

Control of bacterioplankton growth and abundance in deep, oligotrophic Loch Ness (Scotland)

Roger I. Jones*, Judith M. Young

Department of Biological Sciences, Institute of Environmental and Natural Sciences, Lancaster University,
Lancaster LA1 4YQ, United Kingdom

ABSTRACT: Control of bacterial growth and abundance in the plankton of Loch Ness, a deep, oligotrophic lake in northern Scotland, UK, was studied over a 12 mo period. Bacterial intrinsic growth rates and grazing loss rates were estimated from dilution experiments. Growth limitation due to resource availability was determined from nutrient addition experiments. Measured bacterial intrinsic growth rates ranged from zero to 0.69 d^{-1} , these low growth rates being typical of oligotrophic lakes. Bacterial growth rate was stimulated on different occasions by addition of an organic carbon substrate or by addition of mineral nutrients; however, during summer stratification bacterial growth was principally limited by phosphorus availability. Bacterial growth rate fluctuated irregularly, apparently influenced by the influx of resources from the catchment and hence by rainfall patterns. Bacterial growth rarely responded to increased incubation temperature. Although bacterial growth in Loch Ness was generally slow, new bacterial production was removed quite efficiently by grazers and bacterial densities showed only little fluctuation. Heterotrophic nanoflagellates were sometimes an important component of the bacterivore community, but other grazers such as cladocerans also appeared to play an important role in Loch Ness.

KEY WORDS: Bacterial growth · Bacterivory · Nutrients · Temperature · Loch Ness

INTRODUCTION

It is now well established that heterotrophic bacteria play a key role in pelagic food webs in marine and freshwater ecosystems (Pomeroy 1974, Azam et al. 1983). A rapidly expanding literature contains reports that bacteria can account for a large part of the flux of carbon through pelagic systems in lakes (Cole et al. 1988). In productive lakes, and in unproductive clear-water lakes, bacterioplankton are assumed to rely on autochthonous carbon fixed by phytoplankton. By contrast, in unproductive lakes which receive substantial inputs of allochthonous organic carbon, bacteria can provide a critical link whereby some of this carbon is channelled up the food chain (Jones 1992). Thus in some systems much of the bacterial production appar-

ently moves up to higher trophic levels. However, there is still considerable debate as to whether this is always the case or whether bacterial production is often largely respired within the several components of a microbial food web (Ducklow et al. 1986, Weisse 1990). A better knowledge of the factors which control bacterial abundance and production is therefore central to understanding planktonic food webs in lakes.

One widely accepted general observation is that populations of planktonic bacteria show limited variation in abundance, either between lakes or through time within a single lake, with reported densities usually in the region of $10^6 \text{ cells ml}^{-1}$ (Azam et al. 1983, Pace 1988). This apparent low variability clearly implies that bacterioplankton production must be rapidly and effectively exploited so that net population change is minimal. In marine pelagic systems, heterotrophic nanoflagellates (HNF) are considered to be the principal bacterivores (Fenchel 1982a, Sieburth &

*E-mail: r.jones@lancaster.ac.uk

Davis 1982). Although lakes contain a wider variety of zooplankton that may utilise bacteria effectively, including ciliates, rotifers and crustaceans (Børsheim 1984, Kankaala 1988, Sanders et al. 1989), HNF are still usually presumed to be the dominant bacterivores, particularly in oligo- and mesotrophic lakes (Güde 1986, Weisse 1990, Berninger et al. 1991).

The relatively constant bacterioplankton abundance arises despite the fact that bacterial growth and production in lakes can be high and can show considerable variability (Billen et al. 1990). Thus it is possible that the same bacterial abundance could arise when a population is (1) growing rapidly but being grazed rapidly or (2) growing only slowly but suffering little grazing loss. In the first case it might be assumed that the bacterial growth is not restricted by any essential resource (organic carbon substrate, mineral nutrients, temperature) and that the population is controlled primarily by the action of grazers. In the second case the slow growth rate would indicate that the population was controlled primarily by a shortage of one or more resources. In current terminology these 2 cases would be referred to respectively as 'top-down' and 'bottom-up' control (McQueen et al. 1986, Pace & Cole 1994). Although some authors (e.g. Berninger et al. 1991, Sanders et al. 1992) have argued the importance of top-down control, particularly in more eutrophic systems, comparative studies generally point to the importance of bottom-up regulation of bacteria (Billen et al. 1990, Pace & Cole 1994). In fact the 2 possible control mechanisms are not mutually exclusive, and it is quite possible that their relative importance in regulating bacteria might vary seasonally (Ducklow et al. 1992, Sanders et al. 1992). The same is true of the particular resource that might be most limiting to bacterial growth in lakes. Early marine studies led to a widespread view that bacterioplankton growth rates are limited by the release of dissolved organic carbon (DOC) from phytoplankton, and correlations between bacterial production and phytoplankton biomass or production (Bird & Kalff 1984, Marvalin et al. 1989, Robarts & Wicks 1990) suggested that the same might be true in lakes. However, other studies have shown that bacterioplankton effectively compete with phytoplankton for inorganic nutrients in both oligotrophic and eutrophic lakes (Currie & Kalff 1984a, b, Vadstein et al. 1988), indicating that inorganic nutrients might be as important as DOC in regulating bacterial growth rates. Attempts to resolve this argument by studying the response of bacterial growth to enrichment with DOC or inorganic nutrients have been inconclusive, with either resource or both together being required to stimulate

growth during different experiments (Toolan et al. 1991, Coveney & Wetzel 1992, Wang et al. 1992, Bell et al. 1993, Elser et al. 1995).

