PRIMARY PRODUCERS IN SUB–ARCTIC STREAMS AND THE
EFFECTS OF TEMPERATURE AND NUTRIENT ENRICHMENT ON
SUCCESSION

Rakel Guðmundsdóttir

Faculty of Life and Environmental Sciences
School of Engineering and Natural Sciences
University of Iceland
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Rakel Guðmundsdóttir

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Advisors:
Professor Gísli Már Gíslason
Professor Brian Moss
Dr. Jón S. Ólafsson

PhD committee:
Professor Brian Moss
Professor Gísli Már Gíslason
Dr. Jón S. Ólafsson
Dr. Sigurður Reynir Gíslason
Dr. Karl Gunnarsson
Dr. Nikolai Friberg

Opponents:
Professor Richard William Battarbee
Docent Maria M. K. Kahlert

Faculty of Life and Environmental Sciences
School of Engineering and Natural Sciences
University of Iceland
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Effects of nutrients and temperature on primary producers

Útdráttur

Lækir á jarðhitasvæðum geta verið hentugir til tilrauna í vistfræði, m.a. til að meta möguleg áhrif loftslagsbreytinga á vatnavistkerfi. Átta misheitir lækir á afmörkuðu svæði í Hengladölum á Hellisheiði voru notaðir til þess að prófa tilgátur um hugsanleg áhrif hlýnunar á samfélög frumframleiðenda. Einnig voru prófaðar tilgátur um áhrif næringarefna aukningar á sömu lífverusamfélög. Tilgáturnar voru; að aukinn hiti myndi hafa áhrif á uppbyggingu samfélaga frumframleiðenda, m.a. í breytingum á heildar samsetningu frumframleiðenda ásamt breytingum á samsetningu vaxtarforma kísilþörunga; að næringarefna aukning myndi auka frumframleiðni ásamt því að breyta samfélags uppbyggingu frumframleiðenda. Samverkandi áhrif yrðu af næringarefna aukningu og hlýnun sem gæti gefið mynd af því hvernig samfélög frumframleiðenda í lækjum á norðlægum breiddargráðum gætu breyst með hækkandi lofthita, breytingum á landnotkun og fólksfjölgun.

Í ljós kom að í köldum lækjum var þéttleiki lækjarbleðlu *Jungermannia exsertifolia* (Steph.) lítill en lífmassi þörungaskánar á steinum var mikill. Í volgum lækjum var talsverður lífmassi ármosa (*Fontinalis antipyretica* Hedw.) ásamt miklum lífmassa þörungaskánar á steinum. Í heitustu lækjunum var þéttleiki ármosa mikill (*F. antipyretica*) en lítill lífmassi þörungaskánar var á steinum. Í öllum lækjum var lífmassi grænþörunga talsverður. Þættir eins og leiðni, pH og rennsli ásamt þéttleika hryggleysingja og fiska voru metnir með hliðsjón af hita og næringarefna aukningar áhrifum á frumframleiðendur.

Greindir voru 64 kísilþörungahópar og þar af voru 47 greindir til tegunda. Flestar tegundir voru í kaldari lækjunum ásamt fjölbreyttustu vaxtarformunum. Vatnabobbi (*Radix peregra* (Müller)), sem er þörungaæta, virtist hafa mótandi áhrif á vaxtarform kísilþörunga í heitum lækjum en hafði ekki áhrif á þéttleika þeirra. Í heitu lækjunum voru smærri kísilþörungar meira áberandi en í þeim köldu.

Tilraun þar sem magn ammoníumnítrats (NH_4NO_3) var aukið í lækjunum leiddi m.a. í ljós að þekja og lífmassi mosans *F. antipyretica* og þráðkenndra grænþörunga, einkum *Cladophora* spp. jukust marktækt. Aftur á móti minnkaði lífmagn blágrænna niturbindandi baktería (Cyanobacteria), eins og *Nostoc* spp. Lífmagn kísilþörunga jókst marktækt við aukningu næringarefna. Fjölbreytileiki kísilþörunga minnkaði samfara næringarefna aukningu, en TDI (Trophic Diatom Index) breyttist ekki. Vaxtarform kísilþörunga hliðruðust eftir að næringarefnum var bætt í lækina. Þéttleiki *Nitzschia* tegunda minnkaði marktækt við næringarefna aukningu í lækjunum.

Samkvæmt niðurstöðum rannsóknarinnar mun hækkandi lofthiti, aukin áburðarefnanotkun á landi og fólksfjölgun (sem leiðir til aukningar á næringarefnum í vistkerfi) hafa mikil áhrif á samfélög frumframleiðenda í lækjum á háum breiddargráðum. Frumframleiðni eykst og samfélög smárra þörunga og blágrænna baktería mun verða skipt út fyrir samfélög mosa. Samfélög hryggleysingja munu að öllum líkindum breytast ásamt því að þéttleiki fiska mun aukast.

Abstract

Streams in geothermal areas may offer good experimental opportunities for investigating the potential influences of climate changes upon freshwater ecosystems. Eight adjacent streams, varying in temperature, in the Hengill geothermal area, SW Iceland were selected to test hypotheses concerning the effects of nutrient addition and temperature on the community structure of primary producers. These were that: increasing temperature would have major effects on the structure of the primary producer communities, reflected in gross composition, species representation and growth form occurrence; that nitrogen addition would increase production whilst also changing community composition; and that there would be some interaction between temperature and nutrient effects that might give indications as to how sub–Arctic streams might be expected to change with progressive global warming and the associated secondary effects of changing land use.

The density of the liverwort *Jungermannia exsertifolia* (Steph.) was low in the coldest streams but the biovolume of the biofilms on stones was high. In slightly warmer streams the density of the bryophyte *Fontinalis antipyretica* (Hedw.) was high along with high algal biovolume on stones. In the warmest streams the cover of *F. antipyretica* was very high. Green algal biovolume was high in all streams. Cyanobacteria and diatom biovolume was high in the cool and cold streams while it was lower in the warmest streams. Variables such as conductivity, pH and discharge along with the density of invertebrates and fish were examined in a relation to the temperature and nutrient influences on primary producers.

Diatom communities included 64 taxa, 47 of them identified to species level. The coldest streams contained the most diverse diatom communities along with the highest growth–form diversity. The major grazer, *Radix peregra* (Mollusca) influenced growth forms by decreasing the diversity but it did not influence the density of diatoms. Small diatom populations were denser in warmer streams compared with colder streams.

Experiments in which ammonium nitrate (NH_4NO_3) was dripped into each stream over several months showed that biomass of the bryophyte *F. antipyretica* increased significantly when nitrogen was added, along with increased biomass of filamentous algae (mainly *Cladophora*). However, the biomass of nitrogen fixing Cyanobacteria (e.g. *Nostoc*) and *Oscillatoria* decreased significantly with nutrient enrichment. The density of diatoms increased significantly when nutrients were added to the streams. The diversity of diatoms decreased but the TDI (Trophic Diatom Index) did not change, suggesting that this commonly used index may not be a reliable means of detecting nutrient pressures. The growth forms of diatoms were altered by nutrients and the diatom genus *Nitzschia* declined in numbers with nutrient enrichment.

Consequently, the results suggest major changes in the future in subarctic streams as temperatures increase and more land becomes available for stock–keeping, and even cultivation, leading to increased eutrophication. Production will increase and microalgal communities will be displaced by bryophyte growth. There will be effects on invertebrate communities, and increased fish production may lead to additional effects, though these can only be hinted at from the present data.

To my sister Þórey Guðmundsdóttir (1988–2006), father Guðmundur Þór Kristjánsson (1954–2010) and mother Elenborg Helgadóttir

List of papers

The present thesis is based on three published papers and a submitted typescript. The papers will be referred in the text by their respective numbers as following:

Paper 1:

Guðmundsdóttir R, Gíslason GM, Pálsson S, Ólafsson JS, Schomacker A, Friberg N, Woodward G, Hannesdóttir ER, Moss B. 2011. Effects of temperature regime on primary producers in Icelandic geothermal streams. *Aquatic Botany*, **95**: 278–286.

Paper 2:

Guðmundsdóttir R, Pálsson S, Gíslason GM, Ólafsson JS, Moss B. 2012. Variation in diatom and bryophyte communities along a temperature gradient in sub–Arctic streams: model surrogates for trends in larger ecosystems? *Inland Waters*, **2**: 163–176.

Paper 3:

Guðmundsdóttir R, Ólafsson JS, Pálsson S, Gíslason GM, Moss B. 2011. How will increased temperature and nutrient enrichment affect primary producers in sub–Arctic streams? *Freshwater Biology*, **56**: 2045–2058

Paper 4:

Guðmundsdóttir R, Pálsson S, Hannesdóttir ER, Ólafsson JS, Gíslason GM, Moss B. 2012. The influences of experimental nitrogen enrichment on diatom assemblages in sub–Arctic streams. *Ecological Indicators* (**Submitted**).

Other peer reviewed papers not included in the thesis

Paper 5:

Demars B, Manson RJ, Ólafsson JS, Gíslason GM, **Guðmundsdótti R**, Woodward G, Reiss J, Pichler DE, Rasmussen JJ, Friberg NF. 2011. Temperature and the metabolic balance of streams. *Freshwater Biology*, **56**: 1106–1121.

Paper 6:

O'Gorman EJ, Pichler DE, Adams G, Benstead JP, Cohen H, Craig N, Cross WF, Demars BOL, Friberg N, Gíslason GM, **Guðmundsdóttir R**, Hawczak A, Hood JM, Hudson LN, Johansson L, Johansson M, Junker JR, Laurila A, Manson JR, Mavromati E, Nelson D, Ólafsson JS, Perkins DM, Petchey OL, Plebani M, Reuman DC·, Rall BC, Stewart R, Thompson MSA, Woodward G. 2012. Impacts of warming on the structure and functioning of aquatic communities: individual– to ecosystem–level responses. *Advances in Ecological Research* (**in press**)

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Contribution from authors:

Table 1. Contributors to the research results presented in Papers 1–4.

Task	Paper 1	Paper 2	Paper 3	Paper 4
Design of the experiment	Guðmundsdóttir, Moss, Gíslason, Ólafsson	Guðmundsdóttir, Moss, Gíslason, Ólafsson	Guðmundsdóttir, Moss, Gíslason, Ólafsson	Guðmundsdóttir, Moss, Gíslason, Ólafsson
Field work	Guðmundsdóttir, Gíslason, Ólafsson, Hannesdóttir, Friberg	Guðmundsdóttir, Gíslason, Ólafsson	Guðmundsdóttir, Gíslason, Ólafsson	Guðmundsdóttir, Gíslason, Ólafsson, Hannesdóttir
Laboratory analysis	Guðmundsdóttir	Guðmundsdóttir	Guðmundsdóttir	Guðmundsdóttir
Statistical analysis	Guðmundsdóttir, Pálsson	Guðmundsdóttir, Pálsson	Guðmundsdóttir, Pálsson	Guðmundsdóttir, Pálsson
Image production	Guðmundsdóttir, Schomacker, Pálsson	Guðmundsdóttir, Pálsson	Guðmundsdóttir, Pálsson	Guðmundsdóttir, Pálsson
Writing	Guðmundsdóttir	Guðmundsdóttir	Guðmundsdóttir	Guðmundsdóttir
Invertebrate analysis	Friberg, Hannesdóttir, Ólafsson, Gíslason	–	Gíslason, Ólafsson	Hannesdóttir, Gíslason, Ólafsson

Contents

List of abbreviations and symbols

CPOM   Coarse particulate organic matter (woody material and leaves > 1 mm in diameter)

FPOM   Fine particulate organic matter (leaf fragments, invertebrate faeces and organic precipitates < 1 mm in diameter).

ANOVA   Analysis of variance

lme   Linear mixed effect model

RCCA   Regularized Canonical Correlation Analysis

CCA   Canonical Correlation Analysis

°C   Degree Celsius

%   Percentage

=   Equals

±   Plus or minus

<   Less than

>   Larger than

m a.s.l   Metres above sea level

W   West

N   North

º   Degree

P/R   Photosynthesis respiration ratio

Acknowledgements

1. Introduction

1.1 The water cycle and climate change

The transformations of water from freshwater and oceans to atmosphere to earth and back again is generally termed the water cycle. Water can be in the form of vapour, liquid or ice. It moves from one reservoir to another i.e. from the atmosphere to the Earth's surface by precipitation or from rivers to the ocean by runoff. Water movement is driven by physical processes such as evaporation, condensation, precipitation, infiltration, runoff and subsurface flow. The water cycle involves various processes such as the exchange of heat energy, transporting of minerals between various parts of the globe and geological processes (sedimentation and erosion) (Wetzel 2002; Allan 2006).

Climate is closely linked to the water cycle. Solar energy heats the planet and powers the water cycle while evaporation from the oceans and land surfaces cools it. Climate changes have and will cause alterations through the physical processes of the water cycle (Tao et al. 2002; Bouraoui et al. 2004). They have occurred naturally on Earth but recent climate changes are considered to be caused by human activities (IPCC 2007). The Arctic Climate Impact Assessment (ACIA) predicts an air temperature increase of 3–9 °C over the next 100 years in Arctic and Sub–Arctic regions (ACIA 2004; Chapman and Walsh 2007). An air temperature increase of 3–4 °C in these regions has been detected during the last 50 years. Arctic and Sub–Arctic regions are considered to be most sensitive regions to climate changes. Climate changes result in increased mean temperature and increased precipitation with changed seasonality that may lead to increased nutrient loading in freshwater ecosystems (Rouse et al. 1997; Moss et al. 2003; Jeppesen et al. 2010). Changes on organisms have been detected such as range shifts (organisms moving closer to the poles) and migration of plants and animals (Walther et al. 2002; Parmesan and Yohe 2003). Freshwater systems appear to be particularly vulnerable to climate–induced changes.

1.2 Streams and rivers

Freshwater ecosystems are diverse and complex in nature. Running waters are open heterogeneous and hierarchical ecosystems (Soininen 2004). They range from small first order streams to large rivers and occur under widely differing conditions of vegetation, climate, geology and topography (Allan 2006). Abiotic factors affecting rivers and streams include flow, light, temperature, chemistry and substrate, and biotic factors include populations and activities of bacteria, primary producers (algae, cyanobacteria, bryophytes and vascular plants), insects and other invertebrates, fish and other vertebrates. The abiotic factors influence the biotic factors and therefore the structure and function of the ecosystems. Stream water contains many substances and the chemical composition is influenced particularly by the geology of the drainage, climate (i.e. temperature and rain frequency and intensity), volcanic activity and pollution, but also by export of organic matter from the surrounding land. Transported materials in the stream water can be

separated according to whether they are suspended or dissolved, organic or inorganic or by chemical description (Berner and Berner 1987). The role of dissolved nutrients (N, P and Si) on primary producers and on other organisms higher in the food chain is of concern.

1.3 Streams and the river continuum concept

The river continuum concept summarises the biological processes and components of streams and rivers along a longitudinal downstream continuum in forested terrain (Vannote et al. 1980) (Fig. 1).
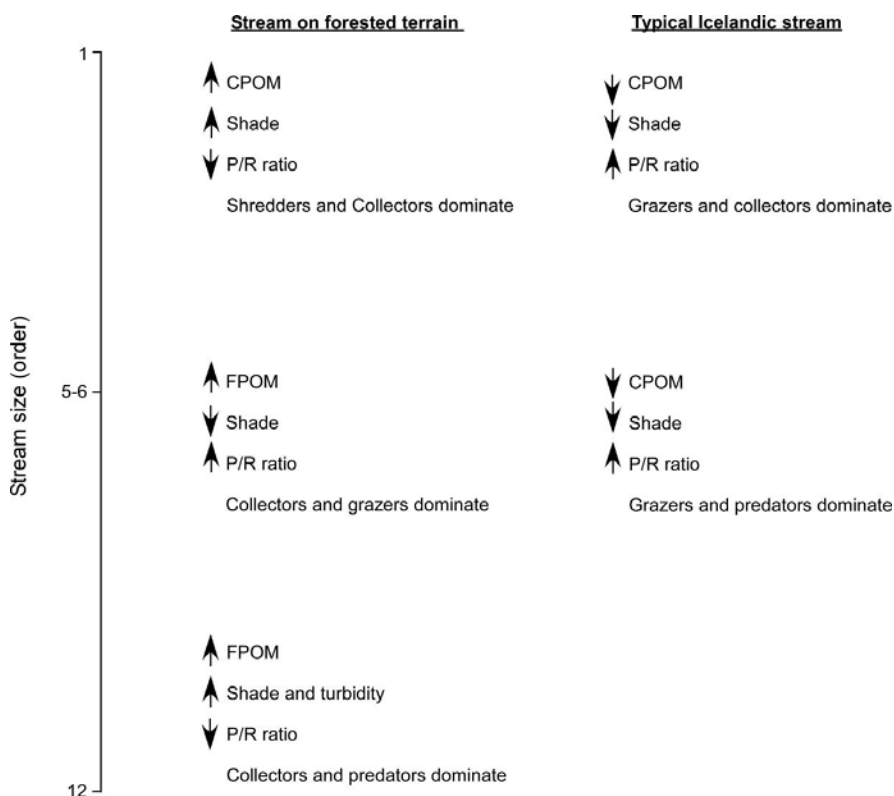
Fig. 1. Schematic drawing showing the main features of a typical stream on forested terrain and a typical Icelandic stream (based on Vannote et al. 1980 and Petersen et al. 1995).

The high reaches or low order streams are usually small and steep with swift, cool water and often shaded by riparian vegetation. Most of the energy is brought in by allochthonous organic matter. Shredders and collectors are the dominant invertebrates in these streams. Because of the shading from the riparian vegetation, primary production is generally low and therefore the P/R ratio is usually less than 1.0. The body shape of the fish in these low order streams allows them to move easily in swift water (Vannote et al. 1980).

In the mid reaches, the channels are exposed to more sunlight than in the high reaches because the riparian vegetation does not shade the middles of the channels. Substrates allow the growth of periphyton (autochthonous production) and the P/R ratio is usually greater than 1.0. Grazers and collectors are the dominant invertebrates in these reaches

(Vannote et al. 1980).

In the lower reaches of the river system, the river gradient is low and the substrates of the river bed are made up by gravel, sand and silt that do not allow for periphyton growth (because of turbidity and abrasion). Most of the energy input is in the form of fine particulate organic matter from upstream sources. Collectors are the dominant invertebrates and the fish are generally more slow–water adapted and feed on zooplankton or in the bottom deposits (Vannote et al. 1980).

Rivers in arctic and alpine environments like Iceland differ from the forested rivers most familiar to Vannote et al. (1980), first in the small size of Icelandic rivers (usually not greater than 5–6th order). They have low nutrient concentrations because they run through a terrain of basalt rock and soils derived from it. The biota is species–poor (in both primary and secondary producers) and the terrestrial vegetation is sparse and thus there is little allochthonous input to these rivers (Petersen et al. 1995). The P/R ratio is greater than 1 because they are clear, with little or no terrestrial vegetation to shade, while it can be below 1 in the typical forested terrain river due to shading by terrestrial vegetation and/or turbidity (Vannote et al. 1980; Petersen et al. 1995).

1.4 Eutrophication in streams and rivers

Along with climate change caused by humans, pollution is also a problem in streams and rivers. Nutrients are used to fertilize crops leading to accumulation of such nutrients in the ground. Rain flushes nutrients through and over the ground leaving them to fertilise streams, rivers and lakes (Cazalas et al. 2004). Also increased temperature increases bacterial activity and therefore decomposition of organic material on land and in waters, leading potentially to increased nutrient supply (Demars et al. 2011). The combined pressures on freshwater bodies have resulted in extension of legislation in Europe to attempt to mitigate the effects of these pressures, and the enactment of the Water Framework Directive.

The European Union´s Water Framework Directive (WFD) requires all water bodies to be of "good ecological status" by 2015 (European Union, 2000; Foster et al. 2001). Defined ecological statuses are five (high, good, moderate, poor and bad) and these are to be determined largely by biological indicators. Various approaches have been developed to monitor the pollution and acidification of streams by the use of biological elements. The WFD defines phytobenthos and macrophytes as biological elements used to monitor streams and rivers. Several member states of the WFD have chosen to use diatoms as proxies for phytobenthos (Kelly 2006; Kelly 2008 a, b). Several diatom–based indices have been developed during the last two decades to provide information on acidification (Battarbee 1999), and nutrients (Kelly and Whitton 1995; Rott et al. 1999; Potapova et al. 2004). One important index used for the WFD is the Trophic Diatom Index (TDI). It was developed to monitor nutrient pollution in streams and rivers and is based on a points system. It allocates diatoms to five groups according to their tolerance towards nutrients with ´s´ scores ranging from 1 (low tolerance) to 5 (high tolerance) (Kelly 2008 a).

Diatoms have frequently been used for monitoring, mainly because their ecology is

relatively well understood and the structure of their silica frustule gives opportunity to identify them accurately to species level. They have been found to be sensitive to various environmental factors such as changes in ionic content, pH, dissolved organic matter and nutrients (Potapova and Charles 2007; Kelly 2008 a).

1.5 Current project

This study is a part of a larger EU project (Euro–limpacs) that aimed at detecting the influences of climate change on all freshwater ecosystem types in Europe. The focus of the study was the succession and structure of primary producers (mainly algae and Cyanobacteria) in small mountain streams in Iceland, varying in temperature due to proximity to geothermal heat. Because of the highly variable heating of the stream water it was possible to detect temperature influences on the primary producers. Experiments were also conducted on the streams by adding ammonium nitrate (NH_4NO_3) in the lower reaches of each stream. Ammonium nitrate was used in the experiments because previous studies by Friberg et al. (2009) revealed that the autotrophic communities in the streams were nitrogen limited. Each stream was divided into a treatment reach (experimental), where the nutrient drip was added to the streams, and a control reach (no treatment), with a buffer reach between them. The structure of these streams was studied before nutrient experiments began. It was important to obtain background information on how the stream communities were structured. After the background study, experiments were conducted to establish how possible changes with increased air temperature and nutrient enrichment would influence primary producers in Sub–Arctic streams and if indices that are frequently used to monitor pollution would apply to the Hengill streams.

1.6 Primary producers in streams

Primary producers acquire their energy from sunlight and their materials from inorganic sources. Macrophytes and various small autotrophs (microscopic algae and cyanobacteria) are the major autotrophs in running waters. Macrophytes include vascular plants (angiosperms), non–vascular plants (bryophytes) and larger filamentous algae (e.g. *Cladophora* spp.). Green algae (Chlorophyta), diatoms (Bacillariophyta) and Cyanobacteria are the most common small periphytic autotrophs in streams (Allan 2006).

Angiosperms are usually found where the depth is moderate and where the current is slow. Bryophytes can be abundant in shaded habitats and cool climates. Periphyton is found on virtually all surfaces receiving light. Diatoms are usually the dominant group in the periphytic community but green algae and cyanobacteria can be dominant under certain circumstances (Allan 2006).

Light, temperature, current, substrate, scouring effects of floods, water chemistry and grazing are important for the succession of primary producers in streams (Hynes 1970; Whitton 1975). Light is a fundamental factor for photosynthesis and therefore important for all primary producers. It influences community structure, population growth and physiology of primary producers (Hill 1996). Green algae are usually associated with high

light levels, while red algae have been found to be most abundant at low light levels (Hynes 1970; Allan 2006). However, some diatom species do not seem to be affected by seasonal changes in light levels (Hill 1996; Allan 2006). Nutrients are essential for primary producers. Phosphate (PO_4^{3-}), nitrate (NO_3^-) and silicate (SiO_3^-) are considered the most critical nutrients for autotrophic production, though some other elements can be limiting under certain circumstances (Hutchinson 1957; Allan 2006). If the N:P ratio is below 5:1 to 16:1 (by atoms) it is conventionally considered that nitrogen limitation may be occurring but above that band phosphorus may be limiting (Redfield 1958; Sterner and Elser 2002).

Some Cyanobacteria are able to fix nitrogen from the atmosphere and can therefore become abundant under otherwise nitrogen–limiting circumstances (e.g. *Nostoc* spp.). Silica can be limiting for the growth of diatoms because of the chemical composition of their walls (Wetzel 2002). Temperature is an important environmental factor influencing primary producers because of its effects on biochemical reactions. In clear and shallow waters, infrared wavelengths may have direct heat influences on the cells but usually the temperature influences are determined by transfer of heat from the surrounding water (DeNicola 1996). Latitude, altitude, continentality, aspect and morphometry determine the overall temperature regime of freshwaters (Hutchinson 1957). Geothermal activity can have influences on the temperature regimes in freshwaters (DeNicola 1996). Current is one of the major influences on the existence and morphological features of primary producers (Biggs and Close 1989; Ghosh and Gaur 1998). Rooted macrophytes are benefitted by slow current while epilithic algae are benefitted by fast current (Allan 2006). Current can also influence the growth forms of algae and Cyanobacteria, e.g. the length and growth form of *Cladophora glomerata* filaments and attachment traits of various diatoms (flat and sticky in fast flowing water and erect loosely attached in slow flowing waters) (Yallop and Kelly 2006). Substrate influences the populations of the periphyton and provides stability against high flows (Allan 2006). Particle size, factors relating to the mix of particles, surface texture and the relative permanence of bed material are critical physical aspects. Small first order streams generally contain large particles (boulders and cobbles) whilst downstream the particle size decreases (Allan 2006).

Virtually all periphyton in running waters serves as food for some herbivores. The extent of herbivore development depends on periphyton growth forms (Yallop and Kelly 2006) and differs among the major taxonomic groups. The diet of herbivores is a result of various characteristics of both the herbivore and the periphytic assemblage. Size, motility, morphological specialization and digestive capabilities are important features of the herbivore determining its feeding behaviour. In general the herbivores are classified in 'functional feeding groups' depending on their feeding behaviour. Scrapers rasp or pick attached periphyton from its surface attachment, collector–gatherers consume loose algae, microbes and detritus (Lamberti and Moore 1984), shredders consume periphyton attached to surface of fallen allochthonous material, and suspension feeders feed on drifting algae (Lamberti and Moore, 1984).

1.7 The main objectives of the study

1. To determine differences in primary producer assemblages that may be related to

differences in temperature but with attention also to other potentially influential conditions such as substrate type, grazers and water chemistry (**Paper 1**).

Three main hypotheses were posed: Firstly, that there would be a shift from epilithic algae and Cyanobacteria in colder streams to dominance of macrophytes and bryophytes in warmer streams; secondly, that at higher temperature the epilithic algal community would change from being dominated by diatoms to Cyanobacteria dominance; thirdly, that macroinvertebrates would influence the structure of primary producer communities in the streams.

2. To determine if streams varying in temperature, vegetation and grazer regime might serve as surrogates (microcosms) for terrestrial ecosystems that might provide convenient and inexpensive models for providing insights to what might happen on landscape scales with future warming. The coldest streams were supposed to resemble tundra, cooler streams were supposed to resemble the tree line while the warmest streams were supposed to resemble boreal forests. The grazers in the streams were much larger than their prey, like grazers in terrestrial habitats (**Paper 2**). Scientists are increasingly using microcosms as surrogates for various studies in ecology (Lawton 1995). These experiments range from using two species in a container competing for some resource to the competition experiment that Gause (1932, 1934) conducted using only three species of *Paramecium* and more complex and controlled laboratory experiments using microorganisms, such as Petchey et al. (1999), which are presumed to simulate complex terrestrial habitats.

Four main hypotheses were posed with this analogy: firstly, that species richness, evenness and the diversity of growth forms would be greater at higher temperatures than at lower temperatures; secondly, that high grazing pressure would restrict the growth forms of diatoms and it would reduce species richness and evenness; thirdly, that bryophytes would be least abundant where grazing was highest; and lastly, that with increased temperature, species with small sized individuals would be more abundant than species with larger individuals. These hypotheses are based on well–known trends in terrestrial habitats.

3. To determine the effect of nutrient additions on the biomass of bryophytes, vascular macrophytes, algae and Cyanobacteria under variable temperature regime and how it might alter the dominant algae and Cyanobacteria groups in the streams (**Paper 3**).

As climate warms, countries at higher latitudes, now covered by coniferous forest or tundra, will become more favourable for settlement. This might lead to those rivers which are currently pristine, or only lightly influenced by anthropogenic impact, becoming more eutrophic or polluted. Industry and agriculture are the main sources of nitrogen enrichment in freshwaters and when human population grows, agriculture and industry will increase with huge impacts on freshwater biota. Agriculture influences the nitrogen cycle because farmers are adding nitrogen–rich fertilizers to increase their crops. Also, as agriculture expands, the animal stocks increase, leading to greater income of ammonium through manure production but also through volatilization of ammonia to the atmosphere. Combined nitrogen in the atmosphere is also increasing with emission of gases containing nitrogen (e.g. from car engines). Human influences have altered the nitrogen cycle by increasing various forms of nitrogen such as NO_3^- that can cause acidification in streams and lakes. Increased nitrogen loadings have also been linked with excessive growth of plants in lakes (Bell et al. 1994).

Three main hypotheses were set up in this study: firstly, biomass of bryophytes, vascular plants, larger filamentous algae and thalloid algae/Cyanobacteria would increase with nutrient enrichment; secondly, that periphytic (epilithic) chlorophyll *a* would increase with nutrient enrichment and that the influences would be strongest in the warmer streams; thirdly, that the biovolume of green algae and diatoms would increase with nutrient enrichment while the biovolume of nitrogen fixing Cyanobacteria (mainly *Nostoc* spp.) would decrease with nutrient enrichment.

4. To determine the effect of nutrient enrichment on diatom assemblages with increased temperature (**Paper 4**).

We estimated effects on diatom diversity (Shannon´s index, Sørensen´s index) and on the Trophic Diatom Index (TDI). Currently, a great effort is being put into using diatoms as indices to assess the ecological state of aquatic habitats due to the implementation of the Water Framework Directive. None of these indices have been rigorously tested experimentally; all have been developed from field correlations, and there is increasing concern that much effort is being wasted to no great gain (Demars et al. 2012). The series of streams used here gave an opportunity for experimental testing of one widely used index.

Four main hypotheses were posed: Firstly, that population density of diatoms would increase significantly with nutrient enrichment; secondly, that diversity estimated with Shannon´s index would fall with nutrient enrichment, and that overlap among communities, estimated with Sørensen´s diversity, would be negatively affected by nutrients (more overlap in enriched reaches); thirdly, that the Trophic Diatom Index (TDI) would be greater when nutrients were added compared with reaches without nutrient enrichment; fourthly, that attachment and growth traits of diatoms would be altered with nutrient enrichment. The changes in traits would be in the form of greater abundance of motile and small species where nutrients were added.

2. Material and methods

2.1 The study area

The study area is located in the Hengill geothermal area, 30 km east of Reykjavík (N64°03´;W21°18´; 350–420 m.a.s.l) in Iceland. Around fifteen streams (groundwater–fed) with different water temperatures, owing to direct or indirect geothermal influences, drain into the River Hengladalsá (Fig. 2). The warmer streams used in the project are not directly fed by hot springs but absorb the heat from the ground they run over (Árnason et al. 1967). Eight streams (Figs 3 and 4) from a total of fifteen were chosen for experiments and observations. The fifteen streams ranged in temperature from 4–42°C but the selected streams ranged in temperature from 4–24°C (Friberg et al. 2009). Other environmental factors were broadly the same. Because the streams are located relatively closely to each other or along a reach of 1.5 km of the River Hengladalsá (Fig. 2) it was presumed that potentially confounding biogeographic effects of differential colonisation were negligible.
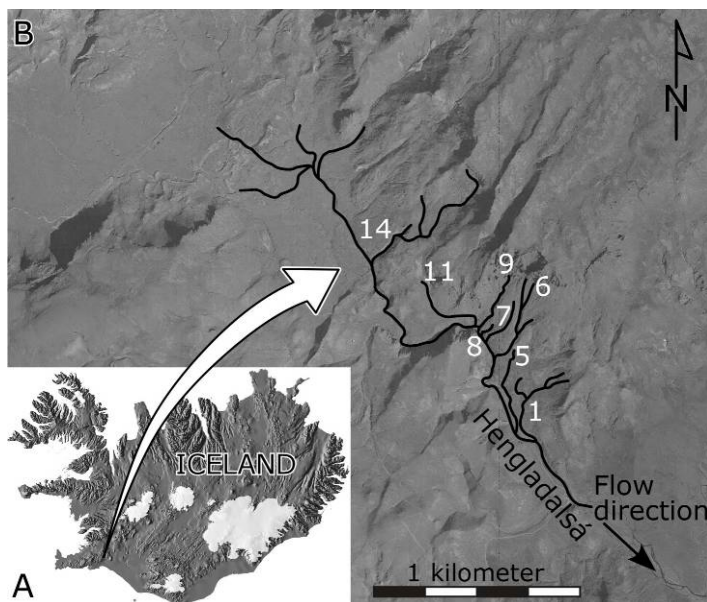
Fig. 2. Map of Iceland (a) and the eight selected streams (b) within Hengill geothermal area. Each stream number is annotated on the map.

The water temperature of the cold streams (Fig. 3) was similar to that expected in high latitude habitats and alpine areas (Ólafsson et al. 2010). The warmer streams (Fig. 4) have regimes comparable to streams at lower latitudes and altitudes in the North Temperate Zone (Allan 2006).

