Protocol development towards using benthic diatom endpoints to determine freshwater ecosystem function and health

Assessing the impact of the environment on the colonisation of substratum by benthic diatoms

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Abstract

Benthic diatom communities have been used for decades in the assessment of the health of freshwater environments. To this end, several benthic diatom metrics have been used to measure the effects of nutrient enrichment, acidification, and organic chemical contamination at the community level. Organic chemicals present in Home and personal care products (HPCPs) have a wide range of functions within household cleaning and personal hygiene products. Despite their prevalence, there is limited research on many of these chemicals, compared to other groups of organic chemicals, such as herbicides or pesticides, which unlike most HPCPs are biologically active. In this thesis, development of a protocol for using benthic diatom community endpoints to determine the effects of organic chemicals on freshwater ecosystem health and function was conducted. The results presented here indicate that microscope slides, ceramic tiles or sandstone substratum could be reasonably used for culturing benthic diatom communities without having a significant effect on the communities that are developed, but ceramic tiles are slightly better at mimicking more developed communities within four weeks. A laboratory-based batch culturing method using communities grown directly from lake water was shown to be capable of developing functioning diatom communities, although longer periods of culturing will likely be required, as these cultures exhibited far lower biomass measurements than field equivalents, and a customized nutrient replacement regime developed using pre-culture tests to determine the usage rate of nutrients within the cultures are recommended. Diatom communities from sites in good ecological quality in the Vale of York were also assessed, and several species of sensitive and tolerant diatoms were identified for a representative community to be cultured using the methodology developed here. Further research studying these sites, and a broader range of others in the region at different times of the growing season are recommended.

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Declaration of independent work

I declare that the thesis presented here is original work and that I am the sole author of this work. The work presented here has not been previously submitted for an award at the University of York, or any other academic institution. All external sources to this work have been acknowledged as references within the text and linked in the reference chapter to the original sources.

Chapter 1: Literature review

1.1. Overview

In this chapter, a review of the literature into the relevance of benthic diatom communities as a representative community for the primary productive trophic level in freshwater ecosystems is conducted. Further to this, the past methods used for tests on diatom communities under ecotoxicological experimentation to develop an understanding of the methods involved, as well as how the effects of the chemicals being assessed affected diatom communities (endpoints), which organic chemicals they have been tested on before, and the possibility of applying these tests to organic chemicals, such as Home and Personal Care Products (HPCPs), in future testing are discussed.

Ecosystems are complex communities of different species that function together as individuals to form a bigger system. Each individual organism within an ecosystem is tied to multiple others through either food web mechanics, or mutually beneficent relationships. However, ecosystems are rarely stable, and are subject to numerous pressures, both external and internal. External forces, including pollution and climate change can force changes in the normal function of an ecosystem, often leading to long term damage. As one of the external pressures, chemicals from various sources can have negative impacts on not just individual species, but the health of the wider communities of related organisms. Although many chemicals are only found in the environment below levels which are considered toxic to organisms, some species are more sensitive to specific chemicals than others, and the mode of action (the pathway through which a chemical affects an individual) that the chemical has may also vary across species in the same ecosystem, or may even have interaction effects with other chemicals.

Diatoms are single celled organisms of the class *Bacillariophyceae*, a common group of brown algae found in virtually all water bodies across the planet. They function as the primary producers in most aqueous ecosystems and contribute to a significant proportion of silica removal from the environment, as well as being a major contributor in aquatic ecosystems to oxygen production. Their abundance and short life cycles mean that any changes within the physical environment around them can be studied in detail relatively quickly. However, the implications of the exposure of diatom communities, and freshwater ecosystems as a whole, to organic chemicals from HPCPs is still largely unknown.

This review aims to assess the use of diatom communities and species as a bio-monitoring tool for the impacts of different types of pollution on freshwater ecosystems. This review will identify many of the organic chemicals found in freshwater environments, as well as the methods of diatom experiments used in prior ecotoxicological studies, in order to prepare for future research into the use of diatom communities to assess ecosystem health in response to these chemicals.

1.2. Freshwater ecosystems and the relevance of diatoms to the assessment of ecosystem health

This section will focus on freshwater ecosystems, the internal pressures that maintain function, and the impacts of pollution on these ecosystems. This section will also cover the importance of diatoms in these ecosystems, as well as the relevance and use of diatoms for assessing the impacts of organic chemicals on these ecosystems.

1.2.1. How freshwater ecosystems work

Ecosystems are a complex assemblage of different organisms, which rely on one another to form an interdependent network, through the movement of nutrients from one species to another or a symbiotic relationship with each fulfilling its own ecological niche important to the health of the

entire ecosystem (Begon *et al.*, 2009). The United Nations Environment Programme defines freshwater ecosystems as lakes, rivers, aquifers and wetland environments that provide important environmental and societal functions, including food, energy production for manufacturing, as well as regulating services, including natural hazard regulation, habitat services, and water purification (UNEP, 2017). Persson *et al.,* (1994) describes how freshwater ecosystems are composed of primary producer organisms (algae, plants), that are then fed upon by herbivores (zooplankton), who in turn are fed upon by carnivores (invertebrates, fish), many of whom feed upon other carnivores (fish), which are categorised by trophic level within the food web.

Within these food webs, Bacillariophyceae (commonly known as diatoms) are single celled organisms that exist in the communities of periphyton and phytoplankton, performing the role of primary producers within the wider ecosystems. They also form their own communities within these ecosystems, composed of dozens, if not hundreds of species depending on the size of the habitat and the variation in its environmental heterogeneity. Diatom communities can develop in almost all known aquatic environments, and act as the primary source of vegetation biomass in marine and freshwater environments, which are believed to produce between 20-40% of the oxygen in the atmosphere through the process of photosynthesis (Field *et al.,* 1998, Virta *el al.,* 2019). Although they are a key food organism for grazing organisms in aquatic food webs, several species, including the species *Thalassiosira rotula* (Ianora and Miralto, 2009), *Melosira varians* (Wendel and Jüttner, 1996), *Skeletonema marinoi* (Miralto *et al.,* 1999), have been observed to produce toxins when digested that have teratogenic impacts on the offspring of grazing herbivores that attempt to digest them (Ianora and Miralto, 2010).

1.2.2. Why are diatoms useful as indicators for ecosystem health?

Diatoms are one of the most important groups of organisms on the planet, responsible not only for a larger share of primary productivity, but also for carbon sequestration through photosynthesis and burial in ocean sediments. They are often grazed upon by organisms of higher trophic levels (grazers, usually zooplankton) and smaller nektonic organisms (usually fish). Diatom-based studies have frequently been used as case studies for the impacts of chemicals on wider ecosystems, and are one of the more effective tools for the monitoring of river ecosystems (Eloranta and Soininen, 2002). Beyene *et al.* (2009) identifies the ubiquity of diatoms as the main reason as to why they are useful for ecology-based assessments in freshwater environments with no or low macroinvertebrate diversity. In freshwater environments the benthic algal communities are much larger than the pelagic communities, accounting for as much as 86% of the total community by biomass and 77% by primary production (Rautio and Vincent, 2006, Ask *et al.,* 2009). As such the benthic communities are more representative of the wider primary production trophic level than the pelagic communities. Although benthic diatoms have been shown to be very effective measures to indicate the ecological health of riverine freshwater bodies in past research (Kelly, 2002, Bellinger *et al.,* 2012, Potapova and Charles, 2012), this is not necessarily true for lakes. Research by Cellamare *et al.,* (2012) on French lakes indicated that although macrophytes, phytobenthos and phytoplankton all reliably predicted the ecological state of French Atlantic lakes, phytoplankton was considered the better measurement. However, the phytobenthos assessments used were based on riverine datasets, and proposed changes to phytobenthos measures for use in lake environments. These recommendations have been implemented leading to the development of measures, including the EQR LTDI2 methods in the U.K (Directive, 2014). Variations in the availability of resources (nutrients and light), have been shown to cause changes in community compositions for diatoms, with many species observed to increase in relative abundance under higher nutrient and/ or light conditions, whilst others may become more prevalent in nutrient poorer, or more shaded water bodies, due to their sensitivity to environmental changes (Kelly *et al.,* 1995, Lange *et al.,* 2011, Cibic *et al.,* 2012).

To make ecotoxicological research on freshwater benthic diatoms universally applicable, representative species need to be identified and selected. Factors including universality, sensitivity to chemical or environmental change, and prevalence across large regions are

important factors for species used for this (Schiffrine *et al.,* 2020). Finlay *et al.,* (2002) identified four common freshwater diatoms from the collation of literature on diatom studies, identifying three globally widespread benthic freshwater species used in the literature: *Gomphonena parvulum*, *Navicula cryptocephala* and *Nitzschia Palea*. This universality makes them useful for cross comparisons of sites from different global settings. However, the age of this document, and the broad usage of species that may not take into account species variants or modern revisions of diatom taxonomic identification which may limit the usage of these results. However, *Gomphonema* and *Nitzschia* have been noted to be more resistant to nutrient enrichment than other genera, although this is not universal to all species of these genera (Larras *et al.,* 2014, Berthon *et al.,* 2011), and will disappear from the communities if there is a decrease in nutrient availability due to their preference for eutrophic conditions (Rimet et al., 2009). As such, these species, and others with widespread distributions, should be used for single species studies. However, in community-based studies the most impacted species should be the focus of the study; Larras *et al.,* (2012) explains that diatoms in benthic communities may not always be the most sensitive to a chemical, as the effects of a chemical varies between species, and some species of chlorophytes or cyanobacteria may be more sensitive than the diatoms present in the community (Schmitt-Jansen and Attenburger, 2005). However, they are still typically the most abundant group of phytobenthos, and thus making the diatoms more representative as the larger group and more useful for community level studies (Surren *et al.,* 2003).

Use of diatoms in scientific studies and environmental assessments

Since the implementation of the EUs Water Framework Directive (WFD), diatoms have become an important indicator organism of water quality. As such, interest in diatoms have seen an increase in use alongside physical and chemical analysis (Arini *et al.*, 2012). This has been seen in work by Cirić *et al.* (2018), where the use of diatoms to determine ecological status of the Lasovačka and Lenovačka streams demonstrated that Lenovačka had a poor ecological status due to lower diatom diversity and trophic classification of the diatoms present, whilst Lasovačka has a moderate ecological status. The authors did however make note that the software used for diatom assessment was based on known measurements of diatom tolerances from Croatia, and that these may be different to those in Serbia, where the communities were sampled. Bowes *et al.,* (2012) made use of the UKTAG trophic diatom index, developed in the United Kingdom by Kelly *et al.,* (1995), to determine the effects of soluble reactive phosphorus (SRP) on periphyton communities in the River Thames, noting that the index was insensitive to the effects of SRP concentrations above 50µg/L. Stenger-Kovács *et al.,* (2007), notes that there are different diatom indexes available, and as such it is possible to use an index more appropriate to the location of study, species distribution and environmental parameters (alkalinity, altitude, lotic/ lentic system). This suggests that although diatoms work well within the context of WFD monitoring programs, there are still gaps in the literature regarding local knowledge of diatom tolerances that need to be addressed to make the results as accurate as possible. Several papers have stated that diatoms are more sensitive to changes in nutrient availability than other commonly used organisms, due to their relative lack of mobility and short life spans compared to macroinvertebrates and fish. This has made them a key species for the monitoring of water quality in the United Kingdom (Hering *et al.,* 2006, UKTAG, 2006, Bae *et al.,* 2014), although Resh (2007) states that this varies based on the characteristics of the environment in question.

Diatoms as indicators of water quality and effects on the wider ecosystem

Fish, macroinvertebrates and diatoms are the most commonly used measures of water quality in Australia Marchant *et al.* (2006). Taylor *et al.,* (2005), Newall *et al*. (2006) and Dalu *et al.,* (2016) concluded that diatoms are more suitable for water quality monitoring than macroinvertebrates. However, research by Eloranta and Soininen (2002) and Soininen (2007) demonstrate that the substratum and bedrock of an ecosystem can have a noticeable impact on diatom communities, with an example of how freshwater ecosystems on or near limestone or other alkaline rocks have more alkaline and conductive water bodies due to these bedrocks, which would then cause a direct preference in the local diatom communities for species that preferred higher pH conditions.

However, this work argued that this affected the water quality, as opposed to having a direct influence on which diatoms can grow on the substratum (Squires and Saoud, 1986). Nonetheless, this can cause issues if comparing sites on varied geology. This issue can be mitigated during site selection and any changes accounted for during the interpretation of any results obtained. Table 1.1. demonstrates the effects of the main physico-chemical attributes of the water column and nutrients used by the diatoms on the diatom community.

Table 1.1. Common physico-chemical measurements for water qualities and their main uses in diatom cells and/ or controls they exhibit on community structure

Physico-chemical measurement	Effects on diatoms/ primary biological uses	References
Nitrate	Primary nitrogen nutrient in diatoms, facilitates growth	Smol and stoermer (2010)
Nitrite	Secondary nitrogen source (favoured by non-diatom algal groups)	Admiraal (1977) Smol and stoermer (2010)
Ammonium	Minor nitrogen source (favoured by non-diatom algal groups), inhibits amino acid assimilation	Admiraal *et al.,* (1987)
Total nitrogen	Sum total of nitrogen sources	Siver (1999), Potapova and Charles (2007)
Phosphate	Primary phosphorous nutrient, facilitates growth	Smol and Stoermer (2010)
PAR availability/ light attenuation	Affects growth rate (lower light attenuation allows for increased light (PAR) availability, lower light levels favour shade tolerant species	Chetelat *et al.,* (1999), Cantonati and Spitale (2009)
Total suspended solids	Reduces growth of by reducing light availability, favours motile species particularly when caused by resuspension of local sediment	Zambrano *et al.,* (2001), Roozen *et al.,* (2007)
Dissolved oxygen	Related to microbial activity, and primary productivity, nutrient required for frustule formation, affects species composition	Dakshini and Soni (1982), Leng and Barker (2006), Tang *et al.,* (2006)
pH	Alters composition of community based on individual species preferences for acidic, neutral or alkaline waters	Smol and Stoermer (2010)
Temperature	Increases diversity and biomass up to 25 degrees celsius, higher temperatures favour chlorophytes and cyanobacteria over diatoms, regulates rate of biochemical reactions	Piggott *et al.,* (2015)
Electrical conductivity	Measure of total ionic strength of the water, effect varies depending on composite ions, but typically alters community structure in favour of species that prefer higher availbility of nutrients	Potapova and Charles (2003)
Alkalinity	Diversity increases with alkalinity, linked to pH control on species distribution	Smucker and Vis, (2011)
DOC	Controls spatial distribution of diatoms by contributing to light attenuation	Evans et al., (2005), Smol and stoermer (2010)
Silicon	Element required for cell wall production	Smol and Stoermer (2010)
Magnesium	Related to pH, alkalinity and conductivity, affects community composition	Soininen (2007)
Potassium	Essential for photosynthesis, co-regulates enzyme reactions with sodium	Overnell (1975), Talling (2010)
Copper	Electron transport, anti-oxidant compounds and response to iron deficiency	Masmoudi *et al.,* (2013)
Fluoride	Micro-nutrient required for growth (can become toxic to some species at concentrations above 100 ppm)	Camargo (2003)
Sodium	Phosphate transportation proteins	Masmoudi *et al.,* (2013)
Chloride	Affects species composition (salinity tolerance of individual species)	Porter-Goff *eat al.,* (2013)
Sulphate	Affects species composition, related to conductivity and pH	Viktor and Szabó (2020)
Zinc	Carbon dioxide fixing, control of gene expressions, antioxidant processes and energy metabolism	Masmoudi *et al.,* (2013)
Calcium	Related to pH, alklainity and conductivity, affects community composition	Soininen (2007)
Iron	Chlorophyll synthesis, Nitrogen fixation, alkalinity	Martin (1990)
Aluminium	Silica shell mineralisation	Vrieling *et al.,* (1999)
Nickel	Hydroylsis of nitrogen from urea	Egleston and Morel (2008)
Lead	No known biological uses, bioaccumulative toxic element	Rivkin (1979)

The response of diatoms to different chemicals varies between the chemicals, and the mode of action they have on them. Response of diatoms to chemicals tends to involve a noticeable shift in the number of and abundance of species, morphological abnormalities, growth and/or reproduction rate, as well as changes to cellular DNA, lipid bodies and other organelles within the diatoms cells (Pandey *et al*., 2017). The variety of modes in which chemicals can affect the diatoms, coupled with the fact that many of these are fairly straightforward to observe, as detailed in later sections, makes diatom analysis relatively cheap and simple as bioassessment organisms. Huerta *et al.,* (2016) discussed the importance of benthic river biofilms (a combination of micro-organisms, including diatoms and other algae, buried within a matrix of extracellular polymeric substances (EPS)) to the health of river ecosystems, whilst also further referencing work by Sabater *et al.* (2007) that evidences these biofilms capacity to sequester chemicals out of the water and store them. Thus making them an excellent location to study the effects of pollution, creating three pathways of exposure: intracellular uptake, cell surface adsorption and adsorption to the EPS (Holding *et al.,* (2003), Morin *et al.,* (2008a)). There has been much work for the potential of benthic biofilms as a form of bioremediation, due to their ability to be monitored for the stress caused by the chemicals they are absorbing by pigments, and their existing use in the bioremediation of heavy metals and persistent organic compounds (Diels *et al.,* 1999, Dorigo *et al.,* 2004, Rodriguez and Bishop, 2008, Mitra and Mukhopadhyay, 2016). These factors make benthic diatoms particularly useful for measuring ecosystem health. As they will be exposed to a greater concentration of chemicals within the biofilm than other organisms free-floating within the water column, as organic contaminants, particularly those with higher molecular weights and surface properties designed to bind to other compounds, readily portioning from the water column to the sediment and biofilms. This is exacerbated by active uptake of the biological communities in the sediment actively taking compounds from the water column (Gobas and MacLean, 2003, Pal *et al.,* 2010).

Impacts of chemicals on diatom communities have also been shown to directly affect organisms higher up the food web. Brust *et al.,* (2001) showed knock-on effects of the herbicide terbutryn, which disrupts photosynthesis, on *L. Variegatus* (Blackworm), due to a scarcity of food resources. Another route through which the impacts of organic chemicals may affect higher organisms is through bioaccumulation and biomagnification. Diatoms and other algal groups may absorb lower quantities of the chemical as individuals, but organisms higher up the food chain will eat multiple contaminated organisms, and will receive a much higher level of exposure. This has been observed with the herbicides triclocarban, triclosan, and propanil (Coogan *et al.,* 2007, Dann and Hontela, 2011, Scholz-Starke *et al.,* 2018), demonstrating the importance of the exposure of diatoms to organic chemicals, and that changes to diatom communities can have significant effects on the wider freshwater communities.

1.2.3. Pressures on ecosystems

The structure and composition of freshwater ecosystems is controlled by pressures exerted on the communities by environmental factors, other organisms within these ecosystems, and anthropogenic influences. A report by Baron *et al.* (2002) defines how the integrity of freshwater ecosystems is dependent on not just water quality, but also the quantity and timing of the seasonal variations in the amount of water flowing through the system. The diagram below (Figure 1.1) is modified from this work and demonstrates the broad variables of flow regime and water chemistry, sediment flux, chemical and nutrient flux, heat (thermal) and light flux, and the biotic assemblage that influence the structure and function of the freshwater ecosystems. Internal factors that control variation and dynamics in freshwater ecosystems are shown in the diagram. The flow regime controls the speed and direction in which water, its associated chemical load, and even pelagic organisms move through the ecosystem; thermal/light input controls primary productivity and the metabolism of organisms; while sediment flux and chemical/nutrient flux controls the basic building blocks of the environment, including the base nutrients for the biotic assemblage, as well as being the controls on water quality, including pH and conductivity. The thermal/light input has been demonstrated to have the strongest influence on algal communities,

when compared to grazing pressure and nutrient availability, based on a predominantly diatom-based study by Lange *et al.* (2011). Dalu *et al.,* (2020a) noted that the turbidity of the water column negatively correlated to species richness in rivers, indicating light availability is a key driver in diatom community structures. Other external pressures have been identified, these include the impacts of climate change (Kundzewicz *et al*, 2009), as well as eutrophication caused by urban development increasing the nutrient and chemical load into freshwater ecosystems (Matthews, 2016, Gao and Zhang, 2010).

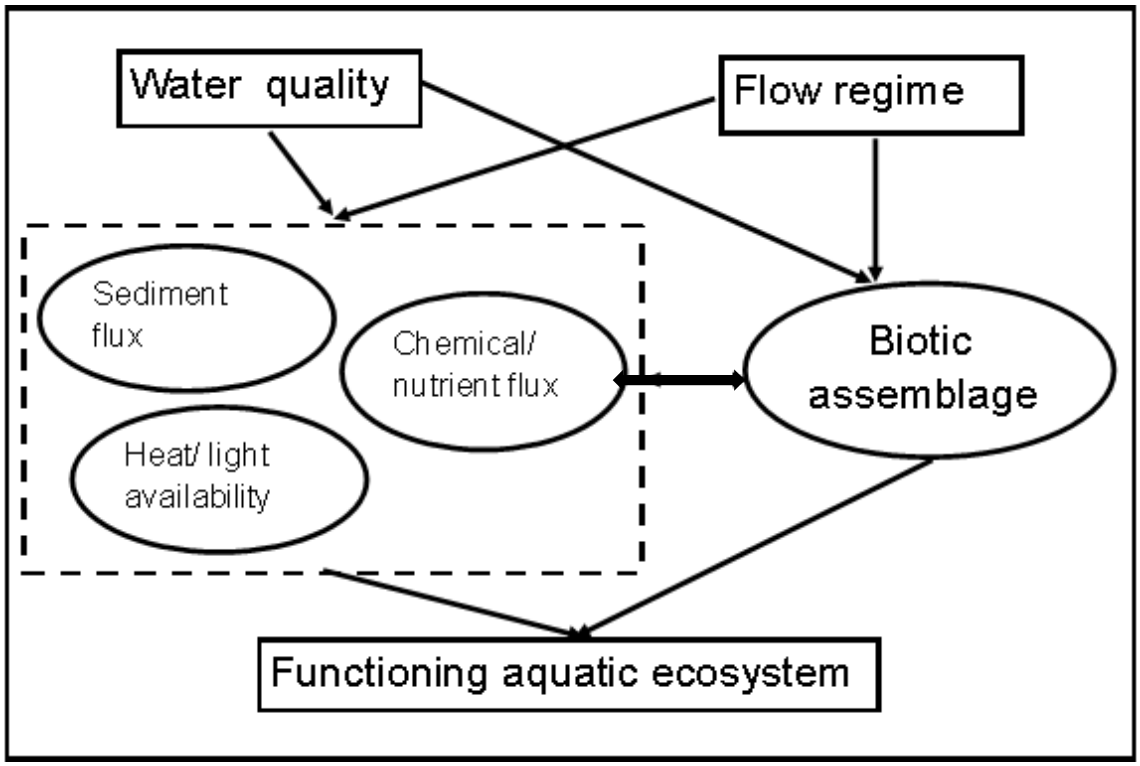


Figure 1.1. Adapted diagram of internal factors affecting functional aquatic ecosystems. Adapted from Baron et al. (2002). The flow regime and water quality are direct drivers of the four elliptical boxes (sediment flux, chemical/ nutrient flux, thermal/ light inputs and biotic assemblage) which then feed into the overall functional aquatic ecosystem.

The conditions of the surrounding environment have noticeable impacts on the communities that inhabit them. Admiraal (1976) tested four diatom species over a temperature range of 4-30^{o}C as well as light availability (four hours, eight hours, and 16 hours in a 24 hour period were tested). *Amphiprora paludosa*, *Nitzschia dissipata* and *Nitzschia sigma* had the highest growth rates at 25^{o}C and higher, and minimal irradiances of 2.5 to 5.0 E.m-2.day-1. However, for the fourth species tested, *Navicula arenaria*, the optimum growth rate was observed at a temperature of 16^{o}C. All four species had an optimum growth rate at 16 hours of light availability. Indicating that for these four species, although they all tolerated the same range of light availability, not all had the same tolerance to temperature, which would allow *Navicula arenaria* to outcompete the other three species in cooler environments, and *vice versa*.

pH is another water quality parameter that is important to monitor on freshwater organisms, including diatom communities, as changes can be potentially harmful to diatom communities and will even alter the composition of these communities (Clements et al., 2000, Hirst et al., 2002, Battarbee *et al.*, 2008, and Luis et al., 2011). Lowered pH values are often associated with heavy metal contamination studies as both are side effects of acid mine drainage (AMD), but can also be caused by precipitation of acid rain due to increased industrial activity (Verb and Vis, 2005, Battarbee *et al.,* 2011).

Alongside the effects of pH alteration caused by AMD, additional effects on the ecosystem due to the increased prevalence of heavy metals, including zinc, chromium, iron and cadmium have been shown to negatively affect freshwater ecosystems. As such benthic diatoms (Bacillariophyceae) are routinely used to monitor the impacts of these chemicals (Renzi *et al.*, 2014, Lelong *et al.*, 2013, Hirst *et al*, 2002, Luis *et al.*, 2011, Verb and Vis, 2005 Belenger *et al*, 1996).

Nutrient availability is also another key pressure on freshwater ecosystems, as excess phosphorus (P) and nitrogen (N) based nutrients can lead to algal blooms that negatively affect higher organisms, whilst an insufficient quantity of these nutrients can limit algal growth, an effect that has repercussions higher up the food chain (Kronvang *et al.,* 2005, Dolman *et al.,* 2016, Reisinger *et al.,* 2016). Additionally, although it has been repeatedly demonstrated that eutrophication (an excess of nutrients in a water body) leads to blooms in photosynthetic algae, including diatoms, that disrupt normal ecosystem functions and lead to the decline of macrophytes (Philips *et al.*, 1978), the inverse can also be true, where environmental contamination causes a reduction in primary producers, particularly in the presence of herbicides, or through bio-magnification of toxicity through the food web (Fleeger *et al*., 2003).

Another pressure on freshwater ecosystems is chemical contamination, caused pre-dominantly from anthropogenic sources. Some of this, as stated earlier, is from urban run-off. However, there are other sources. For example, chemicals can pass through wastewater treatment works and released into the environment (Tarpani and Azapagic, 2018). Huerta *et al.* (2017) identifies pharmaceuticals; chemicals designed to alter biological functions for extended time periods, and endocrine disruptors, chemicals that alter hormone functions, and cause behavioural differences in fish, as major groups of chemicals that are known to enter freshwater environments through wastewater treatment plants. This paper identified concentrations of up to 108 mg/L of Galoxolide, almost double the 59 mg/L EC_{50} value of the marine copepod *Acartia tonsa* for this compound. In laboratory studies these chemicals have been demonstrated to alter behaviour, particularly in regard to activity, aggression, boldness, exploration and sociality in fish (perch) (Brodin *et al.,* 2014). This same paper demonstrates the effects of the pharmaceutical chemical oxazepam on perch (*Perca fluviatilis*) and the invertebrate damselfly (*Coenagrion hastulatum*), with only the fish suffering from behavioural changes, specifically an increase in activity. This demonstrates the difference between species regarding mode of action and chemical sensitivity. This evidences the ability of chemical compounds from external sources to be transported into aquatic systems and have detrimental effects on the local ecosystems.

1.2.4. Summary

In summary, diatoms are useful as bio-indictors for ecosystem health, as they are widespread, reproduce quickly, and easily colonise new substratum within short time frames, making them easily culturable in laboratory settings, and able to show long term, multigenerational impacts of a chemical over much shorter time scales than most other organisms. Their placement at the bottom of the food web, and great enough size to be representative of the primary productivity trophic level, means that they are essential to freshwater ecosystems. As such, any impact on these communities will have a knock-on effect on higher organisms. These organisms have been used for several previous studies on herbicides, pesticides and anti-microbials and as such their sensitivity to several other organic chemicals has been documented. Diatoms have been presented in the literature as a reliable indicator of organic chemicals influencing ecosystem health compared to other biological indicator organisms, as they are least affected by other environmental considerations, and any chemical that negatively affects diatom communities is very likely to have an impact further up the food web due to their position at its base. Furthermore, although much is known about the impacts of many chemical groups, including herbicides, pesticides and even heavy metals on diatom communities, where they are typically more responsive to the effects of these chemicals than other organisms, such as fish and invertebrates, much less is known about the impacts of other types of chemicals, including organic chemicals used in HPCPs.

1.3. Methodology of benthic diatom-based ecotoxicology studies

In this section, the methodologies employed in the literature using benthic diatom communities to assess the impacts of organic chemicals have been reviewed. With a focus on the differences between the use of field samples and laboratory cultures, the experimental set up and the control of environmental parameters *in vitro*.

Diatom samples are collected from field sites by different means, depending on how readily accessible the area is. Most diatom communities that are easy to access, for example coastal waters or river samples, are extracted from these environments by scraping organic biofilms off rocks and other loose natural material by using a sharp blade or a brush (Medley and Clements, 1998, Verb and Vis, 2005, Guasch *et al.*, 1999). However other studies (Gold *et al.*, 2002, Hill *et al.*, 2000) have used artificial substratum, such as glass or ceramic, which have then been left in these environments and allowed to be colonised by benthic microorganisms. These were then brought into laboratory settings submerged in water sourced from the site of colonisation to prevent stress to the communities occurring from the transfer to a different growth medium and setting. Other factors need to be considered for *in vitro* testing, including temperature, photoperiods, and even the composition of the growth medium (Admiraal, 1976). This section will assess the experimental setups used, to inform the development of future laboratory-based tests on benthic diatom communities.

1.3.1. Diatom identification

Identification of the diatoms is based on observations of the morphology of the diatom frustule, the hard silica cell wall. Guasch *et al.* (1998) argued that the identification of environmentally sensitive species has not been consistent across different studies, primarily due to the similarities they share with other species leading to incidents of misidentification. Kociolek (2005) further argued that, due to the difficulty of identifying individual species, additional issues regarding the continuous identification of entirely new species, changes in taxonomic position and names of already well-known species leads to constant issues with consistency of identification over time. This places significant issues with lower taxonomic identification in scientific studies. Rimet and Bouchez (2012) concluded that the level of taxonomic rank identification required will also vary depending on the project at hand. For example, the EU Water Framework Directive will require species level identification, as it requires highly accurate water quality and ecological measurements, whereas studies where the ecology of the individual species is unknown, or this level of detail is not required, then genus or even family level identification of diatoms would be acceptable to use.

Most diatom studies that examine the morphology of the diatoms use a method of chemical cleaning (usually hydrogen peroxide) of the biofilm samples to remove organic matter. Followed by sealing the resultant sample to microscope slides using Naphrax as an adherent, due to its high refractive index, aiding in revealing details in the frustules. Gautam *et al.,* (2017) notes that the use of permanent microscope slides comes with the issue that only one side of the diatom is visible, and as such all other views cannot be examined for deformities. A second issue is that the process of peroxide cleaning and mounting means that these slides cannot be used to differentiate dead or living cells, and information on the 'intactness' of the intracellular organelles, such as the chloroplasts, are lost. However, the longevity of these slides means that recounts of the data is possible, and that the sample will not change its composition over time as with live samples. Microscope images of small quantities of the original sample can be used for this metric (Debenest *et al.,* 2009), although this often leads to difficulties stemming from organic matter obscuring the diatom cells. Diatom counting under the microscope slide is based on each valve identified that is at least 50% intact. Colonies are based on the number of identifiable diatoms in the colony and a whole frustule therefore counting as two (this is where the difficulties about seeing the whole frustule in permanent slides as identified by Gautam *et al.* begins to set in). A total of 300-1000 diatoms, depending on the quantity of chain colonial cells to avoid statistical bias, are then

typically counted per slide to create a statistical count of the species present (Guzkowska and Gasse, 1990). However, the statistical accuracy of the results generally does not improve after 300 frustules and lower densities of the cells may further limit the volume that can be counted within a reasonable timeframe (Soeprobowati *et al.,* 2017). Diatoms are usually identified by using reference books, such as Krammer and Lange-Bertalot (2004) as references (Debenest *et al.*, 2009, Gautam *et al.*, 2017). Or online databases, based upon these works, such as Diatoms of North America (2020), or the Welsh Natural History Museum (Jüttner *et al.,* 2020). However, diatom taxonomy can be convoluted, with new species constantly being added or pre-existing species being redefined as teratological forms, or variants of another species (Falasco *et al.*, 2009).

1.3.2. Community or species studies?

Within the research literature there is a split regarding the way in which benthic diatom studies are undertaken, between those studies that have focused on single species of diatoms under laboratory conditions, and those that study entire communities either *in situ*, or transplanted into laboratory conditions after development in the field. For this latter group, issues may arise due to the natural variation within communities and environmental parameters, creating noise that can obscure the variable being studied. This can lead to complications in experimental methodologies that are difficult to control, particularly in studies covering large areas, where even small changes can have major impacts on the environment's chemical and physical parameters (Kelly *et al.,* 1998). Single species studies are typically employed when the effects of a chemical on individuals is being studied, as species level tests allow for multiple identical organisms to be tested. Multispecies, community level studies, are used to assess the effects of a chemical in a more realistic and natural setting, including competition from other species, making the results more effective at predicting the real-world effects of a chemical (Navarro *et al.,* 2002, Rico *et al.,* 2018, Bautista-Chamizo *et al.,* 2019). Single species studies, however, are less susceptible to the influence of environmental variations, but they do not demonstrate the impacts of a chemical across the ecosystem. This is because they can only be performed in controlled laboratory environments (Jha, 2004). Single species studies have also been shown to produce results indicative of ecotoxicological effects at lower concentrations than community studies, and potentially underestimating the real-world concentrations at which an effect occurs (Franz *et al.,* 2008). Collection methods in the case of single species studies can be more varied. Where community samples tend to be exclusively collected from sample sites for the purpose of assessing larger scale effects of chemicals at the community level, single species studies are designed to assess the effects of a chemical at the individual level, and often use samples taken from culture stocks (Veldhuis *et al.*, 2001), but can also be isolated from community samples (Shishlyannikov *et al.,* 2011).

1.3.3. Control of environmental parameters

For a laboratory method to be reliable and easy to replicate, the control of key environmental parameters needs to be considered. In most cases of *in vitro* experimentation, the environment was controlled to simulate as natural an environment as possible, and maintain control over the conditions of the experiment, and the variables that are altered. Some studies have taken this further than others. For example, by using flowing waters (Rimet and Bouchez, 2011) to simulate natural variation to reduce stress on the samples if they were from riverine systems. It is important in diatom studies to recreate the species' original environment as closely as possible, as it has been shown that even subtle changes in physicochemical parameters, including temperature, light intensity/ duration or water chemistry between natural and artificial conditions can cause stress to occur and cause morphological abnormalities in the frustules or reductions in growth rates that may affect the results of tests, particularly with studies that are looking for these teratological alterations (Admiraal, 1976, Falasco *et al.*, 2009).

Studies that involve culturing within laboratory conditions usually attempt to control the temperature of the environment. In most cases this is a fixed temperature, with most benthic diatom communities being cultured in environments with temperatures between 18-25°C to

mimic natural conditions, with a variability of up to 2°C (Desai *et al.*, 2006, Chang *et al.*, 2011, Wood *et al.*, 2016). The use of incubators or controlled temperature environments has been used in virtually all studies conducted indoors to provide control over the temperatures of the experimental set up. Debenest *et al.,* (2009b) used varying temperature parameters to help simulate changes between daytime and night-time, along with controls on light conditions, which helped to further mimic diurnal patterns. Differences in photoperiods, as well as temperature used during culturing have been observed to influence the growth rate of diatoms, as shown in single species diatom tests performed under such conditions, although this practice has not seen widespread adoption. Lebeau and Roberts (2003) state that the ideal photoperiod for the culturing of diatom communities is between 16:8 and 24:0 hours of light: dark. Photoperiods of 14:10 hour have also been used but is less common, as reduced photoperiods inhibit growth (Admirall, 1976, Yang and Flower, 2012), with papers by Desai et al. (2006), Roubeix et al. (2011) having used this photoperiod. Kiefer (1973), Other work has used a photoperiod of 12 light: 12 dark, and appears to be the more common photoperiod for benthic diatom research (Arini *et al.* 2012, Lelong *et al.,* 2013, Wood *et al.* 2014, and Wood *et al.* 2016). The reasons for the varying photoperiods are never explicitly mentioned within the papers, despite research demonstrating the importance of photoperiod for diatom growth (Li *et al.*, 2017). This variation is likely due to a trade-off between productivity for growth, and accurate simulation of natural environments, particularly seasonal variations in the daily light cycles. The intensity of the received light also has an effect on the diatom communities. Tuji (2000) demonstrated that earlier colonising benthic freshwater diatoms preferred, or were able to tolerate higher light intensities, and later colonising species were more shade tolerant. However, an experiment by Wellnitz and Rader (2003) using daily mean light intensities of 1696µmol/m^2/s and 506 µmol/m^2/s at St. Louis Creek, USA found that, although the decreased light was associated with lower ash-free dry weight concentrations (AFDW), it had no effect on the algal biovolume.

Nutrient availability of the growth medium is also a significant factor in biofilm development and is typically selected to meet the nutrient requirements of as many species of diatoms as possible, to avoid favouring certain species (Ramírez *et al.,* 2015). Many of these mediums, such as DAM are initially created from natural water samples from the sites the samples are taken from, and in the case of long-term cultures, typically modified based on the known nutrient requirements of the test organisms (Gagneux-Moreaux *et al.,* 2007, Wood *et al.,* 2014). Li et al., (2017b) showed that the Nuagli nutrient mixture and a nitrogen to phosphorus (N:P) ratio of 6:1, along with silica enrichment led to the highest cell densities. Debenest *et al.,* (2009b) tested two modified nutrient mediums (Chu no.10, from Nichols and Stein (1973) and Freshwater "WC" medium, from Guillard and Lorenzen (1972)), with the Freshwater "WC" medium, which had fewer micro-nutrients in higher concentrations (including zinc and copper), but also contained extra calcium and sodium macro-nutrients, contributing to the higher growth rate in the laboratory cultures. However, Gérin et al. (2020) found that compared to their novel freshwater medium, and another medium referred to as MCOMBO, the Freshwater "WC" medium provided almost no growth to single species studies of *Sellaphora minima* and *Nitzschia palea*. As such, although there are several diatom mediums available, their effectiveness does vary across cultures, and any long-term laboratory cultures will need to consider this factor.

pH variation also has immediate effects on the availability of many compounds for biological uptake, particularly with metal compounds where higher pH values increase the bioavailability to freshwater diatoms (Liehr *et al.,* 1994, Irving *et al.,* 2009). Lower pH (7.5 compared to 8.3) also increased the rate of bioaccumulation of triclosan on *Navicula* (Ding *et al.,* 2018). All diatoms have a species-specific pH tolerance range (Kilroy *et al.,* 2006). Additionally, due to their short lifespan, changes in diatom communities can be used to monitor relative changes in the pH of the environment based on the abundance of different species in the samples obtained (Hirst et al., 2002, Flower *et al.*, 1993).

1.3.4. Substratum

One of the key considerations in the culturing of diatom communities is the substratum used to develop the biofilms on. The substratum used in the laboratory growth of diatoms do not drastically differ from those used to grow *in situ* samples in work by Gold *et al.*, (2002), Hill *et al.*, (2000). Studies in the lab, such as those by Arini *et al.*, (2012) and Khan, (1991), have been undertaken with glass microscope slides, ceramic plates and analytically clean glass. Arini *et al.*, (2012), continued taking samples from the Riou-Mort river throughout the experiment, and as such the samples were scraped off the surfaces of small rocks within the river channel. The experiments also used an 'artificial stream' made from PVC and cycling water from the bottom of the channel back to the top to simulate fluvial conditions for samples taken at the start of the experiments. The diatoms used here were grown on conventional glass microscope slides. Rimet and Bouchez (2011) used a similar set up to simulate the natural flow of rivers, the only significant difference between this experimental setup and the one used by Arini *et al.* (2012) was that the tubes were made of stainless steel, however since the samples were still being grown on glass slides this had no real impact on the outcomes of the experiments. Conflicting research has shown that there are significant differences between communities grown on different substratum. Investigations by Yang and Flower (2012) using the Round Loch of Glenhead (Scotland) demonstrated that in this oligotrophic lake, substratum material influenced the composition of the community, but diatom total abundances were influenced by depth (0-10 meters), with an increase in depth correlating to a decrease in the total abundance of diatoms. Contrary to this, Barbiero (2000) states that microscope slides only had a 37% similarity in diatom community composition compared to natural stones, whilst other research has shown that the differences between natural and artificial substratum present no more than one degree difference (based on the Bray-Curtis similarity index) in coastal dune lakes (Lane *et al.*, 2000). Other research by Siver (1977) demonstrated that microscope slides tended to overrepresent the species *Achnanthidium minutissimum*, a small and common pennate species, at the expense of *Eunotia incisa* and *Cocconeis placentula* compared to communities on *P. robensii* leaves. Vadoboncoeur (2006) also found that substratum developed on hard surfaces in lakes were positively linked to water column phosphorus, but not on soft substratum, based on chlorophyll-a concentration endpoints. As such, although there is evidence that there are strong similarities between the diatom communities that develop on the commonly used artificial substratum and natural communities, the evidence is not conclusive, and requires further testing.

1.3.5. Addition of test chemicals to exposure experiment

In order to test the effects of a chemical to a diatom community, controlled additions of it to separate replicate vessels at fixed concentrations need to be considered. Typically, this involves using a known concentration of the test chemical in solution, along with separate replicates devoid of the chemical being tested (Debenest *et al.,* 2009b). During ecotoxicological experiments on benthic diatom communities', multiple replicates are exposed to a fixed range of a known concentration of a chemical (usually at least four concentrations). These are used to establish an EC_{50} concentration, where 50% of the maximum potential effects of a chemical on the test organisms are observed. Compared to results from mathematical modelling, and previous range-finding experiments (Sebaugh, 2011). For diatom-based studies, the exposure times typically cover a period of between six hours to twelve days, depending whether the research is focussing on short term or chronic effects, respectively (Debenest *et al.,* 2009b, Proia et al., 2011, Wood *et al.,* 2017). Control replicates without any chemical added are also typically used to monitor community changes due to natural progression of the biofilm community or changes caused by their transfer to the test environment (Ricart *et al.,* 2010, Ding et al., 2018). In experiments where multiple chemicals are used, the chemicals are exposed separately, so the effects of each individual chemical can be observed, rather than their combined effects (Larras *et al.,* 2012, Wood et al., 2016).

1.3.6. Differences between standard ecological monitoring and ecotoxicological endpoints

A further point of importance, is the difference between the methods under which diatom communities have been typically monitored under ecological monitoring programs, which has been codified by the UKTAG methodology in the U.K, and the methods under which ecotoxicological measurements take place. Ecological monitoring methods, which provides the bulk of the usage of diatom-based assessments are conducted in field. As such, a myriad of different interacting factors affects the composition of the community, which are accounted for in the monitoring methods, and many of these key environmental parameters are measured in parallel to the diatom assessments (Kelly *et al.,* 2008, Bere and Tundisi, 2010). Conversely, ecotoxicological measurements, are conducted under strictly controlled conditions, where the only variable is the chemical added to the experimental vessel, and are not subject to the variations observed in field monitoring methods (Wood *et al.,* 2014). Most tests on diatoms at the community level that make use of ecotoxicological methods for the assessment of chemical effects in the past have used pre-existing biofilms from a particular site, or grown on artificial substrates placed at a single site, for the purpose of developing biofilms on controllable, replicable substrates for ecotoxicological testing (Rimet, 2012, Pandey *et al*., 2017). This provides the trade-off of allowing strict ecotoxicological testing on a realistic community, but there is no control over the development of the community, which will be exposed to widely fluctuating and unreproducible field conditions during the development. As such, using current methods of field culturing, these communities cannot be considered useable for standardised studies, and each assessment will be conducted on a completely different community (Debenest *et al.,* 2009b). As such, for fully standardisable ecotoxicological testing on diatoms at the community level to be achieved, a method for culturing stable, long-term diatom communities with a composition representative of more than one water body is needed, similar to what has already been achieved for single species samples. This will allow for the assessment of communities in a holistic way, using ecologically relevant communities that can be grown in a controlled manner, to determine real world effects a chemical may have on these organisms through ecotoxicological testing.

1.3.7. Summary

To conclude, depending on the study requirements, there are different protocols that could be used. The ideal scenario would involve test communities being grown in situ, and then transferred into laboratory conditions for the exposure experiments, to ensure that any testing conducted is an accurate representation as possible of the effects that would occur *in situ* as possible. This is best being conducted on glass or ceramic slides, or even natural substratum from the environment the communities were taken from, although this substratum may cause issues with replicability. Measurements of change in the community will ideally be done via chlorophyll-a growth measurements, as well as measurements in community structure changes (relative abundance, diversity and evenness) to demonstrate statistical changes in structure. From these settings, exposure of singular species or entire communities of diatoms can then be exposed to a broad range of concentrations of a chemical using a variety of biological endpoints to measure the effects. If long term laboratory growth is required, then the biofilms should be tested at a variety of temperatures, light intensities and durations, to identify which combination of parameters will yield optimal growth, whilst the testing of different growth mediums should be conducted to determine which medium will provide the best nutrient sources for the communities being tested.

1.4. Ecotoxicological endpoints for measuring change in diatom communities

Ecotoxicology studies into the effects of chemicals on organisms use an 'endpoint', which is the change being studied in the test organism or community. Endpoints such as abnormalities in diatom frustule shape or the integrity of the cell's DNA are used to measure the physical and biological impacts of a chemical on the functioning and structure of the exposed organisms.

Although certain methods brought up earlier, such as growth rate endpoints measured by chlorophyll-a concentrations, can also be used as endpoints. This section will focus on these endpoints and how they have been used in diatom-based studies on ecotoxicology. Specifically, endpoints that can be applied to measure community level changes. This will include endpoints capable of measuring the size of the biofilm communities, and the diatom communities in particular, as well as the composition and structure of these communities, based on the number and distribution of species (diversity indices) and the abundances of different groups of diatoms adapted to a specific method of surviving in biofilm environments (ecological guilds/ life forms).

1.4.1. Community level ecotoxicological endpoints

Community level endpoints are generally used to measure the structure, composition and sizes of the diatom communities, such as with diversity indices, growth rate, and biomass measurements. These can also be methods traditionally applied to single species studies, but can be scaled up to measure the effects at the community level, such as pigment production and observable damage to intracellular organelles.

Change in net primary productivity

Diatoms are primary producers, as such their activity can be measured as primary productivity. There are various endpoints related to changes in productivity discussed in the literature such as change in biomass, periphyton mass accrual rate, rate of photosynthetic activity, respiration rate and growth rate. These are each discussed below.

Dissolved inorganic carbon (DIC) can be used to measure productivity in a biofilm sample containing diatoms to estimate the rate of photosynthetic activity of the sample, by measuring the rate of uptake of carbon-14 isotopes. This method has been successfully employed by Nielsen *et al.,* (1952) and Birmingham *et al. (*1982).

Algal and diatom biomass are measures of the total mass of algal and diatom communities on a known surface at a point in time. Benrhardt and Likens (2004) measured changes in periphyton algal biomass in a headwater stream in Hubbard Brook experimental Forest, New York, assessing the impacts of nutrient limitation on biomass. Clément and Cadier, (1998), Croce *et al.,* (2017) measured the change in freshwater algal biomass using fluorometers to measure the *in vivo* fluorescence of a known quantity of biofilm that had been thoroughly mixed before removal for chlorophyll-a concentration.

The rate of change of biomass can also be measured. Periphyton mass accrual rate is the rate at which a substratum accumulates periphyton mass (algae that live on surfaces), can be used to measure the rate at which a substratum is colonised. Bowes *et al.* (2012), and Lehosmaa *et al.,* (2018) used a phytobenthos fluorescence measurement instrument to assess the rate of periphyton mass accumulation on unglazed ceramic tiles after seven weeks of incubation at groundwater springs in Finland.

Biomass is another biological endpoint employed in ecotoxicological studies of benthic diatoms. It is typically measured by the use of Ash-Free Dry Weight/ Mass (AFDW or AFDM) measurements, by using a subsample of biofilm to measure its organic matter content and so measure the mass loss of the subsamples' dry weight after combustion (Vera *et al.,* 2010). This measure has been used alongside algal biomass and taxonomic identification by Kang et al., (2018). Hill *et al.,* (2000) and Lozano *et al.,* (2018) used this measure, along with chlorophyll-a content (algal biomass measurement) to create an autotrophic index (AI), which measures the proportion of heterotrophic organisms and organic detritus in the biofilm compared to the chlorophyll producing autotrophs, based on the ratio between the two values. Higher AI values indicating a reduction in the fraction of the algal composition of the biofilm. This indicates what proportion of the biofilm is composed of the algal organisms, and can be used to measure the size of the phototrophic communities in response to the presence of a chemical.

The rate of photosynthesis of a diatom sample can be used as an endpoint, measuring by Biochemical Oxygen Demand, (BOD). This method uses one transparent and one darkened plastic or glass bottle containing diatom samples and a dissolved oxygen probe. The darkened bottle inhibits photosynthetic activity of the diatoms, and so provides a baseline measurement of respiration, whilst the transparent bottle permits it. The dissolved oxygen content at the start of the test is measured using one of the bottles, and the differences between this value and the darkened bottle will determine the volume of respiration in the container, whilst the difference between the initial value and the transparent container will provide an indication of net photosynthesis within the samples (Boyd, 2015). This method is used to assess the rate of photosynthesis for the entire community. The drawback is that it is typically only used for pelagic communities; as it measures dissolved oxygen in the water column. As such, for it to be useful for measuring benthic communities, the water column must be clear of all photosynthetic organisms and a method for developing or transferring the communities into the BOD container for the duration of the measurement will need to be developed. Due to this, only field-based tests on phytoplankton currently employ this method (Keck *et al.,* 2016, Pandey *et al.,* 2018).

Growth rate

Chlorophyll-a concentrations have also been used to measure growth rate by Larras *et al.* (2012), to assess the growth rates of the diatom species in their study before during, and at the end of the organic chemical exposure, using it as a comparison of growth when comparing samples exposed to different levels of the chemical. Despite its usefulness, Pérez *et al.* (2009) cautions against the use of this endpoint, particularly with natural communities, as there are other factors, including the different growth rate of individual species involved that can cause variation in growth rates observed. This could potentially be mitigated by conducting a count using light microscopy to determine the species involved and cross-referencing the proportions of each species identified with their known growth rates from species-based studies. However, if this is unknown then such studies could be time consuming and costly, but the results could still be applied with caution. Kiefer (1973) and Roubeix *et al.,* (2012) used the chlorophyll-a pigment to estimate the biomass of living algal cells, particularly diatoms, as this is the primary photosynthetic pigment used by diatoms, whilst other organisms use it as a secondary pigment. This process measures the *in vivo* fluorescence of chlorophyll-a after extraction in acetone, and then exposed to a laser beam in a darkened chamber which then causes fluorescence in the chlorophyll-a pigment and emits light at a frequency of 614 nm. However, due to the high turnover rate (reproduction and death of individuals) of algae, the results of chlorophyll-a analysis can only work as an estimate, and not an absolute measure of growth (Maxwell and Johnson, 2000, Harris, 2012). This analysis can also be performed by flow cytometry (Franklin *et al.,* 2001, Lelong *et al.,* 2013).

Cell mortality

Several studies (Arini *et al.*, 2012, Wood *et al.*, 2014, Wood *et al.*, 2016) have measured the mortality rates of diatoms in their samples, by staining the intracellular organelles with dyes that will only be taken up by live diatoms, and thus allowing the differentiation between living and dead cells. Chang *et al.* (2011) looked at the impact of β-cyclocitral, a metabolite produced naturally by some species of cyanobacteria, and its capacity to cause cell rupturing in the freshwater diatom species *Nitzschia palea*. This work follows a different approach to diatoms compared to most of the literature surrounding diatoms in ecotoxicology, in that it looks at how to use this chemical to reduce and control populations of algae and cyanobacteria in water bodies, particularly those used for human consumption, rather than as an ecological/ ecotoxicological assessment. This endpoint, although most effective assessing the mortality rates by species, can be applied to the community level, as with Morin *et al.,* (2010), where the effects of triclosan were shown to have a 63% increase in the mortality of the diatom community at 500 ug/L in artificial streams.

Species composition, relative abundance and diversity indices

Diatom cell density, the function of how closely packed the cells of a biofilm are, is a community level measure for assessing diatom community health in response to chemical exposure. In this

method, the total number of frustules in a given area are counted, to identify how many individuals are in this area, which is then directly linked to the productivity of an environment (Soininen and Teittinen, 2019). Seguin *et al.,* (2001) used diatom cell counting to measure the effect of Atrazine, by counting the cells in a known volume of biofilm. Gold *et al.,* (2003) noted a decrease in cell density at freshwater sites polluted by cadmium and zinc. Morin *et al.,* (2008a) used cell density to show that an increase in triclosan concentration (0.05- 500µg/L) lead to an increase in diatom cell counts, due to a reduced competition with bacteria which were more severely affected by the chemical. Franklin *et al.,* (2001) Stauber *et al.,* (2002), Cellamare *et al.,* (2010) and Lelong *et al.,* (2013) used a flow cytometer to count the number of diatom cells present in biofilms.

Relative abundance of diatom species and biodiversity indices such species richness, evenness and Shannon diversity index, are common endpoints for many diatom-based studies to determine ecosystem health, by measuring changes in the community assemblage based upon observed differences between control samples and those exposed to the chemical (Ricciardi *et al.*, 2009, Gold *et al.*, 2002, Verb and Vis, 2005) This method of relative abundance is generally faster and more time and cost effective; as a set value (typically 300-500 frustules) is used, rather than the whole composition of a given volume, which can often reach several thousand cells per sample (Kireta *et al.,* 2012).

Ecological guilds and life forms

Rimet and Bouchez (2011) used a relatively recent method of using diatoms for ecological studies, by dividing the species based on life forms; the ways in which the diatoms live in their environments (e.g. benthic, planktonic and colonial), and by ecological guilds, the method by which an organism exploits available resources (low-profile guild; species that lay close to the substratum surface, high profile guild; species that are attached to the biofilms, but stick out from the surface), and the motile guild (species able to physically move on their own)). This has the advantage of dividing the species by their function within the ecosystems they were taken from. Stenger-Kovács *et al.,* (2013) used this methodology to ascertain that low-profile guilds were the more dominant group during periods of low resource availability (light, silicon, nitrogen and phosphate), high profile guild was dominant during periods of resource richness, and the motile guild was the most sensitive to changes in these factors, although no reasoning is given. Research by Rimet and Bouchez (2011), Larras *et al.,* (2012), and Marcel *et al.,* (2013) states that the motile guild diatoms are less affected by organic chemicals than others due to their ability to move to habitats with thicker exopolysaccharide matrices, similar to how low-profile guild diatoms and species that live in mucous tubes are considered to have protection against dissolved chemicals by this matrix. Although this assumption has yet to be tested, research has shown that in marine diatoms these mucilaginous structures protect against increased salinity in marine diatoms (Steele et al., 2014). Whilst the high-profile guild species are the most exposed to these chemicals in the water column. As such, high-profile guild diatoms species will likely be the most sensitive to chemical exposure, as they will typically be the most exposed in the biofilm, and therefore the percentage of these species compared to those of other ecological guilds in the biofilms could be compared to measure the effects on the wider community structure.

Another method of assessing diatom community structure is through the use of Trophic Diatom Indices (TDIs), to use the structure and composition of the benthic assemblages to interpret the overall ecosystem health (Gomez and Licursi, 2001, Coste *et al.,* 2009). In the UK, starting with work by Kelly and Whittion (1995), a TDI for the U.K was developed, converted using alkalinity data for the site into ecological quality ratios (EQRs) and a nutrient sensitivity score system based on individual species prevalence in eutrophic, chemically impacted sites, or oligotrophic, unimpacted sites, and conducted within the DARLEQ 2 software (UKTAG, 2006). This allows for rapid assessment of ecosystem health and is used to classify the water quality of freshwater ecosystems under the WFD.

Damage to diatom cell organelles

Some efforts have also been made to use chloroplasts in ecotoxicity testing. Although success has been limited, with tests exposing diatom cells to Cd and Zn showing no alteration in chloroplast presence (Arini *et al*, 2012), light and nutrient stress has been shown to reduce the size of chloroplasts in live diatoms. Furthermore, organic chemical contamination has been shown to cause profound differences. Wood *et al.* (2014) has demonstrated the toxicity of atrazine on diatom communities over 48 hours, with the more sensitive genera's, *Amphora*, *Cymbella*, *Gomphonena* and *Ulnaria* showing a significant decline (34-75%) in chloroplast intactness. A follow up study further demonstrated the sensitivity of *Gomphonena* species to multiple different organic chemicals based upon the intactness of the chloroplasts. Panday *et al*., (2017) reviewed the use of this method over recent years and concluded that this method of observing the integrity of benthic diatom chloroplasts is ideal for future rapid and inexpensive ecotoxicological testing, including at the community level, where the percentage of affected individuals can be assessed, and split based upon ecological guilds and life forms.

DNA damage to the diatoms nucleus is a relatively new approach to studying the effects of environmental chemicals on ecosystems. Desai *et al.* (2006) used the comet assay methodology to assess the effect of Cadmium exposure to marine diatoms. One disadvantage of this method observed in this work is the fact that it can only be done at the individual level, thus making DNA damage studies on whole communities very time consuming and expensive. This limiting factor has meant that most studies have isolated key species within the ecosystem that are known for their sensitivity to the chemical being studied, or those that the researchers believed most represented the diatom community in question, based on their greater presence in the environment. Debenest *et al.* (2008) used changes in the size, shape, position and fragmentation of the nucleus of diatoms sampled as community assemblages from the Garonne basin to determine DNA damage. They used a standard light microscopy counting exercise, with the slight alteration of the addition of a chemical staining solution added during culturing. A similar methodology was used by Licursi and Gómez (2013) to identify damage caused by hexavalent-chromium.

Both Debenest *et al.* (2008) and Licursi and Gómez (2013) also used frustule abnormality (teratology), as a percentage of the entire community as an endpoint in their studies, thus demonstrating the impacts of chemicals on both the diatom frustules, as well as their inner cellular organelles at the community level. The split in the use of different methodologies seems to indicate that the use of the Comet assay method is more suited to studying DNA damage caused by chemicals at the species level, whereas examination of nucleus abnormalities is more suited to studies operating at the community level, due to the time intensiveness of the comet assay, whilst the percentage of individuals with nuclear abnormalities can be rapidly assessed by eye and counted as a percentage of either the species, or the broader community, depending on the aims of the experiment. The latter also has the added benefit that such an endpoint can be studied in tandem with other more commonplace diatom assessment methodologies, such as relative abundance, species diversity and frustule abnormality measurements.

Other cellular pigments and compounds as endpoints

Diatoms, as with all other photosynthetic organisms, produce a variety of different pigments in their cells which have a specific function. This subsection will focus on how the changes in the concentrations of compounds within the diatom cells have been used to determine changes in the community structure before and after chemical exposure.

Diatoxanthin is a pigment found in diatoms and some phytoplankton. It is a form of the xanthophyll carotenoid that is responsible for photo-protective dissipation of energy in the cell (Demmig-Adams and Adams, 1996). This pigment, along with Superoxide dismutase, and protein thiols, was linked to increased oxidative stress from nutrient depletion on the diatom *Nitzschia microcephala* (Pinto *et al.,* 2003). Using the marine diatom *Phaeodactylum tricornutum*, Bertrand *et al.,* (2001) conducted a test using axenic cultures using reconstituted seawater to demonstrate

the inhibition of diatoxanthin epoxidation during exposure to cadmium. Further testing on *Phaeodactylum tricornutum* showed that bezafibrate, a drug designed to prevent heart attacks and reduce lipids in the human body, showed a negative effect of these chemicals on the concentrations of diatoxanthin, its progenitor xanthophyll diadinxanthin, as well as chlorophyll-a, chlorophyll-c, pheophytin-a (a breakdown product of chlorophyll-a), fucoxanthin and beta-carotene pigments over a 48-hour exposure experiment (Duarte *et al.,* 2019). However, these pigments may not necessarily be affected by the chemical being tested. For example, Chlorophyll-a concentrations were significantly reduced when *Gomphonema gracile* diatoms were exposed to pesticides (S-metolachor and diuron) by Demailly *et al.,* (2019), as these chemicals directly affected the diatoms' photosynthetic pathways, however the carotenoid concentrations were unaffected by either herbicide. Like this paper, other research frequently groups carotenoid pigments together, rather than looking at individual pigments (Cabrita *et al.,* 2016, Ding *et al.,* 2018, Guasch and Sabater, 1998, Navarro *et al.,* 2002). Despite this, there has still been a large body of research using specific carotenoid pigments. Research on fucoxanthin, another xanthophyll pigment, is typically found in diatoms, and has been found to be present in particularly high quantities in species of the *Asterionellopsis*, *Cyclotella*, *Navicula*, *nitzschia* and *syndera* genera (Li *et al.,* 2017).

Although individual pigments can be useful for the analysis of growth rates or effects of chemicals that inhibit certain functions (such as chlorophyll-a with exposure to herbicides), even more information regarding the exposed diatom community structure and health can be gathered when changes in the different pigments are then compared to one another. The ratios of different pigments can differentiate the abundances of different algal groups. Barrenguet *et al.,* (2003) used the high ratios of fucoxanthin to chlorophyll-c as an indication of biofilm dominance by diatoms, and decreases in the ratios of these two pigments as an indication of a decrease in the size of the diatom communities relative to other groups in experiments using artificial channel systems directly fed by the river Rhine. In another study, zeaxanthin, lutein and beta- carotenoid ratios were used to explain abundances of green algae vs cyanobacteria in community samples from artificial glass substratum cultured in the Osor stream, Spain (Corcoll *et al.,* 2012) The use of pigment ratios could then be used to determine the relative size of communities of different algal groups in a sample without having to manually count hundreds of individuals through light microscopy. Further testing using the ratios of different pigments to measure stress have been studied. Kosakowska *et al.,* (2004) used *Phaeodactylum tricornutum* as a test species to show the variation of diatom pigments (specifically low concentrations of chlorophyll-a, and high concentrations of b-carotene) to the concentration of diadinoxanthin in response to reduced iron content.

Chemical analysis of diatom communities has not been limited to pigments, with other compounds present in the cell having been found useful for assessing diatom community health in the presence of chemical agents. Tests using concentrations of protein thiols as an endpoint for effects of arsenite and dimethylarsenic acid on the diatom *Nitzschia palea*, as well as the effects of different metals (Cu, Zn, Ni, Pb and Ag) on the green algae *Scenedesmus vacuolatus* have shown that these compounds are created in higher concentrations by diatoms to protect the cell from damage (Le faucher *et al.,* 2006, Ding *et al.,* 2017). However, these protein thiols have yet to be tested for their potential uses for monitoring the effects of HPCP chemicals on diatom cellular health.

1.4.2. Summary

This section has identified several endpoints used in ecotoxicological testing of diatom communities which may be applicable to the testing of the effects of HPCP chemicals. Such endpoints should include standard diatom community analysis, identifying the diatoms present to the species level for analysis via diversity indices metrics, to assess the relative abundance of the individual species, how evenly distributed these species are, and to assess the life forms and ecological guilds of the species. Other measurements, including ash-free dry weight, and chlorophyll-a pigment concentration can be used as a measure of biofilm productivity changes in

response to the addition of chemicals, and the measurement of cell mortality can be used to determine if the chemical exposed to has any effect on the rate at which the diatom cells die. Although, these pigments and the cell mortality rates have an underlying issues of species level variations that will not invalidate the results, but will limit their effectiveness. Other more specialised assays, including the monitoring for abnormality in the cell frustules, or the intracellular organelles can be conducted to assess any physical defects to the diatom cell caused by the HPCP chemical. The measurements and comparison of specific pigments and compounds within the cells across the community can be used to measure the overall health of the diatom cells and compare the response of different species within the community without laboriously counting individuals, in response to the additions of these chemical compounds to the replicate vessels. These results, or even a combination of four or five different endpoints, particularly the changes in net primary productivity, species composition, diversity indices, ecological guilds and life forms of the species present could be potentially combined together to provide a broad overview, to demonstrate the effects the HPCP chemicals have at the community level.

1.5. Effects of organic chemicals on freshwater ecosystems.

Schwarzenbach *et al.* (2006) describes chemical pollution of freshwater ecosystems as one of the most important environmental issues of the modern era, with 140 million tons of agricultural runoff (including fertilisers, herbicides and pesticides) and 300 million tons of chemicals from industrial and consumer products entering the aquatic environment, typically through incomplete removal in sewage treatment plants. This section will cover two groups of organic chemicals. Firstly pesticides, herbicides, and anti-microbials, where the impacts of these chemicals on freshwater benthic primary producers are relatively well known, and secondly HPCPs, who's effects on freshwater benthic primary producers are less well understood. Although these sections are not exhaustive, as other chemical groups and compounds exist. The chemicals described here have been selected as they are amongst the most prevalent, and the most studied organic compounds within their classifications. These will act as case studies for how these diatom-based methods and endpoints described in sections 1.3. and 1.4., respectively have been used in the past, and their potential use for the assessment of the ecotoxicological effects of other organic chemicals.

1.5.1. Pesticides, herbicides and anti-microbials

Pesticides, herbicides and anti-microbials are frequently the focus of ecotoxicology studies, particularly on primary producers, including macrophytes, green algae and diatoms. These are chemicals designed to kill unwanted organisms, particularly in agricultural settings. Although some chemicals that fall under these classifications such as triclosan, are used as anti-microbials in HPCPs (discussed in further detail later) (Proia *et al.,* 2011). These studies are often focussed on invertebrates and photosynthetic organisms such as algae, as these are the organisms that these chemicals are designed to target. For example, the herbicide Atrazine and anti-microbial, triclosan, have both been extensively tested on diatom communities *in situ*, measuring a range of chemical concentrations present and how the communities differed over this range (Renzi *et al.,* 2014, Wood *et al.,* 2014 and Guasch *et al.,* 1999). Other work has measured the effects of these chemicals *in vitro* (Roubeix *et al.*, 2011, Renzi *et al.,* 2014). Common ecotoxicology endpoints for pesticides tests often use chlorophyll a pigment concentrations as a measure of diatom growth inhibition (Debenest *et al.*, 2008, Chang *et al.*, 2011, Roubeix *et al.*, 2011), whilst the examination of the abundance of diatom frustules with morphological abnormalities (Debenest *et al.*, 2008, Chang *et al.*, 2011, Roubeix *et al.*, 2011, Wood *et al.*, 2014), and the relative abundances of individual species and diversity indices are used as a measure of community level changes (Ricciardi *et al.*, 2009, Rimet and Bouchez, 2011). These results demonstrated a loss of diversity and a community compositional change when faced with herbicide exposure, with noted examples for diuron (a photosynthesis inhibiting herbicide) reducing the diversity index of benthic diatom communities from 0.7 to 0.3 (taxonomic distinctness), and -0.3 to -1.0 (Shannon

H index), over a concentration gradient of -2.0 to 0.3 (log transformed concentration gradient) (Ricciardi *et al.,* 2009). Further research on triclosan has shown that this chemical causes an increase in the dominance of the diatom species *Achnanthidium minutissimum* and *Achnanthidium biasolettianum*, but also increased diatom mortality in stream biofilms from Barcelona, Spain after a 48-hour pulsed exposure period (Proia et al., 2011). Guasch *et al.* (1999) demonstrated that exposure to triclosan caused damage to the exposed cells' photosynthetic pathways which was the main contributor to changes in diatom community composition in their tests, affecting the diversity and relative abundances, favouring smaller species. Cell mortality rates were also increased within the samples. Research by Ding *et al.* (2018) shows that with the diatoms of the *Navicula* genus, triclosan has a highly toxic effect that inhibited growth and photosynthetic activity that is compounded by lower pH levels (7.5 compared to 8.3) of the surrounding water, believed to be caused by a higher concentration of unionised triclosan in solution, indicating that environmental parameters can contribute to the toxicity of a compound.

1.5.2. Organic chemicals used in HPCPs

HPCPs contain a diverse array of chemicals utilised due to their abilities to improve the effectiveness (e.g. ultraviolet (UV) filters and surfactants) and longevity (preservatives) of numerous products ranging from soaps and shampoos, to cosmetics and washing detergents used for household and personal cleaning on a regular basis (Gouin et al., 2012, Hodges *et al.,* 2012). A review by Lewis (1990) specified that diatoms are more susceptible to the effects of surfactants than they are to lead, and are more sensitive to surfactants than invertebrates. However, the literature of the time was unclear as to whether diatoms were more sensitive than fish. Surfactants are a broad group of compounds used to physically bind chemicals together, that would normally be incapable of mixing, by acting as emulsifiers, detergents and foaming agents (Schmidt *et al.,* 1997, Somasundaran *et al.,* 2004, Ramprasad and Phillip, 2016).

There have been very few studies focussing on the impacts of specific HPCPs on benthic diatom communities, compared to heavy metals, herbicides and pesticides, where the effects on diatom species and wider communities have been well documented, as these compounds are designed to affect these organisms, and are more likely to affect them. Where research does exist, most is focussed on higher trophic levels (grazers and consumers). However, the environmental fate and ecological impacts of some chemicals used in HPCPs are relatively unknown, as although some chemicals, such as triclosan have been extensively studied, many others have not been studied in such great detail. This creates a significant gap in the literature as to their potential effects on freshwater ecosystems. Further chemicals focussed upon in this section have been selected for discussion due to their common usage in HPCP products. These are products generally used externally to the human body, and as such they are not subject to metabolic alterations or absorption. Therefore, they are generally amongst the most common chemicals in surface waters after passing through water treatment plants (Brausch and Rand, 2011). Many of these chemicals have seen extensive assessment for their effects at the species level, for instance through the ECHA REACH system, but these are typically conducted through species level assessments. As such their effects at the community level are largely unknown.

UV filters in Sunscreens

Benzophenones are compounds used in sunscreens to absorb UV radiation. These HPCPs have been observed to reduce growth rates of diatoms of the *Nitzschia* genus (Fent *et al.,* 2008, McCoshum *et al.,* 2016). Multiple products incorporating benzophenone-3 have been identified as being present in marine environments at concentrations above 2280 ng/L, and have been suggested to contribute to coral bleaching (Downs *et al.,* 2016). The majority of research on these chemicals has focussed on their endocrine disruption effects in fish, reducing reproductive capabilities and increasing aggressive behaviour in flathead minnows and Siamese fighting fish, respectively (Fent *et al.,* 2008, Chen *et al.,* 2015). Octocrylene, a UV filter used in sunscreen products, has typically been studied as part of its product mixture (Spisni *et al.,* 2016). However, such methods prevented the accurate identification that octocrylene was the chemical responsible for any changes observed, as the results would have been obscured by the other

chemicals in the mixture. One study noted that in 22 marine sites octocrylene was present at concentrations up to 4450 g/l, and has been detected in 77% of wastewater treatment plant effluents and 14% of surface waters (Brausch and Rand, 2011) Other research has seen concetrations in excess of 3700ug/l in the coastal waters of the United states (Bratkovics *et al.,* 2015). It has been shown that this chemical can produce reactive oxygen species, which can inhibit the growth of phytoplankton. An effect observed to occur at concentrations of 50µM in the ciliated protozoan *Tetrahymena thermophila*. Other UV filters are known to have stronger effects (Brausch and Rand, 2011, Gao et al., 2016, Sendra *et al.*, 2017). Direct effects on marine diatoms using a sunscreen product mixture, including the compound octocrylene (5% of mixture, organisms exposed to 1ml of mixture) has shown that biomass of the test diatom *Nitzschia* genus was only 1.9% of that seen in the controls, thus having the result of reducing the algal biomass (McCoshum et al., 2016). Although as stated earlier, due to the exposure being to a mixture, it is not possible to confirm whether this loss in biomass was due to the effects of octocrylene, another chemical in the mixture, or an effect due to the interaction of several of the chemicals in the mixture.

Surfactants

Benzalkonium Chloride (BAC) is a widely used cationic surfactant, and is believed to have the potential to affect freshwater ecosystems (Lavorgna *et al.,* 2016). Kim *et al.,* (2020) identified concentrations as high as 38.5 ug/l in freshwater sites near a pharmaceutical production facility in South Korea, with EC50 values observed for Daphnia immobilization assays of 41.1 ug/l, indicating that although these sites assessed were near an unusually strong source of contamination, the EC50 concentration in laboratory tests were very close to those seen in the field. Other research, however, has considered 15-200 ug/l of BAC to be environmentally relevant, and observed concentrations in excess of 6 mg/l in hospital wastewater, indicating that the EC50 concentrations for *Daphnia* organisms does occur in some freshwater bodies. (Kümmerer et al., 1997, Pérez *et al.,* 2009). Much of the literature around this chemical is focussed on the effects on specific species. For example it has been shown to cause increased mortality in cyanobacteria at concentrations of 10 mg/l, in the presence of Ethylenediaminetetraacetic acid (EDTA), as EDTA acts as a cell permeabilizer (Alakomi *et al.,* 2006, Vervliet-Scheebaum *et al.*, 2008). Beveridge *et al.* (1998) studied the impact of BAC on nine diatom species and found that growth of cultured samples generally ceased after seven days at concentrations of 10 mg/l, giving EC_{50} values of 0.3 to 3 mg/l, varying between the species studied and their individual tolerances to BAC. The cause of these effects on phytoplankton has been shown to be via the interference of the photochemical system by BAC, and thus the ability of phytoplankton to photosynthesize (Pérez et al., 2009). This will then inhibit the ability of the cells to produce energy, and hinder growth rates, in terms of both reproduction and cell size. One limitation to the effects of BAC in freshwater environments is that it is readily biodegradable, and as such its presence in a habitat is short lived, preventing excessive build up in a location (Ertekin et al., 2016).

The anionic surfactant sodium laureth sulphate (SLES) has been well studied for it's effects on chlorophytes (Drewa *et al.,* 1987, Scholz, 1997). However, the effects of these chemicals are poorly studied for their impact on freshwater diatom communities. Extensive testing has been conducted on the chlorophyte *Desmodesmus subspicatus*, demonstrsting an EC50 of 27.7 mg/l (ECHA, 1993). This surfactant is more toxic to the marine diatoms *Skeletonema costatum* and *Phaeodactylum tricornutum*, with an EC_{50} of 0.37-0.50 mg/l over 72 hours in growth inhibition assays, compared to equivalent results to invertebrates and fish, in marine settings, and to non-diatom freshwater algae (Pavlić *et al.,* 2005). However, it is readily biodegradable, so will degrade in waste water treatment works, which means concentrations are likely to be very low in freshwater ecosystems (Pavlić et al., 2005, Caracciolo *et al.* (2017). There is very limited further research on SLES that is relevant to freshwater benthic diatoms.

Assessments using environmentally relevant concentrations of alkyl sulphates and alkyl ether sulphates on marine diatoms (Belanger *et al.,* 1996) found that concentrations below 0.603 ug L^{-1} used were too low to have a noticeable effect on cell growth and community diversity indices.

Later work by Pavlić et al., 2005 also assessed the effects of these chemicals on marine diatoms, and found 50% growth inhibition occurred within the concentration ranges of 0.27-0.75 mg L^{-1}, indicating that the concentrations found in the environment at the time of writing were too low to have a significant effect on marine diatoms.

Several surfactants have shown effects other than growth inhibition on diatoms. For Alcohol ethoxylates, concentrations between 0.26mg/l and 0.76 mg/l have been shown to increase the relative abundance of motile diatoms as an indirect response. This response is caused by the settling of solids on the substratum (Belanger *et al.,* 2000). This is similar to the responses seen in diatoms exposed to alkyl sulphates, an anionic surfactant, noted by Belanger *et al.* (1996) to be moderately toxic to diatoms. Another surfactant, Linear Alkylbenzene Sulphonate (LAS), has been shown to alter enzyme activity as well as cell membrane permeability in marine diatoms. Tests on five species of marine diatoms showed an EC50 range of 0.24-1.84 mg/l, over a 72-hour exposure period, whilst typical water concentrations of 0.002-0.51 mg/l in the Bay of Cadiz were reported. This experiment also showed that for the herbicide atrazine, these species had an EC50 range of 0.02-0.21 mg/l, indicating that, LAS is less toxic than atrazine for these diatoms. (Moreno-Garrido *et al.,* 2001, Debelius *et al.,* 2008). Other research has seen even higher concentrations, with (Corada-Fernández *et al.,* 2011) reporting concentrations in excess of 2 mg/l in rivers. Research on other species has shown that cyanobacteria are more sensitive to LAS (Belanger et al., 2002).

Cationic polymers

There is limited research on polyquaterniums. Cumming *et al.,* (2011) notes that polyquaternium-42 is known to interfere with cell membranes by disrupting ion channels, while other research has demonstrated an LC_{10} of 0.47 mg/l over 24 hours to the fish *Rasbora heteromorpha*, as it chemically binds to the gills (Cumming 2008).

Chelating agents

Ethylenediaminetetra acetic acid (EDTA), a common chelating agent that degrades by photolysis (Bucheli-Witschel and Egli 2001) has been frequently used as part of the growth medium for diatom-based studies. Knauer et al. (1997), Spaulding (2007), and Liu *et al.,* (2008) used EDTA as an addition to the growth medium during studies into Zinc and Copper impacts on algae to help regulate ion concentrations, although the potential impacts of this chemical was not considered. Dankert and Schut (1976) confirmed that the presence of this compound increased the effectiveness of chloroxylenol against bacteria, by damaging the cell walls of the organisms, indicating potential ecotoxicological effects on micro-organisms.

Fluorescent Whitening Agents

There are also limited data regarding the impacts of Fluorescent Whitening Agents (FWA), compounds used to artificially whiten surfaces. Some FWAs are produced in volume of up to 14,000 tonnes per year (as of 1994), and only 53-89% are removed by sewage treatment plants (Kramer *et al.,* 1996). Work by Managaki *et al.* (2006) monitored the distribution of these chemicals downstream of sewage treatment plants and found that they could be detected up to 10km outside of the bay of Tokyo, and are present in detectable concentrations in freshwater environments. Jensen and Pettersson (1971) tested the FWA chemical 2,5-di-(benzoxaloze-2-yl)thiophene on salmon and demonstrated that 0.01mg/l did not have a significant toxic effect on fish.

To summarise, although there have been attempts to assess the effects of organic chemicals used in HPCPs on standard ecotoxicology species, or as growth inhibition assays on particular marine diatoms species, the full effects they have on diatoms at the community level is still unclear. Furthermore where research has been conducted, it has typically been on marine diatom species, such as with LAS. Or in non-diatom freshwater algae, as with SLES. As such, there is still considerable research gaps on how these compounds influence the effect of diatoms in

freshwater environments, and the best methods on how to measure these effects, with only data on a handful of cosmopolitan species, such as those of the *Nitzschia* genus, to compare to.

1.6. Conclusions and study plans based on this review

1.6.1. Recommendations & Knowledge gaps

To conclude, the research in this review has demonstrated that there are still major knowledge gaps within the literature, specifically regarding the impacts of common HPCP chemicals in freshwater environments on diatom communities and appropriate endpoints for measuring community response to these chemicals. There is also no current standardised method to assess these impacts and testing often uses variations on standard protocol. As such, there is a need for the development of methods that will allow for the holistic assessment of freshwater ecosystem health and function. Several endpoints have been identified for which further investigation into their possible uses for the assessment of organic chemical exposure can be tested, including the growth rate of the biofilms, the species level composition (taxonomic identification and relative abundance, diversity indices, abundance of ecological guilds), and the UKTAG assessment methodology. These endpoints could be applied to an assay to investigate how the structure, composition and growth of biofilm communities are affected by exposure to a chemical, using improved methods similar to those shown in Morin *et al.,* (2008a), or Debenest *et al.,* (2009b), using laboratory cultures developed from live biofilms, to conduct the assays.

In order to tackle this, the overall aim of this project is to develop a methodology for assessing the impacts of organic chemicals on freshwater ecosystem function and health, using endpoints which characterise and measure changes in benthic diatom community structure. Based on the findings of this review, benthic diatoms have been identified as being ideal for such research, as they typically compose the greater proportion of benthic freshwater biofilms, which also contributes more to the primary productivity of the environment than their pelagic equivalents. Their position as primary producers in the food chain further means any impacts a chemical has on these organisms will have ramifications further up the food chain, and they are abundant and cosmopolitan.

Using *in vitro* setups to measure community change in response to chemical exposure, the use of either microscope slides or ceramic tiles as a test substratum is recommended, but further testing of these substratum is advised to assess which material will develop the more natural community composition. Further to this, environmental parameters that best facilitate the natural environment (photoperiod, temperature, light intensity) from which the communities are derived will be determined in order to minimise the effects of stress caused by environmental changes on the diatom cultures.

Based on the findings presented here, the following endpoints should be investigated to measure community structural changes. The use of traditional light microscopy is recommended for the counting and identification of the individual diatoms, incorporating ecological guild and life form organisation instead of genus level groupings to measure changes in community structure, as well as the UKTAG assessment methodology to compare the composition of the community after exposure compared to the composition before exposure, and in comparison, to unimpacted reference sites. For identifying community structural changes, the use of ecological guilds should also be utilised. Ash-free dry weight and chlorophyll-a assessments should also be incorporated as a growth rate/ inhibition assay for the total organic matter content of the biofilm and the size of the algal communities, respectively. These endpoints have been selected due to their prevalence in the literature, and their basis on using the composition of the diatom communities to provide measurements of structural and functional changes in response to chemical exposure.

Furthermore, teratological forms, BOD and DIC should not be used, as they are only applicable in field tests of pelagic algae. Other endpoints looking at damage to diatom cellular organelles and

pigments are not recommended, as effects of a chemical on these endpoints will likely be too specific to species level effects. The use of other pigments, such as diatoxanthin is not recommended at this stage, as the resolution of these compounds will be limited due to the different rates the species in the mixed communities will produce, although these may be added to the assay method at a later time, as a supplement or replacement to chlorophyll-a growth measurements.

It is also recommended that tests should incorporate water quality measures to assess the influence of surrounding physico-chemical parameters, nutrient availability and heavy metal contamination on any community being used for future assessments. This is particularly important when the samples are being taken from the field, although these parameters should still be monitored in controlled conditions to monitor any changes to the culture stock over time.

Based on the findings of this literature review, the following recommendations support the implementation of experiments that can be applied to future ecotoxicology tests using diatoms to assess the effects of HPCP chemicals.

- **Substratum.** Microscope slides or ceramic tiles should be used as a test substratum for the development of benthic diatom communities, as these are typically used in the literature due to their replicability. Further testing needs to be performed to measure the differences between the communities grown on these two substratum. Literature measuring differences in community growth on glass and ceramics is scarce although comparisons with other types of substratum have shown minor differences in the abundances of diatom species between these substratum.

- **Diatom species to measure.** Diatom species with known high sensitivity to organic chemicals, as low abundances of these species are indicative of poor ecosystem health. Furthermore, generalist species that have been identified in the previous literature as useful biomonitoring organisms, such as *Gomphonema parvulum*, should also be considered, as the prevalence of these species makes them highly likely to be present in sites exposed to the chemicals being tested, and their prior use in ecotoxicity testing will aid

- **Measurements of water quality** should be included as part of the analysis. In particular, pH, alkalinity, temperature and light attenuation should be measured, as most benthic diatoms have a clear tolerance for these factors and as such, changes in these factors will need to be measured to monitor the effects they have on the community structure. Nutrient and element analysis should also include key nutrients for diatoms (copper, magnesium, potassium, phosphate, ammonium, nitrate and silica), as the loss or excess of these may influence the relative abundance or presence/ absence of specific species. Electrical conductivity can also be used as an additional measure of ion availability (including nutrient ions, e.g., phosphate) in the water. A broader suite covering other physico-chemical measures, including heavy metals (such as iron and lead) with known effects on diatom communities and individuals, dissolved oxygen and suspended solids concentrations should also be used to increase the understanding of the characteristics of the water bodies in question and their influence on the structure of the benthic diatom communities.

- **Reference sites** used for sample collection should be in good or better ecological quality as rated by the EU Water Framework Directive, and be taken from a standardised depth from as many sources as possible (plant stems and stones) to increase the resolution of the greater benthic community structure of the water body.

- ***In vitro* setups** should use temperatures, photoperiods and light intensities that closely mirror the natural environment as much as possible to reduce external stress to the

communities that may interfere with the results. Growth media used should mirror the water the diatoms originated from to mitigate any effect on community structure (relative abundances of species, growth rates, cell density) due to significant changes in the concentrations in medium nutrient concentrations (Phosphate, ammonium, nitrates, nitrites), alkalinity, pH or electrical conductivity.

- **Chemical exposure.** Communities should be exposed as a series of replicates to a range of chemical concentrations, which should be added as pre-determined concentrations to the replicates' growth medium to ensure adequate mixing and complete dissolution before exposure begins. Exposure should be conducted over a period of three to five days with samples taken daily, placing the substratum with naturally developed diatom communities on microscope slide substratum over a period of up to four weeks in a sterilised container filled with a known concentration of growth medium with a set concentration of the chemical being assessed.

- **Endpoints** recommended for future studies are relative abundance, ecological guilds and diversity indices, which should be used to assess changes in community composition of the diatom communities. The UKTAG assessment methodology should also be incorporated to provide a standardised measurement of the composition of the diatom community against what an unimpacted, reference site community should be, based on the abundance of different diatoms species and their known sensitivity to inorganic nutrients, alkalinity and pH factors. Ash-free dry weights should be used as a measure of organic matter content of the biofilm, and used in conjunction with chlorophyll-a concentrations in the biofilms to identify the growth rate of the biofilm as a whole, and the algal community over the course of the experiment.

These recommendations lay out the groundwork for future tests. Before any *in vitro* culturing can begin, there are still three questions that will need to be answered to inform the experimental set up.

1) Which substratum will be the most effective for ecotoxicological tests?

2) How will these communities differ between those developed in the field, to those developed in a controlled environment, with fixed temperature and lighting (intensity and duration) parameters, where the influx of fresh medium and individuals to the biofilm from the original site is known over a one-month period?

3) What does the structure of diatom communities in Yorkshire look like? This is required to inform the development of a community representative of the region.

To address the first two questions, a field experiment based on at least two different freshwater bodies is recommended to assess how community structure and succession over several months on the substratum identified (microscope slides and ceramic tiles), as well as natural rock comparisons occur. In addition, a simultaneous experiment to assess the differences between communities developed *in situ* to those developed *in vitro* using the same source and water supply will be conducted. To identify a representative community for the third question, a second field experiment will be performed, sampling reference sites rated as being in good ecological status by the EU Water Framework Directive following UKTAG sampling protocols. These experiments will then feed into the development of *in vitro* set ups for diatom cultures which can be used to assess the effects of nutrient addition (as a positive control) and then the effects of HPCP contamination.

1.6.2. Thesis outline

The overarching aim of my thesis is to develop a protocol towards the use of benthic diatom community endpoints to determine freshwater ecosystem function and health in response to organic chemicals

There are three objectives in place to achieve this aim. These are to:

- Develop a laboratory approach to establish representative freshwater diatom community cultures and measure changes in community structure and function;
- Identify a representative diatom community in rivers and streams with low levels of chemical contamination in Yorkshire;
- Quantify the effects water quality parameters have on diatom community structure

In this thesis, I will address these objectives: developing a laboratory approach to establish a representative freshwater diatom community cultures and measure changes in community structure and function, and identify a representative diatom community in rivers and streams with low levels of chemical contamination in Yorkshire. For this, three experiments will be performed to accomplish these objectives. The first two experiments will be conducted for the first objective, and the third experiment will be performed for the third objective assessing the effects of water quality on the diatom community structure. These each correlate to their own chapters.

Experiment 1: Field experiment comparing the succession of diatom communities over a ten-week period on three different substratum and on two lakes with different physico-chemical parameters.
Aim: The overall aim of this experiment was to determine the effects of different substratum on how new benthic diatom communities develop, as well as how these communities develop over time and between two freshwater lakes with different physico-chemical parameters.

The detailed objectives for this experiment were:

- **To determine the effects of different substratum on how new benthic diatom communities develop**. In particular, to determine the structural differences of between benthic diatom communities on three test substratum and where possible, stones taken from the lakes studied as a comparison of what more established communities look like.
- **To assess how the structure of biofilms develop over time** regarding species relative abundance between different algal groups (diatoms, cyanobacteria, chlorophytes, desmids and cryptophytes) and so determine the relative size of diatom communities in the wider primary production trophic level
- **To assess the ecological status of the two lakes** using the UKTAG methodology to the diatom communities developed on the different substratum being tested in each lake
- **To assess how the structure of diatom communities on the substratum develop** over a ten-week period (which species are present, the richness and evenness of the communities, and the productivity of these communities).
- **To relate water quality to diatom community structure.** In particular, to determine the differences between the two lakes in terms of the temporal variation of nutrient (phosphate, ammonium, nitrate, nitrite, chloride and sulphate) concentrations, silica concentrations, metal (Mg, Cu, Ca, Na, Ni, Pb, Fe and K) concentrations, temperature, dissolved oxygen and light attenuation (measured as photosynthetic active radiation (PAR)) and their effects on the structure and composition of the diatom communities.

This experiment will utilise the two largest lakes on the University of York's grounds (designated west campus lake, and known to be in a eutrophic state and east campus lake, which is much newer and believed to be fairly clean), to deploy a series of replicate substratum (microscope slides, ceramic tiles and sandstone) submerged to ten centimetres on a series of rafts to observe how the diatom communities develop over a ten week period on these substratum.

Experiment 2: Comparison of communities developed in field settings to those developed in a controlled laboratory environment
Aim: The overall aim of this experiment is to observe how benthic diatom communities develop in a controlled environment with fixed lighting and temperature, compared to the field sites used in the main field experiment.

Detailed objectives:

- **To determine the differences of the structural development of biofilms between growth in the field and growth in a controlled environment** (with fixed temperature, photoperiod and light availability) over one month by assessing
    - (1) the relative abundances of different algal groups (diatoms, cyanobacteria, desmids and cryptophytes),
    - (2) the species level variation of the diatom communities, as well as
    - (3) the differences in biomass of the biofilms in the field and laboratory cultures.
- **To determine if a 20% replacement of the growth medium is sufficient to replace nutrients** in the replicate vessels used in the controlled temperature environment

This experiment will be used to test a methodology using individual replicate beakers to suspend a substratum (microscope slides used for simplicity and reliability) at 10 cm depths in an environment set to 17^{o}C with a 16-hour light to 8-hour dark photoperiod (300µmol/m/s^{2}) to mirror expected outdoor conditions as closely as possible

Experiment 3: Assessment of the structure and composition of diatom communities across Yorkshire.
Aim: The overall aim of this experiment was to assess the diatom communities in water bodies across Yorkshire with good water quality i.e. that have been rated as 'good' or higher by the Environment Agency under the EU Water Framework Directive in order to establish the composition of a representative community of diatoms from unpolluted rivers in Yorkshire

Detailed objectives:

- **To identify the structure and composition of diatom communities** in five water bodies rated good or higher by the Environment Agency and the EU Water Framework Directive across Yorkshire
- **To explore the relationship between physicochemical parameters and the diatom communities** in these water bodies, to determine which parameters have an influence on driving local variations in these communities
- **To examine the sensitivity of the diatom species present** to the levels of inorganic nutrients measured, and thus determine which species of diatoms could be incorporated into future *in vitro* testing of organic chemicals

Field sampling of diatom biofilm communities will be carried out at a series of sites designated to be in at least "good" biological condition (aiming for "good" overall condition) under the WFD. Samples will be taken from as many different substratum as possible (plant stems, loose sediment and stones) to determine which diatom species are most common and hence representative of Yorkshire diatom communities. The physico-chemical parameters that affect these communities in these sites will also be measured.

Summary of overall results
Following these experiments, a summary chapter of all the results from the above experiments will be produced, to succinctly describe the results of each of the experiments, and the recommendations that they will provide for future tests using freshwater benthic diatoms in assessing the effects of HPCP chemicals and other organic compounds on freshwater ecosystem health and function.

Chapter 2: Field experiment 1: University Campus Lakes

2.1. Introduction:

In past studies, diatoms have been used as indicators of ecosystem health and function in response to contaminants (Cattaneo *et al.,* 2011, Morin *et al.,* 2012, Wood *et al.,* 2019). In ecotoxicology, freshwater diatom communities have been used to test the impacts of a range of organic contaminants, particularly pesticides and herbicides (Ricciardi *et al.,* 2009, Roubeix *et al.,* 2011, Wood *et al.,* 2014). However, these methods tend to grow diatom communities *in situ*, and then transferred them to the laboratory for controlled exposure experiments, preventing the development of a test culture that can be returned to for standardised tests (Debenest *et al.,* 2008). In order to test the responses of diatom communities to these chemicals, a standardised testing methodology needs to be developed so that the direct effects of the contaminant being studied can be measured (Debenest *et al.,* 2008). To achieve this, realistic environmental setting for the growth of these cultures needs to be achieved.

Several types of artificial substrata have been used in the literature for culturing communities of these organisms for testing, and evidence exist that factors, including chemical composition, reactivity, and surface roughness can affect the communities developed (Patil and Anil, 2005). Ceramic tiles and microscope slides being the predominant types used in culturing methods, with a handful of experiments further using acrylic/ PVC substratum, or natural slates (Arini *et al.,* 2012, Khan *et al.,* 1991, Lowe and Gale, 1980, Rimet and Bouchez, 2011, Debenest *et al.,* 2008, Morin *et al.,* 2008b). As such, past research has demonstrated mixed results regarding the similarities between the abundance and diversity of diatom species found on these artificial substratum compared with natural equivalents. Early research (Lowe and Gale, 1980) found that there were many similarities between the communities developed on artificial and natural substrata (up to 80% similarity of percentage of genera). However, more recent research has found that the communities developed on microscope slides tend to favour the diatom species *Achnanthidium minutissimum*, are only 37% similarity to natural communities, and have fewer species in communities on artificial substratum compared to natural substratum (Siver, 1997, Barbiero, 2000). Further research by Dalu *et al.,* (2014), conducted in South African rivers, agrees that substratum has an effect on diatom community structure, with this work demonstrating the species richness on the artificial substratum tested (brick, brown and grey clay tiles) was higher than on the natural substratum (macrophytes, rock and sediment). This difference, however, may be due to the fact that this research was conducted in a river and not a lake setting, and there may have been some interaction caused by the differences in flow regimes or other factors between the lake and river which account for this.

In addition to the direct effects of substrata, the duration over which these substratum need to be exposed to the water body before the communities that colonise them are representative of those found in the wider environment is also an important factor, and how the communities respond to these changes in the physico-chemical parameters around them. Literature sources give a variety of times in which this occurs, ranging from a few weeks to several years. However, most literature suggests that around one month may be sufficient for a freshly exposed substratum to develop a diatom community whose species composition and diversity metrics are representative of its wider environment (Guasch *et al.,* 1998, Belanger *et al.,* 2002, Gold *et al.,* 2002, Larras *et al.,* 2012).

The experiment described in this chapter was designed to test two parameters; type of substratum and duration of exposure, using two lakes with differing nutrient status, age and management practices. The two lakes used were University of York's West campus lake and East campus lake. The experiment was conducted over a ten-week period during the summer growth period, between June and August 2019 to cover the most commonly used exposure durations without becoming restrictively time intensive. The type of test substrata used were microscope slides and ceramic tiles, the most commonly used substratum identified in the literature review.

Along with a natural substratum (sandstone) representative of the local geology of the area. Substratum native to the sites and that have already been exposed for at least a year will also be studied in the experiments to compare existing biofilms in the lakes to those being developed on the fresh test substratum.

2.2. Aim and Objectives:

The aim of this experiment was to determine the effects of different substratum on the development of benthic diatom communities over time in two freshwater lakes with different physico-chemical parameters. This was conducted using standard ecological assessment metrics and biological endpoints (species relative abundance, diversity indices, ecological guild (percentage of motile species)) identified in the literature review to measure these effects. To achieve this, the experiment focused on the following objectives:

- To determine the structural differences in periphytic biofilms, with a focus on their diatom communities, when developed on three substratum (microscope slides, ceramic tiles and sandstone) and, where possible, compare these communities to those already developed on stones native to the lakes.
- To assess how the structure of periphytic biofilms develop over time, focusing on changes in the same endpoints every two weeks over a ten-week period
- To assess the ecological status of the two lakes using the UKTAG working groups (UK organisation created to implement EU WFD protocols) trophic diatom index (TDI) assessment methodology based on diatom communities developed on the different substratum (microscope slides, ceramic tiles and sandstone) and over the ten-week monitoring period.
- To determine the effects of physico-chemical parameters, including nutrient concentrations and physical parameters of the lake water and environment on the structure and health of the diatom communities.

2.3. Methodology:

2.3.1. Experimental setup

To test how diatom communities develop on microscope slides, ceramic tiles and sandstone (the former two selected due to their prevalence in the literature on ecotoxicological testing of diatoms, the latter as a natural comparison), samples of each substratum were exposed to six sites across two different lakes. A series of support structures (rafts) were designed to suspend the substratum to fixed locations in the lakes (Figure 2.2., Figure 2.3.). Replicates were removed every two weeks over a ten-week period from 12/06/2019 to 21/08/2019. The communities were assessed based on the relative abundance of different algal groups and diatom species. Assessments were also conducted using the diversity indices of the diatoms, measurements of diatom biomass (using ash-free dry weight (AFDW) and chlorophyll-a concentrations as surrogate parameters). The UKTAG assessment DARLEQ2 (EQR LTDI2 metric) methodology was also used to predict the nutrient sensitivity of the diatoms within the community to determine the classification of the water body to the EU water framework directives classification system. The physico-chemical parameters of the lake were determined by measuring pH, temperature, dissolved oxygen concentration, alkalinity, electrical conductivity and photosynthetically active radiation (PAR) absorption of the water between the surface and the depth at which the substratum were exposed. Water samples were also taken to measure nutrient concentrations (ammonium, chloride, dissolved organic carbon (DOC), fluoride, phosphate, sulphate, total nitrogen (TN), nitrate, and nitrite), total suspended solids (TSS) concentrations and concentrations of elements known to be required by diatoms (magnesium, copper, calcium, sodium, nickel, lead, iron, silica and potassium, Soininen, 2007, Nagai and De Schamphelaere, 2016, Mu et al., 2018). An additional measurement of the physico-chemical parameters was

conducted during the experimental setup (week zero). The timings of these biofilm and water sampling times are shown in Figure 2.1.

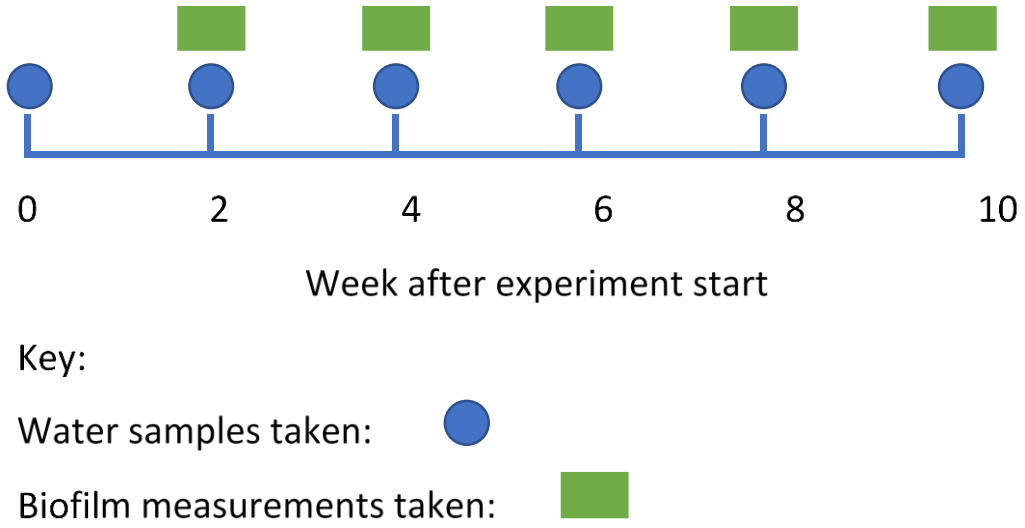


Figure 2.1. Timeline of sample collection during the field experiment for water samples, and biofilm samples over ten weeks, starting on the 12th of June 2019.

2.3.2. Study sites

Two lakes at the University of York campus grounds were used for this experiment. These lakes were chosen due to their known differences in the physico-chemical parameters from research conducted by the University of York and student projects. The first lake (West campus lake; 53.95° N, -1.06° E), is approximately sixty years old, 1 km long and 5-100 m in width, with a highly sinuous and irregular shape. It also has a maximum depth of 3 m, although this has been confirmed to be filled by sediment and decaying leaf matter by up to 1.5 meters in places. This lake has known issues of algal blooms and an unbalanced ecosystem due to a relative lack of invertebrates, macrophytes and predatory fish (University of York, 2019), compared to algae, and grazing birds and fish, It is also considered to be eutrophic. The second lake finished construction in 2010 (East campus lake (53.95° N, -1.03°), is 10 hectares in area, 1200 m long, 200 m wide, and 4 m deep (University of York, 2019). This lake is considered to be relatively oligotrophic.

Three sampling sites on each of the campus lakes were selected, following UKTAG assessment guidelines for phytoplankton sampling, using localities representative of the wider ecosystem which are far enough downstream from discharge points into the lake so that the input is thoroughly mixed with the water body.

The sampling sites chosen are shown in Figure 2.2.

West campus sampling sites

- West campus site one (WC1, Figure 2.2.) is located 5 metres out from the lake edge and is well exposed to sunlight with an approximately 50 cm water column depth. However, the lake bottom is comprised of large (>10 cm) rocks and debris for two metres from the lake shore, with significant visible biofilm growth on the surfaces.
- West campus site two (WC2, Figure 2.2.) is approximately ten metres out from the lakeside, with relatively shallow water and limited shading for the majority of the day, (although the entire stretch of the lake does become shaded from late afternoon). Leaf litter comprises the majority of the superficial surface deposit, intermixed with fallen branches and the roots from trees that grew at the lakeside but have since been cut down.

- Site three (WC3, Figure 2.2.) is three metres offshore. This area of lake is ~1.5 meters deep and the lake edge is vertical, composed of concrete and brick walls. This site also receives ample direct sunlight.

East Campus sampling sites

- The three sites set up on East campus lake (EC1, EC2, EC3, Figure 2.2.) (located on the southern edge of the lake near the car access point to the pumphouse) are relatively shallow with a gentle incline towards the centre of the lake. The area is mainly composed of loose gravel dominated sediment, 5-15cm sized smooth fine-grained rocks and interspersed reed beds. Most areas of this lake are very similar to these sites and, as such, they can be considered representative of the whole lake. This lake is much newer than the West Campus lake, having only been constructed 10 years ago. As such, it is expected that nutrient levels will be much lower in the East campus lake than in the West campus lake, as well as having a less diverse algal community, since there has been less time for microorganisms to colonise this site.

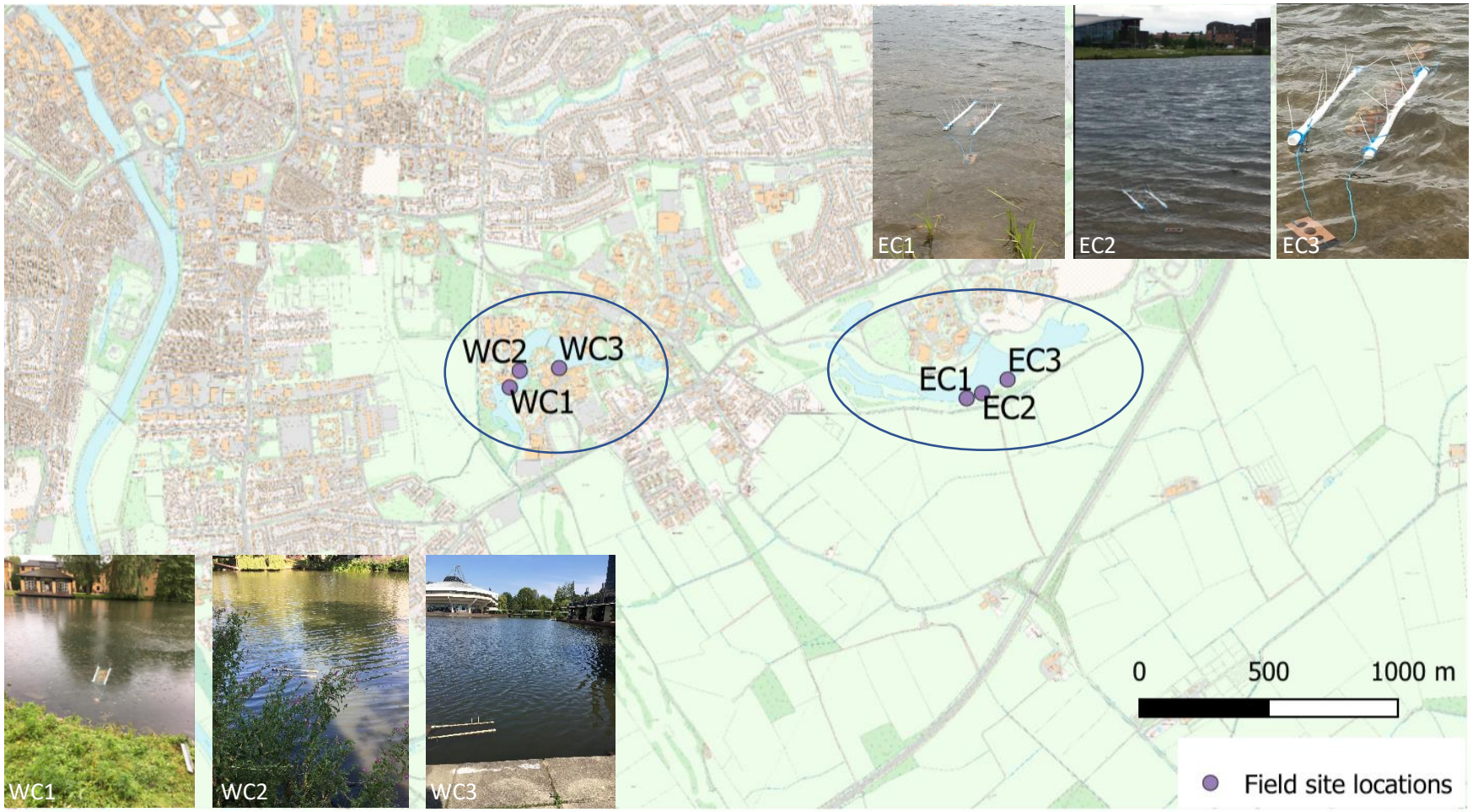


Figure 2.2. Map of the University of York (Ordnance Survey background map, QGIS, Version 3.12.3). Each lake is marked by a blue ring showing the three sites on West campus lake (West campus site 1 (WC1), West campus site 2 (WC2), West campus site 3 (WC3)), and the three sites on East campus lake (East campus site 1 (EC1), East campus site 2 (EC2), East campus site 3 (EC3)).

