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River phytoplankton biological controls on a microscopic level

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Declaration

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

Anna Freeman

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Abstract

This thesis examines biological controls on phytoplankton in temperate lowland rivers on microscopic scale using the River Thames, a major UK river, as an example. The central part of the study explores river zooplankton-phytoplankton interactions in relation to physical environment, water chemistry and spatial patterns in the catchment. The zooplankton were surveyed weekly from 12 sites within the catchment (the Thames, its tributaries and Farmoor Reservoir) during spring-summer months in 2015, a year representative of the long-term seasonal low flow conditions. Six microcosm experiments were conducted to assess zooplankton grazing effect on phytoplankton diversity and abundance. They were supplemented with six laboratory experiments where the zooplankton were adjusted to replicate pre-bloom termination communities. The final part of the study looks at the relationships of phytoplankton, bacteria and chytrids* through experimental work involving incubation in thermal and low nutrients stress.

Evidence was found that apart from water temperature, river flow and travel distance, zooplankton in the Thames are regulated by phytoplankton. In particular the presence of centric diatoms. It was also proposed that plankton may originate in certain tributaries of the Thames, especially those connected to canals, therefore the mixing of waters from these tributaries may be the key control on phytoplankton and consequently on zooplankton, rather than site-specific flow or water quality conditions. Microcosm experiments showed that zooplankton exert seasonal, site specific grazing effect on phytoplankton composition and abundance. Laboratory experiments reinforced the microcosms findings that physical environment is a stronger regulator of phytoplankton dynamics than zooplankton.

Phytoplankton-bacteria-chytrids experiments revealed that both diatom metabolism and presence of attaching bacteria play an important role in diatom bloom termination and recycling. These results indicate a complex interplay between physical and biological environments in terms of nutrient availability and bacteria-diatom interactions. Further investigation is needed to unpick these complex relationships.

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Introduction

1.1 Why studying plankton in rivers?

Large lowland rivers are among some of the most degraded environments on the planet (UN Environment, 2017). Due to flow regulation, high inputs of domestic, industrial and agricultural waste river ecosystems experienced a greater biodiversity loss and had the highest proportion of species threatened with extinction than any other ecosystem type (Millennium Ecosystem Assessmen, 2005). Misguided approach to management practices may lead to abrupt changes at the basis of river ecosystems killing most aquatic animal life and affecting drinking water supply (Henderson *et al*., 2008; Whitehead and Hornberger, 1984). This basis in rivers is formed of microscopic plants (phytoplankton), animals (zooplankton) and protists (can behave as phyto- or zooplankton), defined as plankton (from the Greek πλαγκτός - *planktos*, meaning wanderer or drifter), as they are unable to swim against a current. Phytoplankton are important producers of organic carbon; they photosynthesise generating oxygen and food for zooplankton which in turns are vital sources of food to many larger aquatic animals, such as fish and molluscs (Thorp and Delong, 1994). In many low-gradient rivers, phytoplankton may represent the primary oxygen source (Wehr and Descy, 1998).

When conditions are favourable phytoplankton, tend to develop dense populations, defined as algal blooms, often resulting in the formation of oxygen-depleted zones and high fish mortality. Furthermore, phytoplankton blooms may affect the taste and smell of drinking water and block filtration systems for water treatment (Henderson *et al*., 2008; Whitehead and Hornberger, 1984). Chemical substances, excreted by toxic bacteria, if consumed in sufficient quantities, can cause gastrointestinal disorders, fever and irritations of the skin, ears, eyes, throat and respiratory tract (WHO, 2011). Recently a number of studies raised the concern that predicted future changes in climate would potentially favour longer and more intensive phytoplankton blooms (Hutchins et al., 2010; Cox and Whitehead, 2009; Whitehead, 2009). Therefore, the ability to prevent occurrences of harmful blooms is essential for successful river management.

1.2 Forecasting plankton dynamics. Knowledge gaps

Plankton communities in rivers are regulated by meteorological, hydrological and chemical factors (for example Basu and Pick, 1997; Reynolds, 2000; Chételat, Pick and Hamilton, 2006; Bowes *et al.*, 2016). Biological factors, such as zooplankton grazing (Garnier et al., 1995; Lair & Reyes-Marchant, 1997; Gosselain et al., 1998b), and the effects of pathogenic microbes on algal population have increasingly been considered as important controls on phytoplankton development (Frenken et al., 2016; Maier & Peterson, 2017). However, biological interactions in rivers remain poorly studied, meaning that further research is needed to better conceptualize and

evaluate the relationships between planktonic communities and how they seasonally shape river ecosystems (Waylett et al., 2013).

This study was designed to address these knowledge gaps by exploring the interaction links between zooplankton and phytoplankton in a major river system in the UK - The River Thames. In the Thames, phytoplankton net-growth rates and bloom termination cannot be explained by residence time, water temperature, nutrient chemistry and light intensities alone. This is one of the most intensively monitored river-systems in the UK with readily available historic phytoplankton studies (Lack, 1971; Ruse and Hutchings, 1996; Ruse & Love, 1997), extensive long-term hydrological measurements (Crooks & Kay, 2015), and weekly observations of both water chemistry (Bowes et al., 2018) and bacterioplankton (Read et al., 2015). The Thames is a complex heterogenic system with evident spatial and seasonal variation in planktonic communities (Bowes et al., 2012; Read et al., 2014, 2015).

To achieve aims of this study three objectives were defined. The first was to measure zooplankton community structure and abundance in the Thames and analyse these data to determine key factors regulating zooplankton diversity, distribution and seasonal dynamics with emphasis on zooplankton-phytoplankton interactions. The second was to experimentally *in situ* measure the zooplankton grazing effect and its spatial and seasonal variation in the Thames catchment. The third was to experimentally explore (preliminary design) phytoplankton – bacteria interactions in various temperature conditions. These objectives form four chapters of the manuscript. Chapter 4 and 5 describe zooplankton community distribution and seasonal dynamics (at a weekly resolution) in the Thames catchment. Chapter 6 is based on experimental studies of how zooplankton grazing can influence phytoplankton communities. Chapter 7 describes experimental work on how bacterial and fungal communities affect the diatom dominated phytoplankton communities. Chapters (4-7) is written in a research paper format.

1.3 Chapters summary

Chapter 1 describes the aims and objectives of this study. Chapter 2 reviews the current scientific understanding of biological controls on river phytoplankton with emphasis on the zooplankton grazers and pathogenic microbes. Chapter 3 overviews the study sites with phytoplankton communities previously observed in the area. Chapter 4 summarises results of the weekly plankton survey in the Thames main stem. Chapter 5 describes the spatial and seasonal variability in the zooplankton community in relation to physical environment and phytoplankton

communities. Chapter 6, 7 present results of microcosm experiments, testing negative effects of zooplankton grazing and bacteria-fungi pathogens on algal population. Chapter 8 summarises research results and places study in the wider context of assessing change in phytoplankton dynamics in response to possible environmental change due to flood and water supply management practices. Research strategies and future recommendations were formulated. Appendix. Chapter 9 presents results of laboratory experiments where the zooplankton community was modified to estimate the possibility of post-diatom bloom grazing effect.

1.4 Hypotheses and research questions

Chapter 4

Phytoplankton and zooplankton community structure and interaction along the lowland, eutrophic River Thames

Research questions:

- Do zooplankton abundance and community structure vary significantly along the main river stem. Do the number of species and their population density increase with distance from the source, chlorophyll-a concentration, and water temperature?
- Can the zooplankton community structure be predicted by physical, spatial characteristics, and phytoplankton community composition and abundance?
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Chapter 5

Catchment-scale ecology of riverine zooplankton

Research questions:

- Do zooplankton community composition and abundance vary spatially in the Thames catchment? What are the causes of these variations?
- Do tributaries and Farmoor Reservoir significantly affect zooplankton community composition and abundance in the Thames channel?

Chapter 6

Zooplankton grazing on phytoplankton in a large lowland river. Dialysis microcosm experiment.

Research questions and hypotheses tested:

- Does zooplankton grazing pressure exert significant loss rates of phytoplankton species?
- How does this affect phytoplankton diversity, abundance, distribution and dynamics in rivers?
- What is the importance of zooplankton grazing in comparison with meteorological, hydrological, chemical and other biological regulators?

Chapter 7

Influence of bacteria and parasitical chytrids on planktonic diatoms in the lowland eutrophic river Thames.

Hypotheses tested:

- Warmer water temperatures around 20°C favour bacteria and chytrid parasites
- These pathogens have better success in infecting diatom cells when Si and phosphate levels are low.

- *Chapter 9 (Appendix)*

Hypotheses tested:

- The zooplankton can significantly reduce phytoplankton growth, but this effect is evident when rotifer numbers are higher than 1000 ind m^{-3} replicates.
- The diatom growth can be inhibited by the thermal properties of the environment

Chapter 2 Literature review

2.1 Introduction

This chapter overviews historic-to-present day studies of river plankton. Section 2.2 (Page 8) is focused on phytoplankton modelling techniques. Sections 2.3 - 2.5 describe the factors controlling phyto- and zooplankton development, and temporal dynamics in large lowland rivers. Sections 2.6 - 2.7 are centred around river zooplankton communities. Section 2.8 summarises resent advances in research on phytoplankton associated bacteria and parasitical chytrids. Section 2.9 describes the effect of zebra mussels on phytoplankton in the River Thames.

2.2 Missing links in phytoplankton models

Phytoplankton growth can be simulated using various mathematical approaches. Models that incorporate only physical factors (light intensity, water temperature and flow) tend to significantly under or overestimate phytoplankton biomass during the spring-summer period. This was observed in a study of the Ohio River (US) and the River Thames (UK) when during optimal light, water temperature and residence time conditions chlorophyll-*a* concentrations declined throughout the system (Sellers & Bukaveckas, 2003).The authors hypothesized that at a higher temperature, biological controls could have sufficiently increased algal mortality. A growing number of modelling studies support this idea by adding 'grazing effect' to improve their simulation results (Billen et al., 1994; Kowe et al., 1998; Schöl A., Kirchesch ., Bergfeld T., Schöl F., Borcherding J., 2002; Descy et al., 2003; Waylett et al., 2013; Li et al., 2019) (Table 2-2). Additionally, a small number of studies treat phytoplankton as a community of organisms rather than chlorophyll-*a* biomass (Whitehead et al., 2015).

Table 2-1 Examples of mathematical models with factors considered for simulation of river phytoplankton dynamics

River	Model	Reference	Factors*			
			M	H	C	B
Ohio River	ORACHL (Algal-Chlorophyll model) used with HEC-RAS (One-dimensional hydrologic model)	Sellers and Bukaveckas, (2003)	+	+	-	-
Applied in various rivers	QUAL2K	Chapra et al., (2013)	+	+	+	-
Rivers Meuse, Loire and Mossele	POTAMON	Gosselain et al., (1998) Descy et al., (2012)	+	+	+	+
River Rhine	QSIM 8.3	Schöl et al., (2002)	+	+	+	+
Seine River	RIVERSTRAHLER	Garnier et al., (1995)	+	+	+	+
River Swale	-	Kowe et al., (1998)	+	+	+	+
River Vaal	-	Cloot and Roux, (1997)	+	+	+	-
River Frome	-	Lázár, Wade and Moss, (2015)	+	+	+	+
River Thames	QUESTOR	Waylett et al., (2013)	+	+	+	-

*Factors applied: M – meteorological; H – hydrological; C – chemical; B- biological

Most modelling studies of the River Thames are based on QUESTOR application. The model is set up to utilise weekly water chemistry data from 20 sites in the catchment; it outputs chlorophyll-*a* concentrations at 1-day resolution. Water chemistry is calculated on a reach-by-reach basis moving sequentially downstream and assuming complete mixing for each of the defined 12 reaches (Waylett et al., 2013). The main QUESTOR determinants simulated are chlorophyll-*a*, Biochemical Oxygen Demand (BOD), Dissolved Oxygen (DO), Inorganic and Organic Phosphorus, Nitrate, Particulate Organic Nitrogen, Ammonium, pH, Temperature, Flow

and Photosynthetically Active Radiation in the water column. The processes represented are aeration, BOD Decay, Deamination, Nitrification, Denitrification, Benthic Oxygen Demand, BOD Sedimentation, P Mineralisation, in conjunction with a biological sub-model of Phytoplankton (comprising Growth, Respiration and Death), which includes nutrient uptake and release (Boorman, 2003).

Table 2-2 Phytoplankton representation in models, and parameters for algal losses, including the grazing effect

Model description	Phytoplankton representation	Parameters for phytoplankton losses	Literature
POTAMON One-dimensional non-stationary model simulating phytoplankton dynamic for a whole year, from source to mouth of the main river.	Chlorophyll-*a* *Stephanodiscus hantzschii;* Small centric diatoms; Non-siliceous algae green and golden algae; Large diatoms	Temperature-dependent mortality; Temperature-dependent respiration; Sedimentation; **Grazing by zooplankton** (*Brachionus*-like and *Keratella*-like rotifers)	The Meuse (Gosselain *et al*., 1998) The River Loire (Descy *et al.*, 2012) The River Moselle (Descy *et al*., 2003)
QSIM 8.3 Deterministic model that calculates longitudinal profiles and seasonal cycles of various water quality parameters as well as biomasses of algae, rotifers and mussels.	Chlorophyll-*a* Green algae; Diatoms	Temperature-dependent mortality; Temperature-dependent respiration; **Grazing by zooplankton** *(Brachionus* sp.) and **benthic filter-feeder** (*Dreissena polymorpha*)	The Rhine (Schöl *et al.*, 2002)
RIVERSTRAHLER The process-based algal model used for simulation of diatoms and chlorophytes blooms .	Chlorophyll-a Green algae; Diatoms	Respiration Sedimentation Lysis Grazing by zooplankton as one group	The Seine River (Garnier *et al.*, 1995)
Mathematical model examining the behaviour of the algal community in a fast-flowing river.	Chlorophyll-*a* Phytoplankton: diatoms consisted of centric and pennate diatoms and green algae Benthic algae: pennate diatoms and green algae	Grazing by zooplankton as one group Sedimentation Respiration	The River Swale (Kowe *et al.*, 1998)

2.3 River phytoplankton

River *phytoplankton* (often referred to as *potamoplankton*) can be defined as the assemblage/community of algal species which grow and increase while in the river flow. The word '*algae'* is applied to a broad variety of plants or plant-like organisms of different taxa (Reynolds, 1984). They are photosynthetic – generating complex carbon compounds from carbon dioxide, the energy of light and water, some have become secondarily heterotrophic, but still, retain genetic relations with their photosynthetic relatives. Algae can be free-floating (**planktonic**) or substrate-associated (**benthic/periphytic**). Planktonic organisms drift freely within the main body of water, with some species regulating their position within the water column, so they require low flow rates and optimum light levels. Benthic algae, on the other hand, are substrate-associated, they fix in position or have limited movement, meaning that they can tolerate high flow and low light as long as there is an appropriate substrate to attach, for instance, rocks, submerged water plants, and even macro-invertebrate shells. Some species have both planktonic and benthic stages in their life cycle (Sandgren, 1988). Benthic algae can detach and become planktonic, and planktonic can sink to the bottom when conditions are unfavourable to survive as a dormant metabolically inactive phase.

Benthic species are more suited to small rivers and streams, where they are dominant in algal communities (Reynolds & Descy, 1996). In larger rivers, benthic algae can be found mainly in the headwaters and rarely constitute a significant fraction of the suspended flora in the middle and lower reaches, except some species, such as *Navicula* spp, *Diatoma vulgaris* (Reynolds & Glaister, 1993; Ruse & Hutchings, 1996).'True' planktonic species also originate from benthic environments, but they are better adapted to grow and reproduce in flowing waters (Reynolds & Descy, 1996). Riverine phytoplankton have features that allow them to survive in 'hostile' turbulent flow (Hynes, 1970; Reynolds, 1984). These algae tend to have small bodies with high surface-to-volume ratio for better photosynthesis (Reynolds & Descy, 1996). Phytoplankton genera previously found in large lowland rivers are listed in Table 2-3 (summarized from: Rice, 1938; Holmes and Whitton, 1981; Descy *et al.*, 1987; Reynolds and Glaister, 1993; Garnier, Billen and Coste, 1995; Ruse and Love, 1997; Ha, Kim and Joo, 1998; Hudon, 2000).

Table 2-3 Phytoplankton genera found in large lowland rivers

Phylum	Genera	Other names
Bacillariophyta	*Stephanodiscus, Cyclotella, Skeletonema, Navicula Synedra, Melosira, Asterionella, Nitzschia, Fragilaria, Gyrosigma, Aulacoseira, Cocconeis, Gomphonema*	Diatoms
Chlorophyta	*Chlorella, Eudorina, Pandorina, Pediastrum, Gonium, Scenedesmus, Chlamydomonas, Golenkinia, Actinastrum, Staurastrum, Tetrastrum, Tetraedron*	Green algae
Cryptophyta	*Cryptomonas*	Cryptomonads
Cyanophyta	*Anabaena, Microcystis, Oscillatoria, Planktothrix*	Blue-green algae
Dinophyta	*Peridinium, Glenodinium*	Dinoflagellates
Chrysophyta	*Synura, Dinobryon*	Golden or golden-brown algae
Euglenophyta	*Phacus, Trachelomonas*	Euglenoids

There are two forms of diatoms. The first are 'petri dish'- shaped, with radially symmetrical cells, constituting the Centrales (now called the Biddulphiales). The other are elongate, 'cigar'-shaped or wedge-shaped cell with bilateral symmetry of wall markings, the Pennales (now called Bacillariales) (Sims et al., 2006). Most hypotheses tend to agree that Centrales developed in shallow marine environments, while the Pennales could have originated from pelagic habitats. As a result, centric diatoms are largely planktonic and pennate forms are benthic or periphytic. Some Centrales such as: *Melosira* and *Aulacoseira* are primarily found in benthic habitats, with cells joined in filamentous chains. Both individual cells and filaments may become part of the plankton. Diatoms have a siliceous skeleton (frustule), are non-motile, or capable of only limited movement along a substrate extracting mucilaginous material along their frustules. Being autotrophic, they are restricted to photic zones. Main pigments of diatoms are chlorophylls *a* and c, beta-carotene, fucoxanthin, diatoxanthin and diadinoxanthin. Diatoms store energy as chrysolaminarin (polysaccharide) and lipids. Lipids help to control diatom buoyancy. Diatom cells can be solitary or 'colonial' (attached by mucous filaments or by bands into long chains).

Green algae are a large group of unicellular or colonial photosynthetic eukaryotes which have chloroplasts that contain chlorophyll a and b (giving them a bright green colour) as well as the accessory pigments beta-carotene and xanthophylls in stacked thylakoids. The cell walls of green

algae usually contain cellulose, and they store carbohydrate in the form of starch. There are motile and non-motile cells. The shapes and sizes of green algae are significantly varied from pico-size cells (0.2 and 2 μm) to large colonial and long filamentous forms.

Cryptophytes have one or two chloroplasts containing chlorophylls a and c, together with phycobiliproteins and other pigments. They vary in colour from green, blue, brown to red. Storage material is starch or starch-like. Cryptophytes are unicellular (rarely colonial), often bean-shaped, frequently dorsoventrally flattened with two or more unequal subapical flagella arising in an anterior invagination.

Cyanobacteria are a group of photosynthetic bacteria, some of which are nitrogen-fixing, they range from unicellular to filamentous and include colonial species. Cyanobacteria colonies may form filaments, sheets, or even hollow spheres. Cell internal membranes and organelles are absent, and they are varied in colour from blue-green, grey-green, violet, brown, purple or red dependent on relative proportions of chlorophyll, phycocyanin, phycoerythrin and sometimes brown pigments. Cyanobacteria can form large blooms which often are toxic, and lead to the closure of recreational waters when spotted.

The dinoflagellates are a large group of unicellular, rarely coccoid or filamentous, flagellate eukaryotes. Many dinoflagellates are mixotrophic, combining photosynthesis with ingestion of prey (phagotrophy). They are protists. Cell walls are made of regularly arranged polygonal plates. Cells usually are brown due to the presence of accessory pigments as most photosynthetic species contain chlorophylls-*a* and c2, the carotenoid beta-carotene, and a group of xanthophylls that appears to be unique to dinoflagellates, typically peridinin, dinoxanthin, and diadinoxanthin. A bloom of certain dinoflagellates can result in a visible colouration of the water colloquially known as red tide, which can cause shellfish poisoning if humans eat contaminated shellfish.

Chrysophytes share some similarities with diatoms, their cell walls have silica scales, and the storage material is primarily oil or leucosin. They are mostly unicellular flagellates with golden to yellow-brown cells due to the presence of accessory pigments. Chrysophytes contain the pigment fucoxanthin and were once considered to be a specialised form of cyanobacteria.

2.4 Biological factors regulating phytoplankton

Biological factors generally are associated with natural competition (Ha *et al*., 1998), grazing by zooplankton (reviewed by Sterner, 1989) benthic animals (reviewed by Horne, 2009) and fungal

parasitism (Descy, 1993), accompanied by bacterial and viral infections (Billen *et al.*, 1994; Garnier *et al.*, 1995). Grazing effects on the phytoplankton growth and community structures have received more extensive research attention and are better understood in lakes (as reviewed in Reynolds, 1984; Lehman, 1988; some of the research examples: Lampert *et al.,* 1986; Wu and Culver, 1991) and river estuaries (Kim *et al.*, 2000; Quinlan *et al.,* 2009) worldwide. It has been established that in temperate lakes, seasonal plankton patterns often are initiated by a spring bloom of centric diatoms and cryptomonad flagellates. They, in turn, fuel a rapid population increase of planktonic grazers which peak after a diatom bloom, rapidly deplete their food resource and undergo a crash. In large lowland rivers, however, this subject remains poorly investigated, partly because the zooplankton are represented by rotifers, which are not as efficient grazers as micro-crustaceans found in abundance in lakes. Still, phytoplankton declines and low biomass (clear-water phases) had frequently appeared in temperate rivers when meteorological and hydrological conditions (higher water temperatures, more sunlight over a longer day, less turbidity, slower downstream travel) favoured algal growth. Such declines were observed in the Meuse (Gosselain et al., 1998a), the Moselle (Descy, 1993), the Rhine (de Ruyter van Steveninck *et al.*, 1992), the Seine (Billen et al., 1994), the Spree (Köhler, 1993) and the Thames (Waylett et al., 2013).

Table 2-4 Estimated daily grazing impacts on phytoplankton dynamics in world rivers

Study area	Grazing effect expressed in daily grazing rates (GR) of total algal biomass	Source	References
R. Danube (Austria)	0-13% (w.a.*≤ 10 days) 3-115% (w.a. > 10 days) 0.3–50% (w.a. > 200 days) * w.a. – water age	From literature	Keckeis et al. (2003)
R. Rhine (Germany/Holland)	2-28%, neglecting phytoplankton growth	From literature	De Ruyter van Steveninck et al. (1992)
R. Meuse (Belgium)	1 -32%	Experimental work	Gosselain *et al.*, (1998)
R. Moselle (France and Germany)	3.4 -17.9%		
Nakdong River, (South Korea)	17 ± 31%		Kim *et al.*, (2000)
R. Seine (France)	20 and 50% and occasionally up to 75% for diatoms and chlorophytes.	Modelling exercise	Garnier et al., (1995)

These clear-water phases can be short, as observed in the River Meuse, or span for many weeks affecting long stretches of the River Moselle (Gosselain et al., 1998a). Reynolds and Descy, (1996) highlighted that optimal conditions do not necessarily coincide with the highest net algal increase. In fact, 'these are the conditions under which the phytoplankton dynamics become very sensitive to cell-specific loss processes, particularly where zooplankton development is favoured or where shallow water and accelerated sinking rates contribute to enhanced losses of sedimentation' (citation from Reynolds and Descy, 1996). According to Gosselain et al., (1998) phytoplankton summer variations could be explained as a combination of low growth rates and losses from zooplankton grazing and sedimentation. This study combined grazing measurements from the Meuse over a three year period with measured grazing rates by rotifer-dominated communities and reported phytoplankton loss rates between 1 and 113%, which affected predominantly algae only in the size range < 20 μm, explaining the dominance by larger phytoplankton units in the summer assemblages (Gosselain et al., 1998a). Some examples of estimated daily grazing impacts on phytoplankton dynamics in several world rivers are summarised in Table 2-4.

Garnier et al. (1995) estimated grazing fluxes in the River Seine, values ranged between 20-50% and occasionally up 75% for diatoms and chlorophytes (Table 2-4). Apart from the effect of zooplankton grazing, many reports emphasise the consequences of filtration by benthic filter feeders, especially the zebra mussel Dreissena polymorpha (de Ruyter van Steveninck et al., 1992; Schöl et al., 2002; Descy et al., 2003).

2.5 Other factors

2.5.1 Meteorological factors

Meteorological factors relate to the quality, intensity and duration of irradiance, which effects photosynthesis efficiency. Hutchins *et al.*, (2010) in a study on the River Ouse, North East England showed that increasing riparian shading was more effective at suppressing algal growth than reducing nitrogen pollution. Different algal groups contain various pigments that allow them to adapt and improve photosynthesis efficiency in fluctuating light conditions. Such photosynthetic responses may potentially influence species composition within the planktonic communities. For instance, benthic diatoms and freshwater red algae showed consistent adaptations to low irradiance; these are 'shade-adapted' organisms.

In contrast, most species of green algae were reported as 'sun-adapted' algae, while cyanobacteria and xanthophytes were considered as intermediate groups, with no clear trends of photosynthetic responses to low or high irradiances (Necchi, 2004). When surface irradiance intensity exceeds photosynthetically active radiation, photo-inhibition may occur near the surface level of water column (the process reviewed in Reynolds, 1984; Ferris and Christian, 1991). Necchi, (2004) observed notable photo-inhibition in diatoms and red algae under field and laboratory conditions, but it was less evident among green algae.

2.5.2 Hydrological factors

Water temperature and flow are generally considered as main hydrological constraints. Balbi (2000) in studying the River Nene identified from multiple regression analysis that temperature was the most significant predictor of chlorophyll concentration, followed by discharge and light as a result. Many cellular processes accelerate with an increase in water temperature to maximal values of 25°C and 40°C. These processes are characterised by a non-linear, exponential function and described by Q_{10} values (reviewed by Reynolds, 1984). Various algae show inter-specific differences in the growth rates at different temperatures, although this relationship is subjected to significant interaction with light flux density (Reynolds, 1984; Butterwick *et al.,* 2005).

River flow relates to residence time, which is particularly important for the development of algal communities. The lower the flow, the higher the retention time for planktonic algae to increase biomass (Soballe and Kimmel, 1987; Reynolds and Descy, 1996; Bowes et al., 2012;), as the effect is especially enhanced in rivers with extensive 'dead zones', which are regulated by the river morphology (Reynolds, 2000). Estimated current velocities above which phytoplankton growth and reproduction are severely impaired were previously reported as 0.48 m s^{-1} (reviewed by Bertani *et al*., 2012).

Bahnwart et al. (1998) studying the Warnow River, Germany, concluded that flow velocity can evidently influence phytoplankton community structure and biomass along the river longitudinal profile. The authors observed that in the fast-flowing turbulent environment, cyanobacteria, cryptophytes and diatoms are subjected to large biomass losses, whereas chlorophytes are favoured. Both diatoms and cryptophytes directly benefit from low flow velocity in the lower parts of the river. Desortová & Punčochář (2011) described the important influence of flow on phytoplankton biomass and community seasonal dynamics in the River Berounka (Czech

Republic). Low flow conditions across the catchment resulted in high chlorophyll-*a* concentrations (200 µg l-1) and an unusual shift of species succession to green algae in spring.

Nonetheless, under slow non-turbulent flow, algal cells can sink and are more likely to be grazed by the developing zooplankton population (Reynolds et al., 1982). In a modelling study of the River Lot (France) Thebault and Qotbi, (1999) suggested that sinking is related to a decrease in turbulence and plays a 'primordial role in the disappearance of phytoplankton', while a higher flow, is likely to suspend algae within the water column from the benthic environment (reviewed by Kowe et al., 1998). Two studies on Canadian rivers (Basu and Pick, 1996 and Chételat et al., 2006) found no significant relationship between river flow and phytoplankton biomass.

Lucas et al. (2009) suggested that transport time does not determine whether phytoplankton biomass increases, instead it is regulated by the growth-loss balance. When growth is faster than loss, phytoplankton biomass increases with transport time. On the other hand, when the loss is faster than growth, biomass decreases with the increase in transport time.

2.5.3 Water chemistry factors

Large algal blooms occur in eutrophic ecosystems in response to high nutrient loads, mainly dissolved nitrogen and phosphorus, from fertilisers, detergents and sewage. As a result, chemical constraints have predominantly been related to nitrogen and phosphorus limitations.

Several studies of North American rivers reported a strong positive correlation between total phosphorus concentration in the water column and phytoplankton biomass in rivers (Basu and Pick, 1996; Van Nieuwenhuyse and Jones, 1996; Chételat *et al.*, 2006). Nitrogen also can limit phytoplankton production in temperate eutrophic lakes, especially where phosphate concentrations are relatively high. Some species of cyanobacteria can assimilate nitrogen from the atmosphere (Paerl, 1988). Silicon is required by all phytoplankton in small amounts for protein and carbohydrate synthesis, while diatoms and chrysophytes need silicon to strengthen their cell walls (Reynolds, 1984). When silicon is limited, it is usually followed by a decline in diatom biomass. However, diatom maxima can terminate just as rapidly even when dissolved silica is not limited (Bowes et al., 2012). Özer et al. (2019) in a study of the Ankara Stream in Turkey, showed strong association of phytoplankton composition with spatial gradients in nutrient concentrations.

Nevertheless, other studies showed little relationship between nutrient concentrations and either phytoplankton or periphyton biomass in rivers (Balbi, 2000; Bernhardt and Likens, 2004; Morgan et al., 2006). Descy et *al.*, (1987) suggested that in large lowland rivers with excessive nutrient input, phosphorus and nitrogen typically do not limit phytoplankton production

2.6 River zooplankton grazers

The zooplankton composition, distribution and dynamics in most large lowland rivers follow similar spatial and seasonal patterns (Lair, 2006). In the main river stem above the estuary, the zooplankton are mainly represented by rotifers with a small number of microcrustaceans, and various heterotrophic protists, accompanied by the less generally distributed and occasionally occurring, larval trematode flatworms, mites, gastrotrichs, tardigrades, and the larval stages of certain insects, mussels and fish (Wetzel, 2001).

This study refers to a widely used classification, which separates protists - protozoans from planktonic animals - metazoans (Harris et al., 2000) (Conceptual diagram - Appendix Figure 6. Page 199). Heterotrophic protists (protozoans) are unicellular organisms that feed primarily on bacteria (pico-plankton group 0.2–2 μm), and are known as a food source for metazoans (Pace & Orcutt, 1981; Carlough & Meyer, 1989; Sanders et al., 1989, 1994; Weisse et al., 1990; Carrick et al., 1991; Šimek & Straškrabová, 1992; Arndt et al., 2000; Callieri et al., 2002; Sherr & Sherr, 2002; Joaquim-Justo et al., 2004) Although some species of ciliates and flagellates can consume pico-phytoplankton, and some even prey on metazoan eggs (Sherr and Sherr, 1994, Arndt et al., 2000) their impact on phytoplankton in the freshwater environments is generally considered as negligible in comparison with metazoan activities. Since in freshwater environments, the metazoans are known as dominant planktonic grazers (Reynolds, 1984; Lehman, 1988; Gosselain et al., 1998b; Kim et al., 2000), which actively interact with the phytoplankton community, this research explores only the metazoan component of the zooplankton community.

Zooplankton studies from around the world demonstrated that rotifers are the largest and most diverse group among river metazoans (Table 2-5). Due to their shorter development times, rotifers have clear advantages over micro-crustaceans in lotic environments. For example, in spring rotifer populations in the Rhine, doubled at twice the rate (0.89 day^{-1}) of crustaceans (0.45 day^{-1}) (De Ruyter van Steveninck et al., 1992). Exceptionally large numbers of cladocerans and copepods observed in the St Laurence River (Casper and Thorp, 2007) could be because almost

half of the river flow is originated from the lentic environment, where micro-crustaceans find enough time to grow and reproduce.

Chick et al., (2010) stressed that the sampling technique deployed in most zooplankton studies result in serious underestimation of rotifer abundance. For instance, filtering water through 63-µm mesh may result in significant loss of small rotifers. The study compared two techniques: one required filtering of 180 l of water through 63-µm mesh for macrozooplankton analysis, the other - filtering 18 l through 20-µm mesh applied in microzooplankton studies. The first technique underestimated density and biomass of common rotifers by two to three orders of magnitude. However, this sampling error could have possibly been enhanced due to the use of the larger water volumes, i.e. ten times larger than in microzooplankton collection. Review by Bass and May, (1996) highlighted studies where up to 80% of smaller rotifers were lost using nets and sieves with mesh sizes as small as 45 µm.

The most common river inhabitants worldwide are rotifers of the genera: *Brachionus* and *Keratella*. They are known as 'generalists', filtering small chlorophytes, centric diatoms, detritus, and numerous components of the microbial food web (as reviewed by Lair, 2006). Rotifers families Synchaetidae (*Polyarthra* and *Synchaeta*) are successful due to their ability to thrive on flagellates, chlorophytes, centric diatoms, and cryptophytes of various sizes (with cells length up to 45 µm).

Table 2-5 Examples of metazoan communities and their sampling methodologies from several different studies worldwide

River	Volume (mesh size-µm)	Rotifers	Cladocera	Copepod	Reference
St Laurence River (Canada)	20 l (63)	Not analysed	Dominated by *Bosmina* spp.; *Chydorids*, *Daphnia, Ceriodaphnia*, and *Polyphemus. Calanoid* were found.	Dominated by *Eurytemora affinis*; *Leptodiaptomus*, *Diacyclops* with smaller numbers of *Mesocyclops* and *Acanthocyclops* were found	Casper and Thorp, (2007)

Po River (Italy)	60 l (50)	> 90 % of total zooplankton abundance *Brachionus calyciflorus, Keratella, Lecane, Synchaeta, Polyarthra, Filinia* and *Asplanchna*.	Never exceeded 72 ind l^{-1}, with an apparent prevalence of cyclopoid nauplii		Bertani et al., (2012)
Po River (Italy)	90 l (50)	85 – 99 % of total zooplankton density. *Brachionus calyciflorus*. Also: *Keratella, Lecane, Synchaeta, Polyarthra, Filinia* and *Asplanchna*	Less abundant than copepods *Moina* sp., *Bosmina* sp. Mostly nauplii	Copepod nauplii	Rossetti et al., (2009)
The River Danube, Austria	10-40 l (30)	98 % of the total zooplankton number. Of total density: *Synchaeta >50 %.* *Keratella* >15 % *Polyarthra approx.* 5 % *Brachionus* <5 % *Trichocerca 1.9-8.2%.*	Less abundant than copepods *Bosmina* sp.		Reckendorfer et al., (1999)
The River Danube (Austria)	10 l (37)	84.4 % of the total zooplankton number. *Polyarthra dolichoptera/vulgaris* *Synchaeta oblonga/temula,* *Keratella cochlearis, Brachionus angularis*, *Asplanchna*	*Bosmina* sp. >54% of crustacean biomass *Daphnia* sp.; *Chydorus* sp.	Cyclops and Acanthocyclops, nauplii	Baranyi et al. (2002)

The Rhine (Holland)	20-60 l (37)	97% and 84% at *Brachionus angularis, B. calyciflorus, Keratella cochlearis* and *K. quadrata*.	Cladocerans represented 1%	The relative contribution to total zooplankton was 2%- 15%. Cyclopoid nauplii	Van Dijk and van Zanten, (1995)
River Elbe (Germany)	2.25 l (30)	Of total abundance: *Trichocerca pusilla* (55%), followed by *Keratella cochlearis* (12.6%), *Synchaeta oblonga* (9.5%) and *Keratella cochlearis* (8.1%).	*Bosmina longirostris, Macrothrix laticornis,* and *Alona rectangula*	Cyclopoid nauplii	Zimmermann-Timm et al., (2007)
Saint John River (USA)	1 l (62)	*Brachionus havanaensis* and *Keratella cochlearis*	*Bosmina longirostris*		Leonard and Paerl, (2005)
The River Moselle (France)	15 l (63)	*Brachionus calyciflorus, Keratella cochlearis, Synchaeta and Polyarthra*	*Bosmina* sp.		Viroux, (1997)
The River Meuse (Belgium)	15 l (63)				
Nakdong River System	8 l (35)	98 % of the total zooplankton number. *Brachionus, Polyarthra, Keratella, Asplachna, Notholca*	*Bosmina* sp.; *Diaphanosoma* sp.		Kim et al., (2001)
Waikato River, New Zealand	1035 l (37)	85% of the total zooplankton *Keratella cochlearis* (60 %), *Trichocerca similis* (14 %), *Trichocerca pusilla* (7 %) and *Synchaeta oblonga* (4 %).	*9%* of the total zooplankton number. *Bosmina* sp. (51%); *Chydorus* sp.*; Daphnia* sp.	*6%* of the total zooplankton number. *51% nauplii* *83% Cyclopoids,* 13 % *Calanoids*	Burger et al. (2002)

The dominant cladoceran taxa in rivers are Bosmina and Chydorus. Copepods are generally represented by nauplii and juvenile forms (copepodites and nauplii) of Cyclopoids and Calanoids. Numerically, they tend to subordinate to rotifers (where the latter were sampled correctly). Dense populations of microcrustaceans may develop in connected retentive zones, such as impoundments and off-river waterbodies for instance marinas and gravel pits (as reviewed by Bass and May, 1996). Since the zooplankton communities in most large lowland rivers worldwide are dominated by rotifers, with less than 10% of crustacean (copepod) nauplii, this review is focused primarily on rotifers as the key metazoan grazers.

2.7 Rotifera

Rotifers are the smallest metazoans in freshwater plankton, they feed on most algae, bacteria, and detritus, representing an important pathway of energy flow and nutrient cycling in rivers. These are microscopic or near-microscopic aquatic animals that form a phylum Rotifera. In some old literature, Rotifera is defined as a class of phylum Aschelminthes (Wetzel, 2001), due to morphological similarities with worms. There are approximately 2000 species of rotifers, less than 5% are restricted to brackish and marine environments (Sládeček, 1983). About three-quarters of them are sessile, i.e. attached to the surface and associated with littoral substrates. Rotifers may be planktonic or benthic, and some can divide their life-time between open water and vegetation. Only about 100 species are truly planktonic. Fresh water rotifers are represented by two classes: Bdelloidea and Monogononta.

Rotifers show a wide range of morphological variations and adaptations which were reviewed in some detail by Pontin (1978), Sládeček (1983), Wallace & Smith (2001) and Wallace (2002). Most of the organisms have an elongated body or trunk, head and foot. The cuticle or skin is thin and flexible, although in many species it is thickened and stiffer; and in some, it forms a distinct shell or lorica. The head encloses the brain, from which a system of nerves radiates to all parts of the body. The anterior end or corona, also known as wheel-organ, is ciliated, it is used for swimming and collecting food. The mouth lies ventrally on the head. The digestive system contains a complex muscular pharynx and a set of jaws or *trophy* that functions to seize and disrupt food. The pharynx region with jaws is called *mastax*.

Makarewicz and Likens, (1979) showed that rotifers are significant components of the nutrient cycling and energy transfer within the freshwater ecosystem due to their high intrinsic metabolic rates. Rotifers have higher clearance rates per unit biomass than

cladocerans (5 to 13 times higher) and potentially can excrete phosphorus at a higher rate per unit biomass (Bogdan and Gilbert, 1982).

Unpolluted freshwater ecosystems generally tend to support less than 6000 ind l^{-1}. In contrast, in polluted rivers, rotifers can reach abundances higher than 20000 ind l^{-1} (reviewed by Sládeček, 1983).

2.7.1 Life cycle

The life span of many planktonic rotifers varies between 5 to 11 days in favourable temperatures conditions (19-25°C) Table 2-6. Egg laying and embryonic development intervals range between 1 to 4 days, and juvenile development does not exceed 10 days. Both egg laying and juvenile development are strongly related to temperature conditions, as was observed by Edmondson (1965); Herzig (1983); Galkovskaya (1987); Walz (1987).

Table 2-6 Mean life span of rotifers found in many large lowland rivers. Species names are listed in (Segers, 2007)

Species	T°C	Period of rotifer life span, days	Source
Brachionus calyciflorus	20	11	(Halbach, 1973)
Brachionus angularis	20	5	(Walz, 1987)
Keratella cochlearis	20	9	(Walz, 1987)
Euchlanis dilatata	22	7	(King, 1966)
Synchaeta pectinata	20	5	(Kirk, 1997)

2.7.2 Feeding

Most rotifers are non-predatory. Their food consists of small algae, detritus or bacteria collected either by the coronal cilia or browsed from the surface of vegetation. Predatory species, such as *Asplachna,* are typically large in body size (up to 600 μm) and prey upon small protozoa, rotifers and other micrometazoa of appropriate size (Wetzel, 2001).

Table 2-7 Rotifer functional groups based on their feeding strategies and body size (summarised from Obertegger et al. (2011)). Food preferences were review by (Walz, 1997). Species names are listed in (Segers, 2007)

Functional feeding group Name	Description	Species	Food preference, food cell length
Large microphagous (LM)	Malleate trophy, Large body size (length > 150 µm).	*Brachionus calyciflorus; Brachionus rubens; Brachionus quadridentatus*	*Bacteria, detritus, Chlorococcales, Volvocales, Euglenas and centric diatoms* Food size up to 12 µm
Medium microphagous (MM)	Malleate, malleoramate and ramate trophy. Medium body size (120 µm < length < 150 µm).	*Brachionus angularis; Keratella quadrata; Copepod larvae*	Bacteria, detritus, *Chlorococcales, Volvocales, Euglenales and centric diatoms* Food size up to 10 µm
Small microphagous (SM)	Maleate and malleoramate trophy. Small body size (length < 120 µm).	*Keratella cochlearis; Anuraeopsis fissa; Colurella* spp.*; Lecane* spp.*; Pompholyx sulcata*	Small flagellates, organic detritus, bacteria and algae. Food size up to 10 µm
Raptorial (R)	Virgate trophi	*Euchlanis dilatata*	Organic detritus, bacteria, *Cyanophicea* and *Cyclotella*
		Cephalodella gibba	Unicellular algae, flagellates and ciliates.
		Trichocerca spp.	Filamentous diatoms
		Synchaeta oblonga; Synchaeta pectinata	*Cryptomanas*, centric diatoms and dinoflagellates
		Polyarthra dolichoptera	*Flagellates, Cryptomanas* and *Euglena,* centric diatoms. Food size up to 45µm.
Predatory (P)	Incudate trophi	*Asplanchna priodonta*	Small rotifers and cladocerans, colonial algae and cyanobacteria

Rotifers show a variety of trophi types and coronal shapes, suggesting the importance of niche differentiation in their feeding strategy (Wallace, 2002). *Brachionus* and *Keratella* use ciliary currents to bring food particles into their mouth and can consume a broad variety of particle shapes and sizes, while *Synchaeta*, *Polyarthra*, and *Asplanchna* use a rapid sucking action to capture algae and are restricted to relatively large cells (Stemberger and Gilbert, 1985).

Obertegger et al. (2011) separated rotifers into two principal groups: raptorial and microphagous (Table 2 7). Raptorials grasp, pierce or pump to catch single food items. These are *Ascomorpha, Asplanchna, Collotheca, Gastropus, Ploesoma, Polyarthra, Synchaeta*, and *Trichocerca.* Raptorial rotifers, *Polyarthra* and *Synchaeta*, prefer food in a large size range 1-40 µm. Microphagous rotifers are microfiltrators of all particles ranging between 0.5 to 20 µm and sometimes up to 135 µm. These *are Brachionus, Conochilus, Euchlanis, Filinia, Floscularia, Kellicottia, Keratella, Lecane, Notholca, Anuraeopsis, Testudinella*, and *Trichotria*. Predators *Asplanchnids* are rotifers that feed preferentially on protozoans, small rotifers and all algae larger than 15 µm. Oh et al. (2017) compared trophi-based with taxon-based rotifer composition and concluded that trophies show a clearer relationship with water-quality variables. Every functional group, based on feeding strategies, responded differently to increasing eutrophication.

2.7.3 Ingestion and clearance rates

Clearance rate (*C*), formerly known as filtration rate is the volume of water cleared of food by a consumer organism per unit time and consumer or consumer mass. *Ingestion rate* (*I*) is defined as the amount (number or mass units) of ingested food per unit of time and predator. Calculation of ingestion rate is based on its relationship with clearance rate: $I = C \times d$. In this equation *d* is the mean food concentration, in an open-flow system, this is $I = (d_{out} + d_{in})/2$ (Båmstedt *et al.*, 2000).

There is no well recognised *standard method* to quantify zooplankton grazing, but various techniques have been utilised. These are: radioactive tracers, inert food particles, metabolic inhibitors, the disappearance rate of food, growth kinetics in cultures, preincubation size fraction, gut pigment content, dilution series, egg production (summarised by Hansen *et al.*, 1997). The accuracy of most measurements may be affected by incubation, the content of tracer food particles, biomass estimations and other experimental issues, adding that physiological rates measured in the laboratory conditions may not reflect the norm *in situ.*

To link laboratory results with feeding activities in the 'real' environment, various studies compared gut content analysis with communities of rotifers and phytoplankton or measured feeding rates using radioisotope techniques. Still, the direct field and *in situ* experimental results provide a coarse estimate of grazing (Båmstedt *et al.*, 2000). Hansen *et al*., (1997) suggested that in the absence of direct measurements, laboratory studies could be applied in phytoplankton models, dividing the zooplankton into functional groups to minimise the error of grazing estimation.

Rotifers grazing activity depends on water temperature, food quantity and quality (Stemberger and Gilbert, 1985). Food intake by rotifers increases with temperature (Bogdan and Gilbert, 1982; Galkovskaya, 1987). At low to moderate food concentration, clearances rates are constant, and ingestion rates increase proportionally to food density. At high food concentrations, clearance rate decreases with ingestion rate reaching its maximum. The Q_{10} value for rotifer maximum clearance is 2.4 and for copepods it is 3.2 (temperature change between 10 and 20°C) (Hansen *et al*., 1997). Ingestion rate approaches its maximum at high prey densities; the relationship could be described by Michaelis-Menten (Monod) equation (Boraas, 1983; Hansen *et al.*, 1997) (Figure 2-1).

The decrease in clearance rate could be caused by the hampering of the rotifer feeding apparatus with various floating particles, as a result of which, the ciliary activity gets completely inhibited. *Brachionus* rotifers can form deflecting particles 'screens' with cilia to prevent particular particles from entering rotifers mouth, significantly reducing clearance rates (Gilbert & Starkweather, 1977; Starkweather, 1980).

Ingestion rate $I(d)$:

$$I(d) = I_{max} * d/(K_m + d)$$

where K_m is the half-saturation food density, i.e. $I(K_m) = I_{max}/2$.

Clearance rate $C(d)$:

$$C(d) = I(d)/d = C_{max} * d/(K_m + d)$$

where C_{max} is maximum clearance obtained at low prey density. By inserting $d = K_m$ in Eq.1 and 2, it follows that

$$K_m = I_{max}/C_{max}$$

I_{max}, C_{max} and K_m are illustrated in Fig. 1 (Hansen et al., 1997).

Equation 2-1 Zooplankton ingestion rate I(d), and clearance rate $C(d)$

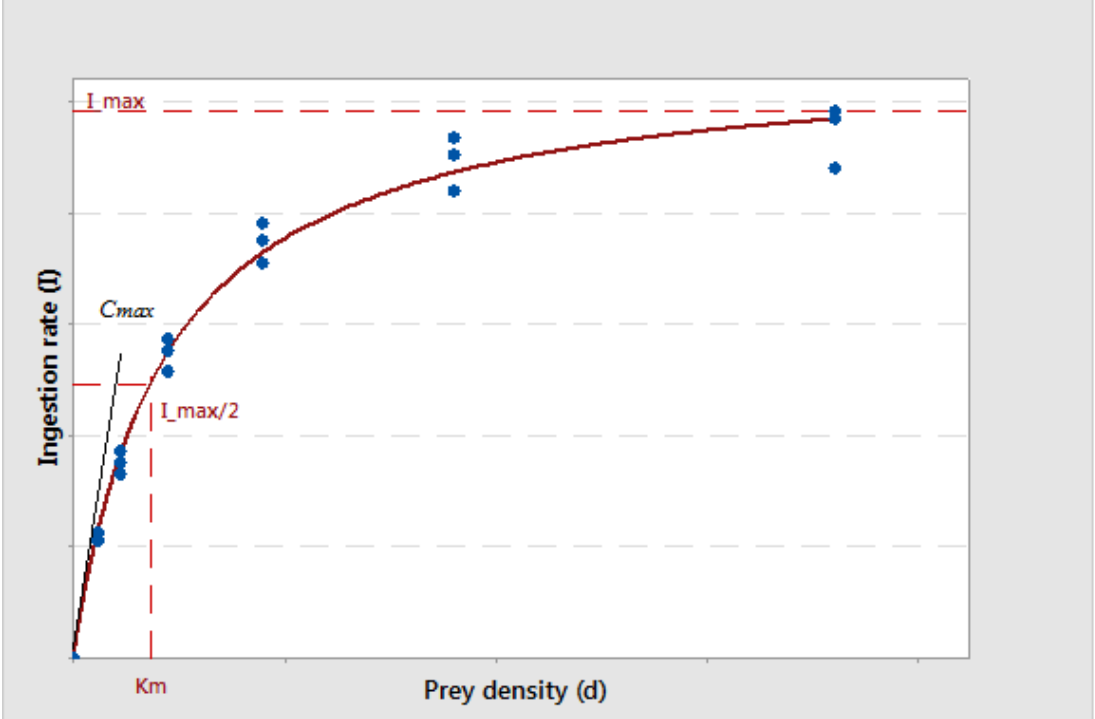


Figure 2-1 Michaelis-Menten kinetics illustrating the maximum ingestion Imax, maximum clearance Cmax and half-saturation constant Km. Equations are listed in Equation 2-1 — conceptual diagram designed using random dataset generated in Minitab 16.

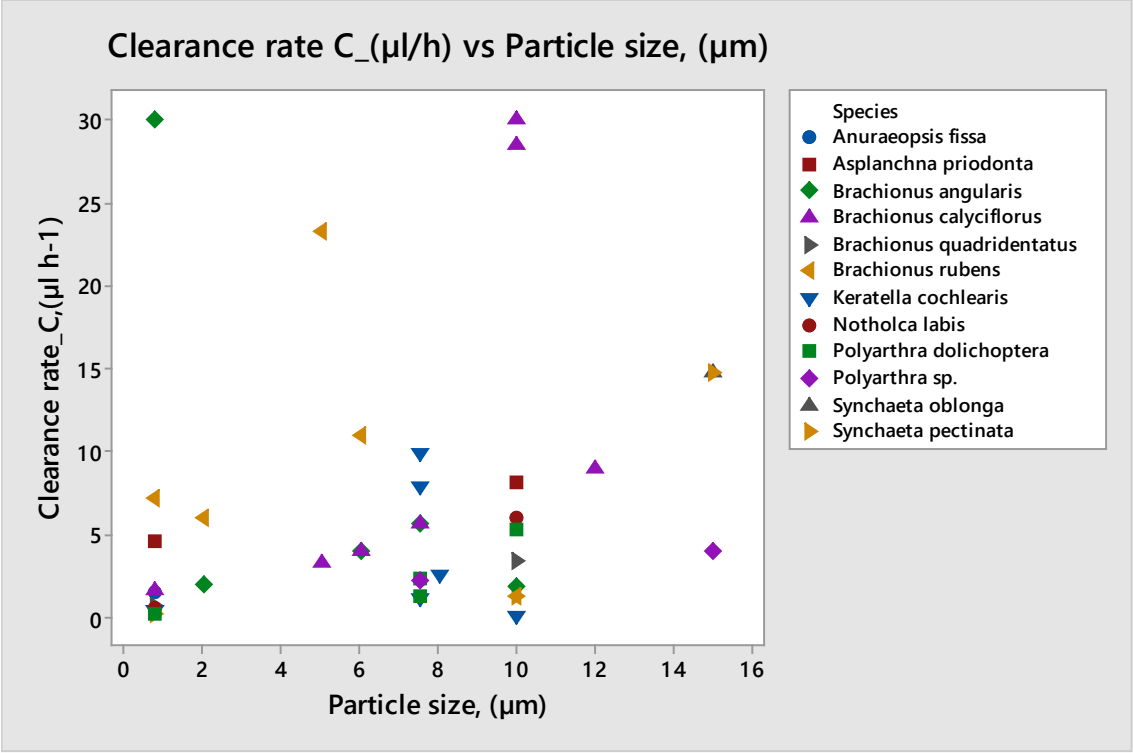


Figure 2-2 Clearance rate in relation to particle sizes for different rotifer species. Clearance rates and literature sources are listed in Table 2-8. Software: Minitab 16

For most of planktonic species found in the River Thames (UK), clearance rates vary between 0.5 and 10 µl h^{-1} (0.012 – 0.24 ml h^{-1}). Studies by Rothhaupt (1990a) and Kim et al. (2000) experimentally determined exceptionally high maximum filtration rates up to 30 ml h^{-1} (0.72 ml h^{-1}) for the Brachionus rotifers (Table 2 8). Still, rotifer filtration rates are significantly smaller than those of cladocerans (Chydorus - 108 ml h^{-1}, Daphnia – 775 µl h^{-1}) and copepods 38 ml h^{-1} but not copepod nauplii.6 ml h^{-1}. The riverine microcrustaceans communities are dominated mostly by copepod nauplii.

Both micro-crustaceans and rotifers feed selectively. Rothhaupt (1990a) established that *Brachionus calyciflorus* prefer small centric diatoms over *Chlamydomonas* (green algae), while *Brachionus rubens* feed mostly on *Chlamydomonas*. Figure 2-2 demonstrates clearance rates of different rotifers in relation to particle sizes.

Table 2-8 Individual clearance rates for rotifers and microcrustaceans (species previously found in the River Thames (UK))

Species	Rotifer Length, µm	Particle size, (µm)	Clearance rate, C (µl h^{-1})	T (°C)	Source	
Brachionus calyciflorus	220-285	*Cyclotella* sp. 10	30 ± 42*SD*	20	Radiotracers experiment	Rothhaupt, (1990a)
		2	nd	20	Experiment (polystyrene spheres and variety of algal cells)	Rothhaupt, (1990b)
		6	4	20		
		12	9	20		
		0.75	1.6	20	Experiment fluorescent microspheres	Kim et al., (2000)
		10	28.5	20		
		5-10	5.6	20	From literature	Keckeis et al., (2003)
			3.3		From literature	Reynolds, (1986)
Brachionus angularis	100-150	2	1.9	20	Experiment (polystyrene spheres and algae)	Rothhaupt, (1990b)
		6	4	20		
		0.75	30	20	Experiment fluorescent microspheres	Kim et al., (2000)
		10	1.8	20		
		5-10	5.61	20	From literature	Keckeis et al., (2003)
Brachionus rubens	200-260	*Chlamydomonas* sp. *5*	23.3	20	Radiotracers experiment	Rothhaupt, (1990a)
		2	6	20	Experiment (polystyrene spheres and algae)	Rothhaupt, (1990b)
		6	11	20		
		0.75	7.2	20		

		10	1.2	20	Experiment fluorescent microspheres	Kim et al., (2000)
Brachionus quadridentatus	200-260	0.75	0.7	20	Experiment fluorescent microspheres	Kim et al., (2000)
		10	3.4	20		
Keratella cochlearis	80-120	0.75	0.4	20	Experiment fluorescent microspheres	Kim et al., (2000)
		10	0.08	20		
		Aerobacter <0.75	0.46-small 0.29-large	25	Experiment radioactive tracer cells	Bogdan et al., (1980)
			0.47-large	21		
		Chlamydomonas sp. 5-10	0.76-small 6.41-large	19		
			1.12-small 6.05-large	20		
			0.75-small 8.13-large	25		
		Rhodotorula, cells 2.7 -8.7 µm long	2.53-small 7.39-large	20		
		5-10	9.9 7.9 4.2 3.3	25* 20* 25** 20**	Experiment *in situ* radioisotope technique Calculated from the regression equation: C = 0.405*T*-0.2088*, May-June C = 0.173*T*-0.119**, Aug - Feb *T*(°C) – temperature	Bogdan and Gilbert, (1982)
Keratella quadrata	120	5-10	3.3	20	From Bogdan and Gilbert, (1982)	Keckeis et al., (2003)
Anuraeopsis fissa	80	0.75	1.5	20	Experiment fluorescent microspheres	Kim et al., (2000)
Notholca labis	100	0.75	0.5	20	Experiment fluorescent microspheres	Kim et al., (2000)
		10	6	20		
Synchaeta pectinata	160-200	0.75	0.13	20	Experiment fluorescent microspheres	Kim et al., (2000)
		10	1.3	20		
		11-20	14.8	3.8	From literature	Keckeis et al., (2003)
Synchaeta oblonga	100-120	11-20	14.8	3.8	From literature	Keckeis et al., (2003)
Polyarthra dolichoptera	100-120	*Chlamydomonas*, 5-10	1.69	19	Experiment radioactive tracer cells	Bogdan et al., (1980)
			1.58	20		
			2.36	25		
		0.75	0.2	20	Experiment fluorescent microspheres	Kim et al., (2000)
		10	5.3	20		
		5-10	0.85-1.3	3.5-6.8	Experiment *in situ* radioisotope	Bogdan and Gilbert, (1982)

					Value calculated from the regression equation: C = 0.130*T*+0.395, Nov-Feb	
Polyarthra sp.	100-120	5-10	2.2	19-25	Value calculated from the regression equation: C = 0.127*T*-0.934, May-June	Bogdan and Gilbert, (1982)
		11-20	4	3.1	From literature	Keckeis et al., (2003)
Asplanchna priodonta	350	0.75	4.6	20	Experiment fluorescent microspheres	Kim et al., (2000)
		10	8.1	20		
Bosmina longirostris	> 1000	11-20	4.3	3.5-25	Experiment *in situ* radioisotope technique Value calculated from the regression equation: C = 0.132*T*+0.999, Aug-Feb	Bogdan and Gilbert, (1982)
Bosmina longirostris		0.75	36	20	Experiment fluorescent microspheres	Kim et al., (2000)
		10	7	20		
Chydorus sp.	> 1000	12-23	21	10	Experiment the isotopic labeling technique	Thompsonm et al., (1982)
		12-23	108	20		
Daphnia spp. Body length *1.3-1.6mm*	1300-1600	20	775	15-20		
Copepod copepodids	>1000	0.75	0.04	15	Experiment fluorescent microspheres	Kim et al., (2000)
		10	12.5	15		
		11-20	38	15	From literature	Keckeis et al., (2003)
Copepod nauplii	~500	0.75	0.1	20	Experiment fluorescent microspheres	Kim et al., (2000)
		10	4.6	20		
		11-20	4.2	15	From literature	Keckeis et al., (2003)

2.7.4 Particle size as an important feature of food quality

Individual clearance rates for rotifers and microcrustaceans are listed in Table 2-8. Consumer size in rotifers is directly coupled with food size. Hansen *et al.,* (1994) estimated a linear size ratio between predators and their optimal prey for *Brachionus* rotifers as 18:1. Nevertheless, this size dependancy rule does not hold for all species. Small *Anuraeopsis* with body length 40-75 µm can feed on *Scenedesmus* sp. (10-13 µm). *Polyarthra* (90-120 µm) consume cells up to 45 µm long. *Synchaeta* feed on large protozoans (100 µm) (Walz, 1997; observed in the study).

Rothhaupt, (1990b) in the experimental study showed that *Brachionus calyciflorus* preferred particles of approximately 10 µm equivalent spherical diameter (*ESD*), while *B. angularis* fed on food items <5 µm (*ESD*). Selective feeding did not occur within one species, at the same time, ingestion rates were not constant over the range of ingestible particle sizes. Rothhaupt (1995) observed *Brachionus* feeding unselectively and concluded that algal nutrient limitation only affects rotifer growth but not ingestion (Rothhaupt, 1995).

2.7.5 Reproduction

Rotifers can reproduce both asexually and sexually. Many planktonic species may alternate these phases (Pontin, 1978). One female rotifer can produce three types of eggs. Amictic females produce *asexual* **diploid** *eggs* which cannot be fertilized; they then develop into amictic or mictic females (if the environmental conditions rapidly changed). Mictic females produce *sexual* **haploid** *eggs*, which develop into males if unfertilized. Male rotifers fertilize sexual eggs restoring the diploid state and forming *resting eggs*. Resting eggs resist extreme conditions and remain dormant for several days, months and even years (Ricci, 2001). These eggs stay within the bottom sediment until environmental conditions (water temperature, flow, oxygen levels, light factors) are favourable again; they then develop into amictic females with hatching rates close to 100% (Ricci, 2001). The period of dormancy extends longer than the persistence of unfavourable conditions, however, resting eggs viability is not affected by increasing duration of dormancy (Pourriot and Snell, 1983). Minimum periods of dormancy vary between species (Table 2-9).

Pourriot and Snell (1983) identified two patterns of resting egg hatching. Rotifers develop either at regular intervals over an extended period or by the synchronous hatching of large numbers of eggs over a short period (a few days) following a phase of inhibition. Massive and synchronised hatching does not happen until some days after applying optimal conditions which suggest that the embryo does not accomplish its development in several stages and remains undifferentiated until it receives an adequate hatching stimulus (Pourriot and Snell, 1983). Monogonont rotifers cannot survive harsh conditions as adults, but bdelloid rotifers can respond to changes in the environment, mainly the evaporation of water, by losing internal water and entering a particular form of dormancy, called anhydrobiosis (Ricci, 2001)

Table 2-9 Length of dormancy and influence of light on some monogonont rotifer species (Pourriot & Snell, 1983)

Species	Minimum duration of dormancy at 18°C, days	Influence of light (0) No effect; (+) Positive effect
Brachionus calyciflorus	2-6	0
Brachionus rubens	14	+
Brachionus angularis	3-10	0
Brachionus plicatilis	28-29	+
Notomatta copeus	55-90	0

2.7.6 Life strategy

Rotifer life strategies vary among species (Walz, 1997). *Brachionus* spp. are known as *r*-strategists due to high population growth and mortality rates. One female can produce up to 23 eggs at a time and low energy cost. During high quality food availability rotifers, r-strategists are at an advantage compared to other metazoans. They have a shorter lag period (2-3 weeks shorter) than crustaceans which allows them plenty of time to explore growing food resources (Lynch, 1980). In contrast, *Keratella* spp. are *K*-strategists with lower growth rates than *Brachionus* and lower mortality rates in pre-productive individuals. *Brachionus* spp. have greater individual body sizes, while *Keratella* spp. have smaller bodies, need less material for the growth of somatic tissues, and allocate their ingested material to form 'energy efficient' eggs. K-strategists are at an advantage when food levels are low. K-strategists tend to live longer and do not need to balance high mortalities with high growth rates (Walz, 1987).

2.7.7 Factor regulating rotifer population dynamics

Temperature

Thermal properties of the environment are one the most important factors determining the population dynamics of rotifers since an increase in water temperature shortens individual egg laying intervals and length of embryonic and post embryonic development times Table 2-10 (Galkovskaya, 1987). Herzig (1983) and Walz (1987) calculated the length of embryonic development (e.d.) in relation to water temperatures for several rotifer species Figure 2-3, 4.

Figure 2-3,4 demonstrate that rotifers life-cycles become quicker in the warming environment. Rotifers have a very wide tolerance to water temperatures occurring between 4 and 25°C Table 2-11 (Berziņš and Pejler, 1989).

Table 2-10 Duration of embryonic development (D, days) in different water temperatures (t,°C)

Species	Temperature, (°C)					Source Regression equation
	5	10	15	20	25	
Brachionus calyciflorus	3.5	1.8	1.1	0.8	0.6	Herzig, 1983 $D=117*(t+4.2)^{-1.583}$
Brachionus angularis	4.6	2.9	1.1	0.7	0.6	Walz, 1987 Determined experimentally
Keratella cochlearis	5.8	1.9	1	0.6	0.5	Herzig, 1983 $D=45*(t-0.84)^{-1.44}$
Keratella cochlearis	4.5	2.5	2	1.3	0.9	Walz, 1987 Determined experimentally
Keratella quadrata	3.5	1.7	1.1	0.8	0.6	Herzig, 1983 $D=28*(t+0.95)^{-1.162}$
Euchlanis dilatata	6.1	2.1	1.4	1.1	0.9	Herzig, 1983 $D=8*(t-3.53)^{-0.705}$
Synchaeta pectinata	3.6	1.5	0.9	0.6	0.4	Herzig, 1983 $D=119*(t+2.64)^{-1.721}$
Polyarthra dolichoptera	5.3	2.1	1.1	0.7	0.4	Herzig, 1983 $D=899*(t+4.78)^{-2.248}$

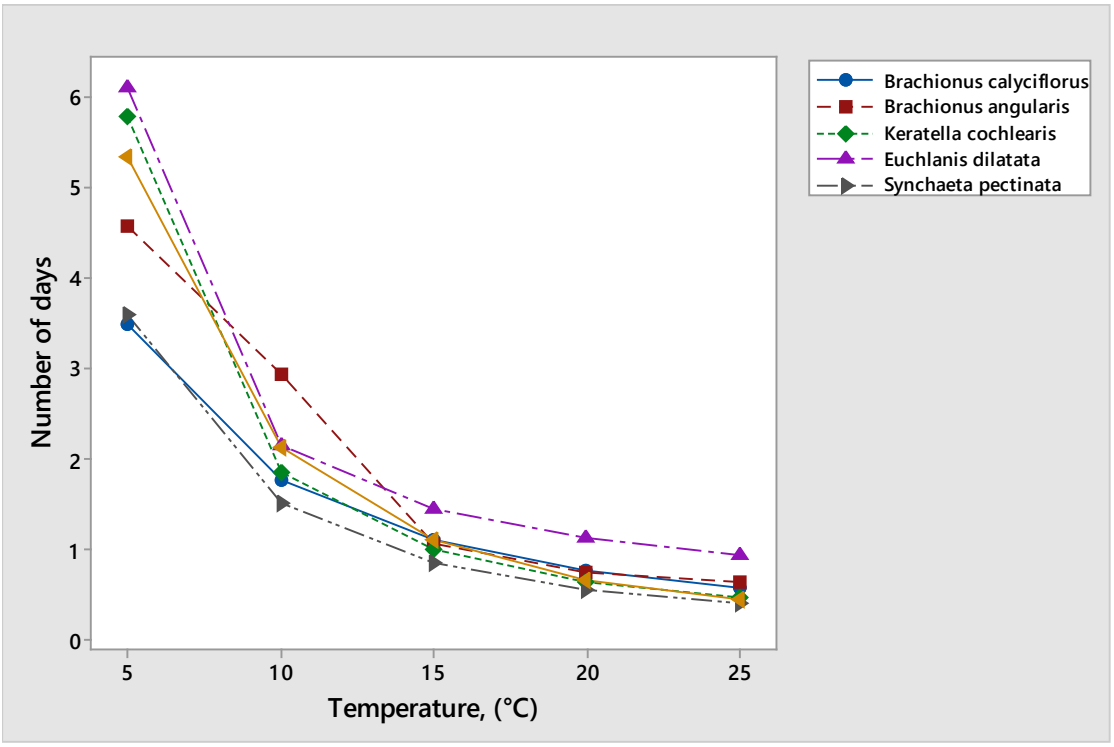


Figure 2-3 Duration of embryonic development of *Brachionus calyciflorus*; *B. angularis*; *Keratella cochlearis*; *K. quadrata; Euchlanis dilatata; Synchaeta pectinata; Polyarthra dolichoptera* (days) in relation to water temperatures (°C)

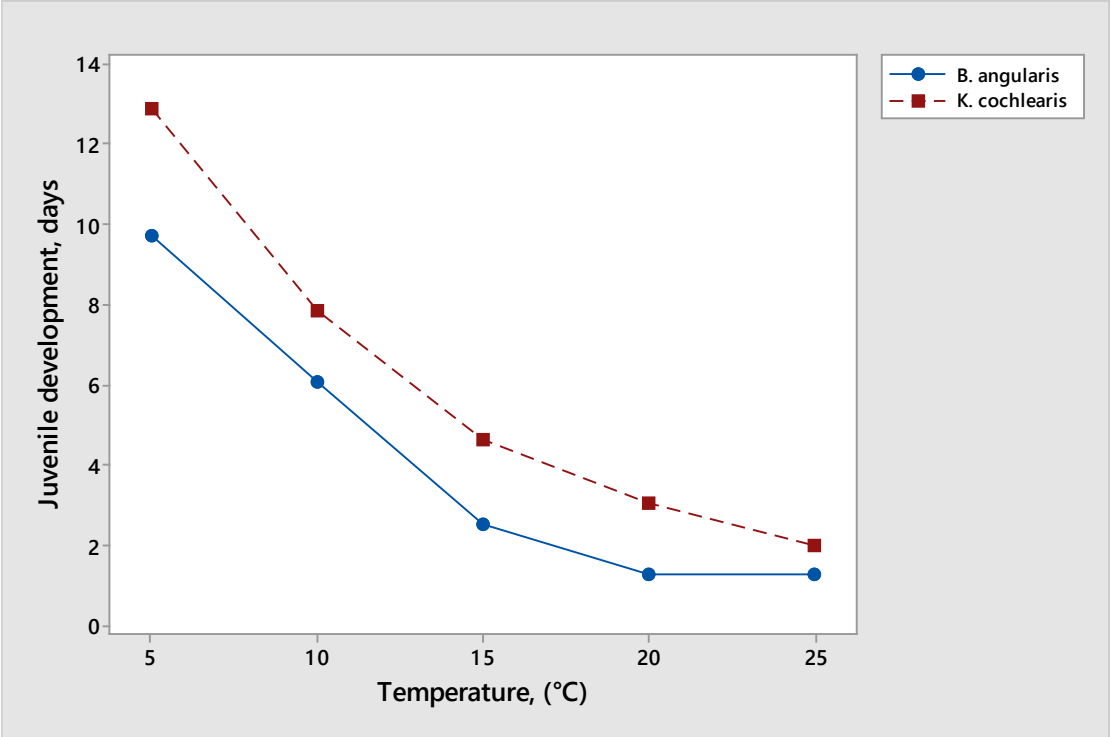


Figure 2-4 Juvenile development of *Brachionus angularis*; *Keratella cochlearis* (days), in relation to water temperature (°C). Data from Walz (1987). Software: Minitab16.

Table 2-11 Occurrence of rotifers in relation to water temperatures. Minimum and maximum temperatures for relatively high abundances, and minimum, optimum and maximum temperature for maximum abundances (Berziņš and Pejler, 1989)

Species	High abundance	Maximum abundance			High abundance
	T_{min} (°C)	T_{min} (°C)	T_{opt} (°C)	T_{max} (°C)	T_{max} (°C)
Brachionus calyciflorus	11.3	13.5	18.0	22.9	23.1
Brachionus angularis	2.6	12.0	15.9	22.5	24.8
Keratella cochlearis	0.0	0.8	14.6	24.4	27.8
Keratella quadrata	0.5	0.5	12.0	21.8	25.5
Anuraeopsis fissa	0.0	1.1	14.4	22.1	29.3
Notholca squamula	0.0	0.0	4.1	11.3	20.6
Euchlanis dilatata	3.4	11.6	18.0	23.6	25.5
Cephalodella gibba	0.0	7.9	15.4	21.0	28.1
Synchaeta oblonga	0.0	0.8	10.7	19.5	25.9
Synchaeta pectinata	0.0	0.8	12.4	22.1	27.0
Polyarthra dolichoptera	0.0	0.8	12.2	19.5	23.6
Asplanchna priodonta	0.0	1.1	14.3	22.1	29.3
Pompholyx sulcata	5.6	11.3	16.1	19.5	25.9

Table 2-11 is based on the study of rotifers in Sweden. Berziņš and Pejler (1989) combined data from 350 lakes, 50 ponds, 20 pools, 15 bogs and 150 running water locations in Southern and

Central Sweden. Samples were collected during 'ice-free' seasons, as a result, winter rotifers were underrepresented. This explains the differences in temperature optimums for *Brachionus calyciflorus* from those described by Herzig, (1983). A study of *B. calyciflorus* from the River Thames (UK) indicated some cold-adapted characteristics, since their reproduction peaked at temperatures between 2°C and 15°C (February-April) (Bottrell, 1977). The Thames study also highlighted the existence of 'thermophilic clone' of *B. calyciflorus* with optimum temperatures higher than 14°C found in Spanish reservoirs (De Manuel, 2000). *Keratella quadrata* also showed some 'climatic' variation in growth and reproduction processes. *Keratella* sp. from the River Thames had shorter development times at temperatures between 5°C and 20°C, than those from Spitsbergen, Norway and Neusiedler See, Austria/Hungary (Herzig, 1983). Barrabin, (2000) summarised data from Spanish reservoirs (Table 2-12). Differences in temperature optimums for the same rotifer taxa in Sweden and Spain is a result of organisms adaptating to their native climatic conditions (Herzig, 1983).

