Invertebrate Diversity in Icelandic Freshwater Springs

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2020

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Dissertation submitted in partial fulfillment of a
Philosophiae Doctor degree in Biology

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Bibliographic information:
Agnes-Katharina Kreiling, 2020, *Invertebrate diversity in Icelandic freshwater springs*, PhD dissertation, Faculty of Life and Environmental Science, University of Iceland, 97 pp.

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Printing: Háskólaprent
Reykjavík, Iceland, July 2020

Abstract

Freshwater springs are thermally stable environments which are largely unaffected by changes in air temperature. They could thus have the potential to buffer rising temperatures and serve as small-scale refugia for aquatic invertebrates in a warming world. To better understand what environmental variables drive invertebrate diversity and community composition, I conducted an extensive field survey on springs in Iceland. Due to rifts in the tectonic plates, large parts of Iceland are of volcanic origin and geothermally active. Thus, a high number of freshwater springs can be found along the edges of the porous lava fields, ranging in temperature from 2°C to boiling hot. Springs also differ in other characteristics, e.g., altitude, spring type, substrate, and vegetation density. I studied the invertebrate community of 49 springs on a spatial scale and followed one of them throughout a year to analyse temporal variability. The most abundant invertebrate groups in Icelandic springs were Chironomidae (Diptera), followed by Ostracoda and Copepoda. One of the main drivers of community composition was temperature, resulting in a distinct species group characteristic for hot springs. Additionally, spring type and geographical position influenced communities. Community composition also differed on a temporal scale within the same spring, with distinctions between summer and winter community. This seasonality in the invertebrate community was unaffected by temperature changes but had implications on the apex predator in the system, Arctic charr. The results of this thesis give some insight into Icelandic spring ecosystems and will contribute to conservation of these unique habitats.

Útdráttur

Fjölbreytileiki ferskvatnslinda er mikill t.d. hvað hita varðar, allt frá því að vera mjög kaldar og sjóðandi heitar. Þrátt fyrir þennan fjölbreytileika er þó hiti í hverjum og einstökum lindum mjög stöðugur og hafa breytingar í lofthita lítil áhrif á hitastig þeirra. Lindir gætu þannig dregið úr áhrifum af hækkandi hita og þannig myndað skjól, á smáum skala, fyrir ferskvatnshryggleysingja í hlýnandi loftslagi. Til þess að skilja betur hvaða umhverfisbreytur hafa áhrif á fjölbreytileika og samfélagsgerðir hryggleysingja þá framkvæmdi ég umfangsmikla rannsókn á lindum víðsvegar á Íslandi. Vegna flekahreyfinga og mikillar eldvirkni eru stórir hlutar landsins nýlegar bergmyndanir þar sem jarðhiti er algengur. Á jöðrum gegndræpra hrauna má finna mikinn fjölda ferskvatnslinda. Lindir eru einnig breytilegar m.t.t. annarra þátta, t.d. hæðar yfir sjávarmáli, lindargerð, botngerð og þéttleika gróðurs. Ég rannsakaði smádýrasamfélög í 49 lindum víðsvegar um landinu. Einnig fylgdi ég eftir breytingum yfir árið í einni lind, til að rannsaka stöðugleika smádýrasamfélags. Algengasti hópur smádýra í íslenskum lindum voru rykmý (Chironomidae, Diptera), þar næst skelkrabbar (Ostracoda) og árfætlur (Copepoda). Einn mikilvægasti þáttur í að skýra uppbyggingu dýrasamfélaga í lindum var hiti og var sérstakur dýrahópur einkennandi fyrir heitar lindir. Einnig skýrðu lindargerð og landfræðileg staðsetning þeirra mikið af þeim breytileika sem sást í samfélagsgerðum hinna ýmsu linda. Greinilegur breytileiki í samfélagsgerð var að finna milli sumars og veturs og var hann ótengdur breytingum í umhverfishitastigi. Hinsvegar hafði árstíðamunurinn áhrif á mikilvægan afræningja í þeim samfélögum, dvergbleikjuna. Niðurstöður rannsóknarinnar gefa upplýsingar um vistkerfi linda. Auk þess bæta niðurstöður rannsóknarinnar við þekkingu okkar á vistkerfum ferskvatnslinda sem m.a. verður hægt að nýta við hverskyns nýtingu og þ.m.t. verndun þessara einstöku búsvæða.

Dedication

To Oliver Bechberger, who encouraged me to start this PhD,
to Leivur Janus Hansen, who encouraged me to continue,
and to Ragnhildur Guðmundsdóttir, who forced me to finish.

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Paper I: Kreiling, A.-K., Ólafsson, J.S., Pálsson, S. & Kristjánsson, B.K. (2018). Chironomidae fauna of springs in Iceland: Assessing the ecological relevance behind Tuxen's spring classification. *Journal of Limnology* 77: 145-154. https://doi.org/10.4081/jlimnol.2018.1754

Paper II: Kreiling, A.-K., Govoni, D.P., Pálsson, S., Ólafsson, J.S. & Kristjánsson, B.K. (in revision). Invertebrate communities in limnocrene and rheocrene springs across a thermal gradient.

Paper III: Kreiling, A.-K., Gíslason, G.M. & Kristjánsson, B.K. (2020). Trichoptera diversity in Icelandic springs. *Zoosymposia* 18: 46-52. https://doi.org/10.11646/zoosymposia.18.1.8

Paper IV: Kreiling, A.-K., O'Gorman, E.J., Pálsson, S., Benhaim, D., Leblanc, C.A.L., Ólafsson, J.S. & Kristjánsson, B.K. (2020). Seasonal variation in the invertebrate community and diet of a top fish predator in a thermally stable spring. *Hydrobiologia*. https://doi.org/10.1007/s10750-020-04409-5

Published manuscripts not included in the thesis:

Guðmundsdóttir, R., **Kreiling**, A.-K., Kristjánsson, B.K., Marteinsson, V.Þ. & Pálsson, S. (**2020**): Ciliate diversity in cold water spring sources in Iceland. *Aquatic Microbial Ecology* 84: 191-203. https://doi.org/10.3354/ame01936

Wurzbacher, C., **Kreiling**, A.-K., Svantesson, S., Van den Wyngaert, S., Larsson, E., Heeger, F, Nilsson, H.R., Pálsson, S. (**2020**): Fungal communities in groundwater springs along the volcanic zone of Iceland. *Inland Waters* 1-10. https://doi.org/10.1080/20442041.2019.1689065

Klinth, M.J., **Kreiling**, A.-K. & Erséus, C. (**2019**): Investigating the Clitellata (Annelida) of Icelandic springs with alternative barcodes. *Fauna Norvegica* 39: 119-132. https://doi.org/10.5324/fn.v39i0.3043

Guðmundsdóttir, R., **Kreiling**, A.-K., Kristjánsson, B.K., Viggó Þór Marteinsson, V.Þ. & Pálsson, S. (**2019**): Bacterial diversity in Icelandic cold spring sources and in relation to the groundwater amphipod *Crangonyx islandicus*. *PLOS One* 14: e0222527. https://doi.org/10.1371/journal.pone.0222527

Alkalaj, J., Hrafnsdottir, Th., Ingimarsson, F., Smith, R.J., **Kreiling**, A.-K. & Mischke, S. (**2019**): Distribution of recent non-marine ostracods in Icelandic lakes, springs, and cave pools. *Journal of Crustacean Biology* 39: 202-212. https://doi.org/10.1093/jcbiol/ruz008

Acknowledgements

I was lucky to have an excellent PhD committee. First of all, I thus want to thank my advisors and committee members: Bjarni K. Kristjánsson, for our tours through the highlands, for never losing patience with me, and for supporting me in all my ideas and stubborn decisions. And even more for his trust and friendship, and for giving me a home. Jón S. Ólafsson, for introducing me to the fascinating world of chironomid larvae, for being a patient teacher in identification, and for his contagious enthusiasm and curiosity. Snæbjörn Pálsson, for the great and very much needed help in molecular labwork, data analysis and statistics, and for answering emails immediatly and at any hour during day and night. Eoin J. O'Gorman, for taking time for discussions via Skype and in person, and for good advice on how to write manuscripts and deal with rejections. Árni Einarsson, through whom I experienced that midges are beautiful. For his naturalist view on the world, and for feeding me with ice-cream, chocolate, and knowledge.

I am grateful for all the people I have met through this PhD, and which have in one way or the other contributed to it and helped me over the past six years:

Dan Govoni, for sharing his great ideas and music with me, for podcast suggestions which made microscope work more entertaining, for the good company in the field. I learned a lot during those long, rainy days in the field with you. Dan, you are a genius.
Kári H. Árnason, for invaluable help with developing sampling methods and with fieldwork in Skagafjörður. To any challenges he found solutions, in all aspects of work and life.
Zophonías O. Jónsson, for the good weather during fieldwork, for both earnest advice and profound bullshit, and for help with PCR optimizations.
David Benhaim and Camille Leblanc for their spontaneity and enthusiasm to start with me a project on the feeding behavior of Arctic charr in springs, for fun hours during fieldwork, and discussions at work, in the pool, and while knitting.

I want to thank all the farmers and landowners who let me sample springs on their land, especially Hjörleifur at Grænavatn, Hólmgrímur at Hraun í Aðaldal, Jóna og Guðbrandur at Staðarhraun, Ottar og Hulla at Enni, and Þóra Ellen Þórhallsdóttir at Hruni.
A bunch of people assisted me during fieldwork: Bjarni K. Kristjánsson, Jón S. Ólafsson, Dan Govoni, Kári H. Árnason, Manon S. Guerin, Ilgin Deniz Can, Sigurður H. Árnason, Zophonías O. Jónsson, Sébastien Matlosz, Jake Goodall, David Benhaim, Camille Leblanc, Milena Kreiling, and Hanna Malhas. Thanks to Kristín Hermannsdóttir and Þorvarður Árnason of the Southeast Iceland Nature Research Centre in Höfn for taking a day of their time to accompany me to springs around Höfn.

For my short adventure into the world of genetics I want to thank Snæbjörn Pálsson and Ragnhildur Guðmundsdóttir for PCR protocols and motivating discussions. At Matís, thanks goes to Viggo Þ. Marteinsson for allowing me to work in his lab and Stephen Knobloch for invaluable help during the labwork, especially in my very first weeks as a "geneticist".

Furthermore, I want to thank Eydís Salome Eiriksdóttir at Hafrannsóknastofnun for advice and assistance in water chemistry analyses. Jovana Alkalaj for Ostracoda identification.

Erling Ólafsson at Náttúrufræðistofnun for help with identification of Ephydridae. Gísli Már Gíslason for help with identification of Trichoptera, general discussions and literature suggestions, and for dragging me to Thailand.

I would like to thank the participants of the Chironomid Symposium 2017 in Trento for welcoming me to the big Chironomid Family, especially Martin Spies, Dimitrija N. Savić, Michelle McKeown, Dubravka Čerba, Djuradj Milošević, Ladislav Hamerlík, Vít Syrovátka, Joel Breil-Moubayed, Paddy Ashe, and Gaston Adamek.

Thanks to Ute Stenkewitz, Will Butler, Hildur Magnúsdóttir og fjölskylda, Jónína Herdís Ólafsdóttir & Jóhann Garðar Þorbjörnsson, Samantha V. Beck and Han Xiao for housing me in Reykjavík during the first years of my PhD. Þóra Ellen Þórhallsdóttir for letting us use her summerhouse at Hruni during fieldwork, and Árni Einarsson and RAMÝ for accommodation at Mývatn.

My colleagues at Ex-Veiðimálastofnun I would like to thank for not only letting me use their microscope but for making me feel welcome among them. Thanks for the great working atmosphere! Thanks to my Hólar/Verið family: Bjarni K. Kristjánsson, Doriane C. Combot, Nicolas Larrañaga, Soizic LeDeuff, Sigurður H. Árnason, Coralie Delarue, Samantha V. Beck, Camille Leblanc, Kári H. Árnason, David Benhaim, Erla Björk Örnólfsdóttir, Skúli Skúlason, Stefán Óli Steingrímsson, Guðmundur B. Eyþórsson, and Bjórsetrið Íslands. And my lab group in Askja: Snæbjörn Pálsson, Hildur Magnúsdóttir, Ragnhildur Guðmundsdóttir, Charles Christian Riis Hansen (thanks for the lasagne!), Elisabeth Knudsen, and Jake Goodall, for support, thought-provoking discussions and comments on manuscripts and conference talks.

A huge thanks to Doriane C. Combot, for her friendship and loyality. Thank you for countless coffee breaks in Verið, for your company in Skagafjörður and on travels, and for always believing in me.

Ég þakka kærlega hattífatti félagar minnir, Hildur Magnúsdóttir og Ragnhildur Guðmundsdóttir, fyrir skemmtilegar stundar í Öskju.

Thanks to my dear friend Oliver Bechberger without whom I would not have fallen in love with Mývatn (where everything began…) and who encouraged me to start this PhD.

Stóra tøkk to Leivur Janus Hansen, for discussions and support, for reading my manuscript drafts over and over again, for his awesome bread, and most of all for reawakening my curiosity for the natural world and reassuring me in being a beetle freak which gets excited about *Aphodius* in sheep dung.

1 Synopsis

1.1 Introduction

Freshwater ecosystems are often described as islands in a terrestrial sea, with many environmental barriers to the dispersal of the organisms that live there (Hagen et al. 2012). Nevertheless, they make a disproportionately large contribution to global biodiversity and face some of the highest extinction risks on the planet (Ormerod et al. 2010). Originally, research in freshwater ecology focused on lakes and rivers but over the past decades, springs and groundwater aquifers have received an increasing attention (e.g., Glazier 1991, Hoffsten & Malmqvist 2000, Barquín & Death 2004, Ilmonen & Paasivirta 2005, Deharveng et al. 2009, Galassi et al. 2009, Bottazzi et al. 2011, Johns et al. 2015, Fattorini et al. 2016 – to name just a few). Springs are important habitats contributing to the overall diversity in freshwaters. Invertebrate abundance and species diversity is often found to be higher in springs than in sites downstream and in nearby non-spring-fed aquatic systems (Glazier 2009). In addition, spring fauna can show peculiar patterns of species distributions as spring and groundwater habitats can act as refugia for endemic or locally extirpated species (Di Sabatino et al. 2003).

Despite the acknowledged importance of springs, there has not been any scientific consensus on the definition of the term “spring”. Freshwater biologists do not tend to discriminate distinctly between spring sources, the points of discharge of groundwater, and the adjacent habitats which they are feeding, i.e., spring brooks or ponds and lakes, due to a lack of clear criteria. The eucrenal zone (the springhead) of spring-fed streams is rather arbitrarily defined as up to 2 m (Wood et al. 2005), 5 m (von Fumetti et al. 2007), 10 m (Erman & Erman 1995; Smith et al. 2003), 20 m (Glazier 1991, Myers & Resh 2002), 40 m (Barquín & Death 2004), 50 m (Lencioni et al. 2011), and even 100 m (Hoffsten & Malmqvist 2000) downstream of the source. Erman and Erman (1995) defined the distance from source to spring brook by temperature increase of 2°C. This definition, however, is based upon physical factors only and does not take into account changes in the ecological community, which may occur over the distance from source to spring brook.

A more recent study therefore aimed to clarify the boundaries between spring source and spring brook based on the spring fauna (von Fumetti et al. 2007). They found changes in the macroinvertebrate assemblages over a range of 5 m from the source and thus proposed this distance could indicate a separation between source and spring brook. Eventually, the border between spring brook and source was defined as the point where temperature differs by 1°C from the temperature at the source (von Fumetti et al. 2007). However, criteria for regional spring zonations in Central Europe do not apply for riparian springs with short spring brooks bordering high order streams (Pešić et al. 2016), and might also be unsuitable for Icelandic springs and spring brooks, especially at high altitudes. Furthermore, defining the border between spring and spring brook based on changes in water temperature might not be applicable for warm streams deriving from geothermal springs, which are common throughout Iceland, or for cold springs in cold climates.

A further complication is that distance to the source cannot be measured linearly for limnocrene springs (which discharge into standing waters, such as ponds and lakes), unlike rheocrene springs (which discharge into spring brooks). Throughout this thesis, the word "source" is used to refer to the discrete point of discharge of groundwater, i.e. to the subsurface part of a spring, whereas "spring" is used to describe the area of a water body containing one or more sources (**Fig. 1**). Due to the difficulties mentioned before, no clear borders were defined for the spring area which differed from spring to spring depending on the local circumstances. It was not the purpose of this thesis to establish a concept of boundaries between eucrenal and non-crenal waters which could be universally applied for all Icelandic springs. Instead, I wanted to focus on two habitats within the spring area, namely the groundwater-influenced source and the surface-influenced area adjacent to it. Thus, two different microhabitats were sampled from each spring: One directly at the source, referred to throughout the thesis as "source" sample. The other sample was collected at the benthic substrate at least 2 m away from the source and referred to as "surface" sample to indicate the more surface water characteristics of the location as opposed to the groundwater nature of the source.

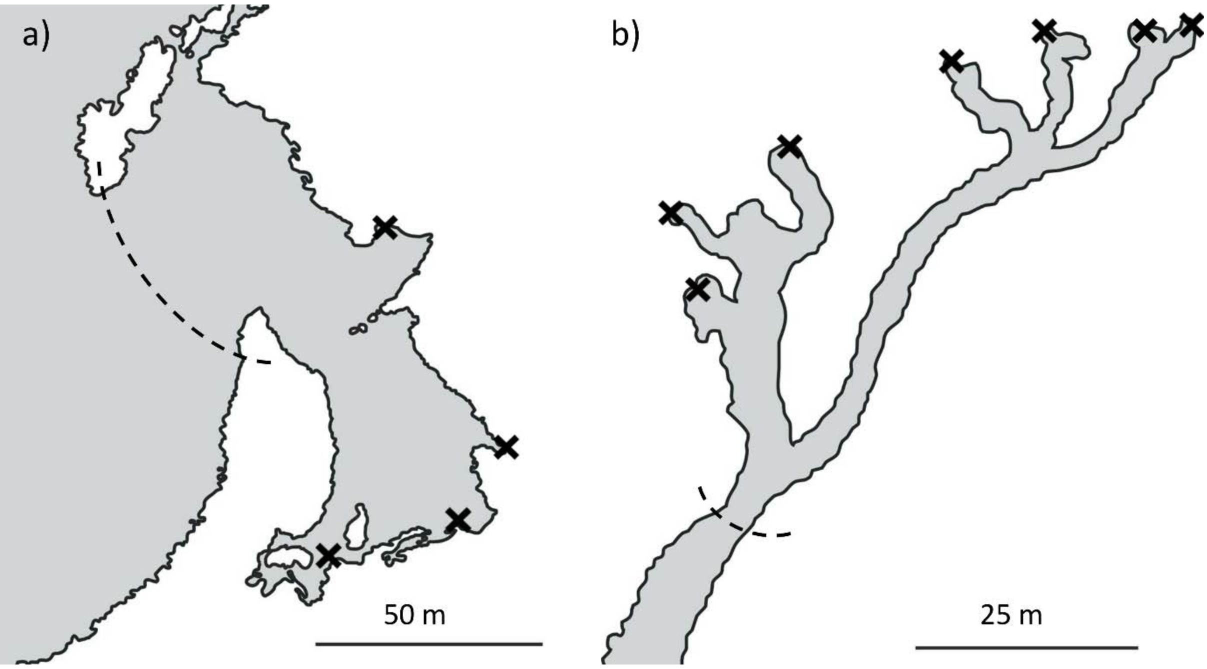

Figure 1. Example of (a) a limnocrene (Kálfaströnd, nr. 24 in Fig. 5) and (b) a rheocrene spring (Skarðslækur, nr. 41 in Fig. 5), illustrating the terms source and spring. Sources (i.e, upwelling groundwater) are indicated with black crosses. The area considered as spring is arbitrarily demarcated by the dashed line. Graphics: L.J. Hansen and A.K. Kreiling

Traditionally, springs have been classified into three different types, which are limnocrene, rheocrene, and helocrene springs (Hynes 1970). Limnocrene and rheocrene springs have a discrete point of discharge (i.e., source), but in the former the out-welling groundwater forms a pool (lentic water) whereas in the latter a stream (lotic water) is formed. In helocrene springs, groundwater is diffusely discharged to the surface and results in the formation of wetlands and marshes. More recently, 12 different types of discharge of springs have been described by Springer and Stevens (2008), but as limnocrene, rheocrene, and helocrene springs are included in their list as categories of their own, the two classifications do not exclude each other. In the thesis, I use the terms limnocrene and rheocrene according to Hynes (1970) in order to describe water outflow characteristics of springs.

It is not only the term "spring" that holds potential for discussion, but one also has to consider how to define groundwater appropriately, i.e., the depth from the source at which the influence of surface water declines. Triska et al. (1989) proposed that the division between groundwater and hyporheic zone in stream systems would be within a reach where less than 10% of the subsurface water originates from the surface water body. However, this might vary in different terrains, such as the porous lava fields of Iceland, where rainwater may easily get mixed with the groundwater, contributing nutrients that are washed out from the lava rock. Practically, the degree of mixing between surface and subsurface water is almost impossible to estimate in the field. In this thesis, all subsurface water is referred to as groundwater. Most springs show a clear water flow out of the point of discharge and I assume that this minimizes the mixing of groundwater and surface water at the source.

As springs are formed where groundwater reaches either surface atmosphere or surface water, the physical and chemical characteristics of spring water are very similar to those of the groundwater from which it derives. In most parts of the world, groundwater habitats are generally characterized by anoxic or hypoxic conditions, thermal stability, low nutrient availability, and permanent darkness (Gibert et al. 1994). Those factors are challenging and promote specialized adaptations of stygobionts, which are the organisms inhabiting groundwater (Gibert et al. 1994). Such characteristic morphological adaptations of groundwater organisms are, for example, lack of pigmentation, a more or less vermiform body shape with numerous long appendages, and a degeneration of the optical organs in favor of an enhanced development of chemical and mechanical receptors (Gibert et al. 1994). The relatively slow metabolic rates of groundwater organisms lead to a prolonged ontogenesis, increased longevity, and less frequent reproduction (Gibert et al. 1994) compared to similar taxa inhabiting surface freshwater. Invertebrates occurring in groundwater have been classified into three main functional groups which are stygoxenes, stygophiles, and stygobites (Gibert et al. 1994). Stygoxens have no affinity for groundwater but are occasionally found in it. Stygophiles are able to spend parts of their life cycles in subsurface water but lack special adaptations for this environment. Stygobites are obligate groundwater dwellers and characterized by the traits described above. They do not have to cope with changes in their local environment as groundwater is commonly constant in physical and chemical conditions and largely unaffected by seasonal changes. Groundwater influence can also moderate temperature variations in surface water bodies (Holmes 2000). Due to the complete lack of light, many groundwater food webs are almost entirely heterotrophic and dependent on nutrient input from the surface (Gibert et al.1994). However, some groundwater food webs are based on chemolithoautotrophic microorganisms as primary producers (Sarbu et al. 1996, Chen et al. 2009). In Icelandic groundwater ecosystems, chemolithoautotrophic bacteria groups have been identified as well, but their role in the food web is still unclear (Guðmundsdóttir et al. 2019). Groundwater has been described as one of the oldest living spaces on Earth with a patchy distribution of subsurface fauna on a local scale due to heterogeneity of physical and chemical conditions (Thulin & Hahn 2008). It also favors the occurrence of relict species, e.g., the groundwater amphipods *Crangonyx islandicus* and *Crymostigius thingvallensis* in Iceland (Kristjánsson & Svavarsson 2007). So far, little is known about the ecological roles that stygobionts play within their community, and about their position in the food web.

Due to their spatial isolation, springs can be seen as "habitat islands" referring to MacArthur and Wilson's (1963, 1967) theory of island biogeography. When MacArthur and Wilson published their Equilibrium Theory of Insular Zoogeography in 1963, they referred to actual oceanic islands. Since then, their theory has been applied to a variety of insular habitats, such as aquatic cave systems, mountain tops, forest fragments, and lakes (e.g., Culver 1970, Brown 1971, Lassen 1975, Zimmermann & Bierregaard 1986). Like islands, lakes are neatly bounded ecosystems which are relatively small and relatively impoverished (Quammen 1996). Accordingly, they can be considered "inverted islands" surrounded by an "ocean" of dry land (Lassen 1975), and the same holds true for springs (Fattorini et al. 2016).

As with islands, the processes of colonization and local extinction of species in springs is shaped by factors such as habitat size and distance to the "mainland", in this case a bigger freshwater body like a lake or a river (MacArthur & Wilson 1963 and 1967). In freshwater ecosystems, the community assembly is shaped by both stochastic and deterministic processes (Chase 2007, Milner et al. 2011). Stochastic processes include ecological drift and colonization by chance, and when they dominate over deterministic processes, the resulting communities are characterized as dispersal-assembled (Hubbell 2001). Deterministic processes, on the other hand, are based on species traits like tolerance and adaptation towards environmental factors, and lead to niche-assembled communities (Chase & Myers 2011). The relative importance of stochastic versus deterministic processes in community assembly might depend on the degree of isolation between springs and on the dispersal abilities of aquatic invertebrates (Bilton et al. 2001, Cid et al. 2020). Communities dominated by insects with a flying terrestrial adult stage might be more stochastically assembled in comparison to communities dominated by crustaceans, whose entire life cycle is confined to aquatic habitats (Bottazzi et al. 2011). Degree of isolation and species dispersal abilities also play a key role in determining the extent of local endemism. In mountainous areas, springs are isolated and often become habitats for rare and endemic species (Erman 1996; Humphreys 2004). Di Sabatino et al. (2003) found 18 endemic species of water mites in springs in Italy. Their disjunct distribution confirms the importance of spring habitats for maintaining diversity in freshwater systems (Di Sabatino et al. 2003). In the same way, spring and groundwater systems can act as refugia for ancient organisms otherwise extirpated from an area (Kristjánsson & Svavarsson 2007). Groundwater aquifers in Western Australia, for instance, contain numerous stygobite species showing endemism on a fine spatial scale (Humphreys 2008).

Springs are not only insular habitats, they also represent transitional zones of environmental gradients (ecotones) between surface water, groundwater, and the surrounding terrestrial habitat. Ecotones are generally characterized by a high biological diversity, since organisms from the adjacent habitats can be found there (Halse et al. 2002), as well as very specialized or relict species (Humphreys 2004). Springs connect the surface world with the otherwise inaccessible world underground, and both stygobiont and surface water organisms can be found there, as well as crenobionts, i.e., species that are specialized at living in spring habitats. In addition to the two adjacent aquatic habitats, a third terrestrial habitat exists in the immediate surroundings of most sources: the moist soil of the shore bank, overhanging riparian vegetation, and semi-submerged mosses. Several invertebrates are specialized towards such semi-terrestrial habitats and contribute to the spring fauna. Semi-terrestrial chironomid species commonly found in Icelandic springs include *Chaetocladius laminatus* Brundin 1947 and *Metriocnemus eurynotus* (Holmgren 1883), which dwell in the wet mosses surrounding springs and streams (Hrafnsdottir 2005). Similar to the invertebrates, the ciliate spring fauna is composed of taxa known from surface water, groundwater, and terrestrial habitats (Guðmundsdóttir et al. 2020).

The invertebrate composition of freshwater springs is shaped by a plethora of environmental and geographical factors such as temperature (Myers & Resh 2002, Glazier 2009, Lencioni et al. 2012), spring type (Cianficconi et al. 1998; Govoni et al. 2018), altitude (Myers & Resh 2002, Barquín & Death 2006, Lencioni et al. 2011, von Fumetti et al. 2017), pH (Bottazzi et al. 2011), flow permanence (Erman 2002, Smith et al. 2003), substrate characteristics (Ilmonen & Paasivirta 2005, von Fumetti et al. 2006), and presence and density of submerged vegetation (Ilmonen & Paasivirta 2005, Glazier 2009). Non-emergent macroinvertebrates (e.g., Crustacea, Gastropoda, and Planaria) might have a competitive advantage over aquatic insects with a terrestrial dispersal stage in springs with constant water temperature and flow, and thus often dominate spring fauna in temperate regions (Glazier 1991, Glazier 2009). However, the spring fauna in high-altitude regions and at northern latitudes is typically dominated by Chironomidae (Diptera), in terms of both abundance and species number (Hoffsten & Malmqvist 2000, Lencioni et al. 2012). Chironomidae are a super-diverse insect family, with an estimated 15,000 species world-wide, and containing taxa that differ in their ecology and habitat preferences (Armitage et al 1995). Because the aquatic larvae are affected by organic content and trace metal load in the sediment, Chironomidae are good bioindicators for water quality (Lencioni et al 2012).

Iceland's location above an upwelling mantle plume on the Mid-Atlantic ridge, where the Eurasian and North American continental plates diverge, has led to a number of tectonic and magmatic processes throughout the island, which, in combination with glaciation, has shaped its landscape (Sigmundsson & Sæmundsson 2008). Due to its volcanic activity and geologically young bedrock, its large glaciers, and high precipitation rates, Iceland has a high number of freshwater springs, ranging in water temperature from very cold (2°C) to boiling hot. They emerge most commonly within and along the edges of the extensive lava fields in the volcanically active zone that crosses the island from the southwest to the northeast. Most of the lava fields were formed following the end of the last glacial maximum some 15,000 years ago (Sæmundsson 1979, Einarsson 1994), and are comprised of barely weathered, highly porous lava rock. The porosity of the substratum allows precipitation to percolate quickly into the groundwater, which often has a long residence time (^{14}C ages are estimated from 3000 BP to modern; Sveinbjörnsdóttir et al. 2000). At the point where groundwater discharges in springs, it is hardly susceptible to fluctuations in outside air temperatures (Einarsson 1994). Consequently, many Icelandic spring systems are very constant in water flow and temperature throughout the year (Einarsson 1994). In contrast to many springs in other parts of the world, Icelandic springs are often extremely shallow (sometimes just a few centimeters deep), with small, narrow sources, and a benthic substrate that is comprised of bare lava rock. The majority of Iceland is not forested, and the vegetation surrounding springs is mostly restricted to shrubs and grasses in the lowlands, and mosses and dwarf shrubs in the highlands. Thus, allochthonous input into the springs can be assumed to be very low. The springs studied in this thesis are quite diverse in appearance (**Fig. 2**) and vary in hydraulic conditions, water temperature, benthic substrate, and type and density of surrounding vegetation.