Stream 7
Temp: 7.2 °C
Cond: 96 µS cm $^{-1}$ at 25°C
pH: 8.35

Stream 14
Temp: 8.4 °C
Cond: 204 µS cm $^{-1}$ at 25 °C
pH: 8.33

Stream 11
Temp: 9.6 °C
Cond: 497 µS cm $^{-1}$ at 25°C
pH: 8.03

Stream 9
Temp: 12.9 °C
Cond: 194 µS cm $^{-1}$ at 25°C
pH: 8.42
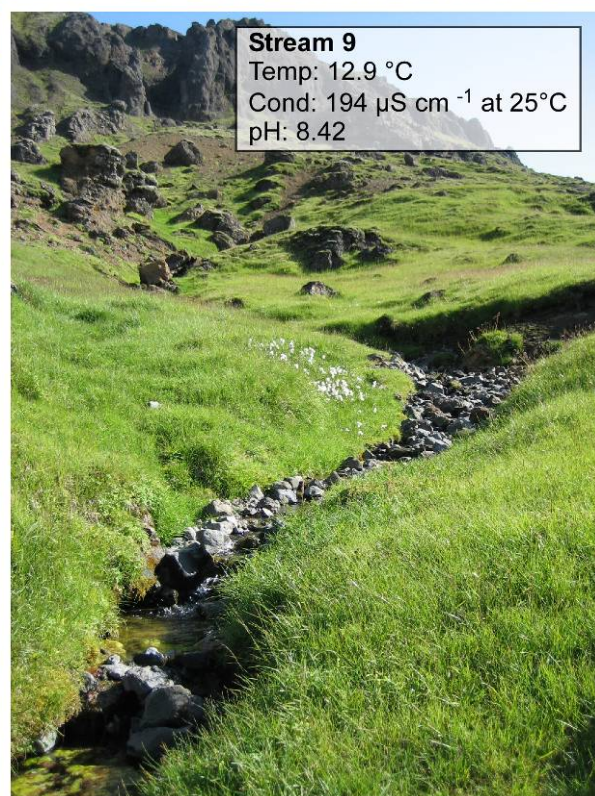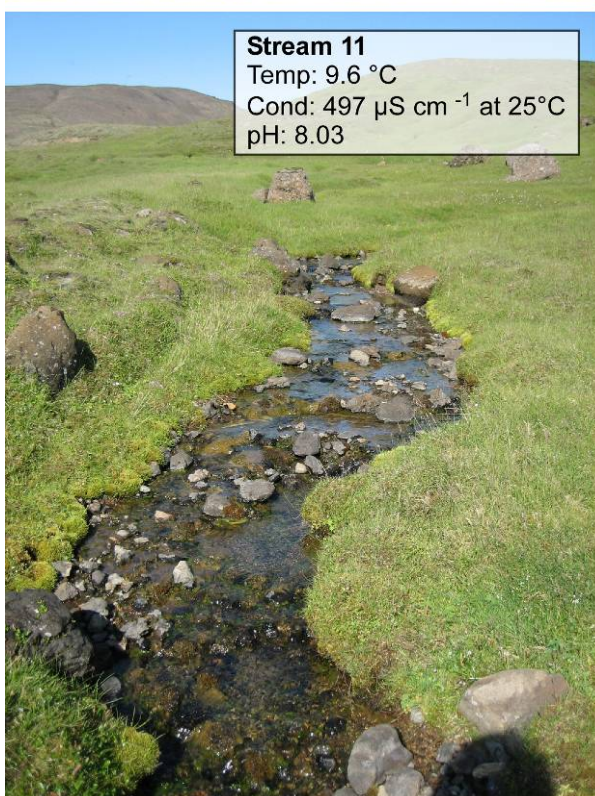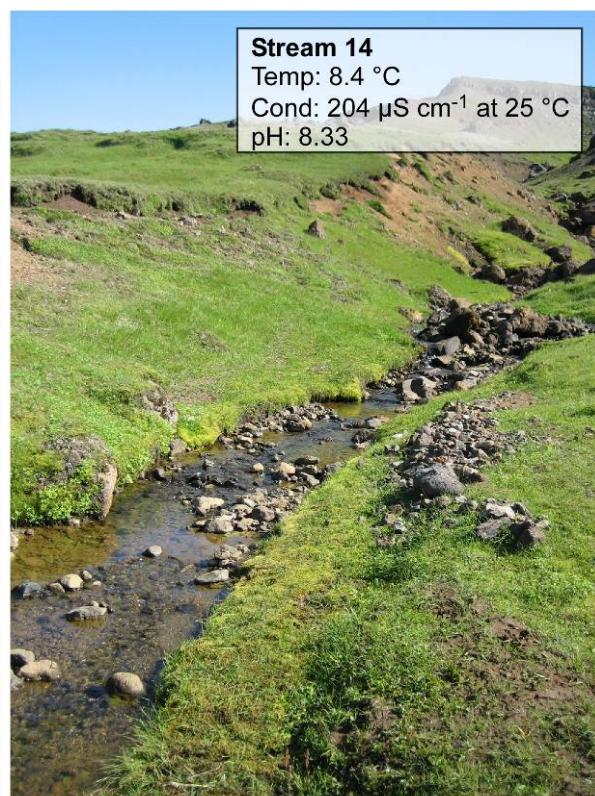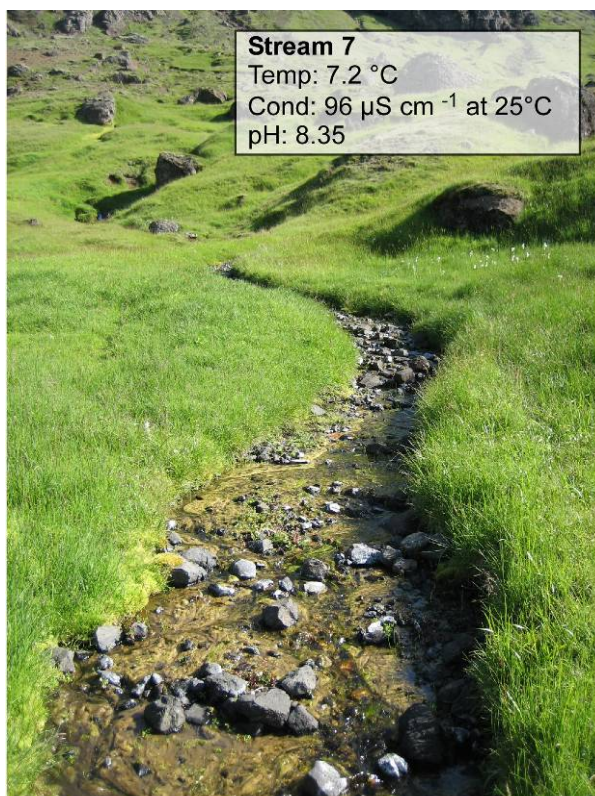
Fig. 3. The four coldest streams. Photos: Rakel Guðmundsdóttir (August 2007).

Fig. 4. The four warmest streams. Photos: Rakel Guðmundsdóttir (August 2007).

2.2 Structural and functional attributes of the streams

The area has a flat valley floor, and slopes with hyaloclastite rocks. The stream gradients range from 0.5 cm m^{-1} to 19.7 cm m^{-1}. The streams are shallow, ranging from 0.05 m to 0.17 m in depth and from 0.63 m to 1.75 m in width. Trees are absent and shrubs are sparse so there is a little or no leaf litter input to the streams (Friberg et al. 2009).

Friberg et al. (2009) studied the structural and functional attributes of the streams. The only fish species observed was brown trout (*Salmo trutta* L.). The abundance of brown trout ranged from 0.02 individuals per m^2 to 0.3 individuals per m^2. Most of the fish have been observed in the intermediate warm streams and the warm streams whilst few have been observed in the coldest streams. Scrapers and filter feeders were the dominant invertebrate functional feeding groups in the four warmer streams (16–24°C), while collectors and gatherers were the dominant invertebrate functional feeding groups in the four colder streams (5–13°C). Thirty–five invertebrate taxa were identified; sixteen of them were Chironomidae. Chironomids, particularly *Eukiefferiella minor* (Edw.) were in their greatest densities in the colder streams compared with warmer streams. The blackfly, *Simulium vittatum* (Müller) was the most abundant macroinvertebrate in the warmer streams (16–24°C) along with the snail *Radix peregra* (Müller). Woodward et al. (2010) found temperature to have strong influences on macroinvertebrate species composition in the streams. They also found greatest diversity (Shannon diversity) to be highest at 10°C with rapid decline as the temperature rose beyond 15°C.

Density of macroinvertebrates was greatest in streams ranging from 13–16°C. The density of individuals ranged from 3000 individuals per m^2 to 16000 individuals per m^2 in the streams and was positively correlated with temperature (Friberg et al. 2009).

2.3 Experimental design

The streams were separated into control reach (no treatment), buffer zone (at the lower end of which the nutrient ran into the stream) and treatment reach. The control and treatment reaches were 15–25 m long and the buffer zone 5 m long. Ammonium nitrate (NH_4NO_3) was pumped into the streams because a previous study by Friberg et al. (2009) indicated that the streams were nitrogen–limited. The aim was to increase the nitrogen levels up to 3–5 times greater in the treatment reaches compared with the control reaches.

On the bank of each stream, two 120 L plastic barrels, containing ammonium nitrate solution were installed. A plastic tube was connected to each barrel through a peristaltic electric pump. The tube extended from the pump to the treatment reach of each stream where nutrients would drip into the stream water. Each pump was powered by a car battery, which was recharged by a solar panel (Figs. 5 and Fig. 6 A, B).

The nutrients were dripped into each treatment reach of each stream for 10 s with 40 s intervals between additions. The rate of nutrient release was determined according to the discharge of each individual stream with an average release of 4–8 ml per minute of an appropriate concentration. The concentration of the ammonium was fixed and inversely proportional to mean stream discharge. The nutrients in the barrels were replenished every 2 weeks and the experiment was conducted from May–September 2006 and from May–August 2007 (**Papers 3** and **4).**
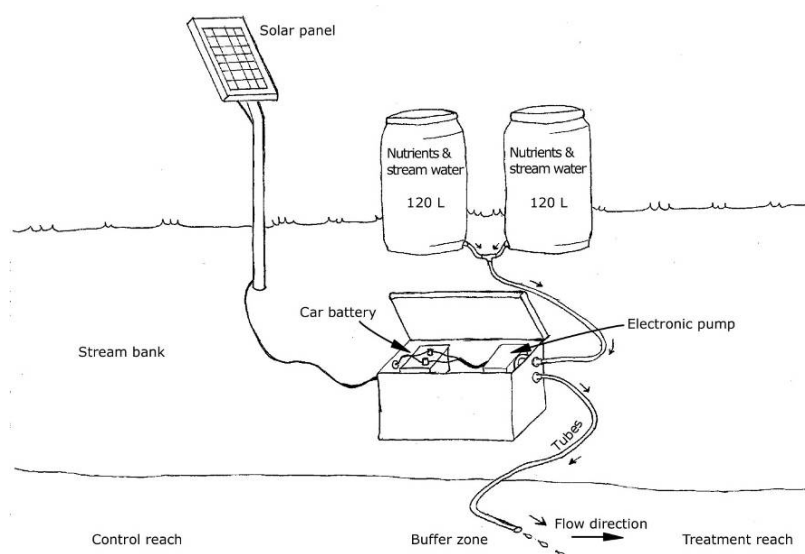
Fig. 5. A diagram showing the experimental setup. On the bank of each stream two 120–L barrels were filled with ammonium nitrate (NH_4NO_3) and stream water. A car battery was used to drive an electric pump which pumped nutrients into the stream's treatment reach. A solar panel recharged the car battery. The control reaches were not treated with nutrients.
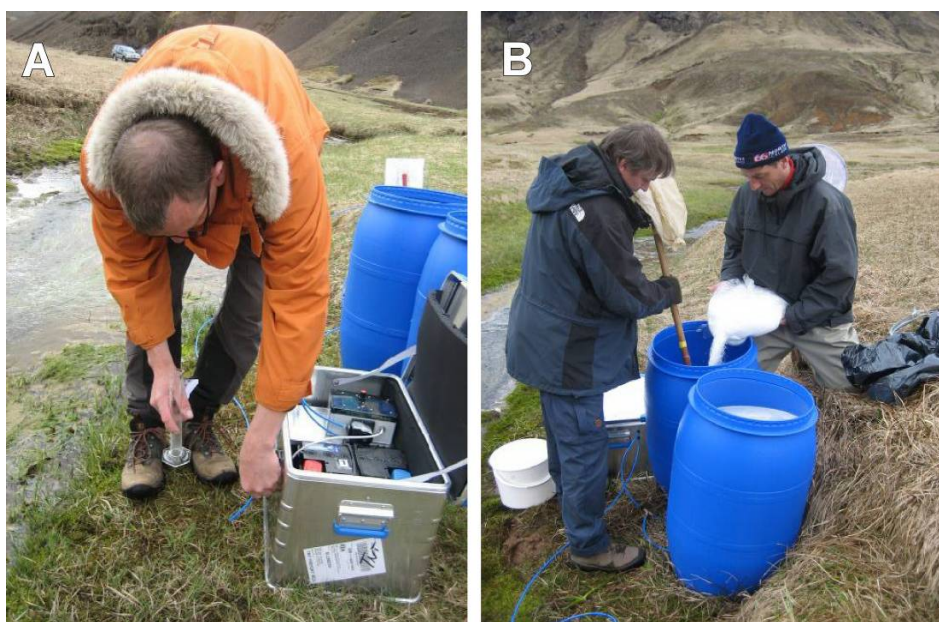
Fig. 6 A–B . The preparation for the enrichment experiments. A: the pumps and the car batteries being connected and the flow from the pumps measured to calibrate the amount of solution needed for this particular stream. B: Ammonium nitrate granules mixed with stream water. The amount of granules was standardized for each stream according to the discharge. Photos: Rakel Guðmundsdóttir (May 2006).

2.4 Sampling

2.4.1 Physical and Chemical variables

Physical and chemical variables were assessed approximately monthly during the summers of 2006 and 2007 (**Papers 1– 4**). However, measurements from the summer of 2006 were used for **Papers 1** and **2** but not from 2007. Discharge (m^3 s^{-1}) was measured using a SonTek Flow Tracker Handled Velocity Meter at the end of July 2006.

On each sampling occasion (summers 2006–2007) temperature, conductivity and pH were measured using a multiprobe sonde YSI 600XLM (Yellow Springs instruments, Yellow Springs, Ohio, USA) standardized to 25 °C (**Papers 1–4**)

Water samples for chemical analysis were collected in 1–L polyethylene bottles from all streams (**Papers 1–4**). The water samples were filtered through GF/C filters before they were frozen (–18 °C) and later sent to the National Environmental Research institute in Denmark for further chemical analysis. Total phosphorus (mg L^{-1}) was analysed by using the method DS 292/DS/EN 6878 (DS–Danish Standard 292, 1985) (**Paper 3**). Total nitrogen (mg L^{-1}) was analysed using the method DS 221/DS/EN 11905, 1975 (**Paper 3**). A UV spectrophotometer (1600 Shimadzu Corporation, Tokyo, Japan) was used to measure TN and TP, following digestion with persulphate to phosphate and nitrate, respectively. Phosphorus was converted to a molybdenum blue complex and measured at 880 nm, and nitrate was determined by reduction to nitrate and diazotisation to a pink dye measured at 545 nm. Silicates (expressed as Si) (mg L^{-1}) were measured at 810 nm as a molybdate complex formed under acid conditions (Koroleff 1983). Samples for ammonium measurements were collected on every sampling occasion in control (**Papers 1–4**) and treatment reaches **(Papers 3** and **4)** and were analysed at the University of Iceland on the same day as the samples were obtained. The ammonium concentration was measured at 630 nm using a salicylate colorimetric method and Lange ion 500 spectrophotometer (Antweiler et al. 1996). Ammonium (NH_4–N) from the stream water was measured because a previous study by Friberg et al. 2009 indicated that the streams were nitrogen limited rather than phosphorus limited.

2.4.2 Biological variables

Sampling was carried out once in a month (May–September 2006 and May–August 2007) when cobbles (5–35 cm diameter) were randomly collected for species identification and functional counting (biovolume of green algae, diatoms and Cyanobacteria (see Hauer and Lamberti 2007) of algae and Cyanobacteria.

Three cobbles were collected from each reach (control and treatment) yielding 48 samples in each month for identification. For chlorophyll *a* analysis, 10–15 cobbles were randomly selected from each reach once per month, yielding around 1800 samples. Each cobble was wrapped in aluminium foil to prevent light damage on the algae and Cyanobacteria before the samples were brought to the lab for further processing.

Sampling of macrophytes was carried out in July 2007. From each reach, 5 samples were randomly harvested (80 samples in total, 40 from control and 40 from treatment) using 14.5 x 14.5 cm quadrats in a random grid. The samples were packed in plastic and frozen in the laboratory for further processing.

Cobbles collected for **Paper 1**, were 120 for chlorophyll *a* analysis (Fig. 7 A) and 24 for the biovolume analysis (Fig. 7 B). Macrophyte and bryophyte biomass was determined to species and estimated from 40 samples (from control). Five to ten samples for invertebrate analysis were harvested from each stream. The invertebrate samples were preserved in 70% ethanol before identification was carried out.
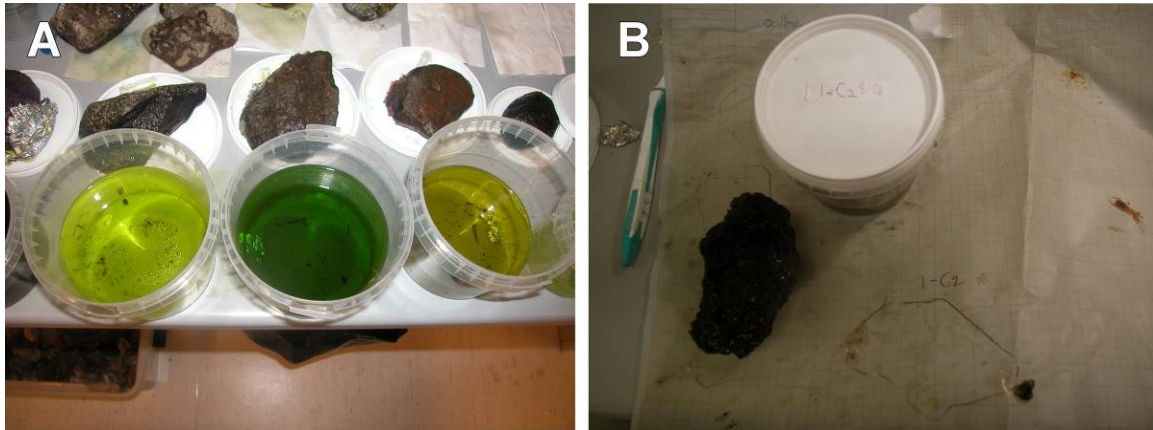
Fig. 7 A–B. Samples processed in the laboratory. A: Stones samples for Chlorophyll *a* analysis placed on the lids from the jars containing the ethanol with extracted chlorophyll after 24 hours in a dark refrigerator. B: A cobble that has been brushed with toothbrush and its surface area drawn on a scaled paper. The jar contains the biofilm brushed from the cobble and Lugol´s iodine solution for preservation. Photos: Rakel Guðmundsdóttir.

For **Paper 2** 96 cobbles were collected (24 samples for each sampling month) for diatom analysis (Fig. 7 B) from May to August 2007 (species and growth form diversity). However when the diatom size categories were estimated, data from nine months (432 samples) were used. Macrophyte and bryophyte biomass was determined to species and estimated from 40 samples (from control). Five to ten samples for invertebrate analysis were harvested from each stream. The invertebrate samples were preserved in 70% ethanol before identification was carried out.

For **Paper 3** 1534 cobbles were collected for chlorophyll *a* analysis and 353 for analysis of biovolume. The cobbles were collected from control and treatment reaches of each stream. Additionally, 80 samples containing macrophyte and bryophyte biomass were harvested. These samples were obtained from both control and treatment reaches.

For **Paper 4** 432 cobbles were collected for diatom analysis, half of them belonging to control reaches and half to treatment reaches of the eight streams. Ten samples for invertebrate analysis were harvested from each stream (5 from control and 5 from treatment). The invertebrate samples were preserved in 70% ethanol before identification was carried out.

Bottom substrate types (i.e. sand, cobbles and pebbles) were estimated visually using five randomly determined areas within a grid in each reach. The bottom structure was described according to the size of the stone particles, with sand smaller than 2 mm, gravel between 2 mm and 1.59 cm, pebbles from 1.6 cm to 6.39, cobbles from 6.4 to 24.9 cm and boulders over 25 cm wide (Giller and Malmquist 2006).

Macroinvertebrates were collected in the first week of August 2004 (Christensen 2006) (**Papers 1** and **2**) and in August 2007 (**Paper 4**). Five to ten samples, each covering 500 cm^2 were randomly taken from each of the eight streams with a Surber sampler, mesh size 200 µm and stored in 70% ethanol.

The data in **papers 1** and **2** were from the years 2006 and 2007, but invertebrate data for six of the eight streams were from 2004 and 2007, the algal samples were correlated to relative abundances of different invertebrate groups. The densities of invertebrates between 2004 and 2007 varied but the proportions were similar. Correlations of invertebrate proportions between streams sampled in 2004 and samples from the corresponding streams from 2007 varied but the maximum values of r^2 ranged from 0.77 to 0.99 per stream, all significant at $p<0.05$. Weather differences in the study area between 2003 and 2006 were inconsiderable and thus unlikely to affect animals in the streams. Temperatures between the 2006 and 2007 summers were similar (Table 1). All these data support the use of the most complete invertebrate data set from 2004 as general drivers in the multivariate analysis for 2006 (**Papers 1** and **2**). For **Paper 4** samples were obtained the same way as the samples from 2004. These data were from both control and treatment reaches of seven streams out of eight.

2.5 Laboratory processing

2.5.1 Chlorophyll *a* analysis

Collected stones were immersed in 96% ethanol and kept at 5°C for 24 h in total darkness. The ethanol was filtered and its volume measured. The area of the stones that had been exposed was covered with aluminium foil, which was then weighed and a mass/area conversion factor was used to determine the area that algae had covered (Hauer and Lamberti 2007).
Absorption of ethanol extracts was measured at 665 and 750 nm using a Lange ion 500 spectrophotometer. A wavelength of 750 nm was used for correction of any residual turbidity after filtration, and the wavelength 665 nm is the absorption maximum wavelength in the red end of the spectrum for chlorophyll *a*. Values for chlorophyll *a*, determined after Talling and Driver (1961), are expressed as µg cm^{-2}.

2.5.2 Diatom processing and analysis

The processing and analysis technique for diatoms is relevant to **Papers 2** and **4**. The collected stones were kept in darkness until laboratory processing took place. In the laboratory the stones were brushed using a medium hardness toothbrush. The slurry of surface material was washed off with distilled water and its volume recorded, and the brushed area was measured by outlining it on scaled tracing paper (mm^2).
Subsamples from the slurry were heated in nitric acid (65%) for up to 24 hours to remove all organic material from the diatom frustules. The volume of the subsample used (usually 10 ml) depended on the algal concentration in individual samples. When no visible organic matter was left (only inorganic sediment), the subsamples were resuspended in distilled water and repeatedly centrifuged (Sorvall ® RT6000) at 3000 rpm for 10 minutes. After

each centrifugation, the pH of the sample was measured. If the pH was low (under 6.0) the sample was re–rinsed with distilled water and re–centrifuged until it had reached pH 6. When pH was greater than 6, a well–mixed sub–sample of 0.5 mL was siphoned off and dispensed onto a circular microscope cover glass were it was left to dry and then mounted with Naphrax® high resolution diatom mounting medium.

Diatoms were identified to species level using a Leica DM 3000 microscope at $\times$ 1000 magnification. A minimum of 200 frustules was counted within defined transects across the cover glass. Keys used for identification were by: Hustedt (1930) and Krammer and Lange–Bertalot (1986, 1988, 1991a and 1991b).

The size categories of diatoms were estimated according to Hauer and Lamberti (2007) (**Papers 2** and **4**). Diatoms that were less than 5 µm in width were considered small, diatoms with width over or equal to 5 µm and shorter than 12 µm in length were classified as medium sized, and those that were 12 µm or more in width, or greater than 25 µm in length, were classified as large diatoms (Hauer and Lamberti 2007).

Growth form structure of the diatoms was estimated and compared with data from Yallop and Kelly (2006). The diatoms were classified in six growth form classes: mucilage stalk (MS), mucilage pad (MP), attached on raphe (AR), motile (MO), chain forming (CF) and diatoms in mucilage tubes (MT) (Yallop and Kelly 2006)

2.5.3 Biovolume of algal/cyanobacterial groups

Biovolume densities of the algal and cyanobacteria communities were obtained from brushings preserved in Lugol´s iodine solution, from standard areas of three stones within each reach **(Papers 1, 3** and **4).** Subsamples of 50–500 µL of the resuspended brushings were counted in sedimentation chambers of 24–mm diameter using an inverted microscope (Wild M50–58257) at 400 $\times$ magnification. Minima of 200 organisms were counted along radial or diametric transects and cell volumes were estimated according to Hillebrand et al. (1999) and Hauer and Lamberti (2007). Densities were expressed as µL cm^{-2}.

2.5.4 Bryophytes and other larger primary producers

Harvested samples of bryophytes, vascular plants and macro algae were frozen for storage, then thawed, rinsed to remove sand and gravel and sorted into groups. Keys from the Icelandic Institute of Natural History were used for bryophyte identifications (Jóhannsson, 1996, 2001). Macrophytes were identified using Stefánsson (1948). After the sorting, the samples were dried for at least 48 hours at 60°C and weighed **(Papers 1** and **3).**

2.6 Statistical analysis

The statistical software R (versions 2.9.2 and 2.11.1) was used for data analysis for all four papers (R Development Core Team). Plots from the R program were edited using the Canvas 11 drawing program.

Paper 1: A linear mixed effect model with the statistical package *nlme* was used to analyse the variation in vascular plant cover (%), bryophytes (%), chlorophyll *a* (µg/cm^2) and

biovolume densities (green algae, diatoms and Cyanobacteria) ($\mu L\ cm^{-2}$) with respect to the environmental differences, taking multiple measurements within streams and variance heteroscedasicity into account.

Variance Inflation Factors (VIF) were calculated to evaluate whether there was linear dependence or collinearity among the environmental variables. If the VIF was smaller than 10, linear dependence or collinearity was not of concern (Quinn and Keough 2002). Spearman´s rank correlation coefficient was used if variables were not linear before and after transformation. It was used to estimate if there was a relationship between the various assemblages of primary producers and invertebrates. Ordination of the primary producer groups with the environmental variables (including invertebrates as drivers) was conducted using Regularized Canonical Correlation Analysis (RCCA) that is implemented in the R package CCA (Gonzales et al. 2008). The RCCA method solves the problem of having a larger number of variables than number of observations.

Paper 2: RCCA was used to ordinate most of the relevant environmental factors (including invertebrates as drivers) and the diatom species composition and growth form diversity.

To estimate if various environmental factors were significantly different between streams a one way ANOVA was used. A linear mixed effect model was used to determinate if there was a relationship between temperature and diatom size categories (Crawley 2002). Pearson´s correlation was used to estimate if there was a relationship between various growth forms of diatoms and invertebrates and also to estimate if there was a relationship between environmental factors, evenness and species richness (Magurran 2004).

Paper 3: Values of chlorophyll *a* ($\mu g\ cm^{-2}$), biomass of larger primary producers (dry weight–gr) and biovolume ($\mu L\ cm^{-2}$) were log–transformed due to their non–normality. Split–plot ANOVA was used to detect possible significant differences between response and predictor variables. In this paper, linear mixed effect models were used in most of the analysis. The reason for the choice of these models was because of the mixture of fixed and random factors and the need to look at time effects on the response variables. These models assume that the fixed effects are unknown constants but the random effects govern the variance–covariance structure of the response variables (Crawley 2002). Individual stream was treated as a random factor because some relationships could be between observations from the same stream as well as relationships between observations from different streams. Nutrient treatment was treated as a binary categorical variable, with 0 as no treatment and 1 as a treatment. Environmental factors such as temperature, conductivity and pH were treated as continuous variables in the linear mixed random effect models. To test if there was a relationship between bryophyte biomass (dry weight–gr) a polynomial regression was used.

Paper 4: The pattern in the diatom communities was explored with the Shannon Weaver diversity index, Sørensen´s index (Magurran 2004) and the trophic diatom index (TDI) (Kelly et al. 2008 a). For the TDI estimation a method described in detail in Kelly et al. (2008 a) was used. The diatoms are categorized in five groups according to their tolerance towards nutrients with ´s´ scores from 1 (low tolerance) to 5 (high tolerance) (Kelly and Whitton 1995). Calculated values range from 0 where there are no impacts from nutrients on diatom communities to 100 where nutrients have maximum impacts on the diatom communities. A linear mixed effect model was used to test if there were nutrient effects on the diversity of diatoms and the TDI. Treatment and years were taken as fixed effects,

environmental factors (temperature, conductivity and pH) as covariates and the samples within streams as nested random effects.

Canonical correlation analysis (CCA) was used to detect if there were relationships between the environmental variables and growth form diversity of the diatom community. The CCA analysis was simplified by amalgamating environmental variables that were highly correlated with each other.

3. Results: summary of Papers

3.1 Environmental features (Papers 1–4)

In August 2006 the stream temperature ranged from 6.6°C (stream 7) to 22.2°C (stream 8) (**Papers 1** and **2**) and the summer (2006 and 2007) mean temperature of the streams ranged from 7.1°C (stream 7) up to 21.6°C (stream 8) (**Papers 3** and **4**) (Table 1). Temperature and conductivity were the main driving variables on the primary producer's communities (**Papers 1** and **2**).

Table 2. Mean temperature (°C) with standard error of the eight streams during the summer 2006 (May–September) and 2007 (May–August)

Stream no.	Temperature (°C)	
	2006	2007
7	7.1 (0.25)	7.3 (0.2)
14	8.35 (0.65)	9.4 (1.7)
11	8.6 (0.72)	10.9 (1.5)
9	13.1 (0.33)	12.8 (0.8)
6	16.5 (0.2)	16.9 (0.7)
1	16.9 (0.61)	18.4 (2.0)
5	17.3 (0.33)	17.6 (0.6)
8	21.6 (0.13)	21.4 (0.3)

Overall the warmer streams had higher conductivity and silicates but lower pH (Tables 3 and 4). When stream 11 was removed as an outlier in conductivity, temperature and conductivity were positively correlated (r = 0.89, t = 4.296, P = 0.008). Temperature and pH were significantly negatively correlated (r = –0.77, t = –2.97, P = 0.025). Conductivity and pH were not significantly different between control and treatment reaches (P>0.05).

Table 3. The conductivity (µS cm^{-1} at 25°C) and pH of the different control and treatment reaches. The sampling was carried out monthly from May until August 2006 and 2007, yielding 8–9 point measurements for each reach within each stream. Values are means with standard errors of the means in parentheses. Streams are in ascending order of mean temperature.

| | pH | | | | Conductivity (µS cm^{-1} at 25°C) | | | |
| | 2006 | | 2007 | | 2006 | | 2007 | |
Stream	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control
7	8.40 (0.13)	8.35 (0.12)	8.62 (0.14)	8.61 (0.17)	108 (10.3)	96 (0.9)	107 (3.0)	102 (3.0)
14	8.35 (0.16)	8.33 (0.18)	8.19 (0.31)	8.26 (0.28)	187 (30.0)	204 (2.8)	241 (21.0)	252 (22.0)
11	8.03 (0.14)	8.04 (0.10)	8.49 (0.11)	8.39 (0.13)	489 (58.1)	497 (55.5)	542 (58.0)	601 (32.5)
9	8.30 (0.03)	8.42 (0.07)	8.42 (0.01)	8.55 (0.09)	207 (0.5)	194 (1.3)	208 (9.5)	209 (11.0)
6	7.99 (0.14)	8.01 (0.15)	8.12 (0.23)	8.38 (0.16)	251 (2.0)	231 (8.9)	238 (12.5)	259 (8.0)
1	7.85 (0.14)	7.88 (0.12)	7.87 (0.32)	7.87 (0.36)	231 (13.9)	229 (13.4)	246 (19.5)	257 (18.5)
5	8.00 (0.13)	7.92 (0.18)	8.47 (0.06)	8.39 (0.09)	235 (7.2)	234 (6.3)	248 (12.5)	258 (8.5)
8	7.99 (0.06)	7.92 (0.09)	8.22 (0.14)	8.17 (0.15)	266 (7.2)	280 (15.2)	293 (7.0)	302 (3.5)

Overall the streams were uniform with low background values of nitrogen and phosphorus (Table 4). Nitrogen and phosphorus were not significantly correlated with temperature (P>0.05). Silicate was correlated with temperature when an outlier (stream 11) was removed from the analysis (r = 0.96, t = 7.52, P = 0.0007). Conductivity and silicate were positively correlated (r = 0.76, t = 2.89, P = 0.03). The discharge was positively correlated with temperature (r = 0.82, t = 3.50, p = 0.01).

Table 4. The discharge and concentrations of N$_{tot}$, P$_{tot}$ and silica in the eight streams studied. The discharge values are based on ten replicate measurements on 11 May 2007, showing the mean and standard errors. The nutrient concentration measurements are based on data from 24 August 2006 in the control reaches of all eight streams. Streams are in order of ascending mean temperature.

Stream no.	Discharge (Ls^{-1})	Total Nitrogen (mgL^{-1})	Total phosphorus (mgL^{-1})	SiO$_3$ –Si (mgL^{-1})
7	0.29 (0.18)	< 0.1	0.021	6.4
14	0.99 (0.29)	< 0.1	0.008	8.7
11	0.43 (0.17)	0.1	0.016	18.7
9	0.04 (0.03)	< 0.1	0.027	9.0
6	0.94 (0.42)	0.19	0.026	14.8
1	1.77 (0.72)	< 0.1	0.022	15.4
5	2.20 (1.03)	0.19	0.023	15.0
8	3.54 (1.42)	< 0.1	0.029	16.1

Adding nutrients to the streams (treatments) gave a consistent pattern of concentrations of NH$_4$–N, typically two to seven times greater than the background value (controls) (**Papers 3** and **4**). Conductivity and pH did not change after nutrient enrichment (**Papers 3** and **4**) (ANOVA, P>0.05).

3.2 Paper 1

This paper aimed to examine the temperature effects on the biomass of bryophytes, chlorophyll *a*, biovolumes of dominant algae groups and Cyanobacteria (species assemblages of diatoms were not included in this paper). Invertebrate assemblages in relation to the primary producers were also examined.

Temperature and discharge explained the variance of the first RCCA dimension, sand and gravel explained the variance for the second dimension and conductivity explained the variance of the third dimension (Fig. 8 a, b).
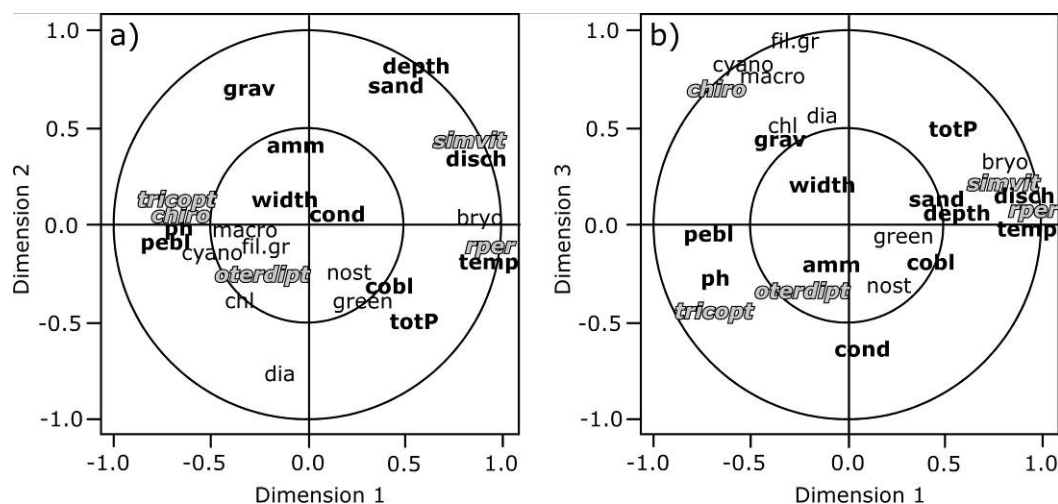
Fig. 8 a–b. A RCCA plot based on Regularized Canonical Correlation Analysis (RCCA) showing the similarities of primary producer assemblages and their relationship with variation in environmental factors: (a) dimensions 1 and 2, (b) dimensions 1 and 3. Environmental variables (abbreviations in bold): totP = total phosphorus, temp = temperature, cobl = cobbles, pebl = pebbles, ph = pH, grav = gravel, amm = nitrogen (ammonium), width = width of the streams, cond = conductivity, sand = sand, depth = depth and disch = discharge. Invertebrate groups also treated as environmental factors (bold italic font): simvit = Simulium vittatum, rper = Radix peregra, tricopt =*Potamophylax cingulatus*, chiro = Chironomidae, otherdipt = other dipterans. Primary producer assemblages (regular font): algal biovolume, dia = diatom biovolume, cyano = cyanobacteria biovolume, fil gr = filamentous green algae coverage (%), chl = chlorophyll *a*, macro = macrophytes (%).

The bryophyte *F. antipyretica* was strongly linked with high temperature and the first dimension (RCCA: r = 0.83) (Fig. 8 a, b). Bryophytes dominated in streams 1, 5 and 8 while epilithic algae and Cyanobacteria were dominant primary producers in streams 6, 7, 9, 11 and 14.

Chlorophyll *a* was variable between streams (ANOVA, F = 81.96, p < 0.001). Temperature did not influence the chlorophyll *a* concentration significantly. However, bryophytes were

negatively correlated with chlorophyll *a* (r = – 0.33, t = – 3.8, p = 0.0002). Chlorophyll *a* was unaffected by invertebrates and not significantly related to any dimension (Fig. 8 a, b).

Diatoms, green algae and Cyanobacteria dominated the epilithic community in the streams. Variability between the groups was high but most of the biovolume was made up by green algae. Where *F. antipyretica* was dense, observed biovolume of epilithic algae was low.

Filamentous green algae were strongly negatively linked with dimension three and thus associated with high conductivity (Fig. 8 b). The biovolume of green algae (mainly small unicellular cells or short filaments) was however not linked to any dimension (Fig. 8 a, b). Identified genera of green algae in the colder streams were *Chaetophora* sp., *Enteromorpha* sp., *Spirogyra* sp. and *Mougeotia* sp., while *Cladophora* sp. was commonest in warm streams.

The biovolume of diatoms was negatively linked (RCCA: r = – 0.75) with the second dimension, indicating that the streams with the coarser stream bed (streams 9, 6 and 8) contained the greatest biovolume of diatoms (Fig. 8 a). The highest diatom biovolume was recorded from the warmest streams. Dominant species were: *Gomphonema rhombicum*, *G. pumilium*, *G. parvulum*, *Rhoicosphenia curvata*, *Fragilaria capucina* and various *Nitzschia* species.

Cyanobacteria biovolume was positively linked with dimension three (RCCA: r = 0.8) also macrophytes (RCCA: r = 0.79) (Fig. 8 b). This indicates that some Cyanobacteria (*Oscillatoria* spp.) and macrophytes are benefitted by low conductivities; they were most frequently observed in stream 7 (cold). Dominant Cyanobacteria in the biovolume were small unicellular cyanobacteria (around 1 µm diameter), *Nostoc* spp. and *Oscillatoria* spp. *Oscillatoria* was the dominant cyanobacterium in the coldest stream. None of the biovolume groups were significantly linked to temperature (lme, p > 0.05).