Table 2-12 Occurrence of rotifers in relation to pH and temperature (Berziņš and Pejler, 1989; Barrabin, 2000)

Species	pH	*T(°C)[1]	*T(°C)[2]
Brachionus calyciflorus	6.6-9.1	7.7-26.1	11.3-23.1
Brachionus angularis	7.7-9	10.2-26.1	2.6-24.8
Brachionus quadridentatus	8.1-9.1	16.3-24	-
Keratella cochlearis	6.3-10.1	6-26.7	0-27.8
Keratella quadrata	6.64-10.18	6.4-26.1	0.5-25.5
Anuraeopsis fissa	8-9.16	23-25.5	0-29.3
Notholca squamula	7.9-8.5	8.4-12.3	0-20.6
Notholca labis	7.53	9.5	-
Epiphanes sp.	7.52	9.87	-
Euchlanis dilatata	6.3-9.6	6.4-24	3.4-25.5
Cephalodella gibba	6.6-8.48	6.4-18.8	0-28.1
Synchaeta oblonga	6.3-8.8	5.9-22.8	0-25.9
Synchaeta pectinata	6.3-9.3	5.9-25.5	0-27
Polyarthra dolichoptera	6.33-9.18	5.9-26.2	0-23.6
Asplanchna priodonta	6.3-9.67	5.9-25.2	0-29.3

*Rotifer study in: [1]- Spain (Berziņš & Pejler, 1989); [2]- Sweden (Barrabin, 2000)

Dong Xi & Zhang (2009) identified that different strains of the same rotifer species may respond differently to similar temperature regimes and food availability. The authors incubated four strains of *Brachionus calicyflorus* from Jinghu Lake (China) and determined their survival and reproduction rates variations.

Table 2-13 Maximum daily growth rates for *Brachionus, Keratella, Synchaeta, Polyarthra* under a range of 'optimal' temperature conditions

Species	Body volume (μm^3) (reviewed by Hansen *et al*. 1997)	T(°C)	μ_{max} 1 day^{-1}	Methodology	Reference
Brachionus calicyflorus	1.61	20	1.3	Lab. experiment. 5 rotifers were placed in 5 tubes with different algal biomass. 20(°C). 14:10 (light:dark photoperiod). After 3-4 rotifer carbohydrate content was measured	Guisande and Mazuelos (1991)
Brachionus calicyflorus	1.15	25	1.3	Chemostat experiment *Chlorella* was used as a food source	Boraas (1983)
Brachionus calicyflorus	1.5	20	1.02	Lab. experiment. *B. calyciflorus* had highest μ_max feeding on *Cyclotella meneghiniana*.	Rothhaupt (1990)
Brachionus calicyflorus	0.97	19	0.82	Lab. experiment. Rotifers were fed *Cryptomonas erosa* v. reflexa	Stemberger and Gilbert (1985)
Brachionus rubens	1.5	20	0.84	Lab. experiment. *B. rubens* had highest μ_max feeding on *Cyclotella meneghiniana*.	Rothhaupt (1990c)
Keratella cochlearis	0.075	19	0.28	Lab. experiment. Rotifers were fed *Rhodomonas minuta*	Stemberger and Gilbert (1985)
Euchlanis dilatata	4.37	22	0.72	Lab. experiment. Rotifers were fed *Chlacmydomonas reinhardti* and *Euglena* sp.	King (1966)
Synchaeta oblonga	0.26	19	0.28	Lab. experiment. Rotifers were fed *Cryptomonas erosa*	Stemberger and Gilbert (1985)
Synchaeta pectinata	0.86	19	0.8	Lab. experiment. Rotifers were fed *Cryptomonas erosa*	Stemberger and Gilbert (1985)
Polyarthra remata	0.304**	19	0.39	Lab. experiment. Rotifers were fed *Cryptomonas erosa*	Stemberger and Gilbert (1985)

**Source: Telesh et al. (1998)

Michaloudi et al. (2018) in a more recent study of *Brachionus calyciflorus* applied the approach of reverse taxonomy and established the existence of four putative species*: B. dorcas, B. elevatus, B. calyciflorus and B. fernandoi* based on molecular species delimitation techniques.

All four species were previously considered as *Brachionus calyciflorus.* The authors highlighted specific morphological traits that were found to be particularly useful for the distinction between species of the *B. calyciflorus* complex.

Reversed taxonomy can successfully be applied to other rotifer species. The *Brachionus calyciflorus* molecular species delamination study demonstrated that the available identification literature and approaches are relatively outdated. Rotifers are small animals, and their morphological characteristics in particular the feeding apparatus are often hard to distinguish without the use of powerful microscopes, such as scanning electron microscopes (SEM) (Hochberg et al., 2015).

Stelzer, (1998) experimentally determined the relationship between water temperature and rotifer population growth rates. Three rotifer species (*Asplanchna priodonta, Brachionus calyciflorus* and *Synchaeta pectinata*) were grown under different temperature conditions at high food concentration (1 mgC l^{-1}) of *Cryptomonas erosa*. *Synchaeta* was better adapted to low temperatures than the other two rotifers and could be the superior competitor below 16 ◦C. Maximum rotifer population growth rates (Table 2-13) increased with body sizes, possibly because larger organisms tend to produce disproportionately smaller eggs (Stemberger et al., 1987).

pH

The effects of pH on freshwater rotifer population dynamics have been summarised based on the field data from various ecosystem types (Bērziņš & Pejler, 1987; Barrabin, 2000; Deneke, 2000). Zhao et al. (2017) in a study of river plankton communities, described pH as one of the key variables explaining rotifer diversity and spatial distribution across the river catchment.

Occurrences of rotifers species in relation to pH, conductivity, alkalinity and water temperature are summarised in Table 2-12. Three ecological groups can be distinguished in relation to pH (Sládeček, 1983).

Alkaline water rotifers are *Keratella cochlearis tecta*, *Brachionus angularis*, *Brachionus calyciflorus*, Brachionus sp., *Synchaeta* sp*., Asplanchna* sp*., Anaraeopsis sp., Mytilina* sp*., Filinia* sp*., Eosphora* sp*., Notholca* sp. Although, some listed genera can show acidophilic species (Table 2-14). For example, *Brachionus sericus* is typically found in lakes with a pH slightly below 3 (Deneke, 2000). **Transition rotifers** occur in both alkaline and acid waters

(most rotifers). **Acidophilic rotifers** are *Cephalodella gibba, Lepadella, Lecane, Monostyla, Trichocerca, Dicranophorus*. Some acidophilic genera show transition and alkaline species. *Cephalodella gibba* was found thriving in lakes with a pH ≤ 3 (Deneke, 2000), and reservoirs with pH > 8 (Barrabin, 2000). According to some studies reviewed by Sládeček (1983), alkaline waters tend to support higher rotifer abundance but smaller diversity than acidic waters. Thus, pH is related to many other chemical parameters, which themselves influence rotifer dynamics.

Table 2-14 Occurrence of rotifers in relation to pH, conductivity, alkalinity and water temperature (Barrabin, 2000)

Species	Conductivity μS cm^{-1}		Alkalinity, meq l^{-1}		pH		Temperature, °C	
	min	max	min	max	min	max	min	max
Brachionus calyciflorus	22	3720	131	3667	6.6	9.1	7.7	26.1
Brachionus angularis	169	3720	1157	3038	7.7	9	10.2	26.1
Brachionus quadridentatus	197	766	1679	3546	8.1	9.1	16.3	24
Keratella cochlearis	14.7	5326	58	4681	6.3	10.1	6	26.7
Keratella quadrata	19.6	5327	98	4682	6.64	10.2	6.4	26.1
Anuraeopsis fissa	183	351	1297	1652	8	9.2	23	25.5
Notholca squamula	189	1126	689.5	4682	7.9	8.5	8.4	12.3
Notholca labis	112	112	779	779	7.53	7.5	9.5	9.5
Euchlanis dilatata	19.6	823	98	3757	6.3	9.6	6.4	24
Synchaeta oblonga	35	5327	58	4682	6.3	8.8	5.9	22.8
Synchaeta pectinata	14	1523	58	4682	6.3	9.3	5.9	25.5
Polyarthra dolichoptera	27	3590	98	3872	6.3	9.18	5.9	26.2

Food quantity and quality

Food limitation is one of the most critical factors for structuring zooplankton communities. There is a positive relationship between food abundance and rotifer reproduction rate (Edmondson, 1965; Galkovskaya, 1987; Guisande and Mazuelos, 1991; Stemberger and Gilbert,

1985). However, rotifers can adapt and respond in many ways to changes in food concentration. For example, they can alter rates of egg production, hatching success, adult and egg sizes, reproduction mode, and lifespan (Boraas, 1983; Guisande and Mazuelos, 1991).

Galindo et al. (1993) experimentally determined that food concentration can significantly affect egg volumes of four rotifer species (*Brachionus calyciflorus; Brachionus angularis, Keratella quadrata, Anuraeopsis fissa).* Below a certain food level, egg and body volumes were small; they increased to a maximal size when food concentrations were raised but then reduced again as more food was available. Therefore, the presence of rotifer eggs and bodies with maximum volumes may define optimal food levels of planktonic species. Below optimal food levels, egg sizes and femail body lengths evidently reduced. Above the optimal level, rotifer life cycle accelerated, and adults matured to smaller sizes with larger number of smaller eggs.

Threshold food levels in rotifers have generally been determined on the population level and are defined as the food concentration at which population growth is zero (Stelzer, 1998). According to Stemberger and Gilbert (1985), threshold food levels can vary considerably (by up to a factor of 17) among species, with larger individuals having higher threshold levels.

The study by Stemberger *et al.* (1987) emphasised that small species of rotifers should experience an energetic advantage over larger ones when resource levels are consistently low. This advantage could be explained by the greater individual mass-specific energy intake compared to mass-specific respiration. At high food concentrations, however, such a relationship may change because large species collect food and divert it into storage and population growth with higher capacity. 'If food resources rapidly fall below the threshold food requirements of small species, then large species, being more resistant to short-term starvation, should have a competitive advantage over smaller ones' (Stemberg*er et al*., 1987). Regression analysis of food thresholds, clearance rates, swimming speeds and respiration demonstrated that food thresholds increase with larger rotifer body sizes (Stemberger et al., 1987).

Rothhaupt (1990a) demonstrated that favorable food concentrations are related to food quality. The daily food intake by rotifers may reach more than 25 times their body mass. However, only 40% of assimilated food tend to be used for growth (Galkovskaya, 1987).

2.8 Pathogens of phytoplankton

Algal-lysing bacteria, fungal parasites and viruses are the primary pathogens of phytoplankton species. Host-specific to some degree, they can cause rapid lysis (rupture of a cell membrane) to a wide range of unicellular and filamentous algae (Reynolds, 1984).

2.8.1 Algicidal bacteria

Diatom-bacteria interactions are a complex subject. These organisms coexisted in common habitats for more than 200 million years developing relation- ships over evolutionary time scale (Amin et al., 2012). Bacteria can be saprophytes, they colonise dead diatoms, decompose organic matter, and play an essential role in silicon regeneration (Bidle and Azam, 2001). In lakes and rivers, saprophytes are generally found in the bottom sediments (Zakharova et al., 2013). Recent studies, reviewed by Amin et al. (2012), indicate that there are also bacteria that consistently associate with living diatoms. These are either free-living cells (Blackburn et al., 1998), attaching to diatoms (Gärdes et al., 2011) or occurring as an intracellular algal symbiont (Schmid, 2003). Bacteria can act synergistically with algae. Croft et al. (2005) described how some algal species obtain vitamin B12 through a symbiotic relationship with bacteria. Some bacteria can compete with algae for nutrients. As a result, indirectly increasing algal mortality. Gärdes et al. (2011) experimentally determined the role of attaching bacteria in stimulating diatom clustering and sinking.

There are some bacteria that specialise in an algal-lysing lifestyle and directly inhibit algal growth (Mayali and Azam, 2004). Jung et al. (2008) isolated and studied the strain of *Pseudomonas fluorescens* HYK0210-SK09 that was successfully suppressing populations of *Stephanodiscus hantzschii* in a controlled environment and an indoor mesocosm experiment. These bacteria attach to the host cells with sticky pili (hair-like external cell appendage), then degrade them with lysozyme-like enzymes (Baker and Herson, 1978). This algicidal activity, however, is inhibited when water temperatures are lower than 10°C, as was described in field mesocosm experiments conducted by Kang et al. (2011). Paul and Pohnert (2011) studied mechanisms by which *Kordia algicida* interacted with host diatoms and emphasised that the release of active enzymes depends on the density of bacteria population rather than diatoms.

2.8.2 Fungal parasites

Fungal parasites and saprophytes of algae belong mainly to the order Chytridiales (Reynolds, 1984). Chytrids are spread as free-swimming, uniflagellate zoospores that seek out suitable hosts to grow on. They penetrate the host cell with a fine mycelial thread and draw nutrients back to the infective zoospores, which then enlarge into a spherical sporangium. Sporangium reaches maturity and releases next generation of zoospores. Infection by fungi usually kills the host cell. The parasite population can rapidly reach 'epidemic' proportion (Kagami et al., 2007). Scholz et al. (2014) identified up to five different species of chytrids within several diatom taxa in marine benthic sediment samples. In rivers, chytrids were observed reducing the abundance of the dominant spring diatoms and infecting multiple species throughout the year (Maier and Peterson, 2017). Frenken et al. (2016) in mesocosm experiments determined that higher water temperatures increase chytrid activity and accelerate the termination of a phytoplankton spring bloom.

2.8.3 Viral pathogens

Recent studies identified several viruses that effectively suppress di- atom populations in the marine environment (Nagasaki, 2008; Tomaru et al., 2012; Kimura and Tomaru, 2015). They accumulate in the host cell cytoplasm and nucleus and trigger cell lysis generally in less than 48 hrs (Nagasaki, 2008). Water temperature (Kimura and Tomaru, 2015) and light intensity (Baudoux and Brussaard, 2008) are essential factors controlling the relationship between algal hosts and their viral pathogens. Kranzler et al. (2019) in a study of marine diatoms and host-associated diatom viruses described how dissolved silicon limitation facilitates virus infection and mortality in diatoms.

2.9 Zebra mussel

Several studies expressed and an opinion that phytoplankton development can be suppressed by zebra mussels (Basu & Pick, 1997; Bettinetti et al., 2000; Scherwass et al., 2010). These studies, however, did not focus on the mussel's population dynamics or feeding activities (filtration rates, and selective feeding).

In the UK, the zebra mussels (*Dreissena polymorpha*) were first recorded in 1826. These organisms have lived in the River Thames for more than 100 years. The unpublished survey conducted by the Centre for Ecology and Hydrology in May 2010 (using the pump-sampling technique) showed that zebra mussels inhabit the lower reaches of the Thames and their

population abundances may spatially vary between 10 ind m^{-2} (Wallingford), 175 ind m^{-2} (Sonning) and 35 ind m^{-2} (Runnymede). These numbers correspond with the Thames survey conducted in 2002 (Aldridge et al., 2004).

At this point it is important to emphasize that in lake and rivers where *Dreissena polymorpha* significantly affected food webs, their abundances reach 10000 ind m^{-2} as was observed in the Shannon River System, Ireland (Minchin & Zaiko, 2013), or over 100000 ind m^{-2} in Lake Erie, US (Bunt et al., 1993). Zebra mussel abundances in the Thames tend to be more than a hundred times smaller than the abundances which may evidently influence the ecosystems they inhabit (some exampels are: Mellina and Rasmussen, 1994 *the St. Lawrence and Hudson rivers and Oneida Lake, New York*; Fahnenstiel et al., 1995; Botts, Patterson and Schloesser, 1996; Vanderploeg *et al.*, 2001 *the Great Lakes*). Seasonal phytoplankton (diatom) blooms in the Thames can be as intensive as in lakes or marine environments (Bowes et al., 2016), so it is plausible to suggest that a small numbers of zebra mussels may exert only a moderate filtration impact (grazing) on the total algal abundances and their seasonal development, if any at all.

2.10 Conclusions

The current understanding of biological controls on phytoplankton blooms in rivers has a speculative nature and is limited to a small number of modelling studies worldwide. In these models, the metazoans are characterised as a prime biological suppressor of algal communities, but only a few studies investigated the actual interactions between river plankton autotrophs and heterotrophs. For instance, the zooplankton grazing may significantly reduce phytoplankton abundances, alter the algal community structure and rapidly recycle essential nutrients back into the water column, supporting further primary production. The zooplankton also represent a fundamental link in river food webs transferring energy to higher trophic levels, essentially feeding fish communities. In large lowland rivers, the zooplankton grazers are dominated by rotifers. These are small animals with relatively short population growth periods, rapidly reaching abundances of up to 50000 ind l^{-1} in eutrophic systems (Bottrell, 1977). Rotifers feed off a wide variety of algal species with one female able to consume thousands of algal and bacterial cells in 24 hr. Therefore, there is a need for research of the riverine planktonic grazers, their spatial and seasonal dynamics in the catchment in relation to the environmental conditions and interactions with phytoplankton communities.

Aside from zooplankton grazers, river ecosystems are home to various microbial pathogens which can rapidly suppress phytoplankton development. This study is focused primarily on metazoan zooplankton grazers with only one chapter (experimental work) exploring some of the bacterial-phytoplankton-fungal relationships.

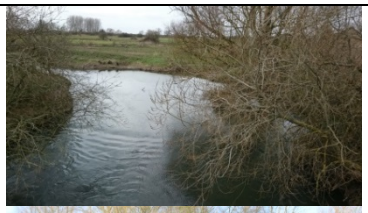
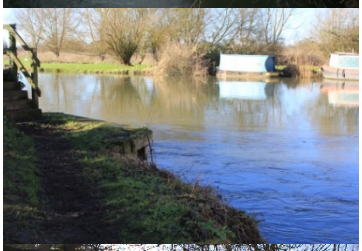
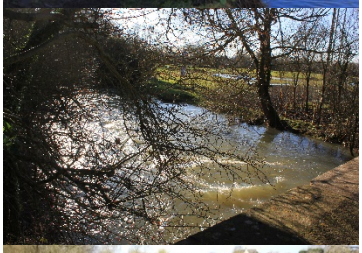
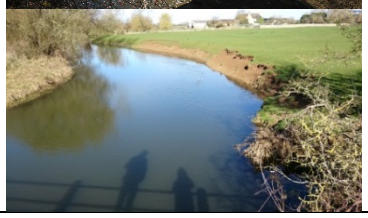
The progress in molecular taxonomy revealed important limitations of traditional rotifer species identification techniques based on body morphology; this allowed the discovery of various new species (previously known as one). To avoid inbuilt errors associated with species identification methods, this study did not apply a diversity index approach, such as estimation of Shannon or Simpson's indexes (Hill, 1973; Tuomisto, 2010) to characterise the zooplankton community. Instead, it describes the total number of rotifer genera/species, the dominant genera or species in the zooplankton community and their weekly abundances as ind l^{-1}.

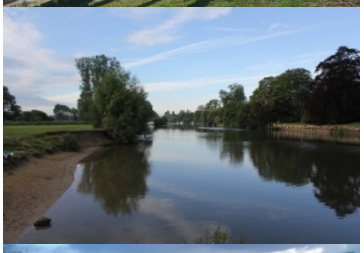
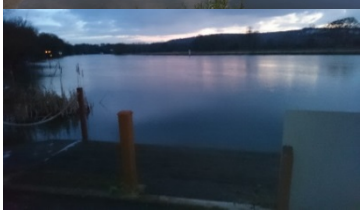
Chapter 3 The River Thames

3.1 Study sites

The 3-year study covers only the non-tidal part of the Thames basin, from Hannington (1) in the upper catchment to Runnymede (9), just upstream of the tidal limit, where tidal movement and salinity do not influence the hydrology and water quality. Sampling points were corresponding with the Centre for Ecology and Hydrology (CEH) Thames Initiative monitoring sites (Bowes et al., 2018) to obtain weekly water quality and flow-cytometry data (Read et al., 2014). Samples were collected at surface level (depth up to 1m) from the riverbank or at midstream as it was previously reported that the zooplankton communities do not differ scientifically across the Thames channel transect (Kiss, 2009). All studied sites are listed in Table 3-1**.** and shown in Figure 3-1**.**. Chapter 4-7 provide geographical, geological and hydrological descriptions of the selected study area.

Table 3-1 Study sites

N	Sampling site	Location within the river channel	Photograph of the location	Study	Coordinates: Latitude Longitude
1	Thames at Hannington	Mid-stream (from the bridge)		Survey Experiments (microcosm)	51.663411° -1.7483843°
2	Thames at Swinford	Right bank		Survey	51.773434° -1.3608063°
3	Evenlode			Survey	51.787779 -1.353355
4	Cherwell at Hampton Poyle	Mid-stream (from the bridge)		Survey	51.833191° -1.27724°

5	Thame at Wheatley	Right bank		Survey	51.740372° -1.1150657°
6	Thames at Wallingford	Left bank		Survey	51.607351° -1.121986°
7	Thames at Goring	Right bank		Survey Experiments (microcosm)	51.5365556° -1.13415°
8	Pang at Tidmarsh	Mid-stream (from the bridge)		Survey	51.467704° -1.0857978°
9	Thames at Runnymead	Right bank		Survey	51.440771° - 0.55397206°
10	The Cut at Paley Street	Bridge		Survey	51.478094° - 0.75007412°
11	Kennet at Woolhampton	Bank/ bridge		Survey	51.39646° -1.1792117°

12	Farmoor Reservoir	Shore		Survey	51.750753° -1.356835°
13	Coln at Welford	Bridge		Experiments (microcosm)	51.690397° -1.7540215°
14	Windrush at Newbridge	Bridge		Experiments (microcosm)	51.709894° -1.4181463°
15	Oxford Canal	Bank		Experiments (*in vitro*) Laboratory incubation	51.782785° -1.283488°

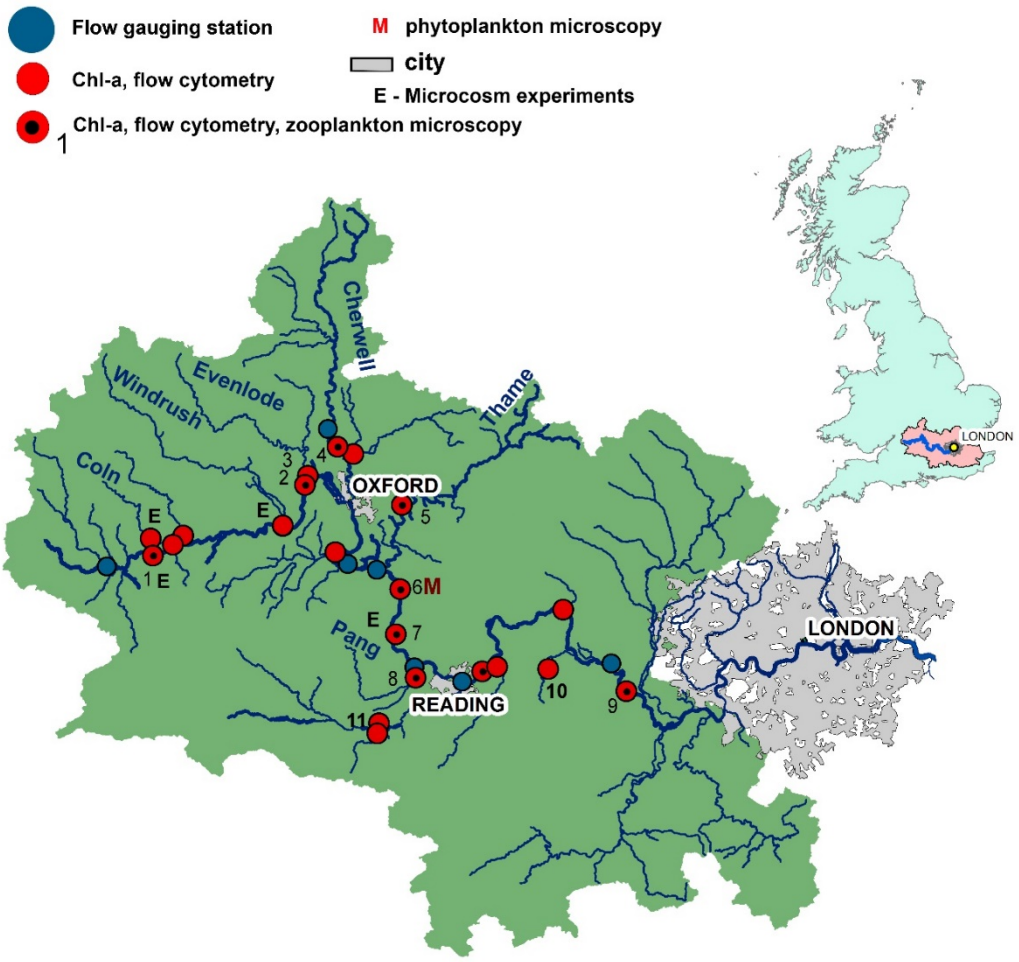


Figure 3-1 Map of the study area and CEH weekly water quality sites. Zooplankton weekly sampling sites 1-Thames at Hannington; 2-Thames at Swinford; 3-Evenlode at Cassington Mill; 4-Cherwell at Hampton Poyle; 5-Thame at Wheatley; 6-Thames at Wallingford; 7-Thames at Goring; 8-Pang at Tidmarsh; 9-Thames at Runnymead;.10-The Cut at Paley Street; 11- Kennet at Woolhampton

3.2 Phytoplankton in the Thames

3.2.1 Introduction

This section aims to provide greater insight into the seasonal and spatial phytoplankton community structure and abundance in the Thames catchment. Data obtained with two fundamentally different methods (chlorophyll-a colorimetry and flow cytometry), were compared to characterise phytoplankton population and its dynamics. Phytoplankton biomass represented as chlorophyll-*a* levels were related to water temperatures, soluble reactive phosphorus, nitrate and silicon concentrations to establish whether nutrient concentrations define spatial and seasonal patterns in plankton dynamics.

3.2.2 Methodology

Weekly flow cytometry data were related to chlorophyll-*a* concentrations obtained from 12 study sites in the Thames catchment between 2013-2015. Data, methods and sources are listed in Table 3-2. Study sites are listed and characterised in Study sites. The R programming environment was used for all statistical analysis (Wickham, 2016; R Core Team, 2017).

Table 3-2 Phytoplankton data type, period collected, methodology and source

Type	Period	Units	Methodology	Source	Reference
Phytoplankton community abundance (10 algal groups)	March 2013-July 2015	cell ml^{-1}	Grab sample flow cytometry	CEH Wallingford	Read *et al.* (2014)
Chlorophyll-*a* concentrations	February 2013-October 2016	µg l^{-1}	Grab sample spectrophotometry	CEH Wallingford	Bowes et al. (2018)

Correlational analysis (package *PerformanceAnalytics* Peterson and Carl, (2018)) related chlorophyll-*a* blooms (concentrations higher than 50 µm l^{-1}) to abundances of 10 algal groups Table 3-3 (described in Read et al. (2014)) in the catchment. These groups are: Large diatoms (G1), Chlorophytes (G2), Large chlorophytes (G3), nano/pico Chlorophytes (G4), Cryptophytes (G5), Large Cryptophytes (G6), Cyanobacteria Microcystis-like (G7), Cyanobacteria (G8), Cyanobacteria Synechococcus-like (G9), Cyanobacteria PE-rich (G10). A generale linear model (GLM, R functions: *glm,* gaussian distribution) was applied to estimate the significance of relationships between chlorophyll and algal (10 groups) abundances (Equation 3-1). Data were tested for normality (with Shapiro–Wilk test), and log transformed (if deviated from the normality assumptions). Diagrams were created with ggplot 2 (Wickham, 2016). All statistically significant differences quoted are at $p \leq .05$ or less.

Chlorophyll-a = Large diatoms (G1) + Chlorophytes (G2) + Large chlorophytes (G3) + nano/pico Chlorophytes (G4) + Cryptophytes (G5) + Large cryptophytes (G6) + Cyanobacteria Microcystis-like (G7) + Cyanobacteria (G8) + Cyanobacteria Synechococcus-like (G9) + Cyanobacteria PE-rich (G10)

Equation 3-1 General Linear Model relating chlorophyll-*a* and phytoplankton groups

Qualitative analysis of phytoplankton was conducted between March and August 2015. 50 ml of water was subsampled from 10 l bulk sample collected from the Thames at Wallingford fortnightly. Samples were left to settle in the sterile petri dishes for 1h in the controlled-temperature environment. Algal cells were studied under the inverted optical microscope Axiovert 40CFL and a DSLR camera Canon 750D at 10x, 20x, 40x and 100x magnification.

Table 3-3 Phytoplankton phenotypical groups distinguished by flow cytometry

Group	Cell length/ Reference culture
G1 Diatoms, Chlorophytes.	12–20 µm /*Chlamydomonas reinhardtii, Stephanodiscus hantzschii*
G2 Chlorophytes	2–5, 5–12, 12 - 20 µm
G3	5–12 µm / *Chlorella vulgaris*, *Raphidocelis subcapitata*, *Scenedesmus vacuolatus*, *S. subspicatus, Cyclotella meneghiniana*
G4 Nano/pico chlorophytes	2–5 µm / *Micromonas pusilla*, *Bathycoccus prasinos, Ostreococcus*
G5 Cryptophytes:	12 µm12–20 µm / *Cryptomonas curvata*
G6 Large cryptophytes, dinoflagellates	>20 µm *Cryptomonas, Peridinium*
G7 Cyanobacteria	5–12 µm / *Microcystis*-type cells
G8 Cyanobacteria	5–12 µm
G9 Cyanobacteria	5–12 µm /*Synechococcus*-type cells
G10 Cyanobacteria	Cell length: 5–12 µm / *Synechococcus*, *Cyanobium*.

Moorhouse et al. (2018) compared identification and enumeration by flow cytometry with results obtained by optical microscopy and HPLC (High-performance liquid chromatography) analysis. All three methods showed relatively coherent and consistent patterns in phytoplankton composition and seasonal dynamics, but there were some discrepancies observed due to limitations of flow cytometry in defining clear boundaries between diatoms and similarly sized chlorophytes, cryptophytes and cyanobacteria, and cryptophytes and dinoflagellates.

3.2.3 Results and Discussion

In the Thames catchment algal blooms tend to occur every year and follow similar spatial rules, but their magnitudes, duration and timing significantly vary inter-annually (Figure 3-3). Relatively high chlorophyll-*a* concentrations (> 50 µg l^{-1}) were observed only in the middle-lower reaches of the Thames (sites: Wallingford, Goring, Runnymede) and some of its tributaries: the Cherwell (up to 250 µg l^{-1}), Kennet, Thame, Evenlode and Cut (around 50 µg l^{-1}). All chlorophyll-*a* blooms sustained below 450 µg l^{-1} (Figure 3-3) and were strongly associated with diatoms ($r = .7$, $p < .05$, $n = 94$, Figure 3-4), large cryptophytes ($r = .38$, $p < .05$, $n= 94$) and phycoerythrin-reach PE-cyanobacteria ($r = .26$, $p < .05$, $n = 94$). High chlorophyll-a concentrations were recorded when diatom abundances exceeded 10000 cell ml-1; in contrast, the sizeable populations of chlorophytes (up to 250000 cell ml-1), and cyanobacteria (100000 cell ml-1) only moderately influenced chlorophyll-a levels (Figure 3 3). Chlorophyll-*a* concentrations tend to peak in late spring/early summer and sometimes in late summer with a

mid-summer decline in-between (Figure 3-3). Diatoms generally dominate in the phytoplankton community in spring/early summer and get succeeded by chlorophytes, cryptophytes, cyanobacteria in July-August. The phytoplankton genera previously observed in the Thames are the same as in other large lowland temperate rivers and are listed in Literature review River phytoplankton Table 2-3 (Fritsch, 1903; Rice, 1938; Ruse & Hutchings, 1996; Ruse & Love, 1997). The GLM showed that diatom abundances were the most significant predictor of chlorophyll-*a* concentrations ($t = 9$; $df = 85$, $p < .05$, $R^2 = 0.57$), followed by cyanobacteria Microcystis-like ($p = .01$) and cyanobacteria *Synechococcus*-like ($p = .02$). Model summary and residual plots are listed in Table 3-4 Figure 3-2. Low phytoplankton biomass in the Thames and its tributaries during the spring/summer growth season 2014, could be explained by prolonged rain events across the catchment. These meteoroloigcal conditons are unfavoriable for algal growth, and result in relatively high river flows and low light intensities (discussed in detail in Bowes et al. (2016)).

Table 3-4 General linear model (Equation 3-1) results: t-statistic and P-value, R software

Predictors	t-Statistic	P-value
(Intercept)	46.570	<0.05
Diatoms	9.059	<0.05
Chlorophytes	-1.358	0.18
Large chlorophytes	-0.012	0.99
nano/pico Chlorophytes	-1.654	0.10
Cryptophytes	-1.123	0.26
Large cryptophytes	1.730	0.09
Cyanobacteria Microcystis-like	-2.454	0.02
Cyanobacteria	-1.002	0.32
Cyanobacteria Synechococcus-like	2.211	0.03
Cyanobacteria PE-rich	1.802	0.08

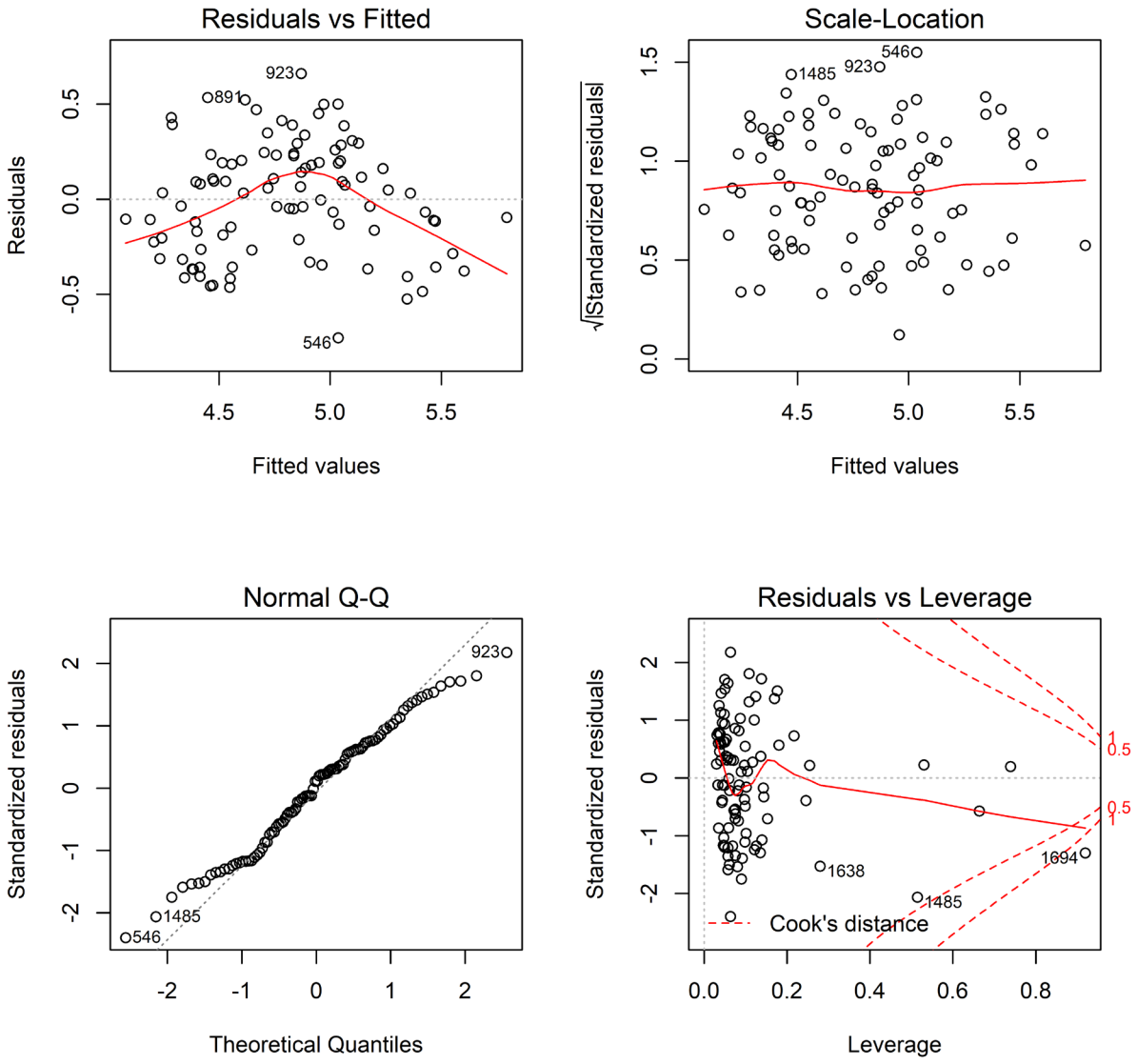


Figure 3-2 Residual plots for Equation 3-1: *Chlorophyll-a = Large diatoms (G1) + Chlorophytes (G2) + Large chlorophytes (G3) + nano/pico Chlorophytes (G4) + Cryptophytes (G5) + Large cryptophytes (G6) + Cyanobacteria Microcystis-like (G7) + Cyanobacteria (G8) + Cyanobacteria Synechococcus-like (G9) + Cyanobacteria PE-rich (G10)*

Chlorophyll-*a* dynamics in relation to water temperatures showed that large phytoplankton blooms tend to occur when temperatures are below 19°C (Figure 3-5). As an exception, in August 2016 when water temperature was almost 20°C there was an intensive (> 400 µg l^{-1}) chlorophyll-a bloom in the Thames at Wallingford. Relating weekly flow cytometry data to water temperature (APPENDIX 1) revealed distinctively different temperature optimums for diatoms, chlorophytes, cryptophytes and cynobacteria. Chlorophyte and cyanobacteria develop dense populations at temperatures between 18-20°C, while diatoms grow better under 15-17°C.

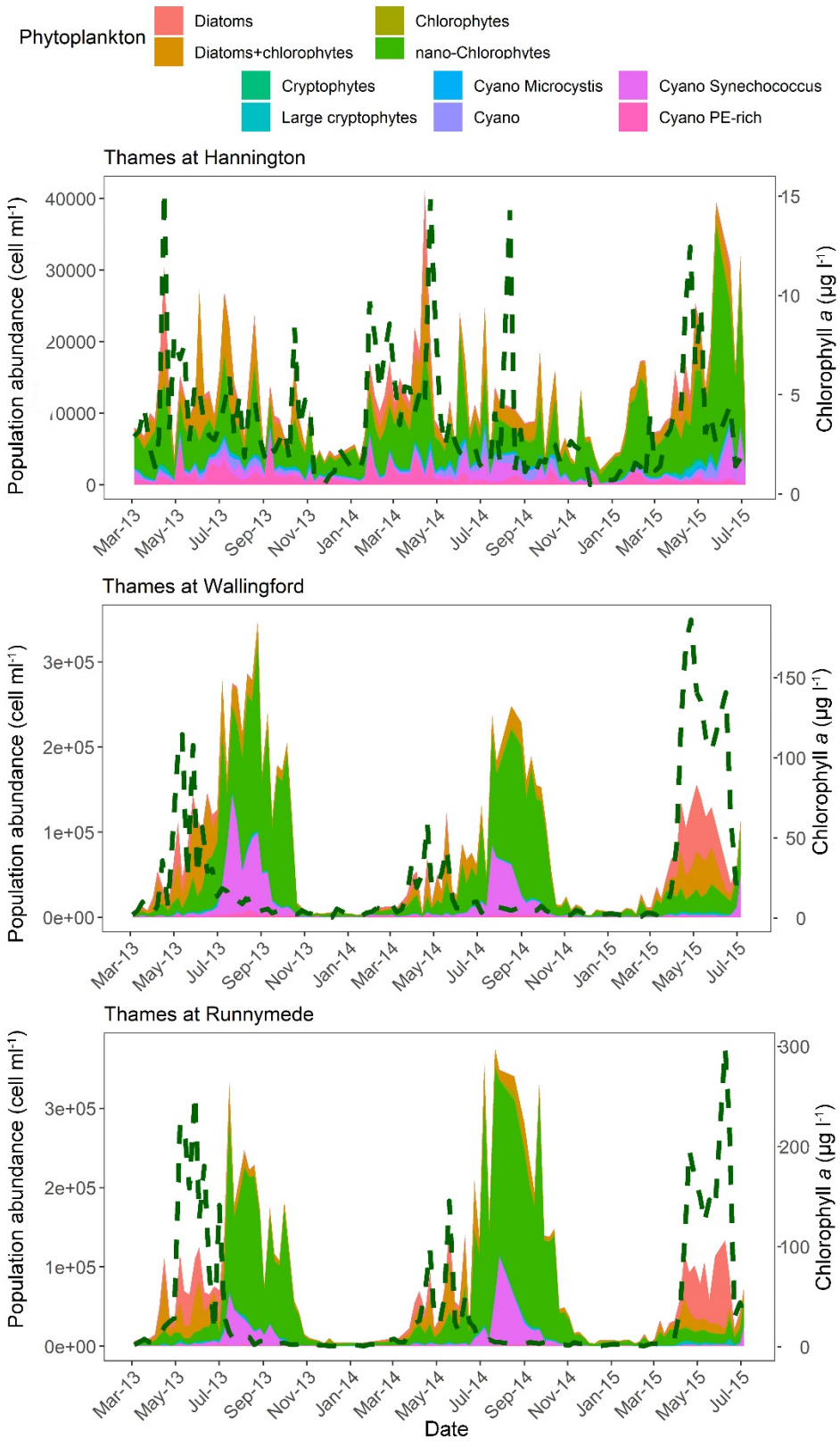


Figure 3-3 (1) Abundances of phytoplankton phenotypical groups based on flow cytometry at 12 sites (Table 2 1) in relation to chlorophyll-a concentrations and in the River Thames catchment (weekly data March 2013-July 2015)

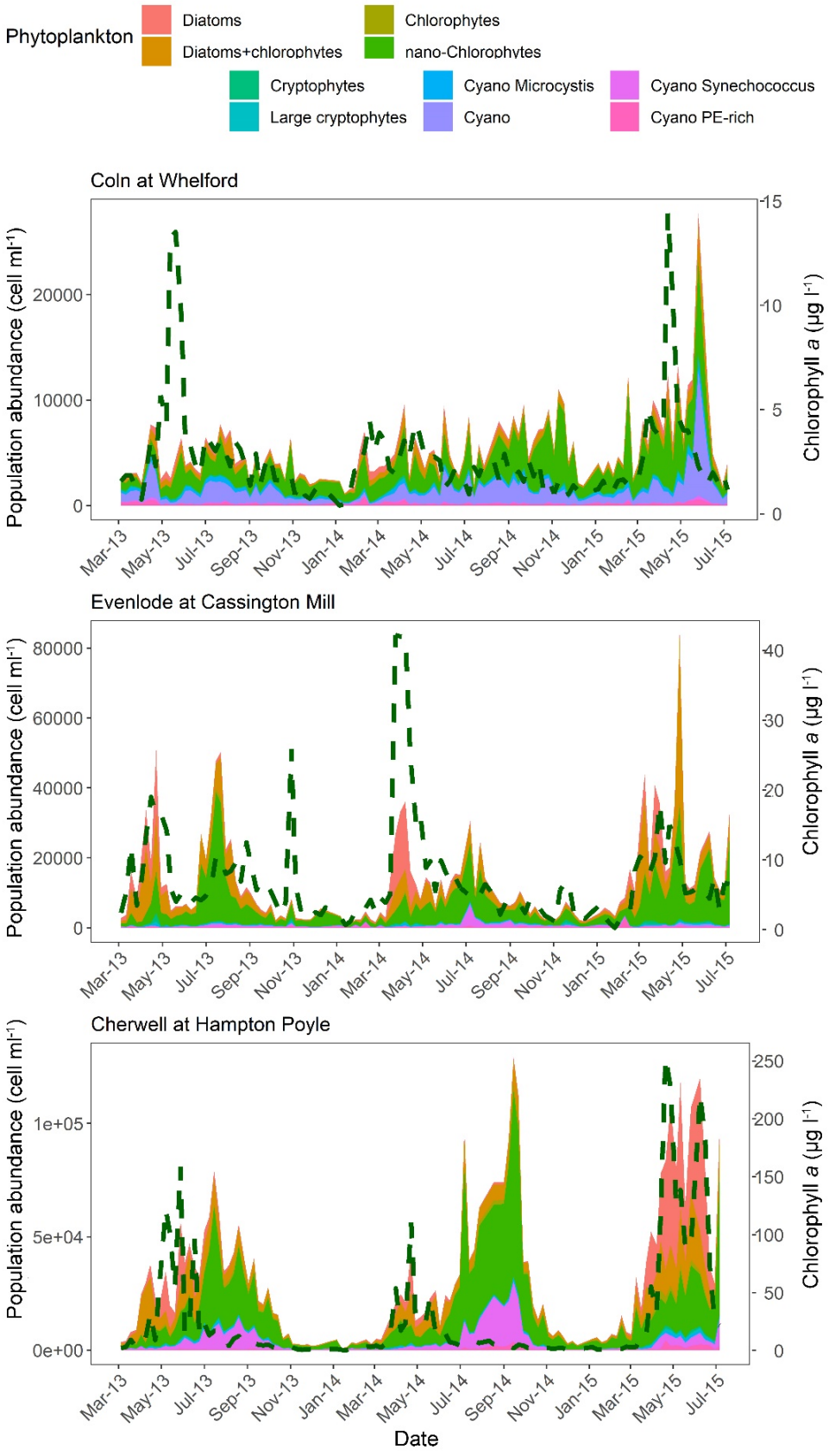


Figure 3-3(2) Abundances of phytoplankton phenotypical groups based on flow cytometry at 12 sites (Table 2 1) in relation to chlorophyll-a concentrations and in the River Thames catchment (weekly data March 2013-July 2015)

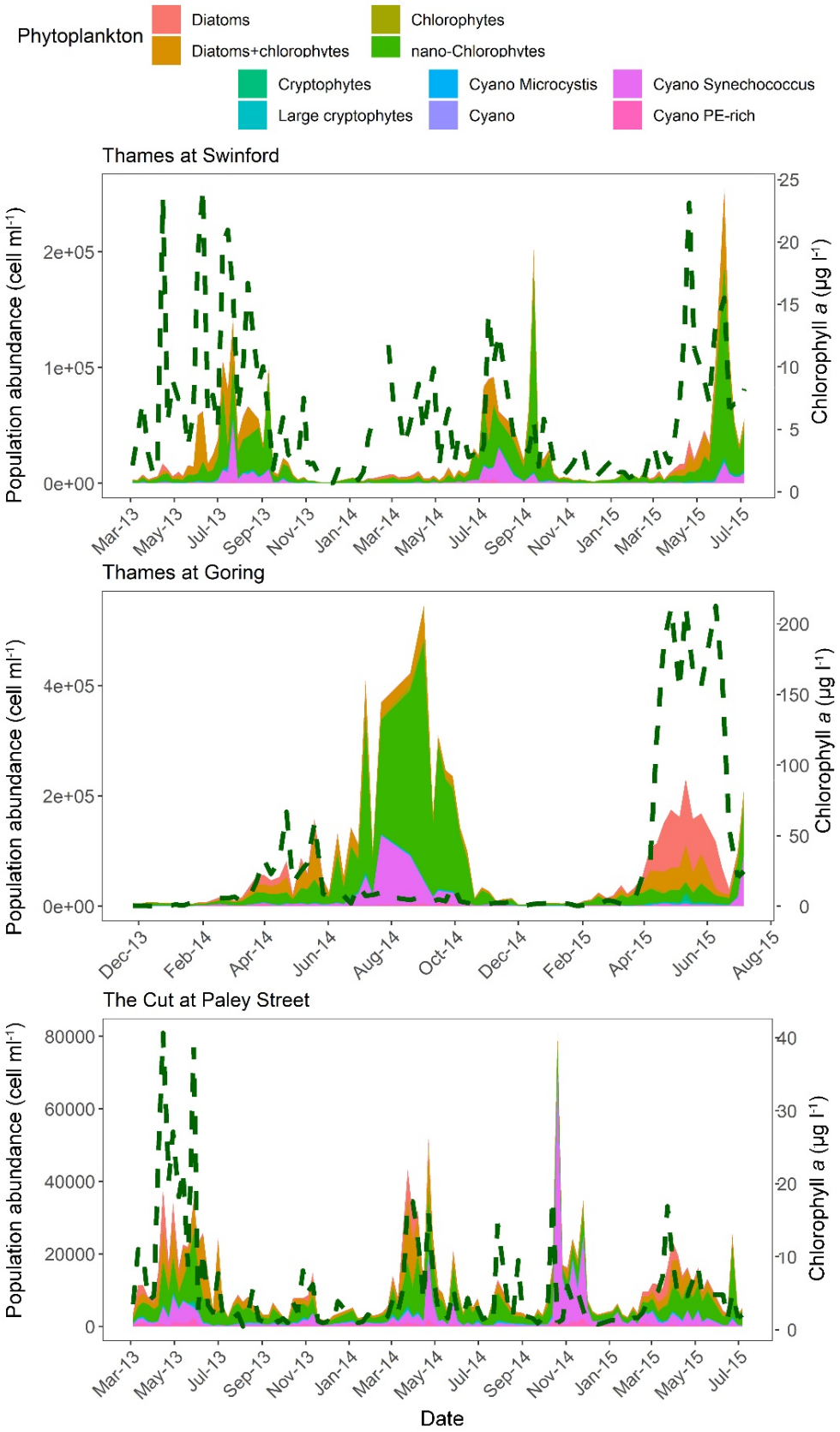


Figure 3-3(3) Abundances of phytoplankton phenotypical groups based on flow cytometry at 12 sites (Table 2 1) in relation to chlorophyll-a concentrations and in the River Thames catchment (weekly data March 2013-July 2015)

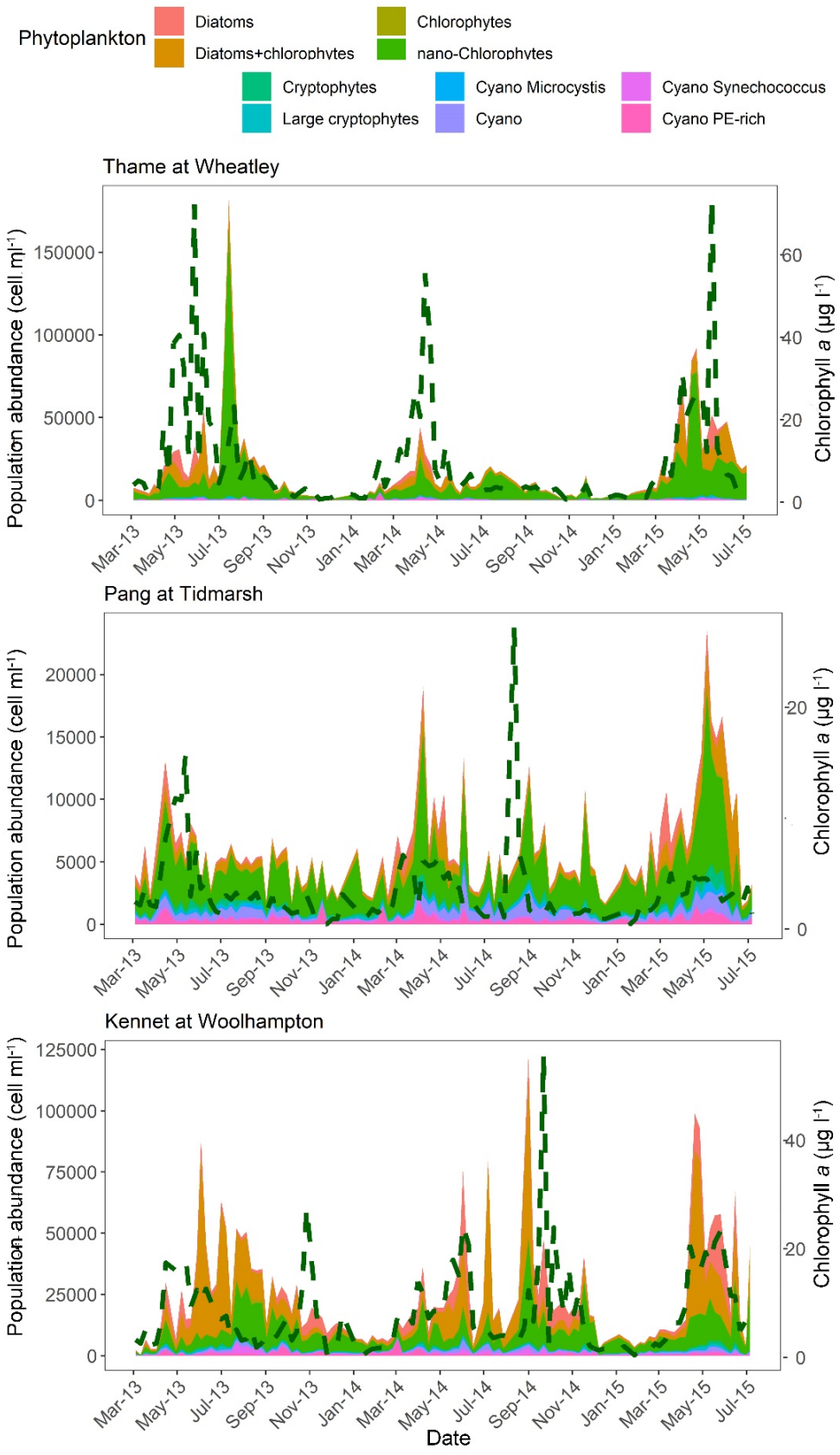


Figure 3-3 (4) Abundances of phytoplankton phenotypical groups based on flow cytometry at 12 sites (Table 2-1 Page 43) in relation to chlorophyll-*a* concentrations and in the River Thames catchment (weekly data March 2013-July 2015)

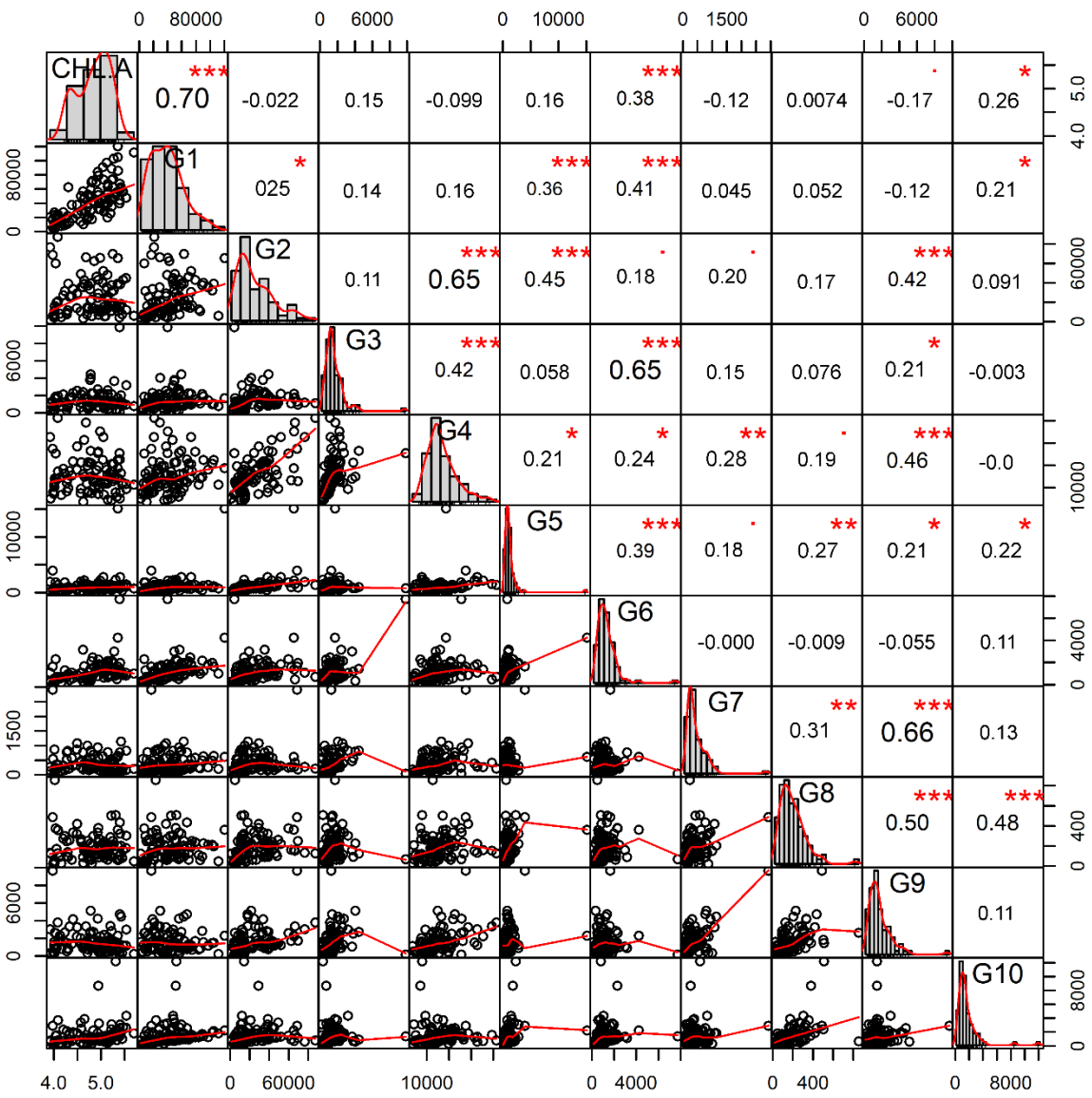


Figure 3-4 Pearson's correlation matrix. From the top left to the bottom right: Chlorophyll-*a* (> 50 µm l^{-1}); Large diatoms (G1); Chlorophytes (G2); Large chlorophytes (G3); nano/pico chlorophytes (G4); Cryptophytes (G5); Cryptophytes (G6); Cyanobacteria Microcystis-like (G7); Cyanobacteria (G8); Cyanobacteria Synechococcus-like (G9); Cyanobacteria PE-rich (G10). Weekly data from 12 study sites March 2013-July 2015.

Planktonic diatoms tend to start seasonal patterns in phytoplankton communities across the Thames catchment (Figure 3-3). They reach high population densities (> 10000 cell ml^{-1}) in early spring and summer and rapidly terminate getting succeeded by chlorophytes, cryptophytes and cyanobacteria. However, there were far less diatoms in the Coln, Pang and Cut than in the Cherwell, Kennet, Evendlode and Thames, indicating that these tributaries were unable to sustain planktonic diatom species and their phytoplankton potentially consisted of dislodged benthic

species (Reynolds & Descy, 1996). The mixed assemblage of pico-green algae, cryptophytes and cyanobacteria characterised phytoplankton of the late summer and early autumn.

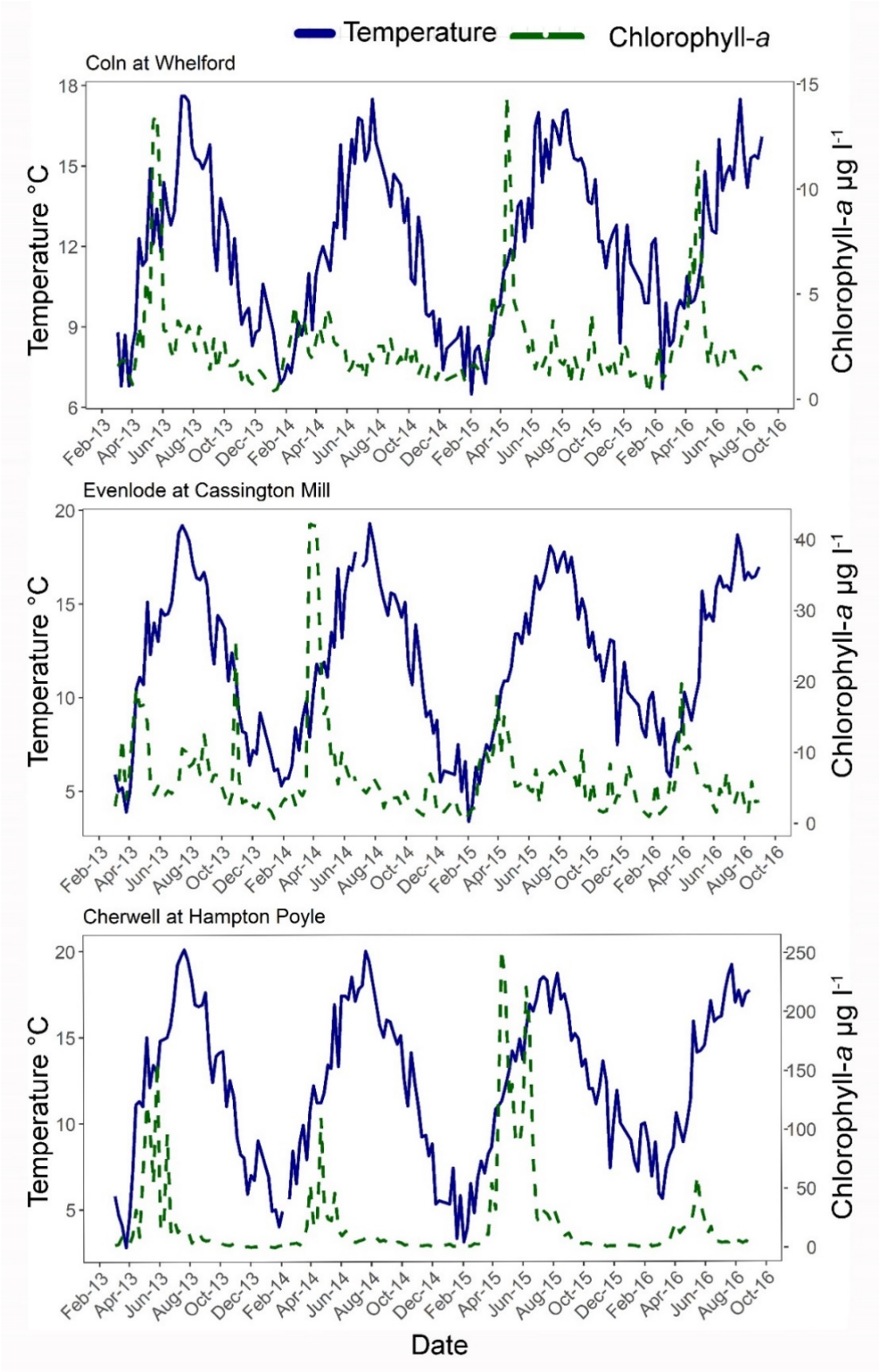


Figure 3-5 (1) Chlorophyll-*a* concentrations and water temperature at 12 study sites in the River Thames catchment (weekly data March 2013-October 2016). see Study sites

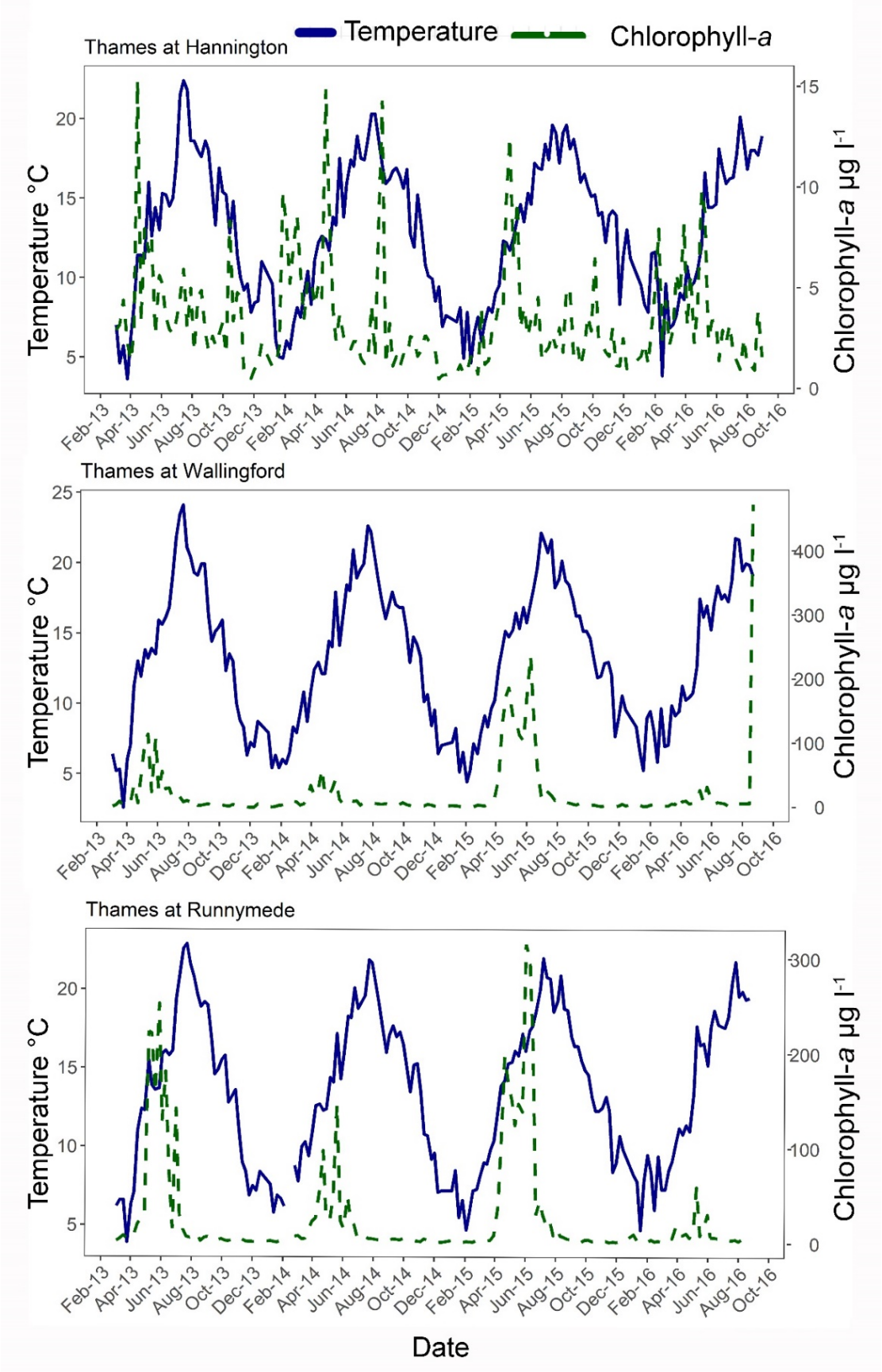


Figure 3-5 (2) Chlorophyll-*a* concentrations and water temperature at 12 study sites in the River Thames catchment (weekly data March 2013-October 2016). see Study sites

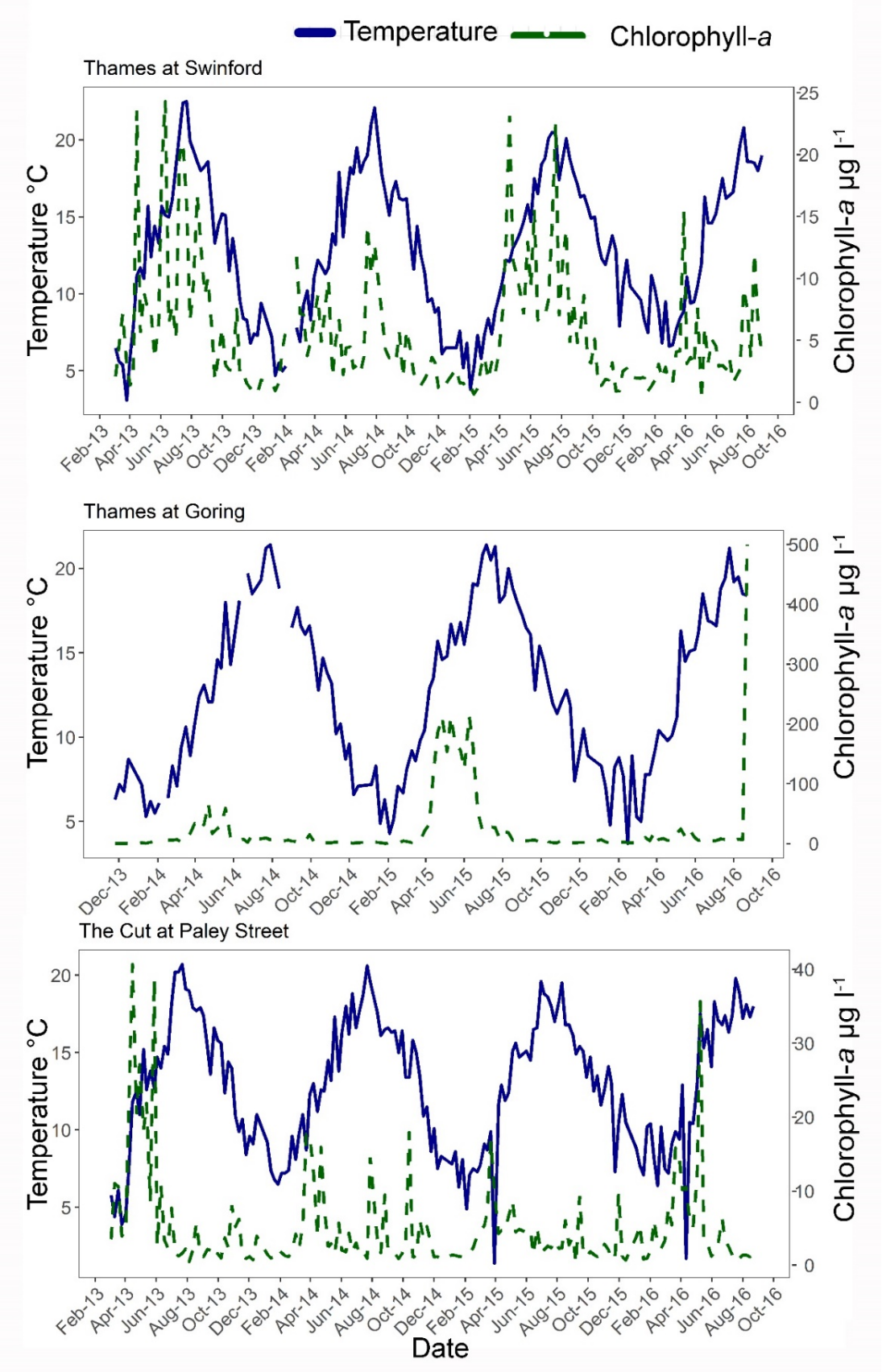


Figure 3-5 (3) Chlorophyll-*a* concentrations and water temperature at 12 study sites in the River Thames catchment (weekly data March 2013-October 2016). see Study sites

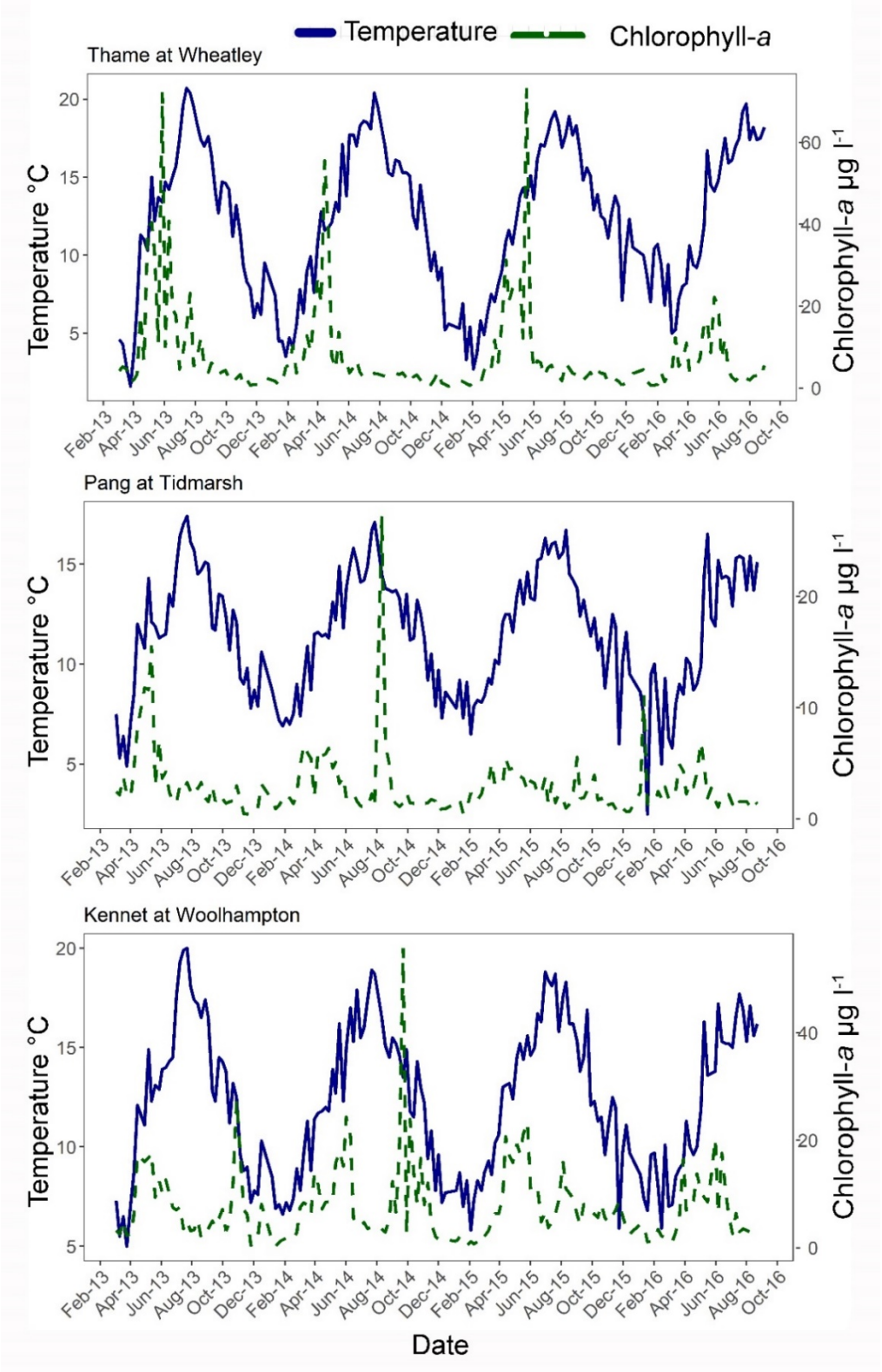


Figure 3-5 (4) Chlorophyll-*a* concentrations and water temperature at 12 study sites in the River Thames catchment (weekly data March 2013-October 2016). see Study sites

Weekly chemistry data collected between March 2013 and September 2016 showed high concentrations of soluble reactive phosphorus (SRP), nitrates and silicon at all studied sites

Figure 3-6 (methodology described in Bowes et al. 2018). These concentrations vary spatially, seasonally and interannually due to the variations in geology, hydrological dynamics, river connectivity, land use, sewage discharge and microbial dynamics across the Thames catchment (discussed in some detail in Bowes et al. 2018).