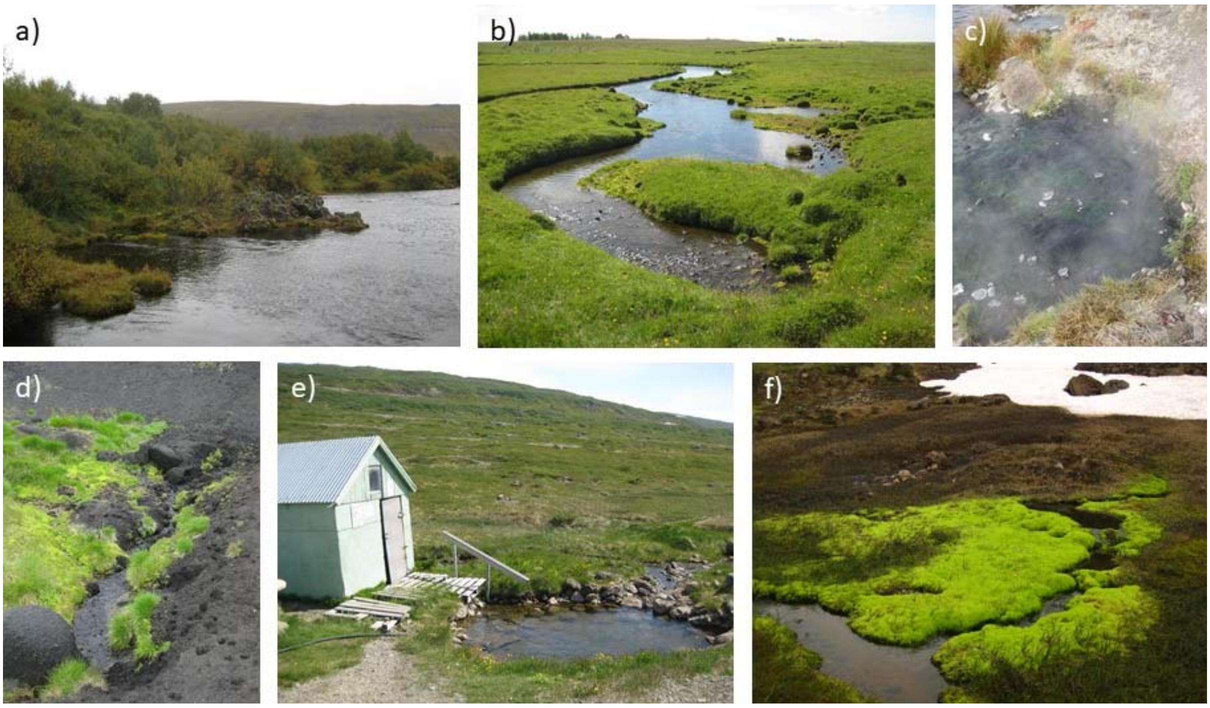

Figure 2. Variety of freshwater springs in Iceland. Examples show (a) the cold limnocrene spring Miðhúsaskógur (nr. 33 in Fig. 5), (b) the cold rheocrene spring Skarðslækur (nr. 41 in Fig. 5), (c) the hot rheocrene spring Steinsstaðir (nr. 44 in Fig. 5), (d) the highland spring Krákárbotnar (nr. 27 in Fig. 5), (e) the hot limnocrene spring Nauteyri (nr. 35 in Fig. 5), and (f) a spring on Dynjandisheiði (nr. 3 in Fig. 5). Photos: A.K. Kreiling

Icelandic freshwater biota is characterized by low species diversity, and the surface freshwater fauna is relatively young (<10,000 years) (Lindroth 1931, Buckland et al. 1986, Coope et al. 1986). Like on the other North Atlantic islands (Shetlands, Faroes, and Greenland), the terrestrial and freshwater fauna has invaded and colonized Iceland from northwest Europe, at the end of the last glaciation period (Buckland et al. 1986, Coope et al. 1986), and thus consists mainly of Palearctic species. In comparison to the continental fauna of Norway and Britain, aquatic insects are poorly represented in Iceland, as species numbers on the North Atlantic islands decline with distance from the European mainland (Gíslason 2005, Gíslason & Pálsson 2020).

A recent study revealed the diverse macroinvertebrate community that occurs in Icelandic cold spring habitats (Govoni et al. 2018), with at least 57 different taxa. The most abundant invertebrate taxa in Icelandic springs are aquatic Diptera larvae, particularly Chironomidae, and small Crustacea belonging to the taxa Cladocera, Ostracoda, and Copepoda (Govoni et al. 2018). In Iceland, Chironomidae are the dominant taxonomic group in freshwater habitats in general (Hrafnsdottir 2005) and particularly in springs (Govoni et al. 2018), and they have been well-studied in Iceland since the 1970s (Lindegaard 1979, Lindegaard 1992, Ólafsson et al. 2002). This is particularly true in Mývatn, which is a eutrophic lake in Northeast Iceland, fed by springs ranging in temperature from 5 to 23°C (Gardarsson & Einarsson 1994, Gardarsson et al. 2004). There are 39 chironomid species that have been reported as adults from the lake (Jonsson et al. 1986) and 30 as larvae (Lindegaard 1979) with *Tanytarsus gracilentus* being the most abundant (Lindegaard & Jónasson 1979, Jonsson et al. 1986, Gardarsson et al. 2004). Chironomidae are a very important food resource both for terrestrial arthropods (Dreyer et al. 2012) and for vertebrates such as fish and ducks (Gudbergsson 2004, Einarsson & Gardarsson 2004). Offspring production of breeding ducks may be positively correlated with chironomid abundance (Gardarsson & Einarsson 1994), and emerging midges also greatly contribute to the food supply of moulting diving ducks (Einarsson & Gardarsson 2004). Similarly, the population size of Arctic charr (*Salvelinus alpinus*) and brown trout (*Salmo trutta*) often follows fluctuations in the population density of midges (Einarsson et al. 2004). Arctic charr is commonly found in Iceland as different morphs, and the small benthic morph is often found in springs and spring-fed streams and ponds (Kristjánsson et al. 2012, Kristjánsson & Leblanc 2018; **Fig. 3**). The maximum body length of small benthic Arctic charr is 15 cm, which makes them especially well adapted to foraging in shallow waters with coarse lava rock substrate, such as narrow spring sources. Together with three-spine stickleback (*Gasterosteus aculeatus*) and brown trout, it represents the top aquatic predator in Icelandic spring brooks and spring-fed ponds.

Figure 3. Small benthic Arctic charr from the spring Skarðslækur, South-Iceland. Photo: A.K. Kreiling

One of the most important factors shaping invertebrate communities in spring systems is temperature (von Fumetti et al. 2007, Glazier 2009). Iceland is one of the most active geothermal regions on earth, together with Rotorua-Taupo on the North Island of New Zealand and Yellowstone in the United States (Þórarinsson 1978). Geothermal areas have been divided into low temperature areas (≤ 150°C at 1km depth) and high temperature areas (≥ 200°C at 1km depth) (Fridleifsson 1979). The high temperature areas are associated with the margin of the active volcanic zones and are characterized at the surface by solfataras and fumaroles, and sometimes by surface waters running from thermal springs (Einarsson 1994, Fridleifsson 1979, Torfason 2003). Since metazoan life is generally restricted to <50°C

(Mitchell 1974, Glazier 2009), high temperature geothermal areas are not suited for an invertebrate faunistic study. The 600 to 700 large- or medium-sized hot and warm water springs in Iceland's low temperature geothermal areas (Einarsson 1994), however, offer a great opportunity to study temperature effects on aquatic invertebrate fauna (O'Gorman et al. 2014). Tuxen (1944) classified Icelandic freshwater springs based on their water temperatures into cold (2°-4°C), tepid (5°-14°C), and hot springs (over 14°C). Another spring classification used by Þórðarson (1981) and based on Schwabe (1933) is more differentiated: Cold springs (<14°C, *kaldar lindir*), warm springs (14°-39°C, *volgrur*), „bathing springs" (30°-70°C, *laugar*), water geysers (70°-100°C, *vatnshverir*), and steam geysers (>100°C, *gufuhverir*). Both classifications consider 14°C as a key transition between categories, which is the average highest temperature of the warmest month of the year in Iceland (Þórðarson 1981). As springs included in this study are not warmer than 45°C, I used Tuxen's classification which is more detailed in this temperature range.

Globally, hot spring fauna differs from that of non-thermal freshwater systems. Hot springs usually only support a few species, and species richness typically decreases with increasing temperature (Pritchard 1991, Ólafsson et al. 2010). Plecoptera, Neuroptera, Odonata, Trichoptera, and Ephemeroptera, which are the dominant insect taxa in European and North American streams, are almost never found in thermal waters (Mitchell 1974). On the other hand, Hydrophilidae (Coleoptera), Chironomidae, Stratiomyidae, and Ephydridae (all Diptera) are common inhabitants of hot springs but less common in colder running waters, apart from Chironomidae (Brues 1927, Mitchell 1974). The Icelandic hot spring community has been described by Tuxen (1944) and is comprised of three "character animals of the absolutely hot springs" (Tuxen 1944): *Radix balthica* Linnaeus 1758 (Gastropoda, Pulmonata), *Scatella tenuicosta* f. *thermarum* Collin 1930 (Diptera, Ephydridae), and *Cricotopus sylvestris* (Fabricius 1794) (Diptera, Chironomidae, Orthocladiinae). Although *R. balthica* and *C. sylvestris* are not restricted to thermal waters and found in a variety of aquatic habitats, they contribute to a species assemblage which is unique as a whole. Due to their distinct communities, hot springs are unique habitats and thus deserve special protection.

Spring habitats in Iceland are getting increasingly threatened. Cold springs serve as a source for drinking water as well as water for industrial purpose. Water abstractions from these sources and transformation into wells irreversibly destroys natural springs and have led to disappearance of spring-fed surface water. Hot springs face even more threats. Geothermal heat has been utilized by humans in Iceland for centuries, mainly for bathing, washing, and heating up houses (**Fig. 4**). The first bore hole was drilled in 1928 at Þvottalaugar in Reykjavík (Einarsson 1994). Nowadays, geothermal heat contributes to heating, to provide hot tap water, and to generate electricity. Also, natural geothermal pools are a big tourist attraction, causing many sensitive hot spring areas to be threatened with severe disturbance due to trampling of the surrounding vegetation, excessive nutrient load, and litter pollution. Assessment of the diversity of invertebrate fauna of cold and hot springs will hopefully affect decisions in nature conservation issues in Iceland.

Figure 4. Postcard printed in Iceland between 1905 and 1910, showing the hot spring Laugar which was used for washing clothes.

1.2 Aims and Objectives of the Thesis

The overall aim of the research was to investigate invertebrate community structure of springs on a spatial (**Paper I, II, III**) and temporal (**Paper I & IV**) scale in relation to ecological factors. I specifically asked:

- Which environmental variables are the main drivers of invertebrate diversity and community composition in springs? (**Paper I, II & III**)
- Is there seasonal variation in the spring community independent of temperature fluctuations, and how does this affect higher trophic levels? (**Paper IV**)

The objective of the study presented in **Paper I** was to establish whether the spring classification based on water temperature proposed by Tuxen (1944) is ecologically relevant, i.e., whether springs in these temperature classes differed from each other in their invertebrate community composition, with a special focus on Chironomidae as the dominant taxonomic group in Icelandic springs. Another aspect of this paper was to compare the hot spring fauna and environmental variables of two springs sampled in 1937 with the fauna of those same springs sampled 80 years later, in 2016, in order to assess stability of springs on long-time temporal scale.

In **Paper II**, invertebrate diversity and community composition in springs were analysed on a spatial scale, encompassing 49 springs in different parts of the country (**Fig. 5**). I assessed the influence of geographical location and environmental variables, especially water temperature, altitude, and spring type, on invertebrate communities, and discussed the findings in the light of assembly theory.

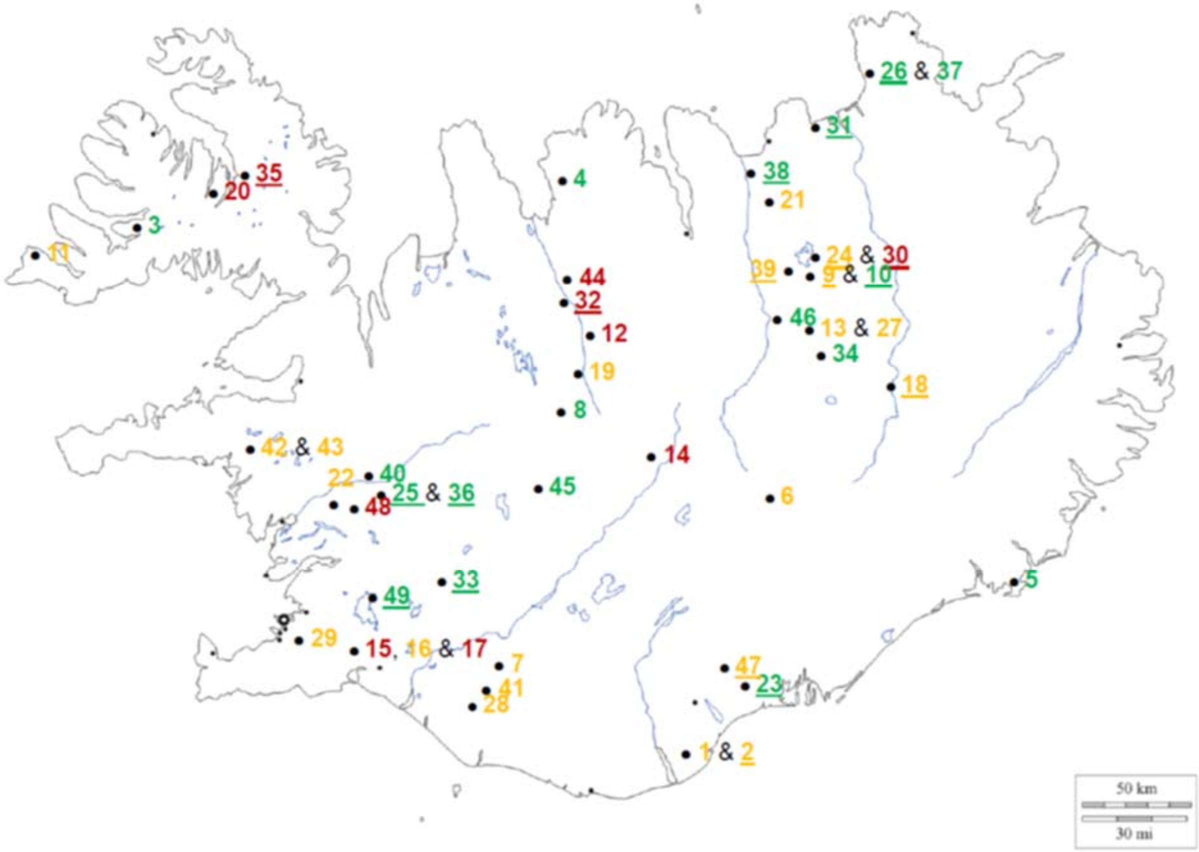

Figure 5. Map of the 49 springs sampled during the study. Springs are grouped by water temperature into cold (green), tepid (yellow), and hot (red), and by spring type into limnocrene (underlined) and rheocrene (not underlined). Springs are numbered as follows: 1 -Botnar I, 2 - Botnar II, 3 - Dynjandi, 4 - Enni, 5 - Friðsæld, 6 - Gæsavötn, 7 - Galtalækur, 8 - Goðdalafjall, 9 - Grænavatn Norður, 10 - Grænavatn Suður, 11 - Hænuvík, 12 - Hafgrímsstaðir, 13 - Hagalækur, 14 - Háöldur, 15 - Hengill IS6a, 16 - Hengill IS7, 17 - Hengill IS8, 18 - Herðubreiðarlindir, 19 - Hofsvellir, 20 - Hörgshlíð, 21 - Hraun, 22 - Hrauná, 23 - Hruni, 24 - Kálfaströnd, 25 - Kiðárbotnar, 26 - Klapparós, 27 - Krákárbotnar, 28 - Lækjarbotnar Hol, 29 - Lækjarbotnar Rvk, 30 - Langivogur, 31 - Lón, 32 - Mælifellslaug, 33 - Miðhúsaskógur, 34 - Mótunga, 35 - Nauteyri, 36 - Oddar, 37 - Presthólar, 38 - Sandur, 39 - Sikið, 40 - Sílatjörn, 41 - Skarðslækur, 42 - Staðarhraun Bær, 43 - Staðarhraun Kirkja, 44 - Steinsstaðir, 45 - Svartárbotnar, 46 - Svartárkot, 47 - Þverá, 48 - Úlfsstaðir, 49 – Vatnsvik

In **Paper III**, I took a closer look at one aquatic insect order, Trichoptera, and studied their distribution across springs in Iceland in relation to spring type, substrate characteristics, and temperature.

After studying a variety of springs on a spatial scale, I wanted to follow the community of one spring on a temporal scale, and the aim of **Paper IV** was thus to analyse seasonal variation in community composition of a spring independent of temperature fluctuations.

Secondly, the paper aimed to assess the influence of this seasonal variation in the invertebrate community on an apex predator in spring habitats, small benthic Arctic charr (*Salvelinus alpinus*). Through a mark-recapture study, I analysed growth rates, feeding selectivity, and individual diet specialization of fish and linked these to invertebrate prey abundance.

1.3 Methodological Remarks

Unlike most springs in the world, the majority of springs in Iceland are of volcanic origin and situated on lava rock as substrate. The source openings are thus generally very small in diameter, and the rough lava substrate makes them difficult to access and almost impossible to sample with traditional sampling methods for springs, like trapping (Husmann 1956, Schwoerbel 1986, Hahn 2003) or pumping (Bou & Rouch 1967, Fiasca et al. 2014). To overcome this challenge, a sampling method was developed called "electrobugging", based on modified electric fishing gear and designed to collect aquatic invertebrates from narrow spring sources (**Paper II**). Most metazoan organisms – with the striking exception of hattifatteners (Jansson 1962) – react to electrical shocks by electronarcosis (Bohlin et al. 1989), during which they temporarily lose "consciousness" and the ability for active locomotion, or they experience partial paralysis and swim towards the electric current (Kristjánsson pers. observation). In freshwater environments, this fact is utilized to easily collect animals in dip or drift nets, and electrofishing gear has increasingly become the method of choice for collecting macroinvertebrates in streams (Taylor et al. 2001, Kruzic et al. 2005, Lento & Morin 2014).

Some minor caveats should be pointed out with regard to electrobugging in springs. Electroshocking affects invertebrate taxa differently (Kruzic et al. 2005, Lento & Morin 2014), and mobile taxa seem to be represented in higher proportions in electrobugging samples than more immobile taxa (Lento & Morin 2014). A possible bias towards some taxonomic groups in an electrobugging sample cannot be excluded, and a direct comparison with an invertebrate sample obtained with a different sampling method (e.g., Surber sampler) is thus not possible. I am aware of these restrictions of electrobugging, but being constrained by the narrow source openings of Icelandic springs which are hardly possible to sample with other methods, electrobugging was the best available option for my study.

One part of the project which ultimately did not contribute to the thesis, consisted in collection and analysis of environmental DNA (eDNA) from freshwater springs. eDNA is a mixture of degraded DNA fragments from different organisms that can be detected in a water or soil sample and which may represent the species currently occupying the respective habitat (Ficetola et al., 2008; Thomsen et al., 2012). eDNA has been established as a powerful tool in advanced monitoring of freshwaters for a range of habitats and taxonomic groups (Bohmann et al., 2014). It has the potential to be a non-destructive sampling method for applied biodiversity assessment and monitoring, and might be especially useful for sites which are vulnerable or not easy to access, such as Icelandic freshwater springs. Data obtained from eDNA collected during this project proved to be successful for the assessment of taxonomy and community composition of some taxonomic groups, e.g., ciliates (Guðmundsdóttir et al. 2020), bacteria (Guðmundsdóttir et al. 2019), fungi (Wurzbacher et al. 2020), and diatoms (unpublished), but was not found adequate for invertebrates. One explanation is that intracellular, - high quality -, DNA from aquatic microorganisms contributes to the eDNA in a water sample, whereas DNA from macroorganisms consists of extracellular, and thus likely to be heavily degraded, DNA only (Taberlet et al. 2018). Before we can rely on eDNA-derived data for invertebrates in Icelandic springs, sequencing protocols (e.g., the use of specific primers targeting shorter DNA fragments) and reference sequence databases need to be improved.

1.4 Main Results and Discussion

During this study, a total of 111 invertebrate taxa were collected from Icelandic springs (**Table 1**). However, not all of those taxa were identified to species level, and the number of the actual species pool in Icelandic springs is thus probably much higher. The dominating invertebrate groups were Chironomidae (67%), Ostracoda (12%), and Copepoda (9%). Chironomidae also dominated in terms of species number, with 28 identified species or species groups belonging to five families: Podonominae (1 species), Tanypodinae (3 species), Diamesinae (at least 4 species), Orthocladiinae (18 species), and Chironominae (2 species). The most common and abundant taxa were *Diamesa* spp. (40% of all Chironomidae), *Eukiefferiella minor* (20%), *Orthocladius frigidus* (13%), *Micropsectra* sp. (7%), *Thienemanniella* sp. (4%), and *Chaetocladius* spp. (4%).

The number of invertebrate taxa varied greatly between springs, ranging from 6 in Lón (site nr. 31 in **Fig. 5**) to 40 in Staðarhraun Kirkja (site nr. 43 in **Fig. 5**) (without taking into account the Ostracoda and Clitellata taxa in these numbers). The highly diverse invertebrate communities of even closely located springs (i.e., high beta diversity) has been emphasised by several authors (e.g., Erman & Erman 1995, Myers & Resh 2002, Buczynski et al. 2003, Lencioni et al. 2011), and could indicate a stochastic community assembly of springs (**Paper II**). However, some functional traits of the spring community are clearly of a deterministic nature, and water temperature and spring type were the key factors influencing the invertebrate community composition (**Paper II**). The benthic community in the surface water of limnocrene springs was characterised by *Arctopelopia* sp. (Chironomidae, Tanypodinae), *Cricotopus tibialis* (Chironomidae, Orthocladiinae), and Cladocera as indicator taxa. The community of rheocrene springs was characterized by *Orthocladius frigidus*, *Thienemanniella* sp. (most likely *T. clavicornis*), and *Chaetocladius* spp. (all Chironomidae, Orthocladiinae), as well as Diptera larvae not identified to species level. Importantly, the community did not differ between limnocrene and rheocrene springs at the source, where the hydrological conditions are similar for both spring types.

Water temperature determined the community composition at both sampling locations within a spring. The validity of the classification by Tuxen (1944), based on the temperature of Icelandic springs, was tested for the Chironomidae community. This revealed distinct assemblages for cold, tepid, and hot springs and thus validated the ecological relevance of the classification (**Paper I**). While the composition of the invertebrate community of cold and tepid springs are less distinct, hot springs are characterized by a core group of invertebrate taxa (**Paper I**, **Paper II**). This core group of Icelandic hot springs consists of *Cricotopus sylvestris* (Chironomidae, Orthocladiinae), *Scatella tenuicosta* (Ephydridae), *Radix balthica* (Pulmonata), and Oribatida (Acari) (Tuxen, 1944 and **Paper II**).

Due to statistical intercorrelation, some environmental variables like substrate type and vegetation density had to be excluded from the main analysis. However, these are variables which have been shown to greatly influence invertebrate diversity and assemblage in springs (e.g., Ilmonen & Paasivirta 2005, von Fumetti et al. 2006). I chose to select one aquatic invertebrate group, Trichoptera, to determine whether its presence and abundance in springs might be associated with substrate type and vegetation density. More Trichoptera larvae were found in springs with sandy substrate and high allochthonus plant input than in springs with lava rock substrate and low vegetation density (**Paper III**). Water temperature was also correlated with Trichoptera abundance, and seemed to be a limiting factor for the distribution of this invertebrate group, restricting the occurrence of Trichoptera larvae to springs with a maximum water temperature of 7.2 °C.

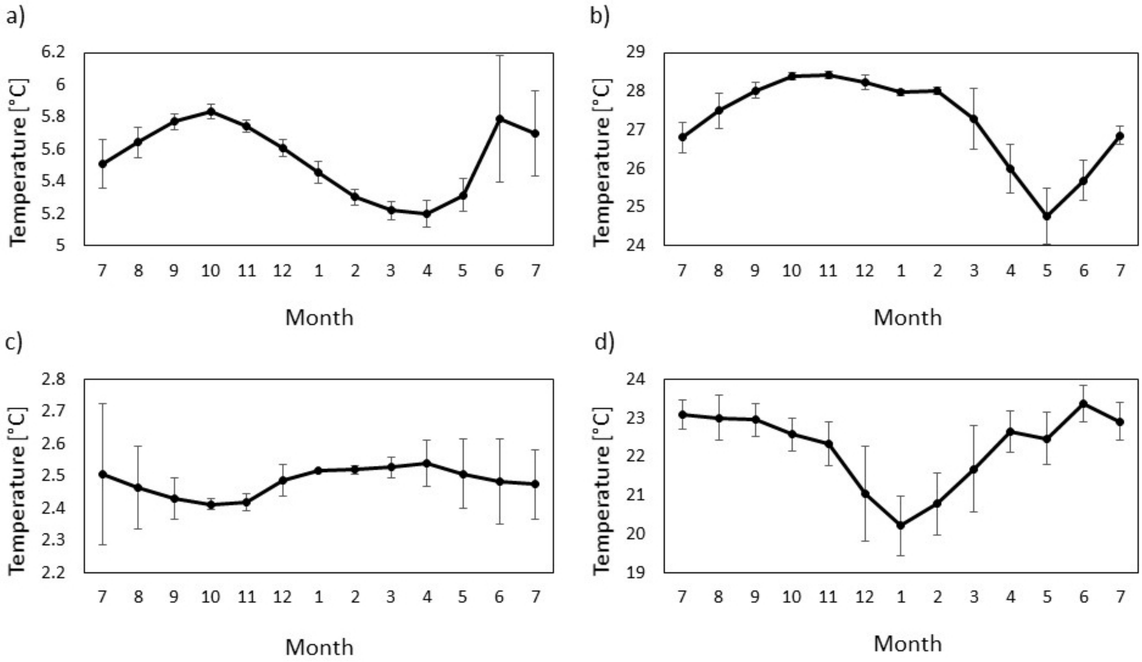

Figure 6. Thermal regime of four springs in Iceland: (a) Skarðslækur (cold rheocrene spring; nr. 41 in Fig. 5), (b) Haöldur (hot rheocrene spring; nr. 14 in Fig. 5), (c) Miðhúsaskógur (cold limnocrene spring; nr. 33 in Fig. 5), and (d) Mælifellslaug (hot limnocrene spring; nr. 32 in Fig. 5). Points show the average monthly temperature and error bars the standard deviation. Temperature was measured at the source in 2 h intervals between July 2016 and July 2017 (a,b,c) and in 6 h intervals between July 2015 and July 2016 (d).

My study confirmed the general stability of springs over time in terms of physical and chemical factors, especially temperature (**Fig. 6**). The extreme thermal stability of Icelandic springs enabled me to study the seasonal variation in the benthic invertebrate community independent of temperature fluctuations (**Paper IV**). I collected benthic invertebrates from one rheocrene spring with multiple sources, Skarðslækur in South-Iceland, over the course of a year. Despite a very constant water temperature of 5.5(± 0.26)°C, the benthic invertebrate community was divided into a winter/spring and a summer/autumn community. These changes in community composition (**Fig. 7**) were mainly driven by the high numbers of Chironomidae larvae from early summer to late autumn, whereas taxa without terrestrial dispersal stage like Ostracoda, Acarina, and Copepoda were relatively more abundant during the winter.

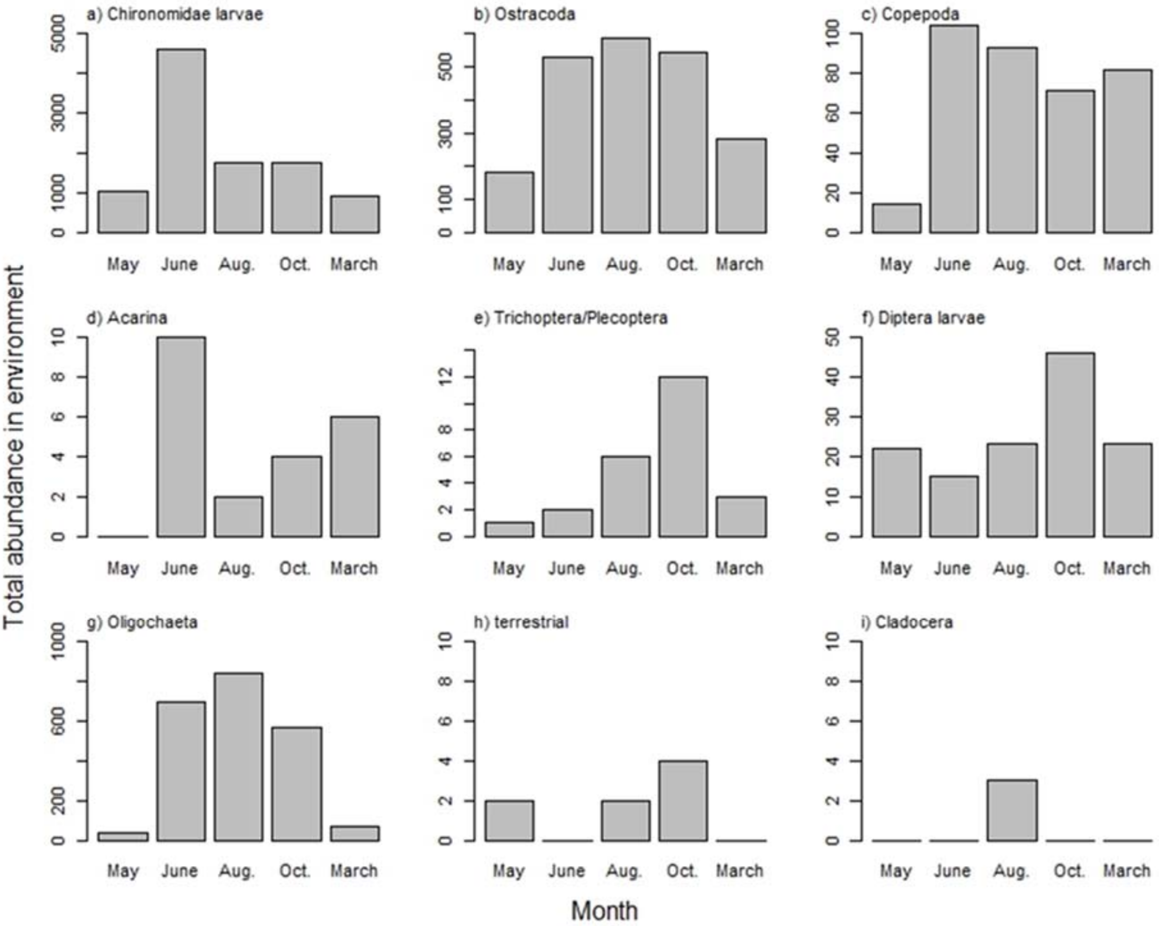

Figure 7. Changes in the community composition of the spring Skarðslækur. Total abundance of invertebrate groups (grey bars) in benthic samples is shown for five sampling events (May, June, August, October, and March) during a year. Invertebrates were grouped into nine groups: Chironomidae larvae (a), Ostracoda (b), Copepoda (c), Acarina (d), Trichoptera and Plecoptera larvae (e), Diptera larvae (f), Oligochaeta (g), terrestrial (h), and Cladocera (i). Note that the y-axis scales differ greatly between groups.

Tuxen (1944) suggested that in hot springs, one generation of insects could follow the other independent of season, but with a slow-down during the dark winter months. It would be interesting to study the seasonal variation in the benthic invertebrate community in hot springs to find out whether their invertebrate communities undergo a similar seasonal pattern as cold springs, since both spring types have limited primary production due to a shortage of daylight during the Icelandic winter.

The seasonal variation in the benthic community was reflected in the diet of the apex predator in the spring, small benthic Arctic charr (**Paper IV**). In summer, when invertebrate abundance in the benthic environment was high, Arctic charr fed mainly on Chironomidae larvae, Copepoda, and Oligochaeta, whereas during times of lower invertebrate abundance in winter, they consumed relatively more Ostracoda, Trichoptera larvae, and Amphipoda. Calculating the average individual diet specialization of fish per month as a measure of intrapopulation niche variation showed that individuals in the population were more specialised in winter than in summer. Body size of fish seemed to determine the preferred prey group, with smaller fish specialising more on Ostracoda, Acarina, Trichoptera larvae, and Plecoptera nymphs, and bigger fish specialising more on Oligochaeta and terrestrial prey in the drift. One interesting observation was the increased occurrence of groundwater amphipods in the Arctic charr diet during winter. This leads to speculations about whether spring-dwelling small benthic Arctic charr might move deeper into the groundwater/surface water ecotone of the spring sources during winter. Possible drivers for such a shift in habitat could be either to switch to a more benthic/groundwater-dependent diet due to a lowered abundance of aquatic insects and drifting prey in the stream, or to seek shelter from harsher weather conditions affecting the surface water of the stream, such as snow and strong winds. In summary, **Paper IV** shows that composition and temporal dynamics of the aquatic invertebrate community in springs can have implications on behaviour and phenotype of higher trophic levels, which should be kept in mind when studying secondary consumers such as freshwater fishes.

