### *Cell Volumes and Cellular Biomass*

Average cell volumes ranged from 0.044 $\mu m^3$, when filamentous forms were lacking, to 0.129 $\mu m^3$ at the beginning of April 1991, when filamentous bacteria comprised up to 10% of total BN (Fig. 4a). Over the year, the average cell volume was 0.065 $\mu m^3$ (SD: 0.022 $\mu m^3$). The average cell volumes of rods and cocci were rather constant over the seasons with mean values of 0.043 $\mu m^3$ (SD: 0.004) and 0.010 $\mu m^3$ (SD: 0.002), respectively. The cell volumes of vibrios varied to a greater extent, namely between 0.056 and 0.089 $\mu m^3$ (mean: 0.069 $\mu m^3$; SD: 0.009). Filamentous cell (Fig. 4b) volumes were approximately one order of magnitude larger (mean: 0.70 $\mu m^3$; SD: 0.26), with the smallest volumes occurring in November (0.24 $\mu m^3$), and the largest in April 1992 (1.33 $\mu m^3$). At the end of summer 1991, filamentous cell volumes

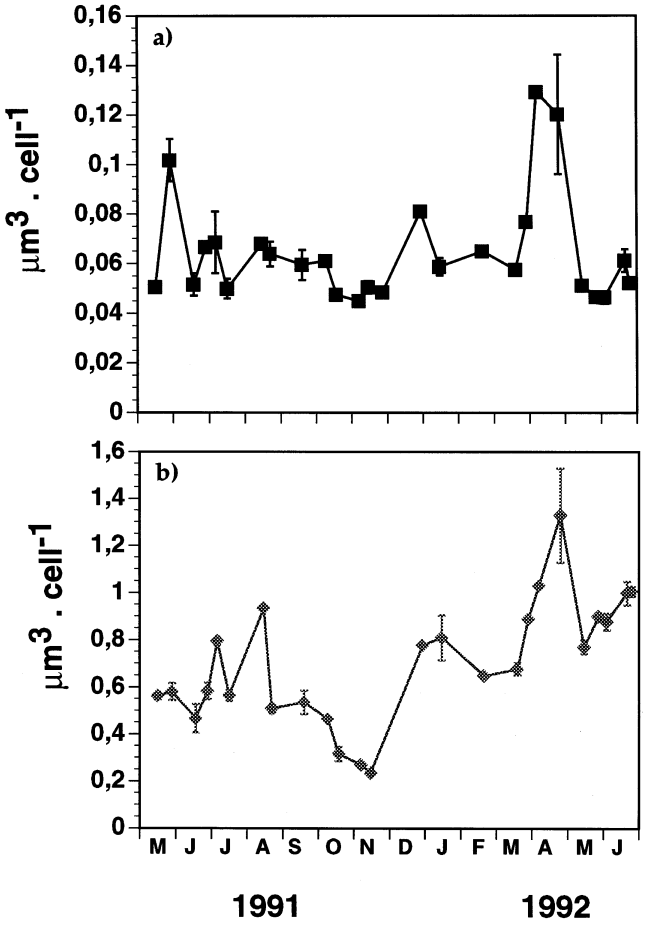


Fig. 4. Variation in bacterial cell volumes in the Kühwörter Wasser during the investigation period; (**a**) mean bacterial cell volumes (weighted according to the relative abundance of each morphotype); (**b**) filamentous bacteria; *bars* represent 2 standard deviations of three replicates.

**Table 1.** Bacterial biomass, bacterial carbon production, and turnover times in the backwater system of the Kühwörter Wasser[a]