Maximum annual SRP concentrations were consistent in the Thames main stem and its eutrophic tributaries: the Evenlode, Cherwell (400 µg l-1) and Cut (> 1500 µg l-1). In contrast, in the Thame, Pang, Kennet and Coln they were below 200 µg l-1. All sites were polluted with nitrates with highest concentrations measured in the Thames headwaters (100 mg l^{-1}), Cut (up to 140 mg l^{-1}), Thame (70 mg l^{-1}) and the Cherwell (50 mg l^{-1}). This could be related to both sewage inflows and historic agricultural contamination of the groundwaters (Smith et al., 2010). Silicon concentrations were relatively even between all studied sites, except for the small predominantly groundwater-fed rural tributary the River Coln.

High nutrient concentrations across the Thames catchment do not always translate into intensive phytoplankton blooms. For instance, despite large presence of SRP and nitrates in the Thames headwaters and the Cut, maximum chlorophyll values there were below 50 µg l-1 indicating a low-to-moderate phytoplankton growth. Intensive and sustained phytoplankton blooms occurred in the middle and lower reaches of the Thames, and in the longer tributaries such as the Cherwell, Evenlode and the Thame. This is explained by phytoplankton biomass positive relationship with travel distances (residence time), longitudinal changes in flow velocity and river morphology (Bahnwart et al., 1998; Bowes et al., 2012). High magnitude chlorophyll blooms in spring-summer 2015 were depleting SRP, nitrate and silicon simultaneously, indicating the dominance of algae which require silicon for growth and reproduction, these are diatoms and chrysophytes. Bloom termination led to rapid recovery of these nutrients. Low magnitude and duration of chlorophyll peaks at all studied sites in 2014 reinforce the idea that nutrient concentrations alone do not trigger active plankton growth in rivers (Desortová & Punčochář, 2011; Waylett et al., 2013). Phytoplankton growth in the Thames is mainly initiated by the favourable physical environment which includes sunlight intensity and duration, optimal water temperature and slow flow velocity (this is fully described by Bowes et al., 2016). When long residence periods in the river channel allow phytoplankton populations to expand, dissolved nutrients are utilized as building blocks for algal cells (Browning et al., 2017). Although high nutrient concentrations alone do not trigger or support peaks in algal abundance, nutrients can limit the amplitude and durations of these events.

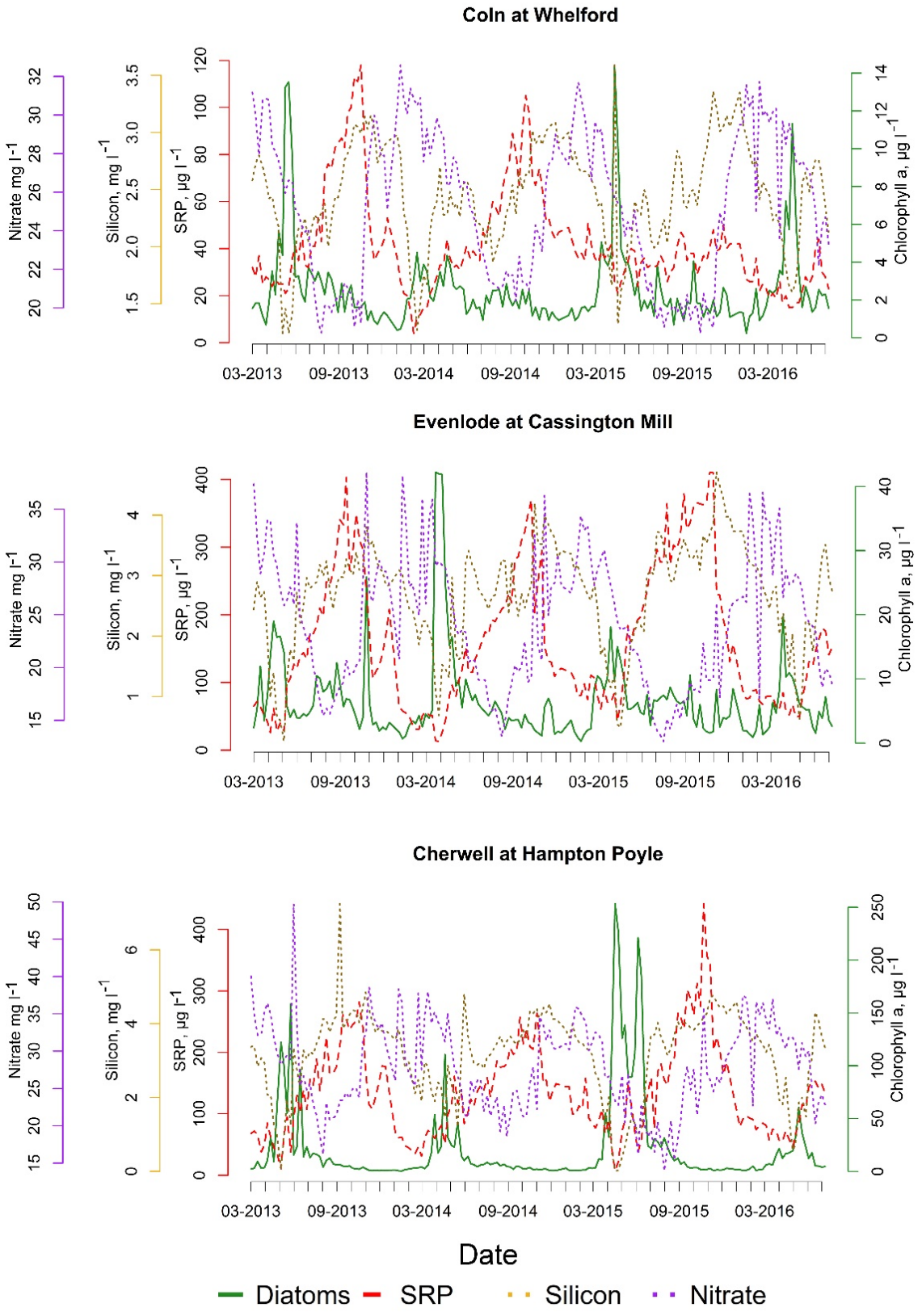


Figure 3-6 (1) Chlorophyll-*a*, soluble reactive phosphorus (SRP), nitrate and dissolved silicon concentrations at 12 study sites in the River Thames catchment (weekly data March 2013-October 2016).

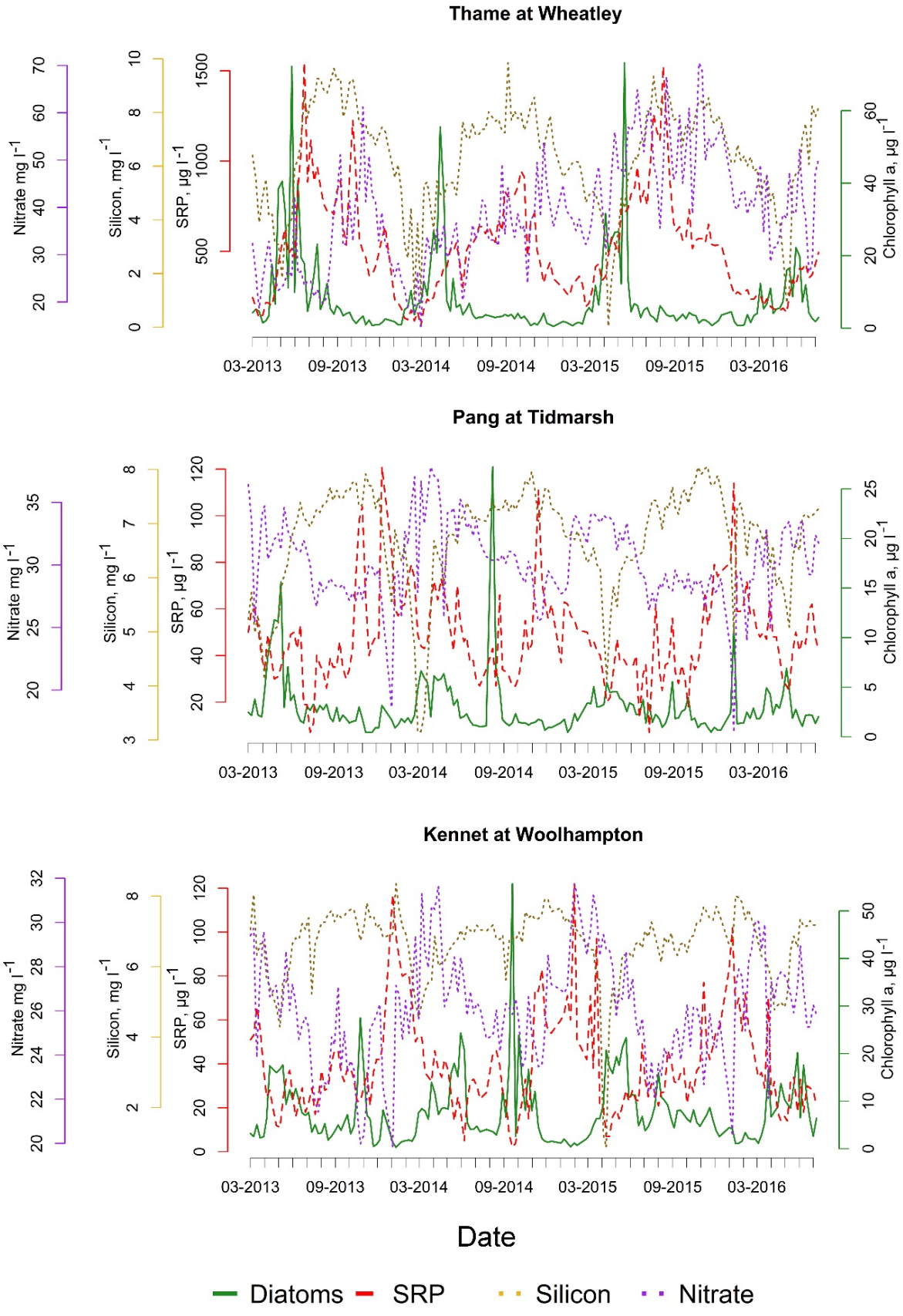


Figure 3-6 (2) Chlorophyll-*a*, soluble reactive phosphorus (SRP), nitrate and dissolved silicon concentrations at 12 study sites in the River Thames catchment (weekly data March 2013-October 2016).

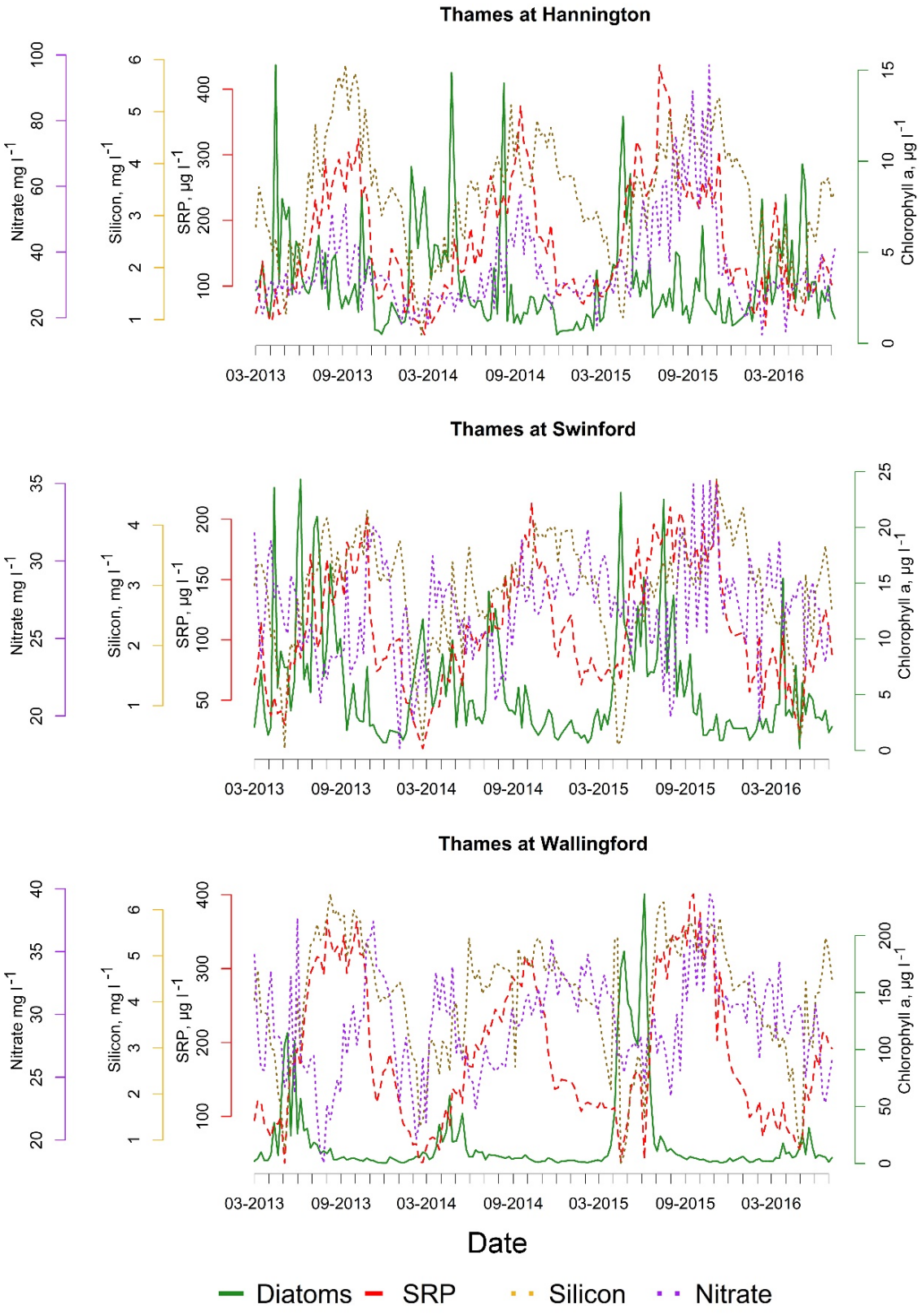


Figure 3-6 (3) Chlorophyll-*a*, soluble reactive phosphorus (SRP), nitrate and dissolved silicon concentrations at 12 study sites in the River Thames catchment (weekly data March 2013-October 2016).

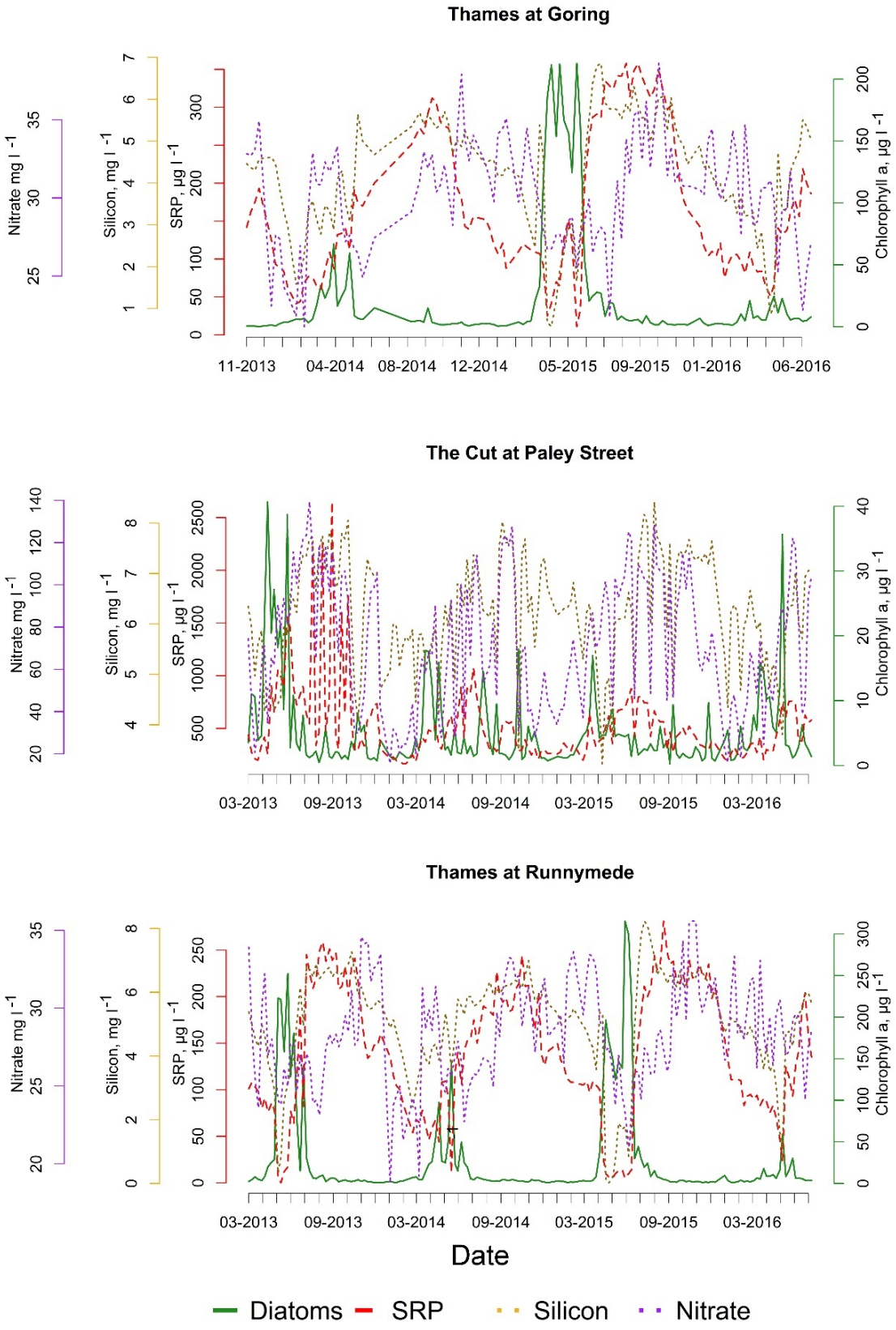


Figure 3-6 (4) Chlorophyll-a, soluble reactive phosphorus (SRP), nitrate and dissolved silicon concentrations at 12 study sites in the River Thames catchment (weekly data March 2013-October 2016). See Study sites

3.2.4 Conclusions

Phytoplankton development in the Thames is initiated and sustained by the optimal physical environment which includes sunlight intensity and duration, favourable water temperature and slow flow velocity. Across the Thames catchment chlorophyll-*a* blooms (> 50 µg l^{-1}) are strongly associated with planktonic diatoms and tend to occur only in the middle-lower reaches and tributaries connected to canals (the River Cherwell, Kennet, and Thame). In small tributaries (the Pang, Coln, and Cut) the annual maximum algal biomass and abundances were approximately 10 times smaller than in the middle and lower reaches of the mainstem Thames and its larger tributaries: the Cherwell, Kennet and Thame which is directly related to travel distances and residence times, longitudinal changes in flow velocity and river morphology

Diatoms initiate seasonal patterns in phytoplankton community in the mainstem Thames and its tributaries, reach high population densities (> 10000 cell ml^{-1}) in early spring and summer and rapidly terminate being succeeded by chlorophytes, cryptophytes and cyanobacteria. Variation in chlorophyll-*a* can be partly explained by cyanobacteria as they become abundant in the Thames main channel and tributaries in late summer and early autumn when water temperatures favour cyanobacteria. Cyanobacteria produce toxins and therefore should be monitored.

Although high nitrate, soluble reactive phosphorus and silicon concentrations alone do not trigger or sustain peaks in algal abundance, nutrients can limit the amplitude and durations of these events. The availability of dissolved silicon sets the upper limit on diatom productivity. Dissolved nutrients are building blocks for algal cell structures and metabolism, lack of their availability makes cells vulnerable to viral, bacterial and fungal infections, slows population growth and reproduction and may rapidly terminate the bloom. High inter-annual variability of algal communities and long periods of low phytoplankton biomass during favourable water temperatures and nutrient concentrations suggest that physical environment and water chemistry alone do not fully explain phytoplankton composition. Other factors, such as zooplankton grazing, or microbial lysis can play an important role in controlling phytoplankton blooms.

Chapter 4 Phytoplankton and zooplankton community structure and interaction along the lowland River Thames

Freeman A., Armstrong L. K., Bowes M.J., Hutchins M. G., Nicholls D., Read D. S., Roberts C., Scarlett P., Thackeray S., Wade A. J., Wickham H.

4.1 Abstract

Zooplankton are an important interactive component of aquatic ecosystems involved in transferring energy and carbon between trophic levels, yet the causes of zooplankton community structure in rivers and their interactions with other organisms, especially phytoplankton, are poorly understood. This study examines the phytoplankton and zooplankton interactions along the River Thames, a lowland river system impacted by farm and farmland runoff and sewage effluent. The phytoplankton-zooplankton communities and water quality were measured at five key sites each week from April to September 2015, a year representative of the long-term spring-summer low flow conditions observed in the river. Based on Canonical Correspondence Analysis, the spatial pattern in zooplankton abundance and community composition was explained by travel distance, phytoplankton and water temperature primarily. Evidence was found that plankton species in the main channel may originate in certain tributaries of the Thames, especially those connected to canals, and therefore the mixing of waters from different tributaries may be the key control on phytoplankton and consequently on zooplankton community composition rather than the site-specific flow or water quality conditions. The frequent appearance of zooplankton species normally found in small ponds and marshes suggests a connection between the river community and those of adjacent floodplains. These results reinforce the need to maintain river-floodplain connection to protect biodiversity and point to the possibility that management measures targeted in specific tributaries may reduce algal blooms in the main channel.

Keywords: plankton, phytoplankton, zooplankton, metazoans, diatoms, cyanobacteria, flow cytometry

4.2 Introduction

Zooplankton are small animals and ‘animal-like’ organisms that are incapable of moving against the water current. They provide food for fish, influence algal community composition and are

involved in the transformation and circulation of energy and organic matter. Plankton grazing can reduce the population of toxic algae (Ger et al., 2016). The fish community is tied closely with zooplankton (Thayer et al., 1974; Medeiros & Arthington, 2008).

Gomes et al. (2019) reviewed recent trends and gaps in scientific literature regarding zooplankton in freshwater environments and emphasized that most studies of zooplankton communities described lentic environments. This tendency occurs because rivers are less favourable to small-bodied animals than lakes and ponds since turbulent flowing waters create an obstacle to feeding and mating processes (Lair, 2006). Due to the high complexity of riverine networks, plankton dynamics in large temperature rivers are difficult to compare. Nevertheless, the zooplankton composition, distribution and dynamics in most of them follow similar spatial and seasonal patterns above the estuary zone (Van Dijk & van Zanten, 1995; Garnier et al., 1995; Kim & Joo, 2000; Viroux, 1997; Baranyi et al., 2002; Burger et al., 2002; Zimmermann-Timm et al., 2007; Rossetti et al., 2009; Bertani et al., 2012). True planktonic communities tend to form in the middle/lower reaches with maximum metazoan numbers fluctuating between 2000 to 10000 ind l^{-1}. Most river studies describe zooplankton as dominated by rotifers, particularly species belonging to the following genera: *Keratella, Brachionus, Synchaeta, Polyarthra, Trichocerca* (Lair, 2006). These patterns are thought to be controlled, in part, by the physical environment including channel morphology (Reynolds, 2000; Schiemer et al., 2001; Casper & Thorp, 2007; Bertani et al., 2012), discontinuities along the river course (Kim & Joo, 2000; Havel et al., 2009), hydrological regime (Bertrand et al., 2001; Baranyi et al., 2002; Galir Balkić et al., 2018) and connectivity with the adjacent floodplain (Górski et al., 2013; Zhao et al., 2017). Pollution caused by human activities can also significantly affect zooplankton on a local scale as was shown in the one-year study by Xiong et al. (2016). The influence of food availability is important and chlorophyll-*a* concentrations have been shown to correlate with zooplankton species richness in lakes (Thackeray, 2007).However, in rivers, zooplankton-phytoplankton interactions have mainly been assessed in terms of the top-down influence of zooplankton upon phytoplankton through grazing (Garnier et al., 1995; Gosselain et al., 1998b; Keckeis et al., 2003) and in terms of biomass rather than community composition (Basu & Pick, 1997; Kobayashi et al., 1998). Few, if any studies, have looked at the combined effects of physical, chemical and phytoplankton-zooplankton interactions in a major river system in detail throughout a growing season.

The aim of this study was to determine the causes for the principal changes in zooplankton composition along the River Thames including the zooplankton-phytoplankton interactions. The Thames is one of the most intensively monitored river-systems in the UK with historic phytoplankton studies (Lack, 1971; Ruse & Hutchings, 1996; Ruse & Love, 1997), extensive long-term hydrological measurements (Crooks & Kay, 2015), and weekly observations of both water chemistry (Bowes et al., 2018) and bacterioplankton (Read et al., 2015) available. To achieve aims of this study we defined two objectives. The first was to measure the zooplankton community structure and abundance along the Thames river continuum from the headwaters to the lower reaches. The second was to analyse these data in terms of the prevailing environmental conditions and phytoplankton composition and dynamics to help determine the most important factors controlling the zooplankton abundance and composition, and to better understand the within river phytoplankton-zooplankton interactions.

4.3 Study area

The River Thames is the second longest river in the UK (346 km from the headwaters to the tidal limit at Teddington). The catchment area to the tidal limit at Teddington in south west London is approximately 9950 km^2. The river rises at Thames Head in the Cotswolds and flows in an easterly direction into the North Sea (Figure 4-1). The geology of the catchment in the northwest consists of Oolitic limestones and clays mainly. Downstream of Wallingford, the Thames flows over chalk until it runs onto sandstones and mudstone at Maidenhead. The Oolites and chalk are productive aquifers. Clays function largely as a solid impermeable area underlying or overlying aquifers. As a result, tributaries arising from different strata have proportionately different inputs of groundwater. Based on estimations of the base flow index value (0.63), the Thames is considered as moderately groundwater-dominated (National River Flow Archive, 2016). The mean annual precipitation is approximately 700 mm (1961-1990) and mean daily air temperature is 11°C. Water discharge in the Thames differs throughout the year, with high flows occurring in the autumn-spring period and low flows in summer (Crossman et al., 2013). This study covers the non-tidal part of the Thames basin from Hannington in the perennial headwaters of the Cotswolds to Runnymede just upstream of the tidal limit. The population in the Thames catchment is over 14 million people, the majority of whom live in London. Despite a high population density of approximately 960 people km^{-2} centred in London (Merrett, 2007), much of the river basin upstream from London is rural and the predominant land cover types are arable (35%), grassland (32%), woodland (16%) and urban (14%) (National River Flow Archive, 2016). Outside London, the cities and towns are Swindon, Oxford, Reading, Slough and

Maidenhead all of which have large Sewage Treatment Works. Sewage discharge and effluent from septic tanks increase concentrations of various organic and inorganic compounds degrading water quality and influencing plankton communities (Environment Agency, 2017). Intensive agriculture results in significant inputs of phosphorus, nitrogen and sediment (Neal et al., 2010). The main channel of the River Thames is intersected by 45 locks with adjacent weirs which are used for navigation and flood control.

The Thames has been intensively monitored in terms of flow and water quality. As part of the Centre for Ecology and Hydrology Thames Initiative, 23 sites have been sampled weekly since 2009 for a broad range of water quality determinands (Bowes et al., 2018). River flow has been monitored at Kingston and Teddington Weir since 1883 and these are data are supplemented by a further 24 upstream monitoring sites all maintained by the Environment Agency. These data are available from the Centre for Ecology and Hydrology's National River Flow Archive following quality control (UK National River Flow Archive (NRFA) which is hosted by the Centre for Ecology & Hydrology (CEH) on behalf of the Natural Environment Research Council © NERC (CEH)).

A zooplankton survey of the Thames was done in 1996 at a two-week interval between April and October (May & Bass, 1998). In this study, maximum zooplankton population densities in the Thames were found to be more than ten times smaller than densities recorded in 1971 (Bottrell, 1977), yet community composition remained relatively constant. Zooplankton were dominated by rotifers which normally inhabit open waters (euplankton or 'true' plankton), and they survive and actively reproduce in the turbulent river environments. The main rotifer species were: *Keratella cochlearis, Keratella quadrata, Synchaeta oblonga, Synchaeta pectinate, Polyarthra dolichoptera*. In late spring and early summer, the number of animals from still eutrophic waters (heleoplankton) increased. These were: *Brachionus* and *Euchlanis, Cephalodella*. Periphytic and benthic rotifers, which are normally found in shallow benthic zones and among macrophytes, were key components in early spring and autumn.

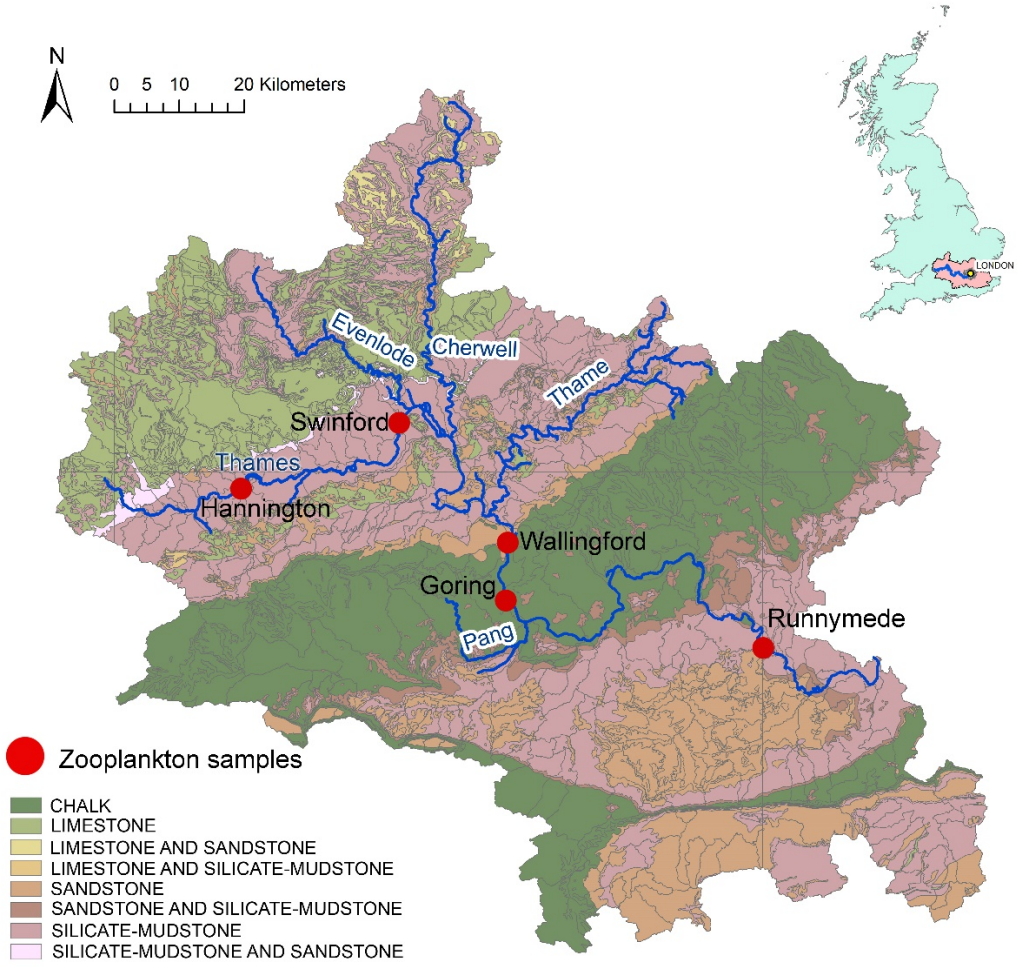


Figure 4-1 The River Thames catchment (non-tidal part only) showing the bedrock and superficial geology (Survey British Geological Survey, 2018) and the five combined flow, water chemistry, phytoplankton and zooplankton study sites: Hannington, Swinford, Wallingford, Goring and Runnymede

4.4 Methodology

4.4.1 Flow, water chemistry and phytoplankton

The flow data from five stations at Cricklade, Eynsham, Day's Weir, Reading and Royal Windsor Park (Figure 4-1; Table 4-1) were used to characterise the flow conditions at the time of water quality and biological sampling during the growing season when zooplankton was measured (April to September 2015) and for a 2-year period commensurate with measurement of the phytoplankton community using flow cytometry. The monthly-mean flows during the sampling period were largely representative of the typical low- to mid-flow conditions in the River Thames ranging between 65% of the long-term mean in April rising to 104% in September.

A sub-set of the CEH Thames Initiative data were used to describe the water quality at the same five sites where flow, phytoplankton and zooplankton were also measured. The water quality was measured weekly for a 2-year period (2014-2015) and included stream water soluble reactive phosphorus, nitrate, silicon, chlorophyll-*a*, water temperatures and suspended sediment. The measurements were made using the methods described in Bowes et al. (2018).

Table 4-1 Studied sites, the corresponding gauging points, and distance from the source of each individual river, mean flow and catchment area

N	Study site	Gauging station**	Distance from the source*, km	Dendritic network length*, km	Discharge $m^3 s^{-1}$ (1972-2015)**
1	Hannington	Thames at West Mill Cricklade	29	193	1.4
2	Swinford	Thames at Eynsham	76	637	14.1
6	Wallingford	Thames at Day's Weir	129	1727	28.8
7	Goring	Thames at Reading	138	1788	38.5
9	Runnymede	Thames at Royal Windsor Park	212	2515	60

* Ordnance Survey (GB), (2016, 2017)

** UK National River Flow Archive (NRFA) which is hosted by the Centre for Ecology & Hydrology (CEH) on behalf of the Natural Environment Research Council © NERC (CEH)

Phytoplankton composition was determined by flow cytometry at the five sites (Table 4-1). This method enumerates six previously defined groups of phytoplankton (listed in Table 4-2) based on cell sizes and pigment fluorescence using a Gallios flow cytometer (Beckman Coulter, UK) equipped with blue (488 nm) and red (638 nm) solid state diode lasers (Read et al., 2014).

The samples (approx. 20 ml) were stored in 30 ml universal tubes at 4°C before analysis within 24 hours of collection. The analysis compares fluorescence from phycoerythrin versus chlorophyll and chlorophyll versus the phycocyanin. A set volume of counting beads FlowCount (Beckman Coulter) was added to individual samples for calibration and testing, each sample was run for five minutes at a high flow rate. Data was processed using the software Kaluza Analysis v1.5a (Beckman Coulter, UK), and exported to a .csv file for plotting and interpretation. This method allows separation and enumeration of diatoms, green algae, cryptophytes and cyanobacteria.

For diatom microscopy, one 50ml sample was collected in May 2015. It was diluted with 40% hydrogen peroxide, then centrifuged with deionised water, and mounted on a clear glass slide with NAPHRAX (Biggs & Kilroy, 2000). Diatom were examined under the optical microscope (x400), centric diatom species were identified based on Krammer & Lange-Bertalot (2000); Taylor et al. (2007).

Table 4-2 Phytoplankton phenotypical groups distinguished by flow cytometry

Group	Cell length/ Reference culture
Diatoms, Chlorophytes.	12–20 µm /*Chlamydomonas reinhardtii, Stephanodiscus hantzschii*
Chlorophytes	2–5, 5–12, 12 - 20 µm *Chlorella vulgaris*, *Raphidocelis subcapitata*, *Scenedesmus vacuolatus*, *S. subspicatus, Cyclotella meneghiniana*
Nano/pico chlorophytes	2–5 µm / *Micromonas pusilla*, *Bathycoccus prasinos, Ostreococcus*
Cryptophytes:	12, 12–20 µm / *Cryptomonas curvata*
Dinoflagellates	12-20 µm/*Peridinium*
Cyanobacteria	5–12, >20 µm/*Microcystis*-type, *Synechococcus*-type cells cells *Synechococcus*, *Cyanobium*.

4.4.2 Zooplankton survey

Zooplankton were sampled in March-September 2015. One-litre samples were collected with a bucket from the bank or mid-stream at the five sites. On the same day, in the laboratory, samples were filtered through sieves with mesh diameters of either 30 µm (under clear water conditions) or 53 µm (during periods of high suspended sediment concentrations) and preserved in formaldehyde solution (4%). Zooplankton (metazoans) were identified and enumerated in sedimentation chambers (after 2 hr of settling) at 100-400 X magnification using an inverted microscope (Zeiss Axiovert 40CFL). Up to 200 individuals were counted and extrapolated to the whole of the original sample. If the total number of organisms was less than 200, all rotifers and microcrustacean nauplii were counted. Identification of rotifers was taken to genus and species level (when possible), using printed guides (Mellanby, 1951; Pontin, 1978; Alekseev, 2010) and web resources (Haney, 2013). For this study, microcrustaceans were differentiated as Cladocerans or Copepods including their reproduction stages.

4.4.3 Data analysis

The R programming environment R 3.4.2 (R Core Team, 2017), with the vegan 2.4-6 package (Oksanen et al., 2017), was used for statistical analysis. Canonical Correspondence Analysis

(CCA) related zooplankton communities and environmental conditions. The explanatory variables tested were: abundance of different algal groups, the distance from source (Figure 4-2) and dendritic network length (the cumulative length of the branching river network upstream of the sampling site). Physical distances (Figure 4-2) were considered to evaluate the changes in zooplankton along the course of the river. In accordance with the classical river continuum concept (Vannote et al., 1980) 'true' planktonic organisms can only appear in the lower reaches of large rivers. Distance from source and dendritic network length were considered as the most appropriate spatial characteristics of the sampling sites because zooplankton cannot swim against a current and complete their entire lifecycle in the water (see comprehensive review by Tonkin et al., 2017).

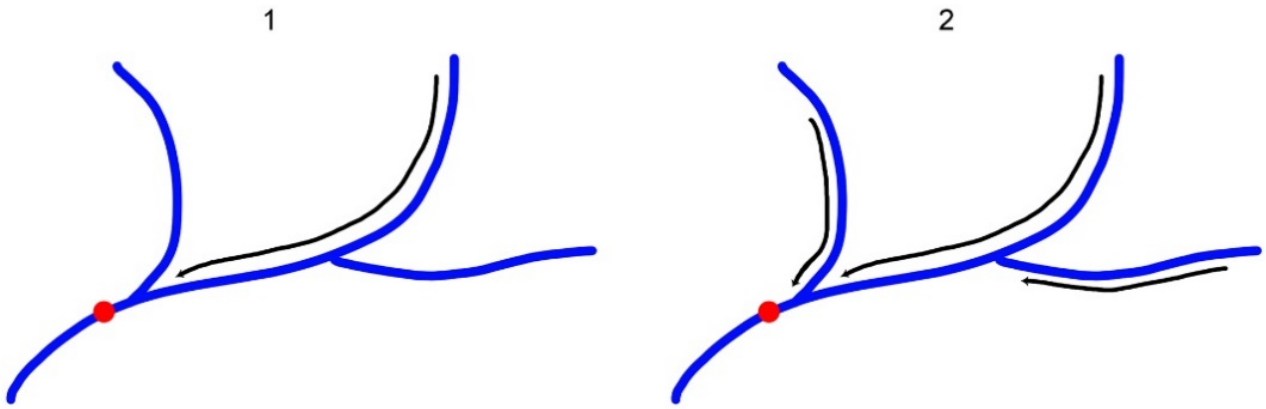


Figure 4-2 Types of physical distances employed in this study: 1- distance from the headwater source; 2- dendric network length

Initially, a model with 11 explanatory variables (dendritic network length, distance from the river source, water temperature, pH, suspended sediment, Chlorophytes, Cyanobacteria, Cryptophytes, Dinoflagellates, Diatoms, Pico-chlorophytes) was used to help determine the key physical and biological factors affecting the zooplankton abundance. This initial model was reduced to 7 variables in a stepwise way based on p–values (function: ordistep). As crustaceans were found in negligible numbers, only rotifers were included in this analysis. Rotifers are known to be more ecologically suited to large rivers because they survive in fast flowing turbulent waters better than crustaceans (Hynes, 1970). Linear dependencies were explored by computing Variance Inflation Factors (VIF), which measure the proportion by which the variance of a regression coefficient is inflated in the presence of other explanatory variables. Permutation tests (n = 999) examined the significance of variables and canonical axes ($P \leq 0.05$) function, anova.cca.

4.5 Results and Discussion

4.5.1 Spatial heterogeneity of the abiotic conditions along the Thames

Spatial and seasonal variation in the abiotic conditions along the Thames was evident (Figure 4-3). Flow rates gradually increased along the channel from maximum of 2 m^3 s^{-1} in the headwaters to 80 m^3 s^{-1} in the lower reaches during winter floods, and 0.2 and 20 m^3 s^{-1} throughout the course of summer correspondingly. During the last week of May there was a peak in flow rates at all sites, caused by rain events. There was also a small increase in flow at the beginning of July. Prolonged summer low flows were benefiting plankton communities.

The Thames upper reaches were generally about 1°C cooler than Runnymede for all seasons (Figure 4-3a). An annual minimum of 4°C was recorded during winter months at Swinford and a maximum of approximately 22°C in summer at Wallingford. Water temperatures favourable for active plankton growth were recorded between early spring and autumn (March-October). The pH along the channel was relatively constant with some evidence for lower values in the headwaters at Hannington and at Runnymede indicating geological influences in the headwaters and possibly lower algal growth at Runnymede. The seasonal variation in pH was approximately 7.4-8.5 across all the sites with the highest pH measured at Wallingford (Figure 4-3b) possibly due to higher summer plankton metabolism. Soluble reactive phosphorus (SRP) concentrations were consistently high (> 200 µg-P l^{-1}) at all studied sites (Figure 4-3c). Nitrate concentrations were twice as high in the headwaters (97 mg-NO_3 l^{-1}) (Figure 4-3d) than in the rest of the river (maximum 35-40 mg l^{-1}). Median concentrations varied between 25-35 mg-NO_3 l^{-1}. High nutrient concentrations in the Thames mainstem are related to both sewage inflows and historic agricultural contamination of the groundwaters (reviewed in Bowes et al., 2018). Low SRP and nitrate concentrations were measured in March-August at all sites. The highest dissolved silicon concentrations were measured in the middle and lower Thames (up to 8 mg-Si l^{-1}; Figure 4-3e). During spring and early summer, silicon was almost depleted by diatoms. Concentrations of suspended solids were higher in the lower Thames (up to 50 mg l^{-1}) (Figure 4-3f) than in the headwaters (25 mg l^{-1}) reflecting the geology, in particular, the presence of mudstones and sandstones in middle and lower parts of the catchment. Seasonal oscillations in nitrate, soluble reactive phosphorus and silicon levels are related to microbial and plant uptake, groundwater inputs, and the flow regime (Wade et al., 2006; Environment Agency, 2009; Neal et al., 2010; Halliday et al., 2014)

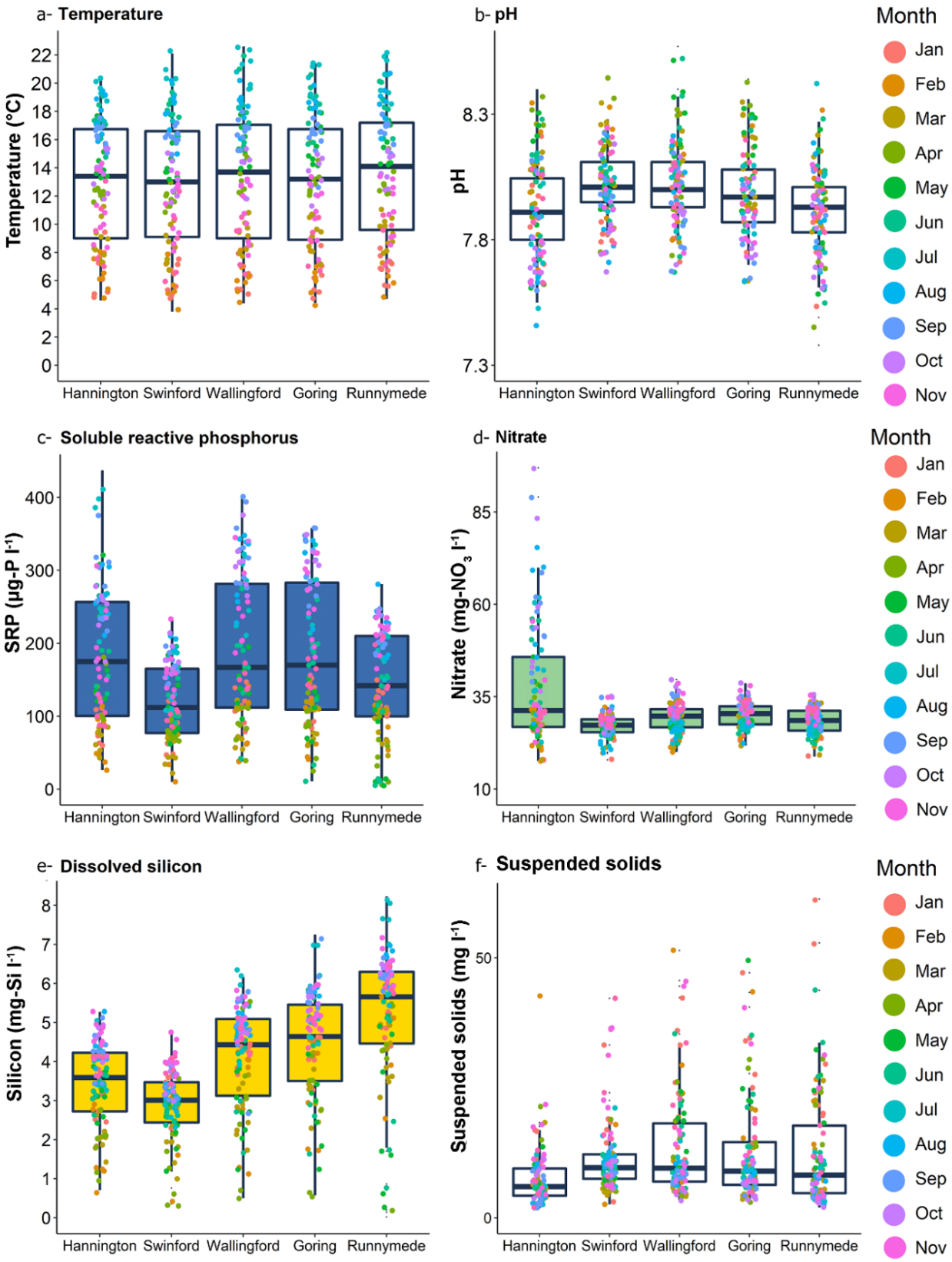


Figure 4-3 Water temperature (a), pH (b), SRP (c), nitrate (d), silicon (e) and suspended sediment (f) along the River Thames. Weekly data between 2014-2015 (January-November) provided by CEH (Thames Initiative Project)

4.5.2 Phytoplankton community succession along the Thames

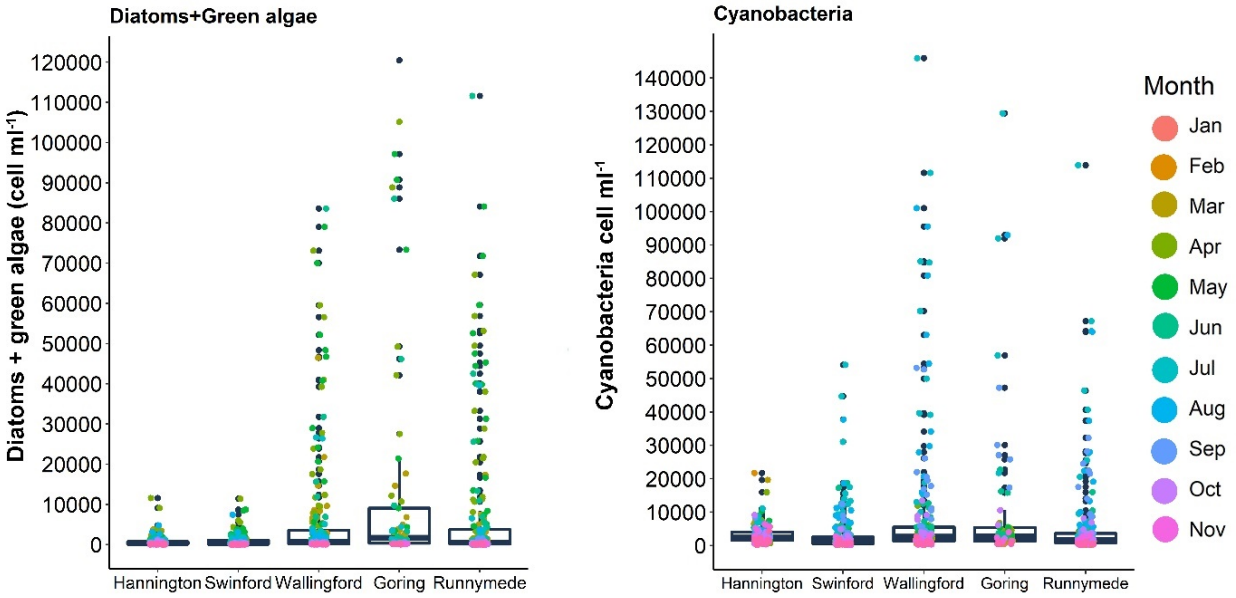


Figure 4-4 ESM 1. Diatoms, chlorophytes and cyanobacteria (cell ml^{-1}) in the Thames channel. Weekly data between 2013-2015 (January-November) provided by CEH.

There were two important successional periods evident in the phytoplankton dynamics in the Thames in 2015. The first was related to April- June diatom blooms (Figure 4-4 ESM 1a,b; Moorhouse et al., 2018), and the second in July to pico-chlorophytes and cyanobacteria. During the first period, in the middle and lower Thames, the diatom population consisted mostly of centric 'true' planktonic species, namely: *Stephanodiscus hantzschianus* Grunow and *Cyclotella meneghiniana* Kützing. These blooms were followed by true planktonic rotifers with a ten-day delay. In the upper Thames, however, diatoms were mostly benthic mixed with small chlorophytes (Figure 4-5)

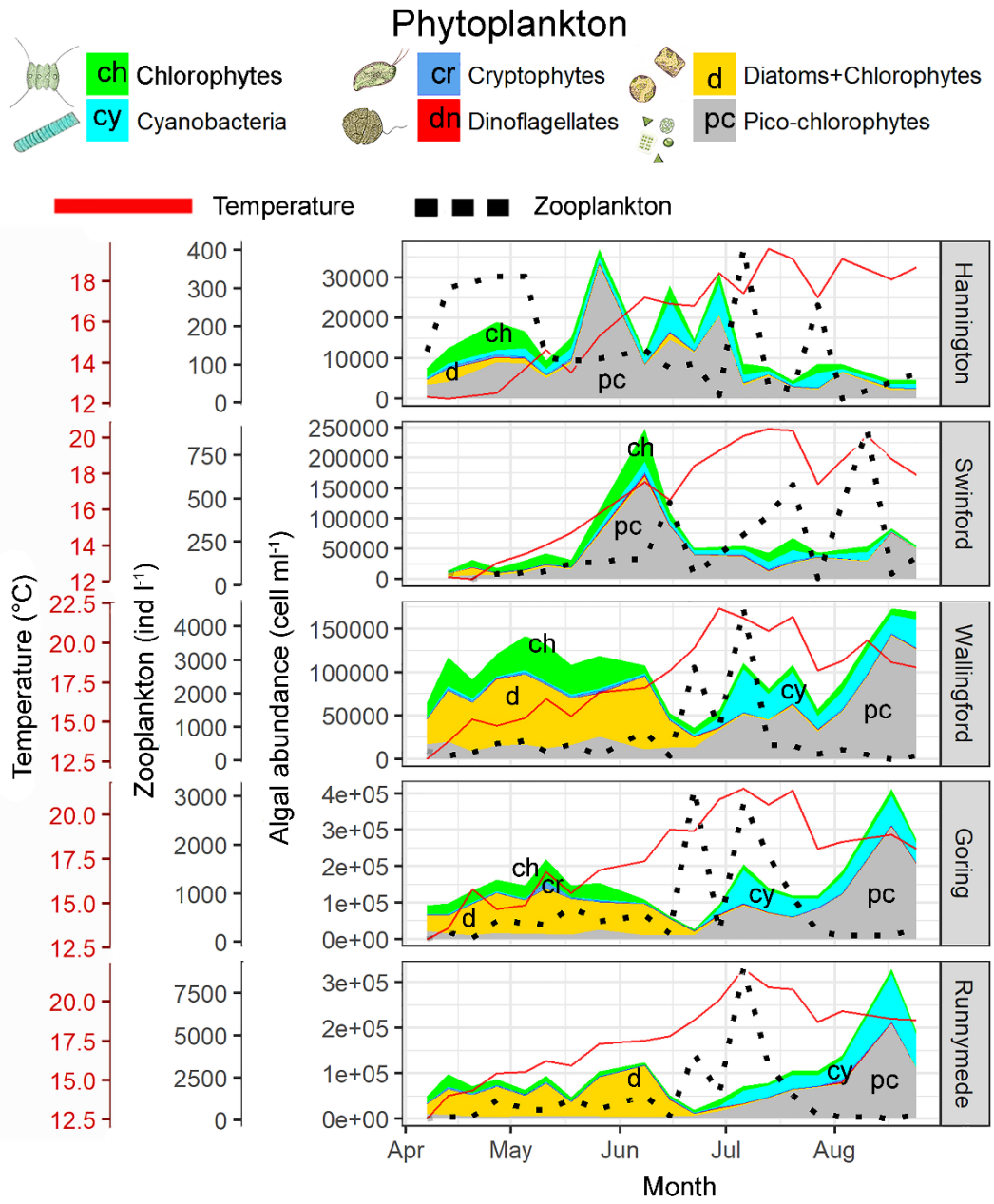


Figure 4-5 Phytoplankton (individual groups listed on the top) population densities (cell ml^{-1}), total zooplankton (dashed line), and water temperature (red line) dynamics during period active plankton growth. Weekly data from the River Thames (April-August 2015)

4.5.3 Zooplankton community diversity and abundance along the Thames

The new zooplankton community data collected simultaneously with the flow, water chemistry and phytoplankton data showed that rotifers formed more than 90% of the total metazoan (animal only) plankton along the river with more than 40 zooplankton species recorded (Appendix. Table 1). Ten species were generally more abundant: *Keratella cochlearis, Keratella quadrata, Synchaeta oblonga, Synchaeta pectinata, Polyarthra dolichoptera, Brachionus calyciflorus, Brachionus angularis, Euchlanis dilatata, Notholca squamula,* and *Notholca*

squamula. Maximum zooplankton population densities were recorded in the middle and lower reaches, with the highest density at Runnymede, followed by Wallingford and Goring.

Zooplankton in the upper Thames were represented mostly by small populations of benthic (bottom sediment), periphytic (by the banks) and epiphytic rotifers (among macrophytes), all of whom were grouped as periphytic, animals (*Lepadella, Lecane, Squatinella, Monostyla, Dicranophorus*, Bdelloid rotifers). In the upper Thames at Swinford, the zooplankton composition was similar to the middle reach, but the population was smaller in size. The sizeable rotifer populations were recorded at Runnymede (9000 ind l^{-1}), Wallingford (4500 ind l^{-1}) and Goring (3100 ind l^{-1}). In the upper Thames, zooplankton numbers did not exceed 1000 ind l^{-1}, at Swinford (750 ind l^{-1}), Hannington (400 ind l^{-1}) (Figure 4-6).

Microcrustaceans, both Copepoda and Cladocera, were observed in small numbers (maximum of 125 ind l^{-1}), along the main channel and 75-90% of them were Cyclopoid juvenile and larval forms. Several adult copepods (maximum 5-10 ind l^{-1}) were observed at every site. Cladocera were rare. *Bosmina longirostris* were found in the headwaters (10 ind l^{-1}), at Wallingford (9 ind l^{-1}) and Goring (16 ind l^{-1}). A small number of *Chydorus sphaericus* appeared at Wallingford (6 ind l^{-1}), Goring (7 ind l^{-1}) and Runnymede (32 ind l^{-1}). The abundance of predatory zooplankton was low overall: rotifers of the genus *Asplanchna* were present only in June in the lower Thames, with peaks lower than 3 ind l^{-1}. Copepodid stages and adults of cyclopoid copepods had negligible abundance.

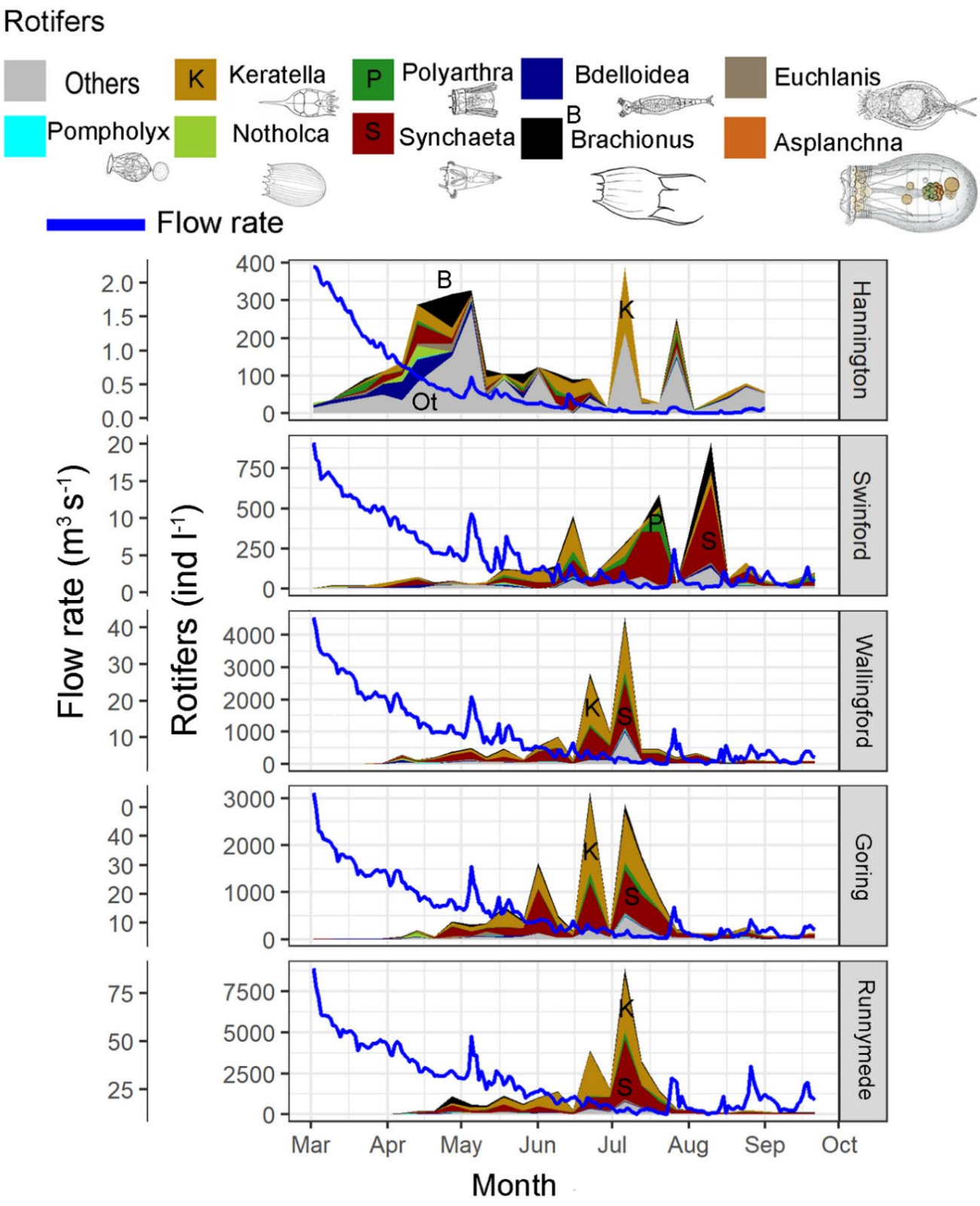


Figure 4-6 Rotifer population and flow dynamics at the five study sites in the River Thames from April to September 2015. Larger and more significant rotifer genera are listed at the top (along their size gradient). Smaller and less abundant *Colurella, Lepadella, Lecane, Anuraeopsis, Dicranophorus, Trichocerca, Trichotria* were grouped as *others*. Less common rotifers in 'others' are listed in Appendix. Table 1

There was a notable difference between the zooplankton community structure in the upper and middle-lower parts of the catchment. The zooplankton communities at Wallingford, Goring and Runnymede were dominated by euplankton ('true' plankton) (Figure 4-6): *Keratella cochlearis, Keratella quadrata, Synchaeta oblonga, Synchaeta pectinate, Polyarthra dolichoptera.* Another predominant group consisted of organisms that prefer still-water environments normally (heleoplankton), in particular ponds or marshes, with elevated pH (genera: *Brachionus, Euchlanis* and *Cephalodella*). The frequent appearance of heleoplankton, particularly after increases in flow suggests connectivity with off-channel, lentic environments for instance wetlands and flood plains (Górski *et al.*, 2013). Riverine wetlands can provide a greater variety of physical structures, which foster higher zooplankton densities and diversity (Mourelatos & Lacroix 199) and organisms can be flushed into a river during flood events from submerged marginal plant-stands in wetlands where they develop high population densities (May & Bass, 1998; Lucena-Moya & Duggan, 2011). However, as floods trigger high zooplankton mortality and removal, sizeable plankton communities can only form during long, low-flow conditions (Figure 4-6; Baranyi et al., 2002; Bertani et al., 2012).

Zooplankton population increase was significantly higher in the summer than spring, which corresponds with algal growth (Figure 4-5). Most rotifers were small filter-feeders which consume centric diatoms, unicellular green algae and bacteria. Spring communities are associated with a spring-early summer diatom bloom, which is closely followed by cold-adapted grazers, such as copepods, and rotifers: *Notholca, Synchaeta, Keratella,* and *Polyarthra.* The mid-summer period was associated with a post-diatom phase when small chlorophytes, cryptophytes and cyanobacteria are more abundant. During this period, rotifers *Brachionus*, *Euchlanis,* along with *Synchaeta, Keratella, Polyarthra* formed dense populations. This temporal difference in zooplankton community composition likely depends on the zooplankton feeding mechanism of different genera. *Brachionus* and *Keratella* create a small current with their cilia to bring food into their mouth, whilst *Synchaeta*, *Polyarthra*, and *Asplanchna* use a rapid sucking action to capture algae (Hochberg et al., 2015), and therefore the shapes and sizes of food particles play an important role in zooplankton diet (Stemberger & Gilbert, 1985; Walz, 1997). Predatory *Asplachna* have large body sizes and prey upon small protozoa and rotifers (Wetzel, 2001). Species of the family *Synchaetidae* were common due to their ability to thrive on the flagellates, chlorophytes, centric diatoms, and cryptophytes which were all observed as present at the five sites. Rotifers can also feed on cyanobacteria, though eventually, cyanotoxins have a negative effect on population although there was no evidence of this effect in this study (Ooms-

Wilms, 1997). The summer zooplankton peak was followed by a sharp fall (August-September), during which the total population was as low as 100-300 ind l^{-1} due to zooplankton mortality.

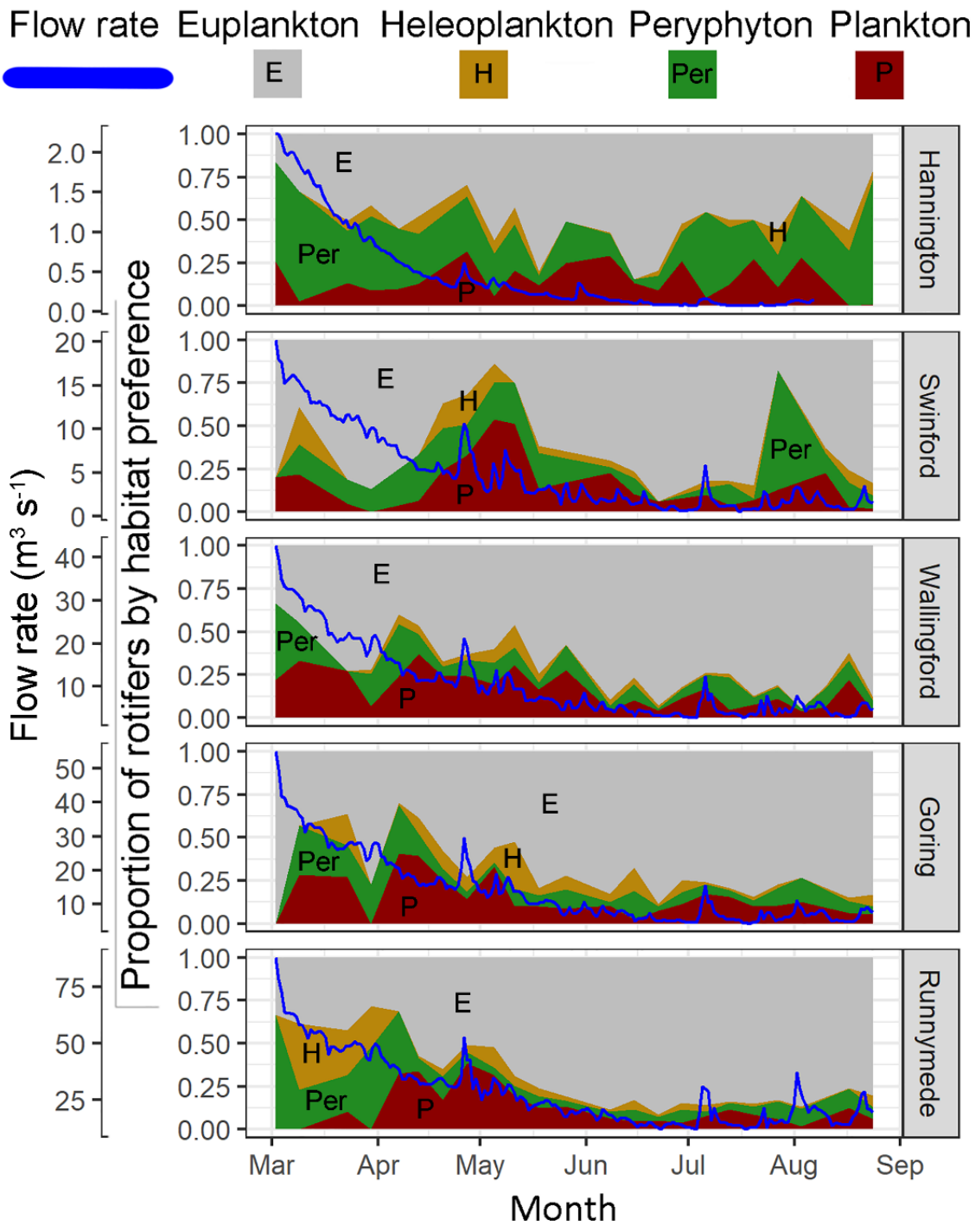


Figure 4-7 Daily mean flow and the relative proportion of rotifers in the River Thames (April-September 2015). The rotifers are grouped by preferred habitats: plankton (found mostly in pelagic zones, but can be grouped as both euplankton, heleoplankton or periphyton); euplankton (true plankton), heleoplankton (eutrophic lakes and ponds); periphytic rotifers (river banks, and among macrophytes)

The peaks in the zooplankton population observed in the middle Thames were similar to those reported by May and Bass (1998) (up to 4000 ind l^{-1}), but much lower than the maxima found in 1971 (65000 ind l^{-1}) (Bottrell, 1977). These results could be affected by different sampling techniques. Given the limited data over the near 50-year period from 1971 to 2015, it is hard to establish whether there was a trend in zooplankton and phytoplankton abundance.

4.5.4 Factors regulating river zooplankton development

The outcome of the Canonical Correspondence Analysis highlighted seven explanatory variables as the key controls on zooplankton community composition: dendritic network length, water temperature, pH, cyanobacteria, cryptophytic, diatoms and pico-chlorophytes (Table 4-3). In the headwaters, *Keratella, Polyarthra* and *Synchaeta* clustered around temperature, small chlorophytes and cyanobacteria whereas *Dicranophorus, Lindia* and *Lecan* grouped away from travel distances being observed at all five sites though with a greater abundance in the headwaters. Species of benthic Bdelloid rotifers were associated with sudden peaks in flow rates (Figure 4-8 a,b). At the scale of an individual river-reach, the relationship between the zooplankton diversity and the local environmental variables representing flow conditions, water chemistry and phytoplankton community composition varied greatly. They ranged from a strong correlation of rotifer community with both spatial and abiotic conditions to exhibiting weak association with these conditions.

Dendritic network length/travel distances

Dendritic network length was the most predictive feature for explaining the spatial variation in the zooplankton composition in this study. This is the same outcome as for bacterioplankton composition in the River Thames where dendritic network length, which is a proxy for travel time, was also the factor that explained the largest variation Read *et al.,* 2015). However, within one catchment different tributaries can differ in their productivity dependent upon geomorphology, baseflow and flood flow (Lair, 2006), and land use (Xiong et al., 2016). Tributaries can equally increase plankton biomass and diversity in the main river or dilute and decrease existing communities (Thorp et al., 1994; Kim & Joo, 2000; Lair, 2005). Thus, dendritic network length may integrate these factors.