At each site, the test substrata were suspended 10 cm below the water surface level in positions where water depth was over 50 cm to minimise any disturbance of the sediment below the rafts which might contaminate the test substrata and to provide ample space to account for evaporation from the lakes over the summer. This depth was used as a compromise, as deeper depths would have caused a loss of light in West campus lake unsuitable for growing algal communities, due to the turbid nature of the lake water. Whilst deeper depths in East campus lake also risked contamination by contact with the lake sediment due to the shallow littoral zone and the risk of evaporation over the summer months lowering the water depth. Shallower depths would have also increased light availability and further reduced the representativeness of the diatom communities developed against those of the natural communities in the lakes. Reference substratum (fine grained sandstones placed throughout the lake during its construction) were selected at depths as close to 10 cm below surface level as possible to mitigate differences in community caused by light attenuation.

Substratum

Rafts 1 m in length and 50 cm wide were used to suspend the test substratum to a standardised depth, with one raft deployed at each of the six sites. The rafts main frame was constructed from wire mesh (Blooma 6-mm gauge welded wire mesh), shaped to create a 1 m long and 50 cm wide rectangular area at the bottom, to attach the replicate substratum. The longitudinal sides of each raft had a10-cm tall vertical edge pointing upwards.

The mesh frame was kept afloat using 1-m long PVC pipes (34-mm diameter) with sealed ends, attached to each vertical side of the wire mesh using plastic cable ties wrapped around the tube and through the upper edges of the wire mesh (Figure 2.3a). The ends of the cable ties were not cut off and instead angled in an upward manner to deter waterfowl from approaching the rafts.

Brick anchors were attached to the ends of the rafts using plastic ropes to tie each end of the brick to the end of one of the pipes, (see Figure 2.3b). This provided extra support of the wire mesh by further reinforcing their connections to the PVC pipes, whilst providing a sturdy connection of the anchors to the rafts, reducing the likelihood that strong winds could detach the rafts from their anchors.

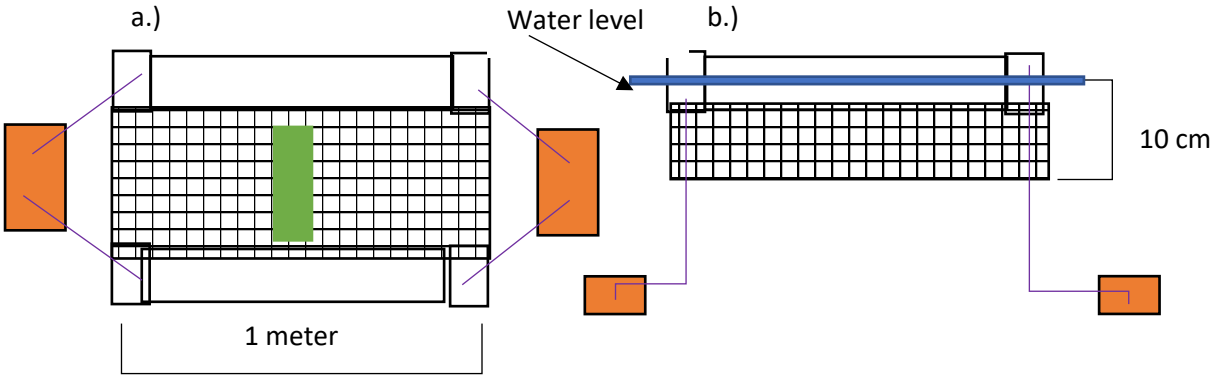


Figure 2.3. Layout of frames. Left diagram is a top-down view of the frame (a), the right is the frame viewed from the side (b). White rectangles represent the PVC floatation tubes, the rectangular grid represents the main frame, the purple lines represent lengths of string and are not to scale for illustrative purposes. The orange blocks are the brick anchors. The green rectangle is an example replicate frame, which held three each (triplicates for replication and to account for any loss/ damage) of the substratum microscope slides, ceramic fragments and lithic fragments (sandstone).

Attached to each raft was a series of replicate frames designed to hold the substratum. These replicate frames were rectangles of wire mesh approximately 9 cm wide and 40 cm long. Seven frames were attached to each raft, with their long edges at right angles to the longer edges of the raft, along the centre of the wire mesh to reduce shading from the PVC pipe when close to dawn

and dusk (Figure 2.3). Each replicate frame contained triplicate samples (for redundancy in case of damage/ loss) of each of the three substrata (Figure 2.4), with a gap of at least 0.5 cm between each substratum. Substratum were attached to the replicate frame by aquarium grade adhesive, and the replicate frame attached to the raft by a series of smaller plastic cable ties attached to each corner of the replicate frame through the rafts mesh frame.

Alongside the three test substrata, an additional set of stones already present in the lake where available (reference substratum) were taken from the immediate area of the raft and from depths as close as possible as the test substratum attached to the raft to prevent variation caused by absorbance of PAR by different water depths. This was only conducted on East Campus lake, as there were very few viable substratum around the sites used on West Campus lake, which had not already been repeatedly removed, scraped for biofilms, and returned by the University for undergraduate teaching purpose. As such, the biofilms could not be considered representative of the wider lake ecosystem.

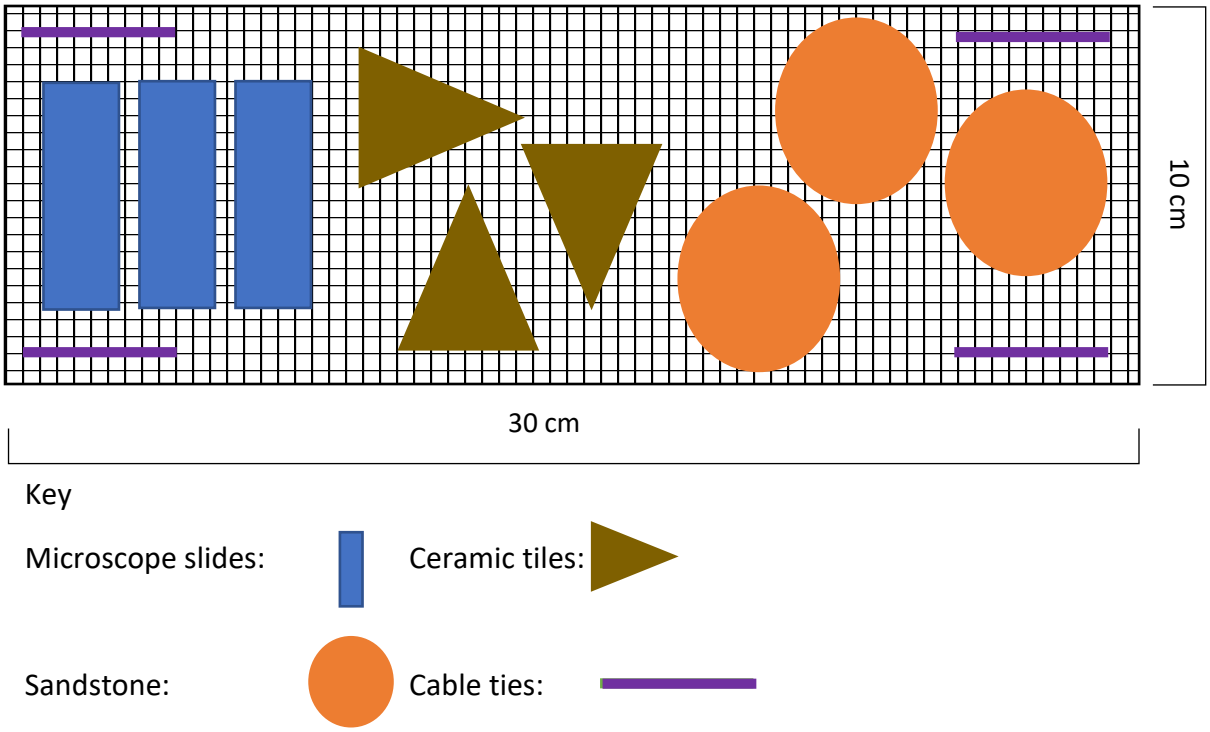


Figure 2.4. Diagram of one of the replicate frames attached to the rafts, showing the positions of microscope slides, ceramic tiles, and sandstone, as well as the location of connectors for the backing material to the raft.

Figure 2.5. Images of the experimental raft at West campus lake, site 1 (WC1) from the lakeside on the day of deployment (a), and from directly above (b) after two weeks of exposure.

2.3.3. Sample collection and field measurements

2.3.3.1. Biofilm sample collection from substratum

After two, four, six, eight and ten weeks of exposure in the lakes, one replicate frame was removed at each of the six sites. Each site was sampled at the same time of the day to within an hour. The biofilm was removed from the smooth substratum (microscope slides, ceramic tiles and reference substratum) using a sharp blade, and from the irregular sandstone substratum using a soft toothbrush. Three replicates of each substratum were removed from each frame, to allow for the later calculation of mean and standard error for the data for each site. The biofilms from each of the three substratum (microscope slides, ceramic tiles and sandstones) on each replicate frame were transferred to one darkened (to prevent growth within the sample) 25-ml HDPE tube and stored at 4^0C for a maximum of 90 days until the end of the experiment, where measurements that were not as time sensitive were performed.

2.3.3.2 Water sample collection

So that the effects of water chemistry could be assessed for their effects on the structure and composition of the diatom communities, at week zero, two, four, six, eight and 10 a series of water samples from 10 cm depth were taken at each site using three high-density polyethylene (HDPE) tubes which were then wrapped in tin foil and kept out of direct sunlight and transferred to a 4˚C cold store within one hour to prevent growth of any organisms within the samples. The water collected in one tube was filtered with pore sizes 0.2 microns attached to a hand pump into another tube, with a second sample processed in the same way but with a 0.45-micron filter. These samples were then stored at -20˚C until further analysis. Additional 500-ml water samples were collected at 10 cm below the water surface from each site for total suspended solids analysis (using HDPE containers) and stored in the dark at 4°C for up to two days.

2.3.3.3. Field measurements of water quality

To measure the effects of other physico-chemical parameters on the development, structure and composition of the diatom communities developed within the lakes, a series of physical and

chemical water quality variables were measured in situ at the three sites in the two lakes at 10 cm depth (the same depth the substrata were exposed to). These were taken at the same time as the biofilm samples (11am-1pm for East Campus lake, and 3-5pm for West Campus lake). pH and temperature were measured using an AP72 probe (Accumet, Massachusetts, US), dissolved oxygen was measured using a ProODO optical dissolved oxygen instrument (YSI, Ohio, US), electrical conductivity was measured using a HI9033 probe (Hanna Instruments, Rhode Island) and alkalinity was measured using a Hanna instruments Alkalinity test kit (Hanna Instruments, Rhode Island).

Photosynthetically active radiation (PAR) was measured just above and 10 cm below the water surface using a PAR special sensor (Skye instruments, Wales, UK). Light attenuation in the top 10 cm of the water column was calculated using the following equation:

$$Light\ attenuation\ (\%) = \frac{PARb}{PARa} X100$$

Where

PARa = Photosynthetic Active Radiation (PAR) (µmol/s/m^2) at water surface

PARb = Photosynthetic Active Radiation (PAR) (µmol/s/m^2) at 10cm depth

Continuous surface PAR (µmol/s/m^2) and water temperature measurements were taken using a Skye instruments PAR special sensor attached to a DL2e datalogger (Delta-T, Cambridge, UK) (surface PAR) and a series of iButtons (Maxim integrated, California, US), with one attached to each of the six rafts. These were deployed one week into the experiment due to delays caused by technical issues.

During week zero, two and four a Natural History Book Service (NHBS, Devon, UK) standard flowmeter was used to measure any flow velocity in the lakes. However, the flow rate of the water in both campus lakes was below the detection limit of the flowmeter (less than 0.1 metres per second) and as such there was no data retained from this instrument.

2.3.4. Analysis of samples and measurements

2.3.4.1. Biofilm analyses

To assess the how the structure and composition of the diatom communities altered throughout the ten weeks of exposure on the test substratum deployed across the two lakes, the total volume of the biofilm suspension was determined using automated pipettes (Gilson Pipetman). Four subsamples were taken after thorough mixing of the suspension, and analysed to determine chlorophyll-a content, Ash-Free Dry Weight (AFDW) relative abundance of algal groups, and diatom analyses (relative abundance, diversity indices and UKTAG LTDI2 assessment). Each of these analyses is explained below.

Chlorophyll-a content

To measure biofilm growth, a volume of 0.1-1 µl (depending on total volume of available biofilm and biofilm viscosity) of the biofilm suspension was filtered over Whatman GF/F filter paper. The filter paper was subsequently wrapped in aluminium foil and stored in a freezer at -80^{0}C. Extraction was performed by placing the filter paper with biofilm in 20 ml of 20% acetone in the dark at 4^{0}C for 24 hours. These extracts were then analysed for the chlorophyll-a content following EPA method 445.0 (Arar and Collins, 1997), using a fluorometer with a chlorophyll-a acidification module attached (Turner Trilogy laboratory fluorometer; extracts 12 mm x 75 mm glass cuvette). A calibration curve over a range of 0-1,000 ug/l was created using stock solutions of a sigma-aldritch chlorophyll-a analytical standard to calculate the chlorophyll-a content of the samples from the fluorometer readings. The calibration curve used the following equation:

$$Chlorophyll\ a\ (ug/l) = \left(\frac{Cc}{(Ar-1)}\right) x\ (Cc)\ x\ (Fb-Fa)\ x\ (Di)\ x\ (Ve)$$

Where

Cc is the gradient of the Calibration curve

AR is the acidification ratio

Fb is the fluorescence before acidification

Fa is the fluorescence after acidification

Di is the dilution factor

Ve is the volume extracted

Ash-free dry weight:
To measure biofilm biomass, a volume of 0.1-1.0 µl of the biofilm suspension was filtered onto pre-weighed Whatman grade 289/3 paper filters using a Millipore chemical duty pump connected to a Thermo Scientific Nalgene polysulfone reusable bottle top filters attached to a 2-litre borosilicate vessel. The filter was placed in a pre-weighed aluminium foil boat and dried in an oven at 40^0C overnight. The weight of the filter and dry biofilm (post-oven weight) was determined. Each sample in a tin foil boat was placed in a ceramic crucible and heated to 500˚C for eight hours in a furnace (Carbolite, Laboratory chamber furnace- CWF) (Steinman and Lamberti,1996). Once cooled in a desiccator, the samples were weighed. The AFDW (organic matter content) of the biofilm was then calculated by the following equation:

$$\text{AFDW (mg/l)} = \frac{A-B\ x\ 1000}{sample\ volume\ (ml)}$$

Where

A is the weight of the biofilm sample + filter dried at 40˚C (mg)

B is the weight of the biofilm sample + filter plus after ignition at 500˚C for 8 hours (mg)

Sample volume is the volume of the biofilm extract (ml)

To express the chlorophyll-a content as ug/cm^2 and AFDW in mg/cm^2 of substratum surface, the surface area of the ceramic tiles, sandstone, and reference substratum of each sample was determined (the surface area of a microscope slide was always 75 mm x 25 mm). The exposed areas of these substrata were wrapped in aluminium foil after the biofilm was removed, with all parts of the tinfoil not in contact with the substratum removed. The remaining foil was then spread out over gridded with 1mm^2 square paper. The outline of the tin foil was drawn, and the surface area determined by counting the number of 1 mm^2 squares within the silhouette. The chlorophyll-a content and AFDW were then converted to a measure of surface area using the following equations:

$$Chla\ sur = \frac{chla\ vol}{SA}$$

Where

Chla sur is the chlorophyll-a concentration per unit of surface area (expressed as ug/cm^2)

Chla vol is the volume of chlorophyll-a present in 1 litre of water (expressed as ug/l)

SA is the surface area of the substratum the biofilm sample was taken from (expressed as cm^2)

And

$$AFDW\ sur = \frac{AFDW\ vol}{SA}$$

Where

AFDW sur is the ash-free dry weight concentration per unit of surface area (expressed as ug/cm^2)

AFDW vol is the volume of ash-free dry weight present in 1 litre of water (expressed as mg/l)

SA is the surface area of the substratum the biofilm sample was taken from (expressed as cm^2)

Relative abundance of algal groups

To determine the size of the diatom community in the biofilm, compared to the communities of other algal groups, a volume of ~0.2 µl of each biofilm extract was used to determine the relative abundance of the different algal groups by placing it on a dipwell microscope slide. Images with algal cells (at least 300 cells per sample) were taken using a light microscope with a camera attachment for imaging (Olympus BX53 upright light microscope with an Olympus T150 camera attachment and using Cellsense entry (Version 3.1) software). For each biofilm sample, 300 algal cells were identified to the algal group level (diatoms, chlorophytes, cyanobacteria, desmids, and cryptophytes), using a modified version of the taxonomic analysis described in Soldo and Behra (2000). The relative abundance of these algal groups was calculated by the following equation:

$$Relative\ abundance\ of\ algal\ group = \left(\frac{count\ of\ individual\ of\ a\ given\ group}{total\ count\ of\ all\ individuals\ of\ all\ groups}\right) x\ 100$$

Diatom analysis (relative abundance, diversity indices, UKTAG LTDI2 analysis)

In order to determine the composition, structure and function of the biofilms sampled during this experiment, a volume of ~0.5 to 5 µl of biofilm extract was used for the diatom metrics. The diatoms frustules were extracted using a hydrogen peroxide method described by Battarbee (1986). To clean the subsample of organic matter and clean the frustules, the biofilm was digested in 15% hydrogen peroxide using borosilicate beakers (Fisher scientific, 50 ml) on a hotplate at 80^{0}C. After all visible chemical reactions had ceased and the fluid in the beaker had turned transparent, the sample was left to cool. The frustules and fluid were then transferred to a HDPE centrifuge tube (Fisher Scientific, 15 ml), and spun in a centrifuge at 1200 RPM for four minutes to separate the diatom frustules from the supernatant.

The cleaned frustules were then mounted onto a microscope slide by evaporating 0.01-0.05 ml of diatom frustules onto a microscope slide coverslip; these were then bound to a microscope slide using Naphrax at 90^{0}C on a hot plate to evaporate the solvent in the Naphrax to seal the slide and cover slip together. Once set, at least 300 individual diatoms in each biofilm sample were identified to the species level using reference guides by the Welsh Natural History Museum and Diatoms of North America (2020), based on work by Krammer and Lange-Bertalot (1986). Species whose abundance was not above 2% in at least one biofilm were removed from the dataset before further analysis was conducted; as they were below the threshold for significance within the community structure (Pla-Rabés *et al.,* 2016).

The relative abundance of each individual diatom species was calculated using the following equation:

$$Relative\ abundance\ of\ taxa = \left(\frac{count\ of\ individual\ of\ a\ given\ species}{total\ count\ of\ all\ individuals\ of\ all\ species}\right) x\ 100$$

Species richness (the total number of species present), evenness and Shannon-H diversity index of each sample were calculated using PAST software (University of Oslo, version 3).

The equation for the Shannon H index is:

$$H = -\sum (Pi * \ln Pi)$$

Where

P_i is the fraction of the entire sample made up of the species measured

The equation for evenness is:

$$Evenness = H \,/\, Ln((s-1) \,/\, Ln(n))$$

Where

s is the number of species observed

n is the total number of individuals in the sample

H is the Shannon diversity Index

The EQR LTDI2 (LTDI2) and percentages of motile and organic tolerant diatom species were calculated using the UKTAG DARLEQ2 software package, using the abundances of the different species identified, as well as the alkalinity data for the sample site and time. The percentage of motile and organic tolerant were calculated based on the software's database of diatoms that fall into either of these two calculations and expressed as the total proportion of the sample composed of species that fall under these classifications as a percentage. The calculation of the sites LTDI2 was conducted using the LTDI2 calculation (Directive, 2014), designed for use with lake diatom communities, and conducted using the following calculation:

$$Observed\ lake\ trophic\ diatom\ index\ (TDI) = (w\ x\ 25) - 25$$

Where W is the following equation:

$$W = \frac{\sum_{j=1}^{n} a_j\ x\ s_j}{\sum_{j=1}^{n} a_j}$$

Where

a_j is the number of valves counted in species of a specific species identified in the DARLEQ2 software taxon list present in the sample

S_j is the nutrient sensitivity score of the corresponding species used for a_j present in the sample

The reference value is calculated as the EQR, using the following equation

$$EQR_{DARLEQ} = (100 - Observed\ lake\ trophic\ diatom\ index\ (TDI)) \div (100 - reference\ value)$$

Where:

reference value is 20 if annual mean alkalinity of the lake is <10 mg/l $CaCO_3$, or 25 is the annual mean alkalinity of the lake is >/= 10 mg/l $CaCO_3$.

If EQR_{DARLEQ} >1, then the value is recorded as 1

*note that as this data was unavailable, the reference value was generated using the concentration observed during time of sampling, which was always far in excess of 10 mg/l of $CaCO_3$, so the reference value was always 25

2.3.4.2. Water quality analyses

Water samples taken from the sites were used to determine the nutrient composition of the lake water. The 0.2 micron filtered water samples were analysed for phosphate, nitrate, nitrite, sulphate, chloride and fluoride concentrations using an ICS2000 Ion Chromatograph (Dionex, California, US), set up to run a sample for 40 minutes using an aqueous potassium hydroxide eluent in a 5 ul loop injection system connected to a DS6 heated (35˚C) conductivity cell (Qian *et al.,* 2003). A multi-nutrient standard (range 0-5 ppm for phosphate, nitrate, nitrite, and fluoride, 0-100 ppm for chloride, 0-25 ppm for sulphate) diluted from stock solutions from Fisher scientific, and Sigma-Aldritch (chloride and nitrite only) was used to construct a calibration curve.

The standard concentration that contained 2ppm phosphate, nitrate, nitrite and fluoride, followed by two ultrapure water samples were added at the end of every 10 samples to act as a drift and blanks, respectively. Dissolved organic carbon (DOC) and total nitrogen (TN) concentrations were determined using a VarioTOC cube (Elementar, Germany) non-dispersive infrared analyser (NDIR) analyser with the 0.45 micron filtered water sample used for this analysis, with pre-prepared standards (constructed from a stock solution of sodium carbonate and potassium phthalate separately diluted to cover concentrations of 1-100 ppm) used to construct a calibration curve. An initial sample of 10% hydrochloric acid was added to acidify the samples, with a blank placed between the standards and the samples, and a mid-range drift, followed by two blanks placed after every 10 samples, and at the end of the run (Sun *et al.,* 2014). Ammonium concentrations were assessed using an AA3 flow calorimeter (Seal Analytical, Hampshire, UK) (Bartelme *et al.,* 2017), using the 0.45 micron filtered water sample, with a series of nitrogen reagents (citrate buffer, sodium salicylate, Dichloroisocyanuric acid, water, Brij solution) and standards (components from Sigma-Aldrtich) created the day before covering a range of 0-2 mg/l, and ultrapure water used between every 12 samples as blanks and additional mid-range standards placed adjacent to the blanks for drift correction.

The 0.45 micron filtered sample was used to determine the concentrations of magnesium, copper, potassium, iron, lead, sodium, zinc, nickel, phosphorus, silicon and calcium, which are known to act as nutrients to diatoms, using an iCAP7200 ICP-OES (Thermo-Scientific, Massachusetts, US). Note these are also contaminants in high enough concentrations (Soininen, 2007, Nagai and De Schamphelaere, 2016, Mu et al., 2018). Calibration curves were constructed for the ICP-OES results using phosphorus and silica standards, with a multi-element standard for the other elements (Merck ICP phosphorus standard, Sigma-Aldritch 1000 mg/l silicon standard, Merck ICP multi-element standard solution IV).

Finally, the concentrations of the total suspended solids (TSS) in the water bodies were calculated. A 500ml sample of water was taken from each site per time point, each of which was then filtered onto a pre-weighed desiccated filter paper (Microglass Fiber Filters, Grade 261(Whatman GF/C grade)) using a filtration unit (pressure 2 psi), and then dried and re-measured. The weight (mg) of the desiccated sediment with filter, as well as the desiccated filter alone were measured. The following equation was then applied to determine TSS:

$$TSS = (weight\ of\ dessicated\ sediment\ plus\ filter - weight\ of\ dessicated\ filter)\ x\ 2$$

2.3.5. Data analysis of biofilm measurements and water quality data

Line graphs were used to graphically represent the results of biofilm measurements (for AFDW, diversity metrics and Chlorophyll-a) as well as the physico-chemical measurements, and bar graphs for algal groups and diatom species, both were conducted in Microsoft Excel, using the replicate results to create a mean and standard error for each dataset.

Statistical analyses were performed for all datasets in IBM SPSS software version 26. For all data, a Kolmogorov-Smirnov test was performed on each dataset to test for normality. If the data were not normally distributed, this was then followed by an initial ANOVA (three-way repeated measures for biofilm results, two-way repeated measures for water quality data), to obtain the Levinnes equality of variance data to test the homoscedasticity of the datasets. If the data was not normally distributed or the variances were not equal, then the data was transformed to improve normality (arcsin square root for algal groups and diatoms variables and log10 or square root if percentage) for the physicochemical water parameters. If the transformations did not yield significance (P) values greater than 0.05, then the data with the highest P value was used, and one way interaction assessments were conducted using non-parametric testing (Friedman tests for the repeated measure time factor, Kruskal-Wallis H test for the lake and substratum factors), as these statistics are more appropriate for data that are not normally distrusted or have equal variances.

The subsequent analyses examine the relationships between the three factors being tested in the analysis, and the biological endpoints of the biofilms developed during this experiment. These factors were the differences between the biological endpoints due to substratum the biofilms were developed on (microscope slide, ceramic tiles, sandstone and the reference substratum), the duration the replicates were developed for (2, 4, 6, 8 and 10 weeks), and the differences between the two lakes the replicates were deployed in (West campus lake and East campus lake).

Three-way repeated measures ANOVAs were then performed to test for any differences in the dataset caused by the substratum type (microscope slides, ceramic tiles, sandstone), duration of exposure (2, 4, 6, 8 and 10 weeks) or lake exposed to (West campus lake and East campus lake) and any interaction between these factors on the various biofilm measurements (Section 2.3.4.) Further two-way repeated measure ANOVAs splitting the dataset by one of the factors at a time were performed if the three-way ANOVAs indicated interaction effects. Further one-way ANOVAs were conducted by splitting the dataset by two of the three factors at a time, if the two-way ANOVAs identified further interaction, or Kruskal-Wallis H tests (if the Kolmogorov-Smirnov test or Levinnes equality of variance tests determined that the dataset was either not normally distributed or the variances were not equal). For East Campus lake data an additional set of replicates for the reference substratum that was excluded from this analysis due to a lack of equivalent data in the West Campus lake results existed. As such, a second analysis discounting the West campus lake results was conducted, and removing 'lake' as a factor, whilst incorporating the data from this additional substratum as a fourth substratum type. All ANOVA analyses were reported with their F (test statistic), df (degrees of freedom of the data) and P (significance of the test statistic) values.

For the physico-chemical parameters (section 3.3.3 and 3.3.6), a two-way repeated measure ANOVA was used to, with only 'time' and 'lake' as factors, as unlike the biofilm measurements, the substratum factor was not relevant to these measurements. If interaction effects were identified between the time and lake factors, then the dataset was split by one factor and the ANOVA/ non-parametric equivalents performed on the other factor separately, to identify the effects at specific times or on a specific lake, as with the biofilm analyses described above.

If there was an effect of the substratum factor (differences caused by biofilms being developed on microscope slides, ceramic tiles, sandstone, or reference substratum), where there were more than two values for this factor, this was followed by appropriate post-hoc tests (Tukey honest significant difference (HSD) (if the data was normally distributed), Mann-Whitney U tests (if the data was not evenly distributed)) to distinguish the exact differences between reported values of the biological measurements of the biofilms developed on the test substratum. For the time factor, this was replaced by the use of the within-subject contrasts, to determine the linear time points between which there was a significant difference No additional testing was performed on the lake factor, as this factor only had two values.

Linear regression analysis was performed on the LTDI2 values for the biofilm, as a measure of predicted trophic state of the lake based on the diatom community composition, against the 27 physicochemical parameters (section 3.3.3 and 3.3.6.) assessed throughout the experiment, with the UKTAG assessment results on the y axis, and the value of the physico-chemical parameter on the x axis, to assess the strength and direction of these parameters on the overall ecological health of the biofilms. MANOVA analysis were performed to assess the effects of all physicochemical parameters on the LTDI2 values of the substratum, with this data split beforehand by the lake factor to differentiate the results between the two contrasting sites. Multiple linear regression was then performed on the LTDI2 values, using the physico-chemical parameters (section 3.3.3. and 3.3.6) identified to significantly affect the ltdi2 of the lake in the MANOVA analysis (P value below 0.050) to determine the combined effect of the most significant factors identified in the MANOVA on the biological quality of the diatom communities (LTDI2) in each lake.

2.4. Results: Biofilm structural measurements

2.4.1. Biomass measurements

Figures 2.6. and 2.7. present the AFDW and chlorophyll-a concentrations, respectively, measured at both lakes. Table 2.1. and Table 2.2. show the results of the three-way and two-way repeated measures ANOVA. These results demonstrate changes in the size of the biofilms and algal communities over time and between the test substrates.

Effect of substratum

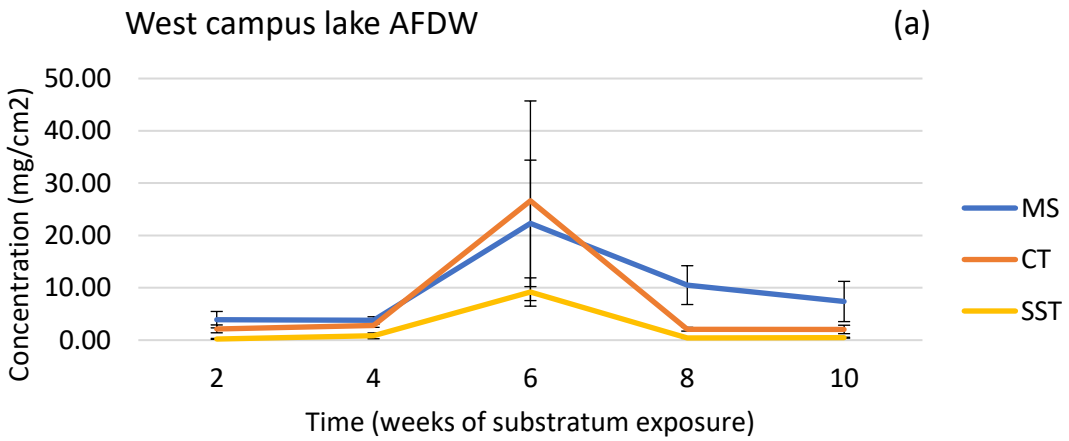
Figure 2.6. shows that AFDW concentrations were lowest on the sandstone substratum and highest on the microscope slides (Figure 2.6., Table 2.1.) ($P>0.050$, Tukey HSD). When incorporating the reference substratum to the East campus lake results only, there was no significant substratum effect on AFDW ($P=0.051$). However, there was a significant substratum effect on chlorophyll-a content, (Figure 2.7., Table 2.2.). Using posthoc testing, chlorophyll-a concentrations on East campus lake demonstrated similarities between biofilms developed on the microscope slides and reference substratum (0-2 $\mu g/cm^2$) ($P>0.050$, Tukey HSD), and lower concentrations than ceramic tiles and sandstone (2-11 $\mu g/cm^2$) ($P<0.050$, Tukey HSD).

Effect of time

AFDW significantly changed over time (Figure 2.6., Table 2.1.), with an increase in AFDW from week 4 to week 6, and then a decrease again to week 8 ($P<0.001$, within-subject contrast, Figure 2.6., Table 2.1). When incorporating the reference substratum data to the East campus data, only AFDW is significantly affected by time (Figure 2.6., Table 2.2.) with within subject contrasts showing that this is due to an increase in concentration (0-5 to 10-50mg/cm^2) between week four and six ($P=0.001$) and a decrease from 10-50 to 0-10mg/cm^2 between weeks six and eight ($P<0.001$).

Differences between lakes

There was no significant difference in AFDW between the two lakes (Figure 2.6., Table 2.1.). However, chlorophyll-a content was significantly higher in the East campus lake than in the West campus lake ($P<0.001$), specifically on the ceramic tiles and sandstone substrata, compared to microscope slide and reference substrata (West campus: 0.1-1 $\mu g/cm^2$, East campus: 2.5-8 $\mu g/cm^2$, Figure 2.7., Table 2.2.) ($P<0.001$ and 0.003, respectively, two-way repeated measures ANOVA), ($P<0.050$, Tukey HSD).



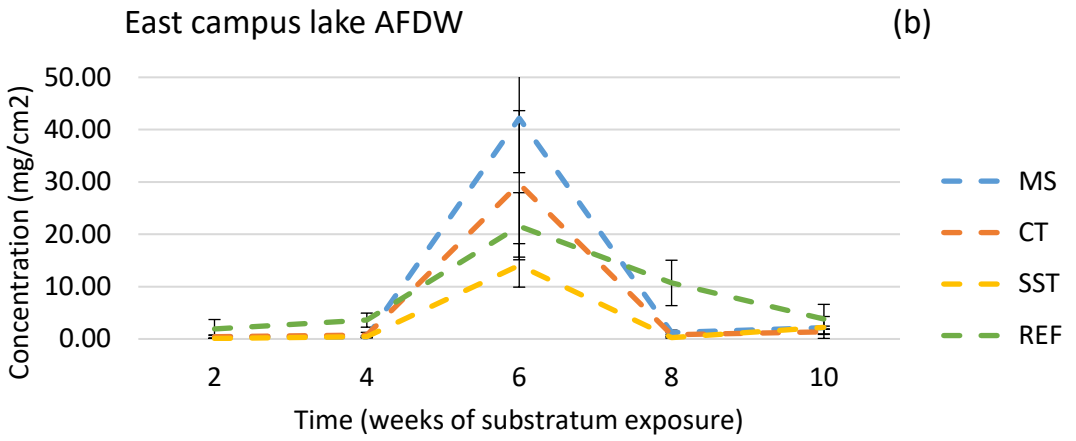


Figure 2.6. Ash-Free Dry Weight (AFDW benthic biofilm at different substratum (MS= microscope slides, CT= ceramic tiles, SST= sandstone, REF= reference stones from lake bottom) over time in West campus lake (a) and East campus lake (b) by substratum over time. Mean ± SE (n= 3).

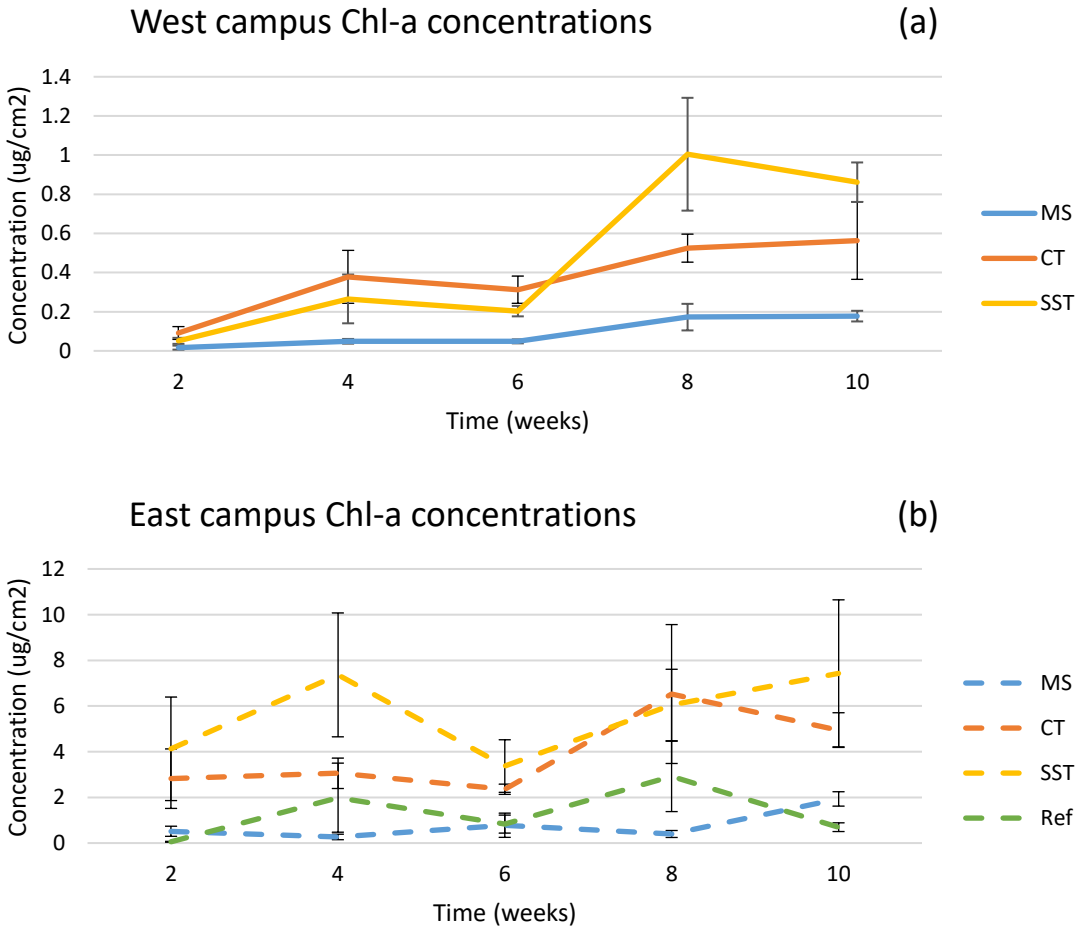


Figure 2.7. Chlorophyll-a concentrations (in µg/cm^2) of biofilms grown on substratum placed in West campus lake (figure 2.7a) and East campus lake (figure 2.6b). Ref is reference substratum, CT is ceramic tile fragments, SST is red sandstone blocks, and MS is glass microscope slide substratum.

Table 2.1. Three-way repeated measures ANOVA for AFDW and chlorophyll-a concentrations, using the three test substratum developed on (substratum), lake exposed to (West campus lake and East campus lake) (lake) as factors, and duration of exposure (2, 4, 6, 8 and 10 weeks) (time) as a repeated measure factor. These are followed by the Interaction effects for the combination of different factors. Bold values show where effects are statistically significant (P<0.050)

	AFDW			Chlorophyll a		
	F	df	P	F	df	P
Substratum	5.411	2	**0.021**	14.024	2	**0.001**
Lake	0.965	1	0.345	106.846	1	**<0.001**
Time	31.142	1.125	**<0.001**	2.111	2.064	0.141
Substratum * Lake	1.289	2	0.311	17.492	2	**<0.001**
Substratum * Time	2.877	2.250	0.086	0.463	4.127	0.767
Lake * Time	2.019	1.125	0.178	1.163	2.064	0.330
Substratum * Lake * Time	0.176	2.250	0.863	0.370	4.127	0.833

Table 2.2. Two-way repeated measures ANOVA for the biomass measurements from East campus lake only, incorporating results from the fourth reference substratum (stones native to the lake) for ash-free dry weight (AFDW) and chlorophyll-a concentrations, using Substratum (microscope slide, ceramic tile, sandstone, and reference substratum) as a main factor, and duration of substratum exposure to the lake (2, 4, 6, 8 and 10 weeks) as a repeated measure factor.

	AFDW			Chlorophyll a		
	F	df	P	F	df	P
Substratum	4.039	3	0.051	22.274	3	**<0.001**
Time	22.789	1.452	**<0.001**	1.680	2.087	0.216
Substratum * Time	1.434	4.355	0.284	0.563	6.262	0.760

To summarise, AFDW concentrations were higher in West campus lake biofilms, and except for week six, there was no overall change in the concentration of AFDW in the biofilms of either lake over time. Conversely, chlorophyll-a concentrations were higher in East campus lake biofilms. Furthermore, biofilms produced on microscope slides contained the highest concentrations of AFDW, whilst the biofilms developed on sandstone substratum contained the highest concentrations of chlorophyll-a, but when compared to the reference substratum in East campus lake, ceramic tiles typically produced the closest AFDW concentrations to the biofilms from the reference substratum, but the microscope slide substratum biofilms was the closest to replicating the chlorophyll-a concentrations in the reference substratum.

2.4.2. Relative abundance of benthic algal groups

Overall, the diatoms and chlorophytes were the most abundant species, with diatoms being more abundant in East campus lake accounting for 43.6% ± 5.84% to 85.77% ± 1.20% of the community there, and 18.3% ± 1.50% to 49.57% ± 2.13% on West campus lake. Whilst chlorophytes were more abundant in West campus lake, where they composed 42.92% ± 7.07% to 73.82% ± 4.36% of the community structure (and 5.30% ± 1.54% to 33.26% ± 12.02% on East campus lake) (Figure 2.8.). Approximately 10% to 20% of the community was comprised of cyanobacteria, whilst the desmids and cryptophytes made up < 2% of the community. For this reason, they were excluded from subsequent analysis. See Figure 2.8, and Tables 2.3 and 2.4.

Substratum and time effects

Overall, there was no significant effect of substratum for diatom, cyanobacteria or chlorophyte abundances (Figure 2.8., Table 2.3.). However, the relative abundances of the three main algal groups did not significantly alter over the ten weeks of exposure as main effects, there were exceptions due to interaction effects. There were two specific effects of time on the abundance of diatoms. The first was higher relative abundance of diatoms observed at week 8 than week 6 in the West campus lake (P<0.050, Figure 2.8., Table 2.4.). The second exception was a lower relative abundance of diatoms at week 4 in the East campus lake, compared to weeks 2 and 6.

Furthermore, significant time* lake interactions occurred for diatoms, which demonstrated that on West campus lake there was a decrease between week two and four (week two: 29.78% ± 1.87% - 47.9% ± 1.45%, week four: 18.3% ± 1.50% - 24.74 ± 2.06%), and an increase on West campus lake between weeks eight (29.36% ± 2.35% to 38.08% ± 1.73%) and ten (32.54% ± 3.81% to 49.57% ± 2.13%), as well as an increase on East campus lake between weeks two (48.49% ± 8.42% to 66.99% ± 16.44%) and four (43.76% ± 5.84% to 85.77% ± 1.20%) (P= 0.002, 0.002, and 0.001, respectively, two-way repeated measures ANOVA between subjects contrast).

When incorporating the reference substratum to the analysis in the East campus lake, there were no significant substratum or time effects on the relative abundance of the cyanobacteria, but there was a significant substratum*time interaction for the diatoms (Figure 2.8., Table 2.4.). However, further statistical analysis confirms there was no effect of substratum or time when the dataset is split by each other, although in Figure 8 it does appear that the diatom abundances were lower at weeks four and eight. These results were therefore not noticeably different to the analysis conducted without the reference substratum.

Lake effect

There were significant overall differences in relative abundances between the two lakes for all three main algal groups, with diatoms and cyanobacteria being more abundant in East campus lake biofilms (diatoms: 18.3% ± 1.50% to 49.57% ± 2.13% for West campus lake, 43.6% ± 5.84% to 85.77% ± 1.20% for East campus lake, cyanobacteria: 0.44% ± 0.36% to 19.92% ± 8.07% for West campus lake, 2.04% ± 1.17% to 27.84% ± 7.41% for East campus lake). In contrast chlorophytes were more abundant in West campus lake biofilms than in East campus lake (42.92% ± 7.07% to 73.82% ± 4.36% for West campus lake, 5.30% ± 1.54% to 33.26% ± 12.02% for East campus lake) (Figure 2.8., Table 2.4.). There were, however, some significant interactions within these broad groupings.

For diatoms, this higher abundance on East campus lake always occurred on both microscope slides (P<0.001, 18.3% ± 1.50% to 35.64% ± 2.89% for West campus, 2.04% ± 1.17% to 27.84% ± 7.41% for East campus) and ceramic tiles (P= 0.013, 19.77% ± 49.57% ± 2.13% for West campus, 48.54% ± 8.21% to 79.06% ± 5.12% for East campus), but on the sandstone substratum, this was only the case in week 4 (23% ± 2% for West campus lake, 78% ± 1% for East campus lake) and week 8 (35% ± 4% for West campus lake, 74% ± 2% for East campus lake) (P=0.050, Kruskal-Wallis H test). Increased abundances on East campus lake also occurred for the cyanobacteria abundances, showing significantly higher abundances on East campus lake for microscope slides (5-12%±1 for West campus, 5%±1 to 25%±2 for East campus) and ceramic tiles (1-7±1 for West campus, 9-19±1-3), but there was no difference between the two lakes at any time point for sandstone (P=0.147, two-way repeated measures ANOVA). For the chlorophytes, the higher abundances developed on microscope slides (55-71±1-7 for West campus, 5-12±1-4 for East campus) and ceramic tiles (49-71±1-8 for West campus, 9-43±1-3 for East campus) were significant. However, biofilms developed on the sandstone typically show a trend towards higher abundances in West campus lake too (43-68±1-7 for West campus, 5-31±1-2 for east campus, all P=0.050, Kruskal-Wallis H test), except for week two (P=0.127, Kruskal-Wallis H test), where although the abundances of chlorophytes on West campus lake were still higher than East campus lake, they were not considered significantly different (West campus lake: 42% ± 1%, East campus lake: 28% ± 2%).

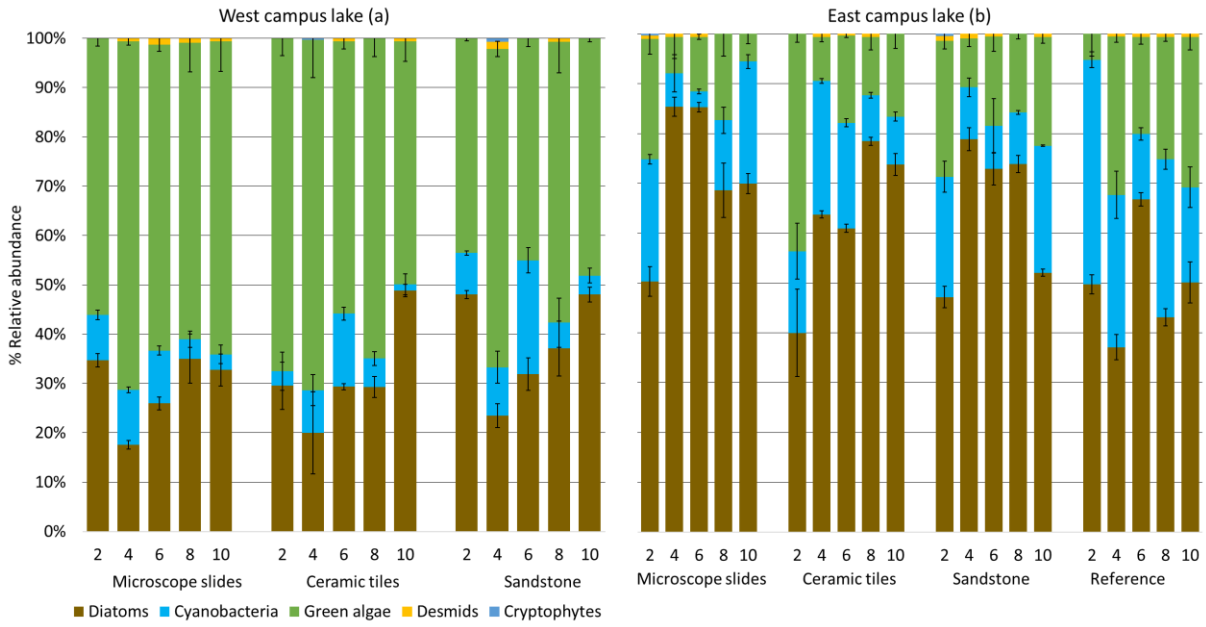


Figure 2.8. Relative abundance of algal groups colonising different types of substratum (microscope slides, ceramic tiles or sandstone, and for East campus also a reference substratum taken from stones already present in the lake) exposed for 2, 4, 6, 8 or 10 weeks in the West (a) and East (b) campus lakes. Mean ± standard error (n=3).

Table 2.3. Three-way repeated measures ANOVA results for the relative abundance of diatoms, cyanobacteria and chlorophytes during the 10-week colonisation period measured at two-week intervals in two lakes.

	diatoms			cyanobacteria			chlorophytes		
Source	F	df	P	F	df	P	F	df	P
Substratum	0.908	2	0.429	0.393	2	0.683	1.812	2	0.205
Lake	148.566	1	**<0.001**	22.642	1	**<0.001**	271.973	1	**<0.001**
Time	2.429	2.542	0.093	0.798	2.320	0.477	1.802	3.106	0.162
Substratum * Lake	2.382	2	0.135	1.331	2	0.301	4.068	2	**0.045**
Substratum * Time	1.798	5.085	0.142	1.659	4.641	0.181	0.645	6.212	0.698
Time * Lake	8.575	2.542	**0.001**	2.639	2.320	0.082	5.545	3.106	**0.003**
Time * Lake * Substratum	1.873	5.085	0.127	1.129	4.641	0.367	0.656	6.212	0.690

Table 2.4. Two-way repeated measures ANOVA results for the relative abundances of the three main algal groups (diatoms, cyanobacteria and chlorophytes) during the ten-week exposure period measured at two-week intervals on the three test substratum plus the reference substratum on East campus lake.

	diatoms			cyanobacteria			chlorophytes		
Source	F	df	P	F	df	P	F	df	P
Substratum	1.264	3	0.350	1.702	3	0.243	0.831	3	0.513
time	2.269	2.470	0.121	0.725	2.212	0.511	0.975	2.739	0.416
Time * Substratum	3.163	7.411	**0.019**	0.981	6.637	0.472	1.577	8.218	0.188

To summarise, substratum type did not strongly affect the abundance of the different algal groups that developed on them, nor did the duration of exposure. However the lake the biofilms were developed in strongly influenced the abundances of the algal groups, with the biofilms developed

in East campus lake being predominantly composed of diatoms with the other two major groups, cyanobacteria and chlorophytes typically composing less than 20% of the community each, whilst in West campus lake the chlorophytes were the largest group, comprising over half the community, with around 30% being composed of diatoms and the rest being almost entirely composed of cyanobacteria.

2.4.3. Diatom species relative abundances

These results show that West campus lake was composed primarily of *G. parvulum*, which began the experiment as the dominant diatom in the community, but as the experiment continued its abundance relative to the other species reduced. Whilst on East campus lake the communities were primarily composed of *Achnanthidium minutissimum*, notable abundances of *Brachysira vitrea*, *Cocconeis disculus*, and *Gomphonema cuneolus*, with the remaining species present in very low quantities. Graphs in figure 2.9. show the abundances by species, divided by lake (West campus lake replicates: green, East campus lake replicates: blue), and sorted by substratum type (microscope slides, ceramic tiles, sandstone and reference substratum (East campus lake only), with a separate bar chart for each time point (Weeks 2, 4, 6, 8 and 10).

The relative abundance of seven diatom species (*B. zellensis*, *C. disculus*, *D. problematica*, *E. adnata*, *F. vaucheriae*, *N. acicularis*, *P. viridis*) were not influenced by the substratum or lake they grew in and did not change over time (P> 0.050, Figure 2.9., Table 2.6.), although most of these were low abundance species (0-3.5%), SE margins did allow *F. vaucheriae*, *P. viridis* and *C. disculus* to occasionally display potential abundances of up to 16, 7, and 7%, respectively.

Differences in abundance between substratum:

The relative abundance of thirty-seven of the forty diatom species observed in abundances over 2% were not affected by the substrate factor. The three species that did exhibit a difference between the substratum was *A. minutissimum* and *B. vitrea* (Figure 2.9., Table 2.6.), with posthoc testing showing that for both species' abundances were different on ceramic tiles to the other two substratum (P<0.050), with *A. minutissimum* being lower on the ceramic tiles than on microscope slides and sandstone, and *B. vitrea* being more abundant on the ceramic tiles than on these other two substratum.

When incorporating the species abundances of the reference substratum to the analysis of East campus replicates, for the two species that showed a significant substratum difference across both lakes (*A. minutissimum* and *B. vitrea)*, the same overall trend was observed. For *A. minutissimum* (P<0.001), the most common species on this lake, abundances were lower on the ceramic tiles and reference substratum (P<0.050, Tukey HSD), than on the microscope slides and sandstones, but that the abundance on these substratum was similar (P>0.050, Tukey HSD). *B. vitrea* (P=0.044) abundances were highest on ceramic tiles and lowest on microscope slides, which despite being significantly different to one another (P<0.050, Tukey HSD), both were similar to the abundances on the sandstone and reference substratum (P>0.050, Tukey HSD). The incorporation of the reference substratum to the East campus lake data, seven species demonstrated a difference by substratum on their abundance. Both *A. daonense* (P<0.001) and *D. problematica* (P=0.015) developed in higher abundances on the reference substratum than on the three test substratum (P<0.050, Tukey HSD). *N. amphibia* (P=0.040) and *E. reichardtii* (P=0.030) also demonstrated higher abundances on the reference substratum compared to the microscope slides and sandstone (P<0.050, Tukey HSD). However the abundances present on the ceramic tile substratum was similar to the other substratum. *N. linearis* was absent on the reference substratum (P=0.031), and most abundant on ceramic tiles (P<0.050, Tukey HSD). Both the microscope slide and sandstone substratum, exhibited similar abundances, compared to those exhibited in the reference and ceramic tile substratum at week two (P> 0.050, Tukey HSD, P=0.025 Kruskal-Wallis H test). *E. prostratum* (P=0.034) also demonstrated similar abundances across the microscope slide, ceramic tile and sandstone substratum. However, the abundance of this species on the reference substratum was significantly higher than on microscope slide and sandstones (P<0.050, Tukey HSD), on week ten only (P=0.024, Kruskal-Wallis H test). *G.*

truncatum abundances were also similar between the ceramic tiles and microscope slides, however the abundances on the ceramic tiles were lower than on the sandstone and reference replicates, whilst the abundances of this species on the microscope slide were also similar those on the sandstone and reference substratum (P=0.020, two-way repeated measures ANOVA) (P<0.050, Tukey HSD) (Figure 2.9., Table 2.7.).

Differences in abundance over time:

For sixteen of the forty species identified, there was no effect of time *(A. inariensis*, *A. modestiforme*, *B. vitrea*, *B. zellensis*, *C. disculus*, *D. problematica*, *E. adnata*, *F. vaucheriae*, *M. varians*, *N. acicularis*, *N. amphibia*, *N. cryptocephala*, *N. dissipata*, *P. viridis*, *R. gibba* and *S. ulna)* (see Figure 2.9., Table 2.6.).

There was an increase in abundance over time for twenty-one diatom species over the course of the experiment. *A. daonense* (between weeks six and eight, P=0.0117), *B. spp* (between weeks eight and ten, P=0.031), *E. neogracile* (between weeks two and four, six and eight, and eight and ten, P=0.016, 0.019 and 0.017, respectively), and *N. palea* (between weeks four and six, as well as weeks six and eight, P=0.001 and <0.001, respectively) (Figure 2.9., Table 2.7.). Further to this, there were decreases in the abundances of *A. pediculus* between weeks two and four as well as weeks eight and ten (both P=0.002), *N. palecaea* (between weeks two and four (P=0.018), and weeks eight and ten (P<0.001) over time. There were a further eighteen species whose abundance significantly changed over time, although only under specific circumstances. These species and specific circumstances are listed below:

- The relative abundances of *A. minutissimum* and *E. reichardtii* shifted over time in both lakes, but under differing conditions. The abundance of *A. minutissimum*, significantly increased over time in West campus lake, where it was a minor species, but only for microscope slides (0.2±0.1 to 4.5±0.5% at week eight) (P=0.040 0.024, two-way repeated measures ANOVA). However, in East campus lake, where it was the primary species in the community, this increase in abundance only occurred between weeks two and four (P=0.012, two-way repeated measures ANOVA). Additionally, *E. reichardtii* abundances increased over time on ceramic tiles (0.5±0.1% to 6.7±0.7%) (P=0.046) and sandstone (0±0.5% to 7±0.7%) (P=0.017, Friedman test) on West campus lakes, but decreased over time on microscope slides (6±0.1% to 0.1±0.3%) (P=0.027) and sandstone (9.8±0.1% to 0.1±0.5%) (P=0.039) on East campus lake.
- Increasing abundances over the course of the experiment limited to West campus lake were also noted for *E. prostratum* (0-1% to 4-8%, P=0.021), *E. sorex* (0-3% to 3-4%, P=0.028), *E. turgida* (4-5% to 5-9%, P=0.008), *G. accuminatum* (0-0.5% to 1-2%, P=0.028) *Gy. accuminatum* (0-1% to 1-2%, between weeks eight and ten only, *P=0.021)*, and *R. abbreviata* (0-1 to 4-5%, P= 0.018, two-way repeated measures ANOVA). Increases in the abundance of *B. brebisonii* over time in West campus lake was also noted across all three substratum (microscope slides: 0±0.5% to 2.8±1%, P=0.031, Friedman test) (ceramic tiles: 0±0% to 4.5±1.6%, P=0.031, Friedman test) and (sandstone: 0.1±0.1% to 5.9±5%, P=0.021, Friedman test). Further to this, two species increased in abundance over time on sandstone substratum on West campus lake. These were *A. daonense* (1±1% to 9.5±2%), and *A. pedicu*lus (0.5±0.5% to 2±1) (P=0.003, two-way repeated measure ANOVA). *N. capitatoradiata* did not have time as a main effect, but its abundance did increase over time on West campus lake ceramic tiles (0±.5% to 2.6±4%) (P=0.031, Friedman test) and sandstone (0±1% to 2±5%) (P=0.038, Friedman test).
- An additional two species abundances did change over time, but only on East campus lake. *G. vibrio* increased from 0.1±1% to 2±1% (P=0.024, two-way repeated measures ANOVA), and the abundance of *N. linearis* likewise increasing over time, but only on ceramic tile substratum deployed on this lake (0.3± <0.1% to 0± 0.1%) (P=0.017, Friedman test).

A further six species were observed to decrease in abundance over time on West campus lake. These were:

- *E. minuta* (6-3% to 3-1%,), *G. truncatum* (2-3% to 0-1%), (P= 0.032 and 0.027, respectively, two-way repeated measures ANOVA), and *G. parvulum* (microscope slides:

72 ± 20% to 1 ± 22%, P=0.022, Friedman test) (ceramic tiles: 55% ±10% to 2.5% ± 1.5%, P=0.022, Friedman test) (sandstone 40% ± 10% to 5% ± 2%, P= 0.043, Friedman test). *N. minuta* also decreased in abundance over time, but only on ceramic tile substratum (P=0.021, two-way repeated measure ANOVA). Effects of decreasing abundance on West campus lake, but limited to the sandstone substratum also occurred for two additional species; *G. cuneolus* (12±<0.1% to 1.1±<0.1%) and *G. olivaceum* (1.5±6% to 4±0.5%) (P=0.043, P=0.043, respectively, Friedman test).

When incorporating the reference substratum into the analysis of East campus replicates, three species demonstrated a significant decrease in abundance over the ten weeks of the experiment. These were *A. pediculus* (week four (1.5-2.5%) and six (0.5-5.1%), P=0.012, Figure 2.9., Table 2.12.), and *E. reichardtii* (between week two and four: P=0.029, weeks four and six: P=0.003, week six and eight: P=0.012, and week eight and ten: P=0.0.15 overall 6-10% to 0-1.9%). *N. linearis* abundances also decreased over time between weeks two and four but only on the ceramic tile substratum (0.5±<0.1% to 0.1±<0.1%, P=0.031, Friedman test). G. cuneolus also demonstrates a decrease in abundance over time on West campus replicates (P=0.004, two-way repeated measure ANOVA, Figure 2.9., Table 2.6.). However, when analysing East campus replicates only with the reference substratum included, *G. cuneolus* abundances also increased on the reference substratum over time (week four and six (week 4-6: 6.9±<0.1 to 1.8±<0.1, P=0.037, Friedman test).

There were a further five species whose abundance increased with time. These were *E. gracile* (week two and four, 0-0.5% to 0.5-1%, P=0.036, Fig 9. Table 7), *B. brebisonii* (increase significant between weeks eight and ten, 0.4±0.7% to 6.1±1.2%, P=0.004), *E. prostratum* (week eight and ten 0±4% to 8.1±0.7%, P=0.038). *N. capitatoradiata* abundances also significantly increased between week six and eight (P=0.017), and week eight and ten (P=0.001) (0.1±<0.1% to 3.2±0.1%).

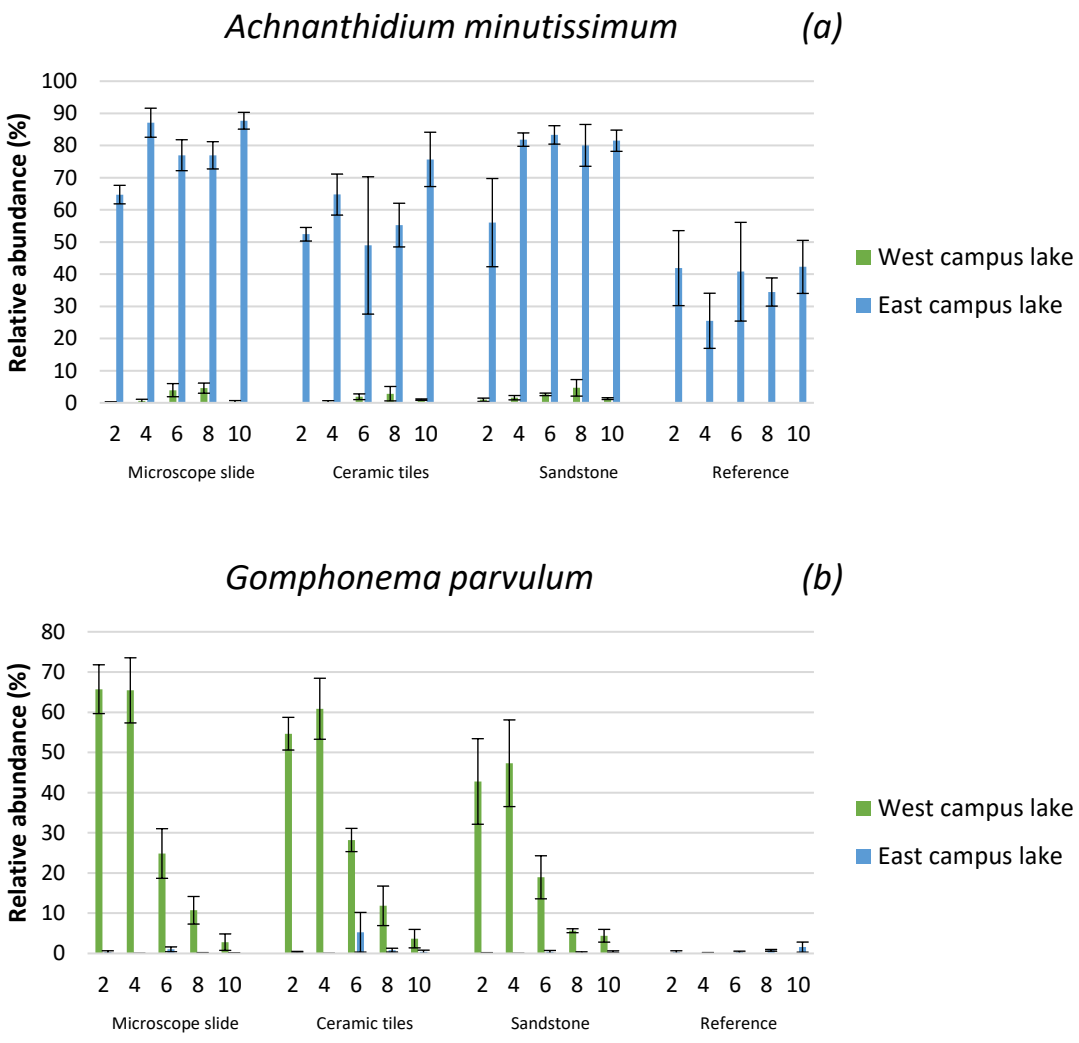
Differences in abundance between lakes:

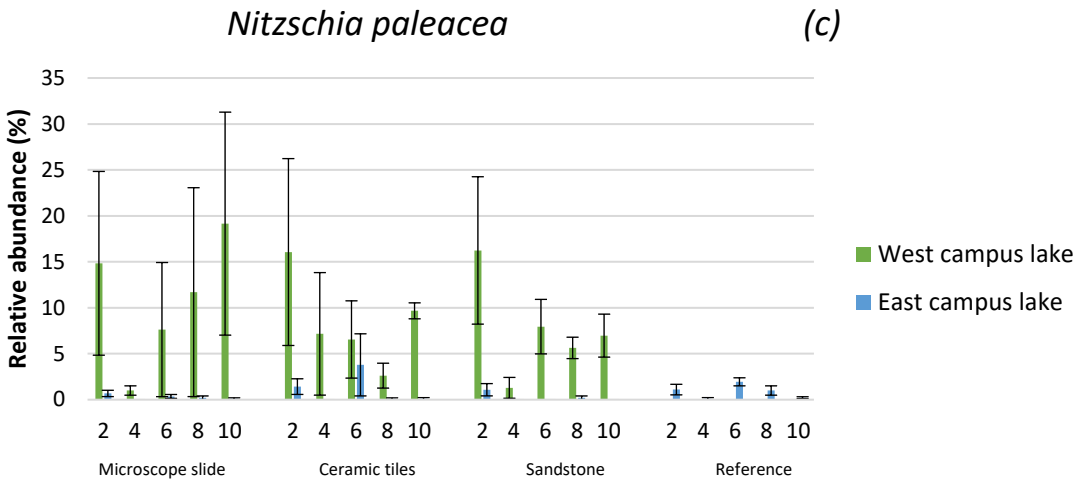
Twenty-eight of the forty species shown in Figure 2.9 and Table 2.6 showed a significant difference in relative abundances between the two campus lakes. In West campus lake, there was a significantly higher abundance (P<0.050, Figure 2.9, Table 2.6) of the species *A. modestiforme*, *B. brebisonii*, *B. spp*, *E. minuta*, *E. prostratum*, *E. reichardtii*, *G. truncatum*, *M. varians*, *N. amphibia*, *N. dissipata*, *N. linearis*, *N. palea*, *N. paleacea*, *R. abbreviata*, *R. gibba* and *S. ulna* (Figure 2.9., Table 2.6.).

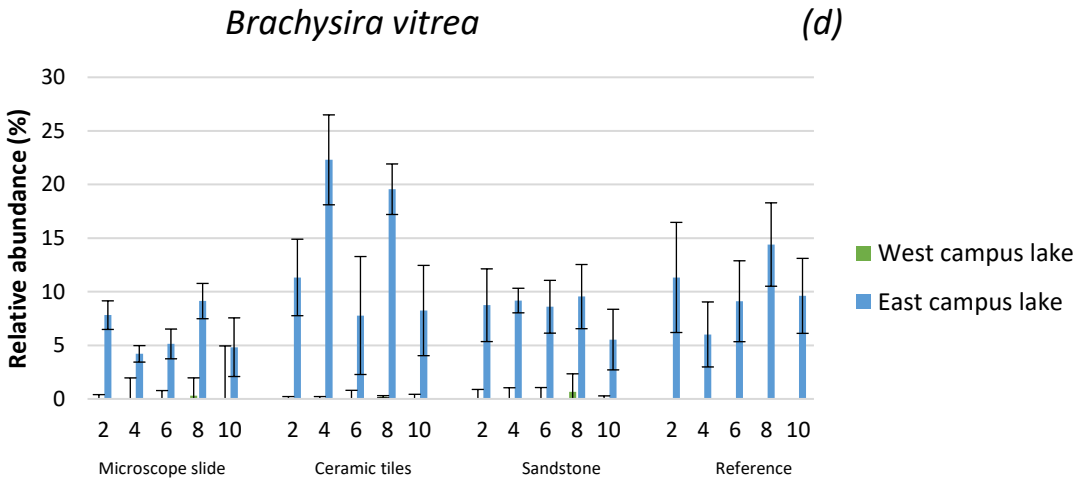
Several other species were also more abundant in West campus lake, but only under specific time and substratum conditions. *A. daonense*, *E. sorex*, *E. turgida* and *Gy. accuminatum* were more abundant in West campus lake, but only in biofilms developed on ceramic tile substratum (P=0.003, 0.079, 0.003, 0.040 respectively, N=1, two-way repeated measures ANOVA). The same effect occurs for *N. capitatoradiata*, but this was further limited to week ten on ceramic tiles and microscope slide substratum (P=0.037, Kruskal-Wallis H test).

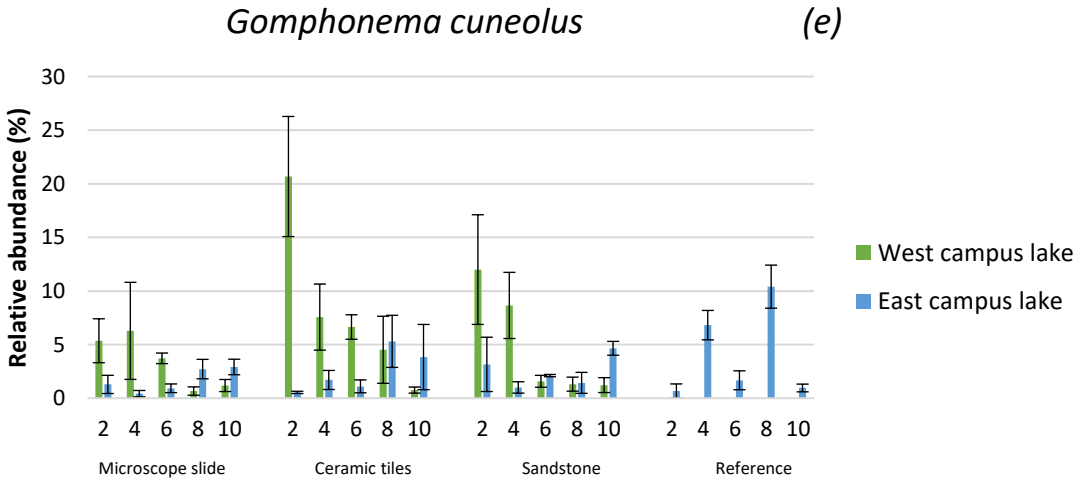
The increased abundances on West campus lake of *G. cuneolus* occurred at all time points for microscope slides (P<0.050, Kruskal-Wallis H test) except for week two (P=0.127). The increased abundance on West campus lake of *A. inariensis* only occurred on the sandstone substratum (P=0.012, N=1, two-way repeated measures ANOVA). For *G. olivaceum*, higher abundances on West campus occurred on microscope slide and sandstone replicates (P=0.026 and 0.031, respectively N=2, two-way repeated measures ANOVA), but for ceramic tiles this was limited to weeks two, four and ten (P<0.050, Kruskal-Wallis H test). *G. parvulum*'s higher abundance on West campus lake was significant for ceramic tiles (P<0.001, two-way repeated measures ANOVA), and all time points on microscope slides and sandstone substratum (P<0.050, Kruskal-Wallis H test), except for week ten on the microscope slides (P=0.246, Kruskal-Wallis H test). Further testing also limits the increased abundance of *E. prostratum* on West campus lake replicates to week eight on ceramic tiles (P=0.046) and sandstone (P=0.046 and 0.037, respectively, Kruskal-Wallis H test). Interactions also limit the increased abundance of *A. pediculus*, on East campus to the microscope slides at weeks two and four (all P=0.037, Kruskal-Wallis H test), and on sandstone (P=0.006, two-way repeated measures ANOVA). *N.*

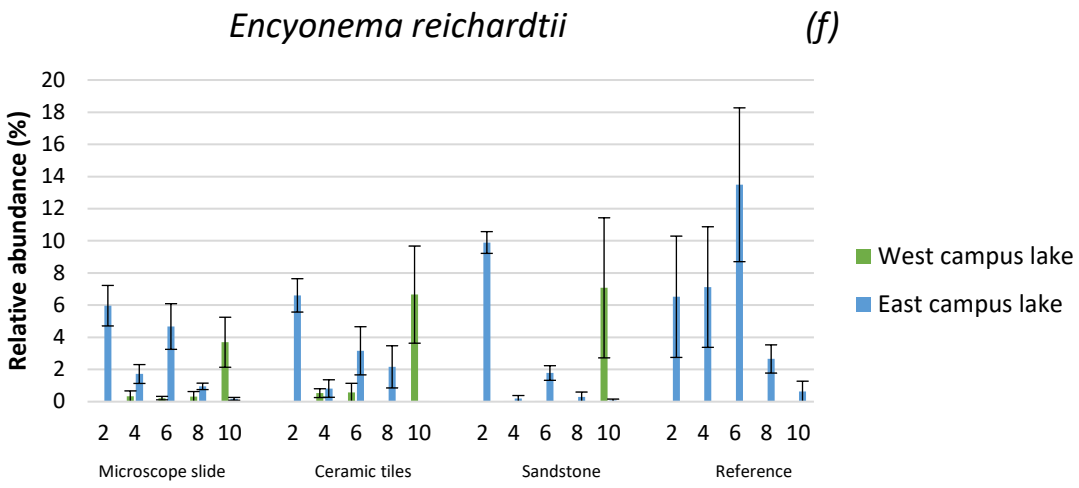
cryptocephala, was also more abundant on West campus lake, but only at week two (microscope slides: P=0.047, ceramic tiles and sandstone: P=0.037, Kruskal-Wallis H test).
Additionally, there were seven species that were more abundant on East campus lake, with five of these species being so regardless of other factors. These were *A. minutissimum*, *A. pediculus*, *B. vitrea*, *E. reichardtii* and *G. vibrio* (Figure 2.7.). *A. minutissimum* and *B. vitrea* comprised the majority of the community structure in this lake (>90%). Higher abundances of *E. minuta* only occurred at week four on microscope slides (P=0.046, Kruskal-Wallis H test), and weeks four to ten on sandstone (P=0.046 for all points except week 10, P=0.036, Krusal-Wallis H test). For *E. reichardtii*, the increased abundance on East campus is limited by interaction effects to all substratum on week two (P=0.037), and week six (P= 0.037 to 0.046). The increased abundance of *G. vibrio* on East campus lake was also limited to week eight on microscope slides, and weeks eight and ten on ceramic tiles (all P=0.037, Kruskal-Wallis H test).

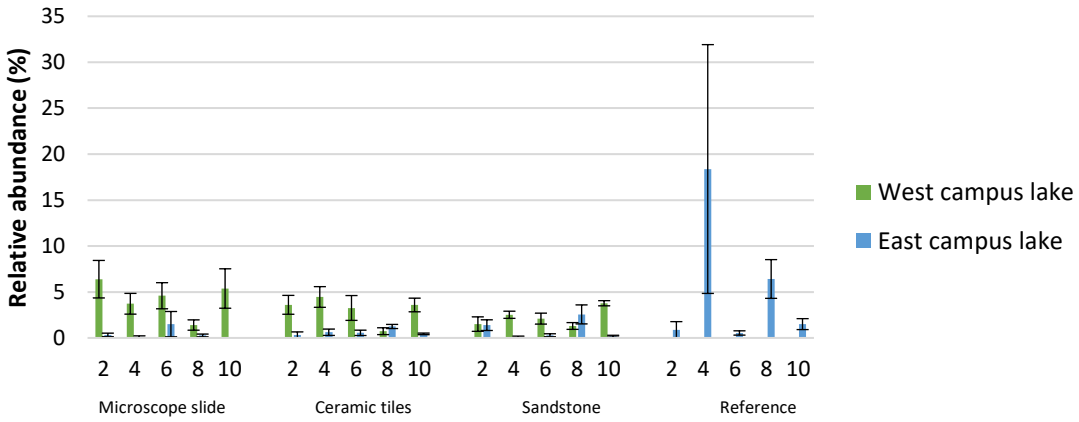


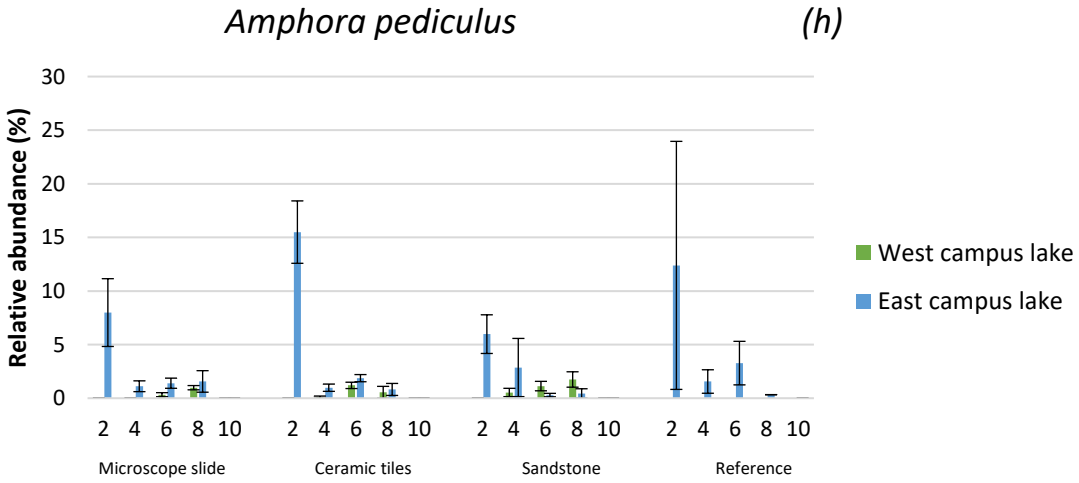

Nitzschia paleacea
(c)
Relative abundance (%)
35
30
25
20
15
10
5
0
2 4 6 8 10 2 4 6 8 10 2 4 6 8 10 2 4 6 8 10
Microscope slide
Ceramic tiles
Sandstone
Reference
West campus lake
East campus lake


Brachysira vitrea
(d)
Relative abundance (%)
30
25
20
15
10
5
0
2 4 6 8 10 2 4 6 8 10 2 4 6 8 10 2 4 6 8 10
Microscope slide
Ceramic tiles
Sandstone
Reference
West campus lake
East campus lake


Gomphonema cuneolus
(e)
Relative abundance (%)
30
25
20
15
10
5
0
2 4 6 8 10 2 4 6 8 10 2 4 6 8 10 2 4 6 8 10
Microscope slide
Ceramic tiles
Sandstone
Reference
West campus lake
East campus lake


Encyonema reichardtii (f)
Relative abundance (%)
20
18
16
14
12
10
8
6
4
2
0
2 4 6 8 10 2 4 6 8 10 2 4 6 8 10 2 4 6 8 10
Microscope slide
Ceramic tiles
Sandstone
Reference
West campus lake
East campus lake


Gomphonema olivaceum (g)
Relative abundance (%)
35
30
25
20
15
10
5
0
2 4 6 8 10 2 4 6 8 10 2 4 6 8 10 2 4 6 8 10
Microscope slide
Ceramic tiles
Sandstone
Reference
West campus lake
East campus lake


Amphora pediculus (h)
Relative abundance (%)
30
25
20
15
10
5
0
2 4 6 8 10 2 4 6 8 10 2 4 6 8 10 2 4 6 8 10
Microscope slide
Ceramic tiles
Sandstone
Reference
West campus lake
East campus lake

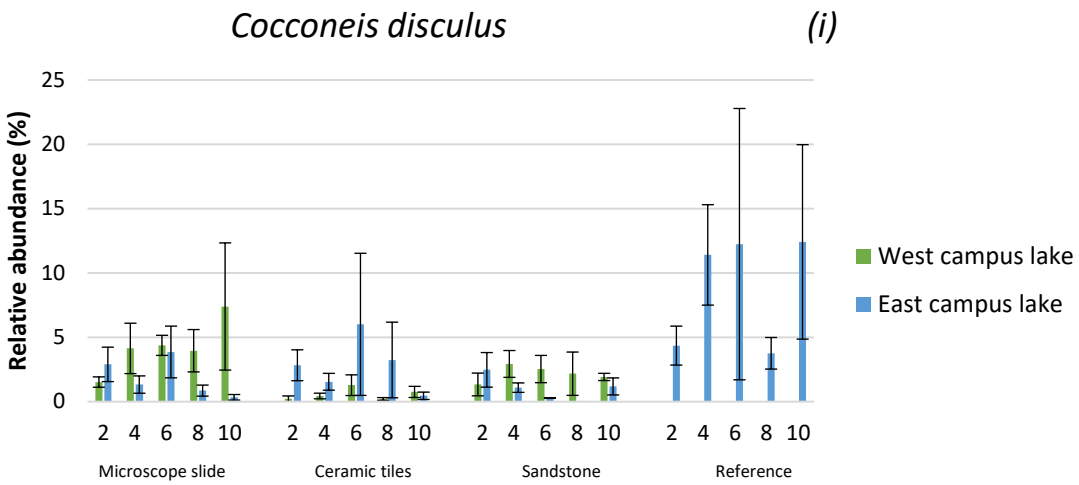


Figure 2.9. Relative abundances of individual benthic diatom species observed in at least one replicate at relative abundances above 2%, split by lake (colour), and sorted by substratum type and time point. Species whose mean value do not exceed 10% are shown in the appendix (Appendix a)

Table 2.5. List of diatom species in Figure 9, and their corresponding graph id letter. Note that species who did not appear in relative abundances of at least 2% on at least one biofilm replicate were not shown here. Full list of species identified an graphs for species with mean abundances below 10% of the diatom community are available in the appendix (Appendix b).

Graph number	Species name
a	*Achnanthidium minutissimum*
b	*Gomphonema parvulum*
c	*Nitzschia paleacea*
d	*Brachysira vitrea*
e	*Gomphonema cuneolus*
f	*Encyonema reichardtii*
g	*Gomphonema olivaceum*
h	*Amphora pediculus*
i	*Cocconeis disculus*
j	*Achnanthes daonenese*
k	*Epithemia turgida*
l	*Fragilaria vaucheriae*
m	*Nitzschia amphibia*
n	*Encyonema prostratum*
o	*Encyonema minuta*
p	*Melosira varians*
q	*Brachysira brebisonii*
r	*Diatoma problematica*
s	*Encyonema neogracile*
t	*Epithemia sorex*
u	*Nitzschia minuta*
v	*Navicula cryptocephala*
w	*Rhoicosphenia abbreviata*
x	*Gomphonema vibrio*
y	*Pinnularia viridis*
z	*Navicula capitatoradiata*
aa	*Nitzschia dissipita*
ab	*Synedra ulna*
ac	*Brachysira zellensis*
ad	*Brachysira spp.*
ae	*Nitzschia paleacea*
af	*Amphora inariensis*
ag	*Epithemia adnata*
ah	*Achnanthidium modestiforme*
ai	*Gomphonema truncatum*
aj	*Gyrosigma accuminatum*
ak	*Rhopaladia gibba*
al	*Nitzschia acicularis*
am	*Gomphonema accuminatum*
an	*Nitzschia linearis*

Table 2.6. Three-way repeated measures ANOVA results for the 39 identified species of diatoms in the field experiment, using substratum, time, and lake as main factors. Species sorted alphabetically

	Achnanthidium minutissimum			*Gomphonema parvulum*			*Nitzschia paleacea*			*Brachysira vitrea*			*Gomphonema cuneolus*			*Encyonema reichardtii*		
Source	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Substratum	13.557	2	**0.001**	2.557	2	0.119	0.053	2	0.948	11.881	2	**0.001**	2.877	2	0.095	0.190	2	0.830
Lake	1603.860	1	**<0.001**	201.772	1	**<0.001**	7.257	1	**0.020**	204.739	1	**<0.001**	12.048	1	**0.005**	9.582	1	**0.009**
Time	3.723	2.133	**0.036**	61.140	1.714	**<0.001**	3.778	1.996	**0.038**	2.758	2.533	0.068	5.687	2.306	**0.006**	8.334	1.743	**0.003**
Substratum * Lake	11.018	2	**0.002**	2.124	2	0.162	0.090	2	0.915	12.157	2	**0.001**	1.562	2	0.249	0.506	2	0.615
Substratum * Time	0.630	4.266	0.655	1.023	3.429	0.411	0.624	3.993	0.650	1.399	5.066	0.252	1.138	4.612	0.362	1.142	3.487	0.360
Time * Lake	3.780	2.133	**0.034**	63.396	1.714	**<0.001**	3.094	1.996	0.064	2.358	2.533	0.100	11.093	2.306	**<0.001**	24.012	1.743	**<0.001**
Substratum * Lake * Time	0.615	4.266	0.666	0.830	3.429	0.506	0.606	3.993	0.662	1.450	5.066	0.234	1.756	4.612	0.159	1.044	3.487	0.402

	Gomphonema olivaceum			*Amphora pediculus*			*Cocconeis disculus*			*Achnanthes daonenese*			*Epithemia turgida*			*Fragilaria vaucheriae*		
	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Substratum	1.566	2	0.249	1.934	2	0.187	1.644	2	0.234	0.559	2	0.586	1.200	2	0.335	0.067	2	0.935
Lake	27.450	1	**<0.001**	36.800	1	**<0.001**	0.269	1	0.613	25.746	1	**<0.001**	13.050	1	**0.004**	4.201	1	0.063
Time	1.732	3.055	0.177	20.002	1.616	**<0.001**	0.722	2.051	0.499	6.836	1.317	**0.013**	9.418	1.598	**0.002**	2.477	1.594	0.119
Substratum * Lake	3.287	2	0.073	2.958	2	0.090	3.015	2	0.087	0.965	2	0.409	0.554	2	0.589	0.033	2	0.967
Substratum * Time	2.074	6.111	0.079	2.730	3.232	0.069	0.769	4.103	0.559	0.327	2.634	0.781	1.119	3.195	0.368	0.567	3.188	0.653
Time * Lake	7.148	3.055	**0.001**	24.828	1.616	**<0.001**	1.736	2.051	0.197	8.040	1.317	**0.008**	9.391	1.598	**0.002**	2.563	1.594	0.112
Substratum * Lake * Time	1.153	6.111	0.352	2.455	3.232	0.090	1.062	4.103	0.397	0.285	2.634	0.811	1.080	3.195	0.384	0.629	3.188	0.614

	Nitzschia amphibia			*Encyonema prostratum*			*Encyonema minuta*			*Melosira varians*			*Brachsira brebisonii*			*Diatoma problematica*		
	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Substratum	0.290	2	0.753	0.841	2	0.455	2.205	2	0.153	0.163	2	0.852	1.831	2	0.202	0.298	2	0.748
Lake	27.857	1	**<0.001**	7.150	1	**0.020**	1.272	1	0.281	7.778	1	**0.016**	9.012	1	**0.011**	0.496	1	0.495
Time	2.070	1.578	0.160	7.098	1.318	**0.012**	6.918	2.364	**0.002**	2.450	1.537	0.124	12.866	1.139	**0.002**	1.250	1.420	0.298
Substratum * Lake	0.664	2	0.533	0.337	2	0.720	0.675	2	0.528	0.127	2	0.882	2.411	2	0.132	1.680	2	0.227
Substratum * Time	0.710	3.156	0.565	1.752	2.635	0.200	1.601	4.728	0.194	0.388	3.074	0.767	1.392	2.278	0.284	0.909	2.839	0.453
Time * Lake	2.418	1.578	0.125	8.185	1.318	**0.008**	5.515	2.364	**0.007**	2.828	1.537	0.096	8.373	1.139	**0.010**	0.280	1.420	0.684
Substratum * Lake * Time	0.588	3.156	0.638	1.948	2.635	0.167	0.873	4.728	0.507	0.394	3.074	0.763	0.967	2.278	0.416	1.078	2.839	0.382

	Encyonema neogracile			*Epithemia sorex*			*Nitzschia minuta*			*Navicula cryptocephala*			*Rhoicosphenia abbreviata*			*Gomphonema vibrio*		
	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Substratum	1.229	2	0.327	0.407	2	0.675	4.100	3.000	**0.028**	0.714	2	0.509	0.107	2	0.899	0.028	2	0.973
Lake	1.341	1	0.269	10.667	1	**0.007**	0.113	1.000	0.742	1.880	1	0.195	15.597	1	**0.002**	12.647	1	**0.004**
Time	9.864	2.181	**<0.001**	4.596	2.299	**0.015**	9.445	2.439	**<0.001**	1.824	1.585	0.192	4.885	2.610	**0.009**	3.241	2.262	**0.049**
Substratum * Lake	0.514	2	0.610	0.206	2	0.817	0.060	2.000	0.942	0.168	2	0.847	0.102	2	0.903	0.484	2	0.628
Substratum * Time	0.487	4.362	0.760	0.774	4.598	0.567	3.492	7.316	**0.006**	0.640	3.170	0.607	1.228	5.220	0.319	1.232	4.525	0.321
Time * Lake	0.933	2.181	0.413	4.326	2.299	**0.019**	2.197	2.439	0.117	5.048	1.585	**0.023**	4.664	2.610	**0.011**	6.304	2.262	**0.004**
Substratum * Lake * Time	0.901	4.362	0.485	0.793	4.598	0.555	0.348	4.877	0.876	0.864	3.170	0.482	1.186	5.220	0.339	0.655	4.525	0.646

	Pinnularia viridis			*Navicula capitatoradiata*			*Nitzschia dissipita*			*Synedra ulna*			*Brachysira zellensis*			*Brachysira spp.*		
	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Substratum	1.000	2	0.397	0.006	2	0.994	0.167	2	0.848	0.143	2	0.868	0.886	2	0.438	0.002	2	0.998
Lake	1.000	1	0.337	1.652	1	0.223	13.446	1	**0.003**	7.826	1	**0.016**	2.800	1	0.120	5.932	1	**0.031**
Time	1.000	1.000	0.337	6.118	1.524	**0.014**	3.446	1.247	0.076	2.843	1.574	0.093	0.674	1.089	0.439	5.932	1.000	**0.031**
Substratum * Lake	1.000	2	0.397	0.448	2	0.649	0.088	2	0.916	0.228	2	0.800	0.886	2	0.438	0.002	2	0.998
Substratum * Time	1.000	2.000	0.397	0.517	3.049	0.678	0.280	2.494	0.805	0.506	3.148	0.691	0.996	2.177	0.402	0.002	2.000	0.998
Time * Lake	1.000	1.000	0.337	11.341	1.524	**0.001**	3.536	1.247	0.073	2.504	1.574	0.118	0.674	1.089	0.439	5.932	1.000	**0.031**
Substratum * Lake * Time	1.000	2.000	0.397	0.310	3.049	0.821	0.161	2.494	0.893	0.565	3.148	0.652	0.996	2.177	0.402	0.002	2.000	0.998

	Nitzschia palea			*Amphora inariensis*			*Epithemia adnata*			*Achnanthidium modestiforme*			*Gomphonema truncatum*			*Gyrosigma accuminatum*		
	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Substratum	3.371	2	0.069	0.334	2	0.723	0.512	2	0.612	0.966	2	0.408	0.234	2	0.795	3.001	2	0.088
Lake	8.634	1	**0.012**	12.931	1	**0.004**	1.976	1	0.185	13.467	1	**0.003**	6.229	1	**0.028**	12.036	1	**0.005**
Time	5.280	2.275	**0.009**	1.740	2.794	0.180	0.756	1.808	0.469	0.997	2.193	0.389	5.043	1.830	**0.018**	3.764	1.869	**0.041**
Substratum * Lake	5.216	2	**0.023**	0.000	2	1.000	0.512	2	0.612	1.045	2	0.381	0.819	2	0.464	2.363	2	0.136
Substratum * Time	5.020	4.549	**0.003**	1.085	5.588	0.389	1.122	3.616	0.369	0.474	4.386	0.770	0.820	3.659	0.517	0.629	3.737	0.637
Time * Lake	7.579	2.275	**0.002**	3.551	2.794	**0.027**	0.756	1.808	0.469	0.807	2.193	0.467	5.461	1.830	**0.014**	3.547	1.869	**0.049**
Substratum * Lake * Time	5.634	4.549	**0.001**	0.951	5.588	0.469	1.122	3.616	0.369	0.516	4.386	0.740	0.642	3.659	0.625	0.836	3.737	0.510

	Rhopaladia gibba			*Nitzschia acicularis*			*Gomphonema accuminatum*			*Nitzschia linearis*		
	F	df	P	F	df	P	F	df	P	F	df	P
Substratum	1.786	2	0.209	0.661	2	0.534	0.050	2	0.951	0.742	2	0.497
Lake	11.748	1	**0.005**	2.098	1	0.173	2.083	1	0.175	5.916	1	**0.032**
Time	2.586	1.168	0.127	0.908	1.217	0.376	4.602	2.412	**0.014**	2.607	2.421	0.081
Substratum * Lake	1.786	2	0.209	1.639	2	0.235	1.657	2.000	0.231	0.330	2	0.725
Substratum * Time	1.077	2.337	0.377	0.607	2.434	0.589	4.825	0.276	0.918	0.729	4.842	0.604
Time * Lake	2.586	1.168	0.127	0.202	1.217	0.708	4.174	2.412	**0.020**	4.250	2.421	**0.018**
Substratum * Lake * Time	1.077	2.337	0.377	1.163	2.434	0.349	1.429	4.825	0.245	1.236	4.842	0.318

Table 2.7. Two-way repeated measures ANOVA results for the east campus diatom species abundance results incorporating the reference substratum results. Species ordered alphabetically. Note Brachysira zellensis and Pinnularia Viridis results are incomplete due to lack of sufficient instances of the species on east campus lake to complete the calculations.

	Achnanthidium minutissimum			*Gomphonema parvulum*			*Nitzschia paleacea*			*Brachysira vitrea*			*Gomphonema cuneolus*			*Encyonema reichardtii*		
Source	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Substratum	53.811	3	**<0.001**	0.979	3	0.449	1.034	3	0.428	4.300	3	0.044	6.203	3	**0.018**	5.038	3	**0.030**
Time	2.000	2.564	0.152	1.274	1.167	0.297	2.635	1.253	0.132	2.323	2.960	0.102	4.471	2.405	**0.020**	11.159	1.472	**0.003**
Substratum * Time	0.881	7.691	0.545	0.896	3.501	0.492	0.929	3.758	0.480	1.232	8.881	0.323	3.058	7.216	0.024	2.138	4.415	0.136

	Gomphonema olivaceum			*Amphora pediculus*			*Cocconeis disculus*			*Achnanthes daonenese*			*Epithemia turgida*			*Fragilaria vaucheriae*		
	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Substratum	2.509	3	0.133	0.616	3	0.624	2.852	3	0.105	66.261	3	**<0.001**	0.984	3	0.447	2.850	3	0.105
Time	1.499	1.052	0.256	8.121	1.119	**0.017**	0.745	1.370	0.448	2.089	1.475	0.173	1.358	2.086	0.285	0.554	1.220	0.508
Substratum * Time	1.665	3.157	0.247	0.470	3.358	0.729	0.708	4.111	0.606	1.364	4.425	0.305	1.450	6.258	0.253	0.368	3.659	0.812

	Nitzschia amphibia			*Encyonema prostratum*			*Encyonema minuta*			*Melosira varians*			*Brachsira brebisonii*			*Diatoma problematica*		
	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Substratum	4.473	3	**0.040**	4.807	3	**0.034**	2.161	3	0.171	0.286	3	0.834	3.490	3	0.070	6.627	3	**0.015**
Time	1.224	2.042	0.320	5.969	1.043	**0.038**	1.851	2.493	0.177	1.122	1.608	0.342	7.226	2.202	**0.004**	2.650	1.948	0.103
Substratum * Time	0.851	6.127	0.551	4.998	3.130	**0.028**	1.030	7.479	0.444	0.959	4.825	0.475	3.584	6.606	**0.015**	2.321	5.843	0.086

	Encyonema neogracile			*Epithemia sorex*			*Nitzschia minuta*			*Navicula cryptocephala*			*Rhoicosphenia abbreviata*			*Gomphonema vibrio*		
	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Substratum	0.777	3	0.539	1.409	3	0.310	2.389	3	**0.144**	2.037	3	0.187	2.352	3	0.148	1.160	3	0.383
Time	7.360	1.789	**0.008**	0.670	1.374	0.477	7.639	1.821	**0.006**	3.418	1.692	0.069	1.266	1.910	0.308	3.334	1.780	0.069
Substratum * Time	0.434	5.366	0.828	1.172	4.121	0.376	2.432	5.462	0.081	0.527	5.077	0.755	0.517	5.730	0.780	1.009	5.340	0.451

	Pinnularia viridis			*Navicula capitatoradiata*			*Nitzschia dissipata*			*Synedra ulna*			*Brachysira zellensis*			*Brachysira spp.*		
	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Substratum	3			2.591	3	0.125	1.901	3	0.208	2.284	3	0.156	3			1.000	3	0.441
Time				7.469	1.840	**0.007**	1.232	1.789	0.316	1.345	1.391	0.286				1.000	1.000	0.347
Substratum * Time				4.537	5.521	**0.009**	1.675	5.366	0.202	0.992	4.172	0.454				1.000	3.000	0.441

	Nitzschia palea			*Amphora inariensis*			*Epithemia adnata*			*Achnanthidium modestiforme*			*Gomphonema truncatum*			*Gyrosigma accuminatum*		
	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Substratum	0.652	3	0.604	0.248	3	0.860	1.639	3	0.256	0.684	3	0.586	5.901	3	**0.020**	3.342	3	0.077
Time	1.277	1.112	0.295	1.348	2.266	0.287	0.607	1.154	0.479	0.763	1.727	0.467	0.214	2.275	0.835	0.206	2.410	0.853
Substratum * Time	0.857	3.337	0.508	1.064	6.799	0.423	0.837	3.463	0.520	1.079	5.182	0.415	1.062	6.826	0.425	0.534	7.229	0.803

	Rhopaladia gibba			*Nitzschia acicularis*			*Gomphonema accuminatum*			*Nitzscshia linearis*		
	F	df	P	F	df	P	F	df	P	F	df	P
Substratum	1.346	3	0.327	3.031	3	0.093	1.325	3	0.332	4.985	3	**0.031**
Time	2.798	1.330	0.118	0.725	1.233	0.444	1.976	1.714	0.179	8.837	1.689	**0.005**
Substratum * Time	0.943	3.989	0.476	0.725	3.699	0.586	0.968	5.143	0.472	6.429	5.068	**0.003**

To summarise, there were very limited differences between the taxonomic composition of the diatom communities between the three test substratum, with this being due to an elevated abundance of *A. minutissimum* on East campus lake microscope slide replicates, that caused a decrease in the abundance of *B. vitrea* in the biofilms developed under these conditions. There were however twenty-seven diatom species whose abundance differed over time. However, this was typically limited to very specific conditions, usually being only between two time points, or on specific substratum and/or times. The majority of the changes associated with this factor were on West campus lake, primarily due to the loss of *G. parvulum* across the full length of the experiment, as well as *E. minuta*, *G. truncatum*, *N. minuta*, *G. olivaceum* and *G. cuneolus* to a lesser extent, and the increase in the abundance of several species (*E. prostratum*, *E. sorex*, *E. turgida*, *G. accuminatum*, *Gy. accuminatum*, *R. abbreviata*, as well as *B. brebisonii*, *A. daonenese*, and *N. capitatoradiata* under specific conditions) replacing the former species in this lake. Finally, the lake factor contributed to the largest share of differences between the data points, with only *A. minutissimum* and *B. vitrea* being more abundant on East campus lake replicates, with the remaining twenty-six species out of the forty identified at abundances greater than 2% being more prevalent in West campus lake biofilms.

2.4.4. Diatom diversity indices results

Overall, these results indicate that there was no significant difference between the results of the three test substratum, although the East campus reference substratum demonstrated lower index results compared to the test substratum. East campus lake was also less even and diverse than West campus lake, but unlike West campus lake whose species richness, evenness and Shannon H index scores increased over the course of the experiment, the diatom communities of East campus biofilms remained relatively unchanged after week two.

Differences between substratum

Although there was no significant difference between the substratum for the species richness or Shannon-H index results, the diatom community's evenness was significantly lower on the microscope slide substratum than the ceramic tiles or sandstone (P=0.011, Figure 2.10., Table 2.8.) (P<0.050, Tukey HSD). When incorporating the reference substratum to the East campus lake results, there was a significant substratum effect on all three of the diversity indices (Figure 2.10., Table 2.9.) due to the differences of the biofilms from this substratum compared to the three test substratum. Using posthoc testing, the community evenness on the microscope slides and sandstone shows similar evenness metrics (0.19-3.5 e^H/S) (P>0.050, Tukey HSD), however unlike, the microscope slides, the sandstone substratum also demonstrated community evenness scores similar to those of the ceramic tiles (0.2-0.31 e^H/S), and the reference substratum demonstrated a higher community evenness than all three test substratum (0.3-0.68e^H/S).

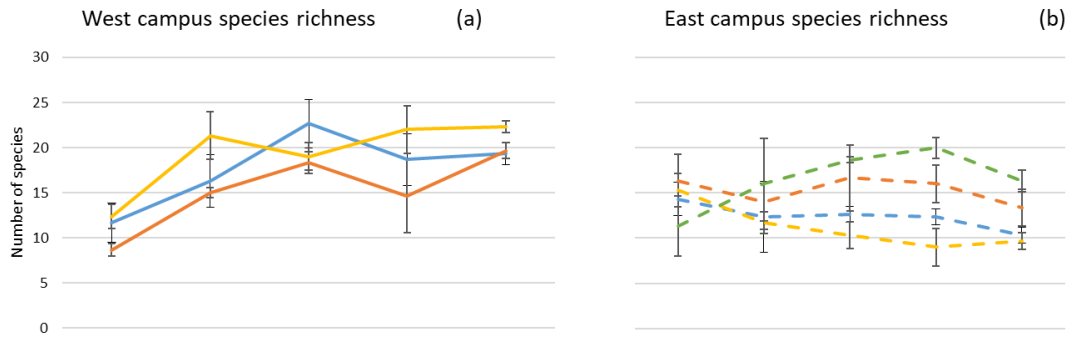
Shannon H index results for all three test substratum (microscope slides, ceramic tiles and sandstone) were all similar to each other (0.5-1.5, Figure 2.10., Table 2.8.), whilst the reference substratum demonstrated a much higher index range (1.5-2.2). Finally, the species richness of the sandstone (7-15 species) was lower than on the reference substratum (8-20 species), but both the biofilms on both of these substratum were similar to the microscope slide and ceramic tile (both 18-10 species) substratum (all posthoc test P<0.050, Tukey HSD tests).

Differences between indices scores over time

Overall, community evenness increased over time (P<0.001), but there was also a significant time*lake effect. This effect to all three substrata on West campus lake (microscope slides: P=0.043, ceramic tiles: P=0.022, sandstone: P=0.048, Friedman test) ranged from 0.25-0.4e^H/S at week two to 2-2.8e^H/S at week ten (Figure 2.10., Table 2.8.). The Shannon H index was also affected by time (P=0.001), with interaction between substratum and lake showing an increase in the diversity index on microscope slides and sandstone on West campus lake (P=0.038 and 0.049, respectively, Friedman test). When incorporating the reference substratum results to the East campus data, there was no temporal change in the diversity indices of the communities.

Differences between indices scores between lakes

Evenness, Shannon H index (both P<0.001) and species richness (P=0.001) were all higher on West campus lake, but all three showed interactions between this factor with substratum and time. Evenness was significantly higher on West campus lake for microscope slides and ceramic tiles at week 10, and on sandstone at week eight (P=0.037, 0.046, and 0.046, respectively, Kruskal-Wallis H test). Further testing limits the higher Shannon H index values to week eight on microscope slides, and week four for sandstones, (both P=0.046, Kruskal-Wallis H test). Interactions with substratum and time for species richness interaction effects demonstrated that higher values only occurred on the sandstone substratum at week 10 (P=0.046, Kruskal-Wallis H test).



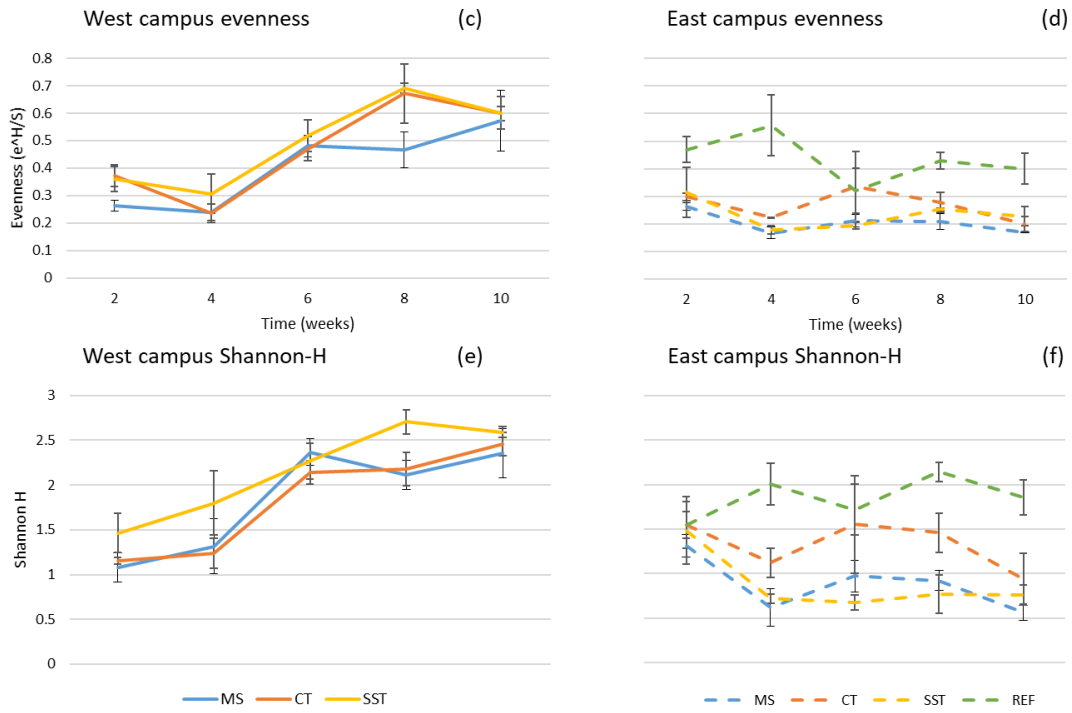


Figure 2.10. Diversity indices of the diatom communities from the biofilm samples. Showing (a) species richness of West campus lake, (b) East campus species richness, (c) West campus biofilms Shannon-H indices, (d) East campus substratum biofilms Shannon-H indices. (e) for West campus biofilm community evenness and (f) east campus community evenness (MS= microscope slides, CT= ceramic tiles, SST= sandstone, REF= reference stones from lake bottom).