| Date | Bacterial biomass (µg C $l^{-1}$) | Bacterial production (µg C $l^{-1}$ $h^{-1}$) | Turnover time (h) |
|---|---|---|---|
| May 15, 1991 | 76.9 (10.9) | 2.6 (0.34) | 30 |
| May 27, 1991 | 79.2 (8.1) | 2.5 (0.72) | 32 |
| June 17, 1991 | 57.5 (14.0) | 2.3 (0.28) | 25 |
| June 27, 1991 | 67.5 (5.9) | 2.3 (0.21) | 29 |
| July 5, 1991 | 61.6 (18.7) | 2.2 (0.11) | 27 |
| July 15, 1991 | 90.7 (18.3) | 2.6 (0.17) | 34 |
| August 14, 1991 | 68.0 (2.3) | 10.0 (1.78) | 7 |
| August 21, 1991 | 61.1 (9.2) | 8.1 (2.75) | 8 |
| September 18, 1991 | 57.4 (9.9) | 1.9 (0.46) | 30 |
| October 8, 1991 | 54.3 (9.1) | 1.0 (0.14) | 52 |
| October 17, 1991 | 51.1 (1.5) | 0.9 (0.05) | 55 |
| November 6, 1991 | 41.0 (1.5) | 0.8 (0.02) | 51 |
| November 14, 1991 | 39.0 (4.3) | 0.6 (0.10) | 69 |
| November 26, 1991 | 27.6 (0.5) | 0.3 (0.03) | 85 |
| December 28, 1991 | 37.1 (0.9) | 0.6 (0.02) | 67 |
| January 14, 1992 | 36.6 (14.4) | 0.5 (0.28) | 71 |
| February 20, 1992 | 51.0 (1.5) | 1.0 (0.17) | 52 |
| March 18, 1992 | 45.2 (6.0) | 1.6 (0.27) | 29 |
| March 27, 1992 | 46.0 (2.1) | 2.3 (0.81) | 20 |
| April 6, 1992 | 55.8 (0.5) | 1.8 (0.30) | 31 |
| April 24, 1992 | 61.7 (26.3) | 1.8 (0.84) | 34 |
| May 14, 1992 | 59.6 (6.0) | 2.2 (0.26) | 27 |
| May 26, 1992 | 58.7 (0.5) | 1.4 (0.41) | 42 |
| May 3, 1992 | 65.5 (7.0) | 2.1 (0.49) | 31 |
| June 20, 1992 | 78.1 (8.0) | 2.8 (0.55) | 28 |
| June 24, 1992 | 121.6 (11.3) | 2.3 (0.48) | 53 |

[a] Values in parentheses represent standard deviations of three replicates

began to decrease and from November to December the average cell volume of the filamentous forms increased from 0.24 µm³ to 0.78 µm³, and remained at this value thereafter.

Average cellular carbon content over all sampling dates and morphotypes was 15.3 fg C $cell^{-1}$ (SD: 2.9), ranging from 12.5 to 24.7 fg C $cell^{-1}$. Mean C content of rod shaped cells amounted to 12.4 fg C $cell^{-1}$ (SD: 0,9), cocci had a mean value of 4.4 fg C $cell^{-1}$ (SD: 0.6), vibrios 17.4 fg C $cell^{-1}$ (SD: 1.6), and filamentous cells 88 fg C $cell^{-1}$ (SD: 31).

### *Total and Morphotype-Specific Bacterial Biomass*

In general, the seasonal variation of total bacterial biomass followed the pattern of total bacterial numbers, with the exception of the periods when filamentous bacteria were abundant (Table 1). Total bacterial biomass ranged from low levels in autumn and winter (28 µg C $l^{-1}$) to maximal values in spring and summer (122 µg C $l^{-1}$), with a mean of 60 µg C $l^{-1}$ (SD: 19 µg C $l^{-1}$) over the year. Each spring, a strong

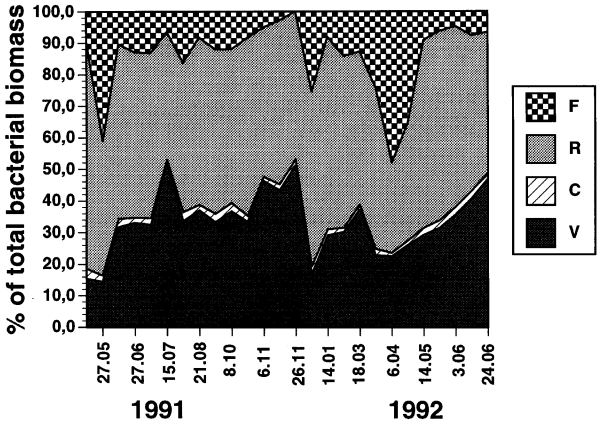


Fig. 5. Variation in the percentage of the four bacterial morphotypes relative to total bacterial biomass in the Kühwörter Wasser during the investigation period; *F*, filamentous bacteria; *R*, rods; *C*, cocci; *V*, vibrios.

