Master's thesis in Geography

Physical geography

Morphological variation of the diatom species *Gomphonema parvulum* Kütz. under nutrient and shading treatment
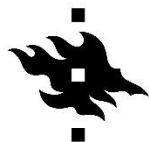
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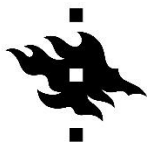
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Morphological variation of the diatom species *Gomphonema parvulum* Kütz. under nutrient and shading treatment

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Tiivistelmä – Referat – Abstract

Diatoms (*Bacillariophyta*) are unicellular microalgae inhabiting nearly all aquatic environments on Earth. Some taxa are endemic to certain regions, whereas some are widely spread or even cosmopolitan. Diatoms' species diversity and habitat selection support their use as bioindicators, and traditional water quality indices are based on species composition and index species. However, trait-based indices have gained interest in recent years and researchers believe that traits could potentially act as a useful tool in environmental assessment. Traits refers to the morphological, physiological and phenological properties of species, and they are closely linked to the species' capacity to grow and reproduce in certain circumstances. Morphological variation in diatoms varies significantly between taxa and species. The possibilities of a diatom to adapt into changing habitat is a result of its capacity to alter its morphological properties.

Urban and agricultural land use affect water resources negatively, and climate change acts as a reinforcing factor creating complex and mixed effects on aquatic environments. Global warming is and will proceed to be strongest near the poles and its unique and harsh habitats. Climate change by anthropogenic activities and environmental pollution has affected and will affect microbial communities and primary producers everywhere. Diatoms have a central role in global productivity and biogeochemical cycle, and changes in microbial cell size could have severe implications for food webs and energy transition of energy in the trophic system.

The aim of this thesis was to monitor the morphological properties, including the size, shape and striae density, of *G. parvulum* and its link to different combinations two stressors: nutrient solution (PO4 and NO3) and limited light availability. Shading treatment had a clear effect on average cell width, but average cell length did not correlate with shading. Nutrient treatment did not alter the cell length but had some effect on striae density. However, it was concluded that striae count or head shape are not most suitable for indicator purposes, as they are affected by cell size. In conclusion, no clear variation patterns according to the nutrient or shading treatment were detected, but result suggest that the increased availability of light could alter the size of *G. parvulum*. Results could be blurred by the small sample size or the presence of cryptic or semi-cryptic species.

Avainsanat – Nyckelord – Keywords Diatom, morphological traits, cell size, climate change
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Piilevälaji *Gomphonema parvulum*in (Kütz) morfologisten ominaisuuksien vaihtelu ravinne- ja varjostusmuuttujien mukaan

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Tiivistelmä – Referat – Abstract

Piilevät (*Bacillariophyta*) muodostavat yksisoluisten organismien äärimmäisen monimuotoisen ryhmän, joiden elinympäristöjä ovat lähes kaikki akvaattiset ekosysteemit. Jotkin taksonit ovat endeemisiä, kun taas toiset lähes kosmopoliitteja. Piilevälajiston monimuotoisuuden ja kasvupaikan valinnan ansiosta ne ovat käyttökelpoisia bioindikaattoreina, ja perinteiset laji-indeksit perustuvatkin indikaattorilajeihin ja lajiston koostumukseen. Viime vuosina lajiston piirteisiin pohjautuvaa (engl. trait-based) indeksejä on alettu kehittää perinteisten rinnalle, ja monet tutkijat uskovat niiden toimivuuteen vesiympäristöjen ekologisen tilan seurannassa. Näillä ominaisuuksilla tarkoitetaan morfologisia, fysiologisia sekä fenotyyppisiä piirteitä, jotka tiiviisti liittyvät lajien kykyyn lisääntyä, kasvaa ja kilpailla tiettyjen olosuhteiden vallitessa. Morfologisten piirteiden lajinsisäinen vaihtelevuus on hyvin erilaista taksonien ja lajien välillä.

Monenlaiset maankäyttömuodot vaikuttavat pintavesien ekologiseen tilaan huonontavasti. Ilmaston-muutos vahvistaa entisestään monia prosesseja ja luo oman paineensa akvaattisiin elinympäristöihin. Lämpeneminen on voimakkainta napa-alueiden läheisyydessä ja niiden karujen ja ainutlaatuisten ja herkkien elinympäristöjen alueilla. Ilmastonmuutos ja saasteet ovat vaikuttaneet ja tulevat vaikuttamaan mikrobitason eliöyhteisöihin kaikkialla. Piilevillä on keskeinen tehtävä globaalissa biogeokemiallisessa kierrossa, ja pieneliöiden solukoon muutoksilla voisi olla tuntuvia seurauksia ravintoverkoille ja energian siirtymiselle trofiatasolta toiselle.

Tässä tutkielmassa tarkasteltiin piilevälaji *G. parvulum*in solukuoren morfologisten ominaisuuksien, kuten koon, muodon ja striojen tiheyden, muutoksia suhteessa kohonneisiin ravinnepitoisuuksiin (PO_4 and NO_3) sekä varjostukseen. Varjostuksella rajoitettiin tulevan valon määrää, ja sillä oli solukuoren leveyteen pienentävä vaikutus. Samankaltaista yhteyttä ei kuitenkaan huomattu pituuden ja varjostuksen välillä. Ravinteilla ja solukoolla ei löydetty korrelaatiota, mutta ravinteilla oli pieni vaikutus striojen tiheyteen. Tämä saattaa kuitenkin johtua muutamasta muista poikkeavasta arvosta. Striojen tiheys tai nuppien muoto ei välttämättä muutenkaan ole hyvä indikaattori, sillä solun koko vaikuttaa kumpaankin. Tuloksiin voi vaikuttaa useat tekijät, mm. kryptiset tai semi-kryptiset lajit sekä mitattujen solujen pieni lukumäärä.

Avainsanat – Nyckelord – Keywords
Piilevä, morfologia, solukoko, ilmastonmuutos

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1. Introduction

Both urban and agricultural land use are affecting water resources negatively, and ecosystem health in aquatic environments is threatened (Foley et al., 2005; Sun et al., 2018). Anthropogenic activities have also altered the cycles of key macronutrients, nitrogen and phosporus, and increased their discharge to freshwaters (Schlesinger, 1997). The impact of multiple stressors varies regionally (EAA, 2012). Furthermore, climate change acts as an additional stressor creating mixed effects and reinforcing several processes, and diatom assemblages' responses to changing climatic factors are complex, too. The intergovernmental Panel on Climate Change (IPCC) predicts a sweltering future, with the global climate being warmed by 2-4,5 °C by anthropogenic activities, and the process is already in action. This will undoubtedly result in consequences for primary producer, an biological communities everywhere will be altered by the environmental changes experienced at all spatial scales.

Diatoms (*Bacillariophyta*) are an extremely diverse group of unicellular microalgae. They have a distinctive feature; a robust silica cell wall called the frustule, which constitutes the foundation for species classification (Round et al. 1990). Other characteristics of division are a unique life cycle and cell division mechanism (Chepurnov et al, 2004). The cell size of diatoms exhibits large variation (Round et al., 1990), but unraveling the relationships between cell sizes and environmental variables has resulted challenging for researchers (Pinto et al., 2020).

Some diatom taxa are widely spread, even cosmopolitan, while others are endemic to certain regions or islands (Vanormelingen et al., 2007). Over 10 000 diatom species have been recognized, the total number being perhaps manifold (Hoek et al., 2009). Diatoms in the genus *Gomphonema* Ehrenberg are the most widely distributed on Earth (Round et al., 1990), inhabiting freshwaters ecosystems with a wide range of ecological conditions. Although some fundamental questions regarding the distribution of diatoms at different scales, microbial biogeography has taken major steps during the past decade (Soininen et al., 2016), and the interest on the biogeography of micro-organisms is resurging towards understanding trait patterns (Green et al., 2008).

Along with macroinvertebrates, phytobenthos is the most extensively used indicator of anthropogenic pressure in rivers (Birk et al., 2012). Diatoms' ubiquitous nature and habitat selection support their use as bioindicators, and they are suggested as a bio-indicators in assessing the ecological status of surface waters in the Water Framework Directive (WFD, European Commission, 2000). They are widely investigated and implemented in monitoring spatial and temporal variations in lakes and rivers (Sun et al., 2018). Diatoms show a relatively quick response to disturbances and changing environments, at the same time reflect water properties from a period of time. They can even form resting stages when circumstances are unfavourable to growth, but this ability is far more common in centric species than pennate (Sahoo & Sechback, 2015). Diatoms are highly selective about habitat characteristics, but response effects vary across species and taxa.

In recent years, diatoms have gained massive interest focusing on global ecological questions, but also on economically relevant matters. Technological solutions inspired by living organisms have become widespread in the 21st century (Zgłobicka et al., 2021). For instance, the application of modified diatom frustule morphology for technological applications has attracted increasing attention lately. Large fossil deposits of diatomaceous earth born from frustules accumulating at the bottom of water are being used by several industries (Sahoo & Sechback, 2015).

Benthic diatoms are applied for indicating the physical and chemical conditions of fluvial ecosystems. As a result of selection pressure by altered freshwater condition, the species composition of algal communities is altered. Species composition is the base of most ecological diatom indices (Tan et al., 2017). However, those indices are biogeographically specific and contain uncertainties of taxonomic identification (tapolzcai et al., 2016). Morphological variation between diatom species is substantial and is closely related to species' tolerance range. Phytoplankton species that have high phenotypic plasticity at the cell level, can cope under a larger variation of abiotic conditions (Groß et al., 2021). The plasticity of singular cells determines the plasticity of strains, and when the strains have low plasticity, the community structure of diatoms is altered as an outcome of shifting environmental conditions.

The response of marine taxa to environmental change is better recorded than that of freshwater taxa, but the mixed effects blur the line between different factors. Ocean acidification due to the uptake of carbon dioxide will likely enhance the growth of larger diatom species, having possible significant consequences for carbon biochemistry (Wu et al., 2014). Due to diatoms' central role in nutrient, silica, and carbon cycles, monitoring their responses to the changing environments is of great significance. Weithoff and Beisner (2019) think that researches would benefit from the use of some process-driven approaches used in marine ecology in unraveling the links between biogeochemical processes and traits. Maintaining the ability of aquatic ecosystems to provide crucial ecosystem services, focally important to humans, is one of the greatest global challenges of the 21st century (Pawlowsky et al., 2018).