Table 2.8. Three-way repeated measure ANOVA results for the diversity indices (evenness, Shannon H indices, and species richness) for microscope slides, ceramic tile and sandstone substratum exposed in both West campus and East campus lakes.

	Species richness			Evenness			Shannon H		
	F	df	P	F	df	P	F	df	P
Substratum	0.022	2	0.978	6.732	2	**0.011**	2.301	2	0.143
Lake	21.297	1	**0.001**	184.447	1	**<0.001**	115.120	1	**<0.001**
Time	2.624	3.013	0.065	13.383	2.473	**<0.001**	7.949	2.682	**0.001**
Substratum * Lake	6.041	2	**0.015**	1.597	2	0.243	6.875	2	**0.010**
Substratum * Time	0.701	6.025	0.651	0.580	4.946	0.713	0.629	5.364	0.689
Lake * Time	10.776	3.013	**<0.001**	13.789	2.473	**<0.001**	18.801	2.682	**<0.001**
Substratum * Lake * Time	0.718	6.025	0.638	0.754	4.946	0.589	0.562	5.364	0.739

Table 2.9. Diversity indices two-way repeated measure ANOVA results for East campus substratum, including the reference substratum.

	Species richness			Evenness			Shannon H index		
	F	df	P	F	df	P	F	df	P
Substratum	4.773	3	**0.034**	52.394	3	**<0.001**	19.540	3	**<0.001**
Time	0.621	2.637	0.589	1.371	2.680	0.278	2.328	2.946	0.101
Substratum * Time	1.197	7.911	0.347	1.108	8.039	0.396	1.379	8.837	0.253

2.4.5. UKTAG assessment results

Overall, there were very limited differences between the UKTAG endpoints between the three substrate types. However, on West campus lake, the LTDI2 and percentage motile species endpoints significantly increased over time, and the percentage of organic tolerant species decreased over time. Whilst for East campus lake after a slight increase in LTDI2 values for the

biofilms between week two and four, these endpoints generally remained static throughout the experiment.

Differences of endpoints between different substratum

There was no effect on the percentage of motile or organic tolerant species found in the biofilms of the different substratum (P=0.677 and 0.128, respectively, Figure 2.11., Table 2.10.). The LTDI2 values were significantly affected by all substratum (P<0.001), although posthoc testing demonstrating that there was no significant difference between the three substratum (P>0.050, Tukey HSD). When incorporating the reference substratum into the analysis of East campus replicates, there was no significant effect of substratum on the percentage of organic tolerant species (Figure 2.11., Table 2.11.), however there was a significant effect of substratum on both TDI and the percentage of motile species (P=0.014 and 0.044, respectively, Figure 2.11., Table 2.11.). Posthoc testing indicated that this was due to the LTDI2 values of all three test substrata being very similar to each other (0.75-0.95, Figure 2.11., P>0.050, Tukey HSD), although the ceramic tiles were typically at the lower end of this range, and were therefore also statistically similar to the lower LTDI2 values observed in the reference substratum (0.65-0.81) (P>0.050, Tukey HSD). For the percentage motility of all four substratum on East campus lake, all three test substratum (microscope slides, ceramic tiles, sandstone) deployed to the rafts retained similar abundances to each other (1-14%), as in the main experiment (P>0.050, Tukey HSD), (Figure 2.11., Table 2.11.) although the percentage of these species were far lower on the microscope slides, which occupied the lower end of this range (1-5%), compared to the reference substratum taken from the lake floor, which has a noticeably higher range in reference (3-14%).

Differences between endpoints over time

The percentage of motile species did not significantly change over time (Figure 2.11., Table 2.11.). However, the percentage of organic nutrient tolerant species decreased over time on West campus replicates from 60-80% at week two to 12-22% at week ten (Figure 2.11., P<0.001, two-way repeated measures ANOVA), with the majority of this reduction occurring between weeks four and eight (P <0.001 (week four and six), and P=0.001 (week six and eight), within subject contrast effects). For LTDI2, an increase was observed across all substratum in West campus (0.1-0.2 LTDI2 at week two to 0.4-0.6 LTDI2 at week ten) (microscope slide: P=0.043, ceramic tiles: P=0.022, sandstone: P=0.038, Friedman test), with this effect also observed in East campus lake, but this was limited to the microscope slide substratum (0.8 LTDI2 at week two to 0.95 LTDI2 at week ten) (P=0.033, Kruskal-Wallis H test).

When incorporating the results from the reference substratum to the analysis of East campus replicates, there was no change over time on the percentage of organic tolerant species (Figure 2.11., Table 2.11.). There was an increase of LTDI2 values (P=0.040) between week two and four (0.61-0.89 at week two to 0.55-0.97 at week four) (P=0.009), as well as an effect of increasing value between week six and eight (0.6-0.95 at week six to 0.89-0.97 at week eight) (P=0.009). There was an overall increase in the percentage of motile species observed (P=0.040), with analysis between the time points showing a significant increase in the abundance of motile species between week eight and ten (P=0.020).

Differences of endpoints between lakes

LTDI2 values were higher on East campus lake (Figure 2.11., Table 2.11.), with the LTDI2 values significantly higher on East campus replicates at weeks two and four on ceramic tiles, as well as week eight on the sandstone substratum (P=0.046, 0.043, 0.046, respectively, Kruskal-Wallis H test). The percentage of motile diatoms was higher in West campus lake, specifically for the biofilms developed on the ceramic tile and sandstone substratum., (P=0.047, 0.011 and 0.080, respectively, two-way repeated measure ANOVA). Organic tolerant species were typically more abundant on West campus lake (Figure 2.11., Table 2.11.) at all time points for ceramic tiles, weeks two, four six and ten for microscope slides, and weeks two, four and ten for sandstones

(All P=0.050, Kruskal-Wallis H test), with the differences at week eight for microscope slides and weeks six and eight for sandstone being significant (all P=0.046, Kruskal-Wallis H test).

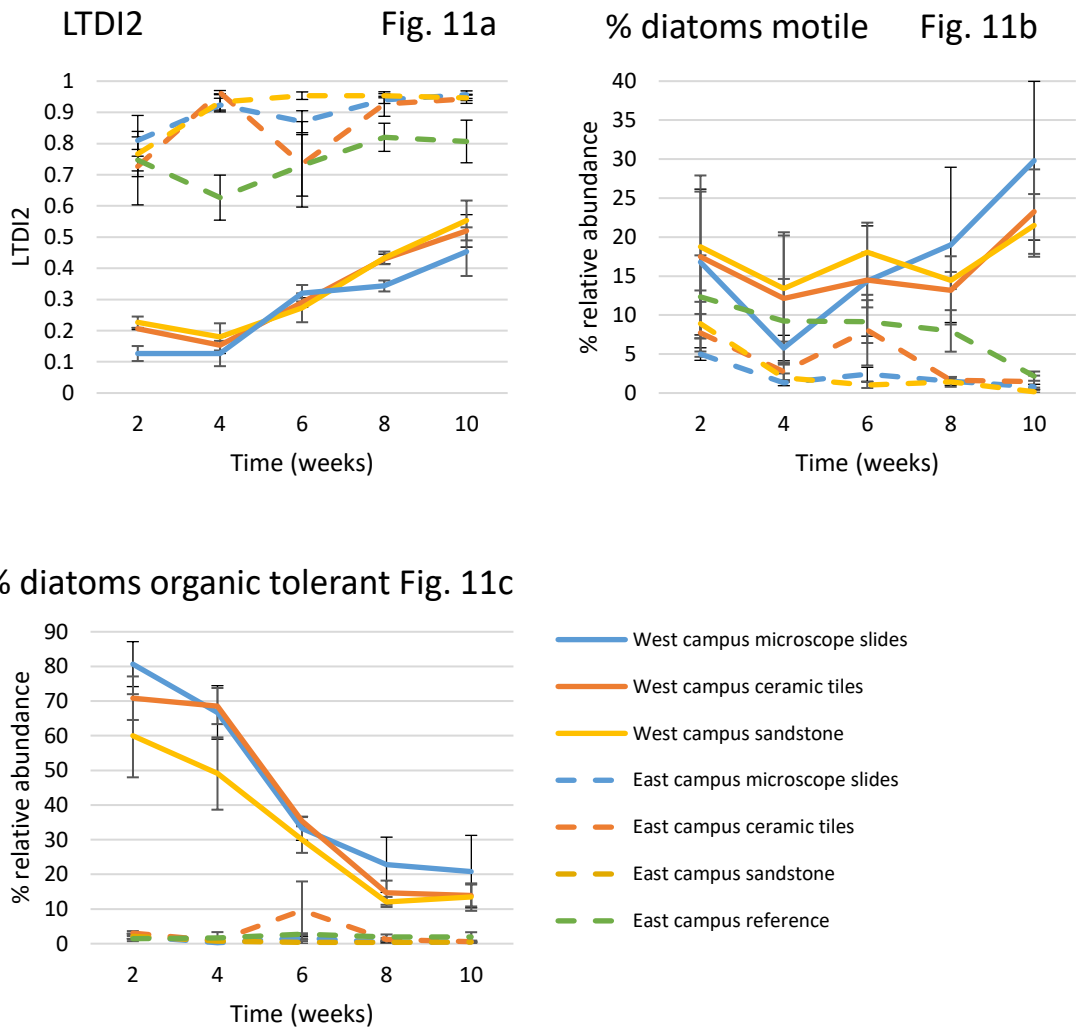


Figure 2.11. UKTAG assessment results for diatom communities on West and East campus lakes, divided by substratum and time point. For West campus there are three substratum, microscope slides, ceramic tiles and sandstone. For East campus lake there is a fourth reference substratum, taken from large pebbles scattered across the sediment at depths of around 10cm below water level. Data has been summarised into LTDI2 value (coloured by corresponding EU WFD water quality classification), and the percentage of motile diatoms and organic tolerant species. DARLEQ 2 software was used for the analysis.

Table 2.10. Three-way repeated measure ANOVA results for the UKTAG assessment LTDI2 index value, percentage of species motile, and percentage of organic nutrient enrichment tolerant species.

	LTDI2			% motile			% organic tolerant		
	F	df	P	F	df	P	F	df	P
Substratum	11.017	3	**0.001**	0.518	3	0.677	2.244	3	0.128
Lake	981.243	1	**<0.001**	22.280	1	**<0.001**	344.668	1	**<0.001**
Time	24.154	2.074	**<0.001**	2.657	1.866	0.092	53.129	2.221	**<0.001**
Substratum * Lake	1.858	2	0.192	0.116	2	0.891	2.760	2	0.098
Substratum * Time	1.411	6.222	0.243	0.496	5.599	0.795	0.535	6.664	0.794
Lake * Time	9.090	2.074	**0.001**	4.021	1.866	0.032	50.392	2.221	**<0.001**
Substratum * Lake * Time	0.903	4.148	0.478	0.500	3.732	0.724	0.739	4.443	0.585

Table 2.11. Two-way repeated measure ANOVA results for the UKTAG assessment LTDI2 index value, percentage of species motile, and percentage of organic nutrient enrichment tolerant species, using only east campus data, including all three test species plus the reference substratum.

	EQR			% motile			% organic tolerant		
	F	df	P	F	df	P	F	df	P
Substratum	6.806	3	**0.014**	4.308	3	**0.044**	1.206	3	0.368
Time	4.789	1.436	**0.040**	5.439	2.505	**0.009**	1.505	1.247	0.256
Substratum * Time	1.374	4.307	0.303	0.619	7.516	0.744	0.888	3.740	0.500

To summarise, there was no difference between the LTDI2 values, percentage of motile diatom, or percentage of organic tolerant diatoms between the microscope slides, ceramic tiles, or sandstone substratum, although in East campus lake the ceramic tiles were better at mirroring the results of the LTID2 and percentage motile endpoints measured for the reference substratum native to the lake. The LTDI2 values were higher in East campus lake, but the percentages of motile and organic tolerant individuals within the diatom communities were higher in East campus lake. Furthermore, although all three of the metrics assessed in this section did not significantly change over time for East campus lake replicates, the LTDI2 and percentage of motile diatoms within the community increased over the ten weeks of the experiment in West campus lake, whilst the percentage of organic tolerant diatoms continually decreased.

2.4.6. Biofilm structural measurements – summary of results

In summary, the biomass measurements showed that for the AFDW and chlorophyll-a concentrations, there were limited differences between substratum, although the ceramic tiles substratum did develop concentrations of organic matter (AFDW) most similar to the concentrations observed on the reference substratum. Biofilms developed on microscope slides were shown to have chlorophyll-a concentrations closest to those of the reference substratum.

Substratum type had minimal effect on the abundance of different algal groups, with the only major effect being that chlorophytes concentrations were typically lower on the microscope slides and ceramic tiles than on the sandstone substratum. Time also had no overall effect on the abundances of different algal groups, with the exception of a slight peak of diatoms on West campus during week eight and a decrease in diatom abundance at week four on East campus substratum. All three of the algal groups were shown to be sensitive to the effects of the lake they were developed in, with diatoms and cyanobacteria being significantly more abundant on East campus lake substratum than their West campus equivalents. chlorophytes were the largest algal group in the West campus lake. For the diatom species results, there was limited effect between test substratum, with *Brachysira vitrea* and *Achnanthidium minutissimum* being the only species to show major differences in abundance between substratum. However, the reference substratum in East Lake gave higher abundances for several species compared to the three

substratum deployed on the rafts. When observing the time factor, most temporal changes in species abundances occurred on West campus lake, with the most abundant species typically becoming less abundant over time. This appears to be due to the reduction of primarily *G. parvulum*, but also many of the other diatoms of the *Gomphonema* genus, in favour of several other species, with the abundances of *A. daonense*, *A. minutissimum, C. disculus*, *N. amphibia* and *N. palea* in particular increasing to replace the disappearing *Gomphonemas*. On East campus lake, changes in abundance occurred for *E. neogracile* and *N. linearis*, the latter of which only occurred on ceramic tiles, however the increase in abundance of *B. brebisonii*, *E. prostratum*, *N. capitatoradiata* and *G. cuneolus* abundances only occurred on the reference substratum native to the lake.

In East campus lake, the diversity indices (species richness, Shannon H index and evenness scores) of the ceramic tile substratum most closely mirrored the results of the reference substratum on East campus lake. However, in comparison to the East campus results the diversity indices were higher on West campus lake, where they increased over time due to the changes in the relative abundances of the different species shown in section 4.3. These changes were likely caused by variations in the physicochemical parameters of the lake (section 5), compared to East campus lake, where they remained static throughout most of the experiment. Biomass measurements (AFDW and chlorophyll-a concentrations) were higher on East campus lake, the former of these two measurements showing a significant increase across both lakes and all substratum on week six, whilst the latter measurement showed a gradual increase over time.

There was generally no significant difference in the LTDI2 values between the three test substrata. Furthermore, the test substratum deployed on East campus lake demonstrated 0.6-0.95 LTDI2 values, indicating good to high quality ecological quality, that did not vary much over the experiment. On West campus the LTDI2 values were far lower, ranging from 0.1 to 0.25 at the start of the experiment, increasing over time to 0.4-0.6. This is indicative of an improvement from poor to moderate ecological quality, driven by the same changes in the relative abundance of the diatom species seen in 2.4.3. that caused the increase in the species richness, evenness and Shannon-H index. The percentage of motile species was not affected by substratum or time, but motile species were more prevalent in West campus lake biofilms, and when comparing the percentage motility of biofilms developed on East campus replicates to the reference substratum taken from the lake, microscope slides developed statistically lower abundances of these species.

2.5. Results: Physico-chemical water quality and effects on LTDI2 values.

2.5.1. Variations in the physico-chemical parameters of the two lakes over time

These results are shown in the appendix (Appendix c).

There was no effect on either factor on the concentrations of TSS within the water body. However, there was a still signficant variation in the readings (0.2-100 mg/l). Lead concentrations were frequently below the detection limit of the ICP-OES (Appendix c(aa), Table 2.12.), as such the results cannot be relied on for analysis and are not discussed.

Influence of time on physico-chemical parameters

There were no significant changes in the concentrations of chloride (95-150 ppm), DOC (7.9-11.9 ppm), fluoride (0.09-0.45 ppm), nitrate (0.07-0.62 ppm), nitrite (0-0.04 ppm), alkalinity (78-156 mg/l), or light attenuation (40%-88 % absorbance) throughout the ten weeks of the experiment (Appendix c., Table 2.12.). There were significant decreases observed in the values of seven of the physico-chemical measurements taken over the course of the experiment. These were:

- Sulphate levels decreased from overall 56-20 ppm at week zero to 38-18 ppm at week ten (Appendix c(u)). Repeated measure ANOVA confirms this decrease only occurred as a trend between weeks two (21.13-48.22 ppm) and four (17.914-6.49 ppm) (P=0.053, within subject contrast)

- TN (Appendix c(c)) concentration decreases were only considered significant between weeks four and six (week four: 1.39-1.07 ppm, week six: 1-1.03 ppm) (P=0.030, within contrast effects).
- Sodium levels decreased from 40-80 ppm at week zero to 20-45 ppm at week ten (P=0.016 and 0.013, respectively, Appendix c(t), Table 2.12.),
- Silicon concentration decreases only occurred as a trend (P=0.050, one-way repeated measure ANOVA, Appendic c(n), Table 2.12.) on West campus lake (35-62 ppm at week zero to <0.01 ppm at week ten, but no change over time was observed on East campus lake.
- EC (Appendix c(k)) also decreased over time, but only on West campus lake (P=0.003, one way repeated measures ANOVA) (860-700µs) (W0 to W2 P=0.005, decrease between W6 to W8 P-0.007),
- Ammonium concentrations decreased in West campus lake between weeks zero (0.709 ppm) and week two (0.016 ppm) (Appendix c(d)) (P=0.019 one-way repeated measure ANOVA, P=0.019, within subject contrast).
- Nickel concentrations decreased over time in East campus lake occurred, however these concentrations were so low (~0.00014 ppm) that this is unlikely to be a significant contributior to water quality (Appendix c(z)).

There were also two physico-chemcal measurements that were shown to increase over time. These factors were:

- Copper (P=0.002, Appendix c(q), Table 2.12) concentrations increased over time from 0.00059 ppm at West campus lake to 0.009 ppm at week 10.
- pH increased over time (Appendix c(i), Table 2.12), with this effect on West campus lake (pH 8-8.9) (P=0.013, one-way repeated measures ANOVA) tending to occur between weeks four and six (P=0.061) and weeks eight and ten (P=0.095), with this increase in pH also occurring on East campus lake, but was only sigificant between weeks six and eight (P=0.046, Friedman tests).

Dissolved oxygen concentrations differed over the course of the experiment, appearing to peak around week four, before decreasing and appearing to increase slightly again by week ten (Appendix c(h), Table 2.12). The intial increase in the concentration of dissolved oxygen for West campus lake occurredbetween weeks two to four (9-19mg/l) (P=0.007, within subject effects), followed by a decreasing concentration from week four to week eight (19-8.6 mg/l) (weeks four to six: P=0.008, week six to eight: P=0.047)). Whereas on East campus lake there was an effect of increasing concentrations between weeks two and four, and weeks eight and ten (week two to four and weeks eight to ten: P=0.008), with a decreasing concentration between weeks four to six (P<0.001). Further similar complex variations occurred in temperature of water, with a pattern similar to that seen with the pH, albeit two weeks behind. There was a significant decrease on West campus lake (Appendix c(j)., Table 2.12.) between weeks six to eight (P=0.007, within subject contrasts), whilst on East campus the increasing temperatures occured between weeks two to four (P=0.048), and weeks four to six (P=0.001), followed by a decrease in temperatures between weeks six to eight (P=0.002), and weeks eight to ten (P=0.020).

Influence of lake on physico-chemical parameters

There was no difference between the two lakes with regards to concentrations of DOC (7.9-11.9 ppm), nitrate (0.07-0.62 ppm), TN (0.04-1.82 ppm) or DO (9-21.9 mg/l) (Appendix c., Table 2.10.). However, eight physico-chemical measurements were higher in East campus lake than on West campus lake. Those parameters were sulphate (East campus: 38.21-56.48 ppm), chloride (East campus: 115.99-148.91 ppm, West campus: 97.78-116.47 ppm), fluoride (East campus: 0.2 ppm, West campus lake: P=0.001 ppm), sodium (East campus: 42-105 ppm, West campus: 20-47 ppm), and calcium (East campus lake: 20-22 ppm, West campus lake: 7.6-20 ppm) (Appendix c., Table 2.12.). The remaining three measurements that were typically higher on East campus lake only occurred at certain times. These were:

- EC was higher in East campus lake (809-950 µs/cm) than West campus lake (700-900 µs/cm) at weeks four, six and eight, but not at weeks two and ten (Appendix c(k)., Table 2.12.)
- Temperature (significant at week zero and week four, trends on weeks six to ten) were higher on East campus lake (17-26^{o}C) compared to West campus lake (17-24 ^{o}C) (Appendix c(j)., Table 2.12.).
- Nickel, at weeks two four and ten (P=0.016, <0.001, and 0.003, respectively, N=1, one-way ANOVA), However, the values observed at West campus lake were typically below the ICP-OES instruments detection level, as were weeks six and eight on East campus, as such accurate comparison for this dataset is not possible (Appendix c(z)).

Nine of the physico-chemical parameters measured were higher in West campus lake than East campus lake. Ammonium (West campus: 0.04 ppm ± 0.003 ppm to 0.71 ppm ± 0.14 ppm, East campus: 0.035 ppm ± 0.008 ppm to 0.054 ppm ± 0.015 ppm), nitrite (West campus: 0.0028 ppm ± 0.0015 ppm to 0.0364 ppm ± 0.014 ppm, East campus: 0 ppm to 0.007 ppm ± 0.0029 ppm), phosphate (West campus: 0.047 ppm ± 0.019 ppm to 0.304 ppm ± 0.097 ppm, East campus: 0.01 ppm ± 0.003 ppm to 0.096 ppm ± 0.051 ppm), light attenuation (West campus: 58.37% ± 4.93% to 87.40% ± 4.00%, East campus: 40.82% ± 0.68% to 44.14% ± 2.15%), and TSS (West campus: 28.73 mg/l ± 8.95 mg/l to 86.73 mg/l ± 43.95 mg/l, East campus: 0.2 mg/l ± 0.3 mg/l to 19.2 mg/l ± 8.41 mg/l), values were all significantly higher in West campus lake, with the remaining four varaibles exhibiting this only at certain times. These were:

- pH tended to be higher in West campus lake at weeks two and four (P=0.050), but was significantly higher in West campus lake at week six (P=0.046, Kruskal-Wallis H test)) (Appendix c(i)., Table 2.12.)
- Alkalinity exhibited a trend towards higher concentrations on West campus lake at weeks 4 and 6, (P=0.050, kruskal-wallis H test, and significantly higher abundances at weeks eight (P=0.043) and week 10 (P=0.025)) (Appendix c(l), Table 2.12).
- Iron exhibited a trend towards higher concentrations in West campus lake at week zero, six, eight and ten for (P=0.050, Kruskal-Wallis H test) (Appendix c(x)., Table 2.12.)
- Silicon concentrations were significantly higher on West campus lake at weeks zero and two, with a trend towards higher concentrations in west campus lake at weeks four and ten (P=0.037, 0.046, 0.050 and 0.068, respectively, Kruskal-Wallis H test) (Appendix c(n)., Table 2.12.).

Table 2.12. Two way repeated measures ANOVA for the physico-chemical parameters, ordered in the same manner as presented in Figure 12, using values from weeks zero, two, four, six, eight and ten as a repeated measure factor (Time). Lake was also used as an additonal factor.

	Nitrate			Nitrite			Ammonium			TBn			Phosphate		
Source	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Time	1.929	1.373	0.226	3.396	1.089	0.133	12.894	2.047	**0.003**	8.434	2.417	**0.006**	7.601	1.061	**0.047**
Lake	1.293	1	0.319	94.203	1	**0.001**	11.393	1	**0.028**	0.581	1	0.488	16.701	1	**0.015**
Time * Lake	0.456	1.373	0.587	2.607	1.089	0.177	12.321	2.047	**0.003**	4.737	2.417	**0.032**	2.094	1.061	0.219

	Light attenuation			TSS			Dissolved oxygen			pH			Temperature		
Source	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Time	2.722	1.720	0.137	1.405	2.539	0.295	17.599	1.749	**0.002**	18.183	2.229	**0.001**	230.897	2.923	**<0.001**
Lake	31.088	1	**0.005**	46.574	1	**0.002**	1.155	1	0.343	16.365	1	**0.016**	125.134	1	**<0.001**
Time * Lake	3.404	1.720	0.097	2.129	2.539	0.164	6.104	1.749	**0.032**	18.936	2.229	**0.001**	4.567	2.923	**0.025**

	EC			Alkalinity			DOC			Silicon			Magnesium		
Source	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Time	77.841	1.624	**<0.001**	0.419	2.231	0.691	0.338	2.710	0.780	9.325	1.668	**0.013**	4.997	1.355	0.066
Lake	16.470	1	**0.015**	125.366	1	**<0.001**	0.627	1	0.473	54.878	1	**0.002**	3.953	1	0.118
Time * Lake	64.362	1.624	**<0.001**	4.853	2.231	**0.035**	2.117	2.710	0.160	5.095	1.668	**0.049**	0.672	1.355	0.493

	Potassium			Copper			Fluoride			Sodium			Chloride		
Source	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Time	4.523	1.604	0.064	40.644	1.165	**0.002**	0.385	1.511	0.641	5.767	2.588	**0.016**	0.455	2.266	0.671
Lake	1.004	1	0.373	0.182	1	0.692	172.943	1	**<0.001**	52.836	1	**0.002**	8.443	1	**0.044**
Time * Lake	0.738	1.604	0.486	4.002	1.165	0.105	0.912	1.511	0.422	2.141	2.588	0.161	1.972	2.266	0.193

	Sulphate			Zinc			Calcium			Iron			Aluminium		
Source	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Time	5.962	1.989	**0.026**	3.459	1.703	0.096	1.122	1.723	0.368	1.840	1.588	0.232	0.563	2.337	0.613
Lake	325.115	1	**<0.001**	0.315	1	0.605	81.853	1	**0.001**	14.875	1	**0.018**	0.610	1	0.479
Time * Lake	3.336	1.989	0.089	1.133	1.703	0.365	1.274	1.723	0.330	7.829	1.588	**0.022**	0.161	2.337	0.882

	Lead			Nickel		
Source	F	df	P	F	df	P
Time	2.887	1.140	0.156	5.971	1.774	**0.032**
Lake	1.989	1	0.231	33.527	1	**0.004**
Time * Lake	0.977	1.140	0.387	8.196	1.774	**0.016**

2.5.2. Regression analysis of the LTDI2 assessment and the physico-chemical parameters of the campus lakes

The results of the regression analysis show that individually, for West campus lake total nitrogen (R^2: 0.628), and electrical conductivity (R^2: 0.620), in particular showing strong negative correlations to the LTDI2, but there was also a strong degree of correlation of the LTDI2 values with chloride (R^2: 0.193), dissolved oxygen (R^2: 0.185), light attenuation (R^2: 0.289) and silicon (R^2: 0.421) in this lake (Figure 2.13., Table 2.13.). Conversely, copper (R^2: 0.718) and magnesium (R^2: 0.498) had the strongest positive correlations to West campus LTDI2, with significant but less well correlated results occurring for pH, alkalinity, zinc, and potassium (Figure 2.13., Tables 2.13.).

Furthermore, far fewer physico-chemical factors had a correlation on East campus lake biofilms, with nitrite (R^2: 0.135), and electrical conductivity (R^2: 0.155) having the strongest negative correlations to the LTDI2, but these were still fairly weak. Likewise, copper had the strongest positive and statistically significant correlation to the East campus lake biofilms LTDI2 (R^2: 0.104) but this correlation was still very low (Figure 2.13., Table 2.13.)

Table 2.13. ANOVA results for linear regression equation of the independent physico-chemical parameters against the dependent LTDI2 results for the (a) West campus lake and (b) East campus lake biofilms

West campus lake

Physico-chemical parameter	R Square	Gradient	F	df	P
Copper	0.718	+	109.603	1	**<0.001**
Total nitrogen	0.628	-	72.638	1	**<0.001**
Electrical conductivity	0.620	-	70.017	1	**<0.001**
Magnesium	0.498	+	42.613	1	**<0.001**
Potassium	0.436	+	33.214	1	**<0.001**
Silicon	0.421	-	31.301	1	**<0.001**
Light attenuation	0.289	-	13.831	1	**0.001**
pH	0.255	+	14.687	1	**<0.001**
Zinc	0.220	+	12.158	1	**0.001**
Chloride	0.193	-	10.291	1	**0.003**
Nitrate	0.188	-	9.945	1	**0.003**
Dissolved oxygen	0.185	+	7.696	1	**0.009**
Alkalinity	0.094	+	4.473	1	**0.040**
Dissolved Organic Carbon	0.077	-	3.602	1	0.064
Sulphate	0.053	-	2.412	1	0.128
Fluoride	0.048	+	2.187	1	0.146
Iron	0.048	+	2.186	1	0.147
Nickel	0.045	+	2.003	1	0.164
Nitrite	0.043	+	1.939	1	0.171
Total suspended solids	0.042	+	1.899	1	0.175
Phosphate	0.035	-	1.559	1	0.219
Lead	0.033	-	1.478	1	0.231
Aluminium	0.023	+	1.027	1	0.317
Ammonium	0.020	+	0.861	1	0.359
Calcium	0.010	+	0.444	1	0.509
Temperature	0.001	+	0.053	1	0.818
Sodium	<0.001	-	0.013	1	0.909

East campus lake

Physico-chemical parameter	R Square	Gradient	F	df	P
Electrical conductivity	0.155	-	10.675	1	**0.002**
Nitrite	0.135	-	9.056	1	**0.004**
Copper	0.104	+	6.719	1	**0.012**
Calcium	0.070	+	4.353	1	**0.041**
Sulphate	0.069	-	4.288	1	**0.043**
Nickel	0.067	+	4.193	1	**0.045**
Sodium	0.060	-	3.684	1	0.060
Total suspended solids	0.036	+	2.172	1	0.146
Aluminium	0.035	+	2.091	1	0.154
Magnesium	0.032	+	1.906	1	0.173
Potassium	0.023	+	1.354	1	0.249
pH	0.021	-	1.241	1	0.270
Temperature	0.021	+	1.240	1	0.270
Silicon	0.018	-	1.062	1	0.307
Dissolved oxygen	0.013	-	0.613	1	0.438
Zinc	0.013	+	0.765	1	0.385
Chloride	0.010	+	0.583	1	0.448
Nitrate	0.005	-	0.285	1	0.596
Alkalinity	0.004	-	0.222	1	0.639
Iron	0.003	-	0.196	1	0.659
Lead	0.002	+	0.099	1	0.754
Light attenuation	0.000	+	0.012	1	0.912
Fluoride	<0.001	-	0.004	1	0.949
Phosphate	<0.001	+	0.003	1	0.956
Dissolved Organic Carbon	<0.001	-	0.026	1	0.871
Total nitrogen	<0.001	-	0.004	1	0.948
Ammonium	<0.001	+	0.007	1	0.932

2.5.3. Multiple regression analysis of the LTDI2 assessment against the physico-chemical parameters of the campus lakes

Table 2.14. summarises the MANOVA analysis of the physico-chemical parameters against the TDI values, and Table 2.15. summarises the model created from MANOVA significant (P<0.050) parameters used to predict the LTDI2 values in a multiple regression analysis for each lake.

The MANOVA analysis indicates that for West campus lake, the correlation of the LTDI2 value with copper, potassium, magnesium, nickel, silicon, electrical conductivity, pH, PAR absorbance, nitrate and sulphate are significant, when analysed together, Whilst for East campus lake only the concentrations of nitrate and nitrite were considered to have a significant effect on the LTDI2 values of the lake.

Table 2.14. MANOVA results of physcio-chemical parameters against LTDI2 values

	West campus lake			East campus lake		
	F	df	P	F	df	P
Nitrate	13.05	23	**0.028**	2.72	22	**0.033**
Nitrite	3.25	23	0.181	6.59	22	**0.001**
Total nitrogen	1.27	23	0.488	0.28	22	0.996
Ammonium	0.33	23	0.948	0.45	22	0.950
Phosphate	1.32	23	0.472	0.35	22	0.985
Light attenuation	16.15	23	**0.021**	0.51	22	0.921
Dissolved oxygen	1.44	23	0.435	0.40	22	0.971
pH	9.24	23	**0.046**	0.64	22	0.828
Temperature	4.52	23	0.119	1.93	22	0.111
Electrical conductivity	28.80	23	**0.009**	2.12	22	0.082
Alkalinity	0.79	23	0.692	0.59	22	0.867
Dissloved organic carbon	0.74	23	0.717	0.51	22	0.918
Silicon	10.77	23	**0.037**	0.41	22	0.967
Magnesium	12.27	23	**0.031**	0.79	22	0.696
Potassium	11.87	23	**0.032**	0.86	22	0.635
Total suspended solids	0.96	23	0.606	1.84	22	0.129
Copper	10.63	23	**0.037**	0.93	22	0.571
Fluoride	0.34	23	0.946	1.21	22	0.372
Sodium	3.32	23	0.176	1.11	22	0.438
Chloride	9.81	23	**0.042**	2.35	22	0.057
Sulphate	44.22	23	**0.005**	2.24	22	0.067
Zinc	4.88	23	0.108	0.22	22	0.999
Calcium	0.25	23	0.979	0.57	22	0.881
Iron	0.22	23	0.987	1.61	22	0.190
Aluminium	1.88	23	0.334	0.67	22	0.806
Nickel	9.50	23	**0.044**	0.30	22	0.993
Lead	0.69	23	0.745	0.68	22	0.794

Using all of the significant parameters for West campus lake from the MANOVA analysis (Table 13, $p<0.05$), the multiple linear regression model created from the nine physico-chemical measurements considered to have a significant effect on the LTDI2 (Table 14) were able to account for 93% ($R^2= 0.93$) of the variation in the LTDI2 values of West campus lake. A similar analysis for East campus lake, incorporating all of the significant values from East campus lake (nitrate and nitrite), accounted for just 14% ($R^2=0.14$) of the East campus lake LTDI2 values (Table 2.14. and 2.15.). Scatter plots of the models are shown in the appendix (Appendix e).

Table 2.15. Model and regression summary of the multiple linear regression model of the physico-chemical parameters shown to have a significant effect on the LTDI2 of the biofilms in lake they were recorded at

	Regression model summary					
Model	F	df	P	R Square	Standard error of the estimate	Physico-chemical parameters used based on MANOVA results
West campus lake	39.05	9	**<0.001**	0.93	0.05	Sulphate, Nickel, Nitrate, Light attenuation, Electrical conductivity, Magnesium, Silicon, Copper, pH
East campus lake	4.52	2	**0.015**	0.14	0.13	Nitrate, Nitrite

2.5.4 Summary of physico-chemical measurement analysis and their effects on diatom community sensitivity:

To summarise, West campus lake had higher concentrations of fluoride, iron and silicon, carbonates (alkalinity) and suspended solids and was more turbid. East campus lake had higher concentrations of calcium and sodium, was warmer, and had a higher electrical conductivity. Temperature spiked at week six in both lakes, pH increased over the first six weeks on West campus lake, but the electrical conductivity decreased on this lake after week six. Dissolved oxygen concentrations increased over the first two weeks, but gradually decreased over the rest of the experiment. Furthermore, elemental concentrations of copper increased over the course of the experiment, but only in West campus lake.

Regression analysis on LTDI2 shows that the diatom communities on West campus lake were more strongly affected by changes in a broad range of the physico-chemical parameters measured (chloride, nitrate, total nitrogen, electrical conductivity, dissolved oxygen, pH, alkalinity, light attenuation, copper, potassium, magnesium nickel, and silicon), whilst for East campus lake only nitrite, sulphate, electrical conductivity, copper and calcium affected the community structure independently. When the influence of these factors was compared simultaneously against the LTDI2 values using Multivariate (MANOVA) analyses, on West campus lake the significant factors were chloride, nitrate, sulphate, electrical conductivity, pH, copper, potassium, magnesium, nickel and sodium, which together accounted for 93.4% of the community variance. East campus lake biofilms were far less sensitive to environmental parameters, with the multivariate analysis showing that together, nitrite and nitrate, along with chloride, sulphate and electrical conductivity were the strongest drivers, but only accounted for 18.5% of the variation in the LTDI2 values of the biofilms. This could indicate that another facto was responsible for the variation in the community, or that the reduction in the variation observed in Figure 2.13. in this lake meant that the fluctuations in these factors were less significant on the structure of the diatom community than they did in the more varied West campus lake.

2.6. Discussion

2.6.1. Effects of substratum

Based on the endpoints measured here, (chlorophyll-a content, algal groups, diatom species, diversity indices, UKTAG endpoints), the results presented here have shown that although there is some variation in the presence of individual diatom species between substratum (AFDW, chlorophyll-a, diatom species), there was no overall significant difference between the type of substratum used on the abundance of different algal groups (diatoms, cyanobacteria, chlorophytes), or the diversity indices and UKTAG assessment endpoints used. The major taxonomic differences being the cosmopolitan species *Achnanthidium minutissimum* and *Brachysira vitrea*, the former of which was observed to grow more abundantly on microscope slides at the expense of the latter, as observed by Lowe and Gale (1980). Variation did occur in diatom abundances in West campus lake between substrata, but they were never consistent, and as such were not likely a direct effect of the substratum by itself.

Research by Hoagland *et al.,* (1982) on glass slides in eutrophic reservoirs in Nebraska found that depending on season and lake, *G. parvulum, G. olivaceum, N. graciloides, N. palea, and N. dissipata* were the dominant species. This is in-line with what was seen in the West campus (eutrophic) lake studied here, with *G. parvulum* being the dominant species on all three substratum tested, at the start of the experiment, decreasing in abundance and being replaced by the rest of the species, except *N. graciloides*. The reference substratum on East campus lake were observed to be more diverse than the three test substrata deployed to East campus lake. Although research by Barbiero (2000) has attributed lower species richness to artificial substratum, based on tests of microscope slides against natural stone substratum at a depth of 50 cm. The sandstone test substratum used here showed statistically similar diversity indices compared to the results of the

microscope slides deployed alongside then, but both differed from the results of the reference substratum taken from the lake, indicating in this case that the difference was more likely due to the age of the biofilms (less than ten weeks compared to ten years).

As such, although there were limited effects shown between the biological endpoints of the biofilms developed on the three test substratum, it can be concluded that any of the three test substratum could be used without affecting the results compared to other types of substratum. However, when the data from East campus lake are assessed alone, and include the reference substratum, the ceramic tiles managed to better replicate the more established biofilms from the reference substratum (fine grained sandstones) taken from the lakes littoral zone. This is based on the closer similarities between the biofilms AFDW concentrations, diversity indices, and UKTAG assessment outputs.

2.6.2. Effects of time

With the exception of an increase in the abundances of diatoms at week ten on West campus lake ceramic tiles, potentially due to an interaction of the water chemistry of West campus lake with the substratum, there was no change in the abundances of diatoms, chlorophytes or cyanobacteria over time. There were also no significant temporal changes in the chlorophyll-a concentrations, and except for the sudden spike in AFDW at week six, no temporal trends in the concentration of this biomass measurement either. The temporary increase across both lakes and all substratum at week six is an unusual and repeatedly present outlier across all replicates. The environmental driver that showed a significant change in line with this increased AFDW concentration is water temperature, which shows a significant increase at this time point, peaking at 23-25 degrees Celsius (Appendix c.), coinciding with unusually warm weather that occurred at this time. However, although increased temperatures are known to increase AFDW content in freshwater biofilms, this increase in temperature would have promoted microalgal activity, which would have been expressed as an increase in chlorophyll-a concentration (Rao *et al.,* 2010), which was clearly not observed in our chlorophyll-a data. This increase in AFDW is likely due to an increase in other groups of organisms in the biofilms around this time. The most likely organisms responsible for this is insect larvae, which would have further increased the biomass by creating protective structures on the biofilms out of mucilage and sediment. Although this was not quantified during the experiment, the abundance of these organisms was noted by visual observations to be higher at this time point and likely caused the increase in AFDW content in the biofilms, with the increased temperatures driving their growth.

When examining the diatom-based metrics and endpoints, there were significant temporal variations in the endpoints shown in West campus lake. Using the diatom species data, this is shown to be caused by a community shift from one where the diatom community is dominated by *Gomphonema parvulum*, along with *Gomphonema cuneolus* and *Gomphonema olivaceum* to a more diverse and even community with greater abundances of *A. daonense*, *A. minutissimum, C. disculus*, *N. amphibia* and *N. palea* as the dominant species in the lake. This shift is likely due to seasonal variations (temperature light availability, nutrient availability, Maraslioglu *et al.,* 2005) in the water column driving the reduction of *G. parvulum*, and its replacement over time with other species, although at this stage the effects of natural succession within the community as it develops cannot be fully ruled out. This ultimately led to the diversity indices indicating that West campus lakes diatom communities became more diverse and even.

The UKTAG assessment also indicated a major improvement in the lake's ecological quality (LTDI2), as this over-abundance of *G. parvulum*, a species typically more prevalent earlier in the growing season, and indicative of eutrophic and polluted sites (Tiwari and Chauhan, 2006), was replaced by more generalist species indicative of higher quality (lower nutrient) water bodies. An issue with using *G. parvulum* does exist, as research has indicated that what is observed as a single species in the field is likely a combination of near identical variants of other *Gomphonema* in long term culture studies (Rose and Cox, 2014). This could potentially mean that individuals of *G. parvulum* identified belonged to other species of this genus. Although the overall trend of this

species and most of the major *Gomphonema* genus species identified were replaced by diatoms of other genus', indicates that the overall change in the diatom community away from this genus remains valid. These changes appeared to occur along with a decrease in electrical conductivity, and the increase of pH and temperature. Multivariate analysis has confirmed that on this lake the pH, light attenuation and the electrical conductivity of the water, as well as the concentrations of sulphate, nitrate, magnesium, silicon, chloride, copper, potassium, and magnesium together account for 93.4% of the variation in the LTDI2 value. As such, it can be assumed that the interaction of these factors with the biofilm communities are the key seasonal drivers in the diatom community structure, affecting the lake's ecological quality over time. However, the inverse relationship between silicon concentrations and the LTDI2 value (Figure 2.13.) is unusual, in that this element is an important nutrient for diatoms used to create the silica frustules. As this was measured as elemental silicon (Si), and diatoms uptake this nutrient in the silicic acid form ($Si[OH]_4$) (Martin-Jézéquel Martin-Jézéquel *et al.,* 2000), it is possible that non-bioavailable silica sources elevated the results seen, and had unseen interaction effects that limited the ability of some species to grow. Ammonium concentrations at the start of the experiment reached a level where they are known to inhibit diatom growth (0.5 ppm) in West campus lake (0.7 ppm ± 0.15 ppm) (Admiraal, 1977, Andersen *et al.,* 2020). However, at this time point (week two) the reverse of this effect occurred with the diatom proportion of the community being elevated in West campus lake, compared to the following weeks. This is likely due to the biofilm communities still being in a late colonisation stage (Barranguet *et al.,* 2005), evidenced by the high abundance of high-profile diatoms such as *G. parvulum* seen at this time point (Figure 2.9.) which, as well as preferring winter/ spring time, are typically one of the first diatom species to colonise a fresh substratum, and are tolerant of nutrient enriched conditions (Sekar *et al.,* 2004, Tiwari and Chauhan, 2006). Furthermore, although the diversity indices of the diatom community appear to stabilise after six weeks, the LTDI2 continues to increase throughout the experiment, as does the increase in the percentage of motile species, and the reduction in species tolerant of organic contaminants. This seems to indicate that, although the structure of the community has fully developed and stabilised by week six, subtle variations in which species are present continue to occur in response to the environmental conditions, favouring more motile species over those that are adapted to high nutrient availability.

On East campus lake, the community was dominated by *A. minutissimum* and *B. vitrea*, with very limited shifts in the community structure over time, with the most notable shift being between weeks two and four, where the abundance of *A. minutissimum* increased, before stabilising at 65-87% of the total diatom community. There was an increase in the abundances of *B. brebisonii*, *E. prostratum*, *N. capitatoradiata* and *G. cuneolus*, but this only occurred on the reference substratum. The two dominant species (*A. minutissimum* and *B. vitrea*) are early colonisers and generalists (respectively) (Cantonati and Lowe, 2014, Dedić *et al.,* 2015), indicating that the biofilms present in this lake are still in the early phases of development after 10 years. The environmental factors measured here do not have as large an effect on the LTDI2 value, and the community structure as a whole, as they did in West campus lake (Appendix c. and 2.13.). Only the electrical conductivity, and concentrations of sulphate, nitrate, chloride and nitrite were confirmed, and only accounted for 18.5% of the LTDI2 variation.

The succession of freshwater benthic biofilm has been observed to begin with bacteria, followed by small pennate diatoms before filamentous algae and high-profile guild diatoms begin to colonise, with the succession culminating in the dominance of cyanobacterial organisms (Cochero *et al.,* 2018, Leflaive *et al.,* 2008, Barranguet, 2005). These early successional organisms (diatoms and bacteria) use nutrients from the surrounding waters to grow, with their growth preparing the substratum for these later colonisers (Brasell *et al.,* 2015). West campus lake appeared to have begun to reach the filamentous algae stage/ high profile diatoms within the first two weeks, based on the high abundance of high-profile diatoms (*gomphonemas*), and an observed, but not quantified high abundance of filamentous chlorophyte and diatoms observed in the live slide images at by week eight. Whereas even at week 10 of the experiment the East campus

communities still appeared to be in the diatom phase, primarily composed of the early coloniser *A. minutissimum*, with very little significant change seen since week four. This would imply that West campus lake's biofilms overall were more developed than the East campus lake communities and, in a later developmental stage (Dedić *et al.,* 2015). The literature regarding the diversity of diatom communities in biofilms indicates that a decrease in species richness over time is expected, as the wider range of early colonisers are lost to competition for resources (Sabater et al., 1998, Hillebrand and Sommer, 2000, Sekar et al., 2002, Sekar et al., 2004). A reduction between week two and four is observed for the species richness, evenness and Shannon-H index of East campus lake, and after week four these appear to have stabilised like the UKTAG assessment results and *A. minutissimum* abundances. However, these values continually increase until week six for West campus lake, which, as mentioned earlier, is due to the loss of *G. parvulum* dominance in favour of more generalist species. Multivariate analysis shows that this variation in the LTDI2 based on changes in the relative abundances of the diatom species observed in Figure 2.9., appears to be driven by this replacement of *G. parvulum,* is shown to be strongly linked (R^2= 0.934, Table 14) to changes in several environmental factors, including electrical conductivity, pH, light availability, as well as silicon, copper, potassium, nickel, chloride, nitrate and nitrite concentrations, trends that have been encountered in other work (Soininen, 2007, Porter-Goff *et al.,* 2013, Pestryakova *et al.,* 2018). Whereas East campus lake was less affected by the physico-chemical factors (R^2= 0.185, Table 2.14.), likely due to the reduced variation in the measurements obtained (Appendix c.).

To summarise, West campus lake communities continually developed for the first six weeks of the experiment, after which the biological endpoints began to stabilise. while this stage was achieved by the fourth week in East campus lake. West campus lake took longer to stabilise because the communities were subjected to larger variations in physico-chemical parameters, and although this was strongly linked to environmental parameters, effects of developmental succession within the diatom communities cannot yet be ruled out.

2.6.3. Effects of lake, influence of physicochemical parameters and interpretation of these parameters in comparison to other freshwater bodies

2.6.3.1. Physico-chemical parameters observed compared to the wider literature's baselines

Compared to the baseline data identified in Table 2.15., alkalinity, as well as the concentrations of fluoride, chloride, sulphate, phosphate, total nitrogen and silicon were within the range identified within the baselines of freshwater rivers and lakes. Nitrite, dissolved oxygen and total suspended solids were also within the baselines seen, but only in West campus lake. In East campus lake, these values were considerably below the baselines, along with the concentrations of copper, zinc, lead, aluminium, iron and nickel. Conversely, the pH, electrical conductivity, temperatures, and concentrations of calcium, potassium, magnesium and sodium were higher in both lakes than the baseline values seen (Table 2.15.). Of these, potassium and sodium concentrations are within the range where in lab experiments, they were reported to have had a significant impact on chlorophyll-a production and biofilm growth, which indicated that these nutrients had an inhibitory effect on the benthic biofilms growth rates (Table 2.15.). Additionally, the electrical conductivity was predominantly driven by chloride, sulphate, calcium and sodium, as well as potassium and magnesium to a lesser extent, based on these elements and ions being present in the highest concentrations in the campus lakes during this experiment (Appendix c.). This correlates with research by Pestryakova *et al.,* (2018) in north-eastern Siberia, who found that for freshwater biofilms, electrical conductivity was the strongest driver of community structure, with thirteen species preferring higher electrical conductivities (>500 us/cm, the category the campus lakes fall into), including diatoms of the *Epithemia* genus, particularly *epithemia adnata*, which was present in both lakes, albeit in very low quantities.

The concentrations of phosphorus (P) and nitrogen (N) nutrients at the levels observed in the two lakes do have significance as reported in the wider literature. Using the trophic state index developed by Ryding and Forsberg (1980), phosphate and nitrogen levels in East campus lake are

indicative of oligotrophic conditions (<1.5 ppm phosphorus (0.5 ppm ± 0.5 ppm phosphate present) and (0.4 ppm of nitrogen (1 ppm ± 0.5 ppm of total nitrogen)). Whilst, for West campus lake, phosphate and total nitrogen concentrations frequently exceed these levels, classifying the lake as mesotrophic (0.2 ppm ± 0.1 ppm of phosphate, 0.12 ppm ± 0.07 ppm total nitrogen). Using national classification schemes, P and N nutrient levels observed in East campus lake are within safe levels for good classification (Phosphate: <0.035 ppm (EPA, 2016), Ammonium: 0.18-0.9 ppm for pH ranges of 8-9 (ANZECC and ARMCANZ, 2000), and 0.25-5 ppm of total nitrogen, depending on lake type (Poikane *et al.,* 2019)). West campus lake saw concentrations that frequently bordered on, and even exceeded these concentrations (maximum phosphate: 0.45 ppm, maximum ammonium: 0.71 ppm) (Appendix c.) at certain time points, indicating that West campus lake contains elevated concentrations of P and N nutrients, significant to the wider literature and national environmental quality programs, which have been shown to have a physical effect on the health of the diatom communities. Further research has shown that increased ammonium concentrations at 20 ppm (no other concentrations were tested) caused stress on two of the three diatom species (*Cyclotella meneghiana* and *Nitzschia spp.*), whilst the third species, *G. parvulum*, which was dominant in the West campus lake biofilms during the start of the experiment, was more resistant to these effects (Zhang et al., 2013). Although regression and multivariate analysis did not confirm that this was a driver of the overall ecological quality of the biofilms, it may have indirectly driven the high abundances of *G. parvulum* at the start of the experiment by inhibiting the growth of less resistant species.

Table 2.16. Table of the physico-chemical measurements performed in this experiment, their mean ± SE for each lake for the whole experiment, baseline measurements found in the literature, as well as any known ecotoxicological effects of the nutrients and any known minimal requirements

Parameter		Observed mean	SE (Standard error)	Baseline	Ecotox effects	References
Fluoride (ppm)	WC:	0.10	0.0020	0.05-3.38 ppm		Neal *et al.,* (2003)
	EC:	0.23	0.0069			
Chloride (ppm)	WC:	108.66	4.3355	0-150 ppm	EC50 1,487 mg Cl/L (5 day growth inihbition)	Neal *et al.,* (2003), Elphick *et al.,* (2011)
	EC:	131.09	4.2322			
Nitrite (ppm)	WC:	0.02	0.0043	0.0002-0.029 ppm (Canada), <0.1 ppm (Danish drinking waters)		RIVM (1993), Pienitz et al., (1995)
	EC:	0.00	0.0009			
Nitrate (ppm)	WC:	0.17	0.0317	0.002-0.892 ppm (Netherlands), 0.1-0.21 ppm (Denmark)		Van Dam and Mertens (1995), RIVM (1993)
	EC:	0.33	0.0961			
Sulphate (ppm)	WC:	18.83	0.6164	0-200 ppm		Neal *et al.,* (2003)
	EC:	45.78	1.6767			
Phosphate (ppm)	WC:	0.30	0.0973	0.1-0.5 ppm		EPA (2016)
	EC:	0.03	0.0103			
DO (mg/L)	WC:	11.71	1.0147	8.2-12.2 mg/l (Naburn Marine, River Foss)		Environment Agency (2020)
	EC:	12.32	1.2137			
Ammonium (ppm)	WC:	0.20	0.0642	0.03-2.4 mg N/L (Humber basin)		Davies and Neal (2004)
	EC:	0.05	0.0042			
Total Nitrogen (ppm)	WC:	1.11	0.1193	1-15 ppm (Chinese rural/ forest lakes), 1.33-2.5 ppm (4/06/2019-06/09/2019) (Tang Hall Beck at Foss island, York)		Xu *et al.,* 2014, Environment Agency, 2020
	EC:	1.00	0.1462			
Electrical conductivity (µs/cm)	WC:	843.11	26.8450	231-359 µs/cm		Environment Agency (2020)
	EC:	908.44	4.4913			
DOC (ppm)	WC:	10.10	0.4999	4.7-44.4 mg/L		Moody (2020)
	EC:	9.99	0.3797			
pH	WC:	8.53	0.0879	7.53 (Gillrudding grange, 2019) 7.89-8.31 (Scarisbrick rail bridge, 2019)		Environment Agency (2020)
	EC:	8.69	0.0382			
Alkalinity (mg/L)	WC:	138.10	4.0155	66-150 mg/l (River Ouse, 2019)		Environment Agency (2020)
	EC:	96.00	3.3796			
Temperature (°C)	WC:	18.61	0.6914	14.6-17.6 degrees Celsius (4/06/2019-06/09/2019) (Tang Hall Beck at Foss island, York)		Environment Agency (2020)
	EC:	20.08	0.8337			
Light attenuation (% absorbance)	WC:	67.50	3.4502	N/A		
	EC:	42.67	1.4343			
TSS (mg/l)	WC:	51.22	10.7157	12-61 mg/l (Norfolk Broads)	200 mg/l reuction in biomass snd filament lengths on periphyton	Baban (1993), Birkett *et al.,* (2007)
	EC:	9.28	2.6921			
Copper (ppm)	WC:	0.01	0.0004	0.2-1.7 ppm (means of five sites (lake district, UK))	EC50 0.21 ppm (Phaeodactylum tricornutum), 7.63 ppm (Amphora coffeaeformis) (Growth inhibition)	Lawlor and Tipping (2003), Masmoudi et al., (2013)
	EC:	0.01	0.0003			
Zinc (ppm)	WC:	0.00	0.0004	1.6-30.4 ppm (means of five sites (lake district, UK))	10.1 ppm	Rachlin et al., 1983, Lawlor and Tipping (2003)
	EC:	0.00	0.0005			
Lead (ppm)	WC:	0.00	0.0003	0.1-2.9 ppm (means of five sites (lake district, UK))	EC50 2.91 ppm (*Surirella crumena*), EC50 15.35 ppm (*Halamphora veneta*)	Lawlor and Tipping (2003), Mu *et al.,* (2018)
	EC:	0.00	0.0002			
Aluminium (ppm)	WC:	0.00	0.0001	3-292 ppm (means of five sites (lake district, UK))	10 ppm (growth inihibition/ chlorophyll a content)	Lawlor and Tipping (2003), Leleyter *et al.,* (2016)
	EC:	0.00	0.0001			
Calcium (ppm)	WC:	11.27	1.0733	0.4-0.3 ppm (means of five sites (lake district, UK))		Lawlor and Tipping (2003)
	EC:	22.44	0.8328			
Iron (ppm)	WC:	0.00	0.0003	75-1664 ppm (30 Swedish rivers, 1987-2010)		Kritzberg, E.S. and Ekström, 2012
	EC:	0.00	0.0002			
Potassium (ppm)	WC:	4.89	0.8253	<0.02 ppm (< 5 umol/L)	>0.098 ppm (>25 umol/L) (growth inhibition)	Talling (2010)
	EC:	5.89	1.0991			
Magnesium (ppm)	WC:	9.72	1.8416	0.2- 0.8 ppm (means of five sites (lake district, UK))		Lawlor and Tipping (2003)
	EC:	13.54	2.8402			
Nickel (ppm)	WC:	0.00	0.0003	0.2-2.8 ppm (means of five sites (lake district, UK))	EC50 0.28 (*Thalassiosira baltica*), 0.69 (*Skeletonema marinoi*) (Marine diatoms)	Lawlor and Tipping (2003), Andersson *et al.,* (2020)
	EC:	0.00	0.0001			
Sodium (ppm)	WC:	31.22	3.7898	3.0-3.4 ppm (means of five sites (lake district, UK))	1.379 ppm (chlorophyll a reduction)	Lawlor and tipping (2003), Natana and Jeyachandran (2007)
	EC:	76.22	5.4427			
Silicon (ppm)	WC:	0.29	0.0565	0-19 ppm (UK surface waters)		Neal *et al.,* (2005)
	EC:	0.13	0.0226			

2.6.3.2. Effects of the different physico-chemical parameters of the biofilms of the campus lakes
The majority of the differences observed in the biofilm structure and composition were between the two campus lakes. The differences observed in the biological endpoints can be summed up as:

- West campus lake benthic biofilms contained more organic matter, and were dominated by chlorophyte algae, as well as a more diverse and even diatom community than East campus lake. It was initially composed primarily of *Gomphonema parvulum*, along with *Gomphonema olivaceum* and *Nitzschia paleacea*, which were gradually replaced with *A. daonense*, *A. minutissimum, C. disculus*, *N. amphibia* and *N. palea*. These diatom communities were indicative of poorer ecological health, based on the UKTAG classification (WFD classification of poor to moderate), and as such demonstrated lower LTDI2 values
- East campus lake benthic biofilms were more productive and primarily composed of the diatoms. The diatom communities observed here were less diverse than West campus lake, being primarily composed of the species *Achnanthidium minutissimum*, along with common occurrences of *Brachysira vitrea*, *Gomphonema cuneolus*, and *Cocconeis disculus*, and the UKTAG assessment results were indicative of higher quality ecosystems (WFD classification of good to high), due to the species present being classified as indicator species of lower trophic states, and therefore a higher LTDI2 value for the water body.

The higher AFDW concentrations observed in the West campus lake biofilms can be attributed to the higher concentrations of suspended solids seen in the water column providing extra material to the biofilms via sedimentation (Appendix c.). Chlorophyll-a concentrations in other studies have shown ranges of 0.05-0.15µg/cm^2 and 0-10 µg/cm^2 after two weeks of growth on Perspex panels in reservoirs and natural streams, respectively (Rao et al., 1997, Sekar *et al.,* 2002, Corcoll *et al.,* 2015). The results here have shown that for West campus lake, the Chlorophyll-a concentrations observed are at the lower end of this range, barely reaching 0.32 µg/cm^2 ± 0.051 µg/cm^2. In contrast, values for East campus lake biofilms were 2.92 µg/cm^2 ± 0.41 µg/cm^2, suggesting that East campus lake biofilms were more productive than West campus lake biofilms. This was not expected as due to East campus lake's younger age the biofilms present in the lake were believed to be less developed. Although, as algal organisms reproduce over very short time scales; the communities are likely to be fully developed and in equilibrium with the water column, but lack of a pre-existing water body flowing into the lake could have helped to limit the species that were able to colonize the site (as this lake was shown to be less diverse than West campus lake), as they would have had to arrive by aerial deposition, animal transport, and overland flow. As shown in Figure 13, the key nitrogen and phosphate nutrients are less concentrated in this lake. All the factors combined are likely to have further limited algal productivity in East campus lake, especially given the lake has known issues of phytoplanktonic algal blooms caused by high nutrient loads (Mallin and Paerl, 1992, University of York, 2019). An effect noted by Cochero *et al.,* (2018), when studying two urban streams on the Big Rib River, Wisconsin, USA, where the stream with higher nutrient concentrations demonstrated lower algal growth, which the authors attribute to a greater concentration of toxic compounds in the river that reduced metabolic activity or the key inorganic nutrients (phosphate and nitrates). Biofilms in East campus lake do have a further advantage in terms of higher light availability. The higher light attenuation, of the water column, as shown by the PAR absorption and TSS concentrations would have reduced light availability to the biofilms (Appendix c). Whilst the AFDW concentrations in the biofilms developed in West campus lakes would have further limited the light availability of algae that were not directly on the surface of the biofilms. These effects are known to be caused by bank erosion and disturbances of the sediment by the lakes oversized fish and waterfowl populations (Manny *et al.,* 1994, University of York, 2019) which would have further reduced the photosynthetic capabilities of benthic biofilms within this lake. With this reduced light availability, West campus lake biofilms should have contained a less diverse diatom biofilm community (Dalu *et al.,* 2020b), however this is not the case.

Over the course of the experiment West campus lake averaged 17.47 ± 0.77 species, and East campus lake presented 13.8 ± 0.56 species, although forty significant species were observed in total. As freshwater diatom communities typically include as few as 9 to as many as 85 species, and an average of 35 (+/- 5) species in UK freshwaters, the results here are a lttile low, but within the expected range (Townsend and Gell, 2005, Yallop *et al.,* 2009, Pla-Rabés *et al.,* 2016)). One factor that can be proven to drive this higher diversity on West campus lake is nutrient availability, as there is evidence that higher availability of P and N nutrients drive an increase in diatom diversity (Schneider et al., 2013). Additionally, the Shannon H index and evenness (overall, West campus, Shannon H: 1.95 ± 0.09, Evenness: 0.46 ± 0.03, East campus, Shannon H: 1.24 ± 0.08, Evenness: 0.29 ± 0.02) metrics further show that the diatom communities developed here were typically less diverse than the communities seen in other studies, where Shannon-H values of 2.0-4.0 have been observed in freshwater diatom communities exposed for up to a month in water bodies of differing trophic states (Sekar *et al.,* 2002, Beltrones *et al.,* 2017, Izagirre *et al.,* 2009, Yallop *et al.,* 2009, Passay *et al.,* 2019). Although in West campus lake the Shannon-H index does not drop below 2.0 after week six, due to continuing changes in the community structure over time.

The high abundance of diatoms in East campus lake biofilms, compared to chlorophytes and cyanobacteria is consistent with previous studies that have reported diatom abundances of over 60% in freshwater environments (Greenwood and Rosemond, 2011, Guerrero and Rodriguez, 1991). This lakes diatom community was primarily composed of *Achnanthidium minutissimum*, with notable quantities of *Gomphonema cuneolus*, *Cocconeis disculus* and *Brachysia vitrea*, as common species, which, are generalist and early coloniser species (Cantonati and Lowe, 2014, Dedić *et al.,* 2015), indicating that even after ten years, the biofilms of this lake are still in the early developmental stage. This is likely related to the limited input of existing communities mentioned earlier. These communities were also consistently classified as high ecological status from week four onward. The health and structure of these communities as well as those of West campus lake, as shown through the multivariate analyses the LTDI2 values of both lakes (Figure 2.14., Tables 2.13.), were dependent on sulphate, nitrate, nitrite and chloride concentrations, as well as the electrical conductivity of the lakes, although this model only accounts for 18.5% of the LTDI2 variation (Table 2.13.), indicating a relatively low effect of the environmental parameters on the ecosystem health. Independently, electrical conductivity, sulphate, nitrite and nitrate had a strong negative influence on the TDI values (Figure 2.13.), whilst chloride concentrations had a positive influence on the TDIs. At the concentrations observed (above 100 ppm), diatom communities are known to begin to adapt, with chlorine resistant species expanding to 30-44% of the community structure when concentrations begin to exceed 35ppm (Patrick, 1977, Porter-Goff *et al.,* 2013). Additional data generated by the UKTAG assessment but not used here (Appendix f) suggested that, aside from East campus lake at week two, these saline tolerant species never comprised more than 4.5% of the community structure. As for nitrite and nitrate, the data on the impacts of these nutrients on the composition of freshwater diatoms community structure is limited, but there is evidence based on estuarine sediment biofilms that cell density and relative abundances of many diatom species present in these environments are negatively correlated to the concentrations of this nutrient (0.8 to 11.3 μM), as well as ammonium (5.5- 270 μM). In addition, an increasing trend of nitrate (6.2 to 424.8 μM) (Under wood *et al.,* 1998), leading to significant changes in the abundance of eight of the 17 diatom species has been identified in this work. The nitrate concentrations in this lake have been shown to have a negative correlation to the LTDI2 in both lakes, as it caused a shift towards an increased quantity of nutrient tolerant species. Although as stated earlier, the low regression values indicate that this change was relatively minor in East campus lake.

The dominance of chlorophytes in West campus lake, as well as that of the *Gomphonema* within the diatom communities, are both reported to correlate with higher P and N nutrient availability (Burkholder *et al.,* 1990, Raschke, 1993, Soininen and Niemelä, 2002, DeNicola *et al,* 2006). Whilst phosphate concentrations were higher in West campus lake, there were also higher

concentrations of nitrogen sources in West campus lake as well, which will have a direct influence on the abundances of the algal groups, as different algal groups known to have a favoured source of nitrogen, which affects their ability to grow in an environment. Diatoms prefer nitrate as their N source, which was more prevalent in East campus lake than West campus lake (Appendix c.), where they accounted for the majority of the benthic organisms. Chlorophytes and cyanobacteria prefer ammonium as N nutrient sources, which was more concentrated in West campus lake, where the former of these two groups is more prevalent. demonstrating the greater availability of the preferred N source nutrient in each lake to their most prevalent algal group (Andersen *et al.,* 2020). The LTDI2 values of West campus lake biofilms were strongly related to the same factors as East campus lake. However, there were further dependant factors; light attenuation, pH, and the concentrations of silicon, copper, potassium and nickel, which together accounted for 93.4% of the TDI variation, indicating that the environmental parameters were very strongly tied to the changes in the environmental conditions. Independently, with the exception of light attenuation which had a negative correlation, all these additional factors had a positive regression to the ecological health of the lake, as measured by the composition of the diatom community. As shown in Table 2.15., the concentrations of these elemental nutrients were below the baseline measurements found in the literature, whilst the pH value is slightly elevated.

To summarise, the water body of origin was the main factor responsible for differences in diatom communities. With the West campus lake being of poorer ecological quality as measured by the LTDI2 assessment, due to increased P and N nutrient availability, reduced light availability, and increased variation in the lake water's electrical conductivity in West campus lake, compared to East campus lake. These factors led to the biofilms being indicative of a higher trophic state, with the function of the diatom communities being reduced in favour of species more adapted to eutrophic conditions.

2.6.4. Conclusions

This chapter has analysed how substratum and duration of exposure affect the development of the primary productive trophic level across two different lakes. This experiment has shown that there were minimal differences between the three test substratum, with the results of the biological endpoints (biomass measurements, relative abundance of the main algal groups relative abundance of the diatom species, diversity indices and UKTAG assessment) indicating that there was no significant difference between the three test substratum used. On East campus lake the chlorophyll-a data did indicate similar productivity to the reference substratum for the microscope slide substratum, whilst the ceramic tiles indicated a closer similarity with the amount of organic matter within the biofilm (AFDW), and diatom species composition. Over time, the communities on West campus lake continually changed, induced by the loss of the dominant diatom, *Gomphonema parvulum*, and its replacement by several generalist species (*A. daonense*, *A. minutissimum, C. disculus*, *N. amphibia* and *N. palea*) indicating an improvement of ecological quality, over the first six weeks of the experiment, or potentially continuing succession of the diatom communities over time. In East campus lake the endpoints remained relatively unchanged starting from the fourth week of the experiment. However, the replicates differed significantly between the two lakes, as West campus lake biofilms contained more organic matter, were dominated by the chlorophyte algal group and had a more diverse and even diatom community than East campus lake. However, these diatom communities were indicative of poorer ecological health (WFD classification of poor to moderate), whilst East campus lake biofilms were more productive, and primarily composed of the diatom algal group. The diatom communities observed in East campus lake were less diverse than West campus lake but were indicative of ecosystems of a higher ecological quality (WFD classification of good to high). These communities were also less heavily influenced by the changes in the physicochemical parameters of the lake, part of which was due to the reduced variation of these parameters over the course of the experiment in East campus lake, compared to West campus lake.

2.6.5. Recommendations

The results of the experiment on the University of York's campus lakes, conducted with the aim of assessing how diatom communities develop on different substratum over a ten-week period, have been described above. Based on these results, the following recommendations should be applied to future experiments studying the effects of chemical contaminants on diatom communities:

- Ceramic tiles are recommended as the substratum to be used for the future ecotoxicological testing of diatom communities, as they provided the closest comparison to the community structure of more established biofilms on naturally occurring substratum in East campus lake. This conclusion was based on the data showing that although species level composition of the communities (diatom relative abundance, diversity indices and UKTAG assessment results), and the ash-free dry weight concentrations of the biofilms were all similar between the three tests substratum, only the biofilms developed on ceramic tile substratum were repeatedly classed as similar to these of the reference substratum.

- Four weeks of exposure in the field is sufficient for biofilms from East campus lake to develop a representative community on artificial substratum, which is supported by much of the existing literature. However, with West campus lake the data is less clear, due to strong temporal variations, with the communities seeming to be structurally stable after six weeks (based on diversity indices), but still continuing to alter at the species level. Further work is required to assess if the changes were fully due to seasonal variations in the environment, or if there was any effect caused by the succession within the community. The data provided here will be further assessed in Chapter 3 to confirm this.

Chapter 3: Seasonality of campus lake benthic biofilm communities

3.1. Introduction

This chapter is a continuation of the analysis of the data collected in chapter 2. Following on the analysis conducted on the time factor analysed, where the conclusion that East campus benthic diatom communities remained relatively stable over time, but West campus lake showed a marked change in the benthic diatom community structure over time. This chapter will explore further whether these changes were due to natural succession within the biofilms, or if they were due to seasonal variations in the surrounding environment. However, these are not the only factors to affect the structure of diatom communities, with other factors such as grazing and altitude known to have significant effects on the structure of diatom communities (Teittinen *et al.,* 2016, de Faria *et al.,* 2017). However, the former factor was not a key consideration of this study, and was considered to be part of the seasonal aspects (alongside other key environmental factors including temperature, light availability and nutrient levels), and, as the two campus lakes are adjacent to each other on flat terrain, this latter factor was also not a key consideration.

Another key temporal driver of diatom community structure is seasonal variation, with communities known to exhibit changes in the species composition across the year in response to changes in temperature, light availability, and nutrient availability (Duong *et al.,* 2008, Passy and Larson, 2011, Dalu *et al.,* 2017). In chapter two, succession within the communities was measured. Although there was no change in the community structure of East campus lake after one-month, West campus lake demonstrated continuous change across the entire ten-week period, due to the continuous reduction of *Gomphonema parvulum*. This was the most abundant species at the experiment's start, and is also known to be tolerant of eutrophic conditions (Tiwari and Chauhan, 2006), but was rapidly replaced by several less organic nutrient tolerant species indicative of better water quality. Research on the River Avon (UK) and Boreal rivers in Finland have demonstrating key seasonal changes in the relative abundances of *Achnanthidium minutissimum* (increase) and *Amphora pediculus* (decrease), as well as increased LTDI2 values being observed in spring to autumn, compared to autumn to winter (Soininen and Eloranta, 2004, Snell *et al.,* 2019). However, other research conducted in blanket peat catchments in Ireland showed no seasonal variation in the diatom communities (O'Driscoll *et al.,* 2014), as the community composition was linked to infrequent flooding in the region that occurred all year round irrespective of the season. Thus, preventing the identification of any discrete seasonal environmental settings that could influence the communities. Similar differences were seen in the results demonstrated in Chapter 2, where one lake was shown to continually improve in water quality, based on the results of the diatom indices over the course of the experiment (West campus lake), whilst the other remained relatively static once the biofilms had become sufficiently developed (East campus lake).

A study performed in Lake Geneva (France/Switzerland) provides evidence for seasonal variation with four distinct diatom communities forming as the seasons change. Firstly, throughout the spring/ early summer growing period, high-profile diatoms dominated biofilms. Later in the summer these were replaced by low-profile diatoms. Over the autumn these species were in turn replaced by motile forms able to actively move across the substratum. Finally, the motile diatoms were then replaced over the winter by high-profile diatoms. Chapter 2 demonstrates that when diatom communities in the West campus lake were removed in succession, the percentage of the high-profile diatom *Gomphonema parvulum* was rapidly replaced by other species, including motile species, indicating that over this given time period diatom communities were following this trend observed by Rimet *et al.,* (2015). However, the substratum the biofilms in this experiment were developed on were all deployed to the sites at the same time. As such the temporal development in the biofilms examined here could have been either due to successional development, or seasonal changes in the environment. This is a

significant distinction, as many of the key species in West campus lake have been observed to be both seasonal, and early colonizers (Rimet et al., 2015, Sekar *et al.,* 2004). As such, as well as understanding how succession of biofilm composition and structure develop over the summer growing period, it is important to understand how the biofilm structure and composition differ at different points in the summer growing period, after identical durations of exposure. This differentiation between the successional stage of biofilms developed on fresh substratum, and seasonal variability in the natural community caused by environmental factors is an important distinction in understanding the development of communities. This will aid in the development of laboratory cultures, allowing the determination of suitable culturing periods of the biofilms before testing organic chemicals on these communities, and providing a greater contextual understanding for species selection in future experiments. As such, this seasonal variation will need to be accounted for in the development of future laboratory cultures, with the timing of the original communities for culturing optimised to account for this variation to include as diverse and representative a community as possible.

This chapter assesses the seasonal variation in the diatom community structure across West and East campus lakes. The structure of the biofilms developed on the three test substratum after four weeks of growth over two different time periods. Early to mid-summer, and mid to late-summer are examined. Data collected as part of the previous field experiment, as well as an additional set of replicates deployed later into the experiment are used.

3.2. Aims and objectives

Aim:

To assess the effect of temporal variations on biofilm and specific diatom community end points developed on three substrata in two lakes with different physico-chemical properties, in a 10-week field experiment.

Objectives:

- To assess the effects of timing of exposure (but with the same exposure length of 4 weeks) on a range of biofilm and diatom community end points by exposing three substrata (microscope slides, ceramic tiles and sandstone) from the 12th of June to the 10th of July (early to mid-summer) or from 24th of July to the 21st of August (mid to late summer) in two lakes with contrasting physico-chemical properties.

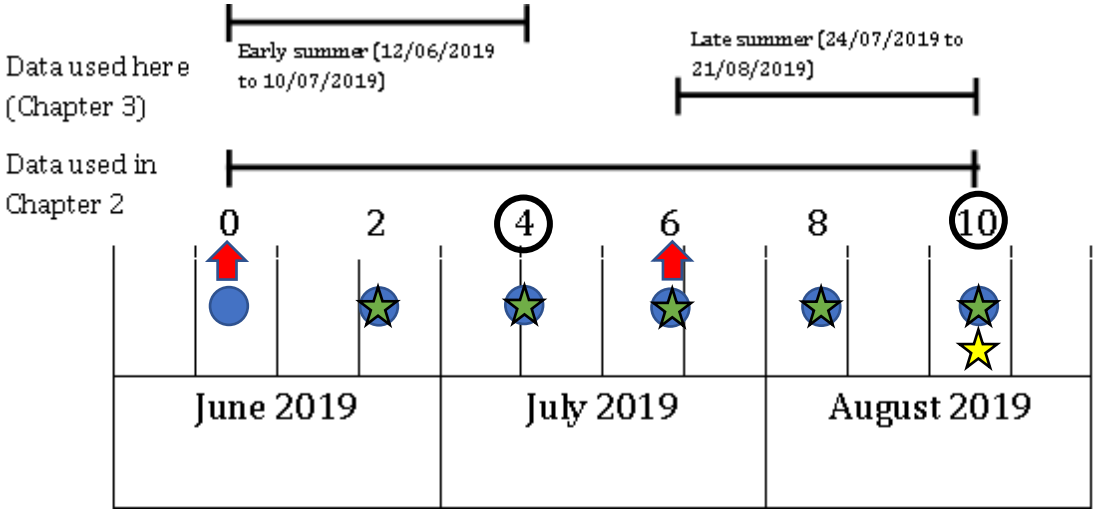
- To explore the potential role of physico-chemical environmental variables in the effects of seasonal timing and length of exposure on a range of biofilm metrics.

3.3. Methodology

The methodology employed for this experiment is described in Chapter 2, including experimental setup, sample collection, and data analysis. Additionally, an extra replicate frame as described in Chapter 2 (Figure 2.3.) was deployed on each of the rafts on the day of the sixth week sampling (24th of July 2019) experiment. These replicates were then left for four weeks until the week ten sampling point (21st of August 2019). This allowed for a direct comparison of the structure of the diatom communities at different points in the summer season, on replicates deployed for identical lengths of time. With one set was deployed in the early summer (12/06/2016 to 10/07/2019) as part of the main series used in the results shown in Chapter 2. The second deployment occurred in the late summer (24/07/2019 to 21/08/2019). The data generated from these two sets of replicates were compared to ascertain differences in the diatom communities across the summer period, when exposed for a fixed duration.

Figure 3.1 shows the timing of data collection from the replicates used in Chapter 2, with the data analysis of the biofilm measurements and physico-chemical measurements of the lakes at these

times. The data from week four is highlighted, as the data used from this time point is used for the analyses conducted in this chapter. The deployment of the late summer replicates is also labelled at week six, as well as their removal at the end of the experiment at week ten.



Key:

= water measurement (probe readings and water samples for laboratory instruments)

= Biofilm sampling for replicates deployed at week zero

= biofilm sampling for replicates deployed at week six

= Replicate deployment

= Biofilm samples used for this chapter

Figure 3.1. Time line of measurements taken for seasonal comparison, showing timings of water quality measurements, biofilm sampling, and deployment times of replicate substrata to each site

The benthic biofilm and diatom community endpoints measured include chlorophyll-a and ash-free dry weight (AFDW) concentrations, the relative abundance of the different algal groups (diatoms, cyanobacteria, chlorophytes, desmids and cryptophytes) and diatom species within the diatom community, and the diversity indices (species richness, evenness and Shannon-H index) and UKTAG assessment endpoints (LTDI2, and percentages of organic tolerant and motile species).

Twenty-six physico-chemical parameters were also measured at these sites during biofilm sample removal, as described in Chapter 2. These included nutrient measurements (nitrate, nitrite, ammonium, total nitrogen (TN), phosphate, chloride, sulphate, fluoride, magnesium, silicon, potassium, sodium, calcium, copper, aluminium, iron, lead, nickel, and zinc), and other water properties (dissolved organic carbon (DOC), dissolved oxygen (DO), total suspended solids (TSS) concentrations, alkalinity, electrical conductivity, temperature and light attenuation (absorbance of photosynthetic active radiation (PAR)) of the water column).

As described in greater detail in Chapter 2, diversity indices were calculated in PAST software (version 3), and UKTAG assessment was calculated in the DARLEQ2 software. Statistical analysis (Kolmogorov Smirnoff test, Kruskal-Wallis H tests, Friedman test, three, two, and one-way repeated measure ANOVA, Tukey HSD tests and Mann-Whitney U tests) were performed in IBN SPSS software (version 26). For the biofilm measurements, although three-way ANOVA methods were used, the Lake factor was not discussed in the results sections unless it was as an interaction effect with the Substrate or Time factor. As this factor has been extensively discussed in Chapter

2, and the primary focus of this chapter is to identify differences caused by the timing of deployment.

3.4. Biofilm results

3.4.1. Biomass measurements

Variations between deployment timing

AFDW of the biofilm did not significantly differ across the timing of the deployment (Figure 3.2, Table 3.1.). There was however a significant time effect on the chlorophyll-a concentration of the benthic algal communities (P= 0.008), but this was not consistent across all substratum and both lakes (Figure 3.2., Table 3.1.), applying only to West campus lake microscope slide and ceramic tile biofilms.

Variations between substratum type

Although there were no significant differences between the three substratum for the AFDW, Chlorophyll-a concentrations in the biofilms demonstrated lower concentrations observed on microscope slide and ceramic tile substratum, compared to the sandstone substratum when developed in East campus lake during the June deployment (P=0.010, one-way ANOVA, P<0.050, Tukey HSD).

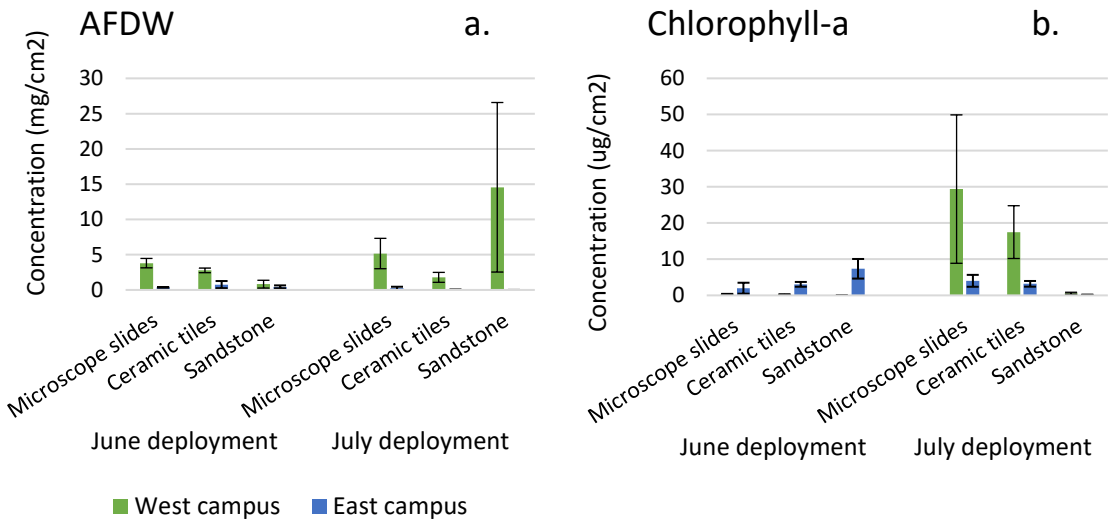


Figure 3.2. Chlorophyll concentrations (a), and AFDW (b) concentrations of the biofilms developed on the three test substratum (MS=microscope slides, CT= ceramic tiles, LM= lithic material (sandstone) after an exposure period of one month starting in June (12th) or July (24th) on west campus and east campus lakes. Mean ± SE (N=3).

Table 3.1. Three-way repeated measure ANOVA results for the ash-free dry weight (AFDW) and chlorophyll-a concentration results, using lake (West campus and East campus), and Substratum (microscope slide, ceramic tiles or sandstone) as factors, and time (4 weeks deployment in early June to early July, and 4 weeks deployment from late July to late august) as a repeated measure.

	AFDW			Chlorophyll a		
	F	df	P	F	df	P
Time	1.041	1	0.328	10.191	1	**0.008**
Substrate	0.577	2	0.577	5.941	2	**0.016**
Lake	5.084	1	**0.044**	0.300	1	0.594
Time * Substrate	1.210	2	0.332	10.846	2	**0.002**
Time * Lake	1.511	1	0.243	24.727	1	**<0.001**
Lake * Substrate	0.655	2	0.537	3.027	2	0.086
Time * Lake * Substrate	1.184	2	0.340	0.203	2	0.819

3.4.2. Algal groups

Variations between deployment timing

There was no effect of substratum or time on the relative abundance of diatoms and chlorophytes as main effects (Figure 3.3., Table 3.2.). For Cyanobacteria there was an interaction between the effects of all three factors, however further testing using posthoc tests and splitting the dataset by the different factors (Time, Substratum and Lake) indicated that there was no significant effect of lake, substratum or time on the relative abundance of these species. However, there were effects that occurred under specific conditions.

Variations between substratum type

For the chlorophytes, there was an increase in relative abundance over time, although this was not a main effect, and was limited to East campus lake (13.8±0.1% in the early summer to 18.6±0.89% in the late summer deployment, P=0.047, two-way repeated measure ANOVA). Diatom variation over time did occur despite not being observed as a main effect (Table 3.2.), but was limited to microscope slide substratum on East campus lake with relative abundances declining from 85.7±0.5% in the June-July period to 73.2±1.7% (P=0.012, one-way ANOVA).

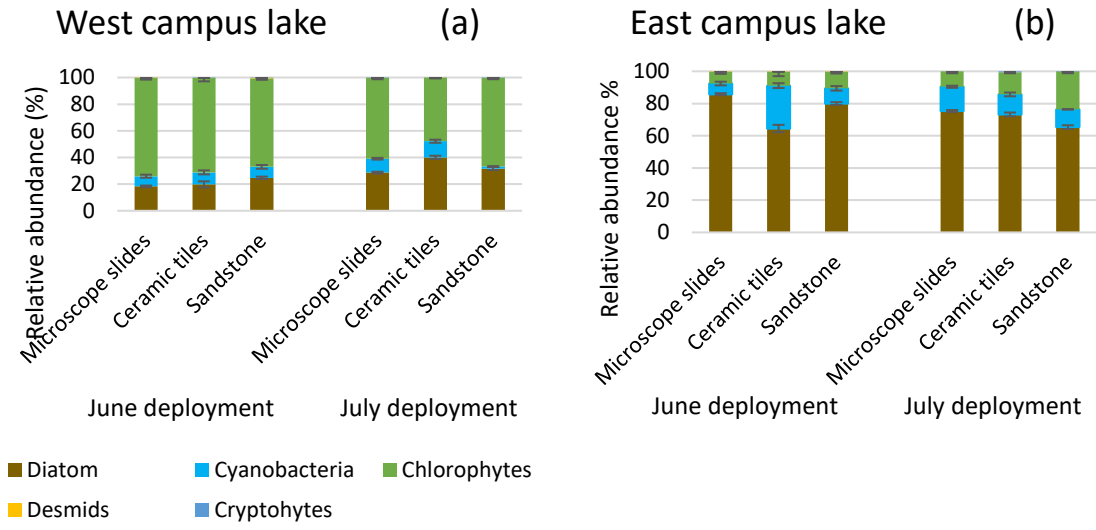


Figure 3.3. Graph showing the abundances of the different algal groups (diatoms, cyanobacteria, chlorophytes, desmids, and cryptophytes), across the three experimental substratum (microscope slides, ceramic tiles, and sandstone) after one month of growth over June to July (12th of June to 10th of July), and July to August (24th of July to 21st of August). Mean ± SE (N=3)

Table 3.2. Three-way repeated measures ANOVA for the three main algal groups (diatoms, Cyanobacteria, and Chlorophytes), using lake (West and East campus lake), and substratum (microscope slides, ceramic tiles, and sandstone) as main factors, and time (June deployment and July deployment) as the repeated measure factor.

	Diatoms			Cyanobacteria			Chlorophytes		
	F	df	P	F	df	P	F	df	P
Time	1.617	1	0.228	0.181	1	0.678	0.825	1	0.382
Substrate	0.130	2	0.879	1.094	2	0.366	1.035	2	0.385
Lake	122.655	1	**<0.001**	1.624	1	0.227	205.455	1	**<0.001**
Time * Substrate	3.994	2	**0.047**	2.723	2	0.106	2.769	2	0.103
Time * Lake	10.289	1	**0.008**	0.740	1	0.790	10.932	1	**0.006**
Substrate * Lake	1.621	2	0.238	0.238	2	0.792	0.839	2	0.456
Time * Substrate * Lake	0.327	2	0.727	4.017	2	**0.046**	0.711	2	0.511

3.4.3. Diatoms

Figure 3.4. shows the percentage relative abundance of each diatom species compared to the overall diatom community in both lakes, across the two deployment periods and for each substratum type. The ANOVA statistics analysis results are shown in Table 3.4.

There was no statistically significant difference in the abundances of twelve of the diatom species between the three test substratum, two lakes, or two deployment times (Figure 3.4., Table 3.4.). These species were *A. pediculus*, *B. spp*, *B. brebisonii*, *D. problematica*, *E. adnata*, *G. accuminatam*, *M. varians*, *N. cryptocephala*, *N. acicularis*, *N. palea*, *P. viridis*, and *R. gibba*.

Variations between deployment timing

Five species were observed to be more abundant in the June deployment, compared to the July deployment. The most significant of these in terms of the overall community structure was the large drop in the abundance of *G. parvulum* in West campus lake between deployment times on microscope slides and ceramic tiles (early summer: 47.30-65.43%, late summer: 3.29-6.25%) (P=0.083, friedman test). The other four species to demonstrate higher abundances in the early summer deployment were *C. disculus*, *G. olivaceum*, as well as *G. truncatum* and *E. prostratum*, but these latter two only demonstrated this effect in West campus lake (Figure 3.4., Table 3.4.), and none of these species exceeded 5% of the total community structure.

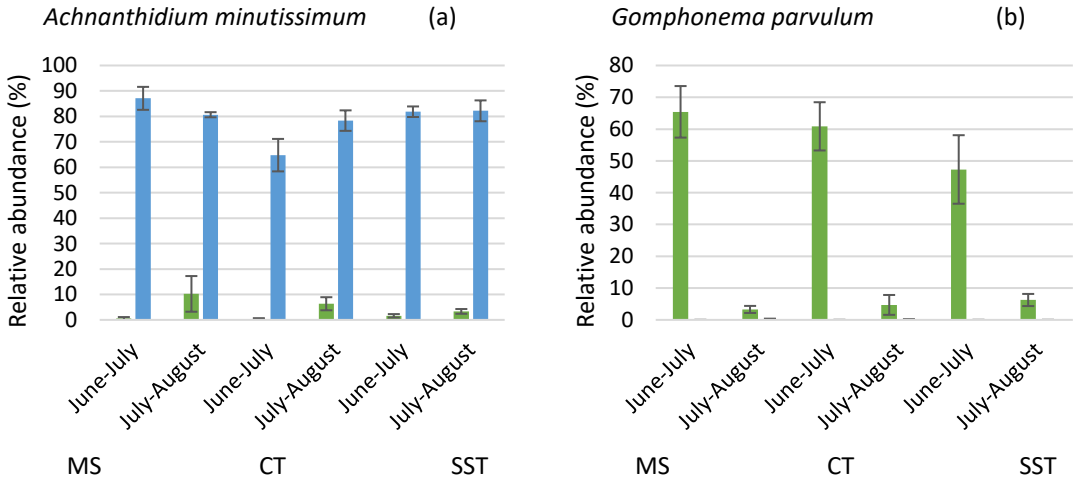
Conversely, an additional six species were observed to be more abundant in the July deployment. Three of these species *S. ulna*, *E. sorex* and *E. turgida*, did not exceed 2% 0f the total community structure (P<0.049 and 0.037, respectively, two-way repeated measure ANOVA) (Figure 3.4, Table 3.4.). *N. dissipata* demonstrated a trend towards higher abundance in the July deployment, but only on West campus lake substratum (all P=0.083, Friedman test) (early summer: 0.11%-0.53%, late summer: 2.03%-8.64%), as does *R. abbreviata* (early summer: 2.81%±0.63, late summer: 0.22%±0.22), and *N. paleacea* (early summer: 0.11%-0.21%, late summer: 28.50%-36.26%), although in the case of this latter species this trend also applies to microscope slides on East campus lake (all P=0.083, Friedman test). These differences are in line with those observed in chapter 2, indicating environmental/ temporal, rather than successional control of the community at four weeks of exposure.

Variation between substratum type

There was no significant effect on the abundance of all but two diatom species. For *A. minutissimum*, microscope slides demonstrated significantly higher relative abundances (87.09±4.51%) compared to ceramic tiles (64.76±6.38%) (P<0.050, Tukey HSD) but both were considered to be similar to abundances observed on sandstone (81.83±2.07%) (P>0.050, Tukey HSD), but only for the June deployment replicates in East campus lake (both P= 0.051 KWH test). Similarly, *B. vitrea* demonstrated differences in abundance between substratum limited to the June deployment in East campus lake (both P= 0.027 KWH test), whereby although microscope slides and sandstone substratum developed similar abundances of this species, (4.21%-9.18%) (P>0.050, Tukey HSD), the ceramic tiles developed much higher abundances of this species (22.30%±4.19) (P<0.050, Tukey HSD). This indicates the effects of substratum is minimal, especially compared to the significant differences seen in most species between the June and July deployment times.

Summary

To summarise, there were significant differences in the composition of the diatom communities between replicates deployed in the early summer period, compared to the late summer period, particularly in West campus lake, where the most common species in the early summer replicates, *G. parvulum*, is replaced in the July deployment replicates by other species, including *A. daonense* and *N. paleacea*, although there is high variation between substratum and even individual replicates as to the exact composition of the replacements. *C. disculus* and *G. cuneolus* also appeared to be less abundant in the July deployment replicates, regardless of lake. Furthermore, as observed in Chapter 2, there was very limited variation in the communities between substratum, with this mainly being due to the increased abundances of *A. minutissimum* on microscope slides and increased abundance of *B. vitrea* on ceramic tiles, but both instances were limited to June deployment replicates in East campus lake. The main difference in the composition of the communities is between the two lakes, based on the significant differences of twenty-one species between the two lakes studied as main effects, with several others showing lake effects at specific times and/or on certain substratum. This indicates that community composition differences seen in West campus lake replicates in Chapter 2 were indeed due to seasonal changes in the biofilms, rather than differences caused by succession in development, and as such four weeks of exposure would be sufficient to grow a representative community of diatoms, as evidenced in Chapter 2 by the East campus lake results.



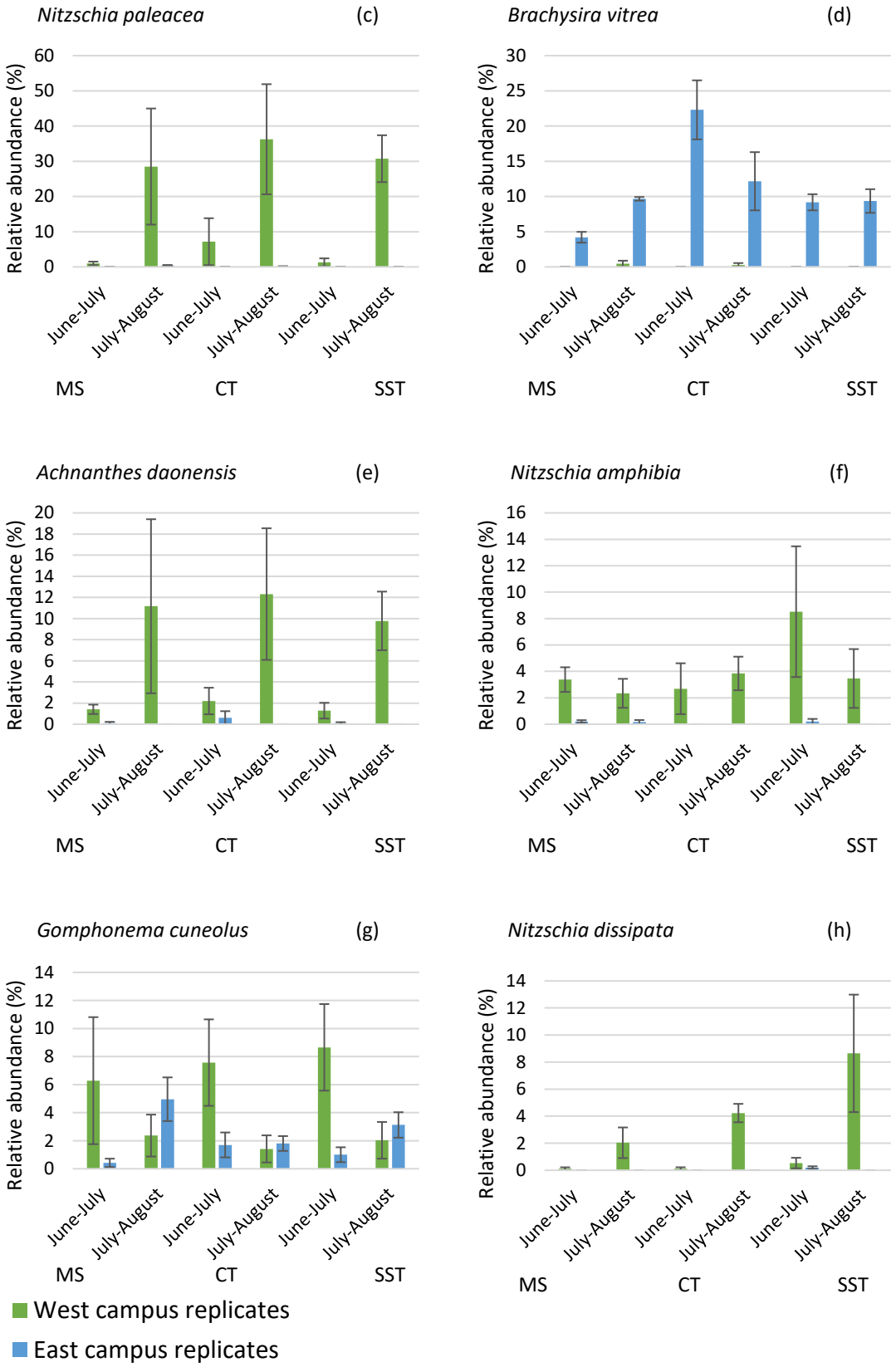

Nitzschia paleacea
(c)
Relative abundance (%)
60
50
40
30
20
10
0
June-July
July-August
MS
CT
SST
Brachysira vitrea
(d)
30
25
20
15
10
5
Achnanthes daonensis
(e)
18
16
14
12
Nitzschia amphibia
(f)
Gomphonema cuneolus
(g)
Nitzschia dissipata
(h)
West campus replicates
East campus replicates

Figure 3.4. Relative abundance (%) of the diatom species present in the two campus lakes (green bars: West campus replicates, blue bars: East campus replicates) and the three test substratum (MS= microscope slides, CT= ceramic tiles, LM = lithic materials (sandstone)), split by replicates deployed for one month starting in early June (June-July deployment), and one month starting in late July (July-August deployment). Mean ± SE (N=3). See Appendix g. for species observed but for whom mean abundance ± SE does not exceed 10%.

Table 3.3. Graph label and corresponding name for the species identified in Figure 4. Sorted by approximate highest abundance.

Graph number	Species name	Graph number	Species name
a	*Achnanthidium minutissima*	u	*Epithemia sorex*
b	*Gomphonema parvulum*	v	*Encyonema neogracile*
c	*Nitzschia palea*	w	*Gomphonema accuminatum*
d	*Brachysira vitrea*	x	*Navicula cryptocephala*
e	*Achnanthes daonense*	y	*Encyonema reichardtii*
f	*Epithemia turgida*	z	*Gomphonema truncatum*
g	*Nitzschia amphibia*	aa	*Epithemia adnata*
h	*Gomphonema cuneolus*	ab	*Gomphonema vibrio*
i	*Nitzschia dissipita*	ac	*Amphora inariensis*
j	*Encyonema minuta*	ad	*Nitzscia palea*
k	*Cocconeis disculus*	ae	*Brachysira brebisonii*
l	*Amphora pediculus*	af	*Nitzschia linearis*
m	*Gomphonema olivaceum*	ag	*Diatoma problematica*
n	*Melosira varians*	ah	*Nitzschia acicularis*
o	*Synedra ulna*	ai	*Gyrosigma accuminatum*
p	*Encyonema prostratum*	aj	*Achnanthidium modestiforme*
q	*Fragillaria vaucheriae*	ak	*Brachysira zellensis*
r	*Rhoicosphenia abbreviata*	al	*Rhopaladia gibba*
s	*Nitzschia minuta*	am	*Pinnularia viridis*
t	*Navicula capitatoradiata*	an	*Brachysira spp.*

Table 3.4. Three-way repeated measure ANOVA for the diatom species observed in the two campus lakes (lake factor), on the three substratum (microscope slides, ceramic tiles, sandstone, substratum factor), deployed at the experiment start (early June), and six weeks later (July deployment), after one month of exposure (time repeated measure factor).

	Achnanthes daonenese			*Achnanthidium minutissimum*			*Achnanthidium modestiforme*			*Amphora inariensis*			*Amphora pediculus*		
Source	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P
time	1.000	5.514	**0.037**	1.000	1.476	0.248	1.000	0.535	0.478	1.000	0.209	0.656	1.000	0.140	0.715
substrate	2	0.124	0.885	2	6.780	**0.011**	2	1.034	0.385	2	1.818	0.204	2	0.225	0.802
lake	1	14.179	**0.003**	1	1303.746	**<0.001**	1	5.573	**0.036**	1	4.775	**0.049**	1	2.700	0.126
time * substrate	2.000	0.011	0.989	2.000	1.650	0.233	2.000	0.221	0.805	2.000	0.678	0.526	2.000	1.368	0.292
time * lake	1.000	6.185	**0.029**	1.000	0.238	0.634	1.000	1.421	0.256	1.000	2.637	0.130	1.000	1.496	0.245
substrate * lake	2	0.067	0.935	2	5.369	**0.022**	2	0.774	0.483	2	2.857	0.097	2	0.170	0.846
time * substrate * lake	2.000	0.026	0.974	2.000	2.400	0.133	2.000	0.814	0.466	2.000	0.132	0.878	2.000	0.420	0.667

	Brachysira			*Brachysira brebisonii*			*Brachysira vitrea*			*Brachysira zellensis*			*Cocconeis disculus*		
Source	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P
time				1.000	0.001	0.975	1.000	0.212	0.654	1.000	0.123	0.732	1.000	5.876	**0.032**
substrate	2			2	1.494	0.263	2	43.334	**<0.001**	2	0.195	0.826	2	1.971	0.182
lake	1			1	0.272	0.612	1	534.551	**<0.001**	1	5.891	**0.032**	1	2.782	0.121
time * substrate				2.000	0.204	0.818	2.000	2.654	0.111	2.000	0.086	0.919	2.000	1.495	0.263
time * lake				1.000	1.124	0.310	1.000	0.391	0.543	1.000	0.123	0.732	1.000	0.032	0.862
substrate * lake	2			2	0.069	0.934	2	43.608	**<0.001**	2	0.195	0.826	2	1.793	0.208
time * substrate * lake				2.000	2.233	0.150	2.000	2.597	0.116	2.000	0.086	0.919	2.000	3.012	0.087

	Diatoma problematica			*Encyonema minuta*			*Encyonema neogracile*			*Encyonema prostratum*			*Encyonema reichardti*		
Source	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P
time	1.000	0.794	0.390	1.000	1.656	0.222	1.000	2.293	0.156	1.000	7.338	**0.019**	1.000	2.398	0.147
substrate	2	0.395	0.682	2	1.792	0.209	2	1.619	0.238	2	1.346	0.297	2	5.168	**0.024**
lake	1	2.210	0.163	1	2.134	0.170	1	7.429	**0.018**	1	4.656	0.052	1	0.074	0.790
time * substrate	2.000	1.103	0.363	2.000	0.932	0.421	2.000	0.526	0.604	2.000	0.920	0.425	2.000	1.178	0.341
time * lake	1.000	0.794	0.390	1.000	11.897	**0.005**	1.000	22.738	**<0.001**	1.000	7.202	**0.020**	1.000	8.601	**0.013**
substrate * lake	2	0.395	0.682	2	2.888	0.095	2	1.345	0.297	2	0.567	0.582	2	0.321	0.732
time * substrate * lake	2.000	1.103	0.363	2.000	1.450	0.273	2.000	0.670	0.530	2.000	0.879	0.440	2.000	2.544	0.120

	Epithemia adnata			*Epithemia sorex*			*Epithemia turgida*			*Fragilaria vaucheria*			*Gomphonema accuminata*		
Source	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P
time	1.000	1.000	0.337	1.000	6.050	**0.030**	1.000	7.411	**0.019**	1.000	0.541	0.476	1.000	3.051	0.106
substrate	2	1.000	0.397	2	0.584	0.573	2	0.298	0.748	2	0.474	0.634	2	2.858	0.097
lake	1	1.000	0.337	1	6.050	**0.030**	1	9.630	**0.009**	1	6.873	**0.022**	1	2.051	0.178
time * substrate	2.000	1.000	0.397	2.000	0.584	0.573	2.000	0.581	0.574	2.000	7.193	**0.009**	2.000	3.104	0.082
time * lake	1.000	1.000	0.337	1.000	6.050	**0.030**	1.000	6.897	**0.022**	1.000	2.325	0.153	1.000	1.544	0.238
substrate * lake	2	1.000	0.397	2	0.584	0.573	2	0.237	0.793	2	0.815	0.466	2	1.405	0.283
time * substrate * lake	2.000	1.000	0.397	2.000	0.584	0.573	2.000	0.503	0.617	2.000	4.269	**0.040**	2.000	1.581	0.246

	Gomphonema cuneolus			*Gomphonema olivaceum*			*Gomphonema parvulum*			*Gomphonema truncatum*			*Gomphonema vibrio*		
Source	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P
time	1.000	4.039	0.067	1.000	5.408	**0.038**	1.000	100.537	**<0.001**	1.000	10.738	**0.007**	1.000	0.051	0.825
substrate	2	0.057	0.945	2	0.658	0.535	2	0.628	0.551	2	1.046	0.381	2	2.364	0.136
lake	1	3.179	0.100	1	47.268	**<0.001**	1	85.089	**<0.001**	1	7.135	**0.020**	1	0.040	0.846
time * substrate	2.000	1.482	0.266	2.000	2.254	0.148	2.000	1.619	0.239	2.000	1.046	0.381	2.000	4.646	**0.032**
time * lake	1.000	22.397	**<0.001**	1.000	3.879	0.072	1.000	101.187	**<0.001**	1.000	7.135	**0.020**	1.000	0.003	0.957
substrate * lake	2	0.114	0.893	2	0.295	0.750	2	0.605	0.562	2	1.739	0.217	2	0.775	0.482
time * substrate * lake	2.000	0.227	0.800	2.000	1.488	0.265	2.000	1.666	0.230	2.000	1.739	0.217	2.000	2.024	0.175

	Gyrosigma accuminatum			*Melosira varians*			*Navicula capitatoradiata*			*Navicula crpytocephala*			*Nitzschia acicularis*		
Source	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P
time	1.000	0.005	0.947	1.000	1.342	0.269	1.000	3.362	0.092	1.000	0.093	0.765	1.000	0.186	0.674
substrate	2	2.396	0.133	2	0.618	0.555	2	4.659	**0.032**	2	0.513	0.612	2	0.752	0.493
lake	1	10.776	**0.007**	1	4.471	0.056	1	1.289	0.278	1	1.889	0.194	1	2.753	0.123
time * substrate	2.000	0.249	0.784	2.000	0.973	0.406	2.000	4.407	**0.037**	2.000	0.264	0.772	2.000	1.380	0.289
time * lake	1.000	0.659	0.433	1.000	4.307	0.060	1.000	2.632	0.131	1.000	1.939	0.189	1.000	1.758	0.210
substrate * lake	2	0.519	0.608	2	0.269	0.769	2	1.987	0.180	2	0.112	0.895	2	2.092	0.166
time * substrate * lake	2.000	0.211	0.813	2.000	1.502	0.262	2.000	1.841	0.201	2.000	0.448	0.649	2.000	0.595	0.567

	Nitzschia amphibia			*Nitzschia dissipata*			*Nitzschia minuta*			*Nitzschia palea*			*Nitzschia paleacea*		
Source	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P
time	1.000	0.484	0.500	1.000	8.571	**0.013**	1.000	3.101	0.104	1.000	0.058	0.814	1.000	17.050	**0.001**
substrate	2	1.933	0.187	2	2.160	0.158	2	0.267	0.770	2	1.121	0.358	2	0.196	0.825
lake	1	30.932	**<0.001**	1	12.474	**0.004**	1	8.528	**0.013**	1	0.576	0.462	1	12.141	**0.005**
time * substrate	2.000	0.570	0.580	2.000	1.219	0.330	2.000	0.165	0.849	2.000	0.458	0.643	2.000	0.006	0.994
time * lake	1.000	0.397	0.540	1.000	9.058	**0.011**	1.000	5.123	**0.043**	1.000	1.395	0.260	1.000	16.675	**0.002**
substrate * lake	2	1.953	0.184	2	1.917	0.189	2	0.354	0.709	2	1.411	0.282	2	0.203	0.819
time * substrate * lake	2.000	0.500	0.619	2.000	1.395	0.285	2.000	0.064	0.938	2.000	1.112	0.361	2.000	0.010	0.990

	Nitzscshia linearis			*Pinnularia viridis*			*Rhoicosphenia abbrev*			*Rhopaladia gibba*			*Synedra ulna*		
Source	df	F	P	df	F	P	df	F	P	df	F	P	df	F	P
time	1.000	2.139	0.169	1.000	1.000	0.337	1.000	5.420	**0.038**	1.000	1.653	0.223	1.000	11.680	**0.005**
substrate	2	0.703	0.514	2	1.000	0.397	2	0.182	0.836	2	0.617	0.556	2	0.341	0.718
lake	1	5.517	**0.037**	1	1.000	0.337	1	7.005	**0.021**	1	1.766	0.209	1	7.181	**0.020**
time * substrate	2.000	1.015	0.391	2.000	1.000	0.397	2.000	4.096	**0.044**	2.000	0.674	0.528	2.000	0.198	0.823
time * lake	1.000	0.719	0.413	1.000	1.000	0.337	1.000	6.143	**0.029**	1.000	1.653	0.223	1.000	3.804	0.075
substrate * lake	2	0.764	0.487	2	1.000	0.397	2	0.340	0.719	2	0.617	0.556	2	0.570	0.580
time * substrate * lake	2.000	0.521	0.607	2.000	1.000	0.397	2.000	2.981	0.089	2.000	0.674	0.528	2.000	1.364	0.293

3.4.4. Diversity indices

Variations between deployment timing

There was no significant change over time on the species richness or the Shannon-H index. However, community evenness was significantly higher in the late summer deployment (0.19 ± 0.02 to 0.45 ± 0.04), than the early summer deployment (0.17 ±0.02 to 0.32 ± 0.06).