**Table 2.** Calculated $K_m$ and $V_{max}$ values derived from the saturation experiments of $^3$H-thymidine incorporation into DNA, and amount of label incorporated into DNA as a percentage of label in the TCA precipitate (% DNA)[a]

| Date | $K_m$ (nM) | $V_{max}$ (pMh$^{-1}$) | % DNA |
|---|---|---|---|
| May 20, 1991 | 0.19 (0.10) | 58.5 (3.7) | 77.3 |
| June 2, 1991 | n.d.[b] | n.d. | 78.3[c] |
| August 22, 1991 | 10.30 (4.50) | 346.5 (53.4) | 67.9 |
| October 8, 1991 | 3.50 (1.33) | 36.4 (3.7) | n.d. |
| November 6, 1991 | 2.37 (0.65) | 21.4 (1.4) | 67.6 |
| December 20, 1991 | 13.10 (1.90) | 35.9 (2.4) | 54.0 |
| March 27, 1992 | 20.81 (1.93) | 56.3 (2.4) | 45.4 |
| April 4, 1992 | n.d. | n.d. | 46.7[c] |
| May 26, 1992 | n.d. | n.d. | 66.7[c] |
| June 3, 1992 | 0.25 (0.07) | 74.8 (3.7) | 75.1 |
| June 24, 1992 | 1.77 (0.78) | 58.5 (3.7) | 58.6 |

[a] Values in parentheses represent standard deviations of the estimate; saturation curves were fitted with the IBM-PC program Enzfitter (Elsevier Biosoft)
[b] n.d., Not determined
[c] Values derived from incubations with $^3$H-thymidine concentrations obtained from the previous saturation experiment

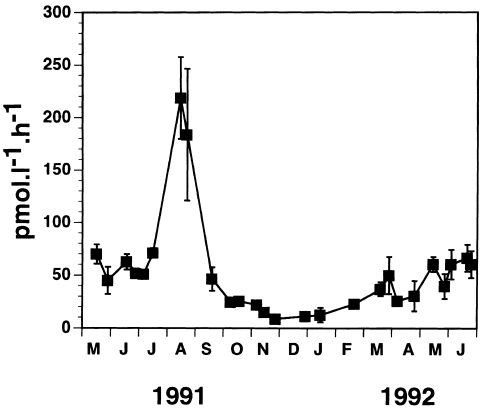


Fig. 6. Variation in the incorporation of $^3$H-TdR into the DNA of bacterioplankton in the Kühwörter Wasser during the investigation period; *bars* represent 2 standard deviations of three replicates, each consisting of three subsamples.

increase in biomass of filamentous bacteria was observed (May 1991 and April 1992), where they contributed 41 and 48% to the total bacterial biomass, respectively (Fig. 5). For a short period during early summer (15 July 1991; 24 June 1992), however, vibrio-shaped bacteria were the dominating morphotype (51.6% and 46.9%, respectively). On average, rods contributed 50.9% (SD: 8.5%), vibrios 33.2% (SD: 10.0%), and filamentous bacteria 13.9% (SD: 11.7%) to total bacterial biomass. For the rods, a maximum value of 72% could be observed in May 1991. At a mean biomass of 2.0% (SD: 0.5%), cocci were negligible throughout the year.

### *Thymidine Incorporation*

TdR incorporation into DNA was found to be linear over at least one hour of incubation (data not shown). The concentration of added thymidine, where a saturation of thymidine uptake into bacterial DNA was reached, changed markedly over the seasons. A low value of 5 nM TdR was sufficient for reaching saturation in late spring. From summer 1991 until early spring 1992, 25–40 nM TdR was necessary. Thereafter, saturation values decreased again to 5 nM. The seasonal variation of the calculated $K_m$ values from these saturation experiments is shown in Table 2. Very low values ($< 1$ nM) were observed in late spring, while, during the rest of the year, $K_m$ values were one to two orders of magnitude higher, with a maximum of 20.8 nM during the winter period.