Invertebrate species linked with high temperature were *Radix peregra* and *Simulium vittatum*. Chironomids were linked with low temperature. *Potamophylax cingulatus* (Stephens) was linked to high conductivity and low water temperature (Fig. 8 a, b).

The streams were separated by environmental and biological features. The warmer streams (Fig. 4 and Fig. 10 A) were positively linked with the first dimension while the colder ones (Fig. 3 and Fig. 10 B) were negatively linked with that dimension (Fig. 9 a, b). One cold stream (stream 7) was strongly positively linked with the third dimension while two other cold streams (streams 11 and 14) were negatively linked with the dimension which was explained by conductivity (Fig. 9 b).
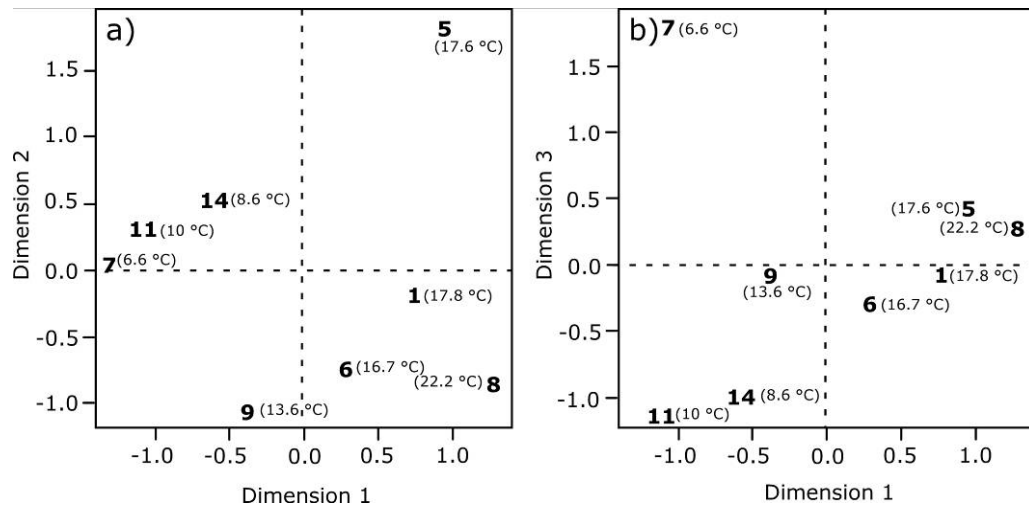
Fig. 9a–b. Streams ordinated by the Regularized Canonical Correlation Analysis (RCCA) on the basis primary producers and environmental factors. Plot a) represents dimension 1 and 2 and b) represents dimensions 1 and 3. The average August (2006) temperatures for each stream are given in brackets close to each stream number.
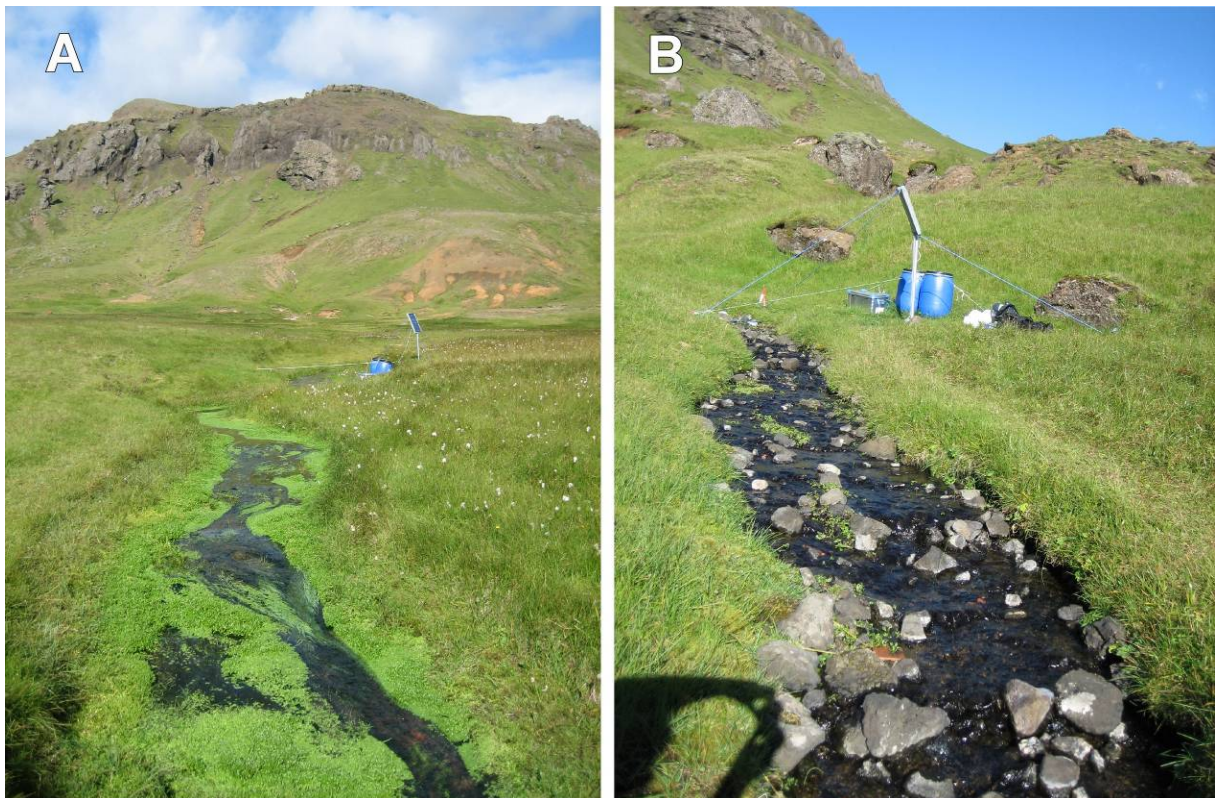
Fig. 10 A–B. Figure A shows one of the warmer streams (stream 1) with cover of *F. antipyretica*, filamentous green algae and *Callitriche stagnalis* (Scop.). Figure B shows one of the colder streams (stream 7) with little vegetation in the stream bed.

3.3 Paper 2

The purpose of this study was to compare patterns in stream primary producers and invertebrates with patterns in primary producers and herbivores in terrestrial habitats to determine if there were common structural features that might be used in model studies.
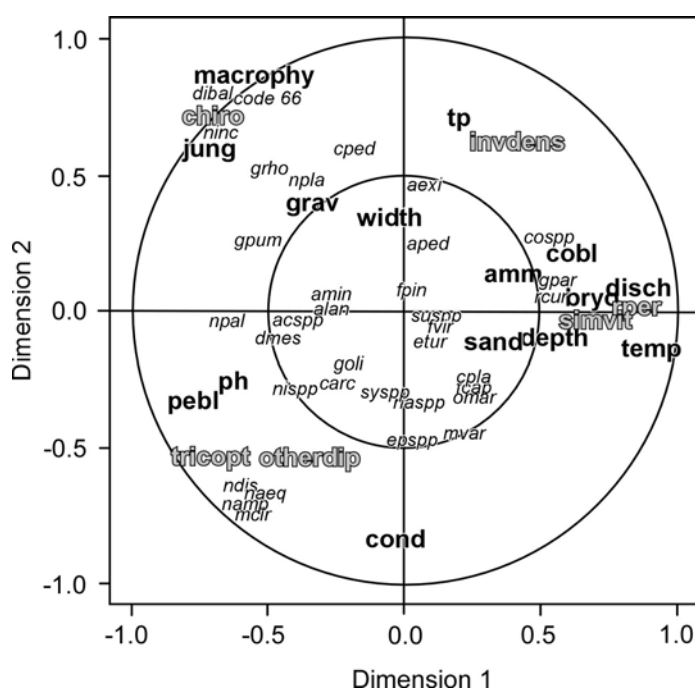
Fig. 11. Results of a Regularized Canonical Correlation Analysis (RCCA) showing the similarities of diatom species (regular letters) and their relationship with environmental factors (bold letters) and dominant macroinvertebrates (large, gray and bold letters). Only species that had over 0.5% abundance in some of the eight streams were included and species that were only found in only one stream were not included. A full list of diatom species is given in Appendix 1 of Paper 2. Abbreviations for environmental features are: jung = *Jungermannia exsertifolia*, macrophy = macrophytes, sand=sand, Cobl= cobbles, depth=depth, width=width, ph=pH grav = gravel, pebl = pebbles, cond= conductivity, bryo = *Fontinalis antipyretica*, tp = total phosphorus, amm= ammonium, temp = temperature, disch = discharge. Abbreviations for invertebrates are: invdens= invertebrate density, tricopt = *Potamophylax cingulatus* (caddisfly), otherdip = other dipteral groups, chiro = chironomids, simvit = *Simulium vittatum* and rper= *Radix peregra*.

Thirty taxa of invertebrates were observed, most of them chironomids. Chironomids were strongly negatively linked with dimension one and therefore negatively linked with temperature. Chironomids were positively linked with dimension two and therefore positively influenced by low conductivity. *Radix peregra* and *Simulium vittatum* were strongly positively linked with the first dimension and high temperature (Fig. 11). The

greatest density of invertebrates was found to be in the warmer streams, though statistical tests revealed that there was no significant relationship between temperature and invertebrate densities.

Vascular macrophytes were very rare but when they were observed it was usually from the coldest stream, though other streams had some vascular macrophytes as well. Therefore vascular macrophytes were positively linked to the second dimension and therefore linked with low conductivity. *Fontinalis antipyretica* was strongly linked with dimension one and high temperature. The liverwort *Jungermannia exsertifolia* was negatively correlated with dimension one and temperature but was positively linked with dimension two and therefore benefitted by low conductivity (Fig. 11).

Sixty–four diatom taxa (Fig. 12 A, B) were identified, most of them to a species level. After the removal of rare species (contributing less than 0.5% of the total abundance) and species that were only observed in one stream, the RCCA separated the diatoms into three main groups. The first group was linked to low water temperature and low conductivity, the second was linked to low water temperature and high conductivity. The last group was observed in the warmest streams and was associated with high discharge (Fig. 11).
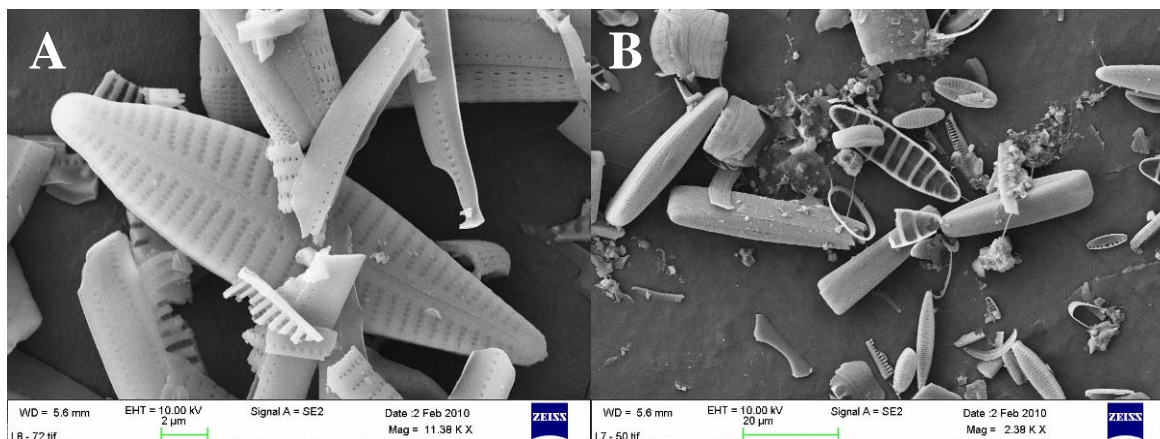
Fig. 12 A–B. Photos from Scanning Electronic Microscope (SEM). Figure A shows commonly observed diatoms from the warmer streams (e.g. *Gomphonema parvulum* (Kütz.) and *Rhoicosphenia curvata* (Kütz.)) whilst Figure B shows common diatoms from a cold stream (e.g. *Gomphonema rhombicum* (Fricke), *Nitzschia inconspicua* (Grunow) and *Diatoma mesodon* Ehrenb.(Kütz)).

Cluster analysis revealed similar patterns as the RCCA did. The major distinctions were based on difference of temperature. Overall three large groups were observed. These groups are the same as those revealed by the RCCA. One group contained high temperature species such as *Gomphonema parvulum, Rhoicosphenia curvata* and *Synedra ulna*. Group number two contained species that were commonly observed in streams with low conductivity and low water temperature. Group two contained the greatest number of species including: *Gomphonema rhombicum* and *Diatoma mesodon* (Fig. 12 B). The third group contained diatoms observed in low conductivity and low water temperature. Species in that group were among others: *Meridion circulare* and various *Navicula* species.

The RCCA analysis showed a syndrome of linked environmental factors consistently affecting the streams. Temperature, depth, flow, bryophytes and invertebrate assemblages were all linked. The aim of this paper was to explore possible similarities between streams varying in temperature and grazing regime and different terrestrial habitats that are influenced by temperature and grazers as well. Shannon´s diversity, Sørensen´s diversity, evenness, species richness and growth form diversity were not significantly affected by temperature though growth forms were more varied in the colder streams with more species as well. Overall, the analogy with terrestrial habitats did not hold though some similarities were observed. The warmer streams that were suggested to be similar to boreal forests had lower biodiversity and lower growth form diversity than the colder streams that were considered to be parallels to tundra. The grazers found in tundra (reindeer mainly) can be very effective grazers like *R. peregra* in the warm streams. Generally boreal forests are diverse compared with tundra. The warm streams resembled tundra regarding the herbivores and diversity of diatoms while the colder streams resembled boreal forests with low grazing pressure and higher diversity. Grazers did not supress the abundance of diatoms in the warmer streams though it was likely that they had influences on what kind of growth forms were observed (low growth form diversity where the grazing pressure was heavy). Higher temperature favoured smaller diatoms.

3.4 Paper 3

The purpose of the paper was to investigate the influences from nutrient enrichment and temperature on various primary producers (diatom species assemblages not included).

Several primary producers were linked to warm streams, including *Fontinalis antipyretica*, *Alopecurus pratensis* (L.), *Equisetum fluviatile* (L.) and *Callitriche stagnalis*. However, *Jungermannia exsertifolia*, *Chaetophora* sp and *Epilobium alsinifolium* (Vill.) were restricted to colder streams.

Interacting influences from temperature and nutrient enrichment resulted in increased biomass of *F. antipyretica*. Vascular plants and *J. exsertifolia* were unaffected by nutrient enrichment while *Nostoc* spp. decreased when nutrients were added. Filamentous green algal populations (mainly *Cladophora* sp.) became denser (Fig. 13 A) when nutrients were added. Chlorophyll *a* increased significantly when nutrients were added. No temperature influences were detected on the chlorophyll *a* concentration.

Most diatom species were linked to the coldest stream (stream 7). Nutrient enrichment had significant positive influences on the biovolume of diatoms (Fig. 13 B). Dominant species were *Gomphonema rhombicum, G. pumilium* (Grunow), *Meridion circulare* Greville (C. Agardh)*, Achnanthes minutissima* (Kütz.)*, Nitzschia palea* (Kütz.), *N. inconspicua* and *Rhoicosphenia curvata.*

Green algae were diverse in the streams. Nutrient influences on the biovolume of green algae were stronger in 2007 compared with 2006. The green algal biovolume was significantly higher in treated reaches compared with control reaches in May and June 2007. Dominant green algal taxa were: *Cladophora* spp. (Kütz.), *Spirogyra* spp. Link in Nees., *Mougeotia* spp. (Agardh.), *Chaetophora* spp. (Schrank) and *Enteromorpha* spp. Link in Nees, 1820.

Cyanobacteria biovolume did not respond as strongly to nutrient enrichment as the green algae and diatom biovolumes. However, large thalloid Cyanobacteria (*Nostoc* spp.) decreased their abundance when nutrients were added in the warmer streams. Dominant taxa of Cyanobacteria were: *Nostoc* spp., *Oscillatoria* spp. and unicellular small cells (around 1 µm diameter).

Fig. 13 A–B. A: Dense mats of filamentous green algae in one of the colder streams (stream 14) after four weeks of nutrient enrichment (photo taken 30th of May 2006). B: tiles to left were placed in control reach of one of the colder streams (stream 7) but the tiles to right were taken from treatment reach of the same stream. Notice the difference in colour of the biofilm on the tiles. Photos: Rakel Guðmundsdóttir.

3.5 Paper 4

The purpose of this study was to detect possible changes from nitrogen enrichment and invertebrate population density on the diatom assemblages in eight mountain streams. Various approaches were used, including diversity indices (Shannon´s index and Sørensen´s index) and the Trophic Diatom Index (TDI).

The population density of invertebrates was not significantly higher in treated reaches compared with control reaches and the density ranged from 7000–7500 individuals per m^2. However, the diatom population density was significantly higher in treated reaches compared with control reaches.

Significant changes in the dominant groups of diatoms were detected (Fig. 14 A). The densities of *Achnanthes* spp. (mainly *A. minutissima*) and *Gomphonema* spp. (mainly *G. pumilium* and *G. rhombicum*) (Fig. 14 B) were significantly greater in treated reaches compared with control reaches. The densities of *Nitzschia* spp. and *Fragilaria capucina* (Desm.) were negatively affected by nutrients. Overall, rarer diatoms were lesser contributors to the diatom communities in treated reaches compared with control reaches. Growth forms were altered by the nutrient enrichment. Mucilage stalk (MS) diatoms (i.e. *Gomphonema* spp.) were rarer in treated reaches compared with control reaches. Mucilage pad (MP) diatoms (i.e. *Achnanthes* spp.) and mucilage tube (MT) diatoms (i.e. *Cymbella* spp.) were more abundant in the treated reaches compared with control reaches.
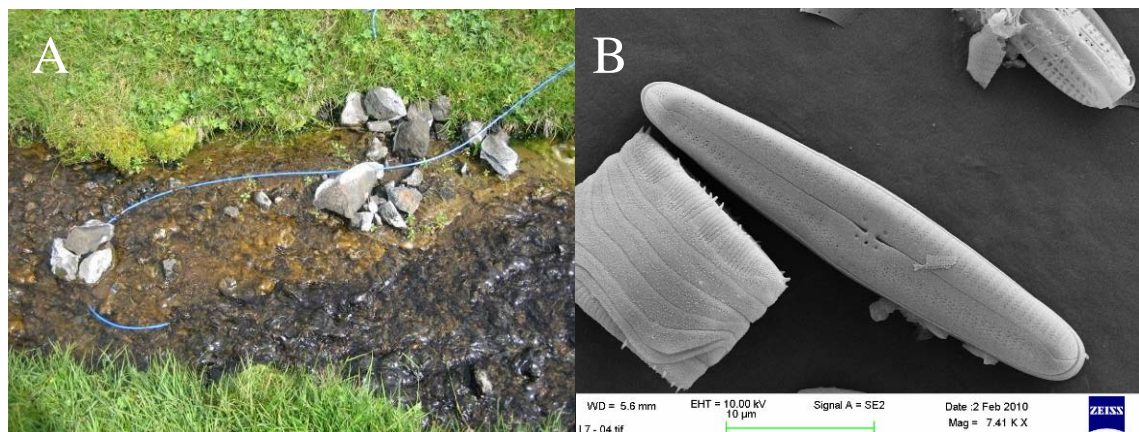
Fig. 14 A–B. A: Nutrient dripping into the lower reaches of a cold stream (stream 7). Notice the dramatic changes on the colour of the biofilm. B: Two species of diatoms (*Gomphonema rhombicum* and *Diatoma mesodon*) that became very abundant with nutrient enrichment mainly in stream 7 and thus perhaps responsible for the changed colour of the biofilm of Fig. A. Photos: Rakel Guðmundsdóttir.

Species diversity (Shannon´s index) of diatoms was significantly lower in treated reaches compared with control reaches. Species overlap (Sørensen´s index) was not affected by the nutrient enrichment. The Trophic Diatom Index (TDI) was typically around 45–47 in all reaches and not affected by nutrient enrichment.

4. Discussion

4.1 Temperature and community composition

Diatoms, green algae and cyanobacteria are usually the most common primary producers in small streams (Allan 2006). Different environmental conditions influence the relative abundances of these groups in different streams.

The present study indicates that stream temperature was the key influential variable on the algal and cyanobacterial assemblages along with the invertebrate assemblages. Both macro– and microvegetation in the streams were found to change along a temperature gradient. The microvegetation change was in the form of less epilithic algae measured by Chlorophyll *a* where the bryophytes were densest. Macrovegetation change was observed as increased cover of the bryophyte *F. antipyretica* as the stream temperature increased, indicating the succession state of the warmer streams was further along than the succession stage of the colder streams (Stream Bryophyte Group 1999). The warmer streams were never observed to be snow covered during the winters. However, the colder streams were snow covered for 4–5 months in 2006 and 2007. Snow cover disturbs the succession of primary producers in streams. It was concluded that the bryophyte *F. antipyretica* was the key primary producer in the warmer streams while epilithic algae and Cyanobacteria were most important primary producers in the coldest streams (**Papers 1** and **2**).

Diatoms have been found to be the dominant primary producers in cold Arctic streams and rivers (Slavik et al. 2004) while green algae may be more dominant in streams at lower latitudes (Biggs et al. 1998). Cyanobacteria are dominant primary producers in geothermally influenced streams in Yellowstone in the USA, New Zealand and Iceland with temperatures from 50–75 °C (Mason 1939; Tuxen 1944; Brock and Brock 1966; Winterbourn 1969). The biovolume of the Cyanobacteria genus *Oscillatoria* spp. was highest in the coldest stream (**Paper 1**) but some species of that genus have been linked with temperatures around 50–68 °C (Winterbourn 1969). The biovolume and cover of *Nostoc* spp. was greatest in the mid temperature ranges (12.8–16.9°) (**Paper 1**). None of the biovolume groups (green algae, Cyanobacteria and diatoms) were significantly linked to temperature (**Paper 1**). The reason for the lack of a relationship between green algae, diatoms, and Cyanobacteria with temperature is not clear but one reason might be competition between these groups and/or competition with the dominant primary producer in the warmer streams, *F. antipyretica*.

A global air temperature increase of 2–4 °C may possibly result in changes in precipitation (increase or decrease depending on latitude), decrease or even disappearance of glaciers, increased release of methane in Arctic areas, sea level rise and changes in distribution of species. Already, there are recorded changes in Arctic and boreal ecosystems including increased precipitation, movement of the treeline to higher altitudes, increased density of various primary producers, changes on the abundance of some grazing animals (e.g. deer),

longer growing season, and disappearance of some species and occurrence of others that were not present before (Moss 2010).

According to the results in this study it is likely that when temperature keeps on rising in the sub–Arctic, the primary producer assemblages in streams will shift dramatically from dominance of diverse epilithic algae communities to more uniform bryophyte dominant communities and thus resemble streams that are currently found in lower latitudes. The epilithic assemblages of the streams were not dominated by diatoms as expected from studies elsewhere. Diatoms were rather equally distributed between the streams of different temperatures. Some diatom species have been observed in warm geothermal pools and streams in New Zealand (Winterbourn 1969). Green algae and cyanobacteria were dominant groups in the biovolume measurements in all streams. The warm streams with the dense bryophyte cover resemble streams at lower latitudes (Stream Bryophyte Group 1999) while the colder streams with rich epilithic algae communities resemble more sub–Arctic streams (Allan 2006) but that can easily change with increasing temperatures.

If temperature increases of 10 °C from present, which is not unlikely within the next century (AICA 2004; Heino et al. 2009), changes on the primary producer assemblages can be very dramatic. These changes can be in the form of loss of species and gain of new ones by shifts towards the poles (Parmesan and Yohe 2003). Greater temperature increase might change the epilithic algal communities to dense cover of bryophytes. The invertebrate assemblages can also change with increased temperatures from Chironomidae dominance to dominance of *S. vittatum* and *R. peregra*. The impacts on primary producers may be in the form of alteration on the importance of different invertebrate functional feeding groups.

4.2 Effects of nutrients

Nutrient enrichment in ecosystems is a problem in at present. As climate warms, land use changes and population density of humans increases in sub–Arctic regions, nutrient enrichment is predicted to increase significantly (ACIA 2004; IPCC 2007; Heino et al. 2009).

In this study, chlorophyll *a*, diatom biovolume and green algal biovolumes increased significantly in the streams when nutrients were added. This is consistent with results indicating increased biomass of primary producers in various ecosystems when nutrient levels increase (Krebs 2001). Nutrient enrichment and high temperature had significant positive influences on the biomass of *F. antipyretica* but no influences on *J. exsertifolia*. Bryophytes have the ability to utilize nutrients in very low concentration (Stream Bryophyte Group 1999) leaving them as strong competitors with other macrophytes and epilithic algae and cyanobacteria. A study by Slavik et al. (2004) on phosphorus enrichment in the Kuparuk River in Alaska revealed increased biomass of bryophytes after seven years of nutrient enrichment. Bryophyte dominance in streams and rivers is considered to be late stage of succession (Stream Bryophyte Group 1999) (**Paper 3**). According to the results in **Paper 3** the combined influences of nutrient enrichment and high temperature lead to significant increase of bryophytes after only 1–2 summers of experiments.

High temperature and nutrient enrichment tends to have positive influences on the growth of green algae in streams and lakes (Lapointe and O'Connell 1989; Biggs 2000). That is consistent with the results in **Paper 3** when the biomass of filamentous green algae (mainly *Cladophora* spp.) and the biovolume of green algae increased significantly with nutrient enrichment and the influences were more dramatic in the warmer streams compared with the colder ones. Green algae and Cyanobacteria are usually most abundant in the summer when the temperatures of streams are highest (Biggs 2000; Moss 2010).

Eutrophication associated with climate change may come from increased livestock grazing and cultivation in Arctic regions (ACIA 2004) as well as from increased nitrogen release from increased burning of fossil fuels. Influences on secondary producers may also be dramatic as their food changes and their temperature tolerance may not be as high as the water temperature increases (**Paper 3**).

4.3 Combined effects of climate change and eutrophication

Based on the results in **Paper 3** it is very likely that eutrophication and a temperature rise of as little as 2 °C will change the communities of primary producers very dramatically. The epilithic community may change from diverse groups of algae and cyanobacteria to dominance of few groups (possibly green algae and/or diatoms). Heino et al. 2009 documented that loss of species diversity in freshwater ecosystems is already occurring. With temperature increase of around 6 °C and eutrophication it is likely that nitrogen fixating cyanobacteria (*Nostoc* spp.) will be replaced by filamentous green algae, diatoms and possible other cyanobacteria groups. The influences may even be more dramatic and lead to the dominance of bryophytes such as *F. antipyretica* whilst the epilithon retreats. *J. exsertifolia* may also disappear from streams if the temperature rises (**Paper 3**).

Terrestrial species diversity is predicted to decrease around 15–37 % in the next 50 years (Thomas et al. 2004). Species diversity in freshwaters has declined drastically since 1970 and is predicted to decline in a similar manner as the predictions for terrestrial habitats indicate (Xenopoulous et al. 2005, Hering et al. 2010). The results presented in **Paper 4** show that the diatom diversity estimated with Shannon´s index decreased significantly with nutrient enrichment. Rarer diatom species (under 1% abundance of the total) were around 40% of the relative abundances in the control reaches while they were 15–18 % in the nutrient enriched sites. Nutrients allow the strong competitors to succeed better than those rare diatoms leading to uniform communities (**Paper 4**).

Changes on the primary producer's assemblages may alter the secondary producer's assemblages because different functional feeding groups of invertebrates are linked with differences in food (algae and Cyanobacteria) assemblages (i.e. particle size) (Allan 2006). The present study show that the abundances of invertebrates did not change with nitrogen enrichment. A difference might have been detected if the experiments had been conducted over a longer period. But the lack of difference of invertebrates between treated and control reaches may indicate that food is not limiting for invertebrates in either control and treatment reaches (**Paper 4**).

4.4 Use of indicator indices

Growth forms were altered by nutrient enrichment. Diatoms attached by mucilage pads (i.e. *Achnanthes* spp.) were favoured by nutrient enrichment while motile diatoms (*Nitzschia* spp. and *Navicula* spp.) were reduced with nutrient enrichment. Motile diatoms are usually considered to be linked with high concentration of nutrients (Kelly 2003; Kelly et al. 2008 a; Kelly et al. 2009) in contrast to these results. It is not clear why there was not a relationship between the motile diatoms and nutrient enrichment in this study but one possible explanation may be that other diatoms such as *G. rhombicum* and *G. pumilium* that were abundant in nutrient enriched sites can have faster growth rates and outcompete the motile diatoms. The size of diatoms was not affected by nutrient enrichment (**Paper 4**).

The Trophic Diatom Index (TDI) in this study (**Paper 4**) was not influenced by nutrients against what was expected. Several studies by Kelly et al. (1995, 1998, 2008 a, b, 2009) have shown that phosphorus pollution influences the TDI. Like the TDI most indices are developed for phosphorus enrichment rather than nitrogen enrichment. They are also based on a correlation between phosphorus and the indices but not experimental work such as in this study. Studies have suggested that it is necessary to calibrate the European indices to regional conditions (Fore and Grafe 2002; Potapova and Charles 2007). An African study by Bellinger et al. (2006) showed an opposite pattern to that found here. They concluded that the TDI was suitable for African streams. The classical indices such as Shannon´s and Simpson–Yule were considered to be inappropriate because they were unsuccessful to show changes in the diatom assemblages. The study by Bellinger et al. (2006) was, as most of the European studies, based on correlation between indices and phosphorus. They concluded that it was important to conduct experiments to understand the influences from nutrient enrichment on diatoms (**Paper 4**).

Eutrophication will be a problem in the sub–Arctic in the near future (ACIA 2004). Eutrophication can be due to increased agriculture, and higher temperature leads to greater decomposition, more settlement and car use and various other factors. Diverse diatom communities will be replaced with simpler communities and thus simpler growth form diversity. Invertebrate assemblages may change also if food is sparse, but the change will likely be later than the change of primary producer communities (**Paper 4**).

The observed differences in primary producer assemblages and invertebrate assemblages allowed a comparison between the streams and terrestrial habitats under different temperature and grazing regimes in **Paper 2**. Dominating grazers in the warm streams were *R. peregra*, a scraping gastropod, while chironomids were dominant in the colder streams.
The results show that temperature did not influence diversity, species richness, evenness or growth form diversity of diatoms significantly though most growth forms and species were observed in the coldest stream. Habitats at lower latitudes generally contain greater diversity than habitats at high latitudes (Krebs 2001). However, studies on the influences of increased air temperature on the diversity of plants have indicated potential loss of diversity even at moderate temperature increase (Thuiller et al. 2005).
Temperature affected the growth forms in various ways. Motile diatoms (*Nitzschia* spp. and *Navicula* spp.) were most common in the coldest stream. Motile diatoms have often been linked to phosphorus enriched streams (Pringle 1990; Kelly 2003) but not directly to a specific temperature regime (**Paper 2**). Smaller sized diatom taxa were favoured by

higher temperature. That finding supports the meta–analysis by Daufresne et al. (2009) which showed that ectothermic species were smaller in warmer climates and larger in cooler climates. It is more beneficial for organisms to have large surface to volume ratio in warmer climates and less surface to volume ratio in colder climates (Freckleton et al. 2003) (**Paper 2**).

4.5 Comparisons with terrestrial habitats

The analogy with terrestrial habitats did not hold. The diversity of diatoms was greater in the cold streams than in the warm streams though not significantly. Tundra habitats were supposed to be analogous to the cold streams with low diversity and high grazing pressure whilst boreal forests were supposed to be analogous to warmer streams with overstory of bryophytes resembling tall trees and lack of large herbivores. The results indicated that the cold streams resembled the boreal forest rather than tundra because of higher growth form diversity and low grazing pressure while the warm streams resembled tundra more due to low growth form diversity and grazing pressure from *R. peregra*. *R. peregra* did not suppress the diatom density though it appeared to shape the community regarding the growth forms (low growth form diversity). Thus, the streams were considered to be bottom up controlled rather than top down (**Paper 2**).

Bryophytes were densest where the grazing pressure was assumed to be greatest, unlike what was expected, because in terrestrial habitats, seedlings are sensitive towards grazing and grazing prevents the potential for the seedlings to become tall trees (analogous to overstory bryophytes) resulting in domination of grassland (analogous to epilithic algae). Overall, the results indicate that using microorganism communities as surrogates for large systems should be not be uncritically accepted (**Paper 2**).

5. Conclusions

The main findings in this thesis are:

- Bryophytes (*F. antipyretica*) were the most abundant primary producer in the warm streams while epilithic algae were dominant primary producers in the colder streams (**Paper 1**).
- Green algae and Cyanobacteria had the highest biovolumes in all streams while diatoms usually had the lowest biovolume (**Paper 1**)
- Temperature was found to be the most influential factor on the community structure of primary producers (**Papers 1, 2** and **3**).
- The streams were considered to be bottom up controlled because the lack of a relationship between grazers and the epilithic primary producers (**Paper 2**).
- Grazing pressure influenced the structure of the diatom communities with lower growth form diversity where the heaviest grazing pressure occurred (warm streams containing *R. peregra*) (**Paper 2**).
- Temperature favoured small sized diatom taxa (**Paper 2**).
- Nutrient enrichment and temperature had significant positive influences on the biomass of *F. antipyretica* but negative influences on cyanobacteria (*Nostoc* sp.) (**Paper 3**).
- The biomass (dry weight) and the biovolume of green algae increased significantly with nutrient enrichment and the influences were temperature related, indicating that nutrient enrichment and high water temperature increase green algae growth (**Paper 3**).
- Diatom diversity estimated with Shannon´s diversity was significantly negatively influenced by nutrients but diversity estimated with Sørensen´s index was unaffected by nutrient enrichment (**Paper 4**).
- The motile genera *Nitzschia* spp. and *Navicula* spp. were negatively influenced by nutrients opposite to many other studies (Kelly 2003) (**Paper 4**).
- Growth forms (attachment modes) were fewer in nutrient enriched sites compared with the control reaches (**Paper 4**).
- The Trophic Diatom Index (TDI) was unaffected by nutrients and the values were low for both control and treatment reaches (**Paper 4**).

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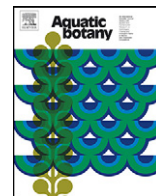
Effects of temperature regime on primary producers in Icelandic geothermal streams

Rakel Gudmundsdottir [a,*], Gisli Mar Gislason [a], Snaebjorn Palsson [a], Jon S. Olafsson [b], Anders Schomacker [c], Nikolai Friberg [e], Guy Woodward [d], Elisabet R. Hannesdottir [a], Brian Moss [f]

[a] Institute of Biology, University of Iceland, Sturlugata 7, IS-101 Reykjavík, Iceland
[b] Institute of Freshwater Fisheries, Keldnaholt, IS-112 Reykjavík, Iceland
[c] Department of Geology, Norwegian University of Science and Technology, Sem Saelands Veg 1, N-7491 Trondheim, Norway
[d] School of Biological and Chemical Sciences, Queen Mary University of London, London E1 4NS, UK
[e] Department of Freshwater Ecology, National Environmental Research Institute, University of Aarhus, Silkeborg, Denmark
[f] School of Environmental Sciences, Nicholson Building, University of Liverpool, Liverpool L69 3GS, UK

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ABSTRACT

Eight adjacent sub-arctic streams with consistently different temperatures but broadly similar chemistry, other than some differences in conductivity and linked pH, were used to investigate effects of temperature regime on the structure of primary producer communities. Grazing by invertebrates was also taken into account to detect possible effects on the primary producers.

The moss species *Fontinalis antipyretica* Hedw. (Bryophyta) was only observed in the warmer streams and its cover was positively linked with temperature. The liverwort *Jungermannia exsertifolia* (Steph.) was absent from the warmer streams and scarce in the colder streams. The biomass of *F. antipyretica* was inversely correlated with concentration of epilithic chlorophyll *a*, possibly due to shading from the bryophyte and/or competition between algae and bryophytes for nutrients. Epilithic algae and bryophyte biomass were not related to density of the main grazer, *Radix peregra* Müller (Gastropoda).

A high biovolume of green algae (Chlorophyta) occurred in all streams. The biovolume Cyanobacteria was greatest in the coldest stream and in the two intermediately warm streams. Diatom biovolume was low compared with the biovolumes of Cyanobacteria and green algae in all streams, except in the two warmest streams, where the highest biovolume of diatom occurred.