The morphological variety of some diatom species has often been considered a taxonomic problem, hampering species diversity estimation or spatial distribution evaluation (Soininen, 2012), and the use of molecular methods has come to show that many species previously defined only by morphological criteria constitute in fact species complexes with cryptic or semi-cryptic species (Kermarrec et al., 2013). Still, there seems to be advantages to the application of trait composition in freshwater monitoring, rather than traditional indices, such as richness, diversity, abundance, and productivity. However, many aspects, such as the seasonal variety of morphological traits, requires further examination in order to be sufficiently understood (Sun et al., 2018).

Diatoms were first incubated in laboratory cultures in the mid nineteenth century (Mann & Chepurnov, 2004). The classification begun some decades later with distinguishing pennate diatoms, with an elongated frustule, from centric ones (Schutt, 1896). Over a period of the last hundred years, laboratory cultures have been providing better insight into the taxonomy of diatoms, based upon the study of cleaned silica valves. Although the centric-pennate-division has been prevailing, phylogenetic analyses on DNA sequence data does not fully support this classification anymore (Alverson and Theriot, 2005; Alverson et al., 2006). Williams and Kociolek (2007) argue that the division between centric and araphid diatoms is not informative nor useful although they seem practical due to their familiarity. In the past decades the comprehension of diatom

reproduction has expanded significantly, particularly on the sexual reproduction. Still, acquiring knowledge on diatom biology, ecology, and geographic distribution has fallen behind when compared to other micro-organisms (Mann, 1999; Chepurnov et al., 2004).

The material used in this thesis was to offered to use by B. Bailet and collegues (B. McKie, A. Truchy, T. Muotka, J. Jyväsjärvi, J. Soininen, A. Huusko, Anette Teittinen & M. Kahlert), who worked as a part of the DESTRESS project (https://www.researchgate.net/project/Disentangling-the-impacts-of-multiple-stressors-on-stream-ecosystems-DESTRESS). The study involved applying an array of different stressors, including nutrients, shading and reduced flow velocity, and their combinations, into an artificial set-up of an oligotrophic system. Stressor refers to a continuously acting agent, whereas disturbance would often implicate some occasional, unpredictable events interfering a community (Tapolczai et al., 2016). The use of an artificial set-up enables excluding several correlating or confounding factors from the experiment. The effect of turbulence, flow velocity and the type of substrate could also be minimised. The aim of this study is to examine how the morphological traits of a cosmopolitan diatom species *Gomphonema parvulum* vary according to two environmental stressors: shading treatment ane elevated nutrient concentrations. *G. parvulum* was chosen due to its high prevalence in the samples and its morphological variety.

Study questions:

1. Do the morphological traits of *Gomphonema parvulum* display variation according to different combinations of nutrient treatment and artificial shading?
2. How is diatom cell size used in assessing environmental change in freshwater environments?

2. Study area

The Kiverjärvi lake is located in the province of Kainuu in Northern Finland. It encompasses a surface area of 2 574 ha and a shoreline extending 89 kilometers (Järvi-

meriwiki). It drains into two different channels, therefore being a bifurcation lake. The study site (Figure 1)(64° 40' N, 27 ° 52' E) is located 1 km from the drainage point to the Varisjoki, a short river stretch that drains into the large Oulujärvi lake. The site is approximately 320 km south from the arctic circle and is an overlapping area for an oceanic and a continental climate. The study site is 135 located metres above sea level. Most of the area forms part of the karelian slate rock district, the most common rocks being quartzite, mica slate, phyllite and granite (Finlandia, 1986). Mineral soil is mostly till, although clay and silt are also typical in river valleys, but bedrock and mineral soil are mainly covered by thick peatland (Finlandia, 1986). The relief and landforms in the area are versatile; the landscape is a mixture of lakes and hills. Kivesvaara hill close to the study site is 294 m asl.
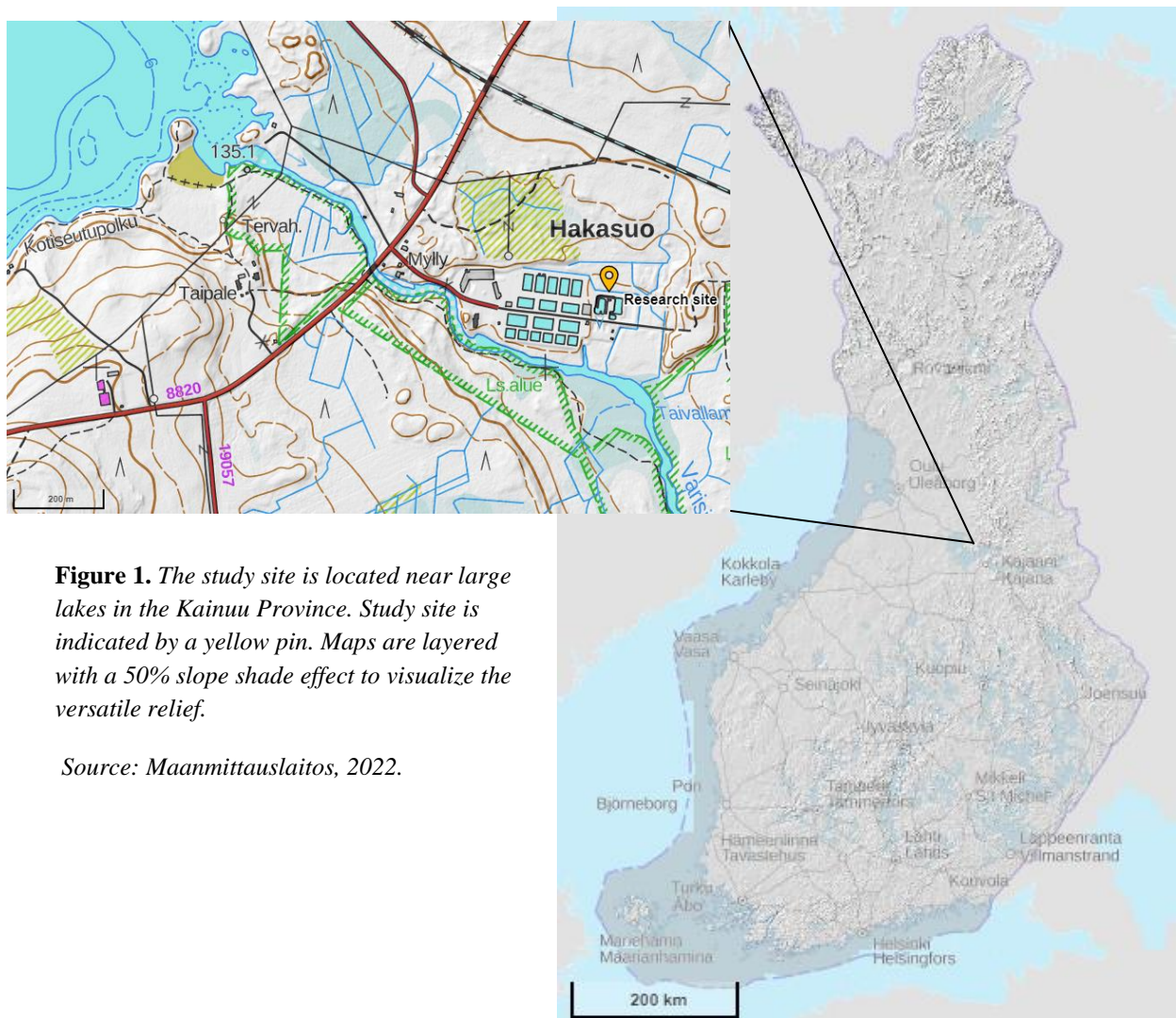

Figure 1. *The study site is located near large lakes in the Kainuu Province. Study site is indicated by a yellow pin. Maps are layered with a 50% slope shade effect to visualize the versatile relief.*

Source: Maanmittauslaitos, 2022.

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The study set up (figure 2) was implemented in a channel construction in the Kainuu Fisheries research station. It consists of a pond where water from the Varisjoki is collected and a channel system where water flow is equally divided into the 24 subchannels.
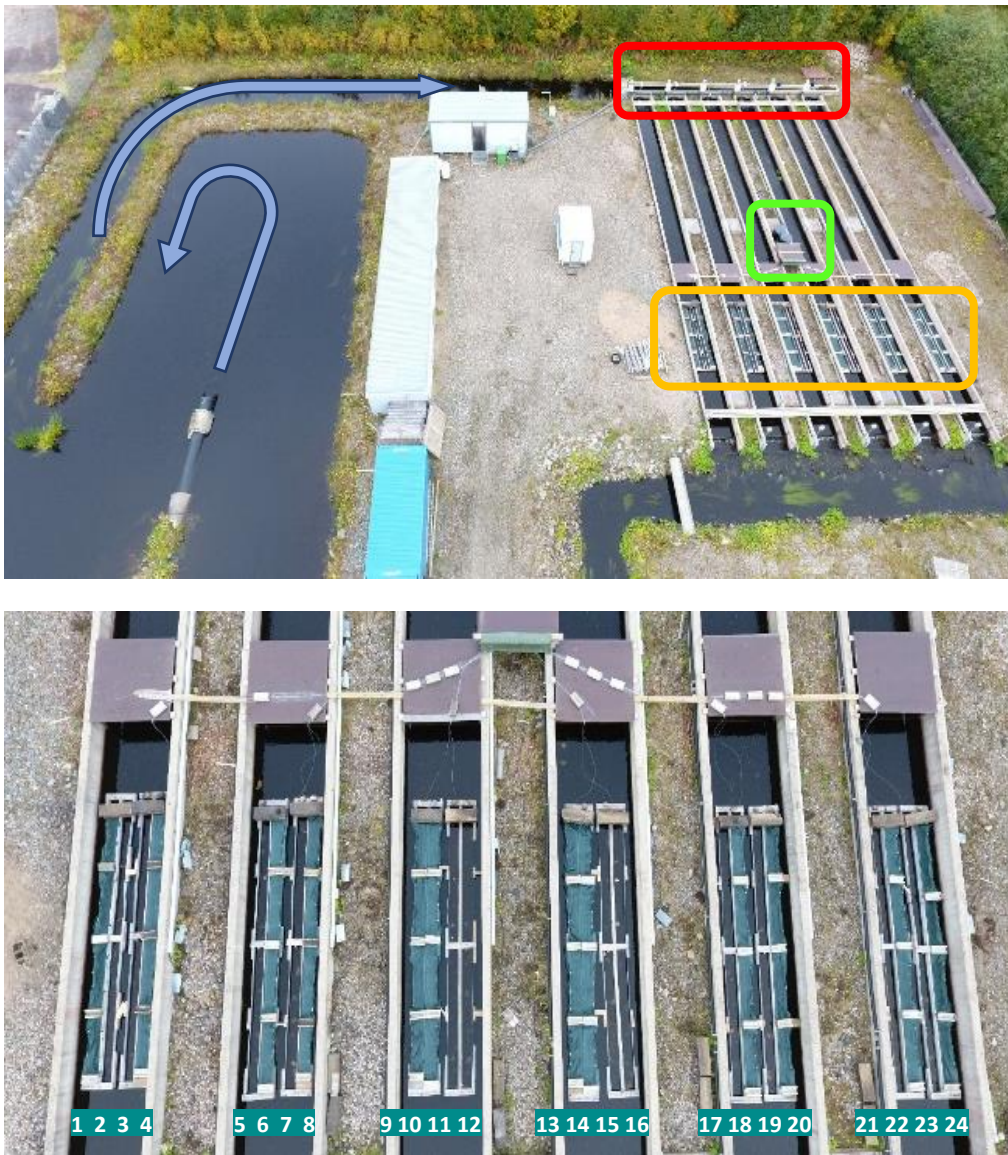