Variation between substratum type

There was also no significant difference in any of the three diversity indices between the substratum (Figure 3.5., Table 3.5.), and observed differences between the two lakes are in line with that observed in Chapter 2.

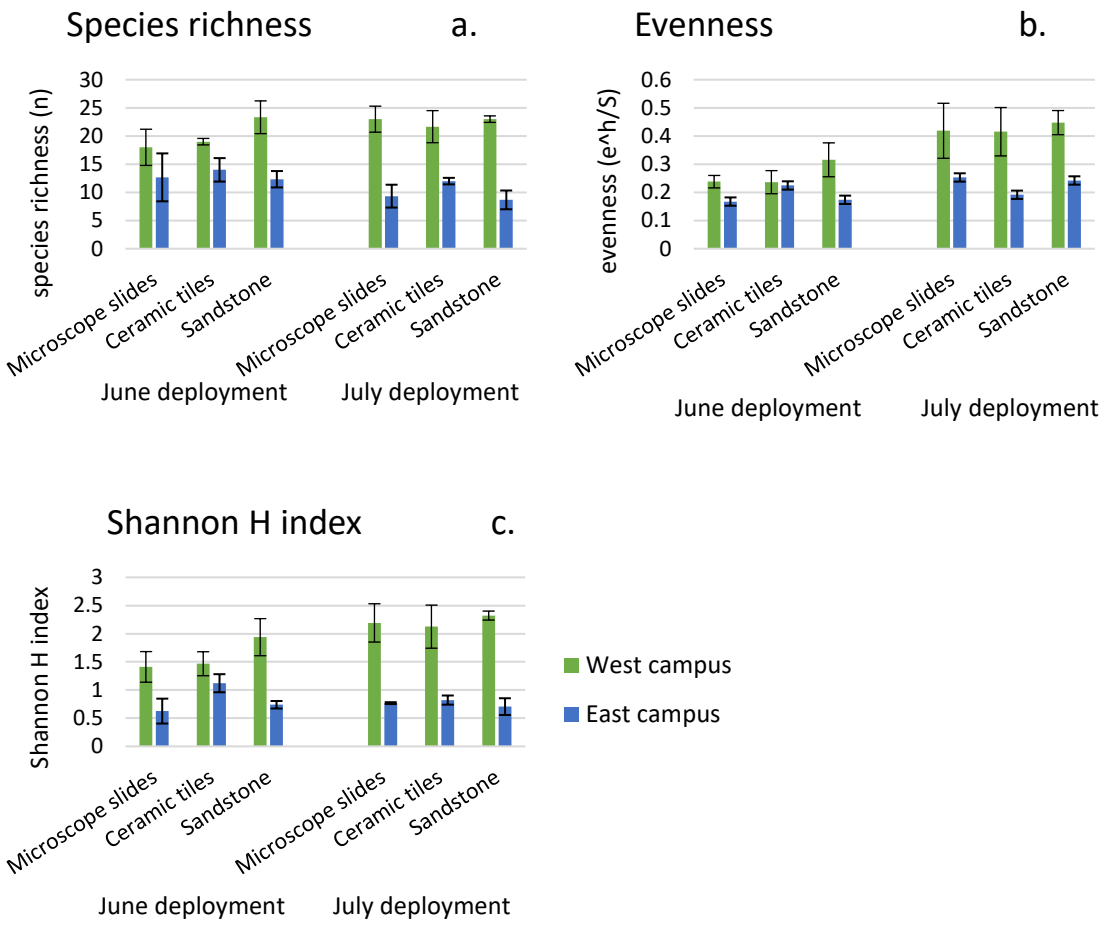


Figure 3.5. Diversity indices (a. Species richness, b. Evenness and c. Shannon H index) for the substratum (microscope slides, ceramic tiles and sandstone) exposed for a one-month period starting in June (June deployment) and July (July deployment) on West campus and East campus lakes. Mean ± SE (N=3).

Table 3.5. Three-way repeated measures ANOVA results for diversity indices (Species richness, Evenness, and Shannon H index) using lake and substratum as factors, and Time as a repeated measure factor (June deployment and July deployment).

	Species richness			Evenness			Shannon H		
	F	df	P	F	df	P	F	df	P
Time	0.057	1	0.815	12.102	1	**0.005**	2.698	1	0.126
Substrate	0.203	2	0.819	0.497	2	0.620	1.133	2	0.354
Lake	43.212	1	**<0.001**	27.187	1	**<0.001**	102.267	1	**<0.001**
Time * Substrate	0.563	2	0.584	0.398	2	0.680	0.680	2	0.525
Time * Lake	5.469	1	**0.037**	3.939	1	0.071	4.244	1	0.062
Lake * Substrate	1.072	2	0.373	0.523	2	0.606	2.519	2	0.122
Time * Lake * Substrate	0.412	2	0.671	0.699	2	0.516	0.349	2	0.712

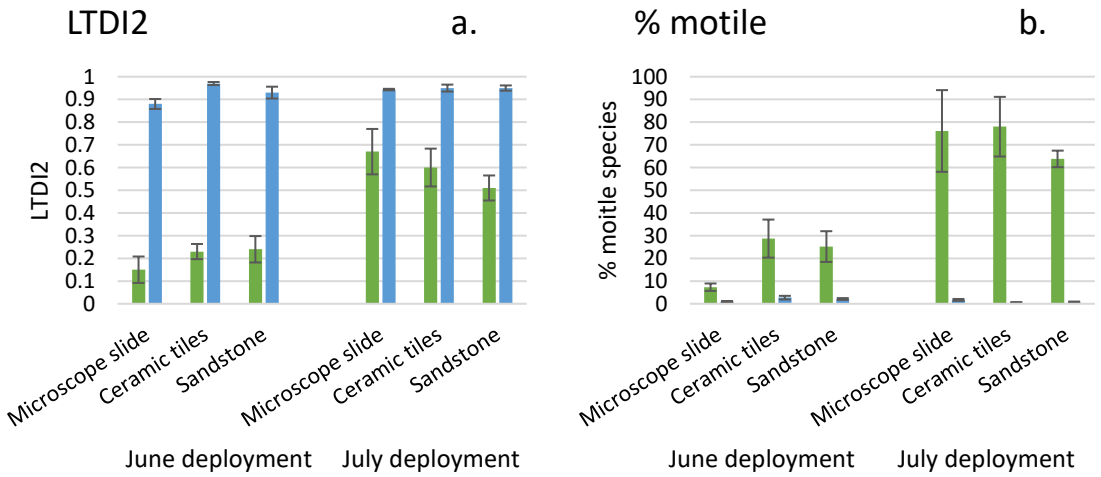
3.4.5. UKTAG results

Variations between deployment timing

There was a significant effect of time on the percentage of motile species and the biofilm LTDI2 values as main effects, although interaction effects occurred, indicating that differences between the deployment times did occur, but only on one of the two lakes (Table 3.6.). The percentage of motile species and the LTDI2 values were higher at the end of the July deployment in West campus lake (motile: 41.6% ± 17.99% to 56.53%± 3.65%, LTDI2: 0.51 ± 0.06 to 0.67 ± 0.1) when compared to the June deployment (motile: 7.3% ± 1.63% to 28.7% ± 8.39%, LTDI2: 0.15 ± 0.06 to 0.24 ± 0.06, both P< 0.001, N=1, two-way repeated measure ANOVA), with the percentage of organic tolerant species was typically higher in the June deployment, compared to the July deployment (June: 44% ± 12.12% to 73.6% ± 3.82%, July: 38.7% ± 18.89% to 46.37% ± 14.33%).

Variation between substratum type

There was no significant effect of substratum on the biofilms' LTDI2 values or the percentage of motile and organic tolerant species present in the biofilms (Figure 3.6., Table 3.6.).



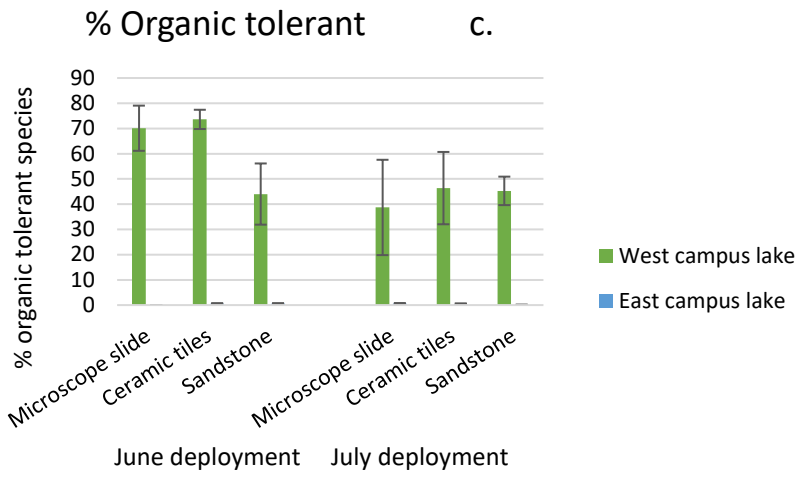


Figure 3.6. Graphs showing the a. LTDI2 values, b. percentage of individuals in the community that are motile, and c. percentage of individuals in the communities tolerant of organic nutrient enrichment, split by deployment period (June deployment and July deployment), and substratum (microscope slides, ceramic tiles, sandstone). Mean ± SE (N=3).

Table 3.6. Three-way repeated measures ANOVA for the LTDI2, % motile, and % organic tolerant results of the biofilm samples shown in figure 3.6, using substratum (microscope slides, ceramic tiles and sandstone) and lake (West campus lake and East campus lake) as factors, and time (June deployment and July deployment) as the repeated measure factor.

	LTDI2			% Motile			% Organic tolerant		
Source	F	df	P	F	df	P	F	df	P
Time	67.288	1	**<0.001**	20.763	1	**0.001**	2.855	1	0.117
Substrate	0.116	2	0.891	0.517	2	0.609	0.503	2	0.617
Lake	258.435	1	**<0.001**	99.261	1	**<0.001**	951.873	1	**<0.001**
Time * Substrate	1.580	2	0.246	0.347	2	0.714	0.010	2	0.990
Time * Lake	63.525	1	**<0.001**	40.366	1	**<0.001**	7.122	1	**0.020**
Lake * Substrate	0.135	2	0.875	0.263	2	0.773	0.072	2	0.931
Time * Substrate * Lake	1.532	2	0.255	0.385	2	0.688	3.105	2	0.082

3.4.6. Summary

To summarise, diatoms were more abundant on east campus lake, and the chlorophytes were more abundant on West campus lake. Chlorophyte abundance was also observed to differ between the June and July deployment biofilm on East campus lake ceramic tiles over time by <5%. West campus lake also had much higher abundances of motile and organic nutrient enrichment resistant diatom species, but had much lower LTDI2 values, indicating dominance of nutrient tolerant species and therefore poorer environmental quality.

Time of initial deployment and substratum used had no effect on the LTDI2 values or the percentage of motile and organic tolerant species on East campus lake, the former of which was consistently high (>0.88), whilst the latter two were consistently low (<5%), indicating a community structure comprised of roughly reference site quality, comprised of diatoms with a preference and adaption to pristine, low nutrient state sites. But on West campus lake, The LTDI2 value increased between the early and late summer deployments, which was followed by a reduction in the percentage of organic tolerant species, indicating a reduction in the species that prefer nutrient rich waters and tolerant of the presence of organic nutrients. However, an increase in the percentage of motile species still indicates a shift towards a more motile

community composition. Furthermore, the between-lake differences in the biofilm and algal/ diatom community measurements used here show the same differences (when present) as seen in Chapter 2.

3.5. Physico-chemical measurements

3.5.1. Variation of the physico-chemical measurements

Graphical representation of the data is available in the appendix (Appendix c), using the same graphs from chapter 2, with the values labelled week four used for the June deployment period, as they were taken simultaneously. The values labelled week ten on Appendix c are the measurements taken during the sample collection for the July deployment replicates.

There was no significant difference in the concentrations of nitrate, nitrite, ammonium, DOC, chloride, sulphate, zinc, iron, aluminium or lead, between the sampling points at the end of the four-week early summer or four-week late summer sampling points, or between the two lakes used at these time points.

Time factor:

Table 3.7 shows a significant difference between the June deployment and July deployment, where higher values were observed in the June deployment for TN (June 1.23 ppm ± 0.13 ppm, July 0.26 ppm ± 0.14 ppm), DO (June: 20.05 ppm ± 0.71 ppm, Jly: 11.17 ppm ± 0.29 ppm), Temperature (early summer: 20.22^{o}C ± 0.47 ^{o}C, late summer: 19.12 ^{o}C ± 0.49 ^{o}C), EC, (June: 905.8 us/cm^2 ± 3.94 us/cm^2, July: 795.17 us/cm^2 ± 42.92 us/), Silicon (June: 0.25 ppm ± 0.05 ppm, July: 0.01 ppm ± 0.004 ppm) (Appendix c, Table 3.7), and sodium, but only in East campus lake (June: 83.14 ppm ± 6.30 ppm, July: 42.68 ppm ± 10.38 ppm, P= 0.035, N= 1, one-way ANOVA).

Meanwhile, higher values were observed in the July deployment for magnesium (June: 6.90 ppm ± 0.62 ppm, July: 26.11 ppm ± 6.32 ppm), potassium (June: 3.58 ppm ± 0.25 ppm, July: 11.01 ppm ± 2.50 ppm), copper (June: 0.006 ppm ± 0.0001 ppm, July: 0.010 ppm ± 0.00008 ppm) (Appendix c, Table 3.7), and nickel, but only in East campus lake (June: 0.000014 ppm ± 0.000005 ppm, July: 0.0007 ppm ± 0.0001 ppm, P= 0.032, N= 1, one-way ANOVA).

Lake factor

Although specific differences did occur between the two lakes, many of these were the same as those described in Chapter 2 (section 5). As such, results described here will focus only on the lake effects that are specific to one of the two deployment times.

The physico-chemical measurements that were higher on East campus lake, meanwhile, were temperature (West campus: 18.68^{o}C ± 0.24 ^{o}C, East campus: 20.65 ^{o}C ± 0.37 ^{o}C), fluoride (West campus: 0.10 ppm ± 0.005 ppm, East campus: 0.24 ppm ± 0.02 ppm), calcium (West campus: 10.32 ppm ± 1.33 ppm, East campus: 23.09 ppm ± 1.53 ppm), EC, but only during the late summer deployment (West campus: 699.67 us/cm^2 ± 9.49 us/cm^2, East campus: 890.67 us/cm^2 ± 1.33 us/cm^2, P <0.001, N=1, one-way repeated measure ANOVA), sulphate (West campus: 18.07 ppm ± 0.62 ppm, East campus: 42.34 ppm ± 2.96 ppm), Sodium, but only at the end of the early summer deployment (West campus: 29.64 ppm ± 7.99 ppm, East campus: 83.14 ppm ± 6.30 ppm, P=0.006, N=1, one-way repeated measure ANOVA), and nickel (West campus lake: 0 ppm ± 0 ppm, East campus lake: 0.005 ppm ± 0.0002 ppm).

Table 3.7. Two-way repeated measures ANOVA of the physico-chemical measurements of the three sites on each lake at week four and week ten of the experiment.

	Nitrate			Nitrite			Ammonium			Total Nitrogen			Phosphate		
	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Time	7.231	1.000	0.055	3.696	1.000	0.127	2.057	1.000	0.225	71.104	1.000	**0.001**	1.556	1.000	0.280
Lake	0.890	1.000	0.399	3.824	1.000	0.122	2.625	1.000	0.181	3.322	1.000	0.142	8.808	1.000	**0.041**
Time * Lake	1.209	1.000	0.333	5.963	1.000	0.071	2.964	1.000	0.160	0.259	1.000	0.638	3.021	1.000	0.157

	Light attenuation			Dissolved Oxygen			pH			Temperature			Electrical Conductivity		
	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Time	0.807	1.000	0.420	171.232	1.000	**<0.001**	1.813	1.000	0.249	15.338	1.000	**0.017**	2593.506	1.000	**<0.001**
Lake	18.349	1.000	**0.013**	3.971	1.000	0.117	3.179	1.000	0.149	35.887	1.000	**0.004**	176.339	1.000	**<0.001**
Time * Lake	0.244	1.000	0.647	3.566	1.000	0.132	12.473	1.000	**0.024**	0.056	1.000	0.824	1677.388	1.000	**<0.001**

	Alkalinity			Dissolved Organic Carbon			Silicon			Magnesium			Potassium		
	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Time	0.014	1.000	0.913	0.085	1.000	0.785	178.759	1.000	**<0.001**	10.768	1.000	**0.030**	10.072	1.000	**0.034**
Lake	82.381	1.000	**0.001**	2.951	1.000	0.161	33.000	1.000	**0.005**	1.924	1.000	0.238	1.350	1.000	0.310
Time * Lake	9.470	1.000	0.037	0.099	1.000	0.769	29.000	1.000	**0.006**	1.064	1.000	0.361	0.848	1.000	0.409

	Total Suspended Solids			Copper			Fluoride			Sodium			Chloride		
	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Time	2.516	1.000	0.188	864.027	1.000	**<0.001**	0.533	1.000	0.506	26.788	1.000	**0.007**	0.012	1.000	0.916
Lake	40.147	1.000	**0.003**	4.256	1.000	0.108	26.106	1.000	**0.007**	16.844	1.000	**0.015**	4.038	1.000	0.115
Time * Lake	0.858	1.000	0.407	7.871	1.000	**0.049**	2.511	1.000	0.188	10.093	1.000	**0.034**	2.442	1.000	0.193

	Sulphate			Zinc			Calcium			Iron			Aluminium		
	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Time	8.074	1.000	**0.047**	6.543	1.000	0.063	2.013	1.000	0.229	0.015	1.000	0.907	0.219	1.000	0.664
Lake	47.649	1.000	**0.002**	1.045	1.000	0.365	41.371	1.000	**0.003**	2.475	1.000	0.191	0.100	1.000	0.768
Time * Lake	9.424	1.000	**0.037**	0.639	1.000	0.469	0.571	1.000	0.492	7.500	1.000	0.052	0.911	1.000	0.394

	Lead			Nickel		
	F	df	P	F	df	P
Time	0.015	1.000	0.907	203.777	1.000	**<0.001**
Lake	2.475	1.000	0.191	179.714	1.000	**<0.001**
Time * Lake	7.500	1.000	0.052	13.411	1.000	**0.022**

3.5.2. Linear regressions of the physico-chemical measurements against the diatom LTDI2 values between early and late summer

All of the LTDI2 values for the biofilms from all of the substratum and from both early and late summer in both lakes were compared against the corresponding physico chemical measurements taken at the sites the biofilm samples were collected from, to ascertain which parameters were driving changes in the diatom community structure (results in Table 3.8). LTDI2 was not significantly affected by any of the physico-chemical measurements from East campus lake, likely due to the more static nature of the biofilms throughout the experiment, as well as the much smaller variations in the physico chemical measurements in this lake, as seen in Chapter 2.

However, seasonal differences in diatom communities developed in West campus lake correlated with several factors. Increased concentrations of TN, temperature, EC, silicon and copper, which demonstrated strong correlations, as well as nitrate, nitrite and phosphate, which demonstrated weaker correlations influenced the diatom community structure, lowering the LTDI2 score of the biofilms. Meanwhile, the LTDI2 scores of the diatom communities were positively influenced by magnesium, potassium, zinc, TSS concentrations, as well as the alkalinity and pH of the lake.

Table 3.8. Linear regression summary of the physico-chemical measurements against the LTDI2 values of communities developed in the two campus lakes in the early summer and later summer.

	West campus lake						East campus lake					
	R Square	Std. Error of the Estimate	Gradient	F	df	P	R Square	Std. Error of the Estimate	Gradient	F	df	P
Nitrate	0.420	0.13298	-	11.569	1	**0.004**	0.037	0.02735	+	0.612	1	0.446
Nitrite	0.449	0.12953	-	13.059	1	**0.002**	0.033	0.02741	+	0.545	1	0.471
Ammonium	0.082	0.16724	+	1.430	1	0.249	0.013	0.02768	-	0.216	1	0.648
Total Nitrogen	0.849	0.06786	-	89.874	1	**<0.001**	0.009	0.02774	-	0.152	1	0.701
Phosphate	0.577	0.11351	-	21.840	1	**<0.001**	0.015	0.02766	-	0.240	1	0.631
Light attenuation	0.156	0.16038	-	2.954	1	0.105	0.120	0.02615	-	2.177	1	0.160
Dissolved Oxygen	0.056	0.10525	-	0.417	1	0.539	0.222	0.01618	+	1.993	1	0.201
pH	0.395	0.13581	+	10.430	1	**0.005**	0.033	0.02740	-	0.554	1	0.467
Temperature	0.753	0.08674	-	48.798	1	**<0.001**	0.011	0.02772	+	0.172	1	0.684
Electrical conductivity	0.766	0.08441	-	52.425	1	**<0.001**	0.029	0.02746	-	0.476	1	0.500
Alkalinity	0.441	0.13048	+	12.636	1	**0.003**	0.035	0.02738	+	0.576	1	0.459
Dissolved Organic Carbon	0.121	0.16362	-	2.211	1	0.156	0.058	0.02705	-	0.988	1	0.335
Silicon	0.801	0.07796	-	64.210	1	**<0.001**	0.063	0.02698	-	1.078	1	0.315
Magnesium	0.668	0.10051	+	32.260	1	**<0.001**	0.005	0.02780	-	0.079	1	0.782
Potassium	0.656	0.10231	+	30.578	1	**<0.001**	0.010	0.02773	-	0.161	1	0.693
Total Suspended Solids	0.547	0.11754	+	19.284	1	**<0.001**	0.085	0.02666	+	1.486	1	0.241
Copper	0.719	0.09254	+	40.929	1	**<0.001**	0.017	0.02763	+	0.282	1	0.602
Fluoride	0.035	0.17144	+	0.586	1	0.455	0.086	0.02665	-	1.501	1	0.238
Sodium	0.045	0.17062	+	0.747	1	0.400	0.072	0.02684	+	1.247	1	0.281
Chloride	0.039	0.17110	-	0.652	1	0.431	0.028	0.02748	+	0.463	1	0.506
Sulphate	0.087	0.16679	+	1.525	1	0.235	0.003	0.02783	-	0.046	1	0.832
Zinc	0.421	0.13279	+	11.646	1	**0.004**	0.122	0.02612	+	2.216	1	0.156
Calcium	0.143	0.16162	+	2.664	1	0.122	0.021	0.02757	-	0.347	1	0.564
Iron	0.057	0.16953	+	0.962	1	0.341	0.038	0.02734	-	0.632	1	0.438
Aluminium	0.008	0.17389	+	0.122	1	0.731	0.024	0.02754	+	0.387	1	0.543
Nickel							0.039	0.02732	+	0.647	1	0.433
Lead							0.086	0.02665	-	1.504	1	0.238

3.5.3. MANOVA analysis of the physico-chemical measurements against the diatom LTDI2 values

Table 3.9 shows the results of the MANOVA analysis, which unlike the previous linear regression analysis that determined how the LTDI2 values correlated to the values of each physico-chemical measurement separately, this analysis was used to determine the effects of the physico-chemical parameters against the LTDI2 values of the biofilms, whilst taking into account covariance of the physic-chemical measurements at the early summer and late summer sampling times for each lake. Note that the df and P values for nitrate at East campus lake, as well as lead and nickel at West campus lake were not calculated due to the readings for at least one time point being uniformly 0 ppm. Furthermore, alkalinity could not be calculated as the values from the late summer deployment were consistent throughout the replicates at each lake, preventing the functioning of the calculation for this factor.

These results indicate that the West campus lake LTDI2 values responded to changes in silicon and aluminium concentrations in the early summer, but in the late summer this was fluoride and iron concentrations. Meanwhile on East campus lake, LTDI2 was not significantly affected by variations in any of the physico-chemical parameters in the early summer, which is consistent with the results for the linear regression (Table 3.8.), but was affected by changes in the concentrations of chloride and sulphate in the late summer deployment.

Table 3.9. MANOVA analysis of the twenty-seven physico-chemical measurements against the LTDI2 results of biofilms removed after four weeks of exposure at the early summer timing (12/07/2019 to 10/07/2019) and late summer timing (24/07/2019 to 21/08/2019).

	West campus June deployment			West campus July deployment			East campus June deployment			East campus July deployment		
	F	df	P	F	df	P	F	df	P	F	df	P
Nitrate	6.807	6	0.134	0.858	6	0.626	0.207	5	0.939	1.758	6	0.406
Nitrite	6.830	6	0.133	1.235	6	0.512		5		2.184	6	0.347
Total Nitrogen	1.337	6	0.487	16.512	6	0.058	1.182	5	0.475	0.686	6	0.695
Ammonium		6		5.635	6	0.158	2.055	5	0.294	1.132	6	0.539
Phosphate	1.111	6	0.545	3.525	6	0.237	0.380	5	0.838	4.370	6	0.198
Light attenuation	0.674	6	0.700	0.745	6	0.670	1.913	5	0.315	7.496	6	0.122
Dissolved Oxygen				0.667	6	0.704	0.379	5	0.838			
pH	0.673	6	0.701	0.776	6	0.658	0.162	5	0.961	0.671	6	0.702
Temperature	1.000	6	0.578	17.000	6	0.057	1.373	5	0.422	5.148	6	0.172
Electrical Conductivity	4.621	6	0.189	1.397	6	0.474	0.200	5	0.942	2.481	6	0.315
Alkalinity	0.720	6	0.681		6			5		1.078	6	0.554
Dissoled Organic Carbon	1.917	6	0.382	1.038	6	0.566	0.739	5	0.643	2.646	6	0.300
Silicon	3105.373	6	**<0.001**	1.629	6	0.428	2.044	5	0.295	0.882	6	0.618
Magnesium	0.674	6	0.701	1.184	6	0.525	0.601	5	0.712	1.053	6	0.562
Potassium	1.547	6	0.443	0.908	6	0.608	0.711	5	0.656	0.990	6	0.581
Total Suspended Solids	0.668	6	0.703	1.912	6	0.383	0.572	5	0.727	9.000	6	0.103
Copper	2.383	6	0.325	2.371	6	0.326	0.177	5	0.954	1.317	6	0.492
Fluoride	0.743	6	0.671	28.598	6	**0.034**	0.311	5	0.879	1.272	6	0.503
Sodium	0.887	6	0.616	5.768	6	0.155	0.289	5	0.892	0.999	6	0.578
Chloride	3.274	6	0.252	0.725	6	0.678	0.557	5	0.735	30.735	6	**0.032**
Sulphate	0.896	6	0.613	2.140	6	0.352	0.536	5	0.747	234.742	6	**0.004**
Zinc	0.798	6	0.649	0.678	6	0.699	1.088	5	0.505	0.825	6	0.639
Calcium	1.711	6	0.414	2.733	6	0.292	1.139	5	0.488	3.336	6	0.249
Iron	2.405	6	0.322	26.431	6	**0.037**	0.331	5	0.868	0.988	6	0.582
Alumnium	22.434	6	**0.043**	0.720	6	0.681	0.823	5	0.605	2.849	6	0.282
Nickel		6			6		0.388	5	0.833	0.803	6	0.647
Lead		6			6			5		1.000	6	0.578

3.5.4. Summary

To summarise, there were significant differences between the early summer and late summer timings for the physico-chemical measurements for both lakes, with higher concentrations in the early summer of TN, DO, silicon and sodium, as well as higher temperatures and electrical conductivity. In the late summer period, higher concentrations of magnesium, potassium, copper and nickel occurred. Although, as demonstrated in the graphs shown in the appendix (Appendix c), the differences were much larger on West campus lake than East campus lake. Furthermore, when using the LTDI2 index to determine how these parameters affected the composition of the diatom community, the communities on East campus lake were not affected by the variations in the physico-chemical parameters, whilst West campus biofilms appeared to be strongly affected by multiple parameters when they were measured independently by linear regression. However, when analysed together to consider the effects of covariance of the physico-chemical measurements, MANOVA analysis showed much more limited effects of the physico-chemical properties of the lakes on the composition of the diatom communities. Furthermore, water temperatures during were also similar during the two sampling times, and the light regime during sampling was only different for West campus lake, based on the light attenuation, but not TSS, and were also not considered significant in the MANOVA analyses.

3.6. Discussion:

As discussed in the previous chapter, there were very limited and consistent differences between the three test substratum, based on the limited differences between the biological endpoints (biomass measurements, algal groups, diatom species, diversity indices, and UKTAG endpoints), indicating that any of the three substratum could be reasonably used without significantly altering the results, as supported by Lowe and Gale (1980), and the differences in the biological endpoints (diatom species, LTDI2, percentage motile, percentage organic tolerant, species richness, Shannon-H index) between the two lakes appears to still be the most significant difference between the replicates within the dataset.

3.6.1. Seasonal variation

The biomass measurements indicated that there was no difference in the concentrations of AFDW or chlorophyll-a in East campus lake replicates regardless of whether they were deployed in the early or late summer. However, replicates deployed in West campus lake demonstrated higher AFDW and chlorophyll-a concentrations than the replicates in the June deployment, but this typically differed between replicates and there were sufficiently large error margins in the July deployment values that any variation observed can be questioned, likely due to measurement errors or unusual activity limited to individual replicates

There was no clear seasonal variation of the relative abundances of the different algal groups for either lake, nor for any of the substratum. Although on West campus lake diatoms were more abundant in the July deployment than the June deployment on West campus lake. As West campus lake was cooler by around 1°C during the sampling time of the July deployment this is likely the cause, as diatoms are known to perform better under cooler conditions (Patrick, 1969, Villanueva *et al.*, 2011). The dominance of the biofilms by diatoms on East campus lake, and chlorophytes on West campus lake observed in the replicates deployed in the early summer were similar to additional replicates deployed in the July deployment, indicating that the relative abundances of the algal groups is linked to the nature of the lake itself i.e. physico-chemical conditions of each lake and pre-existing biofilm structures that provided the seeding material for the replicates, rather than the time of the summer or substratum the biofilms were deployed on. There were also some differences on the East campus ceramic tiles, whereby the abundance of chlorophytes demonstrated a ~5% increase in abundances, but no effect was observed on the microscope slides and sandstone substratum deployed on West campus lake.

Focussing on the diatom specific endpoints (diatom species relative abundance, diversity indices and UKTAG endpoints), East campus lake presented very limited changes between early and late summer, and where difference in the species present did occur, as the communities were overwhelmingly dominated by *A. minutissimum* and *B. vitrea*, these changes were very small, typically being around a 1% difference. As such, the effects these differences had on the community level measurements (diversity indices and UKTAG endpoints) were negligible. These results therefore indicate limited seasonal changes in the diatom communities present over the summer growing period, and as such, sampling of this lake could be conducted at any point in this period. However, there were significant changes in these diatom endpoints observed specific to West campus lake between the two deployment times, with the results indicating that there was seasonal variation in the community size and structure of the diatom communities in this lake. The most prominent of these changes were related to the significant drop in abundance of *Gomphonema parvulum* between the June and July deployments. This difference in abundance mirrors that of the results of chapter 2, with the same effect of samples removed later in the experiment exhibiting lower abundances of these species. This confirms that this is indeed a seasonal effect, linked to changes in the environmental factors, and not related to continued successional development in the biofilm community, and that four weeks of exposure appears to be sufficient time to develop community's representative of environmental conditions.

As noted in chapter two, the differences in the LTDI2 for West campus lake was linked to the loss of *G. parvulum* over time found in West campus lake, and the changes in this metric was strongly

correlated to the changes of the environmental parameters of the two lakes; notably the concentrations of sulphate, nitrate, magnesium, silicon, chloride, copper and potassium, as well as the electrical conductivity, light attenuation and pH of the water column (Chapter 2, section 5.3.). The changes in these parameters would therefore have caused the decrease in this species, and its replacement by more sensitive species that prefer lower nutrient concentrations. This species was not as abundant in the replicates deployed in late summer, and is considered here to be the main cause of the increase in the evenness and Shannon-H indices, as well as the lowered percentages of organic nutrient tolerant species observed in late summer (Figures 3.5b, 3.5c, and 3.6c). Indicating that these communities developed very differently to those developed in the early summer deployment. This is consistent with the overall trends observed in Chapter 2, and as such the difference in community structure observed over time in West campus lake can be concluded to be due to environmental factors, rather than succession within the biofilm, and that four weeks of exposure in the field is sufficient to develop a community of diatoms on artificial substrates representative of the field conditions for both lakes.

The higher diversity index values for West campus lake demonstrate that this lake is more species rich and the abundances of the species more evenly distributed, compared to East campus lake. The results in late summer do however, show a slightly broader range of evenness index values compared to the early summer results, indicating a reduction in the abundance of the dominant diatom species in some biofilms, but this trend was community-wide, and not limited by substratum. The pattern of lower total biomass based on AFDW measurements, but higher proportions of algal biomass, based on chlorophyll-a measurements in the biofilms seen in East campus lake, compared to West campus lake, has also been seen in river-based experiments in New Zealand and Michigan (Pringle, 1990, Suren *et al.,* 2003). Here, lower biomass biofilms with more limited temporal variations were observed in water bodies with lower nutrient availability, whilst water bodies with higher nutrient availability were found to undergo changes in community structure. These studies also noted that communities in nutrient enriched water were more diverse, a trend that is further shown across the results demonstrated here, with the higher nutrient state (based on Phosphate, N nutrients, and silicon, see Appendix c) West campus lake showing higher diversity and evenness metrics than East campus lake, regardless of seasonal variations, or duration of exposure.

3.6.2. Physico-chemical effects on the diatom communities

The results shown in section 3.5.2 and 2.5.3. indicate that there was no significant correlation between the variation in physico-chemical parameters of East campus lake and the variation in the composition of the diatom communities (LTDI2 values). As covered in chapter 2, this is likely due to the limited temporal variation in both the diatom metrics (Figures 3.4., 3.5., and 3.6.), and the physico-chemical parameters of the lake (Appendix c). Similarly, at West campus lake, there was a more significant link between the physico-chemical measurements and diatom community composition (LTDI2). Although multivariate analysis was inconclusive regarding the effects of the physico-chemical measurements, the independent linear regressions performed on West campus lake indicate that there were strong correlations between LTDI2 and several nutrients (nitrate, nitrite, TN, phosphate, zinc, copper, magnesium, potassium, silicon), as well as pH, temperature, EC, alkalinity, and TSS. Increased concentrations of the nitrate, nitrite, silicon and phosphate nutrients, temperature and electrical conductivity were shown to cause a decrease in the LTDI2 values which is in line with the literature (Chapter 2, Table 2.16), as lower LTDI2 values are indicative of increased nutrient availability. Meanwhile, elemental micronutrients (zinc, copper, magnesium and potassium) were shown to increase the LTDI2. The concentration of magnesium was higher in West campus lake than East campus lake, whilst potassium, and zinc at the end of the late summer deployment were higher in East campus lake. Copper concentrations, as well as zinc concentrations in the early summer, were more or less equal, indicating that these nutrients were likely limiting factors in West campus biofilms.

The higher concentrations of TN occurred in the early summer replicates, when *G. parvulum* was most abundant, than in the late summer deployment, when the abundance of this species was less

than 10% of the community composition. The availability of these nutrients at the site is considered seasonal. As several, including phosphate and ammonium are deposited in the lake through the large populations of seasonal bird communities that inhabit West campus lake, but are deterred from in East campus lake through the plantation of shrubbery unsuited to supporting them (University of York, 2019). These nutrients were observed to decrease over the course of the experiment, as shown in Chapter 2, Section 2.5., and the appendix (Appendix c). As such, these nutrients were higher in the spring/ early summer during the breeding season, than the later summer/ autumn when they began to migrate away, thus influencing the diatom community composition earlier in the summer towards species that prefer more eutrophic conditions. Very little research has been conducted into the effects of micro-nutrients on LTDI2 values and other diatom-based indices, as such it is difficult to ascertain the exact reason for the strong effect these nutrients had on the diatom communities, as the LTDI2 index is designed primarily to measure the effects of macronutrients on the diatom communities. However, as these nutrients are known to be vital to the functioning of diatom cells (Van ael, 2014), this could be due to the increased ability of diatoms that prefer conditions of lower nutrient availability to utilise low concentrations of the micro-nutrients.

3.6.2. Recommendations:

Based on the results developed from biofilm samples taken from test substratum in the year early and late summer of the two campus lakes, and the results of chapter 2, there is the possibility that any freshwater benthic diatom community sampled will have different compositions depending on the time of sampling. As shown in West campus lake, but not East campus lake. This has been interpreted as being due to the lower variation in the physico-chemical properties seen throughout the experiment in this lake, as well as lower values seen for key nutrients for algae, and other key physico-chemical measurements, as shown in both this Chapter, and Chapter 2. As such, the following recommendations for future assessment of sites deemed as potential sources for long-term laboratory replicates are suggested:

- Assessment of diatom community composition throughout the growing period is necessary for the determination of what a community representative of a region should look like. Therefore, seasonal sampling for initial assessments of diatom communities using at least three time points is recommended. These should be conducted at late spring/ early summer, mid-summer, and late summer/ early autumn for the determination of a representative community.

- Seasonal sampling must contain a wide range of physico-chemical measurements of the water body, as the physical properties of the water body, as well as the availability of nutrients contained within it can have a substantial effect on the composition of the diatom community. The results presented in this chapter are not conclusive as to how the factors influence the diatom community composition. However, drawing on the larger dataset from Chapter 2, section 2.5.2 and 2.5.3., the determination of the key factors that affected the sensitivity of the species present can be determined. These were nitrate, nitrite, magnesium, copper, silicon, nickel, and sulphate concentrations, as well as the light attenuation, pH and electrical conductivity of the water body.

Chapter 4: Comparison of benthic biofilm communities from an oligotrophic and mesotrophic lake developed under natural and artificial conditions after one month of colonisation

4.1. Introduction

This chapter will focus on the experiment conducted during the summer of 2019, comparing the development of freshwater benthic diatom communities under controlled laboratory conditions designed to mimic natural conditions as possible. This experiment tested a proposed method for assessing the effects of HPCP chemicals on the structure and function of laboratory-based diatom cultures. The structure and composition of the communities developed after one month were measured (biomass measurements, algal groups and diatom species relative abundances, diatom community diversity indices, and UKTAG assessment endpoints), and compared to those of equivalent biofilms developed on the same substratum at the same time in the campus lakes. The temperature, light intensity, pH and nutrient concentrations the communities were exposed to over the four weeks were measured to monitor how the water-quality of the laboratory replicates changed over time.

In ecotoxicological testing, the methods employed to assess the effects of chemicals on the structure and health of freshwater benthic diatom communities have typically been conducted *in situ*, over several weeks on artificial substratum. These replicates were then transferred to laboratory conditions for the immediate exposure of the developed diatom communities to a chemical once they have fully developed. This practice has the advantage that it has been used for decades, and has been proven to be effective at assessing the effects of herbicides using community level endpoints, based on the structure (diversity indices and taxonomic composition) and health (growth rate) of diatom communities (Bérard *et al.,* 2003, Duong *et al.,* 2008, Wood et al., 2016). However, these methods struggle to separate the effects of natural variations in the environment from the effects of the chemical being tested on the highly sensitive diatom communities (Pérès *et al.,* 1996, Medley and Clements, 1998). Other approaches use axenic, single-species, controlled microcosm setups, for both phytoplanktonic and benthic diatoms, as well as other algal groups (Casotti *et al.,* 2005, Lockert *et al.,* 2006, Ivorra et al., 2012).

Past attempts at creating chemostatic, microcosm community cultures of diatoms, similar to those used in single species studies have been attempted, although there are very few examples of it. Early work attempting to develop diatom community cultures by Roeselers *et al.,* (2006) using microscope slides and a flow-lane setup could not produce accurate replication, nor did the communities grown develop communities with similar taxonomic composition or species richness compared to that seen in the original inoculum. Later work by Morin et al., (2008) testing biofilm community growth on glass slides over six weeks found that there were reduced growth rates and differences for the abundances of 13 species, believed to be due to uncontrolled physico-chemical factors (Dissolved oxygen concentrations), but the majority of the environmental effects were due to temperature and light availability. Experiments performed by Debenest *et al.* (2009b) using biofilms from the river Garonne, France were scraped from river stones and then transferred to a closed plastic box filled with a nutrient enriched medium and sterilised microscope slides for three days. The biofilms developed on these slides were then taken and cultivated in batch replicates in artificial growth mediums modified for the nutrient needs of diatoms; based on the Chu No. 10 and Freshwater WC artificial mediums, and grown on the surfaces of Erlenmeyer flasks, centrifuge tubes and microscope slides held within plastic containers over a further period of 10 days. However, these communities were only able to maintain taxonomic similarity to field replicates for a maximum of 96 hours, after which the community shifted towards generalists and species indicative of eutrophic conditions, losing species sensitive to increases in nutrient availability and reduction in oxygenation of the water in the process. Similar processes of community alteration to a composition indicative of eutrophic

conditions were also observed in work by Congestri and Albertano (2011). Therefore, further development of the methodology for community cultures of diatoms that can retain a community representative of real-world benthic biofilms is necessary before they can be used for ecotoxicity tests, but this will be a significant step forward for the community level testing of benthic diatom communities.

This chapter aims to present a potential method for the laboratory-based assessment of organic contaminants on diatom communities, such as HPCPs, using cultured diatom communities as a representative community for the primary productive trophic level in freshwater ecosystems. To achieve this, an experiment has been devised to test a simple batch microcosm approach for growing a diatom community, whilst comparing the community that develops in these beakers after one month.

4.2. Aims and objectives

Aims:

The aim of this experiment is to assess the differences in benthic diatom community endpoints developed *in situ* (field) in a lake and *in vitro* (laboratory) settings using a twice weekly medium (lake water) replacement, and light and temperature parameters, replicating, as closely as possible, the conditions in the lake.

Objectives:

- To compare the structural development (biomass measurements (ash-free dry weight, chlorophyll-a), relative abundance of algal groups and diatom species, diversity indices (species richness, evenness, Shannon-H index) of the diatom communities, and UKTAG assessment endpoints (LTDI2, percentage motile and percentage tolerant of nutrient enrichment)) of biofilms developed in the field with those developed in a controlled environment over a four-week period. Summer conditions (temperature (17^{o}C), photoperiod (16 hours light, 8 hours dark) and light availability (measured as photosynthetic active radiation (PAR) (300 µmol/m^2/s)) will be used for the cultivation.
- To determine if a 20% replacement of the growth medium twice a week is sufficient to replenish nutrients in the water of the lab experiment so as to mimic the water quality observed in the field.

4.3. Methodology

4.3.1. Experimental setup

For this experiment, the benthic diatom endpoints previously described in Chapter 2 were used on a new set of microscope slide replicates deployed to the same field sites, as well as those deployed under laboratory conditions. The aim was to understand how the communities developed after four weeks in the field compared to those which developed under a controlled laboratory environment designed to mimic the field setting as closely as possible.

Lab incubation

For the controlled lab incubation, 600 ml borosilicate beakers containing analytically clean microscope slides and campus lake water (either West or East) were exposed to typical UK summer water temperatures of 17˚C and a daytime photosynthetic active radiation (PAR) of 300 µmol/m^2/s at the water surface level for 16 hours (5:30-21:30) to mimic summer daylight durations. (Grow and Bloom, UFO 150). The LED lamps were suspended to 15 cm above the water surface to avoid an increase of the water temperature. Four beakers were positioned under each LED lamp in a square and each block of four replicates were moved clockwise under the four PAR lights every day. Each beaker contained 600 ml lake water, with eight beakers per lake. One microscopic slide was positioned horizontally in the water at 10 cm depth, similar to the

positioning in the field, by using strings and two bull clips to suspend the slides from (Figure 4.1. and 4.2.). 3.75 L of water from each lake was collected from the three raft locations in equal amounts and mixed. Twice a week, 20% of the water in the beakers was replaced by freshly collected water from the lakes.

The volume of water evaporated over a 3 to 4-day period was determined and an equivalent amount of deionised (DI) water added to each beaker to replace the lost water. DI was specifically used to prevent contamination by new populations and altering the nutrient concentrations in the water column. After careful mixing to fully integrate the DI water without disturbing the biofilms, 20% of the water was removed carefully to avoid disturbing the biofilms on microscopic slides and replaced by fresh lake water taken from within the past three hours. The removed water from each replicate beaker and a subsample of the fresh water from each lake was filtered using 0.45 µm filters and stored at -20˚C for further water chemistry analysis (Chapter 2, water quality analyses).

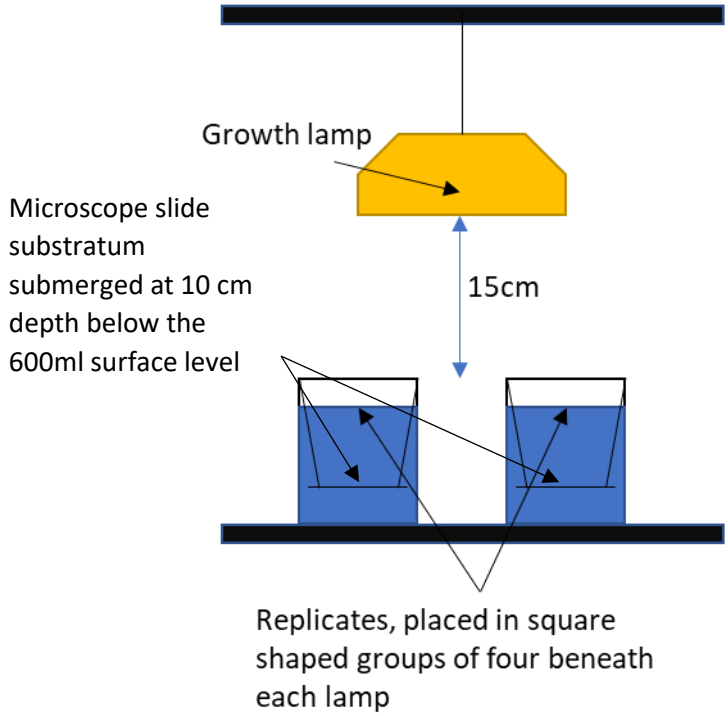


Figure 4.1. Diagram of the layout of the controlled temperature (CT) room replicates, showing the position of the replicate vessels, and the growth lamp, between two shelves of the CT room

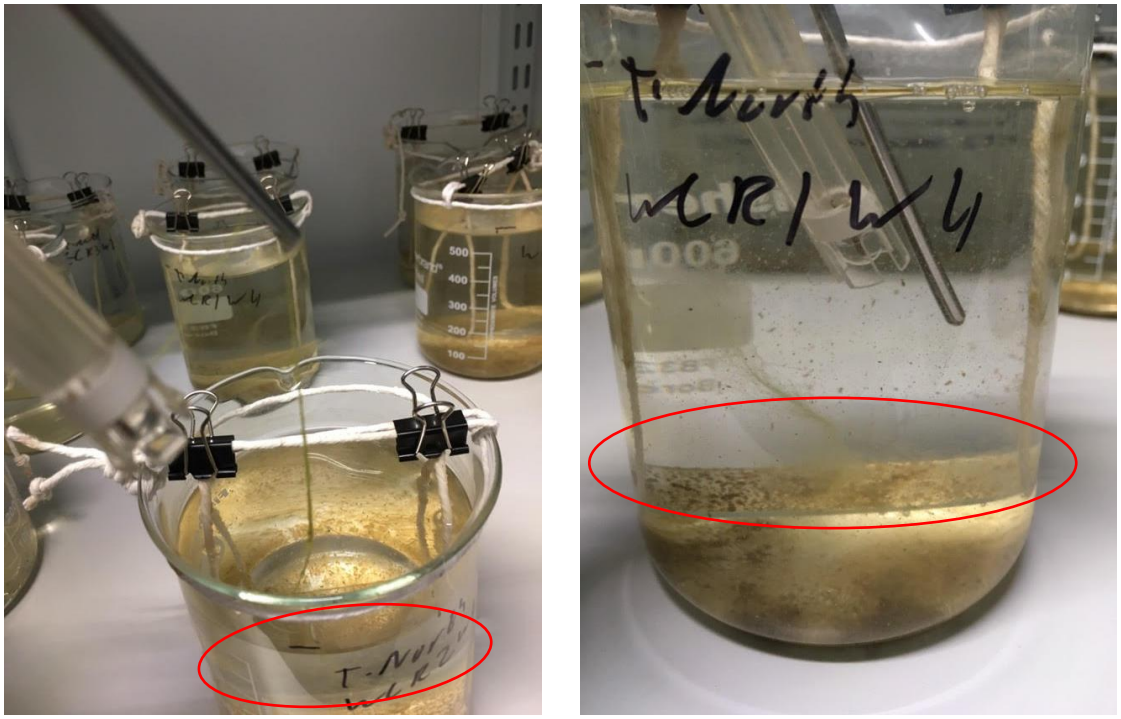


Figure 4.2. Images of the CT room replicates with the position of the microscope slide replicate highlighted by a red ring (a), focusing on a west campus replicate developing macroalgae floating within the water column and (b) close up of a replicate with a pH and temperature probe submerged. Images taken after seven weeks of inoculation.

4.3.2. Sample collection:

Biofilm samples

The same procedures used in Chapter 2 for the removal of biofilms, storage of samples and quantification of total biovolume recovered from the substratum were employed here. However, the biofilm samples of the 16 CT room-based replicates were kept separate, unlike the field where the biofilms retrieved from the triplicate substratum on each raft were combined, as although the triplicates at each site were exposed to the same body of water, the CT room replicates were each kept in an isolated water supply, which may have differentiated over time. Four subsamples were then taken after thorough mixing of the suspension, for chlorophyll-a content. Ash-Free Dry Weight (AFDW), relative abundance of algal groups, and diatom (relative abundance, diversity indices and LTDI2 assessment) analyses. Full details of methods can be found in Chapter 2.

Water quality measurements

Field temperatures were recorded (every hour) using Maxim integrated ibuttons contained within a plastic/rubber holder and attached to the raft using cable ties. Surface PAR at water level was recorded in the field using a Skye instruments PAR special attached to a datalogger and the field PAR continuously measured (every five minutes) at approximately 3 m above the water surface (PAR special sensor, Skye instruments, connected to a DL2e datalogger (Delta-T Devices). For the CT room replicates, the water temperature and pH at 10 cm depth (Accumet AP72 probe) was checked twice a week, and the surface PAR was measured twice a week using a Skye instruments PAR special sensor. The water of both lakes at the start of the lab experiment, the lake water after 3-4 days incubation in each of the beakers, and the fresh lake water used to replace 20% of the water in the beakers were analysed for the same nutrient concentrations measured through water samples taken as in Chapter 2 were used, except for the elemental (magnesium, copper, potassium, iron, lead, sodium, zinc, nickel, phosphorus, silicon and calcium) nutrient concentrations measured using the ICP-OES (Chapter 2, water quality analyses), and total suspended solids.

Data analysis

Results of biofilm biological endpoints measured from both the CT rooms and field sites were graphed as bar graphs in Microsoft Excel. Additional line graphs were plotted for the temperature, pH and Surface PAR measurements for both the CT room and field replicates. Further line graphs were also plotted for the CT room replicates nutrient concentrations, with an additional set of lines for the calculated nutrient concentrations of the replicates after a 20% replacement using the following calculation:

$$Nc\ concentration\ (ppm) = \left(\frac{Nrem}{100} X\ 80\right) + \left(\frac{Nrep}{100} X\ 20\right)$$

Where:

Nc is the calculated concentration of a given nutrient at a specific time

Nrem is the concentration of a given nutrient in 20% medium removed from the replicate at a given time

Nrep is the concentration of a given nutrient in the replacement medium added to the replicates

To compare the nutrient concentrations in the CT room replicates to those in the field sites additional graphs were plotted in Excel for the nutrient concentrations, showing the concentrations for the CT room replicate mediums removed at the fourth and eighth replacement times (end of the second and fourth week of the experiment) (7th and 21st of August 2019), compared against the equivalent field data for those days from the field experiment taken from the data collected for Chapter 2.

For the Surface PAR and temperature data, line graphs were used, using additional data from the iButtons and the PAR special sensor attached to the DL2e datalogger to provide a continuous field comparison to the perceived static values observed in the CT rooms. For temperature, average daily temperature was calculated using the hourly values to create a mean and standard error for each day (N=24). For the surface PAR the mean and standard error for the PAR data were generated using the data recorded every five minutes for the same 16 hours the growth lamps in the CT room were active for (5:30am to 9:30pm).

Statistical analysis

Statistical analyses were performed for all datasets in IBM SPSS software version 26. For all data, a Kolmogorov-Smirnov test was performed to test for normality and a Levene's test to determine whether variances were equal. If this was not the case, data were transformed (arcsine square root for percentages, square root for counts and log10 for continuous data). If the transformed data were still normally distributed and/or variances were not equal ($P \leq 0.05$), then non-parametric alternatives were used (Friedman test instead of a 1-way repeated measures ANOVA, and Kruskal-Wallis H test instead of a 1-way ANOVA) or, if not available, the dataset (untransformed or transformed) with the highest P values for Kolmogorov-Smirnov tests and Levene's tests were used for the ANOVAs.

Two-way ANOVAs were used to assess the effects of exposure outdoors/indoors (factor 'field/lab') and the water quality of the two lakes (factor 'lake') on the algal and diatom endpoints after four weeks of exposure.

For the lab incubation replicates, two-way repeated measure ANOVAs were used to assess the variation of the nutrient concentrations to test the significance of the changes in nutrient concentrations in the replicate vessels over the four weeks of incubation and between the replicate vessels supplied with water from the two lakes.

Further statistical analysis was conducted using a three-way repeated measure ANOVA, incorporating the same factors described above but with an additional lab/field factor for the surface PAR and temperature data, to additionally test the differences of these factors the field

replicates were exposed to, compared to those incubated in the CT rooms. This analysis was also conducted on the nutrient data, however, instead of using direct field comparisons with the nutrient data, as there was only a single combined sample for each lake taken for these results, the third factor was instead a before 20% replacement and after 20% replacement, to compare the changes in nutrient concentrations caused by the replacement of the medium and identify if there is any accumulation or excessive uptake of the nutrients in the CT room replicates during the 3-4 day replacement window.

As the previous two chapters have heavily discussed the differences between the biofilms of the two lakes, the results of this chapter will instead focus on the differences between the biofilms developed on replicates developed in the field or the CT rooms, with specific differences and similarity between lake discussed only if it is strictly relevant to the aim and objectives of the experiment.

4.4. Biofilm results

4.4.1. Biomass

The AFDW (Figure 4.3.) concentrations of the biofilms developed on the microscope slides for four weeks were significantly higher in the field replicates (WC: 1.62 mg/cm^2 ± 0.65 mg/cm^2, EC: 0.48 mg/cm^2 ± 0.17 mg/cm^2) than the CT room replicates (WC: 0.57 mg/cm^2 ± 0.07 mg/cm^2, EC: 0.19 mg/cm^2 ± 0.06 mg/cm^2) (Figure 4.4., Table 4.1.). With a similar effect occurring for the chlorophyll-a (Figure 4.4) concentrations. Although due to the high degree of uncertainty in the West campus field replicates results, this is only statistically significant for East campus lake (Field: 0.39 ug/cm^2 ± 0.12 ug/cm^2, CT rooms: 0.09 ug/cm^2 ± 0.04 ug/cm^2, P=0.041, Mann-Whitney U test).

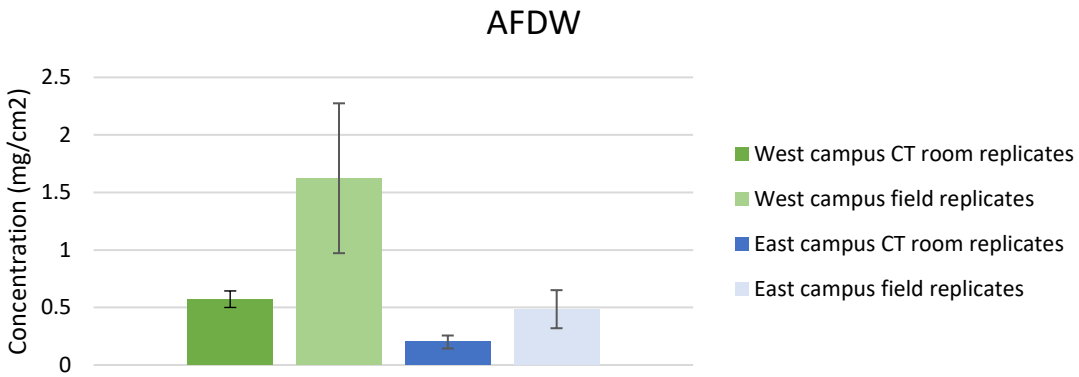


Figure 4.3. Ash-Free Dry Weight (AFDW) concentrations (in mg/cm^2) of biofilms developed on microscope slides for four weeks for replicates in the field (West campus lake or East campus lake, n=3) and replicates in the CT room with water from West campus lake or East campus lake (n=8). Mean ± SE.

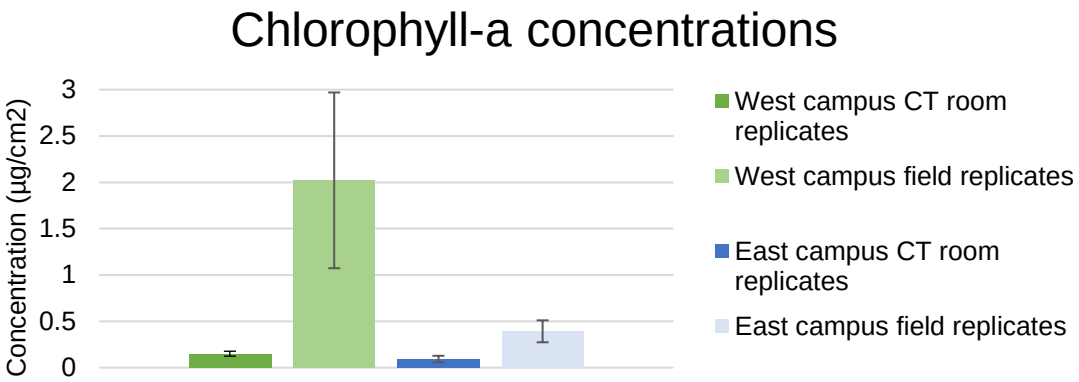


Figure 4.4. Chlorophyll-a concentrations (in µg/cm^2) of biofilms developed on microscope slides for four weeks for, replicates in the field (West campus lake or East campus lake, n=3) and replicates in the CT room with water from West campus lake or East campus lake (n=8). Mean ± SE.

Table 4.1. Two-way ANOVA results for the AFDW and chlorophyll-a concentrations using lab/field setup, and lake of origin as factors.

	AFDW			Chlorophyll a		
Source	F	df	P	F	df	P
Lab/field	11.051	1	**0.004**	16.538	1	**0.001**
Lake	14.099	1	**0.001**	10.002	1	**0.005**
Lab/field * Lake	3.630	1	0.073	8.675	1	**0.009**

To summarise, for both AFDW and Chlorophyll-a, concentrations were higher in West campus lake, compared to East campus lake, but were also significantly higher in the field replicates, compared to the CT room replicates.

4.4.2. Algal group

There was no significant difference in the relative abundance of cyanobacteria between the lab and field incubation (Figure 4.5., Table 4.2.). Furthermore, the differences between the lab and field settings allows the diatom and chlorophyte abundances of East campus lake CT room replicates to be similar to those seen in West campus lake, regardless of lab/ field setting. Specific differences between CT room and field replicates limited to replicates derived from each lake occurred. These were:

West campus lake:

The West campus replicates showed similar abundances regardless of whether they were developed in the field or in the CT room. As such, it can be concluded that there was no significant effect on the abundances of the algal groups caused by the differences between field and laboratory setting in biofilms derived from this lake

East campus lake:

Diatom abundances were higher in the field replicates (74.62% ± 12.33%), compared to the CT rooms (26.73% ± 4.41%) (P=0.014, Mann-Whitney U test), whilst the inverse is true for the chlorophytes, with the abundance of the chlorophytes being higher in the CT room replicates and lower in the field replicates (CT room: 49.47% ± 5.94%, field replicates: 13.04% ± 0.45%, P=0.014, Mann-Whitney U test) (Figure 4.5., Table 4.2.). As such, the chlorophytes replaced the diatoms as the dominant algal group under CT room conditions for East campus lake derived replicates.

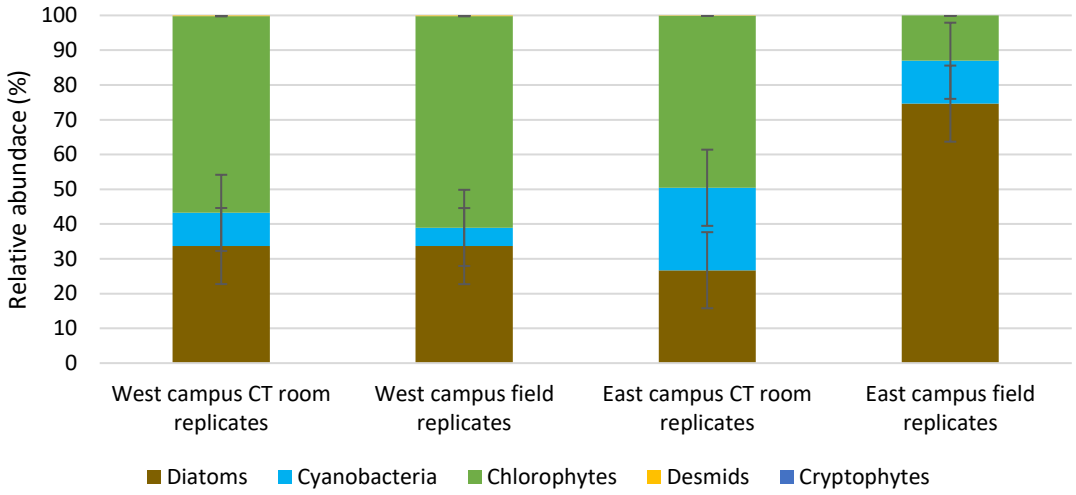


Figure 4.5. Graphs showing the percentage abundance of diatoms, cyanobacteria, chlorophytes, desmids and cryophytes seen in biofilms developed on microscope slides in West campus lake water in the controlled temperature (17°C) room, West campus lake water in situ, East campus lake water in the controlled temperature (17°C) room, and East campus lake).

Table 4.2. Two-way and one-way ANOVA results for the three main algal groups observed in the experiments results, diatoms on the left column, cyanobacteria in the centre column, and cyanobacteria on the right-hand column, starting with the initial two-way ANOVA results, followed by the one-way ANOVAs split by lake, and then the one-way ANOVAs split by lab/field.

	Diatoms			Cyanobacteria			Chlorophytes		
Source	F	df	P	F	df	P	F	df	P
Lab/Field	22.646	1	**<0.001**	1.060	1	0.312	6.453	1	**0.021**
Lake	11.676	1	**0.003**	2.094	1	0.165	18.650	1	**<0.001**
Lake * Field	23.998	1	**<0.001**	0.162	1	0.692	8.405	1	**0.010**

4.4.3. Diatom species results

There were significant changes between the CT room replicates and the field replicates regarding the relative abundance of the individual diatom species. Whilst the composition of the communities when split by lake of origin were similar to those seen in Chapters 2 and 3 at week 10 (East campus lake primarily composed of *A. minutissiumum*, followed by *C. disculus*, *G. cuneolus*, and *B. vitrea*. West campus lake was primarily composed of *N. paleacea*, followed by lower abundances (~10%) of *A. daonenese* and *A. minutissimum*, and the abundances of all other species below <10%).

There was no difference in the relative abundances of eight of the 34 species observed in this experiment between the CT room and lake deployed replicates (Figure 4.6., Table 4.4.). These were *B. brebisonii*, *E. turgida*, *F. vaucheriae*, *Gyrosigma accuminatum*, *H. capitata*, *N. capitatoradiata*, *N. cryptocephala*, and *F. spp.* However, three species were present in significantly higher relative abundances on field replicates than in CT rooms. These included *A. minutissimum*, (field: 12% for West campus and 75% for East campus, CT rooms: 2% for West campus, 22% for East campus) ($P<0.014$, Kruskal-Wallis H test), *A. daonense* (Field: 12.55% ± 7.47% (WC), 0% (EC), CT room: 1.49% ± 0.96% (WC), 0.39% ± 0.24% (EC)) and *G. cuneolus* (for lakes, field: 3-5% field, CT room: 0-3%) (Figure 4.6., Table 4.4.). Further differences between the abundance of the individual species occurred, but these were not consistent between the two lakes. See Table 4.3. to see the full breakdown of species by preference for lake and lab/field setting.

Additional differences between the CT room and field replicates were observed however these occurred only in replicates derived from a specific lake. These were:

West campus lake:

There were 10 species which grew in higher abundances in West campus field replicates, compared to their field eqivalents. These included *N. acicularis*, a major component of field replicates, which virtually disappeared in the CT room replicates, as well as *C. disculus*, *E. neogracile*, *E. prostratum*, *G. accuminatum*, *G. olivaceum*, *G. parvulum*, *R. abbreviata*, *N. amphibia* and *N. dissipata.* These latter four species were only present in the field replicates (Figure 4.6., Table 4.4.). [Furthermore, *N. linearis* was also only identified in West campus CT room replicates.]

Additionally, *N. acicularis* was also more abundant on CT room replicates (West campus CT room replicates: 19% ± 5%, West campus field replicates 0-0.5%) (P=0.001, Kruskal-Wallis H test). Another five species were more abundant in the field for West campus replicates. These were *E. prostratum* (West campus field replicates: 1.2%±0.2%) (all other replicates: 0-0.5%) (P=0.034, Kruskal-Wallis H test), as well as *G. accuminatum*, *G. olivaceum*, *G. parvulum* (West campus field: 5% ± 2%, West campus CT room: <0.5%) (all P=0.046, Kruskal-Wallis H test) (Figure 4.6., Table 4.4.), as well as trend towards this same effect for *S. ulna* S. ulna (West campus field replicates: 5% ± 2%, CT room replicates: 0-0.5%, P=.0.64, Kruskal-Wallis H test. It is also worth noting that all these species in this paragraph shared similar abundances in their least abundant setting to all East campus replicates.

East campus lake:

The relative abundance of *A. pediculus* was significantly higher in the field than in the CT room for East campus lake replicates (Figure 4.6., Table 4.4.).] Furthermore, there were five species which were present in increased abundances in the CT room replicates, compared to their equivalents grown in the field for East campus lake. These species were *G. striata*, which was barely present in the field, but grew to be a major component of the community in the East campus CT room replicates (field: <1%, CT room: 17%, P=0.049, Mann-Whitney U test), with a similar major increase occurring for *B. vitrea*, and minor increases for *E. reichardtii*, *N. dissipata* and *R. gibba*, which were also present in 3-5% of total relative abundance in CT room replicates on East campus lake, but not present in field replicates (Figure 4.6., Table 4.4.).

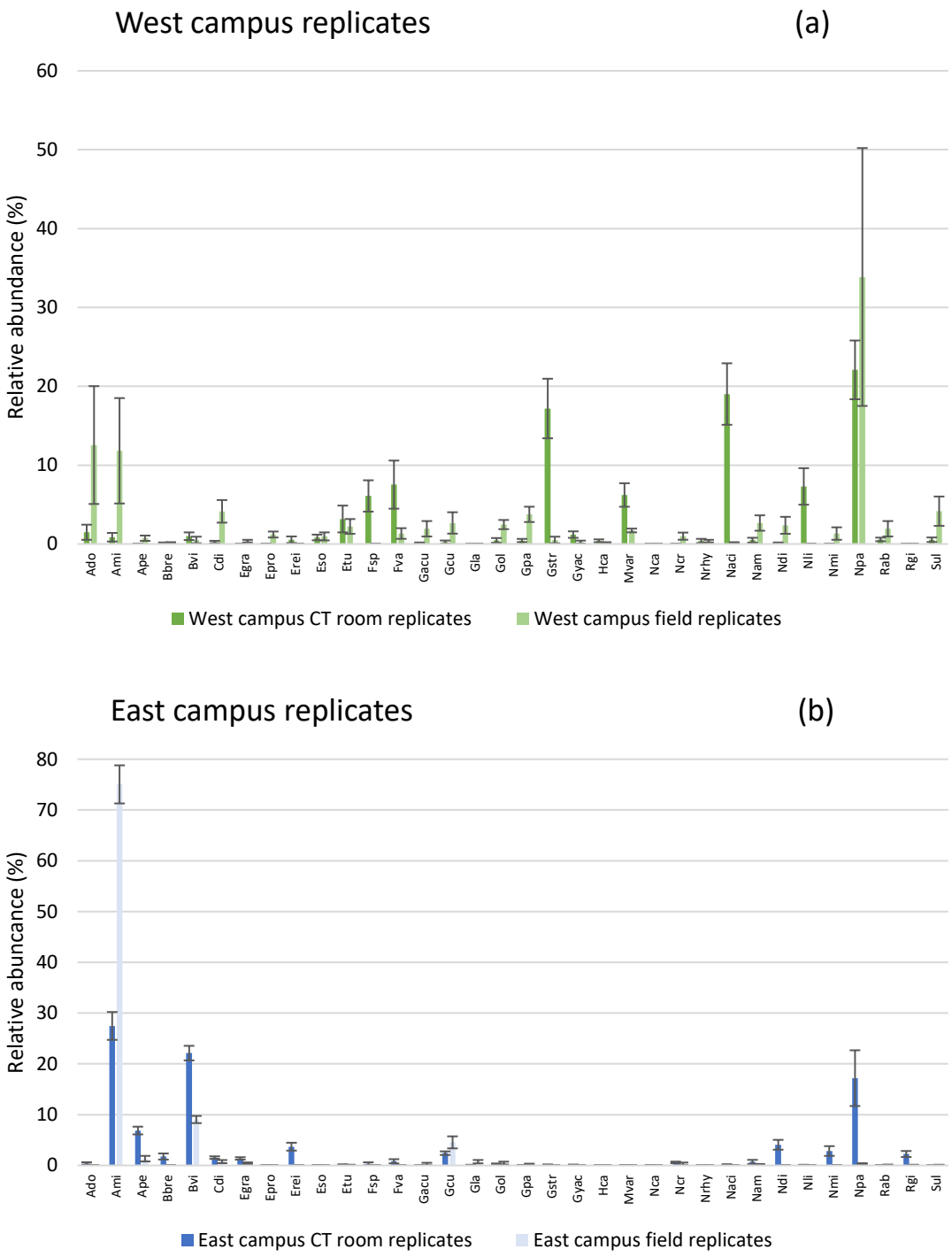


Figure 4.6. Species relative abundance graphs for (a), the west campus lake CT (controlled temperature) room replicates (WCLR) and west campus lake field replicates (WCFR), and (b) the east campus CT room replicates (ECLR) and east campus lake field replicates (ECFR). Species listed alphabetically on x axis, relative abundance (%) on the y axis (see table 13 for species full names). See the appendix (Appendix h) for table with mean ± SE for individual species

Table 4.3. Species id for the species labelled in Figure 6, with the summary of the interaction effects (CT room/ Field effect split by lake (West campus lake (WC) or East campus lake (EC), and lake effect split by CT room (CT) and Field).

Graph name	Species iD	Interaction effects			
		CT room/ Field		Lake	
		WC	EC	CT	Field
Ado	*Achnanthidium daonenese*	> Field	> Field	> WC	> WC
Amin	*Achnanthidium minutissimum*	> Field	> Field	> EC	> EC
Ape	*Amphora pediculus*	-----	> CT	> EC	-----
Bbre	*Brachsira brebisonii*	-----	-----	-----	-----
Bvit	*Brachysira vitrea*	-----	> CT	> EC	-----
Cdi	*Cocconeis disculus*	> Field	-----	-----	-----
Eneo	*Encyonema neogracile*	> Field	> CT	> EC	-----
Epro	*Encyonema prostratum*	> Field	-----	-----	> WC
Erei	*Encyonema reichardtii*	-----	> CT	> EC	-----
Eso	*Epithema sorex*	-----	-----	> WC	> WC
Etu	*Epithemia turgida*	-----	-----	> WC	> WC
Fsp	*Fragilaria species*	-----	-----	-----	-----
Fva	*Fragilaria vaucheriae*	-----	-----	-----	-----
Gac	*Gomphonema accuminatum*	> Field	-----	-----	-----
Gcu	*Gomphonema cuneolus*	> Field	> Field	> EC	> EC
Gla	*Gomphonema lateripunctum*			> EC	-----
Gol	*Gomphonema olivaceum*	> Field	-----	-----	> WC
Gpa	*Gomphonema parvulum*	> Field	-----	-----	> WC
Gacc	*Gyrosigma accuminatum*	-----	-----	-----	-----
Gstr	*Guinardia striata*	-----	> CT	> WC	> WC
Hca	*Hippodonta capitata*	-----	-----	-----	-----
Mvar	*Melosira varians*	-----	-----	> WC	> WC
Nca	*Navicula capitatoradiata*	-----	-----	-----	-----
Ncr	*Navicula crpytocephala*	-----	-----	-----	-----
Nrhy	*Navicula rhynchotella*	-----	-----	> WC	> WC
Nac	*Nitzschia acicularis*	> Field	-----	-----	> WC
Nam	*Nitzschia amphibia*	> Field	-----	-----	-----
Ndi	*Nitzschia dissipata*	> Field	> CT	-----	-----
Nli	*Nitzscshia linearis*	> CT	-----	-----	-----
Nmi	*Nitzschia minuta*	> Field	> Field	-----	-----
Npa	*Nitzschia paleacea*	-----	> CT	-----	-----
Rab	*Rhoicosphenia abbreviata*	> Field	-----	> WC	> WC
Rgi	*Rhopaladia gibba*	> Field	> CT	-----	-----
Sul	*Synedra ulna*	-----	-----	> WC	> WC

Table 4.4. Two-way ANOVA results for the diatom species identified in the field: CT room growth comparison experiment. Lab/field factor compares replicates grown in field or CT room settings, WCEC factor is for west campus or east campus lake of origin.

	Achnanthidium daonenese			*Achnanthidium minutissimum*			*Amphora pediculus*			*Brachsira brebisonii*			*Brachysira vitrea*		
Source	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Lab/field	4.740	1	**0.043**	80.528	1	**<0.001**	10.866	1	**0.004**	2.367	1	0.141	19.446	1	**<0.001**
Lake	5.935	1	**0.025**	176.743	1	**<0.001**	33.391	1	**<0.001**	2.153	1	0.160	170.239	1	**<0.001**
Lab/field * Lake	5.522	1	**0.030**	43.488	1	**<0.001**	17.869	1	**0.001**	2.745	1	0.115	20.208	1	**<0.001**

	Cocconeis disculus			*Encyonema gracile*			*Encyonema prostratum*			*Encyonema reichardtii*			*Epithema sorex*		
Source	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Lab/field	8.365	1	**0.010**	1.181	1	0.292	17.513	1	**0.001**	6.958	1	**0.017**	1.391	1	0.254
Lake	1.357	1	0.259	10.282	1	**0.005**	17.513	1	**0.001**	5.868	1	**0.026**	7.343	1	**0.014**
Lab/field * Lake	16.566	1	**0.001**	5.563	1	**0.030**	20.348	1	**<0.001**	5.868	1	**0.026**	1.879	1	0.187

	Epithemia turgida			*Fragilaria vaucheriae*			*Gomphonema accuminatum*			*Gomphonema cuneolus*			*Gomphonema lateripunctum*		
Source	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Lab/field	0.218	1	0.646	1.278	1	0.273	10.019	1	**0.005**	14.642	1	**0.001**	7.615	1	**0.013**
Lake	4.215	1	0.055	2.153	1	0.160	5.167	1	**0.036**	15.720	1	**0.001**	10.636	1	**0.004**
Lab/field * Lake	0.236	1	0.633	0.167	1	0.687	4.604	1	**0.046**	0.216	1	0.648	7.615	1	**0.013**

	Gomphonema olivaceum			*Gomphonema parvulum*			*Gyrosigma accuminatum*			*Hippodonta capitata*			*Melosira varians*		
Source	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Lab/field	22.525	1	**<0.001**	27.735	1	**<0.001**	1.129	1	0.302	0.013	1	0.909	0.613	1	0.444
Lake	9.951	1	**0.005**	25.982	1	**<0.001**	2.672	1	0.120	3.876	1	0.065	13.515	1	**0.002**
Lab/field * Lake	13.441	1	**0.002**	22.246	1	**<0.001**	0.107	1	0.748	0.013	1	0.909	0.495	1	0.491

	Navicula capitatoradiata			*Navicula crpytocephala*			*Navicula rhynchotella*			*Nitzschia acicularis*			*Nitzschia amphibia*		
Source	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Lab/field	0.351	1	0.561	2.321	1	0.145	0.491	1	0.492	7.483	1	**0.014**	3.373	1	0.083
Lake	0.351	1	0.561	0.151	1	0.702	4.418	1	0.050	7.251	1	**0.015**	2.967	1	0.102
Lab/field * Lake	0.351	1	0.561	5.290	1	**0.034**	0.491	1	0.492	6.797	1	**0.018**	8.026	1	**0.011**

	Nitzschia dissipata			*Nitzscshia linearis*			*Nitzschia minuta*			*Nitzschia paleacea*			*Rhoicosphenia abbreviata*		
Source	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Lab/field	1.290	1	0.271	4.204	1	0.055	0.865	1	0.365	0.114	1	0.740	6.407	1	**0.021**
Lake	1.195	1	0.289	3.702	1	0.070	0.865	1	0.365	2.025	1	0.172	8.018	1	**0.011**
Lab/field * Lake	10.572	1	**0.004**	3.702	1	0.070	5.018	1	**0.038**	7.086	1	0.016	5.367	1	**0.033**

	Rhopaladia gibba			*Synedra ulna*			*Guinardia striata*			*Fragilaria species*		
Source	F	df	P	F	df	P	F	df	P	F	df	P
Lab/field	3.382	1	0.082	10.415	1	**0.005**	5.610	1	**0.029**	2.951	1	0.103
Lake	4.111	1	0.058	11.028	1	**0.004**	6.933	1	**0.017**	1.568	1	0.227
Lab/field * Lake	7.739	1	**0.012**	9.243	1	**0.007**	5.131	1	**0.036**	1.568	1	0.227

4.4.4. Diversity indices results

Overall, the diatom communities (species richness and Shannon-H index) responded differently in terms of measured diversity indices, and as such the biofilms from the two lakes are shown to respond differently to being cultured under controlled environmental conditions (Figure 4.7.). Although the evenness index did indicate that under CT room conditions, the biofilms developed were less even under CT room conditions, specific differences limited by lake of origin did occur between CT room and field settings did occur. These are discussed below.

West campus lake:

Biofilms developed under CT room conditions from West campus lake communities were less species rich (CT rooms: 12 ± 0.94, Field: 21.33 ± 2.96, P=0.018, Mann-Whitney U test) than in the field, but there was no statistically significant difference between the Shannon-H and evenness indices (P=0.414 and 0.153, respectively, Mann-Whitney U test).

East campus lake:

For the Shannon-H and evenness diversity indices, the replicates exposed to East campus lakes demonstrated higher measurements in the CT room replicates, compared to the field replicates (Figure 4.7., Table 4.5.), and although the species richness appears to do the same, the variation within the replicates prevents this from being a statistical effect, indicating a higher overall species richness, and a more even distribution of the species within the community.

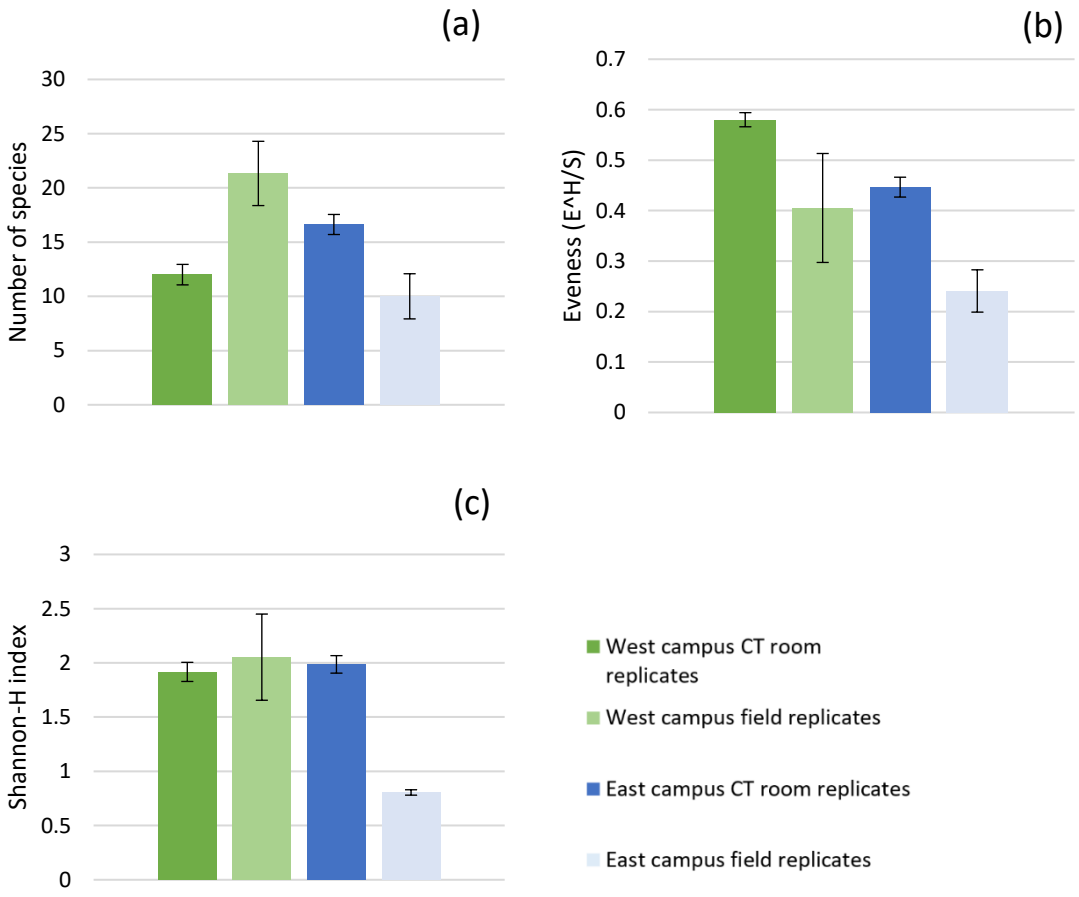


Figure 4.7. Diversity indices ((a) species richness, (b) evenness, and (c) Shannon-H) results for biofilms developed on microscope slides for four weeks in the campus lakes (West campus lake or East campus lake), and in the lab using water derived from one of the two lakes.

Table 4.5. Two-way ANOVA results for the community diversity measures (species richness, Shannon H biodiversity index, and evenness), as well as AFDW as a measure of organic matter content of the biofilm, and the chlorophyll-a concentration, used as a measure of productivity.

	Species richness			Evenness			Shannon H		
Source	F	df	P	F	df	P	F	df	P
Lab/field	0.816	1	0.378	25.156	1	**<0.001**	12.244	1	**0.003**
Lake	5.005	1	**0.038**	15.414	1	**0.001**	15.579	1	**0.001**
Lab/field * Lake	28.326	1	**<0.001**	0.166	1	0.688	19.464	1	**<0.001**

4.4.5. UKTAG assessment results

Regardless of lake, the LTDI2 values of the diatom communities developed after four weeks of exposure on microscope slides was significantly lower on CT room replicates (0.38-0.78) compared to the field replicates (0.5-0.9, Figure 4.8., Table 4.6.). However, effects on the percentage of motile and organic tolerant diatoms differed by the lake the replicates originated from. These are discussed below.

West campus lake:

For West campus lake, an inverse trend occurred where the abundance of organic tolerant species was higher in the field replicates (32.07% ± 15.6%) than the CT room replicates (9.98% ± 1.63%, P= 0.056, Mann-Whitney U test), with the lack of significance due to the wide error margin seen in the field data.

East campus lake:

The percentage of organic tolerant diatom species was higher in biofilms from the East campus lake water derived CT room replicates (18%) compared to the field replicates from East campus lake water (2%) (P=0.014, Mann-Whitney U test). There were also higher abundances of motile species in the CT room replicates (26.6% ± 5.33%) compared with the field replicates (18.03% ± 2.01%, P=0.014, Mann-Whitney U test).

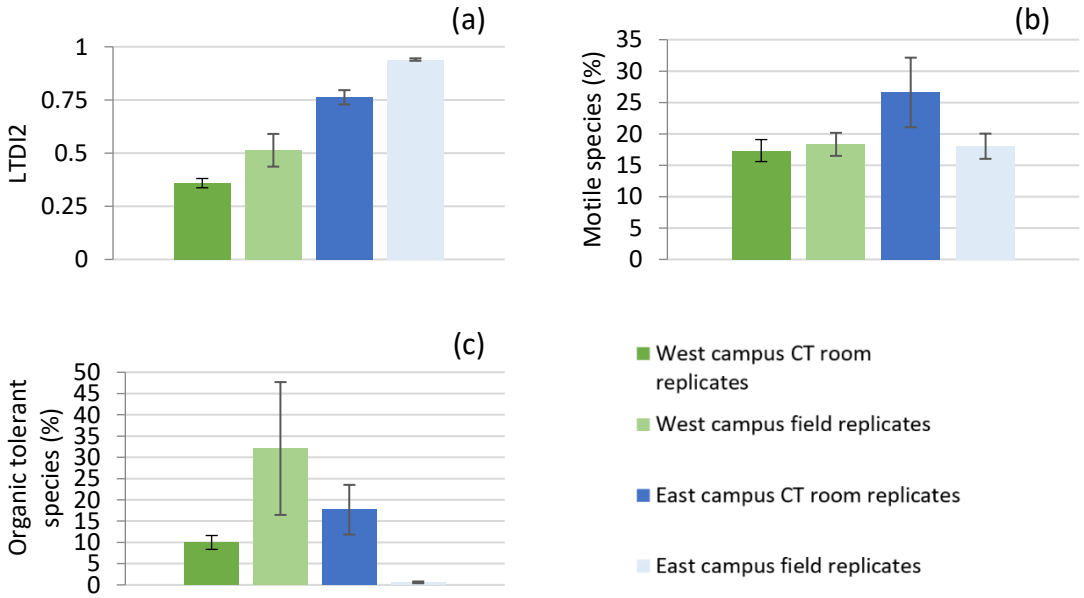


Figure 4.8. UKTAG assessment results for (a) LTDI2 values, (b) percentage of motile species and (c) percentage of organic tolerant species

Table 4.6. Two-way ANOVA results for UKTAG assessment of diatom communities colonising microscope slides *in situ* of the two lakes and in CT rooms. Including the LTDI2 scores, percentage of motile species and percentage of organic tolerant species, using the CT room and field setting as a factor (Lab/Field), and the origin of surrounding lake water as a second (Lake).

	LTDI2			% Motilie			% Organic tolerant		
Source	F	df	P	F	df	P	F	df	P
Lab/field	17.282	1	**0.001**	0.260	1	0.616	0.140	1	0.713
Lake	108.066	1	**<0.001**	4.522	1	**0.048**	3.145	1	0.093
Lab/field * Lake	0.082	1	0.777	13.092	1	**0.002**	8.542	1	**0.009**

4.4.6. Summary

The lower concentrations of AFDW and chlorophyll-a in the CT room replicates compared to the field equivalents indicate that the biofilm communities developed *in vitro* were smaller than their equivalents developed in the field. LTDI2 values for both lakes indicate that the communities developed *in vitro* were indicative of a poorer ecological state than in the field. At the species level for diatoms, the most common species observed in both lakes (*G. striae*, *N. acicularis, N. paleacea* and *N. striae* for West campus, *A. minutissimum* for East campus) were all less abundant in the CT room replicates compared to the field. Further differences limited to specific lakes were:

West campus lake:

There were no differences in the percentage abundance of the algal groups between CT room and field for communities developed using West campus lake water. West campus CT room replicates developed into a community structure that was less diverse and less tolerant of higher nutrient levels. This was due to a loss in the CT room replicates of many of the common species present in the field equivalents, but an increase in the abundance of *Fragilaria species*, *Fragilaria vaucheriae*, *Guinardia striata*, *Melosira varians*, *Nitzschia acicularis*, *Nitzschia linearis* and *Gyrosigma accuminatum* in the CT room replicates.

East campus lake:

Meanwhile, there were significant differences between CT room and field for East campus lake, where there was a diatom dominated community in the field sites, but the replicates in the CT rooms for this lake developed a structure similar the West campus field and CT room replicates, with chlorophytes comprising the majority of the community, with the diatoms comprising the second, and cyanobacteria the third largest algal groups, with the other two groups (desmids and cryptophytes), often composing no more than 2% of the communities. There was also a higher percentage in both the motile and nutrient tolerant diatoms in these replicates, as well as higher abundances in the species richness and community evenness in the CT room replicates, caused by the increased abundance of *Amphora pediculus*, *Brachysira vitrea*, *Encyonema reichardtii*, *Nitzschia dissipata*, and *Nitzschia palea* and a reduction of *A. minutissimum*, which was dominant in the field replicates.

4.5. Physico-chemical measurement results

Measurements shown below are ordered by surface PAR, temperature and pH, followed by the nutrient measurements ordered by those with the most significant effects. Specific differences between nutrient concentrations in the field are not discussed, as this is described in greater detail in Chapter 2, unless they are directly relevant to the analysis of the CT room replicate data.

Effect of 20% replacement:

There was no significant difference between the nutrient concentrations before and after the 20% replacement for ammonium, chloride, fluoride, nitrate, nitrite, or sulphate (Figure 4.9., Table

4.7b.). However, for phosphate and TN, there was a significant increase in the concentrations of these nutrients after the 20% replacement of the growth medium in the West campus derived replicates, with the exception of the 14th and 18th of August, where the replacement decreased the concentration of these nutrients in the replicates (Phosphate: P<0.001, TN: P=0.001, two-way repeated measure ANOVA, Figure 4.9e. and 4.9f.). Lastly, the concentrations of DOC were lower after the 20% replacements (Figure 4.9d., Table 4.7b.).

Changes in physico-chemical measurements over time:

The average daytime PAR intensity over the 3-4 day periods between the CT room medium replacement was higher at the field sites (mean ± se: 440.85 umol/m^2/s ± 5.82 umol/m^2/s, range: 400-800 umol/m^2/s) than in the CT room replicates (mean ± se: 301.72 umol/m^2/s ± 0.76 umol/m^2/s, range: 300-325 umol/m^2/s) (Figure 4.9., Table 4.7.), between both lakes and across all time points (all P=0.009, Kruskal-Wallis H test). Except for the replacement point on the 11th of August for the East campus replicates, where the PAR values experienced by the field and CT room replicates were identical (300 ± 10 umol/m^2/s) (P= 1, Kruskal-Wallis H test). Additionally, the temperatures observed in the field replicates were higher than those seen in the CT room replicates between the 24th of July and the 14th of August (P=0.012, Kruskal-Wallis H test), after which the temperature difference between the field and CT room settings were similar. Where there is comparable pH data between the field and CT room settings, the pH of the field replicates was also either significantly higher, or had a trend towards being higher than the CT room replicates (P<0.100, Kruskal-Wallis H test), except for East campus lake on the 7th of August.

PAR intensity decreased over time in the field when averaging the data between the medium replacement periods (612 ± 0.7 umol/m^2/s to 505 ± 50 umol/m^2/s), however this occurs only between the 31st of July and the 4th of August (P<0.001). However, as seen in Figure 9, the day-to-day averages were much more varied. Water temperature in the CT rooms were lower during the first week of culturing (Figure 4.9., Table 4.7.), caused by the PAR lamps heating the replicates until they were lifted to reduce this effect. The pH of the replicates also decreases from the 24th of July to the 7th of August, from (8.07-8.9 to 7.73-8.52), after which it begins to increase again to a range of 8.51-8.77 (effect for CT replicates: West campus: P<0.001, East campus: P=0.030, trend for field replicates: West campus: P=0.050, East campus: P=0.097, Kruskal-Wallis H test) (Figure 4.9., Table 4.7.).

Four of the nutrients were shown to increase in concentration over the course of the experiment. These were ammonium, TN, fluoride and nitrate (Figure 4.9., Table 4.7., P<0.050, Friedman test). Additionally, four nutrients were shown to decrease in concentration over the course of the experiment as follows:

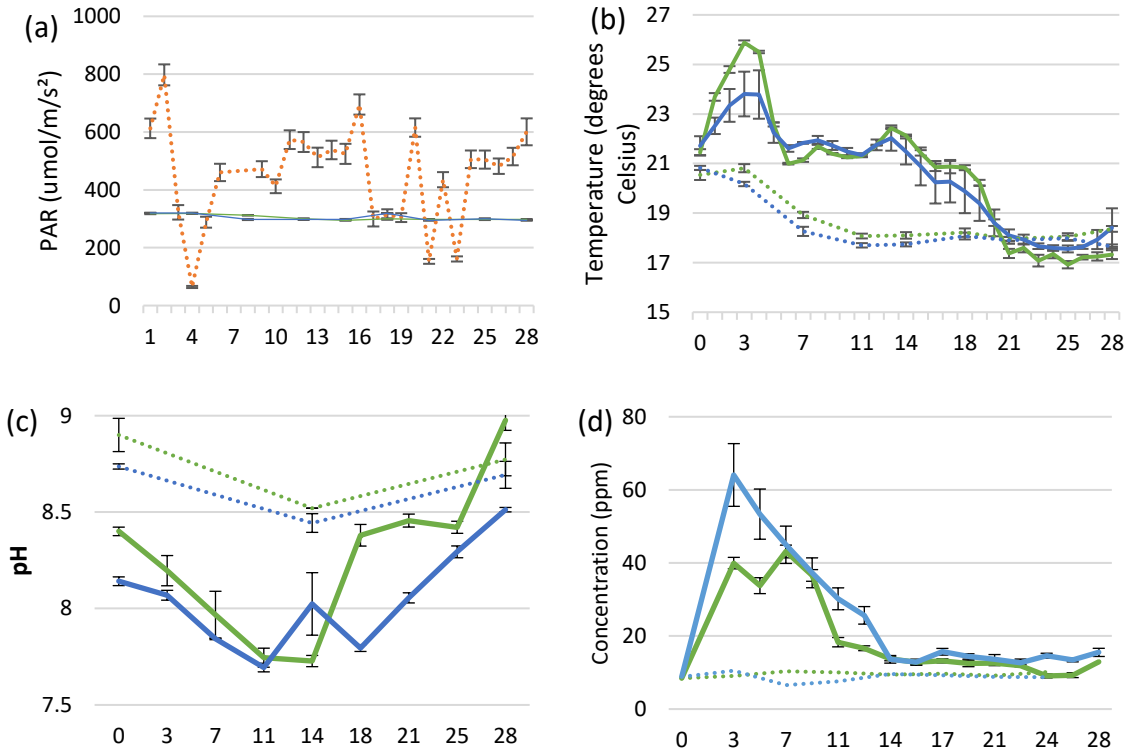
- Chloride (West campus: 130.42 ppm ± 5.45 ppm to 88.14 ppm ± 3.10 ppm, East campus: 113.19 ppm ± 10.47 ppm to 92.12 ppm ± 9.02 ppm)
- Phosphate on West campus lake only (0.054 ppm ± 0.0017 ppm to 0.012 ppm ± 0.011 ppm)
- DOC (West campus: 39.95 ppm ± 1.97 ppm to 12.95 ppm ± 0.67 ppm, East campus: 64.07 ppm ± 8.58 ppm to 15.51 ppm ± 1.12 ppm)
- Nitrite (West campus: 0.02 ppm ± 0.001 ppm to 0.0047 ppm ± 0.001 ppm, East campus: 0.013 ppm ± 0.004 ppm to 0.0024 ppm ± 0.0006 ppm), although for this latter nutrient the concentrations were all very low, as such any variations were likely not significant to the water quality

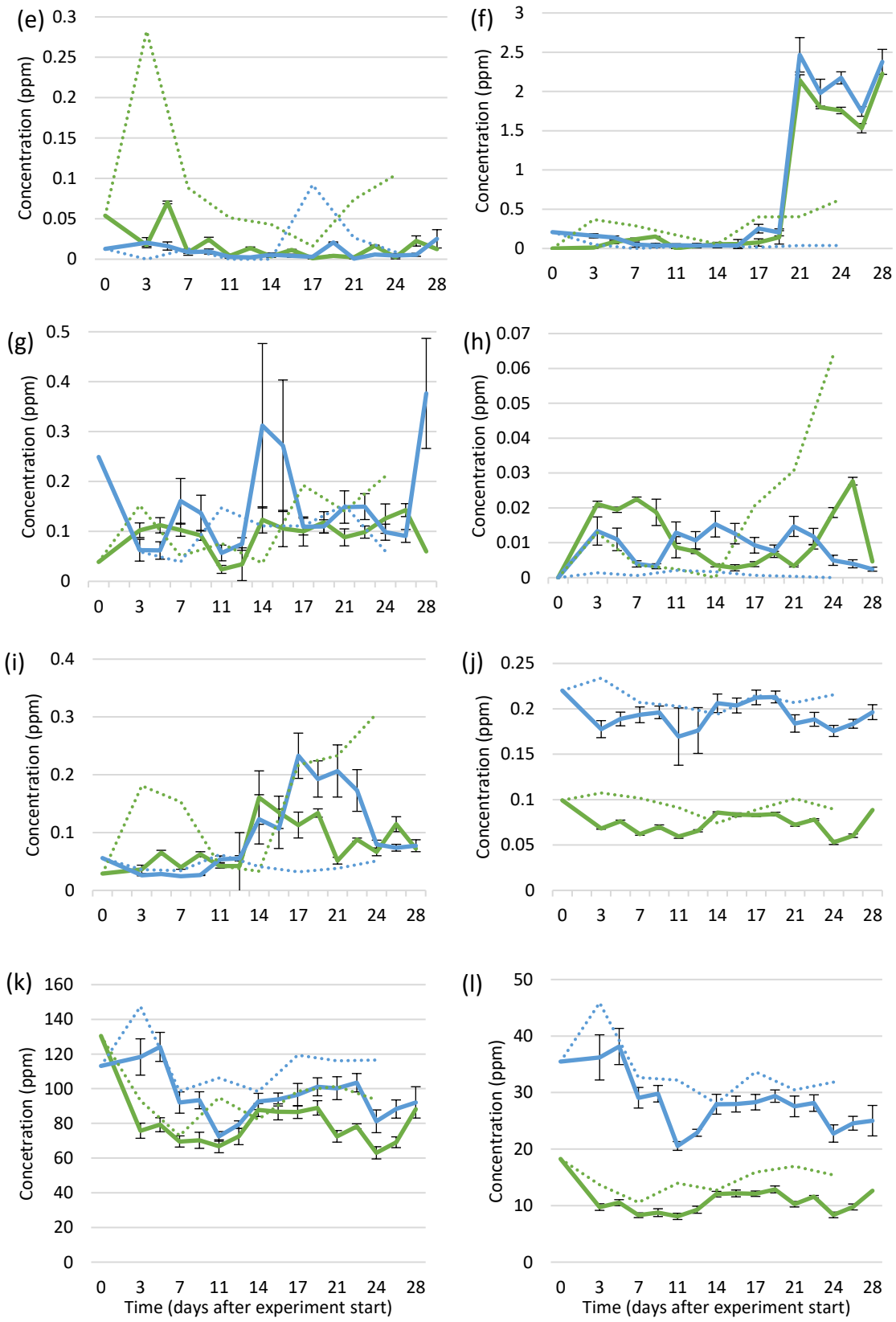
Sulphate concentrations were also affected by time; but the effect differs between lake, with sulphate concentrations increasing over time in West campus CT room replicates (P<0.001, Friedman test), but decreasing over time in East campus CT room replicates (P=0.003, Friedman test).

Differences between CT room replicates derived from the two lakes

PAR values were not significantly different between lakes for the CT room replicates (Figure 4.9., Table 4.7.). However, when split by time points there was a significant difference on the 31st of July, 7th of August, 14th of August and the 21st of August (all P=0.001), caused by East campus replicates receiving higher PAR on the 31st of July and the 14th of August, but PAR was then higher on West campus on the 7th and 21st of August (Figure 4.9., Table 4.7.). This was due to the variation in the light intensity between the different growth lamps. Furthermore, the temperature in the CT room replicates derived from West campus lake water was slightly warmer (18.9-20.8°C) compared to the East campus derived replicates (18.2-2.1°C) between the 27th of July and the 7th of August (P<0.013-0.044, Kruskal-Wallis H test), with East campus replicates being slightly warmer on the 21st of August (West campus 17.5°C ± 0.2°C, East campus: 18.3°C ± 0.5°C). The pH values of East campus lake sourced CT room replicates were lower (7.8-8.5) than those sourced from West campus lake at the start of the experiment, and after the 10th of August (P=0.001, Friedman test), as well as on the 21st of August for field replicates (West campus: 9 ± 0.5, East campus: 8.5 ± 0.01) (P=0.046, Kruskal-Wallis H test).

Between the CT room replicates developed using water sourced from the two lakes, seven of the nutrients were more concentrated in the East campus lake water. These were ammonium (P=0.018), chloride (P=0.001), DOC (P=0.001), Fluoride (P<0.001), nitrate (P=0.012), sulphate (P<0.001) and TN (P=0.007) nutrients (Table 4.9.). Nitrite concentrations were significantly higher in East campus CT room replicates compared to the West campus CT room replicates at week eight (P=0.001), but in the field the nitrite concentrations are higher in West campus replicates at week ten (P=0.037, Kruskal-Wallis H test). Phosphate concentrations were only significantly higher on West campus lake at day eight for CT room replicates (P=0.001). However, no difference between the CT room replicates in the concentrations of phosphate (P=0.288) and nitrite (P=0.451) (Figure 4.9., Table 4.8.).




(e)
Concentration (ppm)
0.3
0.25
0.2
0.15
0.1
0.05
0
0 3 7 11 14 17 21 24 28
(f)
Concentration (ppm)
3
2.5
2
1.5
1
0.5
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(g)
Concentration (ppm)
0.5
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Concentration (ppm)
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Concentration (ppm)
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Concentration (ppm)
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(k)
Concetration (ppm)
160
140
120
100
80
60
40
20
0
0 3 7 11 14 17 21 24 28
Time (days after experiment start)
(l)
Concentration (ppm)
50
40
30
20
10
0
0 3 7 11 14 17 21 24 28
Time (days after experiment start)

Figure 4.9. Physico-chemical variables of the water in the CT room replicates of the 4-week incubation (24 July - 18 August 2019), showing the results measured every 3-4 days before and after replacing 20% of the water in the beakers with fresh lake water, and of the fresh mixed water of each lake (or measured above or in the lakes for daytime PAR and daily water temperature, respectively): including (a) daytime PAR (5:30-21:30; N= 1)), (b) water temperature at 10 cm depth, (c) pH, (d) DOC, (e) phosphate, (f) TN, (g) nitrate, (h) nitrite, (i) ammonium, (j) chloride, (k) fluoride, (l) sulphate.

Table 4.7. a) Three-way repeated measures ANOVA for the surface PAR, temperature, and pH, using the average of the daily measurements for the field surface PAR and temperature aligned to the sampling point in the CT rooms, and pH data for the 24th of July, 7th of July, and 21st of August using CT room/ field setup and lake of origin as factors and time as a repeated measure factor, and b) Two-way repeated measures ANOVA results for the nutrient concentrations, observed in the mediums removed from the CT room replicates using Lake of replicates origin (lake of origin) as a factor and Time as a repeated measure factor.