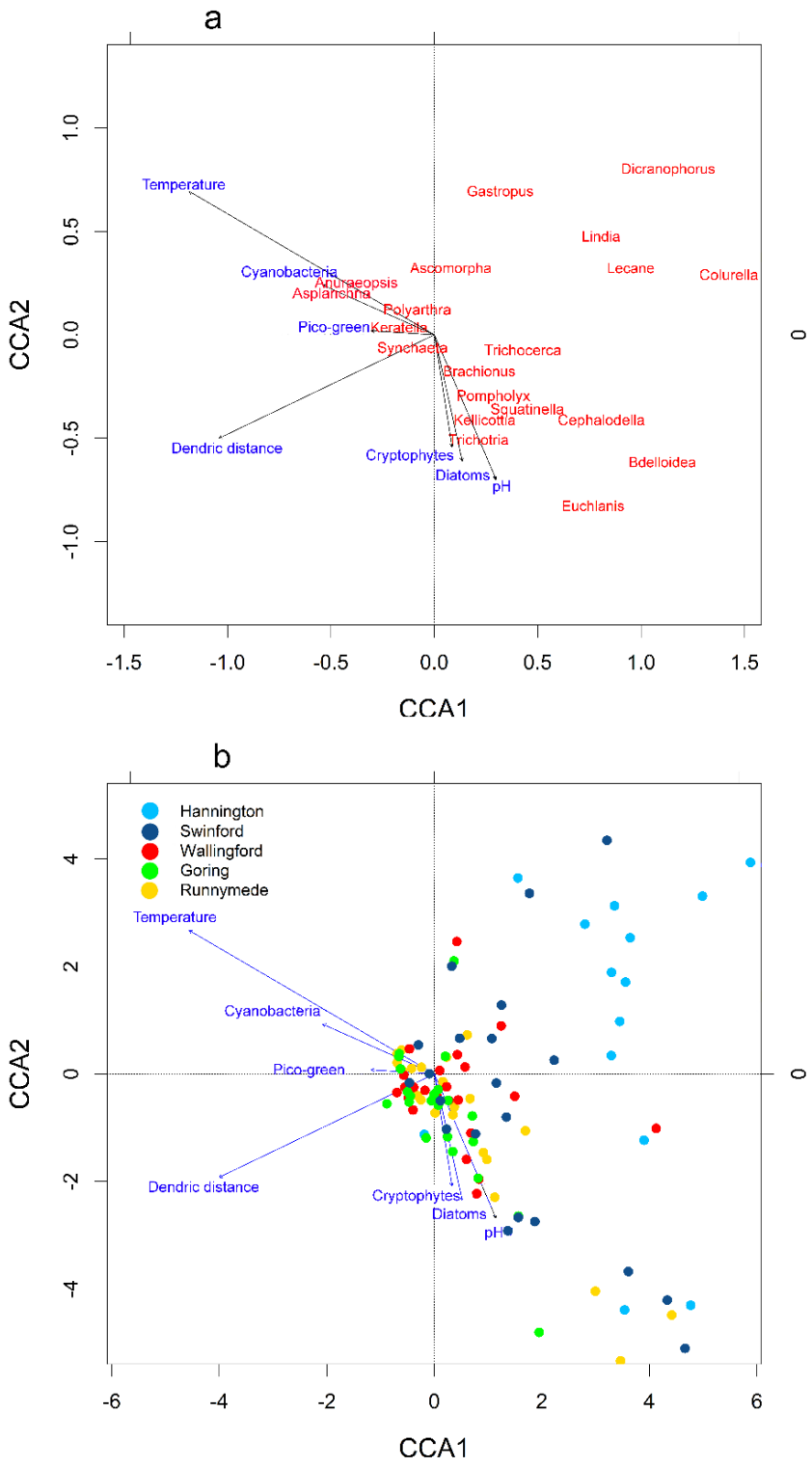


Figure 4-8 Species-conditional triplot (a) based on Canonical Correspondence Analysis of the major rotifer genera data displaying 28% of the constrained inertia. The eigenvalues of axis 1 (horizontally) and axis 2 (vertically) are 0.20 and 0.08, respectively. Species are weighted averages of site scores. Quantitative environmental variables (dendritic network length, temperature, pH, cyanobacteria, cryptophytes, diatoms, pico-chlorophytes) are indicated by arrows. Rotifer data from 5 sites along the River Thames (April-September 2015). (b) Triplot relating rotifer communities at studied sampling sites with significant explanatory variables.

Table 4-3 Significance of the explanatory variables. Permutation test for Canonical correspondence analysis (ANOVA like permutation tests for the joint effect of constraints)

Variable	pseudo-F	p-values
Dendritic network length, km	13.17	0.001 ***
Temperature, °C	12.58	0.001 ***
pH	5.74	0.002 **
Diatoms, cell ml^{-1}	3.33	0.020 *
Cryptophytes, cell ml^{-1}	4.8	0.024 *
Pico-chlorophytes, cell ml^{-1}	2.73	0.079
Cyanobacteria, cell ml^{-1}	3.09	0.049 *

Water temperature

Zooplankton diversity and abundance significantly increased with water temperature (Figure 4-5; 6) and water temperature is known to play an important role in small-bodied animals' metabolism, growth and reproduction (Herzig, 1983; Galkovskaya, 1987; Walz, 1987). Most species were observed within a 15-20°C temperature range. *Brachionus* and *Anuraeopsis fissa, Euchlanis dilatata, Cephalodella gibba* and *Trichocerca pusilla* are known as thermophiles from eutrophic environments (De Manuel, 2000). *Notholca, Synchaeta, Polyarthra dolichoptera* and *Katella cochleasis,* on the other hand, are adapted to low temperatures and are superior competitors below 14 °C (Alekseev, 2010).

Fluctuations in water temperature by 1-2°C were observed to have a significant impact on total zooplankton numbers (Figure 4-5). Water temperature fluctuations reflect changes in other abiotic constraints such as light, flow and water temperature. According to Bowes et al. (2016), light and flow are the key factors identified as controlling phytoplankton dynamics in the Thames. Rotifer populations were observed to grow faster under higher temperatures, since they shorten individual egg laying intervals, length of embryonic and post embryonic development and increase respiration rates and this is probably the case here (Herzig, 1983; Galkovskaya, 1987). In summer, the population of crustaceans was gradually decreasing to less than 10 ind l^{-1} at all studied sites, possibly due to fish predation.

River flow

Spatial variations in flow did not explain observed differences in the zooplankton community composition (Table 4-2). However, at each site, even small changes in flow had a high negative impact on rotifer abundance, although rotifers were able to recover from rapid changes in river

discharge with steep declines in abundance evident at Wallingford, Goring and Runnymede following rain events in late June (Figure 4-6; 4-7). This demonstrates the ability of the zooplankton to self-regulate and to have the resilience to changes in flow. Zooplankton formed abundant and diverse communities when water flow was close to its annual minimum, allowing organisms time to grow, reproduce and interact. The increase in rotifer population density in headwaters during April (up to 400 ind l^{-1}) was potentially associated with flow releasing organisms from the bottom sediment, macrophytes and retention zones, indicated by a high number of Bdelloid rotifers and *Brachionus* spp.

Zooplankton community composition – evidence for dependence upon phytoplankton community

The CCA ordination showed two distinct community clusters and three clusters of species (Figure 4 8). The high abundance of the rotifer genera *Brachionus*, *Trichotria* and *Euchlanis* observed at Wallingford, Goring and Runnymede was explained mostly by the presence of planktonic diatoms, pico-green algae, cryptophytes and high pH. The constrained variance explained 28% of the total variance of the data (Figure 4-8). The first axis accounts for approximately 50% of the constrained variability, and the second - 20% The permutation test showed that both axes were significant ($p < 0.01$; Parkes & Duggan, 2012). Collectively, various issues could be responsible for the low proportion of explained variability: the frequent occurrence of rare species, large geographical area, unmeasured environmental factors, interspecific interactions and sampling errors. It is possible that some undetected local variables affected the rotifer community structure. For instance, fish predation can rapidly decrease zooplankton populations (Thayer et al., 1974; Jack & Thorp, 2002; Medeiros & Arthington, 2008) although this effect needs further detailed study in this case as zooplankton spawning generally begins in spring, so that fish larvae and fry can benefit from feeding on the abundant zooplankton which feed on spring diatom blooms, and fish reproduction is naturally correlated to both phytoplankton and zooplankton development (Thorp and Casper, 2003; Ning et al., 2010). The CCA demonstrated zooplankton-phytoplankton interactions. Further evidence of this relationship is seen in the longitudinal profile (Figure 4-9) that highlights an abrupt increase in phytoplankton biomass based on chlorophyll *a* concentrations and zooplankton abundance between Swinford and Wallingford. The amplitude of these seasonal peaks could not be explained by the inner-channel plankton growth only. Therefore, the influence of tributaries and inner channel storage zones (including locks and weirs) should be considered, as previously described by Bowes et al. (2012). This was reinstated in the study of bacterioplankton by Read et

al. (2015). These profiles confirm that traditional views on gradual longitudinal development postulated in the River Continuum Concept (Vannote et al., 1980) cannot explain zooplankton community distribution and dynamics in highly modified rivers (Le Coz et al., 2017). More recent views include the Riverine Ecosystem Synthesis (RES) framework by Thorp et al. (2006), which conceptualises the importance of catchment heterogeneity and areas with high hydrological retention for plankton development and distribution. This may explain steep plankton biomass and population gradients in the middle of the Thames main stem.

Downstream from Wallingford, the observed stream water chlorophyll-*a* concentrations generally decreased with distance, whereas the zooplankton populations tend to increase. The decrease in chlorophyll-*a* concentration may be related to microbial pathogens, zooplankton grazing, or dilution from less productive tributaries (Bertani et al., 2012; Zimmermann-Timm et al., 2007; Le Coz et al., 2017) as essential for phytoplankton metabolism macronutrients remained available (Figure 4-3). Reynolds (2000) stressed that although lower flow, warmer temperatures and clearer water allow an exponential increase in algal biomass, for planktonic animals the survival opportunities are limited by longer generation times, therefore they are strongly dependent on travel distances and this may explain the general increase in zooplankton abundance with downstream distance. Due to the high number of locks (45) there are retentive zones in the Thames, however each lock has at least one adjacent weir creating a turbulent environment thereby increasing plankton mortality. The increasing current velocity has a negative effect on zooplankton, as they struggle to feed and reproduce (Dickerson et al., 2010) and combined effect of longer residence times and flow over weirs requires further investigation.

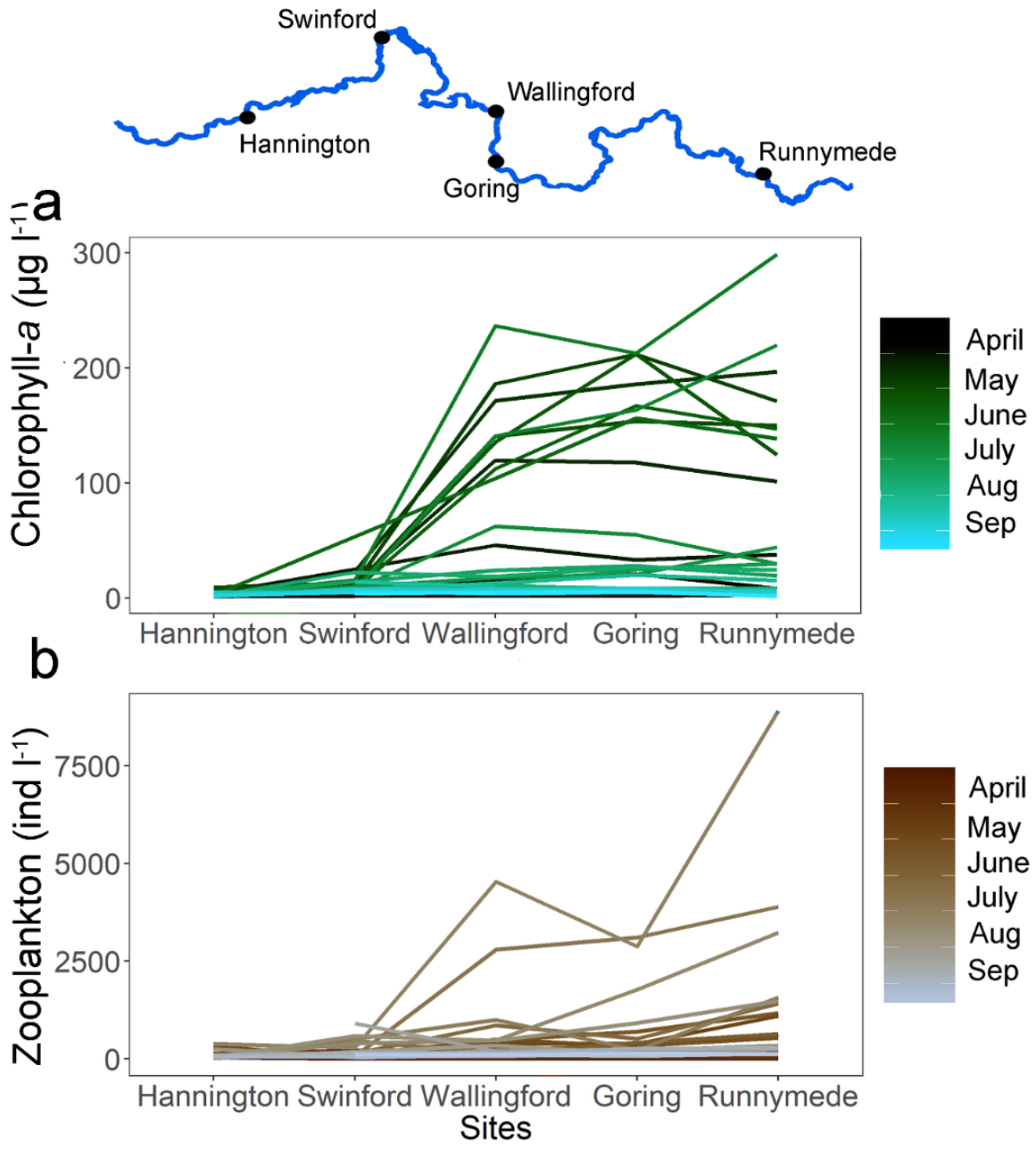


Figure 4-9 Longitudinal profiles: (a) chlorophyll-a and (b) zooplankton abundance at 5 sites along the River Thames during April to September 2015

4.6 Conclusion

The present study shows that under spring to summer low-flow conditions, zooplankton can develop large populations in the middle and lower Thames. During the active growth phase, zooplankton exhibit internal, self-regulatory properties in response to sudden changes in flow and water temperature. Spatial variations in zooplankton community composition in the Thames are regulated by travel distances, phytoplankton communities and water temperatures. There was evidence of connectivity between off-channel, shallow, stagnant environments and the main river

with zooplankton genera of the former found in the main channel. Weekly measurements of phytoplankton composition in parallel to zooplankton data has been a major advantage in this study which demonstrated that zooplankton abundance, community structure and succession were strongly linked to algal biomass and the types of organisms dominating in phytoplankton. Therefore, biological interactions between phyto- and zooplankton should not be ignored when looking to improve our understanding of river ecology. There was a rapid change in phytoplankton and zooplankton between Swinford and Wallingford suggesting a strong influence of the tributaries. Future research should put more emphasis on defining main sources of plankton inoculum in the Thames catchment in the effort to understand and quantify phytoplankton blooms and ecosystem energy and carbon flow. Predation of planktivorous fish or benthic bivalves was not considered in this study and this is needed as the next step to develop an enhanced understanding of the zooplankton community dynamics.

4.7 Acknowledgement

This work was funded by the Natural Environment Research Council (NERC), through the SCENARIO DTP Program and CEH Thames Initiative (NEC04877) and MaRIUS (Managing the risks, impacts and uncertainties of droughts and water scarcity; NERC Grant NE/L010364/1) projects. River flow data was obtained from the NERC National River Flow Archive.

Appendix Table 1 Species of Rotifera. Sites: the Thames and its tributaries (April-June 2015)

Species
Anuraeopsis fissa (Gosse, 1851)
Brachionus angularis Gosse, 1851
Brachionus calyciflorus Pallas, 1766
Brachionus leydigi Cohn, 1862
Brachionus quadridentatus Hermann, 1783
Brachionus rubens Ehrenberg, 1838
Brachionus urceolaris Muller, 1773
Keratella cochlearis (Gosse, 1851)
Keratella quadrata (O. F. Muller, 1786)
Notholca labis Gosse, 1887
Notholca squamula (O. F. Muller, 1786)
Plationus Segers, Murugan and Dumont, 1993
Platyias Harring, 1913
Kellicottia Ahlstrom, 1938
Cephalodella gibba (Ehrenberg, 1832)
Eosphora Ehrenberg, 1830
Colurella Bory de St. Vincent, 1824
Lepadella Bory de St. Vincent, 1826
Squatinella Bory de St. Vincent, 1826
Lecane Nitzsch, 1827
Monostyla Ehrenberg, 1930
Aspelta Harring and Myers, 1928
Dicranophorus Nitzsch, 1827
Ascomorpha Perty, 1850
Gastropus Imhof, 1888
Asplanchna priodonta Gosse, 1850
Conochilus Ehrenberg, 1834
Bryceella Remane, 1929
Proales Gosse, 1886
Epiphanes Ehrenberg, 1832
Euchlanis dilatate Ehrenberg, 1832
Polyarthra dolichoptera Idelson, 1925
Synchaeta oblonga Ehrenberg, 1832
Synchaeta pectinate Ehrenberg, 1832
Pompholyx sulcate Hudson, 1885
Trichocerca pusilla (Lauterborn, 1898)
Trichotria Bory de St. Vincent, 1827
Lindia Dujardin, 1841
Filinia brachiate (Rousselet, 1901)
Scaridium Ehrenberg, 1830
Proales Gosse, 1886
Bdelloidea Hudson, 1884

Chapter 5 Riverine zooplankton at catchment scale

Freeman A., Armstrong L. K., Bowes M.J., Hutchins M. G., Nicholls D., Read D. S., Roberts C., Scarlett P., Thackerey S., Wade A. J., Wickham H.

5.1 Abstract

Zooplankton constitute important links in river food webs. Their communities can be influence by the catchment heterogeneity. This study assessed the effect of tributaries and a large reservoir on the zooplankton in the River Thames, a major UK river. The composition and abundance of metazoans were measured along the Thames, six of its tributaries, and Farmoor reservoir weekly in April-June 2015. These data were analysed with respect to physical geography, water chemistry, and phytoplankton composition to determine the most important factors regulating the zooplankton distribution and dynamics. Multivariate statistical analysis demonstrated that zooplankton in the middle-lower Thames were similar to those in the tributaries with high zooplankton abundance. Farmoor plankton had no visible effect on the Thames plankton. Tributaries with high zooplankton abundance (the Cherwell, Evenlode, Kennet) have longer travel distances and are connected to canals. Both zooplankton and phytoplankton there were mainly true planktonic species. In other rivers (the Thame, Pang, Cut), periphytic and benthic metazoans had greater proportions in the communities. Rotifers formed more than 70% of the zooplankton found in the Thames and its tributaries. Most common were*: Keratella cochlearis, Polyarthra dolichoptera, Synchaeta oblonga* and *Brachionus calyciflorus*. Crustaceans were recorded at all sites, mostly copepods nauplii.

Keywords: plankton, phytoplankton, river catchment, heterogeneity, flow cytometry, metazoans

5.2 Introduction

Zooplankton are an important component of river food webs; they influence the abundance and diversity of, phytoplankton communities by grazing, provide food for fish, and take part in the transformation and circulation of organic matter (Thayer et al., 1974; Medeiros & Arthington, 2008; Ger et al., 2016). Factors regulating zooplankton composition and dynamics have been a focus of river ecology over recent decades (Lair, 2006), with the majority of studies directed at how hydrology, inshore retentive habitats (storage zones), water temperature, chemical composition and food availability drive population abundance, diversity and dynamics (Basu & Pick, 1996; Reckendorfer et al., 1999; Reynolds, 2000; Bertrand et al., 2001; Baranyi et al., 2002; Rossetti et al., 2009; Bertani et al., 2012; Mitrofanova, 2015). Fewer authors have emphasized the importance of tributaries and reservoirs on zooplankton diversity and abundance

(Zimmermann-Timm et al., 2007; Havel et al., 2009; Dickerson et al., 2010; Górski et al., 2013; Zhao et al., 2018).

Thorp *et al.* (1994) and Viroux, (1999) showed that tributaries can significantly influence the zooplankton community in the main river channel. Nevertheless, this effect is highly variable over time and among river systems, as it depends on both abiotic and biotic characteristics of each tributary as well as its contribution to the overall discharge of the main river (Thorp et al., 1994; Wehr & Thorp, 1997; Kim & Joo, 2000; Lair, 2005). Furthermore, Dickerson et al. (2010) observed a significant increase in populations of microcrustaceans, which are commonly found in lakes, in the main river channel downstream from reservoirs. Distance from the nearest upstream reservoir explained more of the overall river zooplankton community pattern than any other combination of environmental factors. Though zooplankton communities are shaped by both regional and local factors (Cottenie et al., 2013), it has been demonstrated that human activities within the floodplain and main river can have a stronger effect on zooplankton composition than dispersal caused by regional factors at the catchment level (Xiong et al., 2016). There is a need for further examination of how catchment environmental heterogeneity and connectivity shape plankton communities in lowland rivers to better understand zooplankton community composition and dynamics with a view to how this will affect food webs and energy and carbon transfer. Therefore, this study aims to assess the influence of tributaries and a reservoir on zooplankton composition and dynamics in the River Thames, a major UK river-system, which has been studied intensively in terms of hydrology (Crooks & Kay, 2015), water chemistry (Wade et al., 2012; Bowes et al., 2014), phytoplankton (Lack, 1971; Ruse and Hutchings, 1996; Ruse and Love, 1997; Read et al., 2014, Moorhouse et al., 2018) and bacterioplankton (Read et al., 2015). Bowes et al. (2012) previously identified that eutrophic tributaries connected to canals had greatly increased phytoplankton biomass entering the Thames. This research question was restated by Read et al. (2015) describing the bacterioplankton spatial distribution in the catchment. The only available survey of zooplankton in the tributary of the River Thames (the River Kennet) was conducted in 1971 (Bottrell, 1977). Maximum metazoan abundance in the Kennet (3500 ind l^{-1}) was almost 20 times smaller than in the Thames (65000 ind l^{-1}). However, in a more-recent study of the Thames in 1996, only 4000 ind l^{-1} were estimated in the middle reach (Bass and May, 1996). This significant fall in the total zooplankton abundance in the Thames could be due to urbanization within the catchment resulting in deterioration in water quality and loss of natural habitats, as alterations in the river

flow such as the construction of new reservoirs or the transfer of water from other catchments impact the zooplankton thereby affecting macroinvertebrates and more importantly fish.

This study aims to define the relationships between the zooplankton community composition and abundance along the Thames channel, some of its major tributaries, and Farmoor reservoir (filled by pumping water from the Thames). The first objective is to measure and characterise zooplankton diversity and abundance in these environments. The second was to relate these data visually and statistically with physical geography, water chemistry, and phytoplankton composition to determine important factors regulating the zooplankton community distribution and seasonal dynamics. This study aims to answer the following questions:

- Do zooplankton composition and abundance vary spatially in the Thames catchment? What environmental factors lead to these variations?
- Do tributaries and Farmoor Reservoir significantly affect zooplankton community composition and abundance in the Thames channel?

5.3 Study area

The River Thames is 346 km long and flows eastwards from the Cotswolds to the North Sea (Figure 5-1). The main channel of the River Thames is divided into reaches by 45 locks with adjacent weirs which are used for navigation and flood control. The catchment population is over 14 million people, and most live in London with other large urban centres in Swindon, Oxford, Newbury, Reading, Slough and Maidenhead. Mean annual precipitation is around 700 mm (1961-1990) and, with a baseflow index (BFI) of 0.63, the Thames can be considered as moderately groundwater-dominated (Whitehead et al., 2015). High flows generally occur in the autumn-spring period, and low flows in summer (Crossman et al., 2013). Water is abstracted from both surface and groundwater resources, and there is a large reservoir at Farmoor (Figure 5-1). The geology of the northwest part of the catchment is limestone and clay mainly. Downstream of Wallingford, the river flows over chalk until it runs onto sandstones and mudstone at Maidenhead. The catchment is mainly rural with the predominant land-cover types being arable (35%), grassland (32%), woodland (16%) and urban and semi-urban (14%) (Appendix. Table 1). Intensive agriculture and sewage inputs result in elevated concentrations of stream water phosphorus, nitrogen and sediment (Neal et al., 2010) and the nutrient inputs have been linked to algal blooms; however, more recent work suggests that water residence time, light and water temperature are more important controls (Bowes et al., 2016). As part of the Thames

Initiative (Bowes et al., 2018) water quality, including chlorophyll-a concentrations, has been measured weekly at more than 20 sites from 2009, and the monitoring programme is ongoing. Most of the water quality sites are co-located at, or very close to, Environment Agency flow gauging stations. The mean daily flow data are available from the National River Flow Archive (National River Flow Archive (© NERC, Centre for Ecology and Hydrology, UK, 2018. http://nrfa.ceh.ac.uk/). The phytoplankton assemblages have been determined at 22 sites between 2013-2015. In this study, 12 sites from the Thames Initiative were sampled for zooplankton. There were five, approximately equidistant, points along the River Thames, two in the upper reaches (Hannington, Swinford) and three in the middle-lower stretch (Wallingford, Goring and Runnymede; Table 5-1). Six tributaries were sampled with three in the upper (Evenlode, Cherwell and Thame) and three in the lower (the Pang, Kennet, Cut) catchment, and Farmoor reservoir was also sampled at the same time. These sites cover different geological, flow, water quality and land cover types (Table 5-1). The Evenlode, Cherwell and Thame are predominately rural with small towns and villages. Larger towns of Aylesbury and Thame are located in the Thame, and Banbury and Bicester are located in the Cherwell catchment which also includes engineered sections and the Oxford Canal (mixes with the Cherwell). The River Pang is a small chalk river, while the River Kennet is rural in its upper part and urban in the lower, flowing through two large towns: Newbury and Reading. The lower reaches are navigable and are known as the Kennet Navigation where the river and Kennet and Avon canal mix. The Cut has a rural headwater but drains Bracknell, and three main Sewage Treatment Works at Bracknell, Ascot and Maidenhead. Water is abstracted from the Thames to supply Farmoor Reservoir during winter high flows, and then water is released during periods of lower flow. The reservoir provides drinking water to Oxford and nearby towns and is also used for recreation.

Zooplankton in the Thames and the Kennet were surveyed in 1971 (Bottrell, 1977) and the Thames only in 1996 (Bass and May, 1996). However, changes in methodology make a comparison with, or between, these studies difficult.

Table 5-1 Studied sites, and distance from the source of each individual river, mean flow and catchment area

Study site	Dist. from the source*, km	Dendric distance*, km	Catch. area**, km^2	Mean flow $m^3 s^{-1}$ **	Bedrock***	Connected canals	Baseflow indices (BFI)*
Thames at Hannington	29	193	185	1.4	Mudstone, Siltstone, Sandstone		0.7
Thames at Swinford	76	638	1616	14.1	Mudstone, Siltstone, Sandstone		0.67
Thames at Wallingford	129	1727	3445	28.8	Chalk		0.64
Thames at Goring	138	1789	4634	38.5	Chalk		0.66
Thames at Runnymede	212	5516	7046	60	Chalk		0.72
Evenlode at Cassington Mill	60	178	430	3.8	Sandstone, Limestone, Argillaceous Rocks (rich in carbonate deposits, corals and shelly faunas)	Old Canal	0.71
Cherwell at Hampton Poyle	81	261	551.7	3.9	Mudstone, Siltstone, Limestone, Sandstone	Oxford canals	0.65
Thame at Wheatley	53	238	533.8	3.8	Mudstone, Siltstone, Sandstone	Grand Union canal	0.55
Pang at Pangbourne	17	37	170.9	0.7	Chalk		0.87
Kennet at Woolhampton	135	206	548	5.1	Chalk	Kennet and Avon canal	0.93
The Cut at Paley Street	14	27	50	0.4	Clay, Silt and Sand		0.46

*Strategy (Ordnance Survey (GB), 2016)

** National River Flow Archive (© NERC, Centre for Ecology and Hydrology, UK, 2018)

*** Geology of Britain viewer. Surface Geology. Bedrock and Superficial 1:625 000 (Survey British Geological Survey, 2018)

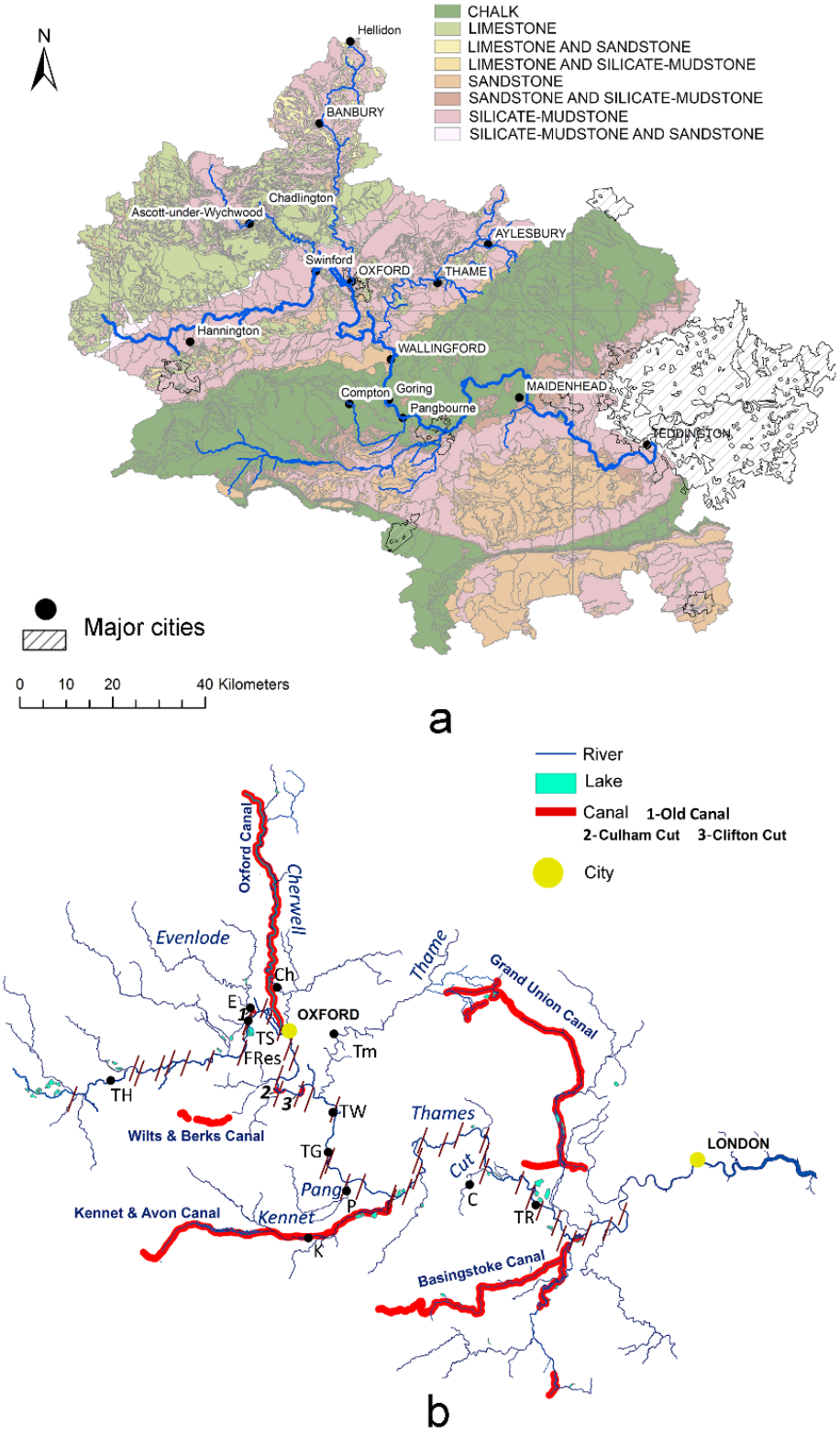


Figure 5-1 Catchment geology (bedrock) and study sites (a). Canals and weirs (b) in the Thames catchment and zooplankton study sites: TH-Thames at Hannington; TS-Thames at Swinford; E-the Evenlode; Ch-the Cherwell; Tm-the Thame; TW-Thames at Wallingford; TG-Thames at Goring; P-the Pang; K- the Kennet; 10-the Cut; TR-Thames at Runnymede; FRes - Farmoor Reservoir

5.4 Methodology

5.4.1 Zooplankton survey

Sampling was conducted weekly in 2015 during the active plankton growth period, April-June. One-litre samples were taken with a bucket from the bank or mid-stream, at the same time as the water chemistry and flow cytometry surveys. Samples were filtered through sieves with mesh diameters 30/53 µm and preserved in formaldehyde solution (4%) on the same day as sampling. Zooplankton (metazoans only) were identified and enumerated in sedimentation chambers (after 2 hr of settling) at 100-400X magnification using an inverted microscope (Zeiss Axiovert 40CFL). Up to 200 individuals were counted and extrapolated on the rest of the sample. If the total number of organisms was less than 200, all rotifers and microcrustaceans were counted. Identification of rotifers was taken to genus and species level (when possible), using printed keys (Mellanby, 1951; Pontin, 1978; Alekseev, 2010) and web resources (Haney, 2013). Body length of minimum of 20 individuals per sample was measured with 'ImageJ' software and digital camera photographs (Rueden et al., 2017). For this study, microcrustaceans were differentiated as Cladocerans or Copepods adults, copepodites, and nauplii.

5.4.2 Water chemistry

To describe water chemistry heterogeneity in the Thames catchment weekly data from a 2-year period (2014-2015) were applied. Measurements of phosphorus, nitrate, silicon, chlorophyll-*a*, water temperatures, suspended sediment, and phytoplankton composition were collected by the CEH Thames Initiative research platform using the methods described in Bowes *et al.* (2018).

5.4.3 Phytoplankton

Phytoplankton composition was analysed using flow cytometry, described in Read *et al.* (2014). This method enumerates algae from the previously defined phenotypical groups of phytoplankton, based on cell sizes and pigment fluorescence using a Gallios flow cytometer (Beckman Coulter, UK) equipped with blue (488 nm) and red (638 nm) solid-state diode lasers. The samples (approx. 20 ml) were stored in 30 ml universal tubes at 4°C before analysis within 24 hr of collection. The analysis protocol compares fluorescence from phycoerythrin versus chlorophyll and chlorophyll versus the phycocyanin. A set volume of counting beads FlowCount (Beckman Coulter) was added to individual samples, each sample was run for five minutes at a high flow rate. Data was processed using the software Kaluza Analysis v1.5a (Beckman Coulter,

UK). This method allows identification and enumeration of diatoms, green algae, cryptophytes and cyanobacteria.

5.4.4 Data analysis

Data were analysed using R 3.4.2 (R Core Team, 2017), with the vegan 2.4-6 package (Oksanen et al., 2017). Dissimilarities in the zooplankton community structure were explored in relation to environmental variables using nonmetric multidimensional scaling (NMDS) (Clarke, 1993). The zooplankton dataset was composed of rotifer genera and crustaceans as cladocerans and copepods and these data were log(x + 1) transformed to reduce the influence of the most abundant taxa. Then data were converted to Bray-Curtis dissimilarity matrix. Significant ($p < 0.05$) environmental variables were plotted as vectors with function 'envfit'. Envfit finds vector averages of environmental variables, and the projections of points onto vectors have maximum correlation with corresponding environmental variables. These variables were: water temperature, pH, suspended sediment, soluble reactive phosphorus, chlorophyll-*a*, nitrate, total dissolved nitrogen, silicon, flow rate, population densities of diatoms, nano-chlorophytes, large chlorophytes, pico-chlorophytes, cryptophytes, large cryptophytes (dinoflagellates), 4 groups of cyanobacteria. The significance of explanatory variables was estimated using permutational multivariate analysis of variance (PERMANOVA, function 'adonis', permutations = 999, method = "bray") (Anderson, 2001). For permutational analysis, two additional variables were considered: travel distances from the source and dendric network length (Figure 5-2). Distance from source and dendric network length (Figure 5-2) were considered as the most appropriate spatial characteristics of the sampling sites because zooplankton cannot swim against a current and complete their entire lifecycle in the water (Tonkin et al., 2017). PERMANOVA analyses the multivariate variance of community data with respect to a set of explanatory variables based on dissimilarity measures, thereby allowing testing of differences at a community level while permitting a wide range of empirical data distributions. To avoid data misinterpretation, when testing significance of travel distances, zooplankton from Farmoor Reservoir were not included.

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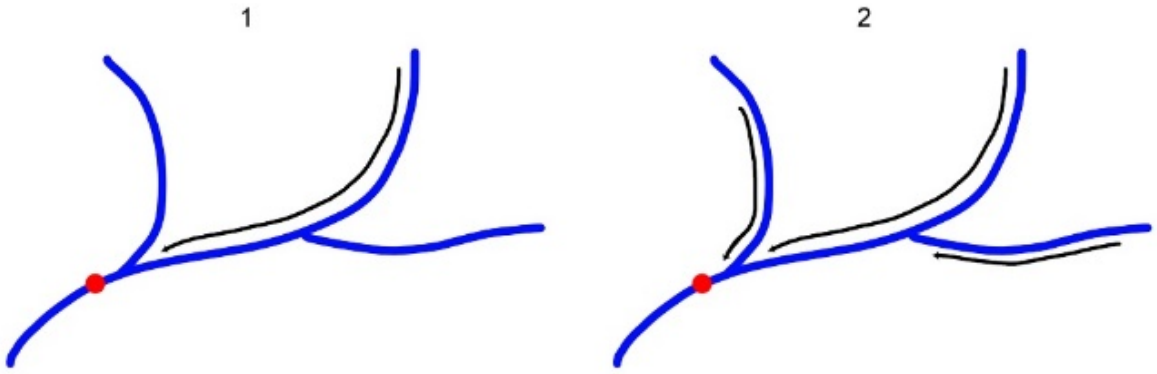

Figure 5-2 Types of physical distances employed in this study: 1- distance from the headwater source; 2- dendric network length

5.5 Results and Discussion

5.5.1 Zooplankton composition and abundance

Zooplankton composition and abundance varied across the Thames catchment, though at all studied sites, the zooplankton were dominated by rotifers (Figure 5-3). More than 40 species were recorded (Appendix.Table 3), and the ten most abundant species were: *Keratella cochlearis, Keratella quadrata, Synchaeta oblonga, Synchaeta pectinata, Anuraeopsis fissa, Polyarthra dolichoptera, Brachionus calyciflorus, Brachionus angularis, Euchlanis dilatata, Notholca squamula, Notholca squamula, Asplanchna priodonta.*

All studied tributaries could be ordered based on their zooplankton maximum abundance and species richness. The most zooplankton-rich tributary was the Cherwell (1133 ind l^{-1}; 44), followed by the Evenlode (993 ind l^{-1}; 39), and Kennet (789 ind l^{-1}; 36), and less zooplankton productive - the Pang (566 ind l^{-1}; 33), Cut (259 ind l^{-1}; 22), Thame (188 ind l^{-1}; 34) (Figure 5-6). In the Thames zooplankton abundance and species richness increased longitudinally from the upper part at Hannington (327 ind l^{-1}; 36) and Swinford (455 ind l^{-1}; 35) to the middle at Wallingford (2800 ind l^{-1}; 43) - Goring (3112 ind l^{-1}; 45), and lower section at Runnymede (3890 ind l^{-1}; 45).Microcrustaceans, both Copepoda and Cladocera, were observed in relatively small numbers (with a maximum of 299 ind l^{-1} in the Cherwell and only 125 ind l^{-1} in the Thames). Over 75-90% of microcrustacean individuals were Cyclopoid copepodites and nauplii. Cladocera were rare; the maximum population densities of adult *Bosmina* spp. (19 ind l^{-1}) were sampled from the Cherwell, and Evenlode (11 ind l^{-1}). *Chydorus* spp. were found in the River Thames main channel, at both upper and lower sites. Adult copepods in the Cherwell and Evenlode were a sign of long residence time in these rivers.

In the main Thames and its tributaries, zooplankton abundance increased in spring or summer, after phytoplankton peaks, with the precise patterns varying among sites. In summer, the magnitude of zooplankton population increase was significantly higher.

The zooplankton community composition in Farmoor reservoir differed from that observed in the main river channel, mostly due to higher abundances of crustaceans. Crustaceans accounted for a large percentage (35-80%) of total zooplankton abundance with more than half represented by adult and juvenile copepods (up to 771 ind l^{-1}). The maximum abundance of more than 1100 ind l^{-1} was recorded in June. Cladocerans found in the reservoir (*Daphnia* spp. Müller, 1785 and *Ceriodaphnia* spp. Richard, 1894) generally inhabit standing waters. They were absent in the Thames and all the studied tributaries. The zooplankton peaked concurrently with the summer diatom and nano- and pico-chlorophytes bloom. In spring phytoplankton there was formed mostly by cyanobacteria (exceeded 105 cells ml^{-1}). Both crustaceans and rotifers tend to avoid grazing on cyanobacteria (Sellner et al., 1993). The maximum zooplankton abundance reached 2500 ind l^{-1}. The rotifer community in Farmoor was composed mainly by *Keratella cochlearis* - 30%, *Synchaeta.*sp - 20%, *Polyarthra dolichoptera* – 14%, *Pompholyx sulcate* – 10%, *Asplanchna priodonta* – 10%, *Keratella quadrata* – 6%. *Asplanchna priodonta* is a predator, some individuals' stomachs contained either adult *Keratella* and *Polyarthra*, or their eggs.

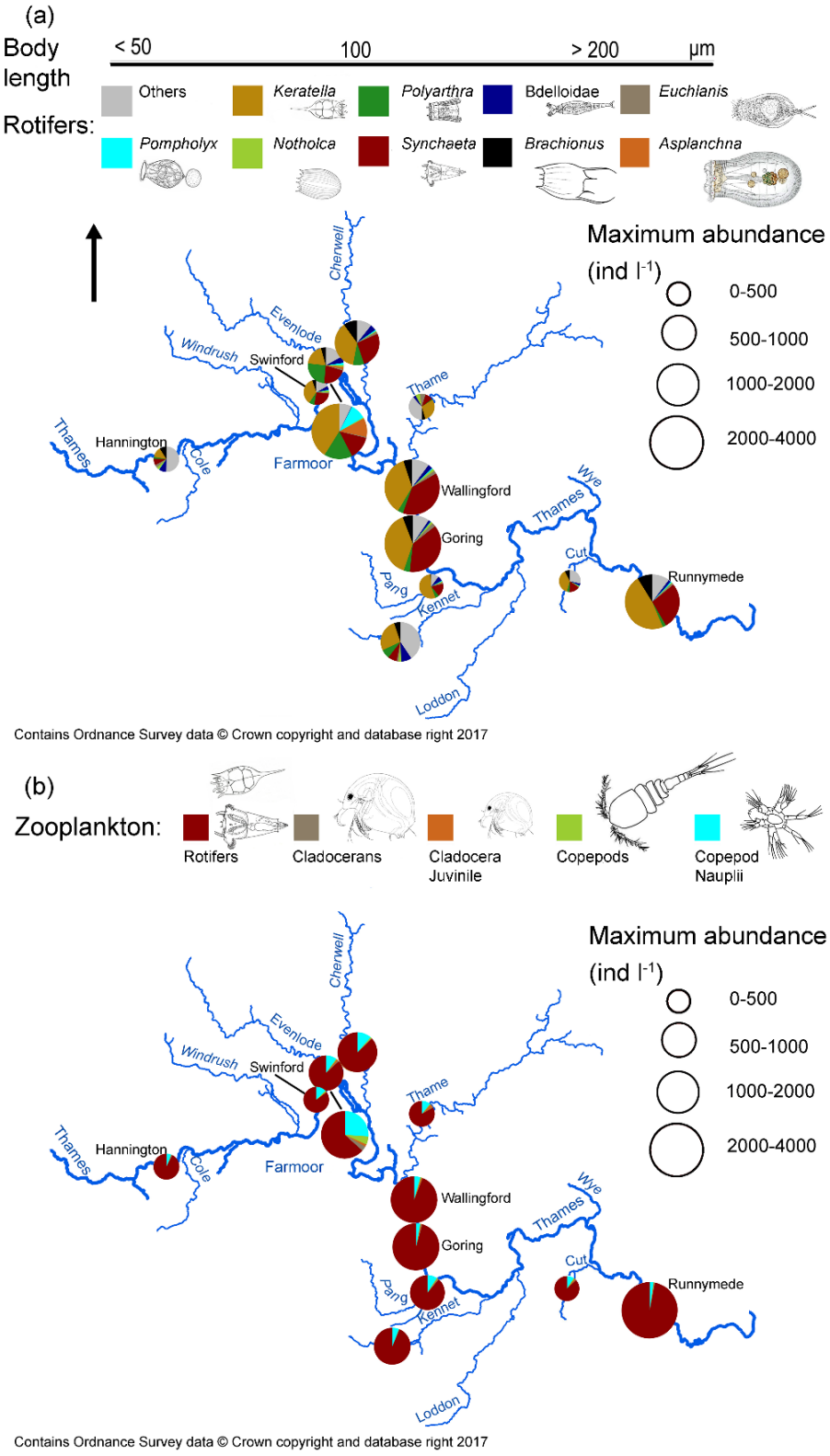

Figure 5-3 Maximum rotifer abundances (ind l^{-1}) and average community structures in April-June across the Thames catchment. Each pie chart: a- mean composition of most abundant rotifer genera; b – mean composition of rotifers and most common microcrustaceans. The size of a circle is related to maximum zooplankton abundances at each individual site. Common rotifer genera are shown along their body size gradient at the top of the diagram. Small and less abundant genera are shown as *others*.

Water temperature in the Thames catchment differs spatially and seasonally. During 2014-2015 between all sites median temperatures varied between 12-15.5°C (Figure 5-4-a). In the spring-summer period, which generally is favourable for phytoplankton growth (Bowes et al., 2016), along the Thames channel, upper reaches were colder than the lower ones by approximately 1°C. At the same time, all tributaries were colder by 2-5°C, particularly the groundwater-dominated Kennet and Pang, which were colder than the Thame, Cut, Cherwell and Evenlode by up to 3°C. Maximum temperatures of 22°C were recorded in the main Thames in summer, followed by 20°C in the Cherwell, Evenlode, Thame, Cut. In the Pang and the Kennet temperature maximums stayed below 18°C. The reservoir's surface layer warmed faster than the Thames most likely due to the absence of groundwater influence. As a result, median and maximum temperatures in the Farmoor were 2°C higher than in the river and its tributaries. The range of pH measured along the Thames is very similar among most sites. In 2014-2015 the highest pH (up to 8.6) was recorded in Farmoor. The Cut was clearly different from other tributaries, where the pH fluctuated in the range 7.1 – 8.0, while at other sites the typical range was 7.5 - 8.3 (Figure 5-4-b).

Soluble reactive phosphorus (SRP), nitrate, dissolved silicon and concentrations of suspended solids in the Thames catchment vary spatially and seasonally. The highest SRP (>1000 µg-P l^{-1}) concentrations were recorded in the Thame and Cut (Figure 5-4-c). In both rivers, these elevated concentrations were related to sewage treatment works inputs (Bowes et al., 2014). In the middle Thames (Wallingford- Goring) – not far downstream from the confluence with the Thame, concentrations were relatively high (up to 500 µg-P l^{-1}). High maximum concentrations were measured in the headwaters of the Thames (max. 430 µg-P l^{-1}) and in the Evenlode (max. 480 µg-P l^{-1}) and Cherwell (max. 442 µg-P l^{-1}). These sites receive both point and diffused pollution. In the Thames at Swinford, Goring and Runnymede, the maximum SRP concentrations were lower (around 300 µg-P l^{-1}) and maximums of 200 µg-P l^{-1} were recorded in the Pang, Kennet and Farmoor. Low spring-summer SRP concentrations (< 20 µg-P l^{-1}) were recorded at all sites partly due to active plankton growth. Similar to SRP, highest nitrate concentrations were in the Cut (120 mg-NO_3 l^{-1}) and Thame (70 mg-NO_3 l^{-1}) tributaries, but also in the Thames headwaters at Hannington (97 mg-NO_3 l^{-1}) related to final effluent inputs from sewage treatment works (Figure 4-d). At other sites, maximum values were less than 45 mg-NO_3 l^{-1}. Throughout the catchment, median concentrations were ranging between 25-35 mg-NO_3 l^{-1}, and only in Farmoor even the maximum was lower than 20 mg-NO_3 l^{-1}.

Highest silicon concentrations were recorded in the Thame (max. 9.8 mg-Si l^{-1}) (Figure 5-4-e). They were followed by the lower Thames tributaries the Cut, Pang and Kennet (up to 8 mg-Si l^{-1}). As a result, downstream from these tributaries, in the Thames at Runnymede, dissolved silicon median 5.5 mg- Si l^{-1} and maximum 8 mg- Si l^{-1} were significantly higher than in the headwaters at Hannington (median - 3.6; maximum – 5 mg-Si l^{-1}), and the middle reach at Wallingford-Goring (median - 4; maximum – 7 mg-Si l^{-1}). In the Thames at Swinford, the Evenlode, and Cherwell overall silicon concentrations (median - 3.5, maximum – 4 mg-Si l^{-1}) were as low as in the headwaters. The lowest median and maximum values (median - 2, maximum – 3 mg-Si l^{-1}) were recorded at Farmoor reservoir where the data are also heavily skewed at each site with minimum values close to 0 in the spring-summer months when dissolved silicon is absorbed by actively growing diatoms.

Concentrations of suspended solids were higher in the lower Thames (up to 50 mg l^{-1}) than in the headwaters (25 mg l^{-1}) (Figure 5-4-f). The upper Thames tributaries the Evenlode, Cherwell and Thame carried high amounts of suspended sediment during late autumn-winter months. These tributaries drain mudstones and sandstones bringing suspended solids to the main Thames, and silts often get washed from agricultural fields in the upper catchment. In contrast, relatively small values were recorded in lower Thames tributaries: The Pan, Kennet and Cut and Farmoor reservoir.

5.5.2 Phytoplankton

There were two important peaks in chlorophyll-*a* concentration in the Thames catchment in 2015, both were related to spring (April-May) and summer (June) centric diatom blooms (Figure 5-5). During the April-June period, chlorophyll-*a* concentrations rapidly increased in the Cherwell and the middle-lower Thames stretch. Chlorophyll-*a* maximums there reached 250-300 µg l^{-1}. In addition, there was a smaller spring increase in chlorophyll-*a* in the Thame (73 µg l^{-1}) and Evenlode (50 µg l^{-1}). In the Kennet, maximum concentrations were registered in September (50 µg l^{-1}). In contrast, in the Pang and Cut chlorophyll-*a* never exceeded 25 µg l^{-1}. In Farmoor, the phytoplankton population visibly increased between July-October, and chlorophyll-*a* reached 50 µg l^{-1}.

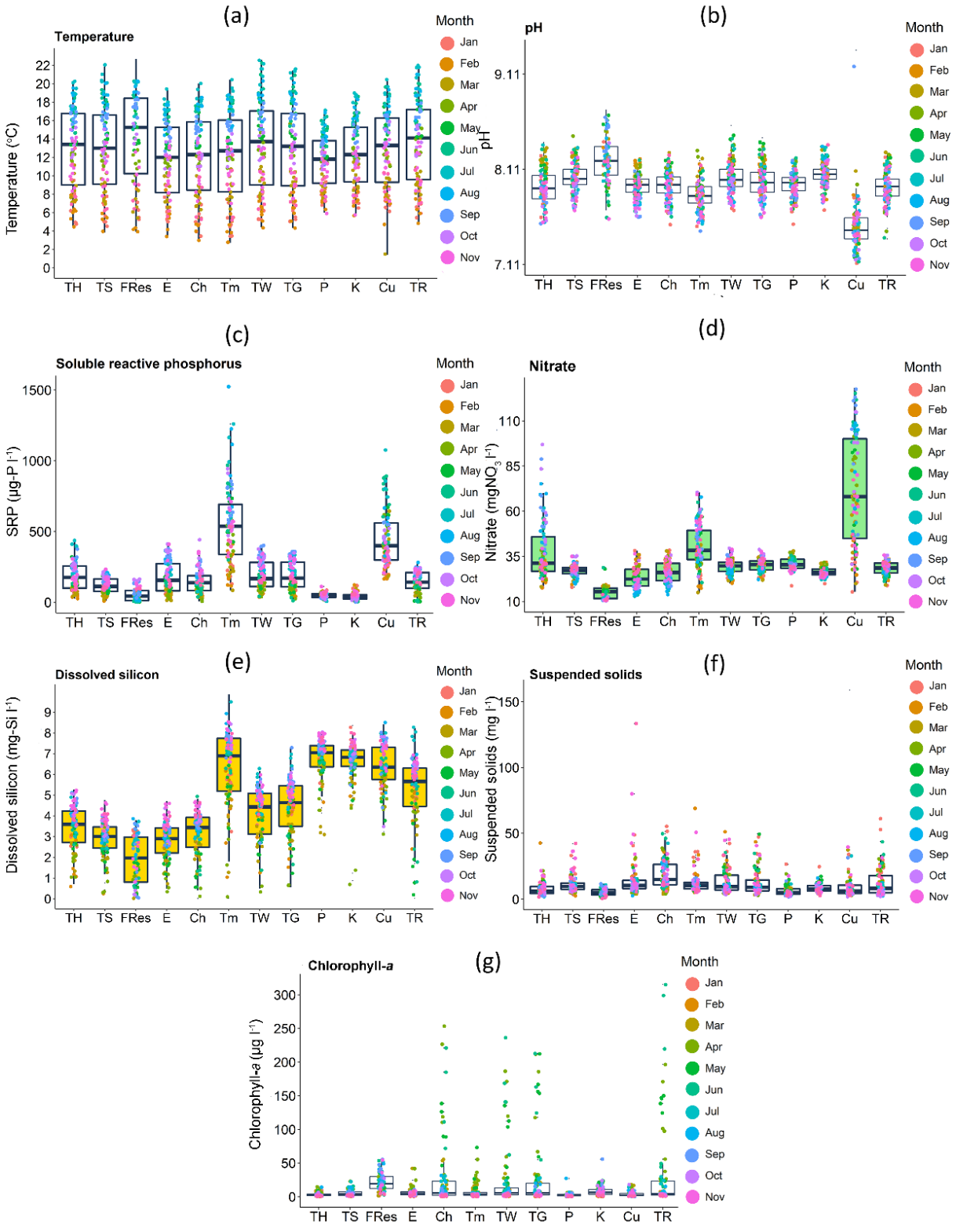

Figure 5-4 Boxplots of water chemistry in the Thames catchment between 2014-2015 (January-November): a - Water temperature; b – pH; c – soluble reactive phosphorus (SRP); d–nitrate; e – dissolved silicon; f – Suspended sediment g – Chlorophyll-a. TH - Thames at Hannington; TS - Thames at Swinford; FRes - Farmoor Reservoir; E - Evenlode; Ch - Cherwell; Tm – Thame; TW – Thames at Wallingford; TG - Thames at Goring; P – Pang; K – Kennet; Cu – Cut; TR - Thames at Runnymede. Weekly data provided by CEH (Thames Initiative Project)

Diatoms were actively growing in the Cherwell (3000-68000 cell ml^{-1}), Kennet (200-20000 cell ml^{-1}), Thame (300-22000 cell ml^{-1}) and the middle-lower Thames (3000-120000 cell ml^{-1}). In the Evenlode, Pang, and Cut their numbers were low (100-1500 cell ml^{-1}), and in the Farmoor ranged between 100-3600 cell ml^{-1}.

Cyanobacteria were ten-times more abundant in Farmoor (up to 140000 cell ml^{-1}) than the main channel Thames where largest populations were recorded at Wallingford (2000-12000 cell ml^{-1}) and Runnymede (600-3000 cell ml^{-1}). The Cherwell was the most favourable tributary for cyanobacteria growth with their population reaching 8000 cell ml^{-1}.It was followed by the Kennet (4000 cell ml^{-1}), Cut (4000 cell ml^{-1}), Pang (3500 cell ml^{-1}), Thame (2800 cell ml^{-1}) and Evenlode (1900 cell ml^{-1}).

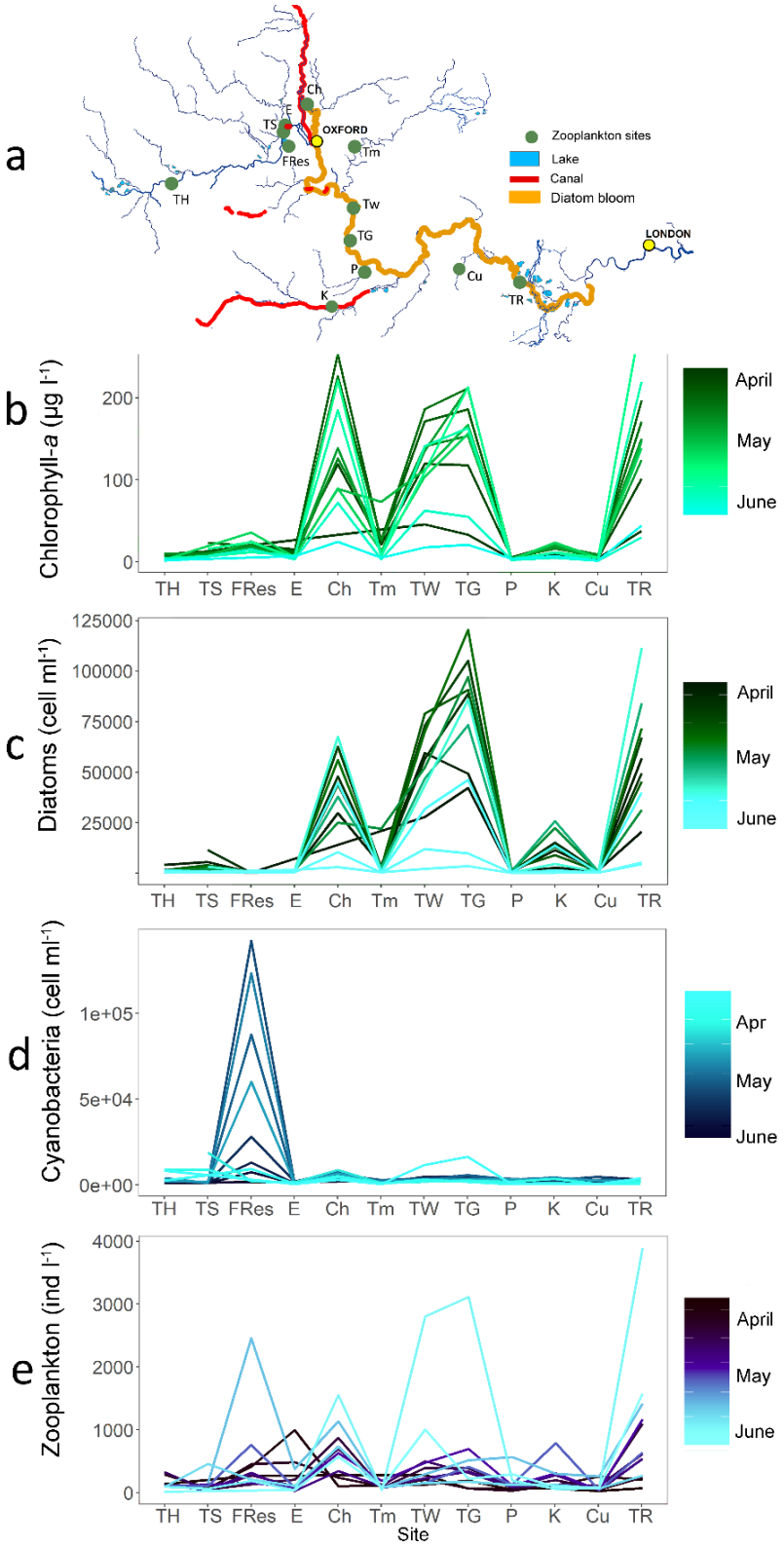

Figure 5-5 Profiles along the Thames catchment: a-Sample location; b-chlorophyll-a concentrations, c- abundance of diatoms, d- cyanobacteria and e- zooplankton between studied sites. TH - Thames at Hannington; TS - Thames at Swinford; FRes - Farmoor Reservoir; E - Evenlode; Ch - Cherwell; Tm – Thame; TW – Thames at Wallingford; TG - Thames at Goring; P – Pang; K – Kennet; Cu – Cut; TR - Thames at Runnymede. Weekly data. April-June 2015. Source: Centre for Ecology and Hydrology, UK

5.5.3 Flow

Flow rates showed a progressive increase from the upper part to in the middle and lower sections of the river (Figure 5-6). In the Thames at Swinford flow rates were below 10 $m^3 s^{-1}$, at the same time at Runnymede they were five times higher 50 $m^3 s^{-1}$. Tributaries can be arranged by their maximum flow rates as: The Pang, Cut (1 $m^3 s^{-1}$), Thame (2.5 $m^3 s^{-1}$), Evenlode (4 $m^3 s^{-1}$), Cherwell (5 $m^3 s^{-1}$), Kennet (6 $m^3 s^{-1}$).

Progressive reductions in flow in April 2015 resulted in longer residence times, and greater plankton growth. During this phase phytoplankton formed dense populations at all sites. Since zooplankton typically require longer residence times to establish a community than phytoplankton, due to their longer generation times, they formed sizeable communities only in the middle-lower Thames, the Cherwell and Evenlode (up to 1000 ind l^{-1}). The April growth phase was followed by a rain event which sharply decreased both phyto- and zooplankton presence across the catchment. However, a week after this event, zooplankton abundance and chlorophyll-a concentrations rapidly increased in the Thame, related to either dislodged benthic organisms or plankton being washed down from ponds upstream. Benthic algae were evidently important part of phytoplankton in the study of the River Enborne, UK by Halliday et al. (2016)

The next important low-flow period was recorded in June. Chlorophyll-*a* concentrations increased significantly in the Cherwell, and the Thames middle-lower stretch. Low flow and high food availability supported dense zooplankton populations. In the Kennet and Farmoor high chlorophyll-*a* concentrations and zooplankton abundances were observed in late May.

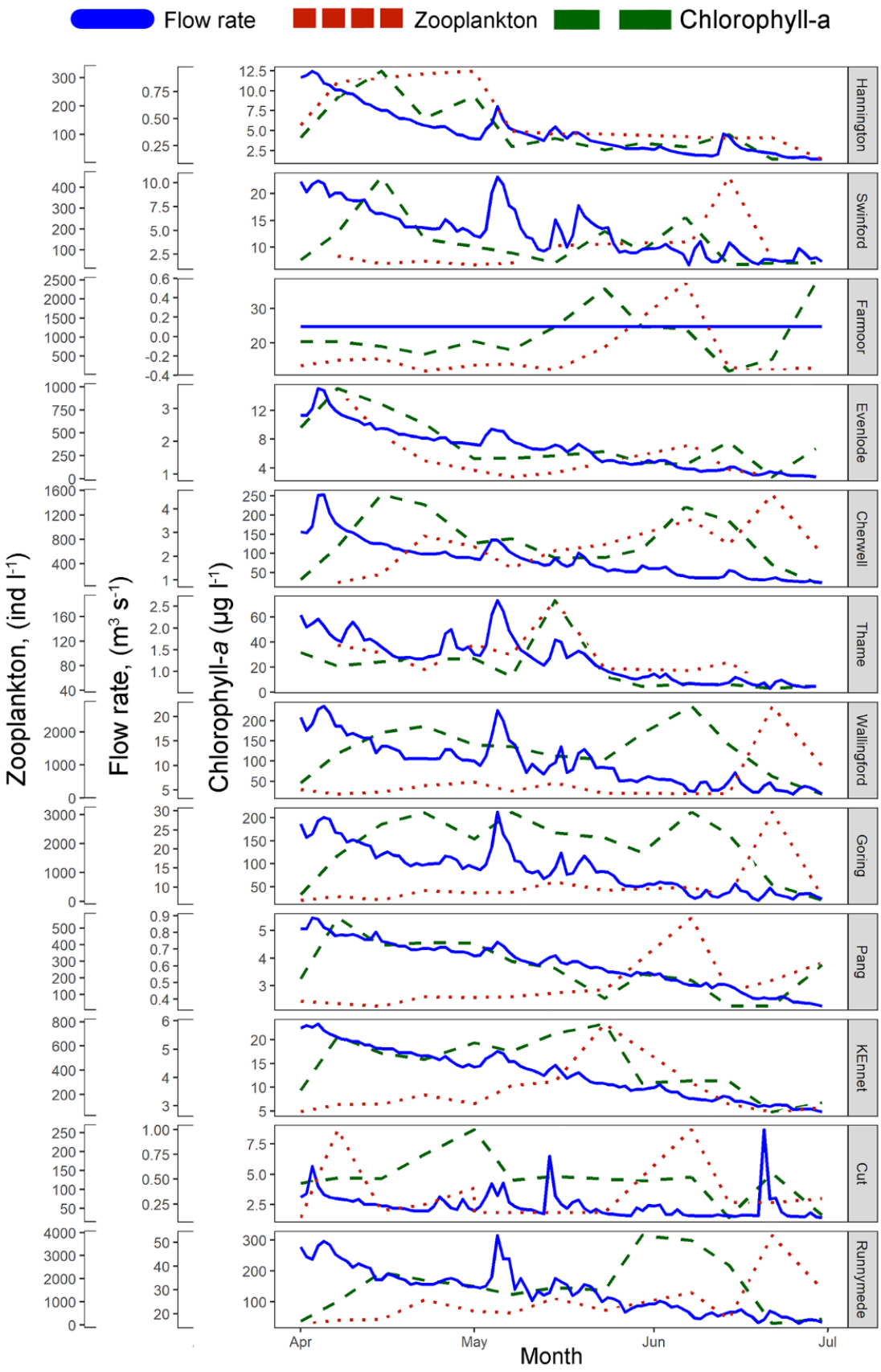

Figure 5-6 Flow rate, chlorophyll-a concentration and zooplankton abundance in the Thames and its tributaries in April-June 2015. Weekly data

5.5.4 Dissimilarities and environmental constrains

Communities are rarely structured by a single factor, instead, they are formed by, and respond to, an array of factors acting simultaneously (Tonkin et al., 2017). In the Thames and its tributaries, even minor increases in flow had a significant negative effect on the community, with some taxa temporarily disappearing. However, during active growth 'phases' most river zooplankton have strong self-regulating mechanisms and were able to rapidly recover (Figure 5-6). This was previously observed in other European rivers, for example, the Po (Bertani et al., 2012) and Danube (Baranyi et al., 2002). Floods trigger high zooplankton mortality and removal, as a result, sizeable plankton communities can only form during long low-flow conditions.

Nonmetric multidimensional scaling (non-metric fit $R^2 = 0.96$; linear fit $R^2 = 0.8$) showed similarities between the tributaries with high zooplankton densities and species richness (the Evenlode, Cherwell, and Kennet) and the sites of the middle and lower Thames (Wallingford, Goring and Runnymede) (Figure 5-7). Rotifer populations there developed under conditions of higher chlorophyll-*a* concentrations (> 50µg l^{-1}) caused by dense populations of diatoms, and cryptophytes. In contrast, communities in other tributaries (the Thame, Pang, Cut) were associated with high nutrient concentrations (SRP, NO_3, and Si) due to more limited phytoplankton growth. Taxa from benthic and periphytic environments made important contributions to the communities found at these sites, independent from the 'true' phytoplankton community and biomass (Figure 5-8). Zooplankton communities from Farmoor reservoir clustered separately from the Thames. They were strongly associated with cyanobacteria populations.

PERMANOVA showed that there were significant differences in zooplankton community composition between all studied sites (PERMANOVA , $F = 3.68$; $R^2 = 0.27$; $P = 0.001$). This may be attributed to higher abundances of cladocerans and copepods in Farmoor Reservoir compared to the main river channel and tributaries, as well as variation in community composition between tributaries with higher and lower zooplankton abundance. The spatial location of sampling sites in the catchment explained a larger part of the variation in the zooplankton community structure than sampling dates (PERMANOVA, $F = 2.73$; $R^2 = 0.17$; $P = 0.001$)

Additionally, PERMANOVA estimated that significant environmental variables explaining variation in zooplankton were dendric network distances, water temperature, suspended

sediment, SRP, and chlorophyll-*a* (Figure 5-2). In the Thames and its tributaries, zooplankton develop diverse communities when travel distances are long, temperature is optimal, and during intensive phytoplankton blooms which absorb SRP. Significance of dendric networks against distances from the source should be treated with caution. For instance, the Thame with small zooplankton abundance and species richness has the largest dendric network length out all tributaries.

Although chlorophyll-*a* and soluble reactive phosphorus (SRP) can be used as proxies to forecast zooplankton development, high concentrations of SRP showed no significant effect on the zooplankton population in the Cut and Thame, suggesting that other factors, such as physical environment and residence time play a more important role. In contrast, even relatively low concentrations of SRP ($< 20 \mu g\text{-}P\ l^{-1}$) allowed intensive cyanobacteria development in the Farmoor. This is an important result, considering that Farmoor's waters originally come from the Thames.

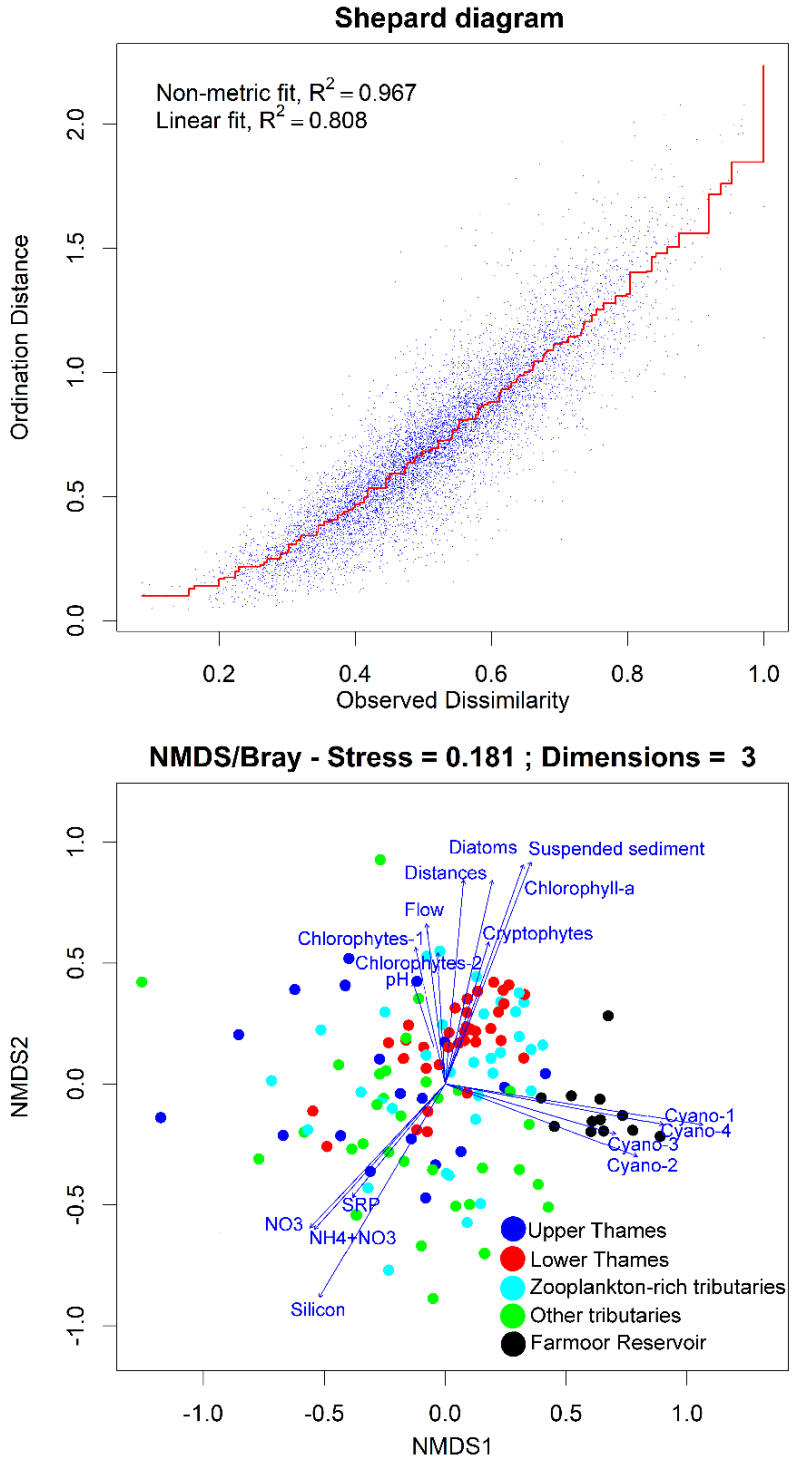

Figure 5-7 Non-metric multidimensional scaling (NMDS) diagrams on rotifer community samples. Transformation: log(x + 1). Bray-Curtis dissimilarity matrix. Number of dimensions = 3, stress = 0.18. Shepard diagram (a) The goodness-of-fit of the ordination is $R^2 = 0.967$; NMDS plot (b) - Upper Thames sites: Hannington, Swinford; Lower Thames: Wallingord, Goring, Runnymede; 'Zooplankton-rich' tributaries: the Evenlode, Cherwell, Kennet; other tributaries: the Thame, Pang, Cut; Explanatory variables (listed in section 3.3). Phytoplankton: Diatoms, Chlorophytes-1 – chlorophytes (mixed with small diatoms); Chlorophytes-2 -large chlorophytes; Cryptophytes – large cryptophytes (mixed with dinoflagellates); Cyano-1 - cyanobacteria Microcystis-like; Cyano-2 – cyanobacteria; Cyano-3- cyanobacteria Synechococcus-like; Cyano-4 - cyanobacteria PE-rich

Table 5-2 PERMANOVA results on zooplankton community data for six tributaries, the River Thames. Weekly zooplankton data April-June 2015

Variable	F.Model	R^2	Pr(>F)
Dendric network length (km)	10.32	0.07	0.001
Temperature (°C)	6.91	0.05	0.001
Suspended sediment (µg l^{-1})	7.91	0.01	0.001
SRP (µg l^{-1})	2.68	0.02	0.006
Chlorophyll-*a* (µg l^{-1})	1.85	0.02	0.046

5.5.5 Do tributaries influence zooplankton in the Thames?

The phyto- and zooplankton data suggest that connectivity with tributaries plays an important role in the ecological structure and function of zooplankton communities in the Thames with the overall effect dependent on both the abiotic and biotic characteristics of each tributary and the contribution to the discharge of the main river. All studied tributaries could be ordered based on their zooplankton maximum abundance and species richness. The most zooplankton abundant tributary was the Cherwell (1133 ind l^{-1}), followed by the Evenlode (993 ind l^{-1}), and Kennet (789 ind l^{-1}), and less zooplankton productive - the Pang (566 ind l^{-1}), Cut (259 ind l^{-1}), Thame (188 ind l^{-1}). The same order applies to rotifer species richness, except for the Thame, where the total number of species observed equalled those in the Pang (Figure 3). It is evident from the data that zooplankton-abundant tributaries seasonally support high phytoplankton abundance: the Cherwell, Kennet and Evenlode (Figure 5).