Figure 2. *Drone pictures of the study set-up. a) Inflow water is divided into six (in red) and further into 24 sub-channels (in yellow). b) Shading treatment (at the sub-channel level, in yellow) is indicated by the presence of green cover on the subchannels. Nutrient treatment (in green) delivered to the sub-channels by plastic tubes.*

3. Diatom background

3.1 Life form and classification

Diatoms are microscopical, unicellular organisms, with a cell size between 10 and 200 μm (Kooistra et al., 2007). They can be found in almost every aqueous environment, and some live as ensymbionts, in a symbiotic relationship, with other single-celled micro-organisms (dinoflagellates and foraminifers)(Round, 1990). The cell casing is an ultralight, solid crust, an inorganic nanostructure produced through biological processes (Shin et al., 2022). These qualities are enabled by the multidimensional and intricate pore structure. Diatoms show great diversity on morphology and structure on various scales (Round et al., 1990). Frustule shell elements can be preserved in sediments for millions of years (e.g., Gersonde and Harwood 1990), which makes the fossil record abundant allowing a deeper insight into the evolutionary history. They have been extensively used in studying the historical conditions both in lakes (e.g. Smol & Cummings) and marine or brackish waters. However, a lot of discussion has revolved around the early stages of diatom evolution (Round, 1981).

Diatoms' contribution to global productivity and nutrient cycling is significant (e.g. Falkowski et al. 2008; Mann 1999). According to estimates, diatoms account for 40 % of the total carbon production in marine environments (Nelson et al., 1995), and around 20–25 % of the total global primary productivity (Hoek et al., 2009). Therefore, the production of organic carbon and oxygen by diatoms is providing air and food for about fifth of whole living world. Being the major producer of phytoplankton mass they lay a foundation to the trophic pyramid in many parts of the oceans (Mann, 1999). Their dominance in phytoplankton blooms is generally accepted to be due to their resistant silica frustule and thus lower mortality rate compared to other algae with similar growing rates (Smetacek, 1999; Hamm et al., 2021).

Taxonomic inventories based on molecular markers, morphological features and crossing experiments have shown for the current geographic distribution of diatoms to manifest immense variation, from global to endemic (Vanormelingen et al., 2007), and that the distribution can be influenced by deterministic and stochastic processes (Green

et al., 2008). The array of factors that affect diatom growth and species distribution, and probably morphological variation also, is extensive. Furthermore, in addition to local climatic variation and local environmental variables, diatoms have a clear response to larger-scale climate (Pajunen et al., 2016).

Williams & Kociolek (2007) have concluded that a natural classification of diatoms is "a long way off", meaning that the phylogenetic relationships among diatoms are deficiently known and documented. Since classifications are based upon phylogeny, the fact that a great part of it remains unsolved, inherintly means that the classification has undergone changes and will further evolve with the emerging of new information and theories.

3.2 Frustule construction

The frustule is formed by two valves named epitheca and hypotheca, and a set of girdle bands (figure 3). It is composed of polymerized silicic acid and contains protein, polysaccharides, and lipids (Hoek et al., 2009), and has an important role of protecting the cell against external pressure, for instance predators such as parasitoid and ingesting protists and crustacean zooplankton (Hamm et al., 2003; Allan,1994). Hamm et al. (2003) suggest that the humbling variety of diatoms in shape and protuberance is a result of responses to specific predator types. The principal dissolved form of silicon in water is silicic acid, which originates from the weathering of rocks and from sewage discharge, and other anthropogenic sources (Allan,1994). Silicic acid is assimilated by diatoms for frustule construction, and released to circulation by chemical dissolution, which can be enhanced by microbial enzymes.

The valve of *G. parvulum* cell ranges from 12-30 µm in length and 4-7 µm in width. The valve shape ranges from lanceolate to oval (Abarca et al., 2014). Striae, consisting of minuscule distinct puncta, are organised in a radial manner. The central area of *G. parvulum* is asymmetrical with one of the striaes on the other side short (Abarca et al., 2014). Among pennate diatoms, one lineage has a longitudinal slit across the frustule crust, called the raphe (Ruck & Theriot, 2011). Its purpose is in the motility of the valve. The number of pennate diatoms with some form of raphe system is close to tens of

thousands, and the diversity of raphe systems is perhaps close to that number (Ruck & Theriot, 2011).
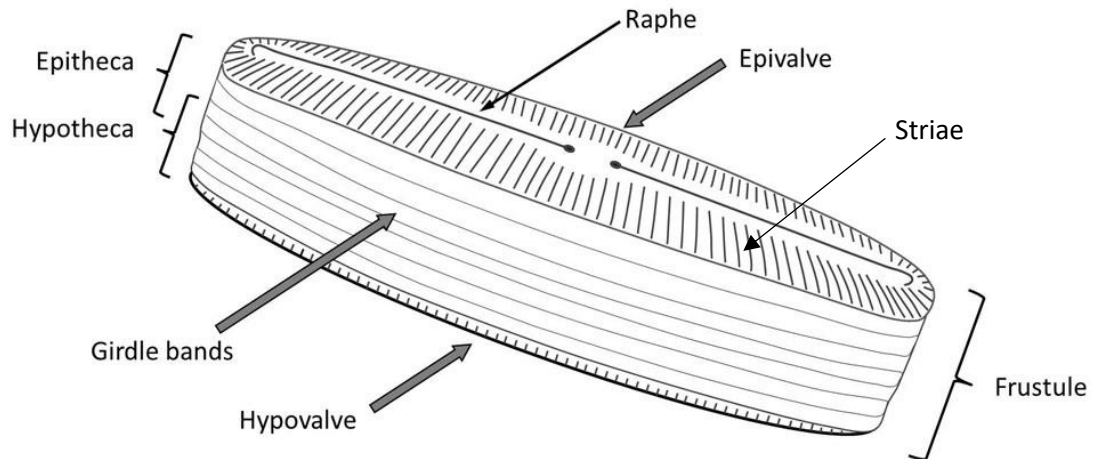

Figure 3. *Illustration of the basic structure of a pennate diatom. Picture modified from: Kacsmarska et al., 2013.*

3.3 Growth and reproduction

Diatoms can be classified on the basis of their growth form and substate. Microalgae exert considerable habitat specialization, and most can be categorized into epilithic (grow on stones), epipelic (grow on soft sediments) and epiphyton (grow on the surface of other plants) (Allan,1994).

Unlike nearly every other algal groups, the life cycle of diatoms is diplontic (Sahoo & Sechback, 2015), meaning that the life cycle is dominated by the diploid stage. Diatoms have two means of cell reproduction: mainly through asexual, the vegetative phase, and occasionally through sexual reproduction (Sahoo & Sechback, 2015). Both are needed for a full life cycle. Mitosis occurs during the vegetative phase, increasing the number of cells, meiosis and genetic recombination in the sexual phase. As a consequence of the vegetative cell division, cells in a growing population constantly decrease in diameter (Round et al., 1990; Kacsmarka & Ehrman, 2021).

Cell size is reduced because the parent cell walls form two new hypovalves, one of which is the same size as the parent, but the other is smaller. Cell size can be restored by

the development of a specialized cell, auxospore, resulting from sexual reproduction. Auxospores are quite complex despite of being single cells. If a cell were to fail to complete sexual reproduction and auxosporulation, it continues dividing mitotically until the cells become critically small and die, after one to several years (Mann, 1999). Moreover, only comparatively small cells can undergo the sexualization process and switch the cycle from mitotic to meiotic. The sexual stage is estimated to last for some hours or days in some cases, whereas the individual genotype has a lifespan of weeks or even years in the asexual stage (Kaczmarska & Ehrman, 2021)

Much of the reproductive biology of diatoms and its evolution remains unknown (Kaczmarska & Ehrman, 2021), and it has been presented that some diatoms species do not undergo the gradual decrease in size followed by cell division. As our comprehension expands, so is our understanding on diatom morphology and the processes behind morphological variation.