In fact there is good reason to suppose that the resource with most influence on bacterial growth might vary seasonally and between lakes, depending on the magnitude of phytoplankton production, inputs of allochthonous DOC and the availability of inorganic nutrients. However, there are few systematic studies of seasonal variation in the factors controlling bacterioplankton growth and abundance in lakes. Here, we present results from a 12 mo study of top-down and bottom-up influences on bacteria in the plankton of a deep, oligotrophic lake with appreciable inputs of allochthonous organic carbon.

METHODS

Experiments were conducted using water samples from Loch Ness. This is the largest volume of water in the British Isles and occupies a simple trench-like basin 39 km long and 1.5 km wide (Maitland 1981). The loch has a maximum depth of 230 m and a mean depth of 132 m. The loch is never ice-covered and is warm monomictic, although the strong winds which frequently blow up the long axis mean that even during summer stratification the depth of mixing extends to 30–40 m. Some summary data on conditions in Loch Ness are given in Table 1.

Between February 1996 and January 1997 samples were collected at approximately monthly intervals from a mid-loch station located over a depth of 200 m. A 30 m flexible tube was used to collect several integrated samples from the 0–30 m water column which were mixed in a clean bucket after straining through a 110 µm mesh to remove large crustacean zooplankton. The mixed sample was used to fill 2 l polypropylene bottles which had been acid washed, rinsed with Milli-Q water and autoclaved. The samples were returned to the laboratory in an insulated box. The water temperature at the time of sampling was measured with a YSI

Table 1. Representative values for some physical and chemical characteristics of Loch Ness

Volume (10 ⁹ m ³)	7.45	(Maitland 1981)
Water residence time (yr)	2.81	(Maitland 1981)
Surface temperature range (°C)	6–13	(This study)
Water colour (mg Pt l ⁻¹)	50	(Jones unpubl.)
DOC (mg l ⁻¹)	3–5	(Jones et al. 1997)
Total phosphorus (µg l ⁻¹)	10	(Jones unpubl.)
Soluble reactive phosphate (µg l ⁻¹)	5	(Jones unpubl.)
Inorganic nitrogen (µg l ⁻¹)	100	(Maitland 1981)
Maximum chlorophyll <i>a</i> (µg l ⁻¹)	1.5	(Jones et al. 1996)

probe and meter. Oxygen measurements were not taken, but previous studies (Maitland 1981, Laybourn-Parry et al. 1994) have shown that the entire water column in Loch Ness is always close to 100% saturated with oxygen.

Estimates of the rate of bacterivory and of the intrinsic (gross) growth rate of bacteria were made using a dilution technique (Landry & Hassett 1982). All glassware was acid washed, rinsed with Milli-Q water and autoclaved. Sample water was filtered through 0.2 μm pore size Anodisc inorganic filters. The filtrate was then mixed with unfiltered sample in the following ratios of unfiltered sample water to 0.2 μm filtrate: 1.0, 0.8, 0.6, 0.4 and 0.2. The mixtures were incubated for 5 d in triplicate in 100 ml glass bottles in the dark in a water bath set to the temperature of the loch at the time of sampling. Bottles were not shaken during the incubation, but agitation has been shown to have no impact on microbial behaviour (Tranvik 1989). Apparent growth rates (AGR) at each dilution were estimated from the change in bacterial density between Day 0 and Day 5. Preliminary trials showed that exponential growth of bacteria in unenriched Loch Ness water samples lasted for around 7 d. Five day incubations were used to maximise changes in bacterial densities while ensuring growth remained within the exponential phase. The intrinsic (gross) growth rate (μ) and grazing loss rate (g) were obtained by linear regression of AGR versus the fraction of undiluted sample (d):

$$\text{AGR} = \mu - gd$$

Possible resource limitation of bacterial growth was also investigated on each occasion by nutrient enrichment experiments. All glassware was acid washed, rinsed with Milli-Q water and autoclaved. Sample water was filtered through 1 μm pore size track-etched polycarbonate (TEPC) filters to screen out most grazers but retain most bacteria. The filtered water was dispensed to 100 ml glass bottles to which nutrient additions were made as follows: no addition (control); 500 $\mu\text{g l}^{-1}$ carbon as glucose (C); 10 $\mu\text{g PO}_4\text{-P l}^{-1}$ (P); 70 $\mu\text{g NH}_4\text{-N l}^{-1}$ (N); and a combined nutrient addition (CNP) with each nutrient added at the same concentration as in the single nutrient treatments. The bottles were incubated (6 d) in the dark in a water bath set to the temperature of the loch at the time of sampling. A separate sample with no added nutrients was incubated at 16°C approximating the annual maximum temperature attained in Loch Ness (Jones et al. 1996). All treatments were performed in triplicate. Samples from each bottle were removed at 0, 4 and 6 d for bacterial counting and a regression of $\ln(\text{bacterial density})$ against time was used to estimate the growth rate. Differences in growth rates between treatments and the

control were assessed by *t*-test following log transformation of the data to normalise the distribution.

