Landsvirkjun

Effects from geothermal effluent on periphyton and invertebrate assemblages in NE-Iceland

Lykilsíða

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Meistaranámsritgerð

Sigurðar Óskars Helgasonar

HAFRANNSÓKNASTOFNUN
Rannsókna- og ráðgjafarstofnun hafs og vatna

Júlí 2017

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90 ECTS thesis submitted in partial fulfillment of a
Magister Scientiarum degree in Biology

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Abstract

Geothermal energy utilization in Iceland is a growing industry in order to fulfil constantly growing energy demand. One of Iceland's geothermal power plant is located in NE-Iceland, the 60 MW Krafla geothermal Power Plant, which currently utilizes energy from 18 boreholes. The primary objective of this study was to investigate possible effects from the effluent from a geothermal energy utilization on the aquatic ecosystem in the stream Hlíðardalslækur. I studied both spatial and temporal variations in the benthic algae and Cyanobacteria assemblages in three geothermally spring-fed streams in the Krafla geothermal area. The diversity and density of invertebrates within the same sites were also studied in September 2015 and January 2016. Periphyton accrual responses to four different nutrient enrichments were also tested in the fall of 2015.

Algae and Cyanobacteria assemblage varied significantly between sampling sites in regards to dominating genera, density and diversity. Relatively high periphyton densities characterized upstream reaches from the power plant, along with the numbers of genera and diversity being relatively high as well. Endosymbiotic diatoms (*i.e.* N_2-fixing genera such as *Epithemia* and *Rhopalodia*) and non-N_2-fixing diatoms (*Caloneis*, *Nitzschia* and *Navicula*) characterized the algae communities, along with the presence of Chlorophyta (*Stigeoclonium* and *Klebsormidium*). The N_2-fixing Cyanobacteria, mainly *Anabaena*, grew spatially upstream, mainly on cobbles near the stream banks. Invertebrates were in low densities and their diversity was also rather poor. The periphyton accrual was significantly increased by adding nitrogen to the streams. There were significant changes in the algae and Cyanobacteria assemblage downstream to the power plant where microbial mats covered the stream bottom. These mats consisted only of heterocystous (N_2-fixing) and non-heterocystous Cyanobacteria, as neither diatoms nor green algae seemed to thrive in that habitat. N_2-fixing genera, *Anabaena* and *Calothrix*, characterized the downstream site closest to the power plant, along with the non-N_2-fixing genera of *Phormidium* and *Lyngbya*. Significantly lower diversity was at sites where the microbial mats had colonized. However, densities and biomass were high. The density of invertebrates was low at the downstream site, closest to the power station with Chironomidae and other Diptera the only taxa present. Phosphorous addition increased biomass significantly and nitrogen had no effects. In general, the effects from the geothermal power plants effluent appear to be highly localized. At a site some three kilometers downstream from the power plant, numbers of taxa and diversity had increased significantly, although biomass and density usually decreased. N_2-fixing diatoms and the N_2-fixing genera of *Anabaena* were in high abundance and partly dominated the streams bottom. At the same site, Chlorophyta (*e.g. Stigeoclonium* and *Microspora*) had reappeared. The density of invertebrates were much higher at this site than it was closer to the power plant. Neither nitrogen nor phosphorous increased the periphyton accrual at this downstream site. The algal and cyanobacterial biomass along with the diversity were significantly higher at the reference site in comparison to the remaining sites, with site KR1-6 as an exception. The assemblage was mainly dominated by non-N_2-fixing diatoms (e.g. *Melosira* and *Rhoicosphenia*) and various species of *Navicula*. The highest density of invertebrates was at the reference site. Nutrient enrichment had no significant effect on the periphyton accrual, which may indicate that the reference site constantly gains nutrients from its nearby source. Generally, algal and cyanobacterial density and biomass differed between seasons, as highest values occurred in late summer, July and September 2015. The temporal differences were also present for the invertebrates, in which density was significantly higher in September in comparison to density in January.

Útdráttur

Nýting orku úr jarðvarma er víða talinn ákjósanlegur kostur til þess að svara sívaxandi orkuþörf mannkynsins. Þrátt fyrir að nýting jarðvarma sé talin nokkuð umhverfisvæn kostur, a.m.k. miðað við bruna jarðefnaeldsneytis, þá hefur verið lítið fjallað um mögulegar afleiðingar slíkra virkjana á nærliggjandi vistkerfi. Aðalmarkmið þessa verkefnis er að rannsaka möguleg áhrif affallsvatns frá Kröflustöð á botnlæga þörunga, blágrænbakteríur og hryggleysingja í Hlíðardalslæk ásamt því að athuga áhrif næringarefnaviðbóta á vöxt botnlægra þörunga og blágrænbaktería.

Helstu niðurstöður leiddu í ljós að marktækur munur var á gerðum samfélaga þörunga og blágrænbakteríaá milli sýnatökustöðva, með tilliti til fjölbreytni, þéttleika og lífmassa. Ofan við Kröflustöð var þéttleiki botnlægra frumframleiðenda fremur lítill. Ríkjandi hópar voru kísilþörungar og niturbindandi blágrænbakteríur. Algengustu kísilþörungarnir voru af ættkvíslunum *Caloneis*, *Nitzschia* og *Navicula*. Niturbindandi kísilþörungarnir *Epithemia og Rhopalodia* voru einnig nokkuð algengir og einkenndu samfélögin. Ættkvíslir grænþörunga sem fundust í lækjum ofan við Kröflustöð tilheyrðu aðallega ættkvíslunum *Stigeoclonium* og *Klebsormidium*. Niturbindandi blágrænbakteríur af ættkvíslinni *Anabaena* fundust í miklum þéttleika en hlutfallsleg tíðni/þekja þeirra var lág. Á sýnatökustöðvum ofan við Kröflustöð var þéttleiki hryggleysingja lítill. Næringarefnaviðbætur gáfu til kynna að nitur væri takmarkandi næringarefni fyrir vöxt þörunga og blágrænbaktería. Fyrir neðan viðbót affallsvatns frá Kröflustöð þöktu blágrænbakteríur botninn og mynduðu þykkar samfelldar breiður. Slíkar breiður drógu verulega úr fjölda ættkvísla og fjölbreytni, en eiga það til að valda verulegri aukningu á þéttleika, þar sem um margar smáar frumur blágrænbaktería er um að ræða. Við nánari athugun kom í ljós að þörungabreiðurnar voru samsettar af niturbindandi blágrænbakteríum af ættkvíslunum *Anabaena* og *Calothrix*. Blágrænbakteríurnar *Lyngbya* og *Phormidium* voru einnig ríkjandi á tímabili, en falla þó ekki undir hóp niturbindandi blágrænbaktería. Fosfór reyndist vera takmarkandi fyrir vöxt blágrænbaktería. Uppbygging samfélaga hryggleysingja var fábreytt og einungis rykmýslirfur og tvívængjur voru þar að finna og í mjög lágum þéttleika. Áhrifin frá affallsvatninu eru því staðreynd en þau reyndust hinsvegar vera staðbundin. Um það bil þremur kílómetrum neðan við virkjunina virtust kísilþörungar og grænþörungar dafna vel. Í kjölfar þynningaráhrifa og viðbóts rennslis frá viðmiðunarstöðinni hækkaði fjöldi ættkvísla og fjölbreytnin þar um leið. Niturbinandi kísilþörungarnar *Epithemia* og *Ropholadia* voru þar áberandi ásamt blágrænþörungunum *Anabaena*. Aðrir kísilþörungar svo sem *Fragilaria*, *Melosira* og *Nitzschia* voru nokkuð algengir ásamt einni ættkvísl grænþörunga, *Stigeoclonium*. Í raun var næst hæsta fjölbreytnin að finna á þessari stöð, KR1-6. Slíkt styður hugmyndina um dvínandi áhrif virkjunarinnar er lengra dregur frá samruna affalsvatnsins við Hlíðardalslæk. Hæsti þéttleiki (september 2015) ásamt fjölbreyttum samfélögum hryggleysingja var þar að finna. Hvorki nitur né fosfór næringarefnaviðbætur juku lífmassa þörunga og blágrænbaktería. Þörungasamfélögin í viðmiðunarælknum, KR2-2, einkenndust af miklum þéttleika, fjölbreytileika og mörgum ættkvíslum þörunga. Ráðandi ættkvíslir voru aðallega *Melosira*, *Fragilaria*, *Rhoicosphenia*, *Navicula og Nitzschia,* sem margar hverjar eru merki um ágætis vatnsgæði. Einnig voru niturbindandi kísilþörungar (*Epithemia* og *Rhopalodia*) nokkuð algengir. Lítill þéttleiki blágrænbaktería einkenndi samfélögin en frekar, þar sem einungis var að finna ættkvíslirnar *Oscillatoria* og *Lyngbya*, sem hvorugar eru niturbindandi. Grænþörungar af ættkvíslum *Klebsormidium*, *Microspora* og *Stigeoclonium* voru nokkuð áberandi innan botngróins mosa, sem einfremur einkenndi viðmiðunarlækinn. Hryggleysingjar á viðmiðunarstöðinni voru með hæstu fjölbreytni ásamt

hæsta þéttleika í janúar 2016. Næringarefnaviðbætur höfðu lítil sem engin áhrif á vöxt þörunga og blágrænbaktería, sem gefur sterklega til kynna að hár styrkur næringarefna er ávallt til staðar sem kemur í veg fyrir takmarkanir næringarefna fyrir vöxt frumframleiðenda.

Rannsókn þessi gefur sterklega til kynna að affallsvatn jarðgufuvirkjanna hafi marktæk áhrif á bæði samfélög botnlægra frumfraleiðenda og hryggleysingja. Þær breytingar sem eiga sér stað er hægt að tengja við breytur á umhverfis- og eðlisþáttum sem er grunnstoðin í mótun vistkerfa. Fyrir neðan affall Kröflustöðvar eru umhverfisþættir afar hentugir fyrir blágrænbakteríur þar sem þær eru hitasæknar lífverur sem að færar eru til þess að binda nitur úr andrúmsloftinu, slíkt er mikill kostur þar sem nitur er takmarkandi. Slík þörungasamfélög eru vel þekkt fyrir að mynda þykkar breiður sem að mögulega geta hindrað útbreiðslu annarra þörunga. Hinsvegar, þá varð einnig vart við það að áhrifin frá affallinu eru afar staðbundin. Áhrifin fara hverfandi eftir því sem neðar dregur, líklegast vegna þynningaráhrifa, þar sem bæði fjölbreytni og fjöldi ættkvísla þörunga og hryggleysingja virðist aukast í samanburði við náttúrulegu skilyrðin fyrir ofan affallið.

I would like to dedicate this thesis to my beloved fiancé and family for nursing me with affection and support

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Abbreviations

Abbreviations	Sampling site
KR1-1	Krafla, Stream 1, Site number 1
KR1-2	Krafla, Stream 1, Site number 2
KR1-3	Krafla, Stream 1, Site number 3
KR1-4	Krafla, Stream 1, Site number 4
KR1-6	Krafla, Stream 1, Site number 6
KR2-2	Krafla. Stream 2, Site number 2
DIP	Dissolved inorganic phosphorous
DOP	Dissolved organic phosphorous
TDN	Total dissolved nitrogen
TDP	Total dissolved phosphorous
DIN	Dissolved inorganic nitrogen
DON	Dissolved organic nitrogen
BT	BenthoTorch
Chl a	Chlorophyll *a*
FO	Frequency of Occurrence
TDS	Total dissolved solids
PCC	Palmer Counting Chamber
HCYA	Heterocyte containing Cyanobacteria
NHCYA	Non-heterocyte containing Cyanobacteria

Acknowledgements

1 General Introduction

1.1 Primary Producers

Autotrophs (primary producers), organisms that acquire their energy from sunlight and nutrients from inorganic sources, are well known for their rapid responses to changes in the environment. The major autotrophs in running waters include macrophytes (larger plants) and various small autotrophs, such as algae and Cyanobacteria. Macrophytes include vascular plants, non-vascular plants and occasionally large filamentous algae, such as *Cladophora* (Allan, 1995). Small autotrophs attached to the substrate are referred to as periphyton, which include benthic algae and bacteria. Macrophytes, especially non-vascular plants, mostly occur where in slow current and moderate depth, while periphyton is known to occur on the water surface in great variety of streams. Benthic algae can be further categorized as macroalgae and microalgae; and by their occurrences, for example, on stones (epilithic), soft sediment (epipelic) and attached to other plants (epiphytic). Lists of epipelic, epilithic and epiphytic species indicate considerable habitat specialization, although some certainly occur in multiple setting (Round, 1964). The morphology of algae varies greatly, ranging from simple single non-motile spheres to large multicellular structures. Cells may come together in small groups or large aggregates but with no definite shape, or may form globular colonies that have a characteristic shape. Another common form of colonized algae is linear colonies, also known as filaments. Phenotypic similarities and dissimilarities of the algae are important in the classification and identification of algal genera/species (Sigee, 2004). The most common divisions of algae in streams include diatoms (Bacillariophyta), green algae (Chlorophyta) and Cyanobacteria (Cyanophyta), also known as blue-green algae (Bellinger & Sigee, 2015).

Algae and Cyanobacteria can serve as ecological indicators of the health of and change in aquatic ecosystems (McCormick & Cairns, 1994; Allan, 2004). The use of algae as bioindicators in Iceland is a relatively young field, which means that there used to be a lack in standardized methods. Earlier algae studies in Iceland have been conducted by variety of scientists which can be traced back to 1770, in which the first data were published for Icelandic algae and Cyanobacteria assemblages by the France botanist Müller (Hallgrímsson, 2007). However, it was not until in the 19th century when studies on periphyton became more common and were identified to modern universal taxonomic species and published in both local and foreign papers (Schwabe, 1933; Krasske, 1938; Sperling, 1975). To begin with, mainly foreign scientists from Denmark, France and Germany investigated the Icelandic flora, followed by the publication of a variety of papers (Hariot, 1893; Børgesen, 1899; Binder et al., 1981). The research centre at Neðri-Ás in Hveragerði, SW Iceland, made a significant contribution to the field of phycology by offering research facilities, which lead to various publication on the Icelandic flora, including the algae and Cyanobacteria.

Algae and Cyanobacteria, can provide relatively unique information as environmental indicators concerning ecosystem conditions due to their nutritional needs and their position at the base of the aquatic food-web (McCormick & Cairns, 1994). As each species of periphyton has its own suite of environmental tolerances and preferences, the species assemblages can give valuable information regarding the condition of their habitat. The short

life-cycles of many algae and Cyanobacteria means that they can respond rapidly to changes in their environment, such as alterations in water chemistry (Hauer & Lamberti, 2006). Environmental factors such as temperature, discharge, nutrients and light availability may also influence assemblages and function (Stevenson, 1997). In most cases, major divisions of algae and Cyanobacteria tend to dominate at different temperature ranges, e.g. diatoms found at 5–20 °C, green algae at 15–30 °C and finally, Cyanobacteria that are usually found in above 30 °C waters (DeNicola, 1996). Along a thermal gradient in streams in California, it was demonstrated that such trends/correlations hold for a variety of ecosystems (Lamberti & Resh, 1985). Such correlations to temperatures might explain seasonal changes in benthic algal assemblages between warm and cold seasons. Current velocity also influences phytobenthic community in streams, as it regulates growth and distribution of submerged autotrophs (Giorgi, 2005). Sources of flow (sources of running waters) have an overriding influence on local habitat conditions which most commonly control the nutrient supply (Biggs & Kilroy, 1994). The renewal of nutrients, gases and other material by currents reduces the risk of nutrient depletion. However, high currents are known to be harmful as sudden increase can scour the substratum, thus preventing periphyton growth (Uehlinger, Kawecka, & Robinson, 2003). Another prime factor that has influence on algae and Cyanobacteria assemblages is the light availability as benthic algae abundance is known to decrease during winter, presumably because of reduced light (Sherman & Phinney, 1971). As a consequence, changes in the dominant growth form might be expected, since motile algae are able to avoid the extremes by movement along the light gradient, while non-motile algae might reduce in abundance. However, some diatoms species do not seem to be affected by seasonal changes in light levels (Fisher, 1995). These factors (temperature, discharge, nutrients, light availability and more) are known to interact in complex ways, making it hard to determine a primary factor regarding periphyton assemblages. In dynamic habitats it is likely that differentiations in periphyton assemblages will occur in correlation with various environmental attributes. There are some indications that seasonal variations are not present in some hot springs communities, as the stable physicochemical conditions and temperatures of the source waters prevent seasonal changes (Mackenzie, Pedros-Alio, & Diez, 2013).

Geothermal hot spring habitats are unique and constant environments (Fagerberg & Arnott, 1981). Geothermal areas are often located on the merges of the tectonic plates, or in close environments to volcanic activities which release hot waters and steam to the earth surface (Glassley, 2010). The geothermal areas in Iceland are divided into two distinct groups, (Gunnarsson, 1973) which are low temperature geothermal fields (<150 °C at 1 kilometre depth) that are found in Quaternary and Tertiary rock formation and high temperature geothermal fields (>200 °C at the same depth) which are located within the active volcanic zone (Kristmannsdóttir & Tómasson, 1978). The communities of geothermally influenced waters has been a source of study for over 60 years, and their presence, distribution and productivity within geothermal ecosystems has received much attention (Vincent & Forsyth, 1987). Geothermal environments are often distinguished from their cool temperate counterparts by steep gradients in temperature, elevated concentration of a range of minerals, extreme pH and different habitats (Boothroyd, 2009). Recognizable separation in geothermal fauna and flora occur longitudinally downstream from geothermal springs. Directly below the source of high temperature waters, the aquatic communities are usually dominated by thermophilic fungi and Cyanobacteria before more diverse array of algae may appear. Knowledge on these ecosystems is an important consideration as these areas will be more and more important in the future, not only for energy extraction but also for recreation and conservation (Elmarsdottir et al., 2003). Studies on geothermal ecosystems in Iceland has

been rather limited until in recent years, when increasing investigations have been carried out for instance on the community structure of the geothermal flora and fauna (Ólafsson et al., 2010). Furthermore, diverse studies have been carried out and are still in progress in the Hengill (Hengladalir) geothermal area, SW Iceland. Williamson and coworkers (2016) studied the structural and functional attributes of biofilms in responses to climate change, as they concluded that temperature can alter how carbon is cycled and coupled to nitrogen and phosphorous. The metabolisms of the geothermal streams of Hengladalir have been studied intensively by Demars and his colleagues (2011). Furthermore, Welter et al. (2015) observed that N_2-fixation would amplify the temperature dependence of ecosystem metabolism. Microbial composition of hot springs in the Krafla area has been investigated in recent years, reflecting high biodiversity of microbial species (Pétursdóttir et al., 2010). It is thus clear that geothermal areas are of growing interest regarding a great variety of studies, including global warming emulation, and their importance are destined to grow both locally, and internationally. Thus monitoring and assessing for Icelandic geothermal ecosystem will be of high value. Algae and Cyanobacteria as bioindicators in geothermal ecosystems are primed to be of great importance, as they are often adapted to the extreme environments and respond quickly to environmental changes (Hauer & Lamberti, 2006).

Algae and Cyanobacteria found in geothermal habitats are sensitive to both temperature and chemical properties of the geothermal waters (Cooper, 1996). The periphyton which generally dominates in hot springs (>74 °C) usually belongs to Cyanobacteria and other microbial organisms (Mackenzie et al., 2013). In the more elevated temperatures, unicellular forms of Cyanobacteria are common and often dominate the benthos. On the contrary, filamentous forms of Cyanobacteria tend to dominate at lower temperatures, with cases of filamentous forms dominating at 57 °C (Boothroyd, 2009). Many algae and cyanobacteria genera are cosmopolitan, meaning that their distribution range extends across all or most of the world in appropriate habitats. Cosmopolitan genera in Iceland include the filamentous genera of *Anabaena*, *Calothrix, Nostoc*, *Oscillatoria* and *Phormidium*. (Hallgrímsson, 2007). Stark and co-workers (1976) found mainly Cyanobacteria within different temperature zones: "Species belonging to the genera *Phormidium* and *Oscillatoria* occurred at the highest temperatures (~53 °C), while *Calothrix* dominated at lower temperatures within the same stream." The ability of thermophilic Cyanobacteria to form mats is a distinctive characteristics of many geothermal ecosystems (Castenholz, 1984), as they frequently occur under certain environmental conditions, usually warm and nutrient rich (Fagerberg & Arnott, 1981). Algal and cyanobacterial mats are also known to occur under extreme condition, such as highly acidic (pH ~2.7) springs in which unicellular Cyanobacteria are the predominant microbial mat-forming phototrophs (Ferris et al., 2005). Even though Cyanobacteria usually dominate geothermally influenced streams at high temperature, diatoms may also dominate at slightly lower temperatures. For example, species of *Pinnularia* are known to dominate at acidic streams up to 29 °C and diatoms, such as *Nitzschia*, *Gomphonema* and *Fragilaria* can be common in streams with around 50 °C (Boothroyd & Browne, 2006).

As well as algae and Cyanobacteria, benthic macroinvertebrates are frequently used as bioindicators. The structure of aquatic invertebrate communities is influenced by temperature among other variables such as current velocity, nutrients, predation and food availability (Burns, 1997; Duggan, Boothroyd, & Speirs, 2007). Temperature is a strong factor influencing the distribution and composition of the invertebrate communities (Friberg et al., 2009), although other factors such as pH and food availability are also considered to

be one of the main factors regulating the faunal distribution (Vincent & Forsyth, 1987; Boothroyd, 2009) . Temperature is also known to increase ecosystem respiration and lower food web stability in warm streams. O'Gorman and his colleagues (2012) observed that snail density increased with increasing temperature in streams, resulting in stronger grazing pressure on diatoms, thus lowering the stability of the food web. Invertebrates are often found in low densities and diversity in high temperature water, but their densities and diversity increases as temperature decreases to a certain extent. In New Zealand it has been shown that fewer invertebrates occurred in close proximity to geothermal springs and other surface manifestation in comparison to cooler neighbouring areas (Boothroyd & Browne, 2006). Similar pattern has been found to occur in geothermal areas in Iceland, where number of invertebrate species decreased as the temperature increased (Ólafsson et al. 2010). They also found that many species living at higher temperatures did not occur in neighbouring colder areas and *vice versa*. In fact, even the modest temperature increases have dramatic effects across a multiple levels of organization, from changes in the organization, from changes in the mean body size of the top predators, to unimodal responses of species population, turnover in community composition, and lengthening of food chains (Woodward, 2010). Furthermore, increase in stream temperature has resulted in that species of Chironomidae have changed from being univoltine to bivoltine in warmer streams (Hannesdóttir, 2012). Geothermal waters generally support simple communities with low species-richness, but often with a high abundance of single species (Boothroyd, 2000). In general, meso- and thermophilic species of invertebrates are fairly rare in comparison with the periphyton, thus algae and Cyanobacteria might be more convenient organisms regarding geothermal assessments. It is of vital importance to investigate and understand the vulnerability of geothermal ecosystems as they often contain highly adapted organisms, which are only capable of surviving at these unique ecosystems (Saha, 1993).

1.2 Geothermal utilization for energy production

As awareness for global warming increases, humans are forced to look for other sources of energy, preferably renewable energy. Renewable energy provide sources of energy that can be naturally replenished such as from the sun, wind, tides, plants and geothermal heat (Demirbas, 2006). Geothermal energy is considered environmentally friendly and emits particularly low levels of greenhouse gases into the atmosphere compared to fossil fuels (Rybach, 2003). The considerable low greenhouse gas emission caused by geothermal energy gives it the potential to play an important role in the transition from fossil fuels to more sustainable fuels (Glassley, 2010). However, any type of energy production impacts the environment, but the degree or extent of this impact depends on the technology used (Rybach, 2003). Generally the environmental impact of geothermal power generation and direct use are minor, controllable or negligible. However, during geothermal power generation various environmental impacts are known to occur, including air pollution, surface water pollution, chemical pollution, high noise levels and solid waste disposal (Rybach, 2003). It is now known that the use of geothermal heat for electricity production produces greenhouse gas emissions of mainly carbon dioxide and sulphur as well as it produces geothermal effluents that need disposal (Kristmannsdóttir & Ármannsson, 2003). It is of immense importance to understand and predict how energy utilization affects the physical, chemical and biological attributes of the environment. Only then can we become truly sustainable on a global scale.

Every geothermal installation produces a unique set of chemical waste waters, which depend intricately upon the geochemistry and the exploitation history of the hydrothermal reservoir and on the individual plant design (Wetang'ula, 2004). Waste water, referred to as effluent water, is perhaps the most threatening pollutant for freshwater environments as it contains separation water, water from boreholes, condensation water along with runoff water from the cooling towers (Guðmundsson, 2009; Guðmundsdóttir, 2016). Currently there are six geothermal power stations operated for producing electricity in Iceland. One of them is the Krafla power station, North East Iceland, which produces energy by harvesting steam from nearby boreholes, 18 which are currently operational (Guðmundsson & Arnórsson, 2002). Steam obtained from steam chambers deep within the earth is led to the surface in order to spin turbines (2 x 30 Megawatts) to produce electricity. In the year 2002 an estimated 40% of the separation water (50–60 L/s) from the Krafla power station was pumped down into boreholes, with the remaining 60% (75–90 L/s) being released into a nearby stream, Hlíðardalslækur which also is called Dallækur (Guðmundsson, 2009a). Significant improvements were made concerning the amount of released effluent waters in 2014. Additional boreholes were put in use in order to pump down up to 87% (125 L/s) of the power plants separation waters. The stream Hlíðardalslækur receives an estimated 115 L/s of the effluent water which comprised mainly of runoff water from the cooling towers (Einarsson, 2014a). Earlier studies state that the natural discharge from the springs that feed the Hlíðardalslækur stream was 87 L/s (Arnórsson & Gunnlaugsson 1976) in the end of summer of 2004 (Ármannsson, 2005b). Given these values are still relevant, the effluent is responsible for approximately 75% of the total discharge below the effluent. The effluent does not only magnify the discharge but it also alters the physical environment, such as temperature regime, chemical composition and even light availability. The effluent water from the cooling towers categorizes as warm-hot (~40 °C), while water from the separation facilities categorizes as hot (~70 °C). Earlier studies state that effluent water contains potentially harmful chemicals such as hydrogen sulfide (H_2S) arsenic (As), boron (B), mercury (Hg), lead (Pb), cadmium (Cd), although in low and harmless concentrations (Ármannsson, 2005a). For instance, in New Zealand, hydrogen sulfide, known to influence the biota caused genotypic variation in the Cyanobacteria *Oscillatoria* that suggest it can tolerate a spectrum of sulfide conditions (Castenholz & Utkilen, 1984). Heavy metal pollution is also quite well known to occur and might affect the biota (Lavoie, Lavoie, & Fortin, 2012). Chances that deviation of most of the previously mentioned chemical and physical parameters (temperatures, discharge, nutrients, light availability, pH, conductivity and additional chemicals) from natural level in the area will affect the stream communities of the geothermal environment, which are often unique ecosystems with highly adapted organisms. Thermophilic (and mesophilic) vegetation including algal mats, thermophilic plants and bacteria, along with various species of invertebrates are characteristic organisms in geothermal environments (Kristmannsdóttir & Ármannsson, 2003).

1.3 Objectives

The overarching objective of this study is to investigate how an operation of a geothermal power plant may impact the ecology of a stream that receives the power plant's effluent. For this purpose I will focus on the ecology of the stream Hlíðardalslækur within the Krafla caldera, NE Iceland. For this I will investigate the possible impact from the power plant's effluent on benthic algae, Cyanobacteria and macroinvertebrate assemblages (Part 1). Furthermore, I will investigate the nutrient budget on these stream systems and the influence on the benthic flora (Part 2). The stream of interest consists of effluent waters from Krafla power plant's cooling towers, along with hot run-off water from the separation facilities. The high temperature and chemical enriched effluent mergers with the natural geothermal stream, with little known consequences on the ecosystem, especially on algal and cyanobacterial assemblages. Results from these studies will improve the knowledge of possible environmental impacts caused by merging geothermal effluents with natural waters.

Part 1: To determine the effects from effluent waters on periphyton and macroinvertebrate assemblages the following hypothesis was made:

Effluent from geothermal utilizations often contains high amounts of potentially harmful chemicals, along with hot waters and various environmental alterations, and thus will be a primary driver in shaping biotic assemblages towards less desirable conditions. It is hypothesized that spatial differences in periphyton and invertebrate assemblages will occur, in which the diversity decreases downstream from the power plant, as pollutant- and thermo tolerant and taxa will become dominant.