Regularized Canonical Correlation Analysis (RCCA) classification based on all primary producer assemblages and invertebrates indicated that warm streams (16.7–22.2 °C) were similar to each other. It also showed that the colder streams (6.6–13.2 °C) were different from the warmer streams and from each other because of differences in conductivity. Temperature and conductivity were the most influential variables in determining overall stream diversity.

1. Introduction

Temperature influences most physiological activities in living organisms, making it one of the most important factors influencing primary and secondary producers in freshwater habitats (Vermaat and Hootsmans, 1994; DeNicola, 1996). Several approaches to predicting the detailed influences of temperature on freshwater ecosystems have been used, including correlation with meteorological variables at different latitudes, space-for-time substitution in predicting possible future changes as climates warm, palaeoecological reconstruction over past periods of climate change, and direct experimentation (for example, Rouse et al., 1997; McKee et al., 2003; Parmesan and Yohe, 2003; Stewart et al., 2005; Forbes and Lamoureux, 2005; Kernan et al., 2010; Woodward et al., 2010b).

Experiments in mesocosms to predict temperature changes in standing waters have been very informative (Moss et al., 2003; McKee et al., 2003; Stephen et al., 2004; Feuchtmayr et al., 2007; Jeppesen et al., 2007; Van Doorslaer et al., 2007; Ventura et al., 2008; Yvon-Durocher et al., 2010a,b, 2011) but owing to the nature of running waters (short retention time and high heating costs) there have been few comparable controlled field experiments in such ecosystems (Hogg and Williams, 1996).

There are, however, correlative and laboratory studies. Some work on the effects of temperature on diatoms suggests that temperature increases diversity and species richness in rivers (Vinson and Rushforth, 1989), but Gudmundsdottir et al. (in press)

demonstrate an opposite effect. Biomass in general might be higher in warmer streams because higher temperatures increase growth and metabolic rates (Allan, 2006; Demars et al., 2011). Similarly, the decomposition rate of organic matter is higher in warmer water, potentially leading to higher nutrient turnover for primary producers (Friberg et al., 2009; Demars et al., 2011).

Vermaat and Hootsmans (1994) conducted a laboratory factorial experiment on the effects of temperature (10, 15 and 20 °C), irradiance and nutrient conditions on periphyton accumulation on glass slides and found temperature to be the key factor influencing the structure of the periphyton. The green algae *Scenedesmus* was dominant at the highest temperature while the diatom genus *Navicula* dominated at lower temperatures and lower irradiances. At the community level, temperature can change the dominance of major algal classes, species composition and diversity, and at the ecosystem level, it can influence the maximum areal primary production (DeNicola, 1996). Usually, diatoms are the most abundant group during the winter and spring, but during summer, other groups, such as green algae and Cyanobacteria, can be more abundant (Patrick et al., 1969; Vermaat and Hootsmans, 1994; Allan, 2006; Biggs, 2000). Cyanobacteria thrive under moderate and high temperature conditions and they can outcompete diatoms under high temperature conditions. Green algae can also tolerate high temperature (Vermaat and Hootsmans, 1994) though not as high as Cyanobacteria and can also outcompete diatoms (Patrick et al., 1969; Allan, 2006). In very hot geothermal streams, diatoms are not as important primary producers as Cyanobacteria, but they are important in cold Arctic and sub-Arctic streams (Tuxen, 1944; Biggs, 1996; Komulaynen, 2009). The thermal growth optima for most diatoms are lower than those of the Cyanobacteria (Patrick et al., 1969).

Other environmental factors than temperature may also, of course, influence the community structure. Boulders can be important for bryophyte attachment, while epilithic algae and Cyanobacteria can thrive on finer substrates (Allan, 2006). Time may also be important. Bryophytes are often considered to be a final succession stage (Slavik et al., 2004), while epilithic diatoms quickly colonize habitats that have been snow-covered or frozen during the winter (Roberts et al., 2004). Macrophytes and many species of bryophytes need longer growing seasons than epilithic algae and are thus not so well adapted to long winters and snow cover (Sand-Jensen et al., 1989). In general, field-based studies are confounded by many simultaneously acting factors, and experimental studies by considerable simplification of the conditions and there is a need to bridge them with field studies in naturally steadier environments.

The present work took advantage of a set of small, comparable, adjacent streams in Iceland, warmed to different temperatures by underlying volcanic rock. The streams were used to test predictions about the influences of temperature on the entire primary producer community, not just the algal communities of many other studies. The primary producers included bryophytes, vascular plants eukaryotic groups of algae, and Cyanobacteria. The majority of studies that are conducted on the effects of specific environmental factors such as temperature are potentially confounded by latitudinal (or altitudinal) gradients, independent of temperature. This makes it impossible to distinguish the influences of biogeography from environmental features of interest (e.g. O'Brien and Wehr, 2010). Biogeography is not considered a problem in our study because the streams are within a confined area (Fig. 1). Woodward et al. (2010a) also found food webs in these streams to be simple. Simple food webs help to minimize confounding factors and make this an appropriate system for isolating the major effects of temperature.

Our underlying intention was to determine differences in primary producer assemblages that might mainly be related
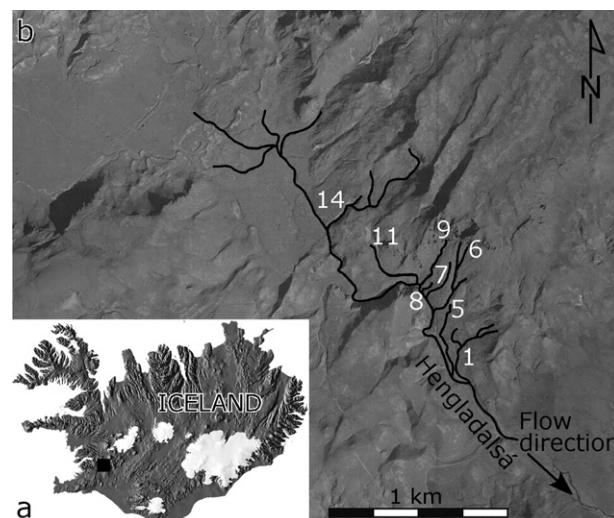
Fig. 1. (a) Map of Iceland. The black square shows the location of the study area. (b) The eight selected streams within Hengill geothermal area. Each stream number is annotated on the map.

to temperature but with attention also to other potentially influential conditions such as substrate structure, water chemistry and grazing. We tested three predictions based on the review above: (1) that there would be a shift from epilithic algae in cold streams towards macrophytes and bryophytes in warm streams; (2) that at higher water temperature, the epilithic algal communities would change from diatoms to Cyanobacteria; and (3) that macroinvertebrates would significantly influence the structure of primary producer communities. We expected to find lower biomass of all epilithic algal groups in the streams that contained *Radix peregra* (Müller) (warm streams) compared with streams that were dominated by Chironomidae larvae (cold streams). *R. peregra* is a gastropod scraper, and similar species elsewhere can have strong influences on epilithic algae. Chironomidae may also be grazers but with less efficiency than snails, though their grazing can be considerable (Steinman, 1996). Chironomidae are generally collectors and gatherers, thus grazing the loosest and largest filaments of the algal/Cyanobacteria assemblages (Mason and Bryant, 1975; Steinman, 1996).

2. Methods

2.1. Study area

The study was carried out in the Hengill geothermal area 30 km east of Reykjavík (N64°03′; W21°18′; 350–420 m a.s.l.) in Iceland (Fig. 1). The area has a flat valley floor and slopes, with hyaloclastite rocks. Several groundwater-fed streams, with different temperatures, owing to direct and indirect geothermal influence, drain into the River Hengladalsá. The warm streams used in the present study are not directly fed by hot springs, but absorb heat from the ground below (Arnason et al., 1967). Mean annual temperature of the streams that were studied was between 4 °C and 24 °C, though some streams in the area are as hot as 42 °C (Friberg et al., 2009). Eight streams varying in temperature and conductivity but having other factors broadly similar were selected for the present study. Since the streams were relatively close to each other within a small area (along a reach of 1.5-km of the River Hengladalsá) we presume that potentially confounding biogeographic effects are negligible. The temperatures of the colder streams were similar to what can be expected in natural alpine streams at similar altitudes in Iceland (Olafsson et al., 2010) and the warmer streams

have regimes comparable to streams at lower altitudes or latitudes in the north-temperate zone (Allan, 2006).

The stream slopes ranged from 0.5 cm m^{-1} to 19.7 cm m^{-1}. The streams were shallow, ranging from 0.05 m to 0.17 m in depth and from 0.63 m to 1.75 m in width, but none of the structural features correlated with temperature (Friberg et al., 2009). For information on the geomorphology of the streams see Friberg et al. (2009) and Woodward et al. (2010a).

The terrestrial vegetation of the catchments was dominated by sedges, grasses and cotton grass (*Carex bigelowii* Torr. ex Schwein, *Carex nigra* (L.) Reichard, *Agrostis capillaris* (L.), *Eriophorum angustifolium* Honckeny), with few low shrub species (*Empetrum nigrum* L. and *Vaccinium uliginosum* L.) (Elmarsdottir et al., 2005; Elmarsdottir and Vilmundardottir, 2007; Gudjonsson and Egilsson, 2006; Kristinsson et al., 2007). Because trees are absent and shrubs are sparse there is only a minimal leaf litter input to the streams (Friberg et al., 2009; Woodward et al., 2010a).

2.2. Structural and functional attributes of the streams

Friberg et al. (2009) and Woodward et al. (2010a) have described the invertebrate and fish communities in the eight streams. Brown trout (*Salmo trutta* L.) was the only fish species found in some of the streams, where its abundance ranged from 0.02 individuals per m^2 to 0.3 individuals per m^2 in the warmer streams. The body mass of brown trout was found to be positively correlated with temperature and the abundance of *Simulium vittatum* Zetterstedt and *R. peregra* but negatively correlated with chironomid abundance (Woodward et al., 2010a). Scrapers and filter feeders were the dominant invertebrate functional feeding groups in the four warmer streams (16–24 °C), while collectors and gatherers were the dominant invertebrate functional feeding groups in the four colder streams (5–13 °C). Thirty-five invertebrate taxa were identified; sixteen of them belonged to Chironomidae. Chironomids, particularly *Eukiefferiella minor* (Edw.), were in higher densities in colder streams compared with warmer streams. The blackfly *S. vittatum* was the most abundant macroinvertebrate in the warmer streams (16–24 °C) along with the pond snail *R. peregra*. Of all measured environmental features, Woodward et al. (2010a) found temperature to have the strongest influences on macroinvertebrate species composition in the streams. They found the greatest diversity (Shannon Index) to be at 10 °C with rapid decline as temperature rose beyond 15 °C.

Density of macroinvertebrates was found to be greatest in streams in the range 13–16 °C. The density of individuals ranged from 3000 individuals per m^2 to 16,000 individuals per m^2 in the streams and was positively correlated with temperature (Friberg et al., 2009). Similar findings had been demonstrated in an earlier study on geothermal streams in Iceland (Olafsson et al., 2010).

2.3. Sampling design

The streams were relatively uniform, each being only a few hundred metres long. A stretch of about 15–25 m was randomly determined in each stream and grids established over the bottom with a spacing of 14.5 cm × 14.5 cm. Bottom samples were taken from this grid using random co-ordinates during summer, May to August 2006. Stretch within stream was not replicated because planned statistical analysis was to use multivariate regression across the continuum of streams and not analysis of variance. Water chemistry did not vary significantly along the stream length and standard samples were taken from the water overlying the middle of each grid. Numbers of samples are specified below.

Physical and chemical variables were assessed approximately monthly. Discharge (m^3 s^{-1}) was measured using a SonTek Flow Tracker Handled Velocity Meter at the end of July, shortly before the biomass sampling in August 2006. Conductivity and pH were measured with a Multiprobe Sonde YSI 600 XLM (Yellow Springs Instruments, Yellow Springs, Ohio, USA) standardized to 25 °C in August 2006. Temperature was monitored in each stream daily in August 2006 using TidbiT^{TM} (StowAway®) temperature loggers, which recorded the stream temperature every 30 min during the sampling period. The temperature within each stream varied very little from upstream to downstream (Ó.P. Ólafsson, personal communication). Temperature was also recorded monthly using a YSI 600 XLM Instrument (Yellow Springs Instruments, Yellow Springs, Ohio, USA) standardized to 25 °C to establish the seasonal changes and variation within each stream. The stream temperatures were relatively constant throughout the year with low standard errors in all seasons except for the autumn when they fluctuated more from day to day (Fig. S1).

Water samples for chemical analysis were collected in 1-L polyethylene bottles from each stream. The water samples were filtered through GF/C filters before chemical analysis. An unfiltered sub-sample of 100 mL was frozen (−18 °C) until it was analyzed at the National Environmental Research Institute, Denmark for total P, which was colourimetrically measured three times in the summer of 2006 by method DS 292/DS/EN 6878) (DS – Danish Standard 292, 1985). The method includes predigestion with persulphate. Samples for ammonium (N–NH$_4$) were collected three times during summer 2006 for analysis using the salicylate method (Antweiler et al., 1996). Ammonium measurements were conducted at the University of Iceland the same day as the samples were obtained. We used data for ammonium in our analysis because it is the main source of inorganic nitrogen for primary producers in the absence of significant amounts of nitrate (Friberg et al., 2009), in these non-agricultural catchments.

Biological variables, including chlorophyll *a*, cover of macrophytes and bryophytes (%), biovolume of algal groups and Cyanobacteria were sampled on 31 August 2006. Eighteen stones (mainly cobbles) were collected from each stream, 15 stones for chlorophyll *a* measurements and 3 stones for diatom identification and biovolume measurements. The stones were kept dark by covering them with aluminium foil during transfer to the laboratory. Macrophyte and bryophyte cover (%) was determined by species and estimated visually at five randomly determined points in the 14.5 × 14.5 cm grid placed over the stream section. Bottom substrate types (i.e. sand and pebbles) were also estimated visually using five randomly determined areas within the grid. The bottom structure was described according to size of stone particles, with sand smaller than 2 mm, gravel between 2 mm and 1.59 cm, pebbles from 1.6 cm to 6.39 cm, cobbles from 6.4 cm to 24.9 cm and boulders over 25 cm wide (Giller and Malmquist, 2006).

Macroinvertebrates were sampled in the first week of August 2004 (Christensen, 2006). Five to ten samples, each covering 500 cm^2, were randomly taken from each of the eight streams with a Surber sampler, mesh size 200 μm and stored in 70% ethanol before analysis.

All eight streams were included in that survey but to justify our use of these data in our 2006 study, we correlated relative abundances of different invertebrate groups available for six of the eight streams with a different year, 2007, for which data were available. The densities of invertebrates between 2004 and 2007 varied but the proportions were similar. Correlations of the invertebrate proportions at streams sampled 2004 with samples from the corresponding streams from 2007 varied but the maximum values ranged from 0.77 to 0.99 per stream, all significant at $p < 0.05$. Results from 2003 (Olafsson et al., 2010) also indicate that the proportions of the dominant invertebrate groups in the streams varied little between years and that the differences in density can mostly be attributed to inevitable patchiness. Climatic variations between 2003 and 2006 in the study area were

Table 1
Mean and standard errors for some key physical and chemical parameters for the Hengill streams. Sampling was carried out three times during the summer 2006.

Stream number	Temperature (°C)	Conductivity (μS cm⁻¹ at 25 °C)	pH	NH₄ (mg L⁻¹)	Total P (mg L⁻¹)
7	6.63 ± 0.02	96 ± 1.2	8.3 ± 0.17	0.035 ± 0.003	0.030 ± 0.004
14	8.56 ± 0.04	204 ± 36.4	8.6 ± 0.27	0.033 ± 0.011	0.014 ± 0.008
11	10.03 ± 0.03	497 ± 71.4	8.0 ± 0.17	0.028 ± 0.005	0.020 ± 0.004
9	13.15 ± 0.04	194 ± 1.6	8.5 ± 0.17	0.021	0.030 ± 0.002
6	16.66 ± 0.02	231 ± 11.7	8.0 ± 0.20	0.034 ± 0.004	0.029 ± 0.002
5	17.61 ± 0.02	234 ± 8.19	7.8 ± 0.25	0.033 ± 0.002	0.029 ± 0.003
1	17.76 ± 0.03	229 ± 17.4	7.8 ± 0.16	0.030 ± 0.001	0.027 ± 0.003
8	22.21 ± 0.004	280 ± 19.4	7.9 ± 0.13	0.028 ± 0.005	0.030 ± 0.001

not considerable and thus not likely to have affected animals in the streams. Temperatures between the 2006 and 2007 summers were similar (Gudmundsdottir et al., in press and Fig. S2). These data all support the use of the most complete invertebrate data set from 2004 as general drivers in the multivariate analyses for 2006. Gudmundsdottir et al. (in preparation) did not find invertebrates to suppress algal and Cyanobacterial growth. They concluded that the streams were bottom up rather than top down controlled by invertebrates.

2.4. Laboratory analyses

Chlorophyll *a* was extracted from each of the 15 stones from each stream by soaking in 96% ethanol at 5 °C for 24 h in darkness, immediately after they had been brought to the laboratory. After 24 h, the ethanol was filtered and its volume measured. The stone surface area that had been colonized by algae was defined and measured by covering it with a single layer of smoothed aluminium foil. The area was later determined by weighing the piece of foil and also measured pieces of the same foil so that weights could be converted to surface area (Hauer and Lamberti, 2007).

The absorption of chlorophyll *a* extracts was measured at 750 and 665 nm. The wavelength of 750 nm was used for correction of any residual turbidity after filtration. Values for chlorophyll *a*, determined after Talling and Driver (1961), are expressed as μg cm⁻².

Biovolume densities of components of the algal (green algae and diatoms) and cyanobacterial communities were made from brushings preserved in Lugol's iodine solution, from standard areas of three stones within each stream. Subsamples of 50–500 μL of the resuspended brushings were counted in sedimentation chambers of 24 mm diameter using an inverted microscope (Wild M50–58257) at ×400 magnification. Minima of 200 organisms were counted along radial or diametric transects and cell volumes were estimated according to Hillebrand et al. (1999) and Hauer and Lamberti (2007). Densities were expressed as μL cm⁻². Hustedt (1930), Prescott (1978), and Krammer and Lange-Bertalot (1986, 1988, 1991a,b) were used for identification of algae, Johannsson (1996, 2001) for bryophytes and Stefansson (1948) for vascular macrophytes.

Macroinvertebrates were identified to species level where possible. The Chironomidae were mounted on glass slides with Hoyer's medium (Anderson, 1954). The larvae were identified at ×400–1000 magnification. The following identification keys were used for invertebrates: Gislason (1979), Cranston (1982), Wiederholm (1983), Dall and Lindegaard (1995), Merritt and Cummings (1996), Nilsson (1996, 1997) and Thorp and Covich (2001).

2.5. Statistical analyses

The statistical software R (version 2.9.2) was used for data analysis (R Development Core Team, 2006). Values for biovolume of algal groups, NH₄–N and total P were log-transformed before linear analysis owing to their non-linearity. A linear mixed effect model using the R-package nlme (Pinheiro et al., 2009) was used to analyze the variation in vascular plant cover, bryophytes, chlorophyll *a* and biovolume densities (green algae, diatoms and Cyanobacteria) with respect to the environmental differences, taking multiple measurements within streams and variance heteroscedasticity into account.

To evaluate whether linear dependence or collinearity among the environmental variables rendered our analysis inappropriate, we calculated the variance inflation factors (VIFs) separately. A VIF value smaller than 10 is generally not of concern (Quinn and Keough, 2002).

We calculated the Spearman's rank correlation coefficient because the relationships of the variables were not linear, to estimate if there was a relationship between the various assemblages of primary producer groups and invertebrates. Ordination of the primary producer groups with the environmental variables, including grazers as environmental factors, used a Regularized Canonical Correlation Analysis (RCCA), implemented in the R-package CCA (Gonzalez et al., 2008). The regularization method solves the problem of having a larger number of variables than number of observations. A prerequisite of an ordinary CCA analysis is that the number of variables is lower than the number of observations.

We followed the recommendation of Gonzalez et al. (2008) in selecting the number of dimensions to present from the RCCA analyses. This entailed looking at the scree graph of canonical correlations versus the dimension, and the scatter plots of variables. The number of dimensions is chosen that reflects a gap between successive values in the scree graph and where correlations between variables and canonical variates are less than 0.5.

3. Results

3.1. Physical and chemical variables

The mean stream temperatures in August 2006 ranged from 6.6 °C to 22.2 °C (Table 1, Fig. S1). The temperature was relatively stable during the winter, spring and summer but fluctuated more during the autumn (Fig. S2). Temperature, depth, sand, and discharge were strongly positively correlated with dimension 1 of the RCCA analysis and were considered to explain the variance in the first dimension (Table 2, Fig. 2a, b).

Sand and gravel (%) were positively correlated with dimension 2 and were considered to be the key variables explaining that dimension (Fig. 2a, Table 2). Conductivity (Table 1) correlated negatively with dimension 3 and was the key variable explaining that dimension (Fig. 2b, Table 2). pH and pebbles substrate correlated negatively with dimension 1 and thus characterised colder streams as slightly more acid and with coarser bottoms (Table 1, Fig. 2a, b). Total P was positively correlated with dimensions 1 and 3 but

Fig. 2. A RCCA plot based on Regularized Canonical Correlation Analysis (RCCA) showing the similarities of primary producer assemblages and their relationship with variation in environmental factors: (a) dimensions 1 and 2, (b) dimensions 1 and 3. Environmental variables (abbreviations in bold): totp = total phosphorus, temp = temperature, cobl = cobbles, pebl = pebbles, ph = pH, grav = gravel, amm = nitrogen (ammonium), width = width of the streams, cond = conductivity, sand = sand, depth = depth and disch = discharge. Invertebrate groups also treated as environmental factors (bold italic font): simvit = *Simulium vittatum*, rper = *Radix peregra*, tricopt = *Potamophylax cingulatus*, chiro = Chironomidae, otherdipt = other dipterans. Primary producer assemblages (regular font): bryo = bryophyte (*F. antipyretica*), nost = *Nostoc* coverage (%), green = green algal biovolume, dia = diatom biovolume, cyano = cyanobacteria biovolume, fil_gr = filamentous green algae coverage (%), chl = chlorophyll *a*, macro = macrophytes (%).

Table 2
Correlations between each of the environmental variables and the first three dimensions of the RCCA analysis.

Predictor variable	Dimension 1	Dimension 2	Dimension 3
Temperature (°C)	0.96	−0.18	−0.02
Conductivity (μS cm^{-1} at 25 °C)	0.08	0.03	−0.64
pH	−0.67	0.11	−0.29
Width (cm)	−0.11	0.11	0.21
Depth (cm)	0.57	0.8	0.03
Discharge (m^3 s^{-1})	0.85	0.39	0.15
Sand (%)	0.46	0.75	0.1
Gravel (%)	−0.33	0.71	0.46
Pebbles (%)	−0.7	−0.02	−0.04
Cobbles (%)	0.42	−0.32	−0.19
NH$_4$ (mg L^{-1})	−0.09	0.04	−0.21
Total P (mg L^{-1})	0.55	−0.49	0.45
Chironomidae (average individuals per m^2)	−0.66	0.08	0.72
S. vittatum (average individuals per m^2)	0.79	0.43	0.18
P. cingulatus (average individuals per m^2)	−0.69	0.12	−0.44
R. peregra (average individuals per m^2)	0.95	0.12	−0.44
Other diptera (average individuals per m^2)	−0.26	−0.26	−0.36

not strongly (Table 2, Fig. 2a, b). Nitrogen (N–NH$_4$) correlated very weakly with all dimensions (Table 2, Fig. 2a, b).

3.2. Classification of streams

The streams were separated by environmental and biological variables as summarized in Figs. 2a, b and 3a, b, which present the first three dimensions from the ordination analysis. Three dimensions present the major canonical correlations. All the warmer streams were positively linked with the first dimension while the colder streams were negatively linked (Fig. 3a, b). Mostly cold streams were positively correlated with dimension 2 while warmer ones were negatively correlated (Fig. 3a, b). In the third dimension (Fig. 3b), the coldest stream (7) differed from the other cold streams mainly owing to low conductivity (Table 1) and was strongly positively correlated while streams with high conductivity were strongly negatively correlated (Table 1, Fig. 3a, b).

3.3. Cover of bryophytes and vascular macrophytes

The bryophyte species *Fontinalis antipyretica* (Hedw.) was most abundant species with up to 80% cover, in the warmest streams (17–22 °C). The liverwort *Jungermannia exsertifolia* (Steph.) was
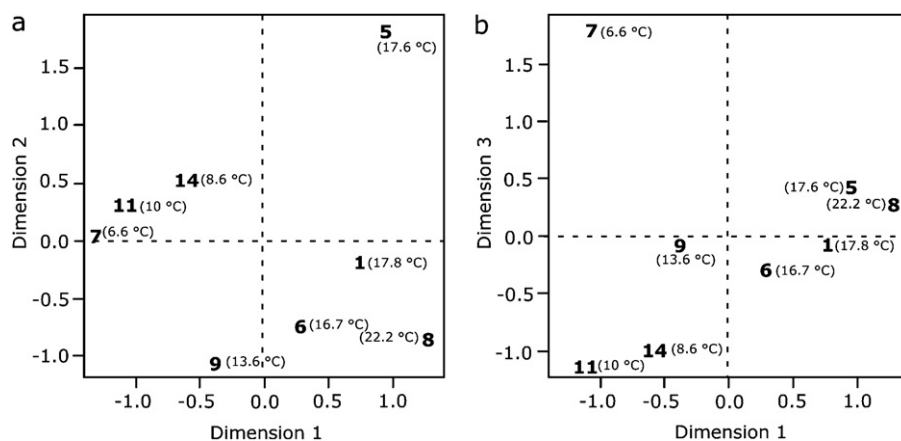

Fig. 3. Streams ordinated by the Regularized Canonical Correlation Analysis (RCCA) on the basis primary producers and environmental factors. Plot (a) represents dimensions 1 and 2 and (b) represents dimensions 1 and 3. The average August (2006) temperatures for each stream are given in brackets close to each stream number.
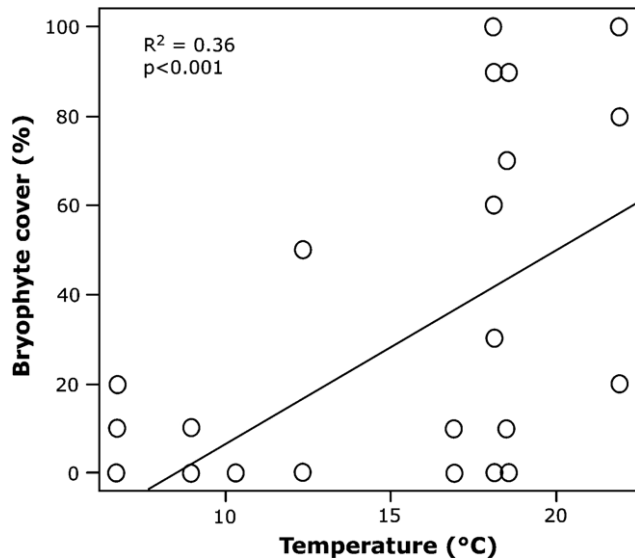
Fig. 4. Observed bryophyte cover (%) in a relation to temperature. Each open circle represents one measurement. A straight line is added on the graph.

Fig. 5. The average concentration of chlorophyll a with 95% confidence intervals (whiskers), in relation to temperature. Stream numbers are annotated by each relevant dot on the figure.

observed only in the coldest streams but only with low cover (<1% usually). The percentage bryophyte coverage increased significantly with temperature (°C) ($b = 4.33$, $t = 3.16$, $p = 0.02$) (Fig. 4). Bryophytes were also closely linked to temperature on the RCCA plot and strongly and positively associated with dimension 1 (Table 3, Fig. 2a, b). Ammonium–N and total P did not have a significant influence on the vegetation cover or total bryophyte cover, though total P correlated positively with dimension 1 (Fig. 2a, b). The bryophyte cover was negatively related to pH ($b = -6.66$, $t = -2.85$, $p = 0.02$ and see Fig. 2a, b). Invertebrate densities overall were not correlated with bryophyte cover, though *R. peregra* density was strongly and positively correlated with the first dimension on the RCCA plots and had no or very weak correlation with dimensions 2 and 3 (Fig. 2a, b).

Among the few vascular macrophytes, *Callitriche stagnalis* (Scop.) was rare (usually <1% cover) but sometimes observed in the warmer streams and *Veronica anagallis-aquatica* (L.) was the only vascular plant species in the colder streams with up to 6% cover in the coldest stream. Invertebrate densities were not significantly correlated with macrophyte cover ($p > 0.05$). Chironomidae and *Potamophylax cingulatus* (Trichoptera) were strongly negatively correlated with the first dimension of the RCCA but the vascular macrophytes to a lesser degree (Tables 2 and 3, Fig. 2a, b). Macrophytes were strongly positively correlated with dimension 3 (Table 3, Fig. 2b). Lower temperatures and lower conductivity favoured vascular macrophytes according to the RCCA analysis.

3.4. Chlorophyll a

In the colder streams, where larger plants were scarce, epilithic algae were the most abundant primary producers (Fig. 5). The concentration of epilithic chlorophyll a varied between 1.13 µg cm^{-2} and 45.3 µg cm^{-2} and was significantly different among streams (ANOVA, $F = 81.69$, $p < 0.001$) (Fig. 5). There was no significant direct correlation between chlorophyll a and temperature or other environmental features. However, there was a significant negative correlation between bryophyte cover and epilithic chlorophyll a ($r = -0.33$, $t = -3.81$, $p = 0.0002$). Densities of none of the invertebrate groups correlated with chlorophyll a ($p > 0.05$) and chlorophyll a was not significantly related to any of the first three dimensions of the RCCA plot (Fig. 2a, b).

3.5. Proportions of Cyanobacteria, green algae and diatoms

Diatoms (Bacillariophyta), green algae (Chlorophyta) and Cyanobacteria dominated the epilithic algal communities. The variability of composition of the community among different streams was high (Fig. 6). Warm, bryophyte-dominated streams had low densities of epilithic algae (Fig. 6). The intermediate temperature streams, with low bryophyte cover, had high biovolumes of all groups of epilithic algae, particularly Cyanobacteria (Fig. 6). The cold streams except for stream 7 (the coldest) had low biovolumes (Fig. 6).

Total biovolume was mainly attributable to green algae (Chlorophyta) and Cyanobacteria while diatoms were not as abundant. Unicellular, unidentified green algae were abundant. Common identified genera of green algae in the colder streams were *Chaetophora*, *Enteromorpha*, *Spirogyra* and *Mougeotia* spp., while *Cladophora* was commonest in warm streams. Filamentous green algae had strong negative correlation with dimension 3 on the RCCA plot but they correlated only weakly with dimensions 1 and 2 (Fig. 2a, b). They were thus associated with increasing conductivity.

Small unicellular cyanobacteria cells (around 1 µm in diameter) were common but larger unicellular Cyanobacteria were also observed, though not as frequently as the small forms. *Nostoc* was most frequently observed in streams ranging from 13.5 °C to 24 °C while *Oscillatoria* spp. were found at a very high density in the coldest stream and often noted at up to 13.5 °C. *Nostoc* coverage (%) was

Table 3

Correlations between each of the biological variables and the first three dimensions of the RCCA analysis.

Response variable	Dimension 1	Dimension 2	Dimension 3
Chlorophyll a (µg cm^{-2})	−0.35	−0.39	0.49
Diatom biovolume (µL cm^{-2})	−0.16	−0.75	0.56
Green algae biovolume (µL cm^{-2})	0.29	−0.39	−0.06
Cyanobacteria biovolume (µL cm^{-2})	−0.54	−0.1	0.8
Bryophytes (%)	0.83	0.05	0.29
Macrophytes (%)	−0.44	0	0.79
Filamentous green algae (%)	−0.24	−0.12	0.94
Nostoc spp. (%)	0.18	−0.24	−0.33

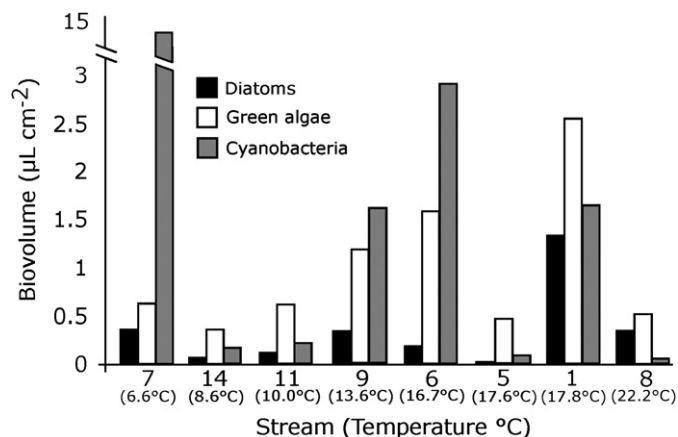**Fig. 6.** The biovolume (μL cm^{-2}) of diatoms (Bacillariophyta), green algae (Chlorophyta) and cyanobacteria. The streams are ranked from the coldest to left to the warmest to the right. The y-axis is broken due to a high biovolume value of cyanobacteria in stream no. 7.

linked to cobbles and conductivity on the RCCA plots (Fig. 2a, b) but not to any of the invertebrate groups ($p > 0.05$).

The highest diatom biovolumes were found in the warmest streams with the most common species: *Rhoicosphenia curvata* (Kütz.) Grunow, *Gomphonema parvulum* (Kütz.) Kütz., *Synedra ulna* (Nitzsch) Ehrenb. and *Cocconeis placentula* Ehrenb. The most common diatoms in the cold and intermediate warm streams were *Gomphonema pumilum* (Grunow) Reichardt and Lange-Bert., *Gomphonema rhombicum* Fricke, *Diatoma mesodon* Kuetzing, *C. placentula* Ehrenb. and *Fragilaria capucina* Desm.

Temperature did not explain Cyanobacteria, diatom and green algae biovolume densities (mixed effect linear model, $p > 0.05$) and neither did conductivity nor any of the other environmental variables (mixed effect linear model, $p > 0.05$). Invertebrates did not influence the biovolume groups (correlation, $p > 0.05$). The Chironomidae plotted close to Cyanobacteria biovolume, with respect to the first two dimensions (Fig. 2a, b). Diatoms were the only group for which biovolume showed strong association with dimension 2 and they also showed a weak association with dimensions 1 and 3 (Table 3, Fig. 2a, b).

3.6. Invertebrate groups

R. peregra and *S. vittatum* densities were strongly and positively correlated with dimension 1 and also positively correlated with temperature ($r = 0.88$, $p = 0.004$, and $r = 0.86$, $p = 0.01$ respectively). Chironomidae and *P. cingulatus* were negatively correlated with dimension 1 (Fig. 2a) and negatively correlated with temperature ($r = -0.90$, $p = 0.005$, $r = -0.79$, $p = 0.02$ respectively). Chironomidae were positively and *P. cingulatus* negatively correlated with dimension 3 (Table 2, Fig. 2b). Other dipterans were marginally correlated with temperature ($p = 0.06$). The macroinvertebrate populations did not correlate with any of the primary producer assemblages.

4. Discussion

Temperature, conductivity and pH differed among the streams and temperature was by far the most influential on the community structure. It varied little along the stream length (Ólafur P. Ólafsson, personal comment) and during the summer within each stream, but gave very different regimes to each stream. According to the RCCA, the warm streams were similar to each other based on their primary producer and macroinvertebrate assemblages but differed greatly from the cold streams. The cold streams were also distinct from each other, mainly because of differences in conductivity. Warm

streams had high bryophyte cover and high populations of snails and blackfly larvae. Colder streams were dominated by epilithic algae and chironomids. The main effect of higher conductivity was to support larger populations of *Nostoc*. Nitrogen and phosphorus concentrations were low and did not differ among the streams, which lie in a landscape little affected by human activities.