The amount of $^3$H-TdR incorporated into DNA, expressed as a percentage of the $^3$H-TdR in the TCA precipitate, varied between 45 and 78% (mean: 64.5%; *SD:* 12.2; $n = 9$; Table 2). Our data appear to follow a seasonal pattern, with high values during late spring and summer, decreasing toward the colder part of the year, with minimal values in April (Table 2).

The seasonal variation of bacterial thymidine incorporation into DNA is shown in Fig. 6. From May to July 1991, the incorporation rate ranged from 45 to 71 pmol l$^{-1}$ h$^{-1}$. In August, a steep increase was recorded, with maximal incorporation rates of more than 200 pmol l$^{-1}$ h$^{-1}$. In September, the incorporation values dropped to a range similar to those observed from May to July. They gradually declined afterward, to a minimum of 9 pmol l$^{-1}$ h$^{-1}$ at the end of November. During the winter period, thymidine incorporation rates remained below 25 pmol l$^{-1}$ h$^{-1}$. From the beginning of spring until the end of the investigation period, TdR incorporation rates ranged from 37 to 66 pmol l$^{-1}$ h$^{-1}$.

### *Bacterial Secondary Production and Turnover Times*

BSP rates are listed in Table 1, and ranged from values below 0.6 μg C l$^{-1}$ h$^{-1}$ at the end of autumn to a maximum of 10 μg C l$^{-1}$ h$^{-1}$ in August 1991. On average, BSP was 2.3 μg C l$^{-1}$ h$^{-1}$ (SD: 2.1 μg C l$^{-1}$ h$^{-1}$). Turnover times for the total bacterial population was approximately 30 h during spring 1991 (Table 1). During the high BSP-rates in August, turnover times decreased to 7–8 h, indicating that, during this short period within the year, bacterial biomass in the water column was turned over three times per day. Turnover time then increased steadily up to values of more than 80 h (3.5 days) at the end of November. During winter, turnover times ranged between 52 and 71 h whereas, in March, they were

again around 30 h (as in spring 1991). Turnover times then stayed constant until the end of June, where an extremely high bacterial biomass led to a high value of 53 h.

## Discussion

### *Bacterial Numbers, Cell Volumes, and Biomass*

The bacterial community of the Kühwörter Wasser exhibited an obvious seasonal dynamic, which became evident at the level of all measured microbial parameters (Figs. 3–6; Tables 1 and 2). Variation in bacterial numbers followed a seasonal pattern which could be related to a characteristic sequence in the morphotype composition of the bacterial population. In both years, the highest bacterial densities occurred in early summer, caused by a rapid increase of rod- and, especially, vibrio-shaped cells. These values increased by more than twice during the rest of the year (Fig. 3). The peak of rod-shaped cells in May 1991, however, was not repeated the following year. Over the year, rods were the predominant bacterial form, contributing, on average, 62% to total BN, followed by vibrio-shaped cells at 29%. Coccal cells comprised only 4–10% of the total BN, and their maximum population density in May 1991 and June 1992 was due to an overall increase of each morphotype at these times rather than to a selective increase in coccal bacterial forms. The percentage of filamentous forms averaged 2.5% over the investigation period, but one pronounced maximum occurred each year during the spring, where their densities were about three to five times higher than the yearly mean. Nevertheless, because of their large cell volumes and, thus, cellular biomass, their contribution to the total bacterial biomass (BBM) was high, with maximal values of 40% during the spring maxima (Fig. 5). However, the bulk of the bacterial biomass in the Kühwörter Wasser consisted of rod- and vibrio-shaped cells during the greatest part of the year, making up 51% (SD: 8.5) and 33% (SD: 10) of BBM, respectively.

Average bacterial cell volumes, varying from 0.045 to 0.129 $\mu m^3$ over the year, were in the size range reported for other freshwater systems [25, 26, 45]. The highest average morphotype cell volumes, with a mean of 0.7 $\mu m^3$ (SD: 0.26) and a range of 0.29 to 1.33 $\mu m^3$ were measured during the occurence of filamentous bacteria. These bacteria reached lengths of up to 60 $\mu m$, thus belonging to the largest bacteria reported in the literature for the planktonic environment [48].