[Note Part 1 is written in a thesis format to capture the detail pertinent to detailed assessment and for future reference. The introduction follows from the thesis introduction and is thus shortened. The goal is to reshape this part as a manuscript for publication in Aquatic Conservation: Marine and Freshwater Ecosystems]

Part 2: To determine the effects of nutrient enrichment on algae and Cyanobacteria response the following hypothesis was made:

Nutrients, especially phosphate and nitrogen, are generally considered to be critical for autotrophic productions. Numerous field studies now provide evidence that nutrient supply can limit periphyton growth in nature. Thus biomass (chlorophyll *a* concentration) is expected to show positive responses to nutrient enrichment, although responses are expected to vary between locations and nutrient treatments. N_2-fixing Cyanobacteria are known to increase under nitrogen limitations because of their ability to fix atmospheric nitrogen. In a pilot study on the periphyton assemblages in the Krafla streams, results showed that the sites downstream from the effluent were dominated by N_2-fixing algae and Cyanobacteria. The presence of N_2-fixing Cyanobacteria downstream from the effluent of Krafla power plant strongly suggests N-limited conditions. Thus I hypothesize that nitrogen treatments will lead to positive responses in periphyton accrual. However, phosphorous treatments are predicted to increase accrual at the reference site, as algae and Cyanobacteria assemblages include few N_2-fixers, therefore not indicating P-limitations.

[Note Part 2 is written in manuscript format for submission to The Journal of Experimental Biology]

1.4 Study site

To determine the effect of the effluent from the Krafla power plant on stream primary and secondary producers, six sampling sites were chosen. Each site consisted of 15 m stream reaches. Sites were selected based on their functional attributes (geothermally influenced, minor-geothermally influenced and effluent influenced) and broad range of environmental conditions, such as pH, temperature, discharge and other habitat attributes (Ármannsson, 2005b). These sites were the same as used in previous studies on benthic invertebrates 2002 – 2003 and 2009 (Ólafsson *et al*. 2010, Ólafsson and Magnúsdóttir in prep.). Three sites were located upstream from the power plant (KR1-1, KR1-2, KR1-3), two were downstream from the power plant (KR1-4 and KR1-6) and the reference site (KR2-2), which is a tributary spring-fed stream, was located downstream from the power plant (Figure 1-1).
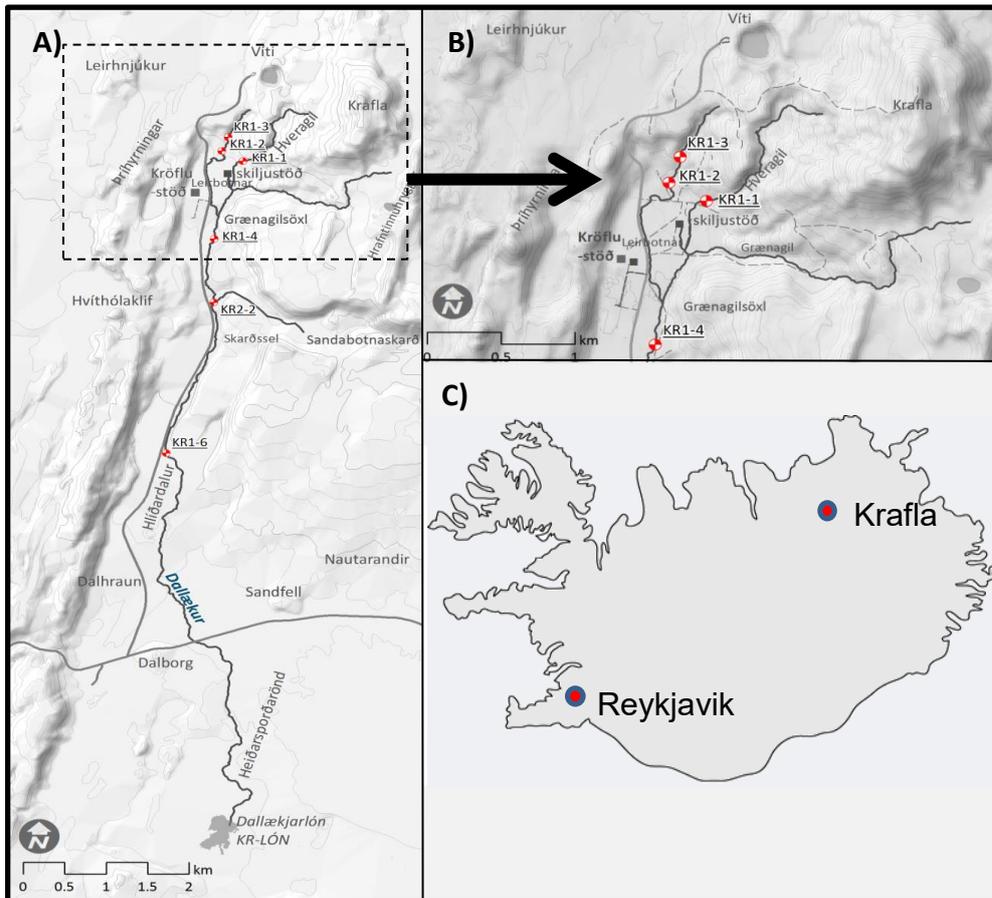
Figure 1-1 Map of the study area. A) The catchment of the stream Hlíðardalslækur showing all the sampling sites with red dots. B) An enlarged area of the sampling sites. C) Map of Iceland showing the location of the study site (Krafla).

All of the study sites are considered to be influenced by geothermal operation, except for the reference site (KR2-2). Site KR1-1 is located in a gully at the mouth of an isolated small valley, Hveragil. The stream originates from warm and cold springs. Its discharge increases significantly early summer due to snowmelt. The benthos is quite dynamic as it mostly contains sand and rough cobbles with unstable riverbed. The stream bank has sparse vegetation, which mainly consists of grasses. Sampling sites KR1-2 and KR1-3 were located in another stream in adjacent valley Hveragil. The stream originates in at least four hot (>40

25

°C) springs and some colder ones (Figure 1-2). The benthos of this stream is covered in precipitates, mainly silica and clay deposits (Arnórsson & Gunnlaugsson, 1976) and the stream banks are well vegetated, mainly with mosses, heather and grasses (Elmarsdóttir & Vilmundardóttir, 2009) but the vegetation cover gets sparser further downstream. Together, these two streams merge and form Hlíðardalslækur. Further downstream Hlíðardalslækur receives wastewater from the separation facilities, condensation and water from the cooling tower. This effluent is rich in various chemicals e.g. Silica, Cadmium, Lead along with various other heavy metals. Downstream from the effluent into Hlíðardalslækur from the power plant is referred here as the impact zone. Within this reach of Hlíðardalslækur there are two sampling sites (KR1-4 and KR1-6). Sampling site KR1-4 is closest to the power plant and thus perhaps under the most critical influence. Further downstream (approximately 3 kilometres) is sampling site KR1-6, which has unique attributes as it is both under the influence from the effluent, along with possible dilutions from the reference site. The control site (KR2-2), which is warm spring-fed stream, which joins Hlíðardalslækur between sampling sites KR1-4 and KR1-6. The stream originates from two nearby springs, which are warm (~17 °C).
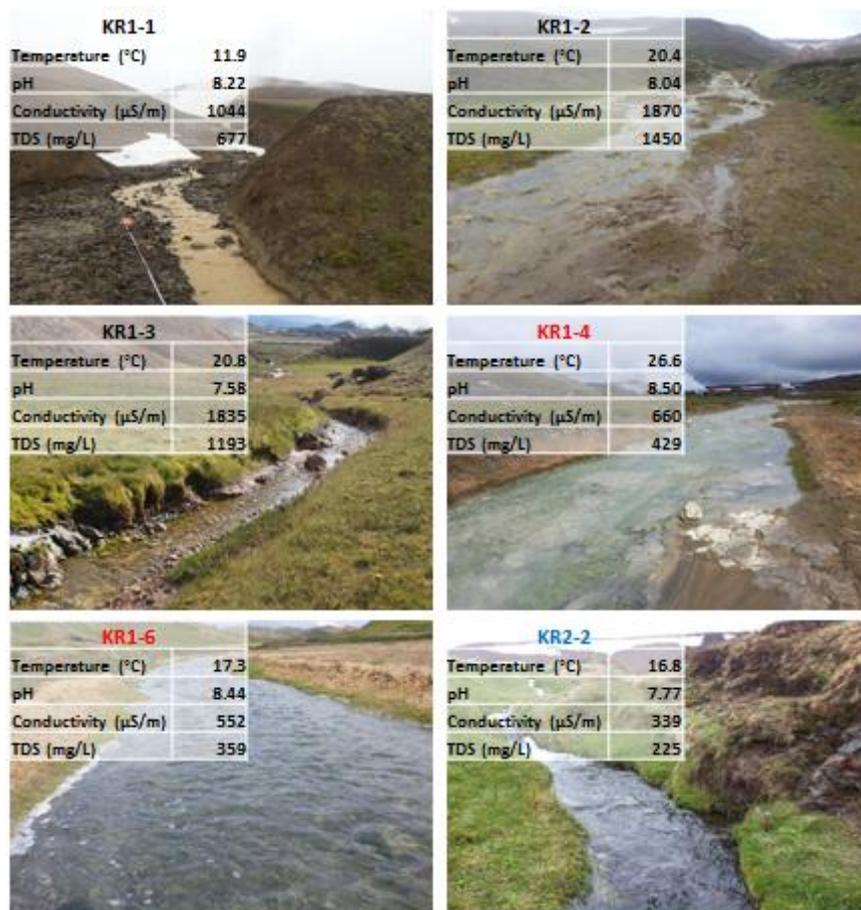
Figure 1-2. Photographs showing all sampling sites, where sites KR1-1, KR1-2, KR1-3 are all upstream from the power plant and KR1-4 and KR1-6 downstream. The site KR2-2 is in a spring fed tributary to the main channel of Hlíðardalslækur and is used as control in this study. At each photo there are information on annual averages for temperature, pH and conductivity.

2 The effect of effluent from a geothermal power plant on periphyton and invertebrate assemblages

2.1 Introduction

Geothermally affected ecosystems, here referred as geothermal ecosystems, are usually discrete and form unique settings for plants and animals. These ecosystems can be regarded as ecological "islands" in biogeographical context. Geothermal ecosystems can be found wherever geothermal activity occur, such as on the merges of the tectonic plates, and on so called continental hot spots were the earth's crust is thin. Iceland is located on the merging of two tectonic plates, hence with numerous geothermal fields. In recent years, the ecology of freshwaters within some of these geothermal fields has been the interest of numerous scientists. The effects of direct solar radiation on algal habitats in the geothermal area of Hengill, SW Iceland, were studied in the early sixties (Sperling, 1975). The invertebrate communities within the same geothermal region, Hengill, were observed in contrast to different temperatures (Ólafsson & Gíslason, 2002). Furthermore, nutrient enrichment under a thermal gradient was observed regarding periphyton growth and assemblages in several streams within the Hengill geothermal area (Guðmundsdóttir et al., 2011). Since then, much water has flowed under the bridge, in which geothermal ecology has become a major subject of variety of environmental impact assessments. However, most preliminary studies in the Krafla geothermal area have mainly focused on geological and geochemical features (Ármannsson, 2003), with lacking studies on the biota and their responses to the local power utilization. However, in 2002, the distribution of vegetation and terrestrial invertebrates were demonstrated in relation to physical and chemical variables in the Krafla area, which concluded the soil temperature to be dominating environmental factor in vegetation distribution (Elmarsdóttir et al., 2003). Ólafsson (2010) and his colleagues studied the ecology of streams at three geothermal fields including Krafla, with aim to investigate the aquatic communities and determine the prime factors in which shape the communities of macroinvertebrates (Ólafsson et al., 2010). Furthermore, in 2007 Pétursdóttir and her colleagues investigated the microbial composition within several hot springs in the Krafla area. Their results showed great variance in microbial diversity between the observed springs, streams and lagoons. The extreme diverse physical environment between those sampling sites are thought to be the main driver in shaping the complex communities (Pétursdóttur, Björnsson, Ólafsdóttir & Hreggviðsson, 2008). It is known that plant and animal communities at geothermal areas consist of variety of organisms, which are well adapted to the unique geothermal environments, in which the biota relies on both the thermal and/or the physicochemical attributes. These communities are often characterised by low biodiversity for aquatic and terrestrial organisms (Elmarsdóttir et al. 2003; Boothroyd & Browne, 2006). Similarly to the geothermal terrestrial environments, geothermal waters support simple communities with low-species richness but often with a high abundance of single species (Tuxen, 1944; Boothroyd, 2000). Studies where periphyton, especially algae-

and Cyanobacteria, are used as environmental indicators can provide relatively unique information concerning ecosystem conditions due to their nutritional needs and their position at the base of the aquatic food-web (McCormick & Cairns, 1994). Understanding the function of geothermal ecosystems and their characteristics is vital to the ability to utilize and allow better sustainable management of the ecosystems.

The main objective of this part of the study was to investigate the possible effects of effluent from a geothermal power plant, on spatial changes in the assemblages of benthic algae, Cyanobacteria (periphyton) and macroinvertebrates in warm spring-fed streams in the Krafla geothermal area, NE Iceland (Figure 1-1). To reach set goals the diversity, density and biomass of benthic algae and Cyanobacteria was investigated over a year. Furthermore, the density and diversity of benthic invertebrates was investigated on two occasions over the study period (September and January). The following hypothesis was made: effluent waters from geothermal power plants often contain high amounts of potentially harmful chemicals, along with hot water and may lead to enhanced fluctuations in many environmental parameters, e.g. temperature and discharge, and thus is a primary driver in shaping stream communities. It is hypothesized that periphyton and invertebrate assemblages will differ spatially. More precise, diversity will decrease downstream from the power plant as few, pollutant tolerant and thermophilic, genera will dominate the assemblages.

Additionally it was predicted that temporal alteration in periphyton and invertebrate assemblages would occur, in which densities and diversity would be higher in summer in comparison to fall and winter.

2.2 Material and methods

The study was conducted at six sampling sites in the catchment of the stream Hlíðardalslækur, NE Iceland (Figures 1-1 and & 1-2). In order to get met my goals I carried out measurements and sampled the six sites seven times over a period of 13 months: June (9–11), July (19–21), September (1–3) and October (6–8) 2015, January (20–22), May (10–11) and June (13–15) 2016. Multiple sampling throughout a year was conducted to mask out confound effect of seasonality in stream flora and fauna.

2.2.1 Field work

Temperature data loggers (HOBO pendant Temperature 64K data loggers, Onset computer corporations, USA and HOBO TidbiT Temperature Logger, Onset computer corporation USA) were placed at each of the six sites and recorded the temperature at 30–60 minute intervals. The loggers were placed inside a PVC tube to prevent them from being damaged. One logger malfunctioned, thus data from that particular logger could not be retrieved.

Conductivity and pH were measured on each sampling occasion with a multiprobe sonde where the readings were standardized to 25 °C (YSI Pro 1030 pH/ORP/Conductivity/Temperature instrument, YSI Incorporated, USA). Temperature was recorded with the same sonde. Discharge was calculated from measurements on the current velocity, which were done at 10–30 cm intervals across the stream at each site using SonTek Flow Tracker Handled Velocity Meter, width and depth. The total discharge was calculated based on these spot measurements by using the Mean Section Method formula retrieved from the SonTek Technical Manual (SonTek, 2006):

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┌─────────────────────────────────────────────────────────────────────┐
│                    Discharge Measurement                              │
│                                                                       │
│      Station Width = $W_i$ = $Location_i$ – $Location_{i-1}$           │
│      Station Area = $Area_i$ = $W_i$ * ($Depth_i$ + $Depth_{i-1}$) / 2 │
│   Mean Station Velocity = $MeanV_i$ = ($Velocity_i$ + $Velocity_{i-1}$) / 2 │
│         Station Discharge = $Q_i$ = $Area_i$ * $MeanV_i$              │
│                                                                       │
│                Total Discharge = Sum($Q_i$)                           │
│                                                                       │
└─────────────────────────────────────────────────────────────────────┘
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Samples for water chemistry were collected in acid washed plastic bottles simultaneously with other sampling. The samples were stored at -20 °C until analysed.

Sampling for biological variables was carried out once a month in the summer (June-August) 2015 and approximately bimonthly over the remaining of the study period (September 2015 - June 2016) in order to obtain a high resolution spatial and temporal data. For chlorophyll *a* measurements and periphyton assemblages, cobbles were removed from the stream bed at randomly chosen coordinates at 15 m stream reach at each sampling site. For algae and Cyanobacteria identification, the biofilm of three cobbles was brushed gently with a nylon brush from an area of 24 x 36 mm over a white plastic tray. The brushed surface of the cobble, the brush itself and the content of the tray was then rinsed with distilled water down to a 100 mL brown glass bottles. The samples were preserved in ~5% Formaldehyde or Lugol's solution (iodine and potassium iodine). For biomass measurements two methods were applied: 1) Periphyton was brushed from an area of 24 x 36 mm from 10 randomly selected cobbles sampled from the streambed at each reach. Each sample was filtered through GF/C filters to retain the periphyton and the filter then immediately frozen in liquid nitrogen and kept frozen until analysed in spectrophotometer. 2) Chlorophyll was measured *in situ* with a fluorometer (BenthoTorch, bbe Moldaenke). Measurements were carried out at 10 cobbles at random coordinates for each reach where the instrument was placed firmly at the surface of a cobble or rock and the chlorophyll measured. The BenthoTorch uses the *in vivo* fluorescence of algal cells: the cell pigments are excited by LEDs of different colours (wavelengths) and emit red fluorescence light as a natural phenomenon with high sensitivity. The intensity of the chlorophyll fluorescence is used to calculate the different algae as chlorophyll *a*, namely green algae, Cyanobacteria and diatoms.

Samples for benthic invertebrates were collected with Surber sampler (14 x 14 cm), with 250 µm net attached which collects the sample. The same random coordinates as for algae and Cyanobacteria were used for invertebrate sampling. Total of 10 replicate samples were collected at each sampling site. The sediment within the frame of the Surber sampler was disturbed with the fingers for approximately 30 seconds to dislodge all animals which were retained in the net. The content of the net was sieved through 125 µm sieve at the bank and the samples preserved in 70% ethanol for storage. Invertebrates were only sampled in September 2015 and January 2016.

2.2.2 Laboratory work

Samples for water chemistry were only analysed for major nutrients (NO_3, NO_2, NH_4, N_{total}, PO_4, P_{total}). All the samples were thawed and filtered through 0.2 µm cellulose acetate membrane filters and kept in acid washed 200 mL PP (polypropylene) bottles. Samples from four sites were analysed, in addition to the effluent water from the cooling towers. For this

purpose only samples from September 2015 and January 2016 were analysed by ALS Skandinavia, Sweden. Nutrient analyses are they key to understand the fundamentals of the periphyton communities, as nutrients often limit the growth of primary producers.

To determine the assemblages and the dominant periphyton genera, I measured the abundance, frequency of occurrence and the diversity of the algae and Cyanobacteria in each sample. The samples were kept in darkness until laboratory processing took place. Each sample was shaken for approximately 30 seconds to get an even distribution of the periphyton before a well-mixed subsample of 0.1 mL was pipetted off and dispensed onto a Palmer-Maloney counting Chamber. Finally, a cover slip was placed carefully on top of the prepared subsample and cover slip was then glued with nail varnish on the edges to prevent samples from drying up.

Algae and Cyanobacteria were identified to genus level using a Leica DM4000 microscope at 200–400 times magnification and individual cells were counted. However, filamentous algae and Cyanobacteria, such as *Anabaena*, *Calothrix* and *Lyngbya* were counted in units (each unit consisted of 10 µm filament), as it was often difficult to distinguish cell partitioning. A minimum of 300 live (chloroplast present) cells/units were counted within a defined number of fields across the Palmer-Maloney counting chamber. The Palmer-Maloney counting chamber was divided into longitudinal transects and the periphyton counted in a left to right direction. Number of fields on each transects were noted to allow for density calculations. When the end of each transect was reached, the field of view was carefully rearranged in a downward motion, and counting continued in a reversed direction until the necessary minimum of cells/units were reached. If the densities of cells/units were low (<300 cells/unit algae and Cyanobacteria) the entire slide was counted. In order to count frequency of occurrence (FO) of each algal and cyanobacterial genera a so called WhippleGrid (NE71 Index pattern, 10 x 10 mm grid with 0.5 mm squares, PYSER-SGI, UK) was used. The WhippleGrid was placed in one of the microscopic oculars to provide grids (cross hairs) to count periphyton occurrences within its field of view. Algae and Cyanobacteria cells were counted along with their occurrences on the WhippleGrid, whereas numbers of cross hairs covered by each cells was counted. For example, single *Epithemia* cell often accounts for 5 occurrences, as the cell might cover 5 cross hairs on the WhippleGrid due to its large size. Frequency of occurrence provides important information on periphyton size and how space-consuming different genera are. Samples from sites KR1-3 and KR1-1 were not processed because of low periphyton density, which meant that those samples would be very time consuming to process. Algae and Cyanobacteria were identified to genera in most cases, and diatoms were identified to species only if obvious characteristics were present and visible at the magnification applied. Based on morphological and physiological characters, Cyanobacteria and diatoms were split into two sub-groups: Nitrogen-fixing (N_2-fixing) and non-nitrogen-fixing (non-N_2-fixing) groups. All of the observed Cyanobacteria were filamentous and were consider to fix nitrogen if a heterocyte was present (heterocyte bearing Cyanobacteria; HCYA) and to not fix nitrogen if heterocyte was absent (non-heterocyte bearing Cyanobacteria; NHCYA), as has been done in various studies (Mackenzie *et al*., 2013).

The densities and frequency of occurrence of algae and Cyanobacteria was calculated with the following formula (Hauer & Lamberti, 2006):

$$Density = \frac{\left(\left(\left(\frac{PCC}{AoF * FoV}\right) x No.C\right) x Vol.T\right)}{Area.T}$$

Density – Density per cm^2.
PCC –Counting Chamber total area (245.5mm^2).
AoF –Area of field of view (0.25mm^2).
FoV – Field of view counted.
No.C – Number of counted cells/units.
Vol. T – Total volume of sample (1000µl).
Area.T – Total area of sampled surface (10.52cm^2).

The identifications was carried out by available keys by: Whitton and Brook (2011); Biggs and Kilroy (2000); Hoef-Emden, and Melkonian (2012); Hoffmann, Werum, and Lange-Bertalot (2013); Kelly (2000) and Bellinger and Sigee (2010).

The chlorophyll measurements were carried out by thawing the CF/C filters and immerse them in 96% ethanol and keep them in a refrigerator for at least 24 hours in total darkness. When all the chlorophyll had been dissolved in ethanol the absorption of the extract was measured at 665 nm and 750 nm using a DR Lange 5000 spectrophotometer in a dim room in 10 x 10 mm cuvettes. A wavelength of 750 nm was used for correction of residual turbidity after filtration and the dissolvent phase. Finally, wavelength of 665 nm was used as the absorption maximum wavelength in the red end of the spectrum for chlorophyll *a*. The absorbance was repeated after acidification with 1N HCL to correct for pheophytins. Chlorophyll *a* values are expressed as µg/cm^{-2} and were calculated according to Lorenzen, (1967), using the extinction coefficient for 96% ethanol from Wintermans & De Mots (1965).

$$Chl\ a = \frac{A * K \left(\left(665_b - 750_b\right) - \left(665_a - 750_a\right)\right) * V}{S * l}$$

$Chl\ a$ − concentration of chlorophyll a (µm/cm^2)
A – 11.99 absorption coefficient (µg/cm^2) of chlorophyll a, derived from reciprocal of specific absorbance of chlorophyll a in 96% ethanol: 83.4 l/(g*cm).
K – 2.43 factor of correction for acidification.
665_b – Absorbance at 665 nm before acidification.
750_b – Absorbance at 750 nm before acidification.
665_a – Absorbance at 665 nm after acidification.
750_a – Absorbance at 750 nm after acidification.
V – Volume of ethanol, used for extraction (ml).
S – Area of stone (cm^2).
l – Length of path light through cuvette (cm).

The proportion of five major algal and cyanobacterial groups (Cyanobacteria, N_2-fixing Cyanobacteria, diatoms, N_2-fixing diatoms, and green algae) was plotted to highlight the periphyton assemblages, along with the correct densities of each algal and cyanobacterial genera for more detailed assemblages structure (Appendix 1). Proportion was plotted based on relative abundance and relative frequency of occurrence (relative FO) from the

identifications and enumerations. Average densities (number of cells/units per cm^2) were calculated for all algal and cyanobacterial genera for four sampling sites, for all occasions.

Invertebrates were identified under a binocular microscope. Once processed the invertebrate samples were preserved in separated tubes in 70% ethanol for further identification at later stage.

In few cases temperature data loggers had been washed up on the banks or frozen due to reduced discharge in the winter. In those cases data were removed for analyses. In few other cases loggers malfunctioned, resulting in non-continuous logging.

2.2.3 Numerical analyses

Owing to non-normality, the data for biomass was log transformed before testing differences using ANOVA (Analyses of Variance). Tukey HSD *post hoc* test (honest significant difference) was plotted to test if difference was significant between biomass or not. Bonferroni correction was applied to counteract the problem of multiple comparisons, thus, significance was present when *P*-values were lower than 0.01.

Temperature data from the loggers were collected and processed in Microsoft Excel. The daily average temperature was calculated as the average value of 24–48 measurements per day. Standard deviations were calculated for each day, along with the coefficient of variation to observe daily temperature stability. Anomalies, such as sudden decrease in temperature due to freezing, were deleted from the loggers.

All statistical analyses were performed using RStudio Version 3.3.1 and Microsoft Excel Version 14.0.7172.5000.

Shannon Wiener Index was used to quantify the diversity of algae and Cyanobacteria.

2.3 Results

2.3.1 Physical Parameters

Temperature was high at the two upstream sites (KR1-2 and KR1-3) and lower at one of the upstream site (KR1-1). The temperature increased right downstream from the power plant and then decreased further downstream. The temperature was warm and constant at the references site. Upstream from the power plant, the average temperature varied between the two streams (KR1-1 versus KR1-2 and KR1-3) according to the average temperature obtained with the multiprobe sonde. The lowest temperature was recorded at site KR1-1 (11.2 °C), but was higher at sites KR1-2 and KR1-3 (19.7 and 19.8 °C). The temperature increased downstream from the power plant with highest values at site KR1-4 (26.8 °C on average). Further downstream from the power plant (KR1-6), temperature decreased, with the average values of 17.9 °C. The reference site (KR2-2) had relatively warm temperatures with stable values throughout the observation, with the average of 16.7 °C.

Table 2-1. The water temperature at all the study sites measured at time of sampling.

	Temperature (°C)					
	KR1-1	KR1-2	KR1-3	KR1-4	KR1-6	KR2-2
9 - 11 June 2015	7.0	15.6	13.9	19.5	15.0	11.8
19 - 21 July 2015	17.5	18.0	17.6	26.4	19.2	17.7
1 - 3 September 2015	14.9	21.6	20.8	35.9	23.7	18.8
6 - 8 October 2015	12.3	21.2	22.0	31.9	16.2	18.5
20 - 22 January 2016	7.7	18.1	N/A	24.2	14.7	16.9
10 - 11 May 2016	11.7	27.9	29.6	21.7	15.2	17.0
13 - 15 June 2016	7.2	15.3	14.9	28.1	21.0	16.1
Average	11.2	19.7	19.8	26.8	17.9	16.7

The continuous recording of temperature revealed that the temperature at the upstream site KR1-2 was higher compared to the other upstream site KR1-1. A great fluctuation was recorded in temperature at the sites upstream from the power plant where the temperature at site KR1-1 was around 10 °C in June and July 2015, but increased suddenly to ~30 °C for about 10 days in middle of August (Figure 2-1). At this site, the temperature decreased rapidly to 10 °C by end of August. Further temperature decrease occurred in the winter (November and December 2015), followed by a rather stable temperature (~8 °C) until the end of May 2016 when there was a decrease for a short period. Temperature was on average higher for the other upstream site, KR1-2, where it was around 15 °C in October 2015 and with relatively constant readings throughout October. By the end of October the temperature had decreased significantly, or down to 0–5 °C. These low values were constant until the end of January 2016 when the temperature increased significantly, or up to 25 °C. From this time, there were great fluctuations at this site, as readings repeatedly changed between 5 and 34 °C throughout the winter. By the end of May 2016, the temperature dropped down to ~10 to 15 °C, but had increased to ~22 °C by the middle of June 2016. Data for the remaining upstream site, KR1-3, could not be retrieved as the data logger tended to malfunction throughout the experiment. Daily fluctuations in temperature were more severe

at site KR1-2 in comparison to KR1-1 according to both standard deviation and coefficient of variation.
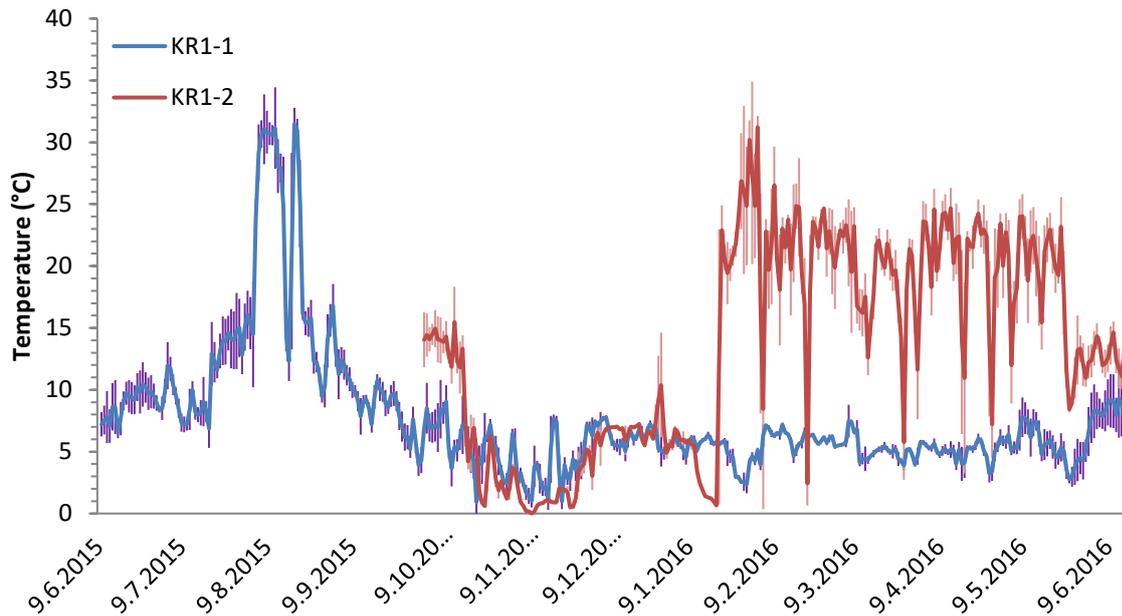
Figure 2-1. The water temperature at two of the upstream study sites measured at 60 minutes intervals. Data is represented as average values per day (24 hrs.) along with standard deviation for each day.