In addition to the main findings of the thesis, there were a number of important faunistic observations. Motaş (1961) claimed that no true water mites (Hydrachnidia) have been found in the Central Highlands of Iceland, not even in hot springs. In our study, however, Hydrachnidia were found in four cold springs in the Central Highlands: Gæsavötn, Goðdalafjall, Hagalækur, and Herðubreiðarlindir, as well as in two high-altitude springs outside of the Central Highlands: Dynjandi and Hengill IS7 (sites nr. 6, 8, 13, 18, 3, and 16, respectively, in **Fig. 5**). Probably all Hydrachnidia found in this study belong to the family Sperchontidae. Four species of the genus *Sperchon* have been recorded for Iceland, and are seemingly the most common water mites in Icelandic waters (Motaş, 1961). In springs, the mite order Oribatida can be represented by aquatic species, hygrophilous species residing in the transitional zone between land and water, and purely terrestrial species (Gerecke & Di Sabatino, 2007). Oribatida were frequently present in both source and surface samples in this study. Three different morphotypes of Oribatida could be distinguished but were not identified to family or lower taxonomic level (**Table 1**).

Similarily, Tardigrada were believed to be absent from spring habitats in Iceland (Tuxen, 1944), but were found in 20 springs during the study, mainly but not exclusively in the surface samples. The numbers of individuals in samples was sometimes very high, with as many as 6,577 individuals in one sample. The current Tardigrada species checklist for Iceland is based on a survey from the 1970s, in which no springs were sampled (Morgan 1980). Tardigrada found in this study are currently being identified with the aim to update the Icelandic species checklist.

Larvae of the chironomid *Diamesa tonsa/cinerella* species group (Rossaro & Lencioni 2015) were found in several springs as a new record for Iceland, although it was mentioned by Motaş (1961) that an adult of the *Diamesa cinerella*-group was collected by sweep-netting from Fljótshlíð, South Iceland, in July 1936 (identified by L. Brundin). Membership to the *Diamesa tonsa/cinerella* group has been confirmed by V. Lencioni, but identification to species was not possible based on larval characters (**Fig. 8**).

This study furthermore shed light on taxonomy and ecology of some invertebrate taxa found in Icelandic springs, resulting in updated species checklists for these taxa, namely Ostracoda (Alkalaj et al. 2019), and Clitellata (Klinth et al. 2019). Data collected during this study also contributed to studies on community composition of aquatic fungi (Wurzbacher et al. 2020), bacteria (Guðmundsdóttir et al. 2019), and ciliates (Guðmundsdóttir et al. 2020) in springs.

Figure 8. Headcapsule of the larva of Diamesa tonsa/cinerella species group. Photo: A.K. Kreiling

1.5 Conclusions and Implications

This thesis contributes to our understanding of freshwater ecology and might give some new impulses to research on springs. The benthic invertebrate community in the surface water of a spring differs from that in the groundwater-surface water ecotone of a spring source, as does the extent to which environmental variables influence those communities (**Paper II**). This finding challenges the misleading terminology used in many studies on springs, which often lack a clear distinction between spring source and surface water of the spring. What we urgently need in research on springs are clear definitions and a consistent terminology.

Furthermore, the thesis poses the idea of using springs as models to test the effects of deterministic and stochastic processes on community assembly. This was attempted in **Paper II** on a rather theoretical basis, but should be verified experimentally and including datasets from other parts of the world.

The importance of following seasonal variation in invertebrate communities for a more complete understanding of diversity was demonstrated in **Paper IV**. Ideally, a routine sampling during winter could be implemented in standard protocols of monitoring programmes. Another important aspect is the effect of seasonal variation in the invertebrate community on higher trophic levels, e.g., fish. Studies on behaviour or phenotype of secondary consumers should keep in mind possible dynamics in composition and abundance of prey organisms when interpreting their data.

Last but not least, results of this study will have implications for nature conservation in Iceland and elsewhere. Springs are sensitive ecosystems and altering their natural state causes irreversible damage. At the same time, springs are island-like ecosystems with the potential to contribute greatly to the overall diversity of an area (gamma diversity). They provide refugia for juvenile stages of invertebrates, which act as key taxa in many food webs, e.g., Chironomidae. Springs often house endemic and relict species, and although this is – with the exception of two species of groundwater amphipods – not the case in Icelandic springs, there are distinct invertebrate assemblages typical for springs. These assemblages are even more clear for hot than for cold springs, and the core invertebrate group of Icelandic hot springs consists of three to four characteristic taxa. Nonetheless, most springs in Iceland are not protected unless they are situated in National Parks. Although the significance of spring habitats has been noted and a few springs have been put on a conservation list (Náttúruminjaskrá 2020), practical actions to protect springs have been lacking so far. Due to the vulnerability of springs, their role in maintaining diversity and ecosystem functions, and the high anthropogenic pressure they are facing, protection of natural hot and cold springs should be one of the priorities in nature conservation in Iceland in the following years.

Table 1 Invertebrates found in freshwater springs in Iceland during this study. Taxa which were included in the data analysis in Paper II are marked with an asterisk (*). The taxa acronyms ("Acrn.") were used in some figures in Paper II and IV. Taxa were mostly identified based on morphological characteristics, except for most clitellata, which were barcoded (COI or 16S).

Taxon	**Acrn.**	**ID Method**	**Reference**
GASTROPODA*	GAS		Paper II
Radix balthica (Linnaeus, 1758)			
CLITELLATA*	OLI		Paper II
Enchytraeidae			
Cernosvitoviella aggtelekiensis Dózsa-Farkas, 1970		16S	Klinth et al. 2019
Cernosvitoviella cf. *minor* Dózsa-Farkas, 1990		16S	Klinth et al. 2019
Cernosvitoviella pusilla Nurminen, 1973		16S; COI	Klinth et al. 2019
Cognettia glandulosa (Michaelsen, 1888)		morpho	Klinth et al. 2019
Cognettia varisetosa (Martinsson, Rota & Erséus, 2015)		morpho	Klinth et al. 2019
Enchytraeus buchholzi Vejdovský, 1879		16S	Klinth et al. 2019
Fridericia dura (Eisen, 1879)		16S	Klinth et al. 2019
Henlea perpusilla Friend, 1911		16S	Klinth et al. 2019
Lumbricillus arenarius (Michaelsen, 1889)		16S	Klinth et al. 2019
Marionina cf. *argentea* (Michaelsen, 1889)		16S	Klinth et al. 2019
Marionina sp.		16S	Klinth et al. 2019
Mesenchytraeus cf. *armatus* (Levinsen, 1884)		16S	Klinth et al. 2019
Lumbricidae			
Aporrectodea caliginosa (Savigny, 1826)		16S	Klinth et al. 2019
Bimastos rubidus s. lat. (Savigny, 1826)		COI	Klinth et al. 2019
Dendrobaena octaedra (Savigny, 1826)		16S	Klinth et al. 2019
Eiseniella tetraedra (Savigny, 1826)		16S	Klinth et al. 2019
Naididae			
Chaetogaster cf. *diastrophus* (Gruithuisen, 1828)		COI; 16S	Klinth et al. 2019
Chaetogaster sp. = langi?		16S	Klinth et al. 2019
Nais communis/variabilis species complex, morphotype A3 (Envall et al. 2012)		16S	Klinth et al. 2019
Nais elinguis Müller, 1773		16S	Klinth et al. 2019
Pristina foreli (Piguet, 1907)		morpho	Klinth et al. 2019
Tubifex cf. *tubifex* (Müller, 1774)		16S	Klinth et al. 2019
Uncinais uncinata (Ørsted, 1842)		morpho	Klinth et al. 2019
TARDIGRADA*	TAR		Paper II
ACARI	ACA		Paper II
Halacaridae*	Hal	morpho	Paper II
Hydrachnidia*	Hyd	morpho	Paper II
Oribatida a,b*	Ora	morpho	Paper II
Oribatida c*	Orc	morpho	Paper II
CLADOCERA*	CLA	morpho	Paper II

COPEPODA*	COP	morpho	Paper II
OSTRACODA*	OST	morpho	
Bradleystrandesia affinis (Fischer, 1851)		morpho	Paper II; Alkalaj et al. 2019
Candona candida (Müller, 1776)		morpho	Paper II; Alkalaj et al. 2019
Cryptocandona reducta (Alm, 1914)		morpho	Paper II; Alkalaj et al. 2019
Cytherissa lacustris (Sars, 1863)		morpho	Paper II; Alkalaj et al. 2019
Fabaeformiscandona sp.		morpho	Alkalaj et al. 2019
Heterocypris incongruens (Ramdohr, 1808)		morpho	Alkalaj et al. 2019
Limnocythere inopinata (Baird, 1843)		morpho	Paper II; Alkalaj et al. 2019
Potamocypris fulva (Brady, 1868)		morpho	Paper II; Alkalaj et al. 2019
Potamocypris pallida Alm, 1914		morpho	Paper II; Alkalaj et al. 2019
Potamocypris villosa (Jurine, 1820)		morpho	Paper II; Alkalaj et al. 2019
AMPHIPODA*	AMP		
Crangonyx islandicus Svavarsson & Kristjánsson, 2006		morpho	Paper II
Crymostigius thingvallensis Kristjánsson & Svavarsson, 2004		morpho	Paper IV
COLLEMBOLA*	COB	morpho	Paper II
PLECOPTERA*	PLE	morpho	Paper II
Capnia vidua Klapálek, 1904			
COLEOPTERA*	COL	morpho	Paper II
Agabus bipustulatus (Linnaeus, 1767)			
TRICHOPTERA	TRI	morpho	
Apatania zonella (Zetterstedt, 1840)*	Azo	morpho	Paper II & III
Limnephilus affinis Curtis, 1834*	Laf	morpho	Paper II & III
Limnephilus griseus (Linnaeus, 1758)*	Lgr	morpho	Paper II & III
Limnephilus sp.	Lph	morpho	Paper II & III
CHIRONOMIDAE			
Podonominae			
Parochlus kiefferi (Garrett, 1925)*	Pki	morpho	Paper II
Tanypodinae			
Arctopelopia sp. (*A. griseipennis* (van der Wulp, 1858))*	Arc	morpho	Paper II
Macropelopia sp.*	Mac	morpho	Paper II
Procladius sp. (*P. islandicus* (Goetghebuer, 1931))*	Pro	morpho	Paper II
Diamesinae			
Diamesa spp.*	Dia	morpho	Paper II
Diamesa bohemani/zernyi gr.			
Diamesa bertrami/latitarsis gr			
Diamesa tonsa/cinerella gr.			
Pseudodiamesa sp.*	Pse	morpho	Paper II
Orthocladiinae			
Chaetocladius spp.*	Cha	morpho	Paper II
Coryoneura fittkaui Schlee, 1968*	Cof	morpho	Paper II
Cricotopus sylvestris (Fabricius, 1794)*	Crs	morpho	Paper II
Cricotopus tibialis (Meigen, 1804)*	Crt	morpho	Paper II
Cricotopus sp.*	Cri	morpho	Paper II
Eukiefferiella claripennis (Lundbeck, 1898)*	Euc	morpho	Paper II

Eukiefferiella minor (Edwards, 1929)*	Eum	morpho	Paper II
Heterotrissocladius sp. (*H. grimshawi* (Edwards, 1929))*	Het	morpho	Paper II
Limnophyes sp.*	Lim	morpho	Paper II
Metriocnemus eurynotus (Holmgren, 1883)*	Meu	morpho	Paper II
Metriocnemus fuscipes (Meigen, 1818)*	Mfu	morpho	Paper II
Orthocladius frigidus (Zetterstedt, 1838)*	Ofr	morpho	Paper II
Orthocladius oblidens (Walker, 1856)*	Oob	morpho	Paper II
Orthocladius sp.*	Ort	morpho	Paper II
Paralimnophyes sp.*	Par	morpho	Paper II
Rheocricotopus effusus (Walker, 1856)*	Ref	morpho	Paper II
Smittia sp.*	Smi	morpho	Paper II
Thienemanniella sp. (*T. clavicornis* (Kieffer, 1911))*	Thi	morpho	Paper II
Chironominae		morpho	
Chironomus sp.*	Chi	morpho	Paper II
Micropsectra sp.*	Mic	morpho	Paper II
SIMULIIDAE*	SIM	morpho	Paper II
EPHYDRIDAE*	EPH	morpho	Paper II
Scatella tenuicosta Collin, 1930			
DIPTERA other*	DIP	morpho	Paper II
NEMATODA/NEMATOMORPHA*	NEM	morpho	Paper II

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2 Paper I

Chironomidae fauna of springs in Iceland: Assessing the ecological relevance behind Tuxen's spring classification

Kreiling, A.-K., Ólafsson, J.S., Pálsson, S. & Kristjánsson, B.K. (2018).

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Authors contribution: AKK, BKK, JSÓ, and SP conceived the study. AKK and BKK conducted the fieldwork. AKK and JSÓ identified invertebrates. AKK and SP analysed the data. AKK, JSÓ, SP, and BKK wrote the paper.

J. Limnol., 2018; 77(s1): 145-154
DOI: 10.4081/jlimnol.2018.1754

Chironomidae fauna of springs in Iceland: Assessing the ecological relevance behind Tuxen's spring classification

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ABSTRACT

In 1937, S.L. Tuxen studied the animal community of hot springs in Iceland, and classified springs according to their relative temperature into cold, tepid, and hot. Eighty years after Tuxen's study, we revisited some of the hot springs in Skagafjörður, Northern Iceland. Our aim was to compare the invertebrate community of 1937 and today, and to assess the stability of hot spring habitats over the years. To test Tuxen's spring classification on an ecological basis, we furthermore collected chironomid larvae from 24 springs of a broad range of temperature, with samples taken both at the surface area of the spring and at the groundwater level. The chironomid species composition of hot springs differed from that of cold and tepid springs. Whereas *Cricotopus sylvestris*, *Arctopelopia* sp., and *Procladius* sp. characterised the chironomid community in Icelandic hot springs, cold and tepid springs were dominated by *Eukiefferiella minor*, *Orthocladius frigidus* and *Diamesa* spp. Community composition analyses and the exclusive occurrence of taxa in one of the temperature classes validated the ecological relevance of Tuxen's spring classification for the chironomid species community. Both environmental parameters and invertebrate community of Icelandic hot springs seem to be the same as 80 years ago. Although springs have the potential to provide stable habitats, they are currently under high anthropogenic pressure, and should be increasingly considered in nature conservation.

Key words: Chironomid larvae; hot springs; invertebrate diversity; groundwater; geothermal areas; water temperature.

Received: January 2018. **Accepted:** April 2018.

This paper was presented at the *20th International Symposium on Chironomidae*, Trento, Italy, 2-8 July 2017.
Session: Ecology and Biomonitoring.

INTRODUCTION

Iceland's location on an up-welling mantle plume on the Mid-Atlantic ridge, where the Eurasian and American continental plates diverge (Sigmundsson and Sæmundsson, 2008), has promoted the formation of a high number of geothermal areas and hot spring systems throughout the island (Friðleifsson, 1979; Sæmundsson, 1979; Einarsson, 1994; Torfason, 2003). Volcanic activity has furthermore resulted in large extents of the country being covered in lava fields (Einarsson, 1994). Groundwater seeps easily through the porous lava rock, leading to the formation of a multitude of cold springs. Despite the fact that cold springs in Iceland are used by humans for water supply, they have received relatively little attention by the scientific community and only few ecological studies on cold springs in Iceland have been published to this day (Govoni *et al.*, 2018).

Since the beginning of settlement of Iceland in the 9th century, warm and hot springs have been used for bathing and washing. Records of hot springs used by humans can be found in the Icelandic sagas, *e.g.* Grettissaga. It is thus not surprising that hot spring research has a long history in Iceland. Most studies have focussed on geological and hydrological characteristics of hot springs and their classification based upon those characteristics (Schwabe, 1933; Þórarinsson, 1978; Friðleifsson, 1979), whereas biological aspects of hot springs have been less studied. Examples of the few faunistic inventories of hot spring communities in Iceland are those by Tuxen (1944) for Skagafjörður, N.-Iceland, and the Central Highlands, and Þórðarson (1981) for Borgarfjörður, W.-Iceland.

Tuxen's work on hot spring animal communities has been quite influential and has been cited widely (*e.g.* Pritchard, 1991; Glazier, 2012). The Danish biologist Søren Ludvig Tuxen stayed at the farm Mælifell in Skagafjörður, N.-Iceland from April to July 1937, and studied the springs in that area to "*find out which were actually the animals characteristic of the hot springs and what were the external factors governing their life*" (Tuxen, 1944, p. 3). Tuxen classified springs based on their temperature into "cold" (below or at the annual mean air temperature, in Iceland 2-4°C), "tepid" (above the annual mean air temperature but below the mean maximum air temperature, in Iceland 5-14°C), and "hot" (above the mean maximum air temperature, in Iceland above 14°C) (Tuxen, 1944). This classification does not take any biotic criteria into account, and may thus be – although convenient and intuitive – not reflecting differences in community composition. As the *"character animals of the absolutely hot springs"* for Iceland, Tuxen listed *Radix ovata* [valid name today *Radix balthica* Linnaeus 1758] (Gastropoda, Pulmonata), *Scatella thermarum* [valid name today *Scatella tenuicosta* f. *thermarum* Collin 1930] (Diptera, Ephydridae), and *Eucricotopus sylvestris* f. *thermicola* [valid name today *Cricotopus sylvestris* (Fabricius 1794)] (Diptera, Chironomidae, Orthocladiinae).

Global climate change models predict an increase in the mean annual global surface temperature of 1-3.5°C during the next 80 years, with a more pronounced warming at higher latitudes (Hughes, 2000). All terrestrial, marine, and aquatic ecosystems are likely to be or are already impacted by these temperature changes. Rare exceptions of these predictions could be groundwater-fed systems such as springs, which for the most part are independent of surface air temperatures (Holmes, 2000). Springs are considered to be stable habitats with little fluctuation in environmental parameters (van der Kamp, 1995). In a world of rapid changes in biodiversity, groundwater habitats and springs could actually represent systems little affected by global warming.

Together with climate change, changes in land and resource use such as the expansion of agricultural areas threatens natural habitats worldwide. Iceland is no exception. In the Southern lowlands of Iceland, area covered by wetlands declined by at least 77% between 1900 and 2010, while agricultural land has expanded 15-fold since 1913 (Wald, 2012). Intensely farmed land shows for example a lower density of breeding waders compared to semi-natural and less intensely cultivated areas (Jóhannesdóttir, 2017). These land use changes, in addition to an increase in industry (*e.g.*, heavy industry and fish farming) and electric production (hydro and geothermal), along with an extreme growth in tourism in Iceland over the last few years, have put an ever increasing anthropogenic pressure on the Icelandic nature. In particular, sensitive habitats such as freshwater springs and geothermal areas are under severe threat of destruction by both physical (*e.g.*, transformation into wells and boreholes, over-use as natural bathing pools, trampling of delicate vegetation in surrounding areas, draining of wetlands for expansion of agricultural land, lowering of groundwater table) and chemical impacts (mainly as excessive nutrient input). Special protection towards spring habitats is needed, as well as simple habitat classifications that can be used for conservation. To this end, we aim to i) validate the ecological relevance of Tuxen's spring classification, and ii) compare the hot spring animal communities in Iceland between 1937 and today.

METHODS

Based on the descriptions provided in Tuxen (1944) we located and sampled two of the hot springs, Hvammkotslaug and Mælifellslaug, in Skagafjörður, N-Iceland (Fig. 1), whose invertebrate fauna and physical properties were studied in 1937 (Tuxen, 1944). Mælifellslaug (N 65°26.557' W 019°20.199', 78 m asl) is a limnocrene, Hvammkotslaug (N 65°26.184' W 019°19.205', 82 m asl) on the other hand a rheocrene. Both are situated a few kilometres apart in an agricultural area, within the boundaries of farm land and on meadows used for horse and sheep grazing, respectively.

In addition, 24 permanent springs with defined source openings were sampled in different parts of Iceland during the summers of 2015 and 2016 (Fig. 1). The sites differed in water temperature (ranging from 3.8°C to 48.2°C at the source opening), spring type (rheocrene or limnocrene), and altitude (from 6m to 928m above sea level). At each site, invertebrate samples were collected from two locations: One directly from the upwelling groundwater at the source opening (in the following referred to as "source" sample), using electrobugging (Lento and Morin, 2014) in combination with a driftnet of 63 µm mesh size. Hereby both invertebrates floating free in the water as well as those on the substratum of the source were collected. The second sample was collected at the benthic substrate few meters downstream of the source (referred to as "surface" sample to indicate the more surface water characteristics of the location as opposed to the groundwater nature of the "source" sample), using a 0.093 m^2 Surber sampler with 63µm mesh. Water temperature and pH were measured with a multi-probe sonde (HYDROLAB DS5).

Invertebrate samples were stored in 70% ethanol and sorted under a low power microscope. Chironomid larvae were mounted on microscope glass slides and fixed in Hoyer's mounting medium (Anderson, 1954). If chironomid number per sample exceeded 250, a subsample of 200 individuals was taken, otherwise all chironomid larvae were processed. Identification to lowest taxonomic level possible was done under a compound microscope (Leica DM4000B). For chironomid larval identifications keys by Andersen *et al.* (2015) and Schmid (1993) were applied.

Statistical analyses were conducted using the package *vegan* (Oksanen *et al.*, 2016) in R (R Core Team, 2016). Sample size was considered through rarefaction. We tested for changes in the hot spring community of the springs Hvammkotslaug and Mælifellslaug by comparing the invertebrate composition in our surface samples for these sites with data provided in Tuxen (1944), using Fisher's Exact test with Monte Carlo simulation for p-values. For a few taxa (*e.g.*, Collembola and *Scatella tenuicosta* f. *thermarum*), Tuxen indicated the number of individuals with "∞" or infinite. As this is not a value which can be included in statistical analysis we replaced it with the number "50" as a measure of large numbers of individuals.

To study whether Tuxen's spring classification based on temperature is ecologically relevant for Chironomidae on the genus/species level, we grouped sites following his grouping into "cold", "tepid" and "hot" and compared their chironomid community composition using adonis, an ANOVA-like permutation test implemented in the *vegan* R package (see Oksanen *et al*., 2016). To detect which taxa were responsible for the differences in community composition between the temperature classes, we conducted an indicator species analysis using the R package *indicspecies* (De Cáceres and Legendre, 2009). Confidence intervals (CI) were obtained after 1000 bootstraps. To display chironomid diversity in relation to temperature classes, we used Shannon Diversity Index (H^1), Taxa number, and Evenness, while differences between the classes were tested with Kruskal-Wallis Rank Sum Test and Wilcoxon Rank Sum Test.

We furthermore performed a Nonmetric Multidimensional Scaling (using metaMDS) on the species data to see whether chironomid communities clustered in a pattern that could be explained with temperature classes of the springs.

RESULTS

We obtained a total of 30,988 invertebrate specimens belonging to 14 orders. Chironomidae larvae were the most dominant group both in terms of abundance (with a total of 16,874 specimens) and in number of taxa (19). The subfamilies Orthocladiinae and Diamesinae were by far the most abundant, represented by 12 and 2 taxa, respectively. Chironominae were represented by only two genera (*Micropsectra* Kieffer 1909 and *Chironomus* Meigen 1803), and Tanypodinae by three genera (*Procladius* Skuse 1889, *Macropelopia* Thienemann1916,

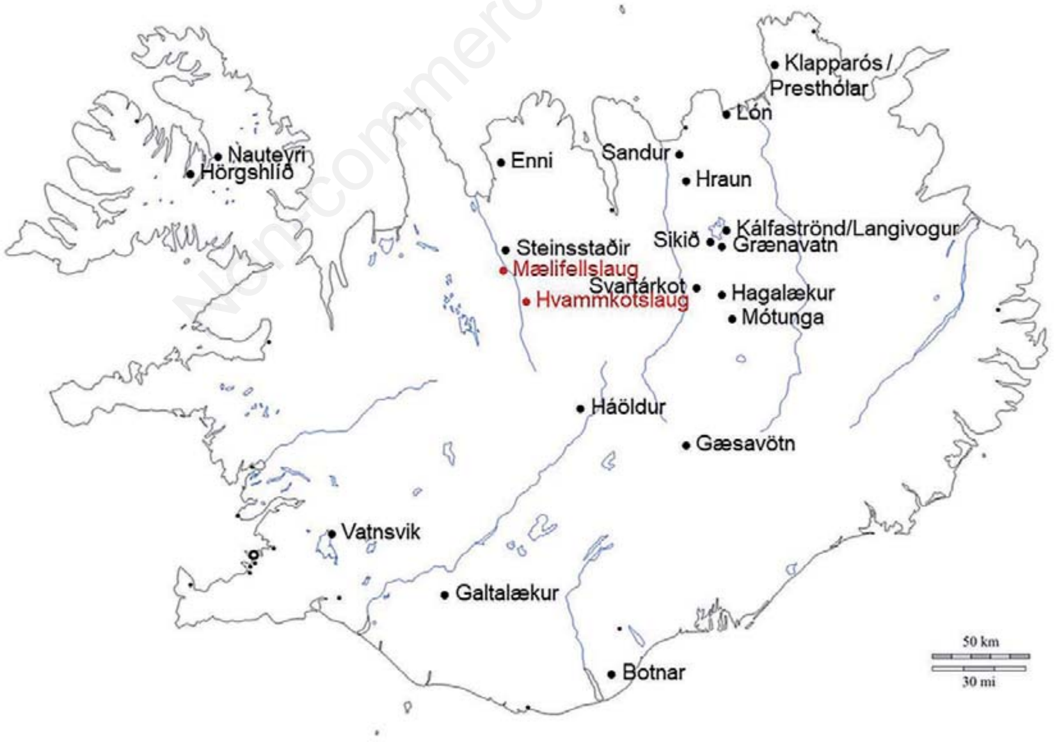

Fig. 1. Sampling sites within Iceland used in this study. The sampling sites of Tuxen (1944) revisited in this study are marked in red.

and *Arctopelopia* Fittkau 1962). Overall, *Eukiefferiella minor* Edwards 1929, *Orthocladius frigidus* Zetterstedt 1838, and *Diamesa* spp. Meigen 1835 were the most abundant chironomid taxa in the samples (Tab. 1). Water temperature and pH of the springs Hvammkotslaug and Mælifellslaug were the same as they were 80 years ago (Tab. 2). Although we found more invertebrate taxa in 2016 than Tuxen recorded, the dominating taxa were the same (Tab. 2), and all taxa reported by Tuxen were found again in 2016. For the spring Hvammkotslaug, overall

Tab. 1. Chironomid composition in Icelandic springs. Percentage of individuals of all samples collected in 2016 within the given temperature class.

Subfamily/Tribe	Species	%		
		Cold	Tepid	Hot
Tanypodinae	Tanypodinae unidentified	0	0.26	0.50
	Arctopelopia sp.	0	0.01	2.11
	Macropelopia sp.	0.27	0.26	2.22
	Procladius sp.	0	0.03	6.04
Diamesinae	*Diamesa* sp.	10.43	22.54	0.40
	Pseudodiamesa sp.	4.70	0.14	0
Orthocladiinae	*Chaetocladius* sp.	0.34	2.13	1.31
	Cricotopus sylvestris	0.07	0	49.14
	Cricotopus tibialis	0	0.38	0
	Eukiefferiella claripennis	0.07	0	0
	Eukiefferiella minor	11.11	12.40	0.40
	Heterotrissocladius sp.	0	0.11	0
	Limnophyes sp.	0	0.86	0
	Metriocnemus eurynotus	0.20	0	0.40
	Orthocladiinae unidentified	10.16	5.00	6.75
	Orthocladius frigidus	46.69	46.84	0.30
	Orthocladius oblidens	1.98	0.03	0
	Rheocricotopus effusus	0.07	0.15	0
	Thienemanniella sp.	6.82	7.90	0.30
Chironomini	*Chironomus* sp.	0	0	2.01
Tanytarsini	*Micropsectra* sp.	7.09	0.93	28.10

Tab. 2. Records of invertebrate taxa and environmental parameters of the hot springs Hvammkotslaug and Mælifellslaug in Skagafjörður, N-Iceland, in 1937 and 2016. Data from 1937 retrieved from Tuxen (1944).

	1937				2016			
Spring	Temperature	pH	Taxa	Individuals	Temperature	pH	Taxa	Individuals
Hvammkotslaug	48°C	>9.0	*Cricotopus sylvestris*	3	48.2°C	9.1	*Cricotopus sylvestris*	4
			Scatella thermarum	∞			*Scatella thermarum*	43
							Diamesa sp.	1
							Orthocladiinae	1
							Acarina	1
							Ostracoda	1
Mælifellslaug	24°C	>9.0	*Procladius* sp.	7	23.2°C	9.5	*Arctopelopia* sp.	6
			Copepoda	2			*Cricotopus sylvestris*	65
			Gastropoda	7			*Micropsectra* sp.	2
			Collembola	∞			Orthocladiinae	6
							Procladius sp.	12
							Acarina	16
							Ostracoda	4
							Copepoda	626
							Cladocera	22
							Oligochaeta	139
							Gastropoda	14
							Collembola	32

invertebrate composition at order or family level was statistically different between years, whereas chironomid composition at genus or species level was not (Invertebrate composition: Fisher's Exact test, P<0.001. Chironomid composition: Fisher's Exact test, P=0.477). For the spring Mælifellslaug, we detected differences in communities between 1937 and 2016 on both taxonomic levels (Invertebrate composition: Fisher's Exact test, P<0.001. Chironomid composition: Fisher's Exact test, P=0.001). Excluding the taxa which had infinite numbers (presented with a number of 50), did not change those results.

