Microbial food webs in oligotrophic arctic lakes

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ABSTRACT

Toolik Lake and nearby lakes are located north of the arctic circle at 68^{o}N on the treeless tundra of Alaska's North Slope. These lakes have an extremely low primary productivity of around 15 g C m^{-2} yr^{-1} yet an allochthonous DOC input results in high bacterial numbers (up to 3.1 x 10^6 ml^{-1} in the summer) and high bacterial productivity (3-8 g C m^{-2} yr^{-1}). Heterotrophic nanoflagellates (1-6 x 10^3 ml^{-1}) are also abundant; these two trophic levels are closely linked and are clearly resource driven or under bottom-up control. Flagellate grazing was shown to remove 14% of the bacteria per hour, an amount roughly equivalent to bacterial production. This indicates simultaneous top-down control of the bacteria. However, the extremely low primary productivity results in a very limited total food resource. The result is that only low numbers of microzooplankton (protozoans, rotifers, copepod nauplii) and zooplankton (cladocera) are supported. These higher levels of the microbial food web are disconnected from the lower levels and exert no top-down control on the flagellates and bacteria. This paper addresses a series of questions concerning the microbial food web structure of Toolik Lake and arctic lakes in the area. A conceptual model describes these links between overall trophy and type of control within the microbial food web.

Background information on the lake and the microbes

Toolik Lake (1 km^2, z_{max} 25 m) lies in hills at 68^o N and 149^o W [12]. The lake is ice covered (1.5-2 m) from late September until late June. It is dimictic; summer temperatures in the deep water reach 6^o C while surface waters may reach 12^o C. Oxygen is generally near saturation even in the hypolimnion during summer stratification and decreases by less than 50% in the deepest part of the lake during the long arctic winter. The annual input of DOC, measured by Whalen and Cornwell [20], is 98 g DOC m^{-2} y^{-1}. Average concentrations of nutrients are 6.4 μg NO_3-N l^{-1}, 2.4 μg NH_4-N l^{-1}, and 7.1 μg total P l^{-1}. Chlorophyll averages 1.3 μg l^{-1}. Nearby Camp Pond has a maximum depth of 0.7 m and freezes completely each winter. Further details on this Long Term Ecological Research site are given in [12].

Methods

Bacteria and flagellates were counted with DAPI [13]. Bacterial productivity was measured as the incorporation of ^{14}C-leucine [17]. Phylogenetic relationships of bacterial 16S rRNA gene clone sequences were inferred by distance matrix analysis [1]. Grazing experiments were based on the size-selective filtration of ^{14}C-labelled bacteria [18].

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Microzooplankton were concentrated from freshly collected water samples by gentle reverse flow filtration [3] with a 20 μm mesh net and enumerated as described in Rublee [14].

Two mesocosm experiments have been conducted. In 1984, 72 m^3 polyethylene mesocosms received a daily addition of nutrients equal to 10 times the natural loading rate [12]. In 1991, dark and light 4.5 m^3 mesocosms received a one-time addition of 2 mg l^{-1} DOC leached from tundra vegetation, an increase to 8 mg DOC l^{-1} of lake water.

Lake N-1, which lies only a few meters from Toolik Lake, is one of several in the area undergoing long-term manipulation (see [15] for a description of the lake, the manipulation and results). This lake is similar to Toolik Lake in its chemistry, biota, and primary productivity. It has an area of 4.4 ha and a maximum depth of 14 m. The manipulation consisted of 6-8 weekly additions of inorganic nitrogen and phosphorus sufficient to increase the summer nutrient loading 5-to 10-fold.