The temperature for the downstream sites was high and correlated between the two sites, with approximately 5 °C difference throughout the study. The temperature increased downstream from the power plant in comparison to the upstream sites (Figures 2-1. and 2-2.). The temperature was higher closer to the power plant (KR1-4) in contrast to the further downstream site (KR1-6). In June 2015, the temperature was around 20 °C for site KR1-4 and rose up to roughly 30 °C in August, with considerable fluctuation throughout the summer. During the first half of the winter (November 2015 – January 2016) the temperature dropped to approximately 15 °C and rose up to ~25 °C in February 2016. For the rest of the study period there were repeated fluctuation. Similar trend in temperature was observed further downstream, at site KR1-6, but the values were lower. In June to October the daily average temperature was between 15 and 20 °C, but by the end of October it was around 10 °C. The daily average temperature dropped down to ~0 °C in February and remained just above freezing, until it started to go up again in May when it rose to approximately 20 °C. A significant decrease in the daily average temperature occurred in the end of May 2016 (10 °C) and then followed by a sudden increase (~20 °C) in June 2016. Daily fluctuations were relatively higher at site KR1-6, often with extremely high peaks according to the coefficient of variation and standard deviation. Temperature at KR1-4 showed more stable conditions regarding the daily average temperature, with minor fluctuations based on both standard deviation and the coefficient of variation.
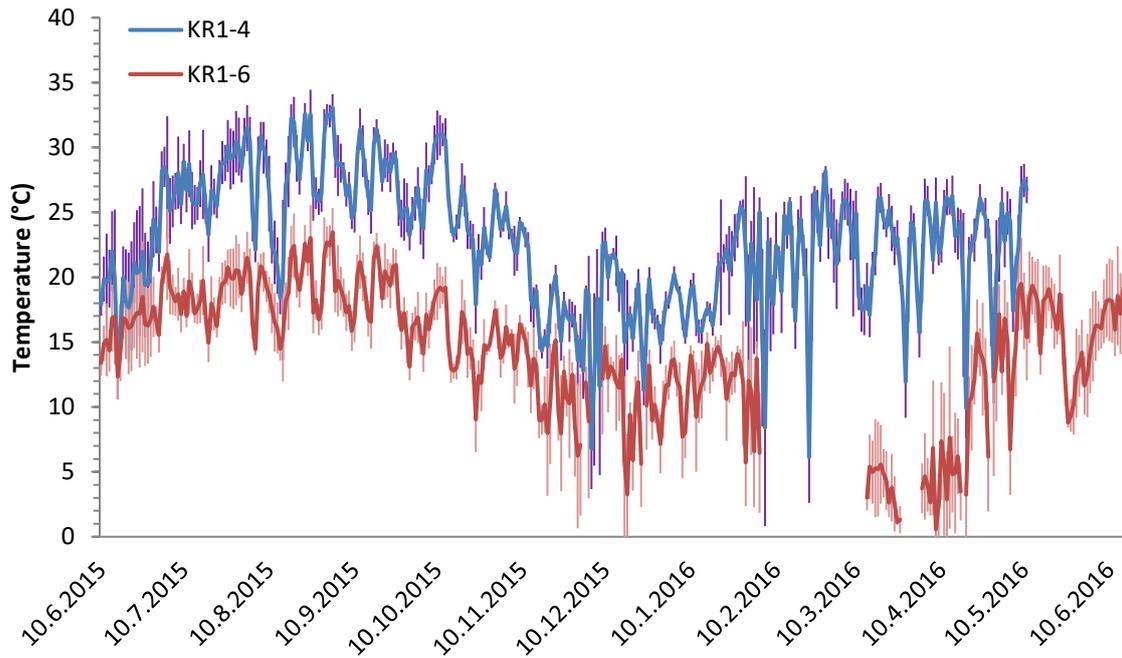
Figure 2-2. The water temperature at two of the downstream study sites measured at 30-60 minutes intervals. Data is represented as average values per day, along with SD for each value.

The reference site (KR2-2) was characterized by relatively warm water, as temperature was approximately 16 °C throughout the study period with minor fluctuation, until a major drop occurred in the spring 2016. The daily average temperature at the reference site KR2-2, ranged between 10 and 14 °C in the spring of 2015 (Figure 2-3). A slight increase was in the daily average temperature during the summer. In August, the daily average temperature was approximately 18 °C and then decreased a bit in October. During the winter and early spring the daily average temperature fluctuated around 16 °C. There was a sudden drop in the daily average temperature in late May, when it was ~10 °C for about two weeks and then by June 2016 it had risen again, reaching 17.7 °C. Daily average temperature was generally stable, as little fluctuations occurred. Standard deviation and coefficient of variation proofed the stability. However, temperature was less stable in the spring.
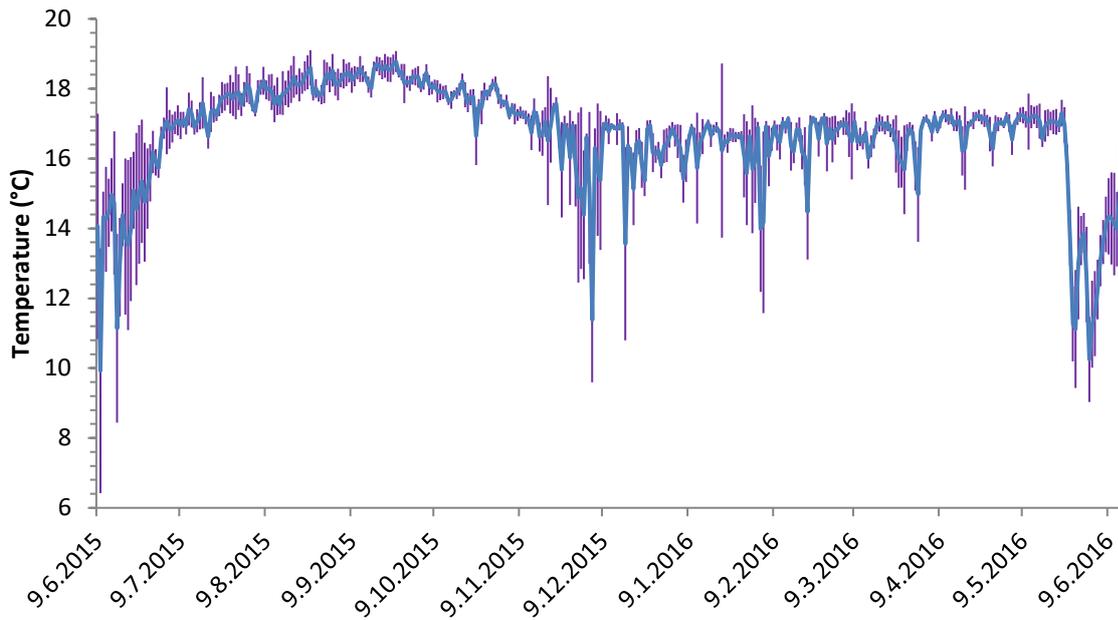
Figure 2-3. The water temperature at the reference site (KR2-2) measured at 60 minutes intervals. Data is represented as average values per day, along with SD for each value.

The stream water was alkaline at all the sites. Downstream from the power plant, values for pH increased in contrast to the upstream sites. The lowest pH values were recorded at the reference site. The pH of the stream water was slightly alkaline on average, ranging from 7.81–8.17 at sites upstream from the power plant. Out of the three upstream sites, lowest pH-value (7.81) was recorded at site KR1-3 while pH-values for site KR1-2 and KR1-1 were higher, 8.17 and 8.09 respectively. At the downstream site, KR1-4, the highest average pH-value was 8.40 and only slightly lower at site, KR1-6, with values of 8.37. The pH values for the reference site were usually lower than for all the other sampling sites (Table 2-2).

Table 2-2. The pH-values all the study sites at the date of sampling 2015-2016.

	pH					
	KR1-1	**KR1-2**	**KR1-3**	**KR1-4**	**KR1-6**	**KR2-2**
9-11 June 2015	8.01	8.09	7.38	7.82	8.24	7.88
19-21 July 2015	8.33	8.03	7.53	8.24	8.45	7.82
1-3 September 2015	8.43	8.07	7.81	8.74	8.37	7.77
10-12 October 2015	8.18	8.02	7.60	8.97	8.49	7.79
20-22 January 2016	8.15	8.00	N/A	8.74	8.63	7.58
10-11 May 2016	7.55	8.97	9.14	8.14	8.04	7.67
13-15 June 2016	8.00	8.01	7.38	8.17	8.37	7.90
Average	8.09	8.17	7.81	8.40	8.37	7.77

The conductivity of the stream water was higher at sites upstream from the power plant than at the downstream sites. The conductivity was lower at the reference site than it was at the other sites. The conductivity was generally high in the upstream sites where the average values were between 937 and 1,870 µS/cm (Table 2-3). The average values for conductivity was lower at the downstream sites from the power plant in comparison to the upstream sites (539–647 µS/cm^2) and the lowest values were recorded at the reference site.

Table 2-3. The conductivity at all the sampling sites measured at dates of sampling throughout the study period.

	Conductivity (µS/cm)					
	KR1-1	KR1-2	KR1-3	KR1-4	KR1-6	KR2-2
9-11 June 2015	312	1,564	1,396	414	374	231
19-21 July 2015	886	1,452	1,295	604	564	348
1-3 September 2015	1,294	2,439	2,475	751	630	358
10-12 October 2015	1,773	2,568	2,634	779	618	378
20-22 January 2016	1,366	2,510	N/A	767	568	361
10-11 May 2016	635	1,537	1,375	644	556	356
13-15 June 2016	296	944	848	570	464	307
Average	937	1,859	1,671	647	539	334

The discharge was low for all the upstream sites except for KR1-2, but it increased significantly downstream from the power plant after the effluent had entered the stream. A further increase in discharge occurred as water from the reference stream merged with Hlíðardalslækur. Information concerning the discharge should be treated with caution, as some of the measured streams were too shallow to obtain reliable readings. Such uncertainties mainly occurred at the sites upstream from the power plant. For the upstream sites, the discharge was highest at KR1-2 (0.028 m^3/sec) and lowest at KR1-3 (0.014 m^3/sec). The discharge for KR1-1 was unstable and the average discharge was 0.016 m^3/sec (Table 2-4). The average discharge was higher downstream from the power plant, with values of 0.221 m^3/sec at site KR1-4 and 0.294 m^3/sec at KR1-6. The average discharge for the reference site was 0.066 m^3/sec. The discharge was over all, highest in the spring (June 2015 and 2016) and decreased throughout the summer. The discharge was not measured in January and May 2016, but it appeared to be lower than in October 2015.

Table 2-4. The discharge at all the sampling sites at five out of seven sampling dates in 2015 and 2016.

	Discharge (m^3/sec)					
	KR1-1	KR1-2	KR1-3	KR1-4	KR1-6	KR2-2
9-11 June 2015	0.028	0.047	0.036	0.308	0.419	0.119
19-21 July 2015	0.004	0.014	0.014	0.216	0.250	0.046
1-3 September 2015	0.005	0.017	0.004	0.206	0.299	0.059
10-12 October 2015	0.005	0.002	0.001	0.147	0.246	0.050
13-15 June 2016	0.041	0.059	0.017	0.229	0.258	0.056
Average	0.016	0.028	0.014	0.221	0.294	0.066

The nutrients concentration generally increased between late summer and winter. All the sites appeared to be phosphorus limited, except the upstream site, KR1-2.

The concentration of total dissolved inorganic nutrient, nitrogen (TDN, NO_3-N, NO_2-N and NH_4-N) and phosphorus (TDP and PO_4-P) varied both annually and spatially (Table 2-5). Total dissolved nitrogen (TND), which is the sum of inorganic and organic nitrogen, was higher upstream from the power plant, at sites KR1-1 and KR1-2, than downstream, at sites KR1-4 and KR1-6. The nutrient concentration in the effluent water was low. The concentration of the dissolved inorganic nitrogen (DIN) was all below detection but dissolved inorganic phosphorus (DIP) was 0.4 µmol/L.

The concentration of dissolved inorganic nitrogen (DIN) at site KR1-1 decreased from 3.11 µmol/L in September to 0.35 in January, on the other hand the concentration of DIP increased from 0.13 to 0.23 µmol/L for the same months. At site KR1-2 the concentration of dissolved inorganic nitrogen increased between September and January, from 1.03 to 4.14 µmol/L and the concentration of DIP increased from 0.03 to 0.10 µmol/L for the same months. The concentration of organic nitrogen was high at site KR1-1 in January which meant that the total concentration of dissolved nitrogen (TDN) was higher in January than in September. The total dissolved nitrogen and phosphorus at site KR1-2 and TDP at site KR1-1 was in lower concentration in the January samples compared with the September samples (Table 2-5).

The concentration of dissolved inorganic nutrient concentration (DIN and DIP) at the downstream sites from the power plant, KR1-4 and KR1-6 was lower than it was at the upstream sites. A relatively high concentration of TDN was observed at the site KR1-4, with values of 10.00 µmol/l in September and 3.86 µmol/l in January. Further downstream from the power plant, at site KR1-6, the concentrations of DIN was relatively high in September with values of 1.04 µmol/L compared with KR1-4. In January the concentration of DIN had increased to 3.32 µmol/L at KR1-6. The concentration of DIP was 0.42 µmol/L in September and 0.77 µmol/L in January. A high concentration of TDN was observed (3.43 and 7.85 µmol/L) along with relatively high concentration of TDP (0.42 and 0.81 µmol/L), due to high concentration of organic N and P.

The concentration of DIN at the reference site, KR2-2, was high in September (2.04 µmol/L) and January (5.81 µmol/L). The concentration of DIP was also relatively high, 0.39 µmol/L in September and 0.97 µmol/L in January. Total dissolved nitrogen was higher than total dissolved phosphorous, with TDN concentration of 5.93 µmol/L September and 9.28 µmol/L in January and TDP was 0.52 in September and 0.94 in January.

Table 2-5. The nutrient concentrations at five of the study sites and the effluent from the cooling tower in September 2015 and January 2016. The concentration are shown for: total dissolved nitrogen (TDN), dissolved inorganic nitrogen (DIN), NO₃-N, NO₂-N, NH₄-N, dissolved organic nitrogen (DON), total dissolved phosphorus (TDP), dissolved inorganic phosphorus (DIP), PO₄-P and DOP = dissolved organic phosphorus (DOP). All concentrations are expressed as µmol/L.

Site	Date	TDN N_{total} µmol/l	NO_3-N µmol/l	NO_2-N µmo/l	NH_4-N µmol/l	DIN µmol/l	DON N_{tot}-DIN µmol/l	TDP P_{total} µmol/l	DIP PO_4-P µmol/l	DOP P_{tot}-DIP µmol/l
KR1-1	Sept	5.85	<0.07	<0.04	3.00	3.11	2.75	0.26	0.13	0.13
	Jan	4.36	0.11	<0.04	<0.20	0.35	4.01	0.23	0.23	0
KR1-2	Sept	3.93	0.14	<0.04	0.86	1.03	2.89	0.09	0.03	0.06
	Jan	6.50	1.00	0.07	3.07	4.14	2.36	0.09	0.10	0
KR1-4	Sept	10.00	<0.07	<0.04	<0.20	0.31	9.69	0.13	0.16	0
	Jan	3.86	<0.07	<0.04	<0.20	0.31	3.55	0.26	0.23	0.03
KR1-6	Sept	3.43	<0.07	<0.04	0.93	1.04	2.39	0.42	0.42	0
	Jan	7.85	1.64	<0.04	1.64	3.32	4.53	0.81	0.77	0.03
KR2-2	Sept	5.93	1.64	<0.04	0.36	2.04	3.89	0.52	0.39	0.13
	Jan	9.28	5.57	<0.04	<0.20	5.81	3.47	0.94	0.97	0
Effluent	June	2.28	0.1	<0.04	<0.20	0.31	1.97	0.387	0.4	0.03

2.3.2 Periphyton diversity

The number of genera was higher in the upstream sites and the reference site than downstream from the power plant. Shannon diversity indices were on average lowest for the downstream site KR1-4 and highest for the reference site. Similar Shannon diversity was at the upstream site, KR1-2, and the downstream site KR1-6. A total of 21 genera were recorded at the upstream site, KR1-2, which was the second highest number of algal and cyanobacterial genera observed (Table 2-6). Of these 21 genera 12 belonged to diatoms (*Aulacoseira, Caloneis*, *Cocconeis, Diploneis*, *Epithemia*, *Fragilaria, Melosira, Meridion*, *Navicula, Nitzschia, Rhoicosphenia, Rhopalodia*), three to Chlorophyta (*Klebsormidium*, *Microspora*, *Stigeoclonium*), one to Ochrophyta (*Tribonema*) and 5 genera belonged to Cyanobacteria (*Anabaena*, *Calothrix*, *Lyngbya, Oscillatoria* and *Phormidium*). The Shannon diversity at the upstream site KR1-2, ranged between 0.16 and 1.63 (Figure 2-4). Although the diversity index reached high values, the diversity was on average lower in contrast to the other sites, except for the downstream site, KR1-4. At site KR1-4 only 9 genera were present, where 5 of them belonged to Cyanobacteria one of Chlorophyta (*Stigeoclonium*), one Tribonemataceae and three genera of diatoms. The lowest Shannon diversity values occurred at this site, KR1-4, where the values ranged between 0.32 and 1.02. Generally, the Shannon diversity was lowest in October 2015 and January 2016 and highest in June 2016 (Figure 2-4). Higher number of genera were found further downstream from the power plant, at site KR1-6, with total of 16 genera, where 8 of them belonged to diatoms, 4 to Chlorophyta and 4 genera of Cyanobacteria (Table 2-6). The Shannon diversity was usually the second highest at site KR1-6, ranging from 0.85–1.81. The highest number of genera was recorded at the reference site (KR2-2) with 22 different algal and cyanobacterial genera, which mainly consisted of diatoms (13), four genera of Chlorophyta and four of Cyanobacteria. The highest Shannon diversity index was found to be at the reference site,

KR2-2 where the values were 1.48–1.95. Seasonal changes in diversity were observed for the upstream site, in which diversity increased from June 2015 to September 2015 (Figure 2-4 & 2-5). In October the diversity decreased followed by a low Shannon diversity index in January 2016. In the summer of 2016 (June), the diversity had increased again. Downstream from the power plant at site KR1-4, seasonal changes were not observed, although some minor changes in diversity occurred between observations. An increase occurred in between January and June 2016. The Shannon diversity for the reference site (KR2-2) was rather stable throughout the study period, except for a peak in July 2015 (Figure 2-4).
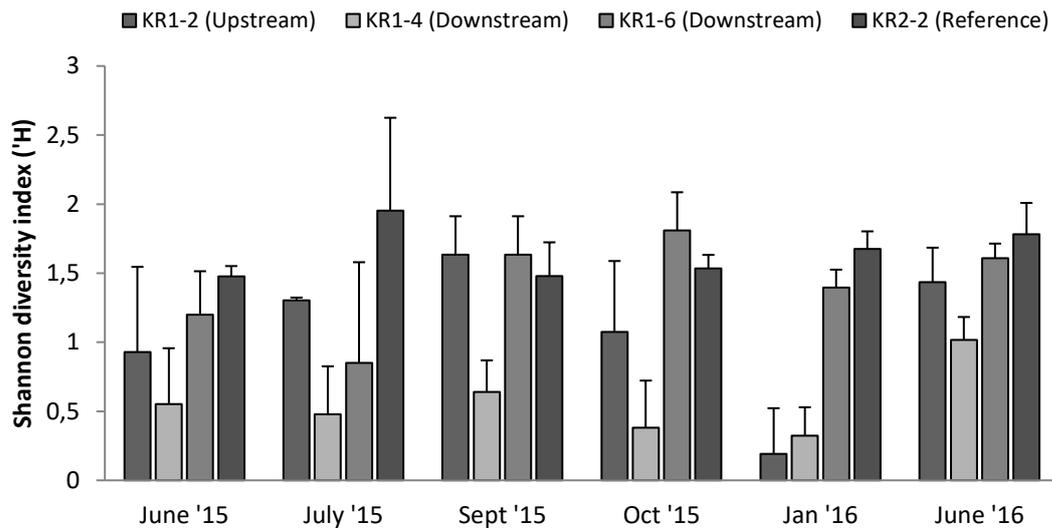
Figure 2-4. The average Shannon diversity index ('H) for four sites 2015–2016 based on 3 replicate samples. Standard deviation is plotted for each column.

Table 2-6. List of taxa (genera, species and different morphs) of algae and Cyanobacteria recorded in one upstream and two downstream sites from the geothermal power plant at Krafla, along with one reference site. X indicates that the particular group was present, hyphens (-) indicates that the genus was not found in the samples and star means that individual algal group is nitrogen fixing. Small cells = S, medium Cells = M, large cells = L.

	KR1-2	KR1-4	KR1-6	KR2-2
Chlorophyta				
Cladophora	-	-	X	X
Klebsormidium	X	-	X	-
Microspora	X	-	-	X
Rhizoclonium	-	-	-	-
Spirogyra	-	-	X	X
Stigeoclonium	X	X	X	X
Cyanobacteria				
Anabaena*	X	X	X	X
Calothrix*	X	X	X	-
Lyngbya	X	X	X	X
Oscillatoria	X	X	-	X
Phormidium	X	X	X	X
Diatoms			-	
Aulacoseira	X	-	X	X
Caloneis	X	-	-	-
Cocconeis	X	-	X	X
Diploneis	X	-	X	X
Epithemia*	X	-	X	X
Fragilaria (m-L)	-	-	X	X
Fragilaria (s-m)	X	-	X	X
Gomphonema	-	-	-	X
Melosira	X	X	X	X
Meridion	X	-	X	X
Navicula (m)	X	X	-	X
Navicula (s)	-	-	-	-
Navicula radiosa	X	-	-	X
Nitzschia (m)	-	X	X	X
Nitzschia (s)	-	-	-	-
Nitzschia linearis/intermedia	X	-	X	X
Pinnularia	-	-	-	X
Rhoicosphenia	X	-	-	X
Rhopalodia	X	-	X	X
Ochrophyta				
Tribonema	X	X	-	-
Total number of observed taxa	22	9	19	24

The number of taxa (genera, species and different morphs) ranged between 4 and 12 per observation at site KR1-2 (Figure 2-5). Usually, numbers of taxa were reasonably constant, with one exception in January, as only four taxa were present. Dominating periphyton were most often N_2-fixing genera of both Cyanobacteria and diatoms. Only 2–6 genera of algae and Cyanobacteria were present throughout the study at site KR1-4, with Cyanobacteria dominating group, but in few cases diatoms and green algae were present. All of the observed Cyanobacteria genera, mainly N_2-fixing genera, were present at KR1-4 at some stage throughout the study (Table 2-8). The second highest number of algal and cyanobacterial taxa was observed at the downstream site further from the power plant, KR1-6, where the number of genera taxa between 8 and 14. For the reference site, numbers of genera were much higher in comparison to the other sites, with 15–20 genera at every single observation.

In general, seasonal fluctuations in number of algae and Cyanobacteria taxa were observed for all the sites, with a noticeable decrease in January at all the sites except for KR1-4 (Figure 2-5). The greatest drop in number of taxa was at KR1-2 between October and January with 13 and 5 genera present respectively. Little change occurred in number of genera at site KR1-4 from autumn till winter, but at KR1-6 the change greater i.e. from 15 to 6 genera from October till January. As for the reference site, seasonal changes were observed, in which number of taxa were stable throughout the summer with 19–21 taxa present. Rather small decrease was observed in the winter as the number of taxa were 16–17.
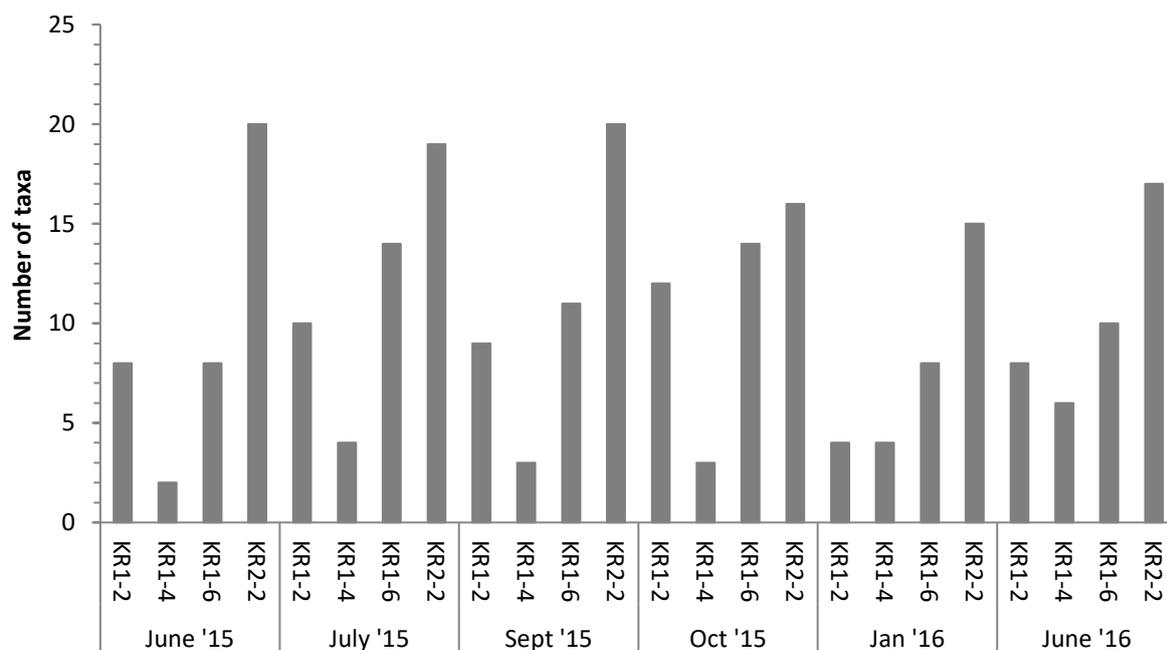
Figure 2-5. The number of algal and cyanobacterial taxa at each site throughout the study period.

2.3.3 Periphyton densities

The density of algae and Cyanobacteria was lowest at the upstream sites. The highest density was at the downstream site, KR1-4. Further downstream from the power plant, the density was also high. At the reference site, the density was high at all sampling occasions. The density of algal and Cyanobacteria was on average lowest for the upstream site KR1-2, with densities ranging from 1,426 to 2,444,112 cells/units per cm^2 (Figure 2-6). The density was higher at the downstream to the power plant where the highest overall density was measured (639,755- 7,159,718 cells/units per cm^2). Further downstream, at site KR1-6, the density was lower, that at KR1-4, as densities ranged from 19,328 to 2,320,016 cells/units per cm^2. The density of algae and Cyanobacteria at the reference site was not as high as it was just below the power plant (KR1-4) with values ranging from 726,612 to 2,224,292 cells/units per cm^2.