After grouping our 24 spring sites based on their temperature as proposed by Tuxen (1944), we obtained eleven sites in the category "cold", six in "tepid" and seven in "hot". Differences in chironomid communities

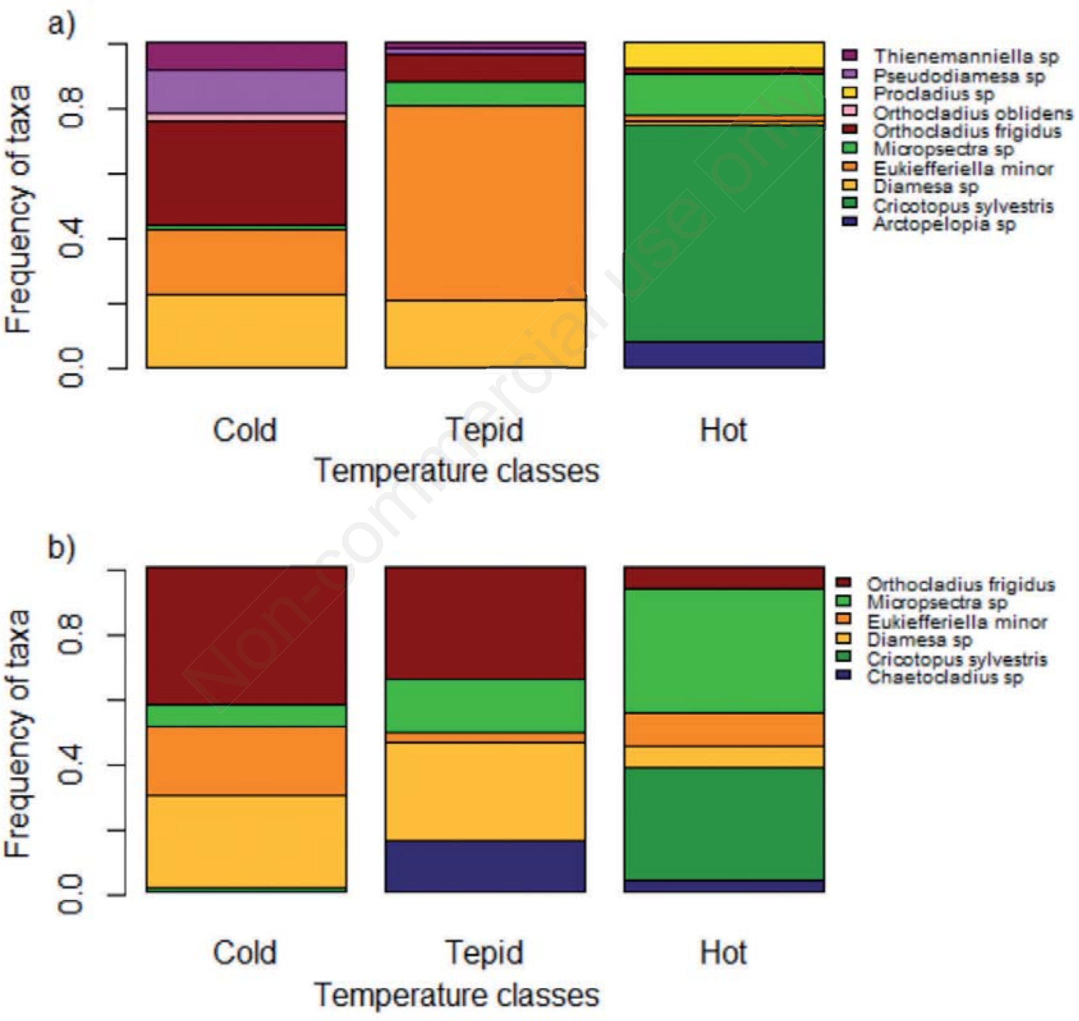

Fig. 2. Chironomid community composition of Icelandic springs in surface (a) and source samples (b). Springs are classified into cold, tepid, and hot (see section "Methods" for explanation). a) ANOVA-like permutation test (adonis), all temperature classes: F Model=4.051, P=0.001, cold *vs* tepid: F Model=0.992, P=0.426, tepid *vs* hot: F Model=5.306, P=0.002, cold *vs* hot: F Model=6.226, P=0.001, after rarefaction). b) ANOVA-like permutation test (adonis), all temperature classes: F Model=1.954, P=0.023, cold *vs* tepid: F Model=0.967, P=0.468, tepid *vs* hot: F Model=2.189, P=0.039, cold *vs* hot: F Model=2.685, P=0.007, after rarefaction).

between temperature categories were highly significant for the surface samples (ANOVA-like permutation test (adonis), F Model=4.051, P=0.001) (Fig. 2a), and also significant for the source samples (ANOVA-like permutation test (adonis), F Model=1.954, P=0.023) (Fig. 2b). To evaluate the differences among the groups we tested all pairwise comparisons, *i.e.* "cold" against "tepid", "tepid" against "hot", and "cold" against "hot". At the surface, differences in the temperature classes were due to differences between "tepid" and "hot", and between "cold" and "hot", even after taking multiple comparisons into account, applying the Bonferroni correction, whereas "cold" *vs* "tepid" was not significant (ANOVA-like permutation test (adonis): Cold *vs* tepid: F Model=0.992, P=0.426, tepid *vs* hot: F Model=5.306, P=0.002, cold *vs* hot: F Model=6.226, P=0.001). A similar pattern was found for the source, with differences between "tepid" and "hot", and between "cold" and "hot" (ANOVA-like permutation test (adonis), cold *vs* tepid: F Model=0.967, P=0.468, tepid *vs* hot: F Model=2.189, P=0.039, cold *vs* hot: F Model=2.685, P=0.007).

The indicator species analysis revealed three taxa with associations with the temperature classes, in either source or surface samples. *Cricotopus sylvestris* was an indicator species for hot springs (Source: IndVal.g=0.53, CI=0.000-0.817, P=0.067. Surface: IndVal.g=0.93, CI=0.707-1.000, P=0.005. Fishers combined probabilities for the two sites is 0.0003). *Diamesa* spp. Meigen 1835 was an indicator species for cold springs (Source: IndVal.g=0.71, CI=0.496-0.866, P=0.011. Surface: IndVal.g=0.64, CI=0.407-0.865, P 0.011) and tepid springs (Source: IndVal.g=0.54, CI=0.295-0.762, P=0.011. Surface: IndVal.g=0.57, CI=0.192-0.810, P=0.011. Fishers combined probabilities for the two sites at both temperature classes is 0.0001). *Eukiefferiella minor* Edwards 1929 associated with tepid springs at the surface (IndVal.g=0.77, CI=0.413-0.960, P=0.035) and with the source of cold springs (IndVal.g=0.80, CI=0.532-0.959,

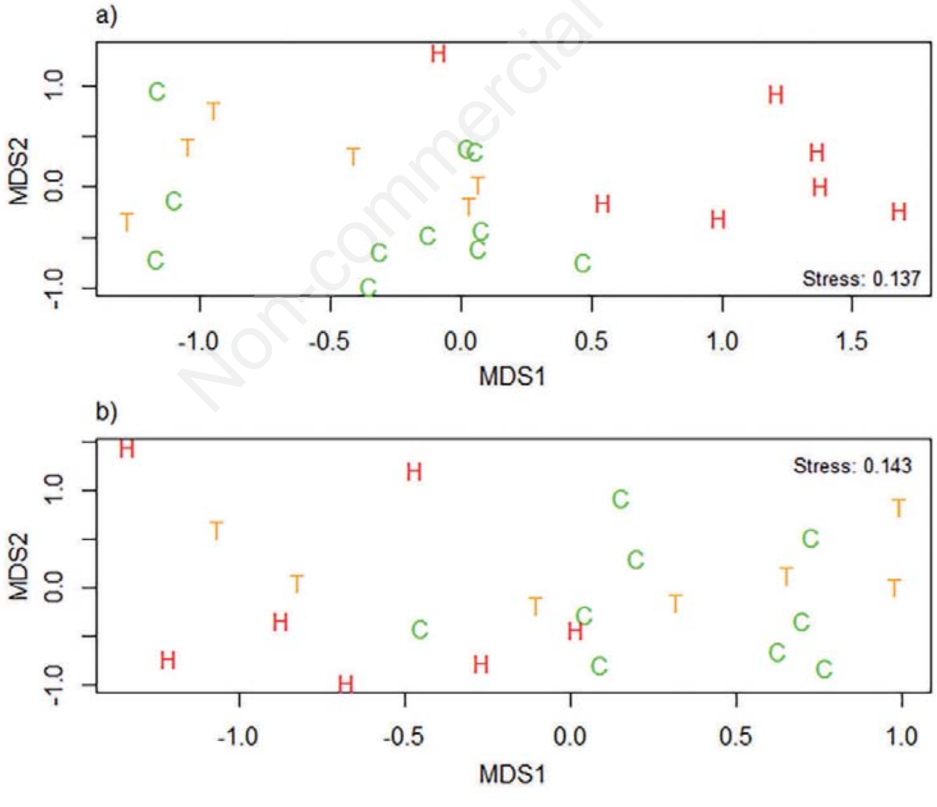

Fig. 3. Ordination of chironomid communities based on nonmetric multidimensional scaling with respect to Tuxen's classification in surface samples (a) and source samples (b). C, cold; T, tepid; H, hot.

P=0.008). Indications of associations to hot springs were furthermore found for *Arctopelopia* sp. and *Procladius* sp., to tepid springs for *Chaetocladius* spp., and to cold springs for *Pseudodiamesa* sp. and *Thienemanniella* sp. Kieffer 1911, but the numbers of individuals obtained for these species were small.

The metaMDS analysis showed that in the surface samples, chironomid communities were clustered according to temperature classes (Fig. 3a), with a clear separation between cold and hot springs and less clear separation for tepid springs, whose communities seemed to group within the cold spring communities. In the source samples, the pattern looked similar but the clustering of chironomid communities into spring temperature classes was less obvious (Fig. 3b).

Higher diversity (Shannon Diversity Index, H^1) was generally observed in the tepid springs than in the cold and the hot springs at the surface, whereas the opposite was the case at the source (Fig. 4). Differences in the Shannon Diversity Index between temperature classes were significant at the surface (Kruskal-Wallis Rank Sum Test, chi-squared=6.555, P=0.038) but not at the source (Kruskal-Wallis Rank Sum Test, chi-squared=2.799, P=0.247). However, when we tested temperature classes in surface samples in pairwise comparison using Wilcoxon Rank Sum Test, no differences could be found. Neither Taxa number nor Evenness differed significantly between temperature classes at either source or surface.

DISCUSSION

The environmental parameters measured for the hot springs Hvammkotslaug and Mælifellslaug have not changed over the past 80 years (Tab. 2). Although all the species found by Tuxen were also found in the present study, there were statistical differences in their community composition between 1937 and 2016. This was the case both for the invertebrate community on order/family level and the chironomid community on genus/species level. However, we do not know what sampling method Tuxen applied in 1937, and how quantitative his sampling was. For Hvammkotslaug and Mælifellslaug he listed only two chironomid species, *Cricotopus sylvestris* (Orthocladiinae) and *Procladius* sp. (Tanypodinae), whereas additionally we found *Arctopelopia* sp. (Tanypodinae), *Diamesa* sp. (Diamesinae) (one individual), and *Micropsectra* sp. (Tanytarsini). Other

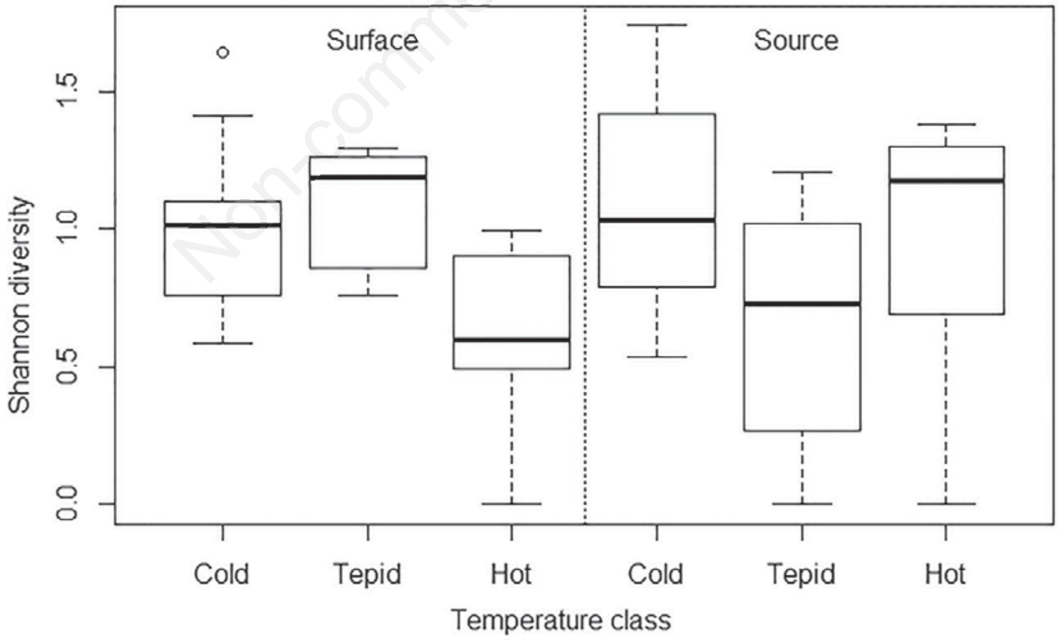

Fig. 4. Shannon diversity of chironomid communities as a function of the temperature class. Diversity differs between temperature classes at the surface but not at the source. Kruskal-Wallis Rank Sum Test, Surface: chi-squared=6.555, P=0.038, Source: chi-squared=2.799, P=0.247.

invertebrate taxa found in 2016, but not mentioned by Tuxen (1944), were Acarina and Ostracoda for both Hvammkotslaug and Mælifellslaug, and Cladocera and Oligochaeta for Mælifellslaug. One has to be careful, though, to draw conclusions based on absence data, especially when lacking information on exact sampling methods. It may be that the four invertebrate groups found now were present at the sites in 1937 but for some reasons were not represented in the samples. However, Tuxen reported all four taxa for other springs, both cold and hot (Tuxen, 1944). Similar is the case of the chironomid taxa *Arctopelopia* sp. and *Micropsectra* sp. that were found now. Tuxen reported *Micropsectra* sp. for a cold spring in Skagafjörður, in close vicinity of Mælifellslaug, so we know that the genus did occur in the area in 1937. In 2016, both *Arctopelopia* sp. and *Micropsectra* sp. were found in very low numbers in Mælifellslaug, namely six and two individuals, respectively. The absence of these genera in Tuxen's samples taken from Mælifellslaug could be attributed to random variance in the samples, or else they represent a more recent colonization. Overall, it seems that hot springs may provide stable habitats over decades and that their dominant invertebrate groups have been the same during the past 80 years.

Tuxen's classification of springs according to their relative temperature is based on the mean annual air temperature of a given place (Tuxen, 1944), and the split between tepid and hot corresponds well to larger areas such as the average temperature in July both for Reykjavík, SW.-Iceland (13.3°C) and Akureyri, N.-Iceland (14.5°C) today. We assigned our springs to Tuxen's temperature classes and compared their chironomid community composition. There are species exclusively found in one of the temperature classes (*e.g.*, *Cricotopus tibialis* Meigen 1804 and *Limnophyes* sp. Eaton 1875 only in tepid and the rare *Procladius* sp. only found in hot springs; Tab. 2). *Cricotopus sylvestris* was found to be an indicator species for the hot springs. *Diamesa* spp. characterize the tepid and cold springs, and *Eukiefferiella minor* was found to be an indicator species for tepid springs at the surface and for cold springs at the source. It should though be noted that several species were represented in the samples in low frequencies and may give stronger associations with temperature with increased sampling. Differences in community composition were highly significant for the samples collected from the surface, but less so for the samples collected at the source openings. A plausible explanation could be that the surface community is generally more diverse than the source community, with a higher number of both species and individuals. A high number of species could promote inter-specific competition and niche partitioning along an environmental gradient (*e.g.,* pH, altitude, spring type, vegetation density). This could lead to a high variability in invertebrate composition among springs with respect to environmental factors such as temperature. It has been shown that spring fauna is highly individual (Erman and Erman, 1995; Lencioni *et al.*, 2011) depending on the occurrence of different microhabitats and local environmental conditions (Bottazzi *et al*., 2011). As opposed to the surface community, the source community is made up of fewer, often more cold adapted species (Ólafsson *et al.*, 2010), *e.g. Orthocladius frigidus*, *Micropsectra* sp., *Eukiefferiella minor*, *Diamesa* spp. The more uniform chironomid communities at the source compared to the surface could result from the mitigating and stabilizing influence of groundwater on the water temperature (Holmes, 2000). The invertebrate community at the source opening could experience less fluctuations in environmental variables, and therefore inter-species competition may be less dynamic and community composition might have more opportunities to stabilize as a result of competition outcome. Additionally, the source opening is generally less vegetated and often quite uniform in terms of substrate, which may result in fewer microhabitats and thus fewer potential for ecological niches. It has been suggested that competitive exclusion would reduce diversity in simple and stable habitats such as hot springs (Mitchell, 1974).

The reported differences in chironomid community composition in "cold", "tepid", and "hot" springs, seem to justify Tuxen's spring classification on an ecological basis, especially for the benthic substrate community of springs. The results of the nonmetric multidimensional scaling suggest a clear separation of sites in the temperature class "hot", but not for the temperature classes "tepid" and "cold" (Fig. 3). This separation is stronger at the surface (Fig. 3a) than at the source (Fig. 3b). However, it is important to note that within the class "tepid" there were two species only found in that class. That "tepid" springs should be treated as a category on its own instead of being merged with the category "cold" can to large extent be justified by the presence of such species only found in that category. It is clear that water temperature is, although a main factor, not the only variable contributing to the community composition in springs. Factors such as pH (Bottazzi *et al.*, 2011; Govoni *et al*., 2018), elevation (Lencioni *et al.*, 2011), geographical location (Di Sabatino *et al.*, 2003), and spring type, *i.e.* hydraulic conditions (Govoni *et al*., 2018) have been shown to influence invertebrate diversity in springs. The springs studied here inevitably differ in those factors additionally to the range in water temperatures, which makes it hard to disentangle interrelations. There have been other classification schemes put forward for hot springs in Iceland, *e.g.*, the spring classification used by Þórðarson (1981) and based on Schwabe (1933) which differentiates between cold springs (<14°C, *kaldar lindir*), warm springs (14°-39°C, *volgrur*), bathing springs (30°-70°C, *laugar*), water geysers (70°-100°C, *vatnshverir*), and steam geysers (>100°C, *gufuhverir*) (Þórðarson, 1981). Tuxen's spring classification reflects differences in invertebrate communities of springs in more details at the colder part of the spectrum than the classification of Schwabe (1933), and is in our opinion a clear and plausible way to transfer ecological data into a message easily understandable by laymen and politicians when protection strategies have to be discussed. Keeping a spring classification as simple as possible by basing it on a single but relevant environmental factor such as temperature can be important in the context of regulations in nature conservation.

CONCLUSIONS

Here we find support for Tuxen's spring classification based on temperature as ecologically relevant for the benthic surface chironomid community of springs, with a clear separation of communities of cold, tepid and hot springs. Comparison with data on hot spring fauna in Iceland collected in 1937 reveals little to no change in both environmental conditions and species composition over the past 80 years. Although springs seem to provide stable habitats over years and decades, their invertebrate communities are not immune to changes. Ecological studies on springs and geothermal areas are necessary in order to emphasize their status as unique and sensitive habitats and consider them in nature conservation regulations.

ACKNOWLEDGMENTS

Thanks to Dan Govoni, Manon S. Guérin and Ilgin Deniz Can for assistance in the field. Special thanks to Kári H. Arnarson who helped to locate and sample Tuxen's springs in Skagafjörður. Oli "Weidenmännchen" Bechberger, Hildur Magnúsdóttir and Charles C.R. Hansen made valuable comments on a preliminary draft. The project is part of a PhD project and was funded by the Icelandic Research Fund (RANNÍS), grant n. 141863-051.

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3 Paper II

Invertebrate communities in limnocrene and rheocrene springs across a thermal gradient

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Authors contribution: AKK, JSÓ, SP, and BKK designed the study. AKK, DPG and BKK developed the sampling method and conducted the fieldwork. AKK processed the samples, AKK and JSÓ identified invertebrates. AKK and SP analysed the data. AKK, DPG, SP, JSÓ, and BKK wrote the manuscript.

Invertebrate communities in limnocrene and rheocrene springs across a thermal gradient

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KEYWORDS
Community assembly; Iceland; indicator species; Chironomidae; temperature;

ABSTRACT

To evaluate how environmental variables shape invertebrate diversity and community composition in freshwater springs, invertebrate samples were collected from 49 limnocrene and rheocrene springs in Iceland. At each site, samples were taken from both the benthic substrate of the spring (“surface”) and from the upwelling groundwater at the spring source (“source”). To collect invertebrates from the spring sources we used a modified method of “electrobugging”. A total of 54 invertebrate taxa were identified, most of them belonging to Chironomidae (Diptera). Chironomid larvae also dominated in terms of abundance (67%), followed by Ostracoda (12%) and Copepoda (9%). Community composition changed with increasing temperature at both the source and surface. Invertebrate communities in the surface samples also differed considerably between spring types, i.e., rheocrene and limnocrene. The results of the study imply that the mechanisms of community assembly in springs might depend on the water temperature, with the source community of hot springs being more niche-assembled (e.g., affected by mechanisms of tolerance and adaptation) whereas the source community of cold springs is more dispersal-assembled (e.g., affected by mechanisms of drift and colonization).

INTRODUCTION

Invertebrate community assembly within freshwater ecosystems is shaped by deterministic and stochastic processes (Chase 2007; Milner et al. 2011). When stochastic processes such as ecological drift and chance colonization are relatively more important, the resulting communities can be characterized as dispersal-assembled (Hubbell 2001), while deterministic processes based on individual species traits, such as tolerance and adaptation towards environmental attributes, lead to niche-assembled communities (Chase & Myers 2011). Niche-assembled communities show more predictable community composition, whereas dispersal-assembled communities result in a higher site-to-site variation (beta diversity) among sites with otherwise similar environmental conditions (Chase 2007).

To our knowledge, assembly processes for invertebrate communities have never been studied in spring ecosystems. Due to their patchy distribution and isolation from each other, springs are considered ecological islands (Fattorini et al. 2016), and are thus interesting ecosystems to study community assembly mechanisms. Springs are usually very stable habitats with little temporal fluctuation in environmental variables such as temperature and chemical composition (van der Kamp 1995; Holmes 2000; Szczucinska & Wasielewski 2013). The great faunistic individuality and "uniqueness" in invertebrate community composition of freshwater springs has been emphasised by many researchers (Erman & Erman 1995; Myers & Resh 2002; Buczynski et al. 2003; Lencioni et al. 2011; Bottazzi et al. 2011), which could indicate that stochastic processes dominate community assembly in springs. Although the patterns may vary depending on the dispersal abilities of invertebrates, e.g., between species with and without winged adults (Bottazzi et al. 2011), there seem to be clear underlying trends of non-random patterns of species diversity and composition in springs, associated to environmental variables such as temperature (Myers & Resh 2002; Lencioni et al. 2012), altitude (Myers & Resh 2002; Lencioni et al. 2011), and spring type (Cianficconi et al. 1998; Govoni et al. 2018), as well as historical and geographical factors (Williams & Williams 1998). Community assembly in springs might differ depending on the dominant taxonomic group. Communities dominated by insects with a flying terrestrial adult stage may be more stochastically assembled in comparison to communities dominated by crustaceans, whose entire life cycle is confined to aquatic habitats. Similarly, the proportion of crenobiont taxa (obligate spring fauna) in a community may influence the assembly processes.

Iceland has a unique abundance of freshwater springs, emerging mostly at the edge of lava fields within or along the volcanically active zone which crosses the country from the southwest to the northeast. Due to the occurrence of geothermal areas, springs with temperatures above 14°C, classified as hot springs (Tuxen 1944; Kreiling et al. 2018), are common. The environmental conditions of Icelandic springs, especially the water temperature, vary among springs, but are temporally very stable within the same spring (Table 1). The high number of springs, expressing thermal stability at a wide range of temperatures, makes Iceland an excellent setting for a large natural experiment, allowing us to study how temperature may shape spring invertebrate communities, and to investigate possible mechanisms behind community assembly in spring habitats. An additional advantage is that the system is comparably simple, as the invertebrate fauna in general and the freshwater invertebrate fauna in particular is depauperate in Iceland (Gíslason 2005). Studies on geothermally heated streams in Southwest Iceland have revealed that macroinvertebrate and meiofaunal community structure change dramatically across a thermal gradient (O'Gorman et al. 2012). Furthermore, warming proved to simplify food-web structure and shorten the pathways of energy flux between consumers and resources (O'Gorman et al. 2019). Warming experiments, in which a stream in Iceland was warmed by 3.8°C, resulted in an increase in biomass and production of large-bodied, slower growing invertebrate taxa, e.g., black-fly larvae (Simuliidae) and snails (Gastropoda) (Nelson et al. 2017).

In addition to temperature, Icelandic springs vary in spring type, altitude, and other physical and chemical factors. Spring type (limnocrene, forming a pond, and rheocrene, forming a stream) influences hydraulic conditions and habitat structure and has been shown to affect the invertebrate community composition in springs, with limnocrene springs having a higher proportion of crustaceans (Govoni et al. 2018). In the present study, springs classified as limnocrene were either located at the shore of shallow lakes or were discrete sources forming a small pond of still or slow-flowing water. The rheocrene springs all discharged into more or less fast-flowing streams and spring brooks, some of them just a few meters long. Assuming that organisms colonize springs from the adjacent aquatic habitat, one would expect lentic taxa to dominate the fauna in limnocrene and lotic taxa in rheocrene springs (Govoni et al. 2018).

Spring communities in Iceland have so far not been studied in relation to altitude, which is an important variable for spring communities in other areas of the world (Myers & Resh 2002; Lencioni et al. 2011). More than 75% of the land area of Iceland is higher than 200 m above sea level (asl) and over one third of the land area is above 600 m asl (Sveinbjörnsdóttir 2007), with the latter mostly located within the desert-like Central Highlands in the interior of the country. In Iceland, botanical studies usually set the division between low- and highland at 300-400 m based on changes in the plant community (Steindórsson 1964; Þóra Ellen Þórhallsdóttir, pers.com.). Thus, a high number of high-altitude springs can be found in Iceland, and the harsher environmental conditions in the highlands might shape the invertebrate community in highland springs.

The definition of spring is often rather blurry in the literature, sometimes defined as the eucrenal zone of a stream, whose length varies depending on the geographical location of the study and researcher, i.e. up to 2 m (Wood et al. 2005), 5 m (von Fumetti et al. 2007), 10 m (Erman & Erman 1995; Smith et al. 2003), 20 m (Glazier 1991; Myers & Resh 2002), 40 m (Barquín & Death 2004), 50 m (Lencioni et al. 2011), and even 100 m (Hoffsten & Malmqvist 2000) downstream of the source. In most studies, samples have been taken from that eucrenal region, generally from the benthic substrate of a stream or pond, using various methods such as Surber sampler (Erman & Erman 1995; Smith et al. 2003; Barquín & Death 2004; Wood et al. 2005; von Fumetti et al. 2007), smaller, often hand-held sweep or drift nets (Hoffsten & Malmqvist 2000; Myers & Resh 2002; Ilmonen & Paasivirta 2005; Lencioni et al. 2011), or by hand picking of invertebrates (Hoffsten & Malmqvist 2000; Myers & Resh 2002), and often combining different methods. Very few studies (e.g, Smith et al. 2003; Fattorini et al. 2016), however, took samples directly at the source of the spring, at the point of groundwater emergence. Spring sources are often hard to access and sample with traditional methods, and this might be the reason why spring studies tend to neglect the source. In this paper we present a new sampling method based on electric fishing gear which allowed us to collect invertebrates from the spring source, i.e., from the ecotone between surface water and groundwater. In addition, we took samples at the benthic substrate within 2 m downstream of the source, which we refer to as "surface" habitat.

Here, we studied invertebrate diversity and community composition in Icelandic springs. Our objectives were to (I) test if invertebrate diversity and community composition were related to environmental variables, both at the spring source and at the surface, and (II) to examine which processes shape invertebrate community assembly in springs and whether such processes may differ among spring source and surface. With respect to the first (I) objective, we predicted that (i) water temperature, spring type, and altitude were the main factors shaping the invertebrate community structure. As stated before, these factors are important drivers of aquatic invertebrate communities in general and in Iceland in particular. We furthermore predicted (ii) a lower alpha diversity at the source due to a generally lower diversity in groundwater ecosystems (Strayer 1994) compared to surface waters. Considering our second (II) objective, we predicted that (iii) invertebrate communities in springs were more influenced by stochastic than deterministic processes resulting in high beta diversity. This prediction is based on the dominant taxa in Icelandic freshwaters being insects, especially Chironomidae (Diptera), which have generally good dispersal abilities. We also predicted that (iv) invertebrate communities at the source show more influence of deterministic processes, reflected in a lower beta diversity, than at the surface. This is based on the source likely having a higher proportion of crenobiont taxa and taxa with limited dispersal abilities.

MATERIAL AND METHODS

Invertebrate samples were collected from 49 springs in Iceland (Figure 1, Table 2). Nineteen of the springs were limnocrene and 30 were rheocrene, and they were grouped into 25 cold (<5°C), 14 tepid (5-14°C) and 10 hot (>14°C at the source) springs, following the classification of Tuxen (1944) and Kreiling et al. (2018). Temperature, conductivity, oxygen saturation, and pH of each sampling location were measured at the time of sampling, using a Hydrolab DS5 multi-probe sonde (Hach Hydromet, Loveland, CO, USA). In addition, HOBO temperature loggers (Onset Computer Corporation, Bourne, MA, USA) were placed at the sites for one year in order to record the thermal regime. Furthermore, spring type, altitude, and presence of fish as top predators at the site were noted. A complete list of sampling sites with their environmental variables is shown in Table 2.

Two samples were collected at each spring site, using different sampling methods: one sample was collected directly from the upwelling groundwater at the source opening (referred to as “source”), using electrobugging (Lento & Morin 2014) based on electric fishing gear. The method was modified specifically to sample invertebrates in spring sources and is described as follows: a drift net (30.5 x 45.7 cm) with a mesh size of 63 µm was removed from its frame to allow for complete flexibility of the opening of the net. Lead weights were tied to the grommets along the bottom of the drift net. The flexibility of the opening of the net allowed it to conform to the contours of the substrate, and the weights held the bottom in place. The top of the net was held upright by two stakes, which had the effect of keeping the net open. The net was placed closely in front of a source in order to catch the outflow. Modified electric fishing gear was used to apply electrical current to the spring. The copper plate serving as the cathode was put into the water body a few meters away from the source. For the anode, we replaced the commonly used pole with a flexible wire connected to an on/off switch on a box. The anode was inserted into the source duct as far as possible into the groundwater. Electricity (300 V, DC) was then applied for one minute. The electricity stuns the organisms within reach and causes them to detach from the substrate and flow into the driftnet. Among the invertebrates caught with this method was *Crangonyx islandicus* Svavarsson & Kristjánsson (Crangonyctidae), an endemic groundwater amphipod. The collection of *C. islandicus* demonstrated that electrobugging in spring sources successfully detaches groundwater organisms. With this method, both invertebrates floating free in the water as well as those on the substrate of the source were collected. The second sample was collected at the benthic substrate approximately 2 m downstream of the source (referred to as "surface" to indicate the more surface water characteristics of the location as opposed to the groundwater nature of the "source" sample), using a 0.093 m^2 Surber sampler with 63 µm mesh. Samples were preserved in 70% ethanol.

Although we would have preferred to employ the same sampling methods at both locations within each spring, we were restricted by the physical structure of the habitat. Collecting a Surber sample within the source was impossible because of the dimensions, and pilot work found that physical scrubbing was too destructive to the source habitat. On the other hand, electrobugging on the benthic surface could have been an effective method only in rheocrene springs, as limnocrene springs lack the flow to carry invertebrates into the net. We were thus confined to use two different sampling methods, each of which we considered to be the most appropriate for the respective location in the spring. The resulting differences between source and surface samples might thus be due to effects of both the location and the sampling method.