Comparable data on bacterial populations in macrophyte-dominated freshwater systems are rare [32], and, to our knowledge, no information exists for backwater systems of large rivers in the temperate climate zone. Only Boon [8] reported bacterial cell numbers of up to $157 \times 10^9$ cells $l^{-1}$, and corresponding biomasses of 10.3 mg C $l^{-1}$ in Australian Billabongs, being more than one to two orders of magnitude higher than our findings. From these data, and the given chlorophyll *a* values, we consider the Billabongs uncomparable to the backwater system of the river Danube.

However, bacterial cell numbers, as well as bacterial biomass, of the Kühwörter Wasser are within the range of values of most other freshwater ecosystems. Sanders et al. [44] listed bacterial densities ranging from $0.4 \times 10^9$ to $34 \times 10^9$ cells $l^{-1}$ for freshwater systems of different trophic states, and bacterial biomass in limnetic systems was reported to vary from 17 to 530 $\mu g$ C $l^{-1}$, with a mean value of 89 $\mu g$ C $l^{-1}$ [50]. Within the range of all reported density and biomass values, our data are in the range of those reported by Lovell and Konopka [30], who determined cell numbers varying from 1.7 to $6.3 \times 10^9$ cells $l^{-1}$ for a small dimictic lake within the temperate climate zone, and displayed chlorophyll values similar to those of the Kühwörter Wasser. Their reported average cell biomass of 14 fg C $cell^{-1}$ led to bacterial biomass values ranging from 24 to 88 $\mu g$ C $l^{-1}$. These results agree with our own findings, with the exception of June 1992, when a bacterial biomass of 121.6 $\mu g$ C $l^{-1}$ (Table 1) was noted.

### *Thymidine Incorporation and Bacterial Secondary Production*

A major problem in quantifying bacterial secondary production using the tritiated thymidine method in aquatic environments is evaluating the appropriate concentration of the isotopes to be added to the samples. Usually it is assumed that the concentration, where maximal incorporation into bacterial DNA is measured, is high enough to inhibit isotopic dilution by extra- and intracellular substrate pools [4]. However, the degree of isotope dilution can vary markedly over the seasons [11]. Thus, the concentration of thymidine added at which a saturation of incorporation into DNA is reached needs to be determined for representative samples. Our results show that the variation of these values follow a consistent pattern over the seasons. During the major part of the year, a final concentration of 25–40 nM thymidine had to be used, while in late spring, 5 nM was sufficient to prevent isotopic dilution by intra- and extracellular TdR pools. This pattern is also reflected in the calculated $K_m$ values, which were extremely low during this period, ranging from 0.19 to 0.25 nM, and from August to May the values were about one to two orders of magnitude higher, varying between 1.8 and 20.8 nM (Table 2). Changes in Michaelis-Menten constants

of natural enzymes are generally difficult to interpret. It must be stressed that $K_m$ determined by the experimental approach reflects the sum of $K_m$ values for all enzymes involved in thymidine uptake, and incorporation into DNA of the different species within the bacterial community. Thus, the observed fluctuations of $K_m$ could result from biochemical modification of the involved enzymes by physiological regulation, from a change of enzymes caused by an alteration in bacterial species composition, or from variations of the magnitude of the maximal uptake rate. In the present study, we found no significant correlation between $K_m$ and $V_{max}$, indicating that variation in the magnitude of the maximal uptake rate is not responsible for the observed $K_m$ fluctuations.

The precentage of radioactivity in the DNA fraction versus the radioactivity in the total TCA precipitate was shown to vary markedly during the year (Table 2). It was stated by Cho and Azam [9] that, under conditions of lower nutrient availability, a larger proportion of the added thymidine might be used as a nitrogen source rather than being incorporated into bacterial DNA. Thus, a higher amount of the radioactivity can be found in the protein fraction. In view of this hypothesis, Hollibaugh [22] concluded from his studies that the metabolic fate of TdR reflects the relative importance of refractory detrital carbon versus freshly exudated carbon to bacterioplankton nutrition. Our findings are consistent with this hypothesis. We found a high proportion of TdR in the DNA fraction during late spring, when enough freshly produced algal substrates are supposed to be available for bacterioplankton. Decreasing values were observed during autumn and winter when primary production values were low, ranging from below detection to 60 $\mu g\ C\ l^{-1}\ day^{-1}$ (Steitz et al., in prep.).