Bacteria were counted by staining and epifluorescence microscopy (Kepner & Pratt 1994). Samples were preserved with buffered glutaraldehyde (final concentration 2%). An appropriate aliquot (0.1 to 10 ml according to the bacterial density) was filtered onto a 25 mm black 0.2 μm TEPC filter mounted on a 0.45 μm cellulose acetate backing filter and stained with DAPI for 7 min at a final concentration of 0.1 $\mu\text{g l}^{-1}$. Small aliquots were diluted to 2 ml with autoclaved Milli-Q water to ensure even distribution of bacteria on the filter membrane. The filter was then sucked dry and mounted on a slide with Cargille type A non-fluorescing immersion oil and bacteria were counted at $\times 1250$ magnification using a Leitz Laborlux epifluorescent microscope with UV excitation. A minimum of 400 cells in at least 5 fields of view were counted. Densities of heterotrophic flagellates in the original samples were also determined using epifluorescent microscopy, but with the primulin staining technique of Caron (1983). Flagellates were considered heterotrophic if they failed to autofluoresce under blue or green light excitation.

RESULTS

Dilution experiments to determine both bacterial intrinsic growth rates and rates of grazing loss were carried out at approximately monthly intervals between February 1996 and January 1997. Examples of 2 of the resultant graphical plots, for February and July

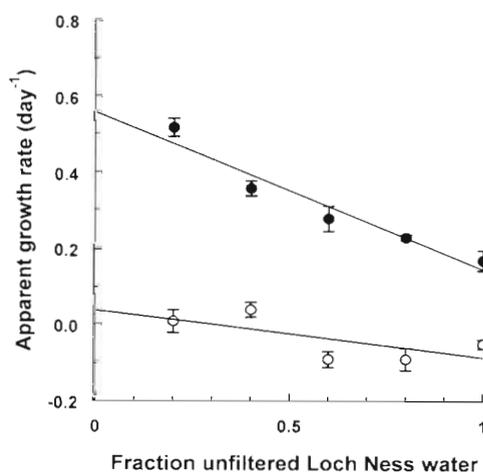


Fig. 1. Examples from February 1996 (○) and July 1996 (●) of results from dilution experiments using Loch Ness water. Data points are means of 3 replicates and vertical bars show standard errors. The fitted regression lines for each occasion are: February, $y = 0.039 - 0.125x$ ($r = 0.670$); July, $y = 0.561 - 0.415x$ ($r = 0.968$)

Table 2. Seasonal changes in Loch Ness samples during 1996/97 of bacterial intrinsic growth rate and grazing loss rate obtained from dilution experiments together with the correlation coefficient (r) from the fitted regression. Also shown are the initial abundances on each occasion of bacteria and heterotrophic nanoflagellates, as well as the mean 0–30 m temperature in the loch at the time of sampling and whether the water column was stratified. Where appropriate, mean values are shown \pm standard errors

	Intrinsic growth rate (d^{-1})	Grazing rate (d^{-1})	r	Bacterial abundance (10^5 cells ml^{-1})	HNF abundance (10^2 cells ml^{-1})	Temperature ($^{\circ}C$)	Stratified water column
February	0.039 ± 0.053	0.125 ± 0.080	0.670	3.88 ± 0.25	2.60 ± 0.12	6	No
March	-0.037 ± 0.056	-0.045 ± 0.084	0.293	2.26 ± 0.27	2.19 ± 0.26	6	No
April	0.228 ± 0.043	0.220 ± 0.066	0.888	2.45 ± 0.11	0.88 ± 0.10	8	No
May	0.637 ± 0.030	0.505 ± 0.046	0.988	4.03 ± 0.63	1.04 ± 0.12	8	No
July	0.561 ± 0.041	0.415 ± 0.062	0.968	9.91 ± 0.34	0	10	Yes
August	0.389 ± 0.080	0.125 ± 0.121	0.513	3.15 ± 0.48	0	12	Yes
September	0.324 ± 0.048	0.030 ± 0.072	0.235	10.12 ± 1.00	0.98 ± 0.19	13	Yes
October	0.547 ± 0.036	0.545 ± 0.055	0.985	8.07 ± 0.23	2.05 ± 0.48	12	Yes
November	0.670 ± 0.045	0.370 ± 0.068	0.953	4.32 ± 0.19	0.87 ± 0.16	8	No
January	0.687 ± 0.077	0.445 ± 0.116	0.911	6.00 ± 0.37	0	6	No

1996, are shown in Fig. 1. In February, the intrinsic growth rate (y -intercept) was extremely low and the grazing rate (slope of line) was also low; in July growth and grazing rates were higher. The monthly data for growth and grazing rates, together with the initial densities of bacteria and HNF, are summarised in Table 2. In general the dilution experiments generated data which were explained well by the fitted regressions, although in 2 experiments (March and September 1996) the fit was very poor.