Rather conventional seasonal shifts were observed for the upstream site KR1-2. In June 552,241 cells/units per cm^2 were present. In July there had been a significant increase in density (2,444,112), followed by a reduction in September (568,267). Density kept constant till October, but reduced down to 1,426 cells/units in January. Densities increased again in June 2016 (1,161,695). Downstream from the power plant, densities were rather stable over the summer and the autumn (ranging between 5,302,428– 7,159,718 cells/units per cm^2). A significant decrease occurred in January, wherein density reduced from 5,305,891 to 639,755 cells/units per cm^2. In June 2016 density increased again, reaching 2,667,028 cells/units. Further downstream from the power plant, site KR1-6, density increased from June (1,347,584) to July (2,302,016) followed by an unexpected decrease in September, in which only 19,328 individuals per cm^2 were present. Another increase occurred in October (947,795), followed by a reduction in January (69,038). Similar to the other sites, an increase occurred between January and June 2016 (1,848,800). Density remained somewhat stable at the reference site (KR2-2) throughout the experiment. Nonetheless, small seasonal shifts were detected as densities increased in July (1,958,133) in comparison to densities in June (1,259,638). Rather stable values were observed throughout the summer and autumn (2,224,292 and 1,973,274), until a reduction occurred in January (724,612). In June 2016 densities had increased again (1,446,899), reaching similar densities as in June 2015.

Table 2-7. Densities for algae and Cyanobacteria (cells/units per cm^2) for the four observed sites over the observation.

Date	KR1-2		KR1-4		KR1-6		KR2-2	
	Average	SE	Average	SE	Average	SE	Average	SE
June 2015	552,241	22,806	5,302,429	73,181	1,347,584	321,415	1,259,638	241,769
July 2015	2,444,112	120,114	6,671,442	1,299,750	2,302,016	389,647	1,958,133	376,763
September 2015	568,267	23,729	7,159,719	1,820,743	19,328	3,076	2,224,292	13,895
October 2015	683,335	394,524	5,305,892	3,063,358	947,795	547,210	1,973,274	1,139,270
January 2016	1,426	1,236	639,755	87,058	69,038	60,124	724,612	28,423
June 2016	1,161,695	176,614	2,667,029	138,583	1,848,800	81,021	1,446,899	9,021
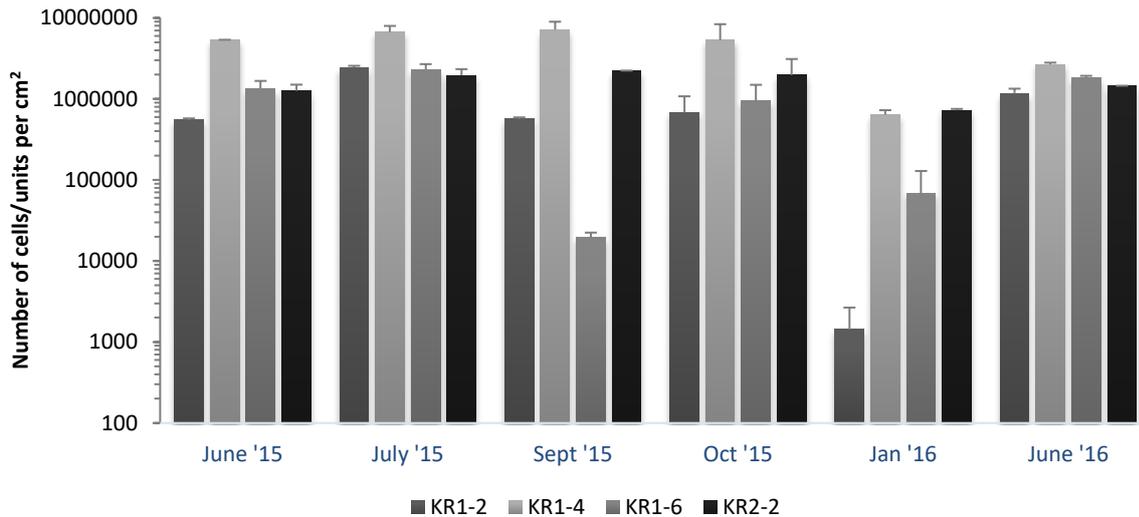
Figure 2-6. Densities for algae and Cyanobacteria for the four observed sites over the observation. Standard error is plotted for each column.

2.3.4 Periphyton assemblages

The upstream site was dominated by diatoms and Cyanobacteria, whereas downstream assemblages were dominated by Cyanobacteria. Further downstream from the power plant the benthic assemblages were more diverse. High relative abundance of diatoms characterised the reference site. Out of the five representative algal and cyanobacterial groups used to get an overview of relative abundance (proportional abundance), it turned out that relative abundance shifted greatly between the sampling sites and over time (Figure 2-7). Green algae were the least common of all the groups and they were only the dominating taxa at site KR1-2 in June 2015 and January 2016. Cyanobacteria and diatoms were consistently the most common of the groups at the streambed. The greatest changes in dominating groups were observed at the upstream site (KR1-2). Green algae dominated the periphyton at KR1-2 in June 2015 (Figure 2-7). In the following month, green algae practically disappeared, and then the periphyton at this site was dominated by diatoms (40%) and Cyanobacteria (60%). N_2-fixing Cyanobacteria were more common than non-N_2-fixing Cyanobacteria at the upstream site in most cases. Relative abundance of diatoms and Cyanobacteria were high in September and October, with rather similar relative abundance. Green algae were the most abundant group regarding the relative abundance (80%) and thus dominated the periphyton is January 2016, with Cyanobacteria being absent. Relative abundance for green algae and diatoms were high in June 2016, were it was around 40% in both cases while it was only roughly 20% for the Cyanobacteria at the same time.

The algae and Cyanobacteria assemblages at site KR1-4 was completely dominated by Cyanobacteria, which were more often than not the only group present (Figure 2-7). Both N_2-fixing Cyanobacteria and non-N_2-fixing Cyanobacteria had high relative abundance. N_2-fixing Cyanobacteria were more abundant in July and September (70% and 60%), but decreased in relative abundances in October and January (20% and 10%), giving way to non-N_2-fixing Cyanobacteria. In June 2016 N_2-fixing Cyanobacteria had increased in relative abundance, with nearly 50% during the same month. The relative abundance for non-N_2-

fixing Cyanobacteria remained 50% as well. In fact, other groups were absent at this site throughout the observation, except for diatoms in September and green algae in October, in which both groups accounted for less than 5% of composition.

The community structure further downstream from the power plant, at site KR1-6 was more complex than at site KR1-4 (Figure 2-7). N_2-fixing diatoms, along with non-N_2-fixing Cyanobacteria were responsible for highest relative abundance. N_2-fixing Cyanobacteria and green algae were present, but their proportion was slightly lower. N_2-fixing Cyanobacteria dominated in July, when their proportion was 80% of the relative abundance. Green algae vanished and diatoms became sparse. The proportion of N_2-fixing diatoms increased in September, however with Cyanobacteria still dominating at the site. All of the five algal and cyanobacterial groups were rather equally abundant in October, although diatoms and Cyanobacteria accounted for approximately 80% of the relative abundance. Green algae did not occur in the January samples and the benthic community was dominated by Cyanobacteria (mainly non-N_2-fixing) and diatoms (mainly N_2-fixing). In June 2016, green algae were found in high relative abundance at the site. However, Cyanobacteria still dominated the algal and Cyanobacteria assemblages, mainly N_2-fixing genera. Diatoms accounted for only approximately 10 % of the relative abundance.

Diatoms accounted for the highest proportion of all the groups at the reference site (KR2-2) throughout the study, thus dominating the assemblages (Figure 2-7.). Rather low alterations in assemblages were observed as diatoms relative abundance was fairly constant, ranging between approximately 65–99%. N_2-fixing diatoms were present at all times but in small proportion, less than 5% of total relative abundance. Cyanobacteria were sparse at the reference site and excessively represented non-N_2-fixing Cyanobacteria. Green algae were present at all occasions, although never exceeding 20% of the relative abundance.
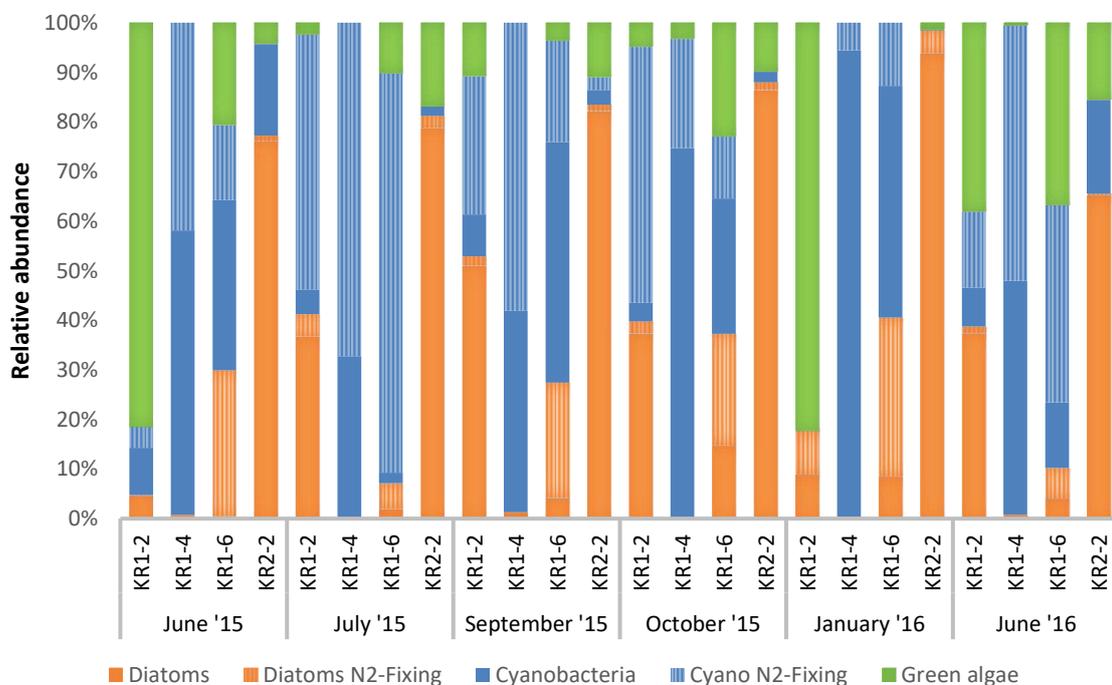
Figure 2-7. Relative abundance of Cyanobacteria and algae at the streambed at four sites in the Krafla area. Each column represents the relative abundance of the five major algal and cyanobacterial groups. Vertical lines = N_2-fixing algae/Cyanobacteria. Filled columns = Non-N_2-fixing algae and Cyanobacteria.

Diatoms dominated in relative frequency of occurrence at all sites except at the upper downstream site, KR1-4. Communities upstream from the power plant were mainly dominated by diatoms according to the relative frequency of occurrence. **The relative FO downstream from the power plant was dominated by Cyanobacteria. Further downstream (site KR1-6), diatoms dominated in relative FO, except when green algae dominated in June 2016. Diatoms dominated the reference site throughout the observation period.** The periphyton community was dominated by diatoms at all the sites based on the relative frequency of occurrence (FO), except for KR1-4 where Cyanobacteria was practically the only group present. Diatom cells can be fairly large hence they can make up for big portion of the relative FO of periphyton. Most of the diatoms were non-N_2-fixing genera, although N_2-fixing diatoms occurred in rather high relative FO in some cases. The relative frequency of occurrence of Cyanobacteria was fairly low, even though they were often extremely high in relative abundance (Table 2-8). The relative FO seemed to be rather similar between N_2-fixing versus non-N_2-fixing Cyanobacteria. Green algae were sparse in comparison to the other groups, they were usually located at all sites, but their FO was low (Figure 2-8). At some of the sites, green algae contributed considerably to the total FO of benthic primary produces where their relative FO was 10–30% (Figure 2-8).

At the upstream site, KR1-2, both N_2-fixing and non-N_2-fixing genera of diatoms were found in similar proportions, ranging between 50–90%. Diatoms dominated based on the relative frequency of occurrence at all but two occasions. The relative FO of Cyanobacteria, mainly N_2-fixing, ranged 0–20%. Green algae dominated in the relative FO in both June 2015 (80%) and June 2016 (40%).

The frequency of occurrence downstream from the power plant (KR1-4) was dominated by Cyanobacteria in spite of their tiny size, they were nevertheless responsible for 90–98% of the relative FO. The relative FO of green algae was little or none and the relative FO of diatoms was 10% at maximum. Relative FO of the Cyanobacteria was rather constant even though significant reduction in densities had taken place in January (Figure 2-8). N_2-fixing Cyanobacteria and non-N_2-fixing Cyanobacteria accounted for equally high relative FO in June and July. N_2-fixing Cyanobacteria dominated over other Cyanobacteria in September as they were responsible for over 60% of the relative FO of benthic primary producers. Non-N_2-fixing Cyanobacteria were responsible for high FO of benthic primary producers in October (80%) and January (90%), thus dominating over other groups. In June 2016, relative FO for N_2-fixing (50%) and non-N_2-fixing (40%) Cyanobacteria were high and diatoms contributed to the remaining 10%. Further downstream from the power plant, at site KR1-6, diatom and green algae relative frequency of occurrence was greater than the Cyanobacteria (Figure 2-8). Diatoms, especially N_2-fixing, usually dominated the communities as their FO ranged from approximately 20–80% over the study period, as they dominated in FO in June, September, October 2015 and January 2016. Green algae were also quite common at this site with high FO in June 2016 (45%). N_2-fixing Cyanobacteria dominated in July, responsible for approximately 40% of the relative frequency of occurrence (Figure 2-8).

All of the five major functional groups of benthic primary producers were present at the reference site (KR2-2) at some point during the study. Non-N_2-fixing diatoms were the dominating functional group for the reference site, accountable for 70–90% of the relative FO, at all observations. Temporal differences were observed, mainly due to seasonal changes for both Cyanobacteria and green algae. On the contrary, minor temporal changes were observed for the relative FO of the diatoms. Cyanobacteria were accountable for small

proportions of the FO (2–12%), in which all the genera were non-N$_2$-fixing. Green algae were responsible for slightly higher frequency of occurrence, ranging from 2–25% over study period.

Figure 2-8. Relative frecuency of occurrence (FO) of different groups of benthic primary producers. Each column represents the relative frequency of occurrence of the five major algal and cyanobacterial groups. Vertical lines = N$_2$-fixing algae/Cyanobacteria. Filled columns = Non-N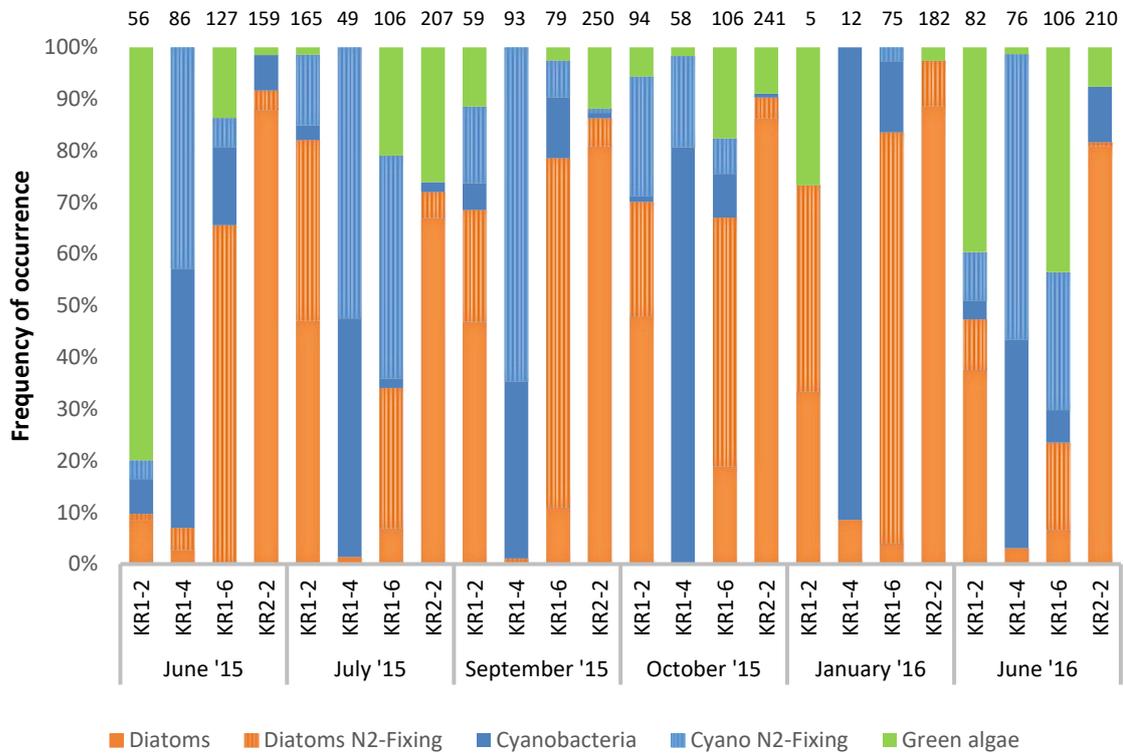$_2$-fixing algae and Cyanobacteria. Numbers of the total frequency of occurrence of periphyton are located above each column.

Table 2-8. Number of cells/units per cm² within each site. * = Nitrogen fixing genera. † = Yellow-green algae. Part 1 of 2.

	June 2015				July 2015				September 2015			
	KR1-2	KR1-4	KR1-6	KR2-2	KR1-2	KR1-4	KR1-6	KR2-2	KR1-2	KR1-4	KR1-6	KR2-2
Chlorophyta												
Cladophora	-	-	-	-	-	-	-	-	-	-	-	7,408
Klebsormidium	404,109	-	-	-	-	-	25,930	-	633	-	-	-
Microspora	-	-	-	3,810	56,664	-	-	162,324	-	-	-	204,998
Rhizoclonium	-	-	-	-	-	-	-	-	-	-	-	-
Spirogyra	-	-	-	-	-	-	5,964	35,388	-	-	-	5,975
Stigeoclonium	26,300	-	284,992	57,560	-	59,525	231,032	36,134	253	-	253	32,597
Tribonema †	11,797	-	-	-	-	-	-	-	-	124,335	-	-
Unidentified green algae	-	-	-	-	-	-	-	-	-	-	634	-
Cyanobacteria												
Anabaena*	26,485	257,274	198,935	-	1,241,410	2,891,397	1,808,564	-	4,943	2,240,872	4,943	66,676
Calothrix*	-	2,490,574	5,806	-	4,224	2,290,970	1,296	-	-	-	-	-
Lyngbya	10,777	20,183	353,155	208,573	92,683	-	37,598	-	5,355	-	5,355	-
Oscillatoria	-	-	-	13,048	-	-	-	28,936	-	13,829	-	-
Phormidium	49,546	2,508,558	106,757	-	25,684	280,762	15,558	-	1,489	2,535,477	1,489	-
Diatoms												
Aulacoseira	-	-	-	29,762	-	-	7,779	228,520	127	-	127	73,893
Caloneis	-	-	-	-	11,031	-	-	-	-	-	-	-
Cocconeis	-	-	-	5,920	-	-	-	8,533	32	-	32	23,372
Diploneis	-	-	-	8,626	2,440	-	-	10,661	127	-	127	3,967
Epithemia*	-	12,385	379,441	8,064	2,184	-	122,905	15,251	5,038	-	5,038	7,934
Fragilaria (m-L)	-	-	-	3,175	-	-	-	62,127	-	-	-	119,538
Fragilaria (s-m)	-	-	-	33,277	-	-	10,372	77,436	-	-	-	130,770
Gomphonema	-	-	-	-	-	-	-	-	-	-	-	-
Melosira	-	6,728	-	645,036	-	-	25,930	979,613	444	-	444	974,039
Meridion	-	-	-	2,393	-	-	-	14,806	32	-	32	62,183
Navicula	2,254	-	4,134	1,639	67,083	10,823	3,889	15,471	-	-	-	4,445
Navicula radiosa	370	-	-	2,200	2,440	-	-	9,464	-	-	-	1,482
Nitzschia	18,709	6,728	2,903	6,482	763,391	-	2,593	8,869	158	-	158	6,452
Nitzschia linearis/intermedia	741	-	-	15,992	65,373	-	1,296	8,869	63	-	63	5,449
Pinnularia	-	-	-	1,004	-	-	-	-	-	-	-	-
Rhoicosphenia	-	-	-	206,595	-	-	-	202,782	-	-	-	467,877
Rhopalodia*	1,143	-	11,455	6,482	109,499	-	1,296	27,421	602	-	602	24,233
Unidentified diatom	-	-	-	-	-	-	-	-	-	-	-	-

Table 2-8. (Continue) Number of cells/units per cm² within each site. * = Nitrogen fixing genera. † = Yellow-green algae. Part 2 of 2.

	October 2015				January 2016				June 2016			
	KR1-2	KR1-4	KR1-6	KR2-2	KR1-2	KR1-4	KR1-6	KR2-2	KR1-2	KR1-4	KR1-6	KR2-2
Chlorophyta												
Cladophora	-	-	6,223	-	-	-	-	-	-	-	4,667	-
Klebsormidium	18,191	-	-	-	-	-	-	-	390,707	-	-	-
Microspora	5,834	-	56,786	78,881	1,077	-	-	-	-	-	-	-
Rhizoclonium	-	-	-	-	-	-	-	-	-	-	-	-
Spirogyra	479	-	9,918	20,293	-	-	-	-	-	-	11,989	10,771
Stigeoclonium	4,634	181,812	87,068	67,642	95	-	32	40,074	15,056	17,780	675,479	208,595
Tribonema†	-	-	-	-	-	-	-	-	34,126	4,445	-	-
Unidentified green algae	-	-	17,503	-	-	-	-	-	-	-	-	-
Cyanobacteria												
Anabaena*	378,877	1,228,868	274,124	-	-	17,004	3,522	-	188,764	261,023	457,121	-
Calothrix*	-	3,142,588	889	-	-	21,608	-	-	-	1,086,074	265,945	-
Lyngbya	2,394	-	158,716	-	-	-	31,783	-	4,862	-	138,555	-
Oscillatoria	18,899	23,923	-	38,296	-	-	-	-	15,056	-	-	291,133
Phormidium	-	727,250	49,229	-	-	597,938	205	759	76,396	1,283,385	109,910	-
Diatoms												
Aulacoseira	1,986	-	2,917	149,333	-	-	3,010	38,894	-	-	-	-
Caloneis	166,516	-	-	-	-	-	-	-	-	-	-	-
Cocconeis	-	-	778	22,218	-	-	-	28,037	972	-	-	8,051
Diploneis	-	-	-	-	-	-	-	10,393	-	-	-	2,393
Epithemia*	1,619	-	159,272	14,569	-	841	19,696	98,894	-	-	111,375	3,336
Fragilaria (m-L)	-	-	29,004	174,933	-	-	-	-	-	-	-	37,425
Fragilaria (s-m)	-	-	-	38,296	-	-	-	25,128	-	-	3,112	58,930
Gomphonema	-	-	-	-	-	-	-	-	-	-	-	-
Melosira	-	-	68,343	1,149,030	-	-	285	366,764	-	-	-	516,882
Meridion	-	-	-	3,902	-	-	-	7,800	-	-	-	29,375
Navicula	4,705	-	-	2,550	-	-	-	3,336	4,862	1,729	3,386	-
Navicula radiosa	-	-	972	-	-	-	32	-	1,414	-	-	27,743
Nitzschia	60,583	-	6,112	10,667	-	-	222	4,005	411,590	12,594	54,818	65,277
Nitzschia linearis/intermedia	3,901	-	1,778	11,551	127	-	32	12,670	2,829	-	12,446	39,964
Pinnularia	-	-	-	4,059	-	-	-	11,594	-	-	-	-
Rhoicosphenia	-	-	-	127,115	-	-	-	133,547	1,004	-	-	126,637
Rhopalodia*	14,721	-	18,169	18,159	127	-	395	12,016	14,055	-	-	943
Unidentified diatom	-	-	-	-	-	864	-	-	-	-	-	-

2.3.5 Periphyton biomass

Biomass values were low for the upstream sites, with the lowest values at KR1-1. Downstream from the power plant biomass increased, but seemed to decrease further downstream at site KR1-6. The reference site had significantly higher biomass in comparison to the remaining sites. Periphyton biomass ($\mu g/cm^2$ chlorophyll) obtained with BenthoTorch was low at site KR1-2 at all observations, including the lowest biomass values of all the sites. Biomass at the upstream site KR1-1 were all under detection limits and was therefore excluded from spatial biomass comparison. Slightly greater biomass was observed for the other upstream site, KR1-3. Biomass at the sites downstream from the power plant (sites KR1-4 and KR1-6) was significantly higher in comparison to the upstream sites (KR1-2 and KR1-3). On the other hand, the values seemed rather low in comparison to the reference site, KR2-2. Biomass was highest for the reference site throughout the year, with values up to 4 folds higher than the second highest site (Figure 2-9). In June 2015 little differences were observed between sites, as significant difference ($P<0.01$) was only recorded between sites KR1-6 and KR2-2. Differences in biomass remained non-significant between all the sites in July ($P>0.01$). Spatial comparison for September and October remained rather similar between sampling sites, resulting in non-significant biomass comparison for all sites, except for sites KR2-2 and KR1-2 in September and KR2-2 and KR1-4 in October. In May, 6 out of 10 comparisons showed significant difference as the reference site differed from all the sampling sites, with KR1-4 being the only exception. Biomass for KR1-2 differed in comparison to all the sites except for KR1-3, which differed in comparison to KR1-4 and KR2-2. In June 2016, both the upper sites (KR1-2 and KR1-3) differed significantly from the rest of the sites. Except for site KR1-2 in comparison to KR1-4 (Appendix 2). It appears as seasonal/temporal changes occurred in which biomass generally increased in late summer and decreased in the autumn/winter (Figure 2-9). Sites upstream from the power plant reached their maximum biomass in October, followed by an obvious reduction in the following winter. In June 2016, the biomass had increased at site KR1-2 but remained low at site KR1-3. The biomass of benthic primary producers at KR1-4 was rather high, 2.3–2.7 $\mu g/cm^2$ and during the first two observations. Then in September it increased to 3.7 $\mu g/cm^2$. Fluctuations were observed for the remaining months, as biomass reduced in October, increased again in May 2016, followed by another reduction in June 2016. At site KR1-6, the biomass reached its highest values in July (3.4 $\mu g/cm^2$). A slight reduction was observed in September as values reduced to 2.5 $\mu g/cm^2$. The biomass at the reference site (KR2-2) also showed temporal variations, as chlorophyll concentrations was 5.5 $\mu g/cm^2$ in June and increased in July (7 $\mu g/cm^2$) and September (10 $\mu g/cm^2$). A decrease was observed in October as concentration was 8 $\mu g/cm^2$. Biomass at site KR1-1 was recorded below 0.2 $\mu g/cm^2$ at all observations, thus data for that site in particular were not tested for significance on spatial or temporal scale. For the upstream site KR1-2, a significant difference in biomass was observed for the month of May in comparison to all other months, as $P<0.01$. Another significant difference was between the month of October and June 2016. At site KR1-3 eight temporal comparisons showed significant difference in biomass. Only two dates were responsible; May 2016 and June 2016, as biomass was low during those months. Downstream from the power plant, at site KR1-4, no temporal significance was observed. Biomass changed between observations further downstream from the power plant, at site KR1-6, as significant difference occurred for three out of fifteen comparison. Biomass at the reference site showed minor changes during the experiment as only non-statistical significant differences occurred on temporal scale throughout the year. *P* values for the comparison are presented in the Appendix.