(a)	Surface PAR			Temperature			pH		
Source	F	df	P	F	df	P	F	df	P
Lab/field	103353.09	1.000	**<0.001**	2657.95	1.000	**<0.001**	50.43	1.000	**<0.001**
Time	1968.96	1.710	**<0.001**	282.41	5.220	**<0.001**	35.66	1.200	**<0.001**
Lake	0.08	1.000	0.780	27.43	1.000	**<0.001**	4.79	1.000	**0.042**
Lab/field * Time	1959.22	1.710	**<0.001**	103.72	5.220	**<0.001**	12.89	1.200	**<0.001**
Lab/field * Lake	0.08	1.000	0.780	10.23	1.000	**0.005**	0.09	1.000	0.769
Time * Lake	2.15	1.710	0.140	4.50	5.220	**0.001**	4.47	1.200	**0.040**
Lab/field * Time * Lake	2.15	1.710	0.140	3.60	5.220	**0.004**	3.91	1.200	0.054

(b)	DOC			Phosphate			TN			Nitrate			Nitrite		
Source	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Rem/Rep	7.943	1.000	**0.009**	141.971	1.000	**<0.001**	18.621	1.000	**<0.001**	0.005	1.000	0.946	0.042	1.000	0.840
Time	123.576	2.090	**<0.001**	60.372	2.864	**<0.001**	739.527	1.713	**<0.001**	4.071	1.584	**0.032**	10.676	3.345	**<0.001**
Lake	28.732	1.000	**<0.001**	59.307	1.000	**<0.001**	19.926	1.000	**<0.001**	4.594	1.000	**0.041**	6.477	1.000	**0.017**
Rem/Rep * Lake	0.481	1.000	0.494	76.654	1.000	**<0.001**	3.957	1.000	0.057	0.174	1.000	0.680	2.518	1.000	0.124
Time* Rem/Rep	1.536	2.090	0.223	8.687	2.864	**<0.001**	7.941	1.713	**0.002**	0.113	1.584	0.848	1.433	3.345	0.235
Time * Lake	8.622	2.090	**<0.001**	18.737	2.864	**<0.001**	4.645	1.713	**0.019**	2.593	1.584	0.097	36.646	3.345	**<0.001**
Time* Rem/Rep * Lake	0.093	2.090	**0.919**	21.062	2.864	**<0.001**	0.257	1.713	0.740	0.013	1.584	0.971	1.394	3.345	0.247

	Ammonium			Fluoride			Chloride			Sulphate		
	F	df	P	F	df	P	F	df	P	F	df	P
Rem/Rep	0.142	1.000	0.709	1.390	1.000	**0.248**	2.171	1.000	0.151	2.347	1.000	0.137
Time	18.135	2.259	**<0.001**	6.726	1.907	**0.003**	16.164	3.689	**<0.001**	17.933	2.497	**<0.001**
Lake	5.460	1.000	**0.027**	904.817	1.000	**<0.001**	56.975	1.000	**<0.001**	689.352	1.000	**<0.001**
Rem/Rep * Lake	4.235	1.000	**0.049**	0.003	1.000	0.959	0.037	1.000	0.849	0.065	1.000	0.800
Time * Rem/Rep	0.362	2.259	0.723	0.201	1.907	0.809	0.262	3.689	0.889	0.200	2.497	0.863
Time * Lake	6.304	2.259	**0.002**	0.855	1.907	**0.426**	7.650	3.689	**<0.001**	12.904	2.497	**<0.001**
Time* Rem/Rep * Lake	0.396	2.259	0.700	0.020	1.907	0.977	0.032	3.689	0.997	0.066	2.497	0.963

4.5.1. Summary

To summarise, temperature and PAR availability were typically far lower in the CT room replicates than in the field, although the field temperature did cool down to similar values to that seen in the CT rooms during the final week of the experiment. Whilst the pH was lower in the CT room replicates, it followed the same temporal trend as for the field sites. For most nutrients, concentrations observed before and after the replacement medium were not significantly different. The exceptions were phosphate, where concentrations were lower in the West campus CT rooms compared to the field throughout the experiment, and total nitrogen, which were significantly higher in all CT room replicates than the field after 21 days. DOC appears to accumulate in the replicates during the 3 to 4-day replacement period. DOC was also frequently higher in the East campus derived replicates. Concentrations of fluoride, chloride and sulphate, as well as nitrate were all higher in the East campus derived CT room replicates compared to the water samples taken directly from the field, indicating nutrient accumulation in these replicates. The remaining nutrients did not show a difference between CT room replicates, although they were typically higher in the West campus lake field replicates (Figure 4.9., see also Chapter 2, Section 5.1, Appendix c.).

4.6. Discussion

4.6.1. Structural differences between CT room cultures and field replicates

The higher benthic algal biomass (chlorophyll-a) and organic matter concentrations (AFDW) observed in the field replicates compared to the CT room replicates is likely related to light intensity, with PAR intensity in the field being twice as high as in the CT room setting (see figure 9). The reduced lighting is an adequate explanation for the reduction in biomass and primary productivity observed in the AFDW and chlorophyll-a concentrations, based on previous microcosm experiments (Thomas *et al.,* 2006, Loiselle et al., 2007). As there were lower light intensities in the CT room (PAR output 301.72 umol/m^2/s ± 0.76 umol/m^2/s) compared to the field sites (440.85 umol/m^2/s ± 5.82 umol/m^2/s). It is likely that the lower diatom abundances and higher chlorophyte abundances seen in the East campus CT room replicate compared to the field is due to this lower light availability, as chlorophytes tend to outcompete diatoms in lower light conditions (Boston and hill, 1991, Biggs, 1995). The different community structures in the CT room replicates compared to the field may be due to several factors, the most important of which is likely the difference in PAR levels. Prior research has indicated that PAR availability affects taxonomic composition by favouring shade tolerant species in lower light conditions (Ledger and Hildrew, 1998). It also has further structural effects on diatom communities, with low light availability correlating to lower nutrient availability and, in turn, favouring low profile diatoms. Meanwhile, higher light availability has been shown to favour high profile and motile diatoms in experiments conducted at 1660–1750 umol/m^2/s and 700–730 umol/m^2/s (Lange *et al.,* 2011). Although Wagner *et al.,* (2015) noted that species diversity was not affected by light availability over a range of 5-125 μmol/ m^2/s, this does not correlate to the results seen here, where the replicates grown in the CT room (under higher light intensity than in Wagner *et al.,* (2015), but lower light intensity than the field sites) were more even, and demonstrated different species richness values to the field replicates grown under stronger natural light. Wood *et al.,* (2016) also noted that light availability is unlikely to affect the outcome of ecotoxicological test results, as in their experiment only four of twenty-six species present ewer affected by light levels, and no overall community effects were noted, although this factor will likely affect the representation of long-term cultures, based on the results presented here. Another significant difference that will have likely affected the biofilm development were the temperature differences the CT room replicates were exposed to compared to the field, in which temperatures were observed to be much higher during the first three weeks of the experiment, although field and CT room temperatures were the same by the third week.

A possibility for the differences in the algal group abundances between CT room and field grown replicates in East campus replicates is nutrient availability. If nitrate availability begins to fall below requirements for the benthic diatom community, or ammonium concentrations increase, then diatoms which prefer nitrate as their nitrogen source, would decrease in abundance, whilst chlorophytes relative abundance would increase with any increase ammonium, as this is their preferred Nitrogen source (Dortch, 1990). Ammonium was observed to increase over time in the CT room replicates, while the concentrations remained stable over time in the field replicates, indicating that this latter explanation is a plausible reason the differences in the algal groups between the CT room and field replicates observed here. Furthermore, experiments by Debenest *et al.,* (2009) found that the Shannon-H index of diatom communities scraped from natural pebbles and transferred to microscope slides found that the Shannon index value was not affected by this transfer. This indicates that the difference observed between the lab and field communities for Debenest *et al.,* (2009) was potentially due to the method of inoculation, using a sterile substratum exposed to a fresh community under an artificial medium optimised for diatom communities, rather than the same nutrient concentrations observed in the field. Therefore, it is likely that for the experiment conducted here, the differences in the nutrient concentrations that developed in the CT room replicate containers, the 20% replacement was not able to fully replace also influenced biofilms in the experiment conducted here, due to lack of or increase in the availability of nutrient sources.

The higher species richness in East campus CT room replicates compared to the field (Figure 4.7.) was due to the lower abundance of *A. minutissimum* (Figure 4.6.) and the increased presence of other species replacing the former species. This increased species richness in the CT room replicates, coupled with the increased evenness caused by the increased abundances of those species that replaced *A. minutissimum* are the factors that led to the elevated Shannon-H and evenness index scores, indicating a more diverse and even community structure in the East campus CT room replicates, compared to their field equivalents. The Shannon-H index was very similar between the West campus and East campus CT room replicates, while the East campus field replicates have a much lower index value. This indicates a shift in the overall community structure of East campus biofilms towards a quantifiably similar structure to West campus lake, although the taxonomic compositions (Figure 4.6.) indicates these communities were still very different. Early experiments by Roeselers *et al.,* (2006) on benthic biofilm communities cultured on polycarbonate slides were unsuccessful in producing community's representative of the source biofilms. Similarly, Morin *et al.,* (2008), used caged microscope slides submerged at 10cm in the Riou-Mort stream, South-West France, which were later transferred for development under laboratory conditions (field and laboratory communities were grown a year apart to the lab replicates). These results found that diatom communities were not strongly affected by the differences caused by the changes between the field and artificial environments, based on species richness, taxonomic composition and species-specific growth rates. Most of the differences in these measurements were linked to the temperature and light intensity. As such, the lower difference between the West campus CT room and field replicates Shannon H index could indicate that the experimental setup in the CT room was similar to the conditions that were present in the field for West campus lake. However, this is not the case, as the nutrient concentrations in West campus CT room replicates, particularly the phosphate concentrations were far lower than in the field (Figure 4.9e. and 4.9f.). As such, the differences to many of the biological endpoints (relative abundance of algal groups and diatom species, percentage of organic tolerant species, and reduction of the species richness) in the CT room replicates of West campus lake compared to the West campus field replicates is likely due to the loss of nutrients compared to the field equivalents, and the lower light intensity compared to that seen in the field.

Twenty one of the 30 species identified in this experiment grew in different abundances over one month, with *E. turgida*, *F. vaucheriae*, *M. varians*, *N. acicularis*, *N. linearis*, *G. striata*, *B. vitrea*, *E. reichardtii* and *N. dissipata* preferentially growing in the CT room conditions, regardless of lake water developed in. A major difference between the work conducted here and in the research that has compared field and lab growth of community cultures (Morin *et al.,* 2008, Debenest *et al.,* 2009) is that we have used a replacement medium taken directly from the lake source, whereas these other experiments used artificial mediums. This allowed for the continuous colonisation of diatoms representative of the field environment throughout the experiment conducted here, whilst these experiments from the literature have been conducted using pre-existing biofilms that were then transferred into the laboratory setting. As such, these replicates would have been continually refreshed with field accurate species during development, which ultimately did not maintain field accurate biofilms in the CT rooms.

The marked lower LTDI2 values in the CT room replicates from both lakes indicate that the differences in the CT room environment have negatively affected the communities compared to the field environment. This could be due to a 2 to 5-fold increase in TN concentrations in the CT room replicates after the three weeks of development (Figure 4.9.). Conversely, for the West campus lake derived CT room replicates, the communities were likely phosphorus limited, as the phosphorus concentrations observed in the replacement mediums added every 3-4 days were far higher than the medium removed from the CT room replicates at that time point (Figure 4.9e.). As nutrient limitation is known to reduce the species diversity of benthic biofilms (Passy, 2008), an effect shown in the species richness of West campus lake CT room replicates, this reduction in P nutrient availability caused by greater uptake by the biofilm communities (Currie and Kalff, 1984) than the medium replacement could add in, can be considered to be a major consideration

in the differences observed in the CT room biofilms from West campus lake, but not in those from East campus lake.

Although the percentage of motile diatoms was not affected between East campus field and CT room replicates, the higher percentage of these on West campus CT room replicates compared to the field replicates indicate a shift in the community towards high motile species. Lange et al*.,* (2011) tested the effects of different light, nutrient and grazing levels using diatoms from the Kauru river, New Zealand cultured in experimental stream channels. The results demonstrated that an increase in light and nutrient availability caused an increase in the abundance of motile diatoms in the community, an effect also observed by Licursi *et al.,* (2016) in Argentinian stream diatoms. Conversely, research by Stenger-Kovács *et al.,* (2013) using biofilms developed on limestone blocks in the Torna stream, Hungary, demonstrated the ability of motile diatoms to perform better in light and nutrient limited conditions, particularly during the winter season, as they can actively migrate to the surface of the biofilms or to more resource rich positions, allowing them to outcompete other diatom guilds that cannot move on their own. This view is further supported by van der Grinten *et al.,* (2005), based on the results of experiments on mixed biofilms of cyanobacteria (*Leptolyngbya foveolarum*) and diatoms (*Nitzschia perminuta*) under different temperature and light regimes. Thus, the increase in motile species observed here could be due to this lower light availability and temperatures observed in the CT rooms compared to the field equivalents (Figure 4.9.), and as such these species had a physical advantage over the other species present in the communities for resource competition, although this cannot be said for certain, due to a continuing conflict within the literature.

4.6.2. Effectiveness of 20% replacement

Phosphorus and nitrogen concentrations appear to be sufficiently replaced in the East campus derived replicates, although the spike of total nitrogen in the final week does cause some concern. In West campus lake these nutrients were not fully replaced by the 20% replacement every 3-4 days. This is shown in Figure 4.9e. and 4.9i. Where even after the addition of the 20% replacement, the nutrient concentrations were still far below the values observed in the pure replacement medium from the field. It is likely that the limitation of these nutrients had an effect on the relative abundances shown in Figure 4.6a and the percentage of organic nutrient tolerant diatoms (Figure 4.8c.), demonstrating a loss of the species that prefer high nutrient conditions Although other factors, such as the lower light availability, may have also contributed to these changes. However, in the final week of the experiment the concentration of TN rapidly increases to 10 times that seen in the previous three weeks. This can be attributed to the invertebrate organisms that began to develop in the replicates at this time point, with visible identification of organisms of the *Daphnia* genus identified. And as the concentrations of inorganic forms of nitrogen were measured here (nitrate, nitrite, and ammonium), this increase will have been caused by the unmeasured organic forms of nitrogen.

The higher concentrations of DOC, as well as ammonium and nitrate, in the CT room replicates growth medium compared to the field sites, particularly in the East campus replicates at the replacement times, were likely due to the accumulation of these compounds in the replicate vessels due to lack of consumption. Evidence for this can be seen in the lower chlorophyll-a concentration results in the CT room replicates (Figure 4.4.). This reduced biomass and hence productivity would equate to a lower demand on these nutrients, which would therefore accumulate over time as fresh lake water added would bring an excess of these nutrients above what the biofilms are capable of using. Furthermore, as mentioned earlier, the P availability appears to have a limiting effect on the development of West campus biofilms, based on the results shown in Figure 4.9. and the interpretation of the species richness data, as well as the significant reduction in algal biomass based on the chlorophyll-a concentrations observed in Figure 4.3. This indicates that the 20% replacement of the growth medium every 3-4 days was not sufficient to maintain field- equivalent nutrient concentrations for the West campus lake. For replicates derived from East campus lake, however, the 20% replacement appears to be more appropriate, as there were very limited differences between the nutrient concentrations of the

replacement and removed mediums. When differences occur, they were not typically statistically significant, but did indicate slight decreases in the concentrations, particularly for phosphate, TN (caused by major increase of unmeasured organic forms), nitrate and nitrite, and minor increases in the other nutrients, with this shown in Figure 4.9. As such, the 20% replacement will have been suitable here, and the changes in the community composition will have more likely been linked to lower light intensity.

4.6.3. Recommendations:

To summarise, one month of culturing *in vitro*, using the methodology employed, will produce working biofilms, however these biofilms are much smaller in size than the field grown equivalents, owing to the changes in the nutrient concentrations and light availability between the field and CT room settings. The method employed was not able to fully account for nutrient usage by the biofilms. Nutrient concentrations were found to differ between the replacement material sourced directly from the campus lakes and the concentrations seen 3-4 days later in the CT room replicates, due to higher consumption rate of nutrients in the West campus lake CT room replicates than the methods ability to replace, whilst also providing slightly more nutrients than were being used by the biofilms in East campus lake CT room replicates. As such the following recommendations are suggested for future culturing using the method developed here.

- Further testing of optimal nutrient replacement regimes of algal communities developed under laboratory conditions. This is to allow for the optimisation of the timing and percentage of medium replaced for the diatom replicates, as the results here have shown that biofilms grown from different sources can have significantly different nutrient requirements. This should be conducted be testing replacement timings (between daily and twice weekly), and higher replacement percentages (between 20% and 50%).
- Additional testing of the method using higher light levels in the lab, and multiple sampling points over the same one-month period should be performed, to assess whether increasing the PAR to more natural levels can fully compensate for the reduced biomass, or if a period longer than four weeks will be required to establish laboratory cultures of equivalent biomass to field replicates. In addition, a method of achieving this increase in light availability without causing unwanted heating of the replicates will need to be devised.

Chapter 5: Biofilm community structure in Yorkshire freshwater bodies

5.1. Introduction

Ecotoxicity tests are typically done with communities developed *in situ* on artificial substratum, which are then either transferred to the lab (Lowe and Gale, 1980), or transferred directly from natural substratum to the lab for culturing, by scraping the biofilm off a natural substratum and transferring them onto artificial substratum (Rimet and Bouchez, 2011, Arini *et al.,* 2012). These communities are then exposed to a graded series of chemical contaminants. However, the benthic diatoms in these natural communities, cultured from a single site, are only representative of that site. This is a considerable issue, considering diatom communities are known to show significant variations across not only environmental gradients, but geographical ranges. Indeed, environmental factors have been shown to account for between 24% and 38% of the variation in benthic diatom community structure (Soininen *et al.,* 2004, Vanormelingen *et al.,* 2008, Teittinen *et al.,* 2015). This variation is noted in several review papers to be caused by local physico-chemical factors, dispersal effects, spatial factors, and local biogeographic factors, and thus create a patchy biogeographical distribution of individual species (Fisher and Dunbar, 2007, Soininen, 2007). This highlights the need to develop a community that is representative of the environment being studied, in order to improve the ecological relevance of ecotoxicological tests on diatoms at the community level. However, the existing literature only assesses communities within singular river reaches, or individual lakes. As such, the possibility of developing a larger scale representative community has not been explored. Furthermore, the development of community diatom cultures under laboratory conditions is still quite rare, due to the inherent difficulties of culturing communities of organisms' sensitive to changes in the surrounding environment (Debenest *et al.,* 2009b, Congestri and Albertano, 2011). As such, most tests have been performed on biofilms developed in the field and then brought into laboratory for immediate testing (Duong *et al.,* 2008, Wood et al., 2014). The understanding of what a representative community for a larger region would look like will greatly inform what kind of community structure and composition should be expected for a laboratory community culture of diatoms, such as in the method proposed in Chapter 4.

As the previous chapter (Chapter 4), has developed the method of how the diatom community cultures can be cultured under laboratory conditions, this experiment was initiated with the aim of identifying what the composition of a community developed within this experimental setup should look like. By developing an understanding of diatom community structure and composition in Yorkshire, with a focus on sites within the Vale of York. These results could then be applied to a stock culturing method from which ecotoxicological tests can return to for a standardised test community. This experiment was designed to assess the benthic biofilm communities of five Yorkshire freshwater bodies in October 2019, with a focus on the diatom communities within those biofilms. The water bodies selected were rated as being in 'good' ecological status by the Environment Agency (Environment Agency, 2020), based on the EU Water Framework Directive designations using ecological measures. This experiment aims to identify the species of diatoms present in the biofilms at these sites, and the abundances they are present in. These results will then be used to determine the structure of a community that would best represent the communities found in the Vale of York, Yorkshire, and the effects the water quality has on the community structure. The results of which can then be used to compare any future communities developed under the methodology presented in chapter 4, using the substrate type and duration of inoculation identified in Chapters 2 and 3.

5.2. Aims and objectives:

Aims:

To assess the benthic diatom communities in water bodies across the Vale of York, at sites where the water quality has been rated as 'good' or higher by the Environment Agency under the EU Water Framework Directives biological and chemical measurements. This experiment is conducted in order to establish the composition of a representative community of freshwater benthic diatoms in Yorkshire that can then be cultured under laboratory conditions as a standardised community for testing the effects of organic contaminants on .

Objectives:

- To identify the structure and composition of diatom communities in water bodies rated good or higher by the EU water framework directive across the Vale of York.
- To explore relationships between physicochemical water quality parameters and the diatom communities in these water bodies.
- To identify which species in these sites are known to be nutrient sensitive, or nutrient tolerant, and therefore may be likely to react to other organic chemicals.
- To use these prior objectives to determine which species of diatoms could potentially be considered for a representative diatom community for the Vale of York, and incorporated into future *in vitro* testing of chemical contaminants.

5.3. Methodology:

5.3.1. Experimental setup

To assess the structure and composition of freshwater benthic diatom communities in Yorkshire water bodies, UKTAG assessment sampling protocols and methodology for macrophytes and phytobenthos (Directive, 2014) were conducted at a series of reference sites identified as being in good ecological status or better by the Environment Agency (2019), using biological metrics (UKTAG macrophytes and Phytobenthos assessment). Additional physio-chemical parameters of the waterbody were measured, including pH, temperature, dissolved oxygen, alkalinity, electrical conductivity and Photosynthetically Active Radiation (PAR) attenuation at the depth at which the substrata were exposed relative to the water surface. Water samples were also taken to measure nutrient concentrations (total nitrogen (TN), nitrate, nitrite, ammonium, dissolved organic carbon (DOC), phosphate, sulphate, potassium, magnesium, silicon and calcium), total suspended solids (TSS) concentrations, and concentrations of elements known to be required by diatoms, but may also act as a contaminant depending on the concentration (chloride, fluoride, copper, sodium, nickel, lead, iron).

5.3.2. Study sites

A series of five sites were selected based upon the Environment Agency results from 2016 (Environment agency, 2019), which demonstrated that the water bodies were in good or higher ecological condition based on biological indicators, particularly the phytobenthos measurements, as well as other biological indicators (macrophytes, invertebrate and fish) and contamination by heavy metals (e.g. manganese, cadmium, lead, arsenic) or other priority compounds (e.g. atrazine, trichloromethane, trichlorobenzenes). A sixth site immediately south of the city of York on the River Ouse was also identified. However, heavy flooding made access to any of the localities at the site with useable substratum and complied with the UKTAG methodology impossible.

These sites were chosen to represent water bodies in the Vale of York that are in "good" or higher ecological conditions/ low trophic level, as defined by the EU water framework directive (EPA, 2016). This assessment was completed using the UK environment agencies catchment data explorer and the most recent data it contained for the classification of water quality, with a specific focus on the biological quality elements (macrophyte and phytobenthos assessments),

and the water quality parameters (physico-chemical measurements) (Environment Agency, 2019).

These sites are shown in the map below (Figure 5.1.), and were identified following the procedures set out in the UKTAG assessment methodology (Directive, 2014). The sites were sufficiently far away from any sources of water into the system (e.g., drainage systems, sewage inputs) that the water will be sufficiently mixed, away from heavy shading where possible, within the main flow of the river to avoid locations where loosely attached diatoms and organic matter can accumulate, and in locations where the substratum have been in equilibrium with the environment for at least four weeks.

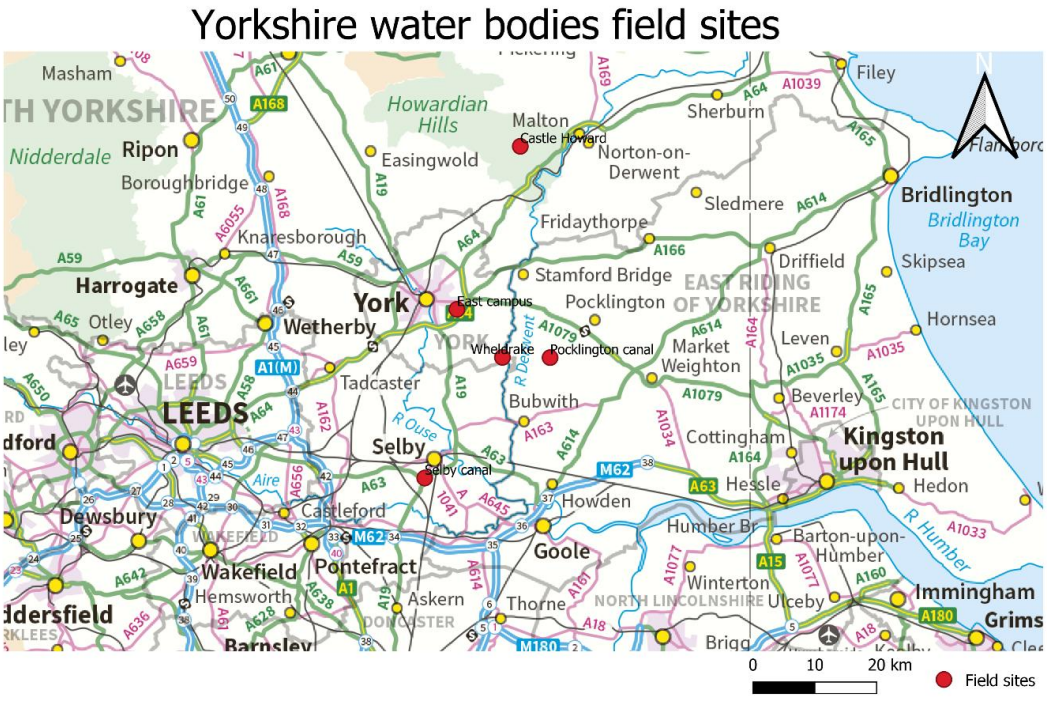


Figure 5.1. Map of the field sites used for sample collection, with the field sites used labelled (From West to East: Selby canal, East campus lake, Wheldrake Ings, Castle Howard great lake, and Pocklington canal)

The sites used were; East campus lake at the University of York (samples were taken from three localities within this site, EC1, EC2, and EC3. These were used to create a mean and standard error for the data in the relevant graphs, and used as replicate values for data analysis in SPSS (ANOVAs and MANOVAs)) This site was previously assessed here (Chapter 2, section 4.5.) and proven to have diatom community's indicative of "good" to "high" TDI, and low levels of inorganic nutrients (Chapter 2, section 5.1.). As such this site was used again here as it meets the requirements of this experiment and can provide a link to the results of the previous experiments. Other sites used include the Great Lake at Castle Howard (due to a large reed bed surrounding the lake, only one site was accessible), which is the main source for Cram Beck, a tributary of the River Derwent identified as being in good ecological quality, Wheldrake ings on the River Derwent (two localities were accessible at this site, WD1 and WD2), Pocklington canal, which connects to the River Derwent and Selby canal, which is part of the River Ouse and Humber. A site on the River Ouse directly south of York city centre was also selected to be used, however, weather conditions significantly elevated the water levels preventing access to useable benthic biofilms.

5.3.3. Sample collection and field measurements

Biofilm samples

Samples of three different substratum types native to the site were taken if they were present, to assess the natural communities that develop here;

- Epilithic (stone) (fine-grained sandstones were used for East campus lake, and taken from 10cm below the water surface, no stone substrata were found at other sites), this substratum was only identified and utilized at East campus lake, due to their high prevalence in the environment and thus the highly representative nature of the biofilms on them within the benthic communities at this site
- Epiphytic (plant) (phragmites used where possible, bullrush used as an alternative, this difference was noted when it occurred. At least three stems 5cm long were taken from 10cm depth), this substratum was identified and sampled at all sites.
- Epipelic (sediment) (detrital material taken from 10cm below the water surface), this substratum was identified and sampled at all sites.

Sediment and stone substrata were taken from areas that were not shaded when possible. These substrata were chosen as they are common substratum in freshwater bodies across the region. Stone substratum were also taken were possible, to add for the comparison to the work in previous chapters. At each location, at least triplicate samples of each substratum were taken from each site and mixed together into a single pooled sample for each substratum. This was performed to provide a more representative sample for the substrata at each site. Plant and stone biofilms were scraped off the substratum using a sharp blade, and sediment biofilm was collected by scraping the top centimetre of surface. The biofilm samples were stored in darkened 25-ml HDPE tube and stored at 4˚C until further analysis.

After thorough mixing of the biofilm suspension, each sample was analysed for;

1. chlorophyll-a content,

2. Ash-Free Dry Weight (AFDW),

3. relative abundance of algal groups,

4. Diatom (relative abundance, diversity indices and UKTAG LTDI2 assessment) analyses as described in detail in the Methods section of Chapter 2.

The TDI values (UKTAG assessment methodology) of the benthic diatom samples from the East campus lake and Castle Howards great lake were still calculated using the lake specific LTDI2 index as in previous chapters, as this metric is specific for lake diatoms. Whereas the TDI values of the benthic diatom samples from the Wheldrake ings, Pocklington canal and Selby canal were calculated using the TDI4 index, which is specific for rivers. As such, in this chapter this metric will be referred to as 'TDI' as opposed to 'LTDI2' used in prior chapters.

Water quality samples

The water samples at each of the field sites were analysed for pH, dissolved oxygen, light attenuation, alkalinity, electrical conductivity, temperature and nutrient concentrations using the same methods as described in Chapter 2 (Chapter 2, section 2.5., Water quality analyses).

Lab based measurements

The same lab-based sample processing and data analyses were conducted for both the biofilm metrics (AFDW, chlorophyll-a, relative abundance of algal groups and diatom species, diversity indices and UKTAG endpoints) and water quality measurements (nutrient analysis) as described in Chapter 2, section 3.4.

5.3.4. Statistical analysis

Statistical analyses were performed for all datasets in IBM SPSS software version 26. For all data, a Kolmogorov-Smirnov test was performed on each dataset to test for the normality of the data. Leven's test was also performed for the datasets to test for equality in the variances. If the data was not normally distributed or the variances were not equal, then the data was transformed (Log10 and square root for physico-chemical measurements, as well as AFDW and chlorophyll-a concentration, and arcsin square root for algal and diatom relative abundances, diversity indices and UKTAG endpoints).

If the data were not normally distributed, this was then followed by an initial ANOVA (three-way repeated measures for biofilm results, two-way repeated measures for water quality data), to obtain the Levenne's equality of variance data. If the data was not normally distributed or the variances were not equal, then the data was transformed to improve normality (arcsin square root for algal groups and diatoms variables and log10 or square root if percentage) for the physicochemical water parameters. If the transformations did not yield P values greater than 0.05, then the data with the highest P value was used, and one-way interaction assessments were conducted using non-parametric testing (Friedman tests for the repeated measure time factor, Kruskal-Wallis H test for the lake and substratum factors).

In order to test the substratum and site effects on the biofilm measurements two-way non-replicated ANOVAs were used. The biofilm variables included the relative abundance of the algal groups and diatom species, diversity indices (species richness, Shannon H index, and evenness), chlorophyll-a and AFDW concentrations, TDI, percentage of motile species and percentage of species tolerant to organic nutrients (calculations described in chapter 2). This was followed by appropriate post hoc tests (Tukey HSD (if the data was normally distributed), Mann-Whitney U tests (if the data was not evenly distributed)) to determine which substratum showed significantly different biofilm variables. Linear regression analysis was conducted using IBM SPSS (version 26) to determine the effect of the physico-chemical measurements on the TDI values. An ANOVA analysis was conducted to test the significance of the regression model. Further MANOVA analysis in the same software was conducted using the TDI value as the dependant value, and the physico-chemical measurements as the independent variables, to determine the effects of these variables on the TDI when considered together. All ANOVA (and MANOVA) stats results were presented using the F (F statistic), df (degrees of freedom), and P (significance level, here $P<0.05$ is considered significant).

Data derived from stone substratum were not used in the ANOVA analyses of the biomass measurements, as this substratum were only present at one site (East campus lake), as such these results were conducted with only the results from the plant and sediment biofilm samples. They were, however, included in the multivariate analysis of the biofilm measurements against the physicochemical parameters of the water bodies. This is shown in table 5.2.

Table 5.1. Usage of biofilm measurement results in the statistical analysis by substratum type (plant, sediment and stone)

Substrate	AFDW/ chlorophyll a	Algal group abundances	Diatom species abundances	Diatom diversity indices	diatom UKTAG endpoints	Multivariate analyses
Plant	Yes	Yes	Yes	Yes	Yes	Yes
Sediment	Yes	Yes	Yes	Yes	Yes	Yes
Stone	No	No	No	No	No	Yes

In order to determine differences in the concentrations and values of the physico-chemical parameters across the five time points and the two lakes, the data from these parameters were tested using one-way repeated measure ANOVAs, using the site taken from as the factor.

Linear regression analysis was performed of the TDI values for the biofilm, as a measure of predicted community health as a dependant factor, against the twenty-seven physicochemical

parameters assessed throughout the experiment as independent factors, with the TDI results on the y axis, and the value of the physico-chemical parameter on the x axis. This was performed to assess the strength and direction of these parameters to the overall ecological health of the biofilms. MANOVA analysis were performed to assess the effects of all physicochemical parameters on the TDI values of the substratum, with this data split beforehand by the site factor to differentiate the results between the five contrasting sites. The parameters with a P value below 0.05 were then used to perform a multiple linear regression, to determine the total effect of these factors on the biological quality of the diatom communities in each site.

5.4. Biofilm measurements results

5.4.1. Biomass

Although the ANOVA analysis do not indicate that there was a significant difference between sites for the AFDW and chlorophyll-a content of the biofilms (Table 5.2a.), visual inspection of the results (Figure 5.2.) suggests that the AFDW concentrations of the sediment substratum from Selby canal (87.12 mg/cm^2), Pocklington canal (68.34 mg/cm^2), and Castle Howard (45.72 mg/cm^2) was much higher than those of East campus lake (19.45 mg/cm^2) and Wheldrake ings (11.16 mg/cm^2). Further to this, the results shown in Figure 5.2b indicate that higher chlorophyll-a concentrations occurred on castle Howard biofilms, although this was limited to the plant substrate (17.9 ug/l), compared to the rest of the dataset (0.9-7.4 ug/l).

There was a statistically significant difference between substratum for both AFDW and chlorophyll-a (Figure 5.2., Table 5.1.). The chlorophyll-a concentrations were higher on the plant substratum (0.5-18 ug/l) than sediment substratum (0.2-4 ug/l), apart from at Selby canal, whilst the AFDW concentrations were higher on substratum removed from sediment substratum (0.5-56.5 mg/cm^2) than the plant substratum (0.35-27.5 mg/cm^2).

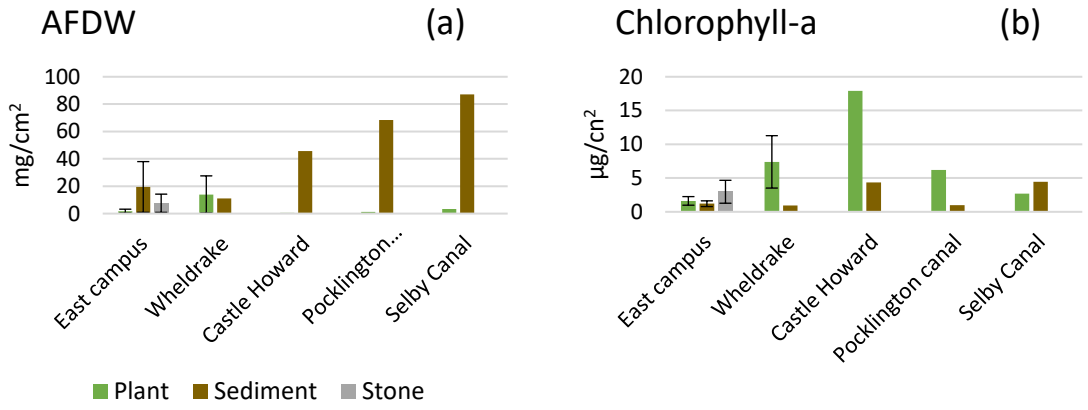


Figure 5.2. biomass measurements using a. AFDW (as-free dry weight) and b. chlorophyll-a concentrations of the biofilms from plant substratum (green), sediment substratum (brown) and stone substratum (grey), sorted by site (EC= East campus, WD= Wheldrake, CH= Castle Howard, SC= Selby canal, PC= Pocklington canal).

Table 5.2. Two-way ANOVA (without replication) results for the biofilm measurements (Chlorophyll-a and AFDW), using the substratum type (plant, sediment, and stone), and site (EC= East campus lake sites, WD= Wheldrake Ings, CH= Castle Howard, SC= Selby Canal, PC= Pocklington Canal)

	Chlorophyll a			AFDW		
Source	F	df	P	F	df	P
Site	4.210	4	0.096	0.933	4	0.526
Substrate	8.603	1	**0.043**	8.774	1	**0.041**
Site * Substrate	2.578	4	0.191	1.260	4	0.414

5.4.2. Algal groups

Overall, diatoms were often the most abundant species, with a further preference towards the sediment substratum. except for at Selby canal, where the percentage of diatoms was extremely low in the sediment biofilms (6.00%), but comprised the majority of the algal community in the plant biofilm at this site (71.92%) (Figure 5.3., Table 5.2.).

Based on the ANOVA results (Table 5.2.), there was no significant effect of site or substratum on the relative abundance of diatoms, cyanobacteria or chlorophytes, although this is likely due to the limited replication available for analysis. Using the mean and standard error as descriptive statistical analysis, diatoms accounted for 54.41% ± 5.10% of the individuals counted. Whilst the cyanobacteria accounted for 13.36% ± 4.41%, and the chlorophytes comprised 32.11% ± 5.38% of the algal communities. The abundances of diatoms in East campus lake (57.95% ± 6.20%) and Pocklington Canal (48.31% ± 11.43%) were close to the mean for the dataset, although both sites also demonstrated fairly high abundances of cyanobacteria (East campus: 20.25% ± 8.30%, Pocklington Canal: 18.02% ± 5.67%), and although the abundance of chlorophytes was close to the mean for the dataset at Pocklington canal (33.67% ± 5.80%), East campus lake demonstrated fairly low abundances of chlorophytes (21.72% ± 4.58%). However, the mean values of the diatom group of the algal community were noticeably lower at Castle Howard (40.61% ± 11.46%), and Selby Canal (38.96% ± 32.96%)), although in both cases they had substantial error margins that overlapped with the mean for the dataset. The percentage of the community that was comprised of cyanobacteria was similar to the mean for the whole dataset at Selby canal (13.38% ± 6.81%), but there were far fewer cyanobacteria at Castle Howard compared to the other sites (1.15% ± 1.15%). Both of these sites exhibited very high abundances of chlorophytes compared to the mean (Castle Howard: 57.92% ± 10.28%, Selby Canal: 47.65% ± 39.77%), although for this latter site there is a large error margin in the results. Wheldrake ings demonstrated unusually high abundances of diatoms in the algal communities (68.57% ± 9.94%), which unlike Castle Howard and Selby canal, did not have an error margin that would bring its lower bounds to the overall mean for diatom abundance in the dataset. Unlike the other sites, there were no cyanobacteria identified at this site, but the chlorophyte presence was close to the overall mean value for the dataset (31.21% ± 9.74%).

Using the mean and standard error of the dataset split by substratum, diatoms appear to comprise a larger proportion of the algal communities on the plant substratum (56.22% ± 8.05%) than the sediment substratum (47.85% ± 9.22%), but the abundance of diatoms on stone substratum was even higher (65.51% ± 4.96%). However, as the availability of these substratum was limited to East campus lake, it cannot be confirmed whether this would have been the case across all sites. The percentage of cyanobacteria was fairly similar on plant and sediment substratum (plant: 13.02% ± 5.62%, sediment: 17.28% ± 9.22%), but their abundance on the stone substratum found on East campus lake were far lower (4.95% ± 3.66%). There were also very similar percentages of chlorophytes present on all three substratum types (plant 30.60% ± 8.37%, sediment: 34.72% ± 10.42%, stone: 29.53% ± 6.88%).

Despite these observed means, at Castle Howard and Pocklington Canal, there were more diatoms and fewer chlorophytes on the sediment substratum (Castle Howard: 52.05%, Pocklington Canal:

59.77%) than the plant substratum (Castle Howard: 29.18%, Pocklington Canal: 36.84%), whilst at Wheldrake, there was only a three percent difference of the abundances of diatoms, cyanobacteria and chlorophytes on the plant (diatoms: 67.42% ± 17.09%, cyanobacteria: 0.00%, chlorophytes: 32.25%) and sediment substratum (diatoms: 70.86%, cyanobacteria: 0.00%, chlorophytes: 29.14%).

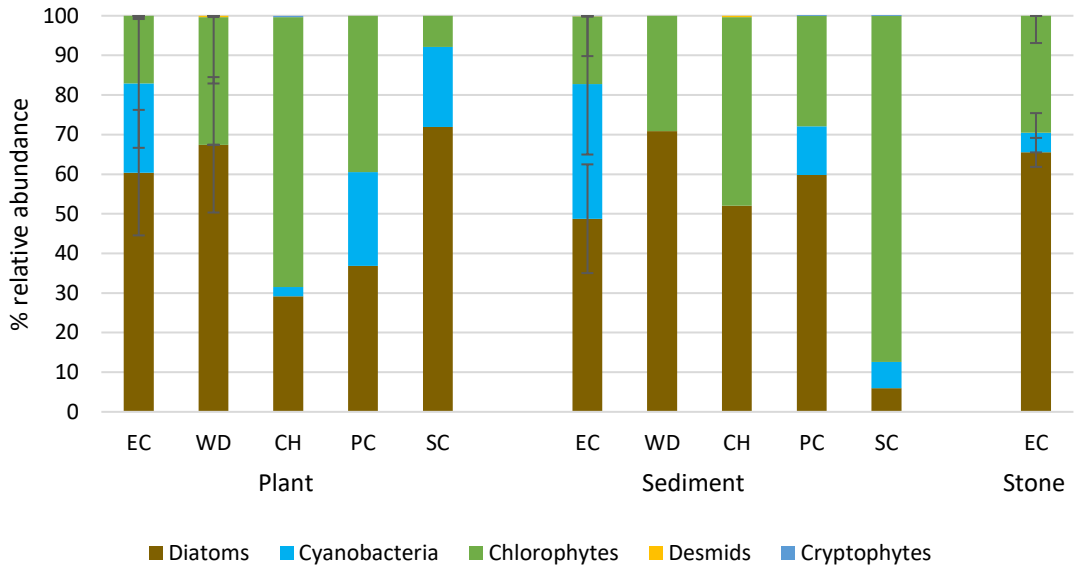


Figure 5.3. Stacked bar graphs showing the percentage abundance of the five main algal groups identified in this experiment (diatoms, cyanobacteria, chlorophytes, desmids and cryptophytes). Graphs clustered from left to right by plant biofilms, sediment biofilms, and stone biofilms, and split by site (East campus, Wheldrake, Castle Howard, Pocklington canal, and Selby canal).

Table 5.3. Two-way ANOVA without replication results for the three main algal groups (diatoms, cyanobacteria and chlorophytes).

	Diatoms			Cyanobacteria			Chlorophytes		
Source	F	df	P	F	df	P	F	df	P
Site	0.650	4	0.657	0.762	4	0.601	2.510	4	0.197
Substrate	0.174	1	0.698	0.049	1	0.836	0.809	1	0.419
Site * Substrate	1.230	4	0.423	0.131	4	0.963	2.857	4	0.167

To summarise, diatoms were shown to be most abundant at Wheldrake, with relatively low abundances observed at Castle Howard and Selby Cabal. Furthermore, although diatoms were shown to be more abundant on plant substratum than sediment substratum overall, this only occurred on specific sites (East campus lake and Selby canal), whilst there more diatoms in sediment substratum on two other sites (Castle Howard and Pocklington canal), whilst there was no difference between substratum on Wheldrake ings. Cyanobacteria were also most abundant at East campus lake and Pocklington canal, but virtually absent from Wheldrake. Chlorophytes were most abundant at Castle Howard and Selby Canal, seemingly as a direct replacement of the diatoms compared to the other sites.

5.4.3. Diatom species relative abundances

Relative abundance of the species is shown in Figure 5.4., and the two-way ANOVA analysis of this data is shown in Table 5.4, using the substratum and site as factors. The full list of species is shown in the appendix (Appendix i), as well as a table demonstrating the mean and standard error (where possible) of the species presented here split by substratum and site. Note that Pocklington

canal plant substratum has no results, as there were no frustules identified on any of the slides created from the remaining biofilm material.

Out of the thirty-six diatom species observed in this experiment, twenty-two species did not demonstrate any specific preference to site or substratum in two-way ANOVA without replication analysis. Of these species, only *Nitzschia linearis* and *Gyrosigma acuminatum* were present at every site. Other species that did not exhibit any statistically significant effects were *Achnanthidium minutissimum*, *Achnathes daonensis*, *Amphora pediculus*, *Amphora inariensis*, *Brachysira brebisonii*, *Brachysira vitrea*, *Diatoma problematica*, *Diatoma vulgaris*, *Encyonema gracile*, *Encyonema reichardtii*, *Gomphonema vibrio*, *Melosira varians*, *Navicula slesvicensis*, *Nitzschia dissipata*, *Rhopoladia gibba*, *Gomphonema* (unknown species), and *Surrirela brebisonii*. Furthermore, *E. prostratum* was only present on stone substratum, which due to the limited availability of these substratum to East campus lake, were only observed at this site. As such, although it met the criteria to be considered for analysis, due to its appearance only on East campus stone biofilms, statistical analysis could not be completed. The most abundant diatoms observed are discussed in detail below.

Gomphonema cuneolus was the only species found in all water bodies in relatively large (>10%) abundances (11.06 ± 2.27) (Figure 5.4.), with particularly high abundances on Selby canal substratum (15.06-21.56) and Wheldrake Lake plant substratum (24.35% ± 10.79%). *G. parvulum* was also present across all sites, but was never particularly dominant within the community, and never exceeded 5% of the communities, except on Wheldrake sediment substratum, where it was present in 9.1% of the community (Appendix j). *Cocconeis disculus* is common to three of the rivers and Castle Howard. It was a primary species in the community from Selby canal (36.88% to 56.41%), as well as dominating the sediment substratum from Pocklington canal (27.27%), and the plant substratum from Castle Howard (7.21% - 29.30%). It was also the second most common species on the plant substratum in East campus lake (33.32% ± 31.71%). *Rhoicosphenia abbreviata* also favoured Selby canal, but only on the sediment substratum (18.43%), and also appeared in reasonable abundances at Pocklington canal (4.54%) (Figure 5.4., Table 5.4.).

Achnanthidium minutissimum was mainly found in the two lakes and is shown to comprise much of the biofilms from East campus lake (3.18% ± 0.69% to 39.31% ± 21.21%) and Wheldrake Lake (1.97% to 48.37 ± 9.58%). It favoured the plant substratum in both lakes, despite the ANOVA results indicating no significant differences.

Melsoira varians was also a major contributor to Wheldrake Lake sediment biofilm (20%), as well as the Castle Howard biofilms on both sediment and plant substratum (15.05-15.74%) and East campus lake stone substratum biofilm (33.16% ± 7.34%). Although as with A. minutissimum, the ANOVA analysis shown in Table 5.4. do not consider these differences to be significant.

With the exception of *Fragiliaria spp.*, and *Navicula rhynchotella*, both of which favour Castle Howard (Figure 5.4., Table 5.4.), and *Amphora pediculus*, which constituted large proportions of the benthic biofilms under very specific conditions, the remaining species identified do not exceed 10% of the communities observed in this experiment (Figure 5.4., Table 5.4.). The differences in abundance of these species between different sites and substratum are discussed below.

Site:
There were statistically significant differences in the abundances of six species between sites. *N. palea var debilis* was present on Wheldrake (1.97%), Castle Howard (0-2.30%) and Selby canal (0-0.97%), but was absent from the two substrata (plant and sediment) statistically analysed here, although the data for East campus lakes shows this species present on the stone substratum at 0 ± 2.63%. *G. parvulum*, was present at all sites, with the highest abundances observed at Wheldrake (0-9.18%), with lower abundances at Castle Howard (3.28-5.65%), Pocklington (0-

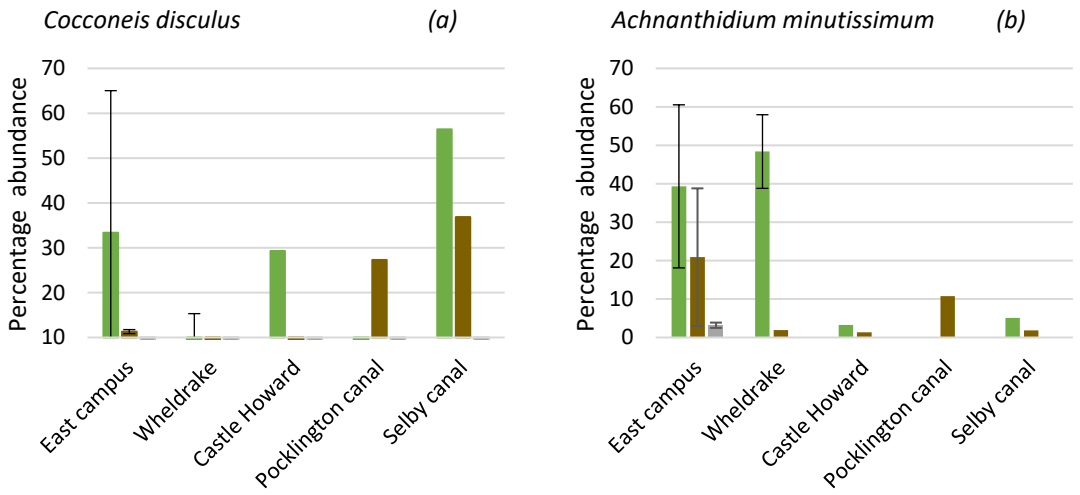
3.57%), Selby canal (0.96-1.25%). East campus (0-1.50%) exhibited the lowest abundances (Appendix j, Table 5.4.).

A further four species demonstrated a preference towards Castle Howard, but only on the plant substratum. These were *A. modestiforme* (3.23%) (also present at Selby canal (0.32%)) (P<0.001, one-way ANOVA without replication), *N. rhynchotella* (5.11%) (also present in very low abundances at East campus lake (0.32% ± 0.32%) and Wheldrake (0.32% ± 0.33%)) (P=0.003, one-way ANOVA without replication), *Nitzschia paleacea* (4.30%) (also present on the plant substratum at Wheldrake (0% ± 0.17%)) (P<0.001, one-way ANOVA without replication), and *Fragilaria spp.* (6.99%) (was also present to a lesser extent at Wheldrake (1.82%)) (P=0.030, one-way ANOVA without replication).

Substratum:
There were four species that demonstrated a preference for specific substratum. Four species preferred to grow on sediment, these were *N. lanceolota*: (plant: 0-1.56%, sediment: 0-8.52%) and *N. palea var debilis* (plant: 0%, sediment: 0-2.30%). The remaining two species that demonstrated a substratum difference only occurred at specific sites. These were *Fragilaria (unknown species)* (plant: 0%, sediment: 3.28%) (only present at Wheldrake and Castle Howard) and *N. paleacea*: (plant: 0% ± 0.17%), sediment: 8.85%) (only at Wheldrake, Pocklington canal and Castle Howard) (P<0.001 and 0.021, respectively, one-way ANOVA without replication), although for this latter species higher abundances on plant substratum did occur at Castle Howard (plant: 4.3%, sediment: 0.6%).

Further substratum effects limited to specific sites occurred for *Gomphonema truncatum* (limited to Pocklington and Selby canal sediment substratum), *Navicula radiosa*, *Nitzschia minuta*, *Rhoicosphenia abbreviata* and *Surrirela roba*. However, due to the lack of replication ANOVA analysis was unable to determine the validity of these effects (Table 5.4.). Additionally, the species *Nitzschia linearis* also tends to prefer sediment substratum (Appendix j, Table 5.4.)

Furthermore, *A. modestiforme* was more abundant on plant substratum, but only at Castle Howard, and was more abundant on sediment substratum at Wheldrake, Pocklington Canal, and Selby canal (Figure 5.4., Table 5.4.). Other species appear to demonstrate a preference for specific substratum based on Figure 5.4. and the graphs shown in the Appendix j, but the ANOVA analysis does not confirm this. *Achnanthidium minutissimum*, *Amphora pediculus* (except at Pocklington canal), *Rhopalodia gibba*, all appear to demonstrate a preference towards plant substratum, whilst a further four species appeared to prefer the sediment substratum. These were *F. spp.*, *N. rhynchotella* (only at Castle Howard), *R. abbreviata*, and *Gyrosigma accuminatum* (only at Wheldrake and Pocklington canal) (Figure 5.4., Appendix j, Table 5.4.).



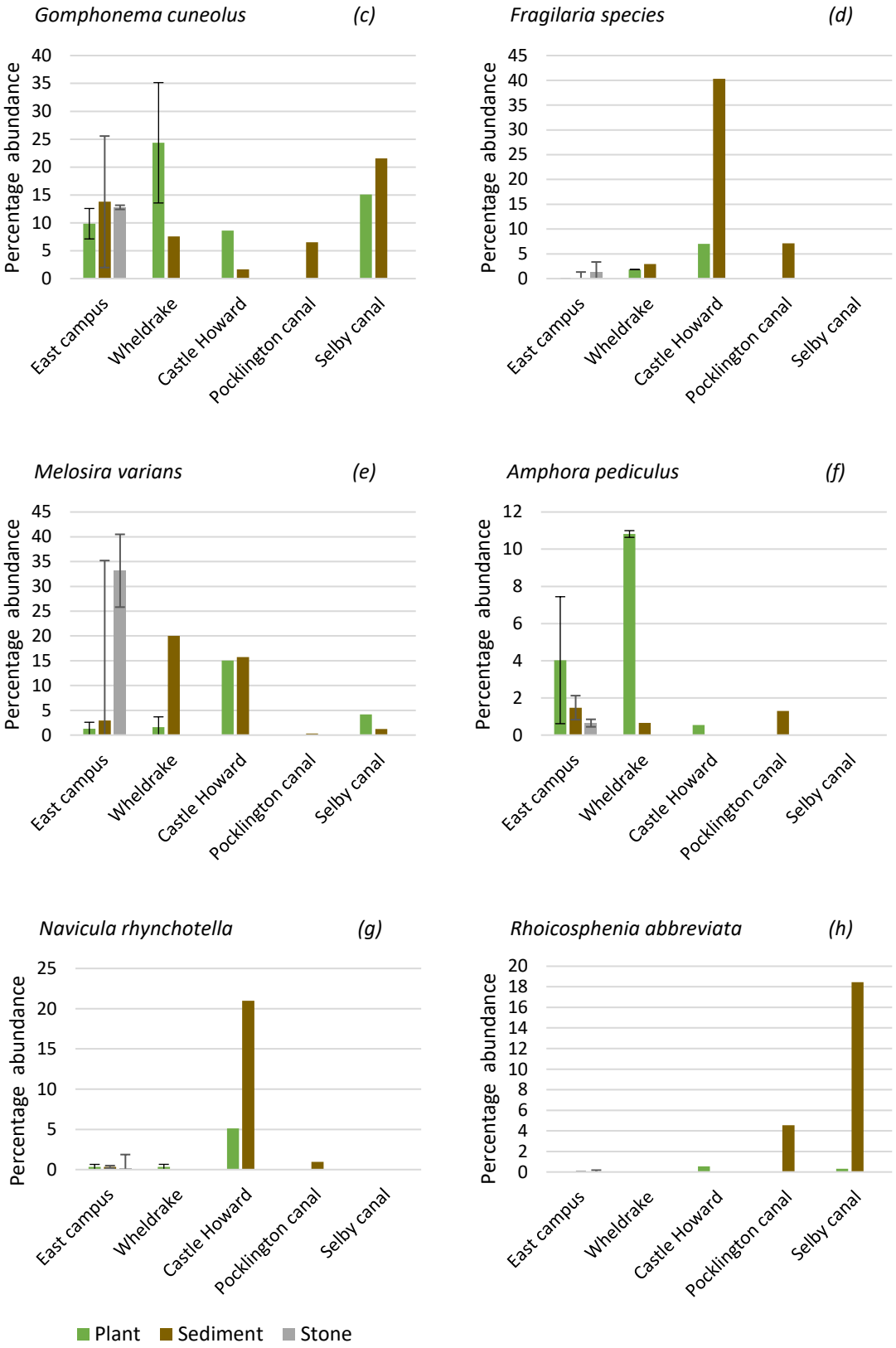

Gomphonema cuneolus (c)
Percentage abundance
East campus
Wheldrake
Castle Howard
Pocklington canal
Selby canal
Fragilaria species (d)
Melosira varians (e)
Amphora pediculus (f)
Navicula rhynchotella (g)
Rhoicosphenia abbreviata (h)
Plant
Sediment
Stone

Figure 5.4. The relative abundances of benthic diatoms species on plant substratum (green), sediment substratum (brown) and stone substratum (c) present across the five sites (East campus, Wheldrake, Castle Howard, Pocklington canal, and Selby canal). Note that although samples were taken for plant substratum from Pocklington canal, no diatom frustules were observed on either of the duplicate slides prepared for this assessment. Samples taken October 2019. Graphs for the remaining species are located in the appendix (Appendix j).

Table 5.4. Two-way ANOVA without replication results for the fifty-four benthic diatom species identified from the October 2020 field sampling period across five reference sites. Note that Eunotia species is not on this table, as although it did appear in abundances above 2% in at least one sample, there was not enough data for the analysis to be conducted.

	Cocconeis disculus			*Achnanthidium minutissimum*			*Gomphonema cuneolus*			*Fragilaria species*			*Melosira varians*		
Source	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Site	0.602	4	0.689	1.168	4	0.468	28.664	4	**0.010**	186.319	4	**0.001**	0.556	4	0.713
Substrate	0.113	1	0.759	0.063	1	0.818	20.085	1	**0.021**	193.426	1	**0.001**	0.066	1	0.814
Site * Substrate	0.260	4	0.887	0.225	4	0.908	12.611	4	**0.032**	77.848	4	**0.002**	0.034	4	0.997

	Amphora pediculus			*Navicula rhynocotella*			*Rhoicosphenia abbreviata*			*Diatoma problematica*			*Nitzschia minuta*		
Source	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Site	0.655	4	0.663	657.624	4	**<0.001**	1573.270	4	**<0.001**	7.800	4	0.062	62.965	4	**0.003**
Substrate	0.008	1	0.934	228.587	1	**0.001**	2472.509	1	**<0.001**	0.518	1	0.524	147.778	1	**0.001**
Site * Substrate	0.004	4	1.000	199.503	4	**0.001**	1539.070	4	**<0.001**	0.637	4	0.672	69.999	4	**0.003**

	Brachysira vitrea			*Nitzschia paleacea*			*Gomphonema parvulum*			*Gomphonema vibrio*			*Navicula lanceolota*		
Source	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Site	0.569	4	0.706	535.586	4	**<0.001**	12.894	4	**0.031**	4.982	4	0.109	5.881	4	0.089
Substrate	0.002	1	0.965	375.113	1	**<0.001**	6.113	1	0.090	8.926	1	0.058	10.500	1	**0.048**
Site * Substrate	0.749	4	0.619	805.586	4	**<0.001**	9.099	4	0.050	4.165	4	0.135	4.757	4	0.115

	Nitzschia dissipita			*Rhopaladia gibba*			*Nitzschia palea var debilis*			*Nitzschia linearis*			*Encyonema gracile*		
Source	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Site	5.974	4	0.087	0.163	4	0.944	11.111	4	**0.038**	5.281	4	0.101	0.282	4	0.873
Substrate	4.818	1	0.116	0.013	1	0.918	38.208	1	**0.009**	9.815	1	0.052	0.003	1	0.959
Site * Substrate	2.775	4	0.214	0.015	4	0.999	8.196	4	0.058	3.503	4	0.166	0.031	4	0.997

	Amphora inariensis			*Eunotia species*			*Fragilaria (unknown species)*			*Amphora modestiforme*			*Gyrosigma accuminatum*		
Source	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Site	2.956	4	0.200	3.329	4	0.175	3.01x10^30	4	**<0.001**	3.93x10^30	4	**<0.001**	2.004	4	0.297
Substrate	0.029	1	0.875	2.975	1	0.183	4.02x10^30	1	**<0.001**	3.99x10^29	1	**<0.001**	5.008	1	0.111
Site * Substrate	0.236	4	0.902	3.859	4	0.148	2.99x10^30	4	**<0.001**	2.32x10^30	4	**<0.001**	3.615	4	0.160

	Surrirela brebisonii			*Achnanthes daonenese*			*Gomphonema (unknown species)*			*Brachysira brebisonii*			*Gomphonema truncatum*		
Source	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Site	6.029	4	0.086	5.271	4	0.102	0.536	4	0.724	0.411	4	0.795	2.50x10^29	4	**<0.001**
Substrate	2.500	1	0.212	0.925	1	0.407	0.019	1	0.899	0.011	1	0.924	6.89x10^29	1	**<0.001**
Site * Substrate	3.416	4	0.170	2.205	4	0.271	0.021	4	0.999	0.069	4	0.987	2.50x10^29	4	**<0.001**

	Navicula radiosa		
Source	F	df	P
Site	x10^30	4	**<0.001**
Substrate	x10^29	1	**<0.001**
Site * Substrate	x10^30	4	**<0.001**

To summarise, twenty-one of thirty-five species identified at abundances above 2% did not show any preference for site or substratum. Additionally, the abundance of fourteen species identified

differed between site, although the abundances of the different species varied between these sites. Eleven species were observed to have a substratum preference, and this was usually towards the sediment substratum. These results indicate that there is no common diatom community structure between the sites analysed here. Therefore, a typical diatom community for the Vale of York cannot be identified for water bodies rated as being in "good" ecological status by the EU water framework directive classification and used here. Further analysis in this chapter will analyse the water quality measurements of the site, and determine which factors drive the local variations in the diatom communities.

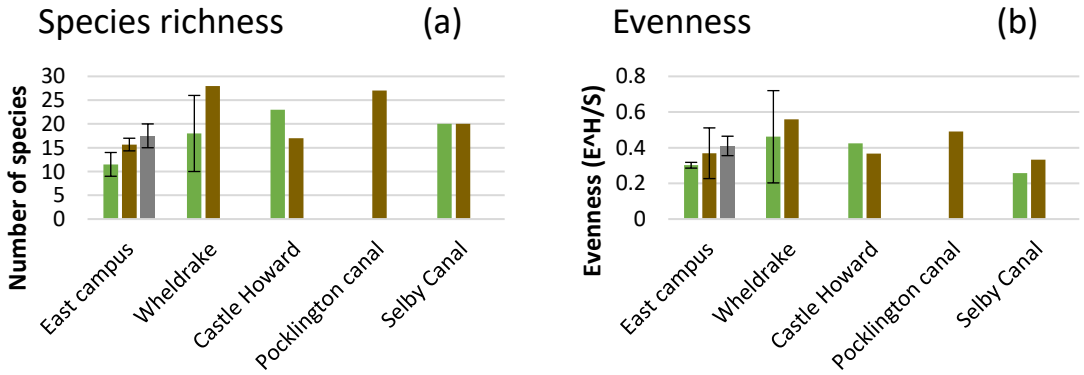
5.4.4. Diversity indices

Site:

Although there was no significant effect of either site or substratum for the species richness or the evenness, there was a trend towards specific effects limited by site and substratum interactions the Shannon-H index (Figure 5.5., Table 5.5.) Further one-way ANOVA analysis was not possible for determining substratum effects at specific sites, but this analysis did confirm that there were specific differences between the sites for the sediment substratum (P=0.049, N= 4, One-way ANOVA). Although the limited dataset prevents the use of Posthoc testing to confirm this, it appears to be due to higher Shannon- H indices observed on Pocklington canal (2.58) and Wheldrake (2.75 ± 0.32), and lower values on East campus lake (1.57 ± 0.2), compared to Castle Howard (1.83) and Selby canal (1.89).

Some observations can be made by comparing the results to the overall mean and standard error for the dataset for the species richness and evenness. For the entire dataset the mean species richness was 18.4 ± 1.51, mean Shannon-H: 1.88 ± 0.12, and the mean evenness was 0.39 ± 0.03. As such, the species richness on East campus plant biofilms is lower than this, but the Wheldrake and Pocklington canal sediment biofilms had a typically higher number of species present than the mean ± SE. The same occurs for the Shannon-H index scores and the evenness index, although for the evenness values of the Selby canal, substratum were also lower than the mean ± SE.

To summarise, these results show that there are between 10 and 27 species in the diatom communities of the biofilms measured, with Wheldrake and Pocklington canal demonstrating the largest range of diatoms (although for the latter this is due to very high species richness on the sediment substratum, and no individuals present in the biofilm samples used for the diatom analysis from the plant substratum for a comparison). There is also very little difference in the evenness and Shannon-H index results between the sediment and plant substratum, or between the sites.



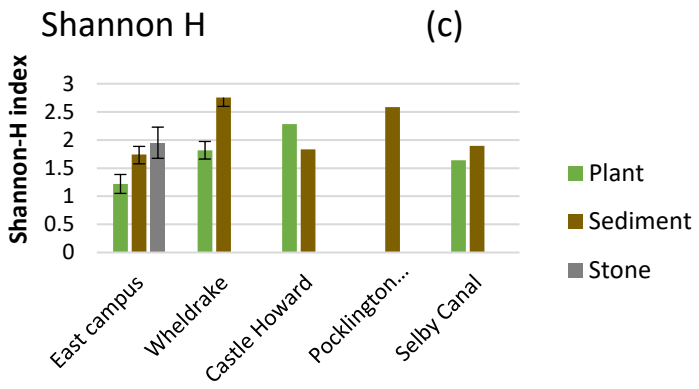


Figure 5.5. Diversity indices (a. species richness, b. evenness, and c. Shannon-H) for the field sites (EC= East campus lake sites, WD= Wheldrake, CH= Castle Howard, SC= Selby Canal, PC= Pocklington Canal) split by the substratum the biofilms were taken on (plant, sediment, and stone).

Table 5.5. Two-way ANOVA (without replication) results for the biofilm measurements (Chlorophyll-a and AFDW), and the diatom diversity indices (species richness, evenness, Shannon-H)

	Species richness			Evenness			Shannon-H		
Source	F	df	P	F	df	P	F	df	P
Site	0.976	4	0.530	0.400	4	0.802	8.671	4	0.053
Substrate	0.296	1	0.624	0.140	1	0.733	8.647	1	0.060
Site * Substrate	0.594	3	0.661	0.064	3	0.975	5.894	3	0.090

5.4.5. UKTAG assessment results

Site:

There were clear differences between the TDI values across the sites, with East campus substratum demonstrating much higher values than the rest, showing a range of 0.75-1, rating the lake as being in good to high ecological quality. While Castle Howard, Wheldrake and Pocklington canal all being within the range of 0.34-0.52, placing these water bodies in poor to moderate classifications, indicating that the water quality of the sites has suffered some deterioration compared to the reference sites due to nutrient enrichment. Finally, Selby canal biofilms had the lowest TDI values (0.20 to 0.23), which correlates to a bad to poor classification, indicating that the communities significantly differ from the composition of the reference sites due to high nutrient concentrations. However, ANOVA analysis (Table 5.6.), does not confirm that these differences between the sites are statistically significant, likely due to the similarity of Wheldrake, Pocklington canal and Castle Howard, and the limited replication in the dataset.

Substratum:

There was a trend towards a substratum effect on the percentage of motile species within the biofilms (Figure 5.6., Table 5.6.) and although post-hoc testing could not identify this, visual inspection of the data shows that there were more motile diatoms on the sediment substratum (3.6-39%) compared to the plant substratum. The sediment substratum show much more motile species at all sites.

There was also an observed tendency towards an effect for the site factor on both the percentage of motile and organic tolerant species. whereby Wheldrake (motile: 10.7-39%, organic tolerant 5.9-33.9%) demonstrated higher percentages of these species compared to the other four sites,

particularly against East campus lake (motile: 0.3-4.5%, organic tolerant: 0-2.9%), as well as Selby canal's organic tolerant species (1-2.5%) (Figure 5.6., Table 5.6.). For this there was no statistically significant P value (P=0.122 for percentage motile and 0.107 for percentage of organic tolerant species), these values are close to 0.1 and may be due to low levels of replication within the dataset.

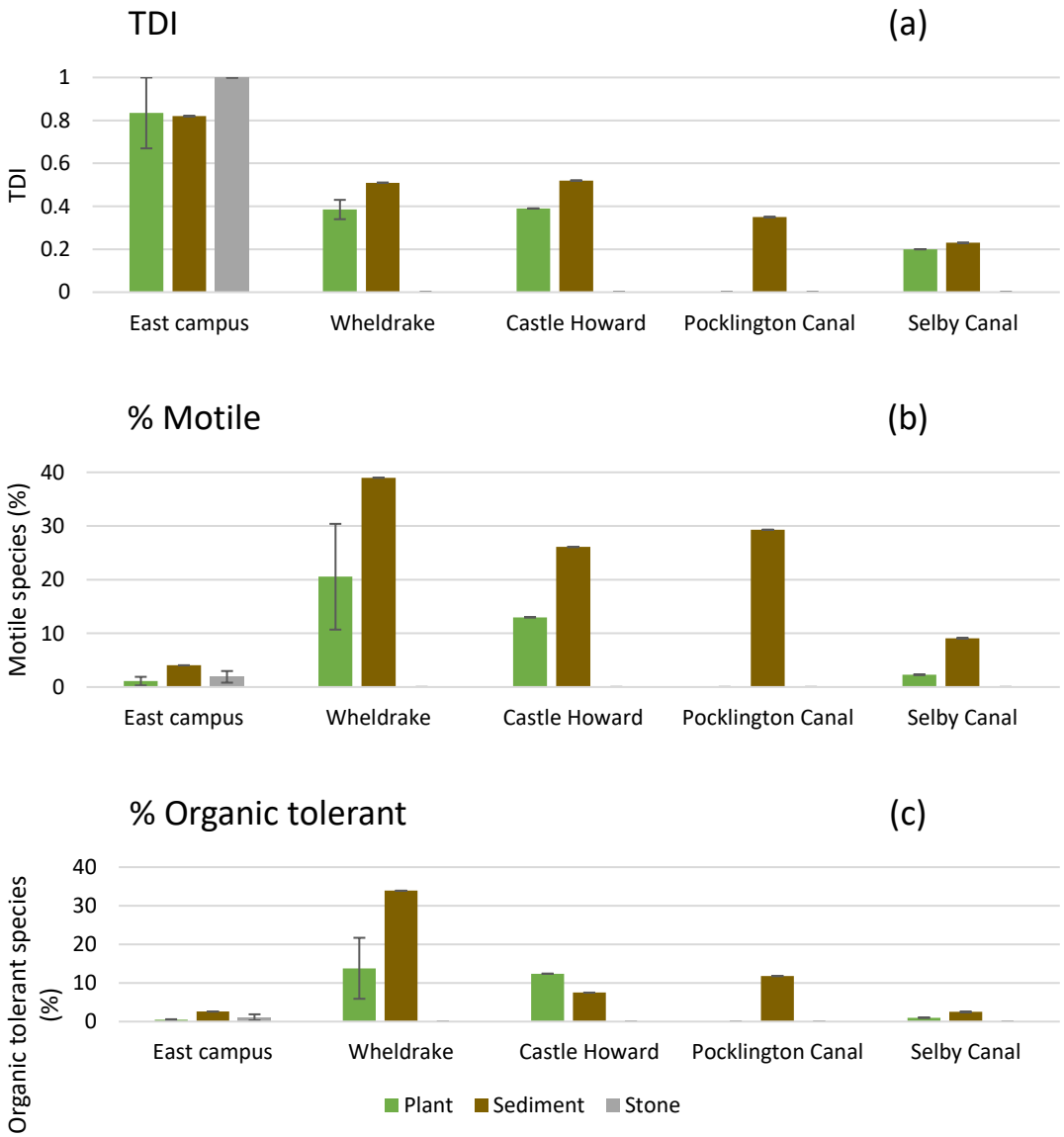


Figure 5.6. Graphs showing the TDI value (a), percentage motility (b), and percentage organic nutrient tolerant (c) for the eight sites visited across the five field sites used. University of York's East campus lake (three localities: EC1, EC2 and EC3 for mean ± SE where N=3), Castle Howard(CH), Selby canal (SC), Pocklington canal (PC), and Wheldrake (two localities: WD1 and WD2 for mean ± SE where N=2).

Table 5.6. UKTAG assessment two-way ANOVA results for the TDI value (using LTDI2 for lakes and TDI4 for rivers), percentage of species motile, and the percentage on species resistant to organic nutrient enrichment.

	EQR LTDI2/TDI4			% Motile			% Organic tolerant		
Source	F	df	P	F	df	P	F	df	P
Substrate	1.681	4	0.349	5.292	4	0.101	5.585	4	0.095
Location	1.107	1	0.370	8.988	1	0.058	2.652	1	0.202
Substrate * Location	0.194	4	0.927	1.044	4	0.506	1.401	4	0.407

To summarise, there was no statistically significant effect between the different water bodies assessed, although clear differences can be observed between some of the sites' TDI values. Motile species were also shown to be more abundant on loose sediment substratum, whilst both motile and organic tolerant species were the most prevalent at Wheldrake, and most limited on East campus lake, where for the latter site the TDI value was also observed to be the highest.

5.4.6. Biofilm analysis summary:

Biofilms taken from plant substratum were typically more productive than those on the other substratum (sediment and stone), based on chlorophyll-a concentrations, however there was usually more organic matter, measured by AFDW, present in the sediment substratum. The latter also demonstrated higher percentages of motile diatom species, compared to biofilms from stone and plant substratum. Although the lack of replication and second sampling period prevented more in-depth analysis of the diatom communities, the communities present during the current sampling period were predominantly composed of diatoms, which comprised between 6.5% and 84.5% of the algal community. The two exceptions to this were the benthic sedimentary biofilms of Castle Howard and Selby canal, where chlorophytes dominated the algal assemblage, comprising 68% and 87% of the algal community, respectively, and the sediment biofilm from the third East campus field sites, whose cyanobacterial composition reached 69%. These communities were typically dominated by the species *A. minutissimum*, *G. cuneolus*, *C. disculus*, and/or *M. varians*. Only the former two species were present on all sites and substratum, and the range of species present at each site varied from nine to twenty-seven.

5.5. Effects of physico-chemical parameters on diatom community health

5.5.1. Analysis of physico-chemical measurements

The concentrations of the different physico-chemical parameters are shown in Figure 5.7., split by the sites the samples were taken from. Table 5.7. shows the results of the one-way ANOVA analysis of the data.

There was a significant effect of site on the concentrations of most physico-chemical parameters measured, with only the concentrations of potassium, magnesium, zinc, calcium, aluminium and lead demonstrating no significant difference between the sites (Figure 5.7., Table 5.7.). However, as posthoc testing could not be completed for any further measurements taken by probe readings or measured using the ICP-OES due to the lack of replication in three of the five sites (Castle Howard, Pocklington canal, and Selby Canal), the interpretation of these differences has been completed using visual analysis of the data presented in Figure 8 alone.

Phosphate concentrations were observed to be below 0.1 ppm at the East campus sites, Wheldrake, Pocklington canal and Castle Howard, Selby canal demonstrated much higher concentrations of phosphate (1.09-1.12 ppm). Ammonium concentrations were highest at Castle Howard (0.31 ppm), compared to the other four sites (0.08 ppm to 0.11 ppm), whilst nitrate concentrations were highest at Pocklington Canal (12.02 ppm to 12.16 ppm). Wheldrake, and Selby canal also showed similar elevated concentrations of TN (4.52 ppm to 5.07 ppm), compared

to East campus lake and Castle Howard (0.38 ppm to 1.33 ppm). As such, nitrate is a key nutrient that differentiates the field sites. Similarly, for both nitrate and nitrite, the highest concentrations were observed at Pocklington canal (nitrite: 0.1-0.13, nitrate: 47.75-47.82 ppm). There were also significantly elevated concentrations of TN (15.27-18.38 ppm) and nitrite (0.02-0.07 ppm) at Wheldrake and Selby Canal, compared to East campus lake and Castle Howard (nitrite: 0-0.04 ppm, TN: 0.06-0.31 ppm).

Wheldrake was the most alkaline site (197.25 ± 3.6 mg/l), followed by Pocklington canal and Selby Canal (153 mg/l). All other sites demonstrated similar alkalinity values (90 to 123 mg/l).

pH and dissolved oxygen (DO) (Figure 5.8., Table 5.8.) parameters of the water in the Castle Howard lake (7.39 pH and 4.42 mg/l) and Selby Canal (7.34 pH, and 7.15 mg/l) were lowest, whilst East Campus lake demonstrated the highest values (8.75 ± 0.034 pH, 11.50 ± 0.16 mg/l). DO and pH vary considerable between the sites.

Similar trends were observed for the electrical conductivity results, as Castle Howard demonstrated the lowest electrical conductivity (514 µs), compared to Wheldrake and Selby canal (716-726 µs), whilst the highest conductivities were observed in East Campus lake (875 ± 2.89 µs), and Pocklington canal (912 µs).

Light attenuation was highest on East Campus Lake (56.10 ± 0.57%), followed by Wheldrake (32.31% ± 14%), which was slightly higher than Pocklington canal and Castle Howard (19.82% to 24.76%). Selby canal had the lowest light attenuation over 10 cm (10.44%). For temperature, all sites demonstrate similar results (14.25-16.20^{0}C) except for East Campus lake, which was shown to be slightly warmer (19.16^{0}C).

Conversely, silicon concentrations were highest at Castle Howard (0.00756 ppm), and lowest in East campus lake (0.007 ppm ±0.00016 ppm), with Wheldrake, Pocklington canal and Selby canal showing roughly similar concentrations in the range of 0.00255-0.00477 ppm.

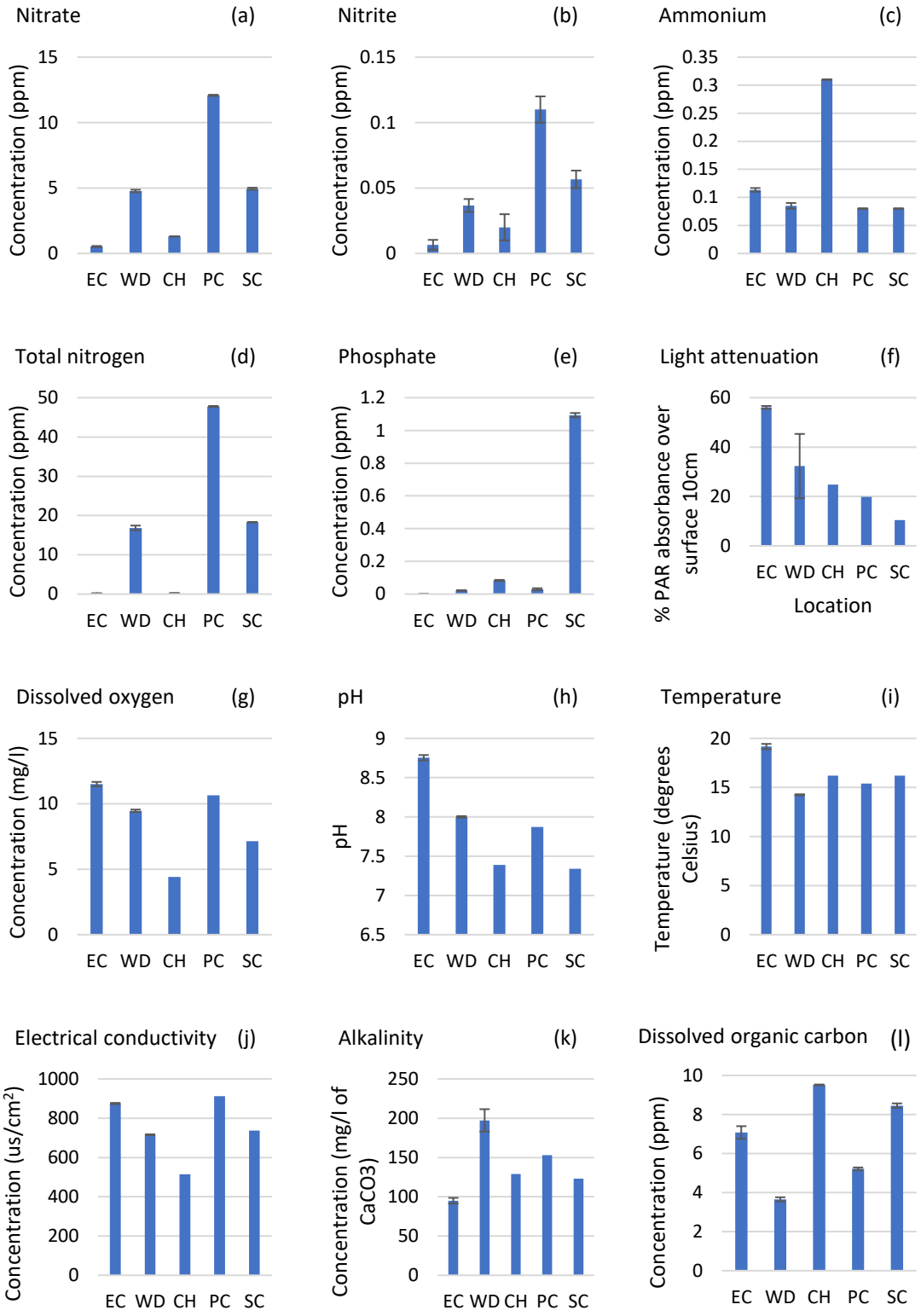
Sulphate concentrations were also lowest at Castle Howard (20.47-20.74 ppm), with slightly higher concentrations in East campus lake (25.24-43.25 ppm). Higher concentrations were observed at Wheldrake ings and Selby canal (63.48-67.0 ppm), and Pocklington canal had the highest concentrations of sulphate (114.84-115.06 ppm).

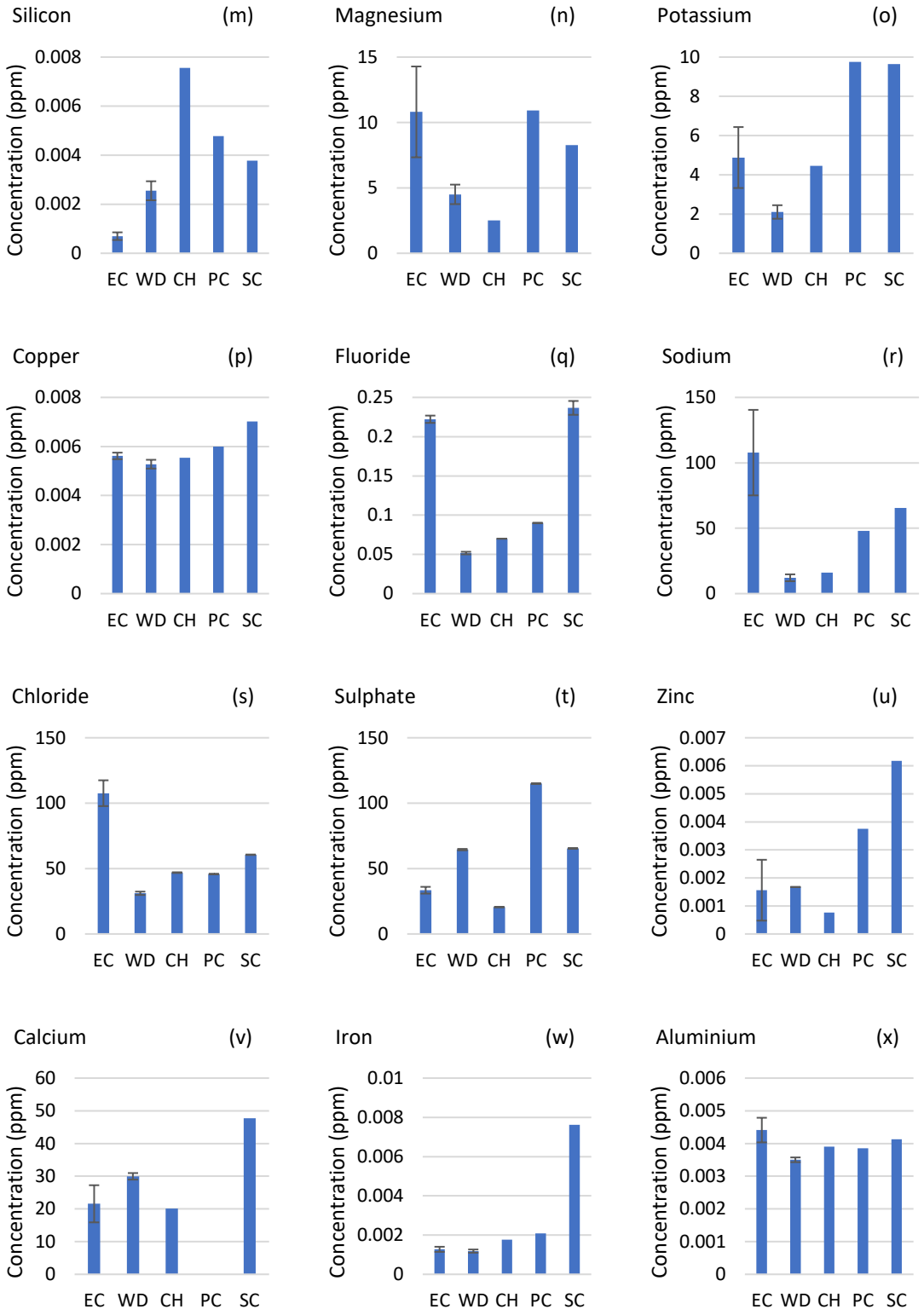
The concentrations of copper were similar across the sites (0.00520-0.006 ppm), although noticeably higher at Selby canal (0.00702 ppm). The same effect was observed for iron (Figure 5.8., Table 5.8.), where iron concentrations were highest for Selby canal (0.00762 ppm), whilst the other sites showed values within the range of 0.0011 ppm to 0.0021 ppm.

Concentrations of nickel at Wheldrake, Castle Howard and Selby canal were below the detection limits of the instrument (0.00001 ppm), whilst East campus contained concentrations of (0.3 parts per billion (ppb)), and Pocklington canal demonstrating much lower concentrations of 0.0927 ppb. However, these values are far below typical freshwater concentrations (Chapter 2, Table 2.16.).

DOC varied considerably across the sites. Wheldrake had the lowest concentrations (3.42-4.06 ppm). Pocklington canal had higher concentrations (5.09-5.34 ppm), followed by East campus (6.04-6.97 ppm), Castle Howard (9.47-9.54 ppm) and Selby canal (8.31-8.68 ppm). The former two demonstrated significantly different concentrations to the latter ($P<0.050$, Tukey HSD).

Fluoride concentrations were also lowest at Wheldrake, as well as Castle Howard and Pocklington canal (0.05-0.094 ppm), with significantly higher concentrations occurring at East campus lake and Selby canal (0.2-0.25 ppm). For chloride concentrations, all sites demonstrate similar results, (31.10-60.50 ppm), except for East Campus lake, which contained higher concentrations of chloride (76.05-245.05 ppm).


Nitrate
(a)
Concentration (ppm)
15
10
5
0
EC WD CH PC SC
Nitrite
(b)
Concentration (ppm)
0.15
0.1
0.05
0
EC WD CH PC SC
Ammonium
(c)
Concentration (ppm)
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
EC WD CH PC SC
Total nitrogen
(d)
Concentration (ppm)
50
40
30
20
10
0
EC WD CH PC SC
Phosphate
(e)
Concentration (ppm)
1.2
1
0.8
0.6
0.4
0.2
0
EC WD CH PC SC
Light attenuation
(f)
% PAR absorbance over surface 10cm
60
40
20
0
EC WD CH PC SC
Location
Dissolved oxygen
(g)
Concentration (mg/l)
15
10
5
0
EC WD CH PC SC
pH
(h)
pH
9
8.5
8
7.5
7
6.5
EC WD CH PC SC
Temperature
(i)
Temperature (degrees Celsius)
20
15
10
5
0
EC WD CH PC SC
Electrical conductivity
(j)
Concentration (us/cm²)
1000
800
600
400
200
0
EC WD CH PC SC
Alkalinity
(k)
Concentration (mg/l of CaCO3)
250
200
150
100
50
0
EC WD CH PC SC
Dissolved organic carbon
(l)
Concentration (ppm)
10
8
6
4
2
0
EC WD CH PC SC


Silicon (m)
Concentration (ppm)
0.008
0.006
0.004
0.002
0
EC WD CH PC SC
Magnesium (n)
Concentration (ppm)
15
10
5
0
EC WD CH PC SC
Potassium (o)
Concentration (ppm)
10
8
6
4
2
0
EC WD CH PC SC
Copper (p)
Concentration (ppm)
0.008
0.006
0.004
0.002
0
EC WD CH PC SC
Fluoride (q)
Concentration (ppm)
0.25
0.2
0.15
0.1
0.05
0
EC WD CH PC SC
Sodium (r)
Concentration (ppm)
150
100
50
0
EC WD CH PC SC
Chloride (s)
Concentration (ppm)
150
100
50
0
EC WD CH PC SC
Sulphate (t)
Concentration (ppm)
150
100
50
0
EC WD CH PC SC
Zinc (u)
Concentration (ppm)
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
EC WD CH PC SC
Calcium (v)
Concentration (ppm)
60
50
40
30
20
10
0
EC WD CH PC SC
Iron (w)
Concentration (ppm)
0.01
0.008
0.006
0.004
0.002
0
EC WD CH PC SC
Aluminium (x)
Concentration (ppm)
0.006
0.005
0.004
0.003
0.002
0.001
0
EC WD CH PC SC

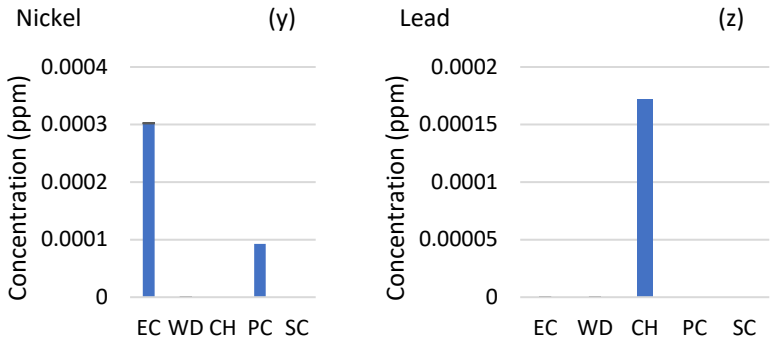


Figure 5.7. Physico-chemical parameters for the eight sites where biofilm samples were taken across the five sites in Yorkshire in September-October 2019. Note that Lead and Nickel values were often below detection limit (0.00001 ppm), as such any negative values recorded were defaulted to zero for these graphs.

Table 5.7. One-way ANOVA without replication results for the physico-chemical parameters from the five field sites in Yorkshire, using site as a factor.

	Total nitrogen			Nitrite			Ammonium			Nitrate			Phosphate		
Source	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Site	1752.972	3	**<0.001**	25.853	3	**<0.001**	382.613	3	**<0.001**	1213.388	3	**<0.001**	14882.501	3	**<0.001**

	Light attenuation			Dissolved oxygen			pH			Temperature			Electrical conductivity		
Source	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Site	6.868	4	0.073	232.163	3	**<0.001**	239.219	3	**<0.001**	21.598	3	**0.016**	2152.164	3	**<0.001**

	Alkalinity			Dissolved organic carbon			Silicon			Magnesium			Potassium		
Source	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Site	20.219	4	**0.017**	47.933	3	**<0.001**	68.670	4	**0.003**	1.105	3	0.468	3.919	3	0.146

	Copper			Fluoride			Sodium			Chloride			Sulphate		
Source	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Site	12.386	3	**0.034**	372.059	3	**<0.001**	1.316	3	0.414	9.041	3	**0.001**	78.417	3	**<0.001**

	Zinc			Calcium			Iron			Aluminium			Nickel		
Source	F	df	P	F	df	P	F	df	P	F	df	P	F	df	P
Site	2.012	3	0.290	1.630	2	0.332	210.859	3	**0.001**	0.550	3	0.682	156.527	3	**0.001**

	Lead		
Source	F	df	P
Site	2.618	3	0.225

5.5.2. Regression analysis

Table 9. shows the correlation coefficient of the equation (R^2), the gradient of the regression line, and the ANOVA analysis of the significance of the model. Graphs of the regression plots of the physico-chemical measurements against the TDI values are available in the appendix (Appendix k).

These results demonstrate that the TDI values of the biofilms had a significant, strong positive correlations were observed between the TDI value and the values for pH (R^2= 0.633), light attenuation (R^2= 0.603), and chloride (R^2= 0.491). There were further negative regressions for seven physicochemical parameters against the TDI values, although their R^2 values were not as strong as the positive correlations. These parameters were total nitrogen (R^2= 0.292), phosphate (R^2= 0.302), nitrate (R^2= 0.272), calcium (R^2= 0.360), silicon (R^2= 0.379), iron (R^2= 0.307) and nitrite (R^2= 0.360) concentrations in the sites assessed, as well as trends towards this effect for zinc, copper and alkalinity. (Table 5.8.).

Table 5.8. Linear regression results of the TDI of benthic diatoms as a function of a range of physico-chemical parameters in five lakes and rivers across Yorkshire in October 2019. Sorted by strength of correlation (R square).

Vale of York freshwater bodies

Model	R Square	Gradient	F	df	P
pH	0.633	+	22.444	1	**<0.001**
Light attenuation	0.603	+	19.771	1	**0.001**
Chloride	0.491	+	12.542	1	**0.004**
Temperature	0.394	+	8.451	1	**0.012**
Dissolved oxygen	0.393	+	8.418	1	**0.012**
Silicon	0.379	-	7.922	1	**0.015**
Nitrite	0.360	-	7.318	1	**0.018**
Calcium	0.360	-	6.741	1	**0.023**
Iron	0.307	-	5.754	1	**0.032**
Phosphate	0.302	-	5.612	1	**0.034**
Nickel	0.295	+	5.451	1	**0.036**
Total nitrogen	0.292	-	5.350	1	**0.038**
Nitrate	0.272	-	4.861	1	**0.046**
Electrical conductivity	0.255	+	4.460	1	0.055
Zinc	0.232	-	3.927	1	0.069
Alkalinity	0.229	-	3.853	1	0.071
Copper	0.217	-	3.605	1	0.080
Sodium	0.172	+	2.695	1	0.125
Sulphate	0.157	-	2.429	1	0.143
Fluoride	0.148	+	2.267	1	0.156
Potassium	0.128	-	1.900	1	0.191
Magnesium	0.068	+	0.946	1	0.348
Lead	0.064	-	0.888	1	0.363
Aluminium	0.056	+	0.765	1	0.398
Ammonium	0.009	-	0.117	1	0.738
Dissolved organic carbon	0.000	+	0.004	1	0.950

5.5.3. Multivariate analysis

Table 5.9 shows the results of the MANOVA tests of each of the twenty-six measurements against the TDI values, whilst Table 5.10. show how the factors considered significant by this analysis

together effect the TDI values. A graph showing the multiple linear regression of this model described in Table 5.10 against the TDI values is shown in the appendix (Appendix l).

The MANOVA results indicate significant negative effects of the concentrations of TN, phosphate, nitrate, lead and alkalinity on the TDI (P= 0.012, <0.001, 0.010, <0.001, 0.040, respectively), as well as a trend towards this effect for silicon, ammonium, iron and nitrite (Table 5.9.). Meanwhile, there were significant positive effects of water body pH, electrical conductivity, and light attenuation to the TDI of the biofilms (P= 0.017, 0.020, 0.020, respectively) (Table 5.9.).

Furthermore, the incorporation of these factors into a multiple linear regression model, retaining ammonium, alkalinity, phosphate, nitrite, silicon, light attenuation and pH can account for 74.3% of the variation in the TDI values observed in the dataset, indicating that there is strong correlation between these combined parameters and the TDI values of biofilms in these water bodies (Table 5.9., Table 5.10.), but the P value was only 0.092 (Table 5.10.). This infers that although there is a strong correlation between the seven physico-chemical parameters used for the model and the TDI of the water bodies assessed, statistical analysis can only conclude that these parameters are likely to influence the TDI in these sites, but cannot confirm this.

Table 5.9. MANOVA results for the TDI values of the biofilms against the physico-chemical parameters of the water bodies the biofilms were retrieved from.

MANOVA (TDI and physico-chemical measurements

Source	F	df	P
Nitrate	6358.007	12	**0.010**
Nitrite	70.155	12	0.093
Total nitrogen	4407.280	12	**0.012**
Ammonium	130.405	12	0.068
Phosphate	2.61x10^29	12	**<0.001**
Light attenuation	1460.739	12	**0.020**
Dissolved oxygen	75.276	12	0.090
pH	2099.476	12	**0.017**
Temperature	15.693	12	0.195
Electrical conductivity	1467.541	12	**0.020**
Alkalinity	385.622	12	**0.040**
Dissolved organic carbon	14.852	12	0.200
Silicon	85.327	12	0.084
Magnesium	0.766	12	0.724
Potassium	1.173	12	0.626
Copper	30.460	12	0.141
Fluoride	37.164	12	0.128
Sodium	1.185	12	0.624
Chloride	4.411	12	0.357
Sulphate	15.850	12	0.194
Zinc	0.839	12	0.704
Calcium	1.651	12	0.549
Iron	62.755	12	0.098
Aluminium	1.933	12	0.514
Nickel	7.905	12	0.272
Lead	2.05x10^29	12	**<0.001**

Table 5.10. Model summary and regression summary of the multiple linear regression model using factors considered significant by the MANOVA analysis. Table includes the R^2 (R square) value, Standard error of the estimate, direction of the linear regression's gradient, and the model's ANOVA F, df, and P values. Note that the model discounted the lead variable.

Model Summary						
Model	R Square	Std. Error of the Estimate	Gradient	F	df	P
1	0.743	0.170	+	2.895	7	0.092

Predictors: Ammonium, Alkalinity, Phosphate, Nitrite, Silicon, Light attenuation, pH

5.5.4. Water chemistry analysis summary:

To summarise, the sites used here covered a wide range of physico-chemical properties, with different sites exhibiting different nutrient concentrations and values of physical properties.

These parameters, when measured against the TDI values, show that increased total nitrogen, phosphate, nitrate, calcium, silicon, iron and nitrite, zinc and copper concentrations, as well as alkalinity, correlated to biofilms whose structure and composition were indicative of lower ecological quality. Concentrations of dissolved oxygen, chloride and nickel, as well as the temperature, light attenuation and electrical conductivity all positively correlated towards diatom community's indicative of higher ecological quality and lower trophic state. When modelling the TDI against the physico-chemical measurements simultaneously, however, the physico-chemical variables that either significantly effect, or tended to affect the TDI, is reduced to total nitrogen, nutrients, phosphate, lead, iron, dissolved oxygen and silicon concentrations. As well as the alkalinity, temperature, light attenuation and pH, which when used to generate a multiple linear model, were further reduced to seven factors (ammonium, alkalinity, phosphate, nitrite, silicon, light attenuation and pH) which were able to predict 74.3% of the TDI variation.

5.6. Discussion

5.6.1. Identification of the structure and composition of diatom communities in water bodies rated good or higher by the EU water framework directive across the Vale of York and exploration of the relationships between the communities and water quality measurements

5.6.1.1. Overall observations of benthic biofilms from the Vale of York

Overall, biofilm indices indicate that there was a range of 0.97 to 17.92 ug/cm^2 of chlorophyll-a in the biofilms, and a range of 1.27 to 87.28 mg/cm^2 of AFDW in the biofilms. Compared to the literature (Sierra and Gomez, 2007), where chlorophyll-a and AFDW concentrations of 0.2 to 60 ug/cm^2 and 2.5 to 20 mg/cm^2, respectively were observed in the Río de la plata estuary, Argentina. The biomass measurements used here varied from being slightly lower to within the lower half of this observed range. Although, as the biofilms sampled in Sierra and Gomez, (2007) were from loose sediment substratum, the lower AFDW seen here, which was observed on plant biofilms would be expected, as these biofilms are less liable to collect sedimented organic matter.

Relative abundance of the algal groups indicates that diatoms were generally dominant in the communities, except at Castle Howard and in the sediment substratum at Selby canal, where chlorophytes were more abundant. Cyanobacteria were also present, but only as 1-10% of the community structure, and never more than 30%. This dominance of diatoms is typical of freshwater diatom communities, as seen on stone biofilms by Pohlon *et al.,* (2010). Although a significant amount of research reviewed by Schnurr and Allen (2015), has concluded that after 20 days of succession from a fresh substratum, filamentous chlorophytes begin to achieve dominance, before eventually being supplanted by cyanobacteria. Although as seen in Chapter 2, with the results of West campus lake showing that this occurred within the first two weeks on

this lake, no evidence of this shift even beginning to occur in East campus lake after ten weeks. As such, this expected successional trend described in Schnurr and Allen (2015) may be limited by local factors, such as nutrient availability. Other research has noted that this diatom abundance is seasonal, with chlorophytes expected to be more abundant in the summer Villanueva *et al.,* (2011). This cannot be confirmed in the work presented here, due to the prevention of field sampling by the Covid-19 lockdown that occurred during the planned sampling of spring and summer biofilms in 2020.

Several species were present across all sites, although not necessarily every substratum. Indicating that only a handful of species may be representative of the communities studied here, whilst also being sensitive to differences in the local physico-chemical measurements observed. These species were *G. cuneolus* (0% to 24.35%), the only highly abundant species that appeared at all sites, with other common species including *C. disculus* (2.06% to 56.41%), *M. varians* (0.32% to 33.16%), *A. minutissimum* (0.88% to 48.37%), *Fragilaria spp* (0% to 40.33%), *A. pediculus* (0-10.82%), and *N. rhynchotella* (0% to 20.96%), which were absent, or almost absent in at least one site. However, other species were common to all sites, albeit at much lower abundances. These were *N. linearis* (0% to 3.95%), *N. minuta* (0% to 13.31%) *G. parvulum* (0% to 9.18%), *A. modestiforme* (0% to 3.23%), and *G. acuminatum* (0% to 1.95%). Further discussion on the relevance of these species to this experiment's objective is in section 5.6.2.

Diatom diversity indices indicate that there were 11.5 to 28 diatom species in the biofilms, with an evenness index score of 0.26 to 0.59 E^H/S. These values indicate that the communities were fairly uneven, and dominated by a handful of species. When developing the TDI method used here, water bodies in England used by Kelly and Whitton (1995) considered 25 species to be a particularly high species richness, indicating that the values seen here, except for the sediment substratum at Wheldrake and Pocklington canal, are typical of the UK. Blanco *et al.,* (2012) reported that diversity indices poorly correlate to abiotic factors in the surrounding environment, but higher nutrient concentrations did allow for the dominance of specific species. Contradictory research by Vilmi *et al.,* (2015), reported that the diversity indices were affected by factors other than nutrients, and other research has shown that species richness is strongly linked to pH (Round, 1991). Research by Kahlert and Gottschalk (2014) using rivers and lakes in Sweden also noted that species richness was typically higher in lakes, which does not support the results seen here, where there was no difference between the species richness of the lakes (East campus lake and Castle Howard), compared to the other river sites.

UKTAG assessment metrics indicate that these sites demonstrated a wide range of TDI values based on the diatom indices, ranging from 'good' to 'poor'. This indicates further analysis is required of the sites, as these sites were used based on the Environment agency (2020) data indicating that as of 2016 these sites were in 'good' or 'high' ecological quality based on the phytobenthos and macrophyte assessment. Analysis of the percentage of motile diatoms and organic nutrient tolerant diatoms indicates an overall range of 1.1 to 39%, and 0.45 to 33.9%, respectively. The abundances of the motile diatoms were strongly influenced by the substratum type, with biofilms from sediment substratum demonstrating significantly higher abundances of motile diatoms compared to the plant substratum, owing to the selective pressures of the environment towards species capable of actively moving through the unstable sediment to reach light and nutrient resources (Méléder *et al.,* 2003).

Table 5.11. Averaged diatom community structure measurements by site and substratum (percentage of diatoms, chlorophytes and cyanobacteria, diversity indices (species richness, evenness and Shannon-H index), and UKTAG endpoints (TDI value, percentages of motile and organic tolerant species)) adjacent to the physico-chemical measurements for the site they were taken from

Site name	Substrate type	% Diatoms	% Cyanobacteria	% Chlorophytes	Species richness	Shannon-H	Evenness	EQR LTDI2/TDI 4	% Motile	% Organic tolerant	Nitrate	Nitrite	Ammonium	Total nitrogen	Phosphate	Light attenuation	Dissolved oxygen	pH	Temperature	Electrical conductivity	Alkalinity	Dissolved organic carbon	Silicon	Magnesium	Potassium	Copper	Fluoride	Sodium	Chloride	Sulphate	Zinc	Calcium	Iron	Aluminium	Nickel	Lead
East Campus	Sediment	132.33	45.67	127.00	17.00	1.89	0.39	0.64	4.05	2.60	0.18	0.01	0.11	0.53	0.00	56.10	11.50	8.75	19.17	875.00	95.00	7.08	0.00	10.81	4.88	0.01	0.22	107.81	107.62	33.45	0.00	21.57	0.00	0.00	0.00	0.00
	Plant	198.00	9.50	102.00	11.50	1.22	0.30	0.62	1.10	0.45																										
	Stone	242.67	33.67	38.33	17.50	1.95	0.41	0.80	1.90	1.10																										
Castle Howard	Sediment	147.00	128.00	55.00	17.00	1.83	0.37	0.48	26.10	7.50	0.21	0.02	0.31	1.30	0.08	24.76	4.42	7.39	16.20	514.00	129.00	9.51	0.01	2.52	4.46	0.01	0.07	16.10	46.92	20.54	0.00	20.14	0.00	0.00	0.00	0.00
	Plant	89.00	7.00	208.00	23.00	2.28	0.43	0.27	13.00	12.40																										
Pocklington canal	Sediment	208.00	43.00	97.00	27.00	2.58	0.49	0.35	29.30	11.80	47.80	0.11	0.08	12.08	0.03	19.83	10.64	7.87	15.40	912.00	153.00	5.21	0.00	10.93	9.75	0.01	0.09	48.03	45.80	114.95	0.00	*2	0.00	0.00	0.00	0.00
	Plant	90.00	216.00	6.00	*1	*1	*1	*1	*1	*1																										
Selby canal	Sediment	231.00	0.00	95.00	20.00	1.89	0.33	0.23	9.10	2.50	18.30	0.06	0.08	4.94	1.09	10.44	7.15	7.34	16.20	737.00	123.00	8.45	0.00	8.28	9.64	0.01	0.24	65.52	60.58	65.41	0.01	47.76	0.01	0.00	0.00	0.00
	Plant	21.00	23.00	306.00	20.00	1.64	0.26	0.20	2.30	1.00																										
Wheldrake ings	Sediment	213.00	47.00	56.00	28.00	2.75	0.56	0.51	39.00	33.90	15.52	0.03	0.08	4.98	0.02	45.34	9.56	8.01	14.20	718.00	183.00	3.87	0.00	5.25	2.45	0.01	0.05	14.74	28.33	64.14	0.00	30.97	0.00	0.00	0.00	0.00
	Plant	163.75	0.00	99.50	14.00	1.90	0.59	0.36	25.48	17.75																										

*1 No frustules identified on any deplicate slides

*2 concentration outside of detection range

Table 5.12. Comparison of the water quality measures used by Kelly *et al.,* (1995), compared to the sites assessed in this chapter, using maximum and minimum values observed at the sites.