At the start of the study, the measured intrinsic growth rate of Loch Ness bacterioplankton was

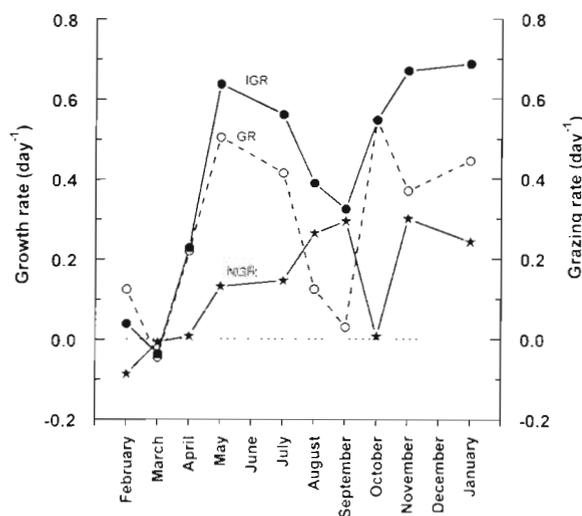


Fig. 2. Seasonal variation during 1996/97 in Loch Ness of bacterial intrinsic (gross) growth rate (IGR, ●) and grazing loss rate (GR, ○) estimated from monthly dilution experiments. Also shown is the estimate of bacterial net growth rate (NGR, ★), the difference between gross growth rate and grazing loss rate

extremely low (Fig. 2), but through the spring of 1996 the rate increased progressively before declining during summer. In the autumn, the intrinsic growth rate increased again and actually reached the highest recorded value of $0.687 d^{-1}$ in January 1997. The estimated rate of grazing loss of bacteria (Fig. 2) showed a similar pattern to that for the bacterial intrinsic growth rate. However, grazing rate only rarely equalled or exceeded the bacterial intrinsic growth rate, so that the estimated net growth rate of bacteria was generally positive (Fig. 2), in which case some net increase in bacterial density should have been possible. In fact, during the early part of the study, when bacterial net growth rate was negative or zero, there was no observed increase in bacterial density (Fig. 3). Bacter-

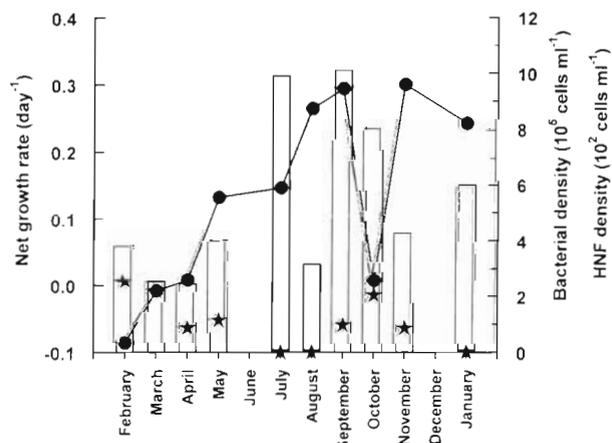


Fig. 3. Seasonal variation during 1996/97 of densities of bacteria (open bars) and heterotrophic nanoflagellates (HNF, ★) in water samples collected from Loch Ness. Also shown are the estimates of bacterial net growth rate (●) obtained from dilution experiments

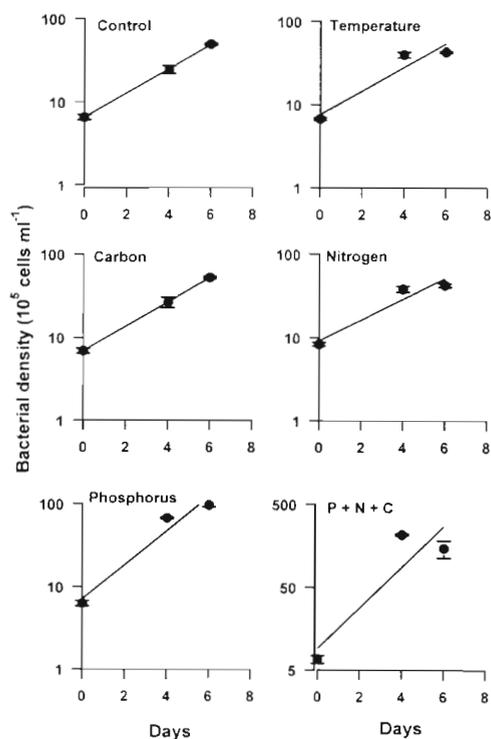


Fig. 4. An example from July 1996 of the changes in bacterial density in water samples from Loch Ness (prefiltered through 1 μm pore size filters) following manipulation of temperature or addition of nutrients. Data points are means of 3 replicates and vertical bars show standard errors. See text for further details

ial density did increase later in the year when the net growth rate increased. The density of HNF (Table 2, Fig. 3) fluctuated through the study period, with only trace densities (insufficient for counting) during summer 1996 and again in January 1997.