We partly accepted our first prediction about the shift from epilithic algae in cold streams to bryophytes and macrophytes in warmer streams. The basis for this prediction was that streams at lower latitudes and higher temperatures have longer growing seasons, capable of allowing build-up of more substantial biomass than in colder streams at high latitudes. Our results showed strong influences from temperature on bryophyte biomass, with the greatest bryophyte cover (50–80%) in the three warmest streams. Epilithic chlorophyll *a* concentration was negatively related to bryophyte cover, suggesting competition between the two. One mechanism leading to bryophyte dominance in warm streams might be suppression of competition from epilithic algae through grazing by snails that were also abundant in warmer streams. Another might be direct shading or greater resistance of bryophytes to erosion during spates. Bryophytes can form very dense mats usually firmly attached to their substrate, thus tolerating spates that might, under circumstances where bryophytes are absent, give epilithic algae opportunity to colonize the stream beds after disturbance. The lack of correlation between bryophyte cover and invertebrate densities suggests that a direct relationship with temperature is more likely than an indirect one through grazing. We accepted the prediction with respect to bryophytes but not to macrophytes. Macrophyte species were few, sparse and very patchily distributed in the stream beds. The stream water was nutrient poor and growth of algae was limited by nitrogen supply (Friberg et al., 2009; Gudmundsdottir et al., in press). However, low nutrient concentration in the stream water may not explain the lack of macrophytes in the streams because macrophytes may obtain substantial amounts of nutrients from the sediment (Sand-Jensen and Borum, 1991). Conductivity did not have any influence on the bryophytes or macrophytes. If future warming brings agriculture to high latitudes, as is not unlikely given the current rapid warming, we might see erosion and transport of enough nutrient-enriched soil to support a greater biomass of vascular plants.

Our second prediction about the dominance of diatoms in cold water versus Cyanobacteria and green algae in warm waters was rejected. We did not find any significant influences from temperature, conductivity, pH, nutrients or bryophytes on the biovolume contributions of diatoms, green algae or Cyanobacteria to the community composition. Cyanobacteria reached their greatest biovolume in the coldest stream (6–7 °C) and the two intermediately warm streams (13.5–16 °C). Diatoms were never the most abundant group of primary producers in the streams and were more abundant in the warmer streams compared with the colder streams, in opposition to the prediction. Both green algae and Cyanobacteria had high biovolumes in all streams. The great abundance of green algae and Cyanobacteria might be explained by the fact that the sampling was carried out in the late summer when temperature and irradiance were high (Allan, 2006). Green algae and Cyanobacteria benefit from high photosynthetically available radiation and can become dominant in late summer or early autumn, while diatoms are usually dominant during winter (Biggs, 2000; Allan, 2006).

The most common taxa of Cyanobacteria in the coldest streams were *Oscillatoria* spp. Studies in south-eastern Switzerland have revealed that *Oscillatoria* sp. can be found in high alpine oligotrophic streams and can even form toxic growths (Mez et al., 1997). Grazing invertebrates did not influence the biovolumes of green algae, diatoms or Cyanobacteria in the streams. Thus it is likely that neither grazers nor temperature influences the balance
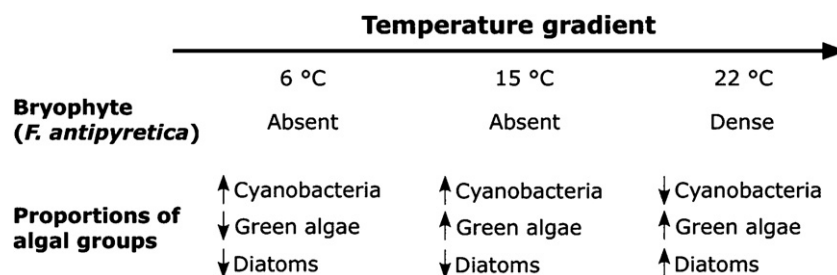
Temperature gradient

	6 °C	15 °C	22 °C
Bryophyte (*F. antipyretica*)	Absent	Absent	Dense
Proportions of algal groups	↑ Cyanobacteria ↓ Green algae ↓ Diatoms	↑ Cyanobacteria ↑ Green algae ↓ Diatoms	↓ Cyanobacteria ↑ Green algae ↑ Diatoms

Fig. 7. The main differences of the primary producers assemblages in the streams. The arrow at the top of the figure points in the direction of increasing temperature. Small arrows in front of individual biological variables represent low (↓) or high (↑) values of relevant variables.

of these primary producer groups. It may be that stochastic factors are important and that there is variation in composition among years.

Our third prediction about the possible influences of macroinvertebrates on primary producers was rejected. Most invertebrate groups (*S. vittatum*, *P. cingulatus*, Chironomidae and *R. peregra*) were strongly influenced by temperature alone. Both *S. vittatum* and *R. peregra* were more abundant in the warmer streams and most chironomids in the colder ones (Woodward et al., 2010a; Olafsson et al., 2010). Because temperature influenced the primary producer dominance (switch from epilithic algae to bryophytes) and invertebrate assemblages concomitantly, it can be concluded that temperature is the key driver for community structure of both invertebrates and primary producers in the streams. Woodward et al. (2010a) found temperature to account by far for the greatest variation in the macroinvertebrate assemblages.

Two of our three predictions (on patterns of epilithic algae and influences of invertebrates on epilithic communities) were rejected and one (on the favouring of larger plants with temperature) was accepted. These findings are of interest because climate change will influence high latitude streams markedly in the future.

A temperature increase of 10 °C from the present, which appears not unlikely within the next century (ACIA, 2004), would change the primary producer assemblages dramatically. With even greater increase of temperature, e.g. of 13–17 °C from present temperature, ecosystems may change dramatically in primary producer assemblages from diverse epilithic algal and bryophyte mixed communities to dense bryophytes. There will also be major effects on the surrounding catchments, so that the subarctic landscape, and its stream systems, will tend much more towards those presently of the cold temperate zone and are likely to be more intensively farmed. With changes in temperature, primary producer assemblages will change, and these will be influenced also by increased nutrient loadings (Gudmundsdottir et al., in press). Our failure to explain, despite the patterns suggested in Fig. 7, the varying compositions of the algal communities by prominent factors like temperature and conductivity, in environments of steady nutrient availability, is of equal interest to our establishment of a link between bryophytes and temperature. It means that detailed prediction of effects of future climate change remains very difficult.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.aquabot.2011.08.003.

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Paper 2

Article

Variation in diatom and bryophyte communities along a temperature gradient in sub-Arctic streams: model surrogates for trends in larger ecosystems?

Rakel Gudmundsdottir[1*], Snaebjorn Palsson[1], Gisli Mar Gislason[1], Jon S. Olafsson[2], and Brian Moss[3]

[1] Institute of Biology, University of Iceland, Sturlugata 7, IS-101 Reykjavík, Iceland
[2] Institute of Freshwater Fisheries, Keldnaholt, IS-112 Reykjavík, Iceland
[3] School of Environmental Sciences, Nicholson Building, University of Liverpool, Liverpool L69 3GS, UK
* Corresponding author email: rakelgud@hi.is

Abstract

Streams within geothermal areas in Iceland that vary in a syndrome of temperature-linked variables, including discharge and potential grazing pressure (snails vs. chironomid larvae), provided a test of how microvegetation structure might change systematically with such drivers. We examined if such streams could form a parallel with biome sequences, for example with similar change in overall structure and organism traits found from tundra to Boreal forest. The warmer streams had an over-story of bryophytes with more open patches of epilithic algae and Cyanobacteria, and a grazer community of snails and blackfly larvae; the vegetation of the colder streams consisted mainly of epilithic algae and Cyanobacteria, dominated by grazing chironomid larvae. Diatom species comprising the epilithic assemblages were generally smaller in the warmer streams compared with the colder streams. Temperature and discharge did not significantly influence the diversity of diatoms (Shannon Index, Sørensen Index, Evenness, Species richness), although more species were observed in the colder streams compared with the warmer streams. Trait diversity was greatest in the coldest streams, but despite high grazing pressure only one growth form (attachment by a mucilage stalk) was predominant in the warmer regimes. Grazers may have influenced diatom species traits but did not significantly suppress the diatom biomass. Analogy with the sequence from tundra, through taiga to boreal forest, over a comparable temperature gradient, showed some parallel features but important differences. Microorganism systems may thus not always be the useful surrogates for larger systems that some ecologists have suggested.

Key words: bottom up, grazing, Iceland, top down, traits, warming

Introduction

Streams harbour primary producers from small unicellular Cyanobacteria, green algae, and diatoms to more obvious bryophytes, liverworts, and macrophytes. Diatoms are often the most diverse and abundant algae in streams (Allan 2006, Gudmundsdottir et al. 2011a), with biofilms made up of various growth forms that may reflect differing niches (Ros et al. 2009). The growth forms are characterised by mode of attachment (mucilage stalks, mucilage pads, or the entire length of the raphe), filamentous modes, and free-living motility (Yallop and Kelly 2006). Size is

also a trait, with some diatoms 2 or more orders of magnitude greater in volume than others. The trait structure of biofilms has been ignored, perhaps because when biofilm is scraped from stones for the purpose of examination, its physiognomy is destroyed.

Currently, microorganism-based laboratory systems are becoming popular as inexpensive surrogates for investigating possible changes, due to warming and other factors, in larger systems where field experimentation is more expensive and for examining theoretical concepts (Harte and Shaw 1995, Lawton 1995, Petchey et al. 1999, Benton et al. 2007). Terrestrial ecosystems vary greatly in

structure and traits, driven by geology, soils, and grazing, but particularly by climate, reflected in temperature and precipitation (Zimov et al. 1995, Krebs 2001). Streams with various temperature regimes might be seen as parallels to temperature influenced-terrestrial habitats for predicting the effects of current warming trends. They are more easily examined and, although more complex and with fewer controls than laboratory systems, may offer greater reality, especially if groups of streams are available that vary strongly in temperature over a small area.

Thus, the diatom microvegetation of cold streams could form an analogue to tundra with its short growth season, low temperatures, low biomass (Bonan et al. 1992), low biodiversity, and frequent disturbance through ice formation and melting (Chapin and Shaver 1981). Warmer streams might be seen as analogues of the tree line vegetation, or taiga, with higher temperature than tundra and scattered trees paralleled by sparse bryophytes in the streams. The warmest streams might be compared with boreal forests, with their evergreen coniferous trees paralleling evergreen bryophytes observed in warm streams (Gudmundsdottir et al. 2011a).

Temperature, however, is not the only factor influencing vegetation structure; grazing may also be important. All land systems have many small grazers, from arthropods to small vertebrates, but some have a substantial part of their grazer biomass in macroherbivores comparable in size to or much bigger than the plants they eat. These macroherbivores have a great potential impact on vegetation structure.

Native macroherbivores on tundra include reindeer and musk oxen. In high densities, reindeer can change the vegetation communities from moss and dwarf shrubs to grass (Oksanen et al. 1995). Boreal forests have higher plant biomass per unit area than tundra and taiga (Bonan et al. 1992) but a lower biomass density of macroherbivores such as moose, wood buffalo, deer, and beaver, yet imposed grazing can have major effects. Introduction of domestic animals in Iceland with the settlement of humans in the 9th century resulted in an almost complete deforestation of Icelandic birch forest (*Betula pubescens* Ehrh.) within a few hundred years (Hallsdottir 1987).

Therefore, in the tundra to boreal forest sequence, temperature tends to increase (Krebs 2001) while there is a reverse trend in macro-herbivore importance. In parallel, streams have the equivalents of macroherbivores, including some insect larvae and snails, which are much bigger than the algae and bacteria on which they feed.

We applied this analogy with terrestrial systems to a set of streams where the relative influences of climate, measured by temperature, and macroherbivore grazing by insects and snails could be assessed. Some of the streambeds were extensively covered by bryophytes, the

analogues of trees; others were covered only by algal films, the equivalents of grasslands and sedge meadows in tundra. Other streams were covered with scattered bryophytes (Gudmundsdottir et al. 2011a), the equivalents of scattered trees in the taiga. Thus, a passable set of parallels exists between the macrovegetation sequence from tundra to boreal forest and the smaller-scale vegetation of the streams. This might be a tempting, inexpensive system to employ to predict effects of temperature increase on key characteristics of ecosystems, with the advantage of increased reality over laboratory systems, which use simple structures and single drivers.

We used 8 adjacent streams in southwest Iceland that varied in temperature and to some extent in conductivity, but other physical and chemical parameters were similar (Gudmundsdottir et al. 2011a, 2011b) and thus avoided the confounding factors of biogeography that are usually problematic in temperature studies. Our overall hypothesis was that changes in vegetation structure, particularly diversity of species and growth forms, would parallel those linked with temperature familiar in the sequence from tundra through taiga to boreal forest. We tested 4 related subhypotheses:

(1) Species richness, evenness, and diversity of diatom growth forms would increase with temperature, which is the usual successional pattern of terrestrial vegetation (Odum 1969).

(2) High grazing pressure would restrict the growth forms of diatoms and reduce species richness and evenness; heavy grazing by macroherbivores tends to produce a more uniform sward (Davidson 1993, Yang et al. 2011).

(3) Bryophytes would be least abundant where the grazing pressure is highest; macroherbivores are very effective in preventing development of tree seedlings and succession to forest (Belsky and Blumenthal 1997), as illustrated by the widespread conversion of forest to grassland by grazing stock in Europe (Arnalds 1987, Hallsdottir 1987).

(4) With increased temperature, species with small-sized individuals would be more abundant than those with larger individuals (Daufresne et al. 2009). This is a well-established phenomenon for homeotherms that may benefit from greater surface to volume ratios in warm habitats and the reverse in colder ones (Freckleton et al. 2003, Hurd and van Anders 2007), but reasons for a similar phenomenon in microorganisms are less clear. Daufresne et al. (2009) consider this feature of size variation in individual species to be important, a third universal ecological response to global warming in aquatic systems in addition to the shift of species ranges toward higher altitudes and latitudes, and seasonal shifts in life cycle events.

Study site

The study was conducted in the Hengill geothermal area in southwest Iceland (64°05′N, 21°30′W; 360–380 m a.s.l.). The area is an active volcanic area covering 173 km^2, with a mean air temperature of 8–10 °C in July (Bjornsson 2003) and a mean annual precipitation of 1870–3080 mm (Crochet et al. 2007).

Within the study area, many small streams emerge from the hillsides and run into the same river (Hengladalsá). The bedrock is mainly hyaloclastite and the vegetation is mostly monocotyledonous (Gudjonsson and Egilsson 2006). Sedges, grasses, and cotton grass (*Carex bigelowii* Torr. Ex Schwein, *Carex nigra* [L.] Reichard, *Agrostis capillaris* [L.], and *Eriophorum angustifolium* Honckeny) dominate, with a few low shrub species (*Empetrum nigrum* L. and *Vaccinium uliginosum* L.) occasionally observed (Elmarsdottir et al. 2005, Gudjonsson and Egilsson 2006, Kristinsson et al. 2007).Trees are absent, so leaf litter and woody debris input to the streams is minimal (Friberg et al. 2009, Woodward et al. 2010).

About 20 small streams are found in the study area; we used 8 small, largely first-order streams for our study (Gudmundsdottir et al. 2011a, 2011b). Their summer mean temperatures vary from 6 to 22 °C, with the warmer streams not fed directly by hot springs, but rather absorbing heat from the warm rocks below (Arnason et al. 1967). The temperature of the colder streams was typical of high latitude and high altitude streams (Olafsson et al. 2010), while the temperature in the warmer ones was comparable with lower altitudes (Allan 2006). The stream slopes ranged from 0.5 to 19.7 cm m^{-1}, the depth ranged from 0.05 to 0.17 m, and the width from 0.63 to 1.75 m. Structural and some major biological features (algal biomass and vascular macrophyte biomass) did not correlate with temperature (Friberg et al. 2009).

We identified 35 invertebrate taxa, most to the species level. Chironomids (mainly *Eukiefferiella minor* Edw.) were dominant and negatively correlated with temperature. Other common invertebrates were *Simulium vittatum* (Zetterstedt) and *Radix peregra* (Müller), which were both positively linked to temperature. Density ranged from 3000 to 16 000 individuals per m^2, and the greatest density was in streams ranging in temperatures from 13 to 16 °C. Collectors and gatherers were the dominant functional feeding groups of the 4 colder streams, while scrapers and filter feeders dominated the 4 warmer streams. The greatest species diversity (Shannon Index) was at 10 °C but declined rapidly as the temperature rose above 15 °C (Friberg et al. 2009, Woodward et al. 2010, Gudmundsdottir et al. 2011b). Woodward et al. (2010) concluded that temperature was the key variable structuring these invertebrate communities.

Methods

Sampling

Most physical and chemical variables were assessed monthly between May and August 2007. The discharge was measured with a SonTek Flow Tracker Handled Velocity Meter 5 times in total from May to August 2006 and May to August 2007. A Multiprobe Sonde YSI 600 XLM (Yellow Springs Instruments, Yellow Springs, Ohio, USA) was used to measure conductivity, pH, and temperature. The temperature varied little from upstream to downstream (OP Olafsson, pers. comm.). Seasonal variation was low, especially in the warmest streams.

Each month water samples for chemical analysis were collected in 1 L polyethylene bottles from each stream. Samples were brought to the laboratory, filtered through GF/C filters, and then frozen (−18 °C) before analysis in Denmark at the National Environmental Research Institute. Samples for ammonium (N-NH$_4$) were obtained 4 times during the summer 2007 and analysed using the salicylate method (Antweiler et al. 1996) on the day of sampling. Ammonium was used as the main variable explaining nitrogen content in our study because it is the main source of inorganic nitrogen for primary producers in the absence of significant amounts of nitrate (Friberg et al. 2009). Total phosphorus was colourimetrically measured 4 times during summer 2007 using method DS 292/DS/EN 6878 (Danish Standard 292 1985), which involves predigestion using persulphate.

Dry weight of vascular macrophytes, bryophytes, macro algae, and macrophytes was assessed on 13 July 2007. At 5 randomly determined points in a grid covering the entire area of each stream, a 14 × 14 cm frame (Surber sampler) was placed on the stream bed and the cover was harvested. The macrophytes were brought to the laboratory, separated into groups, and identified. The macrophytes and mosses were then dried at 60 °C for 24 h before weighing. Mosses were identified using keys for Icelandic mosses by Johannsson (1996, 2001). Stream bed structure (i.e., sand and pebbles) was assessed using the same random points used for the benthic samples. The stream bed was classified according to diameter of stone particles: sand <0.02 cm, gravel 0.02–1.59 cm, pebbles 1.6–6.39 cm, cobbles 6.4–24.9 cm, and boulders >25 cm (Giller and Malmquist 2006).

During the first week of August 2004 (Christensen 2006), 5–10 macroinvertebrate samples were obtained from each stream using a Surber sampler with mesh size 200 μm. The samples were stored in 70% ethanol before identification. To validate whether the data from 2004 could be used to explain diatom communities in August 2007, we correlated relative abundances of different inver-

tebrate groups with data for 6 of the 8 streams from August 2007; correlations between the 2004 and 2007 data for density and community composition ranged from 0.77 to 0.99, all significant at p < 0.05. Climate variations (temperature and precipitation) were small and therefore not likely to have influenced invertebrate densities between different years. Stream temperatures were also similar (Gudmundsdottir et al. 2011b). These data collectively support our use of the more complete invertebrate dataset from 2004 as drivers in multivariate analysis (RCCA) for diatom data from summer 2007 (Gudmundsdottir et al. 2011a).

Diatom samples were obtained by randomly collecting 3 stones (5–35 cm in diameter) from the grids laid out in each stream each month from May to August 2007 (96 samples). This sampling provided the data on community composition and morphological traits, but to estimate the relationship between temperature and diatom size we used monthly data from summers 2006 and 2007, totalling 9 sampling occasions, yielding 216 diatom samples. The stones were wrapped in aluminium foil in the field to prevent light damage, transported to the laboratory, and kept cool until processing began.

Laboratory analysis

Algae were collected by using a toothbrush to brush a delineated area of each stone into a tray. The brushed area was measured by outlining it on an overlain squared tracing paper and was brushed until the surface had a grainy, non-mucilaginous texture. The brushings from each stone were suspended in Lugol's iodine solution. For diatom species identification, subsamples of about 10 mL were heated in nitric acid (65%) for up to 24 h to remove all organic material and then were repeatedly centrifuged and resuspended in distilled water until the acid was removed. A subsample of 0.5 mL of resuspended material was air-dried onto a round microscope cover glass (15 mm wide) and mounted in Naphrax®. Diatoms were identified to species level using standard keys, and up to 2000 frustules were counted within defined transects on each slide.

To determine size category we did not digest the samples with acid. Aliquots of about 50–500 μL (depending on the density of algae) were pipetted into a counting chamber, which was filled with distilled water and left for 20–24 h before identifying, counting, and measuring. Biovolume and the size categories were estimated according to Hauer and Lamberti (2007) and expressed as μL cm^{-3}. Diatoms <5 μm in width were classified as small; diatoms ≥5 μm and up to 12 μm in width were classified as medium; and diatoms >12 μm in width and/or >25 μm in length were classified as large (Hauer and Lamberti 2007).

Macroinvertebrates were identified to species level when possible. Hoyer's medium (Anderson 1954) was used to mount the chironomids on glass slides before identification at 400–1000× magnification with a Leica DM 3000 microscope with Leica DFC 290 camera. Identification keys used were Gislason (1979), Cranston (1982), Wiederholm (1983), Dall and Lindegaard (1995), Merritt and Cummings (1996), Nilsson (1996, 1997), and Thorp and Covich (2001).

Statistical analysis

The R statistical software (version 2.9.2; R Development Core Team 2011) was used for all statistical analyses. All nonlinear data except bryophyte data were log transformed before analysis. Spearman's rank correlation was used to determinate the relationship between bryophytes and temperature. We used Regularized Canonical Correlation Analysis (RCCA) to ordinate most of the relevant variables with the diatom assemblages (species and traits). RCCA with its regularizing step is appropriate when there are few samples but many variables (in this case many diatom species) in the dataset (Gonzalez et al. 2008). A correlation value R = 0.707 is the critical value for 6 df (p ≤ 0.05) and 0.834 for p < 0.01. The RCCA was based on the 8 means from the different streams.

To summarize the association of different diatom species across streams we conducted hierarchical cluster analysis on pairwise distances derived from correlations of the frequencies in different streams (1-r). The package pvclust (Shimodaira 2004) in R (R Development Core Team 2011) was used for the analysis. Clustering confidence was evaluated with 1000 bootstrap samples.

We used one-way ANOVA to test if the various environmental factors were significantly different among streams. A linear mixed effect model, taking the replicate measurements within streams into account as a random effect, was used to determine if there was a relationship between temperature and diatom size categories (Crawley 2002). Correlation was used to estimate if there was a relationship between various growth forms of diatoms and invertebrates and if there was a relationship between evenness and species richness (Magurran 2004) with invertebrates or abiotic factors.

Results

Physiochemical features

The streams differed significantly in temperature (ANOVA, F = 3786, p < 0.001), ranging between 6.6 and 22.2 °C (Table 1). Conductivity was also significantly different among streams (ANOVA; F = 17.27, p < 0.001)

Table 1. Mean and standard errors for key physical and chemical variables of the Hengill streams along with the mean and standard deviations for diatom frustule counts in the streams. Streams are listed in order of increasing temperature.

Stream	Temperature (°C) (n = 3)	Conductivity (µScm⁻¹ at 25 °C) (n = 3)	pH (at 25 °C) (n = 3)	NH_4-N (mgL⁻¹) (n = 3)	Total P (mgL⁻¹) (n = 3)	Discharge (m^3s^{-1}) (n = 5)	Number of diatom frustules (mean cm⁻² ± standard deviation) (n = 3)
7	6.63 ± 0.02	96 ± 1.2	8.3 ± 0.17	0.035 ± 0.003	0.030 ± 0.004	1.3 ± 0.40	$5.0 \times 10^6 \pm 4.8 \times 10^6$
14	8.56 ± 0.04	204 ± 36.4	8.6 ± 0.27	0.033 ± 0.011	0.014 ± 0.008	1.9 ± 0.40	$1.7 \times 10^5 \pm 2.3 \times 10^5$
11	10.03 ± 0.03	497 ± 71.4	8.0 ± 0.17	0.028 ± 0.005	0.020 ± 0.004	1.1 ± 0.58	$5.8 \times 10^5 \pm 2.2 \times 10^5$
9	13.15 ± 0.04	194 ± 1.6	8.5 ± 0.17	0.021	0.030 ± 0.002	0.3 ± 0.04	$2.1 \times 10^6 \pm 1.2 \times 10^6$
6	16.66 ± 0.02	231 ± 11.7	8.0 ± 0.20	0.034 ± 0.004	0.029 ± 0.002	4.1 ± 1.39	$3.9 \times 10^5 \pm 5.2 \times 10^5$
5	17.61 ± 0.02	234 ± 8.19	7.8 ± 0.25	0.033 ± 0.002	0.029 ± 0.003	5.6 ± 1.43	$3.1 \times 10^4 \pm 4.7 \times 10^4$
1	17.76 ± 0.03	229 ± 17.4	7.8 ± 0.16	0.030 ± 0.001	0.027 ± 0.003	2.9 ± 0.49	$2.5 \times 10^4 \pm 1.9 \times 10^4$
8	22.21 ± 0.004	280 ± 19.4	7.9 ± 0.13	0.028 ± 0.005	0.030 ± 0.001	5.0 ± 1.34	$9.2 \times 10^5 \pm 1.6 \times 10^6$

within the range of 96 and 497 µS cm⁻². Most values fell in the range 194–280 µS cm⁻², but one was unusually low and one somewhat higher. Ammonium (NH_4 -N), total P, and pH were not significantly different among streams. Temperature and pH were, however, significantly negatively correlated (r = −0.75, p = 0.03). The values of pH were negatively correlated with dimension 1 (Fig. 1). Ammonium and total P were not significantly related to temperature. The highest discharge was observed in the 4 warmest streams (Table 1) and was positively correlated with dimension 1 (Fig. 1). Discharge was correlated with temperature (r = 0.74, p = 0.05), cobbles (%), and the depth of the streams; conductivity was negatively linked with dimension 2; and temperature was positively linked with dimension 1 (Fig. 1).

Macrophytes and bryophytes

Vascular macrophytes were rare, their density negligible, and they were most commonly observed in the colder streams (Fig. 1). The vascular macrophyte densities were positively linked with dimension 2 (Table 2) and positively correlated with low conductivity (Fig. 1). *Fontinalis antipyretica* (Hedw.), the only observed moss, was abundant in warmer streams but also found in sparse patches in cooler streams. Its cover correlated positively with temperature (S = 2884, p < 0.001, r = 0.73; Fig. 2) and was positively linked to dimension 1 (Table 2; Fig 1). The liverwort *Jungermannia exsertifolia* (Steph.) was very sparse and present only in the coldest streams. Its cover was negatively correlated with temperature (S = 14466, p = 0.02, r = −0.36) but positively linked with dimension 2 (Fig. 1 and 2).

Invertebrates

We identified 30 invertebrate taxa; 16 were larval Chironomidae with *Eukiefferiella minor* (Edw.), the most abundant, and were found in highest densities in the colder streams (Fig. 3). Numbers of chironomids declined significantly with temperature and were positively linked to dimension 2 of an RCCA analysis relating communities to driving variables (Fig. 1). The gastropod species *Radix peregra* dominated the macrobenthos in the warmer streams and was positively correlated with temperature and positively linked to dimension 1 (Fig. 1 and 3). The black-fly species *Simulium vittatum* was also abundant in the warmer streams and was positively correlated with dimension 1 and temperature (Fig. 1 and 3). Other dipterans were sparse, and numbers did not correlate with temperature (Fig. 1 and 3). The only larval caddis observed in the streams was *Potamophylax cingulatus* (Steph.). Its density was low (Fig. 3) and not correlated with any environmental factors, but it was negatively linked to dimension 1 and thus linked to high conductivity and low water temperature. The 4 warmest streams had higher total invertebrate densities than the 4 coldest streams (Fig. 3), but the total density was not correlated with temperature (p > 0.05; Fig. 1).

Diatom community structure

Diatom population density varied between streams (Table 1), and 64 diatom taxa were identified overall; the majority were identified to species level (44 species of 64 taxa). After removal of rare species (<0.5% density and species observed in no more than one stream), 34 diatom species were left for RCCA analysis and cluster analysis (Fig. 1).
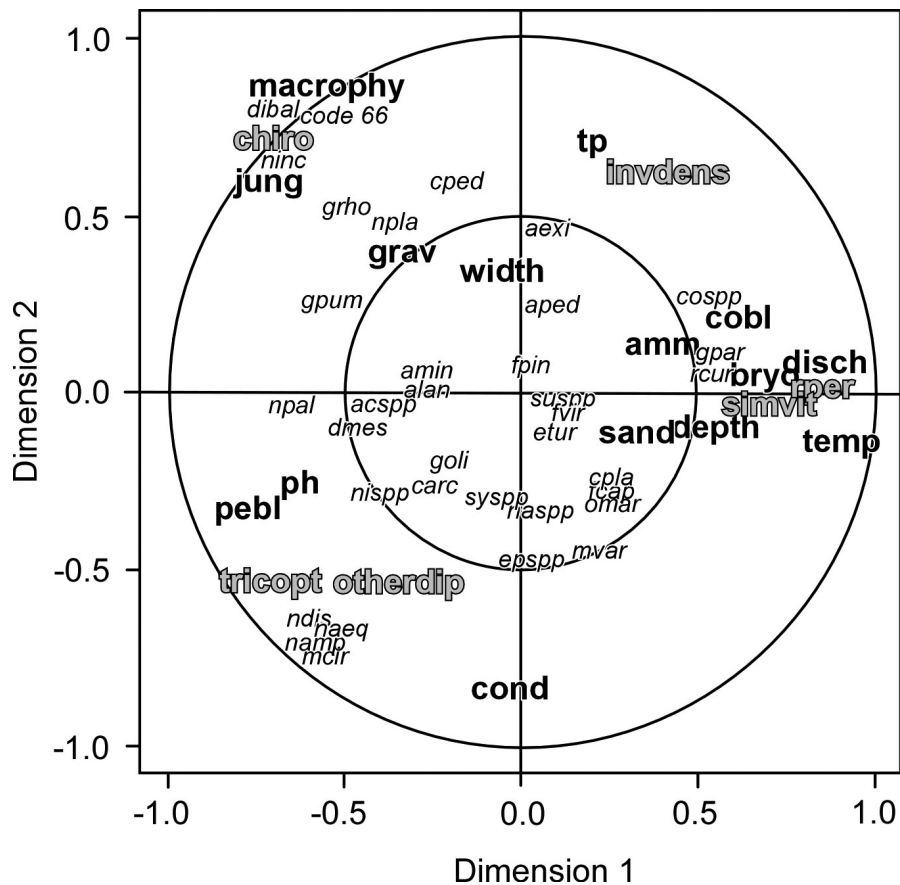
Fig. 1. Results of a Regularized Canonical Correlation Analysis (RCCA) showing the similarities of diatom species (regular type) and their relationship with environmental factors (bold type) and dominant macroinvertebrates (large, gray, and bold type). Only species that had >0.5% abundance in some of the 8 streams were included, and species that were only found in only one stream were not included. A full list of diatom species is given in Appendix 1. Abbreviations for environmental features are: jung = *Jungermannia exsertifolia*, macrophy = macrophytes, sand = sand, Cobl = cobbles, depth = depth, width = width, ph = pH grav = gravel, pebl = pebbles, cond = conductivity, bryo = *Fontinalis antipyretica*, tp = total phosphorus, amm = ammonium, temp = temperature, disch = discharge. Abbreviations for invertebrates are: invdens = invertebrate density, tricopt = *Potamophylax cingulatus* (caddisfly), otherdip = other dipteral groups, chiro = chironomids, simvit = *Simulium vittatum*, and rper = *Radix peregra*.
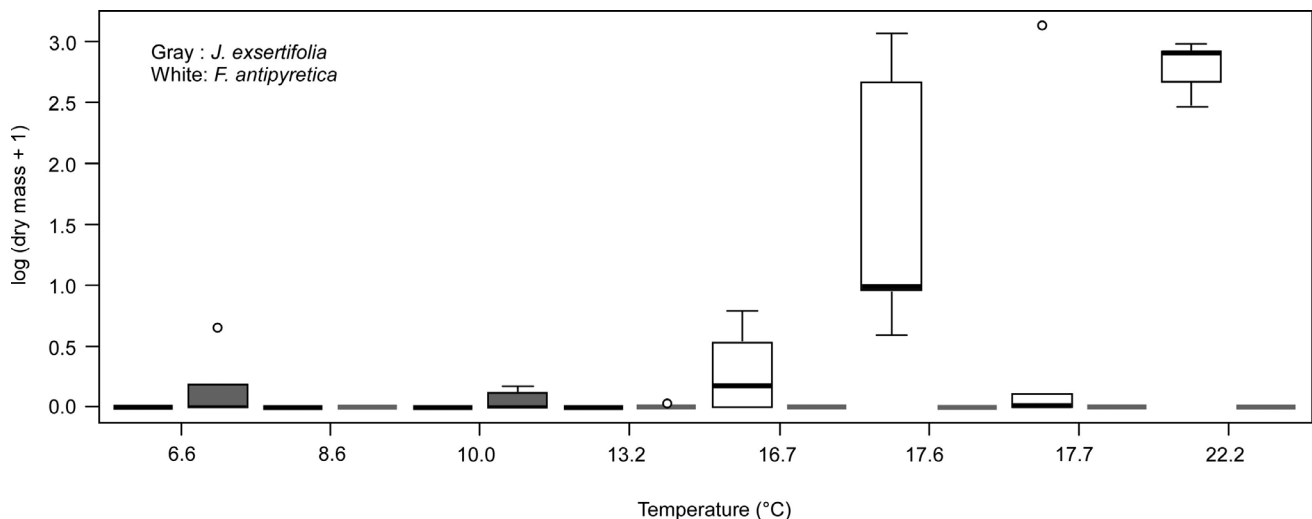
Fig. 2. Observed moss (*F. antipyretica*, white boxes) and liverwort (*J. exsertifolia*, gray boxes) cover (dry weight in g) in the 8 streams. Circles represent outlier values.

Table 2. Correlations between each of the environmental variables (means) and the first 2 dimensions of the RCCA analysis. *: P < 0.05, **: P < 0.01.

Environmental variable	Dimension 1	Dimension 2
Temperature (°C)	0.92**	−0.13
Conductivity (µS cm⁻¹ at 25°C)	−0.01	−0.81*
pH (at 25°C)	−0.62	−0.26
Width (m)	−0.02	0.36
Depth (cm)	0.53	−0.08
Sand (%)	0.36	−0.09
Gravel (%)	−0.33	0.04
Pebbles (%)	−0.77*	−0.31
Cobbles (%)	0.63	0.23
NH_4−N (mg L⁻¹)	0.44	0.15
TP (mg L⁻¹)	0.21	0.70
Discharge (m³s⁻¹)	0.88**	0.09
F. antipyretica (g)	0.68	0.07
J. excertifolia (g)	−0.70	0.61
Macrophytes (g)	−0.55	0.81*
Chironomids (individuals m⁻²)	−0.67	0.72*
S. vittatum (individuals m⁻²)	0.73*	−0.01
P. cingulatus (individuals m⁻²)	−0.70	−0.54
R. peregra (individuals m⁻²)	0.89**	0.03
Other dipterans (individuals m⁻²)	−0.32	−0.53
Invertebrate density (individuals m⁻²)	0.39	0.64

Table 3. Correlations between each of the diatom taxa and species (>0.5% abundance and occuring in more than one stream; mean frustules per cm⁻²) and the first 2 dimensions of the RCCA analysis. *: P < 0.05, **: P< 0.01.

Taxa/species	Dimension 1	Dimension 2
Achnanthes spp.	−0.39	−0.03
A. exigua	0.07	0.48
A. lanceolata	−0.28	0.02
A. minutissima	−0.27	0.07
Amphora pediculus	0.07	0.26
Cocconeis spp.	0.54	0.26
C. placentula	0.23	−0.30
C. pediculus	−0.15	0.59
Ceratoneis arcus	−0.25	−0.26
Diatoma mesodon	−0.47	−0.08
Diatomella balfourina	−0.56	0.81*
Epithemia spp.	0.05	−0.48
E. turgita	0.08	−0.09
Gomphonema pumilum	−0.52	0.25
G. rhombicum	−0.48	0.54
G. parvulum	0.57	0.08
G. olivaceum	−0.20	−0.2
Fragilaria capucina	0.26	−0.29
F. pinnata	0.04	0.08
F. virences	0.13	−0.04
Melosira varians	0.22	−0.45
Meridion circulare	−0.58	−0.74
Navicula spp.	0.05	−0.33
N. placentula	−0.35	0.49
Nitzschia spp.	−0.33	−0.27
N. palea	−0.64	−0.02
N. inconspicua	−0.65	0.69
N. aequorea	−0.51	−0.68
N. dissipata	−0.58	−0.63
N. amphibian	−0.58	−0.70
Rhoicosphenia curvata	0.59	0.07
Synedra spp.	−0.07	−0.32
Surirella spp.	0.14	−0.03
Code 66	−0.56	0.80*

There were 3 main diatom clusters according to RCCA analysis (Fig. 1). The first dimension represented temperature and the second represented conductivity (Table 2). The first diatom community contained *Diatomella balfourina*, *Nitzschia inconspicua*, and diatoms not fully identified and given a code number (66). They were very small *Achnanthes* or *Navicula* spp. and were positively related to dimension 1 (Table 3), and therefore low conductivity, and negatively linked to dimension 2 and temperature (Fig. 1; Table 3). These diatom species were also linked to *J. exsertifolia*, macrophytes, and Chironomids (Fig. 1).