50
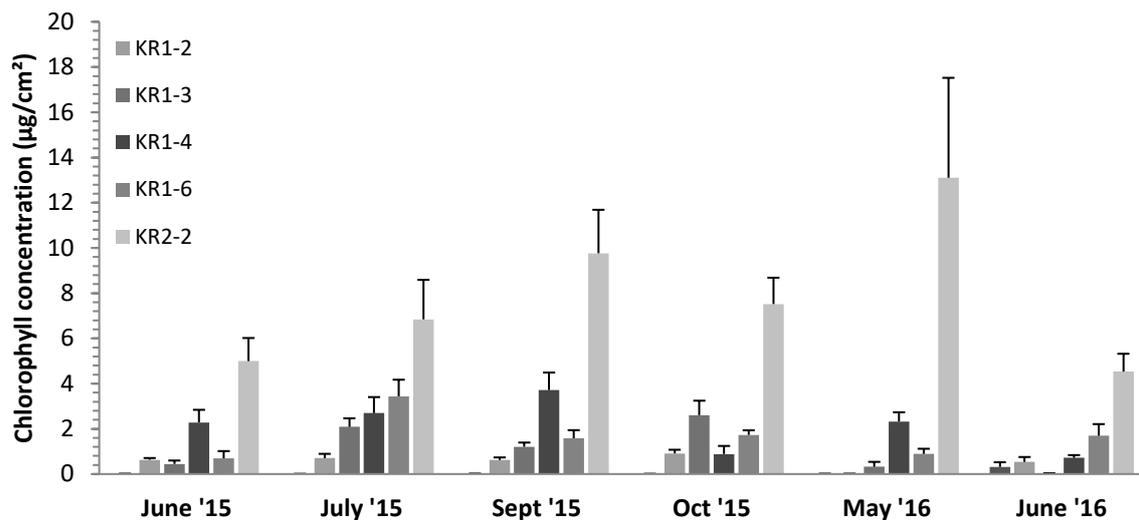
Figure 2-9. Average chlorophyll concentration for the observed reaches throughout the observation. Standard error (SE) bars are plotted on top of each column.

Biomass, obtained with light absorbance via spectrophotometer showed great variations on both spatial and temporal scale, with obvious decline in winter. The biomass was greatest at the reference site on all occasions, whereas, it was low very low at KR1-1. The biomass was also low at the other two upstream sites, much lower than both the sites downstream from the power plant. Periphyton biomass (μg/cm^2 chlorophyll *a*) was low at site KR1-2 at all observations, including the lowest biomass values of all the sites, except for site KR1-1 (Figure 2-10). Similar biomass was observed for the other upstream site, KR1-3. Biomass at the sites downstream from the power plant (sites KR1-4 and KR1-6) was significantly higher in comparison to the upstream sites (KR1-2 and KR1-3), although biomass at site KR1-4 was usually higher than at site KR1-6. On the other hand, the values seemed rather low in comparison to the reference site, KR2-2. Biomass was highest for the reference site throughout the year, with values up to 10 folds higher than the second highest site (Figure 2-10). In June 2015, all but three spatial comparisons differed significantly, as site KR1-4 did not differ from KR1-6 and KR2-2 ($P>0.01$), along with site KR1-2 not differing from KR1-3, $P>0.01$. In July all but four comparisons differed significantly. The reference site, KR2-2, differed significantly in comparison to all the sites. The upstream site from the power plant, KR1-3, differed significantly in all comparisons as well (except KR1-2). In September 2015 major alterations in biomass emerged in which high variations in spatial differences were observed. Only two comparisons did not show significant difference in biomass; KR1-2 in comparison to KR1-3 and KR1-4 in comparison to KR2-2. In October, four comparisons showed significant differences in biomass in which KR2-2 was responsible for three of those comparisons. Observed statistical difference transpired between KR1-4 and KR1-6 as well. Spatial differences were also relatively evident in January, as five out of six comparisons displayed significance in biomass comparison. The only comparison not evident of significant difference occurred between the effluent effected sites, KR1-4 and KR1-6. In June 2016 similar spatial variations transpired as for June 2015. Site KR1-3 differed significantly from all the other sites. The reference site differed only in comparison to site KR1-2 and, as mentioned earlier, KR1-3.

Temporal fluctuations in biomass occurred at the upstream sites (KR1-2 and KR1-3) throughout the year in which biomass reached maximum values in July (5–3 μg/cm^2). A slight reduction took place in the autumn where values kept similar in September (4–2 μg/cm^2) and October (2–5 μg/cm^2). In January 2016 biomass reduced significantly, leaving little evidence of periphyton as biomass reached minimal values, right above 0 μg/cm^2. In June 2016 biomass had increased to 1.5 μg/cm^2 for both sites. Downstream from the power plant a rather unpredictable shifts in biomass occurred. At site KR1-4 biomass values were highest in June 2015 with biomass at 12 μg/cm^2. A small shift took place in July as biomass reduced to ~10 μg/cm^2 and kept fairly balanced throughout the autumn. Values decreased in January as biomass dropped down to ~3 μg/cm^2 (Figure 2-10). Biomass increased again in June 2016 just as for the other sites. Similar trends in biomass values were observed for the further downstream site KR1-6, with an additional biomass decline in September. High biomass was observed at the reference site, KR2-2, during the summer of 2015, with the highest biomass ranging from roughly 38 μg/cm^2 in June to approximately 100 μg/cm^2 in July. In September biomass reduced down to 30 μg/cm^2. Biomass kept relatively balanced until June 2016, in which biomass had increased up to similar values as in June 2015. At the upstream site, KR1-2, trends in seasonal fluctuations were further observed as biomass in January revealed a significant difference ($P<0.01$) in contrast to the remaining months (Appendix). Values also differed significantly for June 2015 in contrast to July, September and October. For KR1-3, significant difference was only observed for June 2016 in comparison to July, September and October. No data could be retrieved for the month of January because of snow coverage, but biomass is expected to differ significantly in relation to site KR1-2. Downstream from the power plant at site KR1-4, biomass kept fairly constant throughout the year, until a major decrease occurred in January, resulting in a significant biomass difference in contrast to all the other months. In June 2016 biomass seemed to have recovered from the decrease in January. Highest fluctuations occurred at site KR1-6, as significant differences occurred for nine out of fifteen comparisons (Appendix 5). January showed significantly different biomass whilst compared to other months, except for October and September. On the contrary, September and October differed as well from the months of June 2015, June 2016 and July 2015. The reference stream remained fairly substantial over the observed period. Significant temporal variations were exclusively recorded for the month of July in comparison to other months.

The upstream site KR1-1 was excluded in all of the comparison due to low values (<0.2 μg/cm^2), resulting in unreliable data for comparison. However, it can be assumed that KR1-1 would differ from the remaining sites. Tukey plots were made which proofed that assumption (see Appendix 2–5 for *P*-values).
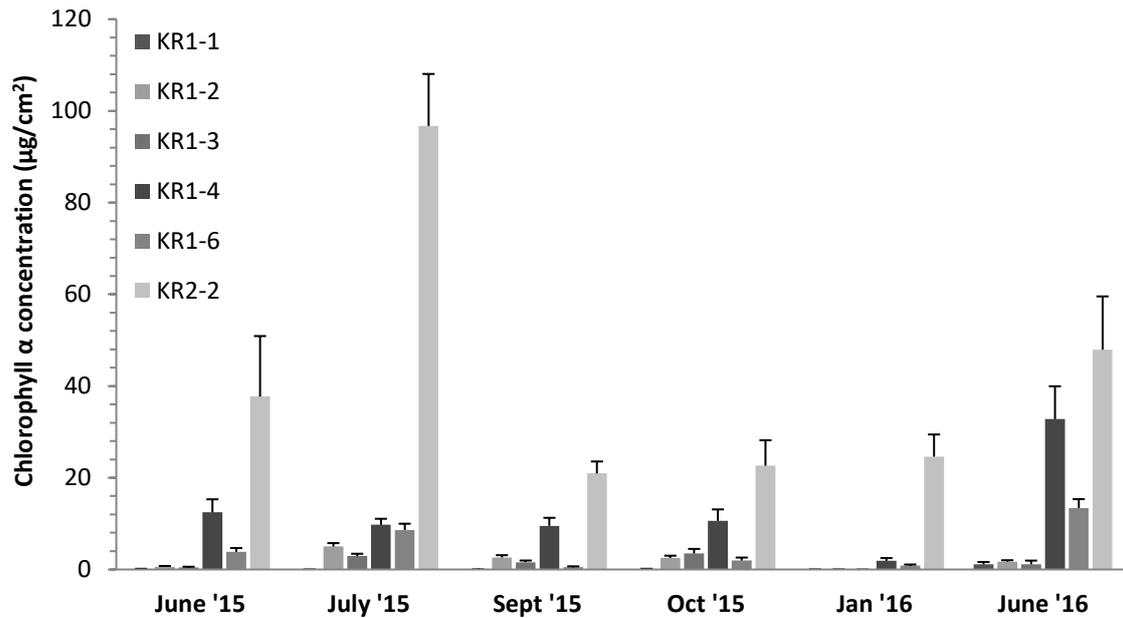
Figure 2-10. Average chlorophyll *a* concentration obtained with a spectrophotometer. Measures apply for all the reaches observed through the year. Positive standard error (SE) bars are plotted on top of each column.

2.3.6 Invertebrates

The lowest number of invertebrate taxa was recorded at the upstream site, KR1-1, in September 2015 with only two taxa present (Figure 2-11). At the other two upstream sites, KR1-2 and KR1-3, there were only 5–6 taxa found. At the downstream sites, below the effluent from the power plant only three taxa were found at site KR1-4, but further downstream at site KR1-6 the number of taxa were 12 in September 2015 (Figure 2-11). The number of invertebrate taxa at the reference site, KR2-2, was 9. The number of taxa at all the sites was lower in January 2016 than it was previously in September (Figure 2-11). Samples at KR1-3 could not be carried out as the site could not be accessed due to snow. Small decrease was observed downstream from the power plant at site KR1-4, as taxa reduced from 3 to 2. Most striking reduction was observed for site KR1-6 in which number of taxa fell from 12 down to only 6 in January. The reference site was the most taxa rich site during the winter, containing 8 taxa. Significant decrease in richness also occurred in winter (January) in comparison to late summer phase (September).
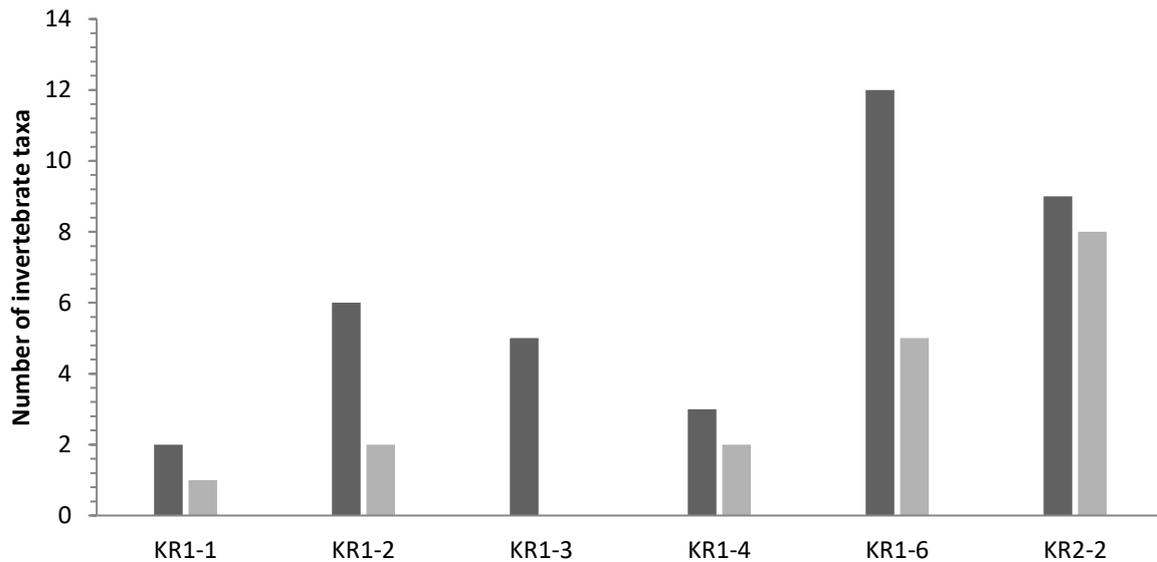
Figure 2-11. Total number of taxa at each site in September 2015 (dark bars) and January 2016 (light bars).

In total 14 invertebrate taxa were observed at all the sites (Table 2-9 & 2-10). The class Insecta contained most of them, 8 out of 14. Three taxa belonged to crustaceans and one to Mollusca, Acarina and Tardigrada. Chironomidae were the most common taxa, being present at all the sites, followed by Simuliidae. Diptera families occurred at all the sites except for KR1-1. Acarina and Copepoda were present at three sites (KR1-2, KR1-6 and KR2-2), while Cladocera, Ostracoda and the mollusc *Radix balthica* were present at two sites (KR1-6 and KR2-2). Tardigrada was the only found at the reference site KR2-2.

Table 2-9. Invertebrate taxa present at the sampling sites in Krafla. Taxa present at specific site at some point at both sampling occasions are indicated by X. Taxa absent at specific sites at both sampling occasions are indicated by hyphens (-). Larvae = (l), pupae = (p).

	KR1-1	KR1-2	KR1-3	KR1-4	KR1-6	KR2-2
Insecta						
Chironomidae (l)	x	x	x	x	x	x
Chironomidae (p)	-	x	-	-	x	-
Diptera	x	x	x	x	x	-
Muscidae (l)	-	x	-	-	x	x
Simuliidae (l)	-	x	x	x	x	x
Simuliidae (p)	-	-	-	-	x	-
Empididae (l)	-	-	-	-	x	x
Trichoptera (l)	-	-	-	-		-
Acarina	-	x	-	-	x	x
Crustacea						
Cladocera	-	-	-	-	x	x
Copepoda	-	x	-	-	x	x
Ostracoda	-	-	-	-	x	x
Mollusca						
Radix balthica	-	-	-	-	x	x
Tardigrada	-	-	-	-	-	x
Total number	**2**	**7**	**3**	**3**	**12**	**10**

Invertebrate densities differed between sampling sites and sampling occasions (Figure 2-12 and Table 2-10). Overall, densities ranged from 34 to 23,902 invertebrate individuals per square metre (invertebrates/m^2). Lowest density for the upstream sites was at KR1-1 in September, with only 93 invertebrates/m^2. At the remaining upstream sites, KR1-2 and KR1-3, densities were higher with 2,279 (KR1-3) and 3,767 (KR1-2) invertebrates occurring per m^2. At sites downstream from the power plant the invertebrate density was 289 invertebrates/m^2 at KR1-4 and 23,902 invertebrates/m^2 at KR1-6 in September 2015 (Figure 2-12 & Table 2-10). Invertebrate density at the reference site was higher in comparison to the others sites, with 19,081 invertebrates/m^2. In January 2016, the invertebrate densities were generally lower than in September 2015 (Figure 2-12). At the upstream site from the power plant, KR1-1, the invertebrate density was 59 invertebrates/m^2 and 34 invertebrates/m^2 at site KR1-2. The invertebrate density at site KR1-4 was as low as it was at the upstream sites in January 42 invertebrates/m^2 and further downstream at site KR1-6, the density was 527 invertebrates/m^2. The highest density in January was at the reference site, KR2-2, with 3,477 individuals/m^2.
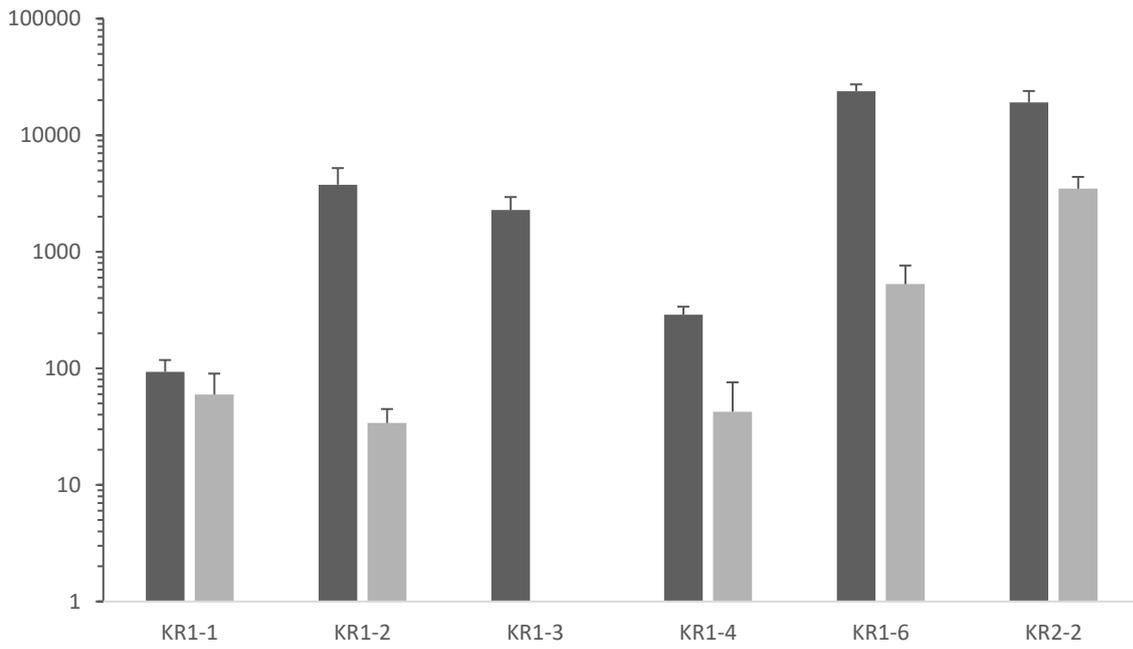
Figure 2-12. Density of benthic invertebrates in September 2015 (dark bars) and January 2016 (light bars). Each column represents average densities along with standard error within each site. Notice the logarithmic y-axis, along with missing values for KR1-3 in January.

Table 2-10. Invertebrate densities in the streams around Krafla (KR). The most abundant taxa are represented with bold numbers. Densities are represented as individuals per square meter (Ind./m^2). Larvae = (l), pupae = (p).

Krafla	September 2015						January 2016					
	KR1-1	KR1-2	KR1-3	KR1-4	KR1-6	KR2-2	KR1-1	KR1-2	KR1-3	KR1-4	KR1-6	KR2-2
Taxonomic groups	ind./m^2	ind./m^2	ind./m^2	ind./m^2	ind./m^2	ind./m^2	ind./m^2	ind./m^2	ind./m^2	ind./m^2	ind./m^2	ind./m^2
Insecta												
Chironomidae (l)	**68**	**3,656**	**2,185**	**163**	**20,408**	**4,426**	**34**	**9**	N/A	**34**	**485**	**995**
Chironomidae (p)	0	9	0	0	**1,471**	0	0	0	N/A	0	9	0
Simuliidae (l)	0	43	43	9	111	**4,375**	0	0	N/A	0	0	94
Simuliidae (p)	0	0	0	0	43	0	0	0	N/A	0	0	0
Diptera	9	9	17	**102**	315	0	0	0	N/A	0	9	0
Muscidae (l)	0	9	26	0	17	102	0	0	N/A	0	9	17
Empididae (l)	0	0	0	0	60	204	0	0	N/A	0	0	9
Trichoptera (l)	0	0	0	0	0	0	0	0	N/A	0	0	0
Acarina	0	0	0	0	51	421	0	**9**	N/A	9	0	**638**
Crustacea												
Cladocera	0	0	0	0	9	13	0	0	N/A	0	0	0
Copepoda	0	17	0	0	17	753	0	0	N/A	0	9	**629**
Ostracoda	0	0	0	0	26	128	0	0	N/A	0	0	**417**
Mollusca												
Radix balthica	0	0	9	0	68	**8,559**	0	0	N/A	0	0	**646**
Tardigrada	0	0	0	0	0	13	0	0	N/A	0	0	0
Total number	77	3,741	2,279	274	22,593	18,992	34	17	N/A	43	519	3,444

2.4 Discussion

The results from this study strongly support the hypothesis in which periphyton and invertebrate assemblages differed significantly on spatial scale, especially downstream to the effluent in comparison to the upstream and the control site. The periphyton biomass upstream from the power plant was rather low and so was the diversity of algae and Cyanobacteria as well. Periphyton density was however rather high, even though biomass values suggested otherwise. Downstream from the power plant (KR1-4) a significant spatial difference was evident as high biomass and very high density of algae and Cyanobacteria characterised the assemblages. However, algae and Cyanobacteria diversity decreased downstream from the power plant as microbial mats, consisting of few Cyanobacteria genera in extremely high densities, dominated the assemblages downstream from the power plant. The low diversity values are likely present due to few, but abundant, dominating genera, which is well known in geothermal environments (Boothroyd, 2000). The absence of other periphyton genera are perhaps because other genera of algae and Cyanobacteria are not able to grow under the effluent effected environment, as high temperature water for cooling facilities is known to limit distribution of aquatic organisms (Hynes, 1960). Further downstream from the power plant, at site KR1-6, assemblages changed significantly. The biomass decreased along with the densities of periphyton, but number of observed genera was high along with high Shannon diversity. The reference site differed from the remaining sites as well, as assemblages consisted of dense periphyton assemblages dominated by diatoms, along with extremely high biomass. Highest biodiversity was observed for the reference site, along with the highest numbers of algal and cyanobacterial genera. Seasonal variations in assemblages was recorded as well, which is likely affected by alterations in physical parameters throughout the year. The results from the invertebrate data also supports the hypothesis. Few taxa of invertebrates and low densities characterised the sites upstream from the power plant along with the diversity values being rather low as well. Out of the three upstream sites, the site closest to the effluent waters (KR1-2) had highest taxa and densities, indicating a longitudinally increase. Downstream from the power plant, at site KR1-4, a significant reduction in numbers of taxa and densities characterised the invertebrate communities. The invertebrate densities and diversities are known to be lower in the warmer stream in the Krafla area according to earlier results by Ólafsson and his co-workers (2010). Both of these results are consistent to previous studies in New Zealand in which Duggan and his colleagues (2007) observed that correlation occurred between increase in geothermal effluent/sources temperature and a decrease in biodiversity, similar to the observed effects from the effluent in present study. Furthermore, many organisms, such as invertebrates and plants, are unable to survive nearby hot effluent waters (Hynes, 1960). Further downstream at site KR1-6 highest numbers of taxa and densities were observed. The communities at the reference site, KR2-2, supported the hypothesis as highest diversity, densities and numbers of invertebrate taxa characterised the site.

Temporal alterations in periphyton and invertebrate assemblages were observed for all the sites, in which densities decreased in the winter. However, diversity increased for some sites but reduced for others. Furthermore, the invertebrate data also supported the hypothesis. The observed data partially supports the temporal predictions concerning the periphyton assemblages and biomass. In an experiment conducted in Canada by Rosemond and her colleagues on seasonally limitation of stream periphyton, it was concluded that both nutrient and light limited periphyton biomass. Light appeared to limit growth in summer and nutrient appeared to be limiting in spring and fall (Rosemond, Mulholland and Brawley, 2000).

Those factors, along with changes in temperature, discharge and dissolved ions surely limited growth of the periphyton in the streams in the Krafla area.

2.4.1 Diversity

The dynamic environmental condition upstream from the power plant was reflected in variety of 22 algal and cyanobacterial genera. Since periphyton were only identified to genus level, I can be rather confident to conclude that numbers of species are relatively higher, as one genus usually includes many species (Bebber, Marriott, Gaston, Harris, & Scotland, 2007). Upstream from the power plant, at site KR1-2, the periphyton diversity was characterised by diatoms and Cyanobacteria. Diatoms contained both N_2-fixing and non-N_2-fixing genera, which are known to thrive well under nitrogen limitations (Guðmundsdóttir et al., 2013). This was true for the Cyanobacteria as well. Of these 22 genera 12 belonged to diatoms (*Aulacoseira, Caloneis*, *Cocconeis, Diploneis*, *Epithemia, Fragilaria, Melosira, Meridion*, *Navicula, Navicula radiosa, Nitzschia linearis, Rhoicosphenia, Rhopalodia*), three to Chlorophyta (*Klebsormidium*, *Microspora*, *Stigeoclonium*), one to Ochrophyta (*Tribonema*) and 5 genera belonged to Cyanobacteria (*Anabaena*, *Calothrix*, *Lyngbya, Oscillatoria* and *Phormidium*). Anabaena and Calothrix might include related species which are hard to distinguish. The Shannon diversity at the upstream site, KR1-2, ranged between 0.16 and 1.63. The variety of Shannon diversity is likely explained by the highly dynamic physical parameters upstream from the power plant. The site is under indirect influence from the geothermal utilization as chances are that excessive water from nearby boreholes might reach the streams, thus altering temperatures and other attributes beyond their natural state. Such incident was observed in previous study in 2002–2003 (Ólafsson et al., 2010) and might explain the observed diversity fluctuations. The daily average temperature also showed high temperature fluctuations, which can further limit distribution of organisms. Shannon index was 0.92 in June and increased to 1.30 in July, leading to the estimation that biodiversity increased in July. Maximum Shannon diversity was recorded in September with values of 1.60. The high diversity might be due to increase in number of genera intertwining with an increase in periphyton densities. The summer peak observed at the upstream site are constant to other studies, in which periphyton growth is known to reach higher values in summer, presumably because if higher temperatures (Allan, 1995). In October diversity had decreased down to 1.10, indicating a slight decrease in comparison to the previous month. Significant decrease occurred in January, in which diversity reduced to 0.16. Those values match the decrease in density and number of genera in the winter. Shannon diversity was again higher in June 2016, reaching 1.40. The observed temporal fluctuations for periphyton and invertebrate assemblages highlight the dynamic environmental conditions in these high altitude and isolated areas upstream from the power plant. However, even though diversity reached high values upstream from the power plant, the diversity was on average lower in contrast to the other sites, except for the downstream site, KR1-4. The effluent water from the power plant is warm and rich in many dissolved elements (Ármannsson, Ólafsson, & Eyjólfsdóttir, 2010) which precipitate as soon as they enter the colder Hlíðardalslækur. Condensation- and separation water from the power plant is also flushed into the stream with similar impacts, causing a significant increase in temperature. Under those circumstances it has been observed that mainly thermophilic bacteria are able to survive (Ferris et al., 2005), with small chances of algae or invertebrates being present (Hynes, 1960; Clements, Arnold, Koel, Daley, & Jean, 2011), which should decrease diversity. Such was the case at site KR1-4 as noticeable decrease in number of genera occurred, as only 9 genera were present throughout the study, in which five genera belonged to Cyanobacteria, two to diatoms, one

to Chlorophyta and finally, one to Tribonemataceae. Not only did the effluent water increase the temperature, but the discharge went from 0.03 m^3/sec from the upstream sites (KR1-1 and KR1-2) to 0.221 m^3/sec at KR1-4, suggesting that the power plant effluents inputs massive amounts of waters. The discharge data for this site is consistent to a previous study, in which the discharge was measured to be 0.215 m^3/sec at the same site (Ólafsson et al., 2010). Shannon diversity index strongly implied reduction in diversity, as values ranged between 0.32 and 1.02, with the average diversity of 0.56. The low diversity values are likely present due to few, but abundant, dominating genera, which is well known in geothermal environments (Boothroyd, 2000). The Shannon diversity for KR1-4 was however relatively high in contrast to the low number of genera. These high values are a strong indication that densities of individuals within its genera were quite even at site KR1-4 for the few genera present. Evenness is equally important to taxa richness when it comes to diversity estimation as explained by Magurran: "The more evenness there is within a community, the more likely it is to recover from various changes, resulting in biodiversity to be considered higher" (Magurran, 2005). The high changes in physiochemical variables unquestionably affects the diversity of the periphyton. Major shifts in periphyton community structure occurred as the periphyton was completely dominated by Cyanobacteria. These Cyanobacteria are one of the most important group in terms of biomass, but the taxonomy of many orders, such as Oscillatoriales, are in a state of chaos, making it difficult to classify them to even taxa (Pentecost, 2003). Cyanobacteria are usually the dominant phototrophic organisms in non-acidic hot environments, such as beneath the effluent, due to their thermal tolerance (Ferris et al. 1996). Further downstream from the power plant, at site KR1-6, it appears as the physical alterations were responsible for striking recovery in periphyton diversity, as diatoms had reappeared along with a few genera of green algae. This site receives water from the same source as KR1-4, thus it was expected to show similarities in algal and Cyanobacteria diversity. However, KR1-6 also receives water from the reference site, which further dilutes the affects from the effluent waters, resulting in alteration in the diversity of the periphyton. In total 19 genera of algae and Cyanobacteria were observed. Diatoms included 11 genera, Cyanobacteria 4 and green algae 4 genera as well. Interestingly, Shannon diversity increased significantly from the upper effluent effected site, ranging between 0.85–1.81, a remarkable increase and a clear indication of community recovery. In fact, the Shannon diversity index was higher than diversity for the sites upstream from the power plant, which strongly suggests that the effects from the effluent are heavily localized to the nearby environments of the power plant. Shannon diversity showed temporal alterations, as the diversity was 1.46 in June. Diversity decreased to 0.85 in July and increased again in September. The diversity remained rather stable throughout the study period. All of the temporal comparison was non-significant, which strongly reflects the constant diversity downstream from the power plant. Numbers of periphyton genera were highest for the reference site, yielding 24 genera in total. Diatoms had the most genera, accounting for 16 of the genera. Cyanobacteria and green algae included 4 genera each. Shannon diversity index was highest for the reference site, with values ranging between 1.47–1.95. High numbers of genera, density and evenness amongst genera are likely to be responsible for significantly higher diversity values in comparison to the upstream sites (KR1-1, KR1-2 and KR1-3) and the upper effluent effected site, KR1-4. Temporal shifts were less obvious for the reference site, likely due to the constant discharge and water temperatures that were observed throughout the year. Number of algal and cyanobacterial genera kept rather constant at all observations, further indicating stable environmental conditions.