3.4 *Gomphonema parvulum* Kützing

Diatoms in the *G. parvulum* complex are common species in freshwaters of a wide range of ecological status, and it is a strong competitor in disturbed conditions (Ivorra et al., 2002). Growth has been even reported to response positively to growth in ammonia (Evenson et al. 1981, Hurlimann and Schanz 1993) and phosphate (Evenson et al. 1981). *G. parvulum* was also found in a site contaminated by a mixture of chemicals originating from textile industries (Goméz et el., 2007). There are species in the genus Gomphonema that are spatially distributed (Kociolek et el., 2004), but *G. parvulum* is considered cosmopolitan. However, a great deal of regional diversity inside the species complex is yet to be revealed (Abarca et al., 2012). The occurrence of cryptic species with differing morphology goes against the theory against the cosmopolitanims of micro-organisms (Abarca et al., 2012; Kermarrec et al., 2013). Different taxa inside the genus are separated based on the length and width of the valve, striae density and the shape of the knobs (e.g. Morales, 2002). Detailed electron microscopy analysis has enabled the analysis of even more detailed insight into the valve morphometrics.

Morphological traits in diatoms demonstrate variation throughout the entire year, and previous studies suggest that morphological features of G. parvulum show variation also along with shifting chemical variables (Sun et al., 2018). Morphological variability among *G. parvulum* is significant, and semi-cryptic species have been identified (Kermarrec et al., 2013). A study by Kermarrec et al. (2013) shows that although *G. parvulum* displays morphological variation, the four semicryptic species that have been specified, cannot be distinguished without applying molecular markers.

Before *Gomphonema parvulum* was specified as a separate species by Friedrich Traugott Kützing in 1849, it was referred to as *Sphenella parvula* (Dawson, 1972). The distinctive feature was the attached growth form of G. parvulum. Later on they were considered as the same species, living attached or free. Several divitions on forms and varieties have been described concerning the two forms, and the variability of *G. parvulum* has arousen discussion at various times. Wallace & Patrick (1950) and Hohn & Patrick (1959) have explored the size reduction and auxospore formation of *G. parvulum*, revealing a pattern of a change in the shape in relation to length.

The genus name originates from the cell's "appearance as a wedge on the end of a mucilage thread (gompho = wedge, nema = thread)" (Dawson, 1972). Looking from the valve view, G. parvulum Kütz. is elongated, the poles attenuating and round, with the plumbness of the heads varying greatly. Raphae extend into terminal fissures near the ends, and slightly curve toward one side in the centre. Striae are shorter towards the centre.

4. Theoretical background

Taxon delineation of diatoms has been relying nearly exclusively on morphological features of the frustule, lacking understanding on the causes of variation patterns. For instance, temperature can cause alterations to valve morphology, and allopatric populations have been on risk at being separated as different species based on morphological variation (Cox, 1995). However, there has been considerable interest towards solving some of the biogeographical questions of functional traits, the "characteristics of an organism that are linked with its fitness or performance", since the

mid-1980s (McGill et al., 2006; Green et al., 2008), and the development of algal identification is headed towards a phylogeny-based system thanks to the advancements in molecular techniques (Tapolczai et al., 2016). One of the central challenges of trait-based methodologies is identifying the characteristics that are most associated with micro-organisms' fitness and performance (Green et al., 2008).

Trait composition reflects the functional response of communities (McGill et al., 2006). It is not as spatially and temporally dependent as species composition and it indicates more consistently the functional adaptation of organisms to environmental conditions (Sun et al., 2018). That being said, trait composition and morphological responses to different environmental factors are not sufficiently studied, and in particular the impact of light and and flow disturbances are poorly known. Additionally, previous studied have mainly focused on marine phytoplankton species, and diatom cell size is often linked to salinity level. The trait-based approach to biogeography carries certain inconveniences: easily recognisable, unambiguous taxa, also called "flag-ship" taxa is often opted for, in order to avoid the need for preparing a detailed morphological analysis of species from various locations. On the other hand, the trait-based approach has many advantages, such as the simplicity of taxon-independent models of community structure, to the use of "common currency" in comparing communities with different species. Furthermore, species composition may be influenced by stochastic forces, whereas trait-based functional groups are determined by environmental conditions (Fukami et al., 2005).

For unicellular organisms, cell size is considered to be the "master trait" (Weithoff & Beisner, 2019). It determines their response to environmental changes by affecting light harvesting and growth rate (Geider et al., 1986,) and nutrient uptake (Lichtman, 2009). The general assumption is that a warming climate will gradually lead to smaller phytoplankton cell size and result in a more rapid turnove rate (Svensson). The question that remains largely unanswered, is the effect of environmental changes (light, nutrient availability) on diatom cells of the same species. Diatoms, just like many other organisms, display more interspecific variation in size than intraspecific variation (Svensson et al., 2004). Other morphological properties that can be altered by the

presence of stressors are pore size and the thickness of the frustule (Soleimani et al., 2020).

4.1 Factors behind morphological variation

Morphological properties of frustules can be influenced by light intensity and wavelength, pH and temperature (Soleimani et al., 2020) as well as nutrients and resource availability (Byllaardt & Cyr, 2010). The main focus in previous literature has nonetheless been in the effects of salinity, and thus the applied diatom species have also been marine taxa. For instance, Svensson et al. (2004) reported size reduction of benthic diatoms and raise the question of whether it is caused by temperature or salinity on their own, and what is the role of other factors.

A large number of studies have also been conducted on the role of heavy metal concentration and diatom morphology (e. g. Falasco et al., 2009a; Pandey et al., 2014), reporting deformities (teratological morphotypes) and morphological variation. That is, deformed frustule outlines, modified striation patterns and raphe alterations. However, the relationship between deformed frustules and heavy metal concentrations have been statistically weak in some studies (Cattaneo et al., 2004; Lavoie et el., 2012). Rimet et al. (2004) reported teratological morphotypes in several taxa, including *G. parvulum*, due to contamination by fluoranthene, a recurringly detected pollutants in rivers. Gautam et al. (2017) suggest that morphologically deformed frustules may be effective indicators of water quality. They reported altered responses related to heavy metal contamination in *Gomphonema Pseudoaugur*, and several other studies showed regularly deformed frustules linked to lead (Pb) contamination. Other factors that can induce abnormalities in the frustule are variations in flow rate, light, pH, nutrient availability, as well as abiotic stresses (Sun et al., 2018, Falasco et al., 2009a). A study by Sun et el., (2018) suggest that chemical variables play a vital role in affecting diatom morphological trait.

The mechanism of how deformities in the valve are induced is not yet understood sufficiently, but teratological morphotypes are malfunctions, not adaptation to changes in the habitat. Sometimes drawing the line between normal cells and teratological forms

is difficult, and each species should be treated according to its morphological variability (Falasco et al., 2009b).

The trait-based approach regarding the algal benthos, particularly diatoms, has increasingly been applied in research (Soininen et al., 2016; Tapolczai et al., 2016), but literature on nutrients' influence on diatom growth in terms of size and shape is restricted. However, it is apparent that diatoms' geographical distribution range is, to some extent, determined by its ability to alter the morphological structure of the frustule valve. Each species' capasity to alter its frustule morphology enables living under, for instance, increased nutrient levels, altered light and temperature conditions, or even elevated toxin and heavy metal levels (Groß et al., 2021). For example the chain length of the diatom *Skeletonema costatum* appears to increase through an enhanced growth rate, which is a consequence of higher temperature and nutrient availability (Takabayashi et al., 2006). The tolerance range of a population is the combined tolerance capacity of individual strains within the community (Groß et al., 2021). The dynamics and stability of benthic diatoms and other benthic algae has been considerably less studied than that of other benthic fauna (Soininen & Eloranta, 2004). Benthic communities are also less studied in terms of seasonality (Tapolczai et al., 2016).

According to Sun et al. (2018) the effects of hydrological variables on morphological traits have been rarely reported. The role of conductivity and organic pollution are also poorly tested (Tapolczai et al., 2016). The effect of hydrological and chemical variables on diatom morphology and its biogeographical variation requires further examination. Delicate, fine-grained morphological analyses have expanded the possibilities for research in terms of distribution patterns, and moreover, revealed exceptional prospects to the study of causalities of factors contributing to diatom growth and valve morphology.

Important physical variables in aquatic environments include pH, conductivity and water temperature. Additionally, in lotic ecosystems the flow velocity and physical disturbances can be crucial elements to benthic assemblages (Allan,1994). Grazers can also have an impact on algae through size-selective grazing (Tall et al., 2006). The role of temperature and other factors on organism size still remains partly undiscovered,

since in laboratory cultures the effects are fully isolated from each other (Byllaardt & Cyr, 2010). Moreover, laboratory studies inherently ignore the long-term evolutionary responses, and exhibit only short-term physiological responses.

4.1.1 Nutrients

Nutrients may affect the morphological traits in some diatoms with an intermediate effect (Sun et al., 2018), but the intensity of the influence and the interference of other factors is still unclear. Contrary to the theory of higher surface-to-volume ratio as the facilitator of better nutrient uptake (Reynolds, 2006), studies on benthic algae rarely support such idea, and the effect of other factors have been observed significantly more clear (Tapolczai et al., 2017). Finkel et al. (2009) found that low nutrient content would benefit the growth of larger diatoms, but their case was conducted on planktonic diatoms. Studying the effects of nutrient enrichment on arctic and sub-arctic freshwater systems is focally important due to the more pronounced warming compared to lower latitudes (Gudmundsdottir et al., 2013). However, Gudmundsdottir et al. (2013) doubt that trait indicators would be better indicators of nutrient pressure than taxonomy-based indicators.

In freshwater environments phosphorus is often a limiting factor affecting the productivity of plants and other autotrophs (Allan,1994; Wetzel, 1983), but in sub-arctic environments and running waters in Finland nitrogen is more commonly the limiting nutrient (Gudmundsdottir et al., 2013; Pietiläinen & Kauppi, 1993). In stream ecosystems dissolved inorganic phosphorus (DIP) is transformed into particulate organic phosphorus (POP) through photosynthesis by plants and microbes. The availability of phosphorus is affected by biological processes with plants, microbes, and bacteria, as well as physical-chemical transformations. Diatoms are also capable of stocking some phosphorus for later use (Sommer ja Kilham 1985).

4.1.2 pH and electrical conductivity

Conductivity refers to the electrical conductance of water, and is a predictor of total dissolved ions (Allan,1994). Ions in the water act as an agent to reduce resistance to

electron flow. Conductivity is often intercorrelated with nutrient content in field measurements, and both conductivity and pH are important variables at explaining diatom composition in streams (Soininen, 2007), electrical conductivity may be the most influential environmental variation locally (Pajunen et al., 2016). Gensemer et al. (1995) found that the average cell length of the diatom Asterionella ralfsii was significally reduced under lowered pH values in a continuous laboratory culture, and they also hypothize that pH can alter the relative distribution of morphotypes with different cell sizes.

pH or conductivity were not altered in the experiment and were consistent throughout the experiment in all subchannels. Both were measured weekly. pH was neutral (7.05-7.1) and conductivity around 2,5 ms/m.