Hydrology and channel morphology are known as key factors determining plankton development (Basu and Pick, 1997). All tributaries differ in plankton travel distances, availability of retentive zones, and flow (Figure 1b). Equally, seasonal variation of discharge leads to rapid fluctuations in plankton communities and adds a dimension of complexity for comparison between tributaries (Figure 6). The Cherwell, Evenlode, and Kennet supplied a diverse and abundant range of zooplankton communities to the main Thames, enhancing its biota (Figure 3). They have a relatively high average discharge (3.8-5 m^3 s^{-1}) and long travel distances (60-130 km) (Table 1). Zooplankton in these tributaries and the middle-lower Thames dominated by true planktonic rotifers (Figure 8) such as *Keratella cochlearis, Polyarthra dolichoptera, Synchaeta oblonga* and

Brachionus calyciflorus. The phytoplankton were also composed of true planktonic taxa; a mixture of small centric diatoms, nano- and pico- chlorophytes, cryptophytes, and planktonic cyanobacteria. Since these tributaries are connected to canals, it is possible that both phyto- and zooplankton originated from these connected canals (Figure 5-1). Equally, adult cladocerans and copepods indicate the long residence periods in these rivers. This is because, compared with rotifers, crustaceans have a longer recruitment period and therefore exhibit a longer lag period between increased residence time and abundance (Lair, 2006). Crustaceans outcompete rotifers and dominate zooplankton assemblages in more lentic conditions. This is due to rapid exponential increases in abundance under conditions of longer residence time (Baranyi et al., 2002; Casper & Thorp, 2007). These results are consistent with the observations of Bottrell (1977) who concluded that zooplankton biomass from the Kennet accounted for 30% of the total zooplankton biomass in the Thames downstream. The Thame is also connected to a canal, but zooplankton numbers there were low. This could be explained by the sampling site location being located far downstream from the connecting lock, meaning that rotifers, even if injected from the canal, could have found river environment unfavourable (Figure 5-1b).

In contrast, in the Thames headwaters phytoplankton communities were formed of benthic/periphytic diatoms, large green algae, pico-plankton and a small number of cyanobacteria. As a result, rotifers there were also predominantly benthic/periphytic (Figure 5-8) of the following genera: *Cephalodella, Dicranophorus, Lecane, Lepadella, Lindia, Philodina, Squatinella*. At the same time, small populations of *Collurella, Euchlanis dilatate*, and *Trichocerca pusilla* were observed. Since they prefer standing eutrophic environments, it implies the connectivity with wetlands and off-channel habitats in the Thames headwaters.

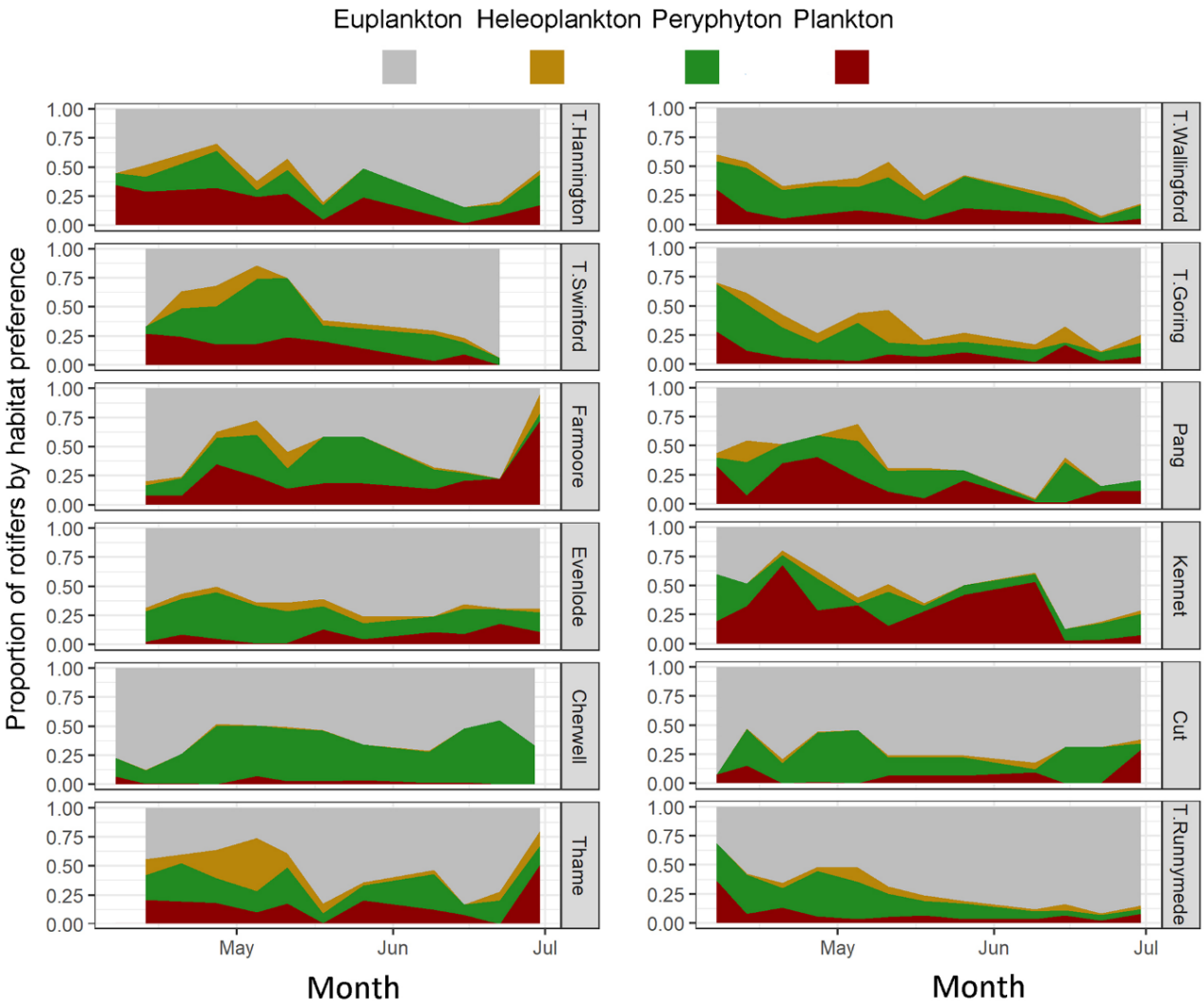

Figure 5-8 Relative proportion of rotifers grouped by preferred habitats: euplankton (true plankton), heleoplankton (eutrophic lakes and ponds); periphytic rotifers (river banks, and among macrophytes); plankton (mixed group found in pelagic zones, can be associated with either euplankton or periphyton).

The suggestion that retentive zones supply the inoculum agrees with findings of Wahl et al. (2008) where off-channel habitats provided a large part of the zooplankton inoculum to the Illinois River. In an even more recent study, Górski *et al.* (2013) showed, for the Waikato River catchment, that connectivity of the main channel with the floodplain governed zooplankton densities and community structure and played an important role in enhancing riverine plankton diversity.

In addition to communities made of actively reproducing organisms, connectivity with tributaries also introduce phytoplankton resting spores and zooplankton eggs to the main river. They are

mixed with the bottom sediment, where the resting stages of plankton are generally stored, affecting the main river's biological productivity (Rice et al., 2006; Palazzo et al., 2008).

The Pang, Cut, and Thame supported small zooplankton communities formed by true-planktonic *Keratella, Colurella, Synchaeta*, and by periphytic *Lecane, Cephalodella* and *Philodina*. It has been observed that rotifers inhabiting submerged aquatic plants and gravel/rocks by the banks often get flushed into the river but cannot reproduce and sustain a population in the turbulent flowing environment (May & Bass, 1998; Lucena-Moya & Duggan, 2011). Bottrell (1977) found that rotifers from the Kennet evidently increased zooplankton biomass in the Thames downstream from the confluence but raised population productivity only by 2%. Adult copepods and cladocera were completely absent. Low diversity and limited zooplankton population densities in these rivers could be associated with a lack of off-channel habitat, primarily - adjacent canals (Figure 5-1), and short travel distances (14-17 km), except the Thame (53 km). These tributaries, particularly the Thame as it has a high relative discharge (3.8 $m^3 s^{-1}$) (Table 5-1), dilute plankton in the Thames. In a study of the smaller River Drawa (Poland) (Czerniawski & Pilecka-Rapacz, 2011) tributaries either had no influence on the shape of zooplankton communities or exerted a potential diluting effect in a much larger river system - the River Elbe (Germany, Czech Republic) (Zimmermann-Timm et al., 2007).

Zooplankton communities in Farmoor Reservoir were significantly different from the Thames (Figure 5-6). Microcrustaceans accounted for 40-90% of the total abundance and included cladocerans (*Daphnia* and *Ceriodaphnia*) that are truly limnetic. The proportion of adults in microcrustacean communities was relatively high, at 10-30%. Among the rotifer species present, there were high densities of the predatory species *Asplanchna priodonta*. However, none of these cladocerans or predatory rotifers were found in the Thames at Wallingford. Even if zooplankton from Farmoor manage to get to the Thames, many would find it difficult to survive. For instance, in the study of the Missouri River (Havel et al., 2009), cladocerans and copepods significantly decreased in abundance downstream from reservoirs due to river turbulence, fish predation and high concentrations of suspended solids. Farmoor phytoplankton enriched with cyanobacteria had a marginal effect on the Thames community downstream (Figure 5-3; and 5-7).

5.5.6 Importance of off-channel habitats

This study agrees with Bowes et al. (2012), suggesting that in the Thames catchment an important proportion of plankton community biomass begin their life in tributaries connected to

canals. Bottrell (1977) also emphasized that locks in the Thames create extra retentive zones in the actual channel. However, each lock has at least one adjacent weir creating a turbulent environment. Since many crustaceans and rotifers find it difficult to feed and reproduce in rapidly increasing water currents, weirs can dramatically elevate zooplankton mortality (Sluss et al., 2008; Dickerson et al., 2010).

Overall rotifer population densities observed in this study are comparable to those reported in the Thames in 1996 (May and Bass, 1998), but are more than ten-times lower than in 1971 (Bottrell, 1977), possibly due to changes in sampling methods. In the Kennet, however, the maximum zooplankton density in 2015 and 1971 did not differ to the same degree suggesting that discrepancies caused by different sampling techniques may not be the sole cause of these differences. Sizeable reductions in zooplankton abundance are often associated with changes in the river catchment morphology, for instance, elimination of off-channel lentic habitats reduces the volume of hydraulic retention zones where plankton populations best develop (Reckendorfer et al., 1999; Reynolds, 2000).

Natural flow provides ecological integrity of river networks (Bayley, 1995; Poff et al., 1997). Off-channel habitats harbour plankton communities (Tockner & Stanford, 2002; Brown et al., 2011) and facilitate high productivity (Amoros & Bornette, 2002; Smith et al., 2017). These complex habitats may also provide refuge from predation by fish, as fish may rapidly decrease zooplankton populations (Thayer et al. 1974; Medeiros & Arthington 2008). Spawning generally begins in spring, so that fish larvae and fry can benefit from feeding on the abundant zooplankton which feed on spring diatom blooms. It is important to emphasize that fish reproduction is naturally well linked to both phytoplankton and zooplankton development. This study opens an opportunity to compare both population dynamics in 2015.

The structural complexity of the catchment habitats plays an important role in boosting productivity in large and middle size rivers, as postulated in the Inshore Retention Concept (Schiemer et al., 2001) and reinforced in the Riverine Ecosystem Synthesis (Thorp et al., 2006). The present study agrees with these concepts, but more work is needed to confirm the influence from the canals in the catchment.

5.6 Conclusion

Riverine structural complexity increase productivity in large lowland rivers. The overall effect of tributaries on zooplankton vary from increasing community diversity and abundance to dilution.

Further research in needed to confirm that canals and other off-channel habitats significantly elevate plankton diversity and abundance in rivers. Farmoor Reservoir showed no influence on either phyto- or zooplankton in the Thames. Future studies should focus on spatially locating major changes in the catchment in relation to decline in retention zones as they are the key sources of both phyto- and zooplankton. In the Thames and its tributaries plankton growth is not driven nor limited by nutrient concentrations alone.

5.7 Acknowledgment

This work was funded by the Natural Environment Research Council (NERC), through the SCENARIO DTP Program and CEH Thames Initiative (NEC04877) and MaRIUS (Managing the risks, impacts and uncertainties of droughts and water scarcity; NERC Grant NE/L010364/1) projects. River flow data was obtained from the NERC National River Flow Archive. I would like to thank Dr Monika Jürgens and Dr Helen Vincent for their help and support during laboratory and field studies.

5.8 Appendix

Appendix.Table 1 Study sites and corresponding flow gauging stations (name, grid reference)

Study site zooplankton	Zooplankton grid reference:	Flow gauging station	Flow grid reference:
Thames at Hannington	SU175961	Thames at West Mill Cricklade	SU094942
Thames at Swinford	SP442085	Thames at Eynsham	SP444087
Evenlode at Cassington Mill	SP447101	Evenlode at Cassington Mill	SP448099
Cherwell at Hampton Poyle	SP499152	Cherwell at Enslow Mill	SP482183
Thame at Wheatley	SP612050	Thame at Wheatley	SP611050
Thames at Wallingford	SU609902	Thames at Days Weir	SU568936
Thames at Goring	SU601823	Reading	SU718740
Pang at Pangbourne	SU636747	Pang at Pangbourne	SU634765
Thames at Runnymede	TQ006723	Thames at Royal Windsor Park	SU980772
Kennet at Woolhampton	SU572667	Kennet at Newbury	SU471671
The Cut at Paley Street	SU869762	The Cut at Binfield	SU853712

Appendix. Table 2 Land cover in the catchment above the study sites

Catchment statistics	Woodland	Arable	Grassland	Urban Extent
		% Land cover		
Thames at Hannington	10.8	46.54	34.15	7.22
Thames at Swinford	10.83	45.55	35.24	6.68
Evenlode at Cassington Mill	14.21	48.7	31.55	4.95
Cherwell at Hampton Poyle	9.22	50.52	33.52	6.44
Thame at Wheatley	9.78	36.34	45.59	8.35
Thames at Wallingford	10.8	46.54	34.15	7.22
Thames at Goring	10.54	44.86	36.02	7.34
Pang at Pangbourne	17.6	45.8	28.36	4.46
Thames at Runnymede	13.21	40.58	34.07	10.4
Thames at Farmoor	10.77	45.76	35.25	6.64
Kennet at Woolhampton	14.49	49.47	29.55	3.38
The Cut at Paley Street	23.50	6.02	29.37	40.69

Appendix. Table 3 Species of Rotifera recorded in the Thames (April-June 2015)

Species
Anuraeopsis fissa (Gosse, 1851)
Brachionus angularis Gosse, 1851
Brachionus calyciflorus Pallas, 1766
Brachionus leydigi Cohn, 1862
Brachionus quadridentatus Hermann, 1783
Brachionus rubens Ehrenberg, 1838
Brachionus urceolaris Muller, 1773
Keratella cochlearis (Gosse, 1851)
Keratella quadrata (O. F. Muller, 1786)
Notholca labis Gosse, 1887
Notholca squamula (O. F. Muller, 1786)
Plationus Segers, Murugan and Dumont, 1993
Platyias Harring, 1913
Kellicottia Ahlstrom, 1938
Cephalodella gibba (Ehrenberg, 1832)
Eosphora Ehrenberg, 1830
Colurella Bory de St. Vincent, 1824
Lepadella Bory de St. Vincent, 1826
Squatinella Bory de St. Vincent, 1826
Lecane Nitzsch, 1827
Monostyla Ehrenberg, 1930
Aspelta Harring and Myers, 1928
Dicranophorus Nitzsch, 1827
Ascomorpha Perty, 1850
Gastropus Imhof, 1888
Asplanchna priodonta Gosse, 1850
Conochilus Ehrenberg, 1834
Bryceella Remane, 1929
Proales Gosse, 1886
Epiphanes Ehrenberg, 1832
Euchlanis dilatate Ehrenberg, 1832
Polyarthra dolichoptera Idelson, 1925
Synchaeta oblonga Ehrenberg, 1832
Synchaeta pectinate Ehrenberg, 1832
Pompholyx sulcate Hudson, 1885
Trichocerca pusilla (Lauterborn, 1898)
Trichotria Bory de St. Vincent, 1827
Lindia Dujardin, 1841
Filinia brachiate (Rousselet, 1901)
Scaridium Ehrenberg, 1830
Proales Gosse, 1886
Bdelloidea Hudson, 1884

Chapter 6 Effect of zooplankton grazing on phytoplankton community in a large lowland riverine microcosm

Freeman A., Armstrong L. K., Bowes M.J., Hutchins M. G., Nicholls D., Read D. S., Roberts C., Scarlett P., Thackerey S., Wade A. J., Wickham H.

6.1 Abstract

Extensive research of lentic environments and estuaries suggests that zooplankton play an important role in reducing algal abundance and changing community structure. In lotic systems the zooplankton grazing effect is underinvestigated. This study examines zooplankton grazing on phytoplankton in the River Thames, UK. Monthly field-based experiments were undertaken in the 2016 growing season at four sites across the Thames catchment. In all experiments, phytoplankton and zooplankton diversity and densities were maintained in dialysis bags exposed to ambient river conditions. Temperature, light and nutrient concentrations were monitored. The zooplankton were rotifer dominated (*Brachionus, Dicranophorus, Trichotria, Keratella, Polyarthra, Synchaeta, Euchlanis, Notholca*) mixed with copepod nauplii. The results demonstrated grazing effect that varied spatially and seasonally in the Thames catchment. In three days, metazoan grazers removed a maximum of 25% of diatoms and chlorophytes, 30% of cryptophytes, and up to 60% of cyanobacteria. The grazing effect on phytoplankton growth rate and community composition was seasonal and site-specific due to high environmental heterogeneity in the catchment. Statistical analysis showed that diatoms, chlorophyte, cryptophytes and cyanobacteria were regulated by water temperature, light, concentrations of important nutrients (mineral phosphorus, nitrogen and silicon) and suspended solids, with grazing occurring when dense phyto- and zooplankton communities were established.

Key words: diatoms, cyanobacteria, chlorophytes, cryptophytes, plankton, flow cytometry, algae

6.2 Introduction

Phytoplankton blooms in rivers may result in high toxin presence, low oxygen concentrations and can ultimately result in fish kills (Paerl, 1988; Hilton et al., 2006). They can block filtration systems of water treatment plants and affect water taste and smell (Henderson et al., 2008). However, the planktonic communities of freshwaters include animals as well as algae. Many of these are herbivores, feeding directly upon algae and bacteria. Extensive research of lentic environments and river estuaries suggested that zooplankton may play an important role in reducing algal abundance and changing community structure (Lampert et al., 1986; Wu & Culver, 1991; Kim et al., 2000; Quinlan et al., 2009). In rivers, zooplankton grazing (herbivory)

was generally neglected since flowing waters are dominated by rotifers, which are thought to be less efficient grazers than the micro-crustaceans found in abundance in lakes (Reynolds, 1984; Lehman, 1988). However, previous river studies have shown that high rotifer population densities were capable of exerting a significant grazing impact, removing between 20-50% of total algal biomass (Garnier et al. 1995; Gosselain et al. 1998 and Kim et al. 2000) and up to 100% of phytoplankton daily production (Lair & Reyes-Marchant, 1997). The zooplankton grazing may explain why phytoplankton typically decline in large rivers when meteorological and hydrological conditions are still favourable for algal development. Such declines were observed in the Meuse (Gosselain et al., 1998), the Moselle (Descy, 1993), the Rhine (de Ruyter van Steveninck et al., 1992), the Seine (Billen, Garnier and Hanset, 1994) and the Spree (Köhler, 1993). The most common river rotifers belong to the following genera: *Keratella, Brachionus, Synchaeta, Polyarthra, Lecane, Filinia*, as reviewed by Lair (2006).Previous studies indicated that important factors such as residence time, water temperature, nutrient chemistry and light alone do not completely explain phytoplankton dynamics in the River Thames (Waylett et al., 2013), and zooplankton grazing may play an important role in phytoplankton net-growth rates and bloom termination. The Thames catchments is a complex heterogenic system with evident spatial and seasonal variation in planktonic communities (Bowes et al., 2012; Read et al., 2014, 2015).

May and Bass (1998) showed previously that significant numbers of small bodied grazers developed directly after an algal bloom, emphasizing a strong link between phytoplankton and zooplankton abundances. In this work, a peak in zooplankton population was followed by low phytoplankton yields until the end of summer. Available data, however, are not sufficient to relate algal bloom cessations with grazing. Our study aimed to address this knowledge gap through assessing to what extent the zooplankton (metazoan only) community influences phytoplankton growth, chlorophyll-*a* production and the structure of algal assemblages, using dialysis membrane microcosms. Five hypotheses were tested:

- Zooplankton grazing decrease net phytoplankton growth rates;
- Grazing effect varies spatially and seasonally within the Thames catchment;
- Zooplankton grazing varies for different algal groups due to selective feeding;
- Grazing effect largely depends on zooplankton population density and community composition, particularly the body size and feeding preferences of the dominant species;
- ‘Top-down’ pressure on algal population is more significant than ‘bottom-up’ effect.

6.3 Methodology

This study focused on the zooplankton (metazoan) grazing on phytoplankton. Six field-based monthly (microcosm) experiments were done in the spring-summer period in the Thames catchment at four sites with contrasting environmental conditions (Figure 6-1). Phytoplankton-zooplankton communities were sampled and incubated in the same stretch of the river in the ambient river conditions, where they were exposed to natural day light, water temperature and constant supply of important nutrients. For 'no grazers' treatment, only the large zooplankton were removed from inoculum by filtering water through the sieve with mesh diameter 53µm. Plankton community abundance and composition were recorded at the start of the experiment (Table 6-2).

These experiments were adapted from dialysis transplant *in situ* studies of environmental factors regulating development of aquatic microorganisms (Gasol et al., 2002; Simek et al., 2003; Štrojsová et al., 2005; Sinistro et al., 2015). Water was maintained inside dialysis bags that allow exposure to ambient nutrient concentrations so both bottom-up (physical environment and water chemistry) and top-down factors (grazing) were assessed simultaneously. Grazing influence on phytoplankton growth was estimated as decrease in chlorophyll-*a* concentrations and population densities of major algal groups. Grazing was compared to other important factors such as: water temperature, light availability, and phosphorus, nitrogen and silicon concentrations. Zooplankton effect on phytoplankton community was assessed based on changes in population densities of diatoms, large and small green algae, cryophytes and various groups of cyanobacteria.

6.4 Study sites

The River Thames is the second longest river in the Britain (346 km). Its catchment area to the tidal limit at Teddington in south west London is around 9950 km^2 (Figure 6-1). The population in the Thames basin is over 14 million people, the majority of whom live in London. Most of the river basin upstream from London is rural and comprised of arable (approximately 35% of the land area) grassland (32%), woodland (16%), and urban (14%) (National River Flow Archive, 2016).

The River Thames catchment is heterogenic with chemical and microbial characteristics varying significantly along the main river and between its tributaries (Bowes et al., 2012, 2018; Read et al., 2015). Intensive agriculture leads to high inputs of diffuse phosphorus, nitrogen and sediment impacting on water quality within the catchment. Sewage discharge and effluent from septic

tanks increase concentrations of various organic and inorganic chemical components which significantly influence algal-bacteria composition in the river (Environment Agency, 2017).

Four sites were chosen to account for the spatial heterogeneity of in water quality identified previously between the upper (Hannington) and lower (Goring) Thames and its tributaries, the Coln and Windrush. The gradient in water quality was used to test the importance of grazing top-down effect in comparison with nutrient concentration, light conditions, and temperature, which have been shown to be important controls on phytoplankton biomass dynamics (Bowes et al., 2016).

Hannington is in the headwaters of the Thames. The catchment draining to Hannington is rural with grassland (40%) and intensive agriculture (38%) the predominant land cover types. Sewage treatment facilities upstream of the site further influence the stream water nitrogen and phosphorus concentrations. The Thames at Goring is located in the middle/lower Thames downstream from Oxford (the major city). At this point on the Thames, the land use composition is similar to that in the headwaters (Bowes et al., 2018).

The River Coln, a tributary of the upper Thames, rises on the edge of the Cotswolds and flows in a south/south-easterly direction through the Cotswold Hills via small towns and villages. The catchment land cover and is also predominately arable and grassland. The river is host to many species of freshwater fish including *Salmo trutta* (brown trout) and *Thymallus thymallus* (grayling). Limestone aquifers are the major source of water in the Coln which has a baseflow index of 0.93 which indicates the predominance groundwater in controlling the flow regime Table 6-1.

The River Windrush drains the Cotswold Hills but to the east of the Coln and flows for about 56 km through rural towns and villages in the upper Thames catchment to meet the Thames at Newbridge. With a population of just over 20000, Witney is the only large town located in the Windrush valley. The River Windrush is host to *Salmo* spp. (trout), (*Thymallus thymallus* (grayling), *Perca* spp. (perch), *Squalius cephalus* (chub), *Rutilus rutilus* (roach) and dace (*Leuciscus leuciscus*).

Table 6-1 Studied sites, their corresponding gauging points, and distance from the source of each individual river, mean flow and catchment area.

N	Study site	Base Flow

		Mean discharge m^3s^{-1} (1972-2015)**	Catchment area**, km^2	Index (BFI)
1	Thames at Hannington	1.4	185	0.7
2	Thames at Goring	38.5	4634	0.66
3	Coln	2.2	130	0.93
4	Windrush	3.4	362.6	0.86

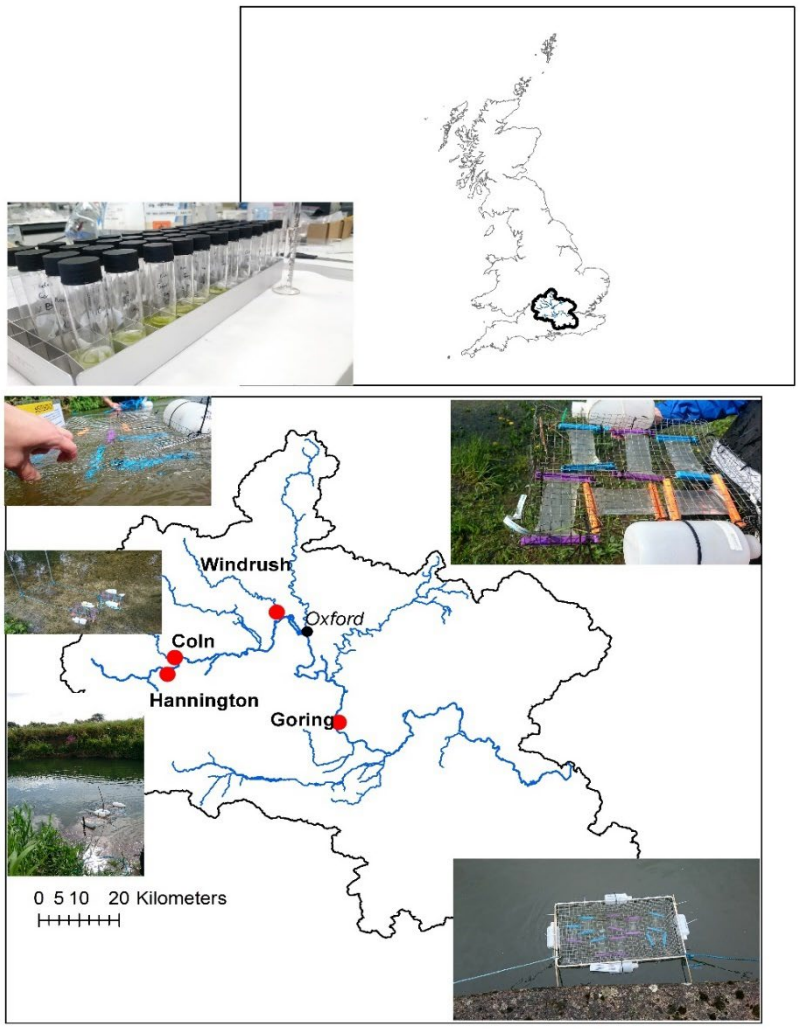

Figure 6-1 Study sites. Dialysis microcosm (experimental design)

6.4.1 Experimental design

To determine the phytoplankton biomass reduction and community composition due to zooplankton grazing under different background nutrient concentrations, microcosm experiments

were conducted between April to September 2016 *in situ* at each of the four study sites. This factorial design involved four study sites, six months, and the grazing factor (filtered/unfiltered water). In the first two experiments (April-May), two replicates per treatment were deployed. This was increased to three replicates for the subsequent experiments.

The six short-term (72 hours) microcosm experiments were done using dialysis tubing, which allow the diffusion of dissolved nutrients and organic carbon but prevent the passage of organisms. Thus, phytoplankton were exposed to ambient environment at studied sites (Gasol et al., 2002). The dialysis bags (BioDesign Dialysis Tubing 14000 MWCO – 49.5mm wet diameter) were cut in lengths of 20–25 cm to hold 200 – 250 ml of water. The bags were washed and left submerged in deionised water overnight, prior to use. In the field, a bulk 10 litre sample was collected at every site by lowering a bucket from the river bank. Half of the collected water was filtered through a sieve with a 53 μm mesh to remove large metazoan (zooplankton) grazers. Both treatments (filtered and unfiltered water) were placed in bags, hermetically sealed, then randomly positioned and cable tied inside metal cages. Light and temperature loggers were attached to the cages at every site with measurements were taken every 30 min (Figure 6-1).

Table 6-2 Environmental variables measured

Variable	Unit	Period measured
Temperature,	°C	Day 1-Day 3: every 30 min
Illuminance	lx	Day 1-Day 3: every 30 min
Soluble reactive phosphorus (SRP)	μg l^{-1}	Day 1
Nitrates	mg l^{-1}	Day 1
Silicon	mg l^{-1}	Day 1; Day 3
Chlorophyll-*a*	μg l^{-1}	Day 1; Day 3
Phytoplankton abundance (10 groups)	ind ml^{-1}	Day 1; Day 3
Zooplankton abundance	ind l^{-1}	Day 1

Due to high water turbulence and turbidity, the number of replicates was increased from two to three per treatment in June-September. The cages were attached to metal poles and fully submerged at a depth of 0.5 m from the water surface, in a position that provided minimum shading. After three days in the river (time was recorded), the cages were brought back to the laboratory where chlorophyll-a and flow cytometer analysis were conducted on each dialysis microcosm within 3 hours. Separate water samples were also collected and analysed at the start of each experiment to measure chlorophyll-*a* (500 ml), phytoplankton composition, zooplankton (1l) and water chemistry (soluble reactive phosphorus (SRP), nitrate (NO_3), silicon (Si)) (Table

6-2) The phytoplankton composition was measured using a flow cytometry based on a 25 ml sample for analysis (as described below). Water chemistry was analysed by the Centre for Ecology & Hydrology using the methods listed in Bowes et al., (2018b).

Phytoplankton growth was estimated based on chlorophyll-*a* and abundance of diatoms, chlorophytes, cryptophytes and cyanobacteria measured with flow cytometry, at the start and end of the experiment. Phytoplankton population growth rate was estimated as (Equation 6-3):

$$k = \ln(g(t)/g0)/t$$

k growth rate
g(t) number of algal cells or chlorophyll concentrations after 3 days;
g0 initial number of phytoplankton cells or chlorophyll concentrations;
t time (hr),
Daily growth rate:
GR = k*24

Equation 6-1 Exponential daily grow rate

In additionally, grazing impact was estimated as difference between net growth in bags with and without grazers. These differences were divided by net growth rates in bags without zooplankton to obtain grazing relative proportions.

$$\textit{Net growth difference} = \text{Filtered}_{g(t)-g(0)} - \text{Unfiltered}_{g(t)-g(0)}$$

$$\textit{Grazing relative proportion} = \text{Net growth difference} / \text{Filtered}_{g(t)-g(0)}$$

g(t) number of algal cells or chlorophyll-*a* concentrations after 3 days;
g0 initial number of phytoplankton cells or chlorophyll *a* concentrations.

Equation 6-2 The three-day difference in phytoplankton abundance between filtered (without large zooplankton) and unfiltered (with large zooplankton) treatments.

6.4.2 Water chemistry

For an overview of the environmental heterogeneity between studied sites, concentrations of soluble reactive phosphorus (SRP), nitrate-nitrogen (NO_3), silicon (Si), chlorophyll-a and water temperature were summarised for the spring-summer period (April-September) of 2014-2016. These data were collected by the Centre for Ecology and Hydrology within the Thames Initiative Project (Bowes et al., 2018).

6.4.3 Zooplankton

One-litre samples were collected with the bucket from the bank or mid-stream. On the same day, in the laboratory, samples were filtered through sieves with mesh diameters 30 μm and preserved

in formaldehyde solution (4%). During the following week, zooplankton (metazoans) were identified and enumerated in sedimentation chambers (after 2hr of settling) at 100-400X magnification using an inverted microscope (Zeiss Axiovert 40CFL). Up to 300 individuals were counted and extrapolated on the rest of the sample. If the total number of organisms was less than 200, all rotifers and microcrustacean nauplii were counted. Identification of rotifers was taken to genus and species level (when possible), using printed guides (Mellanby 1951; Pontin 1978; Alekseev 2010) and web resources (Haney, 2013). Body length was measured with 'ImageJ' software and digital camera photographs (Rueden et al., 2017). For the purpose of this study, microcrustaceans were differentiated as Cladocerans or Copepods.

6.4.4 Phytoplankton

Phytoplankton community composition was measured by flow cytometry. This method enumerates algae from ten previously defined groups of phytoplankton (Read et al., 2014), based on cell sizes and pigment fluorescence using a Gallios flow cytometer (Beckman Coulter, UK) equipped with blue (488 nm) and red (638 nm) solid state diode lasers. These ten groups are: diatoms (G1), chlorophytes and small diatoms (G2), large chlorophytes (G3), nano-pico chlorophytes (G4), small cryptophytes (G5), large cryptophytes (G6), cyanobacteria Microcystis-like (G7), cyanobacteria (G8), cyanobacteria *Synechococcus*-like (G9), cyanobacteria PE-rich (G10).

The samples (approx. 20 ml) were stored in 30 ml universal tubes in the dark at 4 °C before analysis within 24hrs of collection. The analysis protocol compares fluorescence from phycoerythrin versus chlorophyll and chlorophyll versus phycocyanin. A set volume of counting beads (FlowCount; Beckman Coulter) was added to individual samples to determine cell abundances per ml, and each sample was run for five minutes at a high flow rate. Data was processed using the software Kaluza Analysis v1.5a (Beckman Coulter, UK), and exported to a .csv file for plotting and interpretation.

6.4.5 Data analysis

The spatial and seasonal variation in the environmental conditions and the plankton communities were visually assessed prior to statistical analysis. Diagrams were built using R programming environment (Wickham, 2016; R Core Team, 2017). Generalized linear models (GLM, R functions: glm, gaussian distribution) were used to estimate the significance of both continuous and categorical variables. Data were tested for normality (Shapiro–Wilk test) and log

transformation was done on data deviating from these assumptions. Non-significant terms were excluded from the model in a stepwise manner based on P-values.

Model 1 tested three categorical variables (site, month and treatment: filtered/unfiltered) and their interactions. ***Model 2*** tested water temperature, light illuminance, initial size of phytoplankton inoculum, total number of grazers, pH, suspended solids, SRP, Si, and nitrate as major phytoplankton growth predictors. Both equations were individually applied with daily growth rates of chlorophyll-*a* and individual algal groups (G1-G10).

6.5 Results and Discussion

6.5.1 Catchment heterogeneity

Based on weekly spring-summer data from 2014-2016, there were differences in water temperature (Figure 6-2-a) between sites, with the tributaries generally cooler by approximately 1 to 2°C compared to the main channel, due to their increased proportion of groundwater input and high BFI (Table 1). In early spring water temperatures were around 7°C and in summer increased to 22°C (at Goring). pH between sites (Figure 6-2-b) was relatively constant with some evidence for lower values in the main channel at both Hannington and Goring indicative of a geological influence and seasonally low algal growth (Bowes et al., 2014). The spring-summer variation in pH in the catchment was approximately 7.6-8.5 with high values partly reflecting higher summer plankton metabolism and CO_2 depletion. Concentrations of soluble reactive phosphorus (Figure 6-2-c) (SRP) were highest (up to 400 µg-P l^{-1}) in the Thames (Hannington and Goring) and the Windrush (up to 250 µg-P l^{-1}) as these sites are affected by sewage treatment works final-effluent inputs. The lowest SRP concentrations were recorded in the River Coln (up to 100 µg-P l^{-1}). Stream water nitrate concentrations were twice as high in the Thames headwaters at Hannington (97 mg-NO_3 l^{-1}) than in the middle reach at Goring and the Windrush and Coln (up to 35 mg l^{-1}) (Figure 6-2d). The highest dissolved silicon (up to 8 mg-Si l^{-1}) concentrations were observed in the main channel of the River Thames with a maximum of 5.5 mg-Si l^{-1} at Hannington and 7 mg-Si l^{-1} at Goring. The corresponding concentrations in the Coln and Windrush were approximately 3.3 mg Si l^{-1} (Figure 6-2-f). During the late spring and early summer months, silicon was assimilated by diatom growth and almost depleted within the water column. Suspended solids concentrations were higher in the Windrush and lower Thames (up to 50 mg l^{-1}) than in the Thames headwaters and the Coln (less than 20 mg l^{-1}; Figure 6-2-e).

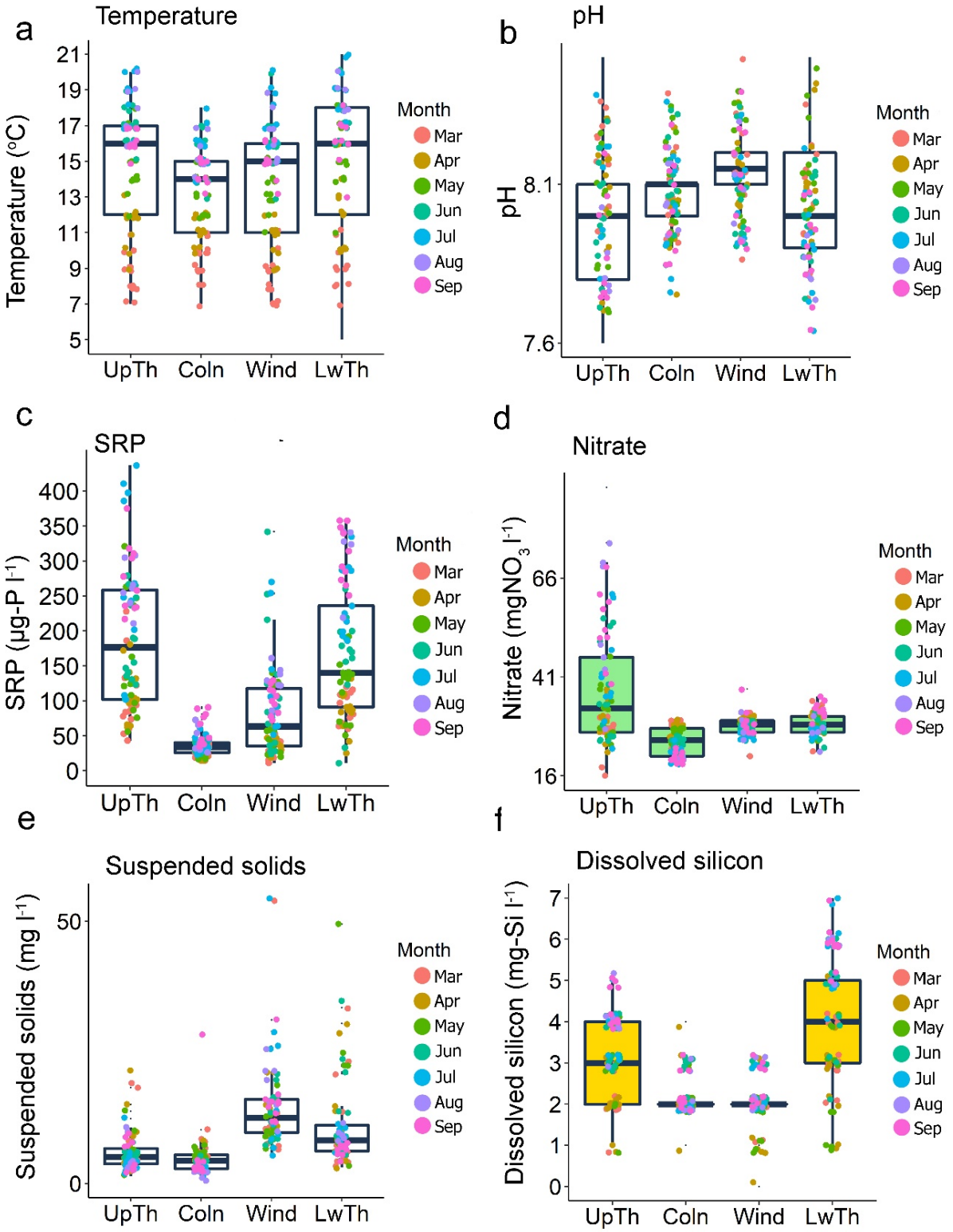

Figure 6-2 Water temperature (a), pH (b), and concentration of SRP (c), dissolved nitrate (d), suspended sediment (e), dissolved silicon (f) in the River Thames. Weekly data between 2014-2016 (March-September) provided by CEH (Thames Initiative Project).

6.5.2 Inoculum

During all six experiments both phyto- and zooplankton abundance and community composition varied spatially and seasonally. They were most abundant in the lower reach of the River Thames (at Goring) (Figure 6-3, 4) where organisms benefited from warmer water temperatures, higher concentration of nutrients and longer travel distances/residence time (Figure 6). These findings agree with the previous study of zooplankton distribution along the Thames (May & Bass, 1998).

The phytoplankton in the Thames headwaters and the tributaries mainly consisted of nano- and pico-chlorophytes mixed with larger chlorophytes, cyanobacteria and a small number of large diatoms (Figure 6-3). *Synechococcus*-like cyanobacteria were common in the Coln and phycoerythrin-rich in the Windrush. During all six experiments, in the Thames at Goring, the large and nano- pico- chlorophytes dominated the phytoplankton community composition mixed with diatoms in April-May (up to 25%) and cryptophytes and dinoflagellates in July-September, whilst proportion of cyanobacteria remained negligible. Similar seasonal patterns in phytoplankton succession in the Thames were previously described by Read et al. (2014) and Moorhouse et al. (2018).

Metazoan grazers have longer generation times than algae and thus exhibit slower numerical population responses. Spring and early summer phytoplankton in the Thames and the tributaries (small unicellular green algae, diatoms in cyanobacteria) were grazed by cold-adapted rotifers such as: *Notholca, Synchaeta, Keratella, Polyarthra* and copepod nauplii. The late summer period is associated with a post-diatom phase, when small chlorophytes, cryptophytes and cyanobacteria were more abundant. During this period, rotifers *Synchaeta, Keratella,* along with *Brachionus* and *Euchlanis,* formed relatively dense populations in the lower Thames (up to 700 ind l^{-1} at Goring in August).

The total numbers of rotifers and microcrustaceans were lower than 200 ind l^{-1} at all sites except in the Thames at Goring where there was a significant increase (up to 600 ind l^{-1}) in rotifer abundance in August-September. In the Coln, the zooplankton populations were less than 100 ind l^{-1}.

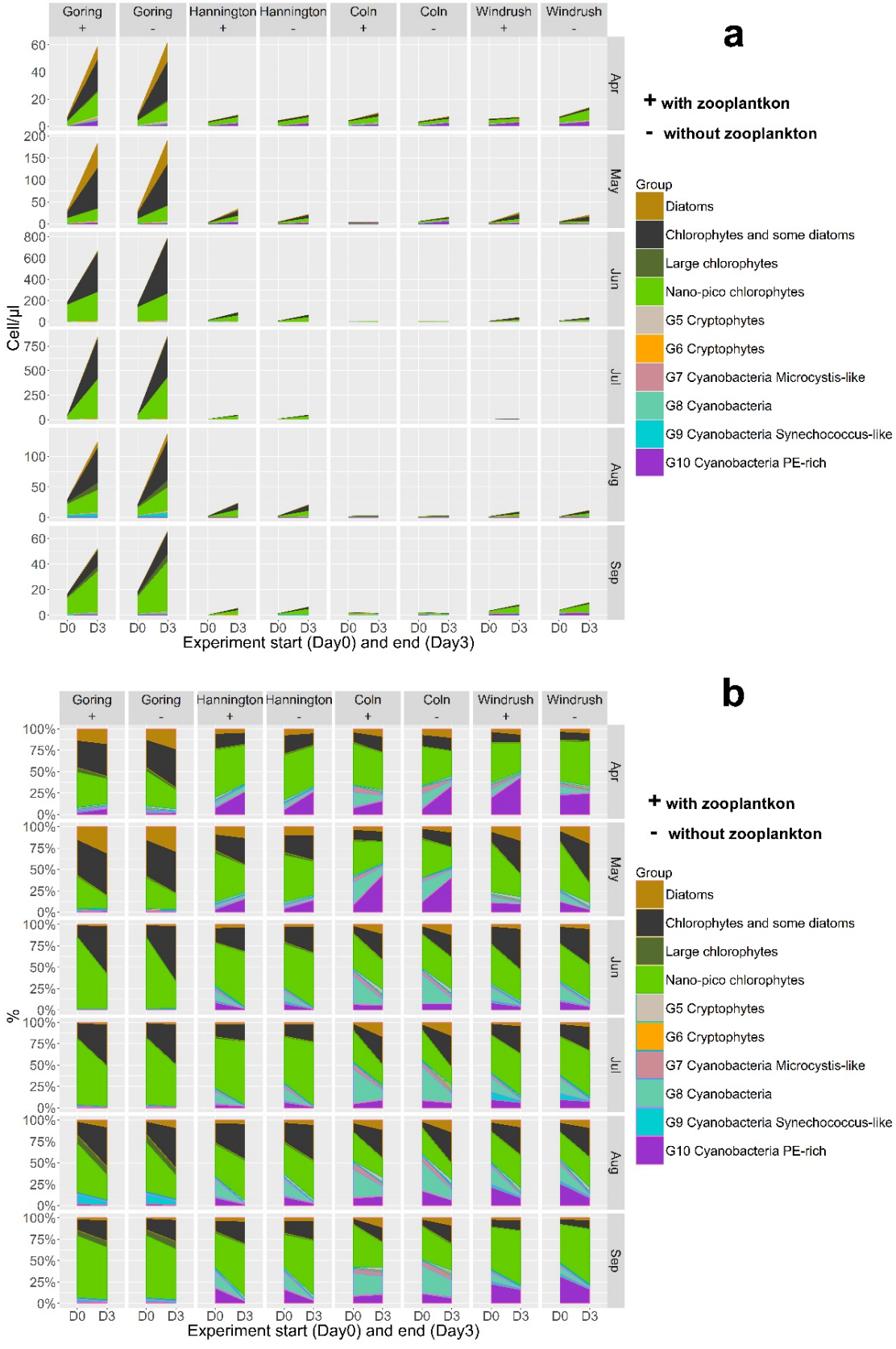

Figure 6-3 Phytoplankton composition in dialysis bags at the start (Day0) and the end (Day3) of experiments: a - based on individual group densities, b - on relative proportion of the different algal group. Treatment: grazers - unfiltered, no grazers – sieved. Flow cytometry data

The zooplankton were predominantly composed by filter-feeding rotifers mixed with a few crustacean nauplii (Figure 6-4). Most rotifers were small with body lengths in the range 100-200 µm or 200-300 µm (Figure 6-4). In the Thames at Goring during the zooplankton population 'bloom' phase, grazers were mainly small (100-200 µm). The commonly observed rotifers belonged to the following genera: *Brachionus, Dicranophorus, Trichotria, Keratella, Polyarthra, Synchaeta, Euchlanis, Notholca.* All bdelloids were grouped as *Bdelloidea.* A small number of crustaceans, mainly copepod nauplii, were found at all sites and *Keratella.*sp. and *Synchaeta.*sp. were dominating in communities in the Thames at Goring in August and early September. Rotifers have a clear advantage over micro-crustaceans in lotic environments due to their shorter development times (Hynes, 1970). For instance, in spring, rotifer populations in the Rhine were observed to double at twice the rate (0.89 day^{-1}) of crustaceans (0.45 day^{-1}) (de Ruyter van Steveninck *et al*., 1992). Rotifers tend to dominate the zooplankton community in many large lowland rivers (Reckendorfer et al., 1999; Viroux, 1999; Kim et al., 2001; Baranyi et al., 2002; Burger et al., 2002; Zimmermann-Timm et al., 2007).

Zooplankton in the Thames and its tributaries originated from diverse habitats and can be sub-divided into the following groups: true-planktonic, which successfully reproduce in the middle of turbulent channels; periphytic, found among rocks and gravel by the banks and near aquatic vegetation, and heleoplanktonic with preference for eutrophic stagnant environments (Figure 6-4). Some rotifers such as: *Brachionus* and *Euchlanis* prefer eutrophic ponds (heleoplankton) whilst many bdelloid species can be found in shallow zones by river banks and around aquatic plants (periphytic) (Pontin, 1978; De Manuel, 2000; Alekseev, 2010) (Figure 6-4). Cyclopoid copepods previously found in the Thames and its tributaries can successfully survive and reproduce in the middle of the channel or near the river banks (Perbiche-Neves et al., 2014). *Keratella* and *Synchaeta* rotifers are true-planktonic species, they developed dense populations in the Thames at Goring in August as a result of longer residence time in this reach and high water temperatures (20°C). In contrast, in the Coln and Windrush, water did not exceed 19°C, due to the significant groundwater dominance at these high BFI sites (Table 1). Species of family *Synchaetidae* were common due to their ability to thrive on the flagellates, cryptophytes, centric diatoms, and chlorophytes. Zooplankton numbers in the Thames headwaters (Hannington) increased in May-June, in contrast in the lower reach (Goring), they peaked in August.

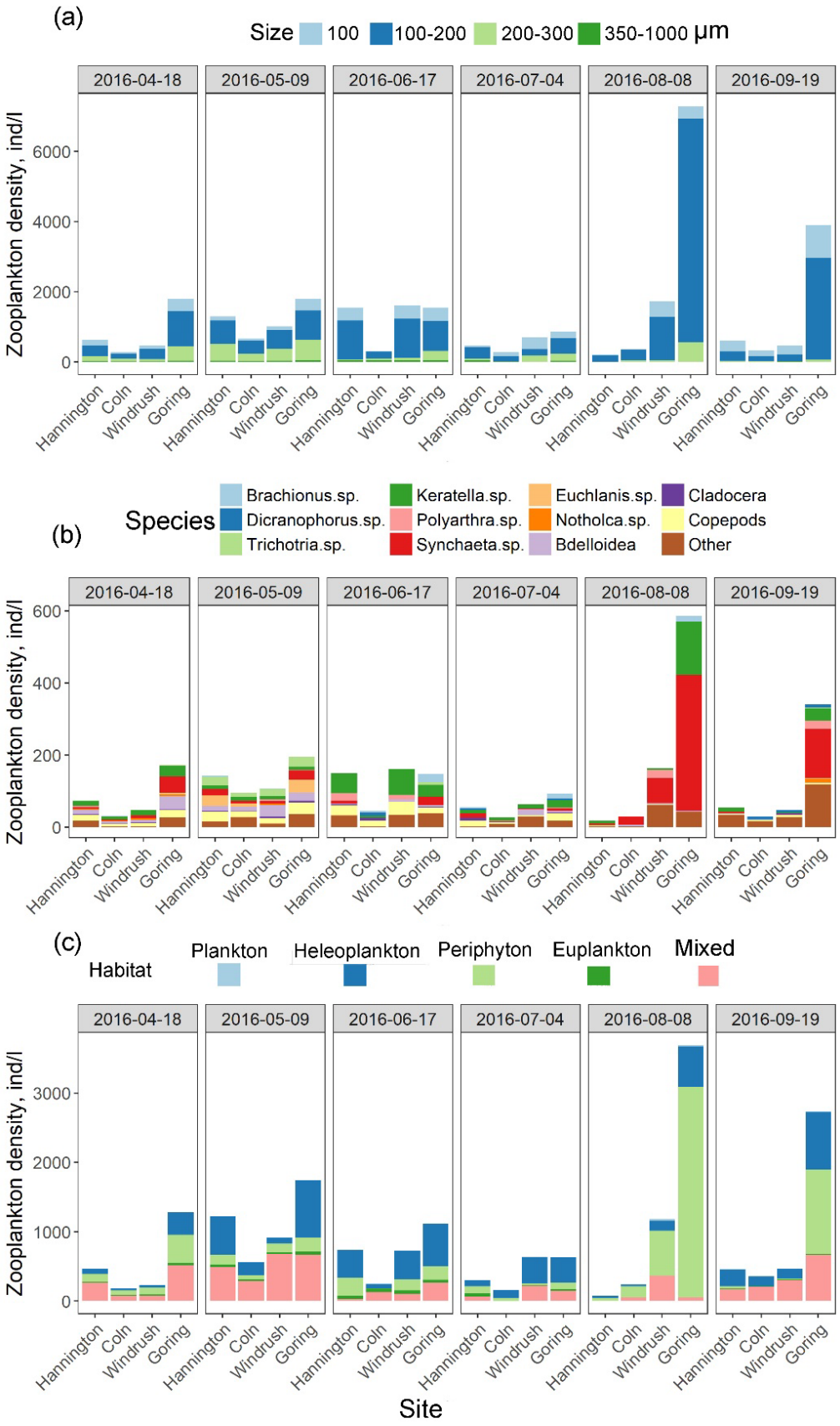

Figure 6-4 Zooplankton communities at studied sites: Thames at Hannington, Goring and the Coln and Windrush. a - body length; b- major genera and taxonomic groups; c - habitat preference. Dialysis bags field microcosm experiments April-September 2016.

Plankton characteristics by habitat should be treated with caution. In the river environment, due to changing connectivity with the floodplain, presence of in-channel retention zones, and high water turbulence, the origin of plankton is often difficult to define (Reynolds, 2000; Schiemer et al., 2001; Górski et al., 2013).

6.5.3 Grazing and selective feeding

Both (Figure 6-3 a,b) showed that metazoan (zooplankton) grazing influenced algal composition and abundance to some degree. There were notable spatial and seasonal variations in the grazing effect, and some evidence of selective feeding (Figure 6-5). Diatoms (G1) and chlorophytes (G2) were actively grazed in May, August and September (Figure 5); however, the grazing impact on diatoms and chlorophytes was a 25% reduction in abundance over three days in the Coln in May. At other sites the grazing impact was less than 10% abundance reduction. Similar patterns were observed with pico-chlorophytes (G4) and cryptophytes (G5, G6). Populations of cryptophytes were reduced by up to 20% in the Windrush and Thames at Goring. Cyanobacteria were evidently reduced in the Coln and the Thames at Hannington (up to 60% in April and 200% in June). In the Windrush and Goring this effect was less pronounced (10-20%). At this point it is important to emphasize that centric diatoms and cryptomonad flagellates contain highly unsaturated fatty acids which are main nutritional constituents of zooplankton diets (Brett and Müller-Navarra, 1997). They are known to determine energetic efficiency across the plant-animal interface, secondary production and the strength of trophic coupling in aquatic pelagic food webs (Vargas et al., 2006). In contrast, phytoplankton communities dominated by green algae and cyanobacteria are often characterised by a low phytoplankton biomass and metazoan abundance (Müller-Navarra & Lampert, 1996).These experiments also show that all phytoplankton types are susceptible to grazing (Figure 6-9) and are likely to face the risk of mortality from not one grazer alone but from the entire zooplankton array (Figure 6-4). Larger abundance of grazers exerted higher pressure on all phytoplankton growth rates in the Thames in August at Goring (Figure 6-6). Rotifers have relatively short generation time, and they are free of dependence on photoperiods and nutrients, their population can largely expand in the presence of

food (Figure 6-4).

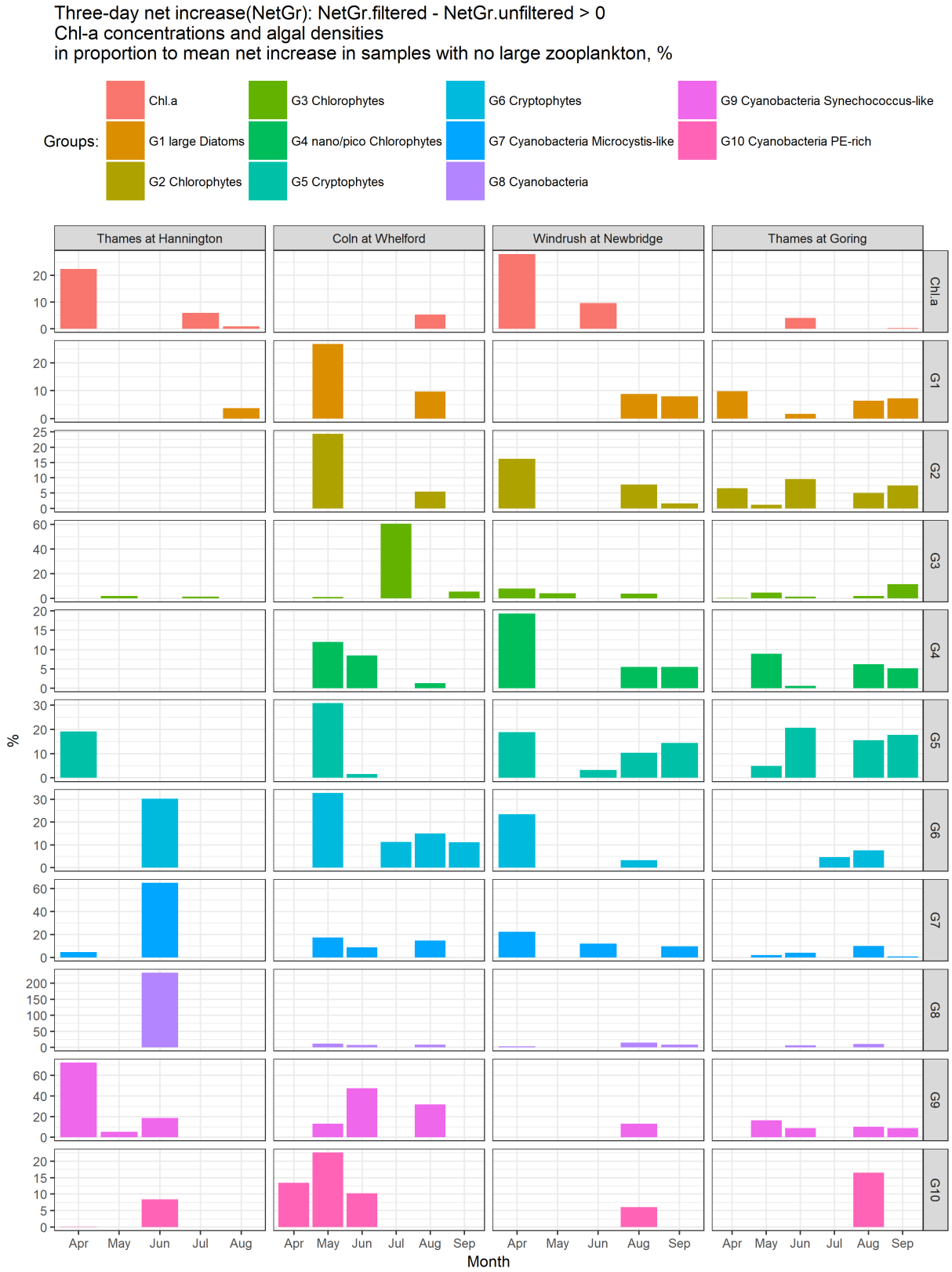

Figure 6-5 Grazing effect estimated as difference in net growth in dialysis bas with and without zooplankton related to net increase in bags without zooplankton. PE-rich. Dialysis bags field microcosm experiments April-September 2016 Equation 6-2

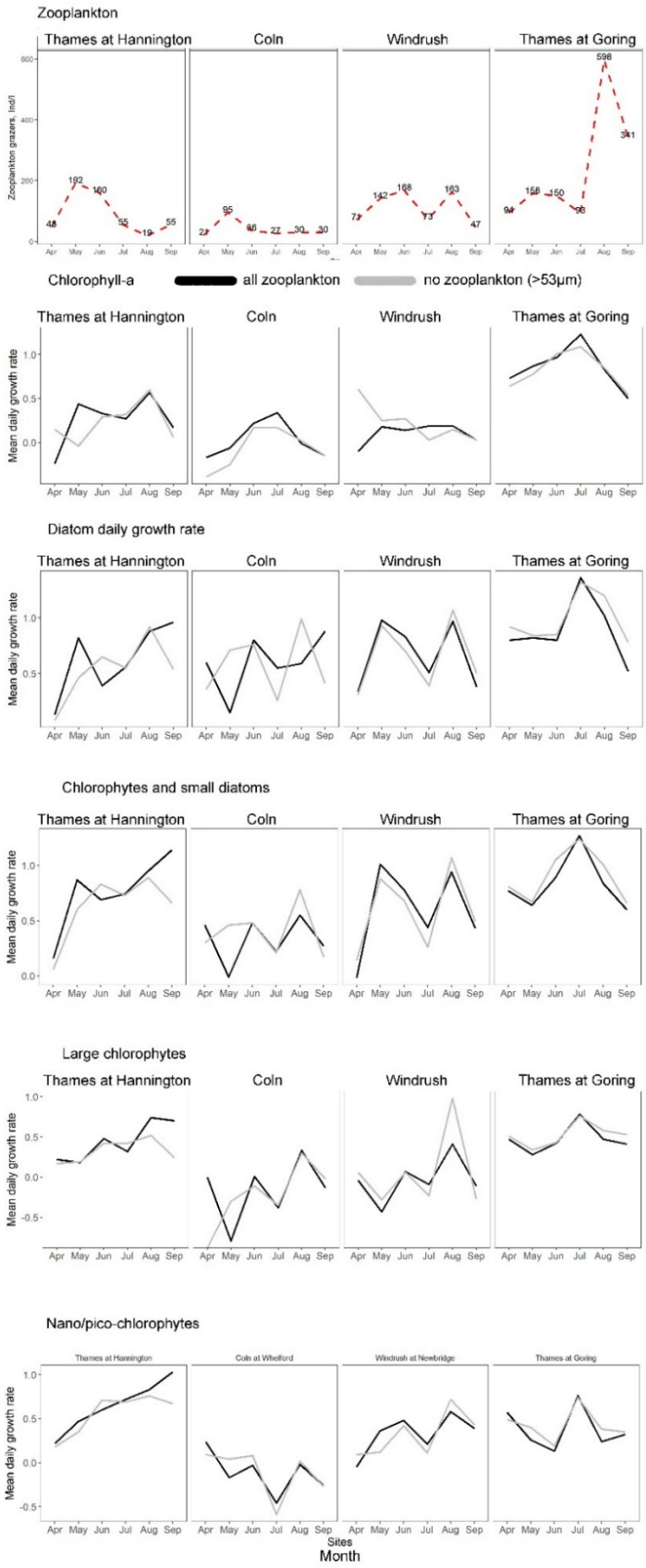

Figure 6-6 (1) Daily growth rates of phytoplankton (Equation 6-1)

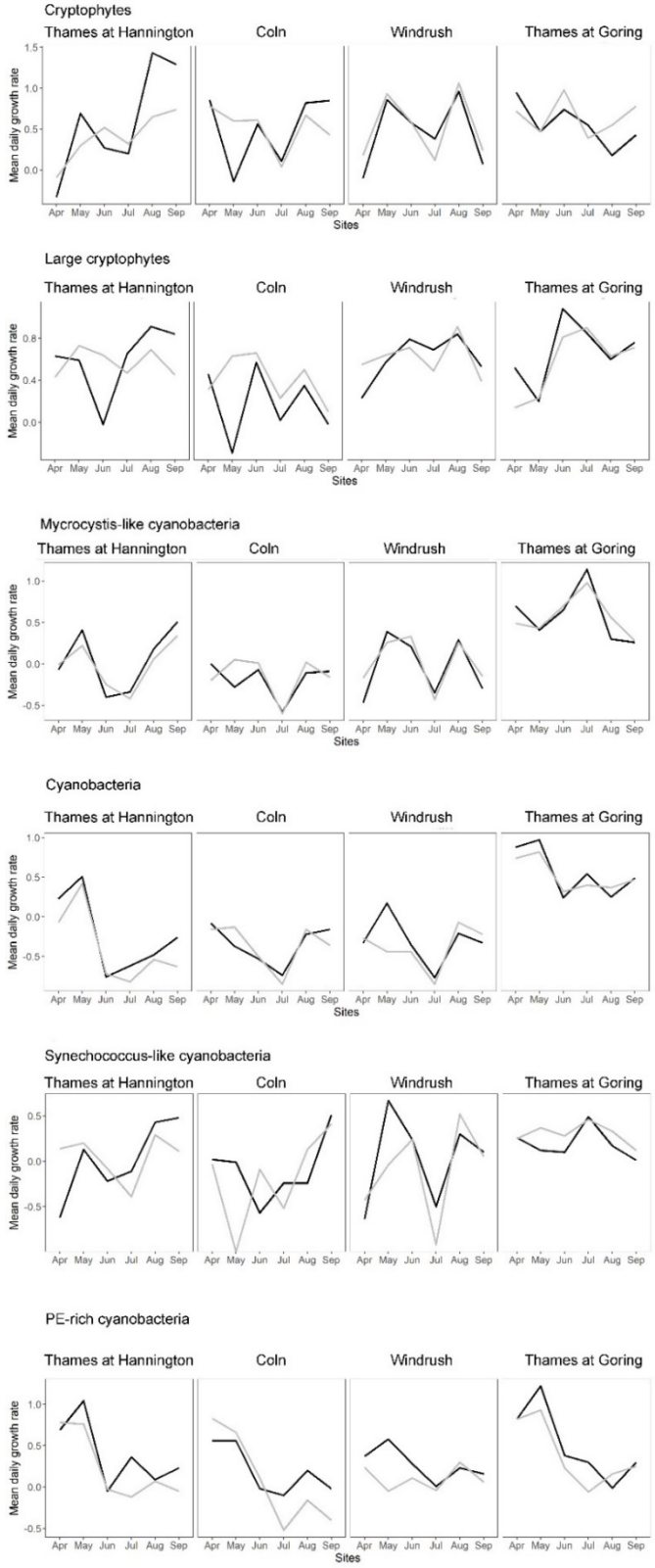

Figure 6 6 (2) Daily growth rates of phytoplankton (Equation 6 1)

6.5.4 Top-down and bottom-up controls

Stepwise selection, two main equations were tested. *Model 1* tested three categorical variables (site, month and treatment: filtered/unfiltered) and their interactions. This model equation was independently applied to all the chlorophyll-*a* growth rates and the growth rates of the individual algal groups (G1-G10).

$$Daily\ growth\ rates = Site + Month + Treatment + Month*Site + Month*Tr + Site*Tr + Month*Site*Tr$$

Site – four studied sites (Thames at Hannington, Goring, the Coln and Windrush);
Month – six months (April-September)
Treatment – filtered/unfiltered

Equation 6-3 Model 1

Statistical models (Equation 3) showed that there were significant spatial and seasonal variations in phytoplankton growth rates. The factor *Treatment* (filtered/unfiltered) was insignificant (P-values > 0.005) (Table 6-3) for chlorophyll-*a* and all phytoplankton groups.

The importance of interaction terms *Site*Treatment*, *Month*Treatment* and *Site*Month*Treatment* for all algal groups demonstrated that grazing is not constant and is largely seasonal and site-specific. In some cases, phytoplankton daily growth rates in bags with zooplankton were higher than in bags without them, suggesting that grazers could have promoted algal growth, possibly due to selective feeding.

Table 6-3 Model 1 Significance (P-values) and improved the coefficients of determination (R^2 adjusted) for chlorophyll-*a* concentrations (Chl.a) and algal groups (G1-G10) Equation 6-3

Predictors/P-values	Chl.a	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10
Site	**	**	**	**	**	0.02	**	**	**	**	**
Month	**	**	**	**	**	**	**	**	**	**	**
Treatment	0.03	0.77	0.58	0.09	0.39	0.22	0.08	0.19	**	0.1	**
Site*Month	**	**	**	**	**	**	**	**	**	**	**
Site*Treatment	**	**	**	**	**	**	**	**	**	**	0.56
Month*Treatment	**	**	**	**	**	**	**	**	**	**	**
Site*Month*Treatment	**	**	**	**	**	**	**	**	**	**	**
R-sq.Adj	0.94	0.90	0.91	0.90	0.93	0.81	0.77	0.94	0.89	0.81	0.84

** - $P < 0.01$

Model 2 tested water temperature, light illuminance, initial size of phytoplankton inoculum, total number of grazers, pH, suspended solids, SRP, Si, and Nitrate as major phytoplankton growth

predictors. This model equation was independently applied to all measured variables including chlorophyll-*a* and individual algal groups (G1-G10).

$$Daily\ growth\ rates = Mean\ temperature + Maximum\ temperature + Mean\ light + Maximum\ light + Inoculum\ size + Total\ number\ of\ grazers + SRP + Si + NO_3$$

Temperature and *light* were estimated from all measurements 30min, 3-day interval)
Inoculum size - initial phytoplankton population abundance
Total number of grazers – total number of rotifers and microcrustaceans
SRP, Si, NO_3 - concentrations of nutrients (Table 2).

Equation 6-4 Model 2

Significant variables for all phytoplankton groups consistently were: mean water temperature, and maximum light illuminance. SRP concentrations were important for phytoplankton biomass (chlorophyll-*a*), chlorophytes, pico-chlorophytes and large cryptophytes/dinoflagellates (Table 6-4). Nitrates were significant predictors of diatoms, pico-chlorophytes and cyanobacteria growth rates, whilst changes in silicon concentrations could be applied to model diatom and cyanobacteria growth. High suspended solids evidently influenced algal development by reducing light penetration into the dialysis bags. The term *Total grazers* was an insignificant predictor for chlorophyll-*a*, chlorophytes, large cryptophytes, and *Synechococcus*-like cyanobacteria (P-values > 0.005) (Table 6-5).

Bowes et al. (2016) previously showed that active chlorophyll-*a* growth in the River Thames can generally be explained by physical factors such as: water temperature and sunshine duration alone. Zooplankton total abundance was significant explanatory variable ($P < 0.05$) for diatoms, pico-chlorophytes, cryptophytes and cyanobacteria. Higher zooplankton abundance in the Thames at Goring in August evidently reduced phytoplankton growth Figure 6-6, 7), but this effect was seasonal and local (Equation 6-3).