A second diatom community was negatively related to both dimensions (Table 3) and thus related to high con-

ductivity and low water temperature (Fig. 1). These diatom species were *Meridion circulare*, *Nitzschia aequorea*, *N. dissipata*, and *N. amphibia*. The caddis larva *P. cingulatus* and dipterans other than simuliids and chironomids were correlated with this community (Fig. 1).

The third community was positively correlated with dimension 1 and therefore with high temperature and high discharge (Fig. 1; Table 3) but not linked to dimension 2. Bryophytes (*F. antipyretica*), depth, high temperature, discharge, ammonium, cobbles (%), *S. vittatum*, and *R. peregra* were the most important correlates for these diatoms, dominated by *Rhoicosphenia curvata*,

Gomphonema parvulum, and *Cocconeis* spp. (Fig. 1). Most of the diatom species had little or no correlation with either dimension and therefore straddled the 3 distinct communities (Fig. 1; Table 3).

We used cluster analysis to establish alternatively how diatom communities were related to environmental factors. The dendrogram showed 2 major distinctions,
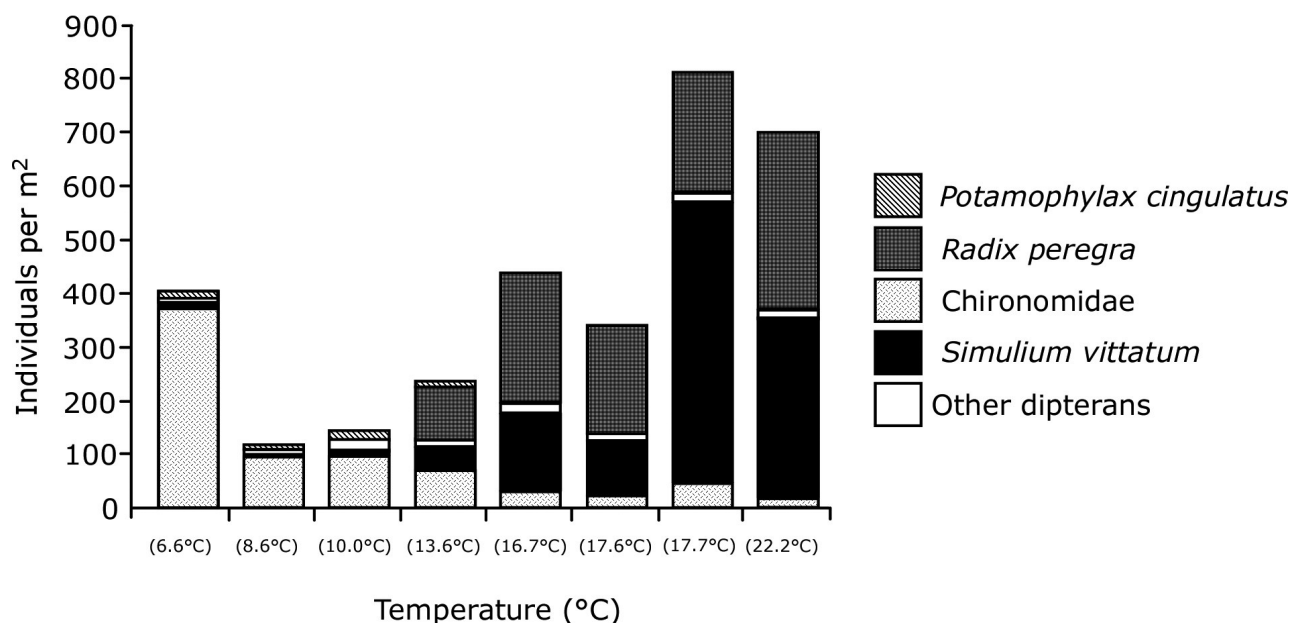
Fig. 3. Abundance of the dominant macroinvertebrates in the 8 streams. The streams are ranked from the coldest to the warmest. Temperature is given below the number of each stream.
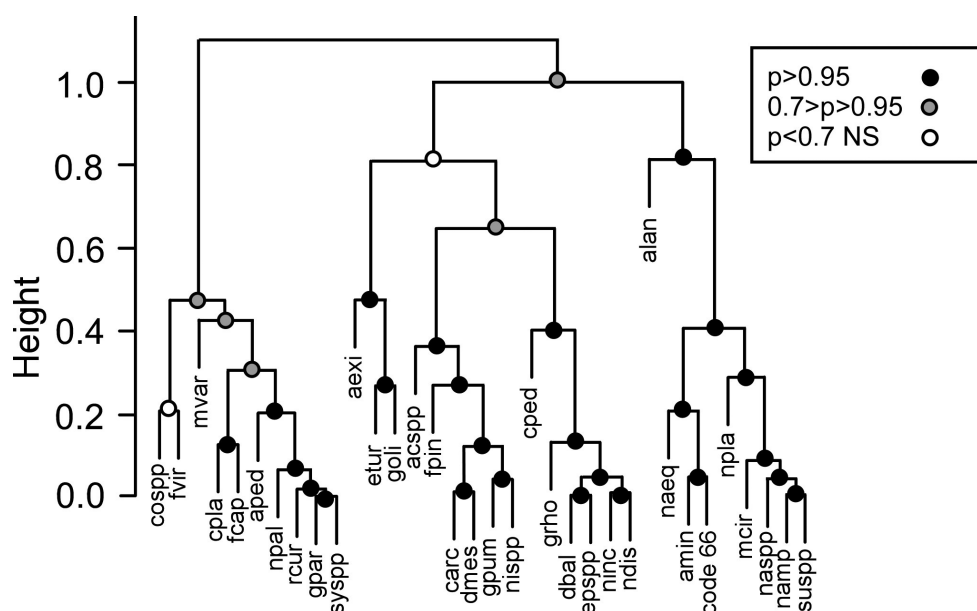
Fig. 4. Dendrogram showing the association of different diatom species in the 8 streams. The support given at each node represents the proportion (p) obtained in 1000 bootstrap samples. The full names of different species are given in Appendix 1.

based on temperature. One of the major clusters (Fig. 4, left) contained 10 species or taxa that were commonly observed in warmer streams. Among those species were *Cocconeis* spp., *Gomphonema parvulum*, and *Rhoicosphenia curvata*, the same species that were clustered around temperature, discharge, bryophytes, *S. vittatum*, and *R. peregra* on the RCCA graph (Fig. 1 and 4). The other major group was linked to lower water temperature and contained 24 species. *Diatomella balfourina*, code 66, *Nitzschia inconspicua*, *N. dissipata*, *N. aequorea*, and *Meridion circulare* were among those 24 species. The cold water species were divided into 2 clusters on the dendrogram. The ones to the right were closely linked to cold water environmental features on the RCCA graph and those species to left (middle branch on the dendrogram) were species scattered between streams on the RCCA graph (Fig. 1 and 4). Invertebrate density had no influence on the diatoms or other environmental factors (Fig. 1).

Diatom taxonomic diversity

Diatom evenness was unaffected by temperature or other important environmental features (conductivity, pH, ammonium, TP, *F. antipyretica*, *J. exsertifolia*, and discharge; $P > 0.05$). Species richness and the Shannon Index showed the same pattern as evenness ($P > 0.05$). Sorensen Index was also unaffected by these environmental features ($P > 0.05$).

Diatom traits and biomass

Diatom traits for individual species and genera (Appendix 1) indicate that motile diatoms (MO) were closely linked to low temperature (Fig. 5), low conductivity, presence of *J. exsertifolia*, and macrophytes. These diatoms were mainly *Nitzschia inconspicua* and other rarer *Nitzschia* spp. (Fig. 1). Mucilage pad (MP) diatoms were also linked to lower temperature but not strongly. Most of the MP diatoms (e.g., *Achnanthes* spp., a common genus
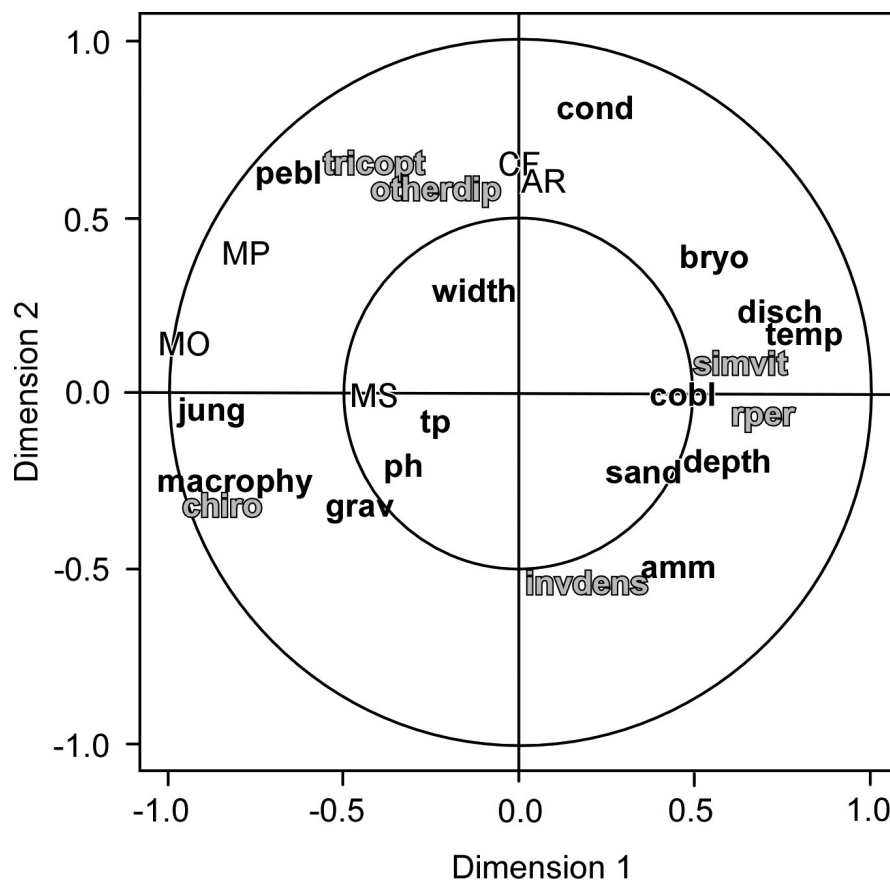
Fig 5. A biplot based on Regularized Canonical Correlation Analysis (RCCA) showing the relationship between various environmental features and growth form traits of diatoms. The growth form traits were MS = mucilage stalk, AR = attached on raphe, MO = motile, CF = chain forming, MP = mucilage pads, MT = mucilage tubes. See Fig. 3 for abbreviations for other environmental features.

in the streets) were found in many streams and therefore not likely to be closely linked to either low or high temperature (Table 4).

Mucilage stalk (MS) diatoms were found in all streams and were mainly *Gomphonema* spp. and *Rhoicosphenia curvata*. Because *Gomphonema* spp. were more often observed in colder streams (except for *Gomphonema parvulum*, which was linked to the warmer streams) than warmer and were more common than *R. curvata*, the RCCA plot showed a closer link between MS diatoms with colder water compared with warmer water. Chain-forming diatoms (CF) and diatoms attached by their raphe (AR) were positively linked to high conductivity and low water temperature. *Meridion circulare* and 3 *Nitzschia* spp. were found where the conductivity was high along with chain-forming *Fragilaria* spp., although they were scattered on the RCCA plot due to rather high occurrence in most streams.

Total diatom biovolume was not significantly related to temperature or invertebrates ($p > 0.05$).

In 2006, temperature had negative influences on population density of large diatoms ($b = -2.02$, $t = -2.15$, $p = 0.035$), and in 2007 population densities of smaller diatoms were close to being significantly favoured by temperature ($b = 4.23$, $t = 2.89$, $p = 0.06$).

Discussion

The RCCA analysis was particularly useful because it showed a syndrome of linked environmental factors consistently affecting the streams. Temperature, depth, flow, bryophyte cover, and features of the invertebrate community were all strongly linked. We argue that the master variable is temperature. The flows were all comparatively low and increased only modestly with temperature, perhaps as a function of lesser permanent ice formation in the soils from which the water percolated. Flow and depth are obviously related, and we cannot argue that shear stress was greatly different among flows as low as these. We assume that opportunities for colonisation of bryophytes and invertebrates were equal because the streams were in a confined area with no obvious biogeographical barriers between them. There is thus a picture of temperature strongly influencing these variables, and an argument for treating them together, as a temperature driven syndrome, in examining the hypotheses posed. Water chemistry was comparatively uniform with only 2 anomalies in conductivity; conductivity formed a separate variable that was separately accounted for in the analyses.

We posed 4 hypotheses, 3 based on parallels with a macrovegetation changing along a temperature gradient, and the fourth relating to recent metaanalyses of organism

Table 4. The relative abundances (%) of attachment traits of the various diatom species in the 8 streams. MP: mucilage pad; AR: attached on raphe; CF: chain forming; MO: motile; MS: mucilage stalks.

	Temperature (°C)							
Trait	6.6	8.6	10.0	13.6	16.7	17.6	17.7	22.2
MP	12.6	20.3	20.4	7.0	6.9	7.5	64.8	6.5
AR	3.4	2.8	9.2	3.4	12.2	26.5	1.4	11.8
CF	15.1	58.0	44.5	26.0	20.3	43.4	8.2	34.1
MO	21.6	13.7	25.0	9.7	10.1	5.7	16.1	3.7
MS	47.2	5.3	0.9	53.9	50.4	16.9	9.6	43.9

size in relation to temperature. The first hypothesis, that temperature would have significant positive influences on diversity of diatoms, growth form diversity, evenness, and community composition, was rejected. Temperature had no influences on diatom evenness, species richness, or Shannon Index. Species richness was generally slightly higher in colder streams compared with warmer streams. Studies on effects of temperature increase within tundra communities have shown loss of plant biodiversity (Walker et al. 2006), although diversity and species richness of many groups of organisms generally increase with decreasing latitude, and therefore average temperature, at comparable altitudes. The discharge was higher in the warmest streams compared with the colder streams but did not have significant influence on the diatom diversity indices and did not affect the density of invertebrates.

Growth form diversity was also greater in the coldest streams because species diversity was slightly higher, although the effect was statistically insignificant. MO diatoms were very common in the coldest streams, and thus we conclude that the motile growth form is highly linked to low temperature and associated environmental factors in our stream system. Although MO diatoms were found in all streams, they dominated where water temperature was lowest, with both low conductivity and high conductivity. Based on the dendrogram, we found these *Nitzschia* species to be linked together in the last cluster (cluster branch to right) where cold water species were clustered together. Gudmundsdottir et al. (unpublished) found motile diatoms to benefit from low nutrient status, and they retreated when nutrient was added to the streams. According to these findings *Nitzschia* spp. and some *Navicula* spp. seem to benefit from low water temperature and low nutrient pollution, in contrast to other studies (Kelly et al. 2001, Kelly 2003, Yallop and Kelly 2006, Yallop et al. 2009). The growth form of MP diatoms was also linked with both dimensions and was mainly found in cold streams with and without high conductivity. These species were also

linked together on the dendrogram, indicating that they were commonly found together in colder streams. *Achnanthes lanceolata*, *A. minutissima*, and code 66 were among those species on both the RCCA graph (Fig. 1) and the dendrogram (Fig. 5). Perhaps MP diatoms are at some disadvantage compared with mucilage stalk diatoms when competition for light in biofilms exists. Because we observed rather few diatom species in the warm streams, the growth form diversity was also low compared with that in the cold streams. Overall, our analogy with the diversity of terrestrial habitats did not hold.

We partly accepted the second hypothesis regarding the negative influences of grazers on growth form diversity. The warmer streams contained fewer growth forms than the colder streams, and no growth form was linked with high temperature on the RCCA graph (Fig. 5). These streams had the highest abundance of the scraper, *R. peregra*, an effective herbivore (Steinman 1996). The grazing from *R. peregra* may be responsible for low growth form diversity in the warmest streams, analogous with reindeer in tundras, which are responsible for lowering biodiversity where they are found (Väre et al. 1995, Austrheim and Eriksson 2001). *R. peregra* was absent from the coldest streams, but Chironomidae were dense. Chironomidae are a diverse group, and many species are collectors and gatherers and feed on the loosely attached and loose periphyton (Allan 2006). The most abundant species was a collector–gatherer rather than an efficient grazer–scraper. Studies on the effects of chironomids on epilithic algae are fewer than those focused on gastropods and show both positive and negative effects on epilithic biomass (Steinman 1996). We found no association between environmental features, diatom assemblages, and density of invertebrates and therefore conclude that grazing did not influence the abundance of diatoms, although it may influence the community composition.

Terrestrial habitats contain macroherbivores that could be regarded as an analogue to *R. peregra* because they are much larger than their prey. In tundra, reindeer and musk oxen are known to strongly impact some areas by grazing if their density is high enough. These animals often occur in large herds on small tundra areas. Reindeer can alter the vegetation by transforming it from dwarf shrubs and moss to grasslands (Olofsson 2006). Macroherbivore grazers in the boreal forest are much sparser and therefore may have less influence on the vegetation. Paradoxically, the warm streams mimicked tundra while the cold streams resembled boreal forest in the effects of large grazers. Warm streams that contain abundant bryophytes had high densities of *R. peregra* and *S. vittatum* in contrast to the forests that contain few large herbivores.

Diatoms attached on their raphe, such as *Cocconeis* spp., and are thus flattened to the substratum, were one of the most common growth forms where grazing was most intense (Steinman 1996, Yallop and Kelly 2006). *Cocconeis placentula* was weakly favoured by higher temperature while *Cocconeis pediculus* was more linked to lower temperature. These diatoms (*Cocconeis* spp., *Rhopalodia* spp., and *Epithemia* spp.) had similar abundances in most streams and were thus not related to grazing pressure or other factors. Diatoms were abundant in all the streams, suggesting that grazing did not significantly limit the production.

The third hypothesis, that in streams with low grazing pressure we would find high abundance of bryophytes, was not accepted. The warmest streams contained the greatest abundance of bryophytes (*F. antipyretica*) but the greatest density of the most efficient grazer, *R. peregra*. Bryophytes are the "trees" of these streams and form dense biomass, potentially shading the underlying vegetation (small algae and Cyanobacteria). *F. antipyretica* overwinters in the streams, as conifers do in boreal forests. Tall trees are usually immune to grazing while the vegetation on the forest floor is easily grazed; thus, grazing does not limit the tall trees of the forests or the bryophytes of the streams, but it affects the vegetation on the forest floor and epilithic algae in streams.

We accepted the fourth hypothesis that higher temperature would favour small diatom taxa while large diatom taxa would be more common in cold streams. With climate change, one of the most prominent effects has been shifts of species toward the poles and higher altitudes. But other effects of climate change have been detected, such as smaller body size and decreased size at maturity (Daufresne et al. 2009). Body size influences fecundity, population growth rate, and competition interaction. Few studies have been conducted on ectotherm species, although they are the majority of all species on earth. Daufresne et al. (2009) conducted a metaanalysis of the effects of increased temperature on ectotherm species and found significant influences from increased temperature on the body size of various organisms (fish, phytoplankton, and copepods). The diatoms in our streams showed a pattern of smaller diatoms in warmer streams and larger diatoms in colder streams. The likely reasons for this are not clear. Smaller organisms have greater surface to volume ratios, and among microorganisms they may have advantages in nutrient uptake; however, the availability of nutrients is likely to be greater with increased temperature owing to higher recycling rates, although denitrification rates may possibly increase. These streams are nitrogen limited (Friberg et al. 2009). Some diatoms with nitrogen-fixing endosymbionts were present (e.g., *Epithemia* sp.), but no

clear relationship between their abundance and nitrogen concentrations was found, largely because the latter were relatively uniform.

Thus, 2 of our hypotheses were rejected and 2, those concerning the lesser growth-form diversity with grazing and the higher incidence of smaller sized diatoms in warmer streams, were accepted. The cold streams paradoxically mimicked the boreal forest rather than the tundra regarding diversity and growth form diversity, and the warm streams mimicked the tundra. Grazing by snails seems to have reduced diatom diversity and growth form diversity, again in contrast to the tundra–Boreal forest analogy where macroherbivore grazing is more significant in the cooler habitat. Grazing did not suppress the abundance of diatoms, however, and thus the streams are considered to be bottom-up controlled rather than top-down controlled. Temperature and grazing pressure are important drivers on the diatom communities because they are on higher plants in terrestrial habitats; however, the effects are not parallel, and our results suggest that using microorganism communities as surrogates for larger systems should not be uncritically accepted.

Acknowledgements

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Appendix

Appendix 1. List of diatom species observed in the eight streams. Traits, trait codes and RCCA codes (for species that had over 0.5% abundance in one or more streams) are given.

Species/taxa	Trait	Trait code	RCCA code
Achnanthes spp Bory	Mucilage pad	MP	acspp
Achnanthes minutissima Kütz.	Mucilage pad	MP	amin
Achnanthes exigua (Pantocsele 1902)	Mucilage pad	MP	aexi
Achnanthes lanceolata Bréb. (Grunow)	Mucilage pad	MP	alan
Amphora spp (Ehrenberg) Pantocsek 1902	Attached on raphe	AR	-
Amphora ovalis Kützing (1844)	Attached on raphe	AR	-
Amphora pediculus (Kützing) Grunow in A. Schmidt, 1875	Attached on raphe	AR	aped
Ceratoneis arcus (Ehrenb.) Kütz. 1844	Mucilage pad	MP	carc
Cocconeis spp. Ehrenb.	Attached on raphe	AR	cospp
Cocconeis placentula Ehrenb.	Attached on raphe	AR	cpla
Cocconeis pediculus Ehrenb.	Attached on raphe	AR	cped
Cymbella spp. C. A. Agardh	Attached with mucilage stalk/ **cells in mucilage tube**	MT	-
Cymbella silesiaca (Bleisch) Krammer & Lange-Bertalot 1986	Attached with mucilage stalk/ **cells in mucilage tube**	MT	-
Cymbella sinuata W. Gregory	Attached with mucilage stalk/ **cells in mucilage tube**	MT	-
Cymbella minuta Hilse	Attached with mucilage stalk/ **cells in mucilage tube**	MT	-
Diploneis ovalis	Motile	MO	-
Diatoma mesodon Kuetzing, 1844	Attached with mucilage	CF	dmes

Species	pad/Chain forms - Filaments		
Diatomella balfouriana (W. Smith) Greville (1855)	Mucilage pad	MP	dbal
Epithemia spp. Kütz.	Attached on raphe	AR	epspp
Epithemia sorex Kütz.	Attached on raphe	AR	-
Epithemia adnata Kütz.	Attached on raphe	AR	-
Epithemia turgida (Ehrenb.) Kütz.	Attached on raphe	AR	etur
Eunotia spp. Ehrenb.	Motile	MO	-
Fragilaria spp. Lyngb.	Attached with mucilage pad/Chain forms - Filaments	CF	-
Fragilaria pinnata Ehrenb.	Attached with mucilage pad/Chain forms - Filaments	CF	fpin
Fragilaria capucina Desm.	Attached with mucilage pad/Chain forms - Filaments	CF	fcap
Fragilaria virescens Ralfs	Attached with mucilage pad/Chain forms - Filaments	CF	fvir
Frustulia spp. Rabenh.	Motile	MO	-
Frustulia rhomboids Ehrenberg	Motile	MO	-
Frustulia vulgaris Thwaites	Motile	MO	-
Gomphonema spp. Ehrenberg	Mucilage stalk	MS	-
Gomphonema acuminatum Ehrenberg(1832)	Mucilage stalk	MS	-
Gomphonema augur Ehrenberg (1840)	Mucilage stalk	MS	-
Gomphonema constrictum Ehrenberg (1832)	Mucilage stalk	MS	-
Gomphonema minutum	Mucilage stalk	MS	-
Gomphonema olivaceum (Lyngbye) Desmazieres	Mucilage stalk	MS	goli
Gomphonema parvulum (Kütz) Kütz	Mucilage stalk	MS	gpar
Gomphonema pumilum (Grunow) Reichardt &	Mucilage stalk	MS	gpum

Lange-Bertalot			
Gomphonema rhombicum Fricke	Mucilage stalk	MS	grho
Gomphonema angustatum (Kütz) Rabenh.	Mucilage stalk	MS	-
Gomphonema trucatum Ehrenberg (1832)	Mucilage stalk	MS	-
Melosira varians C. Agardh	Chain forms - Filaments	CF	mvar
Meridion circulare (Grev.) C. Agardh	Mucilage pad	MP	mcir
Navicula spp Bory	Motile	MO	naspp
Navicula placentula (Ehrenb.) Kütz	Motile	MO	npla
Navicula radiosa Kütz	Motile	MO	-
Neidium spp Pfitzer (1871)	Motile	MO	-
Nitzschia spp. Hass.	Motile	MO	nispp
Nitzschia aequorea Hustedt	Motile	MO	naeq
Nitzschia dissipata (Kütz) Grunow	Motile	MO	ndis
Nitzschia inconspicua Grunow	Motile	MO	ninc
Nitzschia palea (Kütz) W. Sm	Motile	MO	npal
Nitzschia amphibia Grunow	Motile	MO	-
Opephora martyi Hérib.	Mucilage pad	MP	-
Pinnularia spp Ehrenberg (1843)	Motile	MO	-
Rhoicosphenia curvata (Kütz) Grunow	Mucilage stalk	MS	rcur
Rhopalodia gibba (Ehrenb.) O. Müll.	Motile	MO	-
Surirella spp. Turp.	Motile	MO	suspp
Synedra spp Ehrenberg, 1830	Mucilage pad	MP	syspp
Synedra rumpens Kütz	Mucilage pad	MP	-
Synedra ulna (Nitzsch) Ehrenb.	Mucilage pad	MP	-
Synedra arcus (Kütz)	Mucilage pad	MP	-
Code 66 (unidentified very small Achnanthes spp)	Mucilage pad	MP	Code 66
Code 99 (unidentified very small Nitzschia spp)	Motile	MO	-

Paper 3

How will increased temperature and nutrient enrichment affect primary producers in sub-Arctic streams?

RAKEL GUDMUNDSDOTTIR*,†, JON S. OLAFSSON‡, SNAEBJORN PALSSON*,
GISLI M. GISLASON* AND BRIAN MOSS§
*Institute of Biology, University of Iceland, Sturlugata, Reykjavík, Iceland
†Department of Biology, Norwegian University of Science and Technology (NTNU), Trondheim, Norway
‡Institute of Freshwater Fisheries, Keldnaholt, Reykjavík, Iceland
§School of Environmental Sciences, Nicholson Building, University of Liverpool, Liverpool, U.K.

SUMMARY

1. Spring-fed streams, with temperatures ranging from 7.1 to 21.6 °C, in an alpine geothermal area in SW Iceland were chosen to test hypotheses on the effects of nutrients and temperature on stream primary producers. Ammonium nitrate was dripped into the lower reaches of eight streams, with higher reaches being used as controls, during the summers of 2006 and 2007. Dry mass of larger primary producers, epilithic chlorophyll *a* and biovolumes of epilithic algae were measured.

2. Bryophyte communities were dominated by *Fontinalis antipyretica*, and biomass was greatest in the warmest streams. *Jungermannia exsertifolia*, a liverwort, was found in low densities in few samples from cold streams but this species was absent from the warmest streams.

3. Nutrient enrichment increased the biomass of bryophytes significantly in warm streams. No effects of the nutrient addition were detected on vascular plants. The biomass of larger filamentous algae (mainly *Cladophora* spp.) was significantly increased by nutrient enrichment in cold streams but reduced by nutrients in warm streams. Thalloid cyanobacteria (*Nostoc* spp.) were not affected by nutrients in cold streams but decreased with nutrient addition in warm streams. Epilithic algal chlorophyll *a* was increased by nutrients in all streams and to a greater extent in 2007 than in 2006. Nutrient addition did not affect the epilithic chlorophyll *a* differently in streams of different temperatures.

4. There were small differential effects of nutrients, influenced by pH and conductivity, on different epilithic algal groups.

5. As global temperatures increase, animal husbandry and perhaps crop agriculture are likely to increase in Iceland. Temperature will directly influence the stream communities, but its secondary effects, manifested through agricultural eutrophication, are likely to be much greater.

Keywords: bryophytes, Climate change, epilithic algae, *in situ* experiments, nitrogen

Introduction

Arctic and sub-Arctic regions are likely to face more dramatic effects of climate change than lower lati-

tudes. An air-temperature increase of 3–4 °C over the last 50 years has already been reported, and a further increase of 3–9 °C is predicted over the next 100 years (ACIA, 2004; Chapman & Walsh, 2007). Changes associated with existing warming for organisms, such as amphibians, birds, butterflies, alpine herbs and trees (Parmesan & Yohe, 2003; Daufresne, Bady & Fruget, 2007), include range shifts (of organisms

Correspondence: Rakel Guðmundsdóttir, Institute of Biology, University of Iceland, Sturlugata 7, IS–101 Reykjavík, Iceland.
E-mail: rakelgud@hi.is

moving closer to the poles) and migration of animals and plants (Walther *et al.*, 2002; Parmesan & Yohe, 2003). Attention is now turning to the likely future effects of warming by several degrees.

Increase in air temperature will be directly reflected in the temperature of fresh waters (Hogg & Williams, 1996) and indirectly will increase nutrient availability for primary producers (Rouse *et al.*, 1997; Moss *et al.*, 2003; Jeppesen *et al.*, 2010) owing to increased bacterial mineralisation of soils or increased delivery through allochthonous particulate matter derived from the increased growth of riparian vegetation (Rouse *et al.*, 1997; Benstead *et al.*, 2005). In the treeless regions of the Arctic and sub-Arctic, streams are less affected by shade and thus more likely to be nutrient limited than light limited, as are streams in forested areas. There may also be greater eutrophication as areas previously undeveloped become farmed as temperatures rise. The interaction between temperature and nutrient availability is thus of great interest in sub-Arctic streams and is the main topic of this study.

Most of the studies on the combined effects of nutrients and warming have been on lakes at lower latitudes. Some have been conducted in experimental mesocosms (artificial ponds) that could be artificially heated or were sited along temperature gradients (McKee *et al.*, 2003; Moss *et al.*, 2003; van Doorslaer *et al.*, 2007; Feuchtmayr *et al.*, 2007; Ventura *et al.*, 2008). Owing to the nature of streams and rivers, where the retention time is usually short, comparable experimental facilities in artificial stream channels are costly to heat and maintain. This study examines the interaction between temperature and nutrient addition in natural streams where such costs are avoided and can thus give insights into a future scenario of direct warming coupled with the secondary eutrophication effects that warming will have in changing land use towards greater farming use.

Excessive algal and cyanobacterial growth is common in many enriched streams at lower latitudes (Rier & Stevenson, 2006) where, because temperature is higher, nutrients may have greater impacts than in the cold habitats of higher latitudes (Walther *et al.*, 2002; Hudson & Henry, 2009). Thus, the combined effects of temperature and nutrients are of particular interest in sub-Arctic and Arctic landscapes. Changes at a finer scale are also of consequence. For instance, diatoms

(especially stalked ones) tend to respond quickly to increased nutrients owing to their high growth rate and better illuminated position in the algal biofilm (De Nicola *et al.*, 2006). Green algae such as *Cladophora* sp (Kütz.) are also known to respond quickly when nutrients are added (McKee *et al.*, 2003; Veraart *et al.*, 2008). Cyanobacteria tend to respond differently. They may increase or decrease (De Nicola *et al.*, 2006). In nitrogen-limited waters, nitrogen-fixing cyanobacteria tend to be dominant because they can use N_2 when other algae and cyanobacteria cannot (Howarth *et al.*, 1988).

Experimental studies on nutrient effects on primary producers in streams include the studies by Peterson *et al.* (1993), Slavik *et al.* (2004) and Benstead *et al.* (2005). Several methods have been used including point source experiments with nutrient diffusing substrata (Fairchild & Lowe, 1984; Fairchild, Lowe & Richardson, 1985; Friberg *et al.*, 2009), enrichment of stream water by using flow-through enclosures (Triska *et al.*, 1989; Rosemond, 1993), whole stream manipulations (Triska *et al.*, 1989) and integrated bioassays (Pringle, 1987). All of these studies concerned fertilisation but not the joint impact of temperature and nutrient enrichment, and a major disadvantage was their relatively high cost. There was also a risk that naturally interacting factors would not be detected in completely controlled experiments.

Our study minimises these problems, because we conducted nutrient enrichment experiments within a geothermal area containing replicate streams with different water temperatures from natural heat sources (Friberg *et al.*, 2009). Within this natural gradient, we could manipulate the nutrient supply in the lower reaches of each stream, using the upper reaches as controls, without having to manipulate temperature.

We tested three specific hypotheses: (i) The biomass of bryophytes (*F. antipyretica*), vascular plants and larger filamentous and thalloid forms of algae would be increased by nutrient enrichment. (ii) Epilithic chlorophyll *a* accrual would be increased by nutrient enrichment and that the effects would be greater with increasing temperature (Walther *et al.*, 2002; Hudson & Henry, 2009). (iii) The biovolumes of diatoms and green algae would increase with nutrient enrichment, while that of nitrogen-fixing cyanobacteria would decrease.
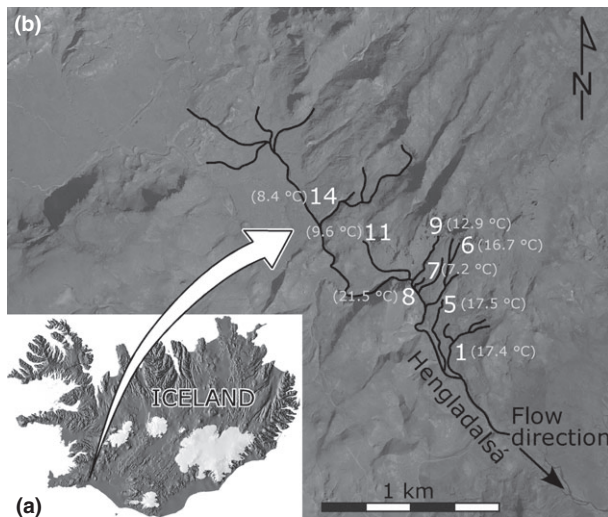
Fig. 1 Map of Iceland (a) and the eight selected streams (b) within Hengill geothermal area. Each stream number is annotated on the map. Streams 1, 5 and 8 are classified as warm, streams 6 and 9 as intermediate and streams 7, 11 and 14 as cold. Mean summer temperatures of individual streams are presented on the map.

Methods

Study area

Experiments were carried out in the Hengill geothermal area SW Iceland (N 64°03; W 21°18; 350–420 m a.s.l.), which is an active volcanic area covering 173 km^2, with a mean air temperature of 8–10 °C in July and annual precipitation of 1870–3080 mm (Elmarsdóttir & Vilmundardóttir, 2009). It has numerous small streams (rheocrenes) that emerge from hillsides and run along a flat valley floor on hyaloclastite bedrock to join the River Hengladalsá (Fig. 1). The terrestrial vegetation is mostly monocotyledonous (e.g. *Carex bigelowii* Torr. ex Schwein, *Carex nigra* (L.) Reichard, *Agrostis capillaris* (L.), *Eriophorum angustifolium* Honckeny), with mosses and few shrubs (*Empetrum nigrum* L. and *Vaccinium uliginosum* L.) (Guðjónsson & Egilsson, 2006). Trees are absent and shrubs sparse, so there is little or no coarse particulate allochthonous input to the streams (Friberg *et al.*, 2009; Woodward *et al.*, 2010); though, there may be a dissolved organic supply from the soils.

There are about twenty small streams in our study area, of which fifteen have been surveyed (Friberg *et al.*, 2009) with eight used in this study (Fig. 1). The selected streams had broadly similar physical and chemical features except for temperature. The stream temperatures ranged from 4 to 42 °C (Friberg *et al.*, 2009). The streams were first order, usually 30–75 m in total length from source to their confluence with the river; three were classified as warm (18.4–21.6 °C), two as intermediate (12.8–16.9 °C) and three as cold (7.1–10.9 °C) according to mean summer temperatures. The higher temperatures, which are above ambient air temperature, are not only attributable to direct upwelling of geothermal water and gases, but also to ground water that absorbs heat from the underlying bedrock (Árnason *et al.*, 1967). The temperature regime of the cooler streams was comparable with that of spring-fed streams at this altitude and latitude, corresponding with typical alpine sub-Arctic streams, whereas the warm streams were considered analogues of streams at lower latitudes under current and warmed conditions. Confounding effects were minimal because the streams were close together in a small area (within a 1.5-km reach along the River Hengladalsá) but our design allowed us to investigate the effects of small differences in chemical composition.