4.1.3 Light and temperature

Light has a direct influence on the performance of phytoplankton photosynthesis (Wagner et al., 2006), and wavelength and light intensity are possible factors in modifying frustule properties (Soleimani et al., 2020). Light, together with temperature and nutrients, has ever greater role in stream and river ecosystems (Reynolds, 2006). Lotic ecosystems have small possibilities to buffer atmospheric temperature variation and are shallow, thus more light is able to reach the bottom (Reynolds, 2006; Allan,1994). Increased nutrient concentrations are often linked to poorer light availability.

Temperature affects the metabolic performance of phytoplankton, and furthermore exerts an indirect effect by altering the availability of several fundamental resources (Reynolds, 2006). According to Finkel et al. (2009) smaller diatoms have more pigment-specific absorption efficiencies and thus light limitation would promote the growth of smaller diatoms. The general trend in phytoplankton cell size is decreasing with warming temperatures, and diatoms display this variation seasonally as well; with larger cells in winter and smaller ones in spring and summer (Sun et al., 2008)

A few recent results suggest that increasing temperature has an increasing effect on frustule size (Fanesi et al., 2016; Groß et al., 2021). Groß et al. (2021) detected a strong

correlation between growth rate and cell size and suggest that permanent changes in temperature within the predicted climate scenarios can significantly enhance the growth rate of diatoms. However, Fanesi et al. (2016) noted that the linearity of the relationship disappeared at temperatures above 19 °C, and that the temperature range that could enhanced growth was species specific. Thus, results are contradictory, and some studies show no clear temperature-size relationship. Byllaardt and Cyr (2010) do not find these mixed results surprising, since there are several factors that follow changes in temperature seasonally and over latitudinal and altitudinal gradients. Their investigations on three diatom species showed larger or similar sized diatoms in 1-1,6 °C warmer water.

In this experiment water derived from the close-by Kivesjärvi lake and therefore it can be assumed that no rapid changes in water temperature occurred, and that temperature was consistent throughout the channels.

5. Material and methods

5.1 Stressor manipulation in Paltamo

Study samples were collected in august and September in 2017 from the 24 artificial stream channels in the Kainuu Fisheries Research Station. The set-up consisted of six artificial streams, each 1.5 m wide, and 24 m long. Channels were further divided with rustproof metal gutters into four sub-channels, resulting in 24 replicates. Different treatment combinations were applied to those replicates. Streams were constantly supplied with water from a pond with water originating from the Kivesjärvi lake. Channel beds, formed by gravel and cobbles, had been naturally colonized over the past 10+ years. Samples we initially acquired for a project carried out by B. Bailet and her colleagues, who studied the diatom community's response to the stressors through three different approaches.

5.1.1 Flow treatment

Water discharge into channels was homogenized in the beginning of the experiment, and then adjusted to three levels of flow. The mean initial discharge of 1.40 ± 0.1 m^3/sec. From the day 21 onwards, discharge was reduced in four channels, in total 16 sub-channels. The two channels affected by the most drastic drought conditions – press disturbance – flow was reduced until only a small leak drained into the channel. When flow disturbances had been applied for 9 days, the discharge was again returned to the initial level, to enable sufficient time for ecological recovery from the drought conditions. Two channels (8 sub-channels) functioned as controls, and thus flow velocity was not manipulated. Flow treatment was not included in the final analyses.

5.1.2 Nutrient treatment

Out of the 24 sub-channels 12 were nutrient enriched using liquid nutrient solutions (Phosphate, PO4; and nitrate, NO3). Nutrients were dissolved into two 500 L tanks filled with filtered stream water (ø 500 µm). Concentrations were targeted at approximately 500 µg NO3-N/L assuming 14µg/L background concentration and 50 µg PO4/L assuming 7µg/L background concentration. The two tanks were filled, and the nutrient solution added every day. Water was pumped continuously into the sub-channels using Watson Marlow 504S peristaltic pumps (Watson Marlow Bredel, Wilmington, MA, USA). Inserting the nutrient solution into the channels increased both NO3 and PO4 concentrations by an approximately 10-fold increase. The nutrient treatment extended from the beginning of the experiment until the biofilm collection.

5.1.3 Shading treatment

Artificial shading treatment was applied to 12 sub-channels using gardening 'shade fabric cloth'. Light intensity was reduced by approximately 70 %, and shading conditions sought to imitate observed shading levels in forest streams of Scandinavia. Cloths were attached over the cub-channels with stables and bulldog clamps. Similarly to the nutrient treatment, shading treatment was applied for 53 days, throughout the whole experiment.

5.2 Diatom biofilm sampling

In the beginning of the experiment a total of 192 tiles (5x5cm) were submerged in the subchannels and left undisturbed for the whole of the experiment. In such way the response to continuous exposure to stressors could be reported. Diatom samples were collected in late September from a total of 96 tiles, four replicas from each sub-channel. Sampling of biofilm was done by rubbing the surface with a toothbrush and rinsing in stream water. Samples were preserved with Lugol (a solution of potassium iodine with iodine in water) and stored in dark for morphological identification.

Hydrogen peroxide (30% H_2O_2) was used to remove organic material from samples, and slides were prepared by using Naphrax (Brunel Microscope Ltd). Samples was examined using light microscopy (LM), a commonly used method for obtaining images of the size and shape of individual frustules (Zgłobicka et al., 2021). Measurements were carried out using the built-in tape measure of a phase contrast light microscope (Olympus BX40, Melville, NY; 1000x magnification). Valve length and width were measured, and density of striae was calculated for 20 valves from each sub-channel. Valves were also categorized into three groups according to head form; 1) no clear "bottleneck", 2) slightly round form, no clear circle-form, 3) well-formed, round shape head (picture 4).
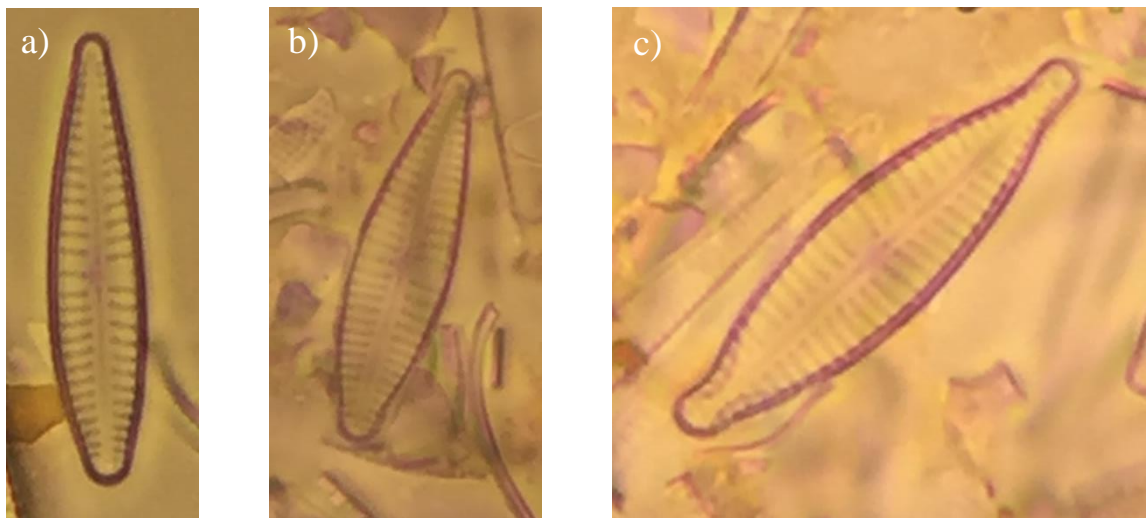

Figure 4. *Examples of the three head form categories a) group 1, b) group, 2 c) group 3.*

5.3 Statistical analysis

Statistical analysis was conducted using R studio (version 2001). Data was first documented and organized in Microsoft Excel (version 2016). Excel was also used to create simple graphs to visualize the data. Generalized linear model (GLM) was used in the analyses because it can be applied to multiple explaining variables. Means for frustule length and width were calculated for each subchannel, GLM was applied to average length and width separately due to their different type of distribution. Geographical data is often count data and not normally distributed, but since average cell length and width are not count data and were considered to be close to normal distribution, they could be treated as normally distributed data in the GLM analysis.

The concentrations of PO4 and NO3 in the nutrient solution were included in the GLM as measured values, and shading was given a value 0 (no shading treatment applied) and 1 (shading treatment applied), since the shading successfully removed about 70 % of incoming light and lighting conditions were quite consistent among the two groups.