Table 6-4 Model 2 Significance (P-values) and improved the coefficients of determination (R^2 adjusted) for chlorophyll-a concentrations (Chl.a) and algal groups (G1-G10) Equation 6-4

Predictors/P-values	Chl.a	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10
Mean temperature, °C	**	**	**	**	**	0.04	**	**	0.1	**	**
Maximum temperature, °C	0.03	0.52	0.69	0.89	0.67	0.65	0.04	**	**	**	**
Mean light, klx	0.03	0.03	**	0.62	0.06	**	0.16	0.68	0.07	**	0.45
Maximum light, klx	0.10	0.19	**	**	**	0.04	0.01	0.03	0.03	**	0.01
Phytoplankton inoculum size, cell ml^{-1}	**	0.38	**	0.16	0.48	0.46	0.30	**	**	0.66	**
Total grazers, ind l^{-1}	0.54	0.30	0.98	0.10	**	0.41	0.51	**	**	0.79	**
SRP, µg l^{-1}	**	0.89	0.03	**	**	0.15	0.00	0.12	0.68	0.44	0.19
Suspended sediment, mg l^{-1}	0.1	0.07	0.02	0.22	**	0.01	0.00	0.01	0.53	0.00	0.01
pH	0.72	0.03	**	0.05	0.04	0.39	0.33	0.99	0.14	0.04	0.01
Silicon, mg l^{-1}	**	0.05	0.15	0.01	**	0.36	0.24	0.27	0.33	0.83	**
Nitrate, mg l^{-1}	**	0.12	0.36	0.89	**	0.05	0.77	0.6	**	0.43	0.15
R-sq.Adj	0.61	0.31	0.6	0.52	0.66	0.23	0.35	0.44	0.63	0.37	0.35

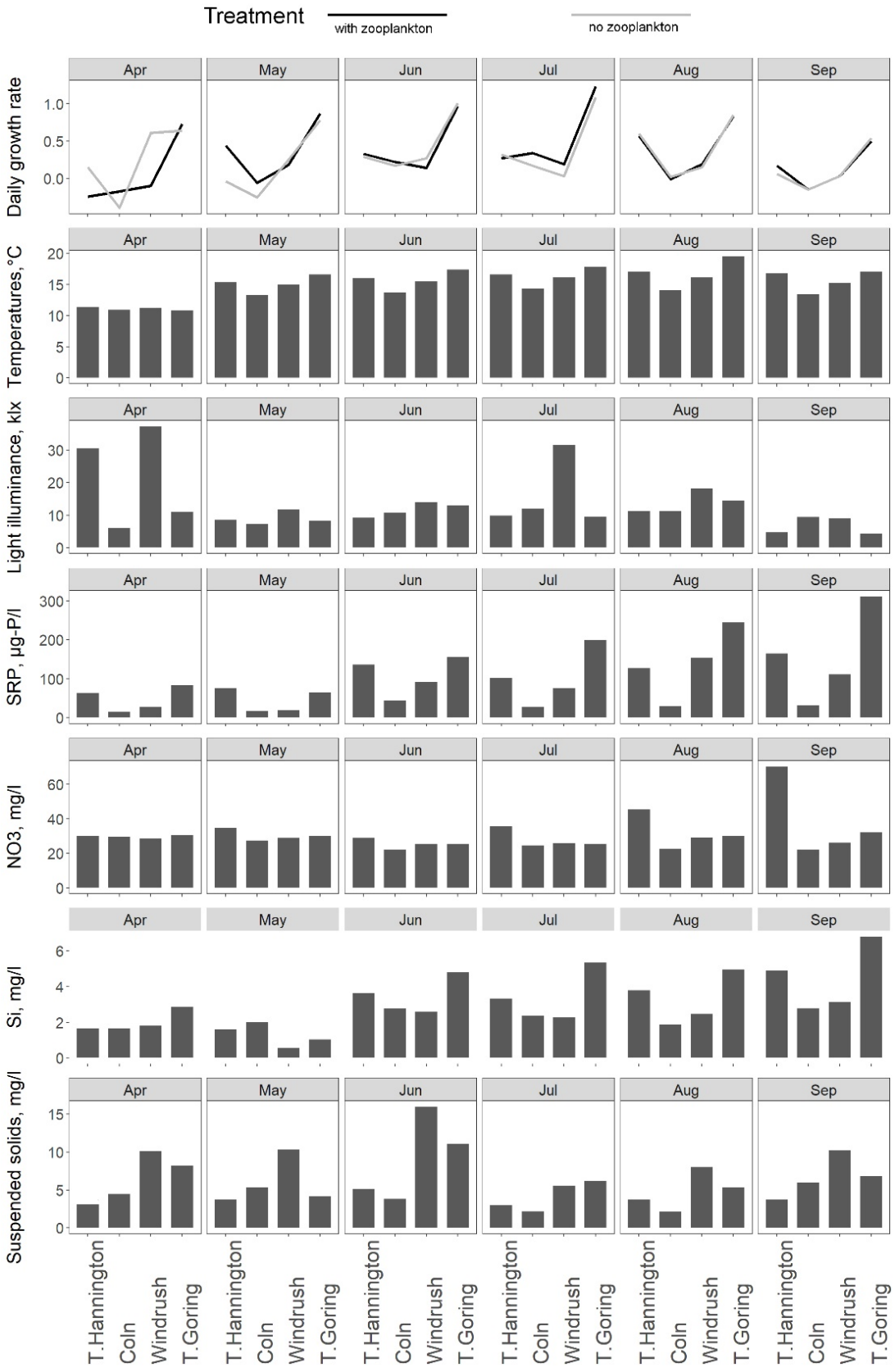

Figure 6-7 a- Chlorophyll *a*; b-growth rate; c-mean water temperature; d- mean light illuminance; e- SRP; f- Nitrate; g- Silicon, e-Suspended sediment. Field grazing experiments April-September 2016. The Thames catchement

These findings agree with Gosselain et al. (1998) where in the River Meuse (the Belgian part) the total phytoplankton biomass declined in summer following the increase in zooplankton biomass and filtration rate. However, in rivers the zooplankton can develop high abundance only during higher water age, in other words, long residence time when water flow and temperature are favourable (Keckeis et al., 2003).

pH was an important factor for diatoms and green algae only. Several studies emphasized pH-dependant absorption of nutrients and limitation of photosynthetic rates of diatoms that limitation at pH > 8.8 for green algae by and (Reynolds, 1984; Hervé et al., 2012). Cyanobacteria can sustain a high photosynthetic rate at pH 9-10 (Unrein et al., 2010).

Concentrations of nutrients, especially SRP, which is an essential component for algal metabolism, were important predictors, but different phytoplankton responded differently. SRP concentrations were significant for phytoplankton biomass (based on chlorophyll *a*).These results comply with a number of studies of North American rivers which reported strong positive correlations between total phosphorus concentration in the water column and phytoplankton biomass in rivers (Basu and Pick, 1996; Van Nieuwenhuyse and Jones, 1996; Chételat *et al.*, 2006).

Nitrogen also can limit phytoplankton production in temperate eutrophic environments, especially where phosphate concentrations are relatively high. Some species of cyanobacteria can assimilate nitrogen from the atmosphere (Reynolds, 1984). Nevertheless, this study showed that nitrate concentrations were an important factor for diatoms, large crypthophytes and a small group of cyanobacteria only. Some studies previously showed little relationship between nutrient concentrations and either phytoplankton or periphyton biomass in rivers (Balbi, 2000; Bernhardt and Likens, 2004; Morgan *et al.*, 2006). Descy *et al.*, (1987) suggested that in large lowland rivers with excessive nutrient inputs of phosphorus and nitrogen typically do not limit phytoplankton production. Bowes et al. (2016) also concluded that increases in nutrient concentrations did not trigger phytoplankton blooms in the River Thames. Some photosynthetic organisms such various species of cryptophytes and dinoflagellates are mixotrophs and are not fully reliant on dissolved nutrient concentrations (Tranvik et al., 1989)

Silicon (Si) was an important explanatory variable of diatom growth. It is required by all phytoplankton in small amounts for protein and carbohydrate synthesis, whilst diatoms and

chrysophytes need silicon to strengthen their cell walls (Reynolds, 1984). When Si is limited it is usually followed by a decline in diatom biomass.

6.5.5 Grazing in lentic and lotic environments

Since based on lentic studies, the zooplankton grazing has often been suggested as an important control factor in phytoplankton dynamics (Schöl A., Kirchesch ., Bergfeld T., Schöl F., Borcherding J., 2002; Waylett et al., 2013), it is important to clarify that lentic and lotic physical environments are different and their plankton communities also differ significantly. The taxonomic composition of planktonic herbivores in rivers is considerably less diverse than that of lentic environments. Rivers generally are dominated by rotifers mixed with copepod nauplii (Lair, 2006). These are small-bodied grazers (body length 50 -500µm) with relatively low clearance rates (0.007-0.1ml d^{-1}) defined as the volume of water cleared of food by a consumer organism per unit time and per consumer or consumer mass (Bogdan et al., 1980; Reynolds, 1984; Rothhaupt, 1990; Harris et al., 2000; Kim et al., 2000). During low flows, warm water temperatures (16-20 °C) and presence of phytoplankton, rotifers rapidly establish dense populations which show some resilience to the rapidly changing turbulent river environment (Baranyi et al., 2002; Lair, 2006; Bertani et al., 2011).

In contrast, lakes are dominated by microcrustaceans (Reynolds, 1984). These animals require longer time to grow and reproduce than rotifers, and they are sensitive to changing river flow and turbulent environment (Hynes, 1970). However, microcrustaceans are larger grazers with up to 100 times higher clearance rate (Thompson et al., 1982; Kim et al., 2000; Keckeis et al., 2003). During spring-summer period in lakes they establish dense populations which exert significant pressure on phytoplankton communities (Lehman, 1988). Although the types of organism inhabiting freshwaters are similar, zooplankton community structures and abundances are different. This means that rules and significance of zooplankton herbivory in lakes should not be applied on rivers or treated with caution.

6.6 Conclusion

This study shows that the river zooplankton community, which is generally composed of rotifers, can remove a quarter of the diatom and unicellular green algae population, and more than half of *Synechococcus*-like and PE-rich cyanobacteria. However, in large, lowland river-catchments, zooplankton grazing occurs only in the area where both phyto- and zooplankton can establish dense populations. In the River Thames, these are middle and low reaches and potentially

eutrophic tributaries that support active plankton growth. Grazing is a seasonal process. The phytoplankton community succession as matched by rapid changes in the zooplankton composition, probably due to the grazers' feeding preferences. Simulation of the grazing process in mathematical models should consider both phytoplankton community diversity and abundance as basic parameters.

The composition of the phytoplankton community of temperate lowland rivers may at times be controlled by grazers. However, significant grazing can occur only when physical constraints are reduced, i.e. when discharge is low, water temperature is high and availability of grazeable algae allow high zooplankton biomass.

6.7 Acknowledgment

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Chapter 7 Bacteria and parasitical chytrids interactions with phytoplankton

7.1 Field observations

This section is a summary of the field observations during the summer of 2016 that led to the research questions and hypothesis tested (thermal regulation and microbial controls) in the laboratory experiment in 2017 (described in Section 7.2)**.** Following a rain event on the Thursday 18th August 2016, a notable rise in the stream water chlorophyll-*a* concentration was observed (Figure 7-1) in the Thames at Wallingford. This increase coincided with residents reporting a change in the river colour from green to brown (Figure 7-1).

Figure 7-1 Change in colour of the River Thames water during an algal bloom. Photographs are taken from the left bank of the River Thames by Wallingford Bridge facing North, during mid-day hours on 18-19/08/2016. Chlorophyll-*a* concentrations: 18/08-8.7µg m-3, 19/08-322.2µg m^{-3}

A water sample was taken at 1 pm on the Friday 19th August 2016 was analysed using optical microscopy and found to contain high population densities of the diatom *Stephanodiscus hantzschii* (Figure 7-2). These are common centric diatoms that have been found in the River Thames since very early studies, more than a hundred years ago (Fritsch, 1903; Rice, 1938).

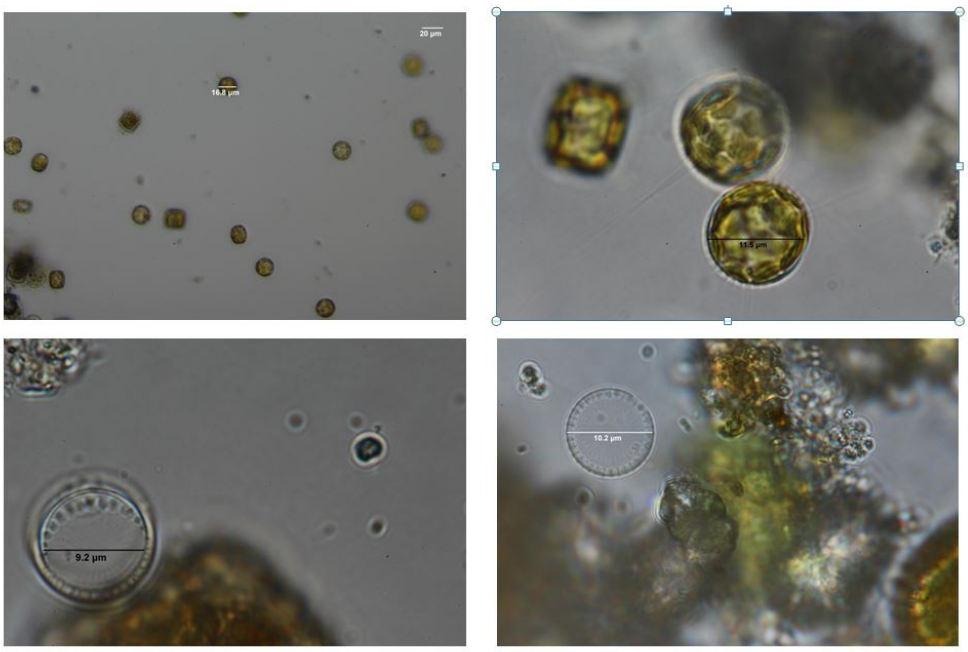


Figure 7-2 *Stephanodiscus hantzschii* diatoms found in water samples Friday 19th August 2016 collected from the River Thames at Wallingford (by the bridge)

The magnitude of the increase in chlorophyll-*a* (Figure 7-3) concentrations from 8.7 to 322.2 µg m^{-3} in less than a day could only be explained by the intensive algal growth upstream or an active population development near the benthic part of the river channel. The water chemistry data sampled five days prior the bloom (15/08/2016) showed no significant change in silicon concentrations along the Thames, in the headwaters at Hannington it was 4.2 mg m^{-3}, which is higher than an annual mean (M_{annual}= 3.3, *SD* = 1.1), in the upper reach at Swinford – 3 mg m^{-3} (M_{annual} = 2.9, *SD* = 0.9), at Wallingford – 4.8 mg m^{-3} (M_{annual}= 4.08, *SD* = 1.3). These data indicated the absence of substantial diatom growth in the Thames channel. Chlorophyll-*a* values measured at Swinford also revealed no sign of rapid diatom growth (12 µg m^{-3}). Furthermore, the hypothesis that centric diatoms reproduced extensively in the benthic area of the river channel, and then were re-suspended during the rain event is not supported by the ecology of *Stephanodiscus hantzschii*. Centric diatoms are floating cells that do not form biofilms; they are considered as truly planktonic algae, sustaining sizeable populations in well-mixed turbulent waters (Gillard, 2010). Such diatoms can, however, be harboured in the river catchment retentive zones (Chapter 5. Importance of off-channel habitats). Still, even if diatoms actively reproduced at the bottom of the main Thames channel, their cells enriched with brown-red carotenoids would have altered water colour days before the first sign of the bloom were observed in the surface area (Stauber & Jeffrey, 1988).

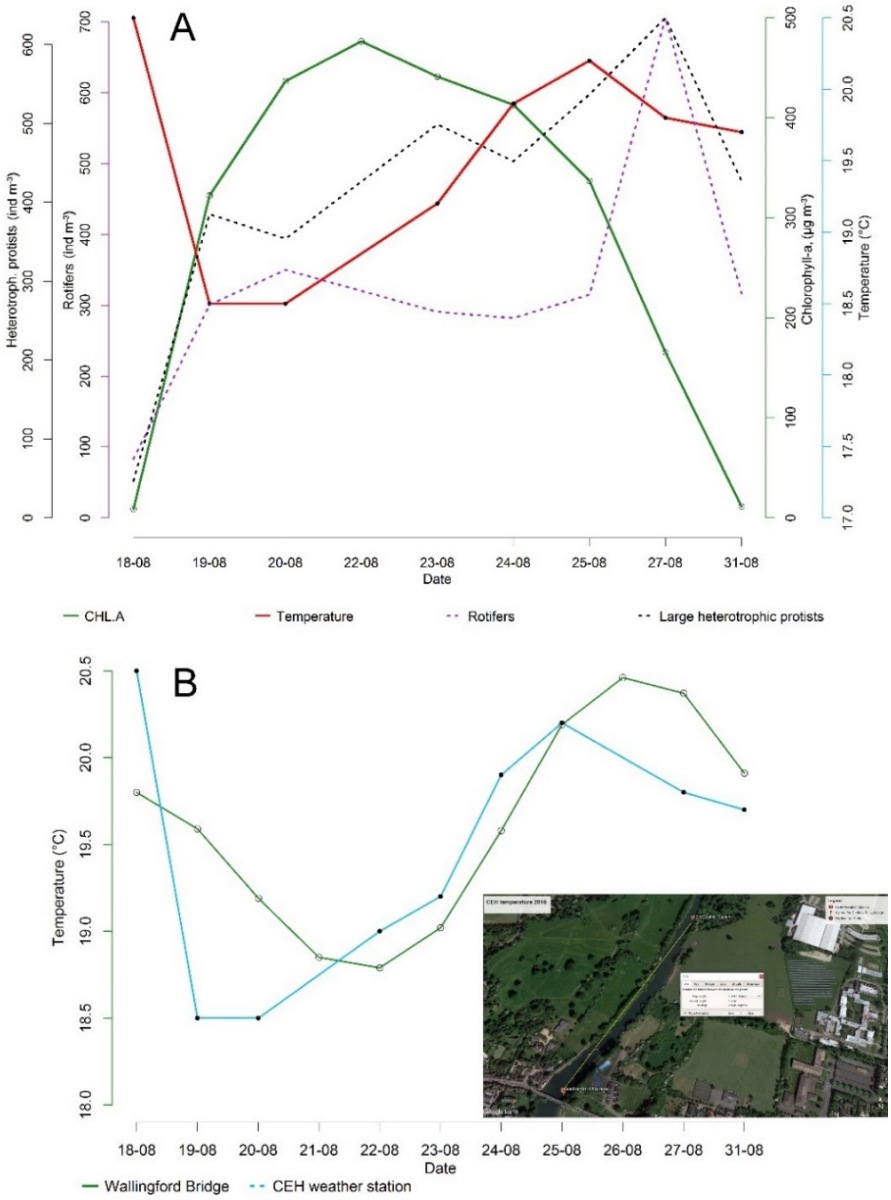


Figure 7-3 A - Water temperature, chlorophyll-a concentrations, rotifer and large heterotrophic protists densities (ind m^{-3}); B – Water temperature measured at Wallingford Bridge and CEH weather station between18-31/08/2016

No significant change in silicon and chlorophyll-a concentrations along the Thames channel prior the bloom and the absence of diatom red colour can only mean that the water parcel enriched with diatoms, could have travelled from the stretch of the river above Wallingford, possibly near Oxford. Weekly phosphorus dynamics along the longitudinal profile of the river revealed a sudden fall in phosphate concentrations in the lower stretch of the Thames during the bloom (Figure 7-4) with no evidence of active consumption five days before the event in Wallingford. Furthermore, water temperatures measured in the Thames at Wallingford by the bridge and the Centre for Ecology and Hydrology (CEH) weather station on the 18th and 19th of

August showed a 1°C variation between these sites (500 m apart). The groundwater fluxes could partly explain these differences. However, they were most evident when diatoms first appeared at Wallingford indicating that the blooming parcel could have developed in a colder environment (1-2°C) than the Thames.

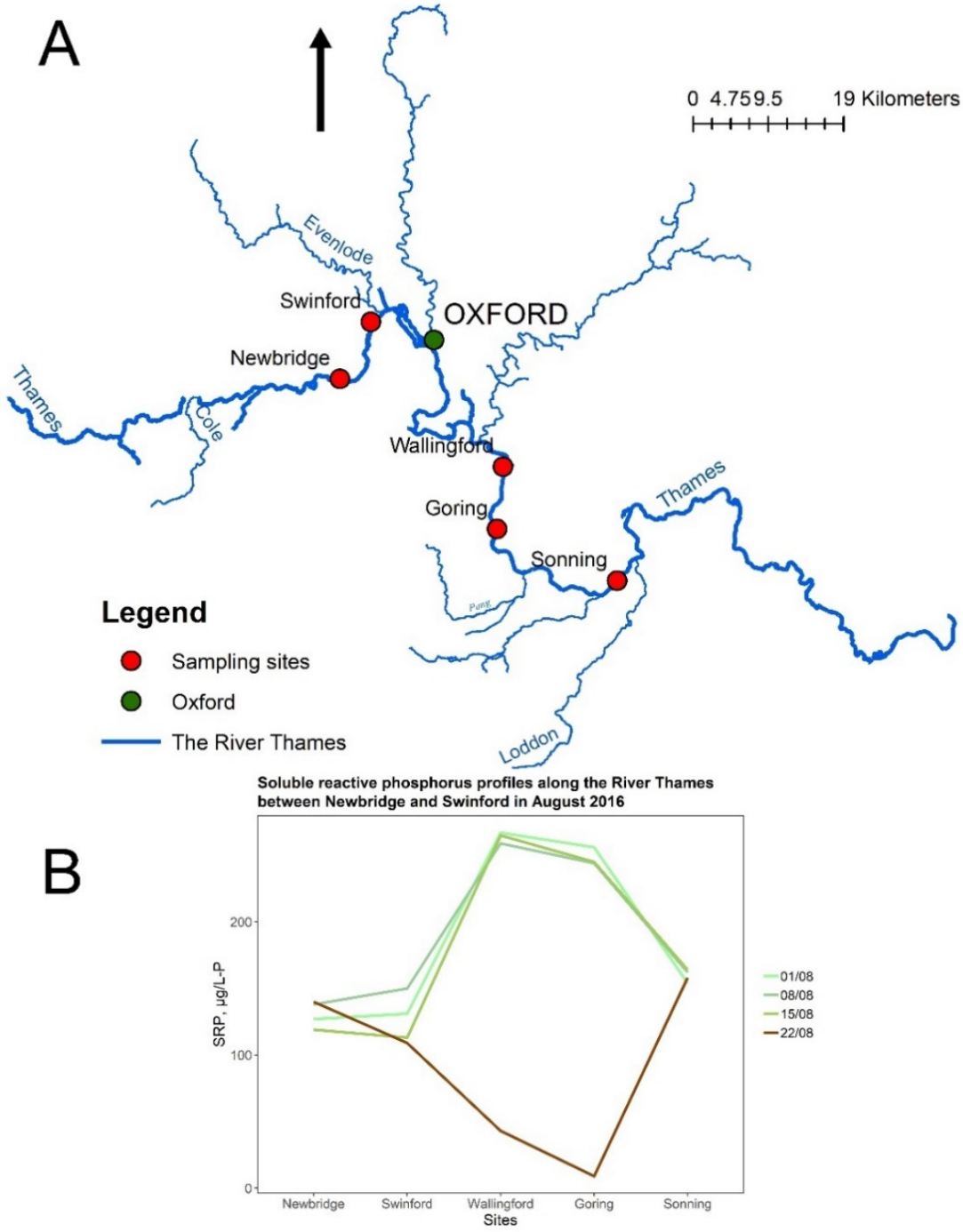


Figure 7-4 Dynamics of the soluble reactive phosphorus in the part of the River Thames in August 2016. A - the River Thames weekly monitoring sites (Newbridge, Swinford, Wallingford, Goring, Sonning). B - Soluble reactive phosphorus profile along the river between Newbridge and Sonning 01-22/08/2016 (weekly measurements)

Finally, it may be concluded the hypothesis that the late summer 2016 centric diatom bloom in the lower Thames originated upstream from Wallingford, potentially in the Oxford large retentive canal system and then entered the Thames carrying a sizeable population (around 200000 ind m^{-3}) of healthy *Stephanodiscus hantzschii*, holds. The bloom advanced downstream (was observed in Windsor) and lasted for about two weeks in total (from the time it was first seen at Wallingford). During its active growth phase in the first week, the numbers of zooplankton grazers were relatively low (around 300 ind m^{-3}). They doubled towards the end of the bloom, taking almost ten days to develop a sizeable community of around 700 ind m^{-3}.

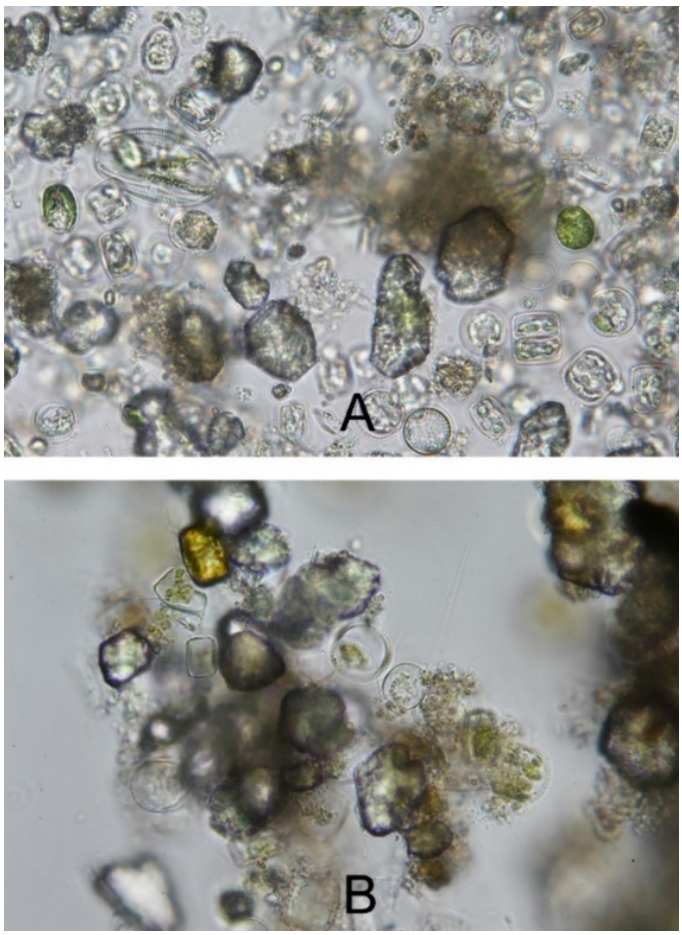


Figure 7-5 Centric diatom (*Stephanodiscus hantzschii*) aggregates observed during extensive blooms in June 2009 (A) and August 2016 (B). Raw, uncalibrated images. Axiovert 40CFL. Canon 650D

Optical microscopy revealed signs of significant diatom mortality unrelated to grazing (Figure 7-5). There were high numbers of empty frustules clustered into aggregates in the shape of amorphic 'glass flakes'; cells with visibly reduced in size chloroplasts, and chytrid sporangia attached to some healthy appearing diatoms, leading to the assumption that diatoms were dying from various microbial infections.

Influence of bacteria and parasitical chytrids on planktonic diatoms in the lowland eutrophic River Thames (UK)

7.2 Abstract

Bactria and fungal community in freshwater ecosystems decompose organic matter and transform nutrients but may also control phytoplankton dynamics by influencing transparent exopolymer particles (TEP) formation. TEP support algal aggregation and sedimentation. This study examines the effect of bacteria and chytrid infection on the diatom-chlorophyte community within the context of temperature change and nutrient limitation stress through experiments with a naturally occurring phytoplankton-bacteria-chytrids culture taken from the River Thames catchment, UK. Phytoplankton cells were identified and enumerated with both optical and scanning electron microscopes (SEM).

To explore the temperature effect, starter culture aliquots were incubated in a temperature-light controlled environment (15.5, 20°C) with added diatom nutrient medium. For nutrient stress at 20°C, deionised water was used instead of the medium. Diatom aggregates (clusters), and individual cells with attached bacteria or chytrids were enumerated at the start, and after 24/48 h of incubation. Data were analyses with Generalised Linear Models. Thermal stress had a significant adverse effect on diatoms at both 15.5 and 20^{o}C and on chlorophytes at 15.5°C. However, at 20^{o}C, the chlorophyte population increased by 25%. After 48 h of incubation, the proportion of attaching bacteria increased up to 80%, at the same time the proportion of chytrids fell by 2-4%.

Under nutrient deficiency stress both diatom and chlorophyte populations declined faster, and the proportions of diatoms infected by chytrids were significantly higher than in the nutrient enriched treatments (but remained unchanged from the starter culture). Low nutrient concentrations had only a moderate positive effect on diatom clustering and attaching bacteria, whereas the incubation period was a significant predictor for both of these variables. Diatom mortalities were positively correlated with the presence of bacteria, chytrids, and proportion of diatom clusters/aggregates. The aggregates were positively assossiated with diatom-attaching bacteria.

Scanning electron microscopy (SEM) revealed a rapid deterioration of frustules within aggregates and the high presence of extracellular polymeric substances (EPS) and transparent exopolymer particles (TEP). X-ray analysis showed that calcium carbonate in river waters may also support diatom aggregation. Overall the results demonstrate that both diatom metabolism and presence of attaching bacteria play an important role in diatom bloom rapid termination and recycling. These results indicate a complex interplay between the physical and biological environments in terms of nutrient availability and bacteria-diatom interactions. Further investigation is needed to unpick these complex relationships.

Key words: plankton, diatom aggregation, extracellular polymeric substances, transparent polymeric substances, bloom termation

7.3 Introduction

In large eutrophic rivers, phytoplankton often form extensive blooms that may affect water-supply quality and result in low oxygen levels or high toxin presence (Millennium Ecosystem Assessmen, 2005; UN Environment, 2017). The onset and decline of phytoplankton blooms are difficult to predict since the relationship between the physical, chemical and biological variables that control algal community structure, and their seasonal dynamics, are not fully explained. Recent advances have been made using long-term weekly flow cytometry, and high-resolution (hourly) chlorophyll-*a* data helped to establish key seasonal and spatial patterns in phytoplankton development in the Thames, but the causes of algal succession and clear water phases remain poorly understood (Read et al., 2014; Bowes et al., 2016, 2018). In large lowland rivers, diatoms initiate seasonal plankton succession, playing an important role in primary production and biogeochemical cycles. In temperate climates, diatom blooms tend to occur in spring/early summer, generally, last for up to two weeks and suddenly terminate, being succeeded mainly by chlorophytes (Reynolds & Descy, 1996). In the River Thames, UK, the community of chlorophytes, mixed with cryptophytes, dinoflagellates and cyanobacteria succeed diatoms in late spring/early summer, at the same time low phytoplankton biomass (clear-water phase) tend to occur in mid-summer, but can also begin in spring and last for most of the growth period (Appendix. Figure 2, 3) (Lack, 1971; Ruse & Hutchings, 1996; Ruse & Love, 1997; Moorhouse et al., 2018). After a seasonal bloom, diatoms rarely restore to high population densities even when environmental conditions (flow, temperature, light, residence time and nutrients) are favourable (Waylett et al., 2013). While in lakes, diatom termination, is often attributed to

zooplankton grazing (Lampert et al., 1986; Wu & Culver, 1991; Kim et al., 2000; Quinlan et al., 2009), in rivers, this process has a marginal to negligible effect (Section 6.5 Conclusion).

Diatom bloom termination and clear-water phases have been related to water temperatures above 19°C (Bowes *et al.* 2016). Still, the exact mechanisms by which temperature and light influence algal community remain uncertain; for example, there is evidence that the 19°C threshold does not always apply to diatom seasonal dynamics in the Thames when, for instance, in August 2016 when water temperatures were fluctuating between 19-20°C there was a sizable centric diatom bloom in the middle and lower reaches (7.1 Field observations Page 143).

Algal cell ruptures (lysis) caused by the microbial activity are an alternative explanation for rapid diatom declines, extended periods of low phytoplankton biomass and nutrient recycling which supports phytoplankton succession (Reynolds, 1984). This explanation is examined through experimentation in this work

7.3.1 Bacteria

Recent studies, reviewed by Amin et al. (2012), indicate that there are bacteria that consistently associate with living diatoms. These bacteria can be saprophytes, colonise dead diatoms, decompose organic matter, and play an important role in silicon regeneration (Bidle and Azam, 2001). The bacteria are either free-living cells (Blackburn et al., 1998), attaching to diatoms (Gärdes et al., 2011) or occurring as an intracellular algal symbiont (Schmid, 2003). Bacteria can act synergistically with algae (Croft et al., 2005) or compete with algae for nutrients, indirectly increasing algal mortality.

There are some bacteria that specialise in an algal-lysing lifestyle and directly inhibit algal growth (Mayali and Azam, 2004). Jung et al. (2008) isolated and studied the strain of *Pseudomonas fluorescens* HYK0210-SK09 that was successfully suppressing populations of *Stephanodiscus hantzschii* in a controlled environment and an indoor mesocosm experiment. These bacteria attach to the host cells with sticky pili (hair-like external cell appendage), then degrade them with lysozyme-like enzymes (Baker and Herson, 1978). This algicidal activity, however, is inhibited when water temperatures are lower than 10°C (Kang et al., 2011). Paul and Pohnert (2011) studied mechanisms by which *Kordia algicida* interacts with host diatoms and emphasised that the release of active enzymes depends on the density of bacteria population rather than diatoms, whilst there is experiment evidence that bacteria stimulate diatom clustering and sinking (Gärdes et al., 2011), the process associated with high concentrations of microgels like transparent

exopolymer particles (TEP). These microgel sticky particles are composed of acidic mucopolysaccharides derived mainly from gelatinous algal cell coatings and bacterial mucus, TEP act as the glue for particle aggregation (Passow, 2002).

7.3.2 Fungal parasites

Another important microbial suppressor of diatoms are fungi. Fungal parasites and saprophytes of algae belong mainly to the order Chytridiales (Reynolds, 1984). Chytrids are free-swimming, uniflagellate zoospores that seek suitable hosts to grow on. They penetrate the host cell with a fine mycelial thread and draw nutrients back to the infective zoospores, which then enlarge into a spherical sporangium. When sporangium reaches maturity, the next generation of zoospores are released. Infection by fungi usually kills the host cell and the parasite population can rapidly reach 'epidemic' proportion (Kagami et al., 2007). Scholz et al. (2014) identified up to five different species of chytrids within several diatom taxa in marine benthic sediment samples. In rivers, chytrids were observed reducing the abundance of the dominant spring diatoms and infecting multiple species throughout the year (Maier and Peterson, 2017) and, in mesocosm experiments determined, higher water temperatures have been shown to increase chytrid activity and accelerate the termination of a phytoplankton spring bloom (Frenken et al.2016).

7.3.3 Aims and objectives

Microbial metabolism is directly regulated by water temperature. It has recently been established that bacteria and chytrids have a narrower thermal tolerance range than that of their host, providing the host with thermal refuges of very low or no infection (Ratkowsky et al., 1983; Kudoh & Tokahashi, 2004; Gsell et al., 2013). Additionally, phytoplankton succession can be triggered by low nutrient stress (Reynolds, 1984; Ljubesic et al., 2007; Wu et al., 2011).

The aim of this study was to quantify the effect of bacteria and chytrids on the Thames diatom-chlorophyte assemblage in the context of water temperature and nutrient availability. To achieve this aim, two objectives were defined. The first was to source representative diatom-chlorophyte assemblages in the Thames catchment, and maintain them in favourable temperature, light and nutrients conditions to reach high population abundances of algae, bacteria and chytrids. The second objective was to assess changes in diatom and chlorophyte abundances, and proportions of algal cells visibly affected by microbial presence through *in vitro* incubation of the algae-bacteria-chytrid community in two different temperature and nutrient conditions. Most visible deformations of algal intracellular content were determined using optical light and confocal

microscopes, whilst external damages to the frustules were assessed with a Scanning Electron Microscopy (SEM). The formation of transparent exopolymer particles (TEP) was statistically related to proportion of diatoms with bacteria attached. This experiment explores the behaviour and interaction of the river phytoplankton-microbial biome.

The main hypotheses tested were:

H1: Given optimum light conditions and nutrient concentrations (Si, N, P), diatom abundances significantly decline in water temperatures higher than 19°C; at the same time the proportion of diatom aggregates (clusters) and diatoms affected by bacteria and chytrids evidently increase, along with the population of chlorophytes.

H2: Low nutrient stress surge diatom population decline yet has a moderate effect on chlorophytes.

H3: Diatom clusters/aggregates and presence of transparent exopolymer particles (TEP) are positively and significantly correlated with the proportion of individual diatom cells with bacteria and chytrids attached.

7.4 Methodology

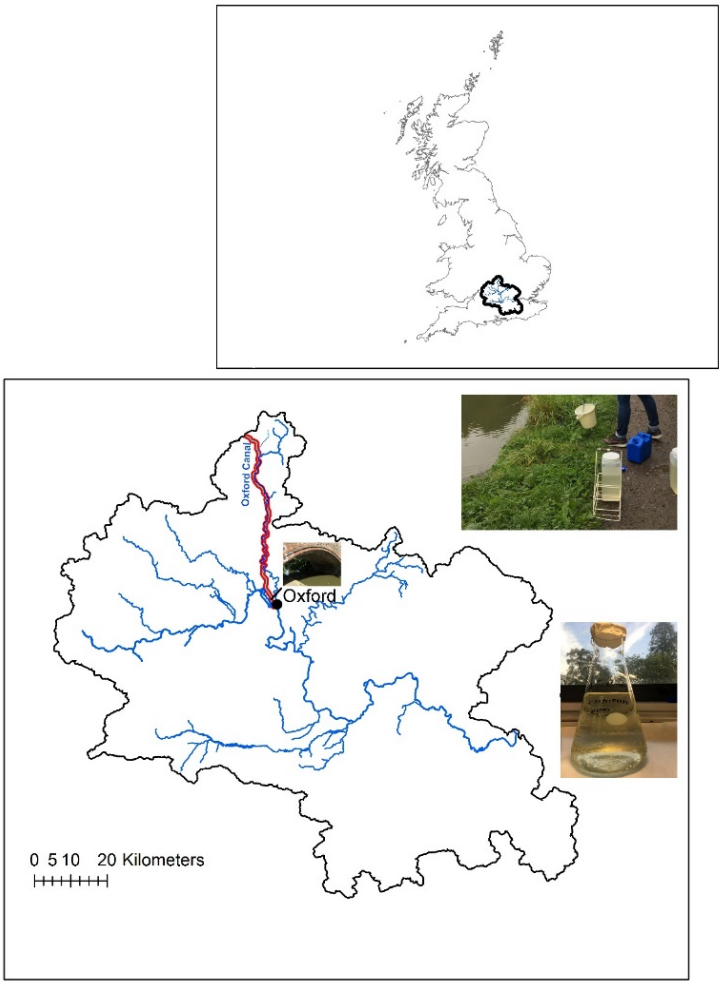


Figure 7-6 Thames catchment. Oxford Canal. Sampling site.
Latitude: 51.782785°; Longitude: -1.283488°)

7.4.1 Starter culture

The algal, bacterial and fungal culture was sourced from the Oxford Canal near its confluence with the River Thames. The Oxford Canal is a 126 km long narrow navigable canal connected to the Thames at Oxford. It is a long semi-lotic environment (Figure 7-6) which has previously been suggested as one of the principal contributors of plankton to the main Thames (5.5.6 Importance of off-channel habitats Page 112). A bulk sample was collected with a 10-litre bucket (prewashed and sterilised), filtered to remove zooplankton and large filamentous algae (Whatman qualitative filter paper, grade 1, 0.11 µm), and incubated under controlled temperature-light conditions to increase the algal-bacteria-fungal population densities to a sizable 'bloom' representative community. A freshwater diatom nutrient medium (Beakes et al., 1988) was added to the filtrate to provide optimum nutrient concentrations, and the composition

is listed in Appendix, Table 1. Filtrates were incubated inside glass flasks (2 l) in the temperature and light controlled environment for three weeks. The glass flasks were covered with filter-paper (to avoid airborne bacterial contamination). Water temperatures were maintained around 16.5°C (s. d. = 0.9) and light illuminance between 10 – 45 klx under a 12/12 h light-dark cycle (replicating the sampling day conditions). Both parameters were measured every 30 min using Hobo Loggers. Diatoms, green algae, parasitical chytrids and bacteria were identified and enumerated under the optical microscope every three days after adding the nutrient medium. The starter algal culture (total cell abundance $3*10^5$ cell ml^{-1}) was dominated by centric diatoms (80%), mixed with green algae, bacteria, amoebas, ciliates. Some centric diatoms (approx. 18%) had bacteria attached to their frustules, and around 5% were infected with chytrids.

Table 7-0-1 Species of centric diatoms and chlorophytes in the phytoplankton starter culture

Centric diatoms	Chlorophytes
Stephanodiscus hantzschii Grunow	*Actinastrum hantzschii* Lagerheim
Cyclotella menenhiniana Kützing	*Chlorella vulgaris* Beyerinck
Melosira varians C.Agardh	*Hindakia tetrachotoma* (Printz) C. Bock, Pröschold et Krienitz
Aulacoseira ambigua (Grunow)	*Keratococcus bicaudatus* (A. Braun ex Rabenhorst) J.B. Petersen
Skeletonema potamos (C.I. Weber) Hasle	*Willea rectangularis* (A. Braun) D.M. John, M.J. Wynne et P. Tsarenko
	Oocystis lacustris Chodat
	Ankistrodesmus falcatus (Corda) Ralfs
	Golenkinia radiata Chodat
	Tetraedron minimum (A. Braun) Hansgirg

7.4.2 Experiment design

To test the effect of changing water temperature on algal, bacteria and fungal communities, the starter culture aliquots (100 ml) were incubated for 48 hours with an added medium (2:1) at 15.5 and 20°C under a 12/12 h light – dark cycle. The light intensities changed between 18 and 39 klx over a 24-hour cycle. For the nutrient stress, the starter culture was incubated at 20°C with added deionised water (2:1) to dilute the nutrient concentrations in the medium. Four replicates were incubated for each treatment (Figure 7-7). Cell condition and signs of lysis in each algal culture were monitored by optical microscopy.

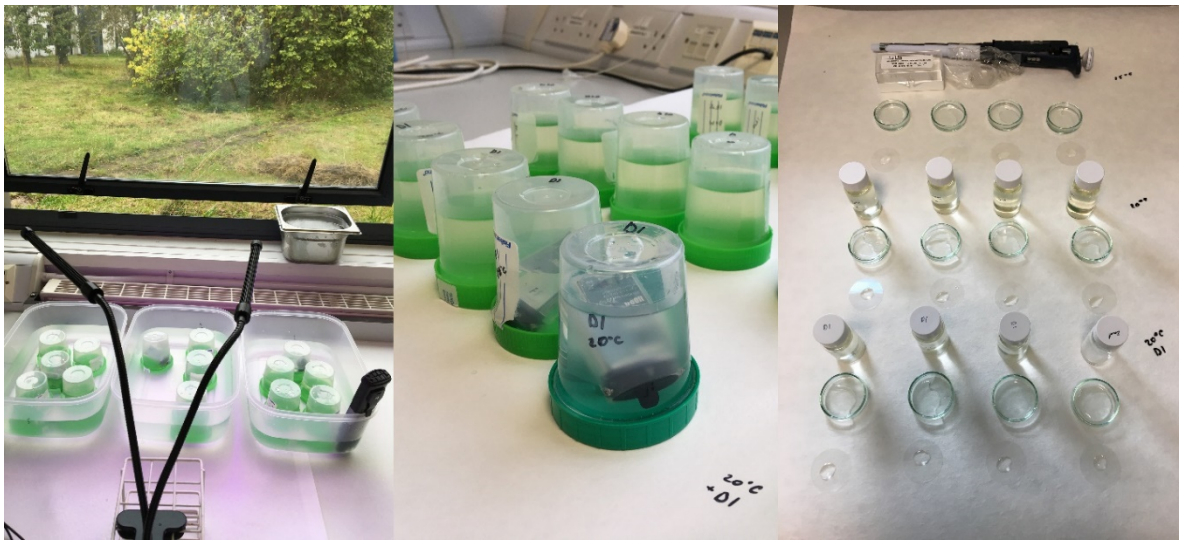


Figure 7-7 Experimental set up. To reach higher light intensities samples were placed by the window during the day with an extra light source. Water temperature was regulated by adding ice to the incubation containers (externally) and recorded every 30min (HOBO loggers inside sterile plastic vials)

Diatoms and chlorophytes were enumerated at the start of the experiment and after 24 hours of incubation. The proportions of clustered diatoms and diatoms with attached bacteria and chytrids were measured after 24 h and 48 h cycles. The experimental variables controlled, tested and measured in this study are listed in Table 7-2.

Table 7-2 List of dependent and independent variables measured and estimated in the study

Variables	Conditions/Units
Independent	
Water temperature	15.5; 20°C
Nutrients	with/without medium
Dependent	
Measured:	
Total number of algal cells	cell ml^{-1}
Total number of diatoms (all cells with 75% of chloroplasts)	cell ml^{-1}
Total number of green algae	cell ml^{-1}
Diatoms with bacteria attached	cell ml^{-1}
Diatoms with chytrids	cell ml^{-1}
Estimated:	
Change algal (diatoms, chlorophytes) abundance: (Abundance24h – Abundance0)/ Abundance0	%
Infected diatoms (bacteria, chytrids)/Total diatoms	%

7.4.3 Optical microscopy

Algae were enumerated and photographed at 10x, 20x, 40x and 100x magnification using an inverted microscope Axiovert 40CFL and a DSLR camera Canon 750D. Diatoms and chlorophytes were enumerated at 10x magnification, bacteria and fungi were studied at 40x and 100x magnifications. Samples were photographed at three microscope magnifications: 10x for algal enumeration; 20x, 40x for diatom aggregates, bacteria and chytrids, 100x for bacterial and fungal infections and signs of cell lysis.

For algal enumeration, samples were left to settle in the sterile petri dishes for 1h in the controlled-temperature environment. Petri dishes were scanned and photographed along their middle area (Figure 7-8). Photographs were processes with ImageJ software (Rueden et al., 2017). Total algal population densities were estimated using automatic cell counting technique, the proportion of diatoms and chlorophytes were estimated using 40x (magnification: x1000) (Grishagin, 2015).

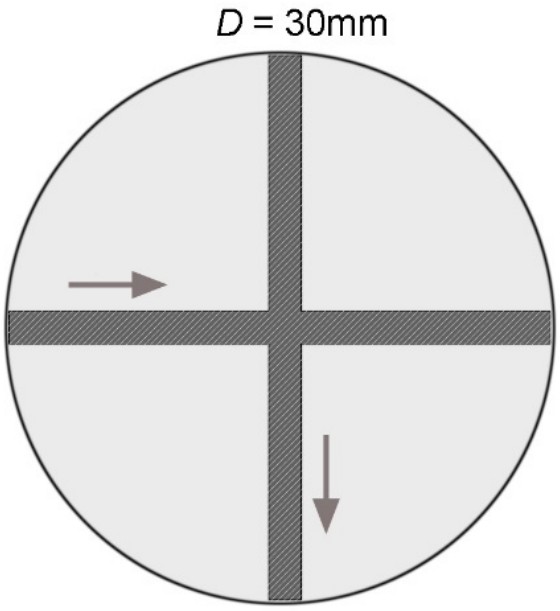


Figure 7-8

Figure 7-8 Optical micrography scanning of a Petri dish. Photographs were calibrated against the area of a petri dish and enumerations were extrapolated accordingly.

A subsample volume of 0.5 ml was placed on a cover slip for identification and enumeration of live and dead diatoms. The live diatoms were defined as those with visible cell contents (cell color – golden-brown, chloroplasts content minimum 80-90% visible from the surface area)

while the dead diatoms were those with empty or half empty frustules (Figure 7-9). A minimum of 500 live diatom cells were identified and counted (Wilson & Holmes, 1981; Gillett et al., 2009). Most of the slide area (75%) was photographed for further identification. All diatoms were identified to the lowest possible taxonomic level (mainly species) at 2500x magnification. The diatom and chlorophytes taxonomy followed predominantly (Cox, 1996; Krammer & Lange-Bertalot, 2000; John et al., 2011; Lange-Bertalot, H., Hofmann, G., Werum, M. & Cantonati, 2017).

Alfie-STORM super resolution (confocal) microscopy was used to visually assess the dead diatoms with chloroplast remaining. Live samples were placed in settling chambers and scanned after 30 minutes of settling.

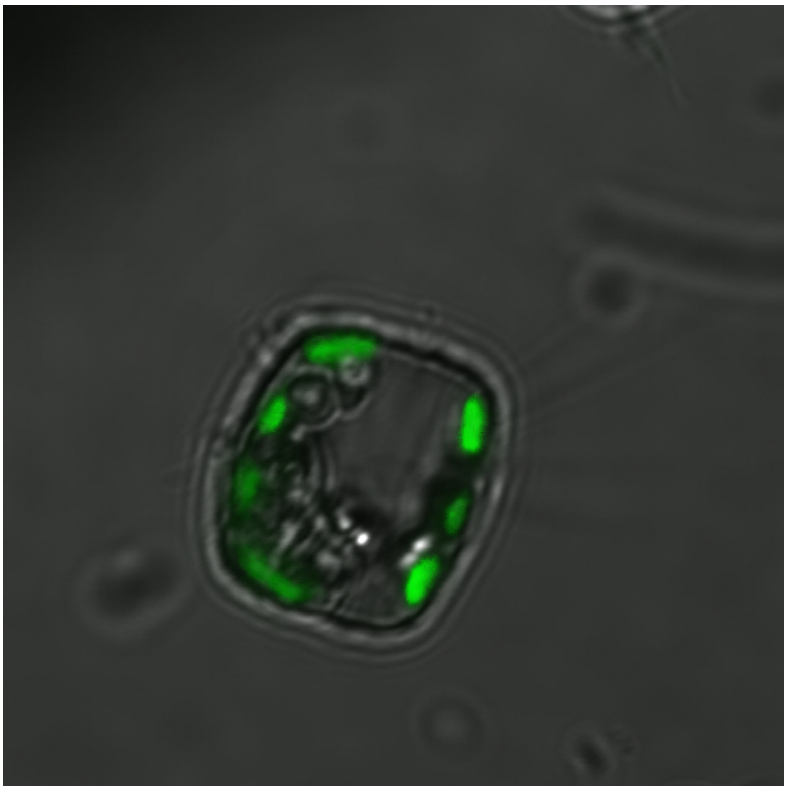


Figure 7-9 Confocal microscopy. Fluorescence of a dead diatom cell with a small number of chloroplasts remaining (Alfie-STORM super resolution microscopy)

7.4.4 Scanning electron microscopy

Diatom mounts were prepared by drying a small drop of the sample (0.5ml) on carbon coated metal stubs under room temperature conditions (18-20°C). In total, 12 stubs were prepared, and samples were taken from every treatment (random replicates). To capture naturally occurring diatom clusters and frustule raptures, chemical preservatives, diatom bleaching substances and

thermal preparation techniques were not included in this study (Krammer & Lange-Bertalot, 2000).Samples were studied under the Quanta FEG 600 Environmental Scanning Electron Microscope (SEM). Compositional analysis of specimens was obtained from X-rays produced by the electron-specimen interaction. This enabled detailed maps of elemental distribution within a selected area of the sample to be produced. Scanning electron microscopy and energy-dispersive X-ray spectroscopy (EDS) were used to determine the chemical composition of diatoms, their organic coating (EPS) and aggregates. The method is based on X-ray analysis of samples and follows a fundamental principal that each element has a unique atomic structure allowing a unique set of peaks on its electromagnetic emission spectrum. EDS can be used to determine which chemical elements are present in a sample and their relative abundance or weight (wt%).

7.4.5 Data analysis

Statistical analyses were performed in R programming environment (Wickham, 2016; R Core Team, 2017). Generalized linear models (GLM, R functions: *glm,* gaussian distribution) were used to estimate the significance of both continuous and categorical variables. Correlation plots were created with packages: PerformanceAnalytics (Peterson, 2015; Peterson & Carl, 2018) and ggplot 2 (Wickham, 2016). Data were tested for normality (Shapiro–Wilk test) and log transformation was done on data deviating from these assumptions (Appendix Figure 0-1). All statistically significant differences quoted are at $p \leq .05$ or less. Adobe Creative Cloud software was used to compose diagrams.

Dissimilarities in the chemical characterization of diatom clusters, TEP and EPS were explored using nonmetric multidimensional scaling (NMDS; Clarke, 1993). The data were converted to Bray-Curtis dissimilarity matrix prior to NDMS application. Significant ($p < .05$) chemical elements were plotted as vectors with function 'envfit'. These elements were: carbon (C), oxygen (O), silicon (Si), sodium (Na), chlorine (Cl), sulphur (S), calcium (Ca). Results and Discussion

7.5 Temperature effect

Diatom cells experienced thermal stress at both 15.5 and 20ºC, though at 20°C the diatom abundances declined significantly faster than at 15.5 ºC ($p = .01$ Table 7-3). Chlorophyte numbers increased with warming to 20 ºC (25%) and decreased with cooling to 15.5 ºC (25%) (Figure 7-10).

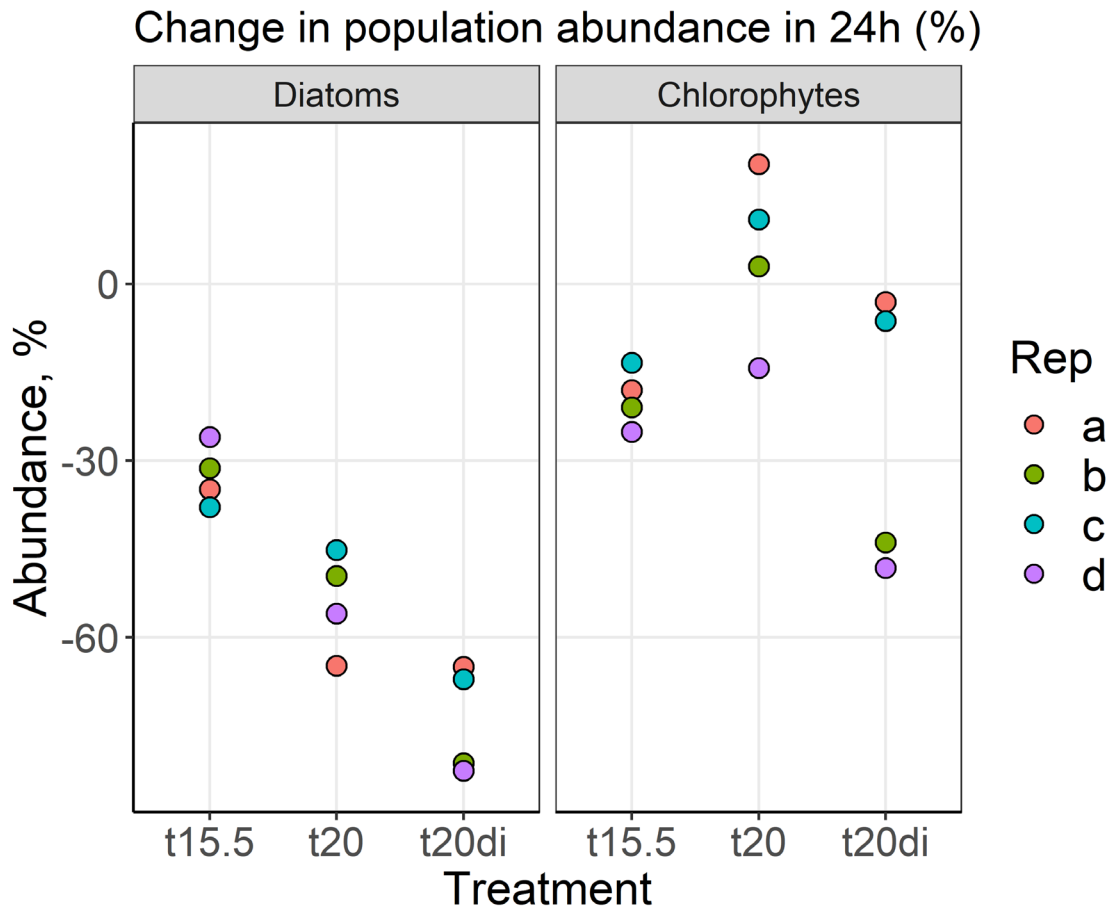


Figure 7-10 Change in population abundance of diatoms and chlorophytes in 24 h. Incubated at 15.5 and 20°C (in nutrient medium) and at 20°C with deionised water (Treatments: t15.5, t20 and t20di, 4 replicates).

Although thermal properties of the environment evidently influenced diatom and chlorophyte abundances, the period/time of incubation played a much greater role in diatom aggregates formation and spread of bacteria and chytrids (Table 7-3). The time of incubation allowed bacteria and chytrids to evidently increase their presence. Water temperatures had a negligible effect on chytrids.

Table 7-3. GLM results for diatom and chlorophyte growth in relation to temperature conditions, and for diatom aggregates, attaching bacteria, and chytrids in relation to temperature and incubation time. Significance t-value (t), degrees of freedom (df), significance (p), and Adjusted R-squared, model significance (p-value)

Dependant variable	Diatoms	Chlorophytes	Bacteria	Chytrids	Diatom clusters
Formula	~ Temperature		~ Temperature + Time		
Temperature 20 t(df) p	-4.31 (6) 0.01*	3.15 (6) 0.02*	-1.19 (13) 0.25	0.52 (13) 0.61	0.17 (13) 0.87
Time (Incubation period 48h) t(df) p	-	-	-5.97 (13) 4.64e-05*	0.17 (13) 0.86	-7.78 (13) 3.03e-06*
Adjusted R-squared	0.71	0.56	0.70	-0.13	0.8
p-value	0.01*	0.02*	0.001*	0.86	1.283e-05*
	Change in population abundance: (Abundance24h – Abundance0)/ Abundance0		The proportion in the sample (Infected diatoms/Total diatoms)		

The effect of water temperature on the diatom-chlorophyte assemblage confirms observations of temperature-related phytoplankton change in the River Thames and its tributaries made weekly from 2011-2015 (Appendix. Figure 2A) (Read et al., 2014; Bowes et al., 2016). Centric diatoms found in the Thames tend to grow better in thermal conditions ranging between 15 – 17 °C, with no dense populations recorded above 20 °C. Chlorophytes, on the other hand, peak at 17-18 °C and can sustain high abundances at 20-24 °C (Appendix. Figure 2B).

The inhibition of diatom population at higher (> 19 °C) water temperature may be due to high bacteria presence. The experimental data suggest that the proportions of diatoms clustered and infected by bacteria increased from less than 10% to more than 80% in 48 h when the water temperature was at 20 °C.

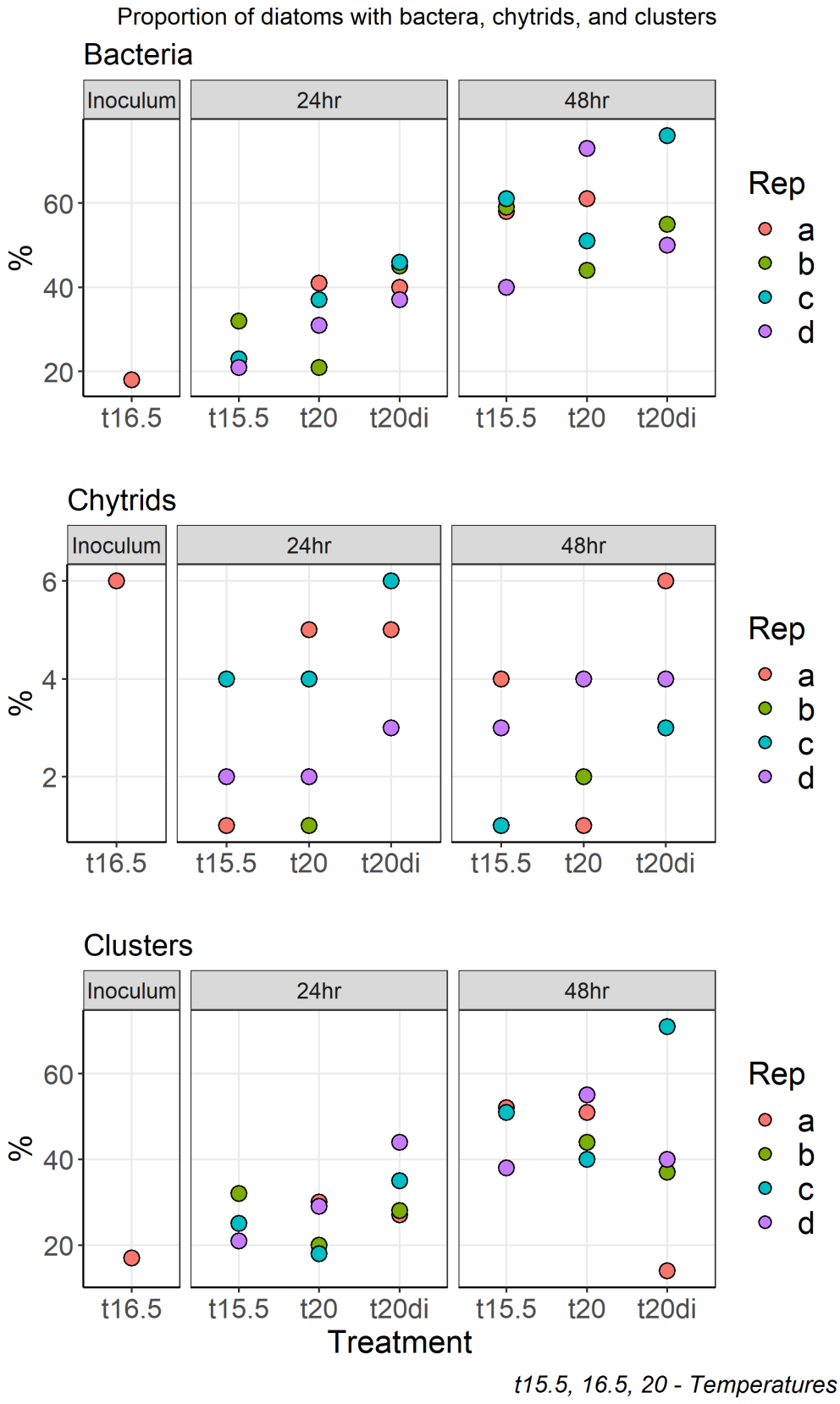


Figure 7-11 Proportions of diatom cells with bacteria or chytrids attached, and diatom clusters/aggregates at the start, 24 and 48 h after incubation at 15.5 and 20°C (in nutrient medium), and at 20°C with deionised water (Treatments: t15.5, t20 and t20di, 4 replicates)

7.6 Low nutrients

Under low nutrient conditions added to water temperature stress, diatom abundances rapidly declined, and at the same time, the proportion of cells with bacteria significantly increased, whilst parasitical chytrids remained similar to the starter culture. (Figure 7-10, 7-11). The nutrient deficiency stress had only a moderate positive effect on diatom clustering and the bacteria population, whereas the incubation period was a significant predictor for both the abundance of both diatom clustering and bacteria (Table 7-4. Figure 7-11).

High diatom-chlorophyte mortality related to nutrient stress is predictable and well explained in the literature (Reynolds, 1984; Hecky & Kilham, 1988). However, the slow algal response during the first 24 hours followed by a sharp increase in attaching bacteria, diatom aggregated and TEP in the next 24 hours, suggests that the period of stress exposure plays a much greater role in algal population dynamics than the environmental stress itself. Algal nutrient uptake is essential for cell metabolism, but it is possible that diatom intracellular reserves may help overcome nutrient deficiency for the 24 hours in this experiment.

Table 7-4 GLM results for diatom and chlorophyte growth in relation to nutrient presence, and for diatom aggregates, attaching bacteria, and chytrids in relation to the nutrient presence and incubation time. Significance (p), t-value and Adjusted R-squared

Dependant variable	Diatoms	Chlorophytes	Bacteria	Chytrids	Diatom clusters
Formula	*~ Nutrient stress*		*~ Nutrient stress + Time*		
Nutrient stress	3.2	2.16	1.63	2.4	.17
20	(6)	(6)	(13)	(13)	(13)
t(df) *p*	.02*	.07	.13	.03*	.87
Time	-	-	4.65	- 0.68	2.25
Incubation period			(13)	(13)	(13)
48h			.0004*	.50	.04
t(df) *p*					
Adjusted R-squared	.57	.34	.6	.22	.17
p-value	.02*	.07	.001*	.08	.12
	Change in population abundance: $(Abundance_{24h} - Abundance_0)/ Abundance_0$		Proportion in the sample (Infected diatoms/Total diatoms)		

This experiment was not focused on the exact chemical component that triggered chytrids, bacteria and diatom clustering, rather it was a pilot to see how long it may take before significant changes in diatom-bacteria-chytrid relationships are observed in an environment that is unfavourable to diatoms with high temperature and low nutrient conditions.

7.7 Diatom clusters/aggregates and transparent exopolymer particles

Since after 24 hours of incubation diatom abundance fell in every treatment, the differences in population numbers before and after incubation ($Abundance_0 - Abundance_{24h}$)/ $Abundance_0$ were defined as diatom mortalities. Diatom mortalities were positively correlated with the presence of bacteria ($t = 3.8$, $r = .77$, $p = .003$, $n = 10$), chytrids ($t = 2.2$, $r = .58$, $p = .05$, $n = 10$), and proportion of diatom aggregates/clusters ($t = 2$, $r(10) = .64$, $p = .07$). Diatom aggregate formation was positively correlated with bacteria presence ($t = 2$, $r = .54$, $p = .07$, $n = 10$) (Figure 7-12).

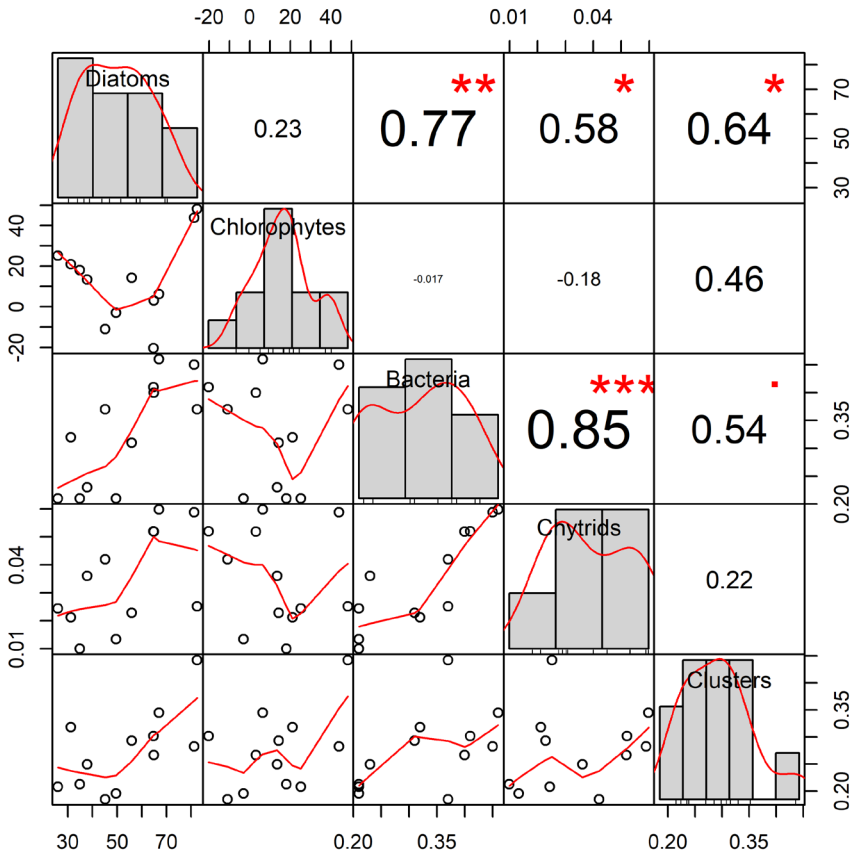


Figure 7-12 Relationships between diatom and chlorophyte mortalities and diatom with bacteria or chytrids and free-floating clusters/aggregates (Pearson's r; * - significance)

Variation in diatom daily mortality is both controlled temperature and nutrient environments and may be explained by the presence of diatoms with attached bacteria (Table 7-5). In contrast, the proportion of clustered diatoms appeared insignificant ($p > .05$) as diatom frustules rapidly deformed and disintegrated into shapeless amorphic flakes within each aggregate, making it hard to estimate the total number of cells forming these clusters (Figure 7-13A).

Table 7-5 Results of the GLM predicting diatom mortality from proportions of clustered diatoms and cells with bacteria attached. Significance (p), t-value and Adjusted R-squared

Explanatory variable	Diatom mortality ~ Bacteria + Clusters
Bacteria t(df) *p*	2.62 (9) 0.03
Clusters t(df) *p*	1.34 (9) 0.21
Adjusted R-squared	0.59
p-value	0.007

The SEM high-resolution images (Figure 7-13) showed diatom frustules coated with extracellular polymeric substances (EPS) and transparent exopolymer particles (TEP). These substances act as a 'glue' for particle aggregation. Diatoms produce EPS (polysaccharide-rich) to cope with the variable and challenging conditions since EPS play important roles in cell protection, adhesion, and ligand binding (Aslam et al., 2018). In response to the presence of phytoplankton, bacteria also produce EPS (polysaccharide-rich) to initiate attachment to algal cell walls (reviewed in Amin, Parker and Armbrust, 2012). Bloom associated bacterioplankton tend to transcribe more copies of genes predicted to increase cell surface adhesiveness through changes in bacterial signalling molecules related to aggregate formation and motility (Rinta-Kanto et al., 2012).

Both diatoms and diatom-attaching bacteria produce sticky organic substances supporting diatom aggregation. Upon exposure to sunlight, these substances tend to rapidly transform into discrete transparent exopolymer particles (TEP) (Shammi et al., 2017). TEP are free-drifting transparent particulate acidic polysaccharides in the form of organic microgels. As a highly surface-active material, TEP are also very sticky and highly foldable in physical structure. As gel-like particles, TEP can enhance the aggregation of solid non-sticky particles and provide surfaces for microbial colonisation. TEP are abundant in the ocean and are often colonised by bacteria (Passow, 2002).

Several strains of bacteria have been found to influence TEP production by attaching to the diatom and subsequently inducing diatom cell aggregation (Gärdes et al., 2011).