2.4.2 Density

The density of algae and Cyanobacteria was on average lowest for the upstream site KR1-2, with the density ranging from 1,426 to 2,444,112 cells/units per cm^2. The periphyton was in fact rather rare at the upstream sites, but the presence of filamentous Cyanobacteria (mainly *Anabaena*) increased the density. Diatoms were low in density, although numbers of diatoms genera exceeded the genera of both green algae and cyanobacteria. The density was highest downstream to the power plant, at site KR1-4. However, as mentioned above, Cyanobacteria were the most common of the periphyton as they grew as continues thick mats in the streams bottom. Cyanobacteria tend to be small in size which might explain the extremely high densities (Whitton et al., 2011). Temporal alterations in periphyton density downstream were rather minor, even though density ranged from 639,755 to 7,159,719 cells/units per cm^2 throughout the study. Density kept rather stable throughout the year, and density reached highest values in September. A significant decrease in density occurred in January. There had been conducted a trial in the purpose of cleaning the cooling towers only two days prior to our sampling in January 2016. Approximately 200 litres of glutaraldehyde had been poured into the cooling towers with the intensions of cleaning it from bacteria and precipitates (Steinn Ágúst Steinsson, Personal communication, 2016). Glutaraldehyde is commonly used as a biocide for system disinfection (mainly to get rid of bacteria) and as a preservative for long term storage, often used to preserve algae and eutrophic organisms (Bellinger & Sigee, 2015; CDC, 2016). Eventually the glutaraldehyde ended up in the effluent and was discharged into the stream. The exact concentration of the diluted glutaraldehyde is not known, but possible influences on the assemblages, especially the Cyanobacteria, must be kept in mind, as the biocide properties of the glutaraldehyde is to eliminate prokaryotic cells (Frayne, 2001). Hence it is impossible to relate the significant decrease in periphyton densities and biomass to the winter conditions as was done for the upstream sites. Bacteria accumulation in hot effluent waters are well known and such growth can be of great nuisance. Use of biocides and/or chlorination to reduce the accumulation is a common practice. Unfortunately, more often than not, parts of the biocides or chlorine end up in the effluent, making the hot effluent even more harmful to living creatures in the receiving waters (Hynes, 1960). Another possible explanation for the decrease in biomass and density might be self-scouring. Thick periphyton mats are vulnerable to dislodgement due to senescence of the bottom-most layers, which weakens their attachment to the substrate and renders the entire mat vulnerable to sloughing. Shading, the build-up of metabolites, and reduced rates of exchange of gases and nutrients all can contribute to the lower-most layers being unable to support the weight of the overlying mat (Allan, 1995). No matter what, the recovery seemed to be rapid, as the algal and cyanobacterial densities had recovered and significant increase had occurred in June 2016, in which Cyanobacteria (*Anabaena, Calothrix* and *Phormidium*) had formed continuous mats anew. Further downstream from the power plant, at site KR1-6, physical variables had undergone various changes. Periphyton density reduced in comparison to KR1-4 but remained significantly higher than sites upstream from the power plant. Microbial mats grew sparsely and in spatial patterns on top of big stones or other stable substrata, but never did they grow in continuous mats. The absence of continuous microbial mats, which characterised the upper site KR1-4, is likely the explanation for lower density. Temporal shifts in densities were quite intense, as densities were relatively high in June and July (1,347,584 and 2,302,016 cells/units per cm^2). In the following month of September 2015 rather remarkable alterations occurred. Significant decrease in density and biomass took place although diversity and number of genera remained rather similar, as density reduced from 2,302,016–19,328 cells/units per cm^2 in

September. No physical evidence supports these alterations, but undocumented disturbances might be the explanation, for example self-scouring as was described earlier. The algae and Cyanobacteria seemed to recover in October, as density reached approximately 900,000 cells/units per cm^2. However, another significant decrease occurred in January were density reduced to roughly 70,000 cells/units per cm^2. Finally, in June 2016, density had increased significantly again, reaching 1,800,000 cells/units per cm^2. The reference site, KR2-2, had the second highest density out of all the sites. Diatoms, mainly *Melosira*, were responsible for the density at this site, unlike the remaining study sites. The high density of the diatoms is a clear indication of high biomass, as diatoms are known to be large in size (Bellinger & Sigee, 2015). As for the temporal alterations of periphyton, a typical summer increase occurred in which densities peaked in July and September 2015 as has often been seen in other studies (Dethier, 1982). In January 2016, periphyton density decreased significantly, followed by increased density in June 2016.

2.4.3 Assemblages

Upstream from the power plant the most common N_2-fixing genera of Cyanobacteria were *Anabaena* and *Calothrix* and the most common non-N_2-fixing genera were *Phormidium, Lyngbya* and *Oscillatoria.* The diatoms contained a great variety of genera, in which both N_2-fixing and non-N_2-fixing genera were common. The most common genera of diatoms were the non-N_2-fixerss *Nitzschia*, *Navicula*, *Caloneis* and *Meridion*. The most common N_2-fixers were *Rhopalodia* and *Epithemia,* both genera are known to prefer moderate to high conductivity streams (Cox, 1999). The high proportions of N_2-fixing genera indicate that lack of inorganic nitrogen might be limiting the growth of other periphyton. However, inorganic nitrogen was 1.03 µmol/L (Table 2-5) and the N:P ratios were high (40:1), suggesting a P-limited growth as phosphorus limitation are known to occur when the N:P ratios are higher than 16:1 (Redfield, 1958). In fact, N_2-fixing genera represented up to 50% of the relative abundance and relative frequency of occurrence at some occasions, which strongly implies that the ability to fix nitrogen is a major advantage at this particular site. Various studies on geothermal ecosystems have indicated that both density and diversity are rather low in comparison to nearby ecosystems were geothermal activities are absent (Vincent & Forsyth, 1987), presumably because of the high temperatures and high amounts of dissolved materials (Robinson & Turner, 1975; Pritchard, 1991). Another explanation for the semi-low density and diversity is the constant precipitations of silica and sediments along with highly fluctuation temperatures (Ármannsson & Ólafsson, 2002). Seasonal assemblages clearly occurred upstream from the power plant. Green algae were responsible for roughly 80% of both relative abundance and relative FO in June. Cyanobacteria and diatoms were responsible for about 10% of the relative abundance. However, due to small size, Cyanobacteria only covered approximately 5% of the relative frequency of occurrence, with diatoms covering about 15%. Cyanobacteria mainly consisted of *Phormidium* and the N_2-fixing genera *Anabaena*, both of which have distinguished small morphology (Cox, 1996). Diatoms were dominated by small groups of *Nitzschia*. In the following month of July, green algae had practically vanished as Cyanobacteria and diatoms became dominant. Cyanobacteria made up for roughly 50% of the relative abundance, with diatoms making up for the later 50%. As expected, Cyanobacteria were responsible for small portion of the occurrences as they only made up for 20% of relative frequency of occurrence. In late summer, September and October, minor alterations took place in which green algae had reappeared and made up for 10–15% of the relative abundance. The relative abundance of both Cyanobacteria and diatoms remained fairly similar as in July. Major changes in algae

community assemblage were observed in January as green algae and diatoms (*Nitzschia* and *Rhopalodia*) dominated in small densities. Most surprisingly, not a single cell of Cyanobacteria was analysed during the harsh winter of January 2016. Vulnerability of the upstream sites might be explained by the fact that environmental conditions are harsh in high altitudes during winter, in which temperature drops far below freezing and snow accumulation hinders light availability which further inhabits growth (Cole & Cloern, 1984; Kocum, Underwood, & Nedwell, 2002).

Assemblages downstream from the effluent, KR1-4, were characterised by Cyanobacteria. The Cyanobacteria formed continuous mats which covered all the benthic, thus out-competing other periphyton in the ecological competition for habitat. Similar microbial mats have been observed in many studies as it is a distinctive characteristic of both mesophilic and thermophilic Cyanobacteria. The ability of mesophilic Cyanobacteria to form mats is a distinctive characteristics of many geothermal ecosystems (Castenholz, 1984), as they frequently occur under certain environmental conditions, usually warm and nutrient rich (Fagerberg & Arnott, 1981). In part, the Cyanobacteria ability to form thick mats arises because of lack of grazing invertebrates (Winterbourn, 1969), but is usually more likely to depend on the micro-environment (Vincent & Forsyth, 1987). N_2-fixing Cyanobacteria and non-N_2-fixing Cyanobacteria were rather equally distributed in terms of relative abundance and frequency of occurrence, except for one observation in which N_2-fixing Cyanobacteria were almost absent. The N_2-fixing Cyanobacteria contained two dominating genera, *Anabaena* and *Calothrix*, while non-N_2-fixing Cyanobacteria contained all of the observed dominating genera for that group, *Phormidium*, *Lyngbya* and *Oscillatoria.* Some species of *Anabaena* are able to produce a range of toxins in waters (Chorus & Bartram, 1999), at this stage it cannot be confirmed if such toxins are present. It turned out to be extremely challenging to distinguish between Cyanobacteria in some cases, as the preservative (Lugol's iodine solution) had deformed the cells, probably by disrupting the cell wall, heterocyte or the sheath of the cells (if present), all of which are crucial characteristics in identifying Cyanobacteria into a correct genus (Whitton et al., 2012). The high relative abundance of N_2-fixing Cyanobacteria must be interpreted carefully since a great variety of factors, or combination of factors, can be responsible for such immense abundance, but temperature and nutrients are known to play significant roles (Welter et al., 2015). The ability to fix atmospheric nitrogen enables them to thrive under nitrogen limited conditions, while other non-N_2-fixing algae may perish due to nitrogen depletion (Bellinger & Sigee, 2010). In fact, a rather low concentration of inorganic nitrogen below the effluent, which might play a significant role of the dominating N_2-fixing Cyanobacteria. Unlike the upstream sites, temporal variations were minor downstream from the power plant at site KR1-4. In fact, periphyton assemblages remained relatively similar throughout the summer. Cyanobacteria made up for roughly 100% of the relative abundance in June, July, September and October, as they formed continuous mats. The mat consisted of several genera, mainly *Anabaena*, *Calothrix* and *Phormidium*, all of which are commonly found in microbial mats (Mackenzie et al., 2013). Relative frequency of occurrence displayed similar values, in which Cyanobacteria dominated the relative FO (frequency of occurrence) (>95%). Assemblages did however shift during the summer in which N_2-fixing genera, such as *Anabaena* and *Calothrix*, declined between observations, giving way to the non-N_2-fixing genera *Phormidium* and *Lyngbya.* Such shifts might be caused by increase in inorganic nitrogen as N_2-fixing Cyanobacteria are known to thrive well under N-limited conditions (Cross et al., 2015). Assemblages kept rather stable until January 2016 where N_2-fixing Cyanobacteria were almost absent and non-N_2-fixing cyanobacteria dominated with 90% of

relative abundance and relative FO. In June 2016, both relative abundance and relative FO were rather equal for N_2-fixing and non-N_2-fixing cyanobacteria, as they dominated the assemblages.

The dominating periphyton further downstream, based on relative abundance and relative FO, were the diatoms and Cyanobacteria. N_2-fixing genera were dominating in relative abundance and relative FO for both groups, which are known to grow rapidly under nitrogen limited conditions (Stancheva et al., 2013). The most common diatoms were the N_2-fixing *Epithemia* and *Rhopalodia* (Deyoe, Lowe, & Marks, 1992), being highest in relative abundance and even higher in relative FO, most likely due to their large size (Bellinger & Sigee, 2015). Non-N_2-fixing genera of diatoms were also quite common, such as *Fragilaria*, *Melosira*, *Navicula* and *Nitzschia*, although always in less relative abundance than the N_2-fixing genera. For the N_2-fixing Cyanobacteria, *Anabaena* and *Calothrix* were highest in relative abundance, along with the non-N_2-fixing genera *Lyngbya* and *Phormidium*. Although Cyanobacteria had the highest relative abundance, ranging from 50–70%, their presence was only responsible for maximum of 30% of the relative FO. Generally, the morphology of the Cyanobacteria were somewhat similar as all genera included extremely small cells forming filamentous spatial mats, making the Cyanobacteria appear high in relative abundance even though they cover only a small portion of the relative FO. Assemblages varied between occasions and the periphyton were dominated by N_2-fixing genera of both Cyanobacteria and diatoms throughout the study, indicating their ability to thrive in nitrogen limiting waters (Stancheva et al., 2013). In June assemblages were dominated both N_2-fixing genera of diatoms (*Epithemia*) and Cyanobacteria (*Anabaena* and *Phormidium*). The green algae genus *Stigeoclonium* was also quite abundant. Minor changes in relative abundance and frequency of occurrence occurred in September, with proportional increase in N_2-fixing diatoms (*Epithemia*), which replaced Cyanobacteria (*Anabaena* and *Lyngbya*) as the dominating genera. In October further alterations took place, as green algae, diatoms and Cyanobacteria were equally distributed regarding to relative abundance, but N_2-fixing diatoms (*Epithemia and Rhopalodia*) dominated according to relative frequency of occurrence, along with *Melosira*. Assemblage alterations in January included reduction of green algae and major proportional increase in N_2-fixing diatoms, which dominated both considering relative abundance (40%) and relative frequency of occurrence (80%). In June 2016 periphyton assemblages looked remarkably similar as to the assemblages in June 2015: Green algae and Cyanobacteria were highest in relative abundance. Rather equal distribution of relative FO of green algae, N_2-fixing diatoms and Cyanobacteria were observed. Based on these data, it is estimated that site KR1-6 reflects seasonal changes, as winter seemed to significantly alter the observed aspects of the stream. However, just as for the upper site (KR1-4), the effects from the glutaraldehyde cannot be excluded, although the glutaraldehyde has most likely diluted to harmless concentration.

At the reference site, KR2-2, diatoms were the dominating periphyton with relative abundances up to 98%. The diatom assemblages were characterized with the leading presence of the genus *Melosira*, which were responsible for more than 60% of diatoms relative abundance and 70% of the relative FO. Species of *Melosira* are well known to occur in slow to medium flowing streams and can dominate the periphyton community in moderately enriched situations (Biggs & Kilroy, 2000). Their species have often been reported as "clean water" species (Cox, 1996), although some species are moderately polluted tolerant (Cooper, 1989). Another frequently occurring genus was *Rhoicosphenia*, a well-known epiphyte that grows on short stalks, often on the surface of other algae and

mosses (Cooper, 1989). The moss covered stream is a suitable habitat for such epiphytes, which might explain the high relative abundance of *Rhoicosphenia*, along with their pollution tolerant attributes (Cooper, 1989). High proportions of various species of *Nitzschia* were present, although their relevant frequency of occurrence was somewhat less due to their small structure. *Fragilaria*, *Pinnularia*, *Navicula* and the clean water genus *Meridion* were frequently noted, but in much less proportions, along with the N_2-fixing diatoms *Epithemia* and *Rhopalodia*. Cyanobacteria were present as a small proportion of the assemblages. Similar portion of branched and unbranched green algae were observed. Cyanobacteria mainly included non-N_2-fixing genera, along with one N_2-fixing genus. Most common genera of Cyanobacteria were *Oscillatoria* and *Lyngbya*. Meagre amounts of *Phormidium* and the N_2-fixing genus *Anabaena* were recorded as well. Various species of *Oscillatoria* are known to thrive under heavy sulphide concentrations (20–70 g/m^2) which is often the scenario in geothermal ecosystems (Vincent & Forsyth, 1987). However, limited growth of Cyanobacteria in this stream is probably due to lower temperatures which are often more ideal for various species of diatoms and green algae (Whitton et al., 2011). Additionally, some diatoms are well known to grow sufficiently in warm waters, hence the high abundance (Bellinger & Sigee, 2015). Of the green algae, *Stigeoclonium* and *Microspora* were the most common genera and *Spirogyra* was also quite abundant. Those three genera are commonly found worldwide in gently flowing streams, in a range of conditions (Cooper, 1989). *Stigeoclonium* are known to withstand high amounts of heavy metals in their environment (Pawlik-Skowronska, 2001), a convenient attribute in geothermal ecosystems. As mentioned earlier, seasonal alterations were minor at the reference site. Diatoms characterized the assemblages in all observations, both concerning relative abundance (75–95%) and relative FO (65–95%). Green algae and non-N_2-fixing Cyanobacteria were present but in low in relative abundances and relative FO. Cyanobacteria had vanished in the winter. Diatoms kept on dominating and in fact increased in relatively abundance, being responsible for up to roughly 95% of the relative abundances and the relative FO. Finally, in June 2016 further alterations in assemblages took place, as green algae and non-N_2-fixing Cyanobacteria reappeared, making up for 15% of relative abundance and 10% of relative frequency of occurrence.

2.4.4 Biomass

Low biomass values reflected the low density at the upstream sites from the power plant. Algae and Cyanobacteria barely grew on the unstable cobbles at site KR1-1 nor on the silica rich precipitates on the bottom of sites KR1-2 and KR1-3. Biomass at the upstream site KR1-1 was in all occasions under detection limits and was therefore excluded from spatial biomass comparison. The extremely low biomass at this site might be caused by several reasons: The streams bottom mainly consists of loose cobbles, which might lead to scouring of the periphyton due to constant overturning of the substrata/cobbles (Robinson & Rushforth, 1987), or maybe because of frequent winding of the streams riverbed. Significant increase in biomass occurred shortly downstream to the Power plant, site KR1-4, further supporting the predictions of the effluent effects. The high density of the microbial mats dominating the stream bottom can be directly related to the increase in biomass. Temporal fluctuations in biomass at site KR1-4 were minor, as statistical differences in biomass were non-significant in all comparisons throughout the year. Biomass usually decreased significantly further downstream, KR1-6, in comparison to the upper site according to the spectrophotometer. However, biomass values according to the BenthoTorch showed non-significant difference for all observations. Biomass showed seasonal fluctuations, in which

biomass was significantly higher in the summer in comparison to biomass in winter. At the reference site, KR2-2, biomass was significantly higher in comparisons to the other sites throughout the year, with a few exceptions as biomass between the reference site and KR1-4 were non-significant. The significantly highest biomass is likely affected by the high fertile waters of the reference site (Demars & Edwards, 2007). The concentration of dissolved inorganic nitrogen was high both in winter and summer, with NO_3 and organic N as the main element. The dissolved inorganic phosphorus concentrations were also relatively high and higher in winter compared with summer. The fact that the reference site is close to its source probably secures the constant nutrient renewal and prevents nutrient depletion (Allan, 1995), Non-significant differences in biomass usually occurred between KR2-2 and the remaining sites according to the BenthoTorch, as only 7 out of 24 BT comparisons showed significant biomass difference ($P<0.01$). However, spectrophotometer indicated that significant difference occurred for 18 out of 24 total comparisons. The reference site is dominated by mosses, which form thick layers in which the BenthoTorch is presumably unable to detect (Khalert & McKie, 2013). Seasonal alteration in biomass were minor at the reference site, as all temporal biomass comparisons were non-significant, except for the month of July, in which biomass peaked and reached extremely high values.

2.4.5 Invertebrates

Invertebrates were commonly found throughout the study sites, but their assemblages highly differed between sampling sites and seasons. The assemblage of invertebrates can be of great importance as species differ greatly in their sensitivity to poisons, such as zinc and other heavy metals (Hynes, 1960). Invertebrate grazing is another issue of great importance, as they tend to feed on primary producers, which might lead to alterations of the periphyton community structure (Rosemond, Mulholland & Brawley, 2000). Organic microlayers occurring on stones and other substrates have been shown to be a food source for various aquatic insects (Rounick & Winterbourn, 1983). Thus the possible grazing effects cannot be excluded, even though the substrates were quite dynamic, maybe preventing microlayers to grow and thus limit the food resources for grazing macroinvertebrates. Results from earlier studies have repeatedly revealed the correlation between grazing invertebrates and decrease in primary production and biomass (Feminella, Power, & Resh, 1989; McIntosh & Townsend, 1996). Density of invertebrates upstream from the power plant was rather low, presumably because of dynamic physical characteristics which most likely limit the distribution of the macroinvertebrates. The unstable daily temperatures upstream from the power plant might have affected the communities, as high temperature fluctuations are known to be harmful to the communities (Cassie, 2006), that goes for both the invertebrates and the periphyton. Not only was the density low, but number of taxa was low as well. Invertebrates in September included rather few individuals. Invertebrates at site KR1-1 were 100 invertebrates/m^2, 3,700 invertebrates/m^2 were present at site KR1-2 and 2,789 at site KR1-3. Chironomidae larvae usually had the highest densities out of all the taxonomic groups. In January a significant decrease in density occurred as 34 individuals were present per square meter at site KR1-1 and 17 at site KR1-2. Only Chironomidae and other Diptera larvae were present in the winter. Invertebrates are known to decrease in density in the winter, although research have shown that species of Chironomidae tend to hatch in the winter as well as in the summer in warm streams (Hannesdóttir, 2012). Invertebrate taxa richness was relatively high at the reference site, KR2-2, including 10 taxonomic groups. In previous studies in the Krafla area, it was concluded that diversity was higher in the reference site in comparison to the warm, effluent effected sites (Ólafsson et al., 2010), similar to

current results of this study. Density of macroinvertebrates was significantly higher in comparison to the remaining sites, except for KR1-6. Temporal alterations regarding invertebrate assemblages were observed, in which higher density occurred in September 2015 in comparison to January 2016. In September density accounted for approximately 19,000 invertebrates per square meter. The grazing invertebrates *Radix balthica* and presumably various species of Chironomidae, along with Simuliidae, dominated the communities. In January 2016, invertebrate density had decreased significantly to 3,500 invertebrates per square meter. Most noticeable alterations were the proportional decrease of Simuliidae larvae and the increase of Crustaceans. Along with the Crustaceans (Copepoda and Ostracoda), *Radix balthica* and Chironomidae dominated the communities. The high and even density might be explained by the low fluctuations of physical parameters. Stable discharge reduced the risk of the stream from drying as well as it prevents scouring of the streams bottom during flash-floods (Fisher, 1982; Allan, 1995). The stable temperature is perhaps the most vital factor, as the monthly and daily average temperature was extremely constant, thus preventing losses due to temperature fluctuations, such as at the upstream sites. The thermal conditions of the waters at the reference site are also likely to have influenced the larval growth of the Chironomidae (Vannote & Sweeney, 1980). It is probable that the high density of snails altered both algae and invertebrate community assemblage through the cascading effects of grazing, as has been observed by Cuker (1983). Herbivory might explain the particularly low abundance of green algae, as they are often consumed by snails and other grazers (Anderson & Foster, 1999). In fact, Rosemond and co-authors observed the top-down control of periphyton by snails, in which snails maintained low biomass assemblages dominated by only a few grazer-resistant species, e.g. basal cells of Stigeoclonium (Rosemond, Mulholland & Brawley, 2000). Additionally, in a study in the aquatic invertebrates around Krafla in 2009 it was observed that approximately 90% of the invertebrates were grazers within that stream (Ólafsson et al., 2010). Macrophytes, such as mosses, are relatively restricted in their occurrence and are rarely consumed by aquatic herbivores in freshwaters (Hutchinson, 1981), due to their low digestibility as has been previously observed (Cummins & Klug, 1979). Macroinvertebrates have been intensively studied in geothermal waters in New Zealand. Boothroyd (2000) stated that geothermal waters generally support simple communities with low species-richness, but often with a high abundance of single species. That description does not fit to the community structure of the reference stream, as the stream appears to be highly fertile, with diverse and dense invertebrate communities. On the contrary, lowest diversity of macroinvertebrates was at the downstream site, KR1-4, closest to the power plant. In fact, the power plant effected site was the only site to include fewer taxa and lower density in comparison to the upstream sites. These results are consistent to previous studies in New Zealand. Duggan et al. (2007) observed that correlation occurred between increase in geothermal effluent/sources temperature and a decrease in biodiversity, similar to the effects from the effluent in present study. Similar results have been found in Hengladalir geothermal area in Iceland, in which invertebrate diversity rapidly declined as temperatures rose over 15 °C (Woodward et al. 2010). Grazing due to invertebrates is estimated to have been highly unlikely, as invertebrate included exceptionally few individuals. Equally important, the only present taxa were Chironomidae and Simuliidae, in which various species of Chironomidae are commonly known grazers in high abundances (Armitage, Pinder, & Cranston, 1995). Even so, the Cyanobacteria dominated benthos is not considered to be suitable food for secondary consumers, although some invertebrates like species of Daphnia are commonly used in biomanipulation in order to reduce Cyanobacteria blooms (Urrutia-Cordero, Ekvall & Hansson, 2016). However, daphnia and other grazers were extremely rare at sites were

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Cyanobacteria dominated. Invertebrate assemblages varied significantly between seasons. Only 270 (per/m^2) invertebrates were observed in September, in which 160 Chironomidae larvae were present, 9 Simuliidae larvae and approximately 100 other Diptera larvae. In January densities reduced down to 43 individuals, as Chironomidae larvae were only observed. Based on the observations it is estimated that the environment downstream to the power plant is rather stable throughout the year due to constant effluent input from the power plant. All in all, the invertebrate assemblages were characterised by few taxa, with low abundances. However, further downstream from the power plant, at site KR1-6, remarkable alterations in macroinvertebrate assemblages took place, as taxa richness was highest for this site with total of 12 taxa being present. Not only was the number of taxa highest, but density was reasonably higher in comparison to other sites, even in comparison to the reference site in which density was higher in September (22,593 individuals/m^2) and second highest in January (519 individuals/m^2). In previous studies in Iceland, many aquatic invertebrates such as adult Chironomidae are known to emerge as adults year-round in warmer streams in contrast to colder streams (Hannesdóttir, Gíslason, Ólafsson, Ólafsson & O'Gorman, 2013), which might explain the rather high densities in the winter. Grazing invertebrates were quite abundant as Chironomidae larvae dominated over other groups and snails (*Radix balthica*) were also common. Both of these are known grazers and might have reduced the biomass and abundances of green algae and diatoms (Arango, Riley, Tank, & Hall, 2009). It appears as the impacts from the geothermal operation effluent dilutes further downstream, in which both diversity and density increases in comparison to site KR1-4.

2.4.6 Variability in temporal community assemblages

The results of this study suggest that the observed geothermal study site experience substantial seasonal changes, although varying considerably between sites. These temporal alterations were more intense in the upper regions (upstream from the power plant), probably as a consequence of the extreme winter conditions of the sub-arctic and the dynamic geothermal activities in the area. Primary producers require minimum light availability for growth and reproduction (Sherman & Phinney, 1971; Allan, 1995) and the snow covers most likely hindered the light in the winter, resulting in extremely low growth of primary producers. It is quite well known that organisms in the sub-arctic undergo shortened daylight on annual scale, which might slow the rate of primary production (Sperling, 1975). Shortened days in January most likely limited the growth of the periphyton in the open riverbed, in which snow did not cover the streams, for both upstream and downstream sites from the power plant. Through bottom-up cascade, reduction in periphyton might be responsible due to the grazing invertebrates (Power, 1992; McIntosh & Townsend, 1996), which might end up in algae depletion, which leads to reduction of grazing invertebrates (Ólafsson et al., 2010). Such cascading effects might explain the reduced invertebrate density in the winter. However, to make such statement one must make detailed investigation on the stomach contents of the invertebrates. Another likely explanation is the well-known reproduction cycle of the dominating invertebrates, Chironomidae and Simuliidae, as they peak in the summer and lay low during the winter (Gíslason & Jóhannsson, 1991). Also, nutrient analysis strongly implied rather intense nutrient uptake (inorganic nitrogen and inorganic phosphorous) over the summer, which might influence the biomass and assemblages when nutrient ratios are altered (Redfield, 1958). To conclude, we observed that various environmental and biological factors undergo seasonal changes and shape the

periphyton and invertebrate assemblages in the Krafla geothermal area. Interactions of these factors are most likely responsible for both spatial and temporal assemblage alterations.

2.5 Conclusions

Physical variables varied greatly upstream from the power plant and showed seasonal fluctuations. The periphyton communities were relatively high in density and usually characterized by the N_2-fixing diatoms *Epithemia* and *Rhopalodia*. However, various groups of small diatoms such as *Caloneis*, *Nitzschia,* and *Navicula* were also high in relative abundance and further characterised the periphyton community. Other genera such as the N_2-fixing genera of *Anabaena* and *Calothrix*, along with the green algae *Klebsormidium* and *Microspora* grew spatially in relatively high abundance. Biomass values for the upstream sites were significantly lower in contrast to the remaining sites.