Results and discussion

Abundance and productivity of the planktonic bacteria and the abundance of the nanoflagellates

Bacteria numbers [7] are high in the summer (1-2 x 10^6 ml^{-1}, maximum of 3.1) and as low as 0.1 x 10^6 ml^{-1} during the winter. Leucine incorporation gave an estimate of 3-8 g C m^{-2} for the annual bacterial production [11], more than half occurring beneath the spring ice cover from allochthonous input of DOC. The total input of DOC (98 g DOC m^{-2} yr^{-1}) is many-fold larger than the primary productivity of the algae (around 14 g C m^{-2} yr^{-1}). Picocyanobacteria numbers are normally low, 10^3-10^4 ml^{-1}, but these autotrophs can briefly increase to 10^5 ml^{-1} in early autumn. The numbers of bacteria in shallow Camp Pond, counted from the time of ice melt through the summer, are 6-10 x 10^6 ml^{-1}.

The number of heterotrophic nanoflagellates (HNF, 2-20 μm) in the lake is usually 1-6 x 10^3 ml^{-1} [12, Hobbie and Bahr unpublished data]. “Typical” numbers are given in the following two samples: in July 1984, a sample from the lake contained 1.2 x 10^6 bacteria ml^{-1} and 2.6 x 10^3 HNF ml^{-1}. In June 1994, a sample contained 1.1 x 10^6 bacteria $ml^{-1}$and 2.7 x 10^3 HNF ml^{-1}. One exceptional series of samples from August 1997, contained up to 100 x 10^3 HNF ml^{-1}. In our study, we have made a careful distinction between heterotrophic and autotrophic nanoflagellates. In this lake, 25-50% of the total nanoflagellates possess some chlorophyll that fluoresces red under excitation by low-blue wavelengths. Some of these flagellates that possess chlorophyll are undoubtedly mixotrophic and consume bacteria. In contrast, Camp Pond has a greater number (2-11 x 10^3 ml^{-1}) of nanoflagellates present. These are predominantly heterotrophic (85-100%).

Species composition of these microbial communities

Some of the bacteria present in the plankton of Toolik Lake have been identified with 16S rRNA analysis [1]. Of the 18 types found, many fall into a beta proteobacterial gene cluster of pseudomonads; another cloned beta proteobacterial gene sequence cluster is related to *Methylophilus methylotrophus*. An alpha proteobacterial gene cluster is a deeply branching relative of the SAR-11 cluster, previously described only in marine environments. Subsequent analyses [9,10] established a high degree of similarity (>97%) among these three clusters of bacterial gene sequences as recovered from Toolik Lake, Adirondack

Mountain Lakes in New York State and Lake Loosdrecht in the Netherlands. These clusters contain only sequences of freshwater bacteria when compared to the current EMBL/Genbank database; the alpha proteobacterial gene sequences are monophyletically related to, but distinct from, the marine sequences.

The identity of almost all of the nanoflagellates is unknown although H. Kling has developed a species list of the algal forms (reported in [12]). Chrysophyceae are dominant and the genera *Chromulina*, *Ochromonas*, *Spiniferomonas*, *Pseudopedinella*, *Pseudokephyrion*, *Paraphysomonas* and *Kephyrion* are present. Some of these have colorless forms or are mixotrophic.

Abundance of the microzooplankton and zooplankton

Microzooplankton, which includes ciliated protozoans, rotifers, and crustacean nauplii, are present in low numbers in Toolik Lake. As reported in O'Brien *et al.* [12] and Rublee [14], for a two year period the July and August values per liter were 20-332 protozoans, 13-53 rotifers, and 3-66 nauplii. Ciliates are mostly oligotrichs and vorticellids. Eight species of rotifers were found, dominated by *Keratella cochlearis*, *Conochilus unicornis*, and *Polyarthra vulgaris*. The two copepod species present are *Diaptomus pribilofensis* and *Cyclops scutifer*.

Adult zooplankton are not abundant in Toolik Lake by the standards of temperate lakes [12]. *Daphnia longiremis* and *Bosmina longirostris* are rarely more abundant than 1 per liter while the copepods *Diaptomus pribilofensis* and *Cyclops scutifer* are more abundant and may reach 10 per liter early in the summer. Late summer numbers are less than 1 per liter.