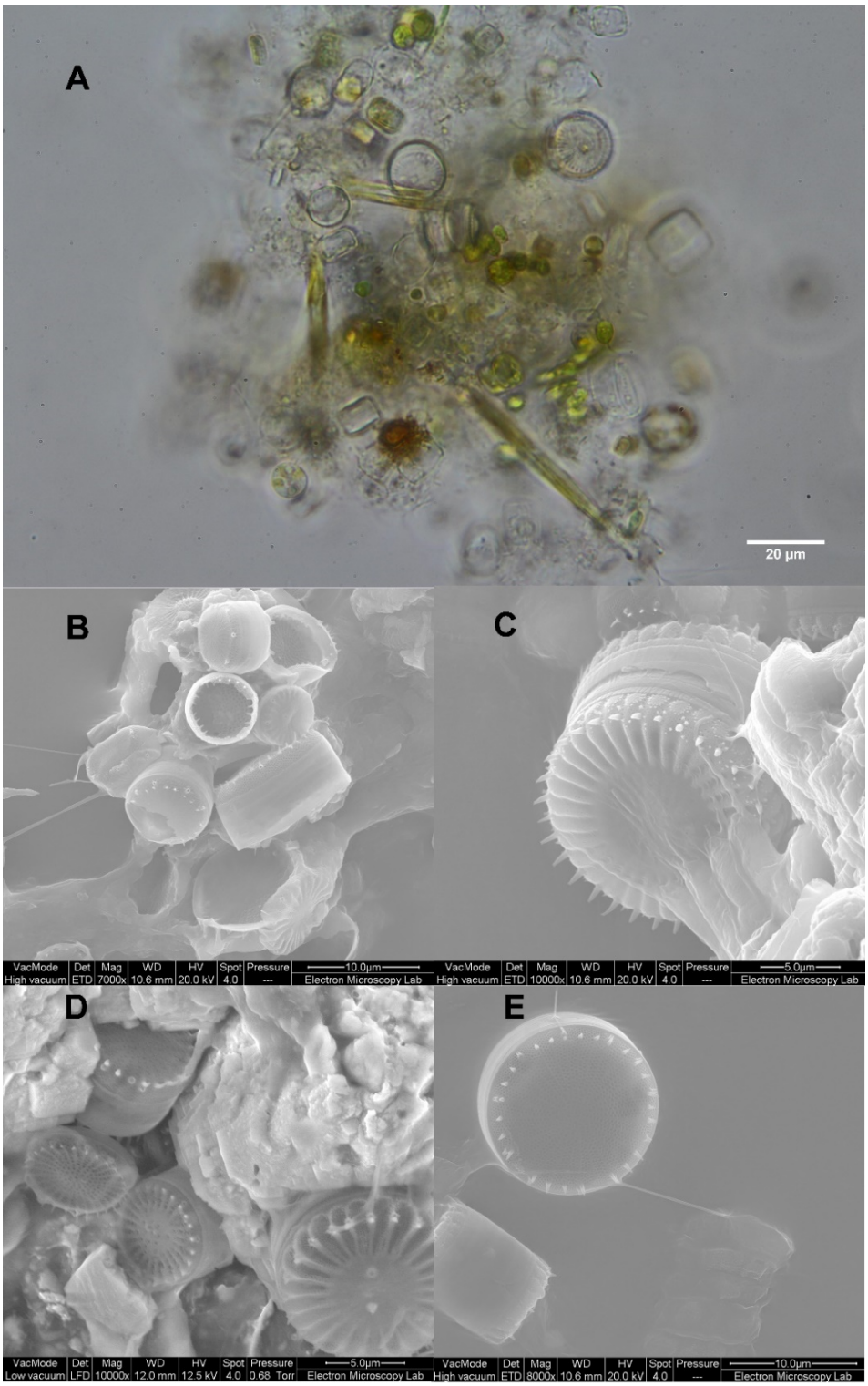
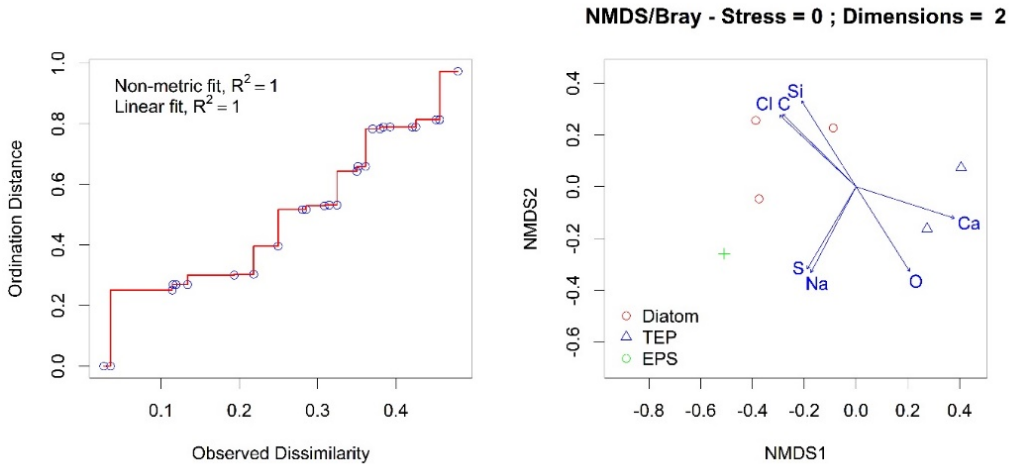


Figure 7-13 Optical and scanning electron micrography. A- Diatom clusters/aggregates, optical microscopy (x1000 magnification); B- Diatom aggregates (SEM); C- Diatom frustule attached to aggregates with TEP; D- Diatom frustules inside of an aggregate; E- Diatoms frustules and their sticky organic coating (EPS)

7.8 Chemical characterisation of diatom frustule aggregates, TEP and EPS

NMDS helped to define the main differences in the elementary composition of diatom frustules, their sticky organic coating and free-floating aggregates (Figure 7-14A). Diatoms have higher carbon and silicon content than free-floating sticky aggregates and TEP, which contain more calcium and oxygen (calcium carbonate, $CaCO_3$). Some aggregates, diatom frustules and their coating contained sulphur (0.7 – 2.8 %) (Figure 7-14B). Sodium chloride crystallised on the surface of a diatom frustule attaching to its surface coating (EPS).

A



B

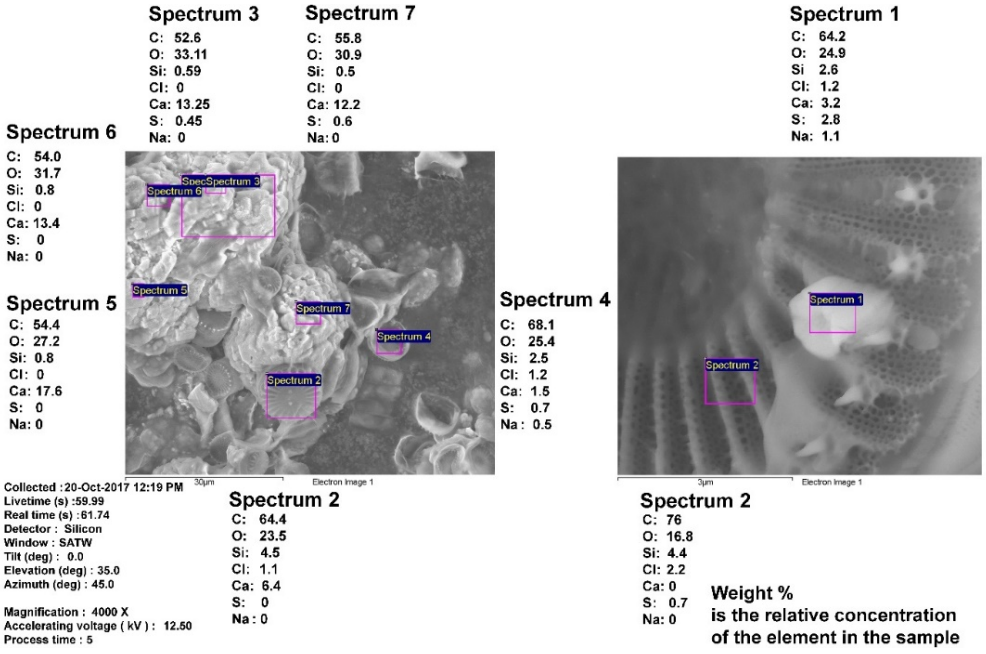


Figure 7-14 Chemical composition of diatom frustule aggregates, TEP and EPS. Scanning electron micrography and energy-dispersive X-ray spectroscopy (EDS)

Transparent exopolymer particles related aggregation stickiness can play a significant role in the sedimentation of centric diatom blooms (Engel, 2000). Thornton and Thake (1998) observed strong positive relationships between diatom aggregate concentrations and water temperature at 10, 15, 20 and 25°C. From X-ray elementary analysis, this study indicated the importance of sulphated polysaccharide diatom coating in binding aggregates together. Gärdes et al. (2011) in the experimental study showed that axenic centric diatom cultures did not form aggregates whereas diatom cultures inoculated with either diatom-attaching or free-living bacteria rapidly aggregated and settled down. Positive correlation between diatom aggregates and diatom-attaching bacteria suggests that aggregate formation is strongly linked to bacteria presence.

The experimental evidence also raised the possibility that, when light intensities are favourable, diatoms bloom may deplete essential nutrients (silicon and phosphates), release a sticky organic coating (EPS), which in turn triggers bacteria to rapidly aggregate and recycle sticky diatom frustules. Taking this a step further, after bloom cessations the remaining it is plausible that healthy cells do not provide a sufficient source to start an immediately growing population unless microbial suppressors are temporarily inhibited in their activities. Since water temperature directly controls microbial metabolism, the pathogens preference to higher temperatures may provide diatoms with a cold thermal refuge (Ratkowsky et al., 1983; Kudoh and Tokahashi, 1990). Calcium carbonate in river water could potentially increase aggregate formation. The bacteria are an important subject for future research into the genetic and molecular targets of elements involved in diatom-bacterium interactions. These are ideas that require further exploration in the next phase of work.

7.9 Conclusion

This study concludes that both diatom cell metabolism and the presence of bacteria may play a key role in rapid bloom termination (48h), which are frequently observed in the River Thames. In laboratory experiments, diatom abundance was observed to decrease rapidly in warmer waters under nutrient stress in conditions similar to those found during blooms in the Thames, and the proportion of cells affected by bacteria in the experiment were observed to increase significantly. In these experiments, chytrids displayed less significant effect on diatom mortality. The experimental results indicate a complex interplay between the physical and biological environments in terms of nutrient availability and bacteria-diatom interactions. Further investigation is needed to unpick these complex relationships.

Appendix

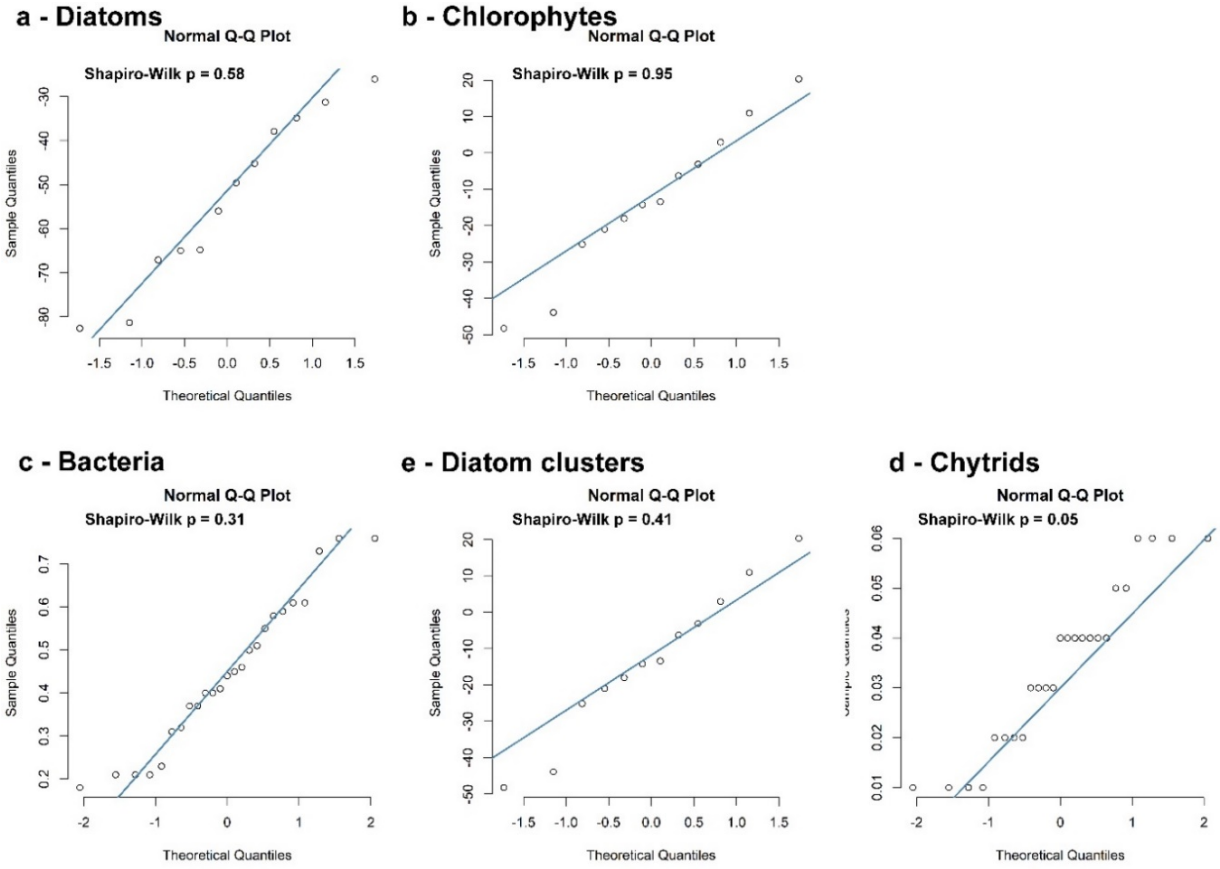
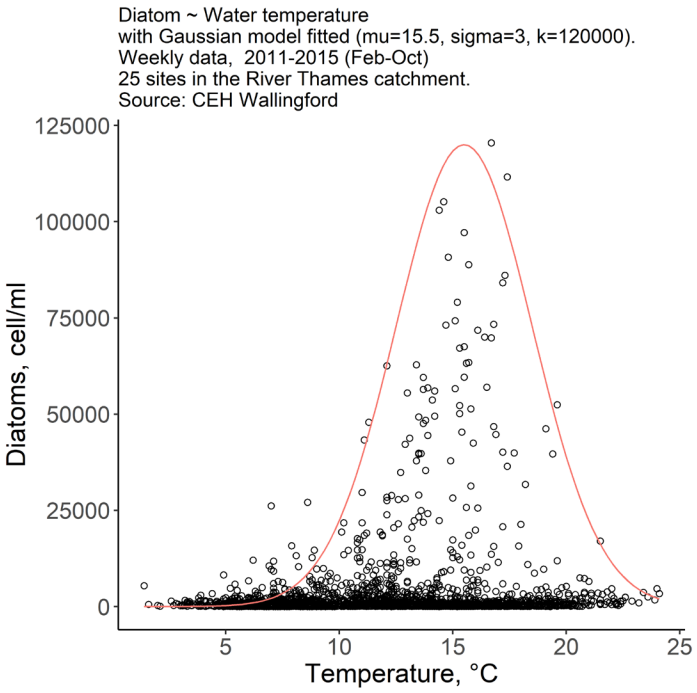


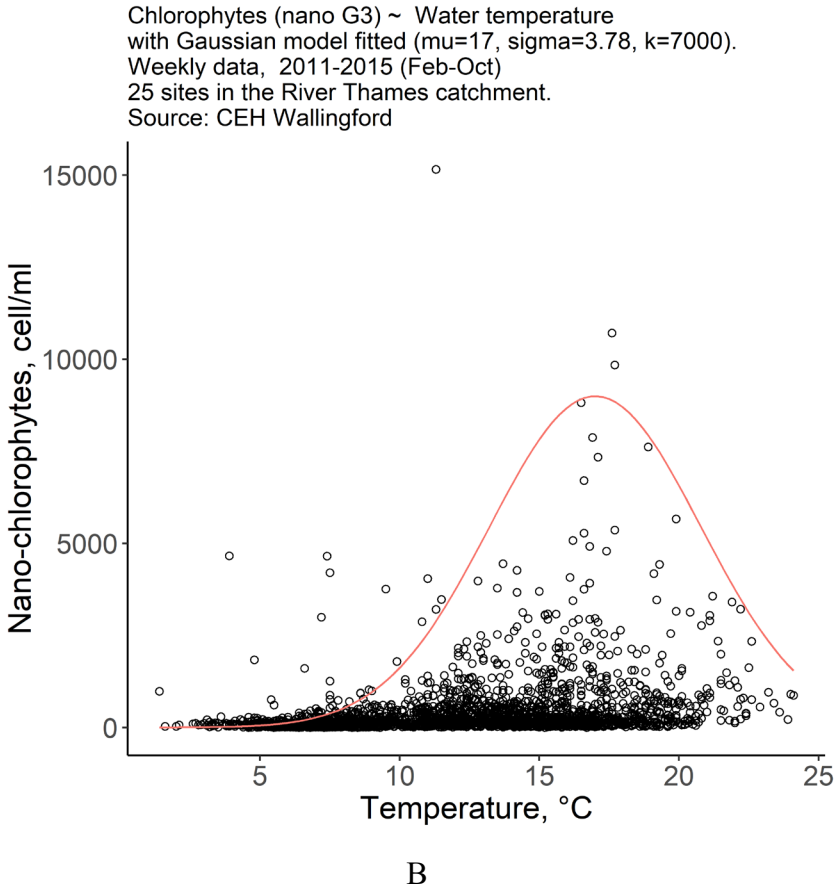
Figure 7-15. Results of Shapiro-Wilks normality test and Q-Q plots for diatom and chlorophyte mortality (%), proportions of diatoms with bacteria, chytrids, and aggregates in samples.

Table 7-6 Diatom nutrient medium

Stocks	Concentration mg l^{-1}
$Ca(NO_3)_2{*}4H_2O$	0.02
KH_2PO_4	0.0124
$MgSO_4{*}7H_2O$	0.025
$NaHCO_3$	0.0159
EDTAFeNa	0.00225
$EDTANa_2$	0.00225
H_3BO_3	0.00248
$MnCl_2{*}4H_2O$	0.00139
$(NH_4)_6Mo_7O_{24}{*}4H_2O$	0.001
Cyanocobalamin	0.00004
Thiamine HCl	0.00004
Biotin	0.00004
$Na_2SiO_3{*}9H_2O$ (Sigma S4392)	0.057
HCl	



A



B

Figure 7-16 Diatom – A and Chlorophyte – B relationships with water temperature in the River Thames. Data collected weekly from 22 sites in the Thames catchment from 2013-2016. Source: Centre for Ecology and Hydrology, Wallingford, Thames Initiative Project

Chapter 8 Wider Discussion and Conclusions

The River Thames is a large important river system and a major freshwater resource in the south-east of UK, yet less than 10% of surface waters in the catchment meet standards of good ecological status (Environment Agency, 2016). This situation is forecast to worsen due to climate change and a growing human population.

Recent studies of climate change impact on water quality in the UK predicted higher water temperatures, intensive rainfall events, and lower river flows in summer (reviewed in Hutchins et al. (2016)). The lower flow velocities will result in longer residence time, which combined with higher water temperature will trigger more severe eutrophication episodes and increase the likelihood of toxic cyanobacteria blooms. With this in mind, river basin managers are seeking innovative solutions to meet water demand and at the same time maintain ecological status. Achieving this requires a complete understanding of how river water quality interacts with the aquatic ecosystem (Hutchins & Bowes, 2018)

This study focused on the biological suppressors of algal growth: mainly zooplankton, but also bacteria and parasitical fungi. These are naturally occurring controls on the phytoplankton community, yet in rivers, the study of these communities and their interactions with phytoplankton are relatively limited in number and scope compared with lentic systems. This final chapter provides an overall summary of findings and considers implications of these in the context of wider scientific literature, policy and practice.

8.1 Zooplankton survey

To determine the current zooplankton community, the River Thames was surveyed from April to September 2015 at weekly intervals, a period representative of the long-term spring-summer low flow conditions. The zooplankton survey was realted to phytoplankton flow cytometry and the parallel water quality monitoring. The main results of this study are summarised in Chapter 4 with further consideration in Chapter 5.

What are the main groups of organisms that compose the zooplankton community in the main River Thames? How do they compare with other temperate lowland rivers?

Rotifers formed more than 90% of the total metazoan (animal only) plankton along the river, with more than 40 species were recorded (Chapter 4 Section 4.5.3 Page 75). The most abundant species were: *Keratella cochlearis, Keratella quadrata, Synchaeta oblonga, Synchaeta pectinata, Polyarthra dolichoptera, Brachionus calyciflorus, Brachionus angularis, Euchlanis dilatata, Notholca squamula,* and *Notholca squamula.* In the headwaters only, rotifers were generally

small in body length, many belonging to genera: *Lepadella, Cephalodella* and *Colurella.* Microcrustaceans were found in small numbers (maximum of 125 ind l^{-1}), over and 75-90% of them were Cyclopoid juvenile and larval forms. Cladocera were rare. *Bosmina longirostris* were found in the headwaters, at Wallingford and Goring. A small number of *Chydorus sphaericus* appeared at Wallingford, Goring and Runnymede. The abundance of predatory zooplankton was low overall; rotifers of the genus *Asplanchna* were present only in June in the lower Thames, with peaks lower than 3 ind l^{-1}; and copepodid and adults of cyclopoid copepods had negligible abundance. All observed organisms were typical for the temperate lowland rivers (Chapter 2. Section 2.6 Page 15).

Did the zooplankton community diversity and abundance increase with distance from the source, chlorophyll-a concentration, and water temperature? How did the zooplankton community respond to the changes in river flow – the key factor regulating phytoplankton dynamics?

Zooplankton communities in the middle and lower Thames were more abundant and diverse than in the upper Thames. This corresponds well with the increase in travel distances, and chlorophyll-*a* concentrations, while the effect of water temperature was less evident, as demonstrated by the CCA analysis, (Chapter 4. Section 4.5.4 Figure 4-7 Page 79) since temperatures in the main river vary only by 1°C. However, water temperature plays an important role in small-bodied animals' growth, and reproduction and high zooplankton abundances and diversity were observed in the Thames following a seasonal increase in temperature (up to 16-20°C) providing further evidence for this wider observation (Chapter 2. Section 2.7 - Rotifera. Page 19). Additionally, water temperature fluctuations corresponded with changes in flow rates due to rain events, which are the main factors controlling phytoplankton dynamics in the Thames (Bowes et al., 2016).

In the Thames, the zooplankton formed sizeable communities during the summer months (Chapter 4. Section 4.5.3 - Figure 4-5 Page75) when the water flow was close to its annual minimum, allowing organisms time to grow, reproduce and interact. Floods trigger high zooplankton mortality and removal as evidenced by a decline in rotifer abundances in the Thames at Wallingford, Goring and Runnymede in late June 2015, meaning that sizeable plankton communities can only form during long low-flow conditions (Chapter 4 Section 4.5.3. Figure 4-6 Page 77, 82). Even minor increases in flow had a negative effect on the zooplankton community, particularly during the active population development 'phase', with some taxa

temporarily disappearing (Figure 4-6, Page 77). However, after prolonged late June rain events rotifer numbers were able to rapidly recover, indicating strong population self-regulating mechanisms. These self-regulating mechanisms may include switching to sexual reproduction mode (for some species), altering hatching success, sizes of adults and eggs, and adapting the lifespan (Chapter 2. Section 2.6 - Rotifera Page 19, 30).

Can the zooplankton community development be predicted primarily by physical, spatial characteristics, and phytoplankton community composition and abundance?

The observed rotifer community was dominated by small filter-feeders which consumed centric diatoms, unicellular green algae and bacteria. The zooplankton composition are strongly associated with phytoplankton composition (Figure 4-4 Figure 4-7 a,b. Page 74, 79). For instance, the first important zooplankton peaks were associated with a spring, and then early summer, diatom bloom. The third abundance peak was related to a mid-summer post-diatom phase, when small chlorophytes, cryptophytes, dinoflagellates and cyanobacteria were more abundant (Figure 4-5; 4-8 a,b. Page 75). Changing food quality has a substantial effect on the river zooplankton population, since rotifers metabolism, growth and reproduction rely on nutritional food resources (Chapter 2. Section 2.7.7 Page 29), such as diatoms and cryptophytes, which are rich in highly unsaturated fatty acids. (Chapter 6 Section 6.4.3 Page 124).

In the Thames headwaters, phytoplankton communities were formed of benthic/periphytic diatoms, large green algae, pico-plankton and a small number of cyanobacteria. As a result, rotifers there were also predominantly benthic/periphytic (Figure 4-4; 4-6, Page 75, 79), but, at the same time, small populations of *Collurella, Euchlanis dilatate*, and *Trichocerca pusilla* were observed. Since *Collurella, Euchlanis,* and *Trichocerca* (Pontin, 1978; De Manuel, 2000; Alekseev, 2010) are known to prefer standing eutrophic environments, their presence in the upper Thames reaches and headwaters implies that the river is well connected with off-channel habitats. In the middle Thames at Wallingford (around 134 km from the source), however, both phytoplankton and zooplankton showed rapid spatial shifts to higher abundances, and changes in community structure characterised by true-planktonic dominance. These shifts cannot be explained by the inner-channel plankton growth rates only (Figure 4-8 Page 81) perhaps emphasizing again the importance of off-channel retentive zones in the catchment. Therefore, it is plausible to suggest that the zooplankton community structure and seasonal dynamics may only be predicted when main spatial sources of the zooplankton-phytoplankton inoculum are defined. Similar to all large lowland rivers, the Thames should be considered as a dynamic

network of the main stem, tributaries, and retentive zones in the main river channel and its catchment.

Do tributaries and Farmoor Reservoir significantly affect zooplankton community composition and abundance in the Thames channel?

Evidence was found that planktonic organisms in the main river stem may originate in certain tributaries of the Thames, especially those connected to canals, and therefore the mixing of waters from different tributaries may be the key control on phytoplankton and consequently on zooplankton community composition rather than the site-specific flow or water quality conditions. The results of this study also reinforce the need to maintain a river-floodplain connection to protect biodiversity and point to the possibility that management measures targeted in specific tributaries may reduce algal blooms in the main channel.

The zooplankton communities dissimilarity analysis (NMDS) from six major tributaries (the Evenlode, Cherwell, Thame, Pang, Kennet, and Cut) and Farmoor Reservoir displayed close zooplankton relations between the middle-lower Thames and long eutrophic tributaries connected to canals (Figure 5-7 Page 108). These results imply that the Cherwell, Evenlode, and Kennet may supply the zooplankton communities to the Thames main stem, enhancing its biota (Figure 5-3 Page 98). The Cherwell, Evenlode, and Kennet are 'zooplankton-abundant' productive tributaries with long plankton travel distances (60-130 km) and a relatively high mean annual discharge, compare other tributaries (the Pang, and the Cut) which have shorter travel distances and residence times for plankton growth (Chapter 5.Figure 5-1 Page 93).

Zooplankton-rich tributaries and the middle-lower Thames were populated by true planktonic rotifers (Section 5.5.1 Page 96 and Figure 5-8 Page 111) such as *Keratella cochlearis, Polyarthra dolichoptera, Synchaeta oblonga* and *Brachionus calyciflorus*. Consistent with the zooplankton communities, the phytoplankton were also composed mainly of true planktonic algae (a mixture of small centric diatoms, nano- and pico- chlorophytes, cryptophytes, and planktonic cyanobacteria). Since the zooplankton and phytoplankton-rich tributaries are connected to canals, it is possible to propose that both phyto- and zooplankton originated from the water column and the bottom sediment of these large semi-lotic retentive zones (Figure 5-1 Page 93). Off-channel environments and connectivity with the floodplain provide a large part of the zooplankton inoculum to the Illinois River (Wahl et al., 2008) and the Waikato River (Górski et al., 2013).

Zooplankton-rich tributaries enhance the biological productivity of the main river primarily by introducing actively reproducing individuals, algal resting spores and zooplankton eggs accelerating plankton community growth within some distance downstream from confluence zones (reviewed in Chapter 5 Section 5.5.5). In contrast, the Pang, Cut, and Thame can dilute phytoplankton populations in the Thames

Farmoor Reservoir exhibited no influence on either phyto- or zooplankton in the Thames. Zooplankton communities in Farmoor Reservoir were significantly different from the Thames (Figure 5-7 Page 108). Microcrustaceans accounted for 40-90% of the total abundance and included cladocerans (*Daphnia* and *Ceriodaphnia*) that are truly limnetic. The proportion of adults in microcrustacean communities was relatively high, at 10-30%. None of these cladocerans or predatory rotifers were found in the Thames at Wallingford (Figure 5-3). Cyanobacteria from Farmoor had no notable effect on the Thames phytoplankton community downstream (Figure 5-5 Page 103).

8.2 Zooplankton grazing on phytoplankton

Does zooplankton grazing pressure exert significant loss rates of phytoplankton species?
Metazoan (zooplankton) grazing was found to influence algal composition and abundance in the Thames, but only to a small degree. Grazing is a seasonal effect, and it is spatially varied. Evident, but marginal, grazing pressure occurred when environmental conditions were favourable for zooplankton growth, that is when the flow is low, water temperature is relatively high (16-20°C), and nutrient-enriched grazable algae (mainly small centric diatoms) are available in abundance. Since rotifers have a relatively short lifespan (around ten days from Section 2.7 Page 19) and are free of dependence on photoperiods and nutrients, when flow and temperature are favourable, their populations rapidly expand in the presence of nutrient-enriched digestible food such as small centric diatoms and cryptophytes (Section 6.4.3. Page 124). These zooplankton community patterns were previously observed in the Thames during spring-summer phytoplankton blooms (Figure 4-5 Section 4.5.2 Page 74 and Section 4.5.4). As a result, populations of rotifers that tend to develop in large lowland rivers (Chapter 4 Section 4.2 Page 65) may exert some grazing pressure on phytoplankton.

In the Thames, the zooplankton 'grazing' mainly occurs in the middle and lower reaches where both phyto- and zooplankton establish dense populations (Figure 6-3 and Figure 6-4 Page 119). Microcosm experiments demonstrated that during summer months, naturally occurring river

rotifer populations (Section 6.4.2 Figure 6-4 Page 130) were able to remove up to a quarter of diatom and unicellular chlorophyte cells in three days, and more than half of *Synechococcus*-like and PE-rich (phycoerythrin-rich) cyanobacteria (Section 6.4.3 Figure 6-5 Page 132). However, as microcosm experiments established, with the overall grazing effect, growth rates of diatoms, chlorophytes, cryptophytes and most cyanobacteria remained positive even when rotifer populations were most abundant (for instance at lower Thames during summer months) (Figure 6-6 Page 133).

Diatoms, chlorophytes, cryptophytes and cyanobacteria (Section 6.4.3 Figure 6 5). were moderately reduced in numbers (up to 25%) but this effect was spatially and seasonally varied. Cyanobacteria abundances were reduced in the Coln and the Thames headwaters (up to 60% in April and 200% in June), but with cyanobacteria, it is not entirely possible to rule out the strong adverse incubation effects.

In the Thames, similar to other freshwater ecosystems, the zooplankton abundances tend to be low when phytoplankton communities are composed of pico-chlorophytes and cyanobacteria. More than 250000 cell ml^{-1} of nano/pico chlorophytes were unable to support rotifer populations, yet less than 10000 cell ml^{-1} fuelled rapid rotifer population growth (Chapter 4 Figure 4-5).

What is the importance of zooplankton grazing in comparison with meteorological, hydrological, chemical and other biological regulators?

The zooplankton grazing overall is a less significant predictor of phytoplankton growth rates than water temperature, maximum light illuminance and concentration of nutrients (P, N, Si) (Section 6.4.4 Page 124). During low phytoplankton biomass phases in the Thames, grazing may be considered as an insignificant process. Microcosm experiments showed evidence of the small zooplankton communities promoting diatom, chlorophyte, cryptophytes, and cyanobacteria growth (Figure 6-6 Page 133). These results agree with wider literature emphasising that small-bodied grazers can support algal growth through rapid recycling of essential nutrients (Literature review Section 2.7 Page 19).

Laboratory experiments described in Appendix 0 demonstrated that during an intensive phytoplankton bloom dominated by centric diatoms, even relatively high numbers (up to 2000 ind l^{-1}) of true-planktonic rotifer grazers (Table 9-4) cannot change algal population growth to negative figures. These findings agree with those from microcosm experiments (Figure 6-6). Daily growth rates of phytoplankton (Equation 6-1) Daily falls in chlorophyll-*a* concentrations

were observed only when phytoplankton starter culture (sourced directly from the River Thames) showed signs of diatom aggregation and high bacteria and parasitical chytrid presence (Figure 7-5 Page 146). The laboratory temperature/grazing experiments confirmed the results of microcosm experiments in which grazing was a less significant predictor of chlorophyll-*a* dynamics than water temperatures (Section 6.4.4 Page 124). However, both grazing and water temperature were less important than '***water age***' when after a week of blooming in the river, diatoms started aggregating surrounded by bacteria and parasitical chytrids (Section 9.5 Page 194).

8.3 Bacteria and parasitical chytrids effect on diatoms in the Thames

The outcome of experiments with naturally occurring phytoplankton-bacteria-chytrid communities signified that in less than 48 h of incubation the numbers of living diatoms can evidently fall (Figure 7-10) through formation if sticky aggregates populated by bacteria (Figure 7-13). Diatom clustering (aggregation) was positively and significantly correlated with the presence of diatom-attaching bacteria and had no strong relationships with parasitical chytrids Figure 7-12).

These experiments also revealed that under thermal stress, bacteria can rapidly explore the diatom population and increase presence from 10% to 80% after 48 hours of incubation (Figure 7-11) in both colder or warmer waters. Adding low nutrient conditions to unfavourable temperatures (20°C) decreased diatom abundances even faster.

Parasitical chytrids, unlike bacteria, did not increase their presence under thermal stress. These observations agree with Gsell et al. (2013), suggesting that the duration and intensity of chytrid parasite pressure on host populations are likely to be affected by the thermal properties of the environment. Adding low nutrient stress to high water temperatures had no notable negative impact on chytrids and might even have a small positive effect.

It is important to emphasise that the algal population responded to nutrient stress only after 48 hours of incubation which was evident from the aggregation processes (Table 7-4 Page 145). The slow phytoplankton response during the first 24 hours followed by a sharp increase in attaching bacteria, diatom aggregated in the next 24 hours, indicated that the period of stress exposure plays a much more significant role in algal population dynamics than the environmental stress itself. Although algal nutrient uptake is essential for cell metabolism, it is possible that diatom intracellular reserves help to overcome nutrient deficiency for up to 24 hours.

Chlorophytes exhibited positive growth under higher water temperatures (20°C) and negative at 15.5 ° (Figure 7-10). Under the optical microscope, their cells appeared to be unaffected by bacteria or fungal pathogens (Figure 7-13). The low nutrient stress caused chlorophyte abundances to fall even under favourable temperature conditions. These findings confirm water temperature optimums for chlorophytes observed during Thames weekly phytoplankton monitoring (Appendix. Figure 1).

Both optical and scanning electron microscopy (Figure 7-13) showed that diatom cells were glued to each other with sticky substances. It has previously been established that when conditions become unfavourable (changing temperature, low nutrient concentrations) centric diatoms produce extracellular (sticky polysaccharide-rich) polymeric substances to protect their cells from external damage (Aslam et al., 2018). However, at the same type, bacteria also produce extracellular polymeric substances (EPS) to initiate attachment to algal cell walls (reviewed in Amin, Parker and Armbrust, 2012). It has been established that bloom associated bacterioplankton tend to transcribe more copies of genes predicted to increase cell surface adhesiveness through changes in bacterial signalling molecules related to aggregate formation and motility (Rinta-Kanto et al., 2012). Under light exposure, these substances convert to free-drifting transparent exopolymer particles (TEP). As a highly surface-active material, TEP are also very sticky and highly foldable in physical structure. Several strains of bacteria have been found to influence TEP production by attaching to the diatom and subsequently inducing diatom cell aggregation (Gärdes et al., 2011).

The main overall conclusion from the phytoplankton-bacteria-chytrids laboratory experiments is that in response to changing the water temperature and nutrient concentrations centric diatom blooms can be suppressed in a short period (less than a week) through bacteria interactions with diatom cells. Diatoms tend to release a sticky organic coating to protect their cells from external stress, at the same time, bacteria release sticky organic substances and actively support diatom aggregation. These aggregation processes accelerate within 48 hours, actively suppressing the diatom population. In this study chytrid parasites showed only a small effect on the diatoms, but this could be explained by the short residence time in experimental setup. Diatom bloom associated bacteria showed no adverse effect on chlorophytes, as a result, when the water temperature was optimal (19-20°C) for chlorophytes, they were succeeding in the phytoplankton community. At the same time, diatoms were deteriorating, and nutrients were being recycled back to the water column. Diatom cell metabolism and bloom associated bacteria can play a key role in

phytoplankton community succession, but these relationships require further investigation. (Section 7.6).

8.4 Further implications

Zooplankton grazing in rivers is important but will not terminate a diatom bloom

This study established that phytoplankton community composition and abundance could be influenced by planktonic grazers and this is generally true in other temperate rivers (Section 6.4 Page 119) However, even relatively high numbers of river "*generalist*" rotifers (Section 2.6 Table 2-3 Page 9) are insufficient to rapidly terminate phytoplankton bloom (Section 9.5 Page 193).

Zooplankton survey data and available literature confirmed that similar to other lowland rivers; the Thames phytoplankton seasonal patterns are initiated by a spring diatom bloom, which in turn, fuels a rapid zooplankton population increase with a lag of 7-14 days, the zooplankton then undergo a crash (Chapter 4 Figure 4-5). In the Thames, as in other temperate lowland river systems, the zooplankton are represented by small rotifers. While some rotifers, such as *Brachionus* spp. Feed on centric diatoms; most are size limited to consume bacteria and small chlorophytes growing on a deteriorating diatom bloom (Figure 6-4 Page 130; Table 2-7 Page 21; Table 2-8 Page 25). Grazers may be able to consume up to a quarter of the diatom population over three days, but this pressure is not enough to terminate a large bloom, for instance, the bloom observed in the middle-lower Thames in August 2016 (Figure 6-6 Page 133).

After a diatom bloom, phytoplankton community is dominated mainly by green algae and cyanobacteria and, as in other rivers, these are often characterised by low phytoplankton biomass and metazoan abundance. In the Thames, there are also dinoflagellates, they appear in spring and summer months during and after diatom blooms (Moorhouse et al., 2018). These are mixotrophs and can consume bacteria and pico-algae (Section 6.4, Page 119).

Implications for mathematical modelling

The zooplankton grazing may be introduced to complex mathematical models predicting phytoplankton dynamics in large lowland rivers. However, this study shows that the overall

impact of the zooplankton on phytoplankton diversity and abundance is marginal, spatial and seasonal (Chapter 6). Defining correct phytoplankton relationships with other factors such as river flow, water temperature, chemical composition, and light are more important and should be prioritised. In fact, the phytoplankton community composition itself should be prioritised over 'zooplankton grazing', as weekly monitoring data showed, the environmental optimums (temperature for instance) are evidently varied between phytoplankton groups (Figure 2-1 Page 52; Appendix. Figure 1). Phytoplankton should be considered as a dynamic community of algal species/groups and not represented as chlorophyll-*a* unless the dominant organisms are defined, and they consistently do not change inter-annually. In the Thames, for instance, chlorophyll-*a* concentrations represent only the diatom population (Figure 3-3 Page 49). In other lowland rivers, however, chlorophytes and cyanobacteria are important groups and are more abundant during the summer months than in the Thames (Kouzminov et al., 2007).

This study also denotes that river catchment environmental heterogeneity may play an important role in river plankton spatial and seasonal dynamics (Chapter 5). For instance, plankton-rich productive tributaries have similar phytoplankton and zooplankton communities to the Thames, implying that they may influence biota in the main river stem (Chapter 3 Figure 3-3; Chapter 5 Figure 5-3 Page 98; Figure 5-7 Page 108). Therefore, mathematical models should account for residence time in the connected retentive zones in the catchment.

Phytoplankton-bacteria-chytrids study (Chapter 7) proposed that even small a change in thermal properties of the environment (by 1-2 °C) might trigger a fast diatom bloom termination associated with diatom-bacteria interactions which result in cell aggregation, deterioration and settling down the water column. Microbiological infections of algal cells and bacteria driven aggregation may be the key component of biological controls on phytoplankton, causing fast mortality of algal cells, exhibiting host-specific behaviour and therefore supporting species succession within plankton communities.

Phytoplankton literature (Chapter 2) often includes the term settling rate (Reynolds, 1984; Sandgren, 1988). Based on the observations described in Chapter 7, it is possible to link this 'settling' term to algal cell metabolic responses to thermal and nutrient stress coupled with bacterioplankton community activities. 'Microbial settling' may describe the process of phytoplankton termination in rivers better than grazing. When conditions become unfavourable, diatom cells cluster and travel down the water column until they reach the bottom sediment. Weekly phytoplankton community data (Section 3.2.3) showed that diatoms in the Thames could

grow to a point where they reach their ‘carrying capacity’, raise pH and deplete essential nutrients. Chapter 7 findings suggest that when conditions become unfavourable, diatoms cluster/aggregate and settle down. During this clustering process, diatom frustules deteriorate and get recycled by the microbial community naturally occurring and associated with diatoms.

During summer months settled diatoms may become resuspended in the water column, but as weekly flow cytometry data demonstrated they could no longer trigger another bloom. It was established that diatoms begin to reproduce after resting spores find a cold temperature refuge unfavourable for the bacterial and fungal community (Ratkowsky et al., 1983; Kudoh & Tokahashi, 2004; Gsell et al., 2013).

Comparing mortality rates related to zooplankton grazing (Section 6.4.3) with mortalities associated with diatom-attaching bacteria and aggregating (Section 7.5), it is feasible to conclude that ‘microbial settling’ may be considered as a prime biological regulator of centric diatom blooms in the Thames. There is also a possibility of algal viral lysis, which was not covered in this study.

Conceptual model

If light intensities and duration are favourable for centric diatom populations (Bowes et al., 2016) (light intensity >20klx; daily sunshine > 5h day^{-1}), they can reach high densities and deplete essential nutrients (silicon, nitrogen and phosphorus), thermal and light conditions also can change, at this point diatoms get suppressed by bacterial, fungal or viral communities within a few days, followed by protozoan, rotifer and micro-crustacean 'grazing'. Parasitical fungi and algicidal bacteria kill diatom cells and recycle empty frustules, as a result after bloom cessations the remaining healthy cells do not provide a sufficient source to start an immediately growing population again unless microbial suppressors are temporarily inhibited in their activities (Figure 8-1, 8-2).

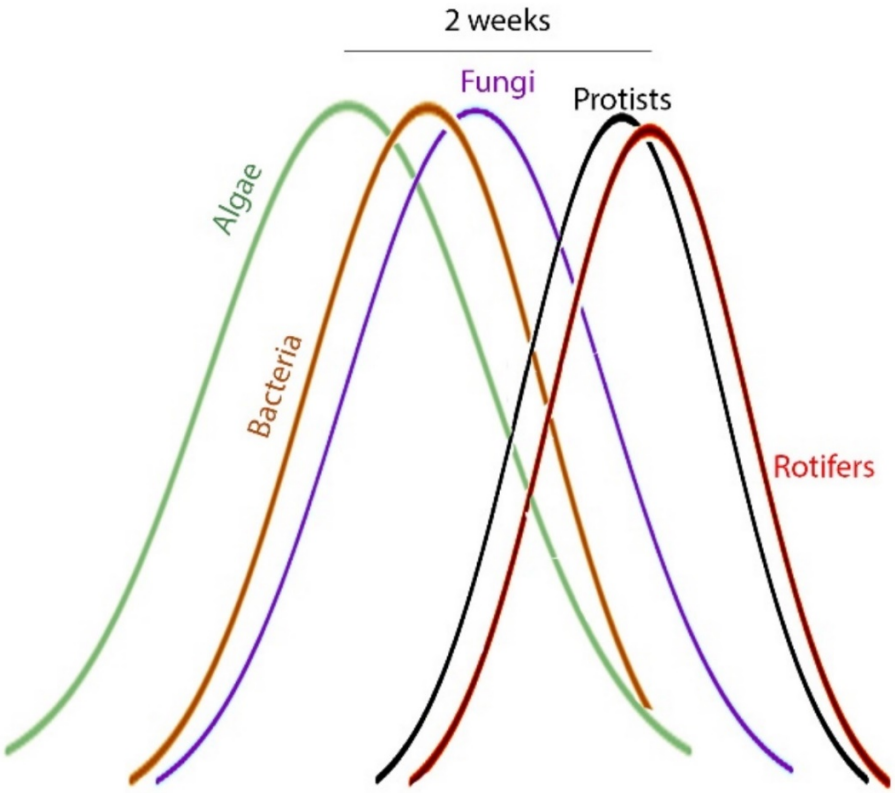


Figure 8-1 Conceptual model of microbiological controls on diatom dominated river phytoplankton

Chlorophytes, cryptophytes and dinoflagellates rapidly succeed diatoms, when water temperature is sustained above 19°C cyanobacteria quickly become more abundant, faster and can dominate within the community. In years of prolonged low flow and drought conditions across the catchment, blue-green algae can develop large toxic blooms (Figure 8 2). During sustained drought events across the Thames catchment, the river channel, in parts, could be compared with shallow eutrophic lakes. In these still and warm environments, cyanobacteria have several competitive advantages to other phytoplankton groups, they are resistance to grazing, have high growth rates and can migrate vertically preventing sedimentation (Lurling et al., 2013). Climate warming will lead to an intensification of cyanobacterial blooms in large lowland river catchments.

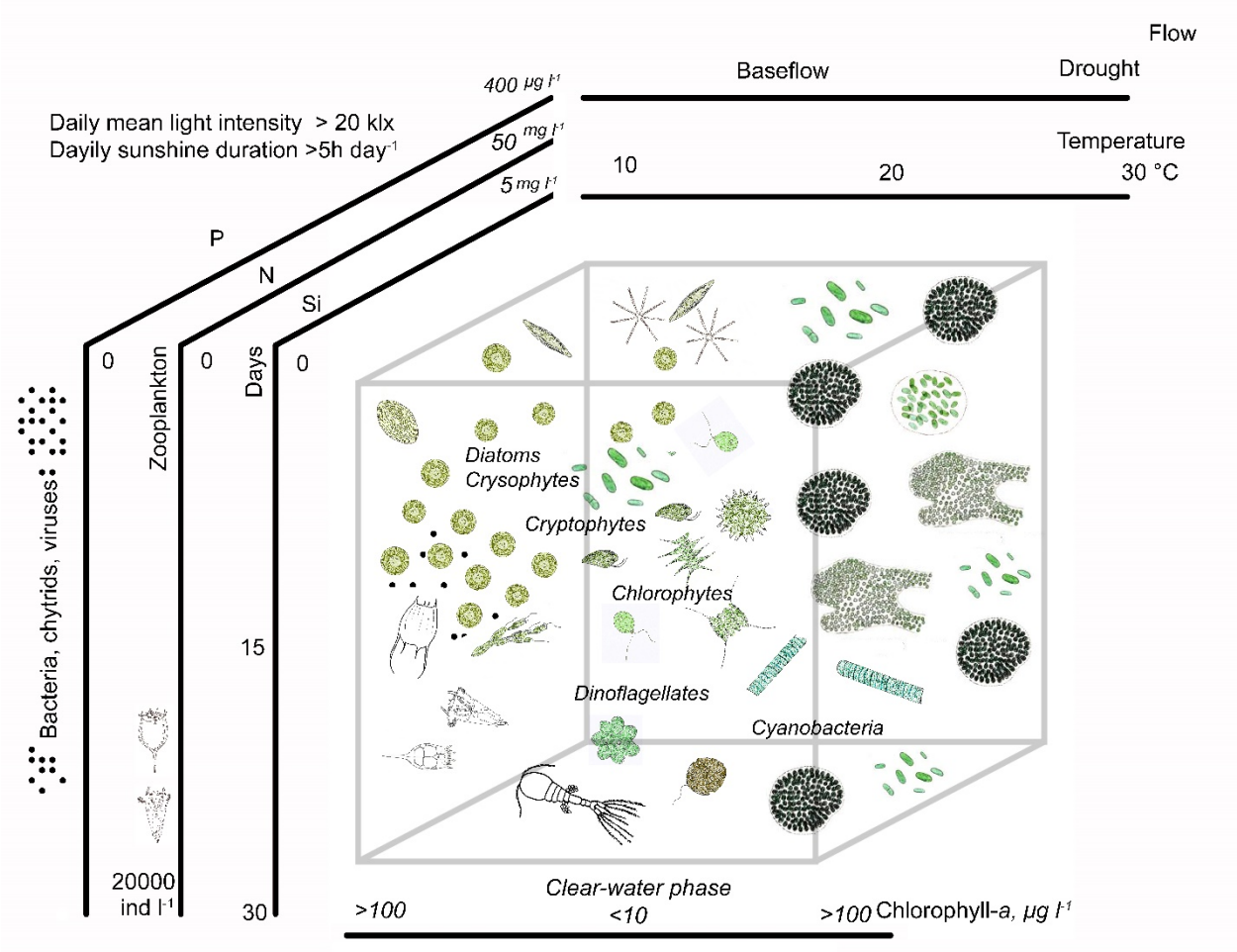


Figure 8-2 Conceptual model of phytoplankton dynamics in relation to flow, water temperature, concentrations of dissolved phosphorus, nitrogen and silicon (SRP, NO_3, Si), residence time (days), presence of microbial pathogens and zooplankton in the optimal sunlight conditions in the River Thames.

8.5 Next steps

8.5.1 Study of algicidal bacteria, fungi and viruses

The experiments (Chapter 7) indicated a complex interplay between the physical environment and algal-bacteria interactions. Further investigation is needed to unpick these relationships. Advanced techniques in genomics and metabolomics can help to identify naturally occurring algicidal bacteria, chytrids or viruses and explain their influence on phytoplankton through very fine-scale biochemical interactions. Microbiological suppressors of phytoplankton can offer a cost-effective solution for algal bloom controls benefiting freshwater management practices.

8.5.2 Catchment connectivity. Monitoring off-channel retentive zones

Catchment connectivity is an important driver of phytoplankton diversity, distribution and dynamics (Hu et al., 2019). Large off-channel retentive environments, such as connected canals and lakes may support harmful algae which include primarily cyanobacteria, but even some diatom species can be harmful to fish. Toxic *Pseudonitzschia* spp and 'spiky' *Chaetoceros* spp. diatoms live in British waters and although they were previously found in marine habitats, some organisms can adapt to freshwater environments as was observed during diatom-bacteria experiments (Chapter 7) when solely marine organisms coccolithophores were found in the Oxford Canal (Appendix Figure 2). *Pseudonitzschia* spp. produce the neurotoxin - domoic acid resulting in poisoning of birds, marine mammals and human shellfish consumers (summerised in: Fehling *et al.* 2006). A non-toxic 'spiky' *Chaetoceros* spp. have barbed, siliceous spines (setae) that penetrate the gill tissue, which then produces excessive mucus that lead to fish asphyxiation.

Cyanobacteria can grow extensively in large lowland rivers, but in the main Thames, they are less successful than other autotrophs, possibly due to low water temperatures (annual maximum below 22°C) and turbulent flows created by the numerous weirs. The influence of weirs on the Thames phytoplankton is poorly understood, but they were observed inhibiting cyanobacteria growth in the Lower Darling River, Australia (Mitrovic et al., 2011). Therefore, any proposal to remove weirs must consider both positive and negative effects on planktonic communities, where negatives could favour cyanobacteria population.

In the Thames catchment cyanobacteria bloom in Farmoor Reservoir (Appendix Figure 3), but they have no effect on the Thames phytoplankton community (Figure 5-5). To limit cyanobacteria blooms in reservoirs it is advisable to control phosphorus concentrations in the river upstream. It is important to emphasize that retentive zones increase river ecosystem productivity, and in the absence of large cyanobacteria blooms, phytoplankton support diverse zooplankton and fish communities. This should be considered if a connected lake or a canal is to be removed from the river floodplain.

8.5.3 Phytoplankton species survey

The River Thames is an important river system which is used as a drinking water supply via a network of reservoirs. However, the last comprehensive study of the Thames phytoplankton was conducted more than 20 years ago (Ruse & Love, 1997). Aside from identifying toxic species,

their spatial variation and seasonal dynamics, studying phytoplankton communities is essential for understanding the zooplankton community patterns (Chapter 4, 5) and fish population dynamics.

Flow cytometry offers only a crude solution to phytoplankton monitoring defining 10 groups of phytoplankton. But even in one grab sample of water from Oxford Canal connected to the Thames, there were various small diatom and chlorophyte species, while larger species were filtered out for the purpose of the experiment (Table 7-1 Appendix Figure 4).

Chrysophytes and dinoflagellates were observed in all zooplankton samples from the middle-lower Thames (Appendix. Figure 5), but they were not included in weekly flow cytometry analysis due to various complications, these may include large cell sizes, the 'overlapping' with diatoms or cryptophytes pigment fluorescence, and optical cell clogging.

Cyanobacteria require more attention. Cyanobacteria in the Thames tend to be sensitive to water temperatures, yet little is known about their response to low light intensities. This is a big gap in knowledge.

In conclusion, there is a need for a comprehensive catchment scale phytoplankton species survey enhanced by the novel microbiological analysis such as DNA sequencing to identify dominant algal species in the catchment, their spatial variation and seasonal dynamics. Phytoplankton can then be accurately related to bacterioplankton and fungal parasites.

8.5.4 Interactive and dynamic river catchment

A dynamic interactive catchment-scale map with phytoplankton, zooplankton, and bacterioplankton communities represented as separate layers on a top of spatial variating in flow, water temperature, nutrient concentrations, riparian shading, and residence time can provide valuable material for the end users improving management strategies and public awareness.

APPENDIX

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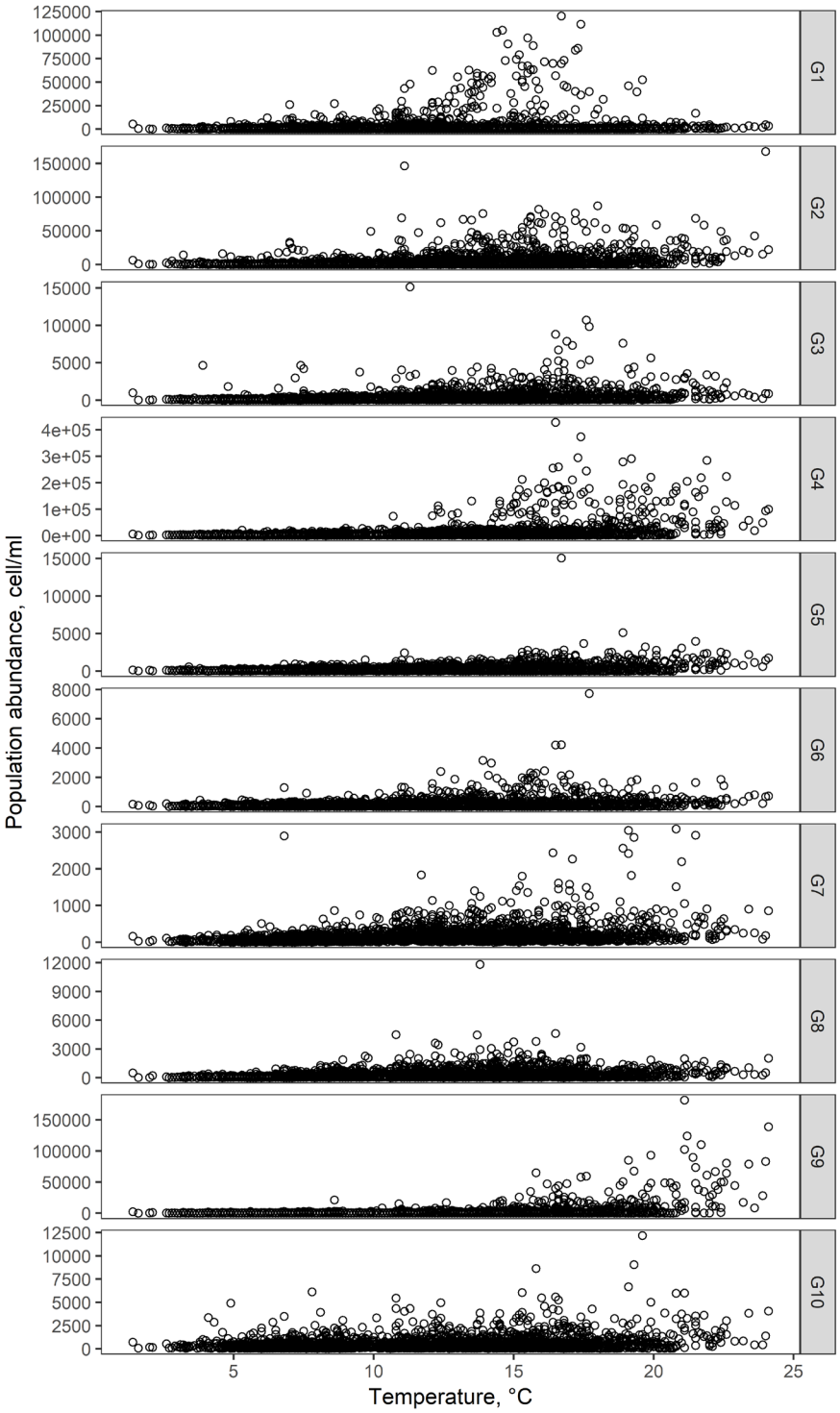
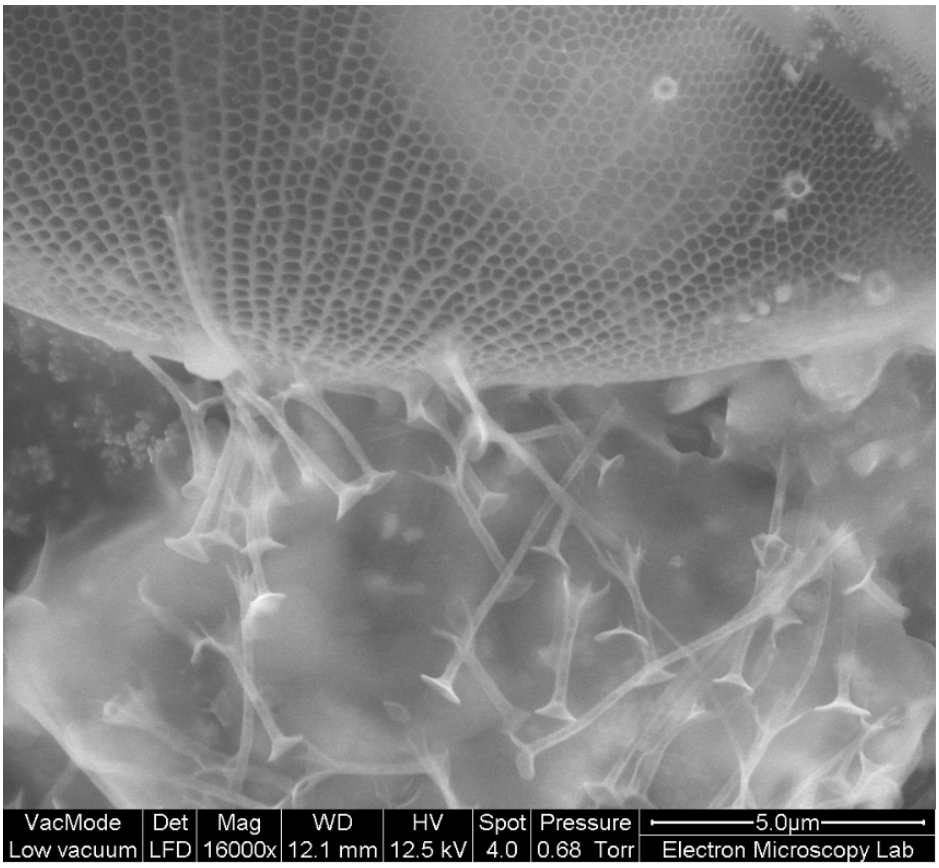
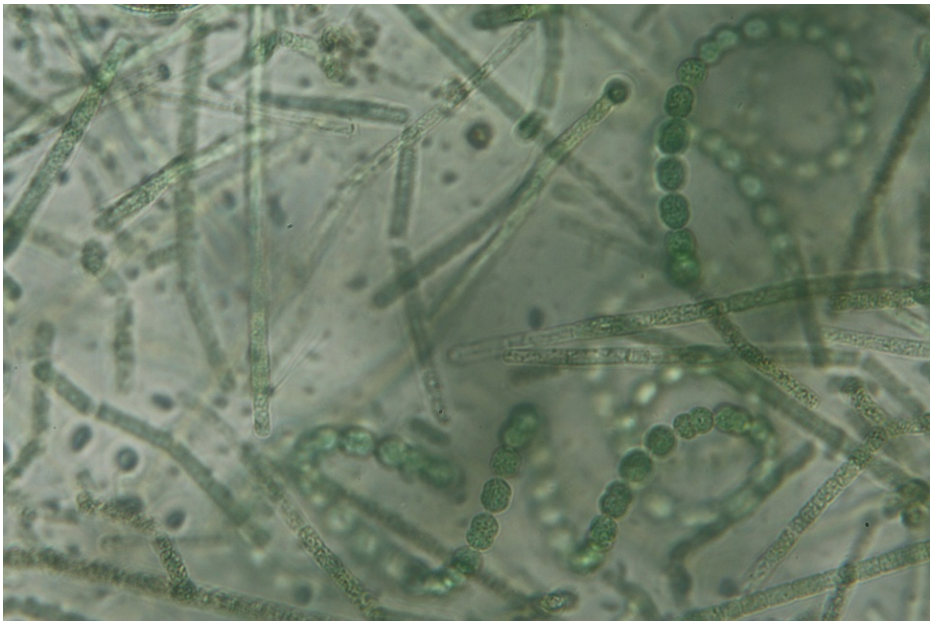


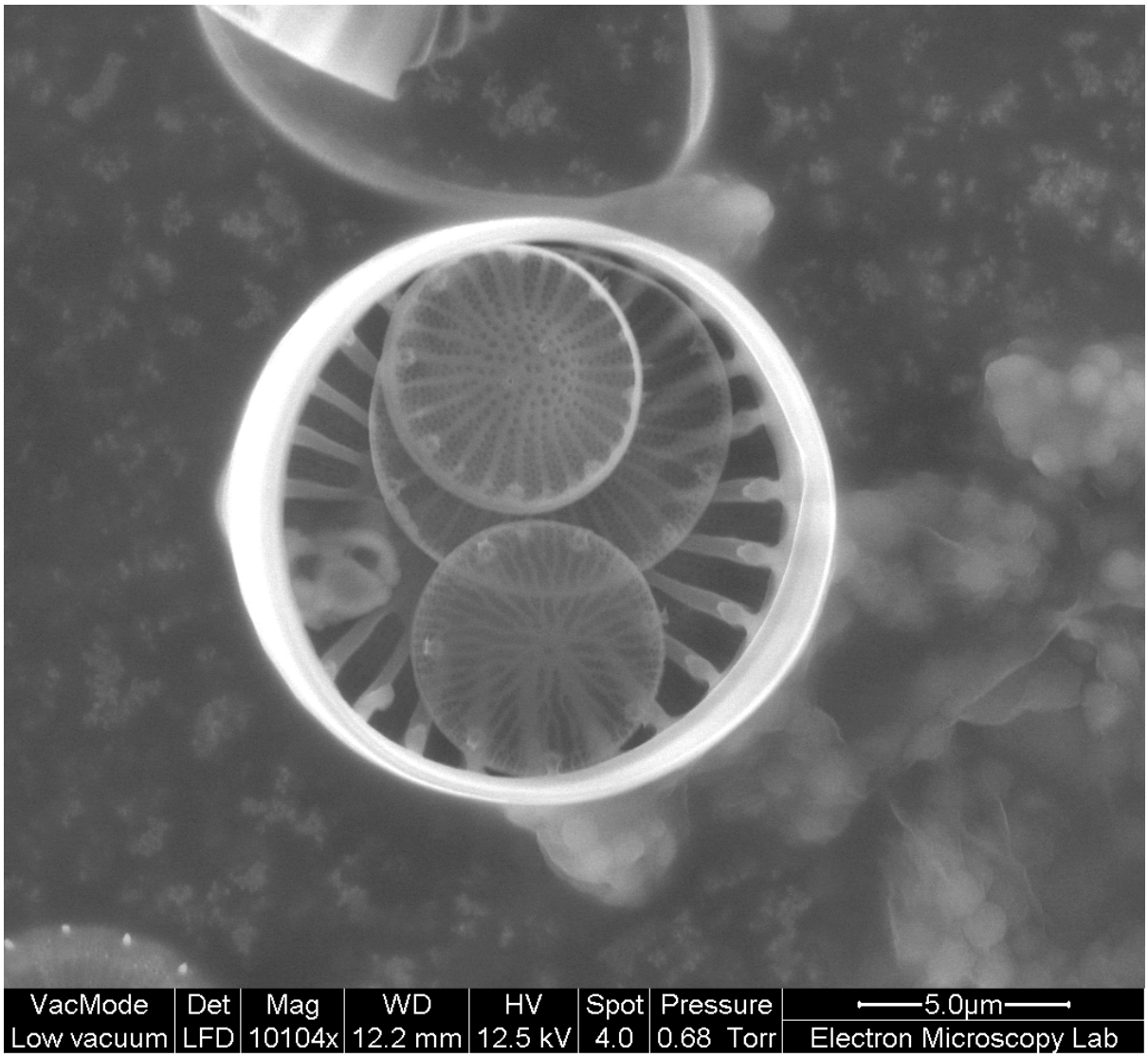
Appendix Figure 8-3 Phytoplankton abundance in relation to water temperature. Weekly flow cytometry data from 22 sites in the River Thames catchment (2013-2015). Large diatoms (G1), Chlorophytes (G2), Large chlorophytes (G3), nano/pico Chlorophytes (G4), Cryptophytes (G5), Large Cryptophytes (G6), Cyanobacteria Microcystis-like (G7), Cyanobacteria (G8), Cyanobacteria Synechococcus-like (G9), Cyanobacteria PE-rich (G10).



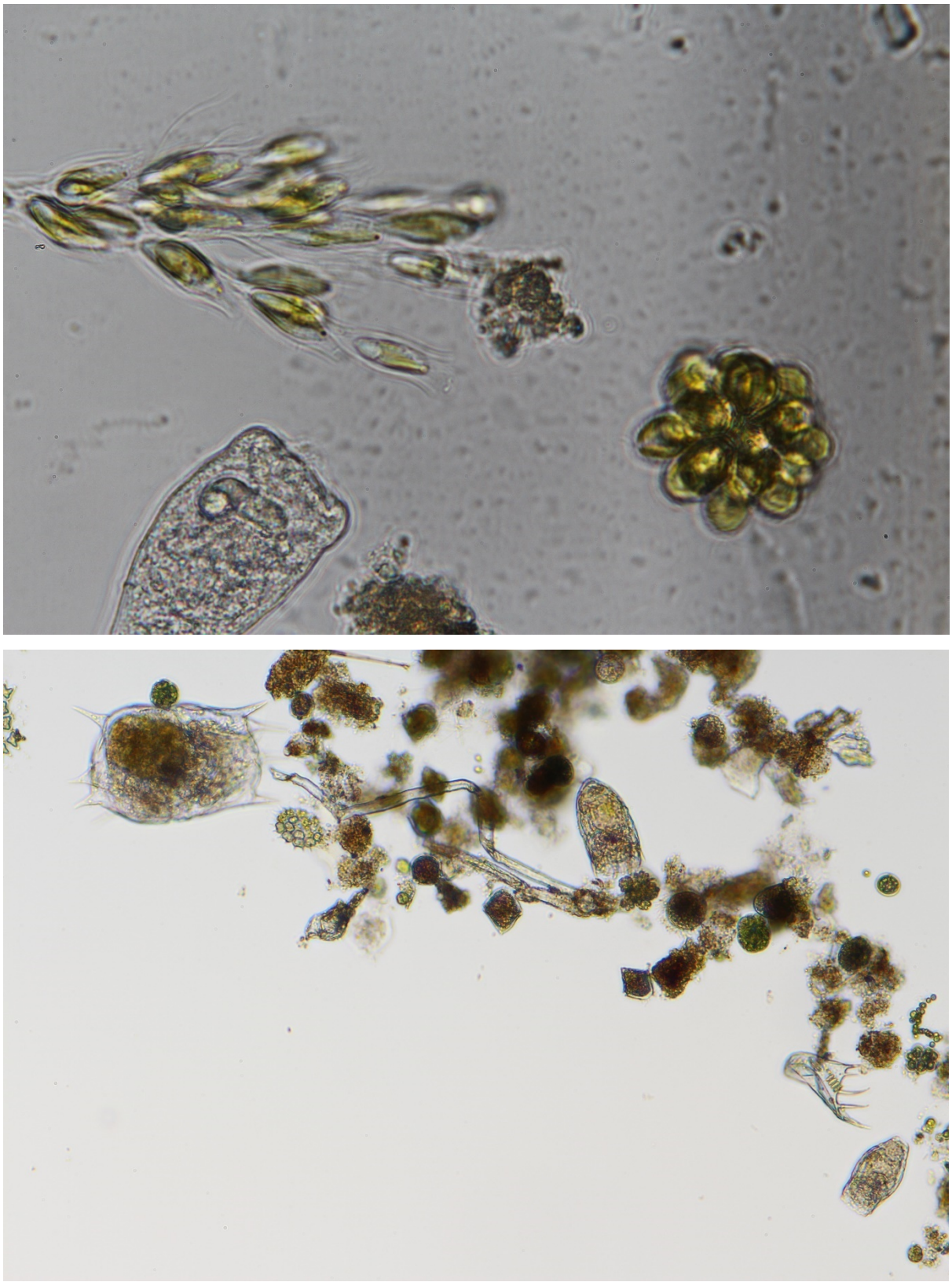
Appendix Figure 2. *Discosphaera tubifera* (Murray & Blackman) Ostenfeld (broken cell) observed in the Oxford canal. Scanning electron microscopy. Method described in Chapter 7. Section 7.4.4 Page 158



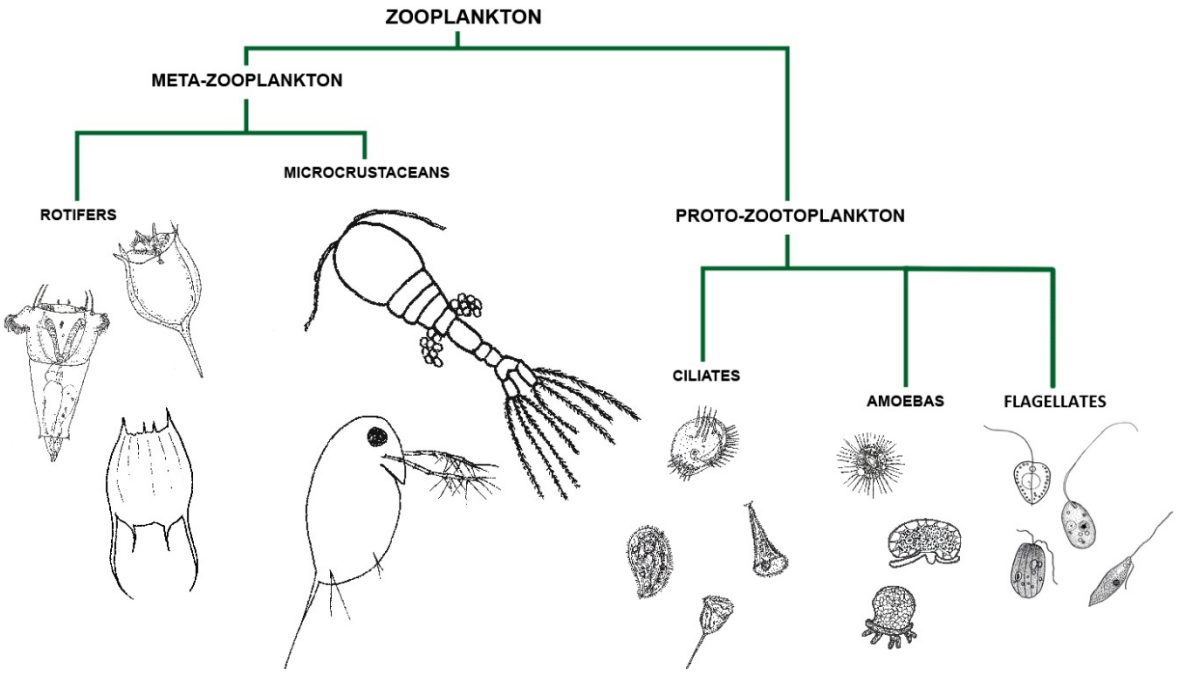
Appendix Figure 3 Cyanobacteria observe in Farmoor Reservoir. Optical microscopy method described in Chapter 4 Section 4.4.2. Zooplankton survey Page 69. Optical microscopy. (18/05/2015). Raw, uncalibrated photograph. No scale.



Appendix. Figure 4 Centric diatoms observed in the Oxford Canal. Scanning electron microscopy. Method described in Chapter 7. Section 7.4.4 Page 158



Appendix. Figure 5 Raw, uncalibrated photograph showing species of Crysophytes (top) (May 2015) and dinoflagellates (bottom) (July 2015). Sampling site: The River Thames at Wallingford Optical microscopy method described in Chapter 4 Section 4.4.2. Zooplankton survey Page 69. No scale.



Appendix Figure 6 Meta-zooplankton (metazoans) and proto-zooplankton (protozoans)

Chapter 9 Appendix

Zooplankton grazing effect on chlorophyll-*a* daily dynamics and thermal regulation of phytoplankton in the River Thames, UK. Results of preliminary laboratory experiments using naturally occurring Thames plankton communities during a large phytoplankton bloom

Continues page numbering

9.1 Introduction

During the 2-week period of an intensive diatom bloom, six preliminary laboratory experiments were conducted to assess the effect of the zooplankton grazing and changing water temperature on the bloom development. The summery of experimental design: factors, levels and replicates are listed in Table 9-1. Rotifer abundances were artificially increased to reproduce population sizes previously observed during chlorophyll-*a* bloom pre-termination phases in the Thames (May and Bass, 1998; Figure 4-5 75). The following hypothesis were tested:

- The zooplankton can significantly reduce phytoplankton growth, but this effect is evident when rotifer numbers are higher than 1000 ind m^{-3} replicates.
- The diatom growth can be inhibited by the thermal properties of the environment.