Parameter	Reference sites for TDI	East campus lake	Castle Howard	Wheldrake ings	Pocklington canal	Selby canal
Total alkalinity (mg/l)	25-6260	90-102	129	19.28-45.34	153	123
Nitrate and nitrite (ppm)	<0.1-15.91	0.08-0.16	0.2267	15.55-18.22	47.91	18.36
Ammonium (ppm)	<0.05-0.41	0.11-0.12	0.31	0.08-0.09	0.08	0.08
Phosphate (ppm)	<0.001-2.035	<0.001	0.08	0.02	0.03	1.09
Electrical conductivity (µs/cm2)	74-1267	870-880	514	714-718	912	737
pH	5.6-8.8	8.71-8.82	7.39	7.99-8.01	7.87	7.34

5.6.1.2. Site specific metrics and key differences in their physico-chemical measurements

As these broad ranges demonstrate, there were significant variations between the sites studied in this experiment, and although they had many species in common, there were very few similarities between the abundances observed. As shown in section 5.5., these differences were driven by differences in the physico-chemical properties of the sites they were exposed to. Table 5.11. shows the biofilm metric results compared to the sites physico-chemical properties. Table 5.12 shows the key measurements used to define reference sites for the UKTAG assessment, compared to the field sites used here. The key points distinguishing each of the sites are:

East campus lake:

This site demonstrated low concentrations of AFDW and Chlorophyll-a in the biofilms, with the lowest concentrations of the latter, particularly on plant substratum. They also had typical relative abundances of the three key algal groups (diatoms, cyanobacteria and chlorophytes). The diatom communities at this site were composed primarily of *Achnanthidium minutissimum*, *Cocconeis disculus*, and *Gomphonema cuneolus*. These communities demonstrated the lowest species richness compared to the other sites, likely due to the 'newness' of the site and the fact it is supplied through surface runoff and aerial deposition, rather than being fed by an established river. However, the evenness of these communities was typical of the dataset. This site also exhibited the highest TDI value of all the sites, and the lowest percentage motile and organic tolerant species. This was the only site designated as being in 'good ecological quality' by the UKTAG assessment index in respect to the EU water framework directive classifications.

Although this site had the lowest alkalinity out of the sites assessed. The alkalinity factor was a component of the multiple linear regression model used in Chapter 2, (section 2.5.3), indicating that alkalinity likely influenced the composition of the diatom community composition. This site also had low concentrations of diatom/ algal macronutrients (nitrate, nitrite, phosphate, silicon), factors which contribute to higher TDI scores (Eloranta and Soininen (2002). This site also exhibited the highest chloride concentrations (107.62 ppm).

Castle Howard:

Castle Howard biofilms contained fairly typical AFDW concentrations of the sites visited, but also exhibited very high concentrations of chlorophyll-a, particularly on the plant substratum, which was three times higher than the next highest value (Wheldrake plant substratum). The abundance of cyanobacteria in these biofilms particularly low, and the relative abundances of chlorophytes were particularly high on the plant substratum, at the expense of the diatoms. The diatom communities are primarily composed of *C. disculus* and *A. minutissimum* (plant), *G. cuneolus*, *F. spp* (primarily sediment), *M. varians*, and *N. rhynchotella*. The diversity indices (species richness, Evenness, Shannon-H) for this site were average for the sites investigate here. The TDI values seen here are 0.39-0.5. This is fairly average for the dataset, but considering these sites were chosen based on Environment Agency (2019) data indicating the sites were in good or higher ecological quality, these are low values, and would be expected to be at least 0.6. This site also appears to have a fairly average percentage of motile diatoms in the sediment substratum, but comparatively high percentage of these species on the plant substratum, and a fairly average percentage of organic tolerant species (~6 to 12%).

This site exhibited very low concentrations of nitrate and nitrite, TN, similar to East Campus lake, but very high concentrations of ammonium (0.3 ppm) and silicon (0.008 ppm), and elevated phosphate concentrations (0.08 ppm) compared to all other sites except Selby canal. Very low, electrical conductivity (500 us/cm^2) and pH (7.39), as well as low dissolved oxygen, magnesium, potassium and sodium concentrations were also observed in this site. Furthermore, although Castle Howards classification was previously unassessed, this lake is the main source for Cram Beck, a small tributary of the River Derwent that as of 2016 was classified as having 'high' ecological quality based on the macrophyte and Phytobenthos data, but was not determined here to be so. However, as there is very limited replication for most sites, limited to a single time point

at the end of the summer due to Covid-19 restrictions, this cannot be considered representative result for the site.

Wheldrake ings:

Wheldrake biofilms observed typically low AFDW concentrations, similar to East campus lake, but the Chlorophyll-a concentrations were typical of the sites visited. As with Castle Howard, the abundance of cyanobacteria at this site was very low, and were practically absent. The diatoms on the other hand were more abundant than other sites (65-70%), with the chlorophytes accounting for the rest of the communities. The diatom communities here are primarily composed of *M. varians* (sediment), A. pediculus (plant). The communities here are both comparatively diverse compared to the other sites (17 to 27 species), and evenly distributed (Evenness index 0.45 to 0.55). This site had an TDI score of 0.4 to 0.5, the same as Castle Howard. This site also has the highest percentage of motile and organic tolerant species, regardless of substratum.

Wheldrake exhibited low concentrations of magnesium, potassium, sodium, and silicon, but had fairly high light attenuation, and extremely high alkalinity values (197.25 mg/l), which is likely the driving factor for its relatively low TDI value, based on similar effects seen by Eloranta and Soininen (2002). Table 5.13. demonstrates that at Wheldrake, as well as at Pocklington and Selby canals there were higher combined concentrations of nitrate and nitrite than the reference sites, with this being especially true at Pocklington canal.

Pocklington canal:

Pocklington canal biofilms demonstrated fairly average AFDW and chlorophyll-a concentrations on the plant biofilms, but the AFDW on the sediment substratum was particularly high (6 mg/cm^2). The algal groups observed here were within the typical range observed for the rest of the sites, although on plant substratum the diatoms demonstrated fairly low abundances, due to a relatively large proportion of the algal community being comprised of cyanobacteria. The diatom communities were primarily composed of *C. disculus* and *R. abbreviata* on the sediment substratum. However, no frustules were recovered from the biofilm during the peroxide digestion for analysis on the plant substratum. The diversity indices of the sediment substratum was particularly high in all three measurements, indicating that compared to the other sites, the diatom community in this biofilm was comprised of a higher number of species with a more even distribution, explaining why even the most dominant species (*C. disculus*) was quite low in abundance (27%) compared to the dominant species of other sites. This site also had a low TDI score (0.35), and an average number of organic tolerant species (~12%). This site also had a high percentage of motile species (28%), although this is skewed by the lack of diatom community analysis on the plant substratum, due to no frustules being present in the sample after peroxide washing.

This site exhibited very high nitrate (12 ppm), nitrite (0.11 ppm) and TN (47.80 ppm) concentrations, as well as fairly high concentrations of zinc.

Selby canal:

Selby canal biofilms typically had very high AFDW and chlorophyll-a concentrations, in comparison to the other sites, but the chlorophyll-a concentrations observed were average for the dataset. Diatoms appear to dominate the plant biofilms here, comprising 65 of the community here, with the majority of the remainder being composed of cyanobacteria. The sediment substratum however, is composed of almost 90% chlorophytes, with the rest split fairly evenly between diatoms and cyanobacteria. *C. disculus*, *G. cuneolus* and *R. abbreviata* comprised the majority of the communities at this site. The diatom communities here demonstrated a slightly high but still relatively average species richness (20), but the communities had a low evenness index score, similar to East campus lake, and average Shannon-H index score. This site, like East campus lake, had very low percentages of motile and organic tolerant diatom species, but

whereas East campus had the highest TDI score (0.8-1.0), Selby canal demonstrated the lowest (0.2-0.2).

This site had the highest concentrations of iron, zinc, calcium, and phosphate of all the sites. This is seen in the diatom communities by the dominance of *C. disculus*, a species known to prefer nutrient rich conditions. This site also exhibited high nitrate, nitrite and fluoride concentrations, but also the lowest light attenuation (10%), low pH (7.34) and fairly low dissolved oxygen concentrations (7 mg/l). Table 5.11 shows that although Selby canal had higher phosphate concentrations than all the other sites, and exceeds the EU water framework directive guidelines on acceptable total phosphorus concentrations for good quality water in lowland high alkalinity waters (0.069 ppm, UKTAG, 2013), it is still within the range of observed concentrations of phosphate at the reference sites.

5.6.1.3. Physico-chemical measurements and TDI of the sites compared to the literature

When comparing the results of the environmental parameters shown in Figure 5.7., to those observed in the wider literature (Chapter 2, Table 2.16.), the concentrations of most of the metals (copper, zinc, iron, nickel, lead, aluminium) were below background measurements of these parameters observed in other freshwater bodies. But, the concentrations of the alkali/ alkali-earth metals (sodium, potassium, calcium and magnesium), as well as the electrical conductivity and alkalinity (at Wheldrake and Pocklington only) were all above background measurements. This is most likely caused by the underlying geology of the region, as the two main rivers connected to these sites (Ouse and Derwent) run through areas of alkaline rocks (chalk, limestone, halite (UKRI, 2020)).

Castle Howard, Pocklington canal and Selby canal are all classed as having a moderate ecological quality based on the TDI values (Table 5.13). This table also indicates that TDIs developed from sediment biofilms typically provide higher TDI classifications than from biofilms taken from plant substratum, as shown in Figure 5.6. The one exception being on East campus lake, where one of the plant replicates was classed as poor, whilst the other plant replicate indicated water quality at the higher end of "good quality".

Table 5.13., Comparison of the TDI classification boundaries (Bennion *et al.,* 2012) to those ascertained from biofilms taken from East campus lake, Castle Howard great lake, Wheldrake ings, Pocklington canal, and Selby canal.

Classification	Lower TDI value boundaries for high alkalinity water bodies	East campus lake	Castle Howard	Wheldrake ings	Pocklington canal	Selby canal
High	0.92					
Good	0.70	0.74-0.9 (stone), 0.91 (plant)				
Moderate	0.46	0.64 (sediment)	0.48 (sediment)	0.51 (sediment)		
Poor	0.23	0.33 (plant)	0.27 (plant)	0.34-0.43 (plant)	0.35 (sediment)	0.23 (sediment)
Bad	0.00					0.20 (plant)

East campus lake, as well as Castle Howard both typically fall within the observed ranges of the key parameters used to identify reference sites. This indicates that the lower classifications observed in the TDI values of Castle Howard were due to the influence of other factors, beyond the key parameters used to designate the reference sites. The remaining three sites, all demonstrated a combined nitrate/ nitrite concentration exceeding the reference site range. This is likely a key factor driving the lower TDI classification of the communities, favouring nutrient

tolerant species at these sites over the nutrient sensitive species found at East campus lake. Multivariate analyses performed here (Tables 5.9 and 5.10) indicate that as well as these factors used by Kelly et al., (1995) to define reference sites, silicon concentrations and light attenuation were key factors in the regression analysis of the TDI against the physico-chemical parameters of the water bodies. This multivariate analysis did not consider nitrate a significant factor, although this may be due to its separation from the nitrite concentrations in the analysis.

Additionally, in the river sites (Wheldrake Ings, Pocklington canal, and Selby canal), the concentrations of nitrate, nitrite and TN were all above the baseline levels for typical concentrations of the nutrients in freshwater bodies shown in Chapter 2 (Table 2.16.) Ammonium was also above the baseline range shown in Table 5.13 at Castle Howard. The phosphate concentrations were lower than the typical range observed in other freshwater bodies, and also safely below the concentrations prescribed by the WFD (EPA, 2016) for "good" quality (0.1 ppm of phosphate) water, with the exception of Selby canal, where the concentrations were twice that of the maximum values observed by EPA (2016), and over five times the limit set by the WFD, which contradicts the sites overall classification of "good quality" by the Environment Agency in 2016 (Environment Agency, 2019). Exploration of the parameters used to assign this classification (Environment Agency, 2020) indicates that the site was classified as being "high quality" for all other measured physico-chemical parameters (Ammonia, temperature, pH), but was still classed as poor for the phosphate concentrations, creating an overall "good" classification, indicating high phosphate concentrations were present, but not considered by the Environment Agency to downgrade the sites classification. The alkalinity was higher in Wheldrake and Pocklington canal than the baseline average. This is most likely due to the canal flowing east-ward into the River Derwent slightly down-river of the Wheldrake site, with the canal originating in an area comprised of calcareous chalk and limestone, whilst sites further upstream on the river Derwent from the Wheldrake site likewise run through similar geology containing chalk and limestone (UKRI, 2020).

Dissolved oxygen concentrations were below the baseline values at Castle Howard and Pocklington, and dissolved organic carbon was below the baseline value at Wheldrake Ings. The pH levels observed in East campus lake were above the baselines using water bodies from the nearby River Ouse, but the pH observed at Castle Howard and Selby canal were below these baseline values seen in Chapter 2 (Table 2.16.). Water temperature was higher in East campus lake compared to nearby sites measured by the Environment Agency (2019). This is most likely due to the site having a larger uncovered surface area with minimal flow and movement in the water column, absorbing a larger amount of heat with limited transport out of the water body. Wheldrake Ings was cooler than all the other sites, likely owing to the high tree cover around the river helping to shade the water. The pH and DO values observed in this experiment at East campus lake were comparatively higher than the other sites measured, although for the other lake (Castle Howard) site these values were amongst the lowest observed in the experiment, and displayed a similar temperature to all other sites bar East campus lake (16.2 degrees Celsius).

Composition of the biofilms at Selby canal were likely affected by nutrient enrichment, with significantly higher concentrations of phosphate, iron, fluoride, copper, zinc and calcium compared to the other sites. This is likely owing to industrial activity in the nearby town of Selby, and the other sites tended to be more isolated from major sources of anthropogenic activity. Another observable effect between the three substratum types is the higher abundance of chlorophyll-a in the plant biofilms, and higher AFDW in the sediment biofilms. This reduced chlorophyll-a in sediment substratum compared to plant substratum has been observed in other research, with data from lakes in Greenland, Canada, and Michigan state US, suggesting that chlorophyll-a production was highest in stone and plant communities, and lowest in sediment communities This lower productivity was ascribed to greater light attenuation in the sediment biofilms, and the increased availability of nutrients on sediment and plant substratum; whilst the higher AFDW in the sediment can be attributed to the nature of this biofilm being composed

detrital matter which has sedimented out of the water, whilst the vertical nature of the plants will have prevented this accumulation. (Vadeboncoeur *et al.,* 2006).

5.6.1.4. Comparisons to results of previous chapters

Comparisons to West campus lake used in the previous experiment, a site that wasn't selected for use in this chapter due to its poor-moderate EU WFD classification based on the results of Chapters 2 and 3, demonstrates several key common physico-chemical controls on the composition of the diatom communities. Linear regressions for West campus lake (Chapter 2, Table 2.13.), as well as the linear regression analysis from this chapter (Table 5.8.) indicate that when considered separately, pH, light attenuation, chloride, dissolved oxygen, silicon, total nitrogen and nitrate all had significant ($P<0.050$) effects on the composition of diatom species in the communities. Both in West campus lake, and across all the sites studied in this chapter. Further comparison of the multivariate analysis (MANOVA and multiple linear regressions) conducted here with the separate analysis conducted for each lake in Chapter 2 (section 2.5.3), confirmed that in both analyses, light attenuation, pH and silicon concentrations were shown to be key factors influencing the diatom communities. These results appear to indicate that light attenuation, pH, and silicon concentrations may be key factors that will always influence the community structures in the region. One point of contention to this assertion is that the results in East campus lake (Chapter 2, Tables 2.13., 2.14., and 2.15.) do not show this effect, and multivariate analysis links the key factors affecting the TDI values on this lake to nitrate and nitrite concentrations only. However, as both this chapter and chapter 2 has shown, East campus lake is very different to the other water bodies used in this thesis, both in terms of the biofilm communities, and physico-chemical properties of the water body. Other research has found that electrical conductivity (Passy *et al.,* 2004), pH (Kilroy *et al.,* 2006), and nutrient load (Dalu et al., 2017) are the predominant factors in the composition of the diatom communities. This difference in the literature is likely due to the inherent differences between the study areas, and the unique factors to the sites, as shown with East campus lake, compared to both West campus lake in the previous chapters, and the other sites assessed here. Although all sites assessed in this thesis do have the commonality of higher-than-expected concentrations of alkali/ alkali-earth metals, electrical conductivity and alkalinity, compared to the background levels identified in Table 2.16, this is due to the nature of the region, with water bodies flowing through carbonate rich rocks further upstream.

A further note to the diatom data collected, is that all of the species identified in this chapter as being either nutrient tolerant, or nutrient sensitive were present in West campus lake replicates in Chapters 2, 3 and 4. In the laboratory culturing experiment, several of the nutrient sensitive species were more abundant in the CT room deployments than the field. A significant difference between the West campus lake results and the field sites studied in this chapter, is the presence of *Nitzschia paleacea*, which by the end of the field and CT room experiment was the dominant species in West campus lake, comprising over 33% of the communities, but was not present in significant quantities in this chapter. As such, incorporation of this lake as one of the additional sites in a follow up assessment may help identify common species in sites below 'good' ecological quality, which most sites in the region appear to be, based on the results presented in this chapter and the Environment agency (2019) dataset.

As such, conclusions drawn from these results indicate that pH, light attenuation and silicon concentrations can be considered key parameters that affect the TDI values, although other factors, including ammonium, alkalinity, phosphate and nitrite were also demonstrated in these results to contribute to the variation in the community sensitivity seen between the sites. The results presented in this chapter also indicate that there was no 'typical' community structure seen across the sites sampled, and the results of most sites do not corresponding to equivalent ecological status presented in the Environment Agency (2019) data from 2016. As such, it is recommended that further sampling across multiple time periods, as originally planned before the COVID-19 lockdown are conducted. This field campaign should further incorporate other sites not limited to the Environment agencies classification of 'good' ecological status, to increase

the number of viable sites used for further assessment of the diatom communities within the Vale of York.

5.6.2. Identification of species present in these sites that are known to be nutrient sensitive, or nutrient tolerant for use in a representative community.

5.6.2.1. Identification of nutrient tolerant and sensitive species present for potential use in future laboratory community cultures

A. minutissimum and *G. cuneolus* were the only species whose abundance exceeded 10% of the community across the sites, and was present on all substratum, and even these species were not particularly common at Castle Howard and Pocklington canal (and Selby canal for *A. minutissimum*). The presence of *A. minutissimum* was expected, as it is a highly abundant species in all freshwater bodies (Wunsan *et al.,* 2002), and was previously observed in extremely high abundances in East campus lake (Chapters 2 and 3). This was among the most common species observed at East campus lake and Wheldrake, but in Selby canal and Castle Howard it was not particularly abundant (<5%), and appeared to show a preference for plant substratum over sediment substratum. The TDI methodology employed here, which uses a scale of one (nutrient sensitive) to five (nutrient tolerant) considers this species to be sensitive to nutrient enrichment (category 2), indicating that this species is not only common to the Vale of York, but is also sensitive to changes in water quality. A. minutissimum is noted as being sensitive to organic chemicals, particularly herbicides in the literature (Stenger *et al.,* 2006, Szczepocka and Szulc, 2009, Larras *et al.,* 2013). Unusually, this sensitivity is described as resistance to these chemicals, and thus responds positively in laboratory testing, compared to other species, with Wood *et al.,* (2016) noting that it was only negatively affected by one of the eight herbicides they tested, and even then, only at the highest concentrations tested. This validates this species future use as an indicator species in a representative community for the region, as it can provide a useful measure of community shift, if this tolerant species begins to increase in abundance, compared to the more sensitive species.

There were other nutrient sensitive species designated by the UKTAG that were observed in the sites, but these were less common, and were shown to respond to specific physico-chemical measurements, demonstrating their potential for a representative community developed under laboratory conditions. These were *Diatoma problematica* (chloride, magnesium, sodium, pH and temperature), *Brachysira vitrea* (magnesium and lead), *Encyonema gracile* (magnesium), *Achnanthes daonensis* (nitrite, phosphate, copper, zinc, calcium, iron and potassium). *Brachysira brebisonii* and *Rhopalodia gibba* are also classified as sensitive, but were not shown to be affected by changes in any of the physico-chemical measurements at the five sites. As such, these latter two species are not recommended for use in a community culture.

The remaining species common to several of the sites were either moderately nutrient sensitive (*Gomphonema cuneolus*, category 3), which would make this species unsuitable for future ecotoxicological tests, as they would be unlikely to respond, or nutrient tolerant species. The two most common of these were *Cocconeis disculus* (which was absent from Wheldrake) and *Melosira varians* (also absent from Pocklington canal). Both of which were classed as category 4 (nutrient tolerant) (Directive, 2014). The presence of these species across several sites at varying degrees of abundances, in some cases almost to the point of virtual absence indicates that these species may were possibly responding to local factors, and are possibly representative of the wider diatom community of the region. This does further confirm their sensitivity to changes in environmental parameters, favouring sites with higher nutrient levels (section 5.6.1). As such, it is recommended that further testing on a greater range of sites, and at different time points of the year should be conducted to better encapsulate the effects of seasonal changes in the water chemistry should be conducted to confirm this. Further MANOVA analysis in the appendix (Appendix m) comparing which physico-chemical measurements affected the abundance of each species indicate that the abundance of *C. disculus* was driven by concentrations of phosphate, calcium, and iron. However, the abundance of *M. varians* was much more complex, with TN,

ammonium, fluoride, nitrite, iron, nickel, pH, temperature, EC, DO, alkalinity, light attenuation and silicon all demonstrating an effect on the abundance of this species, indicating a complex relationship of this species to the environment it is present in.

Using species known to prefer higher nutrient conditions, such as *Cocconeis disculus* and *Melosira varians* (Kelly *et al.,* 2008), the latter of which is further noted to be an indicator species of pollution (Patrick and Palavage, 1994), would be useful for assessing the effects of HPCP organic chemicals. These species could be assessed for increasing abundance in response to the presence of these chemicals, effects which have been observed for other nutrient tolerant species in the literature. For instance, *Nitzschia palea* has been observed to occur in higher abundances in sites with higher phosphate and nitrate concentrations, as well as at sites contaminated with atrazine, or heavy metals (Guasch *et al.,* 1998, Chen *et al.,* 2014).

Other species observed to be tolerant to enrichment by organic chemicals (CEMAGREF, 1982, Kelly *et al.,* 1995), including *E. reichardtii*, *Gy. acuminatum*, *S. brebisonii,* were not affected by any of the physico-chemical measurements used here (Appendix m). There were a further three species that have been designated as tolerant to enrichment by organic nutrients identified in this experiment, were affected by changes in physico-chemical measurements. These were *A. pediculus (chloride)*, *N. lanceolota (Fluoride, alkalinity)*, *A. inariensis (Fluoride, DOC)*. As such, the former three of these species may be useful as tolerant species for a representative community, but these latter three are not recommended due to they are shown to be negatively correlated to the concentrations of certain nutrients.

The final tolerant species identified in the sites used is *G. parvulum*. This species is particularly significant as it is widely used in single species ecotoxicology experiments, such as growth rate assays, due to its prevalence in both healthy and metal contaminated environments, and has a tolerance to metal pollution, particularly with regards to cadmium, copper and zinc contamination (Monteiro *et al.,* 1995), and is also resistant to the effects of herbicides (Ivorra *et al.,* 2002, Larras *et al.,* 2012, Wood *et al.,* 2014). As such, seeing this species abundance correlate to iron, nickel, and copper is unusual. This species should be included in a representative community, as it has been observed to be strongly influenced by environmental factors, and is used in the literature as an indicator species for the effects of organic compounds. Although the issue with identifying this species and separating it from other species that may develop identical appearances to this species will need to be accounted for (Rose and Cox, 2014).

Further species observed in these sites rated as preferring nutrient rich environments, but also as indicators of natural environments unaffected of anthropogenic impacts were *N. dissipata* and *N. linearis* (Patrick and Palavage 1994). These species may also be useful in forming a nutrient tolerant component of the community to measure the loss of sensitive species against, but as they were far less abundant than the previously mentioned species, only small quantities of these species should be considered.

5.6.2.2. What should a representative community look like?

A mixture of nutrient tolerant and nutrient sensitive species would give a balanced community for future testing. If a culture made entirely of sensitive species were to experience an increase in nutrient availability, then there would be little change in their relative abundances, diversity indices or UKTAG endpoints, as these species would likely act in a similar manner. This would then require the use of far more time intensive methods of counting diatoms, such as absolute abundance, which would add to the duration and costs of the experiment. Therefore, including tolerant species to the representative community will allow for the accurate use of community endpoints (relative abundance, TDI, percentage motile, species richness, community evenness), and allow for an assessment where the loss of sensitive species can be observed. Studies by Vidal *et al.,* (2020) found that whilst diatom communities from two different tributary rivers may have shared common genera of diatoms (*Achnanthidium, Fragilaria and Navicula*), the species within these generas showed significant variation in their sensitivity to the chemicals to which they were exposed to (glyphosate, lead, copper sulphate). The exposed site contained more individuals of

species that are more tolerant of chemical contaminants, demonstrating the importance of having contaminant tolerant species as part of the representative community, to provide a contrasting set of species that the sensitive species can be measured against within the community (i.e. percentage of organic tolerant or motile species, TDI index).

As such, the TDI index will play a role in quantifying change in the composition of the community away from or towards those observed in the reference site, with the percentage of organic tolerant species endpoint demonstrating whether the species that become dominant are particularly tolerant of organic compounds. However, the percentage of motile species will be too specific to motile species, which may not necessarily be selected for this representative community, and as such this endpoint will be dependent upon the final community used and the sensitivity of the motile species used, as not all motile species are classed as nutrient tolerant.

Research by Blanco *et al.,* (2012) did not find any relationship between diversity indices and water quality, although sites dominated by singular species were correlated to low total phosphorus contents. Measurements of diversity indices reported here (section 5.4.4.) do not appear to confirm this. As at Selby canal, the site with a phosphate concentration over twelve times higher than the next closest site, fairly average species richness was observed. Earlier work by Leira *et al.,* (2009), Archibald (1972) and Patrick 1973) also notes that the specific index of species richness does increase under increasing nutrient availability. The data presented here shows that Pocklington canal, the site with the highest nitrogen nutrient concentrations, has the joint highest species richness, along with Wheldrake ings, which did also contain relatively high levels compared to both lakes (East campus lake and Castle Howard), although Selby canal also had a similar concentration of these nutrients to Wheldrake, but lower diversity indices. This makes the use of these metrics in further ecotoxicological testing difficult to justify, beyond providing any estimation of the shape of the community, or statistically confirming the loss of a species, which would be a known factor under the usage of laboratory cultures, as the initial species richness will be fixed, and only the loss of species can be quantified. Further to this, the abundance endpoint will be more focussed on the TDI value, as a method of quantifying the abundance of the sensitive species compared to the tolerant species. However, the evenness metric does provide a method for quantifying if there is a shift towards or away from dominance of a handful of species compared to the communities' initial state. As the increased competitiveness of these species under nutrient enrichment stress can be used to infer an effect caused by a HPCP chemical.

To summarise, the species identified here comprise those present in reasonable quantities at the field sites, and confirmed both by the UKTAG database and the data presented in this experiment to be sensitive to increases in nutrient availability or other significant physico-chemical measurements, including electrical conductivity, pH, temperature or alkalinity, and will potentially be negatively impacted by the addition of a novel organic chemical, such as HPCPs. This section also identifies the species present at the sites that were shown to actively prefer higher nutrient conditions, and may therefore be applied to a representative community. This data provides an initial assessment of what a diatom community representative of the Vale of York should look like, and when cultured together will provide a representation of the diatom communities in the Vale of York, consisting of species, both common and rare that are likely to be affected by changes in the environment they are exposed to. As such, *A. pediculus*, *A. inariensis*, *G. parvulum*, *N. lanceolota*, *N. dissipata*, and *N. linearis* can be added to the representative culture, due to being tolerant species to nutrient enrichment, as registered within the TDI endpoints calculation, and they were confirmed here to be affected by changes in the physico-chemical measurements taken between the sites assessed. *G. parvulum* will be especially important, as it is classified as organic nutrient tolerant, and as such is more likely to resist any effects of the organic HPCP chemicals, and under such scenarios the effect can be proven by a community becoming dominated by this species. The nutrient sensitive species, that are known to prefer low nutrient conditions, and were observed to be affected by changes in the environment were: *Achnanthidium minutissimum*, *Diatoma problematica*, *Brachysira vitrea*, *Encyonema gracile*, and *Achnanthes*

daonensis. These should be included as low abundance nutrient sensitive species, with the comparative reduction of these species within the community compared to other tolerant species being indicative of a community level shift towards the nutrient tolerant species. Although, as mentioned earlier, further testing of these sites should still be conducted, with the use of additional sites in the region designated as being in poorer environmental conditions should be conducted to confirm this.

5.6.3. Recommendations:

Based on the results of these experiments, the following recommendations can be made for the development of a community representative of those present in Yorkshire freshwater bodies, and further assessment to improve the identification of these representative communities can be conducted:

- A broader study of Yorkshire water bodies is recommended, including sites not limited to good or higher ecological status to further assess the structure of diatom communities in more detail. These sites should continue to cover both plant (standardised to the use of common *phragmites* stems for sample collection to prevent variation observed in the literature between the biofilms developed on different plant species) and sediment substrates, to aid in identifying the composition of native diatom communities, as the results shown here indicate these substrates produce very different communities. Sampling at each site should be conducted using at least three localities at each site, placed sufficiently far apart (at least 10 meters), to improve the reliability of the results and statistical analysis.

- Further analysis should be conducted at three separate times in the growing season to provide an accurate assessment of how the community structure varies across the summer growing period and also improve the accuracy of representative diatom communities developed from the results. These times should be in late spring/ early summer (May), mid-summer (July), and late summer/ early autumn (late September), as described in the UKTAG methodology for phytobenthos sampling.

- Based on the results produced here, future cultures using benthic diatom communities composed of species that are representative of Yorkshire and sensitive to nutrient changes should be comprised of two groups:

    1. The first group should include nutrient sensitive species with a preference for low nutrient conditions which will tend to decrease in abundance with increasing nutrient availability, and likely organic chemicals. Based on the results shown here the species to be considered for this group are *Achnanthidium minutissimum*, *Diatoma problematica*, *Brachysira vitrea*, *Rhopalodia gibba*, *Encyonema gracile*, and *Achnanthes daonensis*.
    2. The second group should include species that are tolerant to nutrient enrichment, and that can act as a reference for the changes in the sensitive species. Based on the communities examined here, such species include *Cocconeis disculus*, *Melosira varians*, *Gomphonema parvulum*, *Encyonema reichardtii*, *Gyrosigma accuminatum*, and *Surirrela brebisonii*.

- Several physico-chemical parameters have been observed to have a statistically significant correlation to the ecosystem structure and health of the sites investigated, and could affect communities being cultured in laboratory conditions using these species, based on the diatom TDI values at the sites studied here. As such, these parameters are recommended for the additional broader testing in future assessments, expanding on the sites studied here, for further confirmation that the species identified here are representative of the diatom communities in the Vale of York at different times of the year. Parameters that positively affected the TDI, and thus increase the percentage of nutrient sensitive species in the

communities were pH, light attenuation, chloride, temperature, dissolved oxygen and nickel. As such, these factors should be closely monitored during culturing, in order to maintain the required abundances of the nutrient sensitive species. Whilst physico-chemical measurements demonstrated to negatively affect these communities, and as such increase the abundance of nutrient tolerant species were the concentrations of silicon, nitrite, calcium, iron, phosphate, total nitrogen, and nitrate.

Chapter 6: Summary of experimental outcomes and recommendations for future experiments

6. 1. Summary:

In this section, the work conducted in the experimental chapters (Chapter 2-5) will be summarised, looking at how they addressed the aims and objectives set out in Chapter 1, the implications of the results in the continuation of developing a protocol for using freshwater benthic diatoms to assess the effects of HCPC chemicals on freshwater ecosystem function and health at the primary productive trophic level, and the limitations of the methods involved.

6.1.1. How have my aims and objectives been addressed?

The aim of this thesis was to develop a protocol towards the use of benthic diatom community endpoints to determine freshwater ecosystem function and health in response to organic contaminants. The objectives for this identified in the literature review (Chapter 1) were:

- To develop a laboratory approach to establish representative community cultures and measure changes in community structure and function
- Identify a representative diatom community in rivers and streams with low levels of chemical contamination in Yorkshire
- Quantify the effects of water quality parameters on diatom community structure

To achieve these objectives, the following experiments and analyses were conducted:

1. In support of objective one, a field experiment was conducted on the University of York's campus lakes (Chapter 2 and 3) to determine how to best replicate a natural diatom community by:
   (a) Identifying the optimal substratum that should be used, and
   (b) Determining the optimal duration of exposure to a natural environment for the replicates.

   As discussed in Chapter 4, an in vitro approach was then developed by using a batch culturing method and bi-weekly replacement of 20% of the growth mediums to grow benthic diatom communities on microscope slides using water from the campus lakes as the diatom source. This method allowed for the natural development of biofilms on fresh substratum by using replacement medium to simultaneously provide replacement nutrients and bring in fresh diatoms to the replicates, as would occur in the field.

2. The second objective was achieved by assessing diatom communities that develop on benthic substratum in freshwater bodies at a selection of sampling sites in Yorkshire (Chapter 5). The sampled communities were analysed to determine which species were present within the areas sampled, in what abundances, and whether or not they are considered sensitive to nutrient enrichment.
3. To address the third objective, the relationship between the diatom communities' TDI values and the water quality measurements, both taken from the campus lakes and Yorkshire water bodies (Chapters 2, 3, and 5), was determined using individual linear regression and multivariate analyses. This was used to determine whether the TDI, as a quantified value of community sensitivity based on the composition of the diatom community, could demonstrate the effects the physico-chemical measurements of the water bodies being tested affected the community level structure of the biofilms.

6.1.2. What are the implications of the results?

These results provide the next step in the development of a testing protocol for the assessment of the effects of HCPC chemicals on the structure of freshwater benthic diatom communities within controlled laboratory conditions.

The experiments conducted here have identified several key factors for the development of diatom community cultures, based on a field experiment and an initial culturing test. The results have indicated that these cultures should be developed on ceramic tile substratum. Although the biofilm metrics derived from this substratum were similar to the other two substratum tested (microscope slides and sandstone), indicating roughly the same results could be derived from any of the three used, the ceramic tiles provided a closer representation of more developed communities exposed for longer periods of time on sandstone rocks (reference substratum) native to East campus lake, based on the results in Chapter 2 for AFDW, relative abundances of algal groups and diatom species, as well as the diatom diversity indices, and the UKTAG assessment endpoints. This provides a clear answer, compared to the literature sources discussed in Chapters 1 and 2, as there has been an uncertainty as to which artificial substratum will best replicate communities from natural substratum over a short culturing period of a couple of weeks to a couple of months.

A development time of four weeks in the field for the communities from the campus lakes to be suitably representative of those present in the environment was also considered to be sufficient. This is based on East campus lake results, and the stability of the biofilm metrics used in Chapter 2 and 3 after four weeks of exposure until the end of the experiment, and between two different deployment times of equal duration. Results from West campus lake demonstrated continuous community level changes throughout the experiment, increasingly altering towards a community composition indicative of lower trophic states and higher ecological quality. These changes in West campus lake biofilms were observed to be due to changes in physico-chemical measurements, including nutrient concentrations, light attenuation, electrical conductivity and pH, believed to be due to a high level of seasonal variability in West campus lake (see Chapter 3). This was demonstrated by comparing replicates deployed at the start of the experiment for four weeks, to those deployed six weeks in for the same duration. These results indicate that it is the time of year, and the corresponding physico-chemical parameters of the water than influence community structure within a body, rather than the length of time exposed for, that influences longer term community structural changes.

The second experiment (Chapter 4), testing a proposed method for culturing benthic diatom communities *in vitro* demonstrated mixed results. Critically, it was successful in establishing functioning benthic biofilms using the method of fixed replacement of 20% of the culture medium every three to four days. However, as with other attempts to develop similar methods in the literature, the diatom community compositions were very different to those seen in the field., The likely drivers of the difference in composition were identified to be lower than typical light levels in the lab, limiting growth, and the differences in the biofilm nutrient consumption rates, making the 20% replacement far too low in West campus lake-derived replicates. Recommendations to address this are described later in section 6.2.3. This also fits in with the literature sources, where changes in physico-chemical properties in the controlled environment affected the cultured communities.

In the third experiment (Chapter 5), the composition of diatom communities from different Yorkshire sites classified as being of 'good' ecological quality were sampled and analysed, to determine which diatom species were present, and how the community was affected by local physico-chemical parameters. The aim was to develop an understanding of the structure of diatom communities from the wider region (the Vale of York) and derive from this a diatom community culture that would best represent the structure of the communities sampled. This would be used in conjunction with the culturing method proposed here to assess how HCPC chemicals may affect diatom communities. The communities sampled, which came from two lakes, one river and two artificial canals, were found to have no common community structure, and, although several species were common to most of these sites, they were highly variable in their abundances, with many species identified to be limited by the local physico-chemical parameters of the water bodies studied. Despite this, several species were identified that are known to be sensitive to nutrient enrichment. Analysis of these species in the literature in relation

to the UKTAG methodology further confirmed the sensitivity of these species. These species were therefore deemed suitable for use in a representative community for testing the effects of organic chemicals on diatom communities.

It was further determined that, for any representative culture to be effective at determining community level changes, it would also be required to include of species that prefer eutrophic conditions, elevated levels of other physico-chemical properties, such as alkalinity and electrical conductivity, and preferentially, also be tolerant to organic nutrients. These species should be incorporated so that the loss of the sensitive species against these tolerant species can be quantified, and *vice versa*. The combination of both the species sensitive to nutrient enrichment as well as those that are nutrient tolerant would comprise a community which will be representative of the communities studied in the Vale of York, whilst being sensitive enough to ascertain the effects of organic chemicals. However, as will be discussed later (section 6.1.3.), these results were limited due to external factors, and as such further testing on a broader range of sites in the region should be conducted.

6.1.3. What limitations were observed?

There were some limitations in the methodologies employed in the experiments conducted for this thesis. This section discusses in detail the issues that arose during these experiments, and potential remediations for any work continuing on from these results.

Chapters 2 and 3:

A limitation in this chapter is the lack of suitable sediment substratum native to West campus lake to use as a reference, to compare the more developed communities in the lake with those being developed on the test substratum attached to the rafts. This was an issue inherent to the lake, as half of the lake has been sedimented in with organic matter (University of York, 2019), compared to the newer East campus lake, where the original sediment/ pebble substratum is still completely exposed. This limited comparison is what allowed the differentiation of the ceramic tiles from the microscope slides and sandstone substratum as being more suitable for replicating accurate compositions of the native diatom communities, despite all three substrata generating typically similar results without this reference substratum. As such, having an equivalent reliable set of replicates would have been invaluable at confirming the greater representation of the benthic biofilms developed on ceramic tiles.

Chapter 4:

Despite the CT room set up being designed to mirror the environment in the campus lakes as closely as possible, the PAR availability in the CT rooms were far lower than that observed on most days in the field. The effect this had on the results would have at least partly led to the overall reduction of biofilm and algal community size on the lab replicates, as well as affecting the composition of the communities developed. As such, a rethink of the method to achieve more realistic 500-550 $\mu mol/m/s^2$ would need to be conducted. The only method available to elevate the PAR intensity, would have been to suspend the lamps lower and closer to the replicate vessels. This, as seen at the first week of the experiment caused a detrimental heating effect on the replicates, that was uneven due to the different efficiencies of the bulbs that could have affected the results. As such, it is entirely possible that even with a more customised nutrient replacement regime, the differences in light availability may continue to be a challenge in perfecting this method. One solution may be to use a single, long growth lamp to reduce variations in energy efficiency caused by using four smaller lamps split between the sixteen replicates, and also to take into account associated heat emissions and their effect on water temperature when setting the temperature of the controlled environment.

Chapter 5:

Due to the COVID-19 lockdown conducted from mid-March 2020 across the U.K., the full series of field sampling originally planned for autumn 2019, spring 2020, and summer 2020 could not be completed. This has limited the representativeness of the samples that were collected, as it only

represents the community structure at the end of the growth season (autumn 2019). The UKTAG assessment methodology (Directive, 2014) requires that sampling should be undertaken either in late spring/ early summer and early autumn, or as a single sampling point in mid-summer to correctly estimate the diatom community structure and, by extension, the TDI endpoints of the water bodies. As such, the sampling that was conducted was far more limited than intended, which has significantly limited the accuracy of the analysis and limited the findings and conclusions that can be drawn from the dataset.

6.2. Conclusions and recommendations for future experiments:

In this section the recommendations for the future development of the protocol for assessing the effects of HCPC chemicals on freshwater diatom community structure and health, derived from the results of the experimental results (Chapters 2, 3, 4 and 5), are summarised.

From these three experiments, the following recommendations for further optimization of the method used to develop a laboratory culture of diatoms representative of the communities seen in the field are recommended:

6.2.1. Substratum type (Objective 1, Chapter 2):

- Ceramic tiles are recommended for use in future experiments as, although all three tested substratum (microscope slides, ceramic tiles and sandstone) provided virtually identical biofilm communities, when compared against more developed communities on sandstone substratum native to the East campus lake, the ceramic tiles produced much more comparable diatom communities (Chapter 2).

6.2.2. Exposure time (Objective 1 and 3, Chapter 2 and 3):

- Initial findings suggest that four weeks of exposure to environmental conditions may be sufficient to develop representative biofilm communities in the field, and future sampling and culturing methods should adhere to this duration to avoid any variation caused by differences in accumulation and succession over different time frames, and to prevent the accumulation of organic matter that may affect the composition of the community and its sensitivity to the effects of HCPC chemicals. This conclusion is based on the diatom communities from East campus lake as analysed in Chapter 2 retaining stable biofilm endpoints (biomass measurements, algal group and diatom species relative abundances, diatom diversity indices and UKTAG endpoints) after four weeks of development.
- West campus lake communities however, demonstrated continuous change throughout the experiment, but appear to return to similar diversity indices at week ten of the experiment as they exhibited at the week four (Chapter 2). In Chapter 3, it was shown in replicates deployed in West campus lake for the same duration (four weeks), but at different times of the year that significant differences in their composition and structure still occurred, but not in the more stable East campus lake replicates. This, along with statistical analysis demonstrated a strong link in West campus lake of the composition of diatom communities to the physico-chemical conditions lake, rather than continuous succession within the biofilm.

6.2.3. Culturing conditions (Objective 1, Chapter 4):

- The experiment conducted in Chapter 4 indicates that a batch culturing method using a substratum suspended in a borosilicate container using twice weekly replacements of 20% of the culture medium derived directly from the source site can develop functioning diatom communities. However, the method did not provide community compositions or biomass volumes similar to those observed in the field. As such, two recommendations for the further development of this methodology have been identified to address these issues:

    1. Further testing of optimal nutrient replacement regimes of algal communities developed under laboratory conditions. This is to allow for the optimisation of the

timing and percentage of medium replaced for the diatom replicates, as the results here have shown that biofilms grown from different sources can have significantly different nutrient requirements. This should be conducted be testing replacement timings (between daily and twice weekly), and higher replacement percentages (between 20% and 50%).

2. Additional testing of the method using higher light levels in the lab, as well as multiple sampling points over the same one-month period should be performed, to assess whether increasing the PAR to more natural levels can fully compensate for the reduced biomass, or if a period longer than four weeks will be required to establish laboratory cultures of equivalent biomass to field replicates. In addition, a method of achieving this increase in light availability without causing unwanted heating of the replicates will need to be devised.

6.2.4. Composition of a representative community (Objective 2 and 3, Chapter 5):

- A broader study of Yorkshire water bodies is recommended, including sites not limited to good or higher ecological status to further assess the structure of diatom communities in more detail. These sites should be limited to the plant substratum (standardised to the use of common *phragmites* stems for sample collection to prevent variation observed in the literature between the biofilms developed on different plant species), as these communities were observed to have significantly elevated percentages of motile diatoms and lower concentrations of chlorophyll-a, indicating smaller, less productive algal communities due to the inherently different nature of the substratum. Sampling at each site should be conducted using at least three localities at each site, more than 10 metres apart, to improve the reliability of the results and statistical analysis, and conducted at three separate times, during the early, mid and late summer to provide an accurate assessment of how the community structure varies across the summer growing period and also improve the accuracy of representative diatom communities developed from the results.

- Based on the results presented here, future studies using benthic diatom communities composed of species that are representative of Yorkshire and sensitive to nutrient changes should be comprised of two groups:
    1. The first group should include nutrient sensitive species with a preference for low nutrient conditions which will tend to decrease in abundance with increasing nutrient availability. Based on the results shown here the species to be considered for this group are *Achnanthidium minutissimum*, *Diatoma problematica*, *Brachysira vitrea*, *Rhopalodia gibba*, *Encyonema gracile*, and *Achnanthes daonensis*.
    2. The second group should include species that are tolerant to nutrient enrichment, and that can act as a reference for the changes in the sensitive species. Based on the communities examined here, such species include *Cocconeis disculus*, *Melosira varians*, *Gomphonema parvulum*, *Encyonema reichardtii*, *Gyrosigma accuminatum*, and *Surirrela brebisonii*.

- Several physico-chemical parameters have been observed to have a statistically significant effect on the ecosystem structure and health of the sites investigated, based on the diatom TDI values at the sites studied here. As such, these parameters are recommended for the additional broader testing in future assessments, expanding on the sites studied here, for further confirmation that the species identified here are representative of the diatom communities in the Vale of York at different times of the year. Parameters that positively affected the TDI, and thus increase the percentage of nutrient sensitive species in the communities were pH, light attenuation, chloride, temperature, dissolved oxygen and nickel. As such, these factors will need to be closely monitored during culturing, in order to maintain the required abundances of the nutrient sensitive species. Whilst physico-chemical measurements demonstrated to negatively affect these communities, and as such increase the abundance of nutrient tolerant species were the concentrations of silicon, nitrite, calcium,

iron, phosphate, total nitrogen, and nitrate. These latter nutrients will therefore need to be strongly controlled in laboratory tests, as excesses of these nutrients will cause a decrease in the nutrient sensitive species, and increase the abundances of the tolerant species.

Using these recommendations, future ecotoxicological experiments assessing the effects of HCPC chemicals on diatom communities as representative of primary producers in freshwater ecosystems can be further developed. The recommendations of this work provide the basis for developing laboratory cultures of diatom communities which are representative of a chosen site or region. In particular, this work provides an initial assessment of diatom communities within the Vale of York, determining which species could be combined in this culturing method to test their response to organic chemicals.

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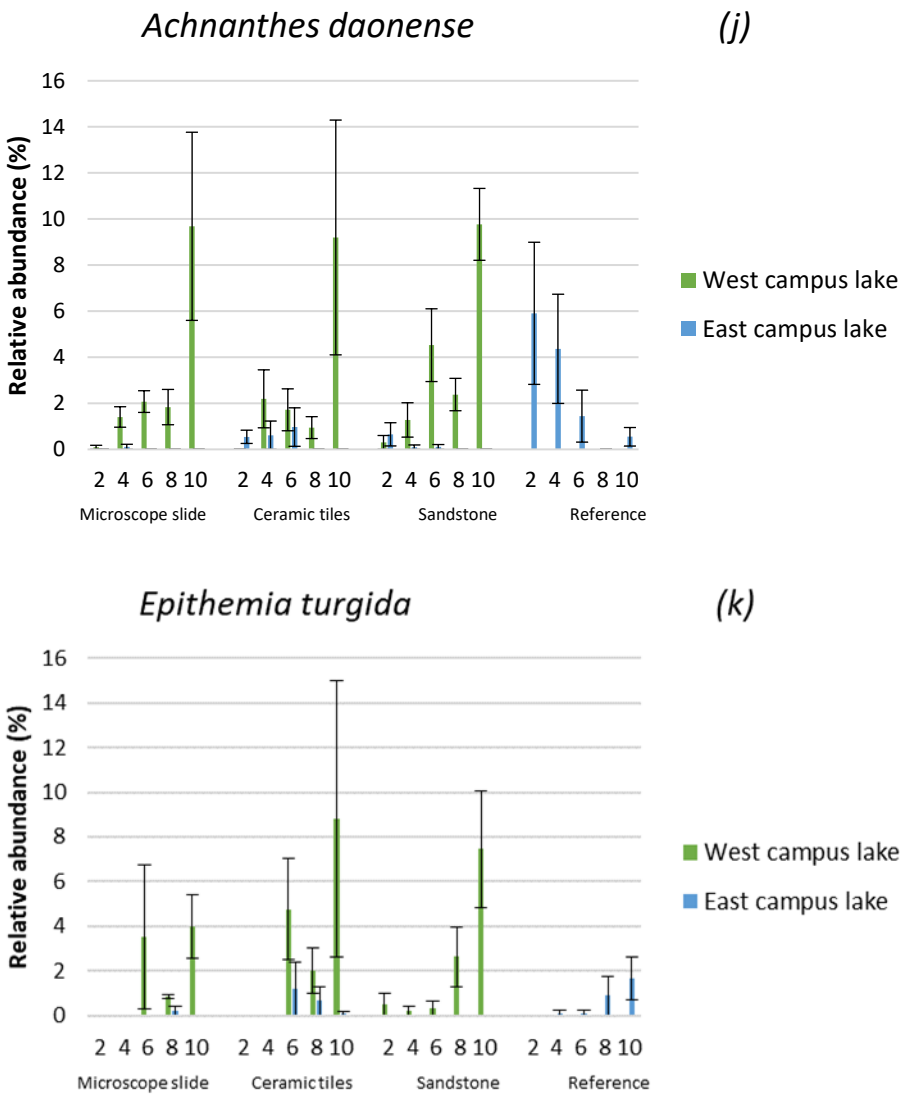
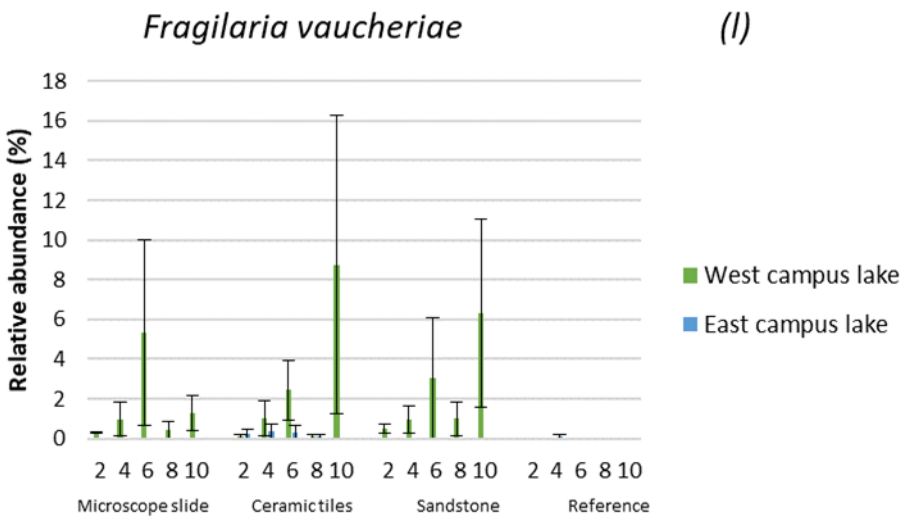
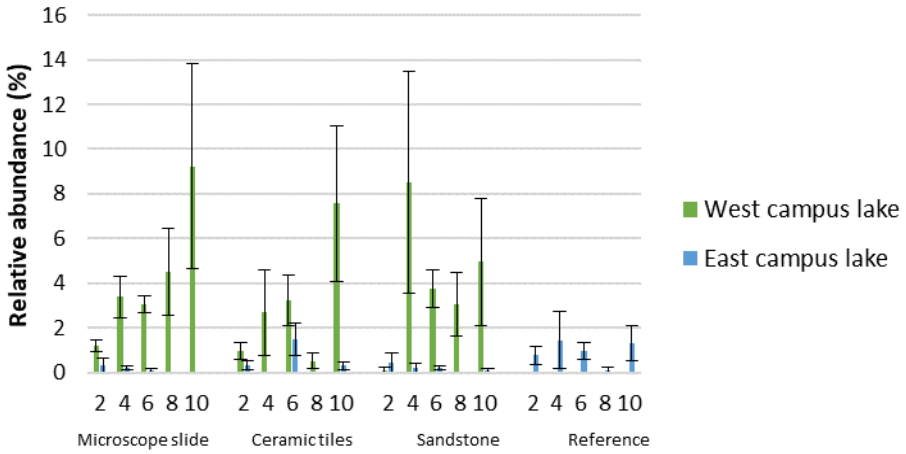
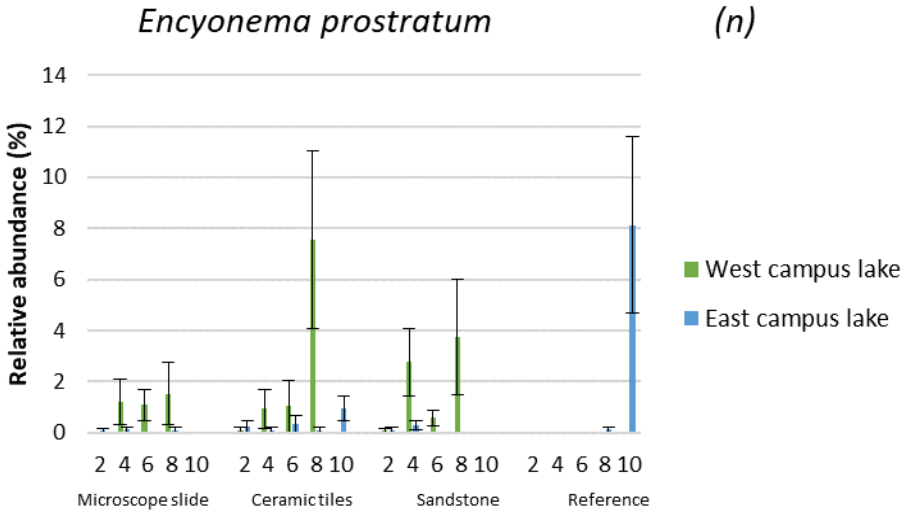
Appendices

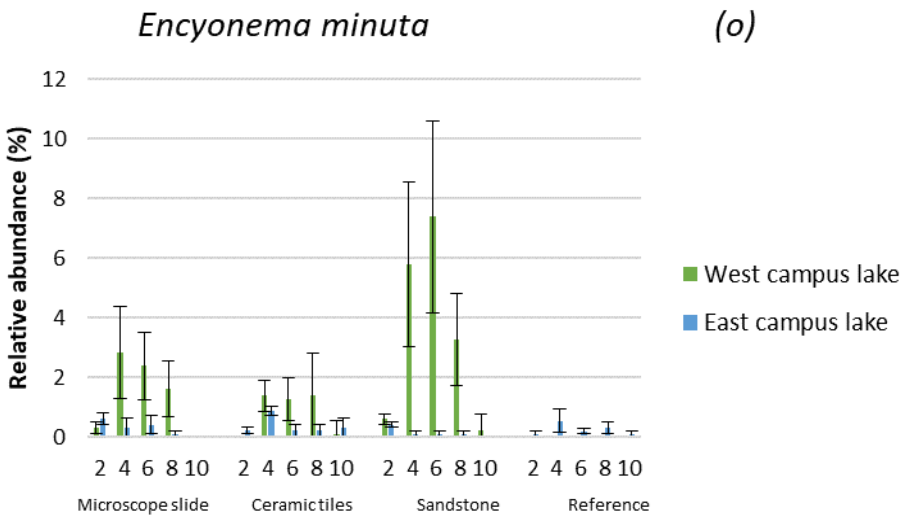
Appendix a. Chapter 2, campus lakes experiment diatom species relative abundances, split by West campus lake (green) and East campus lake (blue), and divided by substratum type (microscope slides, ceramic tiles and sandstone), and ordered by time. Mean ± SE, N=3

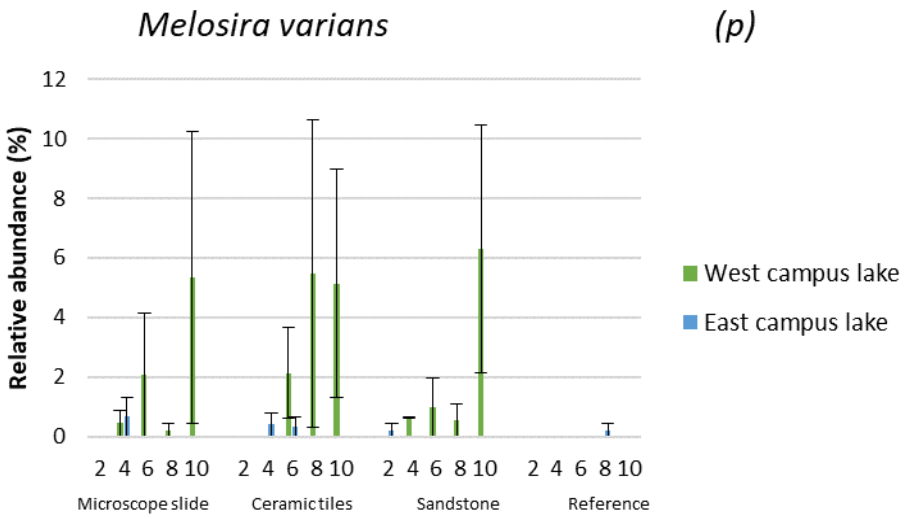


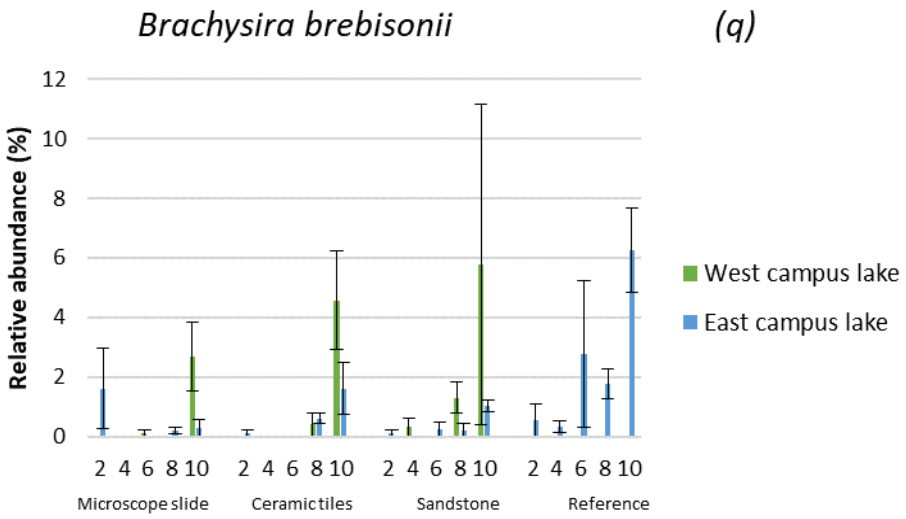

Fragilaria vaucheriae
(l)
Relative abundance (%)
18
16
14
12
10
8
6
4
2
0
2 4 6 8 10 2 4 6 8 10 2 4 6 8 10 2 4 6 8 10
Microscope slide
Ceramic tiles
Sandstone
Reference
West campus lake
East campus lake

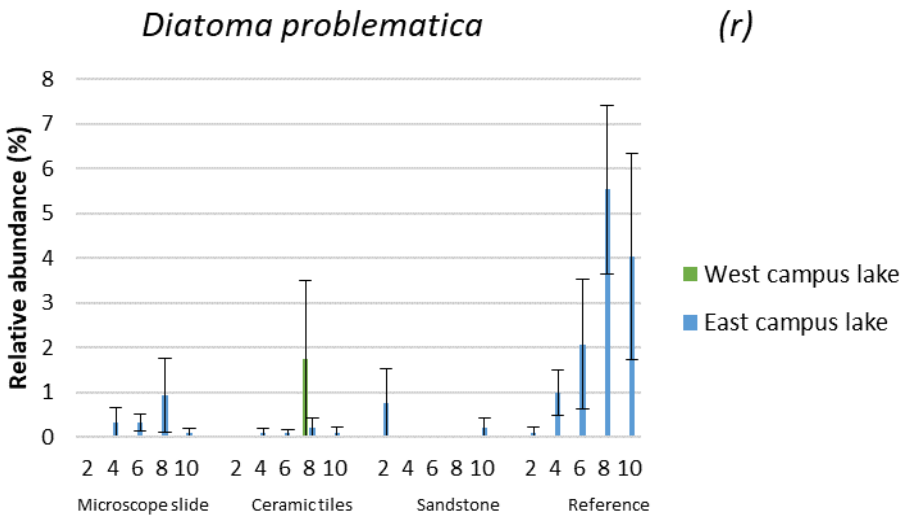

Nitzschia amphibia
(m)
Relative abundance (%)
16
14
12
10
8
6
4
2
0
2 4 6 8 10 2 4 6 8 10 2 4 6 8 10 2 4 6 8 10
Microscope slide
Ceramic tiles
Sandstone
Reference
West campus lake
East campus lake

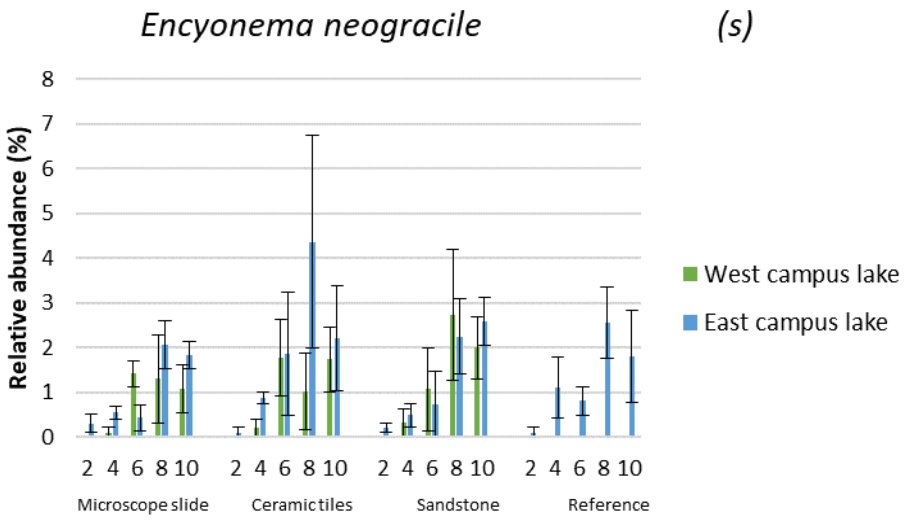

Encyonema prostratum
(n)
Relative abundance (%)
14
12
10
8
6
4
2
0
2 4 6 8 10 2 4 6 8 10 2 4 6 8 10 2 4 6 8 10
Microscope slide
Ceramic tiles
Sandstone
Reference
West campus lake
East campus lake

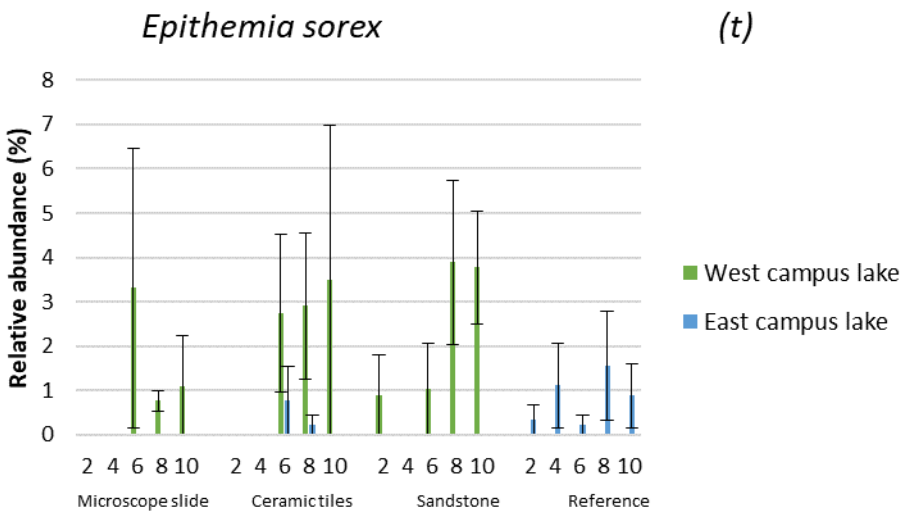

Encyonema minuta
(o)
Relative abundance (%)
12
10
8
6
4
2
0
2 4 6 8 10 2 4 6 8 10 2 4 6 8 10 2 4 6 8 10
Microscope slide
Ceramic tiles
Sandstone
Reference
West campus lake
East campus lake

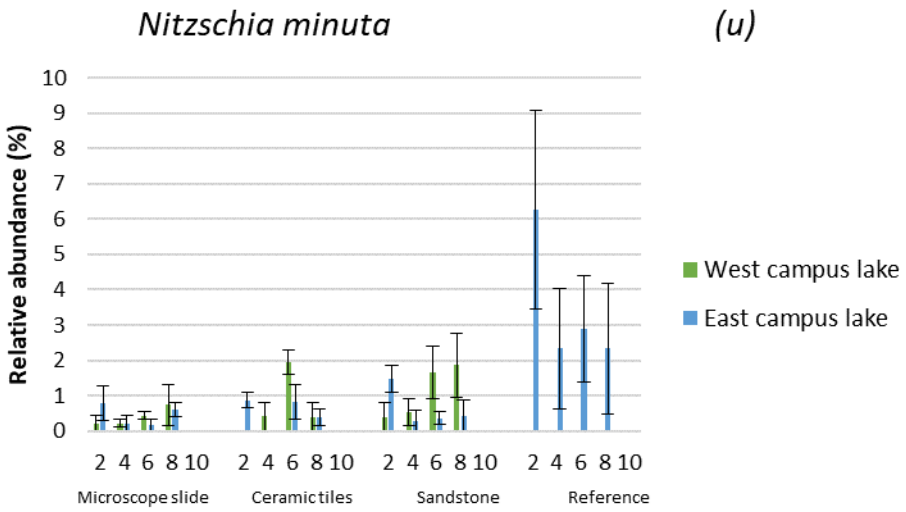

Melosira varians
(p)
Relative abundance (%)
12
10
8
6
4
2
0
2 4 6 8 10 2 4 6 8 10 2 4 6 8 10 2 4 6 8 10
Microscope slide
Ceramic tiles
Sandstone
Reference
West campus lake
East campus lake

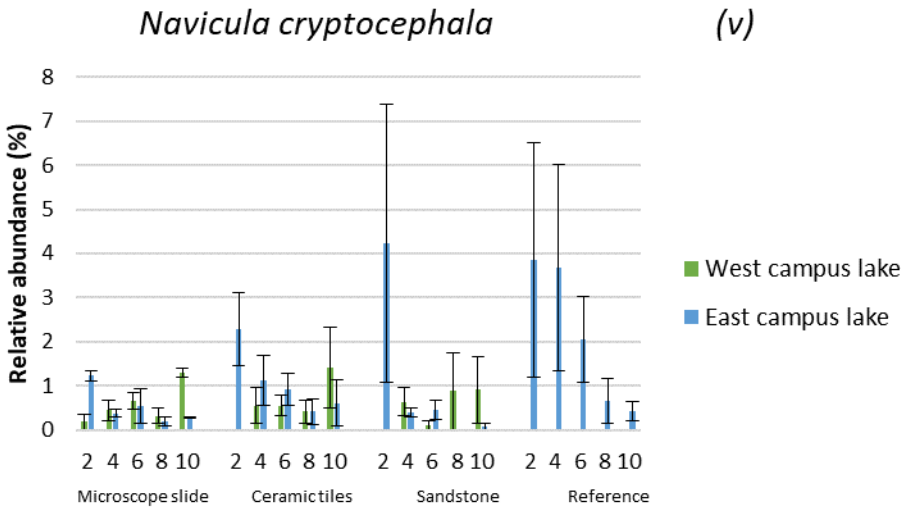

Brachysira brebisonii
(q)
Relative abundance (%)
12
10
8
6
4
2
0
2 4 6 8 10 2 4 6 8 10 2 4 6 8 10 2 4 6 8 10
Microscope slide
Ceramic tiles
Sandstone
Reference
West campus lake
East campus lake

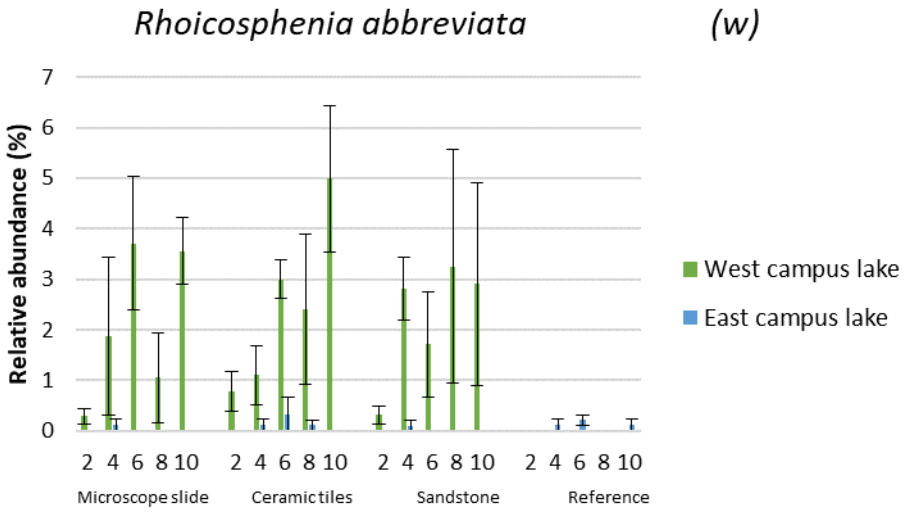

Diatoma problematica
(r)
Relative abundance (%)
0 1 2 3 4 5 6 7 8
2 4 6 8 10 2 4 6 8 10 2 4 6 8 10 2 4 6 8 10
Microscope slide
Ceramic tiles
Sandstone
Reference
West campus lake
East campus lake


Encyonema neogracile
(s)
Relative abundance (%)
0 1 2 3 4 5 6 7 8
2 4 6 8 10 2 4 6 8 10 2 4 6 8 10 2 4 6 8 10
Microscope slide
Ceramic tiles
Sandstone
Reference
West campus lake
East campus lake

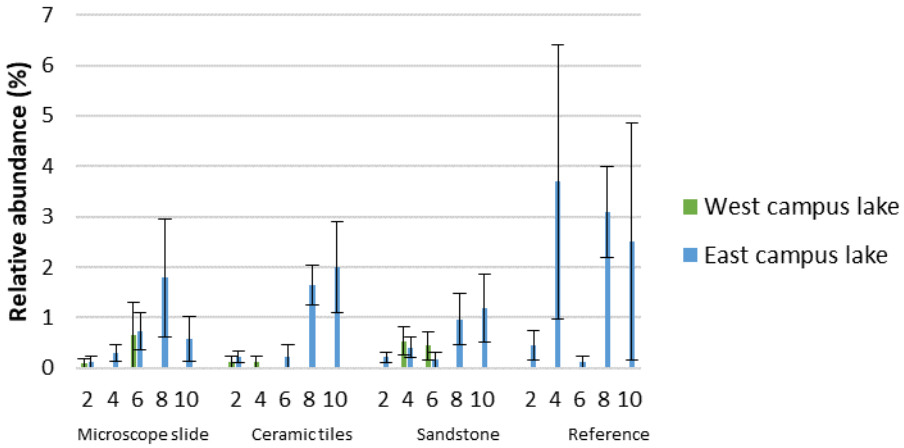

Epithemia sorex
(t)
Relative abundance (%)
0 1 2 3 4 5 6 7 8
2 4 6 8 10 2 4 6 8 10 2 4 6 8 10 2 4 6 8 10
Microscope slide
Ceramic tiles
Sandstone
Reference
West campus lake
East campus lake


Nitzschia minuta
(u)
Relative abundance (%)
10
9
8
7
6
5
4
3
2
1
0
2 4 6 8 10 2 4 6 8 10 2 4 6 8 10 2 4 6 8 10
Microscope slide
Ceramic tiles
Sandstone
Reference
West campus lake
East campus lake

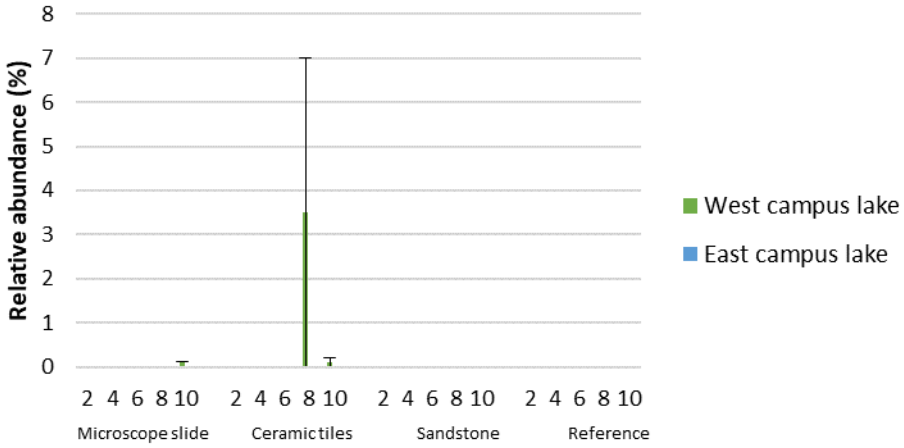

Navicula cryptocephala
(v)
Relative abundance (%)
8
7
6
5
4
3
2
1
0
2 4 6 8 10 2 4 6 8 10 2 4 6 8 10 2 4 6 8 10
Microscope slide
Ceramic tiles
Sandstone
Reference
West campus lake
East campus lake


Rhoicosphenia abbreviata
(w)
Relative abundance (%)
7
6
5
4
3
2
1
0
2 4 6 8 10 2 4 6 8 10 2 4 6 8 10 2 4 6 8 10
Microscope slide
Ceramic tiles
Sandstone
Reference
West campus lake
East campus lake

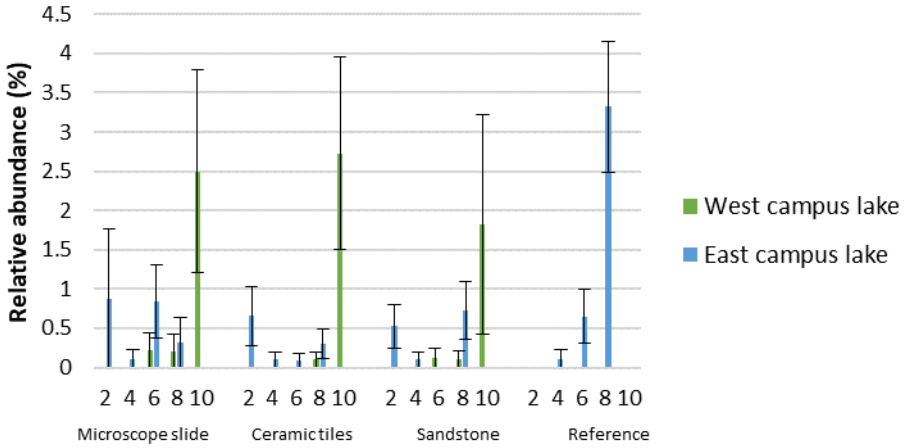
Gomphonema vibrio (x)

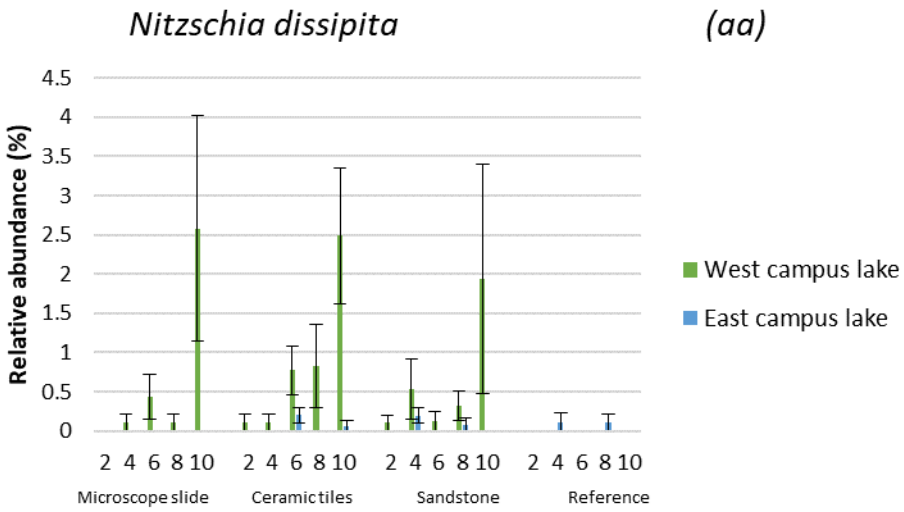


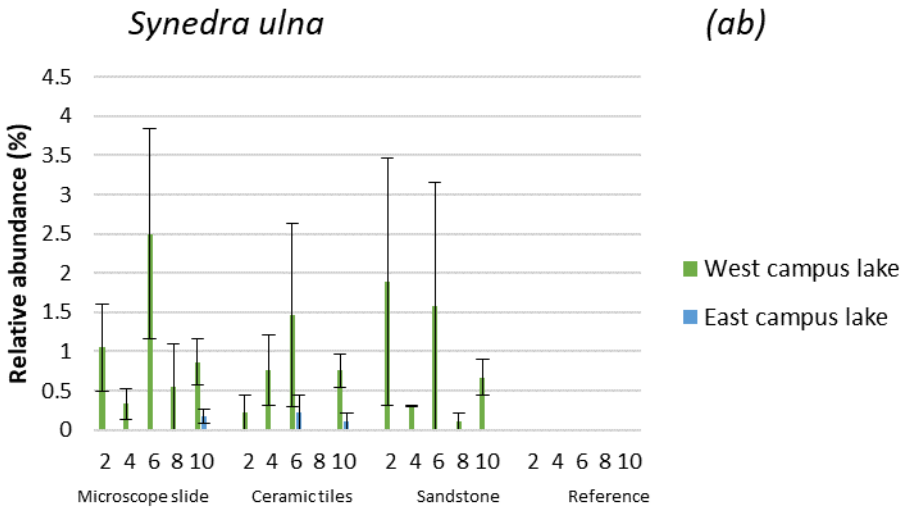
Pinnularia viridis (y)

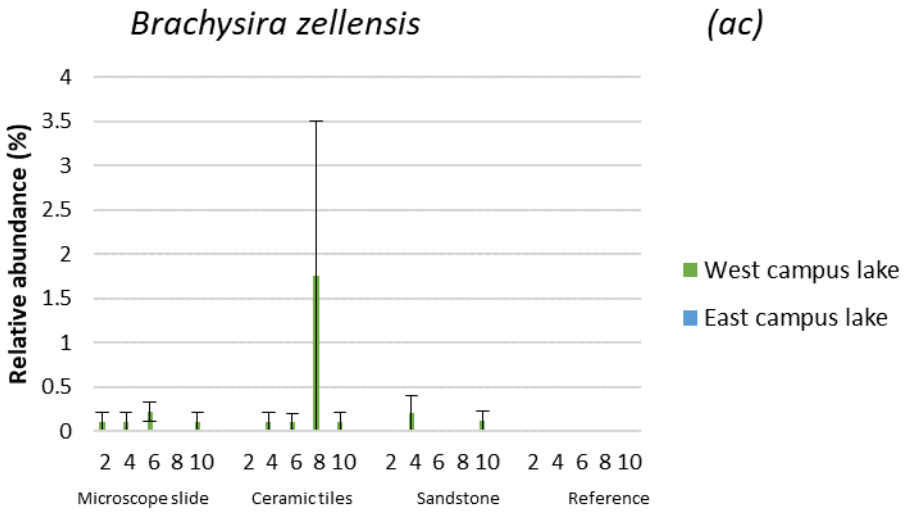


Navicula capitatoradiata (z)

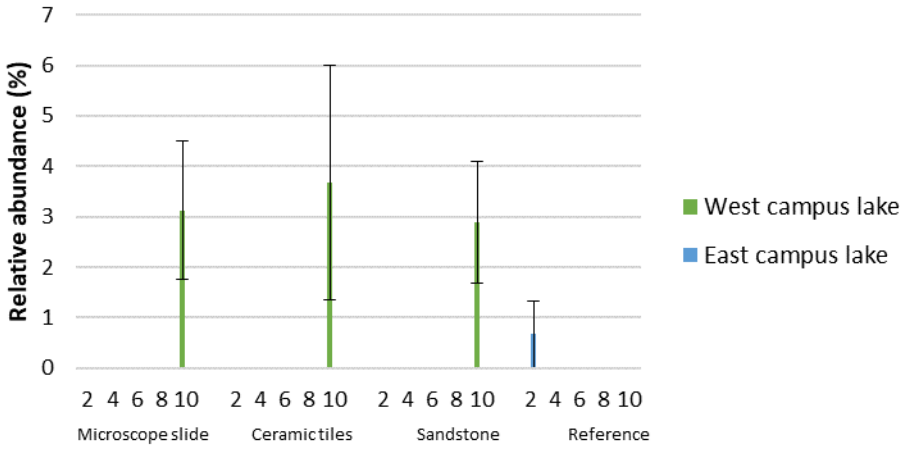



Nitzschia dissipita
(aa)
Relative abundance (%)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
2 4 6 8 10 2 4 6 8 10 2 4 6 8 10 2 4 6 8 10
Microscope slide
Ceramic tiles
Sandstone
Reference
West campus lake
East campus lake

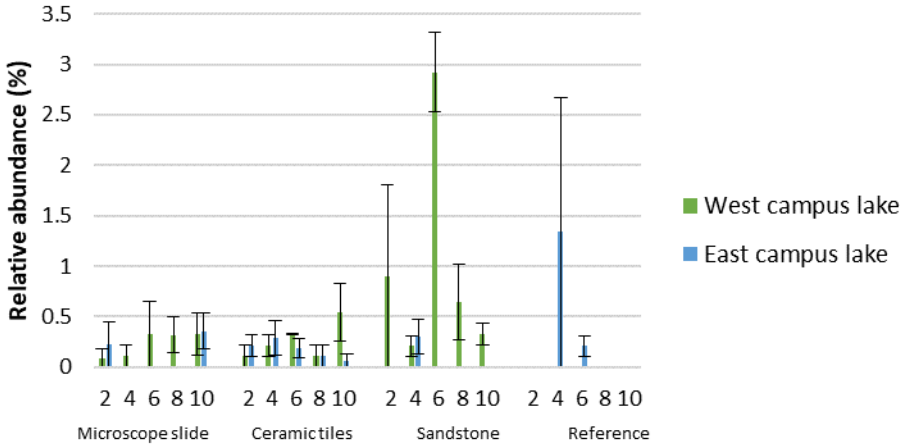

Synedra ulna
(ab)
Relative abundance (%)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
2 4 6 8 10 2 4 6 8 10 2 4 6 8 10 2 4 6 8 10
Microscope slide
Ceramic tiles
Sandstone
Reference
West campus lake
East campus lake


Brachysira zellensis
(ac)
Relative abundance (%)
4
3.5
3
2.5
2
1.5
1
0.5
0
2 4 6 8 10 2 4 6 8 10 2 4 6 8 10 2 4 6 8 10
Microscope slide
Ceramic tiles
Sandstone
Reference
West campus lake
East campus lake

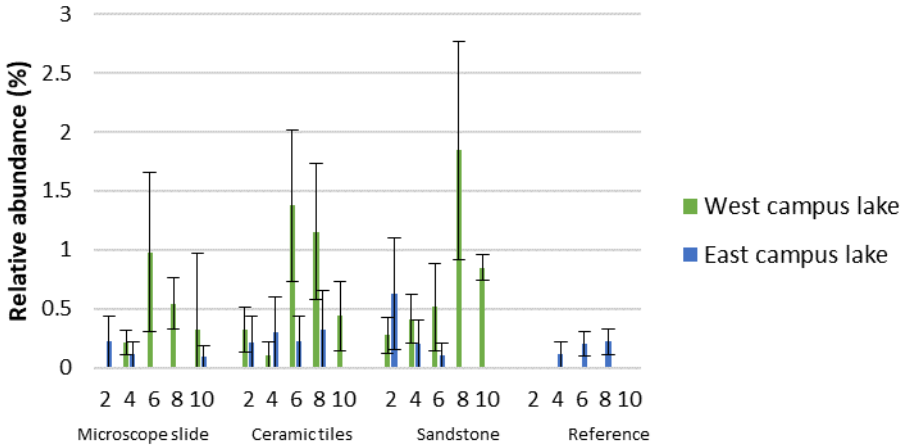
Brachysira spp. *(ad)*

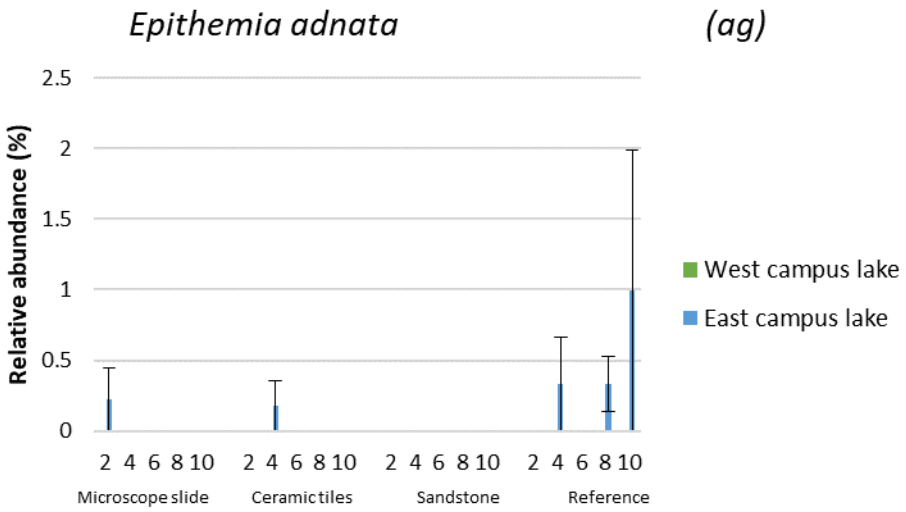


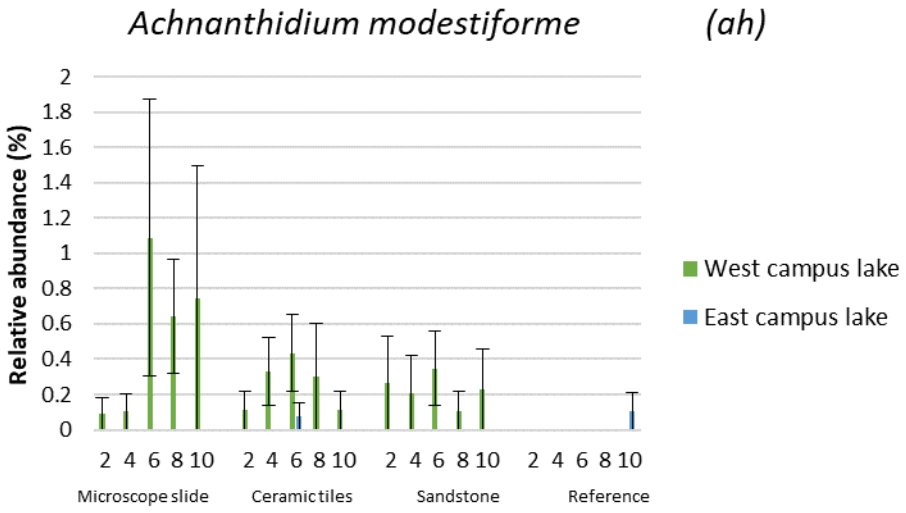
Nitzschia palea *(ae)*

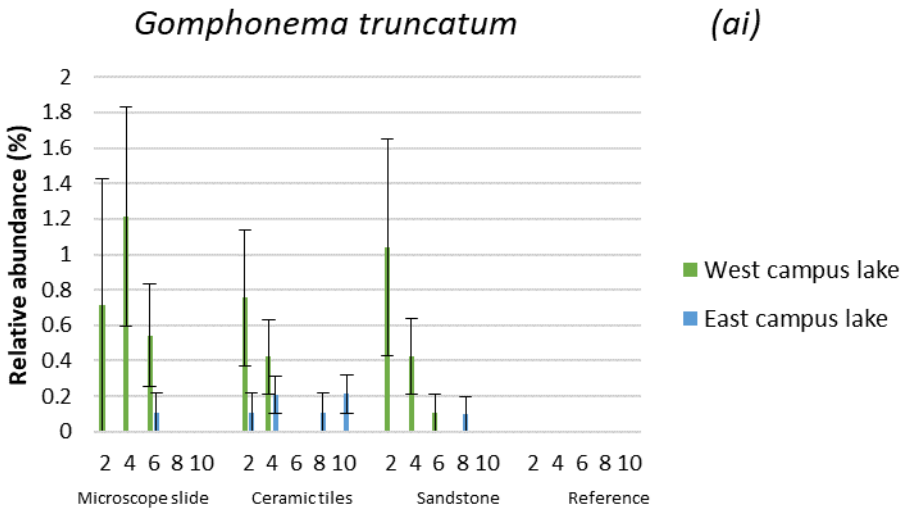


Amphora inariensis *(af)*

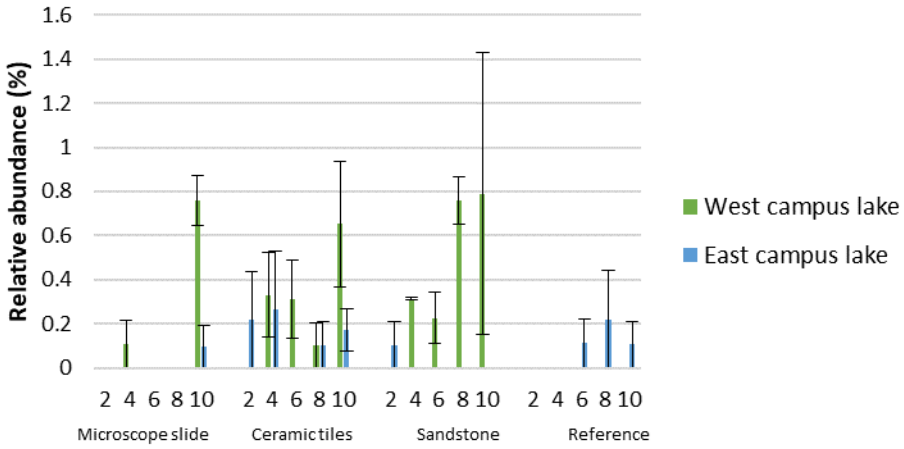



Epithemia adnata
(ag)
Relative abundance (%)
2.5
2
1.5
1
0.5
0
2 4 6 8 10 2 4 6 8 10 2 4 6 8 10 2 4 6 8 10
Microscope slide
Ceramic tiles
Sandstone
Reference
West campus lake
East campus lake

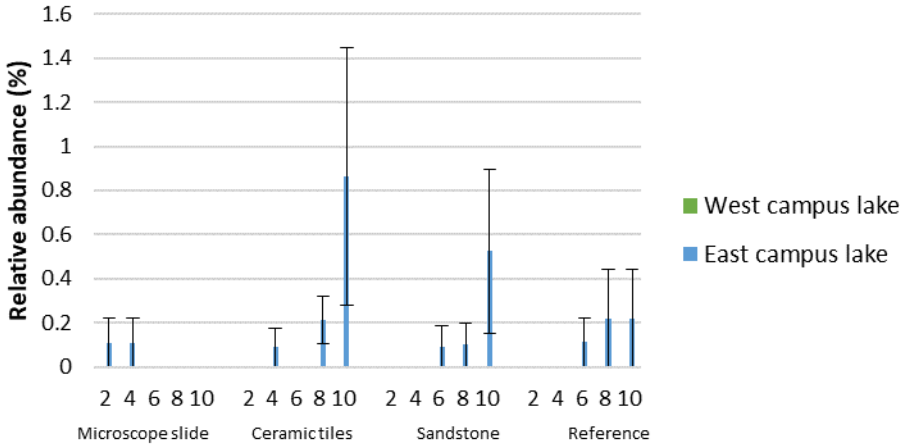

Achnanthidium modestiforme
(ah)
Relative abundance (%)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
2 4 6 8 10 2 4 6 8 10 2 4 6 8 10 2 4 6 8 10
Microscope slide
Ceramic tiles
Sandstone
Reference
West campus lake
East campus lake


Gomphonema truncatum
(ai)
Relative abundance (%)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
2 4 6 8 10 2 4 6 8 10 2 4 6 8 10 2 4 6 8 10
Microscope slide
Ceramic tiles
Sandstone
Reference
West campus lake
East campus lake

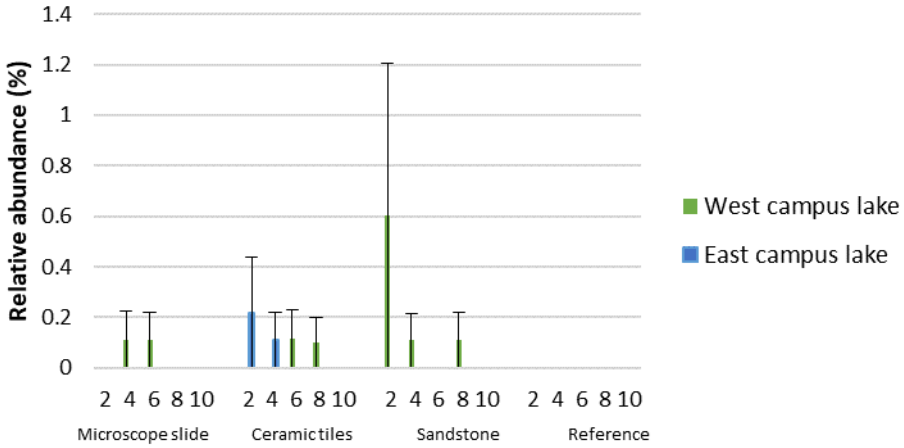
gyrosigma accuminatum *(aj)*

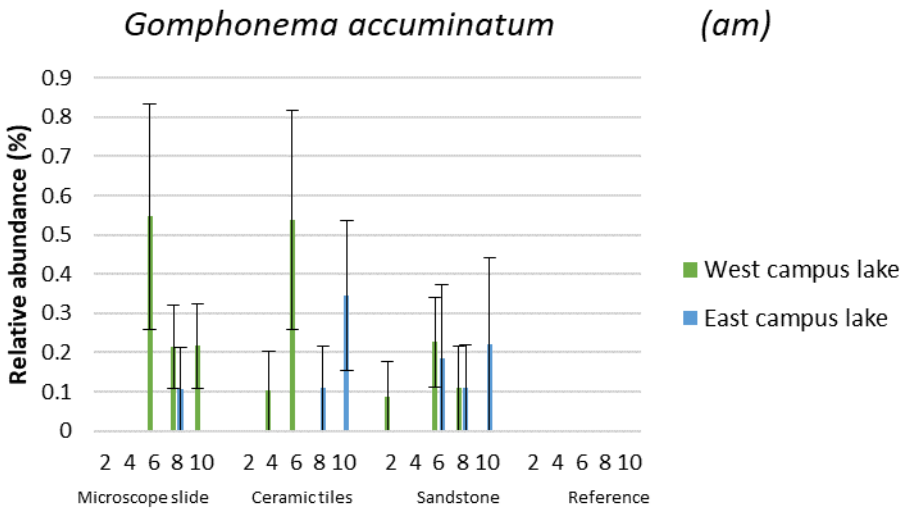


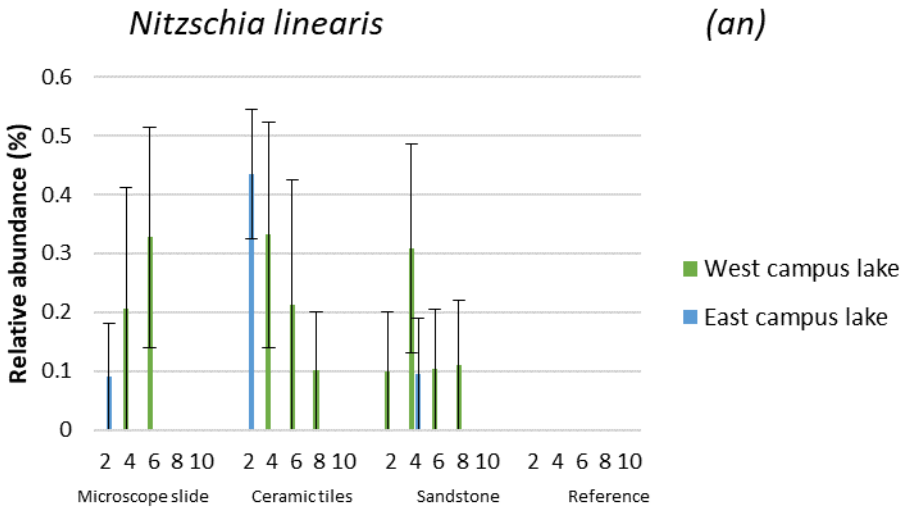
Rhopaladia gibba *(ak)*



Nitzschia acicularis *(al)*



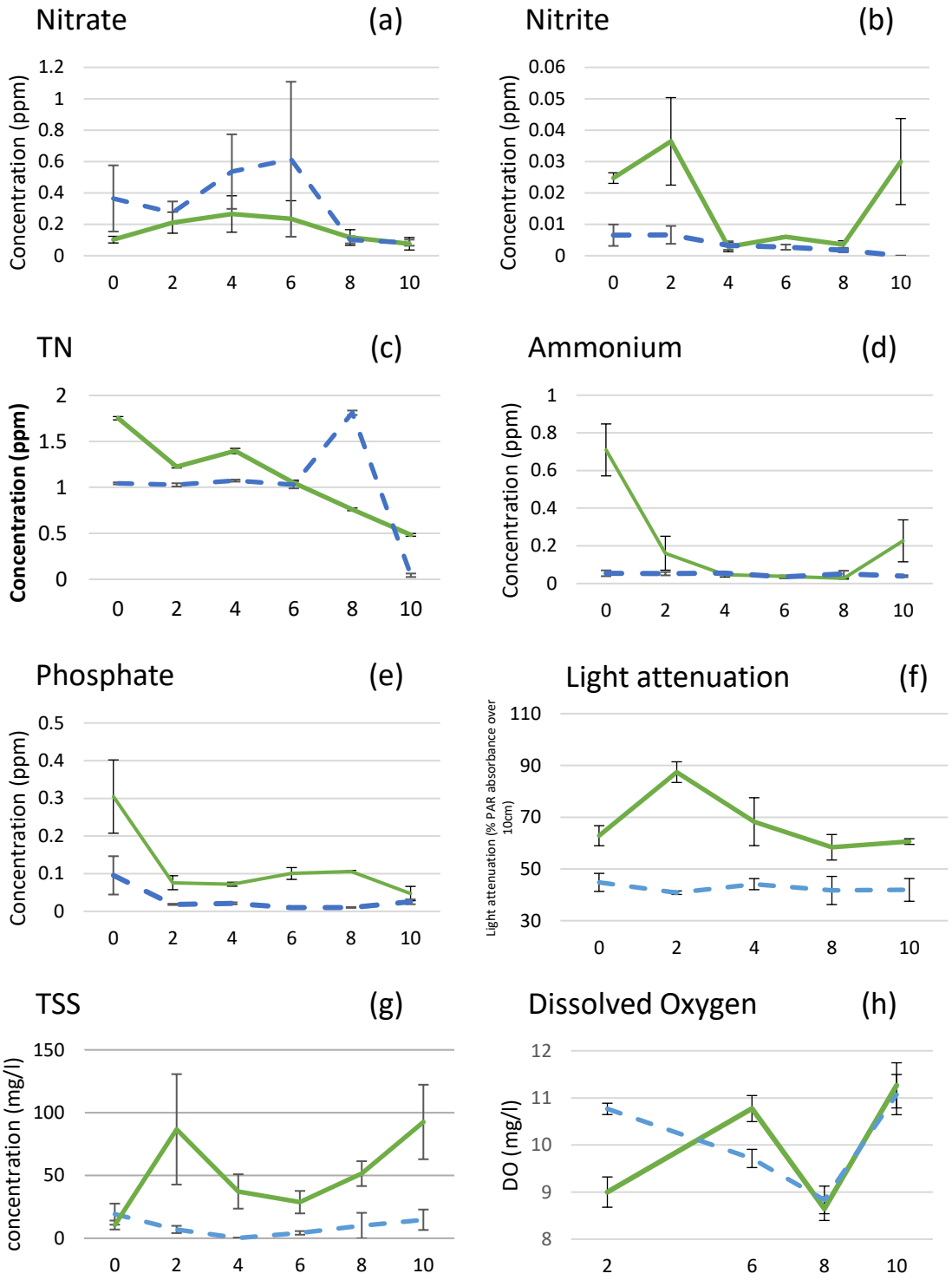

Gomphonema accuminatum (am)
Relative abundance (%)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
2 4 6 8 10 2 4 6 8 10 2 4 6 8 10 2 4 6 8 10
Microscope slide
Ceramic tiles
Sandstone
Reference
West campus lake
East campus lake

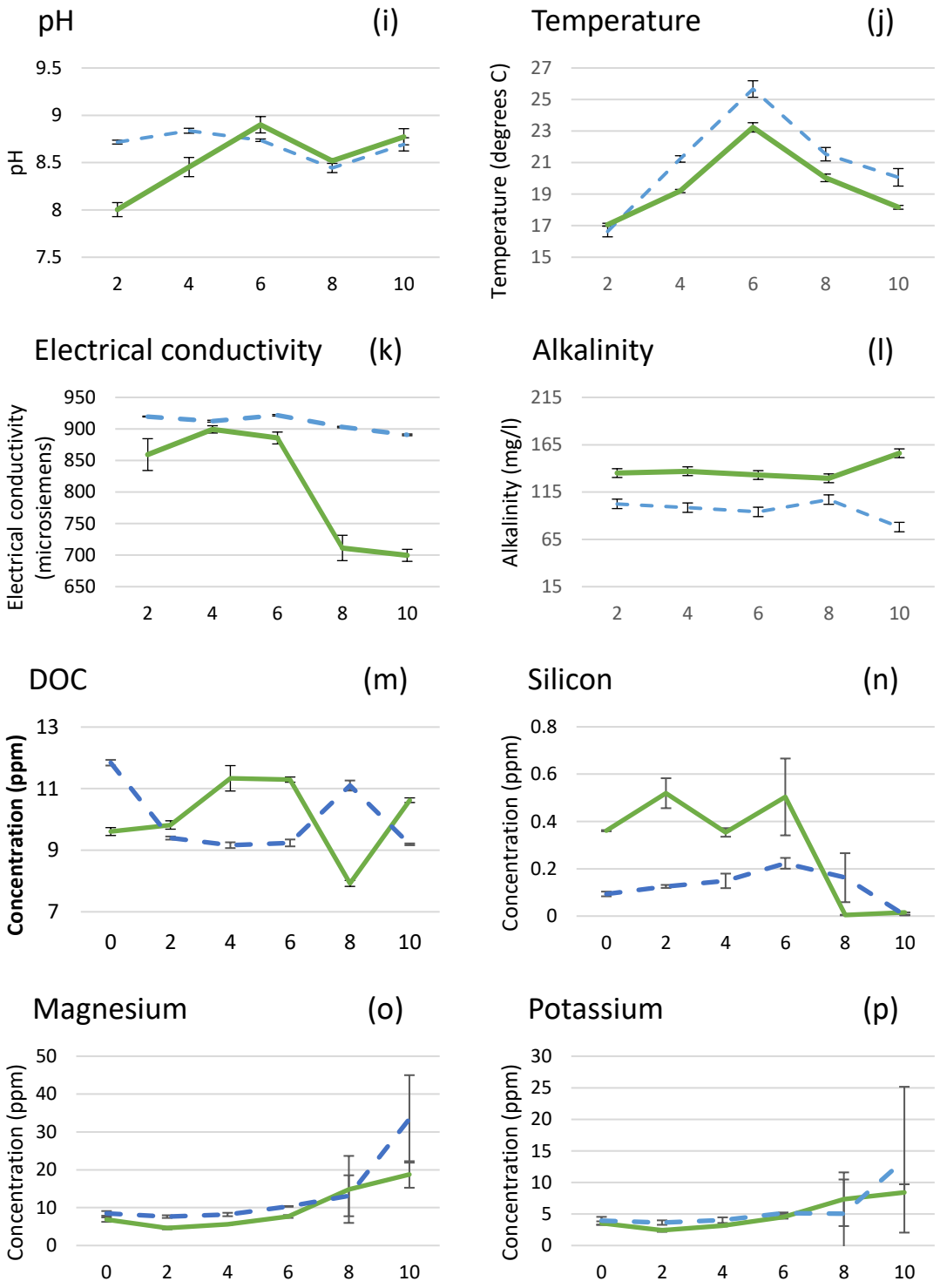

Nitzschia linearis (an)
Relative abundance (%)
0.6
0.5
0.4
0.3
0.2
0.1
0
2 4 6 8 10 2 4 6 8 10 2 4 6 8 10 2 4 6 8 10
Microscope slide
Ceramic tiles
Sandstone
Reference
West campus lake
East campus lake

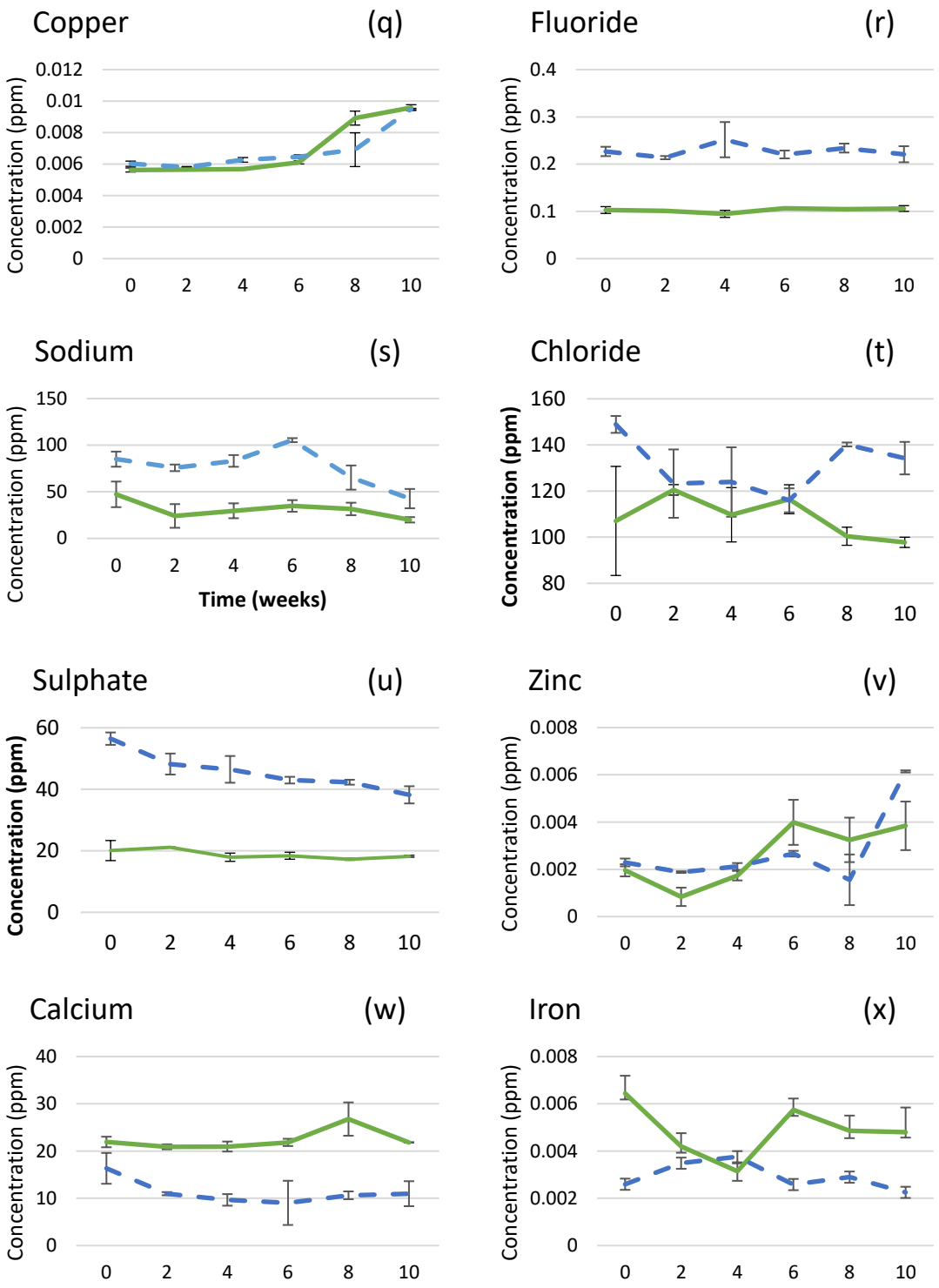
Appendix b. Chapter 2, Full list of diatoms identified in the campus lake experiment

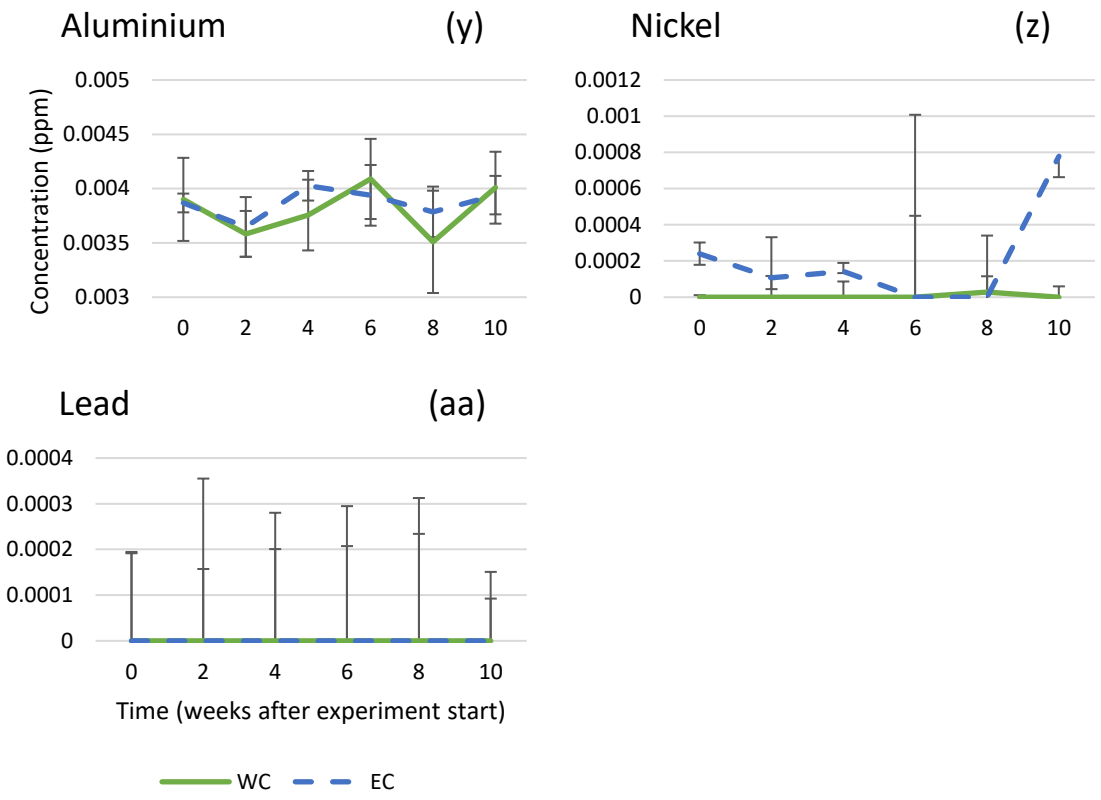
Species name	additional notes
Achnanthes daonensis	
Achnanthidium minutissimum	
Achnanthidium modestiforme	
Amphora inariensis	
Amphora pediculus	
Brachsira brebisonii	
Brachysira (unknown species)	discounted due to low abundance (<2% across all replicates)
Brachysira vitrea	
Brachysira zellensis	
Cocconeis disculus	
Cyclotella meneghiniana	Discounted due to being pelagic
Cymatopleura librile	discounted due to low abundance (<2% across all replicates)
Cymbella helvetica	discounted due to low abundance (<2% across all replicates)
Diatoma problematica	
Encyonema minuta	
Encyonema gracile	
Encyonema reichardtii	
Encyonema prostratum	
Epithemia adnata	
Epithemia argus	discounted due to low abundance (<2% across all replicates)
Epithemia sorex	
Epithemia turgida	
Fragilaria capucina var. gracilis	discounted due to low abundance (<2% across all replicates)
Fragilaria vaucheriae	
Frustulia rhomboides var saxonica	discounted due to low abundance (<2% across all replicates)
Gomphonema accuminatum	
Gomphonema apicatum	discounted due to low abundance (<2% across all replicates)
Gomphonema cuneolus	
Gomphonema lateripunctum	discounted due to low abundance (<2% across all replicates)
Gomphonema olivaceum	
Gomphonema parvulum	
Gomphonema truncatum	
Gomphonema vibrio	
Gyrosigma accuminatum	
Hippodonta capitata	discounted due to low abundance (<2% across all replicates)
Melosira varians	
Navicula capitatoradiata	
Navicula crpytocephala	
Navicula lanceolota	discounted due to low abundance (<2% across all replicates)
Navicula rhynchotella	
Nitzschia acicularis	
Nitzschia amphibia	
Nitzschia capitellata	discounted due to low abundance (<2% across all replicates)
Nitzschia dissipata	
Nitzscshia linearis	
Nitzschia minuta	
Nitzschia palea	
Nitzschia paleacea	
Nitzschia tabellaria	discounted due to low abundance (<2% across all replicates)
Oxyneis binalis var. elliptica	discounted due to low abundance (<2% across all replicates)
Pinnularia microstauron	discounted due to low abundance (<2% across all replicates)
Pinnularia viridis	
Rhoicosphenia abbreviata	
Rhopaladia gibba	
Surirella brebissonii	discounted due to low abundance (<2% across all replicates)
Synedra famelica	discounted due to low abundance (<2% across all replicates)
Synedra ulna	

Appendix c. Chapter 2 and 3, campus lakes experiment physico-chemical measurements between West campus lake and East campus lake recorded over time



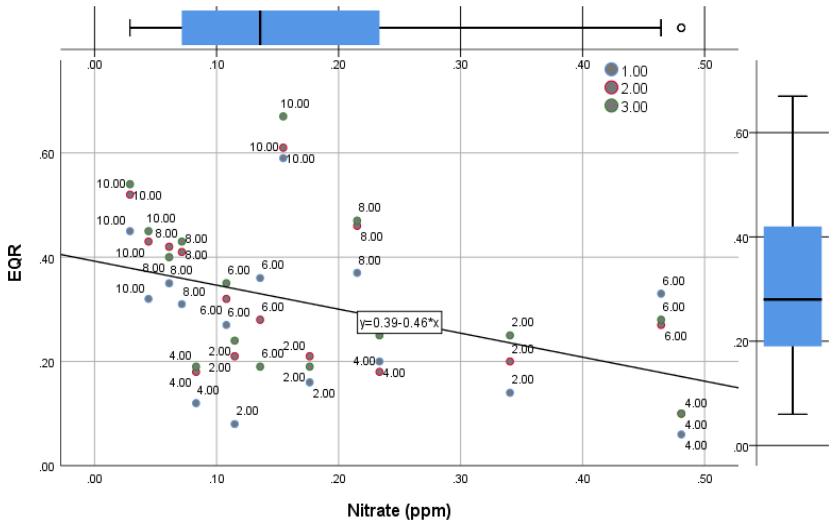

pH
(i)
9.5
9
8.5
8
7.5
pH
2
4
6
8
10
Temperature
(j)
27
25
23
21
19
17
15
Temperature (degrees C)
2
4
6
8
10
Electrical conductivity
(k)
950
900
850
800
750
700
650
Electrical conductivity (microsiemens)
2
4
6
8
10
Alkalinity
(l)
215
165
115
65
15
Alkalinity (mg/l)
2
4
6
8
10
DOC
(m)
13
11
9
7
Concentration (ppm)
0
2
4
6
8
10
Silicon
(n)
0.8
0.6
0.4
0.2
0
Concentration (ppm)
0
2
4
6
8
10
Magnesium
(o)
50
40
30
20
10
0
Concentration (ppm)
0
2
4
6
8
10
Potassium
(p)
30
25
20
15
10
5
0
Concentration (ppm)
0
2
4
6
8
10


Copper
(q)
Concentration (ppm)
0.012
0.01
0.008
0.006
0.004
0.002
0
0 2 4 6 8 10
Fluoride
(r)
Concentration (ppm)
0.4
0.3
0.2
0.1
0
0 2 4 6 8 10
Sodium
(s)
Concentration (ppm)
150
100
50
0
0 2 4 6 8 10
Time (weeks)
Chloride
(t)
Concentration (ppm)
160
140
120
100
80
0 2 4 6 8 10
Sulphate
(u)
Concentration (ppm)
60
40
20
0
0 2 4 6 8 10
Zinc
(v)
Concentration (ppm)
0.008
0.006
0.004
0.002
0
0 2 4 6 8 10
Calcium
(w)
Concentration (ppm)
40
30
20
10
0
0 2 4 6 8 10
Iron
(x)
Concentration (ppm)
0.008
0.006
0.004
0.002
0
0 2 4 6 8 10

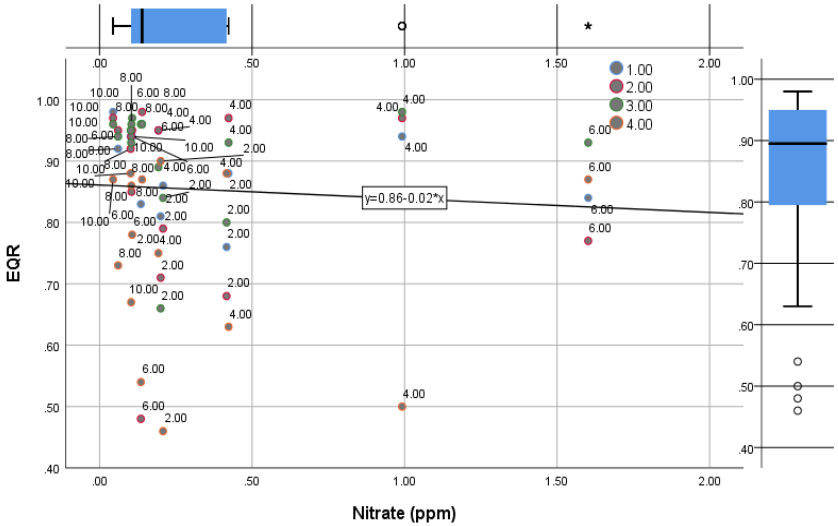

Aluminium
(y)
Concentration (ppm)
0.005
0.0045
0.004
0.0035
0.003
0
2
4
6
8
10
Nickel
(z)
0.0012
0.001
0.0008
0.0006
0.0004
0.0002
0
Lead
(aa)
0.0004
0.0003
0.0002
0.0001
0
Time (weeks after experiment start)
WC
EC

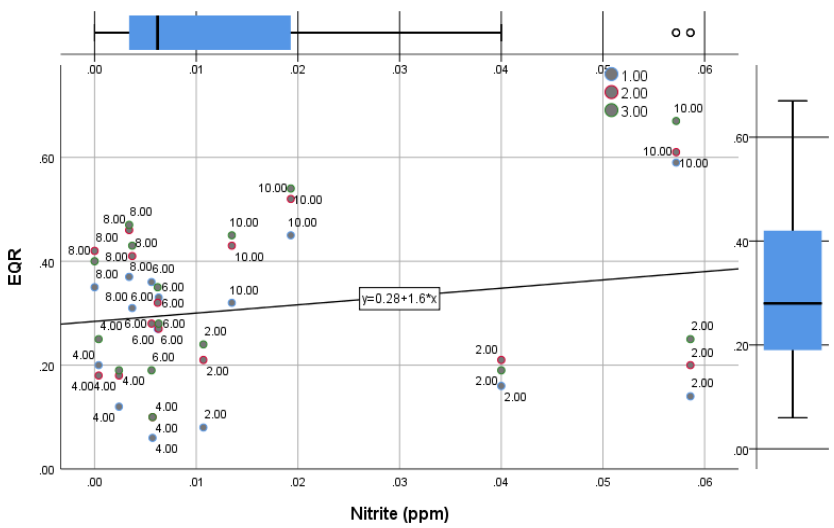
Appendix d. Chapter 2, campus lake experiment regression analysis graphs of the twenty-seven physicochemical parameters measured against the EQR LTDI2 (LTDI2 in chapters) values observed in biofilms taken from artificial substrates deployed on West campus lake (left) and East campus lake (right) biofilms. The latter of which also includes the results obtained from Reference stone substrates native to the site.