The intrinsic growth rate of bacterioplankton is presumably determined mainly by environmental conditions: the availability of an organic carbon substrate, the availability of essential mineral nutrients and the ambient temperature. Bacterial response to these factors was determined by incubations of water samples (filtered through 1 μm filters to remove grazers) supplemented with additions of glucose, phosphorus or nitrogen, or with the temperature increased from that measured at the time of sampling to 16°C, approximating the maximum seasonal temperature attained in Loch Ness. An example of the growth responses observed (from July 1996) is shown in Fig. 4.

The effect of different treatments on the bacterial growth rate relative to that of the control (no nutrient addition, ambient loch temperature) is summarised in Table 3. It is clear that no single factor was controlling bacterial growth rate throughout the study; additions of phosphorus, nitrogen and glucose individually all

stimulated bacterial growth on at least 1 occasion. Additions of nitrogen or glucose alone occasionally stimulated bacterial growth, but not with any recognisable seasonal pattern. However, bacterial growth was consistently stimulated by addition of phosphorus alone during the summer stratified period in the loch. Addition of all 3 nutrients together stimulated growth throughout the summer (May to October), and the stimulation of growth from addition of all 3 nutrients was always significantly greater than that obtained from addition of any one nutrient on its own.

On 2 occasions (May and September), incubations at the seasonal maximum loch temperature produced a statistically significant, but only minor, increase in bacterial growth rate (Table 3). These apparent temperature effects all occurred when the ambient loch temperature was not much below the seasonal maximum; during the winter months, when the loch temperature was lowest (around 6°C), incubation at the higher temperature never produced any stimulation of bacterial growth rate. Indeed, in November a significantly lower growth rate was measured at the higher incubation temperature. This surprising apparent lack of a consistent response of growth rate to temperature was investigated further in February 1997 when the loch temperature was again 6°C. Water samples (filtered through 1 μm filters) were incubated at 4 temperatures from 6 to 22°C either without any added nutrients or with added phosphorus, nitrogen and glucose. At all temperatures, the addition of nutrients produced a significant increase in bacterial growth rate (Table 4). However, temperature again had no significant effect on growth rate, irrespective of the nutrient status of the samples.

Table 3. Summary of seasonal changes in the growth response of Loch Ness bacterioplankton to addition of nutrients or to incubation at the annual maximum loch temperature. Values shown (nd = not determined) are growth rates in treatments as percent of growth rate in the control treatment incubated without nutrient addition and at the *in situ* loch temperature, with the statistical significance (*t*-test) indicated as: **p* < 0.05; ***p* < 0.01; ****p* < 0.001

Month	P	N	C	P+N+C	Temperature
February	144	78	141	nd	70
March	171*	171*	329***	nd	43
April	20	80	20	nd	20
May	144	156	156	275**	219*
July	142**	86	101	167**	98
August	172***	61	144	294***	133
September	144*	94	144*	194***	194**
October	157	79	200*	307**	107
November	100	98	74	88	64*
January	87	54	74	146	nd

Table 4. Bacterial apparent growth rates (\pm SD) in February 1997 water samples from Loch Ness incubated at 4 temperatures without or with added nutrients (P + N + C, see text for details). The temperature in the loch at the time of sampling was 6°C. ND: not determined

Temperature (°C)	Growth rate (d^{-1})	
	Without added nutrients	With added nutrients
6	0.43 \pm 0.05	0.77 \pm 0.03
10	0.51 \pm 0.03	0.78 \pm 0.02
15	0.46 \pm 0.10	0.80 \pm 0.13
22	0.38 \pm 0.01	ND

DISCUSSION

The dilution method was originally introduced by Landry & Hassett (1982) to estimate the impact of grazing by microzooplankton on marine phytoplankton, but has since been applied more widely to estimate grazing impacts on plankton communities (e.g. Weisse 1988, 1990, Tranvik 1989). The method remains controversial because it depends on 3 basic assumptions which can be difficult to evaluate: (1) exponential cell growth; (2) a linear relationship between grazing and the dilution factor; (3) intrinsic (gross) growth independent of the dilution factor. Our preliminary trials confirmed that the first assumption was satisfied even during the rather long incubations necessary because of the low bacterial growth rates. The low bacterial densities in Loch Ness (2 to 10×10^5 cells ml^{-1}) are well below those at which flagellate clearance rates start to become limited by the rate of phagocytosis (e.g. Fenchel 1982b), so the second assumption should also have been valid. The third assumption is more difficult to justify, since it could be argued that in more diluted samples cells would have both a higher initial resource availability per cell and a reduced regeneration of resources from the lower density of grazers. Since our preliminary trials showed that exponential growth was sustained through the incubation under all dilutions we feel that the third assumption probably was satisfied.