There was only one fish species in the streams, brown trout *(Salmo trutta* L.). Scrapers and filter feeders were the main functional feeding groups of invertebrates in the warmer streams (Friberg *et al.*, 2009). However, collectors and gatherers were dominant in colder streams (Friberg *et al.*, 2009). Chironomids comprised 16 of 35 invertebrate taxa and were more common in the colder streams, while the blackfly *Simulium vittatum* (Zettersteth) and the snail *Radix peregra* (Müller) dominated the warmer streams. The density of invertebrates was generally higher in the colder streams than in the warmer ones, whereas taxon richness was greatest in the streams of intermediate temperature (Friberg *et al.*, 2009).

Experimental design

Each stream was divided into an upper control reach (15–25 m), a buffer zone (5 m) between reaches, and a lower treatment reach (15–25 m). Earlier findings indicated that the streams were nitrogen limited (Friberg *et al.*, 2009), and therefore, ammonium nitrate (NH_4NO_3) was used to enrich the treatment reaches. The aim was to increase the nitrogen concentration three to five times compared with background. This is comparable with eutrophic streams elsewhere. On the

bank of each stream, two 120-L plastic barrels, containing ammonium nitrate solution, were installed. A plastic tube ('Legris' polyurethane, 3- or 4-mm inside diameter with 1-mm wall thickness) was connected to each barrel through a peristaltic electric pump (24V 'THOMAS' nr: 2052102) and then from the pump to the stream. Each pump was powered by a car battery, which was recharged by a solar panel. The nutrients were dripped into each stream for 10 s with 40-s intervals between additions. The rate of nutrient release was determined according to the discharge of each individual stream, with an average release of 4–8 mL per minute of an appropriate concentration. The concentration of the ammonium solution was fixed and inversely proportional to stream discharge. For example, 27.5 g L^{-1} solution was used for the stream with the lowest discharge, and 206 g L^{-1}, for the stream with the highest discharge (Table 3). The nutrients in the barrels were replenished every 2 weeks, and the experiments were conducted from May–September 2006 and May–August 2007.

Sampling

All chemical and physical variables and most of the biological variables were measured monthly, from May to September in 2006 and August in 2007. A multiprobe sonde, YSI 600 XLM (Yellow Springs Instrument, Yellow Springs, OH, U.S.A.), was used to measure temperature, pH and conductivity (standardised at 25 °C), on every sampling occasion.

Water samples for chemical analysis were collected in 1-L polyethylene bottles from each reach within a stream and were frozen for storage before further analysis. Samples for total phosphorus (TP), total nitrogen (TN) and silicate (expressed as Si) were analysed at the National Environmental Research Institute in Denmark a few weeks after sampling. Analysis was according to standard protocols (Danish Standard DS 292/DS/EN 6878, 1985) for TP and DS 221/DS/EN 11905, 1975 for TN. A UV spectrophotometer (1600 Shimadzu corporation, Tokyo, Japan) was used to measure TP and TN concentrations, following digestion with persulphate to phosphate and nitrate, respectively. Phosphorus was then converted to a molybdenum blue complex, measured at 880 nm, and nitrate by reduction to nitrite and diazotisation to a dye measured at 545 nm. Ammonium concentration was measured at 630 nm using a

salicylate colorimetric method and a Lange ion 500 spectrophotometer (Antweiler, Patton & Taylor, 1996). Silicates were measured at 810 nm as a molybdate complex under acid conditions (Koroleff, 1983).

On each sampling occasion, 13–18 cobbles or small boulders, 5–35 cm diameter, were collected from each reach within each stream and 10–15 of them were used to measure chlorophyll *a*. The remaining three stones from each reach were used to estimate the biovolume of algae. The stones were wrapped in aluminium foil immediately after they had been retrieved from the streambed to avoid light damage during transfer to the laboratory. Biomass and community composition of bryophytes, macrophytes and large algae were estimated by harvesting all vegetation within randomly placed 14.5 × 14.5 cm quadrats within a larger grid. Five replicate quadrats were harvested within each reach on 24 July 2007. The samples were kept frozen until they were processed in the laboratory.

Laboratory analyses

Chlorophyll a. The stones used for epilithic chlorophyll analyses were immersed in 96% ethanol and kept at 5 °C for 24 h in total darkness. The ethanol was filtered and its volume measured. The exposed area of each stone was covered with a single layer of aluminium foil, which was then weighed and a mass/area conversion factor used to determine the area that algae had covered (Hauer & Lamberti, 2007). Absorption of ethanol extracts was measured at 665 and 750 nm using a Lange ion 500 spectrophotometer. A wavelength of 750 nm was used for correction of any residual turbidity after filtration, and the wavelength 665 nm is the absorption maximum wavelength in the red end of the spectrum for chlorophyll *a*. Values for chlorophyll *a*, determined after Talling & Driver (1961), are expressed as μg cm^{-2}.

Biovolume of algal groups. Attached microalgae were collected by brushing determined areas of three stones from each reach with a medium-firm toothbrush. The brushed area of each stone was measured by outlining it on overlain squared tracing paper. The area was brushed until no more algal film could be detected, and the brushed area had a grainy, non-mucilaginous texture. The brushings were preserved in Lugol's iodine solution. Groups of eukaryotic algae and

cyanobacteria were quantified as biovolume. The sample volume was measured, and a subsample of 50–500 μL (depending on the density of algae) was placed in a counting chamber 24 mm in diameter and left for 20–24 h before identification and counting under a Wild M50–58257 inverted microscope at $\times$ 400 magnification. A minimum of 200 organisms were counted along measured transects. Biovolume was estimated according to Hauer & Lamberti (2007) and expressed as μL cm^{-2}.

Diatom identification was based on Krammer & Lange–Bertalot (1986, 1988, 1991a,b) and Hustedt (1930). Other algal groups and cyanobacteria were identified from the study by Prescott (1978).

Bryophytes and other large primary producers. Harvested samples of bryophytes, vascular plants and macroalgae were frozen for storage, then thawed, rinsed to remove sand and gravel and sorted into groups. Keys from The Icelandic Institute of Natural History were used for bryophyte identifications (Jóhannsson, 1996, 2001). Macrophytes were identified by Stefánsson (1948). After sorting, the samples were dried for at least 48 h at 60 °C and weighed.

Statistical analysis

Chlorophyll *a*, biomass and biovolume values were log-transformed before analysis owing to their non-normality. The statistical software R (version 2.11.1) was used for data analysis (R Development Core Team, 2006). Split Plot ANOVA was used to detect whether there was a significant difference between both response and predictor variables. For most of the analyses, we used mixed random effect models (Crawley, 2002). This was because we had a mixture of fixed and random factors and we wanted to look at time effects on the response variables. It is assumed that the fixed effects are unknown constants but the random effects govern the variance–covariance structure of the response variable (Crawley, 2002). An individual stream was treated as a random factor because we could expect some relationships between observations from the same stream as well as relationships between observations from different streams. Nutrient treatment was treated as a binary categorical variable, with 0 as no nutrients added and 1 as nutrients added. This was carried out, even though exact values (mg L^{-1} NH$_4$–N) were obtained

once per month, because the addition was continuous. Temperature and other physical and chemical features were treated as continuous variables in the mixed effect models. Polynomial regression was used to determine the relationship between bryophyte biomass and temperature.

Results

Physical and chemical features of the streams

Overall, warmer streams had higher pH, conductivity and silicate, but streams were uniform and low in nitrogen and phosphorus content. The nutrient treatments gave the intended differences without significantly changing other variables. The summer mean temperature of the streams ranged from 7.1 °C in stream 7 to 21.6 °C in stream 8 (Table 1). When one stream was removed as an outlier, we found a significant positive correlation between temperature and conductivity ($r = 0.89$, $t = 4.296$, $P = 0.008$) in the control reaches. There was no significant difference between the control and treatment reaches for conductivity (ANOVA, $P > 0.05$) (Table 2).

Streams were of similar pH, and there were no significant differences between control and treatment reaches within each stream (Table 2). Temperature and pH were significantly negatively correlated ($r = -0.77$, $t = -2.97$, $P = 0.025$). Concentrations of the measured nutrients (total *N*, total *P* and Si) were low in control reaches in all streams (Table 3). Silicate was correlated with temperature when an outlier (stream 11) was removed from the analysis ($r = 0.96$, $t = 7.52$, $P = 0.0007$). Silicate was also strongly correlated with conductivity ($r = 0.76$, $t = 2.89$, $P = 0.03$).

Table 1 Mean temperature (SE) (°C) of the eight streams during the summer 2006 (May–September) and 2007 (May–August)

	Temperature (°C)	
Streams	2006	2007
7	7.1 (0.25)	7.3 (0.2)
14	8.35 (0.65)	9.4 (1.7)
11	8.6 (0.72)	10.9 (1.5)
9	13.1 (0.33)	12.8 (0.8)
6	16.5 (0.2)	16.9 (0.7)
1	16.9 (0.61)	18.4 (2.0)
5	17.3 (0.33)	17.6 (0.6)
8	21.6 (0.13)	21.4 (0.3)

Table 2 Conductivity (μS cm^{-2} at 25 °C) and pH of the different control and treatment reaches

| | pH | | | | Conductivity (μS cm^{-1} at 25 °C) | | | |
| | 2006 | | 2007 | | 2006 | | 2007 | |
Streams	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control
7	8.40 (0.13)	8.35 (0.12)	8.62 (0.14)	8.61 (0.17)	108 (10.3)	96 (0.9)	107 (3.0)	102 (3.0)
14	8.35 (0.16)	8.33 (0.18)	8.19 (0.31)	8.26 (0.28)	187 (30.0)	204 (2.8)	241 (21.0)	252 (22.0)
11	8.03 (0.14)	8.04 (0.10)	8.49 (0.11)	8.39 (0.13)	489 (58.1)	497 (55.5)	542 (58.0)	601 (32.5)
9	8.30 (0.03)	8.42 (0.07)	8.42 (0.01)	8.55 (0.09)	207 (0.5)	194 (1.3)	208 (9.5)	209 (11.0)
6	7.99 (0.14)	8.01 (0.15)	8.12 (0.23)	8.38 (0.16)	251 (2.0)	231 (8.9)	238 (12.5)	259 (8.0)
1	7.85 (0.14)	7.88 (0.12)	7.87 (0.32)	7.87 (0.36)	231 (13.9)	229 (13.4)	246 (19.5)	257 (18.5)
5	8.00 (0.13)	7.92 (0.18)	8.47 (0.06)	8.39 (0.09)	235 (7.2)	234 (6.3)	248 (12.5)	258 (8.5)
8	7.99 (0.06)	7.92 (0.09)	8.22 (0.14)	8.17 (0.15)	266 (7.2)	280 (15.2)	293 (7.0)	302 (3.5)

The sampling was carried out monthly from May until August 2006 and 2007, yielding eight-nine-point measurements for each reach within each stream. Values are means with standard errors of the means in parentheses. Streams are in ascending order of mean temperature.

Table 3 The discharge and concentrations of N$_{tot}$, P$_{tot}$ and silica in the eight streams studied

Streams	Discharge (L s^{-1})	Total nitrogen (mg L^{-1})	Total phosphorus (mg L^{-1})	Si (mg L^{-1})
7	0.29 (0.18)	<0.1	0.021	6.4
14	0.99 (0.29)	<0.1	0.008	8.7
11	0.43 (0.17)	0.1	0.016	18.7
9	0.04 (0.03)	<0.1	0.027	9.0
6	0.94 (0.42)	0.19	0.026	14.8
1	1.77 (0.72)	<0.1	0.022	15.4
5	2.20 (1.03)	0.19	0.023	15.0
8	3.54 (1.42)	<0.1	0.029	16.1

The discharge values are based on 10 replicate measurements on 11 May 2007, showing the mean and standard errors. The nutrient concentration measurements are based on data from 24 August 2006 in the control reaches of all eight streams. Streams are in order of ascending mean temperature.

Ammonium (NH$_4$) concentration was low in control reaches and was not correlated with temperature. Overall, there was a consistent pattern of concentrations of NH$_4$–N, typically two to seven times greater in the treatment reaches than in control reaches (Fig. 2).

Nutrient and temperature effects on bryophytes, macrophytes and macroalgal groups

Primary producer species restricted to warmer streams were *Fontinalis antipyretica* (Hedw.), *Alopecurus pratensis* (L.), *Equisetum fluviatile* (L.) and *Callitriche stagnalis* (Scop.). *Jungermannia exsertifolia* (Dum.), *Chaetophora* sp and *Epilobium alsinifolium* (Vill.) were restricted to cold streams. *Nostoc* was found in all temperature regimes but not in all streams. The intermediate streams and the warm streams had the greatest abundance of *Nostoc* sp.

Overall, bryophyte biomass increased with temperature but was reduced by nutrient enrichment (Fig. 3). Biomass of *Jungermannia exsertifolia* was very low, even in the cold streams, and it was absent from the warmer streams. *Fontinalis antipyretica* was very dense in the warmest streams.

Nutrient enrichment significantly interacted with temperature and conductivity such that bryophyte biomass increased with nutrient enrichment but decreased with conductivity in warmer streams (Table S1).

The biomass of vascular plants was low with no significant differences between streams or between control and treatment reaches within streams (ANOVA, $F = 0.459$, $P = 0.501$; $F = 0.332$, $P = 0.567$, respectively). There were no significant influences from any of the environmental variables on the vascular macrophytes. No main effects of nutrient addition or temperature on macroscopic algae were found but biomass declined in the warmer streams when the nutrients were added ($b = -0.055$, $t = -2.41$, $P = 0.019$).

Nutrient addition increased filamentous green algae ($b = 1.9$, $t = 2.16$, $P = 0.03$) and, as for total macroalgal biomass, filamentous algae declined with increasing temperature and nutrient enrichment ($b = -0.11$, $t = -2.27$, $P = 0.03$). Neither conductivity nor pH was correlated with the biomass of macroscopic algae ($P > 0.05$).
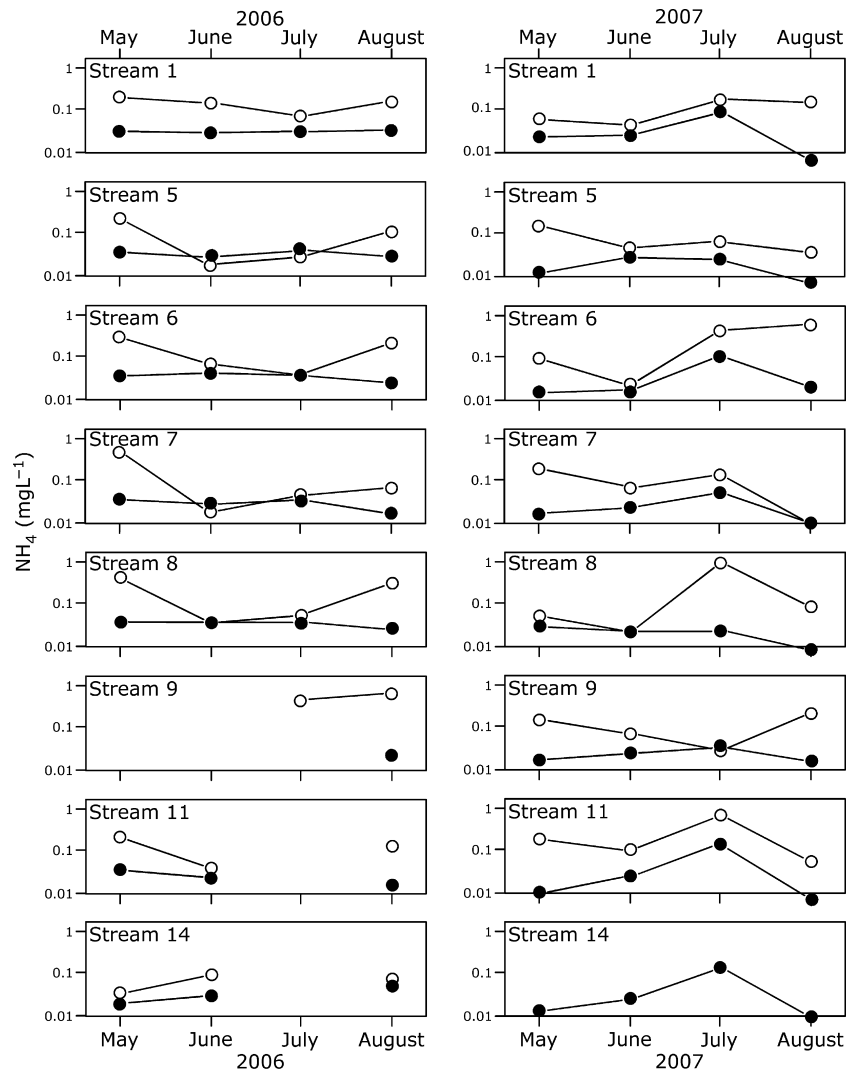
Fig. 2 The concentration of ammonium (NH$_4$-N, mg L^{-1}) in the eight streams. Open circles show ammonium concentration in the treatment reaches, and closed circles show the concentration in the control reaches. Sampling was carried out monthly from May until August.

Nutrient and temperature effects on epilithic chlorophyll a concentration

Chlorophyll *a* was significantly higher in treatment reaches than in control reaches in both years, with stronger effects in 2007 (Table S2; Figs 4 & 5). Temperature had no influence on chlorophyll *a* in either year (ANOVA; *P* > 0.05), and other physical and chemical factors had only minor effects then only in 2007. Chlorophyll *a* was not significantly different between different sampling months in 2006, and there was no interaction between treatment and temperature. There was a more irregular overall trend in concentration in 2007, expressed in both treatment and control reaches (Fig. 5), and an interaction (Table S2) in which temperature reduced the chlorophyll *a* in treatment reaches.

Conductivity had no significant main influence on chlorophyll *a* (*P* = 0.067) in control reaches in either year, but interacted with treatment in 2007 to increase chlorophyll *a* (*b* = 0.0011, *t* = 2.44, *P* = 0.015). pH had no influence on chlorophyll *a* in either year.

Influences on diatom biovolume

Dominant diatom species in the warm streams were *Rhoicosphenia curvata* (Kützing) Grunow, *Gomphonema parvulum* (Kützing) and *Cocconeis placentula* (Ehrenberg). The intermediate streams were dominated by *Gomphonema rhombicum* (Fricke), *Gomphonema pumilum* (Grunow), *Cocconeis placentula*, *Cocconeis pediculus* (Ehrenberg) and *Achnanthes lanceolata* (Brébisson) Grunow. The highest diatom diversity occurred in the coldest streams with dominating species such as
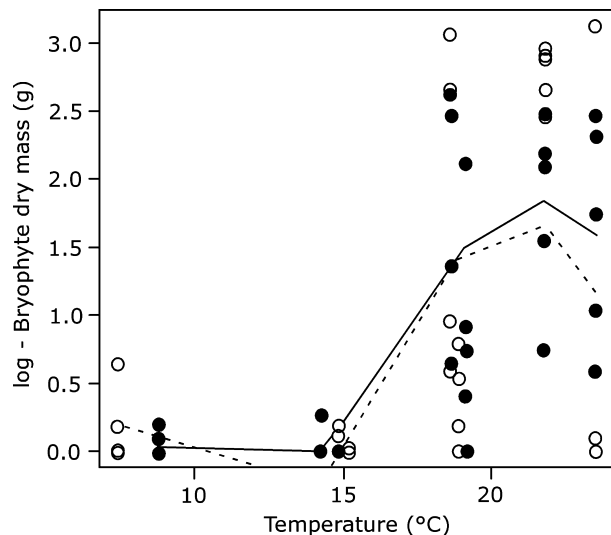
Fig. 3 Log dry mass of bryophytes (g) in relation to temperature in the eight streams. Circles show individual measurements of samples obtained from control (○) and treatment (●). The two lines present predictions based on cubic regression of log-transformed dry mass (g) for control reaches (- - -) and treatment reaches (—). The cubic responses of bryophytes to temperature were controls: $b^1 = 3.37$, $t = 3.78$, $P < 0.001$; $b^3 = -2.27$, $t = -3.11$, $P < 0.004$ and treatments: $b^1 = 4.35$, $t = 6.22$, $P < 10^{-6}$; $b^3 = -2.10$, $t = -3.00$, $P < 0.005$.

G. rhombicum, *G. pumilum*, *Meridion circulare* (Grev.), *Achnanthes minutissima* (Kützing), *Diatoma mesodon* (Ehrenberg) Kützing, *Nitzschia palea* (Kützing), *Nitzschia inconspicua* (Grunow) and *Fragilaria capucina* Desmazière. In all streams, *G. rhombicum* and *G. pumilum* reacted rapidly to nutrient enrichment, and *A. minutissima* was also in one of the coldest streams (stream 11). *Rhoicosphenia curvata* was abundant in the treatment reach of the warmest stream (stream 8).

The diatom biovolume in treated reaches was significantly higher than in the control reaches in both years (ANOVA; $F = 57.60$, $P < 0.001$), but effects of other variables were minor and confined to individual months. Observed changes in diatom biovolume were similar for summers of 2006 and 2007 ($P = 0.56$); we therefore analysed the 2 years together. The diatom biovolume was significantly different between individual months, (ANOVA; $F = 8.11$, $P < 0.001$ and linear mixed effect model; $b = -0.28$, $t = -2.59$, $P = 0.01$) with some interaction between environmental variables and treatment (Table S3).

Influences on green algal biovolume

The assemblages of green algae in the streams were diverse. Most common in the intermediate and warm streams were genera such as *Cladophora* spp., *Spirogyra* spp. and *Mougeotia* spp. *Chaetophora* spp. were found in two of the coldest streams (11 and 14) but were absent from other streams. *Enteromorpha* spp. was found in the cold streams 7 and 9. In all streams, unicellular forms of green algae were common.

Green algae responded differently to nutrient enrichment between years with fewer effects from environmental features in 2006 compared with 2007. In 2006, no significant influences of temperature on the biovolume of green algae were detected (Table S4) but ammonium and pH reduced biovolumes in August. In May 2007, there was a significant negative influence of temperature on green algal biovolume (Table S5) but not in other months. In May and June 2007, the green algal biovolumes increased significantly in treated reaches of the warmer streams (positive interactions between temperature and nutrients), and in May, conductivity reduced green algal biovolume and pH increased it, especially in treatment reaches (Table S5).

Influences on Cyanobacteria

Oscillatoria spp., *Nostoc* spp. and unicellular cyanobacteria were common in the intermediate streams. *Nostoc* spp. was also very common in two of the warmer streams (streams 1 and 5), while *Oscillatoria* spp. were commonly found in the coldest stream (stream 7).

Cyanobacteria were less sensitive to environmental factors than green algae and diatoms, resulting in few significant relationships in the data (Tables S6 & S7). There was a significant difference in the biovolume of cyanobacteria among different months (ANOVA, $F = 13.55$, $P < 0.001$) but no significant influence from temperature (Tables S6 & S7).

Discussion

Our first hypothesis about increased biomass of bryophytes, macrophytes and larger algae with nutrient enrichment was accepted only for bryophytes, which responded to enrichment more in warm streams than in the colder ones. Bryophytes can
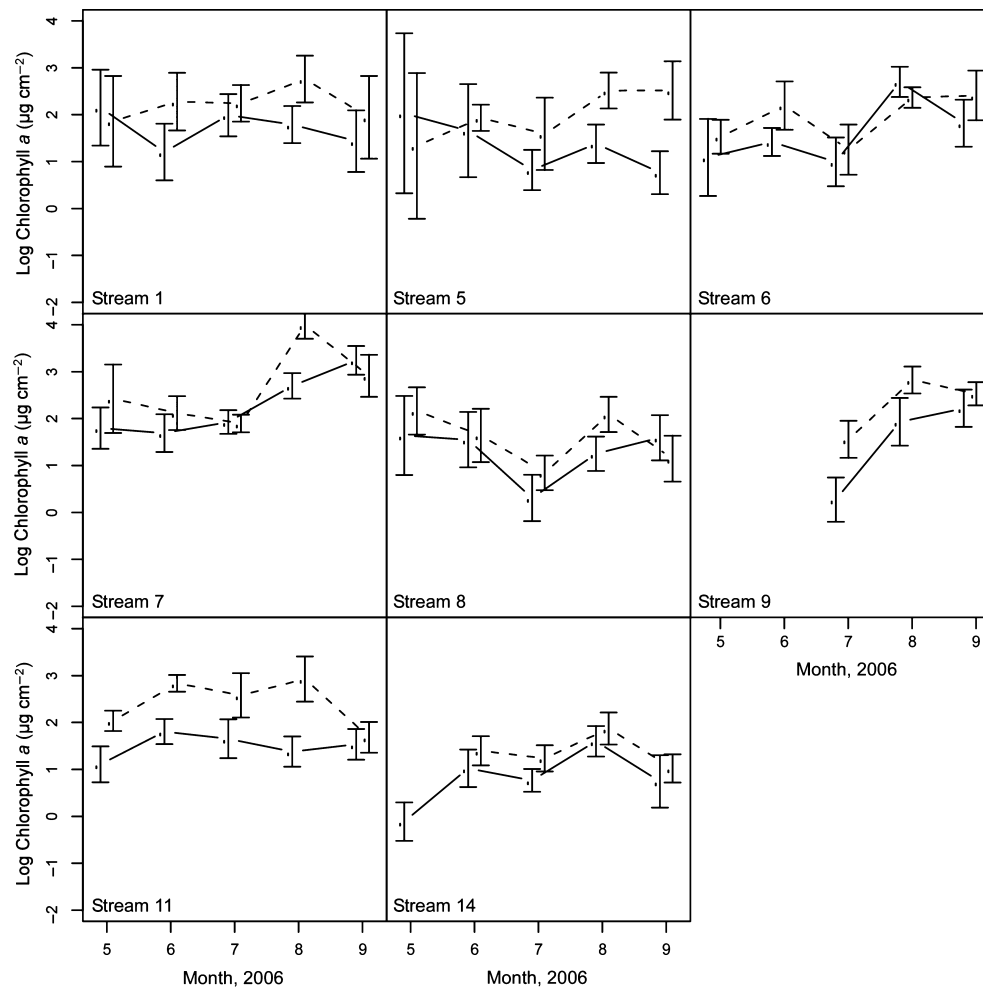
Fig. 4 The concentration of chlorophyll *a* (Log$_{10}$) per cm^2 from stones in each stream. The solid lines represent control reaches, and dashed lines represent the treatment reaches. The sampling was carried out from May until September 2006. Mean and 95% confidence intervals are plotted on the lines.

acquire nutrients even in very low concentrations, which makes them strong competitors of other primary producers (Stream Bryophyte Group, 1999). Slavik *et al.* (2004) conducted long-term experiments with phosphorus enrichment on the Kuparuk River but found responses only after seven years. The increased bryophyte biomass over a short time in our experiment was probably due to the combined effects of high temperature and nutrients.

We rejected the hypothesis that macrophytes would be influenced by nutrient addition. Macrophytes were generally rare and patchy and present in only some of the streams, and no significant influences were detected on their abundance. The macrophytes were rooted and therefore may meet their nutrient demand from the sediment with no reliance on nutrients from

the water column, as is the case for most algae/ cyanobacteria and bryophytes (Sand-Jensen & Borum, 1991). Also, we rejected the hypothesis that the total mass of macroscopic algae would be greater in enriched reaches; the opposite was the case. However, we found positive effects on filamentous algae of nutrient enrichment. *Cladophora* sp. was the most common filamentous algae and has been found to increase its biomass significantly elsewhere with nutrient enrichment (Lapointe & O'Connell, 1989; Biggs, 2000). With nutrient enrichment, in contrast, *Nostoc* sp. almost disappeared from the treatment reaches. *Nostoc* sp. can use N$_2$ and is therefore common in nitrogen-limited habitats but, when we added ammonium nitrate, nitrogen was no longer limiting (Howarth *et al.*, 1988). Bryophytes seemed to
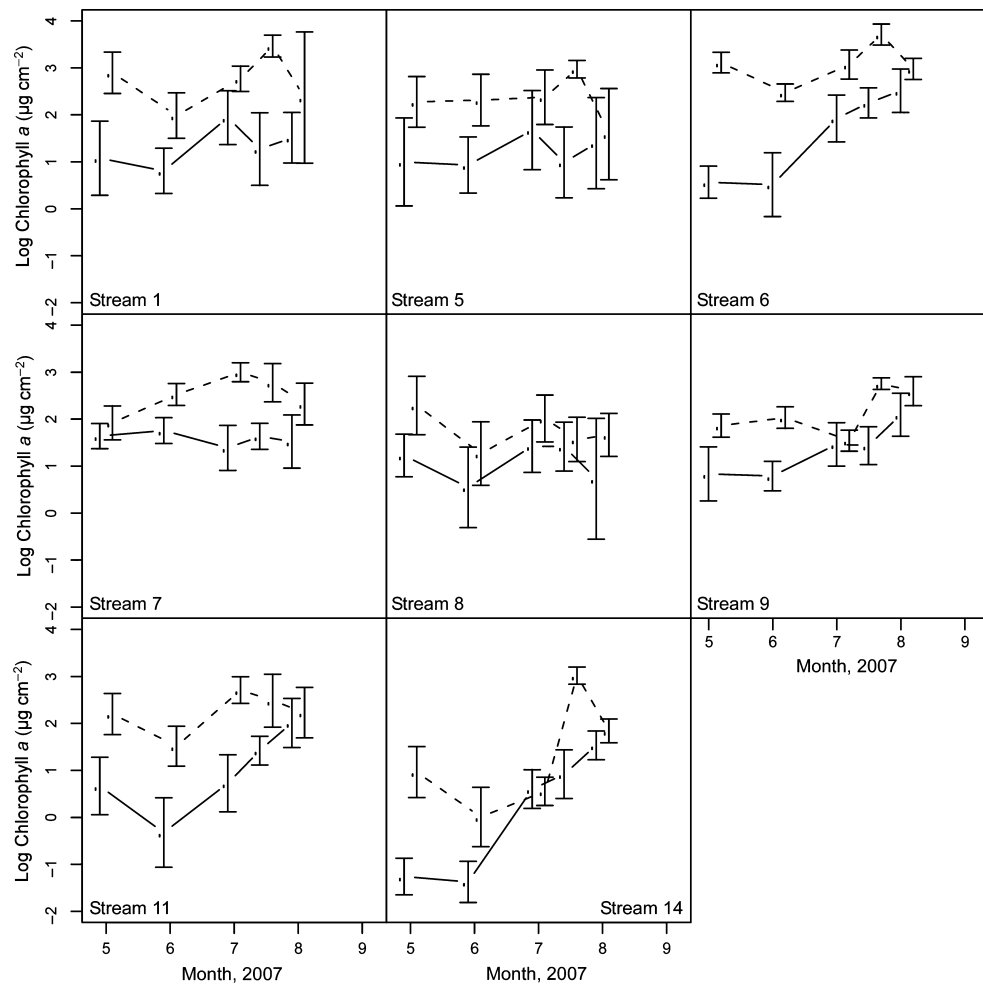
Fig. 5 The concentration of chlorophyll *a* (Log_{10}) per cm^2 from stones in each stream. The solid lines represent control reaches, and dashed lines represent the treatment reaches. The sampling was carried out from May until August 2007. Mean and 95% confidence intervals are plotted on the lines.

outcompete the large algae for resources when nutrients were no longer limiting. The warm streams have relatively steady high temperature throughout the year and were never observed to become snow covered. Bryophytes have long life cycles compared with algae/cyanobacteria, and therefore, stable habitats, such as those in the warm streams, are well suited for their preponderance.

Our second hypothesis was that chlorophyll *a* would increase with nutrient enrichment in all streams, but to the greatest extent in the warmer streams. The argument for the postulated greater increase in warmer waters was that temperature in general enhances metabolic activity and thus biomass (Melillo *et al.*, 1993; Demars *et al.*, 2011). This hypothesis was partly accepted because there was a significant increase in

chlorophyll *a* in all streams when nutrients were added. Even in May, shortly after the experiments started, we saw obvious differences between control and treatment reaches in both years. However, in 2007, chlorophyll *a* concentration was lower in both control and treated reaches in June than in May. A possible explanation for the temporary decline in chlorophyll *a* is heavy rain that can increase flows and scour out the streams. The rapid increase in epilithic algae in treated reaches is likely because of fast and often rather simple life cycles of the algae/cyanobacteria groups dominating the epilithon. It appears that only a slight increase in nutrients could have considerable influences on epilithic primary producers.

We hypothesised that diatoms and green algae would dominate treatment reaches and cyanobacteria

would decline, and this was accepted. Studies on lakes and ponds by McKee *et al.* (2003) also found an increase in the biovolume of green algae, as did several studies on streams and marine habitats (Bach & Josselyn, 1978; Lapointe & O'Connell, 1989; Dodds & Grudder, 1992; Biggs, 2000). However, various studies have revealed an increase in cyanobacterial biovolume with nutrient addition in lakes and ponds (Hudnell, 2010; Poirier, Cattaneo & Hudon, 2010; Savage, Leavitt & Elmgren, 2010). The epilithic cyanobacteria in our streams were mainly *Nostoc* spp. and *Oscillatoria* spp. A likely explanation for the retreat of *Nostoc*, the dominant cyanobacterium in the streams, as discussed earlier, is the loss of its competitive advantage through nitrogen fixation in nitrogen-poor habitats (Grimm & Petrone, 1997).

pH was positively correlated with cyanobacterial biovolume. The pH of fresh waters is closely linked to inorganic carbon supply. If fresh waters are eutrophic, they tend to have higher pH and little free CO_2, possibly leading to changes in species composition and abundance of algae and cyanobacteria. This may be causative, because some algae and cyanobacteria can use bicarbonate readily, or consequential, because the uptake of inorganic carbon tends to increase pH (Moss, 1973). It is not possible to distinguish between cause and effect in this case.

We found diatoms to respond most to nutrient enrichment. The response was more prominent with increasing temperature (Table S3). The increased metabolic rate from rising temperature, and rapid division of unicellular algae with no limiting nutrient resources, could explain the diatom density in the treated reaches. The relative abundance of silicate in warmer waters could also help. Green algal abundance, both unicellular and filamentous, was also influenced by nutrient enrichment. The influences were strongest in May and June in both years, and temperature had a positive effect. The mechanisms are likely to be similar to those for the diatom stimulation, a ready response of organisms with simple life histories and potentially high division rates.

Based on our results, it is very likely that eutrophication and a temperature rise of as little as 2 °C will change communities of primary producers in sub-Arctic streams very considerably. Eutrophication could come from an extension in stock grazing and even some cultivation in these regions as temperature increases. It could also come from atmospheric nitrogen pollution as human activities intensify northwards and vehicle emissions increase. Our results suggest a marked change from streams dominated by diverse but sparse diatom communities to heavy growths of fewer species (R. Gudmundsdottir unpublished data) and increases in filamentous algal growths. The same degree of temperature rise can also lead to a loss of other primary producers, such as the liverwort *J. exsertifolia*. A temperature increase of around 6 °C above the background would mean a likely change from diatoms to bryophyte-dominated communities. The potential consequences on invertebrates and fish from these combined effects of warming and nutrient enrichment would be varied (Hogg & Williams, 1996; Durance & Ormerod, 2007) and yet largely unpredictable for lack of good experimental evidence. Changes in the community composition of primary producers may affect the food web of invertebrates and fish and the niche structure of the system in many ways. We conclude that habitats at high latitudes may be at great risk from global warming through alteration in the dominant primary producers and great increase in total biomass. The wider consequences of this remain to be seen.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1. Effects on bryophyte dry biomass of nutrient additions to streams in Iceland.

Table S2. Results of linear mixed effect regression analyses of the relationships between nutrient enrichment, time and interaction between nutrient enrichment and time on chlorophyll *a*.

Table S3. The effect of nutrient enrichment and environmental variables on diatom biovolume in 2006 and 2007 sampling periods. Environmental variables were: temperature (T), conductivity (Con) and pH.

Table S4. The effects of nutrient enrichment and environmental variables on green algal biovolume from May to September 2006. Environmental variables were: temperature (T), conductivity (Con) and pH.

Table S5. The effect of nutrient enrichment and environmental variables on green algae biovolume from May to August 2007. Environmental variables were: temperature (T), conductivity (Con) and pH.