The invertebrates sampled were sorted under a dissecting stereomicroscope, counted and identified to the lowest possible taxonomic level. Limnocrene Ostracoda, Amphipoda, Plecoptera, Coleoptera, Trichoptera, Chironomidae (Diptera), and Ephydridae (Diptera) were identified to species level. Acarina were classified into Halacaridae, Hydrachnidia, and Oribatida. Chironomid larvae were mounted on glass microscope slides and fixed in Hoyer's mounting medium (Anderson 1954). All chironomid larvae were processed unless the chironomid abundance per sample exceeded 250, in which case a random subsample of 200 individuals was identified. The total number for each taxon in the sample was then calculated based on its proportion in the subsample. Identification to lowest possible taxonomic level was done under a Leica DM4000B compound microscope (Leica Microsystems, Mannheim, Germany). Chironomid larvae were identified using keys by Wiederholm (1983) and Schmid (1993). Other invertebrate groups were identified using keys by Gíslason (1979) for Trichoptera larvae, Lillehammer (1988) for Plecoptera nymphs, Olafsson (1991) for Ephydridae imagines, Gíslason (1977) for aquatic Coleoptera larvae and imagines, and Gerecke & Di Sabatino (2007) for Acarina. Ostracoda of the limnocrene springs were identified using keys by Meisch (2000). For some taxa, e.g., *Diamesa* spp. and Oribatida, we could clearly distinguish several morphotypes/species, without being able to identify them with certainty to a specific taxonomic group. These morphotypes were combined under a higher taxonomic level for analysis, which resulted in more conservative estimates of diversity.

Statistical analysis was done using the software R (version 3.4.1, R Core Team 2016). As a measure of alpha diversity, we calculated taxa richness (N_0), Shannon diversity (N_1), and Shannon evenness (as $E = N_0/N_1$; Hill's ratio) according to Borcard et al. (2018), using the *diversity* function in the *vegan* package (Oksanen et al. 2017). The dependency of the alpha diversity indices as response variables on the environmental variables, spring type, altitude, geographical position (latitude and longitude), and their interactions with the sampling location within the spring (source and surface) were analysed with multiple linear regressions assuming normality of the residuals for Shannon diversity and evenness, but applying generalized linear models (*glm* function in the *stats* package; R Core Team, 2016) with Poisson link function for taxa richness as count data. To assess the collinearity of the environmental variables, correlations between the environmental variables were calculated (function *chart.Correlation* in *PerformanceAnalytics* package; Peterson & Carl 2018), as well as the variance inflation factor (function *vif* in *car* package; Fox & Weisberg 2019). In cases of high collinearity, we retained the independent and most representative variables but excluded the others from subsequent tests. This resulted in initial models with the explanatory variables temperature, spring type, altitude, latitude, longitude, and their interactions with sampling location (source and surface). Initial models were simplified by a step-wise procedure, excluding the least significant variable until the minimal adequate model was reached.

The dependency of differences in community composition, summarized with Bray-Curtis distances (beta diversity), on the same explanatory variables as for the alpha diversity indices, were analysed by running a permutational multivariate analysis of variance (*adonis* function in the *vegan* package). The ordination of samples based on the beta diversity, and the association of the environmental variables to the main axes were visualised with nonmetric multidimensional scaling (NMDS) using the functions *metaMDS* and *envfit* in the *vegan* package. The beta diversity was further assessed by analysing the Sørensen dissimilarity, based solely on the presence or absence of taxa at each site, using the function *betadiver* in *vegan* with method = 1. To evaluate whether the degree of differentiation varied between the source and the surface samples, pairwise differences of the Sørensen dissimilarity between source and surface samples were compared with a Wilcoxon signed-rank test.

To explore preferences of taxa to spring type (rheocrene or limnocrene), we performed an indicator species analysis, as implemented in the functions *multipatt* and *signassoc* of the R package *indicspecies* (De Cáceres and Legendre 2009). The indicator value index is comprised of two components, specificity (A component) and fidelity (B component). Specificity indicates the positive predictive value of a species and is highest when a species is present in the target habitat group but not elsewhere. Fidelity indicates the sensitivity of a species as indicator and is highest when a species is present in all sites of the target habitat group (De Cáceres and Legendre 2009).

RESULTS

In total, 54 aquatic invertebrate taxa were identified (Table 3), the majority (51) of them belonging to the phylum Arthropoda. The most abundant and prevalent invertebrate groups were Chironomidae (Insecta, Diptera) (67%), followed by Ostracoda (Crustacea) (12%), and Copepoda (Crustacea) (9%). Chironomidae larvae was the dominant group both in terms of abundance (with a total of 84,259 individuals) and in number of taxa (26). Ostracoda were identified to species level only for the limnocrene springs, and to avoid bias, those individuals were combined under the higher taxonomic level Ostracoda for analysis. Thus, of the 54 collected taxa, only 47 were included in the data analysis (Table 3), and discussed here.

The different alpha diversity indices varied among the springs, with number of taxa ranging from 1 to 18 at the source and from 1 to 22 at the surface. Both taxa richness and Shannon diversity were on average lower at the source than at the surface, with an average of taxa richness (± standard deviation) of 8 ± 3.8 and 11 ± 4.3, respectively, and an average Shannon diversity of 3.5 ± 1.48 and 4.0 ± 2.00. Mean evenness, on the other hand, was slightly higher at the source (0.5 ± 0.27) than at the surface (0.4 ± 0.21). Diversity indices were similar for rheocrene and limnocrene springs. Taxa richness was negatively correlated with temperature at the surface but was not correlated with temperature at the source (Figure 2b, Table 4a). Furthermore, taxa richness was influenced by geographical position of the spring, and increased slightly westwards (Table 4a). Similarly, Shannon diversity decreased with increasing temperature at the surface, but increased at the source (Figure 2a, Table 4b). Evenness was not influenced by environmental variables or sampling location but decreased westwards (Table 4c).

Community composition was shaped by temperature, spring type, geographical position (latitude), and sampling location within the spring (Table 5). Less variation was observed among the surface samples (mean beta diversity = 0.547) than among the source samples (0.605; V = 231830, p < 0.001). At both source and surface, the community composition changed significantly as temperature increased (Figure 3b and 4b). Generally, *Scatella tenuicosta* Collin (Ephydridae), *Cricotopus sylvestris* Fabricius (Orthocladiinae), Oribatida c, *Macropelopia* sp. (Tanypodinae), *Arctopelopia* sp. (Tanypodinae), *Procladius* sp. (Tanypodinae), *Chironomus* sp. (Chironominae), and Gastropoda were associated with high temperatures and low oxygen saturation. The chironomids *Orthocladius frigidus* Zetterstedt, *Thienemanniella* sp., *Diamesa* spp. (Diamesinae), *Cricotopus tibialis* Meigen, and *Eukiefferiella minor* Edwards (Orthocladiinae), as well as *Apatania zonella* Zetterstedt (Apataniidae, Trichoptera), Hydrachnidia, and *Capnia vidua* Klapálek (Plecoptera) were mainly found at the colder part of the temperature gradient. Community composition differed between rheocrenes and limnocrenes at the surface (*adonis*, F Model = 2.78, p = 0.001) but not at the source (*adonis*, F Model = 0.759, p = 0.737; Figure 5). *Diamesa* spp., *E. minor*, *Micropsectra* sp. (Chironominae), *Orthocladius* spp., and Ostracoda were relatively more abundant in rheocrene, and Tardigrada and Cladocera in limnocrene springs (Figure 5, Table 3). In source samples, communities of rheocrene and limnocrene springs were more similar to each other, with *Diamesa* spp., *E. minor*, *Micropsectra* sp., *O. frigidus*, Ostracoda, and Copepoda as the dominant taxa (Figure 5).

Indicator species (Table 6) for rheocrene springs at the surface were *O. frigidus*, *Thienemanniella* sp., *Chaetocladius* sp. (all Orthocladiinae), as well as *S. tenuicosta* and not further identified Diptera larvae. On the other hand, *Arctopelopia* sp., *C. tibialis* (Orthocladiinae), and Cladocera were indicator species for limnocrene springs at the surface.

DISCUSSION

We predicted (i) that the main drivers of invertebrate diversity and community composition in Icelandic springs were water temperature, spring type, and altitude. The results of our study support this prediction partly, as invertebrate diversity was affected by temperature, but not by altitude or by spring type. The community composition, on the other hand, was affected by temperature and spring type, but not altitude. In addition, geographical position (as latitude and longitude) turned out to influence the diversity and community composition as well. We furthermore predicted (ii) that the source would have a lower alpha diversity than the surface, and this prediction was supported by our results. As the second objective of our study, we examined whether the processes of community assembly differed between spring source and surface, using beta diversity as a proxy to indicate the stochasticity of assembly. We had predicted (iii) that springs have a high beta diversity emphasising the relative importance of stochastic as opposed to deterministic factors during assembly. The results supported this prediction for cold springs, whereas hot spring communities seemed to be restricted by deterministic factors. Lastly, we predicted (iv) a lower beta diversity at the source than at the surface, but our results show the opposite.

Water temperature proved to be one of the most influential environmental variables for both invertebrate diversity and community composition. Invertebrate taxa were distributed along the temperature gradient in line with their reported ecology (e.g., Ólafsson et al. 2010). So were, for example, the cold-stenotherm chironomids *O. frigidus*, *Thienemanniella* sp., and *Diamesa* spp. (Diamesinae) mainly found at the colder part of the temperature gradient, whereas *Arctopelopia* sp., *Procladius* sp., *C. sylvestris*, and *S. tenuicosta* were clearly associated with higher water temperatures. Myers and Resh (2002) found a consistent core group of species occurring repeatedly in warm springs, whereas no such core group existed in cold springs. The core group of Icelandic hot springs seems to be comprised of the chironomid *C. sylvestris*, the ephydrid *S. tenuicosta*, Gastropoda, and the mite order Oribatida. The first three of those taxa were described as the “character animals of the absolutely hot springs” in Iceland by Tuxen (1944, and see Kreiling et al. 2018). Although Tuxen listed one species of Oribatida, *Hydrozetes lacustris* Michael (Hydrozetidae), found in a 16°C warm spring (Tuxen 1944), he did not specifically mention Oribatida in conjunction with hot springs. Our results, however, indicate that Oribatida are common in and characteristic for hot springs in Iceland and should thus be added to the core species group of hot springs.

The community composition of rheocrene and limnocrene springs was clearly different at the surface, but was more similar to each other at the source. Spring type was not reflected in taxa richness, Shannon diversity, and evenness, neither at the source nor at the surface. This is in accordance with a previous study on springs (Govoni et al. 2018), which found that spring type affected community composition but not diversity. This underlines the importance of taking into account measures of both species diversity and species composition in ecological studies, as one might lose crucial information about habitat properties when focusing only on diversity indices.

The indicator species analysis revealed five taxa indicative for rheocrene springs at the surface, three of which, *O. frigidus*, *Thienemanniella* sp., and *Chaetocladius* sp., belong to Chironomidae. This is in accordance with the ecology of these taxa, as they are all three typical inhabitants of fast-flowing waters (Hrafnsdottir 2005). *Scatella tenuicosta* as indicator for rheocrene springs is somewhat controversial, as the species is clearly linked to hot springs. Seven out of the ten hot springs examined in this study were rheocrene, which might result in a bias in the dataset. Indicator species for limnocrene springs were the chironomids *C. tibialis* and *Arctopelopia* sp., which have both mainly been reported from lentic or slow-flowing waters in Iceland (Hrafnsdottir 2005), as well as Cladocera.

Altitude was considered a good predictor for invertebrate taxonomic richness but not for taxon abundances in spring brooks in New Zealand, with lower taxonomic richness at higher altitudes (Barquín & Death 2006). In our study, however, altitude was not related to diversity nor community composition. Altitude did though affect species distribution across Iceland. The chironomids *Chaetocladius* spp., *Limnophyes* sp, and *Metriocnemus* spp. (all Orthocladiinae) were for example mainly found in highland springs (above 300 m asl), and *Parochlus kiefferi* Garrett (Podonominae) was exclusively found in highland springs in this study (data not shown). This distribution pattern could be due to additional characteristics of highland springs other than high altitudes. Springs in the Central Highlands of Iceland are often isolated, both from other water bodies and from human activity in the form of traffic, agriculture, urban areas, etc. Highland springs are thus less exposed to disturbance, which might be reflected in a more intact moss cover surrounding the spring. Moss carpets have been shown to provide important microhabitats in springs which can increase invertebrate abundance and diversity (Lindegaard et al. 1975; Nolte 1991; Ilmonen & Paasivirta 2005). The chironomid taxa predominantly found in highland springs, *Chaetocladius* spp., *Limnophyes* sp, and *Metriocnemus* spp., are all semi-aquatic and thrive on emerging mosses in the transition zone between terrestrial and freshwater habitats. *P. kiefferi* is likewise reported from mosses in springs (Nolte 1991; Hrafnsdottir 2005).

Community composition was linked to geographical position, namely latitude, of the spring. A molecular study on the endemic groundwater amphipod *C. islandicus* found in Icelandic springs (Kornobis et al. 2010) showed that geographical distances between sampling sites were reflected in genetic divergence between monophyletic groups of the species. This provides evidence that geographical position can be an important variable in shaping community composition by determining species distribution, especially in taxonomic groups with slow dispersal abilities (e.g., Crustacea). As discussed for altitude, geographical distances between sampling sites are related to the variable isolation from other water bodies, which has not been analysed in this study but should be included in further analyses.

Fish occurrence was excluded as a variable from the analyses due to intercorrelation with altitude, temperature, and spring type. However, top-down regulation can be an important mechanism in shaping aquatic communities (Sih et al. 1985; Turner & Mittelbach 1990; Ruetz et al. 2002), and presence or absence of fish as top predators affect the structure of invertebrate assemblages in ponds (Fairchild et al. 2000). In Iceland, a small benthic morph of *Salvelinus alpinus* Linnaeus (Arctic charr) is common in the source region of many springs (Kristjánsson et al. 2012), but *Gasterosteus aculeatus* Linnaeus (threespine stickleback) and *Salmo trutta* Linnaeus (brown trout) have as well been observed in springs (Kreiling and Kristjánsson, personal observation). It can be expected that these fish species act as top predators in habitats where they are present, but little is known of their influence on invertebrate communities in springs.

As for all ecological studies, it was not possible to take into account all environmental variables which might influence community composition and diversity, either because we did not measure them or because they got excluded from analysis due to intercorrelation with other variables. Variables such as substrate type, discharge, flow velocity, riparian vegetation, surface area of the spring, and degree of isolation from other water bodies, are likely to influence spring invertebrate communities as well, and could be considered in further studies.

Taxa richness was lower and evenness slightly higher at the source than at the surface, indicating that the source community is made up of fewer and more evenly distributed taxa compared to the surface community. This could be because the source is a more “simple” and more stable habitat and seems to be less affected by environmental variables than the surface habitat. Spring type, which determines the habitat structure and hydraulic conditions around the source, is an important variable for the surface community but not for the source community. At the source, hydraulic conditions for both rheocrene and limnocrene springs are very similar, due to the current of the outflowing groundwater (Govoni et al. 2018).

Controversially to our prediction (iv), beta diversity was higher at the source than at the surface, indicating that source samples were more variable than surface samples. In cold springs, the taxa composition, consisting mainly of common and ubiquitous species of the Icelandic freshwater fauna, e.g., *O. frigidus*, *E. minor*, and *Diamesa* spp., indicates that the source community is less specialized and rather opportunistic, and common species exhibiting good dispersal abilities and a broad tolerance-range towards environmental variables such as temperature, pH, or oxygen availability, have competitive advantage over species with a narrower tolerance range. Chironomids of the genera *Diamesa*, *Orthocladius*, and *Eukiefferiella* were also found to be the initial colonizers in glacial streams in Alaska (Milner et al. 2011). The higher beta diversity at the source could be explained by a stochastic colonization of the source habitat by invertebrates from the adjacent surface habitat, and the low number of stygobiont species found, namely only the amphipod *Crangonyx islandicus*. A high site-to-site variation may indicate that the community assembly is dominated by stochastic rather than deterministic processes, as stated in prediction (iii). Chase (2007) proposed that the relative importance of deterministic assembly processes increases under “harsh” environmental conditions, e.g., high disturbance, low productivity, or predation pressure, resulting in a lower beta diversity (Chase 2007; Chase & Myers 2011). The spring source is characterized by more stable conditions than further downstream, and could thus be considered a less harsh environment. Stochastic processes might dominate the community assembly at the source and lead to a dispersal-assembled community. Which species of a broadly tolerant species pool come first to colonize a spring source might be more a matter of chance than of niche adaptation, as reflected in the higher within-sites variation of source samples. It has been suggested that insects found in springs are not necessarily stenobiotic but instead represent parts of populations with a more flexible ecology (Gerecke & Di Sabatino 2007). These ecologically flexible individuals might be attracted to spring habitats due to ease of oviposition. A colonization of spring sources by broadly tolerant species rather than specialists indicates again that the source community of cold springs is dispersal-assembled. The source community of hot springs, however, is more likely to be niche-assembled and only species which are adapted to high water temperatures and often high concentration of ions can successfully colonize (e.g., Pritchard 1991). This supports the hypothesis that strong ecological filters (“harsh” environments) favour niche-assembly (Chase 2007).

The lower taxa richness in the source samples than in the surface samples could also be attributed to the sampling method, e.g., a possibly lower efficiency of the electrobugging compared to the Surber sampler. Electric stunning of invertebrates might vary with respect to body size, mode of attachment, or complexity of the nervous system. Another possible reason for a lower taxa richness at the source is due to the fact that Crustacea were only partially identified to species. Many Crustacean species are known to be creno- or stygobiotic (Galassi et al. 2009), which could underestimate the diversity at the source as opposed to the surface. However, the known Crustacean fauna of Icelandic springs consists – with the exception of two groundwater amphipods – mostly of ubiquitous aquatic taxa than obligate groundwater dwellers, although some Ostracoda (e.g., *Potamocypris pallida*) might be crenobiontic in Iceland (Alkalaj et al. 2019).

It has been proposed that species diversity is generally low in springs and spring brooks due to their temperature stability which consequently reduces thermal niches and potentially taxa richness (Pritchard 1991; Barquín & Death 2006). Thus, springs might have a low alpha diversity, but beta diversity on the other hand might be high and contribute greatly to the overall freshwater diversity of a region (Pritchard 1991). Taxa richness of the springs in this study varied between 1 and 22 taxa (on average 11) in the surface samples, and between 1 and 18 taxa (on average 8) in the source samples. Those numbers are on average indeed lower than invertebrate taxa richness found in studies in other water bodies in Iceland, e.g., 16 invertebrate taxa collected from one sampling station in the glacial river Vestari-Jökulsá, 14 from the run-off river Svartá, and 33 from the spring-fed river Laxá (Gíslason et al. 1998). However, the total number of invertebrate taxa found in our study (gamma diversity) was 54, which is comparable to the overall number of invertebrate taxa reported from Icelandic rivers, e.g., 52 invertebrate species from the river Laxá (Gíslason 1994).

CONCLUSION

The main drivers of invertebrate community composition in Icelandic springs were water temperature, spring type, and geographical position (latitude). Although alpha diversity was similar for limnocrene and rheocrene springs, there were differences in their invertebrate community composition, and indicator taxa could be assigned to each spring type. Invertebrate composition and diversity differed between sampling locations within a spring, with greater alpha diversity in the surface and greater beta diversity in the source habitat. Furthermore, we found evidence that the assembly mechanisms of the source communities might differ depending on the water temperature, with hot springs being more niche-assembled, whereas cold springs are more dispersal-assembled.

Due to their temporal stability in chemical and physical variables, springs could act as refugia for freshwater organisms in rapidly changing environments, and understanding the processes governing community structure is becoming increasingly important. Although Icelandic waters are relatively depauperate, their natural temperature gradients make them an excellent place to test theory, and while individual springs may have low diversity, a large portion of the Icelandic freshwater fauna is represented in the entirety of the spring ecosystem. Therefore, more emphasis should be put on keeping spring habitats intact on a large scale because in the words of Erman & Erman (1995): *Whether springs are high or low in species diversity is immaterial to their importance as habitats for species that can live nowhere else.*

ACKNOWLEDGEMENTS

We want to thank Jovana Alkalaj for identifying the ostracod species of the limnocrene springs. Gísli Már Gíslason helped with the identification of Trichoptera larvae and commented on the manuscript. Thanks to Leivur Janus Hansen, Hildur Magnúsdóttir, and Ragnhildur Guðmundsdóttir for valuable comments and suggestions on an earlier draft. The project was funded by the Icelandic Research Fund (RANNÍS), grants nr. 141863-051 and 185083-051.

DATA ACCESSIBILITY STATEMENT

Data deposited in the Dryad repository, Dryad doi: XXXXX.

AUTHOR CONTRIBUTIONS

AKK, JSO and BKK designed the study, AKK, DPG and BKK developed the sampling method and conducted the fieldwork, AKK processed the samples, AKK and JSO identified invertebrates, AKK and SP analysed the data, and all authors contributed to the writing of the manuscript. The authors declare no conflict of interest.

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TABLES

Table 1 Thermal stability of springs in Iceland. The water temperature was measured over the course of one year, and the mean, minimum, maximum, and standard deviation (SD) in °C are given. Data for ten of the 49 springs studied are shown here as example.

Spring	**Mean**	**Minimum**	**Maximum**	**SD**
Dynjandi	2.7	1.7	6.7	1.03
Galtalækur	5.4	5.1	6.6	0.17
Góðdalafjall	3.6	3.5	5.6	0.16
Háöldur	27.3	22.7	29.1	1.23
Hruni	3.6	3.2	5.7	0.29
Kálfaströnd	5.3	5.2	5.7	0.04
Krákárbotnar	5.5	2.3	13.5	0.96
Mælifellslaug	22.3	15.1	24.3	1.31
Miðhúsaskógur	2.5	2.4	3.7	0.10
Staðarhraun Kirkja	4.7	4.3	5.1	0.25

Table 2 Environmental characteristics of the springs studied. The environmental variables were measured at the time of sampling, both at the source and the downstream benthic substrate (i.e. surface) of the investigated springs.

Spring		Coordinates	Altitude [m asl]	Type	Fish presence	Location	Temperature [°C]	pH	Oxygen [%]	Conductivity [µS/cm^{-1}]
1	**Botnar I**	N 63°38.707'	36	rheocrene	yes	Source	5.7	8.11	72.5	113.7
		W 018°14.749'				Surface	5.61	8.03	74.2	115.9
2	**Botnar II**	N 63°39.275'	33	limnocrene	yes	Source	7.35	7.91	78.5	108.4
		W 018°15.142'				Surface	7.19	7.96	77.4	108.9
3	**Dynjandi**	N 65°44.604'	305	rheocrene	no	Source	2.31	7.63	47.6	50.9
		W 023°09.302'				Surface	2.81	7.64	48.1	50.9
4	**Enni**	N 65°53.371'	151	rheocrene	no	Source	4.65	7.44	76	81.6
		W 019°19.755'				Surface	4.45	7.27	75.9	80
5	**Friðsæld**	N 64°17.753'	27	rheocrene	no	Source	4.9	7.44	49.7	131.4
		W 015°14.764'				Surface	4.83	7.37	49.8	129.7
6	**Gæsavötn**	N 64°46.697'	928	rheocrene	no	Source	7.18	7.49	1.7	201
		W 017°30.687'				Surface	7.97	7.48	13.7	201
7	**Galtalækur**	N 64°00.453'	128	rheocrene	no	Source	5.4	7.99	73.1	151.8
		W 019°55.148'				Surface	5.09	7.93	72.3	151.8
8	**Goðdalafjall**	N 65°10.792'	570	rheocrene	no	Source	3.55	8.03	31.5	148.2
		W 019°03.972'				Surface	3.57	7.96	31.5	147.6
9	**Grænavatn Norður**	N 65°32.905‘	291	limnocrene	yes	Source	6.49	8.92	65.5	138.9
		W 016°58.908‘				Surface	6.49	8.93	60.6	139.5
10	**Grænavatn Suður**	N 65°32.205’	285	limnocrene	yes	Source	4.23	8.82	67.7	100.5
		W 017°00.477’				Surface	4.45	8.98	63.3	100.4
11	**Hænuvík**	N 65°36.420'	45	rheocrene	no	Source	5.02	7.37	46.3	106.3
		W 024°11.776'				Surface	5.76	7.24	46.5	102.3
12	**Hafgrímsstaðir**	N 65°26.184‘	82	rheocrene	no	Source	48.2	9.09	5.1	224
		W 019°19.205‘				Surface	43.19	9.21	3.9	248
13	**Hagalækur**	N 65°20.022'	441	rheocrene	no	Source	7.27	8.6	69.3	NA
		W 017°03.430'				Surface	5.52	9	70.5	NA

14	**Háöldur**	N 64°55.627'	767	rheocrene	no	Source	25.84	9.31	2.1	130.2
		W 018°16.947'				Surface	25.5	9.25	1.9	129.9
15	**Hengill IS6a**	N 64°03.426‘	406	rheocrene	no	Source	14.42	7.59	70.2	209
		W 021°18.244‘				Surface	11.49	7.43	63.7	214
16	**Hengill IS7**	N 64°03.407‘	384	rheocrene	no	Source	5.77	7.64	74.5	86
		W 021°18.391‘				Surface	5.38	8.19	74.8	86.9
17	**Hengill IS8**	N 64°03.414‘	381	rheocrene	no	Source	17.15	7.44	66.8	281
		W 021°18.439‘				Surface	16.59	7.54	66.1	287
18	**Herðubreiðarlindir**	N 65°11.548'	493	limnocrene	yes	Source	5.6	6.8	66.3	137.8
		W 016°13.508'				Surface	5.93	6.78	65.6	137.2
19	**Hofsvellir**	N 65°17.013’	354	rheocrene	no	Source	6.12	6.28	47.6	145.9
		W 019°02.850’				Surface	6.61	6.38	47.9	161.7
20	**Hörgshlíð**	N 65°49.885'	20	rheocrene	no	Source	32.43	8.35	39.1	13.4
		W 022°37.657'				Surface	32.23	9.05	58.1	190
21	**Hraun**	N 65°50.414'	34	rheocrene	yes	Source	5.21	7.46	70	156.2
		W 017°21.289'				Surface	4.8	7.52	65.4	156.3
22	**Hrauná**	N 64°42.261'	71	rheocrene	yes	Source	6.25	8.09	75	66.5
		W 020°59.870'				Surface	5.58	8.45	76	57.6
23	**Hruni**	N 63°51.547'	43	limnocrene	yes	Source	3.42	7.95	77.3	635
		W 017°44.486'				Surface	3.11	8.11	78.7	637
24	**Kálfaströnd**	N 65°33.759'	283	limnocrene	yes	Source	5.95	9.38	60	109.1
		W 016°56.710'				Surface	5.13	9.22	54	110.8
25	**Kiðárbotnar**	N 64°42.073'	128	limnocrene	yes	Source	3.39	9.43	78	46.4
		W 020°52.805'				Surface	3.12	9.47	77.7	46.4
26	**Klapparós**	N 66°16.481'	9	limnocrene	yes	Source	4.05	7.69	79	83
		W 016°24.530'				Surface	3.82	7.53	78.3	82.4
27	**Krákárbotnar**	N 65° 19.852'	430	rheocrene	no	Source	8.58	8.77	69.5	46.3
		W 017°04.654'				Surface	5.91	8.67	69.1	112
28	**Lækjarbotnar Hol**	N 63°57.422'	78	rheocrene	yes	Source	5.36	8.17	77.1	120

		W 020°15.892'				Surface	4.71	7.94	76.2	120
29	**Lækjarbotnar Rvk**	N 64°04.287'	121	rheocrene	no	Source	5.32	8.69	80.6	75.4
		W 021°40.107'				Surface	3.67	8.59	78.7	74.8
30	**Langivogur**	N 65°37.012’	286	limnocrene	yes	Source	19.79	8.34	75.1	434
		W 016°55.000’				Surface	13.41	8.37	62.4	300
31	**Lón**	N 66°05.785'	6	limnocrene	yes	Source	4.92	8.02	77.8	78.3
		W 016°55.514'				Surface	4.37	8.18	78	78.7
32	**Mælifellslaug**	N 65°26.557'	78	limnocrene	no	Source	23.44	8.72	3.9	182.3
		W 019°20.199'				Surface	21.59	7.69	3.2	192.8
33	**Miðhúsaskógur**	N 64°17.373'	184	limnocrene	no	Source	3.36	9.38	80.2	36.3
		W 020°30.706'				Surface	2.4	9.29	78.1	35.8
34	**Mótunga**	N 65°17.942'	437	rheocrene	no	Source	4.5	8.96	66.5	96.4
		W 017°07.114'				Surface	4.11	8.87	62.6	96.4
35	**Nauteyri**	N 65°55.039'	31	limnocrene	no	Source	25.5	8.66	27.4	162.7
		W 022°20.500'				Surface	29.77	8.93	41	167.5
36	**Oddar**	N 64°42.110'	124	limnocrene	yes	Source	3.71	9.41	78	46.4
		W 020°53.787'				Surface	3.11	9.38	77.6	46.4
37	**Presthólar**	N 66°15.477'	28	rheocrene	yes	Source	4.11	7.95	76.7	93.7
		W 016°24.081'				Surface	3.78	7.92	76.8	92.6
38	**Sandur**	N 65°57.223'	8	limnocrene	yes	Source	3.94	7.57	66.1	154.8
		W 017°32.701'				Surface	3.77	7.42	55	151.9
39	**Sikið**	N 65°35.210’	284	limnocrene	no	Source	5.05	7.7	51.5	156
		W 017°05.573’				Surface	4.67	7.65	44.6	155.7
40	**Sílatjörn**	N 64°42.810'	100	rheocrene	yes	Source	3.99	7.6	75.3	57.1
		W 020°58.600'				Surface	3.85	7.72	75.3	56.6
41	**Skarðslækur**	N 64°00.306'	103	rheocrene	yes	Source	5.2	6.7	46.8	112.7
		W 020°07.110'				Surface	5.24	6.92	47.2	112.9
42	**Staðarhraun Bær**	N 64°44.610'	62	rheocrene	yes	Source	5.4	5.26	78.9	68.2
		W 022°05.647'				Surface	3.98	5.68	79.7	69.9

43	**Staðarhraun Kirkja**	N 64°44.855'	62	rheocrene	yes	Source	5.21	5.31	81.3	71.8
		W 022°05.812'				Surface	4.57	5.26	79.2	69.4
44	**Steinsstaðir**	N 65°28.162'	62	rheocrene	no	Source	42.62	8.48	80.1	265
		W 019°21.390'				Surface	40.24	8.47	86.3	266
45	**Svartárbotnar**	N 64°44.768'	568	rheocrene	yes	Source	3.24	9.75	37	86.9
		W 019°25.801'				Surface	3.3	9.83	39.2	86.9
46	**Svartárkot**	N 65°20.153'	382	rheocrene	no	Source	3.8	8.9	71.5	83.8
		W 017°13.926'				Surface	3.66	8.96	70.8	83.9
47	**Þverá**	N 63°52.396'	53	limnocrene	yes	Source	5.11	7.45	76.1	66.3
		W 017°49.199'				Surface	4.76	7.51	75.2	65.7
48	**Úlfsstaðir**	N 64°39.911'	56	rheocrene	no	Source	26.24	10.03	43	0.3
		W 021°14.963'				Surface	42.96	9.82	2.9	282
49	**Vatnsvik**	N 64°14.757'	109	limnocrene	yes	Source	3.71	8.89	74.6	53.1
		W 021°03.304'				Surface	3.64	8.71	76.1	52.5

Table 3 Invertebrates found in freshwater springs in Iceland. Numbers represent total number of individuals found in rheocrene and limnocrene springs at the source and the surface. Taxa which were included in the data analysis are marked with an asterisk (*). The taxa acronyms ("Acrn.") refer to the ones used in Figures 3, 4, and 5.