In principle, the intrinsic growth rates from our dilution experiments should be comparable to the growth rates measured in the (unenriched and undiluted) controls of our enrichment experiments, provided that the 1 μm prefiltration removed all grazers from the latter. The 2 separate measures of growth rates were actually significantly correlated ($r = 0.814$, $p < 0.001$), although the intrinsic growth rates measured from the dilution experiments were mostly higher than the growth rates from the controls in the enrichment experiments. This difference may be attributable to incomplete removal

of grazers by the 1 μm prefilter producing residual grazing losses in the enrichment experiments; it is well known that 1 μm filtration does not remove all HNF (e.g. Chrzanowski & Simek 1993). Conversely, if higher resource availability in the more diluted samples did produce higher intrinsic growth rates, this would lead to slightly steeper regression slopes and, consequently, overestimates of both intrinsic growth rate and grazing rate. Any errors in this direction would actually strengthen our conclusions about the low rate of bacterial growth in Loch Ness and the relatively small impact of HNF grazing.

The bacterial intrinsic growth rates determined by the dilution technique for water samples from Loch Ness ranged from $0.687 d^{-1}$ down to not significantly different from zero (Table 2). These values are comparable with those reported for other freshwater and marine plankton (e.g. Tranvik 1989, Billen et al. 1990, White et al. 1991, Chrzanowski et al. 1995). That the maximum values recorded from Loch Ness are low compared with many maximum values reported from elsewhere is consistent with the view that bacterial growth rate reflects bacterial production, which tends to be lower in oligotrophic lakes like Loch Ness (Billen et al. 1990, Laybourn-Parry & Walton 1998).

Variation through the year in the bacterial intrinsic growth rate in Loch Ness showed no evidence of a particular seasonal pattern (Fig. 2). At the start of the study, during the winter of 1996, the bacterial growth rate was very low, but the highest rates recorded during the study actually occurred in the following winter of 1997. Low values actually occurred during the late summer when the phytoplankton biomass is maximal in Loch Ness (Jones et al. 1996). This is in marked contrast to other lakes (e.g. Lake Constance, Germany; Simon 1987, Güde 1990) in which bacterial abundance and production (and hence presumably growth rates also) show a pronounced seasonality, with low values during winter and high values during summer or associated with the spring phytoplankton bloom. The lack of such a pattern in Loch Ness suggests that bacterial growth is not principally determined by seasonal changes in insolation, either directly, by effects on temperature, or by dependence on DOC produced by phytoplankton.

The deep mixing and poor light penetration into the moderately humic water mean that the underwater light climate experienced by the phytoplankton in the loch is exceptionally unfavourable, and the maximum daily rates of areal phytoplankton photosynthesis achieved in Loch Ness are only around $100 mg C m^{-2} d^{-1}$ (Jones et al. 1996). This very low phytoplankton production in Loch Ness (Jones et al. 1996) is clearly insufficient to support the bacterial production (Laybourn-Parry et al. 1994), implying that bacteria in the

loch are dependent on the influx of allochthonous DOC from the catchment. In practice, the total organic carbon content of the loch is dominated by detrital carbon of allochthonous origin (Jones et al. 1997). Laybourn-Parry et al. (1994) suggested that this dependence of bacterioplankton in Loch Ness on allochthonous inputs of DOC would lead to fluctuations in bacterial growth and abundance being closely linked to patterns of rainfall and drainage from the catchment.

The period of our study actually coincided with an extended period of unusually low rainfall throughout much of the British Isles, including the Loch Ness catchment. The fluctuations in monthly rainfall at the loch (Fig. 5) do appear to link to the possible control of bacterial intrinsic growth rate by resource availability (Table 3). Prior to the start of the study, the rainfall during most of 1995 had been well below that of previous years. February 1996 was wetter, and at this time no nutrient limitation of bacterial growth was observed (Table 3). March was dry, and phosphorus, nitrogen and particularly organic carbon (as glucose) were each observed to stimulate bacterial growth. April and May were a little wetter and nutrient addition no longer stimulated bacterial growth. Through the summer months, when rainfall was low and the loch was stratified, phosphorus limitation of bacterial growth was consistently observed. October and November brought appreciable rainfall, and the associated flux of materials from the catchment into the loch appeared to remove mineral nutrient limitation of bacterioplankton, although organic carbon addition did produce a stimulation in September and October. Despite December 1996 and January 1997 being unusually dry months, bacterial growth in winter appeared to be unconstrained by nutrient availability. However, February 1997 was particularly wet but a combined PNC addition did stimulate bacterial growth (Table 4).