Plotting the calculated $K_m$ values from the saturation experiments against the percentage of labeled thymidine in the DNA, we found a strong negative correlation ($r = -0{,}83$; $P < 0{,}01$; $n = 7$) between these two parameters. Because of the mentioned difficulties in interpreting changes of Michaelis-Menten constants, no satisfying explanation can be offered for this relationship.

BSP rates in the Kühwörter Wasser were within the upper range observed for other freshwater ecosystems [12]. The authors summarized data from 24 freshwater habitats with values from 0.017 to 6.38 $\mu g\ C\ l^{-1}\ h^{-1}$. Higher values, up to 16 $\mu g\ C\ l^{-1}\ h^{-1}$, are reported from several authors for eutrophic environments [13, 17, 43]. In our study, we found a pronounced maximum of 10 $\mu g\ C\ l^{-1}\ h^{-1}$ in August, coinciding with a peak of chlorophyll *a* and with the maximal biomass of submersed macrophytes (105 g C $m^{-2}$ $year^{-1}$; Kirschner et al., in prep.). During the rest of the year, values remained around or below 2 $\mu g\ C\ l^{-1}\ h^{-1}$. Values below 1 $\mu g\ C\ l^{-1}\ h^{-1}$ were recorded during autumn and winter, when temperature is thought to be the limiting factor for bacterial growth [24, 44, 61]. As Pomeroy et al. [39] pointed out, bacteria require higher substrate concentrations at lower temperatures, and, therefore, postulated that the activity of the microbial loop is controlled by the interaction of two factors, temperature and substrate concentration, especially in cold water ecosystems.

Turnover times reached minimal values in August, when the bacterial population of the Kühwörter Wasser was calculated to be renewed three times per day, while in late autumn and winter, bacterial turnover times were approximately three days.

### *Considerations on the Seasonal Development of the Bacterial Community in the Kühwörter Wasser*

Although there is general agreement that, apart from physical and chemical factors such as temperature, pH, and solar radiation, bacterial populations are controlled by bottom-up (nutrients), as well as by bottom-down forces (predation) [e.g., 18, 19, 37]. The degree to which each of these factors influence bacterial communities in different aquatic ecosystems is still under debate [e.g., 37, 39, 40, 46, 61]. The Kühwörter Wasser, a shallow mesotrophic backwater branch, is characterized by a distinct seasonal rhythm typical for freshwater ecosystems in the temperate climate zone [58]. Thus, it must be assumed that the influence of the different shaping factors varies widely over the year. Temperature is considered to be a major factor limiting bacterial growth [1, 36, 61]. In the Kühwörter Wasser, temperature correlated significantly with bacterial numbers ($r = 0.74$; $P < 0.0001$), thus explaining 55% of the seasonal variation. BSP rates (log-transformed) were significantly correlated with temperature ($r = 0.72$; $P < 0.001$), as well as with chlorophyll *a* ($r = 0.61$; $P < 0.001$). Multiple regression analysis revealed that both temperature and chlorophyll *a* explained 69% of the seasonal variation of BSP rates ($r = 0.83$; $P < 0.00001$).

When the water temperature fell below 10°C (November–March), cell densities decreased to values of $2–3 \times 10^9$ cells $l^{-1}$, corresponding to bacterial biomass values ranging from 27 to 40 $\mu g\ C\ l^{-1}$. Also, BSP rates were rather low ($\leq 1$ $\mu g\ C\ l^{-1}\ h^{-1}$). Because primary production of algae and submersed macrophytes tend toward zero at this time of the year, bacteria are supposed to be both substrate and temperature limited during this period [46]. Loss rates of bacteria were probably