Taxon	**Acrn.**	**Surface samples**		**Source samples**	
		rheocrene	**limnocrene**	**rheocrene**	**limnocrene**
GASTROPODA*	GAS	637	70	2	9
OLIGOCHAETA*	OLI	1512	1437	56	65
TARDIGRADA*	TAR	697	7976	4	8
ACARI	ACA				
Halacaridae*	Hal	12	5	13	7
Hydrachnidia*	Hyd	65	36	5	12
Oribatida a,b*	Ora	74	48	19	14
Oribatida c*	Orc	104	0	11	0
CLADOCERA*	CLA	8	258	0	41
COPEPODA*	COP	6804	1841	1712	834
OSTRACODA*	OST	10085	997	3908	97
Bradleystrandesia affinis (Fischer, 1851)		0	1	0	0
Candona candida (Müller, 1776)		11	53	0	0
Cryptocandona reducta (Alm, 1914)		1	0	0	0
Cytherissa lacustris (Sars, 1863)		0	4	0	0
Limnocythere inopinata (Baird, 1843)		0	3	0	0
Potamocypris fulva (Brady, 1868)		82	110	0	2
Potamocypris pallida Alm, 1914		0	72	2	5
Potamocypris villosa (Jurine, 1820)		0	0	0	2
AMPHIPODA*	AMP				
Crangonyx islandicus Svavarsson & Kristjánsson, 2006		0	0	4	2
COLLEMBOLA*	COB	16	36	99	54
PLECOPTERA*	PLE				
Capnia vidua Klapálek, 1904		45	3	16	5
COLEOPTERA*	COL				
Agabus bipustulatus (Linnaeus, 1767)		4	1	5	0
TRICHOPTERA	TRI				
Apatania zonella (Zetterstedt, 1840)*	Azo	14	4	0	0
Limnephilus affinis Curtis, 1834*	Laf	5	0	0	0
Limnephilus griseus (Linnaeus, 1758)*	Lgr	19	1	3	3
Limnephilus sp.	Lph	0	1	0	0
CHIRONOMIDAE					
Podonominae					
Parochlus kiefferi (Garrett, 1925)*	Pki	58	3	1	0
Tanypodinae					
Arctopelopia sp. (*A. griseipennis* (van der Wulp, 1858))*	Arc	0	12	0	10
Macropelopia sp.*	Mac	66	7	7	21
Procladius sp. (*P. islandicus* (Goetghebuer, 1931))*	Pro	5	38	0	22
Diamesinae					
Diamesa spp.*	Dia	22201	941	4382	3822

Pseudodiamesa sp.*	Pse	64	24	0	0
Orthocladiinae					
Chaetocladius spp.*	Cha	2671	33	343	30
Coryoneura fittkaui Schlee, 1968*	Cof	116	0	0	0
Cricotopus sylvestris (Fabricius, 1794)*	Crs	365	69	13	45
Cricotopus tibialis (Meigen, 1804)*	Crt	5	80	0	56
Cricotopus sp.*	Cri	58	3	0	39
Eukiefferiella claripennis (Lundbeck, 1898)*	Euc	7	0	13	34
Eukiefferiella minor (Edwards, 1929)*	Eum	13067	586	1073	922
Heterotrissocladius sp. (*H. grimshawi* (Edwards, 1929))*	Het	0	16	0	0
Limnophyes sp.*	Lim	124	12	16	0
Metriocnemus eurynotus (Holmgren, 1883)*	Meu	236	0	16	18
Metriocnemus fuscipes (Meigen, 1818)*	Mfu	58	0	0	0
Orthocladius frigidus (Zetterstedt, 1838)*	Ofr	6917	158	1597	1427
Orthocladius oblidens (Walker, 1856)*	Oob	959	218	5	327
Orthocladius sp.*	Ort	3252	468	1942	1437
Paralimnophyes sp.*	Par	3	0	0	0
Rheocricotopus effusus (Walker, 1856)*	Ref	230	7	1	0
Smittia sp.*	Smi	0	0	10	0
Thienemanniella sp. (*T. clavicornis* (Kieffer, 1911))*	Thi	3069	60	97	67
Chironominae					
Chironomus sp.*	Chi	0	2	0	18
Micropsectra sp.*	Mic	4398	239	258	371
SIMULIIDAE*	SIM	13	1	4	0
EPHYDRIDAE*	EPH				
Scatella tenuicosta Collin, 1930		479	0	20	1
DIPTERA other*	DIP	120	9	13	20
NEMATODA/NEMATOMORPHA*	NEM	336	85	17	10

Table 4 Dependency of alpha diversity indices on environmental variables and sampling location within each spring (source or surface). Slope (b), standard error (SE), t-, and p-values are shown. Asterisks (*) behind p-values indicate level of significance. a) Taxa richness. The minimal adequate model used was *glm(Taxa richness~Temperature*Location+Longitude, family="poisson")*. $R^2 = 0.199$. b) Shannon diversity. Minimal adequate model: *lm(Shannon~Temperature*Location)*. $R^2 = 0.178$. c) Shannon evenness. Minimal adequate model: *lm(Evenness~Temperature*Location+Longitude)*. $R^2 = 0.143$.

Diversity index	Variable	b	SE	t-value	p-value
a) Taxa richness					
	Temperature	-0.0003	0.005	-0.07	0.945
	Location	0.454	0.090	5.04	<0.001 ***
	Longitude	0.037	0.015	2.44	0.015 *
	Temperature*Location	-0.015	0.007	-2.23	0.026 *
b) Shannon diversity					
	Temperature	0.054	0.023	2.40	0.018 *
	Location	1.747	0.443	3.94	<0.001 ***
	Temperature*Location	-0.130	0.031	-4.15	<0.001 ***
c) Shannon evenness					
	Temperature	0.006	0.003	1.79	0.077 .
	Location	-0.075	0.064	-1.17	0.247
	Longitude	-0.028	0.011	-2.49	0.015 *
	Temperature*Location	-0.005	0.005	-1.18	0.240

Table 5 Dependency of the invertebrate community composition on environmental variables and sampling location within each spring (source or surface). The minimal adequate model used was *adonis(CommunityMatrix~Temperature*Location+Type*Location+Latitude +Longitude)*. Asterisks (*) behind p-values indicate level of significance.

Variable	F Model	r^2	p-value
Location	3.654	0.033	0.001 ***
Temperature	5.377	0.049	0.001 ***
Type	2.311	0.021	0.005 **
Latitude	4.129	0.037	0.001 ***
Longitude	1.476	0.134	0.082 .
Temperature*Location	1.776	0.016	0.021 *
Type*Location	1.737	0.016	0.030 *

Table 6 Indicator species of rheocrene and limnocrene springs at the surface. A multilevel pattern analysis was used to obtain indicator species (see text). An explanation of the components specificity and fidelity is given in the Methods section. Asterisks (*) behind p-values indicate level of significance.

Indicator species	**Specificity (A component)**	**Fidelity (B component)**	**Indicator value index**	**p-value**
Rheocrene springs:				
O. frigidus	0.962	0.742	0.85	0.007 **
Diptera larvae other	0.886	0.645	0.76	0.004 **
Thienemanniella sp.	0.967	0.581	0.75	0.015 *
Chaetocladius spp.	0.979	0.452	0.67	0.042 *
Ephydridae	1.000	0.290	0.54	0.039 *
Hydrachnidia	0.782	0.355	0.53	0.114
Plecoptera	0.897	0.290	0.51	0.099 .
R. effusus	0.950	0.161	0.39	0.432
L. griseus	0.917	0.129	0.34	0.372
Oribatida c	1.000	0.097	0.31	0.380
M. eurynotus	1.000	0.065	0.25	0.525
M. fuscipes	1.000	0.065	0.25	0.508
C. fittkaui	1.000	0.032	0.18	1.000
E. claripennis	1.000	0.032	0.18	1.000
Paralimnophyes sp.	1.000	0.032	0.18	1.000
L. affinis	1.000	0.032	0.18	1.000
Limnocrene springs:				
Cladocera	0.980	0.333	0.57	0.019 *
Arctopelopia sp.	1.000	0.222	0.47	0.014 *
C. tibialis	0.965	0.222	0.46	0.024 *
Procladius sp.	0.929	0.111	0.32	0.127
Chironomus sp.	1.000	0.056	0.24	0.387
Heterotrissocladius sp.	1.000	0.056	0.24	0.379

FIGURES

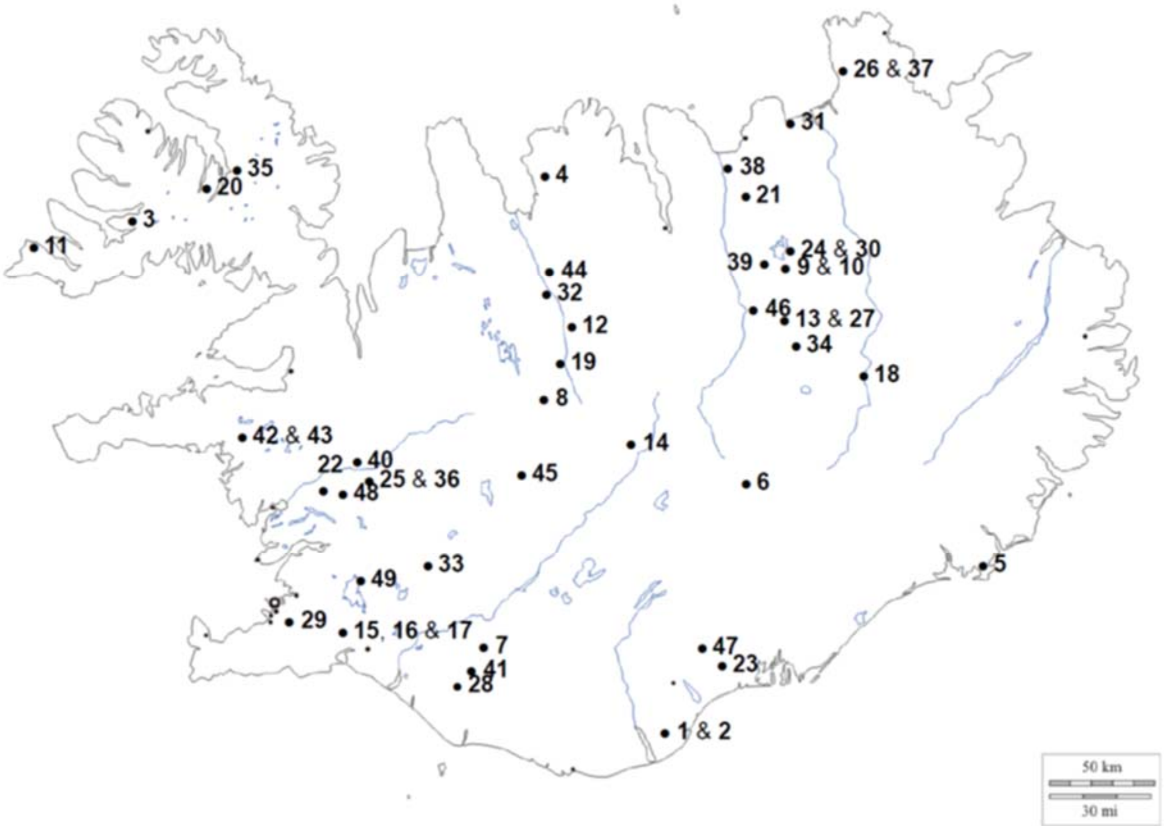


Figure 1. Location of the 49 freshwater springs in Iceland investigated in this study. Numbers refer to springs as listed in Table 2.

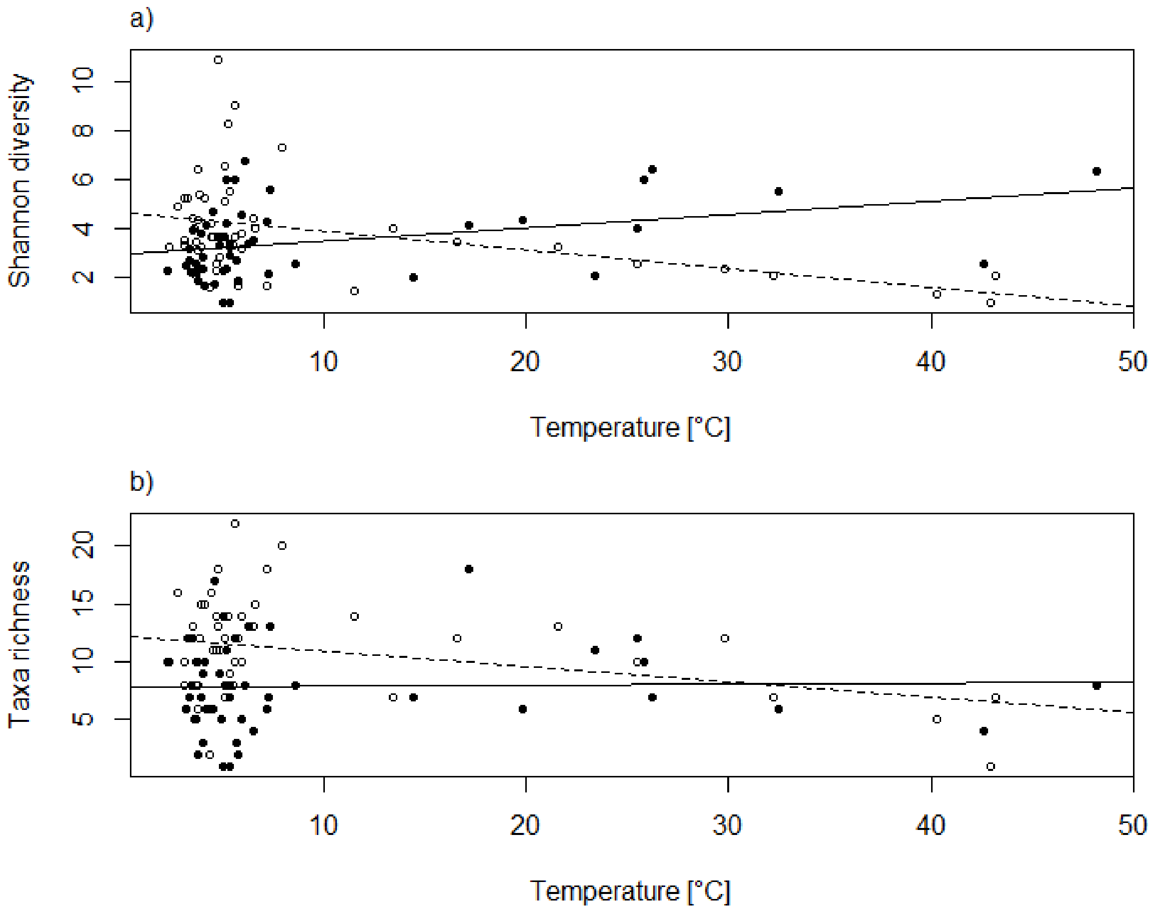
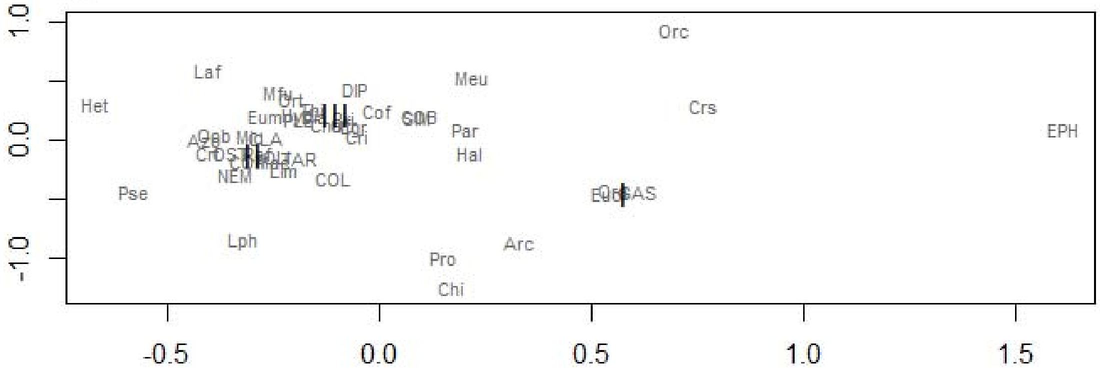


Figure 2 a-b. Diversity of invertebrates in Icelandic springs with respect to temperature. Shannon diversity (a) and Taxa richness (b) are shown in relation to spring temperature at the source (black dots) and the surface (white dots). Regression lines for the source samples are shown as continuous line and for the surface samples as broken line. The regression analysis is summarized in Table 4.

a) Taxa



b) Sites

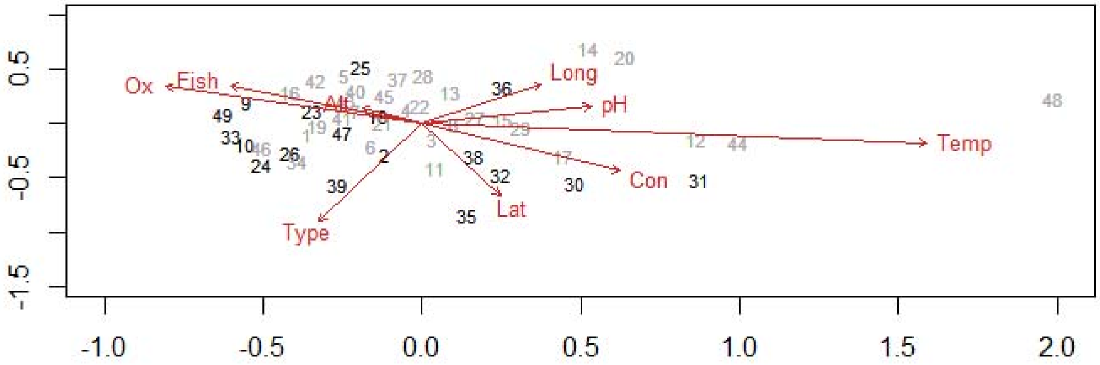


Figure 3 a-b. Non-metric multidimensional scaling of invertebrate taxa (a) and sites (b) for surface samples. Non-linear stress was 0.210. The association of the environmental variables to the axes are shown with arrows (Ox = oxygen saturation, Fish = fish presence, Alt = altitude, Type = spring type, Temp = temperature, pH, Con = Conductivity, Long = Longitude, Lat = Latitude). Acronyms of invertebrate taxa (a) are listed in Table 3. Three clusters (I – III) of overlapping data points were defined for clarity reasons, containing the following taxa: cluster I – Euc, Ora, GAS; cluster II – Crt, Lim, Mac, Mic, Oob, Ref, OST, COP, CLA, Azo, NEM, OLI, TAR; cluster III – Cha, Cof, Cri, Dia, Eum, Mfu, Ofr, Ort, Pki, Thi, Hyd, SIM, DIP, Lgr, PLE, COB. Spring sites (b) are labelled in grey for rheocrenes and in black for limnocrenes, site numbers as in Table 2. Note that the axis scales differ between (a) and (b).

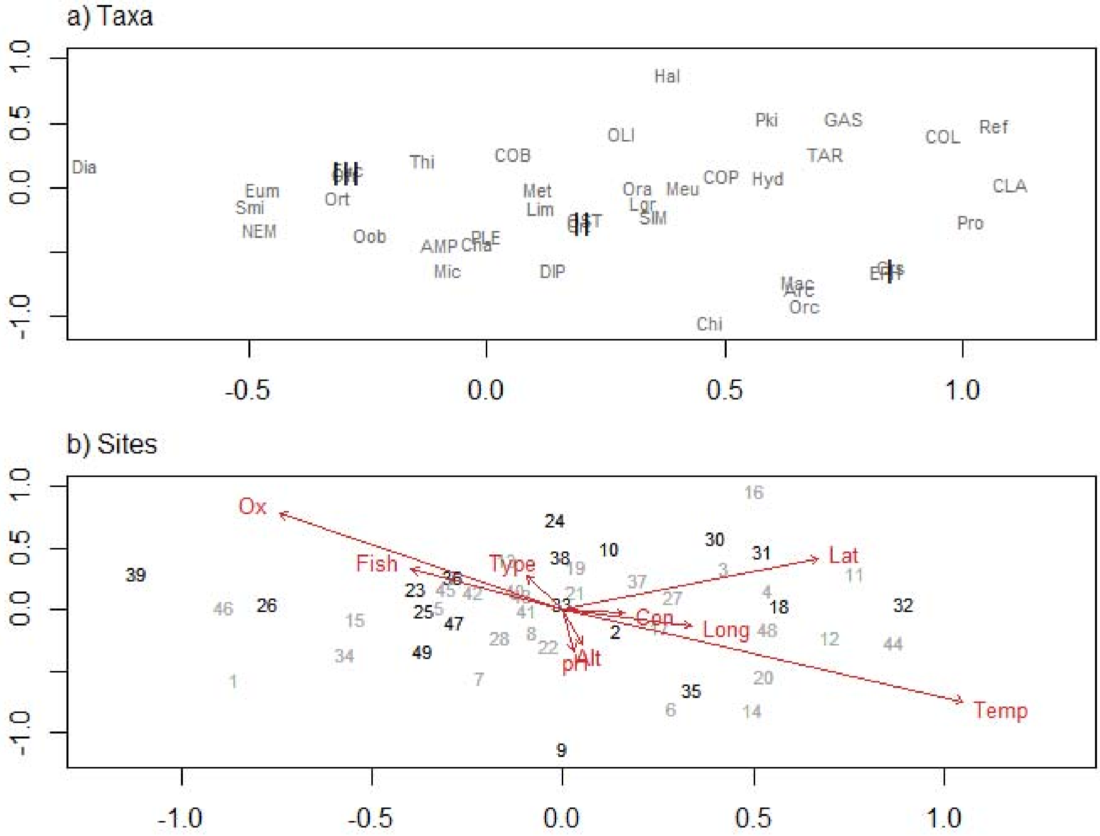


Figure 4 a-b. Non-metric multidimensional scaling of invertebrate taxa (a) and sites (b) for source samples. Non-linear stress was 0.239. The association of the environmental variables to the axes are shown with arrows (Ox = oxygen saturation, Fish = fish presence, Alt = altitude, Type = spring type, Temp = temperature, pH, Con = Conductivity, Long = Longitude, Lat = Latitude). Acronyms of invertebrate taxa (a) are listed in Table 3. Three clusters (I – III) of overlapping data points were defined for clarity reasons, containing the following taxa: cluster I – Crs, EPH; cluster II – Crt, OST; cluster III – Euc, Ofr. Spring sites (b) are labelled in grey for rheocrenes and in black for limnocrenes, site numbers as in Table 2. Note that the axis scales differ between (a) and (b).

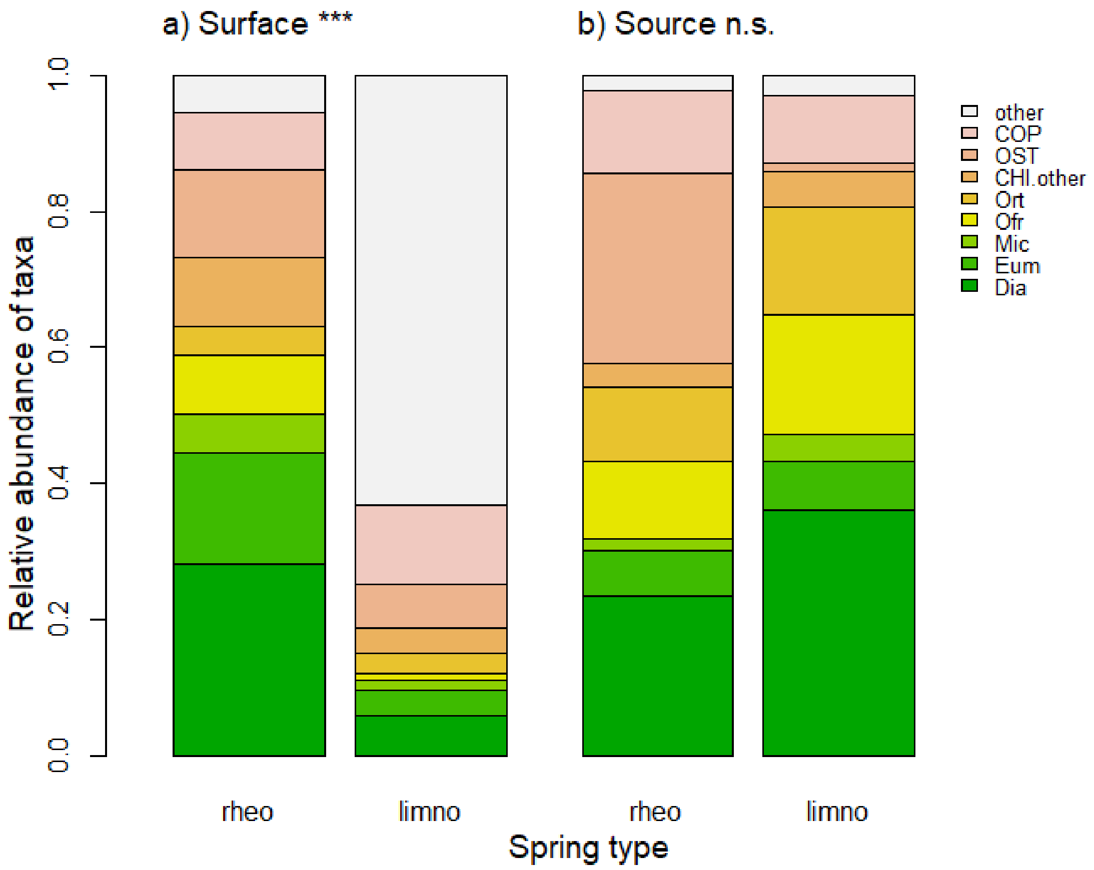


Figure 5. Simplified community composition of rheocrene and limnocrene springs for surface (a) and source (b) samples. Taxa with a total relative abundance of lower than 5% were grouped together into “other”, or “CHI.other” for chironomid species. Asterisks (*) above the graphs indicate level of significance. Taxa acronyms listed in Table 3.

4 Paper III

Trichoptera diversity in Icelandic springs

Kreiling, A.-K., Gíslason, G.M. & Kristjánsson, B.K. (2020).

© *Zoosymposia* 18: 46-52. Doi:10.11646/zoosymposia.18.1.8. Open Access.

Authors contribution: AKK, GMG, and BKK conceived the idea for this study. AKK and BKK conducted the fieldwork. AKK and GMG identified Trichoptera. AKK analysed the data. AKK, GMG, and BKK wrote the paper.

Zoosymposia 18: 046–052 (2020)
https://www.mapress.com/j/zs

ISSN 1178-9905 (print edition)
ZOOSYMPOSIA
ISSN 1178-9913 (online edition)

https://doi.org/10.11646/zoosymposia.18.1.8
http://zoobank.org/urn:lsid:zoobank.org:pub:0FDB2227-66A8-4235-B341-9D7773175CC1

Trichoptera diversity in Icelandic springs

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Abstract

The aim of this study was to characterize the Trichoptera fauna of freshwater springs in Iceland and to relate distribution of caddis larvae to environmental properties of the springs. Out of a total of 48 springs sampled, Trichoptera larvae were found in only eleven. Larval densities were low, as was species diversity. Only three of the 12 species known to occur in Iceland were found: *Apatania zonella*, *Limnephilus griseus*, and *Limnephilus affinis*. The occurrence of *A. zonella* in springs in North-Iceland may suggest that springs might play a role as refugia for this species that is otherwise excluded from the area by larvae of the predatory caddis species *Potamophylax cingulatus*, which seems to be absent from spring habitats. Caddis larval abundance was higher in rheocrene springs and in springs with sandy substrate, and decreased with increasing water temperature. Presence or absence of Trichoptera larvae, on the other hand, was not associated with any of the environmental variables measured.

Keywords: Caddis larvae, *Apatania zonella*, *Limnephilus griseus, Limnephilus affinis*, temperature

Introduction

Iceland is probably the country with the highest number of freshwater springs relative to its area. Eight of the ten largest springs in the world are found in Iceland (Óskarsdóttir 2011). Many of the numerous smaller springs emerge from the edge of porous lava fields and are highly variable in appearance as well as physical and chemical properties. One evident feature is the variability in hydraulic conditions, reflected in different spring types, i.e. limnocrene (pool-forming) and rheocrene (stream-forming) springs. Another peculiarity of Icelandic springs is their extreme range in temperature, from as cold as 2°C to geothermally heated hot springs. The benthic substrate in the immediate vicinity of the spring sources is often comprised of bare lava rock, and sometimes sand or gravel. Allochthonous input into the spring from surrounding terrestrial vegetation is usually low, as the majority of Iceland is not forested.

The aquatic insect fauna of Iceland is species-poor (Gíslason 2005; Hrafnsdottir 2005). Odonata are absent, and Plecoptera and Ephemeroptera are each represented by only one species (Tuxen 1938; Lillehammer *et al.* 1986). Chironomidae (Diptera) is by far the most abundant taxon in Icelandic freshwaters, as well as being the most species-rich taxon (Hrafnsdottir 2005). Trichoptera are represented by twelve species, belonging to the families Apataniidae (1), Limnephilidae (10), and Phryganeidae (1) (Ólafsson & Gíslason 2010). Distribution and habitat preferences of Icelandic Trichoptera have been studied for a wide range of freshwater habitats including spring-fed rivers and pond/pool habitats (Gíslason 1981a) but spring sources themselves had not been sampled in a systematic manner.

The invertebrate community in springs is influenced by a number of ecological factors, such as water temperature (Myers & Resh 2002), spring type (Govoni *et al.* 2018), and the type of benthic substrate (Ilmonen & Paasivirta 2005; von Fumetti *et al.* 2006). For case-bearing Trichoptera larvae, substrate type is especially important as a source of case-building material (Hanna 1961). Likewise, the amount of plant detritus falling into the spring from the surrounding vegetation (allochthonous material) might be a limiting factor not only as case-building material but also as food source for detritus-feeding species.

Submitted: 13 Jun. 2019; Accepted by Pongsak Laudee: 28 Aug. 2019; published: 12 Jun. 2020

The objectives of this study were 1) to assess the Trichoptera fauna of springs in Iceland, and 2) to determine if habitat characteristics could explain the distribution of caddis larvae in springs. We hypothesized that a) Trichoptera presence and b) abundance in springs is controlled by spring type, water temperature, predominant substrate, and availability of allochthonous plant material.

Methods

Samples were collected at 48 spring sites around Iceland in the summer months (June to August) of 2015 and 2016. One sample per site was collected from the benthic substrate of the spring, approximately 2 meters downstream of the spring source, using a 0.093 m^2 Surber sampler with 63 µm mesh. In the laboratory, Trichoptera larvae were identified from the samples under a Leica MZ12.5 dissecting stereomicroscope (80–100x magnification) using a key by Gíslason (1979).

The sites were classified according to spring type (limnocrene or rheocrene), predominant substrate (lava rock or sand/organic matter), and potential amount of allochthonous input into the water body based on surrounding vegetation (high or low). The 48 sampled springs were distributed among those categories as follows: 30 rheocrenes and 18 limnocrenes, 19 with mainly lava rock and 29 with mainly sandy substrate, and 14 with high and 34 with low amounts of surrounding vegetation. The water temperature of the springs was measured with a multi-probe sonde (HYDROLAB DS5) at the time of sampling, revealing that temperatures ranged from 2.4 to 43°C among sites.

All statistical analyses were conducted in the software R (R Core Team 2016, version 3.4.1). To test whether environmental characteristics of springs differed between sites in which Trichoptera larvae were present and sites in which they were absent, we developed a generalized linear model with binomial distribution, using the function *glm* in the R package *stats*. To relate Trichoptera abundance to environmental variables, we developed a generalized linear model with poisson distribution, in which we included only sites where caddis larvae were present. Larvae of all species were pooled, because the small sample sizes did not allow for statistical analysis at the species level.