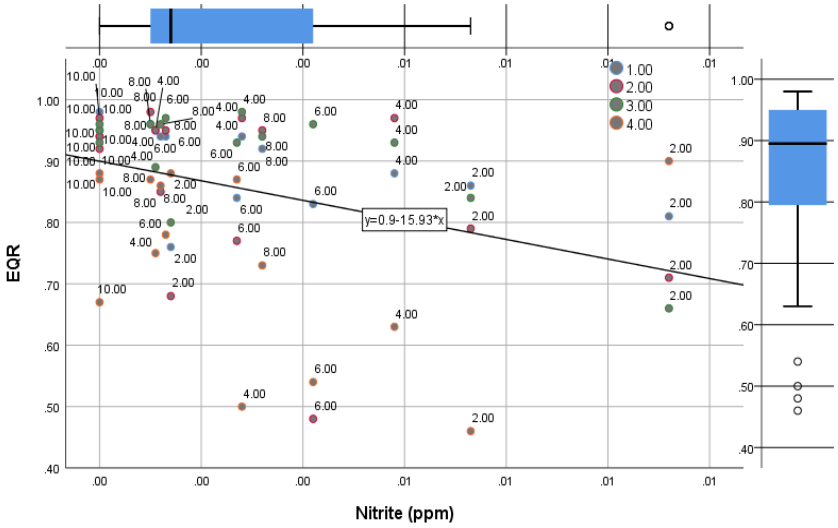
West campus lake

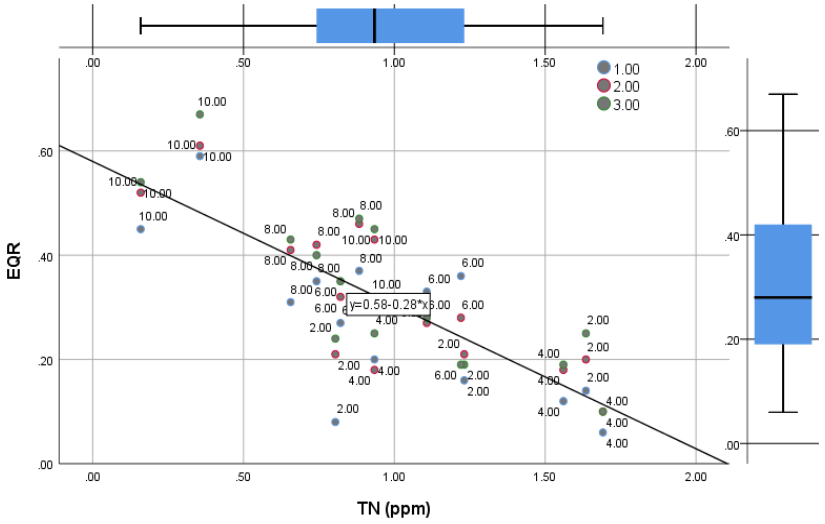


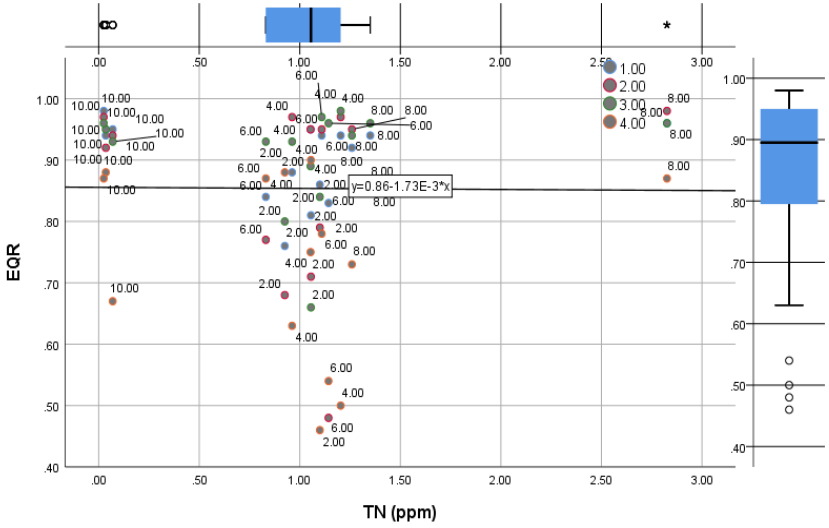
East campus lake

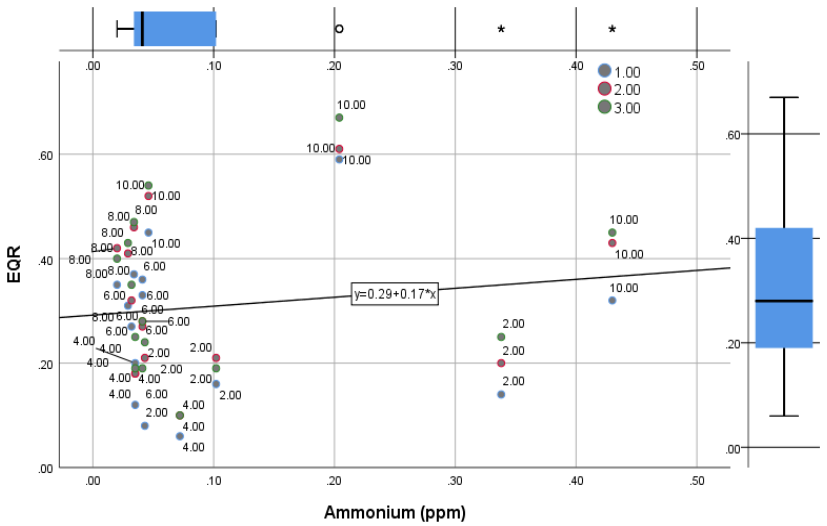


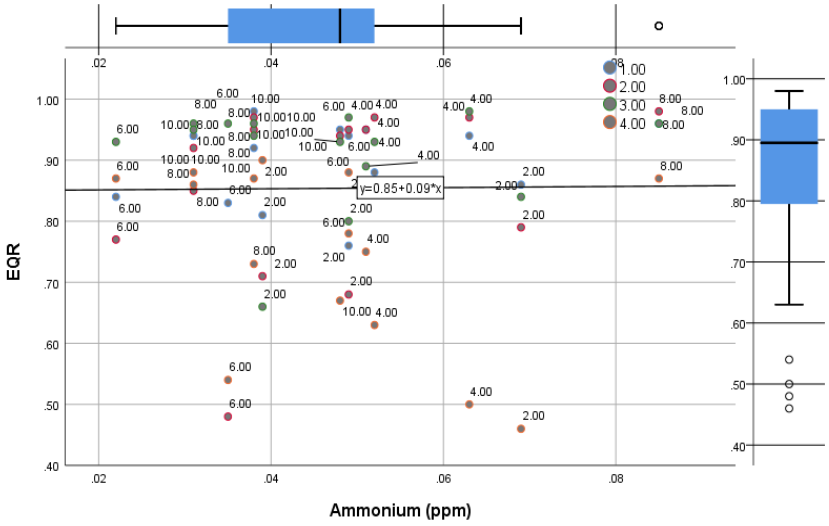

EQR
Nitrite (ppm)
y=0.28+1.6*x
1.00
2.00
3.00

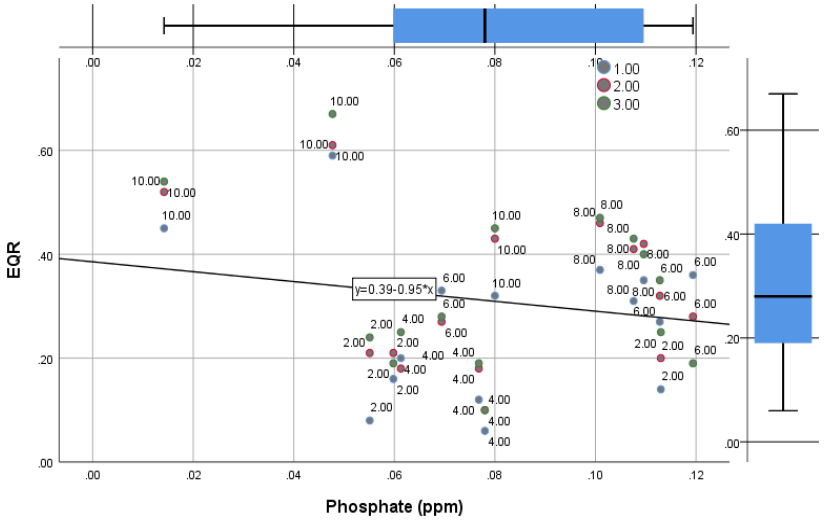

EQR
Nitrite (ppm)
y=0.9-15.93*x
1.00
2.00
3.00
4.00

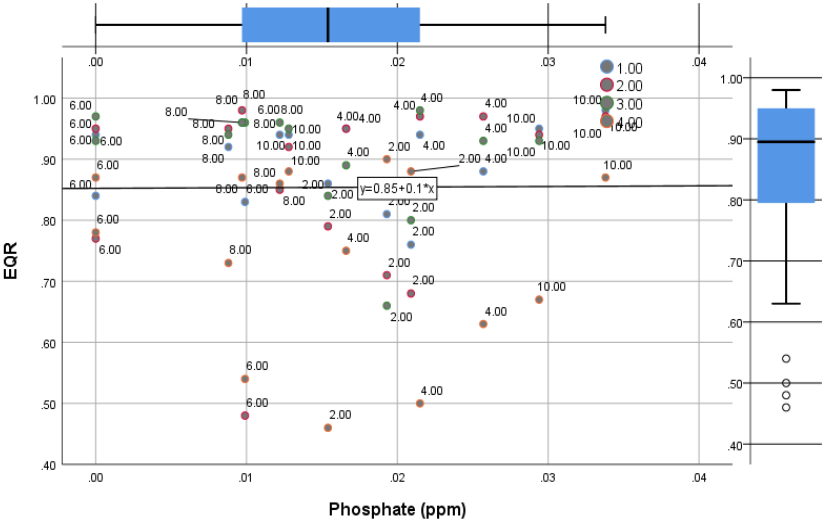

EQR
TN (ppm)
y=0.58-0.28*x
1.00
2.00
3.00

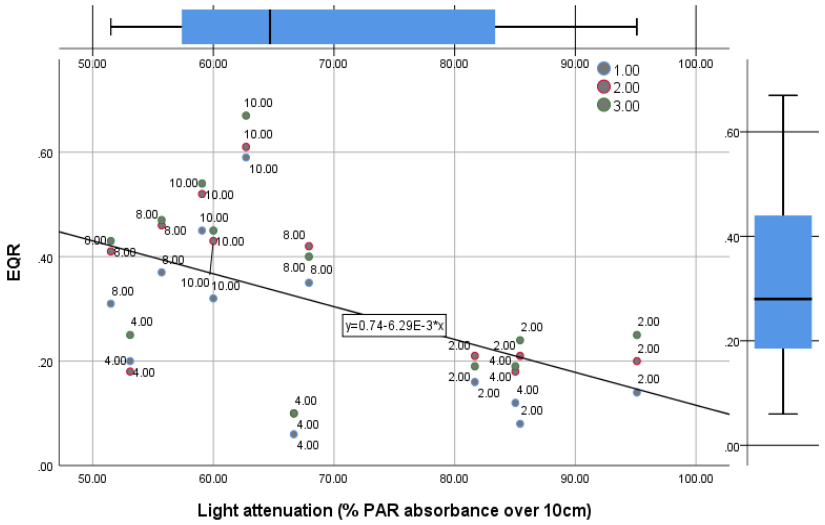

EQR
TN (ppm)
y=0.86-1.73E-3*x
1.00
2.00
3.00
4.00

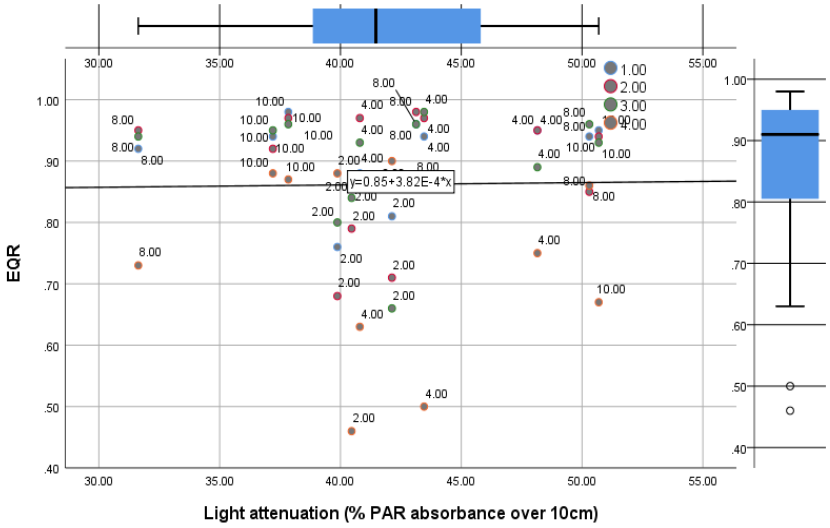

EQR
Ammonium (ppm)
y=0.29+0.17*x
1.00
2.00
3.00

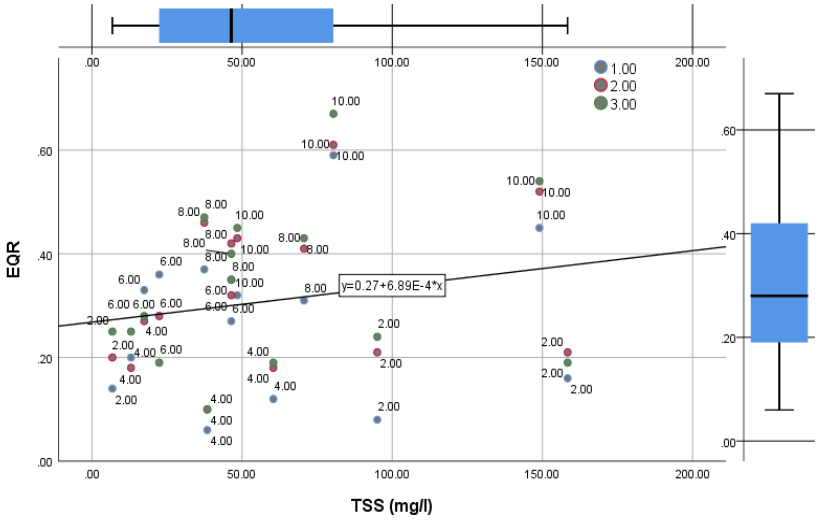

EQR
Ammonium (ppm)
y=0.85+0.09*x
1.00
2.00
3.00
4.00

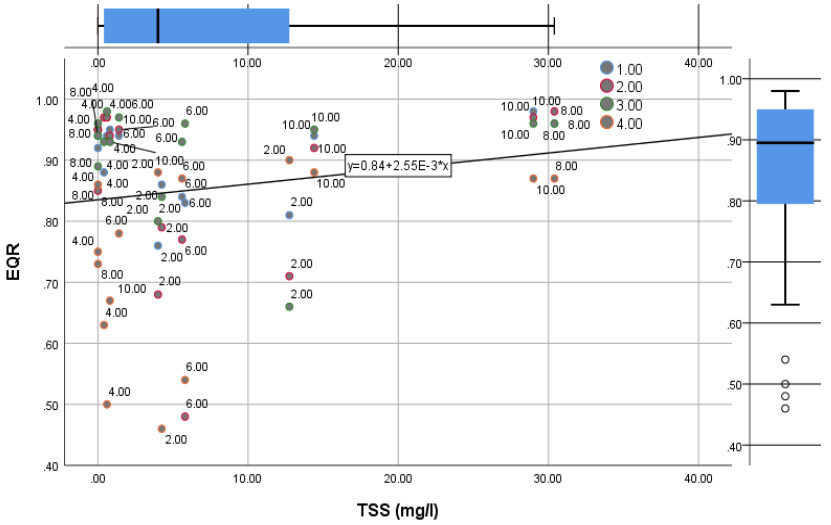

EQR
Phosphate (ppm)
y=0.39-0.95*x
1.00
2.00
3.00

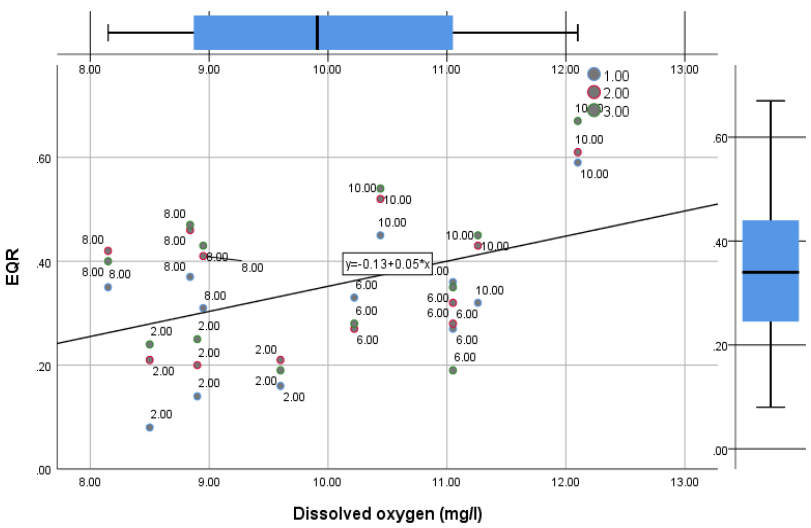

EQR
Phosphate (ppm)
y=0.85+0.1*x
1.00
2.00
3.00
4.00

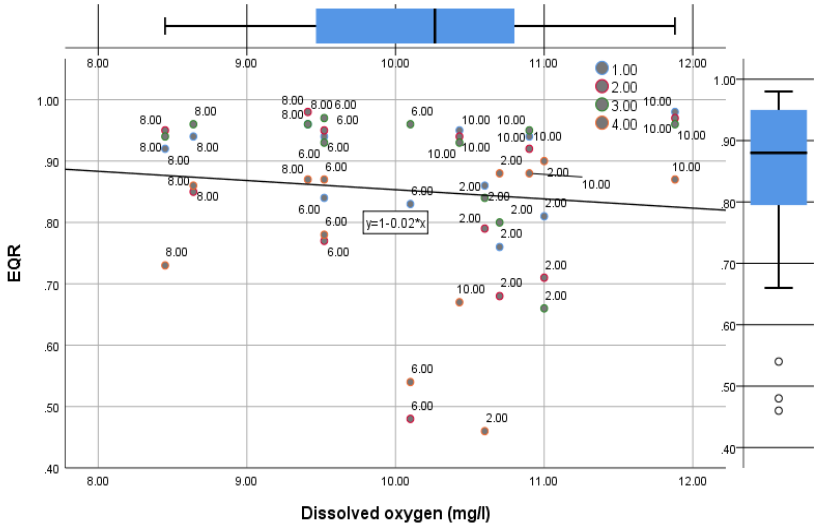

EQR
Light attenuation (% PAR absorbance over 10cm)
y=0.74-6.29E-3*x
1.00
2.00
3.00

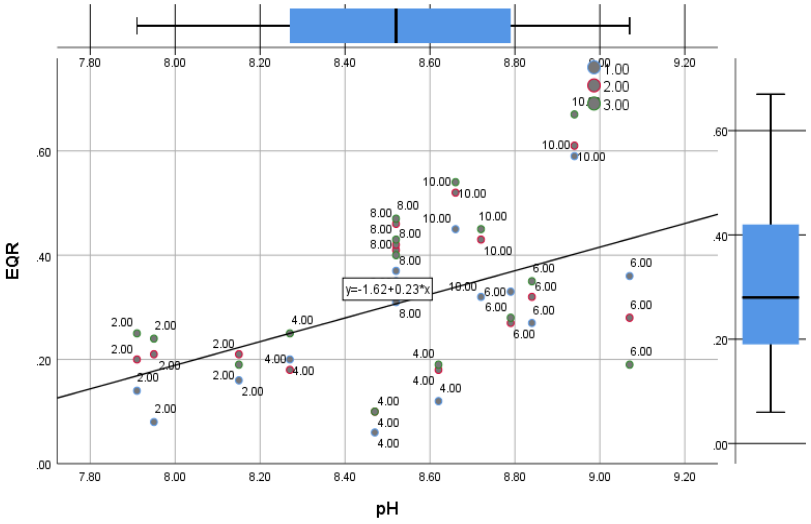

EQR
Light attenuation (% PAR absorbance over 10cm)
y=0.85+3.82E-4*x
1.00
2.00
3.00

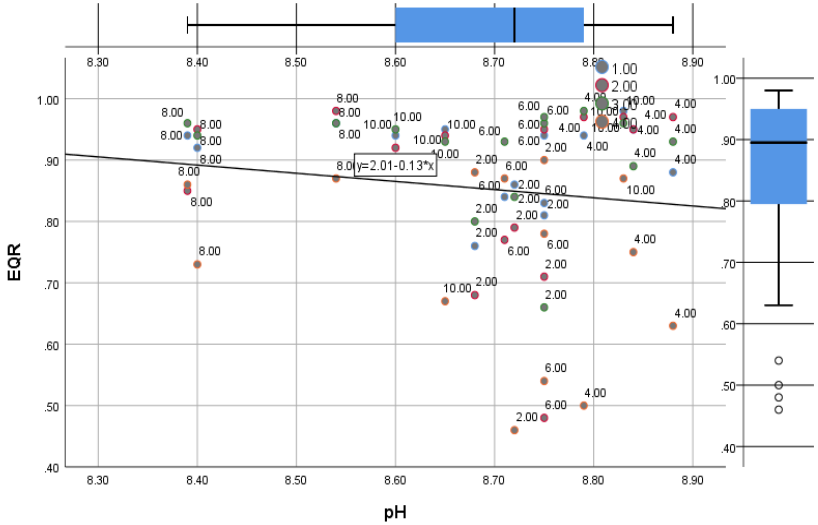

EQR
TSS (mg/l)
y=0.27+6.89E-4*x
1.00
2.00
3.00

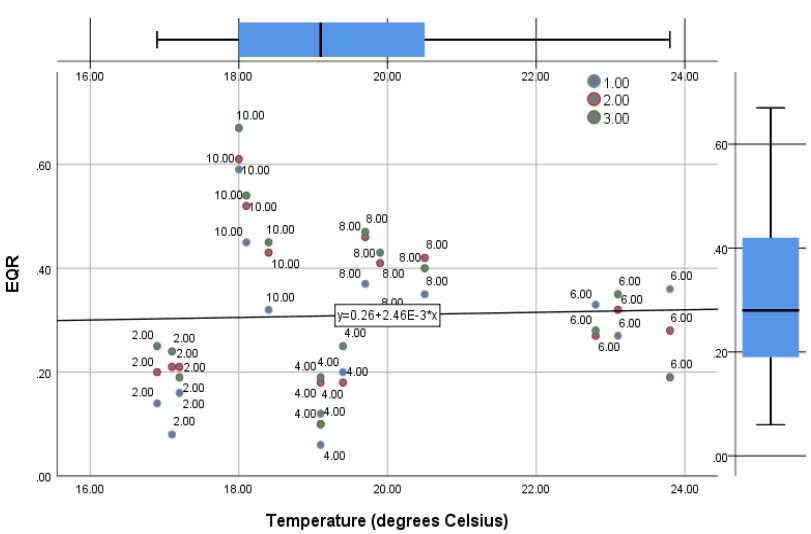

EQR
TSS (mg/l)
y=0.84+2.55E-3*x
1.00
2.00
3.00
4.00

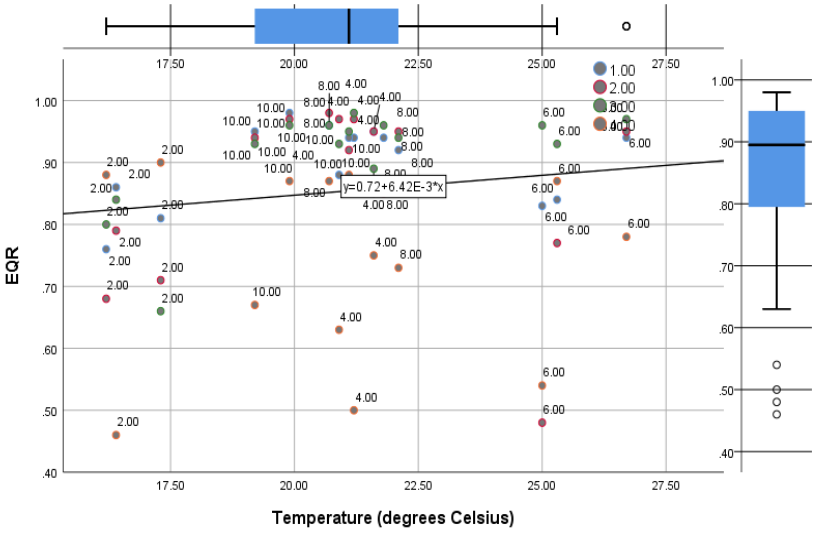

EQR
Dissolved oxygen (mg/l)
y=-0.13+0.05*x
1.00
2.00
3.00

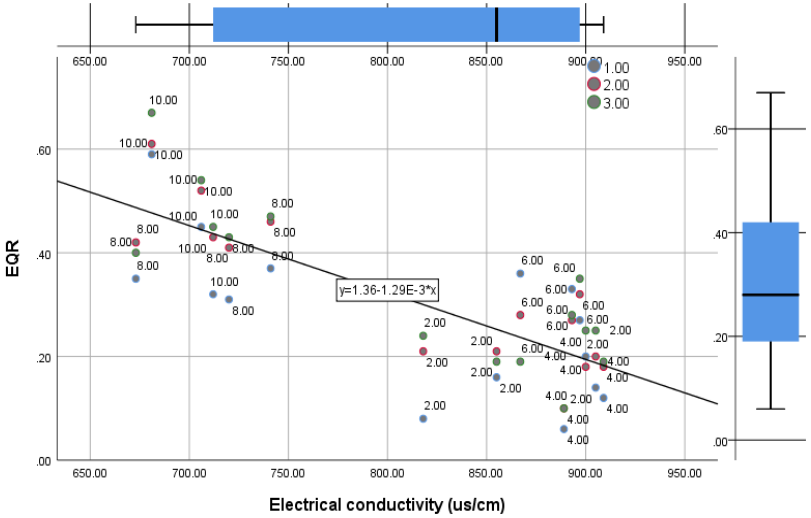

EQR
Dissolved oxygen (mg/l)
y=1-0.02*x
1.00
2.00
3.00
4.00

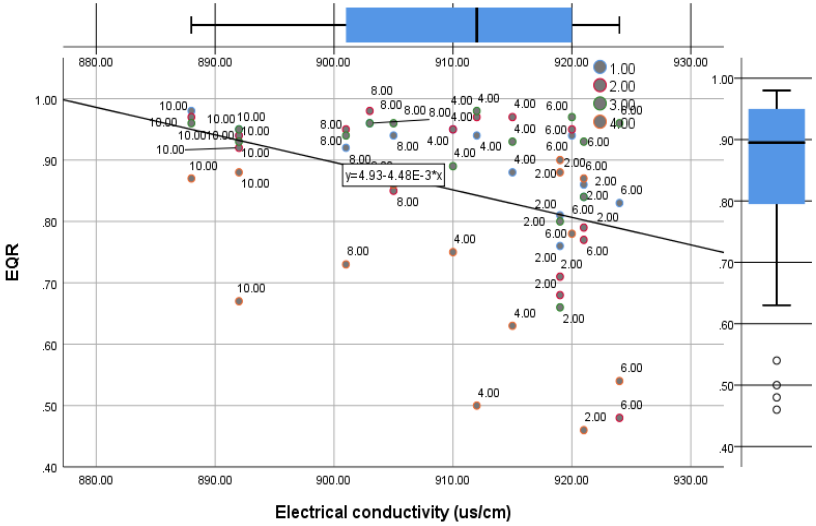

EQR
pH
y=-1.62+0.23*x
1.00
2.00
3.00

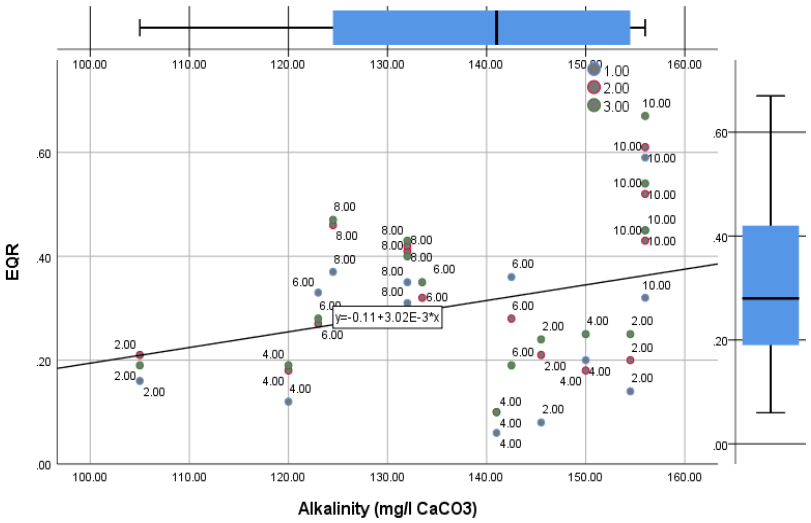

EQR
pH
y=2.01-0.13*x
1.00
2.00
3.00
4.00

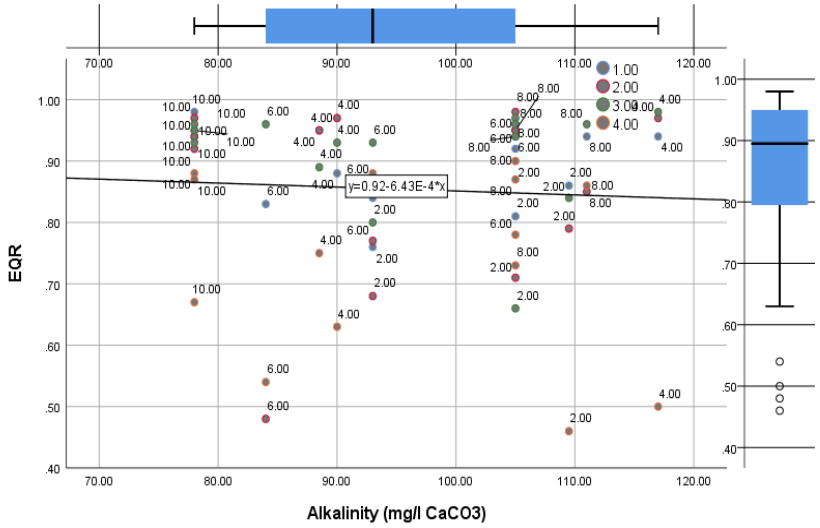

EQR
Temperature (degrees Celsius)
y=0.26+2.46E-3*x
1.00
2.00
3.00

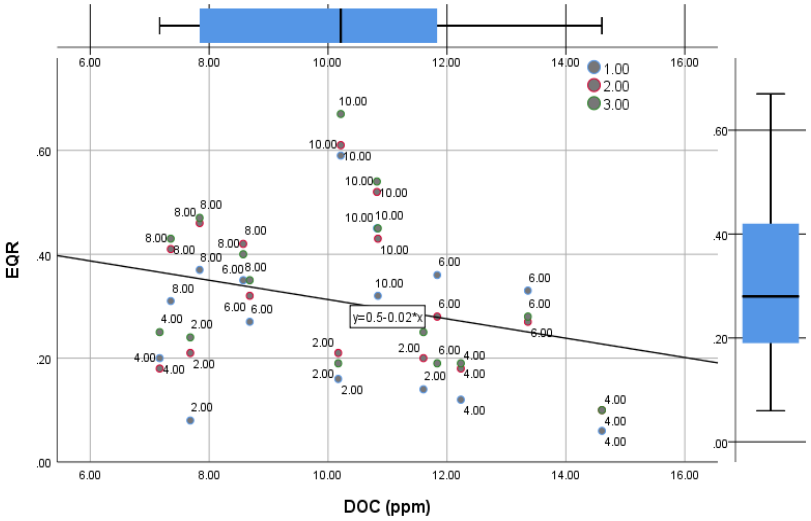

EQR
Temperature (degrees Celsius)
y=0.72+6.42E-3*x
1.00
2.00
3.00

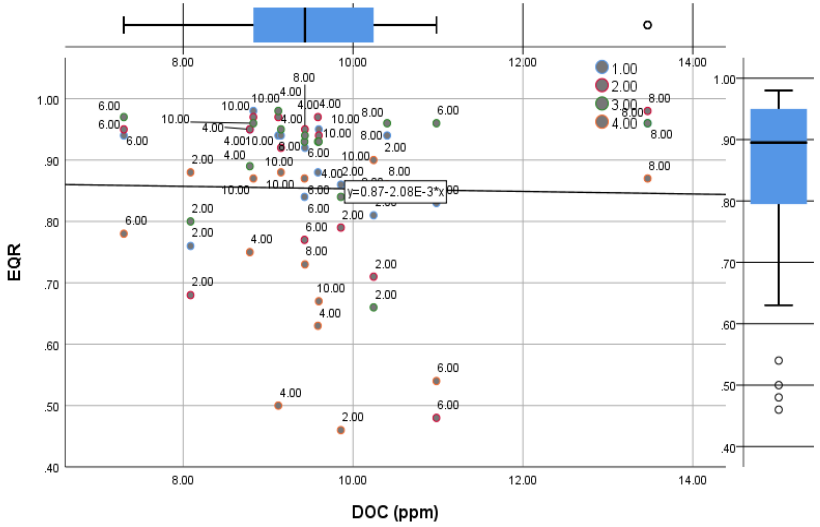

EQR
Electrical conductivity (us/cm)
y=1.36-1.29E-3*x
1.00
2.00
3.00

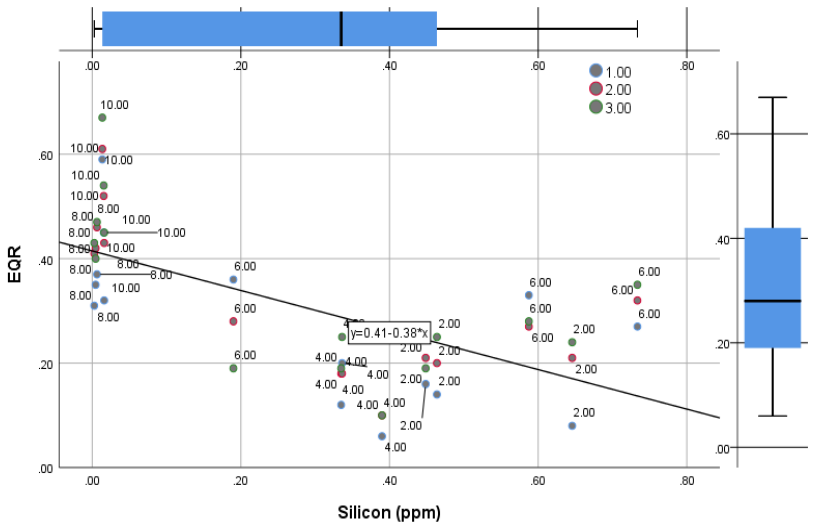

EQR
Electrical conductivity (us/cm)
y=4.93-4.48E-3*x
1.00
2.00
3.00

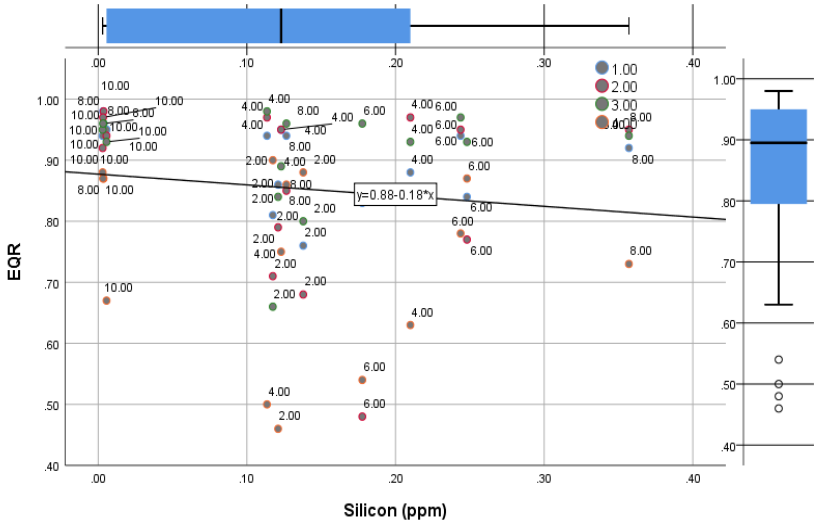

EQR
Alkalinity (mg/l CaCO3)
y=-0.11+3.02E-3*x
1.00
2.00
3.00

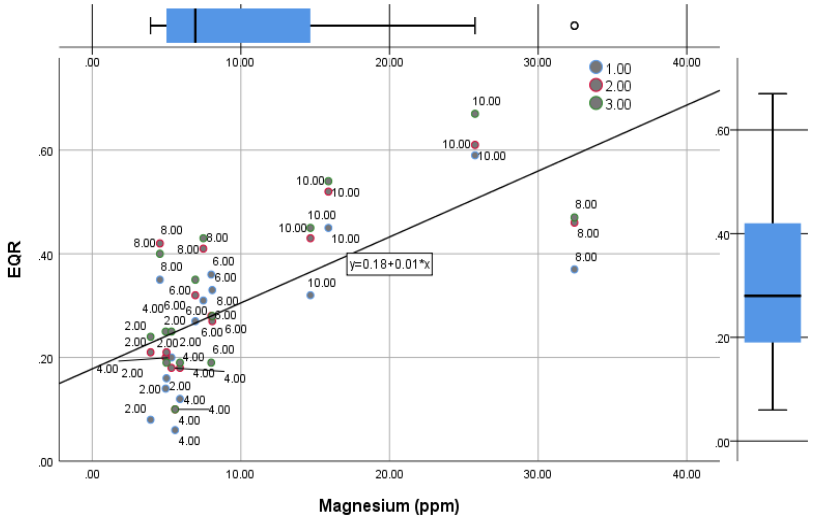

EQR
Alkalinity (mg/l CaCO3)
y=0.92-6.43E-4*x
1.00
2.00
3.00
4.00

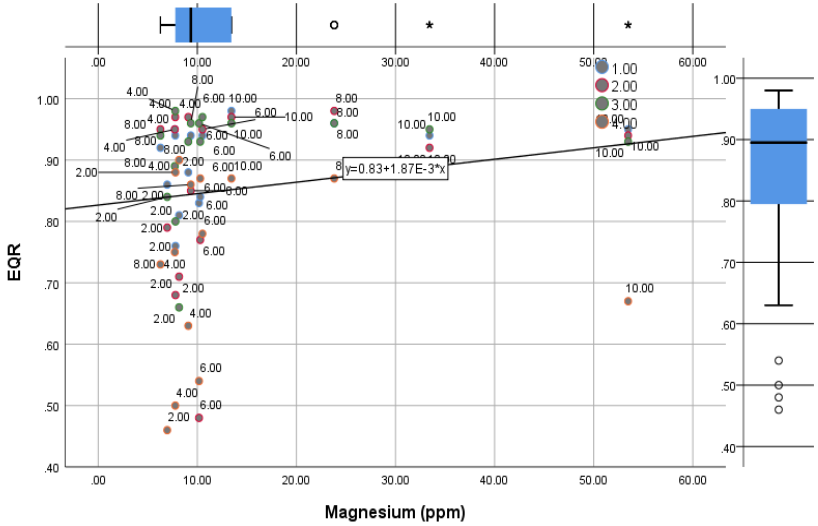

EQR
DOC (ppm)
y=0.5-0.02*x
1.00
2.00
3.00

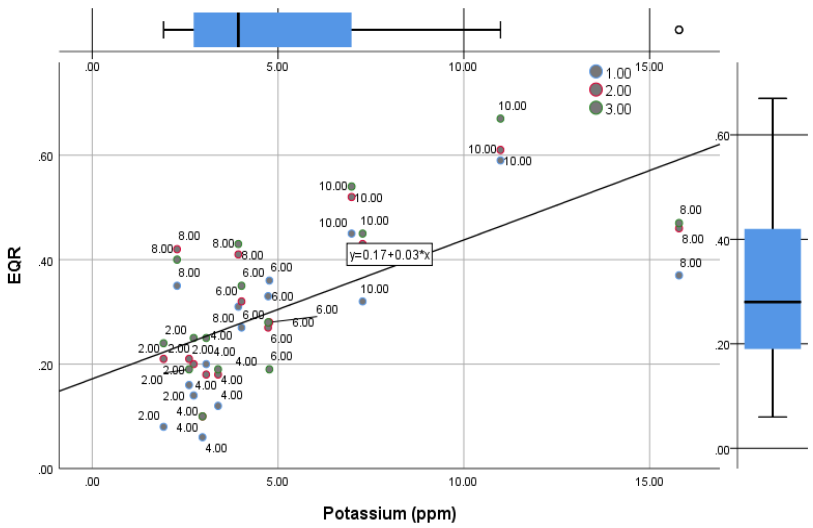

EQR
DOC (ppm)
y=0.87-2.08E-3*x
1.00
2.00
3.00
4.00

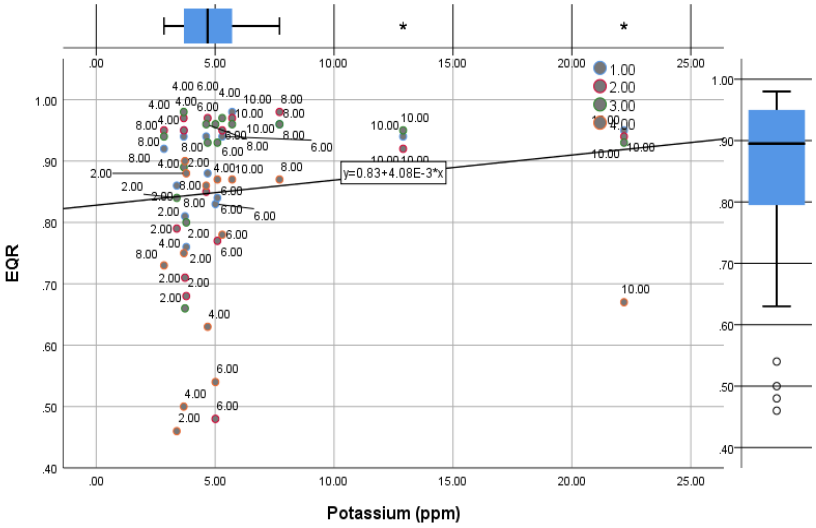

EQR
Silicon (ppm)
y=0.41-0.38*x
1.00
2.00
3.00

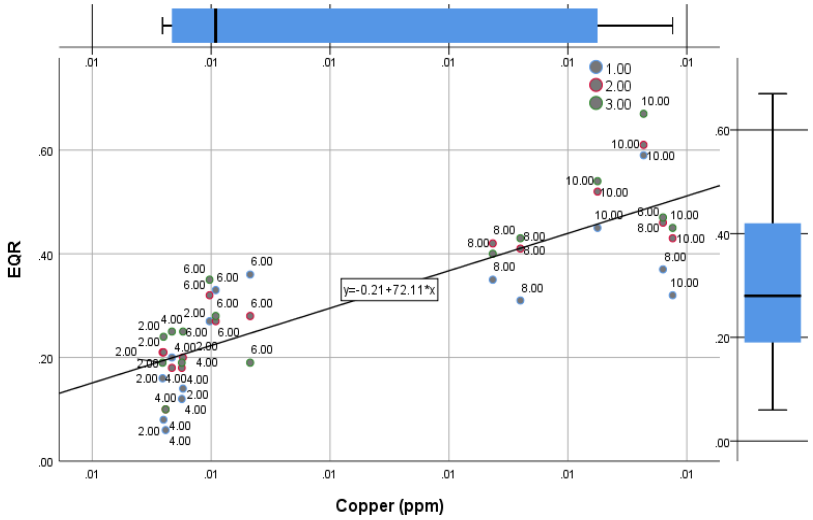

EQR
Silicon (ppm)
y=0.88-0.18*x
1.00
2.00
3.00

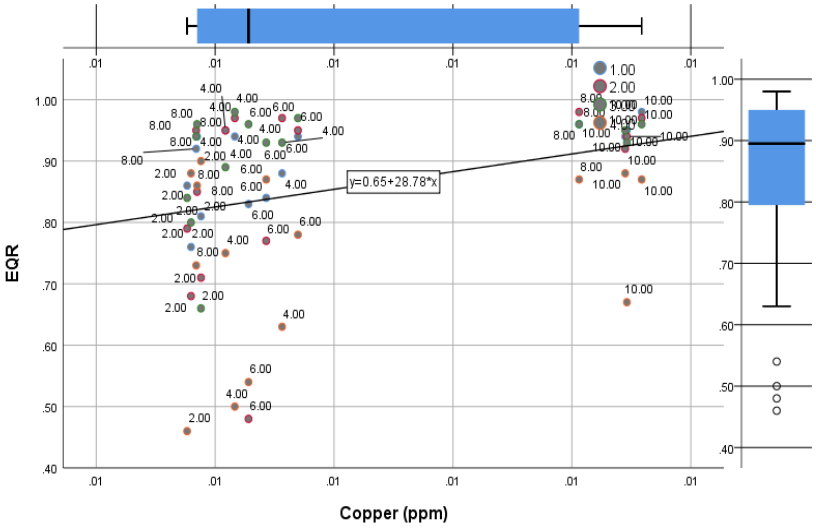

EQR
Magnesium (ppm)
y=0.18+0.01*x
1.00
2.00
3.00

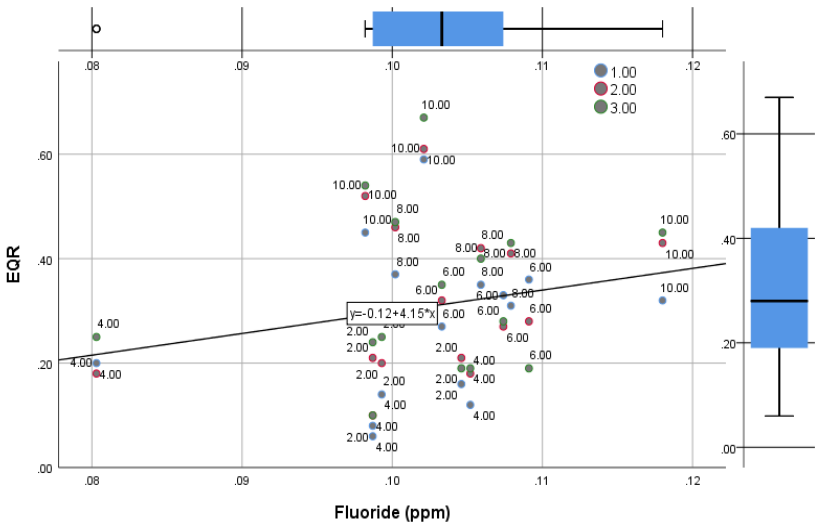

EQR
Magnesium (ppm)
y=0.83+1.87E-3*x
1.00
2.00
3.00

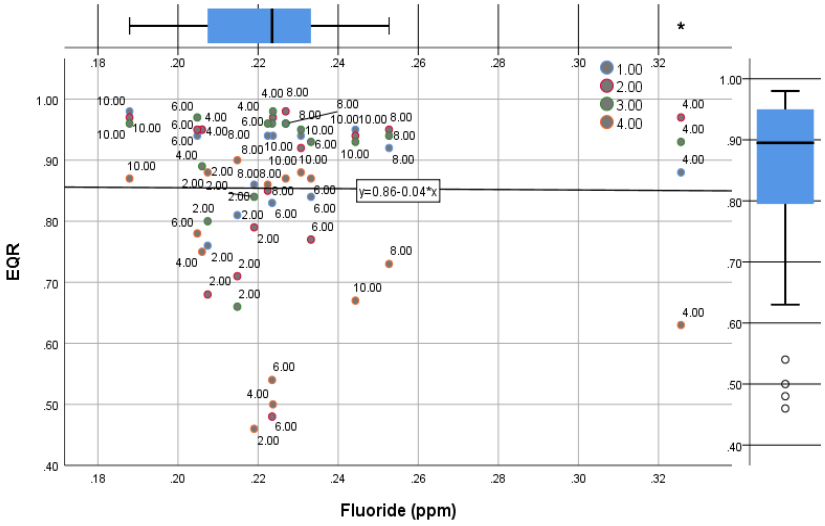

EQR
Potassium (ppm)
y=0.17+0.03*x
1.00
2.00
3.00

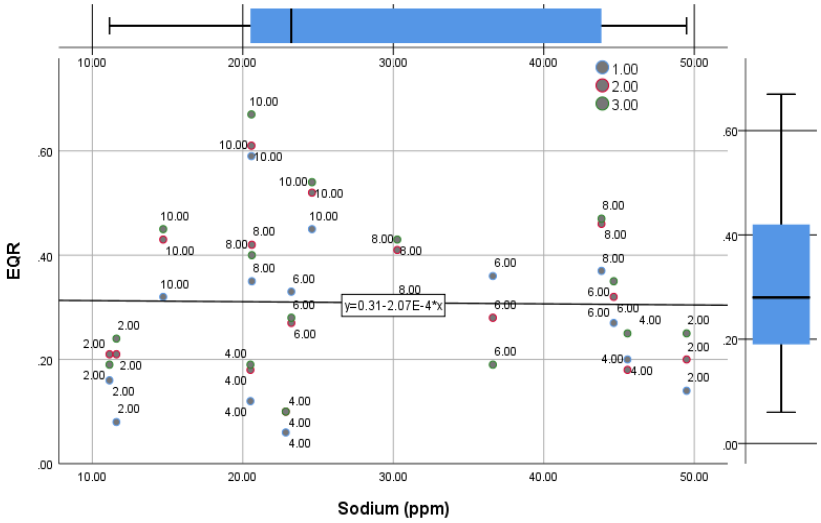

EQR
Potassium (ppm)
y=0.83+4.08E-3*x
1.00
2.00
3.00

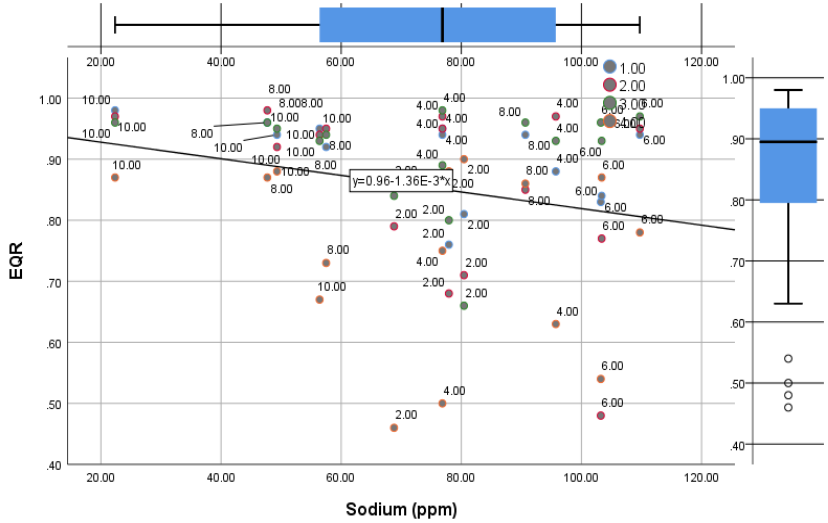

EQR
Copper (ppm)
y=-0.21+72.11*x
1.00
2.00
3.00

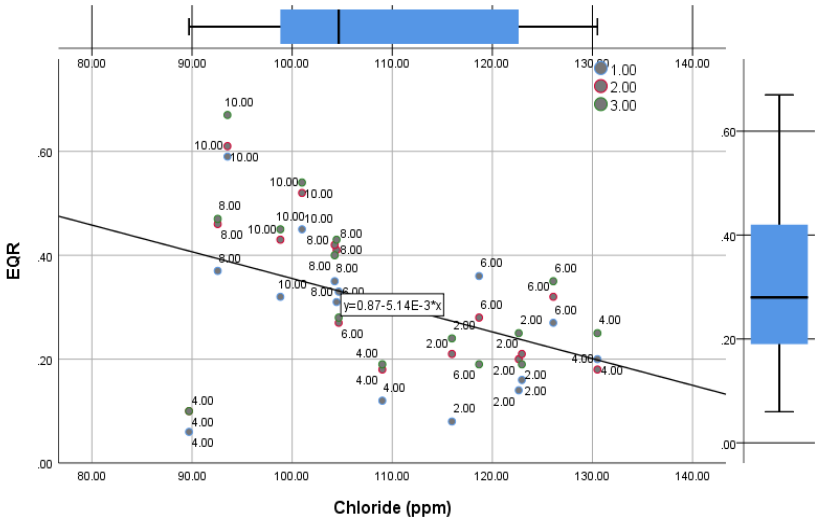

EQR
Copper (ppm)
y=0.65+28.78*x
1.00
2.00
3.00

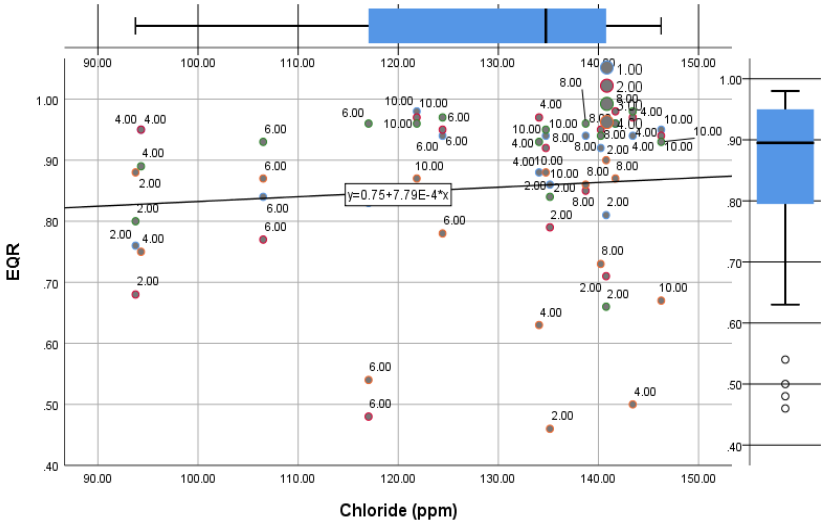

EQR
Fluoride (ppm)
y=-0.12+4.15*x
1.00
2.00
3.00

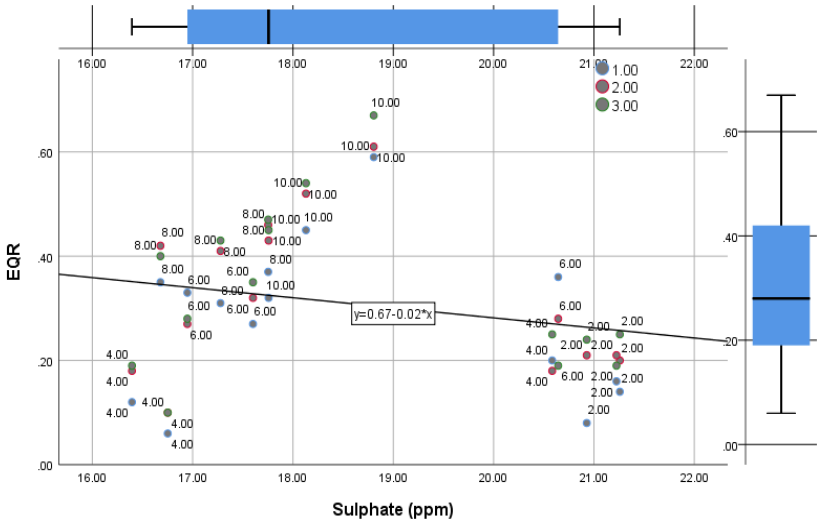

EQR
Fluoride (ppm)
y=0.86-0.04*x
1.00
2.00
3.00
4.00

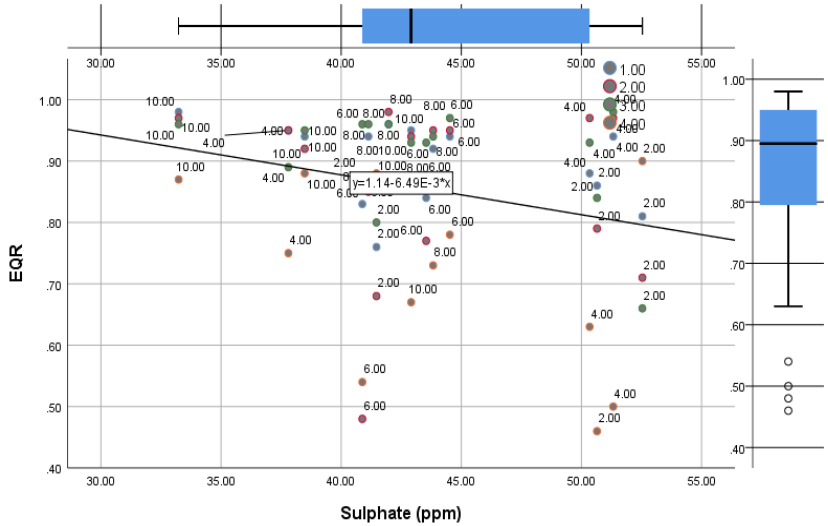

EQR
Sodium (ppm)
y=0.31-2.07E-4*x
1.00
2.00
3.00

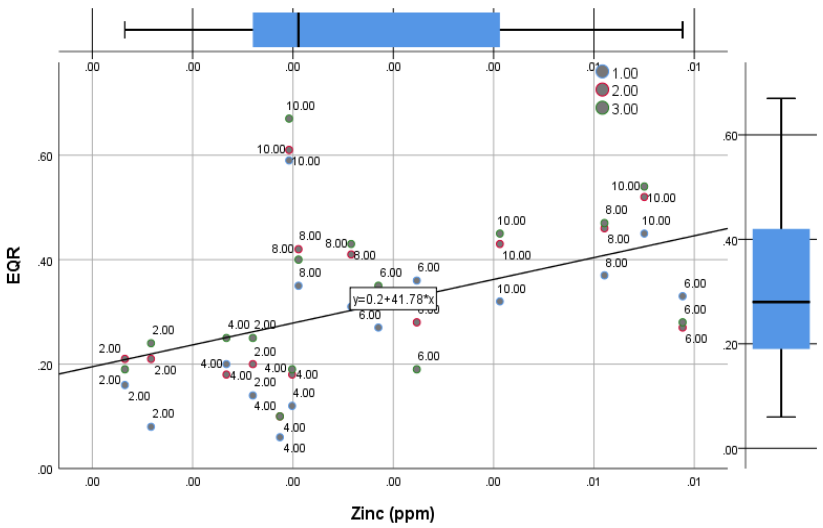

EQR
Sodium (ppm)
y=0.96-1.36E-3*x
1.00
2.00
3.00
4.00

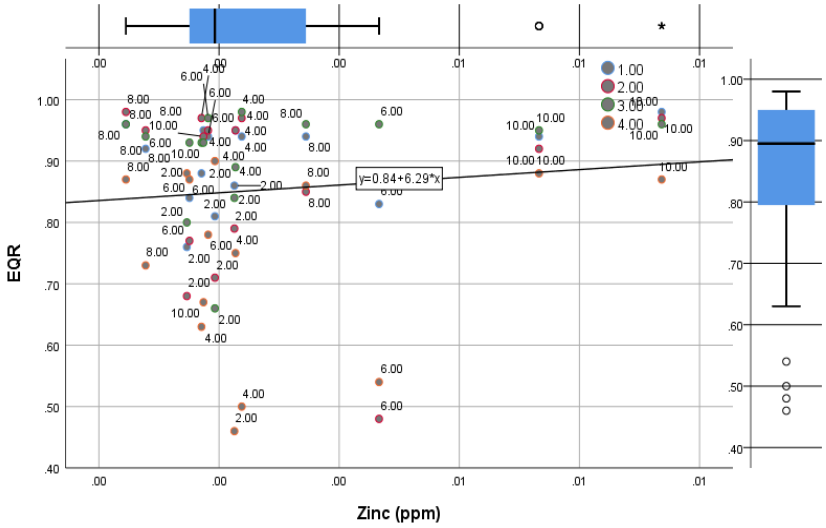

EQR
Chloride (ppm)
y=0.87-5.14E-3*x
1.00
2.00
3.00

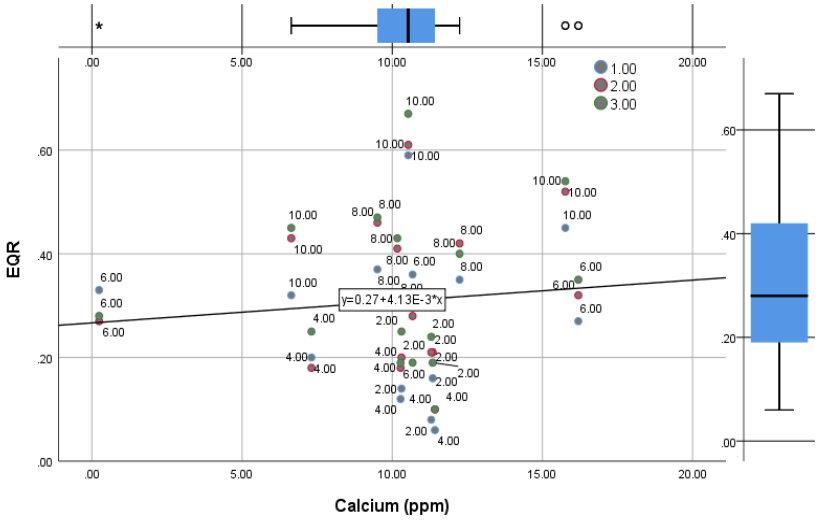

EQR
Chloride (ppm)
y=0.75+7.79E-4*x
1.00
2.00
3.00

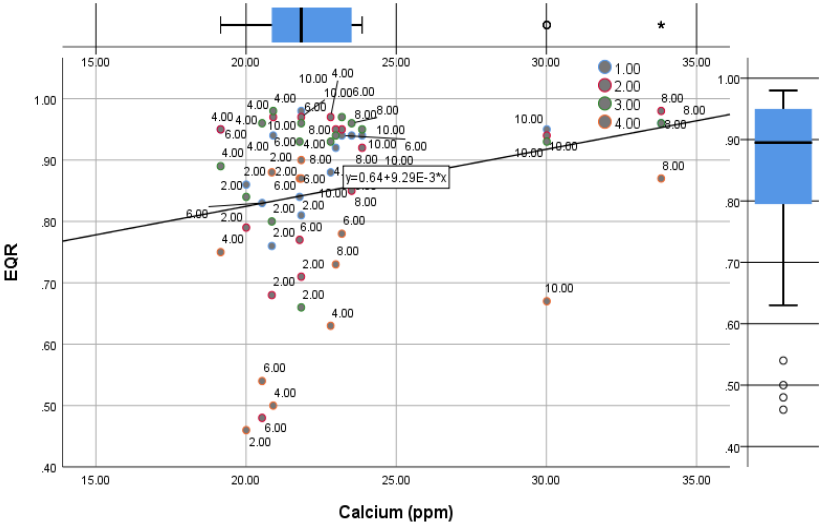

EQR
Sulphate (ppm)
y=0.67-0.02*x
1.00
2.00
3.00

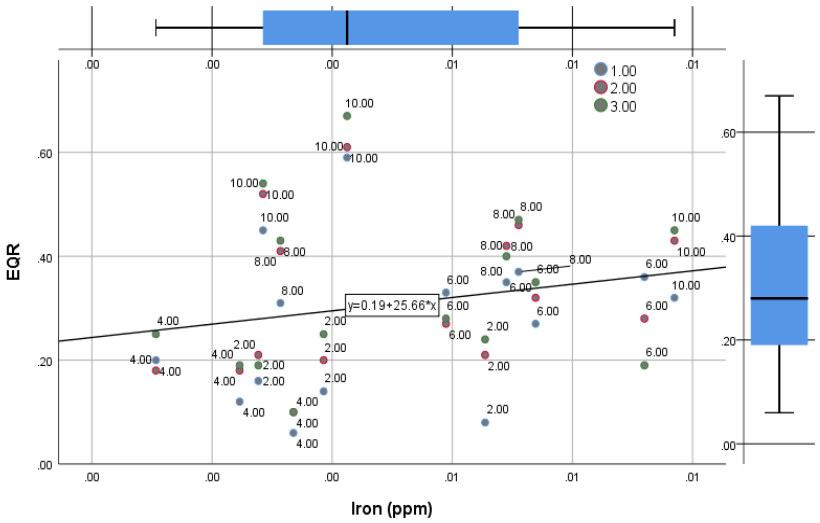

EQR
Sulphate (ppm)
y=1.14-6.49E-3*x
1.00
2.00
3.00

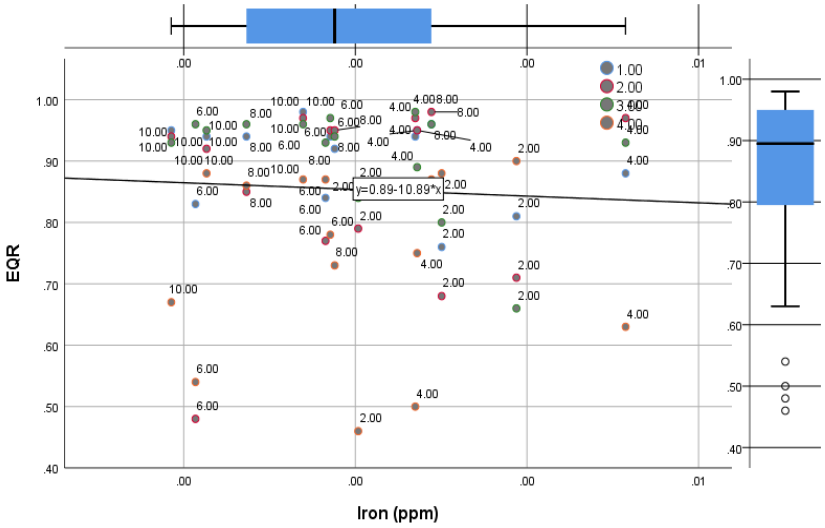

EQR
Zinc (ppm)
y=0.2+41.78*x
1.00
2.00
3.00

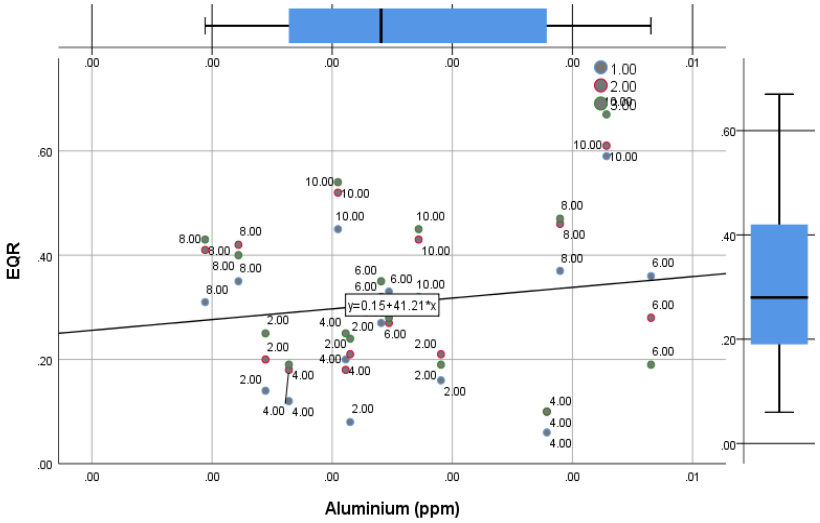

EQR
Zinc (ppm)
y=0.84+6.29*x
1.00
2.00
3.00
4.00

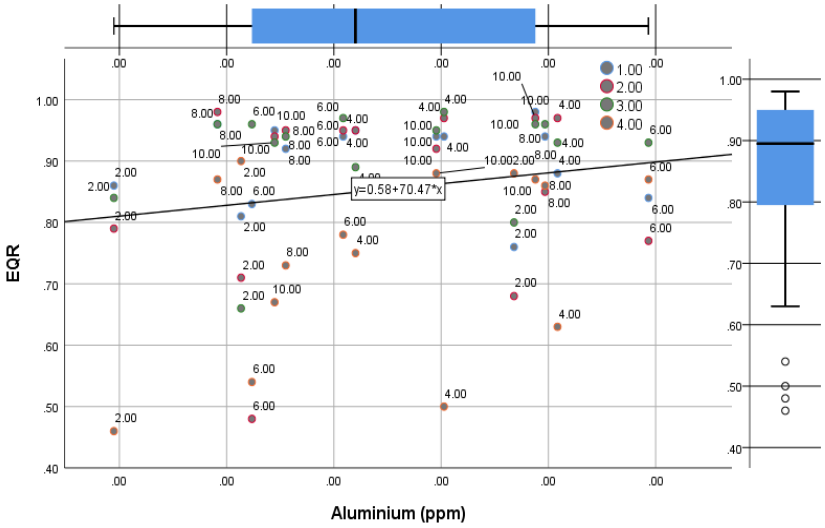

EQR
Calcium (ppm)
y=0.27+4.13E-3*x
1.00
2.00
3.00

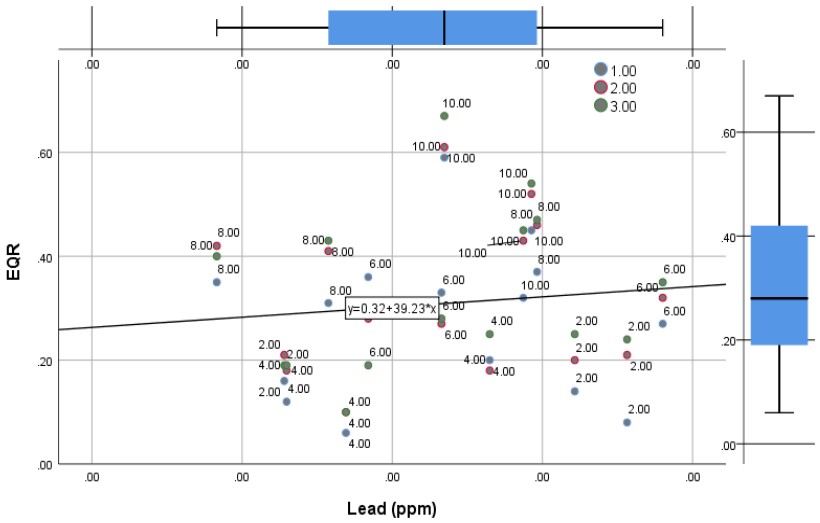

EQR
Calcium (ppm)
y=0.64+9.29E-3*x
1.00
2.00
3.00
4.00

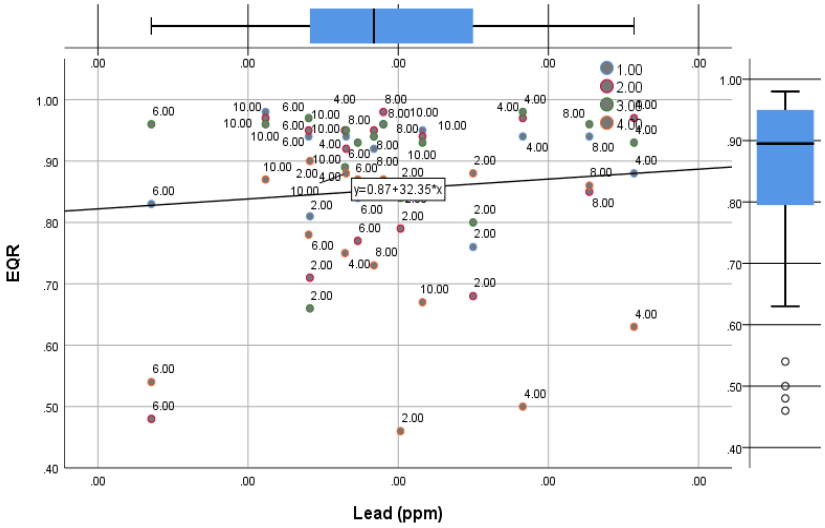

EQR
Iron (ppm)
y=0.19+25.66*x
1.00
2.00
3.00

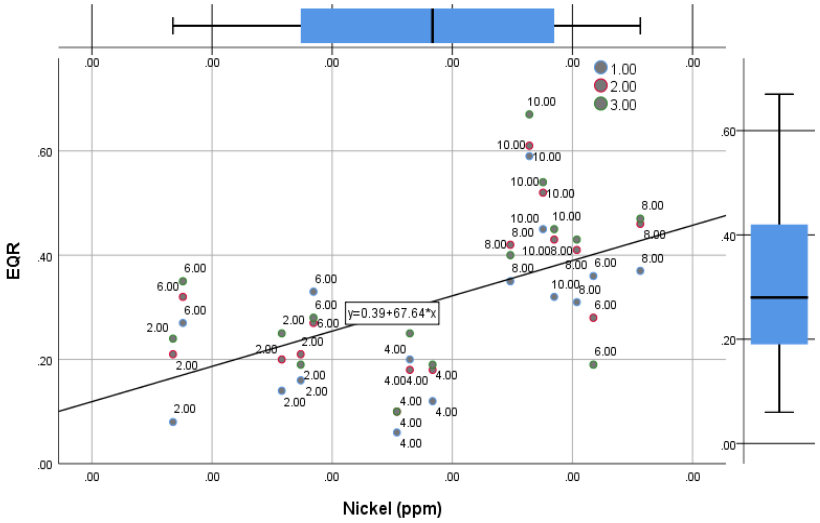

EQR
Iron (ppm)
y=0.89-10.89*x
1.00
2.00
3.00

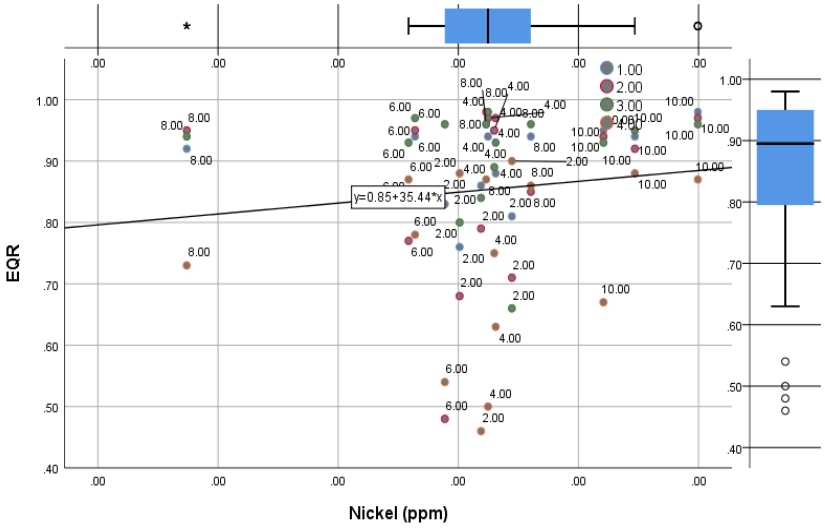

EQR
Aluminium (ppm)
y=0.15+41.21*x
1.00
2.00
3.00


EQR
Aluminium (ppm)
y=0.58+70.47*x
1.00
2.00
3.00
4.00

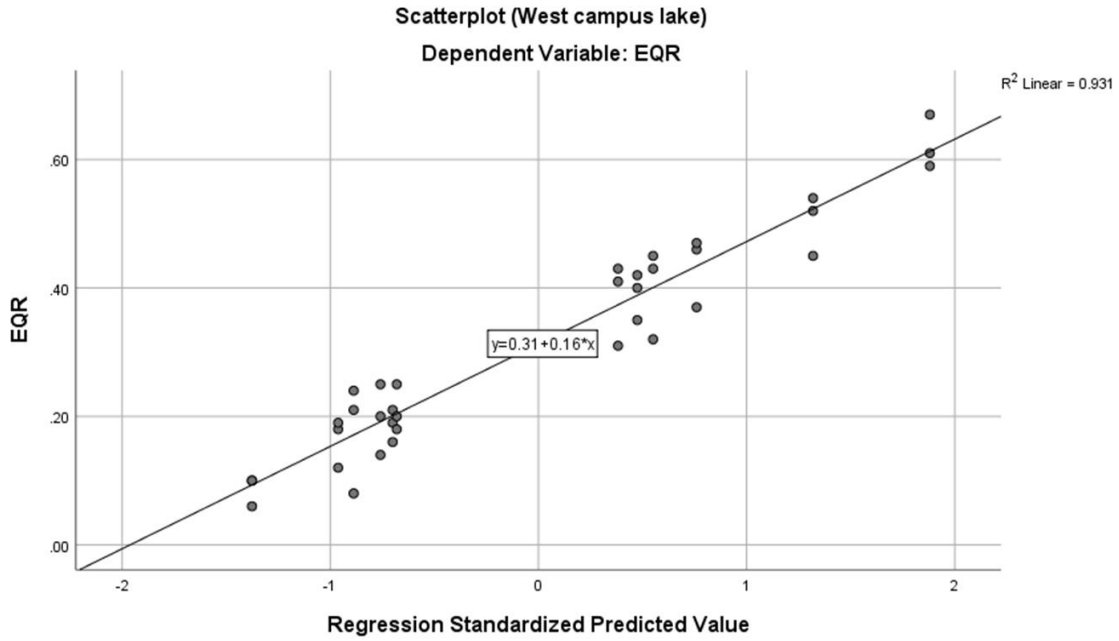

EQR
Lead (ppm)
y=0.32+39.23*x
1.00
2.00
3.00

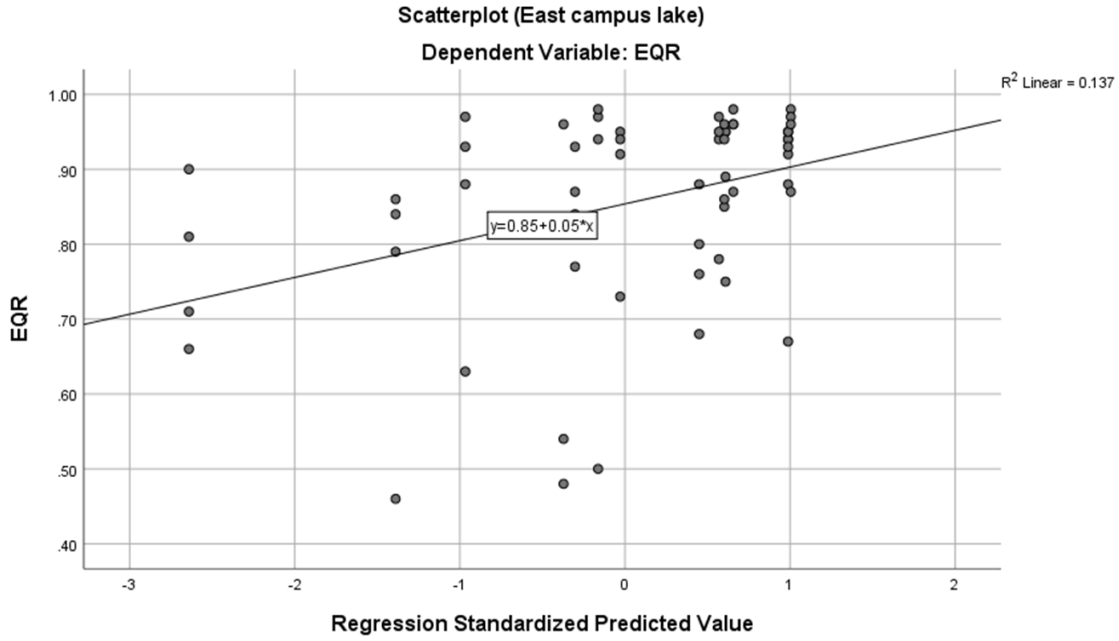

EQR
Lead (ppm)
y=0.87+32.35*x
1.00
2.00
3.00


EQR
Nickel (ppm)
y=0.39+67.64*x
1.00
2.00
3.00

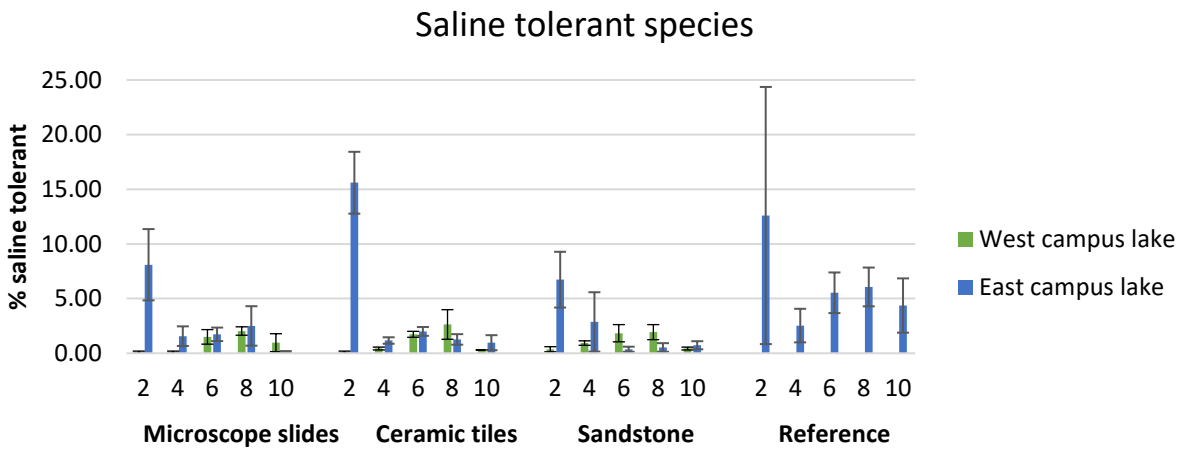

EQR
Nickel (ppm)
y=0.85+35.44*x
1.00
2.00
3.00

Appendix e. Chapter 2, multiple linear regression model for the physico-chemical parameters modelled against the EQR LTDI2 values considered significant MANOVA analysis.