**Table 3.** Comparison of phytoplanktonic gross primary production (PP), bacterial carbon production (BCP), and estimated bacterial carbon demand (BCD) for four seasons (see text) in the backwater of the Kühwörter Wasser

| Period | Months | PP[a] ($\mu g\ C\ l^{-1}\ day^{-1}$) | SD[b] | $n$[c] | BCP ($\mu g\ C\ l^{-1}\ day^{-1}$) | SD | $n$ | BCD[d] ($\mu g\ C\ l^{-1}\ day^{-1}$) | BCD/PP (%) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | March–May | 540 | 34 | 10 | 48 | 10 | 8 | 155 | 29 |
| 2 | June | 187 | 74 | 6 | 58 | 7 | 5 | 186 | 99 |
| 3 | July–September | 408 | 145 | 9 | 120 | 91 | 5 | 387 | 95 |
| 4 | October–February | 38 | 15 | 4 | 17 | 7 | 8 | 54 | 142 |

[a] Data from Steitz et al. (in prep.). Primary production was measured by the $^{14}C$ method, $NaH^{14}CO_3$, 4 μCi (Carbon 14 Centralen, Denmark) added to 100-ml Winkler bottles, 4 h incubation time
[b] SD = standard deviation
[c] $n$ = Number of sampling events per season
[d] BCD was calculated assuming a bacterial carbon growth efficiency of 31% [27]

not just due to grazers, which are usually not very active at such low temperatures [19]. It was shown recently [31] that bacteriophages in the Kühwörter Wasser are responsible for 15.8 to 30.1% of the loss rate of bacterial production, with highest mortality rates for temperatures at or below 5°C. Toward spring, bacterial biomass and BSP rates increased with increasing temperature (Fig. 1), primary production (Tab. 3), and increasing chlorophyll *a* values (Fig. 2). During this period, the bacterial community structure was completely altered, and filamentous bacteria were contributing up to 40% of the bacterial biomass (Fig. 5). Such high biomass values for filamentous bacteria in temperate freshwater ecosystems were also observed by others [24, 40, 48] and were interpreted as the occurence of grazing-resistant bacteria, arguing that they are simply too big to be consumed by heterotrophic nanoflagellates. On the other hand, it was stated that bacteria of this cell size would not be able to grow under nutrient-limited situations [24], as is probably the case during the winter season.

A phase of lower algal biomass and primary production, usually referred to as clearwater phase [52], followed the spring maximum when densities of filamentous bacteria dropped again and the morphotype composition of the bacterial community was similar to the situation before the spring bloom. During this period, usually metazooplankton organisms (primarily cladocerans) were reported to feed efficiently on both phytoplankton and HNF [15, 52] and, as was recently proposed by Jürgens and Güde [24], also on filamentous bacteria, which fit well into the prey-size spectrum of many Daphnia species.

A second peak of bacterial numbers at the beginning of summer was caused mainly by a strong increase in vibrio-shaped cells, where primary production of phytoplankton began to reach a second maximum (Table 3). We can only speculate about the reasons for the strong increase of this morphotype. Chemical resistance against viral attack or against grazing is possible [24], although no experimental evidence is available, so far, to support this hypothesis.

The August maximum in BSP rates coincided with the second peak in chlorophyll *a*, as well as with high temperatures above 24°C, while bacterial numbers decreased from greater than $7 \times 10^9$ to $4 \times 10^9$ cells $l^{-1}$. At the same time, submersed macrophytes exhibited their maximum annual biomass (105 g C $m^{-2}$; Kirschner et al., in prep.). It is expected that the release of macrophyte leachates increases the pool of metabolizable dissolved organic carbon thus sustaining high bacterial growth rates [32, 33, 55, 58]. High BSP rates (Table 1), despite a decrease in bacterial numbers (Fig. 3), indicated, again, a strong predation pressure and/or virus-induced lysis rate for this period.

During late summer and the first half of autumn, bacterial numbers and production rates remained between 0.9 and 1.9 $\mu g\ C\ l^{-1}\ h^{-1}$, with lower biomass concentrations than in spring and midsummer (41–54 $\mu g\ C\ l^{-1}$). During this period, chlorophyll *a* (4–6.5 $\mu g\ l^{-1}$), as well as temperature (15–17.5°C), decreased markedly to reach their minimum values in winter.