Overall this pattern of bacterial growth response to added nutrients in relation to rainfall patterns supports the view that bacterial growth in Loch Ness is mainly determined by the influx from the catchment by river discharge of both organic carbon substrates and mineral nutrients. A similar seasonal linkage between increased bacterial (and zooplankton) production and periods of snow-melt or rainfall was found in humic lake Kjesåspalten, Norway (Hessen 1998). In Loch Ness this relationship is unlikely to be precise, since the large volume of the loch and the relatively long water residence time will dampen the impact of discharge on concentrations of nutrients in the water column. The availability of nutrient resources relative to their stoichiometric requirement by bacterial cells is probably finely balanced in Loch Ness so that any one resource may appear as most limiting at different times. However, during the summer stratified period in

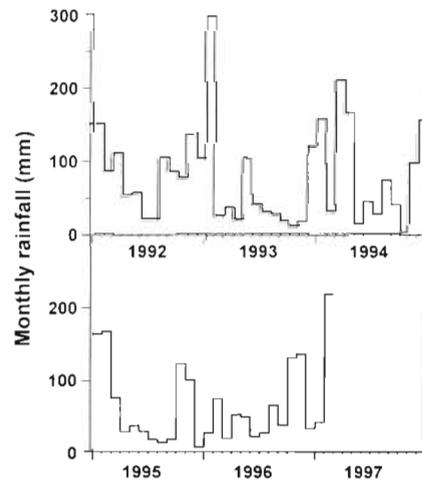


Fig. 5. Variation in total monthly rainfall measured in the grounds of Glen Urquhart High School, Drumnadrochit, by the northwest shore of Loch Ness

the loch, when phytoplankton production of organic carbon is highest and demand for nutrients within the epilimnion by phytoplankton is also maximal, availability of phosphorus is clearly the single most important factor determining bacterial intrinsic growth rate.

At times when resource availability appeared not to be limiting bacterial growth in Loch Ness, notably during the autumn and winter period of higher rainfall and more efficient circulation in the water column, it might be expected that bacterial growth rate would be temperature dependent. Such an effect was clearly reported from a eutrophic lake by Felip et al. (1996), and temperature has been shown to be a strong determinant of bacterial growth rates in freshwater and marine habitats (White et al. 1991). However, this was not evident in Loch Ness. Bacterial intrinsic growth rates determined from dilution experiments showed no significant regression on temperature ($r = 0.232$). Moreover, during those periods when the ambient loch temperature was lowest, bacterial growth did not respond at all to higher temperature in experimental incubations, either in the monthly tests with the seasonal maximum loch temperature or in the more detailed test in February 1997. This was an unexpected finding and one which is difficult to explain, since bacterial intrinsic growth rate must be determined by either chemical or physical factors. Of course it is possible that some nutrient other than those tested was limiting bacterial growth, but in view of the abundant evidence for P and N limitation of planktonic processes this does not seem likely. It also seems unlikely that the forms in which the nutrient supplements were supplied (organic carbon as glucose, phosphorus as phosphate, nitrogen as ammonium) would have hindered

their utilisation by the bacteria. One possible explanation is that the winter bacterial assemblage was adapted to lower temperatures and could not immediately respond to the much higher temperatures in the experiments. In the monthly tests (Table 3), bacterial growth rates at the higher incubation temperature were actually lower than at the control temperature in February, March, April and November 1996, although only in November was this reduction statistically significant. In addition, the February 1997 assemblage appeared to show an increased growth rate at 10°C compared to that at the *in situ* temperature of 6°C, but growth rate declined at 15°C and declined further at 22°C (Table 4), although only the difference between the 10°C and 22°C treatments was statistically significant and when nutrients were added growth rate was constant at all temperatures.

Another possible explanation for the lack of an observed temperature response could be that the pre-filtration through a 1 µm pore size filter did not always remove all the grazers, and that in the higher temperature treatments any increased growth by bacteria was offset by increased grazing so that no significant difference in bacterial growth was observed between the treatments. This would be a particular problem if smaller flagellate cells were predominant in the community at lower lake temperatures. In fact, during incubations of samples from the winter of 1996/97, appreciable numbers of flagellates were noticed in some treatments despite 1 µm pre-filtration; no such growth of flagellates during the incubations was observed in any other experiments. Although we did not assess the size of HNF in our samples, Weisse & Müller (1990) noted that small flagellates (<2 µm) were numerically dominant in the HNF community of Lake Constance and, given the plasticity of many flagellates, it is quite possible that such cells might pass through the 1 µm pores of the prefilter (e.g. Chrzanowski & Simek 1993).