Flagellate grazing as a control on bacteria in the lake

In the 1984 mesocosm we found a classic predator-prey oscillation between the HNF and their bacterial prey. A number of these oscillations occurred over 37 days and we even found that the bacterial productivity was at its highest when the bacteria were grazed so much that their numbers fell drastically [8]. These mesocosm experiments established that the HNF had the potential for exerting control on the bacterial numbers.

We have been able to extend these conclusions to the lake in the experiment using ^{14}C-labelled lake bacteria. Here we found that 14% of the bacteria were grazed by the HNF per hour. This removal rate is roughly equivalent to bacterial production. In addition, grazing by larger zooplankton and microzooplankton was not important.

Top-down control is not exerted on nanoflagellates by microzooplankton

The microzooplankton of these lakes, ciliates, rotifers, and crustaceans nauplii, have been extensively studied (details of abundance and seasonal distribution reported in [14] and [15]; preferred prey size and clearance rates reported in [12]). In the 1991 light mesocosm, we found that these organisms appeared to be exerting control on the HNF community [6]. The microzooplankton numbers were high when the HNF numbers were low. Clearly, based on mesocosm data, the microzooplankton have the potential for top-down control of the flagellates . However, in the lake microzooplankton are always present in extremely low numbers. Consequently, the top-down control of HNF by microzooplankton is not important under the oligotrophic conditions of Toolik Lake. To test what happens in a more productive lake, we also examined microzooplankton in N-1, a fertilized lake. Here eutrophication increased the abundance of microzooplankton, changed the dominant species, and caused emergence of new functional groups, bactivorous protozoans and predacious rotifers [15, 2].

A conceptual model of the controls of the microbial food webs of arctic lakes

Case I (Fig. 1) represents an ultra-oligotrophic lake where there is practically no input of organic matter from land or atmosphere and low loading of inorganic nutrients to the lake. Bacteria are present, growing on organic matter from the algae, but their numbers are so low (less than 0.1-0.2 x 10^6 ml^{-1}) that HNF can not exist and the control of the microbial system is from the bottom, or resource, end of the food web. There is some evidence that grazing does occur because bacteria population numbers do not slowly increase over time in Case I lakes and picocyanobacteria numbers decrease sharply between mid and late summer in some Antarctic lakes [W. Vincent, personal communication]. Mixotrophy, that is, the consumption of bacteria by algae, is likely to exist. This process may be important in these systems.

In an ultra-oligotrophic lake that receives a significant amount of dissolved organic carbon (DOC) from land (Case II in Fig. 1), the algal growth is the same as in Case I but the bacteria receive more than half of their organic carbon from land. This subsidy of organic matter from outside the lake is a strong stimulus to bacterial growth and allows significant numbers of HNF to exist; both bacteria and HNF are resource controlled. But there is also simultaneous top-down control of bacterial numbers by HNF. The microzooplankton are present but in such low numbers that they exert no top-down control on bacteria and HNF. Toolik Lake is in this category.

When more inorganic nutrients enter a lake, it becomes slightly more productive and is