Table S6. The effect of nutrient enrichment and environmental variables on Cyanobacteria biovolume from May to September 2006. Environmental variables were: temperature (T), conductivity (Con) and pH.

Table S7. The effect of nutrient enrichment and environmental variables on Cyanobacteria biovolume from May to August 2007. Environmental variables were: temperature (T), conductivity (Con) and pH.

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Paper 4

1 **Diatoms as indicators: the influences of experimental nitrogen**

2 **enrichment on diatom assemblages in sub-Arctic streams**

3

4 [a*]Rakel Gudmundsdottir (rakelgud@hi.is), [a]Snaebjorn Palsson (snaebj@hi.is), [a]Elisabet

5 Ragna Hannesdottir (erh@hi.is), [b]Jon S. Olafsson (jsol@veidimal.is),[a]Gisli Mar Gislason

6 (gmg@hi.is) and [c]Brian Moss (brmoss@liverpool.ac.uk)

7

8

9 [a]Institute of Biology, University of Iceland, Sturlugata 7, IS 101 Reykjavík, Iceland

10 [b]Institute of Freshwater Fisheries, Keldnaholt IS 112 Reykjavík, Iceland

11 [c]School of Environmental Science, Nicholson Building, University of Liverpool L69

12 3GS, UK

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19
20 **Correspondence**: Rakel Gudmundsdottir, Institute of Biology, University of Iceland,

21 Sturlugata 7, IS–101 Reykjavík, Iceland. E-mail: rakelgud@hi.is

22

23 **Key words:** *In situ* experiments, streams, nitrogen, diatoms, diversity, Trophic Diatom

24 Index.

25 **Summary**

26   1. We measured effects of experimental nitrogen enrichment on diatom assemblages

27   in sub-Arctic streams to test the validity of approaches commonly used to assess

28   of nutrient enrichment influences on communities.

29   2. Diatom densities increased significantly in the experimental streams but there was

30   no significant difference in invertebrate density between control and treatment

31   reaches.

32   3. The diversity within streams (Shannon´s Index) was significantly reduced by

33   nutrient addition but the Sørensen´s Index did not change. The Trophic Diatom

34   Index (TDI), which is presumed to reflect nutrient status, was not influenced by

35   nutrient addition and generally the values were low in both control and treatment

36   reaches.

37   4. The diatom genera *Achnanthes* and *Gomphonema* increased significantly while

38   *Nitzschia* and *Fragilaria* density decreased significantly. Less abundant diatom

39   species, which constituted around 40% in relative abundance in the control

40   reaches, were around 15-18% in treatment reaches.

41   5. Growth forms were altered by the nutrients, e.g. diatoms attached by mucilage

42   pads were more abundant in treated reaches compared with control reaches. On

43   the other hand, motile diatoms became scarcer. The size of diatoms was

44   unaffected by nutrient enrichment.

45

46

47

1. Introduction

Diatoms often dominate the algal communities in streams and rivers (van den Hoek, 1995; Allan, 2006); they are dynamic and diverse, and their ecology is relatively well understood. They are claimed to be sensitive towards many environmental factors such as ionic content, pH, dissolved organic matter and nutrients, and therefore have been used to monitor streams and rivers in many regions (Potapova and Charles, 2007; Kelly et al., 2008 a,b). Furthermore, diatoms have been found to be better correlated with chemical measures of water quality than other algae (Kelly et al., 2008 a,b).

Studies on the effects of nutrient enrichment on stream algae have suggested that diatom species richness declines (Peterson et al., 1985) and algal biomass increases (Bothwell, 1989) with increasing phosphorus. Gudmundsdottir et al. (2011b) manipulated nitrogen in eight Icelandic streams, varying in temperature, and found that biofilm algal chlorophyll *a* and bryophyte (*Fontinalis antipyretica* Hedw.) biomass increased with increased nitrogen. Pringle (1990) showed that addition of inorganic and organic phosphorus increased numbers of motile diatoms, mainly *Nitzschia* (Hass.) and *Navicula* (Bory), on sandy substrates and *Cocconeis placentula* (Ehrenb.) and *Achnanthes minutissima* (Kütz) on glass slides. With this and other evidence and the general acceptance that stream diatom communities reflect nutrient status, various diatom-based indicators and indices have been developed (Kelly et al. 1995), whilst more widely used diversity indices, for example the Shannon´s Index and the Sørensen's Index (Magurran, 2004), can be readily applied to diatom communities. Comparison among indices using field data, however, suggests that different indices do not always give similar results (Besse-Lototskaya et al., 2011).

71

72   One of the requirements of the European Water Framework Directive (European Union,

73   2000) is the use of benthic algal communities for the assessment of ecological status of

74   flowing waters and many member states have turned to specific diatom indices to do this

75   (Kelly et al., 1998). The Trophic Diatom Index (TDI), which uses a scoring system based

76   on indicator species (Kelly and Whitton, 1995), is being used to assess eutrophication

77   pressures in several States. The index is based on correlations among phosphorus

78   concentrations and diatom communities, derived from field correlations (e.g. Borchardt,

79   1996; Potapova et al., 2004), but has not been tested experimentally in a rigorous way.

80   There are many factors acting simultaneously with phosphorus, not least combined

81   nitrogen, grazing and flow effects that could confound such correlations. Most

82   freshwaters are probably co-limited by both phosphorus and nitrogen (Moss et al., 2012),

83   and even when either is limiting, concentrations of the other are generally correlated.

84   Phosphorus is conventionally considered to be the limiting nutrient on primary producers

85   in freshwaters but nitrogen may be limiting, especially where human settlement has

86   developed or where phosphorus is abundant naturally in the soils e.g. in neo-volcanic

87   Icelandic bedrock (Ritter, 2007).

88

89   All indices depend on the species composition of communities. There are disadvantages

90   to this in that species substitution is common in many communities as a result of natural

91   fluctuations and random effects. There is a possibility, however, that changes in traits,

92   such as mode of attachment and size, may give more accurate reflection of change.

93   Daufresne et al. (2009) found that that size, in a range of organisms, is reduced with

94   temperature, but trait analysis has not been used in the assessment of anthropogenic

95   impact. A system of streams in Iceland has given us the opportunity, not only to make an

96   experimental test of how effective various indices of diatom community composition are,

97   in reflecting change in nutrient loading, but also to assess the use of traits as well as

98   taxonomic indices.

99

100   Our hypotheses were first that the diatom cell density per unit area would increase with

101   increased nutrient concentration in the streams. Secondly, we hypothesized that diversity,

102   expressed by the Shannon´s Index (Magurran, 2004) would fall with increased nutrient

103   concentration. We also expected high overlap between species i.e. β-similarity, calculated

104   according to Sørensen´s Index, to increase with nutrient enrichment (Magurran, 2004).

105   When nutrients are abundant, fast-growing diatom species are likely to benefit whilst

106   many slow-growing species are likely to become rarer, and may even disappear from the

107   streams, resulting in lower diversity. Thirdly, we hypothesized that the Trophic Diatom

108   Index (TDI) (Kelly, 1998) would reflect the experimental nutrient enrichment in the

109   streams (Kelly et al., 2008a) with higher TDI (more tolerant species observed) with

110   nutrient enrichment. Studies have linked high phosphorus levels in streams and rivers to

111   higher TDI values, but the index is widely assumed to reflect nutrient pressures in

112   general. Lastly we hypothesized that nutrient enrichment would change the trait

113   characteristics (size, nature of attachment and motility) of the diatom community and that

114   this approach might then be valuable in indicator studies. We expected to find more

115   motile species where nutrients were abundant (because such unattached species are

116   vulnerable to washout but might be able to build up populations able to compensate for

117   such losses if nutrients support increased growth rates), and increased numbers of smaller

118   diatoms with nutrient enrichment, owing to the advantages of their potentially shorter life

119   cycles, when resources are not limited (Moss, 1973). The experimental streams varied in

120   conductivity, temperature and invertebrate community (see Gudmundsdottir et al., 2011a)

121   but our design included control and treatment reaches within each of these short and

122   uniform streams so that other variables were controlled for.

123

124   **2.   Methods**

125   *2.1 Study area*

126   Nutrient enrichment experiments were carried out in the Hengill geothermal area in SW

127   Iceland (N 64°03; W 21°18; 350-420 m a. s. l.) (Fig 1), an active volcano covering 173

128   km^2. The study area is compact (Gudmundsdottir et al., 2011a,b) with streams that are

129   very close to each other, thus minimizing confounding factors. Friberg et al. (2009)

130   carried out experiments with nutrient diffusion substrates in the streams and found that

131   the algal communities were nitrogen-limited. The mean air temperature in July is 8-10 °C

132   and the annual precipitation is 1870-3080 mm (Bjornsson, 2003; Crochet et al., 2007).

133   Numerous small streams (rheocrenes) emerge from the hillsides and run along a flat

134   valley floor on hyaloclastide bedrock to join the River Hengladalsá. Vegetation in the

135   area is mainly monocotyledonous with *Carex bigelowii* Torr. ex Schwein, *Carex nigra*

136   (L.) Reichard, *Agrostis capillaris* (L.), and *Eriophorum angustifolium* Hockney. Mosses

137   and shrubs (*Empetrum nigrum* L. and *Vaccinium uliginosum* L.) (Gudjonsson & Egilsson,

138   2006) are also found, but because trees are absent and shrubs are sparse, little or no

139   allochthonous particulate organic matter enters the streams (Friberg et al., 2009;

140   Woodward et al., 2010).

141

142   For the enrichment experiment, eight streams were used, each 30-75 metres long with no

143   detectable longitudinal trends in chemistry of biological communities. Their annual mean

144   temperature ranged from 4-24 °C (Friberg et al., 2009). Their conductivity was between

145   96 and 497 (μS cm^{-1} at 25 C°) and pH 7.92-8.42, whilst nutrient concentrations were

146   much more uniform (TP: 0.008-0.029 mg/L^{-1}, TN: <0.1-0.19 mg/L^{-1}) (Gudmundsdottir et

147   al., 2011b). Brown trout (*Salmo trutta* L.) was the only fish species observed in these

148   streams. The main invertebrate functional feeding groups were scrapers and filter-

149   collectors. *Simulium vittatum* (Zettersteth) and the gastropod *Radix peregra* (Müller)

150   were dominant in the warmer streams while chironomids were dominant in the colder

151   streams. Chironomids comprised 16 of 35 invertebrate taxa in the streams (Friberg et al.,

152   2009).

153
154
155   *2.2 Experimental design*

156   Each of the eight streams (Fig. 1) was divided into an upper control reach (15-25 m), and

157   lower treatment reach (15-25 m) with a 5-m buffer zone between the control and

158   treatment reaches. The study area is not settled by humans and there is no agriculture,

159   resulting in near pristine reference (in the definition of the Water Framework Directive

160   (2000)) sites to compare as controls with the treated reaches. Finding reference sites is

161   difficult or impossible in most parts of Europe because of strong influences from human

162   settlement and agriculture (Kelly et al., 2008a). On the bank of each stream we placed

163   two 120-L plastic barrels containing stream water in which ammonium nitrate (NH_4NO_3)

164   had been dissolved, along with an aluminum box that contained an electric pump for

165   continuously delivering the solution. The pump was driven by a car battery recharged by

166   solar power. More detailed description of the technology is given in Gudmundsdottir et

167   al. (2011b). The experiments were carried out from May-September 2006 and from May-

168   August 2007.

169
170

171   *2.3 Sampling*

172   <u>2.3.1 Physical and chemical variables</u>

173   Physico-chemical variables were measured monthly during the experimental periods. A

174   multiprobe sonde, YSI 600 XLM (Yellow Springs Instrument, Yellow Springs, Ohio

175   USA) was used to measure temperature, pH and conductivity (standardized at 25 °C).

176   Water samples for total phosphorus (TP), total nitrogen (TN) and silicate (SiO_3-Si) were

177   obtained on each sampling occasion and were frozen in 1-L polyethylene bottles prior to

178   later analysis at the National Environmental Research Institute in Denmark. Detailed

179   descriptions of chemical measurements are given in Gudmundsdottir et al. (2011b).
180

181   <u>2.3.2 Diatoms</u>

182   Stones were collected randomly from the stream-bed for diatom analysis each month.

183   Three stones were randomly collected from each treatment and each control reach (48

184   stones monthly for nine months, yielding 432 samples). Stones which were colonized by

185   bryophytes were not used. The size of stones was between 5 and 35 cm in diameter. Each

186   stone was wrapped in aluminum foil in the field to avoid light damage during

187   transportation to the laboratory.

188

189   2.3.3 Invertebrates

190   Invertebrates were sampled in the last week of August 2007. All streams except one

191   (stream 14) were included. From each reach, five Surber samples (14 x14 cm and mesh

192   size 200 µm) were taken at random coordinates. After the samples were brought to the

193   laboratory they were sieved using a 125 µm sieve and preserved in 70% ethanol.

194   Invertebrates were counted and identified to generic or species level. Chironomidae

195   larvae were mounted on microscopic slides in Hoyer´s medium (Anderson 1954) and

196   identified under a high power microscope. Other taxa were identified under a dissecting

197   microscope.

198

199   *2.4 Laboratory processing*

200   2.4.1 Diatoms

201   The algal biofilm was removed from each stone, with a medium-hardness toothbrush.

202   The slurry of surface material was washed off with distilled water collected in a tray, and

203   its volume recorded; the brushed area was measured by outlining it on scaled tracing

204   paper. Subsamples from the slurry were heated in nitric acid (65%) for up to 24 hours to

205   remove all organic material from the diatom frustules. The volume of the subsample used

206   (usually 10 ml) depended on the algal concentration in individual samples. When no

207   visible organic matter was left, the subsamples were repeatedly resuspended in distilled

208   water and centrifuged (Sorvall® RT6000) at 3000 rpm for 10 minutes until the

209   supernatant water had reached pH 6. A well-mixed sub-sample of 0.5 mL was then

210   pipetted onto a circular microscope cover glass where it was left to dry, and then mounted

211   with Naphrax® high resolution diatom mounting medium. Diatoms were identified to

212   species at 1000 times magnification with a Leica DM 3000 microscope. Around 600-700

213   frustules, with a maximum of 2000 and a minimum of 200 only where samples were very

214   sparse, were counted within defined transects across the cover glass. Keys used for

215   identification were-: Hustedt (1930) and Krammer & Lange-Bertalot (1986, 1988, 1991a,

216   & 1991b).

217   Diatoms that were less than 5 µm in width were considered small, diatoms with width

218   over or equal to 5 µm and shorter than 12 µm in length were classified as medium-sized

219   and those that were 12 µm or more in width, or greater than 25 µm in length, were

220   classified as large diatoms (Hauer and Lamberti, 2007). Growth form of the diatoms was

221   estimated and compared with data from Yallop and Kelly (2006). The diatoms were

222   classified into six growth-forms: mucilage stalk (MS), mucilage pad (MP), attached on

223   raphe (AR), motile (MO), chain forming (CF) and diatoms in mucilage tubes (MT)

224   (Yallop and Kelly, 2006).

225

226

227   *2.5 Statistical methods*

228   The statistical software R (version 2.11.1) was used for all data analysis (R Development

229   Core Team, 2006). The pattern in the diatom communities was partly explored with the

230   Shannon´s Index and Sørensen´s Index (Magurran, 2004).

231

232   To calculate the trophic diatom index (TDI) we used the method described in detail in

233   Kelly et al. (2008a). Diatoms are categorized in five groups according to their tolerance

234   towards nutrients with ´s´scores from 1 (low tolerance) up to 5 (high tolerance) (Kelly

235   and Whitton, 1995). Values ranged from 0 (no impacts from nutrients) to 100 (maximum

236   impacts from nutrients).

237   To summarize the effect of the nutrient enrichment we applied meta-analysis, developed

238   by Hedges et al. (1999) for: Shannon´s Index, Sørensen´s Index, growth-forms, TDI

239   values, the number of diatom frustules per unit area, and number of invertebrates. The

240   method is based on the natural logarithm of the response ratio of the averages in the

241   experimental and control reaches, (L). By considering variances within and among

242   samples, and the sample numbers it allows the calculation of confidence intervals and

243   testing of differences among L-values (Q-statistic). Combined L-values were calculated

244   for each statistic, and when differences among L-values were significant those values

245   were further explored. To find out if there was a difference in the composition of growth

246   forms of diatoms between control and treatment reaches we used chi-square tests.

247
248
249   3. Results

250   **3.1 Physio-chemical variables**

251   Ammonium concentration was about 5.5 times greater in treated reaches compared with

252   control reaches. Total phosphorus and silicate concentrations were similar between

253   reaches (Table 1). Conductivity was unaffected by the nutrient enrichment but pH was

254   significantly slightly higher in control reaches compared with treated reaches (Table 1).

255   Discharge was measured between control and treatment reaches (Table 1) and among the

256   streams fell within a narrow range with a mean around 2.9 m^{3}sec^{-1}.
257

258   **3.2 Invertebrates**

259   Mean invertebrate density was between 7000 and 7500 individuals per m^2 but with high

260   variability between samples. Chironomids and *Radix peregra* had the highest densities in

261   both control and treated reaches. None of the invertebrate groups were significantly

262   different in density between control and treatment in the streams (Table 2).
263

264   **3.3 Diatoms**

265   The population density of diatoms in treated reaches was significantly higher than in

266   control reaches (Fig. 2 shows the dramatic effects of nutrient addition). Standard

267   deviations were high for all diatom groups, as expected in stream systems. Relative

268   abundances of *Achnanthes* sp. (mainly *A. minutissima*) and *Gomphonema* (*G. pumilium*

269   and *G. rhombicum*) were significantly higher in treated reaches compared with control

270   reaches, whilst the relative abundances of *Fragilaria* spp. and *Nitzschia* spp. were

271   reduced. *Rhoicosphenia curvata* was not influenced by nutrient addition but the

272   abundance of other rarer diatoms was decreased significantly in treatment reaches (Table

273   3). Thus, nutrient enrichment had strong and significant influences on the diatom groups

274   in the streams (Fig. 3).

275
276

277   **3.4 Growth forms**

278   Overall, the distribution of growth forms was significantly altered by nutrients ($\chi^2 = 24.4$,

279    $p = 0.0002$) but the size classes of diatoms were unaffected ($p > 0.05$). Mucilage stalk

280   (MS) diatoms were proportionally fewer in treated reaches compared with control

281   reaches while mucilage tube diatoms (MT) were proportionally more abundant in

282   treatment reaches (Table 4) though the absolute changes were low. However, proportions

283   of mucilage pad diatoms (MP) (e.g. *Achnanthes* spp.) were much greater in treated

284   reaches than in control reaches. Diatoms attached by their raphe (AR) (e.g. *Cocconeis*

285   spp. and *Epithemia* spp.) and chain-forming diatoms were not significantly affected.
286

287   **3.5 Diversity indices**

288   The Shannon´s Index for diatom communities was significantly reduced by nutrients

289   (Table 5). Rarer diatom species were significantly fewer in treated reaches compared

290   with control reaches according to the meta analysis (Table 3) (Fig. 3). Sørensen´s Index

291   was not altered by the nutrient enrichment. The Trophic

292   Diatom Index (TDI) was unaffected by nutrients (Table 5). The values derived from the

293   TDI ranged from around 45-47 in both sets of reaches.

294
295
296   **4. Discussion**

297   Nitrogen enrichment significantly increased the diatom abundance in the streams (Fig 2).

298   Diatoms in general have short life cycles, allowing rapid population growth when

299   nutrients or other factors are not limiting. Nutrient enrichment had negative influences on

300   cyanobacteria such as *Nostoc* spp. and *Oscillatoria* spp. (Gudmundsdottir et al., 2011 b),

301   but green algae (mainly *Cladophora* spp.) became more abundant when nutrients were

302   added to the streams. Thus nitrogen enrichment changed the dominant primary producer

303   groups significantly by replacing cyanobacteria with filamentous green algae and

304   diatoms. Possible reasons for the change may be that cyanobacteria tend to have lower

305   growth rates than diatoms and green algae (Moss et al., 2012). Also, the observed

306   cyanobacteria were nitrogen fixers (Gudmundsdottir et al., 2011 b) and better able than

307   most diatoms and green algae to thrive under nitrogen limitation (Friberg et al., 2009).

308   When nitrogen is not limiting, nitrogen fixers are easily replaced with fast growing

309   diatoms and green algae. For the purposes of monitoring streams, however, diatom

310   communities offer greater ease of use because of their greater species richness and better

311   defined taxonomy. There are difficulties in defining taxa consistently among the

312   prokaryotic Cyanobacteria and accurate identification of green algae often requires

313   reproductive stages that are often not present (John et al., 2002).

314   Diatom diversity estimated with the Shannon´s Index decreased significantly with

315   nutrient addition. Diatom species that are strong competitors, having the ability to

316   become very abundant when nutrients are not limiting, are likely to succeed under these

317   changed circumstances, leaving the rare and slow growing species behind. Thus greater

318   nitrogen load is likely to alter the diatom community from high diversity to relative

319   uniformity. Changes in the secondary producer communities might be anticipated if there

320   is high specificity in choice of food taken; however, invertebrate densities were

321   unaffected by the nutrient treatment, suggesting a lack of such specificity and possibly an

322   absence of food limitation even under control conditions in these streams. Shifts in food

323   availability may have influences on invertebrates, especially because invertebrates at

324   specific ages need specific foods (e.g. different particle size) (Allan, 2006). Changes in

325   food availability may have influences as well as competition between invertebrates. Also

326   predatory fish can modify invertebrate communities (Allan, 2006). Other factors like

327   stream flow characteristics (magnitude, frequency, duration, timing and variation) may

328   limit abundance, diversity, evenness, richness, individual taxa and functional feeding

329   groups (Konrad et al., 2008).

330   We hypothesized that the Sørensen´s Index would be greater in treated reaches compared

331   with control reaches, indicating greater overlap among communities in nutrient-treated

332   sections but no such effect was found. However, the decline in Shannon´s Index

333   suggested that rarer diatoms became relatively less abundant in treated reaches (around

334   15-18%) while they contributed around 40% of the relative abundance in control reaches.

335   Streams are likely to contain more uniform community structures when nutrients are

336   raised compared with streams with lower nutrients, though the Sørensen's index was not

337   sufficiently sensitive to detect this.

338

339   We anticipated that the Trophic Diatom Index (TDI) would have higher values with

340   nitrogen enrichment compared with control reaches because it is intended to be a nutrient

341   indicator and was designed as such. However we did not find any significant relationship

342   between the TDI and nitrogen enrichment. Several observational studies have suggested a

343   relationship between phosphorus and TDI (Kelly et al., 1995, 1998, 2008 a, b) and most

344   diatom indices have been calibrated to phosphorus pollution but not nitrogen pollution.

345   The cosmopolitan nature of diatoms suggests a consistent trophic preference but studies,

346   mainly conducted in North America, have indicated that it is necessary to calibrate the

347   European indices to regional conditions (e. g. Fore and Grafe 2002; Potapova and

348   Charles, 2007). Moreover, European phycologists have argued that indices developed in

349   certain parts of Europe may not be suitable for other European regions (Kelly et al., 1998;

350   Pipp, 2002; Rott et al., 2003; Besse-Lotostkaya et al., 2011). A study by Besse-

351   Lotostkaya et al. (2011) compared performance of various diatom based indices and

352   revealed considerable differences in the results. They concluded that even though diatom

353   based indicator indices served as powerful biomonitoring tools, they should be applied

354   with caution. Our study being experimental rather than correlative adds weight to the

355   need for such caution. More experimental work is needed to establish deeper

356   understanding on the use of various indices.

357   A study on African streams in forested and deforested habitats by Bellinger et al. (2006)

358   showed an opposite pattern to that of our study. The deforested streams were presumed to

359   be nutrient polluted by erosion and other factors while the forested streams were control

360   sites. Their findings suggested that the TDI which is designed in Europe was a good

361   indicator for tropical African streams but classical diversity indices (Shannon H, J and

362   Simpson−Yule D, E) were not useful. *Achanthes*, *Gomphonema* and *Navicula* were the

363   most common genera in the deforested streams. *Achnanthes* and *Gomphonema* were also

364   dominant in the treated reaches of our experimental work. *Navicula* did not respond

365   significantly in our study and that may have strong influences on the differences of the

366   outcomes of these studies. The study by Bellinger et al. (2006) was, like most of the

367   studies in Europe, not conducted on experimentally enriched streams but used

368   correlation. Its authors concluded that more controlled studies (experiments) were needed

369   to better understand how light and nutrients (proximate factors), and land use and

370   anthropogenic inputs (ultimate factors) affect algae species (Bellinger et al. 2006).

371

372   If there is now some doubt as to the consistent behaviour of indices using taxonomic

373   units, there may be more potential for using growth forms. Although nutrients did alter

374   the growth form diversity of diatom communities in our experiment, size categories, the

375   simplest aspect of trait determination, were unaffected by nutrients. Diatoms attached by

376   mucilage pads, for example *Achnanthes* spp., benefited from nutrient enrichment.

377   Proportions of mucilage stalk diatoms in general changed little but one such genus,

378   *Gomphonema*, increased significantly in the treated reaches. The reasons are not clear but

379   the result suggests that there may be a great deal of individual specificity which makes it

380   unlikely that trait indicators will be any more successful than taxonomic indicators of

381   nutrient pressures. Contrary to our hypothesis, diatoms that are motile (e.g. *Nitzschia* and

382   *Navicula*) became scarcer in treatment reaches, compared with controls. Other studies

383   have shown that these diatoms benefit by nutrient enrichment (Kelly, 2003). Again this

384   suggests an element by which particular species that are present may influence apparent

385   trait behaviour and warns against too much generalisation.

386

387   The present study supports the need for greater caution in the use of specific indices that

388   may be developed for certain habitats and particular nutrients. The failure of the Trophic

389   Diatom Index (TDI) to reflect a substantial nitrogen addition is particularly worrying,

390   though several studies conducted in Europe have revealed a correlation between the TDI

391   and phosphorus enrichment and this suggests that the circularity in deriving such indices

392   from correlations rather than from controlled experimentation has led to a confidence that

393   may be premature. Work carried out in connection with the Water Framework Directive

394   on links between other groups such as macrophytes and invertebrates and simple pressure

395   drivers (e.g. Johnson et al., 2006; Penning et al., 2008; Irvine, 2009; Hering et al., 2010;

396   Demars et al., 2012) has also suggested that simple relationships do not occur and calls

397   into question the whole basis of assessment of ecological quality through such indicators

398   (Moss, 2008).

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400

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595

596   **Figure captions**

597

598   **Fig 1.** Map of Iceland (a) and the eight selected streams (b) within Hengill geothermal

599   area.

600

601   **Fig 2.** Changes in algal biofilm when nutrients were dripped into the lower reach of a

602   cold stream. The tube dripped ammonium nitrate into treated reach. Notice the dramatic

603   difference in the colour of the biofilm. The flow direction is left to right.

604

605   **Fig 3.** The relative abundances (%) of dominant diatom groups in control and treatment

606   of the eight streams.

Tables.

Table 1. Physio-chemical variables within the control and treatment reaches of the eight streams. 'Sign' means there is a significant difference between reaches at P<0.05. N is number of samples. NS means no significant difference at P = 0.05. Meta-analysis was applied for all variables except Si and discharge.

	Total phosphorus (mg L⁻¹)	Ammonium-N (mg L⁻¹)	Si (mg L⁻¹)	Conductivity (µS cm⁻² at 25°C)	pH	Temperature (°C)	Discharge (m³ sec⁻¹)
Mean ± SD (Control)	0.08 ± 0.03	0.031 ± 0.027	12.95 ± 4.3	262.8 ± 126.2	8.22 ± 0.25	13.8 ± 6.0	2.92 ± 1.96
Mean ± SD (Treatment)	0.03 ± 0.04	0.171 ± 0.200	12.62 ± 3.1	256.1 ± 113.4	8.20 ± 0.24	13.4 ± 6.3	-
N (Control)	85	33	8	16	16	47	40
N (Treatment)	72	33	8	16	16	47	-
L - value	-0.05	1.50	-	0.03	0.02	0.02	-
Significance	NS	Sign	NS	NS	Sign	NS	-

Table 2. Mean density (m⁻²), standard deviation, standard error and number of samples within control and treatment reaches of the seven streams for prominent invertebrate groups, genera and species. Relative abundances (%) are given for each genus or species. Also, L values from meta-analysis are given for the absolute abundance of each taxon used. Differences in L values are: Sign, significant at P<0.05; NS not significant at P=0.05.

Statistic	All inverts	Chironomidae	Simulium	Radix peregra	Other invertebrates
Control					
Average density (m⁻² x 10⁻³)±SD	7.2 ± 12.1	13.7 ± 16.6	3.8 ± 7.6	10.5 ± 12.5	0.8 ± 0.75
Standard error	1.037	2.852	1.297	2.142	0.13
Samples (n)	35	35	35	35	35
Relative abundance (%)		47.5	13.2	36.4	3.0
Treatment					
Average density (m⁻² x 10⁻³)±SD	7.4 ± 12.5	12.5 ± 13.5	3.8 ± 5.1	12.5 ± 17.8	0.81 ± 0.74
Standard error	1.072	2.312	882	3.060	126
Samples (n)	34	34	34	34	34
Relative abundance (%)		42.2	12.7	42.3	2.74
L value	0.105	-0.10	0.32	-0.32	-0.05
Significance	NS	NS	NS	NS	NS

Table 3. Mean density (cm⁻²), standard deviation, standard error, number of samples within control and treatment reaches of the eight streams for diatom genera and species. Relative abundances (%) are given for each genus or species. Also, L values from meta-analysis are given for the relative abundance of various diatom genera and species. Sign, significant difference at P<0.05; Ns not significant at P=0.05

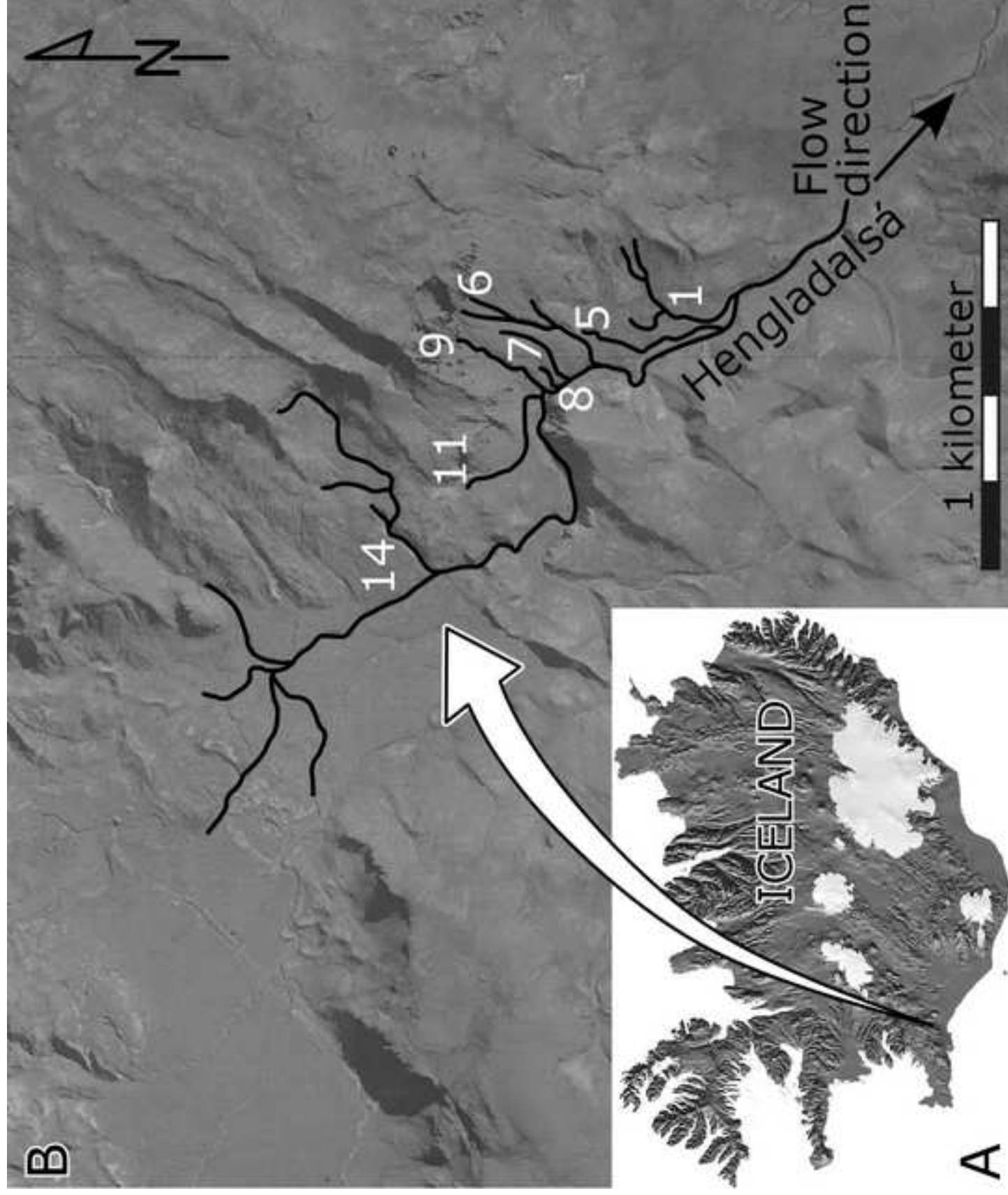| Statistic	All diatoms	Achnanthes spp.	Gomphonema spp.	Fragilaria spp.	Nitzschia spp.	Rhoicosphenia curvata	Other rarer diatoms
Control							
Average density (cm⁻²) ± SD	$1.5 \times 10^5 \pm 4.1 \times 10^5$	$1.2 \times 10^5 \pm 1.8 \times 10^5$	$1.0 \times 10^5 \pm 1.1 \times 10^5$	$1.4 \times 10^5 \pm 2.8 \times 10^5$	$2.0 \times 10^5 \pm 2.9 \times 10^5$	$1.3 \times 10^5 \pm 3.8 \times 10^5$	$4.8 \times 10^5 \pm 7.9 \times 10^5$
Standard error	3.4×10^4	3.6×10^4	2.3×10^4	5.6×10^4	5.9×10^4	7.8×10^4	1.6×10^4
Samples (n)	24	24	24	24	24	24	24
Relative abundance (%)		11	19	12	13	6	40
Treatment							
Average density (cm⁻²) ± SD	$1.2 \times 10^6 \pm 4.4 \times 10^5$	$2.8 \times 10^6 \pm 8.6 \times 10^6$	$2.4 \times 10^6 \pm 3.3 \times 10^6$	$3.3 \times 10^5 \pm 5.2 \times 10^5$	$1.0 \times 10^6 \pm 1.8 \times 10^6$	$3.6 \times 10^4 \pm 1.1 \times 10^5$	$9.0 \times 10^5 \pm 1.1 \times 10^6$
Standard error	3.6×10^4	1.8×10^6	6.8×10^5	1.1×10^5	3.6×10^5	2.3×10^4	2.3×10^5
Samples (n)	24	24	24	24	24	24	24
Relative abundance (%)		27	31	6	10	8	18
L value	1.37	0.81	0.62	-0.55	-0.54	-0.2	-0.82
Significance	Sign	Sign	Sign	Sign	Sign	NS	Sign

Table 4. Mean percentage and standard deviations for various growth forms of diatoms. Abbreviations for growth forms: MP=mucilage pad, AR=attached on raphe, MT=mucilage tubes, MO=motile, CF=chain forming, MS=mucilage stalks.

Growth form	Control		Treatment		Significance at P<0.05
	Mean (%)	SD (%)	Mean (%)	SD (%)	
MP	11.5	5.1	32.3	34.5	Sign
AR	15.5	23.1	3.3	1.7	NS
MT	0.6	0.7	1.2	1.7	Sign
MO	31.8	5.5	16.6	12.2	Sign
CF	9.2	5.5	15.7	11.7	NS
MS	31.4	34.1	30.9	24.1	Sign

Table 5. Mean, standard deviations, number of observations (N), L values from meta-analysis and significance for Shannon Index, Sørensen´s Index and TDI in control and treated reaches of the eight streams. Sign, significant difference at P<0.05; NS not significant at P=0.05.

Statistic	Shannon´s Index (LN)	Sørensen´s Index	TDI
Mean SD (Control)	1.95 ± 0.44	0.65 ± 0.08	45.64 ±10.47
Mean SD (Treatment)	1.77 ± 0.48	0.66 ± 0.07	46.79 ±10.34
N	409	476	409
L - value	-0.1	0.01	0.03
Significance	Sign	NS	NS