Results

Trichoptera larvae were found in 11 of the 48 spring sites sampled (Fig. 1). They occurred in low numbers, ranging from one to 20 individuals per site, resulting in densities between 10 and 215 individuals/m². Only three of the 12 Trichoptera species recorded from Iceland were found in the sampled springs: *Apatania zonella* (Zetterstedt 1840), *Limnephilus affinis* Curtis 1834, and *Limnephilus griseus* (L. 1758). The first of these species has a Holarctic distribution, the latter two Palaearctic distributions. *Limnephilus griseus* was the most common species, with a total of 28 individuals found at eight sites (Fig. 1). *Apatania zonella* was found at six sites, with a total of 20 individuals (Fig. 1). At only one site, Staðarhraun in Western-Iceland, 12 larvae of *L. affinis* were found (Fig. 1). The spring Staðarhraun was also the only site in the study where all three Trichoptera species occurred together. *Apatania zonella* and *L. griseus* co-occurred at three sites and *A. zonella* and *L. affinis* co-occurred at one site out of 48. In most cases, only a single species was found: in two sites there was only *A. zonella*, and in four sites only *L. griseus*.

Trichoptera larvae occurred exclusively in the colder springs studied, ranging in water temperatures from 2.4 to 7.2°C. However, presence or absence could not be linked to any of the measured habitat properties of the site (Table 1).

Abundance of caddis larvae, on the other hand, was associated with water temperature, predominant substrate, and plant input (Table 1). Trichoptera abundances were higher on sandy substrate (6.7 ± 6.67; average number of individual per sample ± standard deviation) than on lava rock (3.5 ± 2.69) (Fig. 2b) and in springs with potentially high plant input (7.7 ± 6.70) rather than low input (3.0 ± 2.76) (Fig. 2c). Abundance decreased with increasing water temperature (Table 1).

Although spring type was not statistically significant in explaining either Trichoptera presence/absence or abundance (Table 1), caddis larvae were more often found in rheocrene (7) than in limnocrene (4) springs, and were also more abundant in rheocrenes (Fig. 2a), with on average 7.7 ± 6.21 individuals per Surber sample in rheocrenes compared to 1.8 ± 1.30 in limnocrenes.

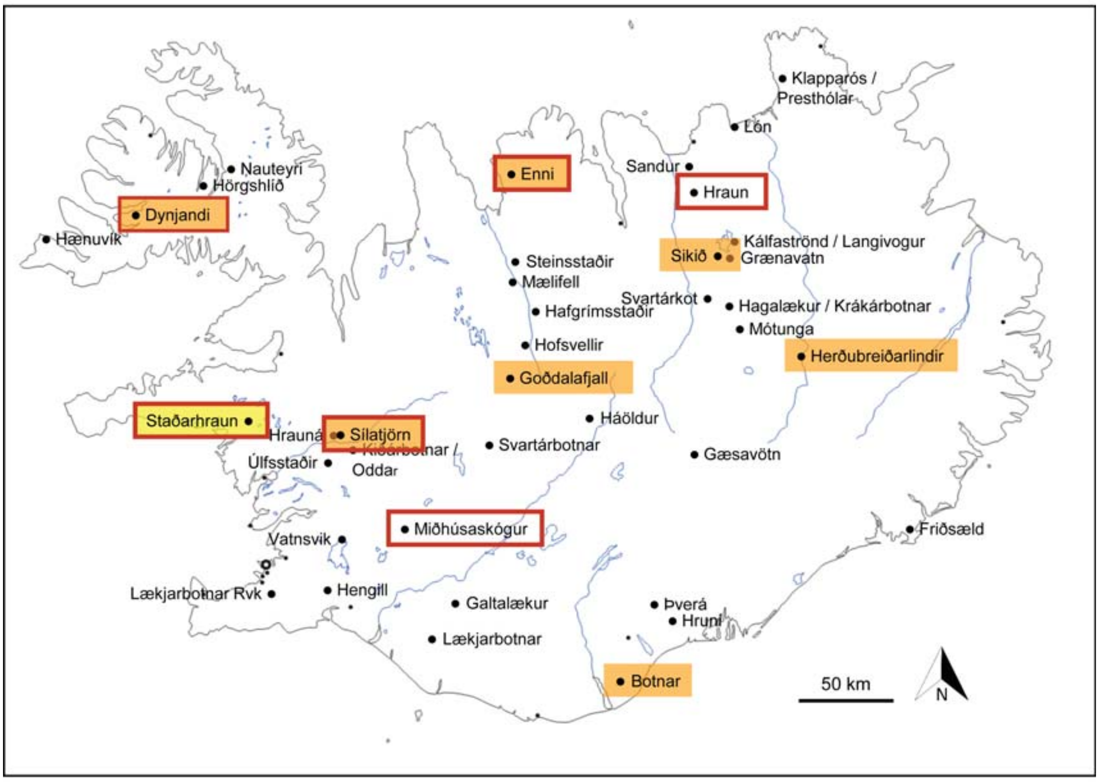

FIGURE 1. Map of sampling sites in springs in Iceland. Springs where Trichoptera were found in this study are highlighted. Orange fill indicates occurrence of *L. griseus*, yellow fill occurrence of both *L. griseus* and *L. affinis*, and red outline indicates occurrence of *A. zonella*.

TABLE 1 Relationship between presence/absence and abundance of Trichoptera larvae from Icelandic springs and environmental properties of the spring. Generalized linear models with binomial distribution were used for presence/absence, and with poisson distribution for abundance (see text). The slope b of the regression, standard error, z-values and p-values are shown. Asterisks (*) behind p-values indicate level of significance, where * > 0.01%, and ** > 0.001%.

Variable	**b (slope)**	**Std. Error**	**z**	**p**
Presence/absence:				
Spring type	-0.036	0.8226	-0.044	0.965
Temperature	-0.312	0.3031	-1.030	0.303
Substrate	-0.869	0.8460	-1.027	0.304
Plant input	1.373	0.7785	1.764	0.078
Abundance:				
Spring type	-0.658	0.4422	-1.488	0.137
Temperature	-0.732	0.3037	-2.412	0.016 *
Substrate	-1.107	0.3914	-2.828	0.005 **
Plant input	1.824	0.6293	2.899	0.004 **

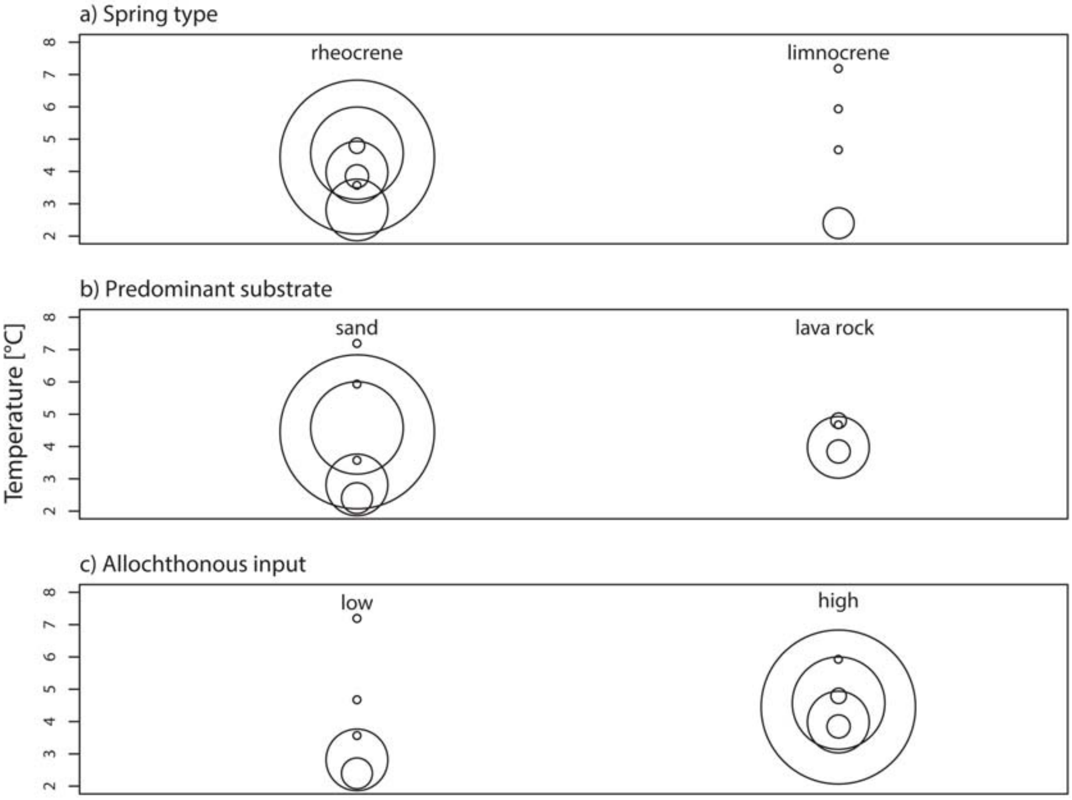

FIGURE 2. Abundance of Trichoptera larvae in springs of different water temperatures depending on (a) spring type, (b) predominant substrate and (c) amount of allochthonous plant material at the site. Water temperatures of the springs plotted on the y-axis. Circles are proportional to the number of Trichoptera larvae in the samples.

Discussion

All three Trichoptera species found in springs in this study were previously recorded for spring-fed rivers in Iceland (Gíslason 1981a). In a study in Þjórsárver in the Central Highlands of Iceland (Gíslason *et al.* 1990), *A. zonella, L. affinis*, and *L. griseus* were the only caddis larvae found in rheocrene springs and their effluent streams with temperatures around 5°C. The most common Trichoptera species, *L. griseus*, is known to inhabit a wide range of habitats in Iceland. It occurs mainly in lakes and rivers but also in ponds and marshes (Gíslason 1981a), and feeds on plant material and detritus. The second limnephilid species, *L. affinis,* has likewise a wide choice of habitats in Iceland and has been recorded from springs especially at freshwater springs with influence from the sea (Gíslason 1978). The main food item of the latter species is plant material and detritus. The third species found in springs, *A. zonella*, is very frequent in Icelandic running waters (Gíslason 1981a), feeding mainly on diatoms (Gíslason & Sigfússon 1987).

The occurrence of *A. zonella* in springs in North-Iceland is presumably associated with the fact that the omnivorous limnephilid *Potamophylax cingulatus* (Stephens 1837) does not occur in springs. Predation pressure from *P. cingulatus* may exclude *A. zonella* from rivers and streams in large parts of Iceland (Gíslason 1981b). *Potamophylax cingulatus* was first found in East-Iceland in 1959 and has spread from there, expanding its distribution over East- and Northeast-Iceland in the 1970s and by 2008 occurred all over the country with the exception of the Central Highlands (Gíslason *et al.* 2015). Although it is now common in spring-fed streams in Iceland, *P. cingulatus* has not been found there in springs. This could be due to limited food availability in springs. In Iceland, *P. cingulatus* preys on Chironomidae larvae and larvae of *A. zonella*, likely causing the exclusion of this species in streams formerly inhabited by it (Gíslason 1981b). Icelandic springs are oligotrophic, especially close to the source, with generally low primary production. Chironomidae larvae are present and abundant, mostly in early instars (Kreiling, personal observation), but might not be sufficient as a food source for *P. cingulatus*, for which the larva is now Iceland's largest stream-dwelling caddis. For the diatom-feeding larvae of *A. zonella*, springs could act as refugia from predation by *P. cingulatus* in areas where *A. zonella* has been otherwise displaced.

On the global scale, Trichoptera are one of the most species-rich groups of aquatic insects found in springs (Erman & Erman 1995; Myers & Resh 2002; Maiolini *et al.* 2011). The low Trichoptera diversity found in Icelandic springs in this study can partly be explained by the low regional species pool. In general, Trichoptera diversity on islands in the North Atlantic decreases with latitude and with distance to the mainland (Gíslason 2005): One caddis species is reported from Svalbard, 12 from Iceland, 20 from the Faroes, 37 from the Shetlands, compared to 193 species known from Norway. Furthermore, one has to bear in mind that the three species found in springs in Iceland represent 25% of the country's Trichoptera fauna.

Whether Trichoptera larvae were present or absent in a spring was not associated with the measured habitat properties (Table 1). However, the presence of caddis larvae in springs was clearly limited by water temperature. No Trichoptera were found at temperatures above 7.2°C, whereas other aquatic insects such as Chironomidae larvae are common and abundant in Icelandic springs even at temperatures as high as 40°C (Kreiling *et al.* 2018).

Although this study was limited to the low number of springs in which caddis larvae were found, the abundance of Trichoptera larvae could be linked to certain habitat properties. Abundance was higher in colder springs and decreased with increasing temperature (Table 1).

Trichoptera were more abundant in springs with sandy bottom compared to springs with lava rock as the predominant substrate (Fig. 2b, Table 1). In Iceland, all three species found in this study are quite opportunistic in their choice of case building material, especially in the early instars, and sand grains are commonly used (Gíslason, personal communication). Appropriate case building material is thus literally everywhere in springs with predominantly sandy substrate but rare on bare lava rock. Substrate composition, including amount of leaf litter, has been shown to influence the macrofauna diversity in springs (von Fumetti *et al.* 2006). Larval abundance was also related to the amount of potential allochthonous material entering the springs, with more caddis larvae in springs with high plant input (Fig. 2c, Table 1). However, the classification of springs into sites with low and high allochthonous input, respectively, was based only on estimation of vegetation density around the spring and allochthonous material in the spring itself was not directly measured in this study. Although Trichoptera abundance was not statistically associated with either of the spring types (Table 1), it was clearly higher in rheocrene than in limnocrene springs (Fig. 2a). Invertebrate abundances are often reported to be higher in rheocrene than in limnocrene springs (Maiolini *et al.* 2011), and rheocrene springs hold higher species richness compared to limnocrene springs, which is the case for both aquatic invertebrates in general (e.g., Ilmonen & Paasivirta 2005; Maiolini *et al.* 2011) and Trichoptera in particular (Cianficconi *et al.* 1998).

In this study, we found a very simple Trichoptera community in springs in Iceland, and were able to link caddis larvae abundance to some habitat properties. However, we were restricted to very few environmental variables, and did not measure other potentially important factors such as flow velocity, algal production, or isolation from other water bodies. It could be worth studying this extremely simple faunistic system in more detail, to shed light on the mechanisms structuring species distribution in freshwater springs.

Acknowledgements

Thanks to Snæbjörn Pálsson for advice in statistical methods and to Leivur Janus Hansen, Ragnhildur Guðmundsdóttir and Hildur Magnúsdóttir for helpful discussions and comments on the manuscript. The project was funded by the Icelandic Research Fund, grant nr. 141863-051.

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5 Paper IV

Seasonal variation in the invertebrate community and diet of a top fish predator in a thermally stable spring

Kreiling, A.-K., O'Gorman, E.J., Pálsson, S., Benhaim, D., Leblanc, C.A.L., Ólafsson, J.S. & Kristjánsson, B.K. (2020).

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Authors contribution: AKK, EJO, SP, DB, CAL, JSÓ, and BKK contributed to the design of the study. AKK, DB, CAL, and BKK conducted the fieldwork. AKK processed the invertebrate samples. AKK and SP analysed the data with contributions from EJO and BKK. AKK, EJO, SP, DB, CAL, JSÓ, and BKK wrote the manuscript.

Hydrobiologia
https://doi.org/10.1007/s10750-020-04409-5

PRIMARY RESEARCH PAPER

Seasonal variation in the invertebrate community and diet of a top fish predator in a thermally stable spring

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Received: 7 January 2020 / Revised: 28 August 2020 / Accepted: 7 September 2020

Abstract Many life-history events in aquatic invertebrates are triggered by seasonal changes in water temperature, but other ecological factors may be important as well. To rule out the confounding effects of changing water temperature, we studied the seasonal dynamics of an aquatic invertebrate community and their effect on a top fish predator in a thermally stable freshwater spring in South Iceland. We sampled benthic invertebrates five times over a year and conducted a mark-recapture study on the top predator in the system, small benthic Arctic charr, *Salvelinus alpinus* (L.). We assessed variation in diet composition and feeding preferences by calculating the electivity and individual specialisation of each fish at each sampling time. There was a clear separation of winter and summer communities for the benthic invertebrates. The variation in prey availability was also reflected in the fish diet, with higher feeding selectivity in summer than in winter for the highly abundant Chironomidae larvae. In contrast, individual specialisation as a measure of intrapopulation niche variation was higher in winter when prey availability was lower. We furthermore found that groundwater amphipods might play an important role in the winter diet of spring-dwelling Arctic charr. In conclusion, seasonal variation in the invertebrate community is an important factor to consider and has the potential to alter the phenotype (e.g. growth rates) and behaviour (e.g. feeding preferences) of higher trophic levels.

Keywords Feeding selectivity · Individual specialisation · Arctic charr · *Salvelinus alpinus* · Phenology

Handling editor: Katya E. Kovalenko

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Introduction

Temperature determines the metabolic demands of all ectothermic organisms (Brown et al., 2004). Water temperature is thus a crucial factor shaping individual life-histories, community composition, and food webs in freshwater ecosystems. Seasonal temperature change is a cue that triggers life-history events in many aquatic invertebrates (e.g. emergence and diapause) (Ward & Stanford, 1982). Altered temperatures can also lead to changes in community composition and food web structure in freshwater ecosystems (Petchey et al., 1999; Burgmer et al., 2007; Shurin et al., 2012; O'Gorman et al., 2017, 2019). For example, increasing temperature can lead to greater fish production, driven by behavioural changes in feeding selectivity and altered energy flow through the food web (O'Gorman et al., 2016).

Because temperature affects organisms on so many different levels, it can be hard to disentangle temperature effects from other variables in ecological field studies. Here, we take advantage of a natural setting in which temperature is independent of major seasonal changes, a thermally stable groundwater-fed spring, to study the seasonal variation in its invertebrate community and the diet of a fish predator. Freshwater springs are stable environments with little seasonal fluctuation in temperature, pH, conductivity, and oxygen concentration (van der Kamp, 1995; Szczucinska & Wasielewski, 2013). Nevertheless, there are clear seasonal differences in the aquatic invertebrate communities of springs (Nolte, 1991; Bottazzi et al., 2011; Berlajolli et al., 2019). These seasonal differences are mostly driven by aquatic insects with an aerial dispersal stage (e.g. Diptera, Trichoptera, Plecoptera), whose larvae dominate the invertebrate community in spring and summer, whereas wholly aquatic taxa (e.g. Ostracoda, Copepoda, aquatic Coleoptera) are relatively more abundant during the winter months. Thus, the summer and winter invertebrate communities in springs are notably different (Berlajolli et al., 2019). Seasonal variation in invertebrate abundance and community structure can have implications for higher trophic levels. Prey availability can affect a predator in various aspects and at various life stages, for example in relation to morphology (Kristjánsson et al., 2012; Kristjánsson & Leblanc, 2018), growth rates (Amundsen et al., 2007; O'Gorman et al., 2016), and feeding behaviour (Emlen, 1966; O'Gorman et al., 2016).

Optimal foraging theory predicts feeding strategies to be a trade-off between resource quality, distribution, and abundance (Emlen, 1966; Stephens & Krebs, 1986). When prey availability in the environment is scarce, a generalist predator could consume a broad selection of the mixed quality prey it encounters, minimising time spent foraging; or it may invest more effort into searching for nutrient-rich prey, maximising the energy gained from a smaller quantity consumed (Schoener, 1971). When prey availability is high, it might be even more beneficial to actively select prey items with the highest energetic gain (Emlen, 1966). In that case, we would expect a consumer to feed more selectively when prey abundance is high. Selective feeding behaviour, where consumers actively choose high quality resources over more abundant, lower quality resources has been documented for a wide range of animals, including insects (Haslett, 1989; Klecka & Boukal, 2012), birds (Zwarts & Blomert, 1990; Schaefer & Schaefer, 2006), mammals (Evans et al., 2006; Jones & Norbury, 2011), and fishes (Uieda & Pinto, 2011; Tófoli et al., 2013; O'Gorman et al., 2016).

To evaluate resource use at the individual level, one can compare the diet of an individual to the food availability in the environment, for example using indices of feeding selectivity. Another approach is to calculate the individual diet specialisation, which measures the proportion of the dietary niche of the whole population used by an individual (i.e. the intrapopulation niche variation) (Bolnick et al., 2002). To estimate individual diet specialisation, an individual's diet is compared to the population's diet, rather than to food availability in the environment. Quantifying diet with both methods gives the best overview of individual variation in resource use. Individual diet specialisation is common in many animal groups (Araújo et al., 2011) and likely ubiquitous in nature. But the levels of individual specialisation may vary among populations of the same species (Bolnick et al., 2003, Araújo et al., 2011, Kristjánsson & Leblanc, 2018), and depend on a variety of ecological factors such as intra- and inter-specific competition, predation, parasitism, and diversity and availability of resources (Darimont et al., 2007; Costa et al., 2008; Araújo et al., 2011; Svanbäck et al., 2011; Tinker et al., 2012; Britton & Andreou, 2016). There is evidence that individual diet specialisation is flexible within the same individual and varies with environmental conditions (Zango et al., 2019). For example, diet specialisation has been found to be higher when resource availability is low (Svanbäck et al., 2011).

Individual specialisation has been studied in northern freshwater fishes, including Arctic charr [*Salvelinus alpinus* (Linnaeus, 1758)] (Kristjánsson & Leblanc, 2018). Arctic charr is known for its high diversity (Klemetsen, 2013), and several discrete morphs often occur in sympatry, differing in morphology (Skúlason et al., 1989), genetics (Skúlason et al., 1996), preferred habitat (Kristjánsson et al., 2012), and diet (Malmquist et al., 1992). One of the morphs, the small benthic (< 15 cm adult length), is often found in springs and spring-fed streams and ponds (Kristjánsson et al., 2012), and represents one of the top predators in Icelandic freshwater springs.

In this study, we investigated how seasonal variation in abundance and composition of an aquatic invertebrate community affects a top fish predator, independent of the confounding effects of seasonal temperature change. We hypothesised that: (1) the benthic invertebrate community varies between seasons, with distinct winter and summer communities; (2) changes in invertebrate community composition are reflected in the diet of Arctic charr; and (3) feeding selectivity of Arctic charr is higher and individual specialisation lower in seasons with high invertebrate abundance.

Materials and methods

Sampling site

The study site was the spring of the small stream Skarðslækur (64°00.306′ N 20°07.110′ W, 103 m asl) in South Iceland. Groundwater emerges from several distinct sources and runs off as shallow streams (depth < 0.5 m), merging into a single main channel a few meters downstream. The substrate of the stream is mainly comprised of lava rock near the spring sources and smaller stones, gravel, and sand farther downstream. Small benthic Arctic charr are common in the spring region and even seek shelter in the sources (Kristjánsson et al., 2012). Arctic charr are seemingly scarce in the main channel, where the substrate is predominated by sand. The total surface area fished at each sampling occasion was 333 m^2, subdivided into six smaller sampling zones: a, b, c, d, e, and f (Fig. 1), which differed in substrate characteristics and number of spring sources. Temperature, electric conductivity, oxygen saturation, and pH of each sampling location were measured on each sampling occasion, using a Hydrolab DS5 multi-probe sonde (Hach Hydromet, Loveland, CO, USA). In addition, a HOBO temperature logger (Onset Computer Corporation, Bourne, MA, USA), measuring at 2-h intervals, was placed at the site for 1 year to provide a detailed thermal profile.

Fish processing

We conducted a mark-recapture study between May 2017 and March 2018, visiting the site five times during that period (in May, June, August, October 2017, and March 2018). On each sampling occasion, fish were captured by electrofishing and processed on-site. Fish were anesthetised with 2-phenoxyethanol (300 ppm), and fork length and wet weight were measured for every individual. Stomach contents were collected for diet analysis of fish with a fork length ≥ 55 mm by inserting a plastic catheter attached to a syringe through the mouth as far as the anterior part of the stomach and flushing its content out with clear water (Meehan & Miller, 1978). Invertebrates in the stomach samples were preserved in 70% ethanol until identification. Fish with a fork length ≥ 65 mm were then tagged by implanting a Passive Integrated Transponder (PIT tag, 12 mm HDX, Oregon RFID, Portland, OR, USA) through a small incision into the abdominal cavity upon first capture. Each PIT tag has a unique code which can be read by a handheld field scanner (Tags4all Inc., Mitchell, Ontario). After processing, the fish were allowed to recover before they were released back into the spring at the location where they were caught.

Population density, biomass, and growth rate of Arctic charr

Population size (N) of Arctic charr was estimated using the Petersen estimator $N = \frac{m \times c}{r}$, with m = total number of marked fish in the system, c = number of fish captured, and r = number of marked fish recaptured (Robson & Regier, 1964). Population size was calculated for June, August, and October, and averaged. We did not estimate population size for March, due to a very low recapture rate of only four fish, and because numbers would have been biased due to removal of fish from the spring for behavioural studies in the laboratory after the sampling in October. Population density (D) in the spring was then estimated as $D = N/A$, with A = surface area fished. Population biomass (B) was calculated as $B = \bar{M} \times D$, with $\bar{M}$ = mean body mass during sampling event.

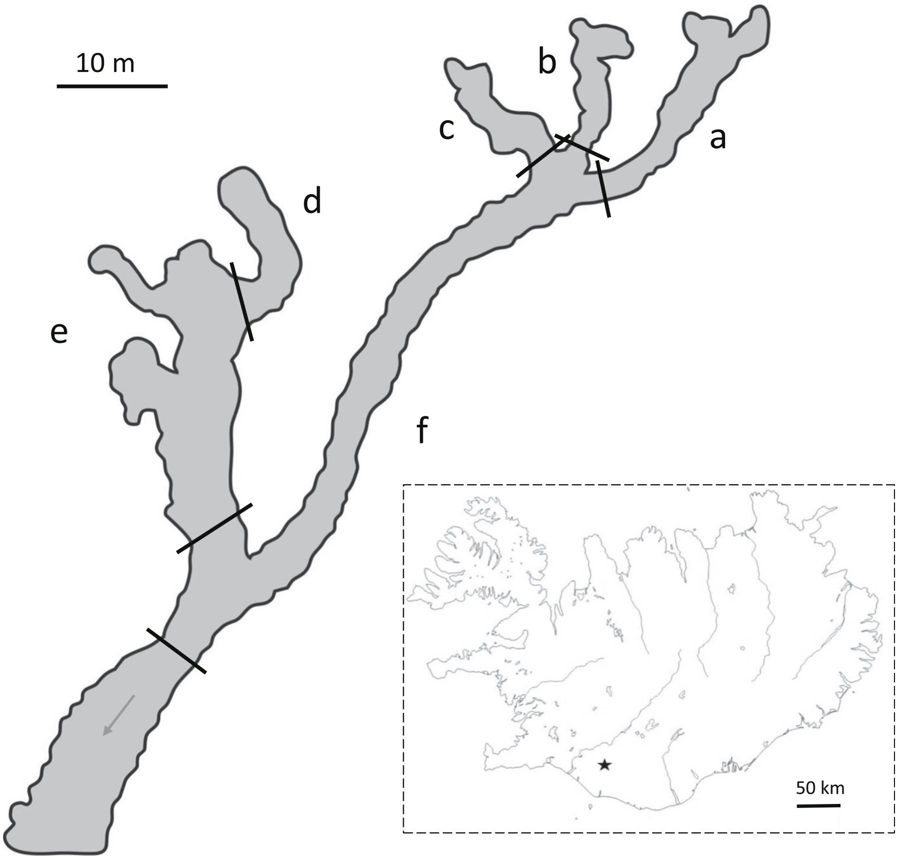

Fig. 1 Map of the spring region of the study stream, Skarðslækur in South Iceland. Lines and letters (a, b, c, d, e, f) indicate division of the site into different sampling zones. The star in the figure inset shows the location of the sampling site in South Iceland

Growth rate (G) between sampling events was calculated as $G = \frac{M_2 - M_1}{t}$, with M_1 and M_2 as initial and final weight of recaptured fish in grams, and t = number of days between sampling events. We calculated the body condition of the fish according to Barnham & Baxter (1998) as $K = \frac{10^5 \times W}{L^3}$, with W = weight in grams and L = fork length in millimetres.

Invertebrate availability and diet composition

To study seasonal variation in the invertebrate community and estimate prey availability in the habitat, we collected invertebrates from the benthic substrate of the spring. Due to logistical reasons, samples were only taken at two zones of the spring (a and d, Fig. 1) on each sampling occasion, using a 0.093 m^2 Surber sampler with 63 μm mesh. Arctic charr feed in a variety of microhabitats in streams, such as stony substrate, macrophytes, and near the banksides. Thus, one Surber sample was collected from each of these three microhabitats and pooled, resulting in a sampled surface area of 0.279 m^2 per sample. Samples were stored in 70% ethanol until processing.

Invertebrates in the samples were counted and pooled into nine groups to facilitate comparison between the benthos and the diet of Arctic charr, i.e. there would be too many zeros in the dataset if comparisons were performed on a species-level basis (see also O'Gorman et al., 2016). The nine prey groups were: Chironomidae larvae, Ostracoda, Copepoda, Acarina, Trichoptera larvae/Plecoptera nymphs, predatory Diptera larvae [not including Chironomidae, and largely consisting of *Limnophora riparia* (Fallén, 1824)], Oligochaeta (including earthworms), aerial insects (largely consisting of adult stages of Diptera, Plecoptera, and Trichoptera), and miscellaneous rare taxa (including Cladocera, Amphipoda, aquatic Coleoptera larvae and adults, and

Collembola). Note that organisms in the miscellaneous group were occasionally found in fish stomachs, but were absent in the benthic samples (except for Cladocera). Since the aerial and miscellaneous groups were not systematically sampled and only stochastically represented in the benthic samples, a comparison between their relative abundance in the fish diet and in the environment was not possible, and they were thus excluded from the analysis of feeding selectivity. Fish with empty stomachs ($n = 61$ out of 692) were also excluded from further analysis.

The selectivity (S_i) of Arctic charr feeding on each of the prey groups was calculated as:

$$S_i = \frac{d_i/b_i}{\sum_{j=1}^{n} d_j/b_j},$$

where d and b were the relative abundance of each prey group in the diet and in the benthic invertebrate samples, respectively (Chesson, 1983). We then calculated the Relativized electivity index E_i (Vanderploeg & Scavia, 1979; Lechowicz, 1982), as:

$$E_i = \left(S_i - \frac{1}{n}\right) / \left(S_i + \frac{1}{n}\right),$$

where n = number of prey groups available. Values of E_i range from -1 to $+1$, with negative values indicating avoidance of prey, positive values indicating selection, and a value of zero indicating random feeding.

To estimate individual specialisation (i.e. the diet overlap between an individual and the population as a whole), we used the proportional similarity index:

$$PS_i = 1 - 0.5 * \sum_j |p_{ij} - q_j|$$

where p_{ij} is the proportion of the j-th prey group consumed by the population which is also consumed by individual i, and q_j is the proportion of the j-th prey group in the population's diet (Schoener, 1968; Feinsinger et al., 1981; Bolnick et al., 2002). PS_i values range from 0 (strong specialisation) to 1 (generalisation). We then calculated the population-wide prevalence of individual specialisation, IS, as the average of an individual‘s PS_i values for each sampling month. S_i, E_i, and PS_i were calculated only for fish with prey items in their stomachs.