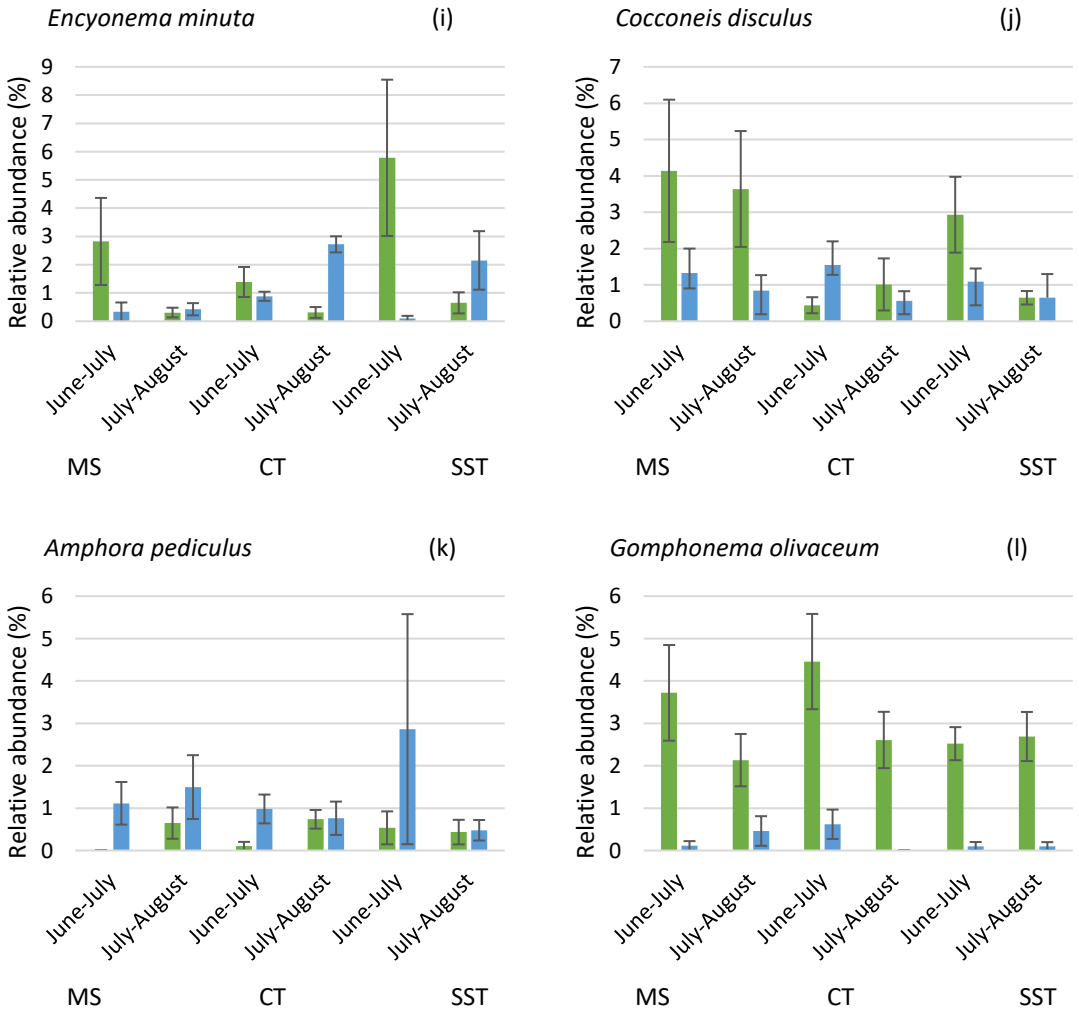


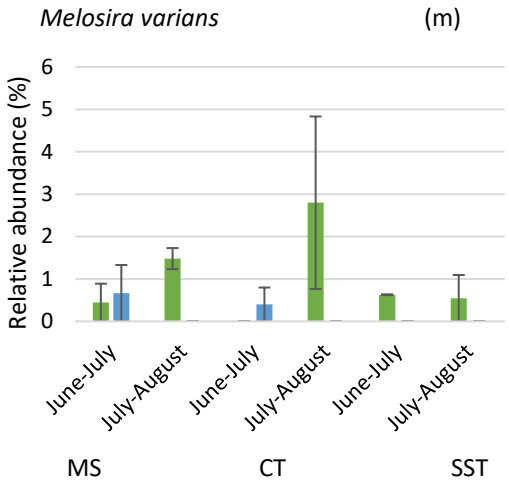


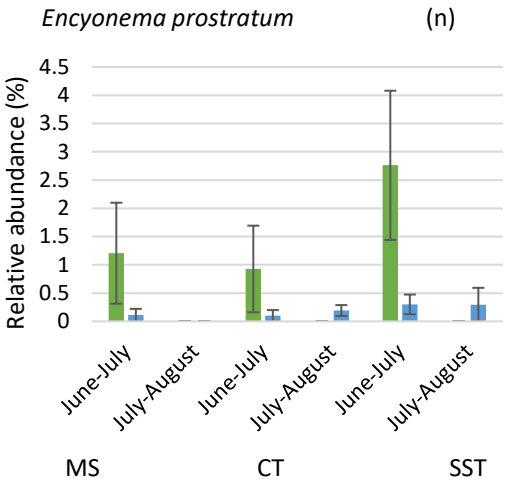
Appendix f. Chapter 2, saline tolerant species calculated from UKTAG assesment endpoints

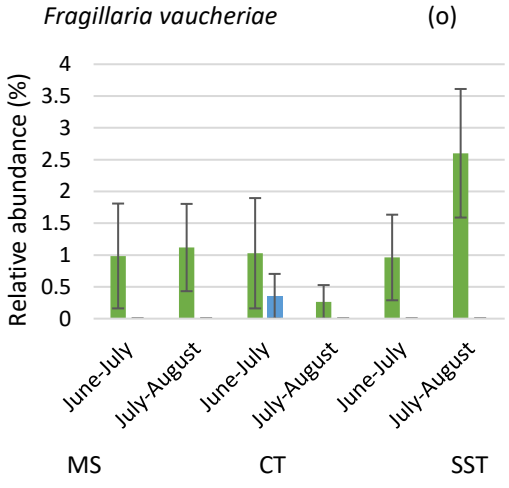


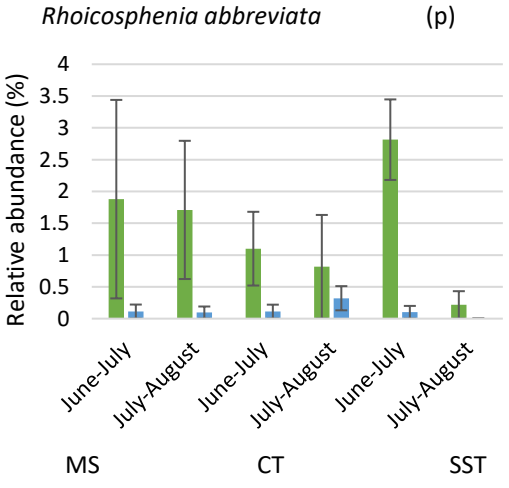
Appendix g. Chapter 3, Relative abundances of diatom species present in the campus lakes during the late and early summer deployment times

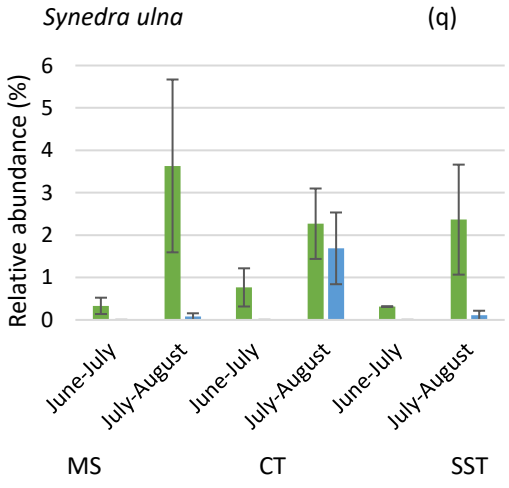


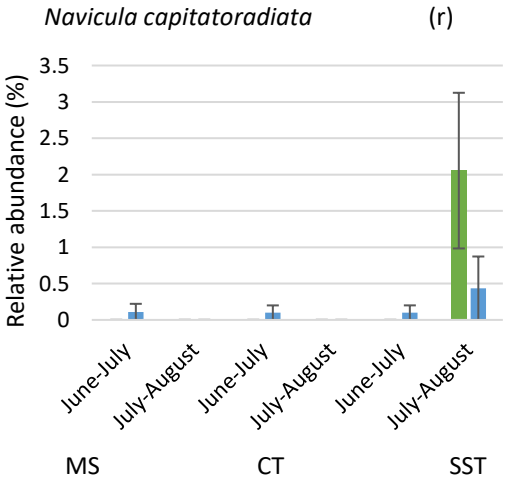

Melosira varians
(m)
Relative abundance (%)
0
1
2
3
4
5
6
June-July
July-August
June-July
July-August
June-July
July-August
MS
CT
SST

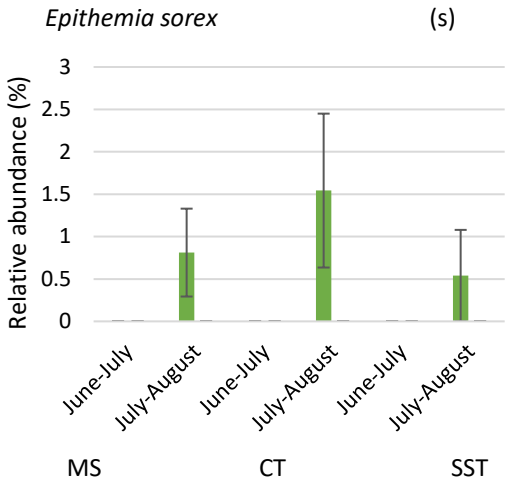

Encyonema prostratum
(n)
Relative abundance (%)
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
June-July
July-August
June-July
July-August
June-July
July-August
MS
CT
SST

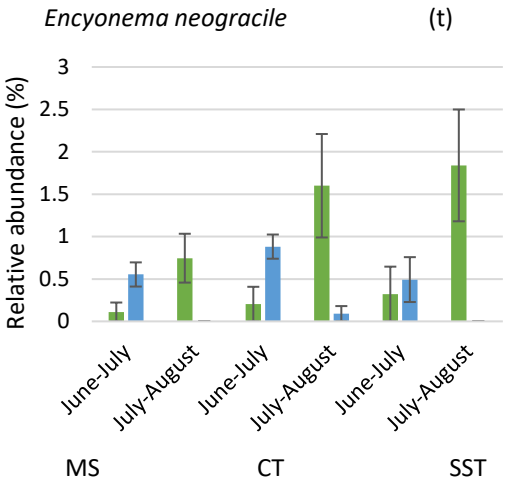

Fragillaria vaucheriae
(o)
Relative abundance (%)
0
0.5
1
1.5
2
2.5
3
3.5
4
June-July
July-August
June-July
July-August
June-July
July-August
MS
CT
SST

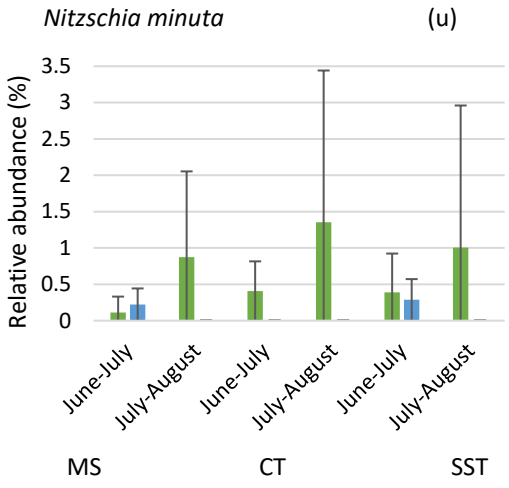

Rhoicosphenia abbreviata
(p)
Relative abundance (%)
0
0.5
1
1.5
2
2.5
3
3.5
4
June-July
July-August
June-July
July-August
June-July
July-August
MS
CT
SST

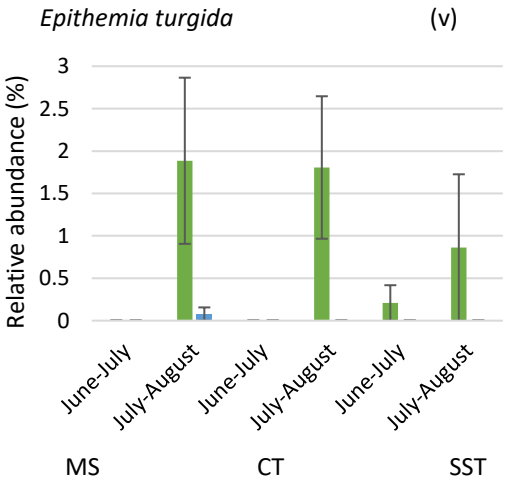

Synedra ulna
(q)
Relative abundance (%)
0
1
2
3
4
5
6
June-July
July-August
June-July
July-August
June-July
July-August
MS
CT
SST

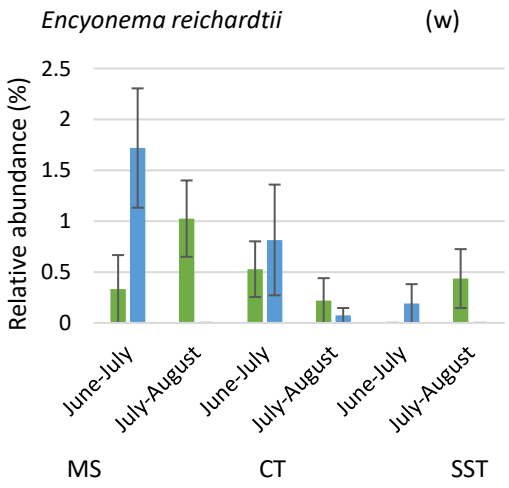

Navicula capitatoradiata
(r)
Relative abundance (%)
0
0.5
1
1.5
2
2.5
3
3.5
June-July
July-August
June-July
July-August
June-July
July-August
MS
CT
SST

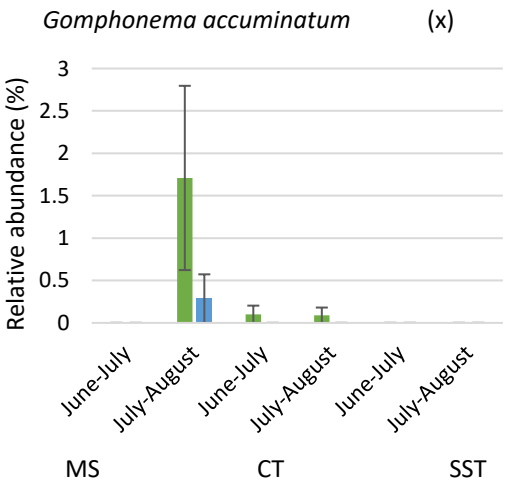

Epithemia sorex
(s)
Relative abundance (%)
3
2.5
2
1.5
1
0.5
0
June-July
July-August
June-July
July-August
June-July
July-August
MS
CT
SST


Encyonema neogracile
(t)
Relative abundance (%)
3
2.5
2
1.5
1
0.5
0
June-July
July-August
June-July
July-August
June-July
July-August
MS
CT
SST

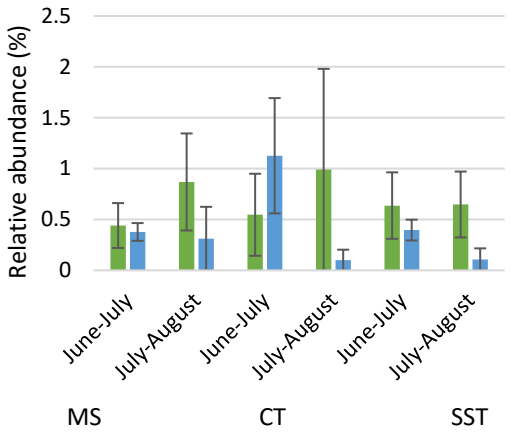

Nitzschia minuta
(u)
Relative abundance (%)
3.5
3
2.5
2
1.5
1
0.5
0
June-July
July-August
June-July
July-August
June-July
July-August
MS
CT
SST


Epithemia turgida
(v)
Relative abundance (%)
3
2.5
2
1.5
1
0.5
0
June-July
July-August
June-July
July-August
June-July
July-August
MS
CT
SST

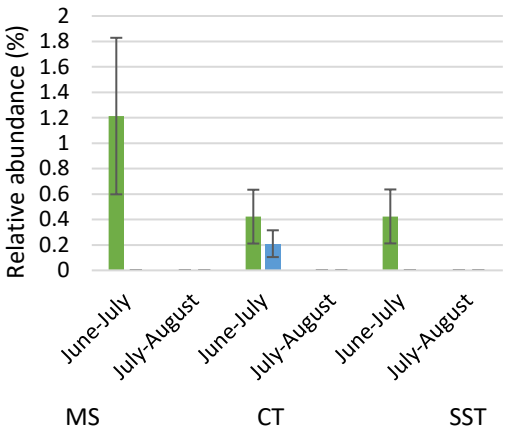

Encyonema reichardtii
(w)
Relative abundance (%)
2.5
2
1.5
1
0.5
0
June-July
July-August
June-July
July-August
June-July
July-August
MS
CT
SST


Gomphonema accuminatum
(x)
Relative abundance (%)
3
2.5
2
1.5
1
0.5
0
June-July
July-August
June-July
July-August
June-July
July-August
MS
CT
SST

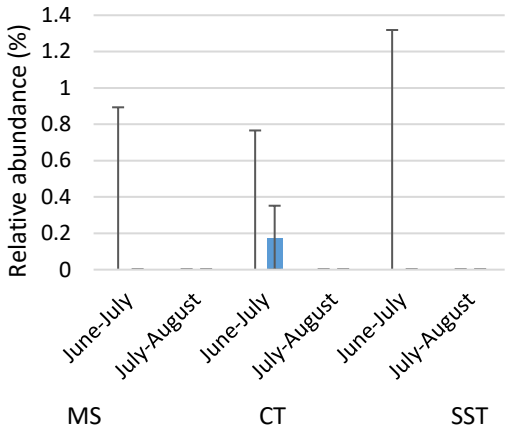
Navicula cryptocephala (y)



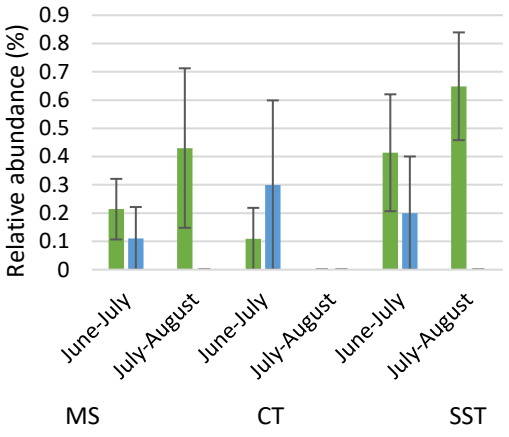
Gomphonema truncatum (z)



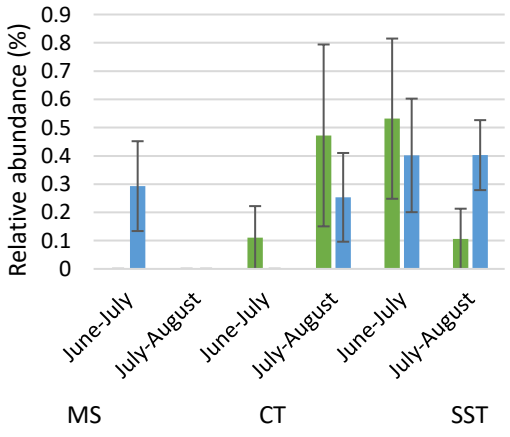
Epithemia adnata (aa)



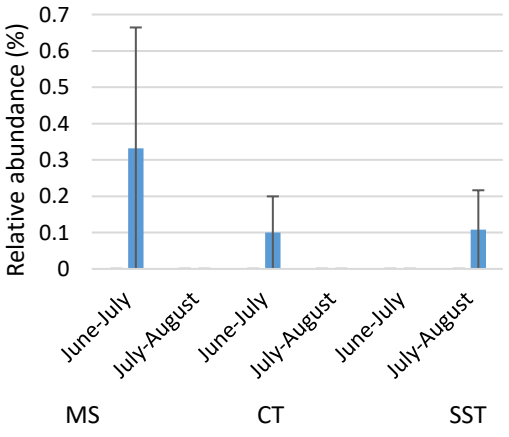
Amphora inariensis (ab)

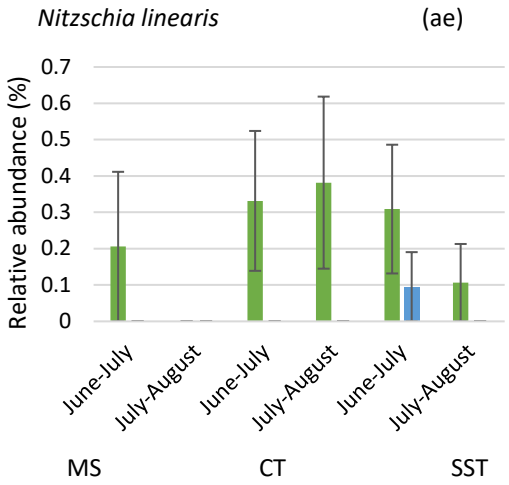


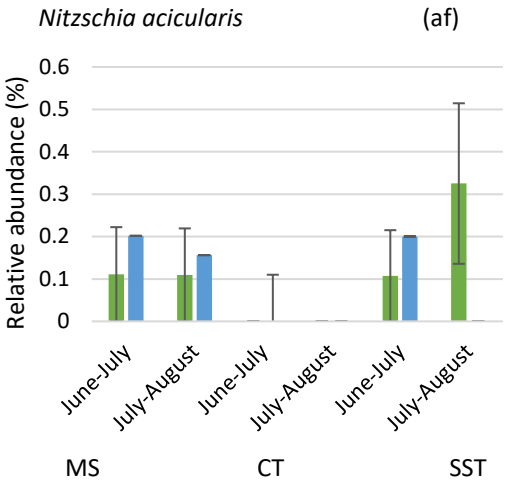
Gomphonema vibrio (ac)

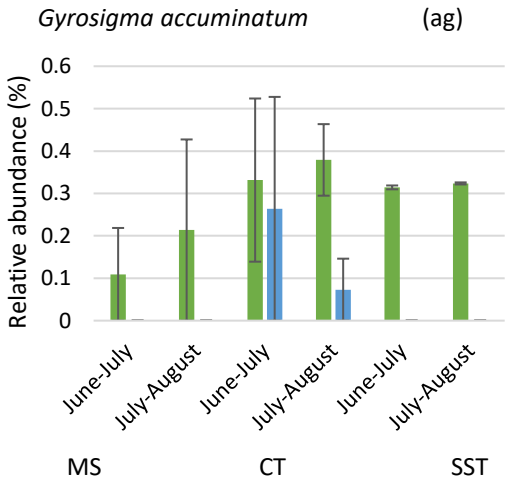


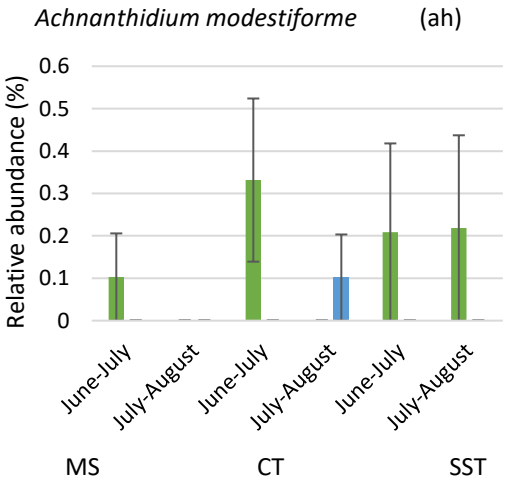
Diatoma problematica (ad)

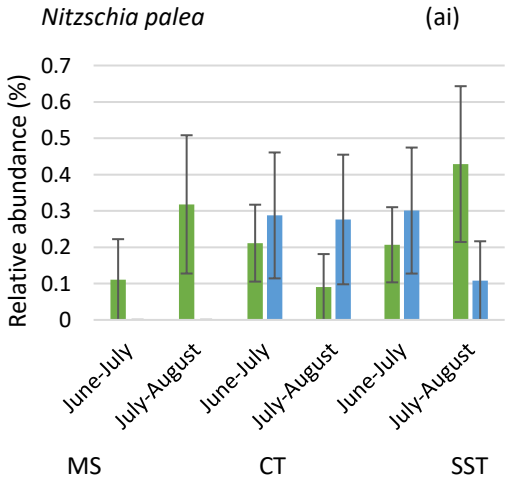


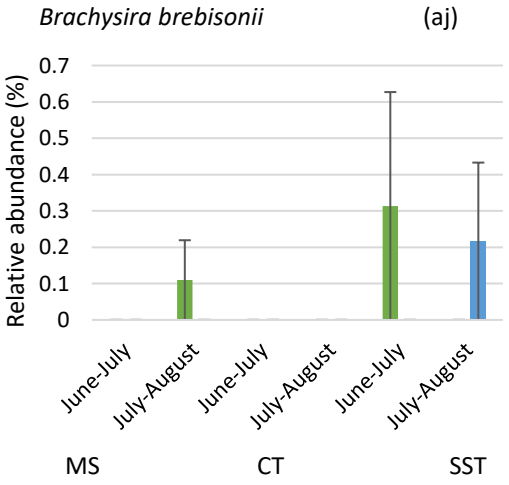

Nitzschia linearis
(ae)
Relative abundance (%)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
June-July
July-August
June-July
July-August
June-July
July-August
MS
CT
SST

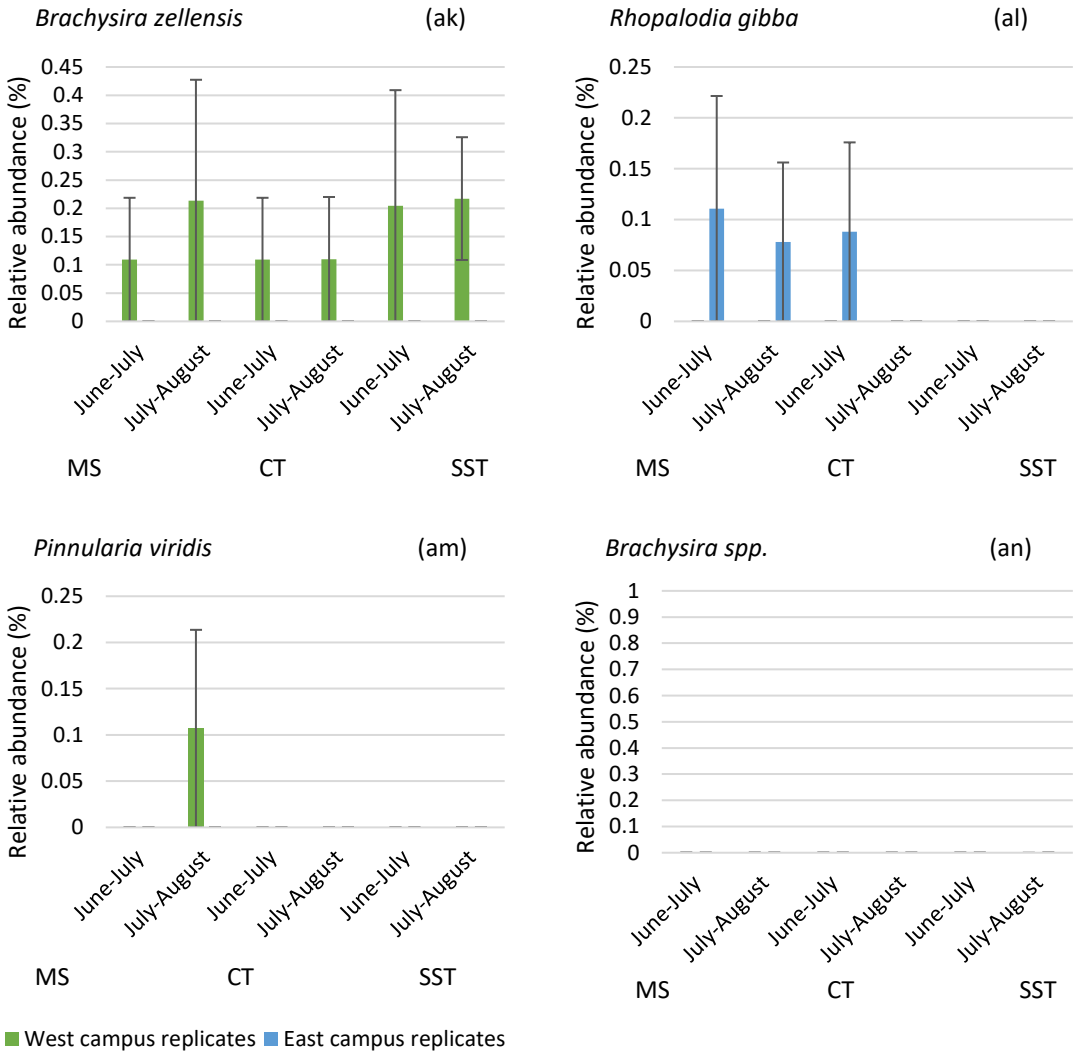

Nitzschia acicularis
(af)
Relative abundance (%)
0.6
0.5
0.4
0.3
0.2
0.1
0
June-July
July-August
June-July
July-August
June-July
July-August
MS
CT
SST


Gyrosigma accuminatum
(ag)
Relative abundance (%)
0.6
0.5
0.4
0.3
0.2
0.1
0
June-July
July-August
June-July
July-August
June-July
July-August
MS
CT
SST


Achnanthidium modestiforme
(ah)
Relative abundance (%)
0.6
0.5
0.4
0.3
0.2
0.1
0
June-July
July-August
June-July
July-August
June-July
July-August
MS
CT
SST


Nitzschia palea
(ai)
Relative abundance (%)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
June-July
July-August
June-July
July-August
June-July
July-August
MS
CT
SST


Brachysira brebisonii
(aj)
Relative abundance (%)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
June-July
July-August
June-July
July-August
June-July
July-August
MS
CT
SST


Brachysira zellensis
(ak)
Relative abundance (%)
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
June-July
July-August
June-July
July-August
June-July
July-August
MS
CT
SST
Rhopalodia gibba
(al)
Relative abundance (%)
0.25
0.2
0.15
0.1
0.05
0
June-July
July-August
June-July
July-August
June-July
July-August
MS
CT
SST
Pinnularia viridis
(am)
Relative abundance (%)
0.25
0.2
0.15
0.1
0.05
0
June-July
July-August
June-July
July-August
June-July
July-August
MS
CT
SST
Brachysira spp.
(an)
Relative abundance (%)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
June-July
July-August
June-July
July-August
June-July
July-August
MS
CT
SST
West campus replicates
East campus replicates

Appendix h. Chapter 4, lab-field growth comparison, diatom taxonomic composition of the replicates. Mean ± SE, N= 3 (field replicates), N=8 (CT room replicates).

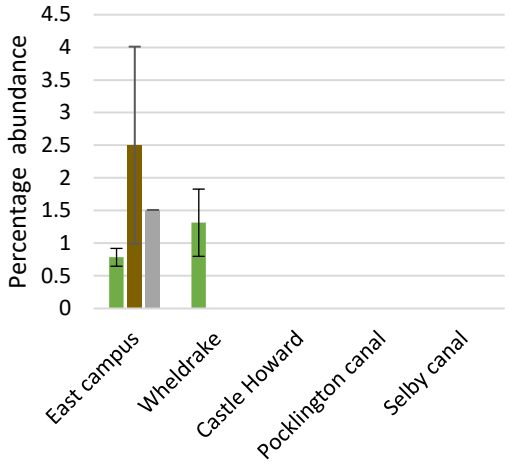
	West campus CT room replicates		West campus Field replicates		East campus CT room replicates		East campus Field replicates	
Species	Mean	Standard error	Mean	Standard error	Mean	Standard error	Mean	Standard error
Achnanthidium daonensis	1.49	0.96	12.55	7.47	0.39	0.24	0.00	0.00
Achnanthidium minutissimum	0.86	0.55	11.82	6.68	27.47	2.74	75.05	3.75
Amphora pediculus	0.00	0.00	0.73	0.34	6.87	0.76	1.33	0.55
Brachsira brebisonii	0.10	0.09	0.13	0.10	1.76	0.60	0.00	0.00
Brachysira vitrea	0.99	0.49	0.51	0.42	22.12	1.44	9.04	0.71
Cocconeis disculus	0.25	0.16	4.14	1.43	1.55	0.25	0.75	0.31
Encyonema gracile	0.00	0.00	0.36	0.17	1.38	0.24	0.43	0.18
Encyonema prostratum	0.00	0.00	1.21	0.38	0.04	0.04	0.00	0.00
Encyonema reichardtii	0.54	0.41	0.00	0.00	3.69	0.77	0.00	0.00
Epithema sorex	0.83	0.36	0.97	0.51	0.04	0.04	0.00	0.00
Epithemia turgida	3.17	1.70	2.23	0.94	0.11	0.11	0.08	0.06
Fragilaria species	6.09	1.99	0.00	0.00	0.32	0.30	0.00	0.00
Fragilaria vaucheriae	7.53	3.05	1.33	0.68	0.82	0.42	0.00	0.00
Gomphonema accuminatum	0.10	0.09	1.93	0.98	0.00	0.00	0.29	0.23
Gomphonema cuneolus	0.23	0.21	2.67	1.36	2.42	0.36	4.54	1.19
Gomphonema lateripunctum	0.00	0.00	0.00	0.00	0.08	0.05	0.74	0.33
Gomphonema olivaceum	0.46	0.30	2.47	0.58	0.28	0.11	0.46	0.29
Gomphonema parvulum	0.44	0.21	3.76	0.97	0.04	0.04	0.19	0.16
Guinardia striata	17.18	3.78	0.51	0.42	0.12	0.08	0.00	0.00
Gyrosigma accuminatum	1.20	0.41	0.24	0.19	0.12	0.06	0.00	0.00
Hippodonta capitata	0.40	0.19	0.13	0.10	0.00	0.00	0.00	0.00
Melosira varians	6.22	1.49	1.72	0.24	0.04	0.04	0.00	0.00
Navicula capitatoradiata	0.00	0.00	0.00	0.00	0.04	0.04	0.00	0.00
Navicula crpytocephala	0.00	0.00	1.00	0.45	0.60	0.16	0.31	0.25
Navicula rhynchotella	0.39	0.27	0.37	0.17	0.00	0.00	0.00	0.00
Nitzschia acicularis	19.02	3.90	0.13	0.10	0.16	0.08	0.00	0.00
Nitzschia amphibia	0.49	0.31	2.66	0.98	0.70	0.40	0.16	0.13
Nitzschia dissipata	0.10	0.10	2.37	1.08	4.07	0.97	0.00	0.00
Nitzscshia linearis	7.30	2.32	0.00	0.00	0.08	0.05	0.00	0.00
Nitzschia minuta	0.00	0.00	1.33	0.79	2.84	0.96	0.00	0.00
Nitzschia paleacea	22.08	3.73	33.86	16.35	17.19	5.49	0.35	0.08
Rhoicosphenia abbreviata	0.58	0.23	1.93	0.98	0.04	0.04	0.10	0.08
Rhopaladia gibba	0.00	0.00	0.00	0.00	2.25	0.59	0.08	0.06
Synedra ulna	0.54	0.29	4.16	1.86	0.00	0.00	0.08	0.06

Appendix i. Chapter 5, Yorkshire water bodies experiment. Relative abundance of all species observed in the field sites split by substratum and site. Mean ± SE (where available).

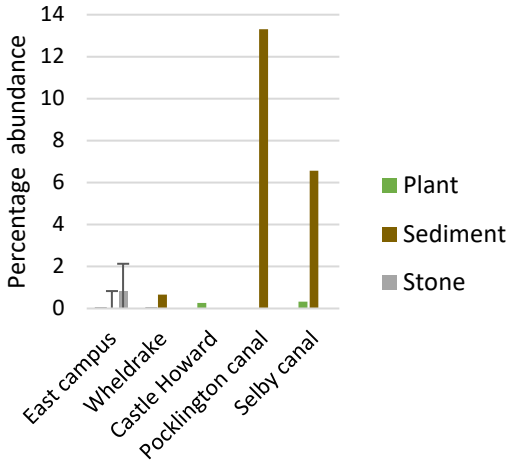
		Plant		Sediment		Stone		Plant		Sediment	Plant	Sediment	Plant	Sediment	Plant	Sediment
	Graph	East campus		East campus		East campus		Wheldrake		Wheldrake	Castle Howard	Castle Howard	Pocklington canal	Pocklington canal	Selby canal	Selby canal
Species	letter	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	Mean	Mean	Mean	Mean	Mean	Mean
Achnanthidium minutissimum	*b*	39.31	21.21	20.89	17.90	3.18	0.69	48.37	9.58	1.97	3.23	1.31	0.00	10.71	5.13	1.88
Achnanthidium daonensis	*ab*	0.00	0.00	0.00	0.50	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.64	2.19
Amphora modestiforme	*y*	0.00	0.00	0.00	0.17	0.17	1.03	0.00	0.00	0.33	3.23	1.64	0.00	1.30	0.32	2.81
Amphora pediculus	*f*	4.03	3.41	1.47	0.65	0.65	0.20	10.82	0.18	0.66	0.54	0.00	0.00	1.30	0.00	0.00
Amphora inariensis	*v*	0.00	0.00	0.00	1.33	1.33	2.51	0.00	0.00	2.62	0.27	0.00	0.00	0.00	0.00	0.00
Anomoeoneis sphaerophora	*aj*	0.00	0.00	0.17	0.00	0.00	0.00	0.66	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Brachysira brebisonii	*ad*	0.00	0.00	0.00	1.33	1.33	0.22	0.00	0.00	0.66	0.00	0.00	0.00	0.00	0.00	0.31
Brachysira vitrea	*k*	6.26	4.42	3.38	1.39	2.06	1.75	6.00	0.07	5.25	0.00	0.00	0.00	0.00	0.96	2.50
Cocconeis disculus	*a*	33.32	31.71	11.31	0.45	0.88	8.98	4.44	10.88	5.57	29.30	7.21	0.00	27.27	56.41	36.88
Cocconeis pediculus	*ai*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.62	0.00	0.31
Cyclotella meneghiana	-----	0.00	0.00	0.00	0.17	0.17	4.30	0.00	0.00	0.00	12.90	0.66	0.00	1.30	1.60	0.31
Diatoma problematica	*i*	0.78	0.14	2.50	1.51	1.51	0.00	1.31	0.52	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Diatoma vulgaris	-----	0.00	0.00	0.00	0.00	0.00	1.45	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Encyonema brevicapitatum	*an*	0.00	0.00	0.00	0.00	0.00	0.22	0.50	0.00	0.66	0.00	0.00	0.00	0.32	0.00	0.00
Cymbella neocistula	*ar*	0.00	0.00	0.00	0.83	0.83	0.22	0.00	0.00	0.66	0.00	0.00	0.00	0.00	0.00	0.00
Encyonema minuta	*aw*	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Encyonema neogracile	*u*	0.00	0.00	1.97	0.65	0.65	0.23	1.16	0.68	0.33	0.81	0.00	0.00	0.65	0.00	0.00
Encyonema prostratum	-----	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Encyonema reichardtii	*w*	0.00	0.00	0.22	0.22	0.22	0.00	0.00	0.22	0.00	0.00	0.00	0.00	3.57	0.00	0.00
Epithemia sorex	*av*	0.16	0.16	0.43	0.43	0.43	0.00	0.16	0.43	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Epithemia turgida	*am*	0.15	0.15	0.43	0.43	0.43	0.18	0.17	0.43	0.00	0.54	0.33	0.00	0.00	0.00	0.00
Eunotia	*q*	0.00	0.00	1.24	1.08	1.08	0.00	0.00	0.91	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Eunotia bidenta	*as*	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fragilaria species	*d*	0.00	0.00	0.00	1.33	1.33	2.03	1.82	0.00	2.95	6.99	40.33	0.00	7.14	0.00	0.00
Fragilaria vaucheriae	*ag*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.92	0.31
Frustulia rhomboides	*ax*	0.00	0.00	0.00	0.33	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.32	0.00	0.00
Gomphonema accumintatum	*au*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.32	0.64	0.00
Gomphonema cuneolus	*c*	9.85	2.73	13.77	11.79	12.78	0.37	24.35	10.79	7.54	8.60	1.64	0.00	6.49	15.06	21.56
Gomphonema exilissium	*aq*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.96	0.00
Gomphonema parvulum	*m*	0.00	0.00	0.00	1.50	1.50	1.44	0.00	0.00	9.18	5.65	3.28	0.00	3.57	0.96	1.25
Gomphonema truncatum	*ae*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.65	0.00	0.63
Gomphonema vibrio	*n*	0.96	0.66	10.56	10.56	10.56	0.18	0.81	10.56	0.00	0.54	0.00	0.00	0.00	7.37	0.31
Gyrosigma accuminatum	-----	0.00	0.00	0.00	0.17	0.17	0.19	0.17	0.00	0.66	0.27	0.00	0.00	1.95	0.64	0.63
Melosira varians	*e*	1.29	1.29	2.91	32.29	33.16	7.34	1.62	2.05	20.00	15.05	15.74	0.00	0.32	4.17	1.25
Navicula lanceolota	*o*	0.15	0.15	0.17	0.50	0.50	2.69	1.16	0.17	8.52	0.54	0.33	0.00	2.60	0.00	0.00
Navicula radiosa	*af*	0.00	0.00	0.00	0.00	0.00	0.16	0.00	0.00	0.33	0.54	0.00	0.00	0.65	0.00	0.00
Navicula rhynocotella	*g*	0.32	0.32	0.33	0.17	0.17	1.70	0.32	0.33	0.00	5.11	20.98	0.00	0.97	0.00	0.00
Naviucla slesvicensis	-----	0.00	0.00	0.00	0.00	0.00	2.90	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Nitzschia acicularis	*at*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.64	0.00
Nitzschia amphibia	*ao*	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Nitzschia dissipita	*p*	0.32	0.32	0.22	1.45	1.88	2.73	0.32	0.22	8.20	0.00	0.00	0.00	2.27	0.00	0.31
Nitzschia linearis	*t*	0.32	0.32	0.00	0.66	0.66	1.27	0.32	0.00	3.93	0.27	0.66	0.00	0.97	0.00	1.25
Nitzschia minuta	*j*	0.00	0.00	0.00	0.83	0.83	1.30	0.00	0.00	0.66	0.27	0.00	0.00	13.31	0.32	6.56
Nitzschia palea var debilis	*s*	0.00	0.00	0.00	0.00	0.00	2.63	0.00	0.00	1.97	0.00	2.30	0.00	0.97	0.00	0.00
Nitzschia paleacea	*l*	0.00	0.00	0.17	0.17	0.17	2.56	0.00	0.17	8.85	4.30	0.66	0.00	3.57	0.00	0.00
Rhoicosphenia abbreviata	*h*	0.00	0.00	0.00	0.00	0.00	0.18	0.00	0.00	0.00	0.54	0.00	0.00	4.55	0.32	18.44
Pinnularia viridis	*ak*	0.00	0.00	0.00	0.17	0.17	0.11	0.00	0.00	0.33	0.00	0.00	0.00	1.30	0.32	0.31
Rhopaladia gibba	*r*	2.75	2.75	0.22	0.22	0.22	0.00	3.25	0.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fragilaria (unknown species)	*x*	0.00	0.00	0.00	0.00	0.00	1.05	0.00	0.00	3.28	0.27	0.98	0.00	0.00	0.00	0.00
Gomphonema (unknown species)	*ac*	0.00	0.00	0.00	1.50	1.50	0.33	0.00	0.00	0.98	0.00	0.00	0.00	0.00	0.00	0.00
Delphineis surriela	*ah*	0.00	0.00	0.00	0.33	0.33	0.09	0.00	0.00	0.00	0.27	1.64	0.00	0.00	0.00	0.00
Ulnaria ulna	*al*	0.00	0.00	0.00	0.17	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.28	0.00
Surirella angusta	*ap*	0.00	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.98	0.00	0.00	0.00	0.00	0.00	0.00
Surirella roba	*ay*	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00
Surrirela brebisonii	*aa*	0.00	0.00	0.00	0.50	0.50	0.87	0.00	0.00	2.62	0.00	0.33	0.00	0.00	0.32	0.00

Appendix j. Chapter 5, Yorkshire water bodies experiment diatom species relative abundances between the five sites and across the three substratum types used.

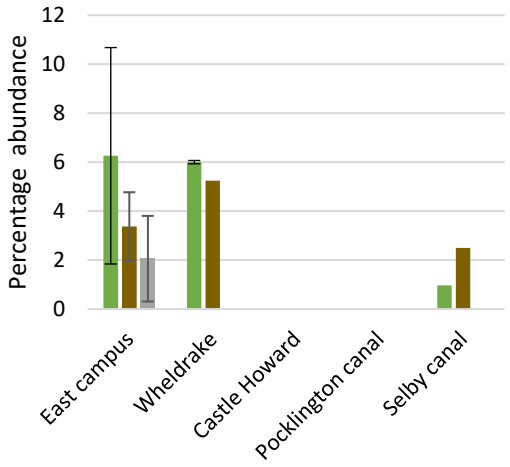
Diatoma problematica (i)



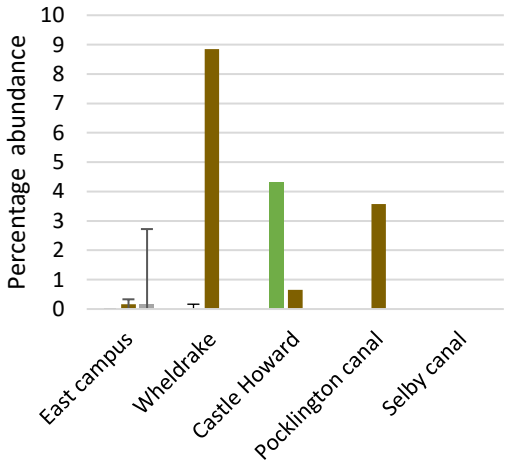
Nitzschia minuta (j)



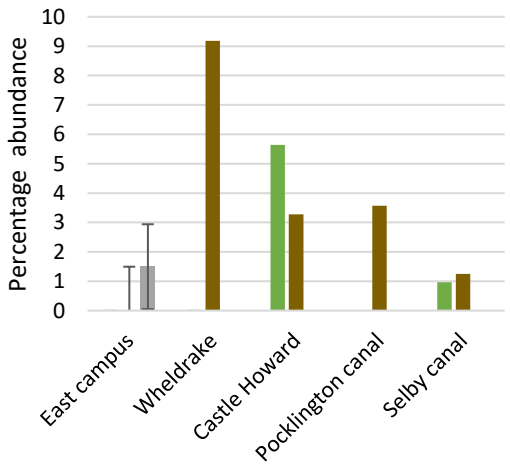
Brachysira vitrea (k)



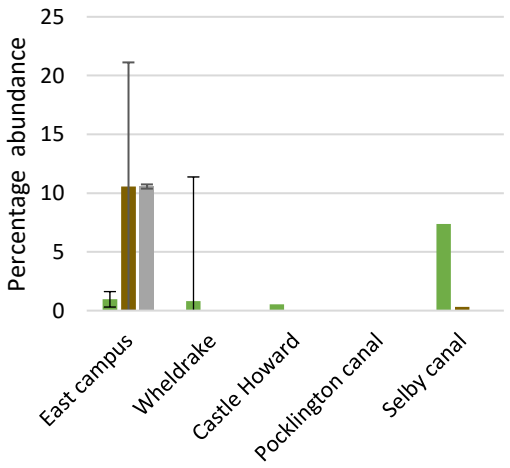
Nitzschia paleacea (l)



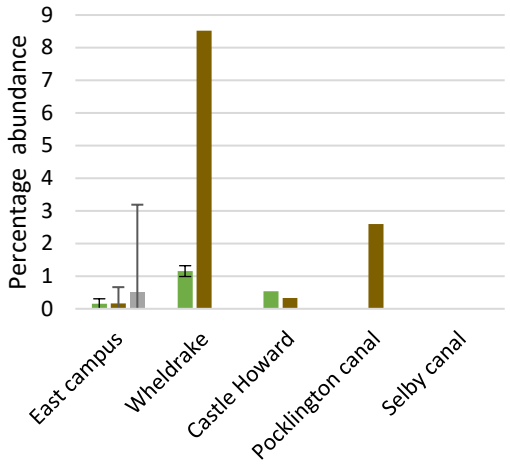
Gomphonema parvulum (m)



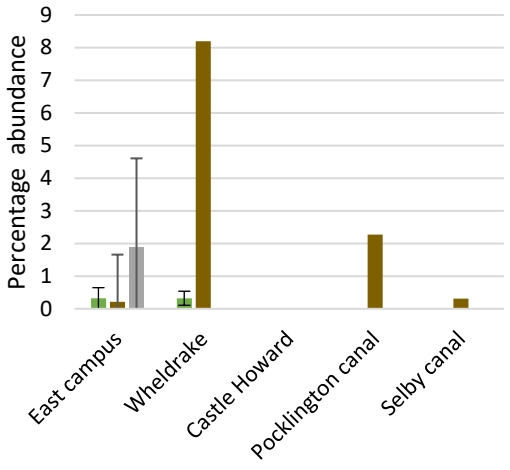
Gomphonema vibrio (n)



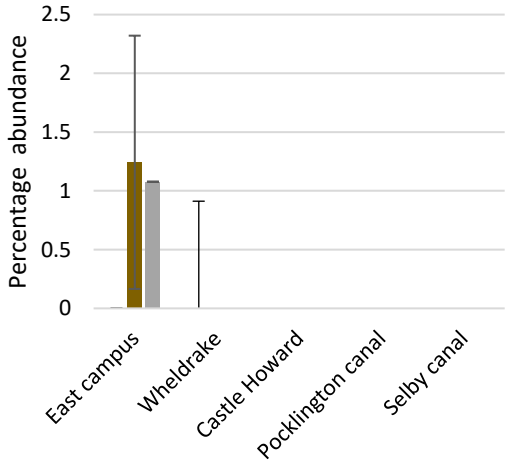
Navicula lanceolota (o)



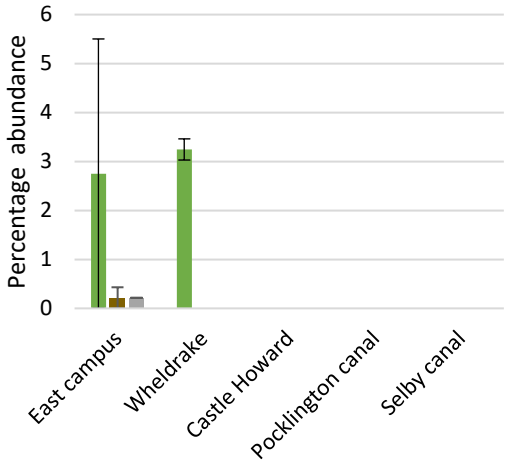
Nitzschia dissipita (p)



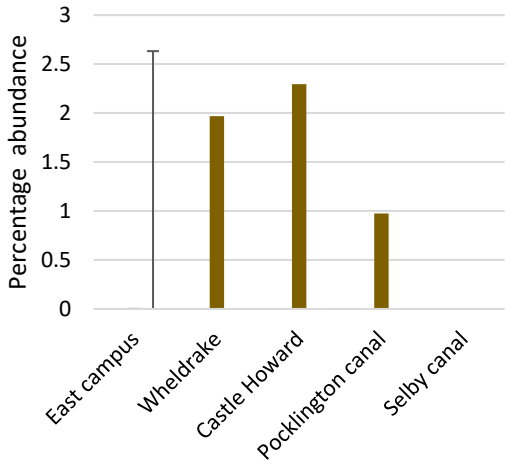
Eunotia spp. (q)



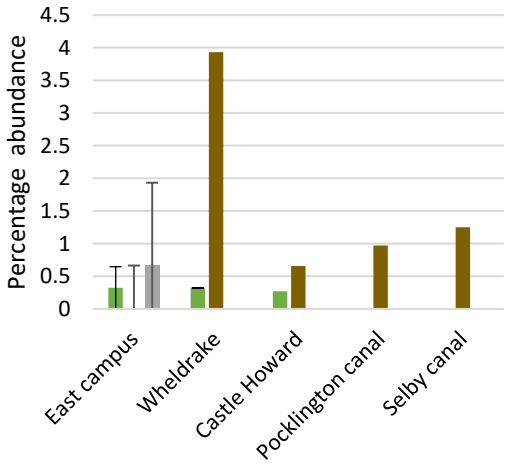
Rhopalodia gibba (r)



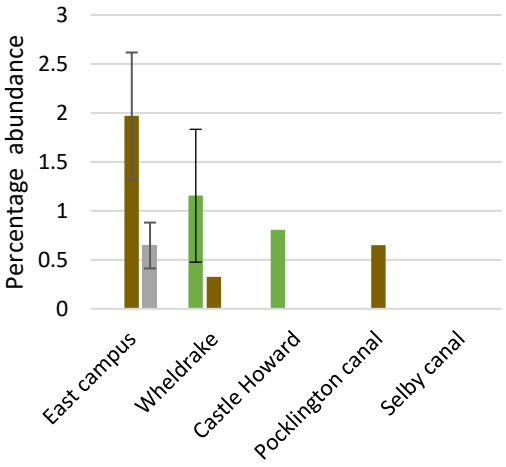
Nitzschia palea var debilis (s)



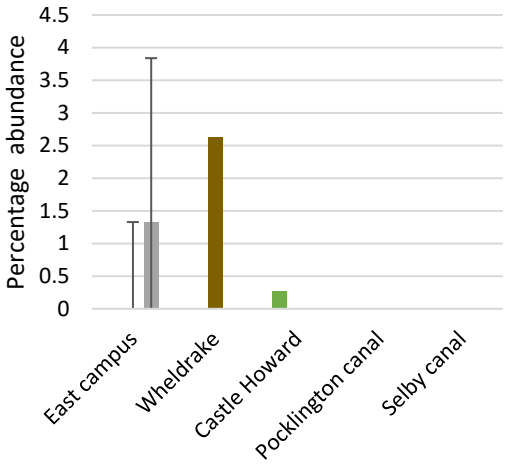
Nitzschia linearis (t)



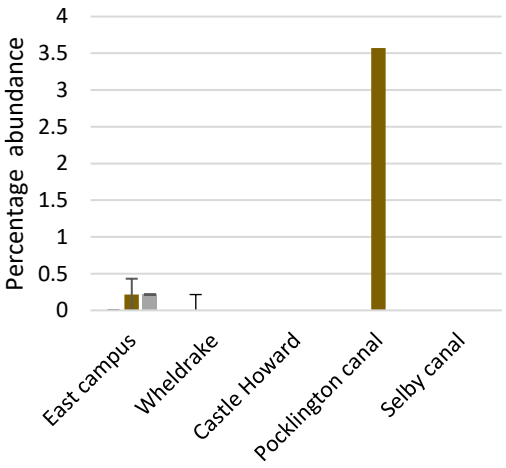
Encyonema neogracile (u)



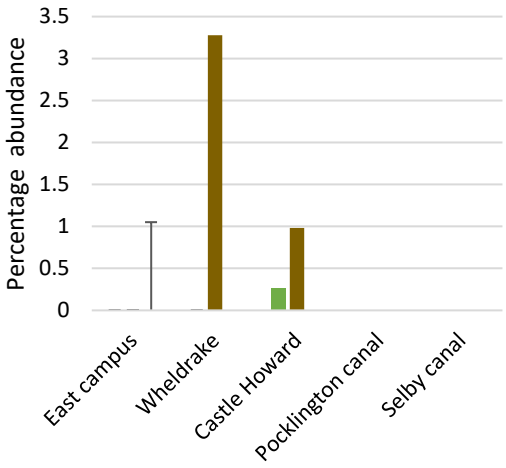
Amphora inariensis (v)



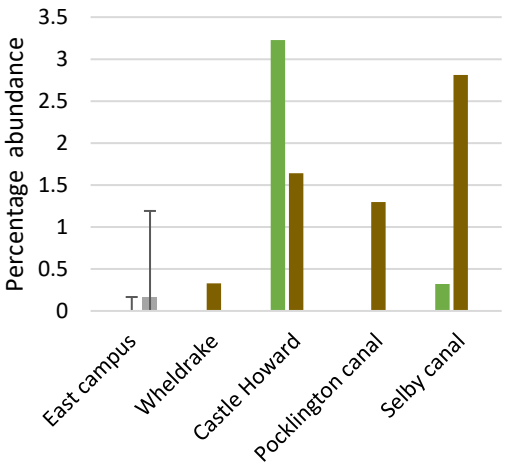
Encyonema reichardtii (w)



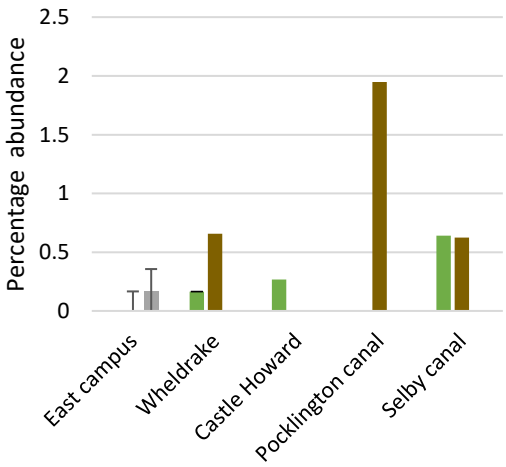
Fragilaria (unknown species) (x)



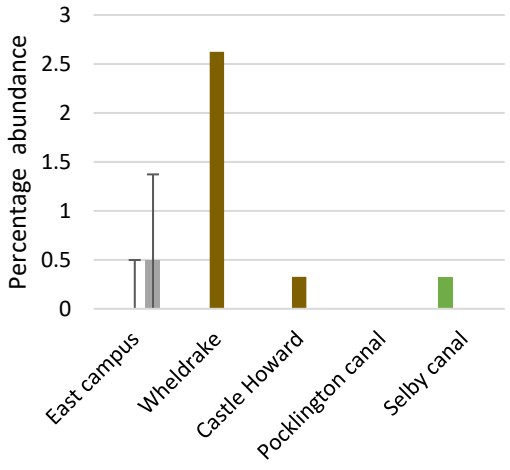
Amphora modestiforme (y)



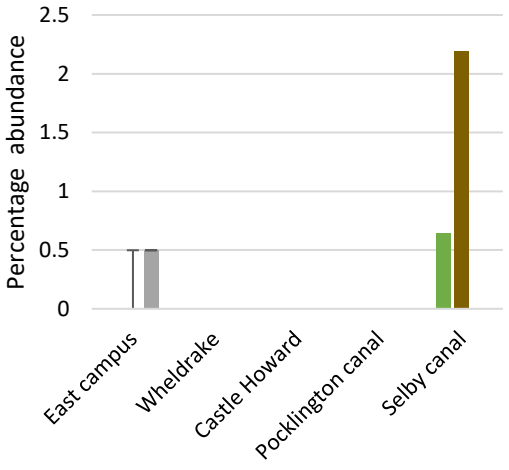
Gyrosigma accuminatum (z)



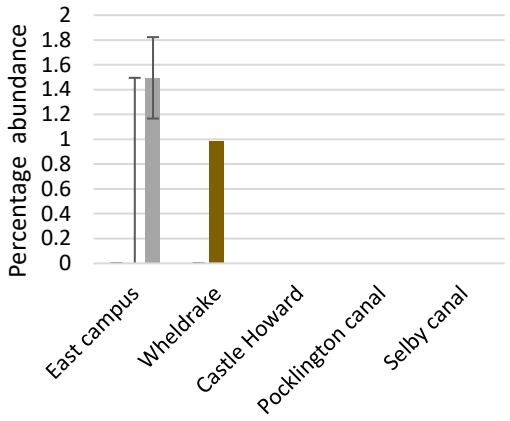
Surrirela brebisonii (aa)



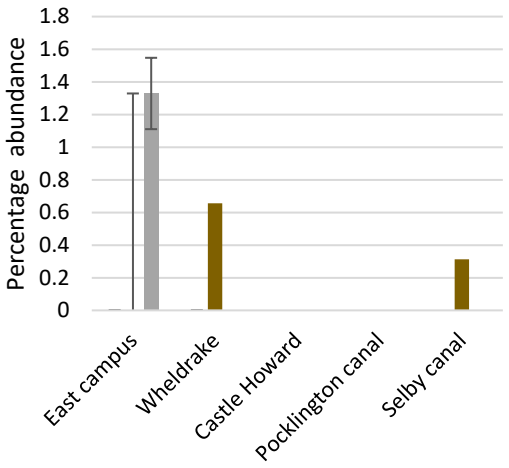
Achnanthes daonense (ab)



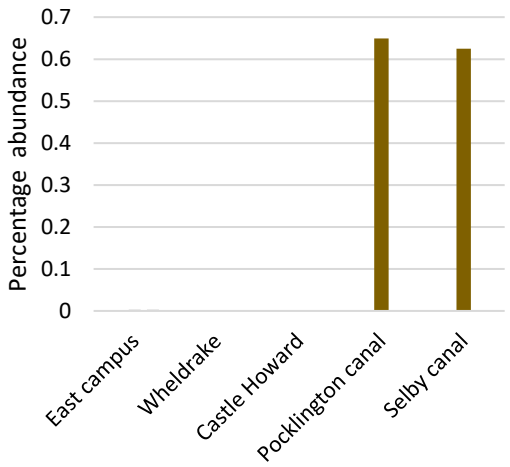
Gomphonema (unknown species) (ac)



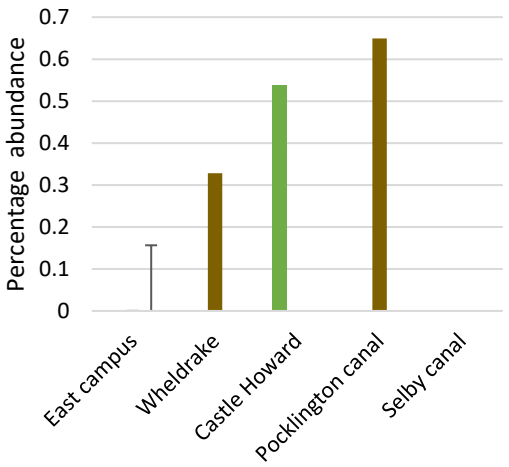
Brachysira brebisonii (ad)



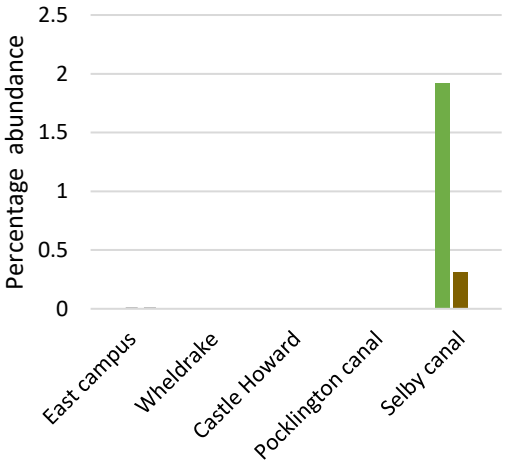
Gomphonema truncatum (ae)



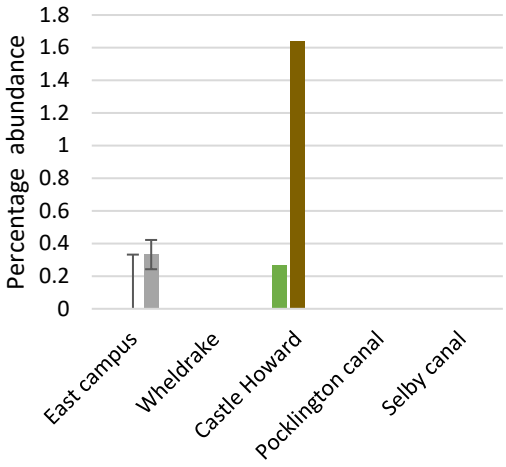
Navicula radiosa (af)



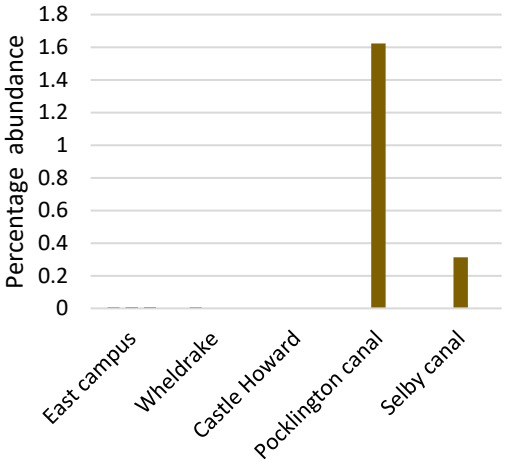
Fragilaria vaucheriae (ag)



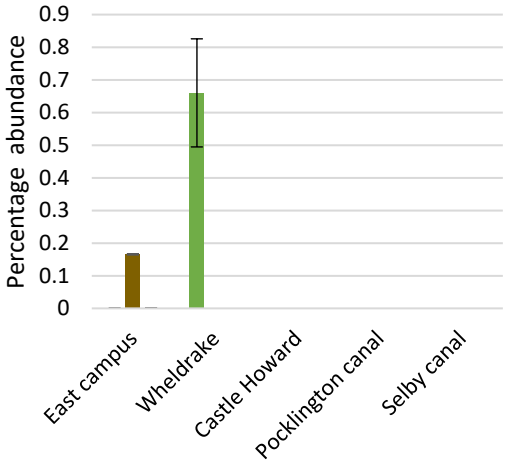
Delphineis surriela (ah)



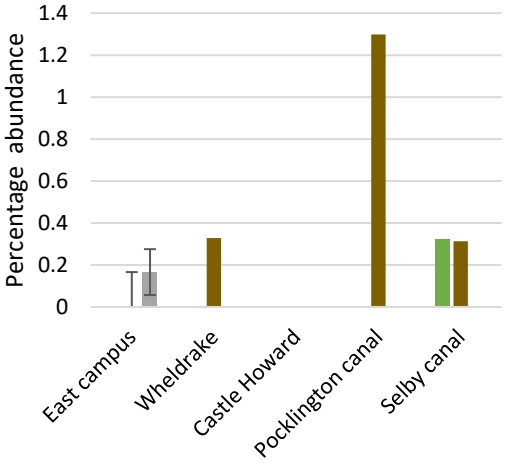
Cocconeis pediculus (ai)



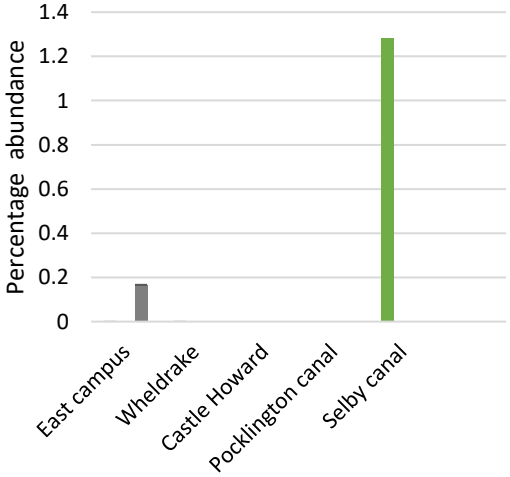
Anomoeoneis sphaerophora (aj)

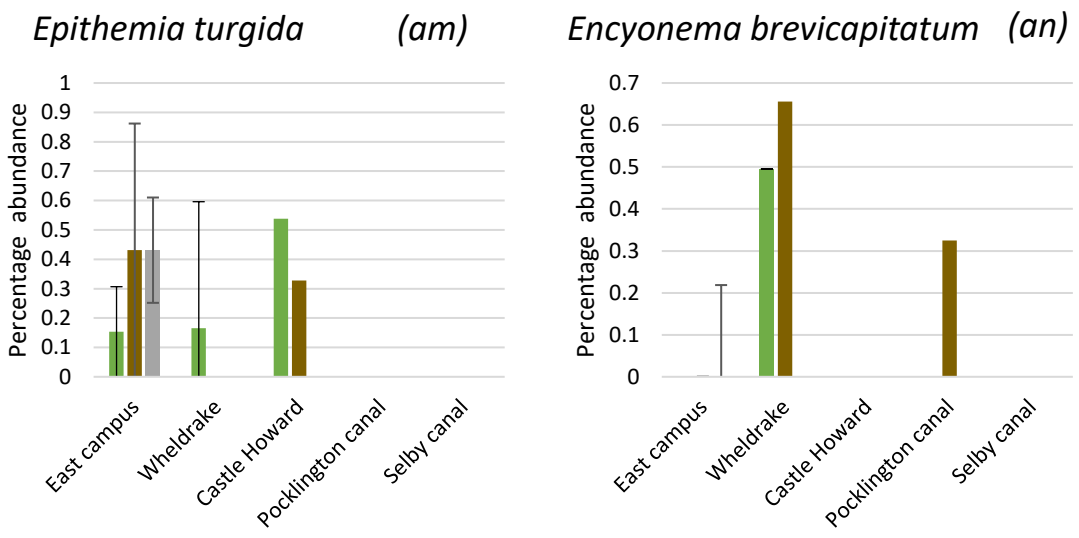


Pinnularia viridis (ak)

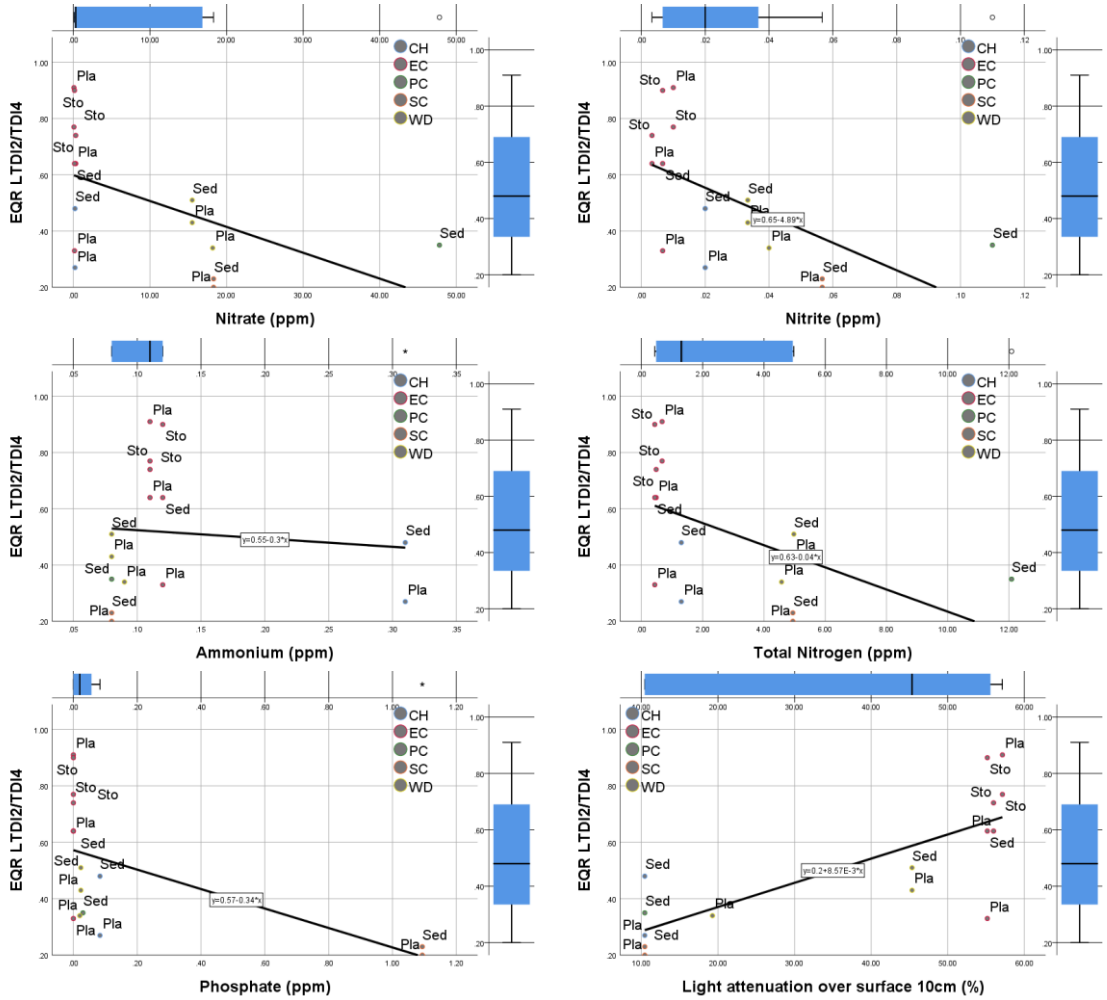


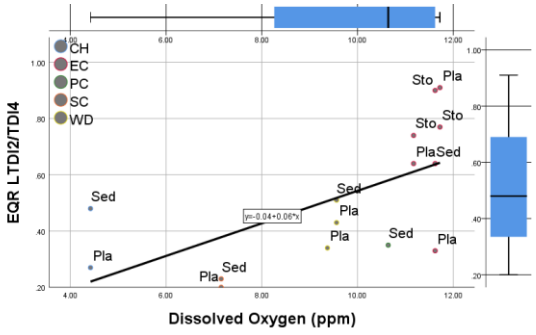
Ulnaria ulna (al)

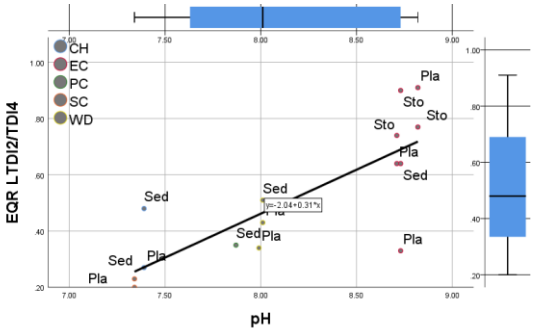


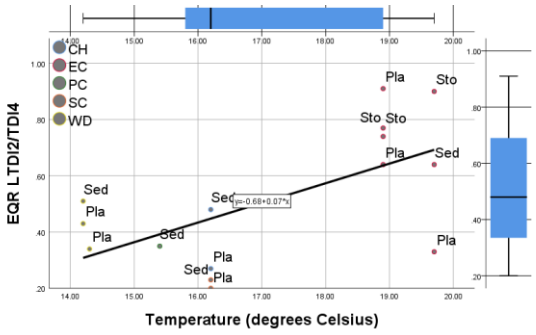


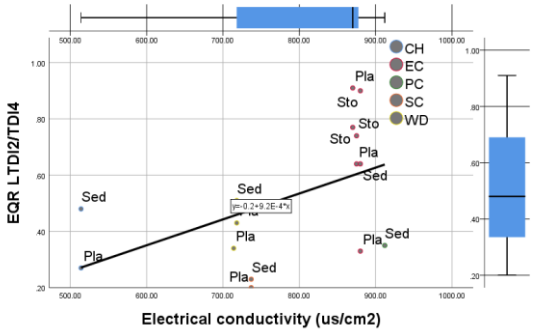
Appendix k. Chapter 5, physico-chemical measurements of the Yorkshire sites plotted against the TDI of the biofilms from these sites.

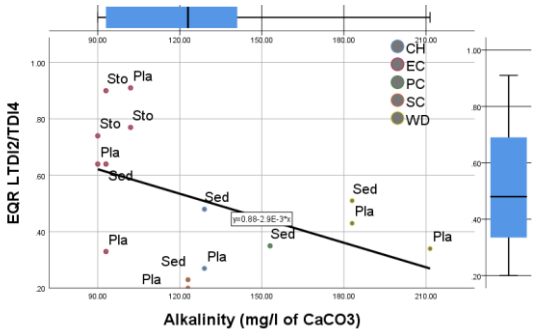


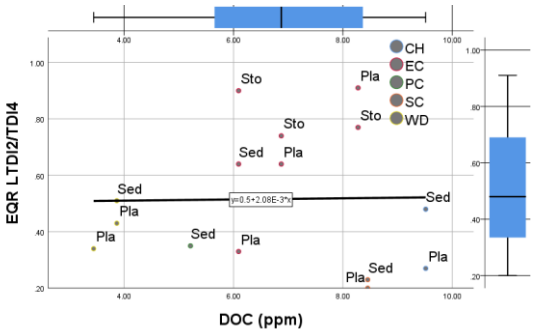

EQR LTDI2/TDI4
Dissolved Oxygen (ppm)
CH
EC
PC
SC
WD

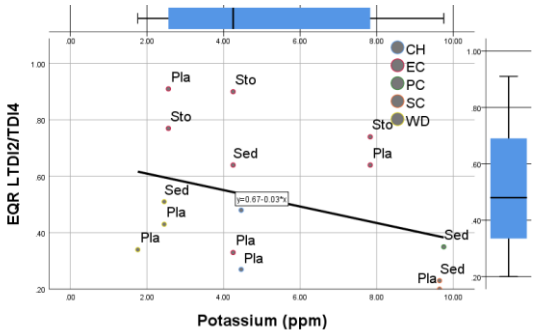

EQR LTDI2/TDI4
pH
CH
EC
PC
SC
WD

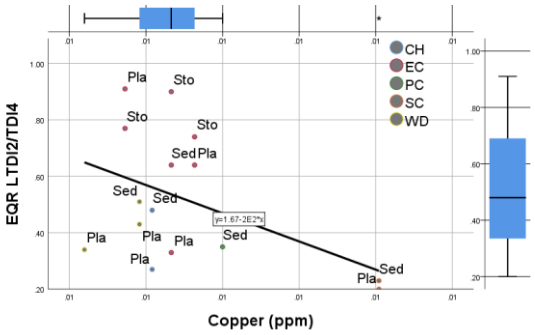

EQR LTDI2/TDI4
Temperature (degrees Celsius)
CH
EC
PC
SC
WD

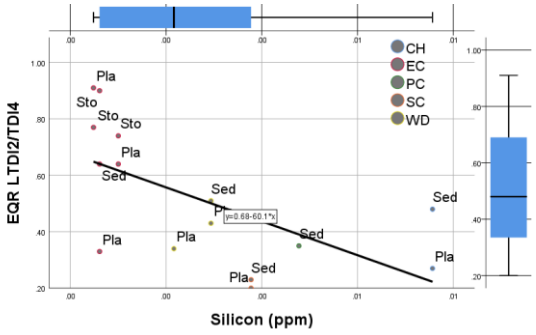

EQR LTDI2/TDI4
Electrical conductivity (us/cm2)
CH
EC
PC
SC
WD

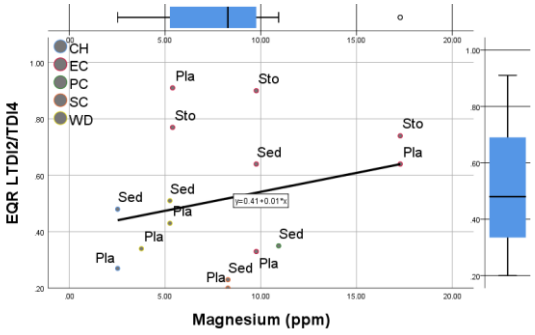

EQR LTDI2/TDI4
Alkalinity (mg/l of CaCO3)
CH
EC
PC
SC
WD

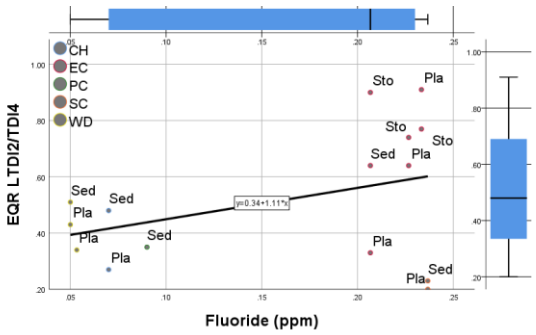

EQR LTDI2/TDI4
DOC (ppm)
CH
EC
PC
SC
WD

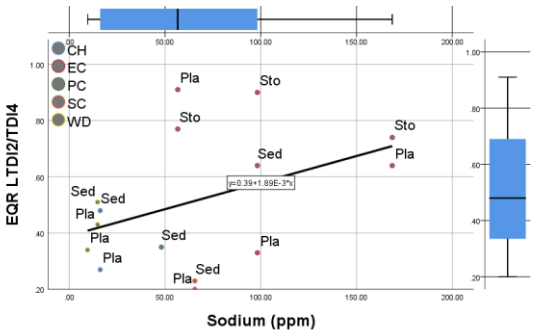

EQR LTDI2/TDI4
Potassium (ppm)
CH
EC
PC
SC
WD

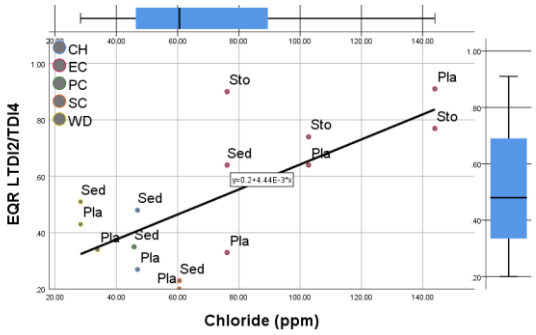

EQR LTDI2/TDI4
Copper (ppm)
CH
EC
PC
SC
WD

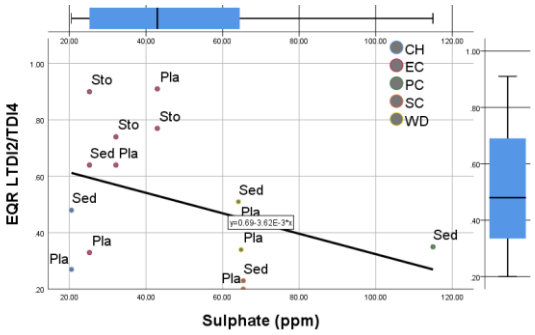

EQR LTDI2/TDI4
Silicon (ppm)
CH
EC
PC
SC
WD

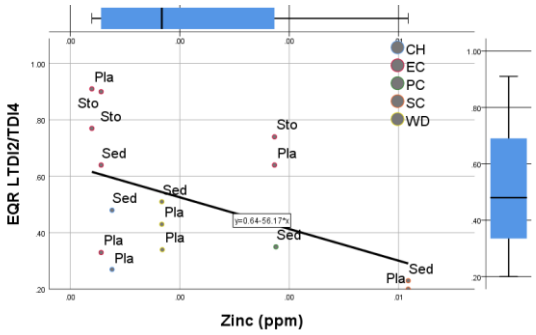

EQR LTDI2/TDI4
Magnesium (ppm)
CH
EC
PC
SC
WD

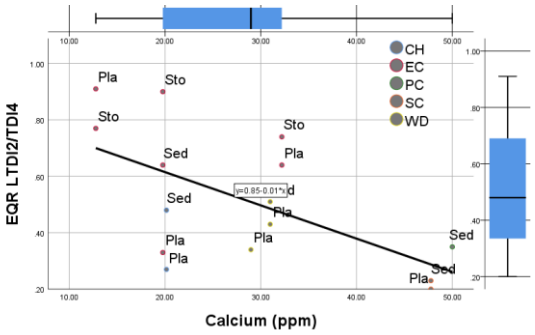

EQR LTDI2/TDI4
Fluoride (ppm)
CH
EC
PC
SC
WD

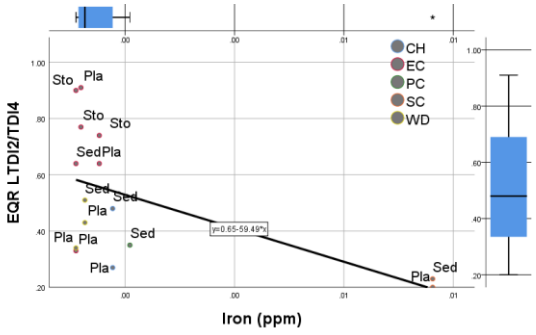

EQR LTDI2/TDI4
Sodium (ppm)
CH
EC
PC
SC
WD

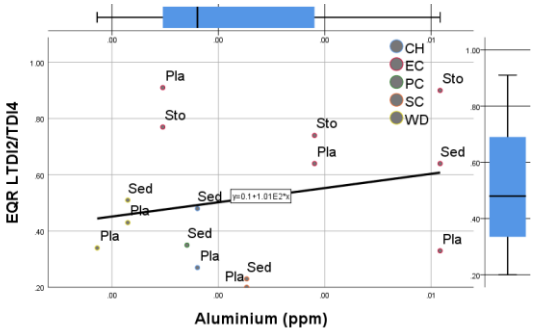

EQR LTDI2/TDI4
Chloride (ppm)
CH
EC
PC
SC
WD

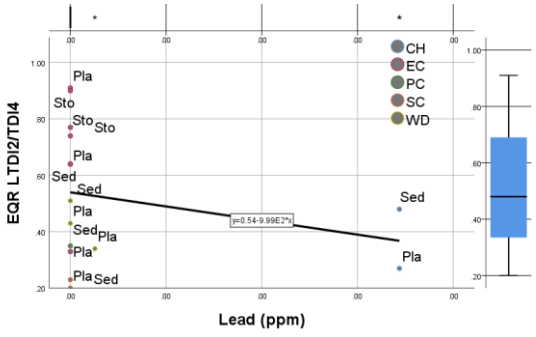

EQR LTDI2/TDI4
Sulphate (ppm)
CH
EC
PC
SC
WD

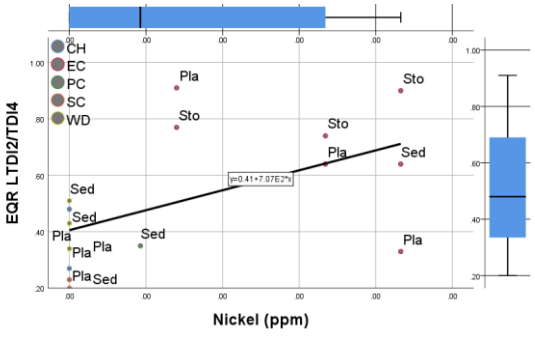

EQR LTDI2/TDI4
Zinc (ppm)
CH
EC
PC
SC
WD


EQR LTDI2/TDI4
Calcium (ppm)
CH
EC
PC
SC
WD

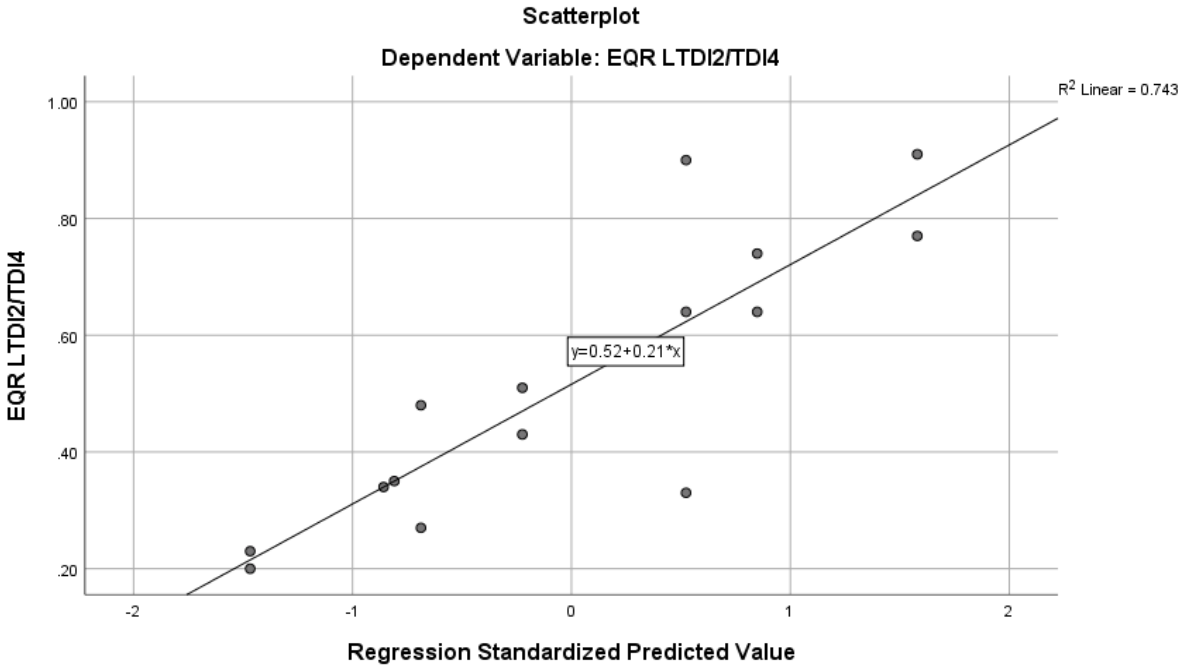

EQR LTDI2/TDI4
Iron (ppm)
CH
EC
PC
SC
WD


EQR LTDI2/TDI4
Aluminium (ppm)
CH
EC
PC
SC
WD


EQR LTDI2/TDI4
Lead (ppm)
CH
EC
PC
SC
WD


EQR LTDI2/TDI4
Nickel (ppm)
CH
EC
PC
SC
WD

Appendix l. Chapter 5, scatterplot showing TDI (EQR LTDI2/ TDI4) values versus physico-chemical measurements model



Appendix m. Chapter 5, MANOVA analysis for the diatom species present in the Yorkshire water bodies against the physicochemical measurements of the site

	Achnanthidium minutissimum			*Achnanthes daonensis*			*Achnanthidium modestiforme*			*Amphora pediculus*		
	F	df	P	F	df	P	F	df	P	F	df	P
Nitrate	1.366	10	0.444	3.477	3	0.058	5.038	4	**0.021**	1.538	6	0.292
Nitrite	0.623	10	0.751	6.806	3	**0.009**	5.387	4	**0.017**	2.873	6	0.097
TN	1.401	10	0.434	3.426	3	0.060	6.697	4	**0.009**	1.905	6	0.209
Ammonium	29.060	10	**0.009**	0.478	3	0.705	253.981	4	**<0.001**	0.107	6	0.993
Phosphate	0.210	10	0.974	608.817	3	**<0.001**	3.607	4	0.051	0.574	6	0.742
Light attenuation	0.772	10	0.671	1.623	3	0.246	4.238	4	**0.034**	2.154	6	0.169
DO	1.830	10	0.338	0.522	3	0.677	22.656	4	**<0.001**	1.209	6	0.400
pH	0.970	10	0.580	1.884	3	0.196	11.596	4	**0.001**	3.026	6	0.087
Temperature	1.937	10	0.320	1.057	3	0.410	3.171	4	0.069	2.300	6	0.150
EC	6.047	10	0.083	0.096	3	0.960	19.059	4	**<0.001**	1.305	6	0.364
Alkalinity	2.477	10	0.246	0.718	3	0.564	0.983	4	0.463	1.075	6	0.456
DOC	0.929	10	0.597	1.158	3	0.373	1.778	4	0.217	0.074	6	0.997
Silicon	3.983	10	0.141	0.170	3	0.914	66.368	4	**<0.001**	0.939	6	0.522
Magnesium	14.912	10	**0.024**	0.090	3	0.964	1.104	4	0.412	4.118	6	0.043
Potassium	0.418	10	0.871	4.543	3	**0.030**	0.776	4	0.568	0.637	6	0.701
Copper	0.295	10	0.938	27.078	3	**<0.001**	2.328	4	0.135	0.261	6	0.939
Fluoride	1.201	10	0.494	1.195	3	0.361	1.896	4	0.195	0.782	6	0.610
Sodium	5.910	10	0.085	0.299	3	0.825	1.467	4	0.290	3.887	6	0.049
Chloride	8.617	10	0.051	0.577	3	0.643	1.870	4	0.200	3.949	6	**0.048**
Sulphate	1.393	10	0.436	2.565	3	0.113	5.603	4	**0.015**	0.678	6	0.674
Zinc	0.344	10	0.913	8.287	3	**0.005**	2.124	4	0.160	1.071	6	0.458
Calcium	0.605	10	0.761	6.888	3	**0.009**	3.499	4	0.055	1.495	6	0.304
Iron	0.197	10	0.978	313.844	3	**<0.001**	3.432	4	0.058	0.528	6	0.773
Aluminium	1.920	10	0.323	0.323	3	0.809	0.661	4	0.635	0.839	6	0.577
Nickel	15.671	10	**0.022**	0.074	3	0.973	40.132	4	**<0.001**	0.536	6	0.768
Lead	1.815	10	0.341	0.621	3	0.617	1.663	4	0.241	0.948	6	0.518

	Amphora inariensis			*Brachysira brebisonii*			*Brachysira vitrea*			*Cocconeis disculus*		
	F	df	P	F	df	P	F	df	P	F	df	P
Nitrate	1.311	3	0.324	2.512	3	0.118	0.388	9	0.890	1.290	11	0.516
Nitrite	0.238	3	0.868	2.028	3	0.174	0.772	9	0.658	1.398	11	0.490
TN	1.741	3	0.222	3.483	3	0.058	0.520	9	0.810	0.818	11	0.668
Ammonium	3.343	3	0.064	0.478	3	0.705	0.594	9	0.763	0.588	11	0.773
Phosphate	0.297	3	0.827	2.913	3	0.087	1.026	9	0.533	96.340	11	**0.010**
Light attenuation	0.642	3	0.606	0.628	3	0.613	1.702	9	0.320	0.807	11	0.673
DO	1.451	3	0.286	0.221	3	0.880	0.964	9	0.561	0.430	11	0.856
pH	0.672	3	0.588	0.758	3	0.543	0.960	9	0.562	0.620	11	0.757
Temperature	3.231	3	0.069	2.386	3	0.130	0.827	9	0.629	0.460	11	0.840
EC	2.019	3	0.175	0.130	3	0.940	1.567	9	0.352	0.323	11	0.914
Alkalinity	4.149	3	0.038	2.091	3	0.165	0.607	9	0.755	0.580	11	0.777
DOC	6.110	3	**0.012**	2.244	3	0.146	0.451	9	0.852	0.774	11	0.687
Silicon	2.001	3	0.178	0.092	3	0.963	0.788	9	0.649	0.352	11	0.899
Magnesium	0.873	3	0.487	0.200	3	0.894	7.892	9	**0.031**	0.350	11	0.900
Potassium	0.632	3	0.611	1.850	3	0.202	0.916	9	0.583	1.030	11	0.591
Copper	0.544	3	0.663	2.527	3	0.117	0.792	9	0.647	10.441	11	0.091
Fluoride	4.651	3	**0.028**	2.024	3	0.175	2.743	9	0.172	0.470	11	0.835
Sodium	1.308	3	0.325	0.715	3	0.565	4.279	9	0.088	0.269	11	0.941
Chloride	1.764	3	0.217	1.246	3	0.344	1.345	9	0.414	0.903	11	0.636
Sulphate	1.882	3	0.196	2.402	3	0.128	0.430	9	0.865	1.403	11	0.488
Zinc	0.183	3	0.906	1.631	3	0.244	0.935	9	0.574	4.537	11	0.194
Calcium	0.259	3	0.854	1.726	3	0.225	0.538	9	0.798	357.886	11	**0.003**
Iron	0.300	3	0.825	2.920	3	0.087	1.010	9	0.540	47.828	11	**0.021**
Aluminium	1.330	3	0.319	0.781	3	0.531	0.992	9	0.548	0.558	11	0.788
Nickel	2.646	3	0.106	0.064	3	0.978	1.167	9	0.475	0.323	11	0.914
Lead	1.053	3	0.412	0.328	3	0.806	30.437	9	**0.002**	1.686	11	0.431

	Diatoma problematica			*Diatoma vulgaris*			*Encyonema gracile*			*Encyonema prostratum*		
	F	df	P	F	df	P	F	df	P	F	df	P
Nitrate	1.435	6	0.322	2.352	1	0.151	1.244	5	0.372	0.536	1	0.478
Nitrite	2.755	6	0.105	0.977	1	0.342	1.279	5	0.360	0.677	1	0.427
TN	2.479	6	0.130	1.431	1	0.255	1.590	5	0.266	0.731	1	0.409
Ammonium	0.061	6	0.998	0.280	1	0.606	0.428	5	0.818	0.019	1	0.893
Phosphate	0.311	6	0.912	0.154	1	0.702	0.342	5	0.874	0.197	1	0.665
Light attenuation	2.947	6	0.092	0.924	1	0.355	0.725	5	0.624	0.632	1	0.442
DO	2.258	6	0.155	0.001	1	0.977	0.535	5	0.745	0.705	1	0.418
pH	7.214	6	**0.010**	0.108	1	0.748	0.736	5	0.617	0.784	1	0.393
Temperature	8.992	6	**0.005**	2.286	1	0.156	1.627	5	0.257	1.369	1	0.265
EC	2.846	6	0.099	0.188	1	0.672	0.716	5	0.629	0.753	1	0.403
Alkalinity	1.810	6	0.228	7.685	1	**0.017**	1.262	5	0.366	0.654	1	0.435
DOC	0.133	6	0.987	3.528	1	0.085	0.816	5	0.571	0.132	1	0.723
Silicon	1.791	6	0.232	0.029	1	0.868	0.531	5	0.748	0.689	1	0.423
Magnesium	4.118	6	**0.043**	0.817	1	0.384	8.321	5	**0.005**	0.159	1	0.697
Potassium	0.365	6	0.880	1.467	1	0.249	0.650	5	0.670	0.054	1	0.820
Copper	0.132	6	0.988	1.447	1	0.252	0.170	5	0.967	0.022	1	0.886
Fluoride	1.106	6	0.443	2.111	1	0.172	0.632	5	0.682	0.259	1	0.620
Sodium	5.859	6	**0.018**	1.282	1	0.280	6.325	5	0.012	0.328	1	0.577
Chloride	13.530	6	**0.002**	1.161	1	0.302	0.619	5	0.691	0.005	1	0.944
Sulphate	0.717	6	0.649	1.652	1	0.223	1.124	5	0.419	0.876	1	0.368
Zinc	0.460	6	0.818	0.034	1	0.858	0.642	5	0.676	0.545	1	0.474
Calcium	1.000	6	0.492	0.036	1	0.853	0.620	5	0.690	0.418	1	0.530
Iron	0.285	6	0.926	0.243	1	0.631	0.300	5	0.900	0.243	1	0.631
Aluminium	6.338	6	0.014	1.854	1	0.198	2.323	5	0.139	3.031	1	0.107
Nickel	0.877	6	0.556	0.004	1	0.951	0.569	5	0.723	0.513	1	0.487
Lead	0.987	6	0.498	1.230	1	0.289	0.308	5	0.895	0.231	1	0.640

	Encyonema reichardtii			*Eunotia spp*			*Fragilaria spp*			*Fragillaria (unknown species)*		
	F	df	P	F	df	P	F	df	P	F	df	P
Nitrate	0.553	2	0.590	0.589	3	0.636	0.690	5	0.645	0.685	3	0.581
Nitrite	0.753	2	0.494	0.803	3	0.520	0.192	5	0.957	0.105	3	0.955
TN	0.647	2	0.542	0.745	3	0.549	0.894	5	0.528	0.653	3	0.599
Ammonium	0.071	2	0.932	0.053	3	0.983	61.034	5	**<0.001**	89.271	3	**<0.001**
Phosphate	0.200	2	0.822	0.203	3	0.892	0.119	5	0.984	0.083	3	0.968
Light attenuation	0.806	2	0.471	0.826	3	0.509	0.884	5	0.533	1.623	3	0.246
DO	0.634	2	0.549	0.722	3	0.561	3.326	5	0.064	6.204	3	**0.012**
pH	0.986	2	0.404	1.042	3	0.416	0.952	5	0.498	1.579	3	0.255
Temperature	0.602	2	0.565	0.977	3	0.442	1.722	5	0.235	1.057	3	0.410
EC	0.687	2	0.523	0.787	3	0.528	6.612	5	**0.010**	9.486	3	**0.003**
Alkalinity	0.578	2	0.577	0.656	3	0.597	1.179	5	0.397	0.758	3	0.543
DOC	0.238	2	0.792	0.172	3	0.913	2.400	5	0.130	2.559	3	0.114
Silicon	0.639	2	0.546	0.719	3	0.563	7.107	5	0.008	11.832	3	**0.001**
Magnesium	2.848	2	0.101	1.905	3	0.193	0.817	5	0.570	1.302	3	0.327
Potassium	0.941	2	0.419	0.588	3	0.637	0.345	5	0.872	0.271	3	0.845
Copper	0.219	2	0.807	0.144	3	0.931	0.165	5	0.969	0.181	3	0.907
Fluoride	0.689	2	0.522	0.567	3	0.649	3.595	5	0.053	2.645	3	0.106
Sodium	2.260	2	0.151	1.664	3	0.237	1.102	5	0.429	1.256	3	0.341
Chloride	2.965	2	0.093	1.853	3	0.201	1.074	5	0.441	0.972	3	0.444
Sulphate	0.135	2	0.875	0.368	3	0.778	1.519	5	0.285	1.575	3	0.256
Zinc	0.613	2	0.559	0.562	3	0.652	0.291	5	0.905	0.281	3	0.838
Calcium	0.934	2	0.422	0.765	3	0.539	0.216	5	0.946	0.256	3	0.855
Iron	0.149	2	0.864	0.182	3	0.906	0.133	5	0.980	0.082	3	0.968
Aluminium	0.365	2	0.702	1.184	3	0.365	1.036	5	0.458	0.522	3	0.677
Nickel	0.348	2	0.714	0.425	3	0.739	39.975	5	**<0.001**	36.922	3	**<0.001**
Lead	1.557	2	0.254	0.984	3	0.439	1.713	5	0.237	2.361	3	0.133

	Gomphonema cuneolus			*Gomphonema exilisium*			*Gomphonema parvulum*			*Gomphonema truncatum*		
	F	df	P	F	df	P	F	df	P	F	df	P
Nitrate		13		2.409	1	0.147	14033.249	7	**<0.001**	1.359	2	0.297
Nitrite		13		4.619	1	0.053	89.449	7	**<0.001**	2.523	2	0.125
TN		13		1.983	1	0.184	679.096	7	**<0.001**	1.261	2	0.321
Ammonium		13		0.440	1	0.520	390.607	7	**<0.001**	0.219	2	0.807
Phosphate		13		10.182	1	**0.008**		7		4.775	2	0.032
Light attenuation		13		2.131	1	0.170	1057.406	7	**<0.001**	1.279	2	0.317
DO		13		0.795	1	0.390	220.217	7	**<0.001**	0.674	2	0.529
pH		13		2.218	1	0.162	303.304	7	**<0.001**	1.405	2	0.286
Temperature		13		0.264	1	0.617	46.471	7	**<0.001**	0.731	2	0.503
EC		13		0.065	1	0.803	1553.098	7	**<0.001**	0.364	2	0.703
Alkalinity		13		0.002	1	0.969	111.122	7	**<0.001**	0.305	2	0.743
DOC		13		0.655	1	0.434	7.295	7	**0.014**	0.347	2	0.714
Silicon		13		0.249	1	0.627	204.959	7	**<0.001**	0.413	2	0.671
Magnesium		13		0.007	1	0.936	0.840	7	0.593	0.079	2	0.925
Potassium		13		4.046	1	0.067	1.968	7	0.214	1.862	2	0.201
Copper		13		8.345	1	0.014	15.990	7	**0.002**	3.826	2	**0.055**
Fluoride		13		0.797	1	0.390	77.507	7	**<0.001**	0.541	2	0.597
Sodium		13		0.002	1	0.969	1.649	7	0.279	0.150	2	0.862
Chloride		13		0.112	1	0.743	2.103	7	0.192	0.052	2	0.949
Sulphate		13		1.748	1	0.211	9.513	7	**0.007**	1.223	2	0.331
Zinc		13		5.866	1	0.032	2.318	7	0.163	3.034	2	0.089
Calcium		13		4.971	1	**0.046**	2.675	7	0.126	2.507	2	0.127
Iron		13		10.086	1	**0.008**	257.773	7	**<0.001**	4.773	2	0.032
Aluminium		13		0.001	1	0.971	0.908	7	0.555	1.394	2	0.289
Nickel		13		0.099	1	0.758	237.951	7	**<0.001**	0.304	2	0.744
Lead		13		0.906	1	0.360	1.669	7	0.275	0.504	2	0.617

	Gomphonema vibrio			*Gomphonema (unknown species)*			*Gyrosigma accuminatum*			*Melosira varians*		
	F	df	P	F	df	P	F	df	P	F	df	P
Nitrate	0.570	7	0.761	1.499	2	0.266	1.056	2	0.381	14552.730	10	**<0.001**
Nitrite	0.805	7	0.613	0.383	2	0.691	1.035	2	0.387	108.067	10	**0.001**
TN	0.515	7	0.797	2.526	2	0.125	0.995	2	0.401	570.801	10	**<0.001**
Ammonium	0.809	7	0.610	0.457	2	0.645	1.004	2	0.398	201.024	10	**0.001**
Phosphate	1.971	7	0.213	0.148	2	0.864	2.179	2	0.160		10	
Light attenuation	0.882	7	0.569	0.097	2	0.909	0.146	2	0.866	725.230	10	**<0.001**
DO	0.976	7	0.520	0.002	2	0.998	0.280	2	0.761	195.904	10	**0.001**
pH	1.056	7	0.482	0.088	2	0.917	0.591	2	0.570	349.897	10	**<0.001**
Temperature	0.536	7	0.783	3.232	2	0.079	0.289	2	0.755	24.040	10	**0.012**
EC	1.017	7	0.500	0.163	2	0.851	0.452	2	0.648	990.347	10	**<0.001**
Alkalinity	0.448	7	0.842	3.399	2	0.071	0.112	2	0.895	134.075	10	**0.001**
DOC	0.669	7	0.696	3.330	2	0.074	0.077	2	0.927	5.564	10	0.092
Silicon	0.956	7	0.530	0.022	2	0.978	0.392	2	0.684	208.282	10	**<0.001**
Magnesium	0.785	7	0.625	0.329	2	0.726	0.686	2	0.524	1.531	10	0.401
Potassium	1.255	7	0.399	0.920	2	0.427	2.186	2	0.159	2.605	10	0.233
Copper	2.141	7	0.186	0.271	2	0.768	3.217	2	0.079	20.555	10	0.015
Fluoride	1.306	7	0.380	2.881	2	0.099	0.944	2	0.418	42.801	10	**0.005**
Sodium	0.605	7	0.738	1.152	2	0.351	0.462	2	0.641	2.414	10	0.253
Chloride	2.582	7	0.134	1.819	2	0.208	1.366	2	0.295	1.764	10	0.351
Sulphate	0.408	7	0.867	1.797	2	0.211	0.731	2	0.503	6.945	10	0.069
Zinc	1.302	7	0.382	0.035	2	0.966	3.125	2	0.084	2.184	10	0.282
Calcium	1.632	7	0.284	0.139	2	0.872	3.457	2	0.068	3.483	10	0.166
Iron	1.867	7	0.232	0.178	2	0.839	2.254	2	0.151	163.741	10	**0.001**
Aluminium	0.647	7	0.710	1.258	2	0.322	0.174	2	0.843	0.869	10	0.624
Nickel	1.103	7	0.460	0.071	2	0.932	0.794	2	0.476	174.883	10	**0.001**
Lead	1.882	7	0.229	0.159	2	0.855	1.321	2	0.306	1.093	10	0.532

	Navicula lanceolota			*Navicula radiosa*			*Navicula rhynocotella*			*Navicula slesvicensis*		
	F	df	P	F	df	P	F	df	P	F	df	P
Nitrate	1.045	6	0.470	0.834	2	0.460	1.741	4	0.225	2.352	1	0.151
Nitrite	0.985	6	0.499	0.172	2	0.844	1.201	4	0.374	0.977	1	0.342
TN	1.628	6	0.268	0.999	2	0.399	1.735	4	0.226	1.431	1	0.255
Ammonium	0.640	6	0.698	4.704	2	**0.033**	77.178	4	**<0.001**	0.280	1	0.606
Phosphate	0.763	6	0.621	0.099	2	0.907	0.410	4	0.797	0.154	1	0.702
Light attenuation	0.708	6	0.655	1.004	2	0.398	2.279	4	0.140	0.924	1	0.355
DO	0.457	6	0.820	2.337	2	0.143	6.047	4	0.012	0.001	1	0.977
pH	0.489	6	0.799	0.980	2	0.406	2.310	4	0.137	0.108	1	0.748
Temperature	3.212	6	0.076	1.400	2	0.287	1.636	4	0.247	2.286	1	0.156
EC	0.875	6	0.557	2.914	2	0.096	10.378	4	**0.002**	0.188	1	0.672
Alkalinity	3.906	6	**0.049**	1.211	2	0.335	1.322	4	0.333	7.685	1	**0.017**
DOC	1.359	6	0.346	2.283	2	0.148	1.869	4	0.200	3.528	1	0.085
Silicon	0.470	6	0.812	3.075	2	0.087	10.532	4	0.002	0.029	1	0.868
Magnesium	1.132	6	0.432	0.916	2	0.428	2.448	4	0.122	0.817	1	0.384
Potassium	0.484	6	0.802	0.416	2	0.670	0.216	4	0.923	1.467	1	0.249
Copper	0.543	6	0.763	0.202	2	0.820	0.207	4	0.928	1.447	1	0.252
Fluoride	5.837	6	**0.018**	2.232	2	0.154	2.338	4	0.133	2.111	1	0.172
Sodium	1.926	6	0.206	1.117	2	0.362	2.817	4	0.091	1.282	1	0.280
Chloride	1.318	6	0.360	1.092	2	0.369	1.612	4	0.253	1.161	1	0.302
Sulphate	2.204	6	0.162	1.450	2	0.276	2.151	4	0.156	1.652	1	0.223
Zinc	0.268	6	0.935	0.213	2	0.812	0.205	4	0.929	0.034	1	0.858
Calcium	0.135	6	0.987	0.223	2	0.804	0.302	4	0.869	0.036	1	0.853
Iron	0.746	6	0.632	0.106	2	0.900	0.326	4	0.854	0.243	1	0.631
Aluminium	3.337	6	0.070	0.670	2	0.531	0.487	4	0.746	1.854	1	0.198
Nickel	0.746	6	0.631	4.041	2	0.048	32.584	4	**<0.001**	0.004	1	0.951
Lead	2.798	6	0.102	1.350	2	0.299	1.995	4	0.179	1.230	1	0.289

	Nitzschia dissipita			*Nitzschia linearis*			*Nitzschia minuta*			*Nitzschia palea var debilis*		
	F	df	P	F	df	P	F	df	P	F	df	P
Nitrate	0.885	4	0.510	2.225	4	0.147	7.461	4	**0.006**	1.465	3	0.283
Nitrite	0.457	4	0.765	1.146	4	0.395	9.804	4	**0.002**	0.426	3	0.738
TN	1.362	4	0.320	2.614	4	0.106	12.740	4	**0.001**	1.274	3	0.336
Ammonium	0.419	4	0.791	1.990	4	0.180	0.360	4	0.831	2.998	3	0.082
Phosphate	0.502	4	0.736	0.549	4	0.705	3.865	4	**0.043**	0.118	3	0.948
Light attenuation	0.224	4	0.918	0.256	4	0.899	4.669	4	**0.026**	1.058	3	0.410
DO	0.154	4	0.956	0.674	4	0.626	1.380	4	0.315	1.436	3	0.290
pH	0.240	4	0.909	0.584	4	0.682	3.735	4	**0.047**	0.678	3	0.585
Temperature	1.453	4	0.294	1.512	4	0.278	9.495	4	**0.003**	2.269	3	0.143
EC	0.201	4	0.931	0.811	4	0.549	1.173	4	0.385	2.039	3	0.172
Alkalinity	1.452	4	0.294	0.971	4	0.469	5.458	4	**0.016**	5.554	3	**0.017**
DOC	1.851	4	0.203	1.618	4	0.252	1.519	4	0.276	3.711	3	**0.050**
Silicon	0.107	4	0.977	1.030	4	0.443	0.811	4	0.549	1.864	3	0.200
Magnesium	0.294	4	0.875	0.585	4	0.682	0.672	4	0.628	0.996	3	0.434
Potassium	0.693	4	0.615	0.433	4	0.782	1.285	4	0.345	0.824	3	0.510
Copper	0.454	4	0.768	0.485	4	0.747	1.994	4	0.179	0.629	3	0.613
Fluoride	1.902	4	0.194	0.720	4	0.599	2.629	4	0.105	3.221	3	0.070
Sodium	0.576	4	0.687	0.767	4	0.573	1.624	4	0.250	1.415	3	0.295
Chloride	2.988	4	0.080	0.828	4	0.540	2.714	4	0.098	1.322	3	0.321
Sulphate	1.431	4	0.300	2.197	4	0.150	3.375	4	0.060	1.652	3	0.239
Zinc	0.276	4	0.886	0.596	4	0.674	1.637	4	0.247	0.148	3	0.929
Calcium	0.426	4	0.787	1.184	4	0.380	2.333	4	0.134	0.144	3	0.931
Iron	0.508	4	0.732	0.507	4	0.732	3.647	4	**0.050**	0.151	3	0.926
Aluminium	1.107	4	0.410	0.901	4	0.502	1.373	4	0.317	1.242	3	0.346
Nickel	0.149	4	0.959	1.209	4	0.372	0.440	4	0.777	2.511	3	0.118
Lead	1.603	4	0.255	0.097	4	0.981	0.478	4	0.752	1.573	3	0.257

	Nitzschia paleacea			*Rhoicosphenia abbreviata*			*Rhopaladia gibba*			*Surrirela brebisonii*		
	F	df	P	F	df	P	F	df	P	F	df	P
Nitrate	0.479	4	0.751	0.826	3	0.509	0.968	3	0.445	0.966	3	0.446
Nitrite	0.081	4	0.986	1.335	3	0.317	0.955	3	0.451	0.383	3	0.768
TN	0.497	4	0.739	0.592	3	0.634	1.173	3	0.368	1.667	3	0.236
Ammonium	63.672	4	**<0.001**	3.018	3	0.081	0.064	3	0.978	0.434	3	0.733
Phosphate	0.167	4	0.950	4.920	3	**0.024**	0.306	3	0.820	0.380	3	0.770
Light attenuation	1.211	4	0.371	1.665	3	0.237	1.384	3	0.304	0.452	3	0.722
DO	4.188	4	**0.035**	2.068	3	0.168	1.443	3	0.288	0.587	3	0.637
pH	1.066	4	0.427	1.589	3	0.253	2.012	3	0.176	0.589	3	0.636
Temperature	0.973	4	0.468	0.318	3	0.812	2.046	3	0.171	2.260	3	0.144
EC	6.639	4	**0.009**	1.865	3	0.199	1.297	3	0.329	0.447	3	0.725
Alkalinity	0.647	4	0.643	0.359	3	0.784	0.841	3	0.502	2.062	3	0.169
DOC	2.287	4	0.139	1.053	3	0.411	0.370	3	0.776	2.655	3	0.106
Silicon	8.158	4	0.005	2.130	3	0.160	1.206	3	0.357	0.533	3	0.670
Magnesium	1.060	4	0.430	1.261	3	0.340	0.244	3	0.864	0.255	3	0.856
Potassium	0.183	4	0.941	4.203	3	**0.036**	0.619	3	0.619	1.513	3	0.271
Copper	0.168	4	0.949	6.344	3	**0.011**	0.348	3	0.792	0.581	3	0.641
Fluoride	2.461	4	0.120	1.445	3	0.287	0.829	3	0.508	1.759	3	0.218
Sodium	0.875	4	0.515	1.025	3	0.422	0.212	3	0.885	0.723	3	0.561
Chloride	0.798	4	0.556	0.191	3	0.900	5.836	3	0.014	1.252	3	0.342
Sulphate	1.105	4	0.411	0.953	3	0.452	0.587	3	0.637	1.092	3	0.397
Zinc	0.206	4	0.929	4.019	3	**0.041**	1.128	3	0.384	0.607	3	0.626
Calcium	0.235	4	0.912	2.761	3	0.098	2.424	3	0.126	0.605	3	0.626
Iron	0.143	4	0.962	5.135	3	**0.021**	0.350	3	0.790	0.468	3	0.711
Aluminium	0.457	4	0.766	0.559	3	0.654	3.047	3	0.079	0.800	3	0.521
Nickel	29.070	4	**<0.001**	2.806	3	0.094	0.639	3	0.607	0.288	3	0.833
Lead	1.600	4	0.256	1.275	3	0.335	1.599	3	0.251	0.098	3	0.959