6. Results

Nutrient measurements and cell size means used in the GLM analysis are listed in table 1. Cell size range was 11-30 in length and 4-7 in width, and means by subchannels varied from 18,95 to 22,3 in length and 4,5 to 5,5 in width. Nutrient solutions were the same for subchannels 1 & 5, 2 & 3 and 4 & 6. Stress by nutrients in the enriched subchannels was approximately ten-fold for both NO3 and PO4.

channel	subchannel	Nutrients	av. length, μm	av. width, μm	NO3, μg/L	PO4, μg/L	Shading
	1	Enriched	20,55	5,1	96,9	41,1	1
1	2	Control	19,05	5,5	16,8	4,9	0
	3	Enriched	19,09	5,2	103,5	50,1	0
	4	Control	20,45	5,1	17,1	4,7	1
	1	Enriched	21,45	5,25	121	60,1	0
2	2	Enriched	21,45	4,9	151	69,9	1
	3	Control	21,6	5,4	16,5	5,4	0
	4	Control	21,1	4,5	18	5,5	1

3	1	Enriched	18,95	5,05	151	69,9	1
	2	Control	21,65	4,65	18	5,5	1
	3	Enriched	20,5	5,3	121	60,1	0
	4	Control	20,45	4,95	16,5	5,4	0
4	1	Enriched	21,05	4,8	192,3	84	1
	2	Control	21,65	4,8	19,4	5,1	1
	3	Control	20,5	5,1	19,4	5,2	0
	4	Enriched	20,4	5,4	134,2	70,9	0
5	1	Enriched	21,15	5,4	103,5	50,1	0
	2	Enriched	19,8	4,85	96,9	41,1	1
	3	Control	21,5	5,45	16,8	4,9	0
	4	Control	20,65	5	17,1	4,7	1
6	1	Control	22,15	4,95	19,4	5,2	0
	2	Enriched	21,6	5,05	192,3	84	1
	3	Enriched	22,3	5	134,2	70,9	0
	4	Control	21,85	4,6	19,4	5,1	1

Table 1. *In total, half of the subchannels were enriched with nutrient solution, but the order is not the same between channels. Nutrient solution concentration varies between enriched channels but is notably higher than in control subchannels.*

A connection was detected in GLM results between average frustule size and shading. The subchannel with the shortest average length frustules received shading treatment and the two subchannels with the longest frustules were measured form a channel with no shading treatment. However, the relatioship was statistically insignificant for frustule length and and shading. Frustule width and shading on the other hand shared a statistically significant relationship, meaning that the shading treatment seemed to result in less thick cells. With no shading cloth over the subchannel, average cell width increased consistently, which can be seen clearly from the plot (figure 5a) Mean cell length is not linked to mean cell width (figure 5b), although the plot shows that in subchannels where average cell was taller, cells were quite narrow, but no subchannel had on average short and thin cells.

Boxplots in figure 6 represent the cell width-length ratio in enriched and control channels. In control subchannels the cell length seem to increase in a more linear and consistent manner as the cell width increases. The range in the cell length is also larger in control cubchannels. However, it cannot be said that the nutrient treatment would have increased or decreased cell sizes, but perhaps the nutrient solution has somehow disturbed growth.
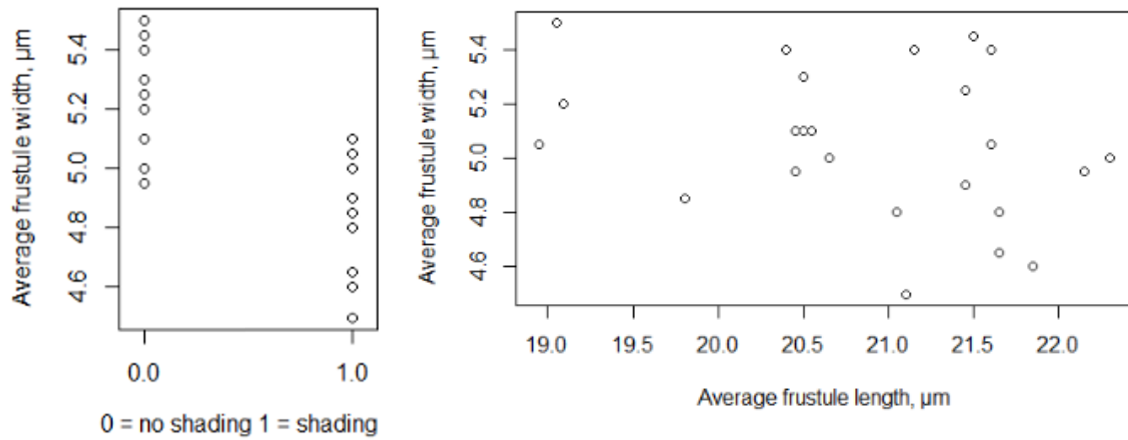
Figure 5. *a) Shading treatment seemed to have a clear effect on cell width, but cell length did not show similar connection with shading treatment and b) mean cell length and width did not correlate with each other.*

Figure 6. *a) Shading treatment seemed to have a clear effect on cell width, but cell length did not show similar connection with shading treatment and b) mean cell length and width did not correlate with each other.*
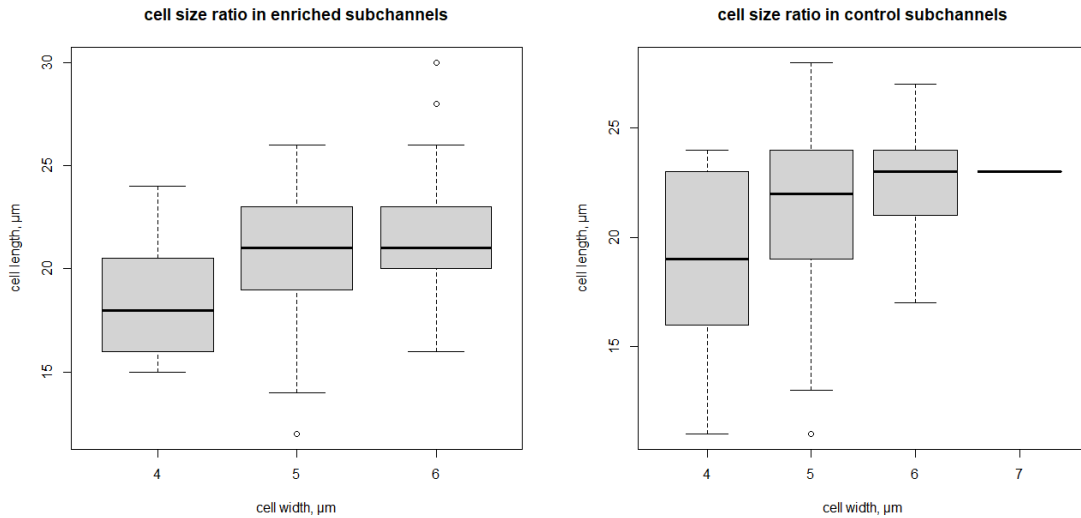
Frustule lengths are illustrated by boxplots (figure 7) and different colours to indicate the stressor treatment that diatoms in each subchannel received. Shading treatment is indicated in light blue, nutrient treatment in pink and subchannels with both treatments in dark green. Subchannels with nutrient treatment seem to have less short valves than in other channels, but apart from that the cell lengths do not follow any patterns according to the treatment.
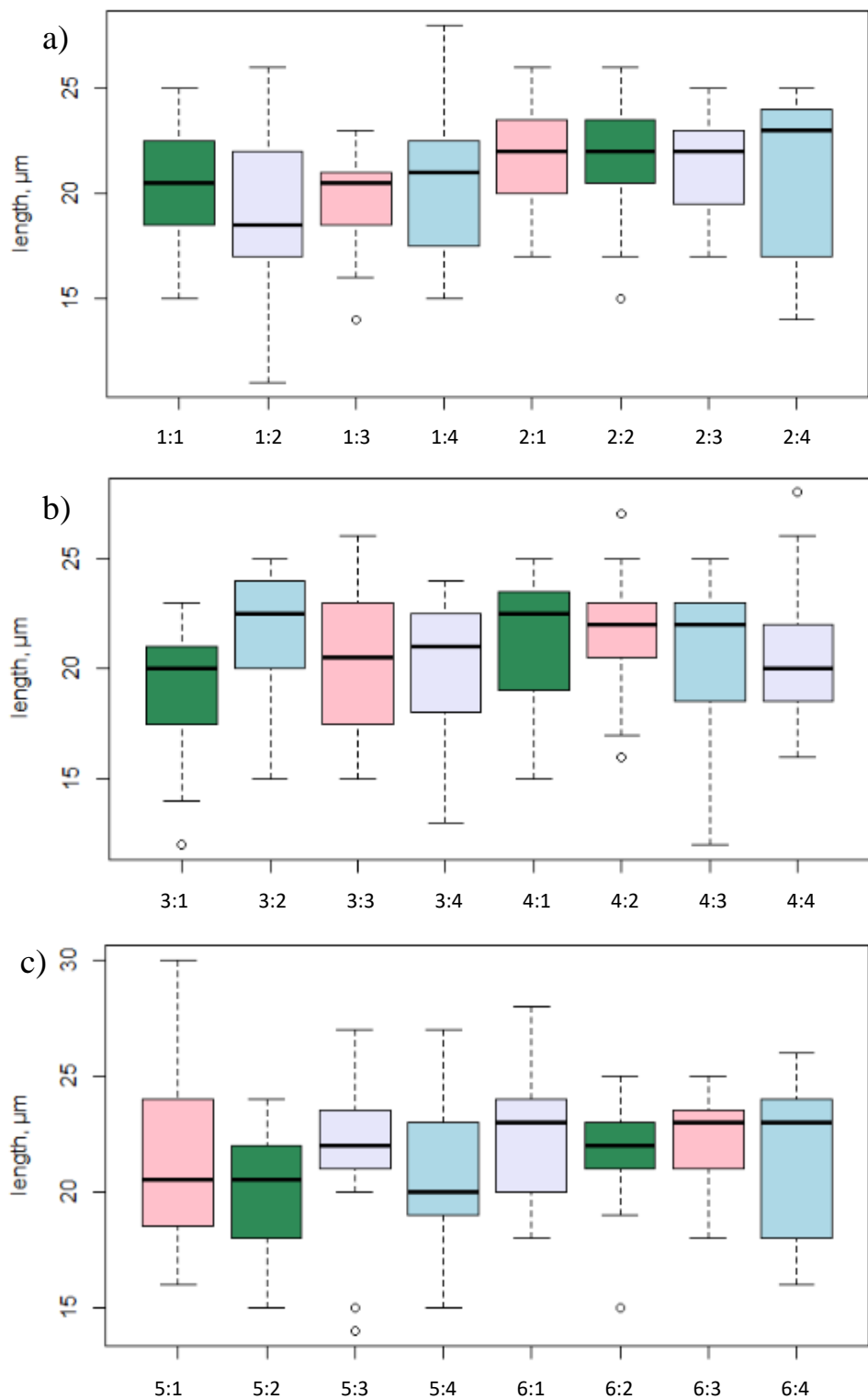
Figure 7. *Frustule length variation in each subchannel: a) channels 1 & 2, b) channels 3 & 4, c) channels 4 & 5. Outliers are indicated with dots and colors indicate the combination of shading and nutrient treatment. Largest frustules were measured in the first subchannel of fifth channel, which is why the scale is larger than in two ones above.*