Periphyton assemblages for the reference site included the highest number of genera. The vast numbers of genera, along with immense densities and biomass reflected in exceedingly high diversity in comparison to the remaining sites. The stream was characterized by benthic moss, which served as a substrate for algal epiphytes such as *Rhoicosphenia* and *Cocconeis*. *Stigeoclonium*, *Klebsormidium* and *Spirogyra* are some of the green algae which further characterized the communities. High nutrient concentration resulted in diverse assemblages and high biomass. High densities of invertebrates were observed, which were dominated by species of Chironomidae and the grazing snails *Radix balthica.*

Downstream from the power plant the assemblages were dominated by Cyanobacteria, all of which were capable of forming microbial mats which reflected in high biomass. Dominating genera were *Phormidium* and *Lyngbya* along with the N_2-fixing genera of *Calothrix* and *Anabaena*. Neither green algae nor diatoms grew on the benthos. However, the observed effects from the effluent appeared to be highly localized. Approximately three kilometres downstream from the effluent input, a striking recovery in periphyton assemblages took place. On one hand biomass and densities decreased significantly, but on the other numbers of genera and diversity increased. Assemblages consisted out of 19 genera, 11 belonging to diatoms, 4 to Cyanobacteria and 4 to green algae. Just as for the upstream sites, *Anabaena*, *Lyngbya* and *Phormidium* dominated in relative abundance, closely followed by the N_2-fixing diatoms *Epithemia* and *Rhopalodia*. *Stigeoclonium* represented the green algae, a genus known for its tolerance for heavy metals. Other characteristic green algae were *Klebsormidium* and *Spirogyra*.

General seasonal trends were observed for the biomass, as biomass values usually peaked in the summer / late summer and decreased significantly in the winter. Such was evidential for all the sites except for the reference site. Diversity showed little relations to seasons, except for the upstream site KR1-2, in which diversity decreased significantly in the winter. Periphyton assemblages did not show temporal alterations regarding the dominating genera. However, a significant decrease in periphyton densities was observed for all sites in January. Discharge reduced in the upstream sites in the winter, causing streams to dry up, as well as the conductivity and pH increased significantly. Such incidents have been noted and are known to alter the assemblages of periphyton (Townsend et al. 2002). Densities were also generally higher in mid-summer in comparison to densities in the spring (June 2015 and 2016), autumn (September 2015) and, as mention above, in the winter (January 2016). The predictions regarding temporal alterations are partly approved, as significant temporal differences in densities and biomass were mainly observed between winter and summer, but not between every sampling occasion.

It is now known that effluent waters from geothermal power plants causes significant alterations on periphyton and invertebrate assemblages. The alterations observed in this study can be related to the changes in the physical attributes due to the effluent input and seasonality. The physical alterations from the effluent waters give huge advantage for Cyanobacteria to bloom, as they are mesophilic and capable of fixing nitrogen out of the atmosphere under nitrogen limited conditions. Such cyanobacterial assemblages are able to form dominating mats which might further limit the distribution of other algal genera. However, it is also rather clear that the effects are strictly localized, in which the negative impacts dilute further downstream and even enhances diversity.

3 Periphyton biomass responses to nutrient enrichment

3.1 Introduction

Nutrients are one of the foundations of autotroph communities in streams and lakes (Dodds, 2006), especially the major ones like phosphorous and nitrogen which are known to have critical influence on autotrophs (Allan, 1995) as well as on various herbivores (Sterner & Hessen, 1994). Abundant data indicate that the growth and reproduction of autotrophs as well as large scale ecosystem primary production are frequently limited by supplies of nutrients (Hecky & Kilham, 1988; Elser et al., 1990; Elser et al., 2007). Nutrient limitation plays an important role in shaping community structure and ecosystem processes in aquatic environments (Capps et al., 2011). Phosphate, nitrogen and silica are usually the most critical nutrients for freshwater primary producers, such as the algae and Cyanobacteria (Hutchinson, 1967). In low nutrient waters, phosphorus is often the limiting nutrient. Icelandic streams generally have low nutrient concentrations because they run through a terrain of basalt rock and soils which has derived from it (Gíslason & Arnórsson, 1988). In terrestrial environments, soil age is of key importance because P becomes limited due to mineralogical transformation over time, thus old soils are more frequently P-limited (Schimel, 2004). However, geothermal fields are known to be rich in phosphorus due to high volcanic activities and young soils, thus many geothermal streams are considered as not being phosphorous limited (Friberg et al. 2009, Porder & Ramachandran, 2013). In nitrogen limited waters, N_2-fixing Cyanobacteria and diatoms, tend to be dominant because they can use atmospheric nitrogen when other algae and Cyanobacteria cannot (Howarth, Marino, Lane, & Cole, 1988).

Experimental studies on nutrient effects on primary producers in streams have been conducted by numerous scientists, such as nutrient enrichment of nitrogen and phosphorus (Slavik et al., 2004; Gudmundsdottir et al., 2011; Cross, Hood, Benstead, Huryn, & Nelson, 2015; Welter et al., 2015). Several methods have been used to assess responses to nutrient enrichment on streams such as nutrient diffusing substrata (Friberg et al., 2009; Capps et al., 2011) and whole stream manipulations (Triska, Kennedy, Avanzino, Zellweger, & Bencala, 1989). Capps and coauthors (2011) compared different methods of nutrient enrichment in order to clarify differences between various methods. Their results indicated that responses to nutrient enrichment varied between the applied methods. However, due to low costs and convenience, the nutrient diffusing substrata (NDS) method was applied in our experiment. The chemistry of the waters in Hlíðardalslækur stream has been monitored frequently since the utilization begun in 1978 (Ármannsson, 2003, 2005b; Einarsson, 2014a), but the monitoring has mainly been focused on heavy metals and potential toxic chemicals in the effluent waters and excessive waters from the boreholes. Low concentrations of heavy metals such as arsenic and lead have been detected in the streams, being below threatening threshold to the environment, <0.0005 mg/l (Óskarsson, 2015). However, information concerning nutrients in effluent waters is rather limited, thus adding to the knowledge is of vital importance. The Landsvirkjun power company has official permission to release effluent waters into the environment as long as annual monitoring shows no effect of contamination in the groundwater. Based on those terms it is necessary to add nutrient monitoring to the list, especially the highly effective ones, such as nitrogen and phosphorus.

The study site is under variety of influences from a geothermal utilization with little known consequences on the aquatic ecosystems. The effluent waters from the Krafla power plant are of main concern as little is known about their nutrient composition. My aim was to investigate biomass responses to nutrient diffusing substrata (NDS) containing four different nutrient treatments (control, nitrogen, phosphorus and nitrogen + phosphorus). Two NDS experiments were located upstream from the power plant and two downstream. Nutrients, especially phosphate and nitrogen, are generally considered to be critical for autotrophic productions. Numerous field studies provide evidence that nutrient supply can limit periphyton growth in nature, thus biomass (chlorophyll *a* concentration) is expected to show positive responses to nutrient enrichment. N_2-fixing Cyanobacteria and diatoms are known to increase under nitrogen limitations because of their ability to fix atmospheric nitrogen. In a pilot study on the periphyton assemblages in the Krafla streams, results showed that the sites downstream from the effluent were dominated by N_2-fixing algae and Cyanobacteria. The presence of N_2-fixing Cyanobacteria and diatoms downstream from the effluent strongly suggests N-limited conditions. I hypothesize that nitrogen treatments will cause positive responses in periphyton accrual downstream from the effluent. However, phosphorous treatments will increase accrual at the reference site and upstream from the effluent, as periphyton assemblages include fewer N_2-fixers, suggesting P-limitations.

3.2 Material and methods

The catchment of the stream Hlíðardalslækur was divided into four reaches based on their position around the Krafla geothermal power plant. Preliminary studies regarding limiting nutrients in the observed streams are lacking, and therefore, both ammonium nitrate (NH_4NO_3) and potassium phosphate (KH_2PO_4) were used to enrich the treatment reaches. Nutrient diffusion substrates (NDS) consisted of 90 mL plastic pots containing either 2% agar (controls) or agar to which N, P or N + P were added. Phosphorus was added as 0.1 M KH_2PO_4 and nitrogen as 0,5M NH_4NO_3. 20 cm^2 area consisted of 200 µm nylon mesh marked the colonization surface at each pot (Hauer & Lamberti, 2006). A total of 24 pots were placed at each reach, containing six replicates of each treatment. The pots were randomly placed in stainless steel frames, which were anchored to the stream benthos with stainless steel rails in September 2015. The aim was to increase nutrient concentration in order to observe benthic communities responses through biomass alterations.

Water samples for chemical analysis (total nitrogen and total phosphorous) were collected in 1-L acid washed polyethylene bottles from each reach in September 2015 and January 2016 (Figure 1-1). Additionally, a sample from the effluent was collected in order to obtain data on nutrient inputs from the geothermal utilization. Samples were frozen for storage until further analysis. Samples for total phosphorus (TP) and total nitrogen (TN) were analysed at the laboratory of ALS Scandinavia in Sweden a few months after sampling.
In October 2015, trays were collected after being immersed in the streams for approximately 4 weeks. The periphyton growing on the nylon mesh at the top of the pots was carefully removed and the mesh split in half. One half of each nylon mesh was used to measure chlorophyll *a,* thus those samples were frozen for laboratory analyses. The other half of each replica was preserved in approximately 10% Lugol's iodine solution for possible algae and Cyanobacteria analysis, which was not carried out in this study.

Chlorophyll *a*. The periphyton from each replicate of the nutrient diffusing substrata were immersed in 96% ethanol and kept in a refrigerated dark place for at least 24 hours. The volume of the ethanol was measured. After chlorophyll *a* had dissolved, the sample was filtered in a centrifuge before measuring the light absorption. The absorption of the ethanol extract was measured at 665 nm and 750 nm using a spectrophotometer, this was done in a dim room to prevent chlorophyll breakdown. A wavelength of 750 nm was used for correction of residual turbidity after filtration and the dissolvent phase. Finally, wavelength of 665 nm was used as the absorption maximum wavelength in the red end of the spectrum for chlorophyll *a*. The absorbance was repeated after acidification with 1N HCL to correct for pheophytins. Chlorophyll *a* values are expressed as µg/cm^{-2} and were calculated according to Lorenzen, (1967), using the extinction coefficient for 96% ethanol from Wintermans & De Mots (1965).

$$Chl\ a = \frac{A * K\left((665_b - 750_b) - (665_a - 750_a)\right) * V}{S * l}$$

Chl a − concentration of chlorophyll *a* (µg/cm^2)
A – 11.99 absorption coefficient (µg/cm^2) of chlorophyll a, derived from reciprocal of specific absorbance of chlorophyll a in 96% ethanol: 83.4 l/(g*cm).
K – 2.43 factor of correction for acidification.
665$_b$ – Absorbance at 665 nm before acidification.
750$_b$ – Absorbance at 750 nm before acidification.
665$_a$ – Absorbance at 665 nm after acidification.
750$_a$ – Absorbance at 750 nm after acidification.
V – Volume of ethanol, used for extraction (ml).
S – Area of stone (cm^2).
l – Length of path light through cuvette (cm).

The biomass data was log-transformed before analysis as owing to their non-normality. ANOVA (Analyses of Variance) was applied to calculate significance levels (*P*-values) in biomass between treatments. Tukey HSD (honest significant difference) *post hoc* was plotted to highlight if differences were significant or not.

All statistical analyses were performed using RStudio Version 3.3.1 and Microsoft Excel.

3.3 Results

At the upstream site KR1-2 an increase of dissolved inorganic nutrients was observed between September and January. DIN increases from 1.03 to 4.14 µmol/L and DIP increased from 0.03 to 0.10µmol/L. The total dissolved N and P at site KR1-2 were in lower concentration in the winter samples compared with the summer samples (Table 3-1).
Downstream from the power plant (KR1-4 and KR1-6) a dilution of dissolved inorganic nutrient concentration (DIN and DIP) was observed after mixing with the effluent water from the power plant and thus the nutrient concentration was lower there than upstream. DIN concentration did not vary between the two months at site KR1-4 but concentration of DIP was higher in winter than in the summer, 0.23 µmol/L and 0.16 µmol/L, respectively. High concentration of TDN was observed at site KR1-4, with values of 10.00 µmol/l in September and 3.86 µmol/l in January, due to high concentration of organic N. Concentration of TDP was much less, ranging from 0.13 to 0.26 µmol/L. Further downstream from the power plant, at site KR1-6, DIN concentrations were high in September with values of 1.04 µmol/L. In January DIN concentration had increased to 3.32 µmol/L. The DIP concentrations were 0.42

and 0.77 µmol/L in September and January, respectively. A high concentration of TDN was observed (3.43 and 7.85 µmol/L) along with high concentration of TDP (0.42 and 0.81 µmol/L), due to high concentration of organic N and P.

The nutrient concentration and the ratio of nutrients at the reference site, KR2-2, were different from what was observed at the upstream and downstream sites, and the water was high in nutrients. The concentration of DIN was high both in summer and winter, (2.04 and 5.81 µmol/L), with NO_3 and organic N as the main nutrient. The DIP concentrations were also high and lower in summer compared with winter (0.39 and 0.97 µmol/L). TDN was in 5.93 September and 9.28 in January and TDP was 0.52 in September and 0.94 in January.

Table 3-1. The nutrient concentrations at five of the study sites and the effluent from the cooling tower in October 2015 and January 2016. TDN = Total dissolved nitrogen. (DIN) Dissolved inorganic nitrogen = Sum of NO_3-N, NO_2-N and NH_4-N. (DON) Dissolved organic nitrogen = TDN - DIN. TDP = Total dissolved phosphorus. (DIP) Dissolved inorganic phosphorus = PO_4-P. DOP = Dissolved organic phosphorus. Nutrient concentration is expressed as µmol/l.

Site	Date	TDN N_{total}	NO_3-N	NO_2-N	NH_4-N	DIN	DON N_{tot}-DIN	TDP P_{total}	DIP PO_4-P	DOP P_{tot}-DIP
		µmol/l	µmol/l	µmol/l	µmol/l	µmol/l	µmol/l	µmol/l	µmol/l	µmol/l
KR1-2	Sept	3.93	0.14	<0.04	0.86	**1.03**	2.89	0.09	**0.03**	0.06
	Jan	6.50	1.00	0.07	3.07	**4.14**	2.36	0.09	**0.10**	0
KR1-4	Sept	10.00	<0.07	<0.04	<0.20	**0.31**	9.69	0.13	**0.16**	0
	Jan	3.86	<0.07	<0.04	<0.20	**0.31**	3.55	0.26	**0.23**	0.03
KR1-6	Sept	3.43	<0.07	<0.04	0.93	**1.04**	2.39	0.42	**0.42**	0.00
	Jan	7.85	1.64	<0.04	1.64	**3.32**	4.53	0.81	**0.77**	0.03
KR2-2	Sept	5.93	1.64	<0.04	0.36	**2.04**	3.89	0.52	**0.39**	0.13
	Jan	9.28	5.57	<0.04	<0.20	**5.81**	3.47	0.94	**0.97**	0
Effluent	June	2.28	<0.1	<0.04	0.2	**0.31**	1.97	0.387	**0.4**	0.03

Observed measurement showed that inorganic nutrient increase took place over the winter in most cases (Figure 3-1). The upstream site KR1-2 seemed to be the only inorganic phosphorus limited site according to the Redfield ratio. In September inorganic nutrients were low, with values close to a co-limited state between DIN and DIP. A significant increase had occurred in January. For the downstream sites (KR1-4 and KR1-6) along with the reference site (KR2-2), inorganic nitrogen seemed to be the limiting nutrient. In all cases nutrient concentration moved away from the Redfield line and never crossed it. In other words, no shifts in the limiting nutrients due to nutrient increase were observed. The effluent waters appeared to be highly N-limited.
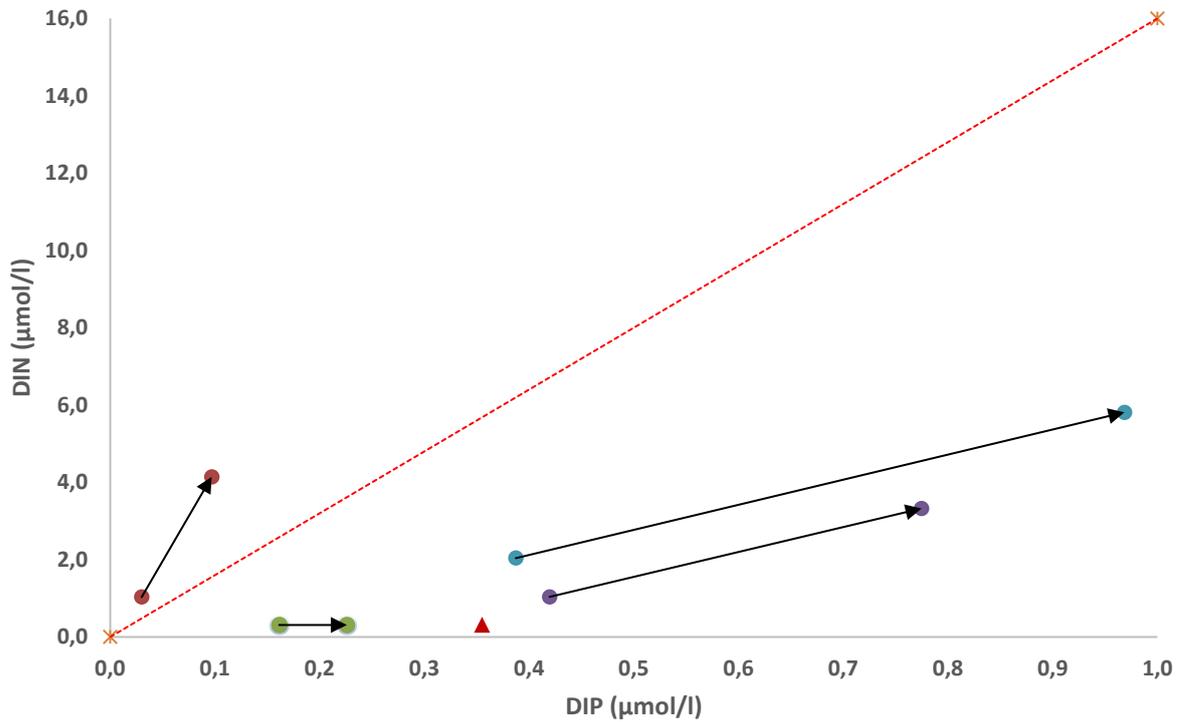
Figure 3-1. The ratio of N and P at the observed sites. The lines represent the increase of nutrient (inorganic nitrogen and phosphorus) from September to January. Red dotted line = Redfield ratio, N:P =16:1. Values beneath the Redfield line are classified as nitrogen limited. Values above the line are classified as phosphorus limited. Red dots = KR1-2. Green dots = KR1-4. Purple dots = KR1-6. Blue dots = KR2-2. Red triangle = Effluent.

At the upstream site (KR1-2) accrual growth was observed in contrast to the nitrogen treatment. Similar responses were observed for the N+P treatment. However, phosphorous alone did not lead to an increase in accrual in comparison to the control treatment. Although periphyton accrual often responded positively to numbers of the treatments, the increased accrual was not always statistically significant. For the upstream site KR1-2, the N+P treatment was the only treatment to differ significantly to the control treatment ($P<0.05$). Significant difference was observed between the phosphorous treatments in comparison to the N+P treatment as well. Very high accrual was recorded at the reference site, for all of the treatments, with the control = 50 µg/cm^2 (Figure 3-2). Nitrogen treatment seemed to lead to an increase (20%) in accrual, as values increased to roughly 60 µg/cm^2. The N+P treatments had equally high accrual when compared to the control treatment. Phosphorous treatment alone did however not lead to an accrual increase in comparison to the control treatment (Figure 3-2). However, non-significant difference was observed between all the treatments ($P>0.05$). Rather low chlorophyll concentration (4.5 µg/cm^2) was measured in the control treatment downstream from the power plant at site KR1-4. Nitrogen addition did not in any way alter the periphyton accrual. On the other hand, the phosphorous along with the N+P treatments led to increased accrual. Statistical significance was observed in 4 out of 6 treatment comparisons. Both phosphorous alone and the N+P treatment differed significantly in contrast to the control treatment. As the matter of fact, same results were

obtained for the P and N+P treatments in comparison to the N treatment as well. The accrual at site KR1-6 was rather low for the control, similar responses were observed for the nitrogen treatment as well. Phosphorous and N+P treatments did however enhance accrual, similar to the upper downstream site (KR1-4). Even so, there was no-significant difference between all of the treatments at site KR1-6 (Figure 3-3). Effluent waters contained practically no inorganic nitrogen (Table 3-1). Inorganic phosphorus input on the other hand was quite high.
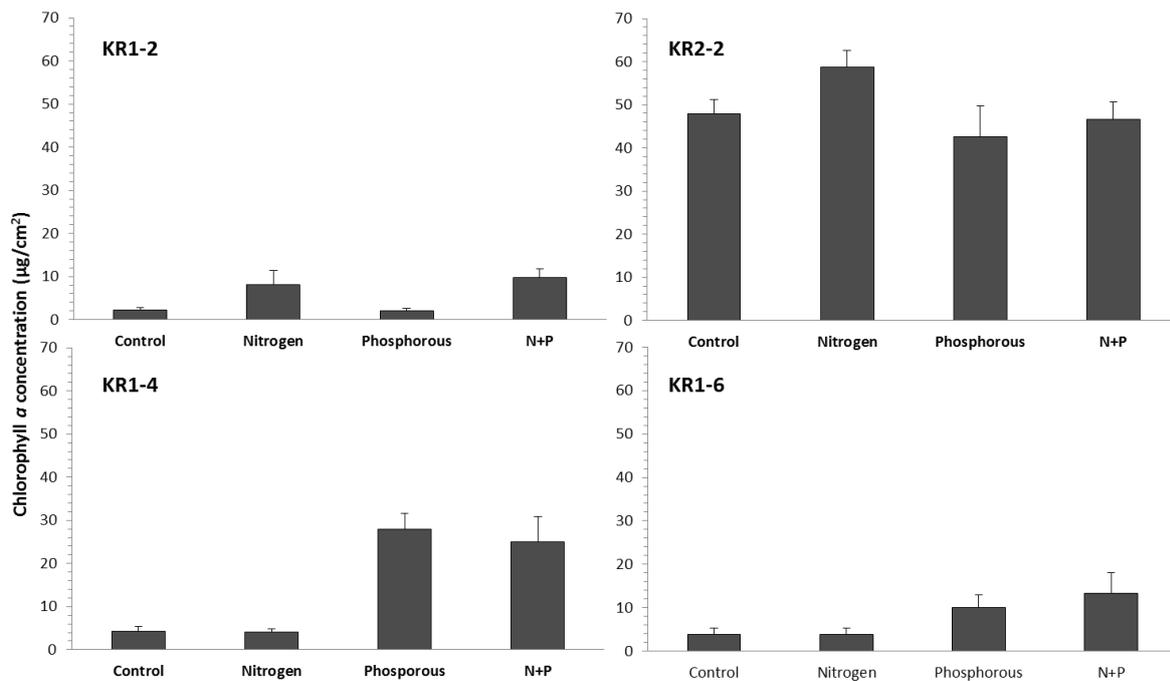
Figure 3-2. Periphyton accrual (biomass) to four nutrient treatments. Positive standard error bars are plotted for each treatment. N+P = Treatment including both nitrogen and phosphorous.
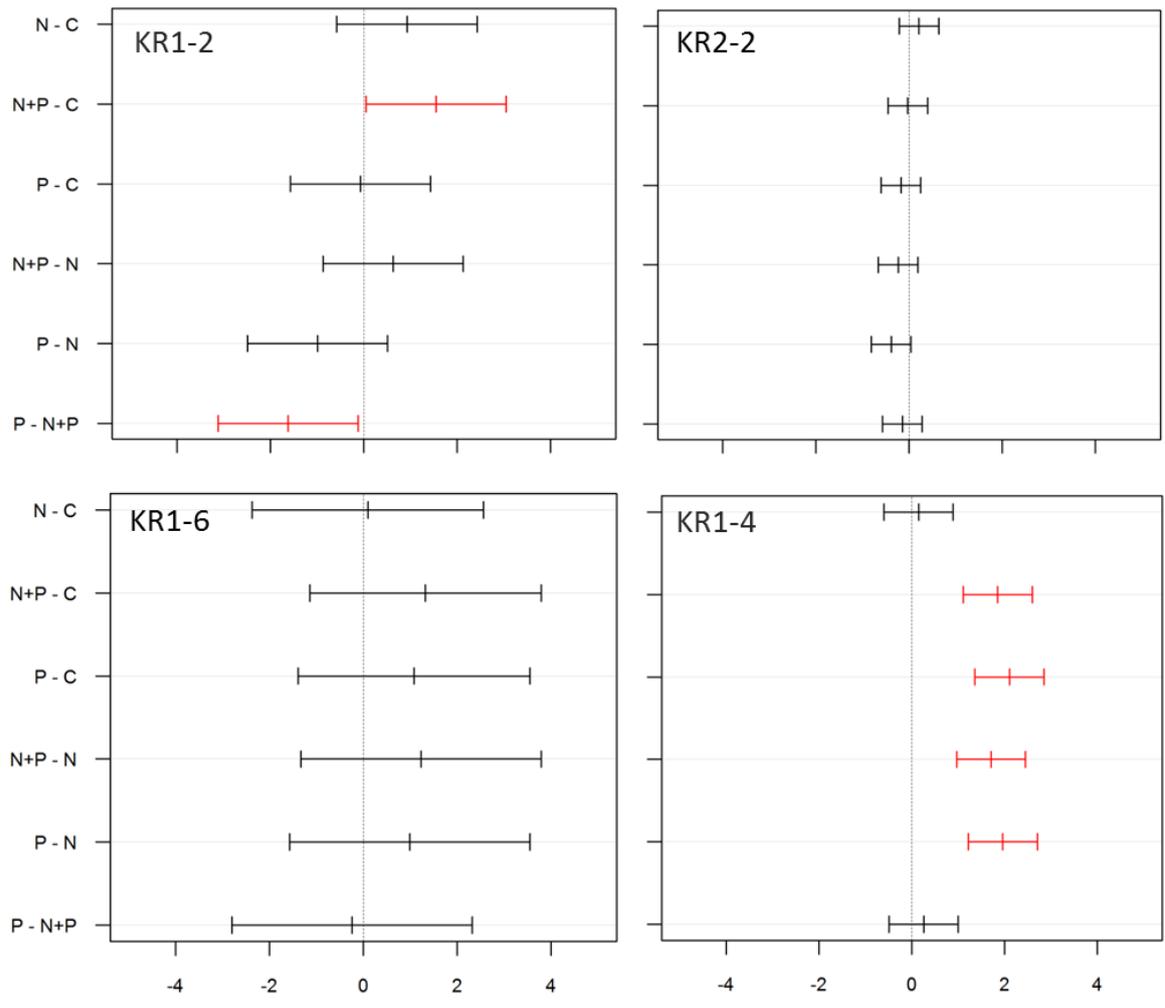
Figure 3-3. Tukey HSD plots showing the outcome of testing four nutrient enrichment. Black lines represent non-significant difference between compared treatments (95% confidence levels). Red lines represent significant difference between treatments. Differences in mean levels of biomass are presented on the x-axis.

3.4 Discussion

The nutrient diffusing substrata were successful for all the reaches with high variety of periphyton growth responses. Generally, nitrogen appeared to be the limiting nutrient upstream from the power plant as increased accrual responses were observed when N treatment was added. Phosphorus treatment led to enhanced accrual below the effluent (only P enrichment enhanced accrual) at site KR1-4, leading to the conclusion that phosphorus may be a limiting nutrient downstream from the power plant. Based on the obtained data, the hypothesis must be rejected, as periphyton accrual responses to nutrient enrichment were opposite to the prediction. In order to increase the understanding of nutrient addition in geothermal streams every single reach and their responses to the nutrient treatments will be discussed independently hereafter.