Statistical analysis

All statistical analyses were performed in R 3.4.1 (R Core Team, 2016). Multivariate analyses were required throughout because changes in the relative abundance of, electivity for, or individual specialisation on a particular prey group will implicitly result in altered relative values of one or more of the other prey groups. Differences in the benthic invertebrate community in the spring and in the diet composition of Arctic charr between sampling months were analysed using a permutational multivariate analysis of variance (PERMANOVA; *adonis* function in the *vegan* package; Oksanen et al., 2017). A similarity percentage analysis (SIMPER; *simper* function in the *vegan* package) based on Bray–Curtis distances was performed to compare taxonomic differences in invertebrate community and diet composition between the five sampling months. The effects of sampling month, sampling zone in the spring, body length, and body condition of fish (explanatory variables) on the feeding electivity, E_i, and individual specialisation, PS_i, (response variables) were tested with a PERMANOVA and visualised with non-metric multidimensional scaling (NMDS). The weighted averages of the prey groups were calculated (*wascores* function in the *vegan* package; Oksanen et al., 2017) and shown in the NMDS plots to identify prey groups that were driving electivity and individual specialisation. Dissimilarity matrices were based on Euclidean distances, which is appropriate when zero-values (here: no selectivity) are relevant to the hypothesis being tested (Clarke et al., 2006).

Results

Environmental variables

The water temperature of the spring was extremely stable over the course of the year at 5.5 ± 0.26°C (mean ± standard deviation). The lowest temperature measured was 4.8°C and the highest was 7.4°C. The daily fluctuation in water temperature ranged from 0.03 ± 0.048°C in January to 0.60 ± 0.487°C in July. Other environmental variables were also similar on the five sampling occasions, with average oxygen saturation of 75.2 ± 0.99%, pH of 7.3 ± 1.03, and conductivity of 138 ± 29.7 μS cm^{-1}. This confirms the environmental and thermal stability of the Skarðslækur spring, providing a semi-controlled experimental setting.

Population density and growth rate of Arctic charr

Among a total of 472 tagged fish, we obtained 55 recaptures, with a few individuals recaptured multiple times. The population size (*N*) of Arctic charr in the spring region of Skarðslækur was estimated to be 1920 (± 244) fish, resulting in a population density of 5.8 fish m^{-2}. Fish were not captured evenly across the sampled area but mainly close to the sources. Population biomass (*B*) was highest in May at 32.48 g m^{-2}, and lowest in March at 22.04 g m^{-2} (Table 1). Average growth rates were highest between June and August at 0.13 ± 0.103 mm d^{-1}, as opposed to 0.09 ± 0.060 mm d^{-1} between August and October, and 0.04 ± 0.031 mm d^{-1} between May and June.

Seasonal variation in invertebrate community

The total abundance of invertebrates in the benthic samples varied over the year, ranging from 1306 in May to 5967 in June (Table 1). Chironomidae larvae were by far the most abundant group on all five sampling occasions, reaching up to 4591 individuals in June. The second most abundant group were Ostracoda, with a maximum abundance of 587 in August. Other invertebrate groups were less abundant but still exhibited clear seasonal changes, for example larvae of Trichoptera/Plecoptera and predatory Diptera were most abundant in October, while Copepoda and Acarina peaked in June. The invertebrate community in the spring differed between sampling months, supporting our first hypothesis ($Fp = 3.80$, $r^2 = 0.75$, $P = 0.022$, $Df_{Month} = 4$, $Df_{Residuals} = 5$; Fig. 2a). The highest average between-groups dissimilarities based on the SIMPER analysis were between June and March (65.9%) and May and June (64.8%), the lowest between August and October (15.7%). Dissimilarities of pairwise comparisons between the other months ranged from 31.7% (May–March) to 45.7% (August–March). The taxa contributing most to these differences were Chironomidae larvae, followed by Oligochaeta (Fig. 2a).

Diet composition, feeding selectivity, and individual specialisation of Arctic charr

The most abundant prey groups in the stomach samples were Chironomidae larvae (with a total of 15 233 in 635 Arctic charr stomachs), followed by aerial insects (1714), the miscellaneous group (150), predatory Diptera larvae (1226), and Ostracoda (826). As for the benthic invertebrate community, there was seasonal variation in the abundance of prey groups in the diet, for example the highest abundance of Ostracoda and Acarina occurred in March (Fig. 2b). The miscellaneous group in the diet was dominated by groundwater amphipods in March, but consisted mainly of Cladocera and aquatic Coleoptera in all other sampling months. The proportion of fish caught with empty stomachs was higher in winter and early summer, with 12% in March and 16% in May, compared to 6–7% in June, August, and October. Note that these patterns mirror total invertebrate abundance in the environment (Table 1). For fish with

Table 1 Temporal distribution of invertebrates in the benthic habitat of the spring and in the stomachs of Arctic charr

Month	Total invert.	Total stomachs	Prey items	Prey groups	*B* [g*m^2]	PS_i
May	1306	99 (16 empty)	34 ± 44.5	2.3 ± 1.12	32.48	0.71 ± 0.22
June	5967	181 (13 empty)	22 ± 42.6	2.2 ± 1.22	29.00	0.69 ± 0.16
August	3318	146 (11 empty)	44 ± 75.2	2.6 ± 1.23	30.74	0.76 ± 0.14
October	3000	183 (11 empty)	33 ± 50.9	3.0 ± 1.47	31.32	0.65 ± 0.16
March	1379	83 (10 empty)	19 ± 28.9	2.6 ± 1.28	22.04	0.44 ± 0.16

Columns include the total number of invertebrates in the benthic samples (Total invert.), the total number of fish stomachs examined (with number of empty stomachs in brackets), average number of prey items per fish (± standard deviation), average number of prey groups per fish (± standard deviation), population biomass (B) [g*m^2], and average individual specialisation (PS_i ± standard deviation) in the diet. The values of PS_i range from 0 (strong specialisation) to 1 (generalisation)

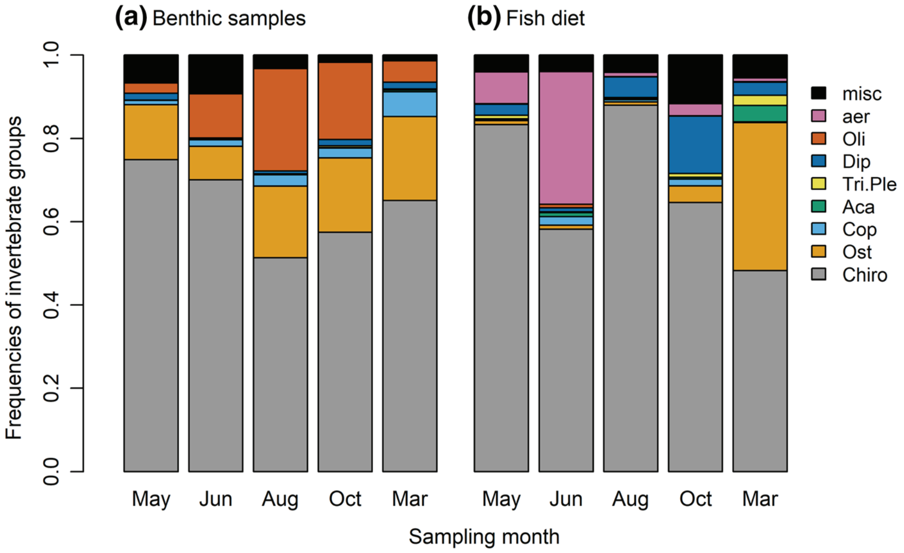

Fig. 2 Invertebrate composition in **a** the benthic samples and **b** Arctic charr stomachs over the five sampling months. The number of stomachs sampled in each month is shown in Table 1. Prey groups are abbreviated in the legend as follows: Chiro (Chironomidae larvae), Ost (Ostracoda), Cop (Copepoda), Aca (Acarina), Tri.Ple (Trichoptera/Plecoptera larvae), Dip (predatory Diptera larvae), Oli (Oligochaeta), aer (aerial insects), and misc (miscellaneous)

prey in their stomachs, the average number of prey items per individual (± standard deviation) was 31 ± 53.6, belonging to 2.5 ± 1.32 of the nine invertebrate groups (Table 1). The diet composition of Arctic charr differed between sampling months, supporting our second hypothesis (Fp = 27.56, $r^2 = 0.16$, $P = 0.001$, $\mathrm{Df}_{\mathrm{Month}} = 4$, $\mathrm{Df}_{\mathrm{Residuals}} = 599$; Fig. 2b). The highest dissimilarities in the diet composition were between May and June (64.9%) and June and March (65.9%), and the lowest between August and October (15.7%) (SIMPER analysis).

The large number of Chironomidae larvae in the fish stomachs reflected their abundance in the benthic environment, and they were often proportionally higher in the diet compared to the benthos (Fig. 3a). Invertebrate groups such as Acarina, Trichoptera/Plecoptera larvae, and predatory Diptera larvae, were also proportionally high in the diet compared to the benthic samples (Fig. 3d, e, f). On the other hand, Ostracoda and Oligochaeta were proportionally low in the diet on all sampling occasions (Fig. 3b,g) despite their high abundance in the benthic samples. This suggests selective feeding of fish, but median values of E_i were negative (indicating avoidance of prey) for all prey groups and all sampling months, except for Chironomidae larvae in June (Fig. 4). However, there was large variation in E_i among fish (Fig. 4), and several individuals showed feeding preferences for Chironomidae larvae throughout the year (Fig. 4a), for predatory Diptera larvae in August, October, and March (Fig. 4f), and for Trichoptera/Plecoptera larvae (Fig. 4e) in March. There was no clear general pattern linking feeding selectivity to total invertebrate abundance in the environment, in contrast to our third hypothesis (Table 1). While fish preferred some prey groups such as Chironomidae, Copepoda, and Oligochaeta in June (when total invertebrate abundance was high), there was a stronger preference for Ostracoda and Trichoptera/Plecoptera in March (when total invertebrate abundance was low).

Sampling months, sampling zone in the spring, and body length of fish contributed to the individual variation in relative electivity between fish (Table 2a). Body length was positively correlated with a preference for predatory Diptera larvae (Fig. 5a). Although body condition was marginally non-significant in the PERMANOVA (Table 2), it was positively correlated with a preference for Trichoptera/Plecoptera larvae and predatory Diptera larvae (Fig. 5a).

Average individual specialisation (PS_i) was much higher in March than during the summer, as reflected by a low PS_i value (Table 1), indicating that individuals in the population became more specialised in winter than in summer. The highest individual

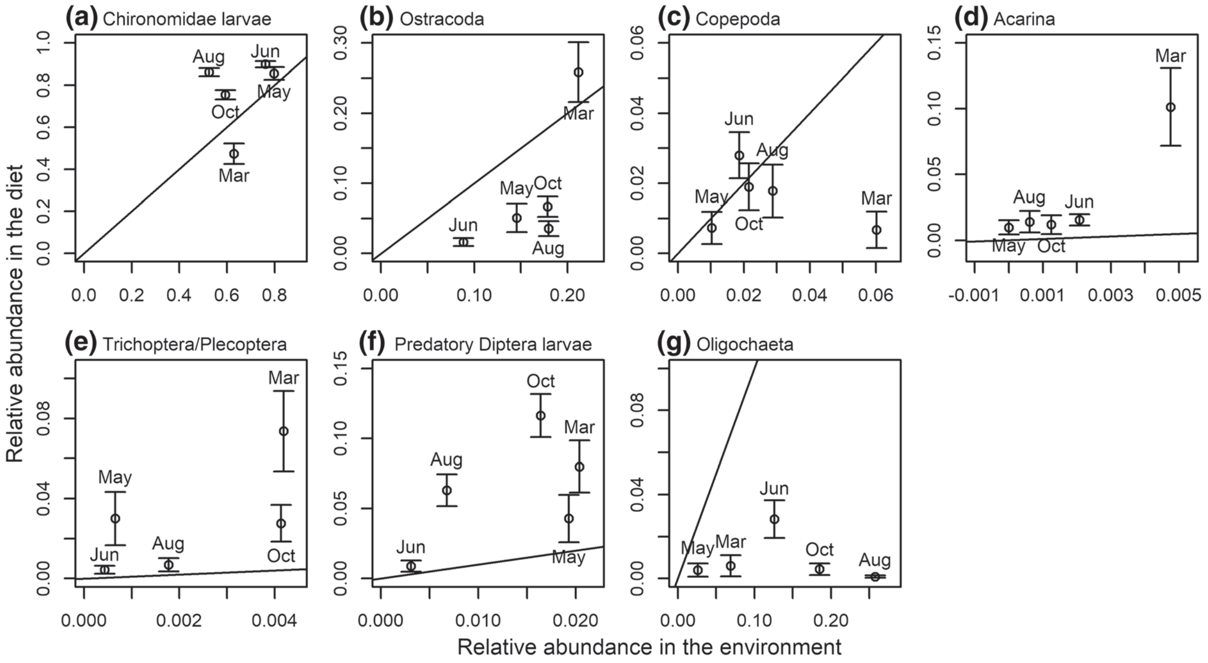

Fig. 3 Relationship between invertebrate groups in the diet of Arctic charr and their availability in the benthic environment. The average proportion of invertebrate groups in the stomach samples (mean ± standard error) are plotted against the average proportion of those groups in the benthos for each of the five sampling months, which are indicated with a label next to the data point that corresponds to each sampling month. The black line is a regression line with a slope of 1; points below the line indicate that the item is more abundant in the environment than in the diet; points above the line indicate that the item is more abundant in the diet than in the environment. Note that aerial insects and the miscellaneous group were not adequately sampled in the environment and thus are not shown in the figure

specialisation in March coincided with the time of the lowest invertebrate abundance, partially supporting our third hypothesis (Table 1). Variation in individual specialisation for the nine prey groups was influenced by sampling months, sampling zone in the spring, and body length of fish, but not by body condition (Table 2b). Smaller fish in the population specialised more on Ostracoda and Acarina as food items, whereas bigger fish specialised more on Oligochaeta, particularly large earthworms (Fig. 5b).

Discussion

The benthic invertebrate communities in Skarðslækur in winter (March) and spring (May) were analogous to each other, but very dissimilar from the communities in summer (June and August) and autumn (October), which were in turn very similar. This grouping into winter/spring and summer/autumn communities supports our first hypothesis and is in line with the results of a study on the seasonality of invertebrate communities in mountain springs in Kosovo (Berlajolli et al., 2019). The high dissimilarity between consecutive months in May and June suggests a rapid transition between spring and summer, possibly triggered by emergence and oviposition of aquatic insects with an aerial adult stage.

The seasonal variation in abundances seen in the benthic invertebrates can mostly be explained by the life-histories of the taxa. Many crustacean species (e.g. Cladocera and Ostracoda) undergo a clear seasonal periodicity, with very low abundances during the winter when reproduction ceases and populations consist mainly of resting stages (eggs and diapausing individuals) (Pennak, 1953). Similarly, many water mites (Hydrachnidia) are less active during winter months and most adults are generally found in spring and early autumn, though seasonal dynamics vary a lot among species (Pennak, 1953; Schmidt, 1969). Many aquatic insects with an aerial adult stage emerge in early spring to late summer, and oviposit shortly after.

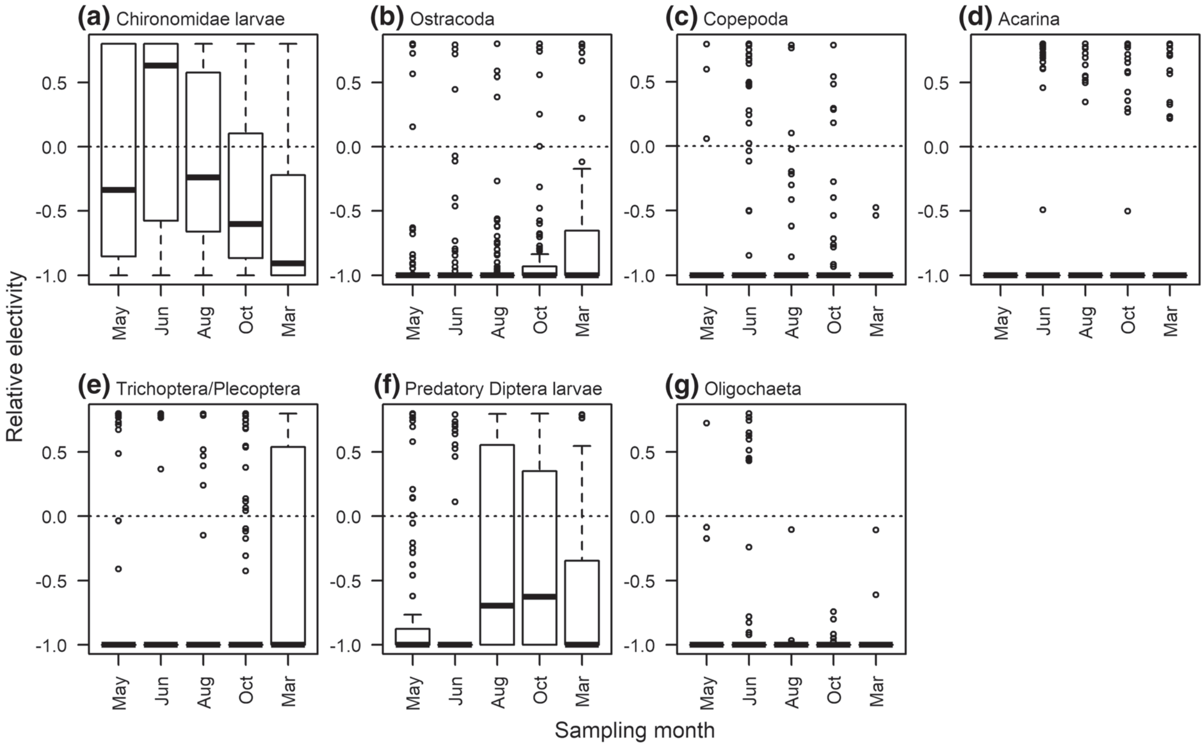

Fig. 4 Distribution of relative electivity E_i of Arctic charr on five sampling occasions (May, June, August, and October 2017, and March 2018). Boxplots show the interquartile range (box) of the data point distribution, minimum and maximum values (whiskers), the median (black line), and outliers (dots). A positive value (above the dotted line) of E_i indicates selectivity for the prey group, whereas a negative value (below the dotted line) indicates avoidance of the prey group. Note that aerial insects and the miscellaneous group were not adequately sampled in the environment and thus E_i was not calculated for these prey groups

Table 2 Dependency of explanatory variables on (a) relative electivity (E_i) and (b) individual specialisation (PS_i) of Arctic charr

Response variable	Explanatory variable	Df	Fp	r^2	*P*-value
(a) E_i					
	Sampling month	4	13.853	0.087	<0.001***
	Sampling zone	5	2.387	0.019	<0.001***
	Body length	1	5.533	0.009	<0.001***
	Body condition	1	1.970	0.003	0.082
	Residuals	559		0.882	
(b) PS_i					
	Sampling month	4	31.139	0.178	<0.001***
	Sampling zone	5	1.653	0.018	0.043*
	Body length	1	5.592	0.008	0.002**
	Body condition	1	0.527	0.001	0.727

Asterisks (*) indicate the level of significance for each *P*-value (where * < 0.05, ** < 0.01, and *** < 0.001)

For example, the flight and oviposition periods in Iceland for the dominant Trichoptera in the benthic samples, *Limnephilus griseus* (Linnaeus, 1758) and *Limnephilus affinis* Curtis, 1834, are between late March and October (Gíslason, 1992). All larval instars can be found during most of that time, and the October peak in abundance of Trichoptera larvae seen in our data marks the end of the reproductive season before the winter mortality of larvae begins (Gíslason, 1978, 1992).

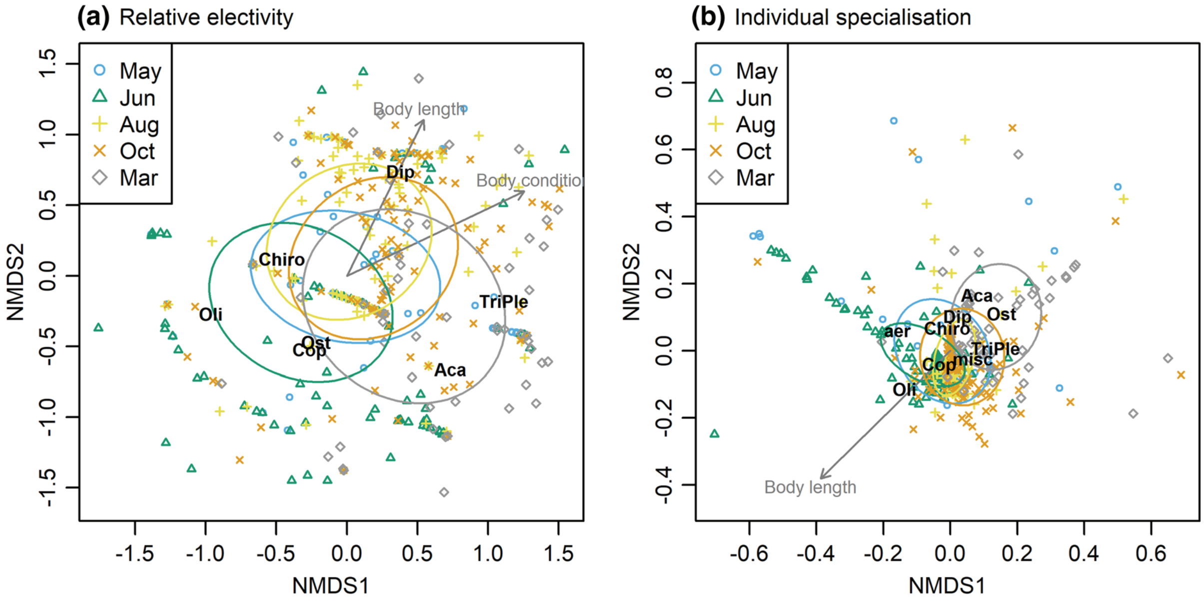

Fig. 5 Non-metric multidimensional scaling (NMDS) plots illustrating the (a) relative electivity (E_i) and (b) individual specialisation of Arctic charr in the study stream. The five sampling months are coded by symbols and colours (defined in the legends of each panel), with coloured ellipses indicating the confidence region for the centroids of each month. The weighted averages of prey groups are indicated with black text, and their correlations with body length and body condition of fish are indicated with grey vectors and labels. Body condition did not explain significant variation in individual specialisation (see Table 2), so no vector is shown. Note that the direction of a vector indicates whether prey groups are positively (or negatively) correlated with it and the further the centroid of a group is along (or in the opposite direction to) the vector, the stronger the correlation. Prey groups are abbreviated as follows: Chiro (Chironomidae larvae), Ost (Ostracoda), Cop (Copepoda), Aca (Acarina), Tri.Ple (Trichoptera/Plecoptera larvae), Dip (predatory Diptera larvae), Oli (Oligochaeta), aer (aerial insects), and misc (miscellaneous)

Since water temperatures were stable over the year in the studied spring system, invertebrate life-history events like hatching, emergence, or end of dormancy must be triggered by other cues. Multiple variables, both internal (e.g. sex, body size) and environmental (e.g. photo- or hydroperiod, chemicals in the environment), can interact to determine the onset of life-history events in invertebrates (Nylin & Gotthard, 1998; Shama & Robinson, 2006). Possible candidates for environmental cues acting on aquatic insects are changes in photoperiod (Lutz, 1974), primary production, and thus food availability for primary consumers (Anderson & Cummins, 1979).

The SIMPER analysis revealed the same pattern of pairwise dissimilarities between the diet composition of Arctic charr in different months as for the benthic invertebrate communities in those months, supporting our second hypothesis. The diet composition of Arctic charr was similar during winter/spring (March and May) and during summer/autumn (June, August, and October), but differed between these two periods. Dominant prey groups in the fish stomachs, such as Chironomidae larvae and Copepoda, partly reflected invertebrate availability in the environment (Fig. 3). The high proportion of aerial insects in the fish diet in June (Fig. 2b) coincided with emergence and oviposition of most aquatic insects (Hannesdóttir et al., 2013), during which fish can easily pick them off from the water surface. Mass emergences of Chironomidae are known to be a critical energy subsidy for fish and ducks (Einarsson & Gardarsson, 2004; Einarsson et al., 2004).

There was no clear evidence that Arctic charr exhibited stronger feeding selectivity for invertebrate groups when their availability in the environment was high, in contrast to our third hypothesis. One clear exception to this was for Chironomidae in June, coinciding with their highest absolute abundance in the environment (Fig. 4a). Note that many species of Chironomidae larvae are in their last and largest larval stage before pupation around this time of year (Hannesdóttir et al., 2013), and thus are potentially more attractive prey items. In contrast, the feeding preference of fish for Trichoptera/Plecoptera larvae was highest in March, when general invertebrate availability was low (Figs. 3e,4e). Arctic charr also tended to prefer predatory Diptera larvae in August and October (Figs. 3f,4f), even though they were among the rarest invertebrate groups in the environment (Fig. 2). The protein-rich diet of predatory Diptera larvae may make them a more profitable food source than primary consumers (Cummins & Klug, 1979; O'Gorman et al., 2016). In support of this, fish with a better body condition were found to prefer predatory Diptera larvae, whereas fish with a feeding preference for Chironomidae larvae were associated with a poorer body condition (Fig. 5a). It seems that fish are not achieving the best body condition if they feed on the small, abundant prey in the system, but rather when they feed selectively on large, rare, and more energetically valuable resources. Similarly, brown trout (*Salmo trutta* Linnaeus, 1758) has been shown to invest more time in finding large, rare prey in energetically demanding environments (O'Gorman et al., 2016). Body condition could thus be a direct result of the choice of prey, or the choice of prey may be dictated by constraints imposed by body condition, mediated for example through competition as discussed below.

Seasonal variation in the abundance of the miscellaneous group in the fish diet was likely due to differences in the ecology (e.g. timing of reproduction, microhabitat use) or phenotype (e.g. body size) between the constituent taxa. Cladocera and aquatic Coleoptera were prevalent in the fish diet in October and groundwater amphipods in March. All amphipods found in the stomach samples belonged to *Crangonyx islandicus* Svavarsson & Kristjánsson, 2006, with the exception of a single *Crymostigius thingvallensis* Kristjánsson & Svavarsson, 2004. Both amphipod species are endemic to Iceland, but whereas the former is relatively common in cold springs across the country (Kristjánsson & Svavarsson, 2007), the latter is rare. This is only the second time *C. thingvallensis* has been recorded outside of the lake Þingvallavatn (Kristjánsson & Svavarsson, 2007). The dietary shift to groundwater amphipods during the winter might imply that spring-dwelling small benthic Arctic charr moves deeper into the groundwater during that time of year. This could either be due to a food shortage in the surface stream, or the more favourable stable thermal conditions in the groundwater, which is unaffected by snowfall and wind. The higher preference for Ostracoda, Trichoptera larvae, and Plecoptera nymphs in winter also indicates a shift towards benthic prey. Similar results have been shown for brown trout in Minnesota streams, which rely more on benthic prey than on drift during the winter (Anderson et al., 2016), but mostly consume drifting prey in summer (Bachman, 1984).

In accordance with our third hypothesis, the population-wide prevalence of individual specialisation was highest in March when total invertebrate abundance was low (Table 1). It could be advantageous for an individual to specialise on a prey group that its conspecifics do not feed on at times when food availability is low to avoid intrapopulation competition. In a study on benthic isopods, the degree of diet specialisation was negatively correlated with resource abundance, not forager densities, suggesting that exploitative competition is the driver behind diet specialisation (Svanbäck et al., 2011). Competition for resources could be mediated by body size, as larger fish in our study showed stronger individual specialisation (Table 2b). A good example here was the preference of larger fish for Oligochaeta (see Fig. 5b), which were dominated by larger earthworms in our study. Earthworms are likely to be too large a prey item for smaller fish, which specialised instead on tiny Ostracoda and Acarina. The importance of Oligochaeta is often overlooked in the diet of freshwater fish, even though their energy content per unit of dry mass is almost as high as for insects (Brey et al., 1988). Oligochaeta of both aquatic and terrestrial origin (washed into streams following heavy rainfall) have also been shown to play a potentially important role in the diet of salmonids during summer months (Aarefjord et al., 1973).

Another explanation for greater differences in diet among individuals at times of lower prey abundance could be the higher stochasticity in the resources that individuals encounter, and diet differences would thus be driven more by prey availability rather than individual choice.

Kristjánsson & Leblanc (2018) found that spring type influenced individual specialisation of Arctic charr, with higher diet similarity among individuals of a population in streams than in ponds. The more variable diet of fish in ponds was due to a higher diversity of crustaceans associated with that habitat, although their study did not take seasonal changes in community composition into account (Kristjánsson & Leblanc, 2018). Our results show that crustaceans are also important food for Arctic charr in stream-associated springs during the winter, which might contribute to the stronger individual specialisation in March. This implies that both habitat variability and temporal variability of individual specialisation could be driven by similar mechanisms (i.e. composition of invertebrate prey).

The growth rates of recaptured fish were much lower between May–June than between June–August and August-October. This was not surprising, since the invertebrate abundance in the benthic substrate of the spring was highest between June and October (Table 1), and fish had plenty to feed on. Somatic growth rate is positively correlated with quantitative food intake in Arctic charr, and influenced by intraspecific competition for limited resources (Amundsen et al., 2007).

The patchy distribution of fish in the sampled area was striking. Most fish were caught in or near the spring sources, a few from under the overhanging stream bank, but almost none were found in the open area of the stream. The main channel of the stream, which does not have spring sources, contained a lot less Arctic charr than the other, source-rich sampling zones (Fig. 1), indicating that the fish prefer the spring sources as habitat. This may be due to the stable groundwater temperature and/or a higher abundance and diversity of invertebrate prey (e.g. groundwater amphipods make an important contribution to the winter diet of Arctic charr). The variety of microhabitats around the sources (e.g. mosses, macrophytes, lava rock) may also offer protection from predators such as brown trout, which occurs in the stream and feeds on small benthic Arctic charr. During early summer, a colony of Arctic tern (*Sterna paradisaea* Pontoppidan, 1763) uses the meadows around the spring as a nesting site, and terns have been observed feeding on Arctic charr in the open stream during the June sampling. High predation pressure in the summer months could also contribute to the lower individual specialisation during that time of the year (Aráujo et al., 2011). Under high predation pressure, prey might seek shelter in the same protected microhabitat and thus use a similar dietary niche (Werner et al., 1983).

Conclusion

The observed division into a winter and summer community, even though water temperature remained constant, emphasises the need for repeated sampling over more than one season for a complete characterisation of spring invertebrate communities. This seasonal variation in invertebrate availability also affected the diet, feeding selectivity, individual specialisation, growth rates, and biomass of Arctic charr. Feeding preferences for the dominant Chironomidae larvae indicated that the population preferred the most abundant prey during summer, which resulted in a narrower population niche width. The proportion of individual specialisation within the population, on the other hand, was higher in winter, which could be a strategy to relax intra-species competition at times when food resources are less abundant. This resulted in a broader population niche width, but a narrower individual niche width during winter, highlighting the value of studying both population-level selectivity and individual-level specialisation in studies on fish diet. Overall, we have demonstrated that seasonal variation in invertebrate communities can affect higher trophic levels, which should be considered when interpreting the behaviour (e.g. feeding ecology) and phenotype (e.g. growth rates) of fish in field studies.

Acknowledgements Thanks to the people involved in the fieldwork for this project: Zophonías O. Jónsson, Jake Goodall, Sebastien Matlosz, and Marlène Kundruhn. Leivur Janus Hansen created the map of the sampling site in Fig. 1 and contributed comments and discussions. Thanks to Gísli Már Gíslason for commenting on the manuscript. The project was funded by the Icelandic Research Fund (RANNÍS), Grants nr. 141863-051 and 185083-051, and by Náttúruverndarsjóður Pálma Jónssonar.

Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

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