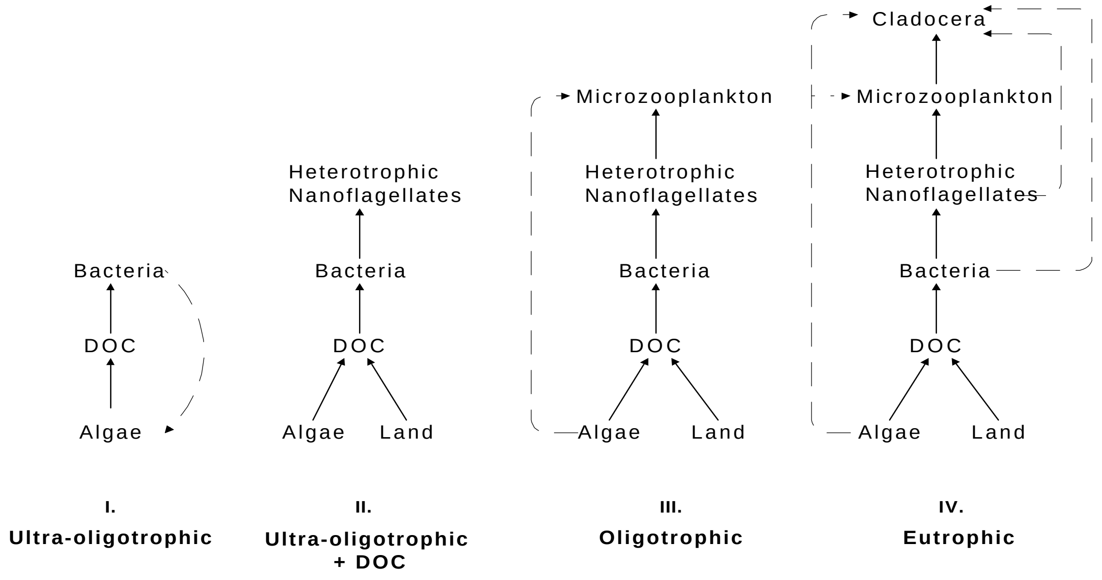

Fig. 1. Conceptual model of microbial food webs throughout a range of freshwater trophic systems (reprinted from Archiv Hydrobiol with permission).

raised to oligotrophic status (Case III in Fig. 1). The bacteria now receive most of their organic matter from algal production (bottom-up control of bacterial productivity by resources). The HNF are present and exert control on the bacteria numbers by their grazing (top-down control). There is now enough productivity in the whole lake system that ciliates, rotifers, and copepod nauplii can exist. This was the situation reported by Rublee and Bettez [15] after the fertilization of Lake N-1. These microzooplankton now are able to control the HNF by their grazing (top-down control).

At the next stage of enrichment of arctic lakes (Case IV in Fig. 1), the cladocera become important in the top-down control of microzooplankton, HNF, and bacteria. These zooplankton are present in Cases II and III but are so rare that they exert no top-down control. They have the potential to be abundant in mesotrophic and eutrophic lakes, but these are not common in the Arctic. Cladocera do become abundant in shallow ponds in the arctic where they obtain some food from the planktonic food web but probably obtain most of their food from the benthic algae and bacteria. One example of this is the 2-9 *Daphnia middendorffiana* per liter found in ponds near Barrow [19]. Planktonic primary productivity was very low, around 1 g C m^{-2} y^{-1}, yet zooplankton were abundant and filtered the entire volume of water every two days.

This conceptual model agrees with the basic premises of Fenchel [4] and Sanders *et al.* [16] who suggested that bottom-up control was more important in regulating bacterial abundances in oligotrophic environments and that top-down control was more important in mesotrophic and eutrophic environments. The data from oligotrophic Toolik Lake are an exception in that grazing by HNF is capable of removing all of the bacterial biomass several times per day. We conclude that there is regulation from the HNF back onto the bacteria numbers but that this single link is the only instance of top-down control in the microbial food web. Evidently the input of DOC from land into the lake enriched only a part of the food web, the bacteria and the nanoflagellates.

This limitation of top-down control is caused by the oligotrophic conditions that allow only small numbers of zooplankton and microzooplankton to exist; the animals are too rare to exert top-down control. These results are consistent with those of Gasol *et al.* [5] in their study of heterotrophic flagellates. Their analysis of temperate lakes concluded that the relative importance of resource availability and predation in determining HNF abundances varied with seasonality and food web structure. These arctic lakes eliminate the superimposed variability of food web structure and provide an example of the importance of resource availability at the extreme end of nutrient and temperature gradients.

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