Most of the measured valves, 66%, were categorized according to head shape into group 2. 23% of frustules had well formed knob (group 3) and only 11% of frustules were in the group 1, which was the least knob-shape. Differences between the subchannels are presented in figure 8. Most of the group 1 valves were detected from the samples from the first channel, subchannel 3, which was an enriched subchannel with no shading treatment. However, nutrient concentrations were less than in other enriched subchannels (103 μg/L NO3 and 50 μg/L PO4), and other subchannels with high proportion of valves fron group 1 were not enriched. Teratological forms did not occur in the samples, which was expectable, since presumably no unusual concentrations of heavy metal or other pollutants were present.
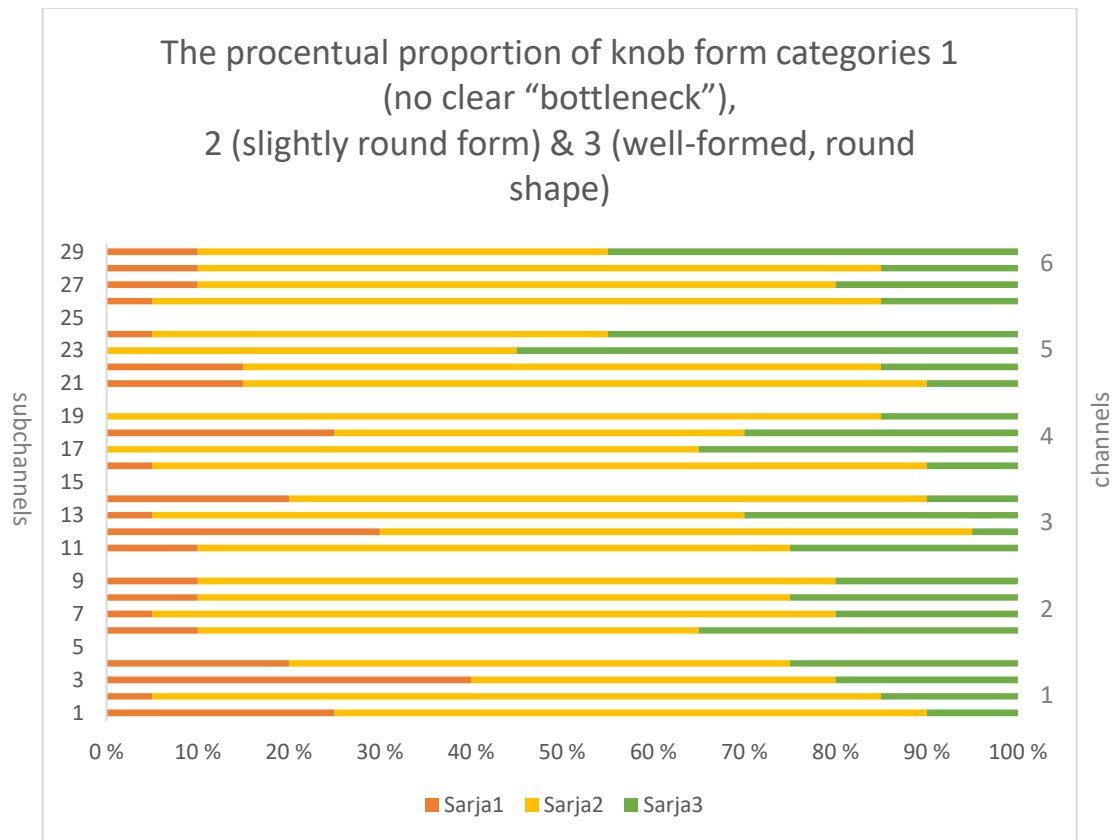

Figure 8. *The distribution of head forms in three categories. Orange = group 1, yellow = group 2, green = group 3. Variation between channels is substantial, which could not be explained by the data.*

The striae density in measured cells varied between 11 and 15 µm, not counting the two frustules with a striae density of 10 and 16 per 10 µm. Striae in the enriched subchannels were less dense than striae in the control channels. Medians were 13 for both and mean values 13,02 for control channels and 12,76 for enriched. Histograms (figure 9) visualize the unimodal distributions of striae count in the subchannels according to the nutrient treatment. The profile is fairly similar apart from the higher number of cells with small striae count in the enriched subchannels.
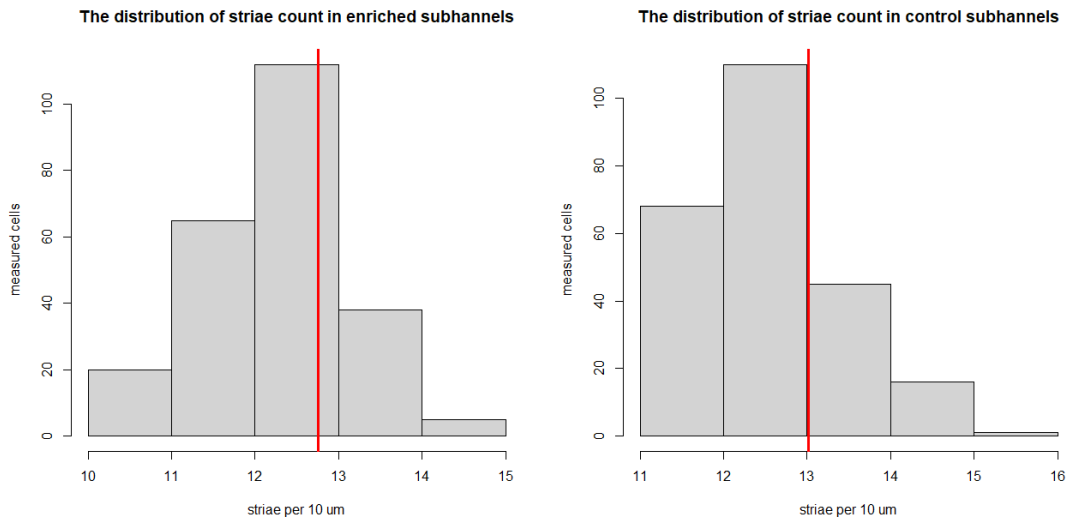

Figure 9. *Histograms for striae count for subchannels a) with and b) without nutrient treatment. Red horizontal line indicates mean value and smaller striae count per 10 µm indicates less dense striae.*

7. Discussion

The use of similar study set-ups in studying morphological or other type of traits is recommendable because many of the variables are easy to control, but circumstances are not as isolated as in laboratory studies. Although no clear patterns between morphological properties and the two variables (nutrients and shading) were detected with this data, results could have been different with more than one species, larger sample size or different criteria.

Striae density per 10 alone is a poor measure in terms of comparison; the smaller the valve, the denser the striae appear, because denser striae closer to valve ends are included. Therefore, more useful would be to measure the distance of individual striae, their length or positioning. Dawson (1972) noted that the number of striae per 10 was not useful in identifying the four varieties of *G. parvulum*. The frustule size is linked to pole shape as well. As Kermarrec et al. (2013) noted, pole shape is affected by cell size reduction, and therefore propose supplementing the criteria with for instance the shape of the central area and the distance of raphe endings. Dawson (1972) observed that the cell shape of *G. parvulum* remains fairly constant as size reduction takes place, apart from a slight rounding of the cell as the breadth to length ratio decreases, a feature noted by Geitler (1932). A further confounding factor to the discussion is the wide use of the term "trait". Traits refer to the morphological, physiological and phenological properties of species, and they are closely linked to the species' possibilities to grow and reproduce under certain circumstances (Violle et al. 2007).

Even judging from this brief review on the topic, it is quite clear that including other criteria would bring additional value into the evaluation. In environmental assessing it could result useful including both trait-based monitoring as well as more traditional bioindicators, such as species richness and similarity indices. Tracking some easily measurable traits, such as cell size, can enhance and support understanding of diatom assemblages and environmental variables (B-Berés et al., 2017).

7.1 Research questions and brief answers

The research questions were:

1. Do the morphological properties of *Gomphonema parvulum* display variation according to different combinations of nutrient treatment and artificial shading?

Results suggest that nutrient treatment and shading did have some effect on the morphological properties of *G. parvulum*, but the patterns were not clear nor statistically significant for most of the figures. Furthermore, results could be blurred by aspects

related to the possible presence of cryptic species or the inaccuracy of the average values used in the analyses. In conclusion, no clear pattern between the size and shape of the valve and the multiple stressors was detected for this particular species. Perhaps another species with a less wide range of ecological conditions that can be inhabited would have responded with more pronounced changes in cell size.

Interestingly the cell width and shading treatment showed a strong negative correlation (-0,86), but cell length did not follow the same trend. That is, cells were wider when shading treatment was not applied and more light could reach the substrate. Clues on why such a trend emerged could not be found in the literature.

Results by Soininen and Kokocinski (2006) suggest that the average maximum cell length is weakly correlated with species richness locally, a trend that supports earlier findings on other organism groups. The species richness was not considered in the analysis, but since the relative abundance of *G. parvulum* showed substantial variation, it could be interesting to see if some of the variation could be explained by species statistics.

2. How is diatom cell size used in assessing environmental change in freshwater environments?

The web of science search for the search words diatom (all fields), cell size (all fields) and light (all fields) showed 487 results. When the key word "marine" was applied to exclude articles on marine taxa, results dropped to 153 articles. 31 articles inside this group were under the category of oceanography. Furthermore, when "cell size" was coupled together by using apostrophes, a mere 50 articles appeared. When "light" was replaced with "nutrient*", similar number of results appeared. While the search does not completely cover all relevant studies, it provides a clue of the overall trend in the literature on the topic. Trait-based approaches have indeed been a challenge when it comes to the ecology of unicellular algae, but gradually these challenges are being unravelled (Tapolczai et al., 2016)

Morphological traits are not yet widely used in the assessment of the ecological status of freshwater environments. Views on the matter are both in favour and against it. The general conception is that while tracking microbial responses, in terms of cell size and other key morphometry as well, is focally important, morphology may not be an optimal indicator of water quality in freshwaters. Additionally, the responses of benthic diatom communities to changes in trophic status in freshwater systems appear to be more variable than in marine environments (Finkel et al., 2009).