The estimated rate of grazing loss of bacteria varied through the study in a way that closely followed the variations in the bacterial intrinsic growth rate (Fig. 2). This suggests that bacterial production in Loch Ness is exploited rather efficiently by consumers. It has been argued that the heterotrophic nanoflagellates which are often presumed to be the principal consumers of planktonic bacteria require bacterial densities around 10^6 ml⁻¹ in order to sustain their populations (e.g. Andersen & Fenchel 1985). In Loch Ness, bacterial densities only rarely reached 10^6 ml⁻¹ (Fig. 3). However, prey density thresholds for some flagellates as low as 2×10^4 bacteria ml⁻¹, which are well below the bacterial densities in Loch Ness, have recently been reported from culture studies (Eccleston-Parry & Leadbeater 1994). The density of HNF in the loch was also

low compared to values commonly reported from other lakes (e.g. Lake Constance; Weisse & Müller 1990). However, the density of HNF relative to that of bacteria in Loch Ness was broadly consistent with the correlation for global freshwaters presented by Berninger et al. (1991). Assuming a typical clearance rate for HNF of 10 nl cell⁻¹ h⁻¹ (Weisse 1990, Eccleston-Parry & Leadbeater 1994), the initial HNF and bacterial abundances can be used to estimate the contribution of HNF to the grazing rate determined on each experimental occasion (Table 2). Such estimates range from 0 to 100% with a mean value for the 10 experiments of 26%. Moreover, assuming the same typical clearance rate by HNF of 10 nl cell⁻¹ h⁻¹ and a typical bacterial density in Loch Ness of 5×10^5 ml⁻¹ would allow a daily ingestion of 120 bacteria per flagellate. At a typical yield of 2×10^{-3} flagellate cells per bacterium (Eccleston-Parry & Leadbeater 1994), this would mean a flagellate growth rate of only 0.17 d⁻¹. Such a growth rate is consistent with estimates from *in situ* incubations carried out in the loch previously (see Laybourn-Parry et al. 1994) and would be at the lower end of the range reported in the literature (Eccleston-Parry & Leadbeater 1994). These simple calculations suggest that HNF have only limited impact on bacterial populations in Loch Ness, consistent with the findings of Laybourn-Parry & Walton (1998).

In fact, grazing rates obtained from the dilution experiments were not correlated with HNF densities, so other groups of grazers must have been important bacterivores at times in Loch Ness. Mixotrophic flagellates are very scarce in the phytoplankton of Loch Ness (despite their frequent prevalence in the plankton of oligotrophic and dystrophic lakes). However, both ciliates (typically around 500 ind. l⁻¹, mainly *Strombidium viride* and *Halteria grandinella*) and heliozoans (typically around 10 ind. l⁻¹, mainly *Actinosphaerium* sp.) are present in the loch throughout the year without any marked seasonal pattern (Laybourn-Parry et al. 1994) and are likely to have been actively grazing bacteria in the dilution experiments. Rotifers (mainly *Polyarthra* spp. and *Conochilus hippocrepis*) are also an important component of the zooplankton community in Loch Ness during the spring and summer period, reaching densities of almost 200 ind. l⁻¹ (Fulcher 1996), and smaller types might have passed the 110 µm pre-screening mesh used to remove larger zooplankton prior to the dilution experiments. The cladoceran zooplankton which predominate in Loch Ness in late summer (*Daphnia longispina* and *Bosmina coregoni*) can also ingest bacteria but should have been effectively excluded from the dilution experiments by the pre-screening. Thus any grazing by these cladocerans (as well as by larger rotifers and copepods) would be additional to the grazing rate measured in the dilution

experiments. This additional, undetermined grazing pressure could, therefore, explain why net bacterial growth indicated by the dilution experiments did not always relate to observed fluctuations in bacterial density in the loch. A large population of *D. longispina* did develop in the loch during summer 1996, reaching a maximum recorded density of 6700 ind. m⁻² in October (A. Shine pers. comm.). This might also partly account for the virtual disappearance of HNF in the summer, since these small flagellates would have been efficiently grazed by the filter feeding cladocerans (e.g. Sanders & Porter 1990). Thus it appears that the grazing pressure on bacteria in Loch Ness derives from a diverse group of consumers (cf. Pace et al. 1990, Pace & Cole 1994), in contrast to what appears to be the case in many other lakes in which grazing by HNF is the predominant cause of bacterial loss (Sanders et al. 1989, Tranvik 1989, Weisse 1990).

In conclusion, our results indicate that bacterial density in Loch Ness is rather low (<10⁶ cells ml⁻¹) and exhibits the same limited annual variability that has been widely reported from other lakes. However, because the phytoplankton production in the loch is so low, bacterial growth rate does not show same the kind of marked seasonality that is commonly reported from temperate lakes. The low bacterial growth rates measured for Loch Ness (Table 2) and the frequent limitation of growth by 1 or more nutrients, particularly phosphorus (Table 3), show that bacterial growth in Loch Ness is mainly 'bottom-up' controlled by shortage of resources. There is also some evidence that bacterial growth is influenced by the influx of fresh resources from the catchment and hence by rainfall patterns. Although bacterial growth in Loch Ness is generally slow, new bacterial production is nevertheless removed quite efficiently by a variety of grazers so that bacterial densities show only little fluctuation. HNF are an important component of the bacterivore community at some times, but do not appear to have the overwhelmingly dominant role that they have been reported to play in other lakes.

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