Periphyton accrual at the upstream site from the power plant, KR1-2, increased significantly when N+P enrichment were added, but N enrichment alone did not lead to significant accrual. Such effects are less common in stream studies but have been observed consistently by Stockner and Shortreed (1978) and Friberg and his colleagues (2009). They observed that additional phosphorous and nitrogen lowered the N:P ratios and had stimulating effect on growth, especially N_2-fixing Cyanobacteria. Such might be the case for this study as it emerged that periphyton assemblages at that site included a relatively high portions of both N_2-fixing Cyanobacteria and diatoms. The N+P treatment led to a significant accrual increase in comparison to the phosphorus treatment as well, suggesting that nitrogen is the main limiting nutrient, but might be co-limiting along with phosphorous. Co-limitation by N and P may be a common finding because the periphyton includes multiple species that have different nutrient requirements (Tank & Dodds, 2003). Phosphorous alone did not lead to accrual increase, as accrual was equal to the control treatment. The benthos was completely covered in precipitations which is known to have high silica concentration (Arnórsson & Gunnlaugsson, 1976), which might include high phosphorus concentration as well. Phosphorus is an immobile element which tends to co-precipitate or be adsorbed on the surface of precipitates, decreasing the concentration of dissolved phosphorus (Domagalski & Johnson, 2012). Thus, there is a change that periphyton absorbed phosphorus from the precipitation.

High accrual characterized the reference site which was a reflection of high nutrient concentration. This site receives water which is not influenced directly by the geothermal utilization and should therefore reflect natural conditions. All of the nutrient treatments had high accrual, although treatments did not differ from each other and N treatment led to highest accrual of all. No other nutrient enrichment treatment exceeded that accrual, in which both P alone and N+P treatment showed little to no responses and all accrual responses were non-significant. It appears as neither nitrogen nor phosphorus are limiting for the growth of the moss and diatom dominated reference site, at this given time. The limited accrual responses to nutrient enrichments might be because the reference site is only about 50 meters long. Due to the short distance from its origins, nutrients are constantly renewing. Nutrient depletion for such a short distance might be unlikely as currents bring a renewal of nutrients, gases and other material which reduces the risk of nutrient depletion (Uehlinger et al., 2003). Hence, nutrients might be constantly of sufficient needs which might explain the stable biomass concerning all the treatments.

Responses in periphyton accrual to nutrient enrichment downstream from the power plant were of a different sort. Inorganic nitrogen seemed to have no effect on the biomass at site KR1-4, even though nitrogen was practically absent according to the nutrient analysis (Table 3-1). The lack of growth responses regarding nitrogen addition was proofed whereas non-significant difference was obtained between control and N treatments ($P>0.05$). On the contrary, phosphorus treatment led to roughly 6 fold periphyton accrual in comparison to the control. In fact, so did the N+P treatment and the positive increases were highly significant ($P<0.05$). The fact is that communities at this site are dominated by thick mats of N_2-fixing Cyanobacteria, which are capable of fixing nitrogen from the atmosphere (Tsygankov, 2007; Stancheva et al., 2013; Pinto et al., 2016). As a consequence, inorganic nitrogen does not limit the growth of the communities. However, phosphorus appeared to enhance periphyton accrual, leading to the estimation that P might be the limiting nutrient downstream from the effluent. Autotrophs usually require low concentration of phosphorus, however, P limitation is known to occur under certain conditions. Bothwell (1989) stated that thin films of periphyton communities reach saturation regarding cellular uptake of phosphorus at low levels. However, thick mats impede diffusion so that saturation of uptake by the community might require concentration much greater. This might help explaining why periphyton accrual continues in response to phosphorous enrichment well after saturations conditions for individuals cells should have been surpassed, such as at site KR1-4 (Perrin et al., 1987). My suggestions are that N_2-fixing Cyanobacteria obtain their nitrogen through their heterocyte, thus they do not depend on dissolved inorganic nitrogen compounds for growth, giving them an advantage over other autotrophs. Hence, I estimate that N is not the limiting nutrient, even though the Redfield ratio suggests so. On the other hand, inorganic phosphorus seemed to be moving towards depletion by the time the NDS were immersed in the streams (Figure 3-1). The possible P depletion in September and the need for Cyanobacteria for dissolved inorganic phosphorus for growth heavily imply that P is the limiting nutrient downstream from the power plant, as the communities are dominated by N_2-fixing Cyanobacteria. Similar responses were observed further downstream from the power plant at site KR1-6. Nitrogen did not enhance growth in any ways, in which accrual for the nitrogen treatment and control treatment showed no difference. Both P and N+P treatments resulted in non-significant ($P=0.62$ and $P=0.46$) accrual as well. Also, site KR1-6 receives nutrient rich waters from the reference stream, which might provide the periphyton with additional nutrients which are not present at the upper downstream site (KR1-4). Results from a pilot study showed that periphyton assemblages were found to include high densities of algae and Cyanobacteria, in which both N_2-fixing Cyanobacteria and N_2-fixing diatoms played a major role in the assemblages downstream from the power plant. The presence of N_2-fixing algae and Cyanobacteria partly explains why inorganic nitrogen addition does not cause enhancements in growth, in similar ways as at the upper site, KR1-4. In the light of increased periphyton growth responses to both the P and N+P treatment at site KR1-4, I estimate that inorganic phosphorus is partly limiting downstream from the effluent, as the enrichment enhanced periphyton growth only at site KR1-4 but not further downstream, KR1-6.

Nutrient diffusing substrata are useful detectors of limiting nutrients in aquatic systems (Pringle, 1987). However, Capps and coworkers (2011) tested the methodological differences between various types of NDS. Results indicated that further research are needed to clarify differences between methods, until then, results obtained with NDS methods should be interpreted with caution. The observed data for this experiment are no exception, although the results should indicate which nutrients are limiting for periphyton growth

within the observed reaches. Further testing, such as identification and enumeration of algae and Cyanobacteria, would improve the important knowledge of geothermal ecosystem function. Such knowledge is of vital importance if to improve geothermal utilization in the most sustainable way as possible.

3.5 Conclusions

Nutrient enrichments (N, P and/or N+P) led to significant increase in periphyton accrual (increased chlorophyll *a* concentration) at all the sites, except for the reference site and the downstream site, KR1-6. As expected, accrual responses varied greatly between reaches and between treatments. Nitrogen + phosphorus enrichment resulted in significant biomass increase at site KR1-2, whilst phosphorus enrichment alone had no effect. Very high periphyton accrual took place at the reference site, most likely due to the nutrient rich waters. Nitrogen enrichment led to 20% accrual increase. However non-significant difference occurred between all of the nutrient enrichments. Downstream from the power plant, phosphorus enrichment did lead to significant increase in accrual at site KR1-4, and so did the N+P enrichment. The fact that nitrogen enrichment alone did not increase the accruals implies that phosphorus is the main limiting nutrient at that site. Further downstream (KR1-6), the accrual did not increase significantly with phosphorus nor nitrogen enrichments. Nitrogen seemed to have no effect on periphyton accrual beneath the effluent, probably due to dominating microbial mats of N_2-fixing Cyanobacteria.

The obtained results from this experiment disapprove the hypothesis, as nitrogen appears to be the limiting nutrient above the effluent (site KR1-2) and phosphorus limits the growth downstream from the effluent (KR1-4), contrary to the predictions, thus the hypothesis is rejected.

4 General discussion

From the obtained data presented in this study it was possible to compare both spatial and temporal periphyton and invertebrate assemblages within the geothermal regions of Krafla, NE Iceland. The most common periphyton in streams are usually diatoms, green algae and Cyanobacteria (Allan, 1995). Communities in freshwaters are shaped by a variety of physical variables, such as temperature, pH, discharge, light availability and nutrients. According to the results in the first part of this thesis, the observed physical variables varied greatly between sampling sites, and between sampling occasions. Based on the high dynamics in physical variables, it was expected for the periphyton and invertebrate assemblages to be quite diverse.

Effluent waters from the Krafla power plant affect the assemblages of the stream periphyton and invertebrates. The effluent from the power plant altered the temperature and the nutrient composition of the stream, along with increasing the discharge. Increase in temperature is perhaps the most important parameter, as each species of periphyton has its own range of temperature tolerances and preferences (Maitland, 1978). Hot effluent waters are produced by many industries which use the water for cooling purposes. Such effluent can be poisonous and/or too hot for living creatures, thus killing organisms near their input in streams (Hynes, 1960). The temperature downstream from the power plant in Krafla was on average high, which most likely hindered the growth of green algae and diatoms. Assemblages were dominated by thermal tolerant genera of Cyanobacteria, which are known to thrive well in hot waters (DeNicola, 1996). The dominating genera were *Anabaena*, *Calothrix*, *Phormidium* and *Lyngbya*. All of these genera are known to frequently occur in warm-hot geothermal waters and have been observed in various studies (Fernandez-Turiel et al., 2005). In fact, the Cyanobacteria formed continuous mats which covered the entire bottom of the stream. The ability of thermophilic Cyanobacteria to form mats is a distinctive characteristics of many geothermal ecosystems (Castenholz, 1984), as they frequently occur under certain environmental conditions, usually warm and nutrient rich (Fagerberg & Arnott, 1981). However, little is known about nutrient input from the Krafla power plant, although heavy metals and silica are known to occur in various concentrations in effluent waters and excessive waters from the boreholes (Arnórsson & Gunnlaugsson, 1976). In this study, chlorophyll *a* concentration increased at the sites when nutrients were added, although varying between treatments and location of the enrichment. The waters downstream from the power plant appeared to be nutrient poor, as inorganic nitrogen was practically absent. It turned out that the effluent water was extremely poor in nutrients, which might have further limitations on the growth of the periphyton. In a way, nitrogen limited environments are convenient for N_2-fixing algae and Cyanobacteria, as they are able to fix atmospheric nitrogen, which gives them an advantage to dominate the assemblages (Bellinger & Sigee, 2015). N_2-fixing Cyanobacteria, *Anabaena* and *Calothrix*, belonged to the mats and thus partly dominated the assemblages. Those results are consistent to previous studies, wherein N_2-fixing genera/species dominate under N-limited conditions (Stancheva et al., 2013).

Results from the nutrient enrichment showed positive responses to the phosphorous treatment downstream from the effluent, while nitrogen enrichment had no effect. Nitrogen fixing Cyanobacteria obtain their nitrogen through their heterocyte, thus they do not depend on inorganic nitrogen for growth, giving them an advantage over other autotrophs. Hence, it is estimated that N is not the limiting nutrient, even though inorganic nitrogen was extremely low. On the contrary, P-limitation is likely the case downstream from the power plant, as the

N$_2$-fixing Cyanobacteria rely on dissolved inorganic phosphorous for growth. This is consistent with results indicating high growth responses (increased chlorophyll *a* concentration) of periphyton in various ecosystems when nutrients levels are increased (Scrimgeour & Chambers, 1997; Stephens, Brasher, & Smith, 2012).

The alterations of the nutrient along with the temperature in favour of N$_2$-fixing Cyanobacteria are not the only effects caused by the power plant. As Cyanobacteria dominated, other periphyton, such as diatoms and green algae, might not have been able to grow. Thus the biodiversity decreased significantly. The few genera of Cyanobacteria present were all filamentous, which means that densities were extremely high due to their tiny cells (Pinto et al., 2016). The thick microbial mats were also most likely responsible for significantly higher biomass in comparison to other sites, except the reference site. The invertebrate density was low downstream from the power plant, which further indicates possible thermal or chemical pollution from the power plant, as warm effluent often harm or kill nearby organisms (Hynes, 1960). Not only were the invertebrates sparse, but only three taxonomic groups were recorded, in which Chironomidae was the most abundant group. The zone downstream to the effluent seems to be Polysaprobic. Hynes (1960) defined Polysaprobic as: "the zone of gross pollution with organic matter of high molecular weight, very little or no dissolved oxygen and the formation of sulphides. Here bacteria are abundant, as are other organisms, but there are few species of animals and these all live on decaying organic matter or feed on bacteria". The high biomass along with extremely high densities of Cyanobacteria and low densities of invertebrates fits Hynes definition. The increased temperatures from the effluent, along with the lack of inorganic nitrogen might be responsible for the lack of more diverse assemblages, as has been observed in earlier studies. Densities of aquatic invertebrates are known to be less in the warmer streams of Krafla in comparison to the colder ones (Ólafsson et al., 2010). Similar results have been observed in New Zealand, in which fewer aquatic invertebrates occurred in close proximity to geothermal resources, with higher temperatures, in part probably resulting from the lack of diverse habitats (Boothroyd & Browne, 2006).

Further downstream from the power plant physical variables kept rather similar, except for a relatively high increase in discharge and a decrease in temperatures. Average temperatures were 17.9°C, which is an optimal temperatures for many periphyton (DeNicola, 1996). Periphyton assemblages included all five of the observed groups, including the Cyanobacteria, diatoms and green algae. The most common diatoms were the genus *Melosira* and the N$_2$-fixing *Epithemia* and *Rhopalodia*. N$_2$-fixing Cyanobacteria (*Anabaena* and *Calothrix*) along with non-N$_2$-fixing Cyanobacteria (*Oscillatoria*, *Lyngbya* and *Phormidium*) had the highest relative abundance. However, Cyanobacteria accounted for small portion of the relative frequency of occurrence, presumably because of their small size as has been repeatedly noted (Whitton et al., 2011). In fact, highest Shannon diversity occurred for this particular site, along with the reference site. However, biomass reduced significantly in contrast to the upper site, implying more sparse assemblages. However, I state that the communities fit Hynes definition of Mesosaprobic zone: "zone where recovery has proceeded further and condition are suitable for many algae, and tolerant animals and some rooted plant may occur" (Hynes, 1960). The fact that high density and diversity of aquatic invertebrates were present at this site further supports the statement. Highest number of invertebrate taxa occurred at the site further downstream from the power plant, as Chironomidae, Diptera and *Radix balthica* characterized the communities. In fact, the density and diversity were higher in comparison to the upstream sites. Recovery of the

diversity might persist due to longitudinal dilution gradient of harmful environmental attributes (most likely temperature and dissolved metals) in coexistence with the input of clean-water from the reference stream and other sources such as precipitation.

The reference site was the ideal image of a healthy environment in which various clean-water species of diatoms, such as *Melosira* and *Meridion* (Cooper, 1989), dominated the communities. The thick moss layers might explain the tremendous biomass values, which were responsible for the reference site to be significantly higher regarding biomass in comparison to the remaining sites. Grazing might have altered the assemblages, but further observations are needed to tests that possibility. Periphyton growth did not show significant increase in accrual to any of the nutrient treatments (in comparison to control treatment) at the reference site, thus indicating that neither nitrogen nor phosphorous limits the growth of the periphyton. This is consistent to previous studies regarding the beneficial attributes of currents, as the flow of water brings continual renewal of gases and nutrients which benefits periphyton growth by enhancing nutrient uptake (Allan, 1995). Also, the sampling site is located about 10 meters downstream to the source, thus decreasing the risk of nutrient depletion. Even so, the periphyton seemed to respond positively to N-treatment, although the difference was not significant in contrast to the control treatment. There seemed to be no correlation between temporal alterations and dominating genera/taxa. This was true for both periphyton and invertebrate communities.

Temporal alterations in both periphyton and invertebrate assemblages were observed throughout the study. Generally, algae and Cyanobacteria density along with biomass differed between seasons, as highest values occurred in late summer (July and September). The observed summer peaks in density and biomass match the results from other studies, in which periphyton growth is known to peak in summer, presumably because of higher temperatures (Allan, 1995). However, the upstream sites seemed to be more vulnerable to seasonal changes in comparison to the lower altitude sites. In the winter of 2016 (January) a significant decrease in periphyton densities and biomass were observed at all sites. Organisms in the sub-arctic undergo shortened daylight on annual scale, which might slow the rate of primary production (Sperling, 1975), resulting in lower density and biomass. In June 2016 periphyton density and biomass had reached similar values as in June 2015. As for the invertebrates, density was high in late summer (September) and decreased significantly in January.

It was observed that the effluent waters from the power plant causes significant effects shortly below the merging with the stream Hlíðardalslækur. Variation of the observed physical parameters reflected the vast degree of impact caused by the introduction of the effluent. Temperature increases, along with the pH and discharge. The lower conductivity implies that the amount of dissolved materials reduced significantly below the effluent. Diversity decreases, algal and cyanobacterial assemblages changes and becomes dominated by a few genera of Cyanobacteria in high extremely high abundance, which approves the hypothesis. However, the results strongly suggest that the effects are highly localized to the nearby habitats of the power plant. As a matter of fact both diversity and assemblages showed remarkable recoveries as the effluent waters diluted, resulting in significantly higher values in comparison to the sites upstream to the power plant. Ármannsson and Ólafsson (2002) stated that various alterations due to the effluent are highly localized in Hlíðardalslækur, close to the effluent sources. Further from the sources, extremely rapid dilution of the stream eliminates the affects from the power plants, and thus the effluent is unlikely to cause long distance disturbances.

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Appendix A
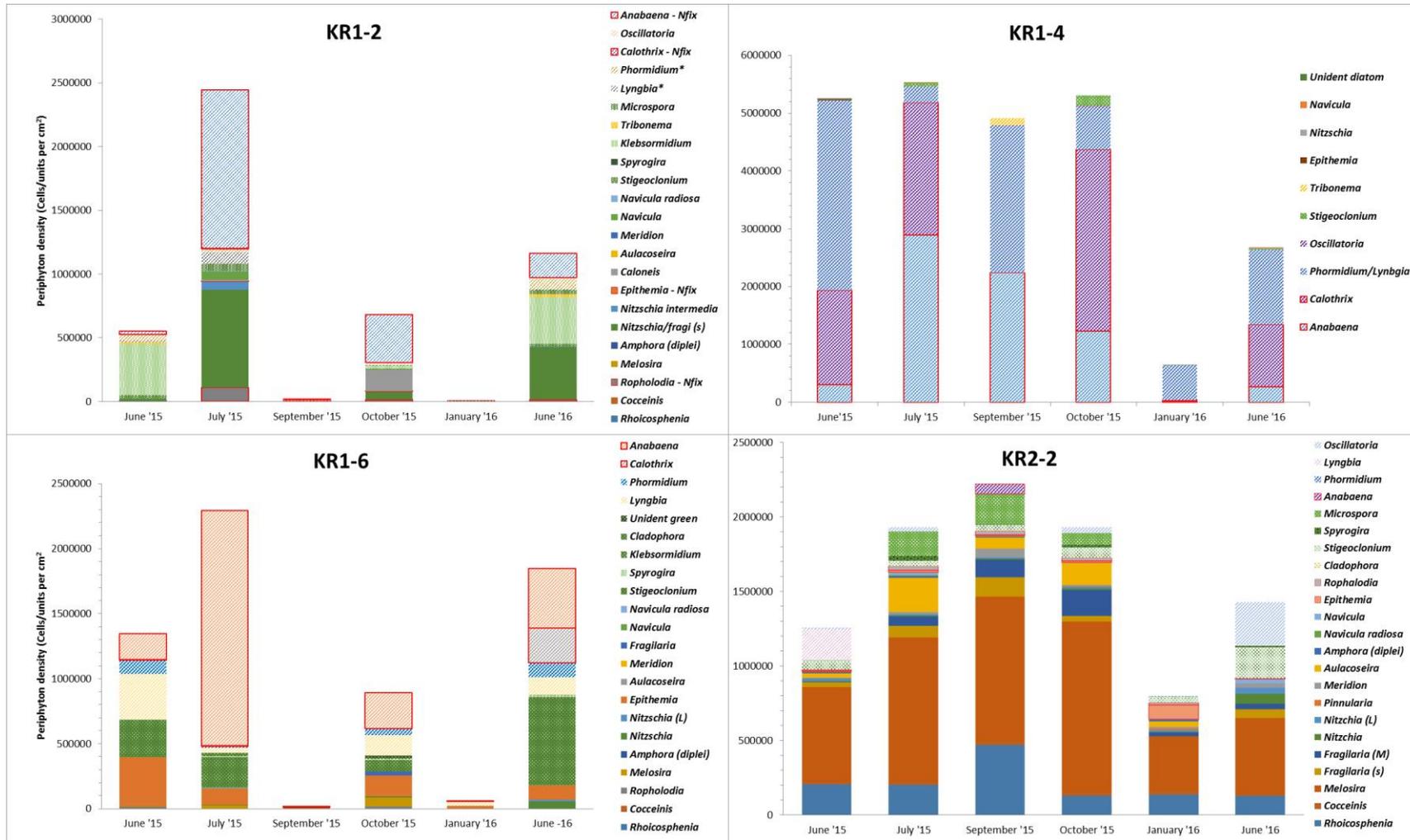
Appendix 1. Algae and Cyanobacteria assemblages at the four sites in the Krafla area. Red boarders represent N$_2$-fixing genera.

	June 2015				July 2015			
	KR1-2	KR1-3	KR1-4	KR1-6	KR1-2	KR1-3	KR1-4	KR1-6
KR1-3	0.9719	-	-	-	0.3099	-	-	-
KR1-4	0.9793	0.7621	-	-	0.3123	1	-	-
KR1-6	0.1276	0.3812	0.0347	-	0.0526	0.9040	0.9022	-
KR2-2	0.6104	0.2608	0.9076	**0.0032**	0.0903	0.9673	0.9664	0.9992

	September 2015				October 2015			
	KR1-2	KR1-3	KR1-4	KR1-6	KR1-2	KR1-3	KR1-4	KR1-6
KR1-3	0.8287	-	-	-	0.6615	-	-	-
KR1-4	0.2056	0.8056	-	-	0.4501	0.0280	-	-
KR1-6	0.8492	0.9999	0.7821	-	0.6780	0.9999	0.0300	-
KR2-2	**0.0068**	0.1059	0.6034	0.0962	0.0107	0.2464	**<0.0000**	0.2350

	May 2016				June 2016			
	KR1-2	KR1-3	KR1-4	KR1-6	KR1-2	KR1-3	KR1-4	KR1-6
KR1-3	0.7073	-	-	-	**0.0006**	-	-	-
KR1-4	**0.0000**	**<0.0000**	-	-	0.2200	**<0.0000**	-	-
KR1-6	**0.0002**	**0.0107**	0.0385	-	**0.0046**	**0.0000**	0.5506	-
KR2-2	**0.0000**	**0.0000**	0.1822	**<0.0000**	**<0.0000**	**0.0000**	0.0207	0.4720

Appendix 2. Results of biomass (Chlorophyll concentration) comparison between sampling sites. Bolded *P*-values represent significant difference (*P*<0.01) between compared sites. BenthoTorch data.

	KR1-2					KR1-3				
	June '15	July '15	Sept '15	Oct '15	May '16	June '15	July '15	Sept '15	Oct '15	May '16
July '15	0.9998	-	-	-	-	0.1603	-	-	-	-
Sept '15	0.9999	0.9999	-	-	-	0.4525	0.9902	-	-	-
Oct '15	0.9902	0.9493	0.9815	-	-	0.1607	1	0.9902	-	-
May '16	**0.0000**	**0.0000**	**0.0000**	**0.0000**	-	**0.0093**	**<0.0000**	**<0.0000**	**<0.0000**	-
June '16	0.0351	0.0731	0.0450	**0.0056**	**<0.0000**	**0.0003**	**0.0000**	**<0.0000**	**0.0000**	0.8558

	KR1-4					KR1-6				
	June '15	July '15	Sept '15	Oct '15	May '16	June '15	July '15	Sept '15	Oct '15	May '16
July '15	0.9906	-	-	-	-	**0.0010**	-	-	-	-
Sept '15	0.9255	0.9992	-	-	-	0.0224	0.9146	-	-	-
Oct '15	0.3171	0.0932	0.0318	-	-	**0.0061**	0.9932	0.9977	-	-
May '16	0.9625	0.9999	0.9999	0.0421	-	0.4304	0.1011	0.6149	0.3251	-
June '16	0.9184	0.5745	0.3147	0.8241	0.3888	**0.0076**	0.9027	0.9999	0.9981	0.4612

	KR2-2				
	June '15	July '15	Sept '15	Oct '15	May '16
July '15	0.9999	-	-	-	-
Sept '15	0.9513	0.9289	-	-	-
Oct '15	0.9236	0.8952	0.9999	-	-
May '16	0.6815	0.6265	0.9908	0.9975	-
June '16	0.9999	1	0.9112	0.8707	0.5470

Appendix 3. Results of biomass (Chlorophyll concentration) comparison between sampling occasions. Bolded *P*-values represent significant difference (*P*<0.01) between compared occasions. BenthoTorch data.

	June 2015				July 2015			
	KR1-2	KR1-3	KR1-4	KR1-6	KR1-2	KR1-3	KR1-4	KR1-6
KR1-3	0.9736	-	-	-	0.4145	-	-	-
KR1-4	**0.0000**	**0.0000**	-	-	**0.0400**	**0.0003**	-	-
KR1-6	**0.0001**	**<0.0000**	**0.0177**	-	0.1383	**0.0014**	0.9722	-
KR2-2	**0.0000**	**0.0000**	0.3752	**0.0001**	**0.0000**	**0.0000**	**0.0000**	**0.0000**
	September 2015				October 2015			
	KR1-2	KR1-3	KR1-4	KR1-6	KR1-2	KR1-3	KR1-4	KR1-6
KR1-3	0.5065	-	-	-	0.9996	-	-	-
KR1-4	**0.0007**	**<0.0000**	-	-	**0.0115**	**0.0436**	-	-
KR1-6	**<0.0000**	**0.0005**	**0.0000**	-	0.8159	0.7623	**0.0005**	-
KR2-2	**<0.0000**	**0.0000**	0.0557	**0.0000**	**0.0003**	**0.0018**	0.7058	**<0.0000**
	January 2016				June 2016			
	KR1-2	KR1-3	KR1-4	KR1-6	KR1-2	KR1-3	KR1-4	KR1-6
KR1-3	N/A	-	-	-	**0.0010**	-	-	-
KR1-4	**0.0000**	N/A	-	-	**0.0171**	**0.0000**	-	-
KR1-6	**0.0000**	N/A	0.5924	-	0.1722	**<0.0000**	0.8591	-
KR2-2	**0.0000**	N/A	**<0.0000**	**<0.0000**	**0.0064**	**<0.0000**	0.9964	0.6663

Appendix 4. Results of biomass (Chlorophyll *a* concentration) comparison between sampling sites. Bolded *P*-values represent significant difference between (*P*<0.01) compared sites (). Spectrophotometer data.

	KR1-2					KR1-3				
	June '15	July '15	Sept '15	Oct '15	Jan '16	June '15	July '15	Sept '15	Oct '15	Jan '16
July '15	**<0.0000**	-	-	-	-	0.0107	-	-	-	-
Sept '15	**0.0017**	0.5618	-	-	-	0.1662	0.7743	-	-	-
Oct '15	**0.0016**	0.5714	1	-	-	0.0354	0.9999	0.8915	-	-
Jan '16	**0.0000**	**0.0000**	**0.000**	**0.0000**	-	N/A	N/A	N/A	N/A	-
June '16	0.0316	0.1020	0.9189	0.9140	**0.0000**	0.6921	**0.0003**	**0.0086**	**0.0016**	N/A
	KR1-4					KR1-6				
	June '15	July '15	Sept '15	Oct '15	Jan '16	June '15	July '15	Sept '15	Oct '15	Jan '16
July '15	0.9979	-	-	-	-	0.1759	-	-	-	-
Sept '15	0.9750	0.9997	-	-	-	**0.0000**	**0.0000**	-	-	-
Oct '15	0.9930	0.9999	0.9999	-	-	0.3489	**0.0007**	**0.0064**	-	-
Jan '16	**<0.0000**	**<0.0000**	**<0.0000**	**<0.0000**	-	**0.0008**	**<0.0000**	0.7660	0.1859	-
June '16	0.0766	0.0311	0.0108	0.0181	**0.0000**	0.0163	0.9222	**0.0000**	**<0.0000**	**0.0000**
	KR2-2									
	June '15	July '15	Sept '15	Oct '15	Jan '16					
July '15	**0.0060**	-	-	-	-					
Sept '15	0.9999	**0.0029**	-	-	-					
Oct '15	0.9330	**0.0003**	0.9803	-	-					
Jan '16	0.9999	**0.0034**	1	0.9734	-					
June '16	0.69588	0.2233	0.5453	0.1761	0.5775					

Appendix 5. Results of biomass (Chlorophyll *a* concentration) comparison between sampling occasions. Bolded *P*-values represent significant difference (*P*<0.01) between compared occasions. Spectrophotometer data.

KR1-2				KR2-2		
	C	**N**	**N+P**		**C**	**N**	**N+P**
N	0.3418442	-	-	**N**	0.5313624	-	-
N+P	**0.0417765**	0.6518112	-	**N+P**	0.9965791	0.4111713	-
P	0.9989582	0.2773268	**0.0310713**	**P**	0.6479820	0.0826549	0.7699971
KR1-4				KR1-6		
	C	**N**	**N+P**		**C**	**N**	**N+P**
N	0.9535715	-	-	**N**	0.9995870	-	-
N+P	**0.0000008**	**0.0000035**	-	**N+P**	0.4601775	0.5507334	-
P	**0.0000001**	**0.0000003**	0.7927642	**P**	0.6238513	0.7099050	0.9932782

Appendix 6. P-values for the comparisons of four nutrient treatments in Hlíðardalslækur. Bold numbers represent significant differences ($P<0.05$) between treatments.

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