Climate change and its consequences on the physiochemical processes, mainly temperature, pH and nutrient cycling, has had and will have an effect on the biological community and affect species composition and overall primary production and biodiversity (Smol, 2010). Diatom cell size has already been used to track climate trends in the past. E. g. Chen et al. (2014) used paleolimnological data to investigate how diatom communities and organism size in an oligotrophic lake system had changed in an area particularly sensitive to climate change. They detected a consistent change in diatom cell size according to temperature trends, a finding that was supported by previous studies. In addition to the decrease in cell size in the phytoplankton community, warmer temperatures and higher nutrient content may sometimes also benefit smaller species, perhaps because they have higher growing rates (Sorvari et al., 2002). Finkel at al., (2009) too, believe that the cell size structure of diatoms could help in reconstructing past environmental change and reveal changes in water quality and macronutrient concentration. So the question that follows is that why it could not be used in water quality assessment in present or to predict future scenarios.

7.2 Potential sources of error

Possible sources of error are mostly associated with humane errors in measurement and the small number of measured samples. The measurements per se contain only a small chance for mistakes, but despite the efforts to perform random sampling, a change for unintentional selection of cells with a certain shape or size exists. This could be due to the lack of identification skills and the variety in the morphology of this particular species. It is unlikely that cells from species other than G. parvulum were included in measurements.

The number of measured cells from each sample was moderate, and does not accurately represent the size and shape distribution of the samples. In the near future perhaps, artificial intelligence (AI) together with more accurate microscoping techniques could unravel some difficulties for systematic measuring of cells and enhance resource-efficiency.

Hydrological aspects were consistent, with the exception of the flow regulation, and were not taken into consideration in analysing the results. Although their role in diatom growth is likely important and perhaps even as defining as physio-chemical variables (Allan, 1995; Burfeid-Castellanos, 2020), it is questionable wether alterations of such small magnitude as the flow treatment in the experiment, could have an impact in the cell size for example. It is more likely that hydrological aspects, such as turbulence and flow velocity, would affect diatoms through growth form selection. Additionally, nutrient and other resource availability is often linked to flow regime, and therefore it can be assumed that flow regulation would have not altered the results.

7.3 Conclusions and future prospects

Since gradually increasing stress promotes the development of stress-adaptation strategies (Tapolczai et al., 2016), morphological traits and trait-composition could result useful in assessing the long-term influence of stressors. Trait-based techniques can also be applied to support traditional indices. Rimet et al. (2004) investigated the responses of diatom assemblages and diatom morphology to pollution by Polycyclic aromatic hydrocarbons (PAH) and suggest that a variety of methods should be implemented to assess the sensitivity of different taxa to pollution. Falasco et al., (2009b) believe that the number of teratological forms in diatoms will serve as a useful tool in the evaluation of water quality in lotic ecosystems.

One of the challenges with indices based on cell sizes is the demand for time-consuming measuring work. Taxon-based indices are not an exception, on the contrary, since they require advanced identification skills are no less time-demanding. Spaulding et al. (2012) believe that size analytics could potentially serve as a highly useful tool in understanding for instance population dynamics and phylogenetic relationships, and that

alternative techniques could be harnessed to efficiently measure diatom cells from varying sizes and shapes. They tested two measuring techniques and found that the measurements agreed well with manual light microscopy measurements. The captured high-resolution images could be used to quantify 30 different morphological measures.

More delicate analysis of the frustule is enabled by electron microscopy (EM); scanning and transmission electron microscopy (SEM, TEM) (Zgłobicka et al., 2021). Even more detailed morphological information can be obtained by the Focused Ion Beam (FIB) technique, and it has already been applied on freshwater diatom species. These detailed techniques will uncover possibilities for expanding our understanding of frustule morphology and different environmental stressors' role in it.

Although some studies have found in situ evidence that benthic algae would increase as a result of higher temperatures, no conclusion about how global climate change could affect benthic assemblages in the long run can be made. In the era of rapid and unpredictable environmental change, it is extremely valuable to establish different future scenarios and address the role of micro-organisms, their biogeography and morphological adaptability. The size of organisms in aquatic environments is linked to trophic systems (Allan,1994; Svensson et al., 2014), and the systematic reduction in body size could affect the energy flow in the trophic web.

Since there is lacking evidence on diatom morphometry and traits in freshwater research, it would be useful to conduct comparative studies to assess both latitudinal and altitudinal gradients in freshwater phytoplankton (Weithoff & Beisner). As the consequences of climate change are already beginning to manifest, tracking microbial responses to the changing local environmental factors and large scale is of focal importance. Valve morphology is closely related to the adaptability of different species and therefore studying their morphological properties should be of our interest.

8. Acknowledgements

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Appendix

Appendix 1. Graph showing the relative abundance of all identified cells from the samples by subchannels (1-24)

Relative abundace of *Gomphonema parvulum* in the samples

subchannels

Relative abundance of identified species
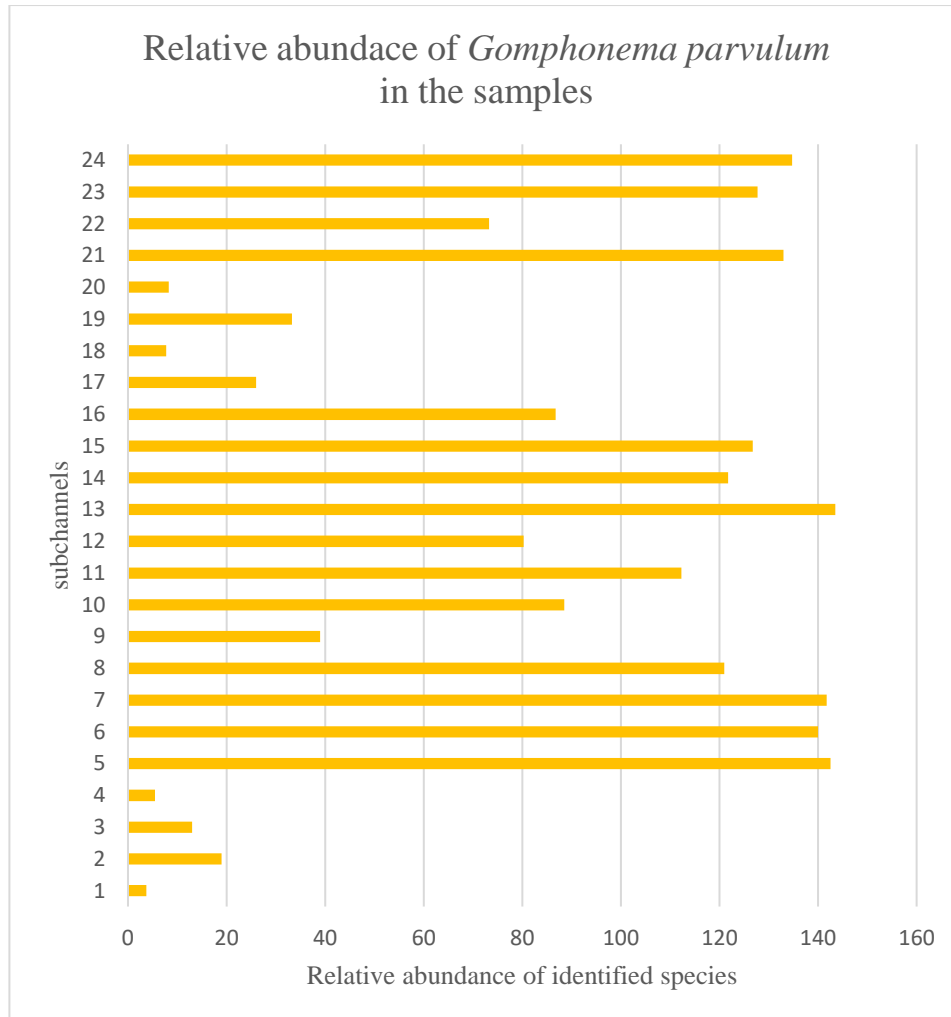
Appendix 2. GLM-results and ANOVA-test for cell width

```
glm(formula = lev_ka ~ NO3 + PO4 + Shade, family = "gaussian",
    data = diatom_env)

Deviance Residuals:
    Min       1Q    Median       3Q       Max
-0.35610  -0.09052  -0.00613   0.16285   0.29201

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)  5.297e+00  7.073e-02  74.893  < 2e-16 ***
NO3          9.025e-05  6.933e-04   0.130 0.897718
PO4         -5.880e-04  2.217e-03  -0.265 0.793564
Shade       -4.083e-01  8.473e-02  -4.819 0.000104 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for gaussian family taken to be 0.0401732)

    Null deviance: 1.73958  on 23  degrees of freedom
Residual deviance: 0.80346  on 20  degrees of freedom
AIC: -3.416
```

```
> anova(norm_glm, test = "F")
Analysis of Deviance Table

Model: gaussian, link: identity

Response: lev_ka

Terms added sequentially (first to last)


      Df Deviance Resid. Df Resid. Dev       F    Pr(>F)
NULL                     23     1.73958
NO3    1  0.00323        22     1.73635  0.0805 0.7795954
PO4    1  0.00001        21     1.73634  0.0003 0.9872872
Shade  1  0.93288        20     0.80346 23.2214 0.0001044 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> (1.73958-0.80346)/1.73958
[1] 0.5381299
```

Appendix 3. GLM-results and ANOVA-test for cell length

```
glm(formula = pit_ka ~ NO3 + PO4 + Shade, family = "gaussian",
    data = diatoms_env)

Deviance Residuals:
    Min       1Q    Median       3Q       Max
-1.8712   -0.4412    0.2143    0.6493    1.4438

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept) 20.955948   0.376923  55.597   <2e-16 ***
NO3         -0.009911   0.036264  -0.273    0.787
PO4          0.017353   0.073304   0.237    0.815
Shade        0.148804   0.531269   0.280    0.782
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
> anova(norm_glm, test = "F")
Analysis of Deviance Table

Model: gaussian, link: identity

Response: pit_ka

Terms added sequentially (first to last)

      Df Deviance Resid. Df Resid. Dev      F Pr(>F)
NULL                    23     20.470
NO3    1 0.155465        22     20.315 0.1537 0.6992
PO4    1 0.006152        21     20.308 0.0061 0.9386
Shade  1 0.079350        20     20.229 0.0785 0.7823
```