### *Bacterial Carbon Requirements*

To assess the magnitude of carbon fluxes in the water column of the Kühwörter Wasser, we integrated pooled data of the phytoplanktonic primary production (Steitz et al., in prep.) into our budget. The bacterial carbon demand (BCD) was estimated, assuming a constant bacterial carbon growth efficiency of 31% over the year according to Kristiansen et al. [27]. So far, a wide range of conversion efficiencies has been reported, varying from 6 to 33% for microcosm experiments

[29, 34, 54], from 20 to 30% for continuous culture experiments [5] and oxygen chambers [3], or from 40 to 90% when pure cultures were used [23]. However, carbon conversion efficiencies of approximately 50% have typically been used for most budget calculations [14, 41, 42]. We used a value of 31% [27] because the data from which this conversion efficiency is derived is from natural bacterial communities in a continuous culture system, making it possible to measure bacterial net production and respiration directly. Thus the experimentation remains independent of radiolabeled substrates.

Our data set was divided into four classes, according to four major seasonal periods of phytoplanktonic development. Period 1 (March–May) was attributed to the phytoplankton spring maximum, period 2 (June) to the following decline of the phytoplankton bloom (clearwater phase), period 3 (July–September) to the phytoplankton summer maximum, and phase 4 (October–February) to the chlorophyll *a* winter minimum. Table 3 provides a basis for speculations on the magnitude of the carbon flux in the microbial compartment. The BCD over the seasons ranges from 29% of the phytoplanktonic carbon production in spring to 142% in winter, indicating that carbon from sources other than phytoplanktonic primary production is necessary to account for bacterial carbon requirements from late spring to winter. Considering potential grazing rates of the zooplankton, and assuming a conservative grazing rate of 50% for spring and summer [15, 28, 58], it becomes obvious that phytoplanktonic primary production can potentially cover the carbon demands of both the bacterial and the grazer compartment only from March to May, with an excess of 115 $\mu$g C $l^{-1}$ $day^{-1}$ for the detritus pool. However, one has to consider that only the exudated dissolved organic carbon from primary production represents a food source for microheterotrophs. Exudates from actively growing phytoplankton are often dominated by a wide range of low molecular weight compounds, including amino acids, and thus easily consumable by heterotrophic bacteria [6, 53, 57]. The amount of extracellular carbon released from phytoplankton may reach 12% of the primary production in eutrophic lakes [51], and, on average, 11.6% (with a range of 3–32%) of the primary production for mesotrophic lakes [20]. According to a seasonal study from Sell and Overbeck [47], the exudated organic carbon constituted, on average, 65% of the phytoplankton primary production, with a range of 30.5–99%, consisting mainly of easily consumable organic carbon in early summer, and high molecular weight compounds in late summer and early autumn. Adopting an average exudation range from 12 to 65%, according to the above findings, we would expect an estimated release of utilizable dissolved organic carbon (DOC) by the phytoplankton compartment ranging from 32.4 to 175.5 $\mu$g C $l^{-1}$ $day^{-1}$, derived from the primary production left over by the grazers from March to May. This means that either a subsidy of 122.6 $\mu$g C $l^{-1}$ $day^{-1}$ is required from other carbon sources to cover the bacterial carbon demand, or that 20.5 $\mu$g C $l^{-1}$ $day^{-1}$ are left over for the detritus pool. In contrast, a subsidy of utilizable DOC from other carbon sources making up 125.2–174.7 $\mu$g C $l^{-1}$ $day^{-1}$ must be provided for the clearwater phase (June), some 254.4–362.5 $\mu$g C $l^{-1}$ $day^{-1}$ during the period of the phytoplankton summer maximum, and between 41.5 and 51.7 $\mu$g C $l^{-1}$ $day^{-1}$ in late autumn and winter, when the exudated phytoplanktonic organic carbon consists mainly of high molecular weight compounds [10, 47]. In conclusion, our estimations of the magnitude of the carbon flow within the microbial compartment indicate that, for most of the year, the carbon demand of the bacterioplankton cannot be covered by phytoplanktonic primary production, and relies on exudates, decomposition products of macrophytes, and benthic algae, as well as an import of terrestrial organic matter.

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