9.2 Methodology

The original algal communities were sourced from the River Thames at Wallingford. For the starter culture, a bulk sample was collected on the day of every experiment by lowering a 10-litre bucket (prewashed and sterilised) from the Thames riverbank and immediately transported to the laboratory. For the nutrient medium, water was sourced from the Thames at Sonning before a parcel of water with diatom bloom reached this part of the river (Figure 9-1). Nutrient medium was filtered through filters with 0.22 μm pore diameter to remove algae and bacteria and refrigerated (4°C). The concentrations of the important for algal growth micro-and macro-elements in the nutrient medium are listed in Table 9-2. The medium was added to starter culture aliquots (1:1) to provide optimum nutrient concentrations.

9.3 Experiment design

To measure temperature and grazing effect aliquots of the starter culture were incubated inside glass beakers (250 ml) placed on algal shakers at low speed in a temperature/light-controlled environment under a 12/12h light-dark cycle. Light illuminance was maintained between 10-30klx, water temperatures and light intensities were measured every 30 min using Hobo Loggers. In all six experiments, phytoplankton dynamics were assessed as daily changes in chlorophyll-*a* concentrations.

Table 9-1 The summery of experimental design: factors, levels and replicates (exp-t 1-6)

		Factor		
Experiment	Date	Grazers, ind m-3	Water Temperature, °C	Water age, (conceptual term, counted from the first sampling day)
Experiment 1	19 – 20/08	Levels: 0; 350; 1000 Incubated at 19 - 22°C Replicates: 3	-	1
Experiment 2	20 – 21/08	Levels: 0; 1500 Incubated at 18 - 19°C; 19 -22°C	Levels: 18 - 19°C; 19 -22°C Rotifers: 250 ind m^{-3} Replicates: 3	2
Experiment 3	22 – 23/08	-	Levels: 18 - 19°C; 19 - 22°C Rotifers: 250 ind m^{-3} Replicates: 4	4
Experiment 4	24 – 25/08	-	Levels: 16 – 17°C; 18 - 19°C; 19 - 22°C Rotifers: 250 ind m^{-3} Replicates: 3	6
Experiment 5	25 – 26/08	-	Levels: 16 – 17°C; 18 - 19°C; 19 - 22°C Replicates: 3	7
Experiment 6	25 – 26/08	Levels: 0; 2000 Incubated at 18 - 19°C Replicates: 3	-	7

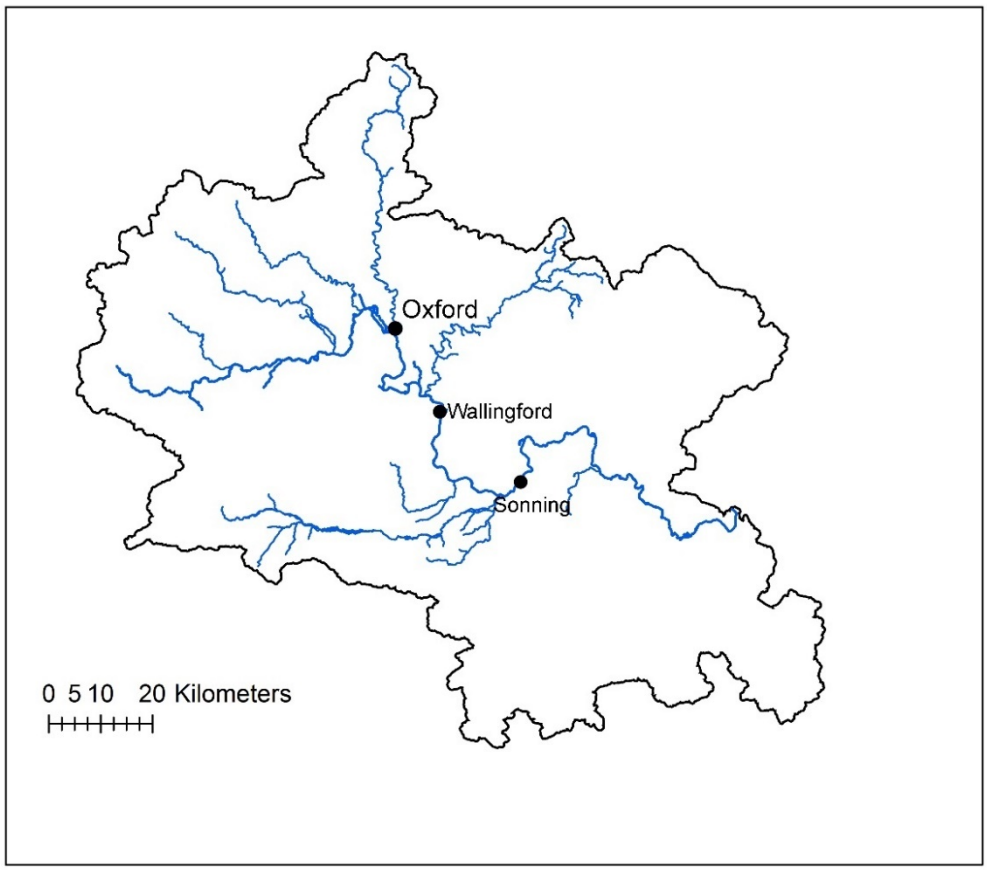


Figure 9-1 Thames at Wallingford (51.596995°; -1.133410°) and Sonning (51.466520°; 1.283488°)

To estimate the grazing effect, for the "null zooplankton" treatment, rotifers were filtered out using a sieve with 53µm mesh diameter, these rotifers were added to treatments with highest rotifer abundances. The zooplankton were identified and enumerated before and after the experiment using the optical microscope. The starter algal culture (total cell abundance $2*10^5$ cell ml^{-1}) was dominated by centric diatoms (95%), mixed with a small number of green algae, bacteria, amoebas, and ciliates. Chlorophyll-*a* concentrations were measured at the start of the experiment and after 24h of incubation.

Optical microscopy

Algae were enumerated and photographed at 10x, 20x, 40x and 100x magnification using an inverted microscope Axiovert 40CFL and a DSLR camera Canon 750D. Diatoms and chlorophytes were examined at 20x magnification, bacteria and fungi were studied at 40x and 100x magnifications. Samples were photographed at three microscope magnifications: 10x for zooplankton enumeration; 40x for diatom aggregates, bacteria and chytrids, 100x for bacterial

and fungal infections and signs of cell lysis. For zooplankton enumeration samples were left to settle in the sterile Petri dishes, scanned and photographed. Photographs were processed with ImageJ software (Rueden et al., 2017). More than 300 individuals were counted per one sample, they were then extrapolated. Rotifers were identified to species and genus levels (see Chapter 4-Methodology Page 51) For diatom identification, a small subsample was prepared using Hydrogen peroxide (30%), a method described in (Krammer & Lange-Bertalot, 2000).

Table 9-2 Concentrations of main chemical components in the nutrient medium. Measured at CEH Wallingford (mean values from measurements on 15/08/16 and 24/08/2016)

Variable	Unit	Value
pH		8
Suspended solids	mg m^{-3}	3.4
Soluble reactive phosphorus (SRP)	µg m^{-3}	164
Total phosphorus (TP)	µg m^{-3}	184
NH4	mg m^{-3}-NH_4	0.049
Si	mg m^{-3}-Si	6
Chlorophyll-a	µg m^{-3}	2.96
NO3	mg m^{-3}-NO_3	28.70
SO4	mg m^{-3}-SO_4	47.9
Na	mg m^{-3}	33
K	mg m^{-3}	6.93
Ca	mg m^{-3}	104
Fe	mg m^{-3}	13
Cu	mg m^{-3}	4.95
Conductivity	µS cm^{-1}	681

9.4 Data analysis

All statistical analyses were performed in the R programming environment (Wickham, 2016; R Core Team, 2017). Generalized linear models (GLM, R functions: *glm,* gaussian distribution) were used to estimate the significance of categorical variables. Diagrams were created with ggplot 2 (Wickham, 2016). Equations for statistical models are listed in Table 3.

Chlorophyll-a = Grazing (Experiment 1, 6)

Chlorophyll-a = Temperature + Grazing (Experiment 2)

Chlorophyll-a = Temperature (Experiment 3, 4, 5)

Chlorophyll-a = Temperature + Grazing + Age of water (Experiment 1-6)

Data were tested for normality (with Shapiro–Wilk test), and log transformation was performed on data deviating from the normality assumptions. All statistically significant differences quoted are at $p \leq .05$ or less. For correlational analysis package *PerformanceAnalytics* was used (Peterson & Carl, 2018).

9.5 Results and Discussion

Experiments 1, 2 showed that although the presence of rotifers can explain some variation in chlorophyll-*a* dynamics, grazing has less impact on diatom growth than water temperature which was tested in Experiments 2-5 (Table 9-3). The grazer mean community composition is listed in Table 9-4.

Diatoms growth was persistently suppressed at temperatures above 19°C (Figure 9-3). Rotifer grazing had an adverse effect on phytoplankton growth (Table 9-3. Experiment 1, 2, 6), but did not cause any significant falls in chlorophyll-*a* concentrations (Figure 9-3. Experiment 1, 2, 6), even when rotifer abundances were relatively high (> 1000 ind m^{-3}). Experiment 6 showed that when rotifer numbers were as high as 2000 ind m^{-3}, they still did not exert any notable pressure on chlorophyll-*a* concentrations (Table 9-3). Rotifers, particularly the ones with large bodies, consume small centric diatoms, but find their silicate frustules difficult to digest. Consequently, rotifers tend to develop dense populations after the diatom bloom, because they then filter out pico-plankton and bacteria growing in the post-diatom nutrient environment (Arndt, 1993; May & Bass, 1998; Bmstedt et al., 2000)

The last experiment was done during the bloom pre-termination phase, as less than a week later, the diatoms have completely settled down in the Thames channel and were washed out of the system (Figure 7-5 Page 146). When the starter culture was incubated with and without grazers and under different water temperature conditions, both temperature and the rotifers could not explain daily falls in chlorophyll-a concentrations (Table 9-3. Experiment 5). As a result, data from all six experiments were combined into one statistical model (Table 9-3. Experiment 1-6) to compare the importance of the age of bloom (“water age”) against the presence of grazers and thermal effect. It was difficult to estimate the actual age of the bloom since the area in the catchment (whether diatoms travelled from a lake or a canal) and the time when centric diatoms first reached high population densities (over 50000 ind m^{-3}) could not be established. Thus, a simple approach was taken. Age was estimated in days the bloom was first seen at Wallingford.

Water age" was the only significant factor that explained most of the variation in phytoplankton growth/mortality (expressed as change in chlorophyll-*a* concentration).

Table 9-3 Results of the GLMs predicting chlorophyll-a daily dynamics. Factors, equations, t-value (t), degrees of freedom (df), significance (*p*), Adjusted R-squared, and model significance (p-value)

Experiment		1	2	3	4	5	6	1-6
Equation Dependent variable	Treatment	~ Grazing	~ Temp + Grazing	~ Temperature			~ Grazing	~ Rotifers + Temp + Age of water
Temperature	16-17°C		-4.3		-4.3	-.45		-.93
t			(9)		(6)	(6)		(42)
(df)			.005*		.01*	.66		.36
p								
	18-19°C		8	-3.8	-1.4	-.13		.61
			(9)	(6)	(6)	(6)		(42)
			< .05*	.008*	.2	.9		.55
	19-22°C		-3.7	-9.7	-2.54	-1.31		.53
			(9)	(6)	(6)	(6)		(42)
			.004	< .05*	.05*	.24		.6
Rotifers ind l^{-1}	0	17.2	8					-.93
Grazing		(6)	(9)					(42)
t		< .05*	< .05					.36
(df)	350	-8.4					-.369	.25
p		(6)					(6)	(42)
		.0001*					.731	.8
	1000	-2.1						1.1
		(6)						(42)
		.08						.27
	1500		-.51					-.2
			(9)					(42)
			.62					.8
	2000						-.059	-.39
							(6)	(42)
							.956	.7
Age of water t(df) *p*								-3.12 (42) .003*
Adjusted R-squared		.9	.52	.93	.36	.01	-.2	.45
p-value		.0003	.01	< .05	.11	.4	.7	< .05

The effect of "the bloom age" or "water age" concludes that even when nutrient concentrations and water temperature remain favorable for diatom growth, and the population of grazers is marginally important, the aged bloom will still rapidly terminate, and the concentrations of chlorophyll-a will fall. The factor behind the actual aging remains uncertain, except that under an optical microscope, diatom cells were forming amorphic aggregates populated by various bacteria, while some were visibly recycled. Optical microscopy revealed active microbial population "blooming" along the diatoms, meaning that they could be the main drivers of the diatom bloom termination (Figure 7-5).

Figure 9-2 Preliminary experiments. Water was collected from the River Thames at Wallingford. Chlorophyll-*a* concentrations were analysed on the same day and 24 h after the incubation. Rotifer community diversity and abundances were examined on the first day of experiments.

Water age was previously applied to characterise plankton dynamics in the Danube River relating phytoplankton mortalities to increased abundance of zooplankton grazers (Baranyi et al., 2002; Keckeis et al., 2003). Indeed, the zooplankton population tends to increase with water age/residence time, and grazing is known as an important control of phytoplankton biomass in the lentic and marine environment (reviewed in: Reynolds, 1984; Bmstedt *et al.*, 2000). Nevertheless, in lentic environments, grazer abundances, body sizes and filtration rates are higher than in rivers. For instance, the number of metazoan grazers in a lake upper layer can reach up to 100000 ind m^{-3} (examples in: Ferrara, Vagaggini and Margaritora, 2003; Khalifa *et*

al., 2015) which is 10 folds higher than the commonly observed maximum of less than 9000 ind m^{-3} in lowland rivers (reviewed in: Lair, 2006).

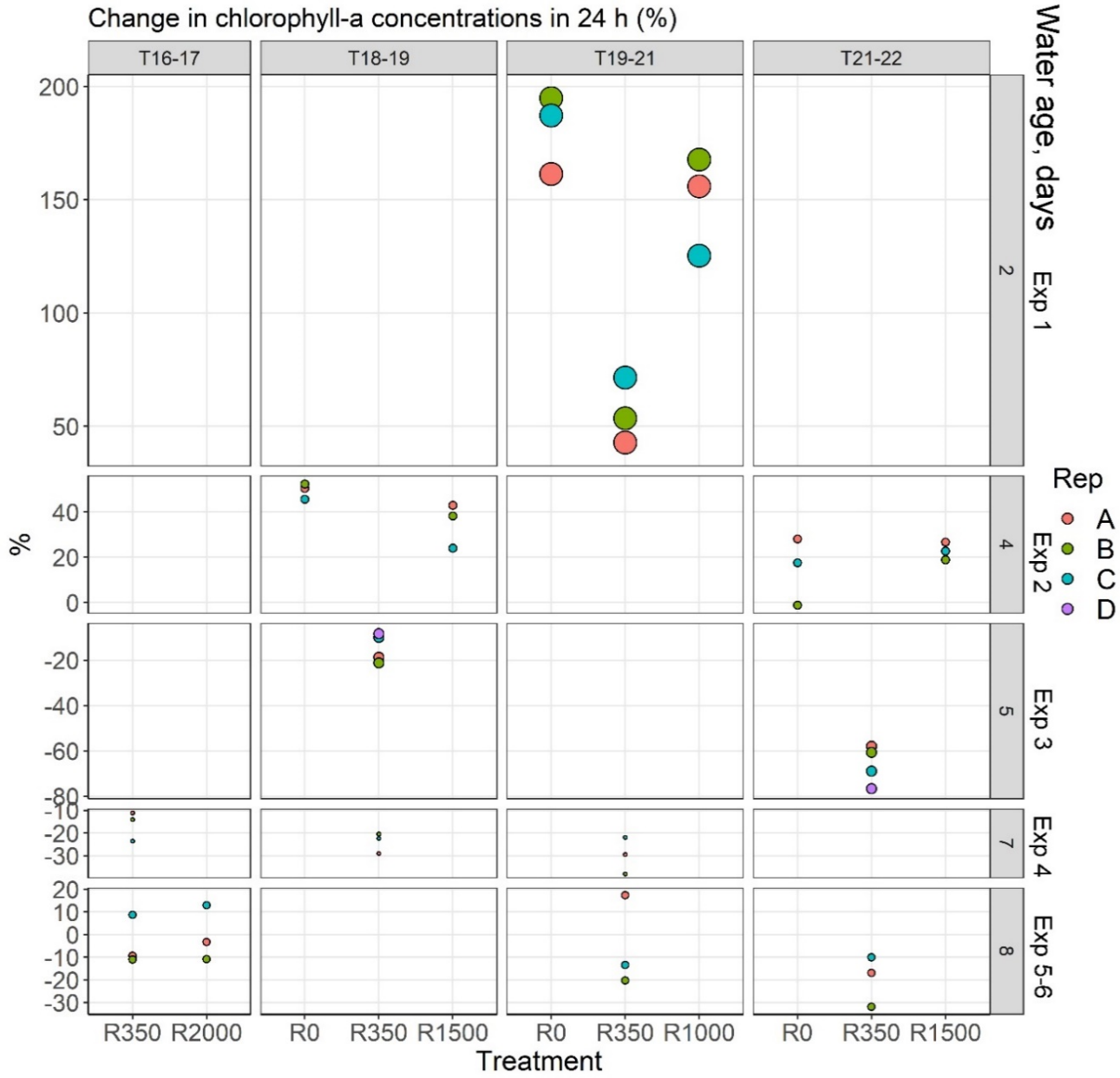


Figure 9-3 Daily change in chlorophyll-a concentrations (Day_1 – Day_0)*100/Day_0, (%). Treatments: R0 – filtered, (0 grazers); R350, 1000, 1500, 2000 – unfiltered, (rotifer abundances: 350,…2000 ind m^{-3}). Water age, (days)

Table 9-4 Rotifer community composition used in the experiments

Rotifer genus/species	Proportion in the community, %
Brachionus calyciflorus	4
Brachionus angularis	1
Brachionus rubens	1
Brachionus quadridentatus	1
Brachionus urceolaris	1
Squatinella sp.	1
Keratella cochlearis	21
Keratella quadrata	1
Anuraeopsis fissa	1
Euchlanis dilatata	2
Trichotria sp.	1
Colurella sp.	2
Lecane sp.	1
Lindia sp.	1
Eosphora sp.	2
Cephalodella gibba	1
Cephalodella sp.	2
Trichocerca sp.	2
Ascomorpha sp.	1
Synchaeta oblonga	29
Polyarthra dolichoptera	13
Pompholyx sulcata	3
Dicranophorus sp.	4
Lepadella sp.	1
Bdelloid rotifers	3

References

Aldridge, D. C., P. Elliott, & G. D. Moggridge, 2004. The recent and rapid spread of the zebra mussel (Dreissena polymorpha) in Great Britain. Biological Conservation 119: 253–261.

Alekseev, V. R., 2010. A guide to zooplankton and zoobenthos in European Russia, Vol. 1: Zooplankton [in Russian]. KMK, Moscow - Saint Petersburg.

Amin, S. A., M. S. Parker, & E. V. Armbrust, 2012. Interactions between diatoms and bacteria. Microbiology and Molecular Biology Reviews 76:.

Amoros, C., & G. Bornette, 2002. Connectivity and biocomplexity in waterbodies of riverine floodplains. Freshwater Biology 47: 761–776.

Anderson, M. J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology 26: 32–46.

Arndt, H., 1993. Rotifers as predators on components of the microbial web (bacteria, heterotrophic flagellates, ciliates) - a review. Hydrobiologia Kluwer Academic Publishers 255–256: 231–246.

Arndt, H., D. Dietrich, & B. Auer, 2000. Functional diversity of heterotrophic flagellates in aquatic ecosystems. The Flagellates 240–268.

Aslam, S. N., J. Strauss, D. N. Thomas, T. Mock, & G. J. C. Underwood, 2018. Identifying metabolic pathways for production of extracellular polymeric substances by the diatom Fragilariopsis cylindrus inhabiting sea ice. ISME Journal Springer US 12: 1237–1251.

Balbi, D. M., 2000. Suspended chlorophyll in the River Nene, a small nutrient-rich river in eastern England: long-term and spatial trends. Science of The Total Environment 251–252: 401–421.

Båmstedt, U., D. J. Gifford, X. Irigoien, A. Atkinson, & M. Roman, 2000. ICES Zooplankton Methodology Manual. ICES Zooplankton Methodology Manual. Elsevier.

Bahnwart, M., T. Hübener, & H. Schubert, 1998. Downstream changes in phytoplankton composition and biomass in a lowland river-lake system (Warnow river, Germany). Hydrobiologia 391: 99–111.

Baranyi, C., T. Hein, C. Holarek, S. Keckeis, & F. Schiemer, 2002. Zooplankton biomass and community structure in a Danube River foodplain system : effects of hydrology. Freshwater Biology 47: 473–482.

Basu, B. K., & F. R. Pick, 1996. Factors regulating phytoplankton and zooplankton biomass in temperate rivers. Limnology and Oceanography 41: 1572–1577.

Basu, B. K., & F. R. Pick, 1997. Phytoplankton and zooplankton development in a lowland river. Journal of Plankton Research 19: 237–253.

Bayley, P. B., 1995. Understanding Large River: Floodplain Ecosystems. BioScience 45: 153–158.

Beakes, G. W., H. M. Canter, & G. H. M. Jaworski, 1988. Zoospore ultrastructure of Zygorhizidium affluens and Z. planktonicum, two chytrids parasitizing the diatom Asterionella formosa. Canadian Journal of Botany 66: 1054–1067.

Bernhardt, E. S., & G. E. Likens, 2004. Controls on periphyton biomass in heterotrophic streams. Freshwater Biology 49: 14–27.

Bertani, I., I. Ferrari, & G. Rossetti, 2012. Role of intra-community biotic interactions in structuring riverine zooplankton under low-flow, summer conditions. Journal of Plankton Research 34: 308–320.

Bertani, I., H. Segers, & G. Rossetti, 2011. Biodiversity down by the flow: New records of monogonont rotifers for Italy found in the Po river. Journal of Limnology 70: 321–328.

Bertrand, C., V. Siauve, S. Fayolle, & A. Cazaubon, 2001. Effects of hydrological regime on the drift algae in a regulated Mediterranean river (River Verdon, southeastern France). Regulated Rivers: Research & Management 17: 407–416.

Berziņš, B., & B. Pejler, 1989. Rotifer occurrence in relation to temperature. Hydrobiologia 175: 223–231.

Bērziņš, B., & B. Pejler, 1987. Rotifer occurrence in relation to pH In May, L., R. Wallace, & A. Herzig (eds), Rotifer Symposium IV SE - 16. Springer Netherlands: 107–116.

Bettinetti, R., G. Morabito, & A. Provini, 2000. Phytoplankton assemblage structure and dynamics as indicator of the recent trophic and biological evolution of the western basin of Lake Como (N. Italy). Hydrobiologia 435: 177–190.

Biggs, B. J. F., & C. Kilroy, 2000. Stream Periphyton Monitoring Manual. NIWA for Ministry for the Environment.

Billen, G., J. Garnier, & P. Hanset, 1994. Modelling phytoplankton development in whole drainage networks: the RIVERSTRAHLER Model applied to the Seine river system. Hydrobiologia Kluwer Academic Publishers 289: 119–137.

Bmstedt, U., D. J. Gifford, X. Irigoien, A. Atkinson, & M. Roman, 2000. Feeding. ICES

Zooplankton Methodology Manual Academic Press 297–399.

Bogdan, G., & J. J. Gilbert, 1982. Seasonal patterns of feeding by natural populations of Keratella, Polyarthra, and Bosmina: Clearance rates, selectiveties, and contributions to community grazing. Limnology Oceanography 27: 918–934.

Bogdan, K., J. Gilbert, & P. Starkweather, 1980. In situ clearance rates of planktonic rotifers. Hydrobiologia 73: 73–77.

Boraas, M. E., 1983. Population-Dynamics of Food-Limited Rotifers in 2-Stage Chemostat Culture. Limnology and Oceanography 28: 546–563.

Bottrell, H. H., 1977. Quantitative studies on the Cladocera, Copepoda and Rotiferae of the river Thames and river Kennet at Reading. University of Reading.

Botts, P. S., B. A. Patterson, & D. W. Schloesser, 1996. Zebra Mussel Effects on Benthic Invertebrates: Physical or Biotic?. Journal of the North American Benthological Society [University of Chicago Press, Society for Freshwater Science] 15: 179–184.

Bowes, M. J., L. K. Armstrong, S. A. Harman, H. D. Wickham, P. M. Scarlett, C. Roberts, H. P. Jarvie, G. Old, E. Gozzard, N. Bachiller-Janeno, & D. Read, 2018. Weekly water quality monitoring data for the River Thames (UK) and its major tributaries (2009-2013): The Thames Initiative research platform. Earth System Science Data Discussions 1–24.

Bowes, M. J., E. Gozzard, A. C. Johnson, P. M. Scarlett, C. Roberts, D. S. Read, L. K. Armstrong, S. A. Harman, & H. D. Wickham, 2012. Spatial and temporal changes in chlorophyll-a concentrations in the River Thames basin, UK: Are phosphorus concentrations beginning to limit phytoplankton biomass?. Science of The Total Environment 426: 45–55.

Bowes, M. J., H. P. Jarvie, P. S. Naden, G. H. Old, P. M. Scarlett, C. Roberts, L. K. Armstrong, S. a. Harman, H. D. Wickham, & A. L. Collins, 2014. Identifying priorities for nutrient mitigation using river concentration-flow relationships: The Thames basin, UK. Journal of Hydrology Elsevier B.V. 517: 1–12.

Bowes, M. J., M. Loewenthal, D. S. Read, M. G. Hutchins, C. Prudhomme, L. K. Armstrong, S. A. Harman, H. D. Wickham, E. Gozzard, & L. Carvalho, 2016. Identifying multiple stressor controls on phytoplankton dynamics in the River Thames (UK) using high-frequency water quality data. Science of the Total Environment Elsevier B.V. 569–570: 1489–1499.

Brett, M. T., & D. C. Muller-Navarra, 1997. The role of highly unsaturated fatty acids in aquatic food web processes. Freshwater Biology 38: 483–499.

Bunt, C. M., H. J. Maclsaac, & W. G. Sprules, 1993. Pumping rates and projected filtering impacts of juvenile zebra mussels (*Dreissena polymorpha*) in western Lake Erie. Canadian Journal of Fisheries and Aquatic Sciences 50: 1017–1022.

Burger, D. F., I. D. Hogg, & J. D. Green, 2002. Distribution and abundance of zooplankton in the Waikato River, New Zealand. Hydrobiologia 479: 31–38.

Butterwick, C., S. I. Heaney, & J. F. Talling, 2005. Diversity in the influence of temperature on the growth rates of freshwater algae, and its ecological relevance. Freshwater Biology 50: 291–300.

Callieri, C., S. M. Karjalainen, & S. Passoni, 2002. Grazing by ciliates and heterotrophic nanoflagellates on picocyanobacteria in Lago Maggiore, Italy. Journal of Plankton Research 24: 785–796.

Carlotti, F., J. Giske, & F. Werner, 2000. Modeling zooplankton dynamics. ICES Zooplankton Methodology Manual Academic Press 571–667.

Carlough, L. a., & J. L. Meyer, 1989. Protozoans in two southeastern blackwater rivers and their importance to trophic transfer. Limnology and Oceanography 34: 163–177.

Carrick, H. J., G. L. Fahnenstiel, E. F. Stoermer, & R. G. Wetzel, 1991. The importance Lake Michigan of zooplankton-protozoan trophic couplings in Lake Michigan. Limnology and Oceanography 36: 1335–1345.

Casper, A. F., & J. H. Thorp, 2007. Diel and lateral patterns of zooplankton distribution in the St. Lawrence River. River Research and Applications 23: 73–85.

Chapra, S., G. Pelletier, & H. Tao, 2013. QUAL2K: A Modeling Framework for Simulating River and Stream Water Quality, Version 2.11: Documentation and Users Manual. 109.

Chételat, J., F. R. Pick, & P. B. Hamilton, 2006. Potamoplankton size structure and taxonomic composition: Influence of river size and nutrient concentrations. Limnology and Oceanography. , 681–689.

Clarke, K. R., 1993. Non-parametric multivariate analyses of changes in community structure. Australian Journal of Ecology 18: 117–143.

Cloot, A., & G. LE Roux, 1997. Modeling Algal Blooms in the Middle Vaal River : a Site Specific Approach. Wat. Res. 31: 271–279.

Cox, B. a, & P. G. Whitehead, 2009. Impacts of climate change scenarios on dissolved oxygen in

the River Thames, UK. Hydrology Research 40: 138.

Cox, E. J., 1996. Identification of freshwater diatoms from live material. Journal of the Marine Biological Association of the United Kingdom Cambridge University Press 76: 1117.

Croft, M. T., A. D. Lawrence, E. Raux-Deery, M. J. Warren, & A. G. Smith, 2005. Algae acquire vitamin B12 through a symbiotic relationship with bacteria. Nature 438: 90–93.

Crooks, S. M., & A. L. Kay, 2015. Simulation of river flow in te Thames ober 120 years: Evidence of change in rainfall-runoff response. Journal of Hydrology: Regional Studies Elsevier B.V. 4: 172–195.

Czerniawski, R., & M. Pilecka-Rapacz, 2011. Summer zooplankton in small rivers in relation to selected conditions. Central European Journal of Biology 6: 659–674.

De Manuel, J. B., 2000. The rotifers of spanish reservoirs: Ecological, systematical and zoogeographical remarks. Limnetica 19: 91–267.

de Ruyter van Steveninck, E. D., W. Admiraal, L. Breebaart, G. M. J. Tubbing, & B. Van Zanten, 1992. Plankton in the River Rhine: Structural and functional changes observed during downstream transport. Journal Of Plankton Research 14: 1351–1368.

Deneke, R., 2000. Review of rotifers and crustaceans in highly acidic environments of pH values ≤ 3. Hydrobiologia 433: 167–172.

Descy, J.-P., E. Everbecq, V. Gosselain, L. Viroux, & J. S. S. Smitz, 2003. Modelling the impact of benthic filter-feeders on the composition and biomass of river plankton. Freshwater Biology 48: 404–417.

Descy, J. P., 1993. Ecology of the phytoplankton of the River Moselle: effects of disturbances on community structure and diversity. Hydrobiologia 249: 111–116.

Descy, J. P., M. Leitao, E. Everbecq, J. S. Smitz, & J. F. Delige, 2012. Phytoplankton of the River Loire, France: A biodiversity and modelling study. Journal of Plankton Research 34: 120–135.

Descy, J. P., P. Sevais, J. S. Smits, G. Billen, & E. Everbecq, 1987. Phytoplankton biomass and production in the River Meuse (Belgium). Water Research 21: 1557–1566.

Desortová, B., & P. Punčochář, 2011. Variability of phytoplankton biomass in a lowland river: Response to climate conditions. Limnologica 41: 160–166.

Dickerson, K. D., K. A. Medley, & J. E. Havel, 2010. Spatial variation in zooplankton

community structure is related to hydrological flow units in the Missouri River, USA. River Research and Applications 26: 605–618.

Dong, L.-L., Y.-L. Xi, & L. Zhang, 2009. Effects of food concentration on life history traits of Brachionus calyciflorus clones in Jinghu Lake. The journal of applied ecology China 20: 370–375.

Edmondson, W. T., 1965. Reproductive Rate of Planktonic Rotifers as Related to Food and Temperature in Nature. Ecological Monographs. .

Engel, A., 2000. The role of transparent exopolymer particles (TEP) in the increase in apparent particle stickiness (alpha) during the decline of a diatom bloom. Journal of Plankton Research 22: 485–497.

Environment Agency, 2009. River Basin Management Plan - Thames River Basin District. Bristol, UK.

Environment Agency, 2016. Water for life and livelihoods Part 1: Humber river basin district River basin management plan. 107.

Environment Agency, 2017. Thames Water ordered to pay record £20 million for river pollution. Press release. , https://www.gov.uk/government/news/thames-water-ordered-to-pay-record-20-million-for-river-pollution.

Everbecq, E., V. Gosselain, L. Viroux, & J. P. Descy, 2001. Potamon: A dynamic model for predicting phytoplankton composition and biomass in lowland rivers. Water Research 35: 901–912.

Fahnenstiel, G. L., G. A. Lang, T. F. Nalepa, & T. H. Johengen, 1995. Effects of Zebra Mussel (Dreissena polymorpha) Colonization on Water Quality Parameters in Saginaw Bay, Lake Huron. Journal of Great Lakes Research Elsevier 21: 435–448.

Fehling, J., K. Davidson, C. Bolch, & P. Tett, 2006. Seasonally of Pseudo-nitzschia spp. (Bacillariophyceae) in western Scottish waters. Marine Ecology Progress Series 323: 91–105.

Ferrara, O., D. Vagaggini, & F. G. Margaritora, 2003. Zooplankton abundance and diversity in Lake Bracciano, Latium, Italy. Journal of Limnology 61: 169–175.

Ferris, J. M., & R. Christian, 1991. Aquatic primary production in relation to microalgal responses to changing light : a review. Aquatic Sciences 53: 187–217.

Frenken, T., M. Velthuis, L. N. De Senerpont Domis, S. Stephan, R. Aben, S. Kosten, E. Van

Donk, & D. B. Van de Waal, 2016. Warming accelerates termination of a phytoplankton spring bloom by fungal parasites. Global Change Biology 22: 299–309.

Fritsch, F. E., 1903. Further observations on the phytoplankton of the River Thames. Annals of Botany 17: 631–647.

Galindo, M. D., C. Guisande, & J. Toja, 1993. Reproductive investment of several rotifer species. Hydrobiologia 255–256: 317–324.

Galkovskaya, G. A., 1987. Planktonic rotifers and temperature. Hydrobiologia 147: 307–317.

Gärdes, A., M. H. Iversen, H.-P. Grossart, U. Passow, & M. S. Ullrich, 2011. Diatom-associated bacteria are required for aggregation of Thalassiosira weissflogii. The ISME journal 5: 436–445.

Garnier, J., G. Billen, & M. Coste, 1995. Seasonal succession of diatoms and Chlorophyceae in the drainage network of the Seine River: Observation and modeling. Limnology and Oceanography 40: 750–765.

Gasol, J. M., M. Comerma, J. C. García, J. Armengol, E. O. Casamayor, P. Kojecká, & K. Šimek, 2002. A transplant experiment to identify the factors controlling bacterial abundance, activity, production, and community composition in a eutrophic canyon-shaped reservoir. Limnology and Oceanography 47: 62–77.

Ger, K. A., P. Urrutia-Cordero, P. C. Frost, L. A. Hansson, O. Sarnelle, A. E. Wilson, & M. Lürling, 2016. The interaction between cyanobacteria and zooplankton in a more eutrophic world. Harmful Algae 54: 128–144.

Gillard, J., 2010. The pennate diatom life cycle: a genetic, physiological and biochemical study using Seminavis robusta as a new experimental model. Ghent University.

Gillett, N., Y. Pan, & C. Parker, 2009. Should only live diatoms be used in the bioassessment of small mountain streams?. Hydrobiologia 620: 135–147.

Gomes, L. F., H. R. Pereira, A. C. A. M. Gomes, M. C. Vieira, P. R. Martins, I. Roitman, & L. C. G. Vieira, 2019. Zooplankton functional-approach studies in continental aquatic environments: a systematic review. Aquatic Ecology 8: 191–203.

Górski, K., K. J. Collier, I. C. Duggan, C. M. Taylor, & D. P. Hamilton, 2013. Connectivity and complexity of floodplain habitats govern zooplankton dynamics in a large temperate river system. Freshwater Biology 58: 1458–1470.

Gosselain, V., J. P. Descy, L. Viroux, C. Joaquimjusto, A. Hammer, A. Metens, & S. Schweitzer,

1998a. Grazing by Large River Zooplankton - A Key to Summer Potamoplankton Decline - The Case of the Meuse and Moselle Rivers in 1994 and 1995. Hydrobiologia 369/370: 199–216.

Gosselain, V., L. Viroux, & J. P. Descy, 1998b. Can a community of small-bodied grazers control phytoplankton in rivers?. Freshwater Biology 39: 9–24.

Grishagin, I. V., 2015. Automatic cell counting with ImageJ. Analytical Biochemistry Elsevier Inc. 473: 63–65.

Gsell, A. S., L. N. de Senerpont Domis, E. van Donk, & B. W. Ibelings, 2013. Temperature alters host genotype-specific susceptibility to chytrid infection. PLoS ONE 8:.

Guisande, C., & N. Mazuelos, 1991. Reproduction pattern of Brachionus calyciflous Pallas at different food concentrations. Journal of Plankton Research 13: 279–286.

Ha, K., H. W. Kim, & G. J. Joo, 1998. The phytoplankton succession in the lower part of hypertrophic Nakdong River (Mulgum), South Korea. Hydrobiologia 369–370: 217–227.

Halbach, U., 1973. Life Table Data and Population Dynamics of the Rotifer Brachionus calyciflorus Pallas as Influenced by Periodically Oscillating Temperature In Wieser, W. (ed), Effects of Temperature on Ectothermic Organisms SE - 19. Springer Berlin Heidelberg: 217–228.

Halliday, S. J., R. A. Skeffington, M. J. Bowes, E. Gozzard, J. R. Newman, M. Loewenthal, E. J. Palmer-Felgate, H. P. Jarvie, & A. J. Wade, 2014. The water quality of the River Enborne, UK: Observations from high-frequency monitoring in a rural, lowland river system. Water (Switzerland) 6: 150–180.

Halliday, S. J., R. A. Skeffington, A. J. Wade, M. J. Bowes, D. S. Read, H. P. Jarvie, & M. Loewenthal, 2016. Riparian shading controls instream spring phytoplankton and benthic algal growth. Environmental Science: Processes & Impacts 18: 677–689.

Haney, J. F., 2013. An-image-based Key to the zooplankton of North America. version 5.0. , http://cfb.unh.edu/cfbkey/html/index.html.

Hansen, B., P. K. Bjørnsen, & P. J. Hansen, 1994. The size ratio between planktonic predators and their prey. Limnology and Oceanography 39: 395–403.

Hansen, P. J., P. K. Bjørnsen, & B. W. Hansen, 1997. Zooplankton grazing and growth: Scaling within the 2-2,000-µm body size range. Limnology and Oceanography 42: 687–704.

Harris, R. P., P. H. Wiebe, J. Lens, H. R. Skjoldal, & M. Huntley, 2000. Zooplankton

methodology manual. .

Havel, J. E., K. a. Medley, K. D. Dickerson, T. R. Angradi, D. W. Bolgrien, P. a. Bukaveckas, & T. M. Jicha, 2009. Effect of main-stem dams on zooplankton communities of the Missouri River (USA). Hydrobiologia 628: 121–135.

Hecky, R. E., & P. Kilham, 1988. Nutrient limitation of phytoplankton in freshwater and marine environments: A review of recent evidence on the effects of enrichment. Limnology and Oceanography .

Henderson, R., M. Chips, N. Cornwell, P. Hitchins, B. Holden, S. Hurley, S. a. Parsons, A. Wetherill, & B. Jefferson, 2008. Experiences of algae in UK waters: A treatment perspective. Water and Environment Journal 22: 184–192.

Hervé, V., J. Derr, S. Douady, M. Quinet, L. Moisan, & P. Lopes, 2012. Multiparametric Analyses Reveal the pH-Dependence of Silicon Biomineralization in Diatoms. PLoS ONE 7:.

Herzig, A., 1983. Comparative studies on the relationship between temperature and duration of embryonic development of rotifers. Hydrobiologia 104: 237–246.

Hill, M. O., 1973. Diversity and Evenness: A Unifying Notation and Its Consequences. Ecology 54: 427–432.

Hilton, J., M. O'Hare, M. J. Bowes, & J. I. Jones, 2006. How green is my river? A new paradigm of eutrophication in rivers. Science of The Total Environment 365: 66–83.

Hochberg, R., R. L. Wallace, & E. J. Walsh, 2015. Soft Bodies, Hard Jaws: An Introduction to the Symposium, with Rotifers as Models of Jaw Diversity. Integrative and comparative biology 55: 179–192.

Holmes, N. T. H., & B. a. Whitton, 1981. Phytoplankton of four rivers, the Tyne, Wear, Tees and Swale. Hydrobiologia. , 111–127.

Horne, J., 2009. The influence of grazing macroinvertebrates on the structure of benthic diatom assemblages: implications for biomonitoring. University of Sheffield.

Hu, J., S. Chi, & J. Hu, 2019. An attempt to measure longitudinal connectivity based on the community structure of phytoplankton. Environmental Monitoring and Assessment Environmental Monitoring and Assessment 191:.

Huang, W. E., A. Ferguson, A. C. Singer, K. Lawson, I. P. Thompson, R. M. Kalin, M. J. Larkin, M. J. Bailey, & A. S. Whiteley, 2009. Resolving genetic functions within microbial populations:

In situ analyses using rRNA and mRNA stable isotope probing coupled with single-cell raman-fluorescence in situ hybridization. Applied and Environmental Microbiology 75: 234–241.

Hudon, C., 2000. Phytoplankton assemblages in the St. Lawrence River, downstream of its confluence with the Ottawa River, Quebec, Canada. Canadian Journal of Fisheries and Aquatic Sciences 57: 16–30.

Hutchins, M. G., R. J. Williams, C. Prudhomme, M. J. Bowes, H. E. Brown, A. J. Waylett, & M. Loewenthal, 2016. Projections of future deterioration in UK river quality are hampered by climatic uncertainty under extreme conditions. Hydrological Sciences Journal Taylor & Francis 61: 2818–2833.

Hutchins, M. G., A. C. Johnson, A. Deflandre-Vlandas, S. Comber, P. Posen, & D. Boorman, 2010. Which offers more scope to suppress river phytoplankton blooms: Reducing nutrient pollution or riparian shading?. Science of The Total Environment 408: 5065–5077.

Hynes, H. B. N., 1970. The Ecology of Running Waters. Liverpool University Press.

Jack, J. D., & J. H. Thorp, 2002. Impacts of fish predation on an Ohio River zooplankton community. Journal of Plankton Research 24: 119–127.

Joaquim-Justo, C., C. Detry, F. Caufman, & J. P. Thomé, 2004. Feeding of planktonic rotifers on ciliates: A method using natural ciliate assemblages labelled with fluorescent microparticles. Journal of Plankton Research 26: 1289–1299.

John, D., A. J. Brooks, & B. Whitton, 2011. The Freshwater Algal Flora of the British Isles An Identification Guide to Freshwater and Terrestrial Algae. .

Keckeis, S., C. Baranyi, T. Hein, C. Holarek, P. Riedler, & F. Schiemer, 2003. The significance of zooplankton grazing in a floodplain system of the River Danube. Journal of Plankton Research 25: 243–253.

Khalifa, N., K. A. El-Damhogy, M. Reda Fishar, A. M. Nasef, & M. H. Hegab, 2015. Vertical distribution of zooplankton in Lake Nasser. Egyptian Journal of Aquatic Research National Institute of Oceanography and Fisheries 41: 177–185.

Kim, H.-W., S.-J. Hwang, & G.-J. Joo, 2000. Zooplankton grazing on bacteria and phytoplankton in a regulated large river (Nakdong River, Korea). Journal of Plankton Research 22: 1559–1577.

Kim, H. W., & G. J. Joo, 2000. The longitudinal distribution and community dynamics of

zooplankton in a regulated large river: A case study of the Nakdong River (Korea). Hydrobiologia 438: 171–184.

Kim, H. W., G. J. Joo, & N. Walz, 2001. Zooplankton Dynamics in the Hyper-Eutrophic Nakdong River System (Korea) Regulated by an Estuary Dam and Side Channels. International Review of Hydrobiology 86: 127–143.

King, C. E., 1966. Food, age, and the dynamics of a laboratory population of rotifers. Ecology 48: 111–128.

Kirk, K. L., 1997. Life-history responses to variable environments: Starvation and reproduction in planktonic rotifers. Ecology 78: 434–441.

Kobayashi, T., R. J. Shiel, P. Gibbs, & P. I. Dixon, 1998. Freshwater zooplankton in the Hawkesbury-Nepean River: Comparison of community structure with other rivers. Hydrobiologia 377: 133–145.

Köhler, J., 1993. Growth, production and losses of phytoplankton in the lowland River Spree. I. Population dynamics. Journal of Plankton Research 15: 335–349.

Kouzminov, A., J. Ruck, & S. A. Wood, 2007. New Zealand risk management approach for toxic cyanobacteria in drinking water. Australian and New Zealand Journal of Public Health 31: 275–281.

Kowe, R., R. E. Skidmore, B. a. Whitton, & a. C. Pinder, 1998. Modelling phytoplankton dynamics in the River Swale, an upland river in NE England. Science of the Total Environment. , 535–546.

Krammer, K., & H. Lange-Bertalot, 2000. Süßwasserflora von Mitteleuropa. Bacillariophyceae. Centrales, Fragilariaceae, Eunotiaceae. Teil 3. Süßwasserflora von Mitteleuropa. .

Kranzler, C. F., J. W. Krause, M. A. Brzezinski, B. R. Edwards, W. P. Biggs, M. Maniscalco, J. P. McCrow, B. A. S. Van Mooy, K. D. Bidle, A. E. Allen, & K. Thamatrakoln, 2019. Silicon limitation facilitates virus infection and mortality of marine diatoms. Nature Microbiology Springer US.

Kudoh, S., & M. Tokahashi, 2004. Fungal control of population changes of the planktonic diatom Asterionella Formosa in a shallow eutrophic lake. Journal of Phycology 26: 239–244.

Lack, T. J., 1971. Quantitative studies on the phytoplankton of the Rivers Thames and Kennet at Reading. Freshwater Biology 1: 213–224.

Lair, N., 2005. Abiotic vs. biotic factors: Lessons drawn from rotifers in the Middle Loire, a meandering river monitored from 1995 to 2002, during low flow periods. Hydrobiologia 546: 457–472.

Lair, N., 2006. A review of regulation mechanisms of metazoan plankton in riverine ecosystems: aquatic habitat versus biota. River Research and Applications 22: 567–593.

Lair, N., & P. Reyes-Marchant, 1997. The potamoplankton of the Middle Loire and the role of the “moving littoral” in downstream transfer of algae and rotifers. Hydrobiologia 356: 33–52.

Lampert, W., W. Fleckner, H. Rai, & B. E. Taylor, 1986. Phytoplankton control by grazing zooplankton: A study on the spring clear-water phase. Limnology and Oceanography 31: 478–490.

Lange-Bertalot, H., Hofmann, G., Werum, M. & Cantonati, M., 2017. Freshwater Benthic Diatoms of Central Europe: over 800 Common Species Used in Ecological Assessment. English edition with updated taxonomy and added species. Koeltz Scientific Books.

Lázár, A. N., A. J. Wade, & B. Moss, 2015. Modelling Primary Producer Interaction and Composition: an Example of a UK Lowland River. Environmental Modeling & Assessment Environmental Modeling & Assessment 21: 125–148.

Le Coz, M., S. Chambord, P. Meire, T. Maris, F. Azémar, J. Ovaert, E. Buffan-Dubau, J. C. Kromkamp, A. C. Sossou, J. Prygiel, G. Spronk, S. Lamothe, B. Ouddane, S. Rabodonirina, S. Net, D. Dumoulin, J. Peene, S. Souissi, & M. Tackx, 2017. Test of some ecological concepts on the longitudinal distribution of zooplankton along a lowland water course. Hydrobiologia 802: 175–198.

Lehman, J. T., 1988. Selective herbivory and its role in the evolution of phytoplankton growth strategies Growth and reproductive strategies of freshwater phytoplankton. Cambridge, USA: 369–387.

Lehman, J. T., & C. D. Sandgren, 1985. Species-specific rates of growth and grazing loss among freshwater algae. Limnology and Oceanography 30: 34–46.

Leonard, J. A., & H. W. Paerl, 2005. Zooplankton community structure, micro-zooplankton grazing impact, and seston energy content in the St. Johns river system, Florida as influenced by the toxic cyanobacterium Cylindrospermopsis raciborskii. Hydrobiologia 537: 89–97.

Li, Y., H. Liu, R. Yang, & L. Tang, 2019. Dynamics in a diffusive phytoplankton–zooplankton system with time delay and harvesting. Advances in Difference Equations The Author(s) 2019:

14–16.

Ljubesic, Z., I. Cetinić, D. Viličić, K. Mihalić, M. Caric, & G. Olujić, 2007. Spatial and temporal distribution of phytoplankton in a highly stratified estuary (Zrmanja, Adriatic Sea). Marine Ecology. .

Lucas, L. V., J. K. Thompson, & L. R. Brown, 2009. Why are diverse relationships observed between phytoplankton biomass and transport time?. Limnology and Oceanography. , 381–390.

Lucena-Moya, P., & I. C. Duggan, 2011. Macrophyte architecture affects the abundance and diversity of littoral microfauna. Aquatic Ecology 45: 279–287.

Lurling, M., F. Eshetu, E. Faaseen, S. Kosten, & V. Huszar, 2013. Comparison of cyanobacterial and green algal growth rates at different temperatures. Freshwater Biology 58: 552–559.

Lynch, M., 1980. The Evolution of Cladoceran Life Histories. The Quarterly Review of Biology, 55: 23–42.

Maier, M. A., & T. D. Peterson, 2017. Prevalence of chytrid parasitism among diatom populations in the lower Columbia River (2009–2013). Freshwater Biology 62: 414–428.

Makarewicz, J. C., & G. E. Likens, 1979. Structure and Function of the Zooplankton Community of Mirror Lake , New Hampshire. Ecological Society of America 49: 109–127.

May, L., & J. a Bass, 1998. A study of rotifers in the River Thames, England, April-October, 1996. Hydrobiologia 387: 251–257.

Medeiros, E. S. F., & A. H. Arthington, 2008. The importance of zooplankton in the diets of three native fish species in floodplain waterholes of a dryland river, the Macintyre River, Australia. Hydrobiologia 614: 19–31.

Mellanby, H., 1951. Animal life in fresh water. A guide to British fresh-water invertebrates. Methuen & Co. Ltd, London.

Mellina, E., & J. B. Rasmussen, 1994. Patterns in the Distribution and Abundance of Zebra Mussel (Dreissena polymorpha) in Rivers and Lakes in Relation to Substrate and Other Physicochemical Factors. Canadian Journal of Fisheries and Aquatic Sciences 51: 1024–1036.

Michaloudi, E., S. Papakostas, G. Stamou, V. Neděla, E. Tihlaříková, W. Zhang, & S. A. J. Declerck, 2018. Reverse taxonomy applied to the Brachionus calyciflorus cryptic species complex: Morphometric analysis confirms species delimitations revealed by molecular phylogenetic analysis and allows the (re)description of four species. PLOS ONE Public Library

of Science 13:.

Millennium Ecosystem Assessmen, 2005. Ecosystems and Human Well-being: Synthesis. Island Press, Washington, DC.

Minchin, D., & A. Zaiko, 2013. Variability of Zebra Mussel (Dreissena polymorpha) Impacts in the Shannon River System, Ireland. Quagga and Zebra Mussels 587–598.

Mitrofanova, E., 2015. Seasonal patterns of Potamoplankton in a Large Lowland River of Temperate zone (Upper Ob as a case study, Russia). Int. J. Environ. Res. 9: 697–710.

Moorhouse, H. L., D. S. Read, S. McGowan, M. Wagner, C. Roberts, L. K. Armstrong, D. J. E. Nicholls, H. D. Wickham, M. G. Hutchins, & M. J. Bowes, 2018. Characterisation of a major phytoplankton bloom in the River Thames (UK) using flow cytometry and high performance liquid chromatography. Science of the Total Environment Elsevier B.V. 624: 366–376.

Morgan, A. M., T. V Royer, M. B. David, & L. E. Gentry, 2006. Relationships among nutrients, chlorophyll-a, and dissolved oxygen in agricultural streams in Illinois. Journal of environmental quality 35: 1110–1117.

Müller-Navarra, D., & W. Lampert, 1996. Seasonal patterns of food limitation in Daphnia galeata: separating food quantity and food quality effects. Journal of Plankton Research 18: 1137–1157.

National River Flow Archive, 2016. Catchment Spatial Data. NERC (CEH) Contains Ordnance Survey data © Crown copyright and database right 2012.

Neal, C., H. P. Jarvie, P. J. A. Withers, B. A. Whitton, & M. Neal, 2010. The strategic significance of wastewater sources to pollutant phosphorus levels in English rivers and to environmental management for rural, agricultural and urban catchments. Science of the Total Environment Elsevier B.V. 408: 1485–1500.

Necchi Jr, O., 2004. Light-Related Photosynthetic Characteristics of Lotic Macroalgae. Hydrobiologia 525: 139–155.

Obertegger, U., H. Smith, G. Flaim, & R. L. Wallace, 2011. Using the guild ratio to characterize pelagic rotifer communities. Hydrobiologia 662: 157–162.

Oh, H. J., H. G. Jeong, G. S. Nam, Y. Oda, W. Dai, E. H. Lee, D. Kong, S. J. Hwang, & K. H. Chang, 2017. Comparison of taxon-based and trophi-based response patterns of rotifer community to water quality: applicability of the rotifer functional group as an indicator of water

quality. Animal Cells and Systems 21: 133–140.

Ooms-Wilms, A. L., 1997. Are bacteria an important food source for rotifers in eutrophic lakes?. Journal of Plankton Research 19: 1125–1141.

Özer, T., İ. Açıkgöz Erkaya, M. A. T. Koçer, A. U. Udoh, & D. Yalçın Duygu, 2019. Spatial and temporal variations in composition of algae assemblages with environmental variables in an urban stream (Ankara, Turkey). Environmental Monitoring and Assessment 191: 387.

Pace, M., & J. D. Orcutt, 1981. The relative importance of protozoans, rotifers, and crustaceans in a freshwater zooplankton community. Limnology and Oceanography 26: 822–830.

Paerl, H. W., 1988. Growth and reproductive strategies of freshwater blue-green algae (Cyanobacteria) In Sandgren, C. D. (ed), Growth and reproductive strategies of freshwater phytoplankton. Cambridge University Press, Cambridge, USA: 261–315.

Palazzo, F., C. C. Bonecker, & A. P. C. Fernandes, 2008. Resting cladoceran eggs and their contribution to zooplankton diversity in a lagoon of the Upper Paran?? River floodplain. Lakes and Reservoirs: Research and Management 13: 207–214.

Parkes, S. M., & I. C. Duggan, 2012. Are zooplankton invasions in constructed waters facilitated by simple communities?. Diversity and Distributions 18: 1199–1210.

Passow, U., 2002. Production of transparent exopolymer particles (TEP) by phyto- and bacterioplankton. Marine Ecology Progress Series 236: 1–12.

Perbiche-Neves, G., C. E. F. da Rocha, & M. G. Nogueira, 2014. Estimating cyclopoid copepod species richness and geographical distribution (Crustacea) across a large hydrographical basin: comparing between samples from water column (plankton) and macrophyte stands. Zoologia (Curitiba) 31: 239–244.

Peterson, B., 2015. Performance Analysis in R. .

Peterson, B. G., & P. Carl, 2018. PerformanceAnalytics: Econometric Tools for Performance and Risk Analysis. .

Poff, N. L., J. D. Allan, M. B. Bain, J. R. Karr, K. L. Prestegaard, B. D. Richter, R. E. Sparks, & J. C. Stromberg, 1997. The natural flow regime: a paradigm for river conservation and restoration. BioScience 47: 769–784.

Pontin, R., 1978. Freshwater planktonic and semi-palnktonic rotifera of the British Isles. .

Pourriot, R., & T. W. Snell, 1983. Resting eggs in rotifers. Hydrobiologia 104: 213–224.

Quinlan, E. L., C. H. Jett, & E. J. Phlips, 2009. Microzooplankton grazing and the control of phytoplankton biomass in the Suwannee River estuary, USA. Hydrobiologia 632: 127–137.

R Core Team, 2017. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Ratkowsky, D. A., R. K. Lowry, T. A. McMeekin, A. N. Stokes, & R. E. Chandler, 1983. Model for bacterial culture growth rate throughout the entire biokinetic temperature range. Journal of Bacteriology 154: 1222–1226.

Read, D. S., M. J. Bowes, L. K. Newbold, & A. S. Whiteley, 2014. Weekly flow cytometric analysis of riverine phytoplankton to determine seasonal bloom dynamics. Environmental Science: Processes & Impacts 16: 594.

Read, D. S., H. S. Gweon, M. J. Bowes, L. K. Newbold, D. Field, M. J. Bailey, & R. I. Griffiths, 2015. Catchment-scale biogeography of riverine bacterioplankton. ISME Journal Nature Publishing Group 9: 516–526.

Reckendorfer, W., H. Keckeis, G. Winkler, & F. Schiemer, 1999. Zooplankton abundance in the River Danube, Austria: the significance of inshore retention. Freshwater Biology 41: 583–591.

Reynolds, C. S., 1984. The ecology of freshwater phytoplankton. Cambridge University Press, Cambridge, UK.

Reynolds, C. S., 2000. Hydroecology of river plankton: the role of variability in channel flow. Hydrological Processes 14: 3119–3132.

Reynolds, C. S., & J.-C. Descy, 1996. The production biomass and structure of phytoplankton in large rivers. Arch. Hydrobiol. , 161–187.

Reynolds, C. S., & M. S. Glaister, 1993. Spatial and temporal changes in phytoplankton abundance in the upper and middle reaches of the River Severn. River Systems 9: 1–22.

Ricci, C., 2001. Dormancy patterns in rotifers. Hydrobiologia 446–447: 1–11.

Rice, C. H., 1938. Studies in the phytoplankton of the River Thames (1928-1932). II. Annals of Botany 2: 559–581.

Rice, S. P., R. I. Ferguson, & T. B. Hoey, 2006. Tributary control of physical heterogeneity and biological diversity at river confluences. Canadian Journal of Fisheries and Aquatic Sciences 63: 2553–2566.

Rinta-Kanto, J. M., S. Sun, S. Sharma, R. P. Kiene, & M. A. Moran, 2012. Bacterial community

transcription patterns during a marine phytoplankton bloom. Environmental Microbiology 14: 228–239.

Rossetti, G., P. Viaroli, & I. Ferrari, 2009. Role of abiotic and biotic factors in structuring the metazoan plankton community in a lowland river. River research and applications 24: 941–959.

Rothhaupt, K. O., 1990a. Changes of the functional responses of the rotifers Brachionus rubens and Brachionus calyciflorus with particle sizes. Limnol. Oceanogr. 35: 24–32.

Rothhaupt, K. O., 1990b. Differences in particle size-dependent feeding efficiencies of closely related rotifer species. Limnology and Oceanography 35: 16–23.

Rothhaupt, K. O., 1990c. Population growth rates of two closely related rotifer species: effects of food quantity, particle size, and nutritional quality. Freshwater Biology Blackwell Publishing Ltd 23: 561–570.

Rueden, C. T., J. Schindelin, M. C. Hiner, B. E. DeZonia, A. E. Walter, E. T. Arena, & K. W. Eliceiri, 2017. ImageJ2: ImageJ for the next generation of scientific image data. BMC Bioinformatics BMC Bioinformatics 18: 1–26.

Ruse, L., & A. Love, 1997. Predicting Phytoplankton Composition in the River Thames, England. Regulated Rivers: Research & Management 13: 171–183.

Ruse, L. P., & A. J. Hutchings, 1996. Phytoplankton composition of the River Thames in relation to certain environmental variables. Arch. Hydrobiol. 113: 189–201.

Sanders, R. W., D. Leeper, C. H. King, & K. G. Porter, 1994. Grazing by rotifers and crustacean zooplankton on nanoplanktonic protists. Hydrobiologia 288: 167–181.

Sanders, R. W., K. G. Porter, S. J. Bennett, & A. E. Debiase, 1989. Seasonal patterns of bacterivory by flagellates, ciliates, rotifers, and cladocerans in a freshwater planktonic community. Limnology and Oceanography 34: 673–687.

Sandgren, C. D. (ed), 1988. Growth and reproductive strategies of freshwater phytoplankton. Cambridge University Press, Cambridge, USA.

Scherwass, A., T. Bergfeld, A. Schol, M. Weitere, & H. Arndt, 2010. Changes in the plankton community along the length of the River Rhine: Lagrangian sampling during a spring situation. Journal of Plankton Research 32: 491–502.

Schiemer, F., H. Keckeis, W. Reckendorfer, & G. Winkler, 2001. The “inshore retention concept” and its significance for large rivers. River Systems 12: 509–516.

Schöl A., Kirchesch ., Bergfeld T., Schöl F., Borcherding J., M. D., 2002. Modelling the Chlorophyll a Content of the River Rhine – Interrelation between Riverine Algal Production and Population Biomass of Grazers, Rotifers and the Zebra Mussel, Dreissena polymorpha. Internat. Rev. Hydrobiol. , 295–317.

Segers, H. H., 2007. Annotated cheklist of the rotifers (Phylum Rotifera), with notes on nomenclatures, taxonomy and distribution. Zootaxa. .

Sellers, T., & P. Bukaveckas, 2003. Phytoplankton production in a large, regulated river: A modeling and mass balance assessment. Limnology and Oceanography 48: 1476–1487.

Sellner, K. G., D. C. Brownlee, M. H. Bundy, S. G. Brownlee, & K. R. Braun, 1993. Zooplankton Grazing in a Potomac River Cyanobacteria Bloom. Estuaries 16: 859.

Shammi, M., X. Pan, K. M. G. Mostofa, D. Zhang, & C. Q. Liu, 2017. Photo-flocculation of microbial mat extracellular polymeric substances and their transformation into transparent exopolymer particles: Chemical and spectroscopic evidences. Scientific Reports Springer US 7: 1–12.

Sherr, E. B., & B. F. Sherr, 2002. Significance of predation by protists. Antonie van Leeuwenhoek 81: 293–308.

Simek, K., K. Hornak, M. Masin, U. Christaki, J. Nedoma, M. G. Weinbauer, & J. R. Dolan, 2003. Comparing the effects of resource enrichment and grazing on a meso-eutrophic reservoir. Aquatic Microbial Ecology 31: 123–135.

Šimek, K., & V. Straškrabová, 1992. Bacterioplankton production and protozoan bacterivory in a mesotrophic reservoir. Journal of Plankton Research 14: 773–787.

Sims, P., D. Mann, & L. Medlin, 2006. Evolution of the diatoms: Insights from fossil, biological and molecular data. Phycologia 45: 361–402.

Sinistro, R., M. L. Sánchez, F. Unrein, M. R. Schiaffino, I. Izaguirre, & L. Allende, 2015. Responses of phytoplankton and related microbial communities to changes in the limnological conditions of shallow lakes: a short-term cross-transplant experiment. Hydrobiologia 752: 139–153.

Sládeček, V., 1983. Rotifers as indicators of water quality. Hydrobiologia 100: 169–201.

Sluss, T. D., G. A. Cobbs, & J. H. Thorp, 2008. Impact of turbulence on riverine zooplankton : a mesocosm experiment. Freshwater Biology 1999–2010.

Smith, J. T., R. T. Clarke, & M. J. Bowes, 2010. Are groundwater nitrate concentrations reaching a turning point in some chalk aquifers?. Science of the Total Environment Elsevier B.V. 408: 4722–4732.

Soballe, D. M., & B. L. Kimmel, 1987. A large-scale comparison of factors influencing phytoplankton abundance in rivers, lakes, and impoundments. Ecology 68: 1943–1954.

Starkweather, P., 1980. Aspects of the feeding behavior and trophic ecology of suspension-feeding rotifers. Hydrobiologia 73: 63–72.

Stauber, J. L., & S. W. Jeffrey, 1988. Photosynthetic pigments in fifty-one species of marine diatoms. Journal of Phycology 24: 158–172.

Stelzer, C., 1998. Population growth in planktonic rotifers. Does temperature shift the competitive advantage for different species?. Rotifera VIII: A Comparative Approach 387/388: 349–353.

Stemberger, R. S., & J. J. Gilbert, 1985. Body Size, Food Concentration, and Population Growth in Planktonic Rotifers. Ecology 66: 1151–1159.

Stemberger, R. S., J. J. Gilbert, J. J. Gilbert, & R. S. Stemberger, 1987. Rotifer Threshold Food Concentrations and the Size-Efficiency Hypothesis. Ecological Society of America 68: 181–187.

Sterner, R. W., 1989. The Role of Grazers in Phytoplankton Succession Plankton Ecology SE - 4. Springer Berlin Heidelberg: 107–170.

Štrojsová, A., J. Vrba, J. Nedoma, & K. Šimek, 2005. Extracellular phosphatase activity of freshwater phytoplankton exposed to different in situ phosphorus concentrations. Marine and Freshwater Research 56: 417–424.

Survey British Geological Survey, 2018. 1:625 000 scale. Surface Geology. Bedrock and Superficial. Geology of Britain viewer. NERC, http://mapapps.bgs.ac.uk/geologyofbritain/home.html.

Taylor, J. C., W. R. Harding, & C. G. M. Archibald, 2007. An Illustrated Guide to Some Common Diatom Species from South Africa An Illustrated Guide to Some Common Diatom.

Telesh, I. V., M. Rahkola, & M. Viljanen, 1998. Carbon content of some freshwater rotifers. Hydrobiologia 387/388: 355–360.

Thackeray, S. J., 2007. Crustacean zooplankton species richness and productivity: To what extent do the conclusions depend upon the choice of metrics?. Oikos 116: 614–628.

Thayer, G. W., D. E. Hoss, M. A. Kjelson, W. F. Hettler, & M. W. Lacroix, 1974. Biomass of Zooplankton in the Newport River Estuary and the Influence of Postlarval Fishes. Chesapeake Science 15: 9.

Thebault, J.-M., & A. Qotbi, 1999. A model of phytoplankton development in the Lot River (France). Water Research 33: 1065–1079.

Thompson, J. M., A. J. D. Ferguson, & C. S. Reynolds, 1982. Natural filtration rates of zooplankton in a closed system: the derivation of a community grazing index. Journal of Plankton Research 4: 545–560.

Thorp, J. H., A. R. Black, K. H. Haag, & J. D. Wehr, 1994. Zooplankton Assemblages in the Ohio River: Seasonal, Tributary, and Navigation Dam Effects. Canadian Journal of Fisheries and Aquatic Sciences 51: 1634–1643.

Thorp, J. H., & M. D. Delong, 1994. The Riverine Productivity Model: An Heuristic View of Carbon Sources and Organic Processing in Large River Ecosystems. Oikos 70: 305.

Tonkin, J. D., F. Altermatt, D. S. Finn, J. Heino, J. D. Olden, S. U. Pauls, & D. A. Lytle, 2017. The role of dispersal in river network metacommunities: Patterns, processes, and pathways. Freshwater Biology 141–163.

Tranvik, L. J., K. G. Porter, & J. M. Sieburth, 1989. Oecologia a common freshwater phytoplankter. Oecologia 78: 473–476.

Tuomisto, H., 2010. A consistent terminology for quantifying species diversity? Yes, it does exist. Oecologia 164: 853–860.

UN Environment, 2017. A Framework for Freshwater Ecosystem Management. .

Unrein, F., I. O'Farrell, I. Izaguirre, R. Sinistro, M. dos Santos Afonso, & G. Tell, 2010. Phytoplankton response to pH rise in a N-limited floodplain lake : relevance of N2 -fixing heterocystous cyanobacteria. Aquatic Sciences 179–190.

van Dijk, G. M., & B. van Zanten, 1995. Seasonal changes in zooplankton abundance in the lower Rhine during 1987–1991. Hydrobiologia 304: 29–38.

Van Nieuwenhuyse, E., & J. Jones, 1996. Phosphorus-chlorophyll relationship in temperate streams and its variation with stream catchment area. Canadian Journal of Fisheries and Aquatic Sciences 53: 99–105.

Vanderploeg, H. A., J. R. Liebig, W. W. Carmichael, M. A. Agy, T. H. Johengen, G. L.

Fahnenstiel, & T. F. Nalepa, 2001. Zebra mussel (Dreissena polymorpha) selective filtration promoted toxic Microcystis blooms in Saginaw Bay (Lake Huron) and Lake Erie. Canadian Journal of Fisheries and Aquatic Sciences 58: 1208–1221.

Vannote, R. L., W. G. Minshall, K. W. Cummins, J. R. Sedell, C. E. Cushing, G. W. Minshall, K. W. Cummins, J. R. Sedell, & C. E. Cushing, 1980. The River Continuum Concept. Canadian Journal of Fisheries and Aquatic Sciences NRC Research Press 37: 130–137.

Vargas, C. A., R. Escribano, & S. Poulet, 2006. Phytoplankton food quality determines time windows for successful zooplankton reproductive pulses. Ecology Wiley-Blackwell 87: 2992–2999.

Viroux, L., 1997. Zooplankton Development in 2 Large Lowland Rivers, the Moselle (France) and the Meuse (Belgium), in 1993. Journal of Plankton Research 19: 1743–1762.

Viroux, L., 1999. Zooplankton distribution in flowing waters and its implications for sampling: case studies in the River Meuse (Belgium) and the River Moselle (France, Luxembourg). Journal of Plankton Research 21: 1231–1248.

Wade, A. J., D. Butterfield, & P. G. Whitehead, 2006. Towards an improved understanding of the nitrate dynamics in lowland, permeable river-systems: Applications of INCA-N. Journal of Hydrology 330: 185–203.

Wahl, D. H., J. Goodrich, M. A. Nannini, J. M. Dettmers, & D. A. Soluk, 2008. Exploring riverine zooplankton in three habitats of the Illinois River ecosystem: where do they come from?. Limnology and Oceanography 53: 2583–2593.

Wallace, R. L., 2002. Rotifers: exquisite metazoans. Integrative and comparative biology 42: 660–667.

Wallace, R. L., & H. A. Smith, 2001. Rotifera eLS. John Wiley & Sons, Ltd.

Walz, N., 1987. Comparative population dynamics of the rotifers Brachionus angularis and Keratella cochlearis. Population (English Edition) 213: 209–213.

Walz, N., 1997. Rotifer life history strategies and evolution in freshwater plankton communities Evolutionary Ecology of Freshwater Animals. Springer Basel AG: 199–151.

Waylett, A. J., M. G. Hutchins, A. C. Johnson, M. J. Bowes, & M. Loewenthal, 2013. Physico-chemical factors alone cannot simulate phytoplankton behaviour in a lowland river. Journal of Hydrology Elsevier B.V. 497: 223–233.

Wehr, J. D., & J.-P. Descy, 1998. Use of phytoplankton in large river management. J. Phycol. 34: 741–749.

Weisse, T., H. Mueller, R. M. Pinto-Coelho, A. Schweizer, D. Springmann, & G. Baldringer, 1990. Response of the microbial loop to the phytoplankton spring bloom in a large prealpine lake. Limnology and Oceanography 35: 781–794.

Wetzel, R. G., 2001. Limnology. Limnology. Elsevier.

Whitehead, P. G., G. Bussi, M. J. Bowes, D. S. Read, M. G. Hutchins, J. A. Elliott, & S. J. Dadson, 2015. Dynamic modelling of multiple phytoplankton groups in rivers with an application to the Thames river system in the UK. Environmental Modelling & Software Elsevier Ltd 74: 75–91.

Whitehead, P. G., & G. M. Hornberger, 1984. Modelling algal behaviour in the river Thames. Water Research 18: 945–953.

Whitehead, P., R. L. Wilby, R. W. Battarbee, M. Kernan, & A. J. Wade, 2009. A review of the potential impacts of climate change on surface water quality. Hydrological Sciences Journal 54: 101–123.

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.

Wilson, C. J., & R. W. Holmes, 1981. The ecological importance of distinguishing between living and dead diatoms in estuarine sediments. British Phycological Journal 16: 345–349.

World Health Organization, 2011. Guidelines for drinking-water quality, fourth edition. WHO chronicle. World Health Organization.

Wu, L., & D. Culver, 1991. Zooplankton Grazing and Phytoplankton Abundance: an Assessment Before and After Invasion of Dreissena Polymorpha. Journal of Great Lakes Research Elsevier 17: 425–436.

Wu, N., B. Schmalz, & N. Fohrer, 2011. Distribution of phytoplankton in a German lowland river in relation to environmental factors. Journal of Plankton Research 33: 807–820.

Zhao, K., K. Song, Y. Pan, L. Wang, L. Da, & Q. Wang, 2017. Metacommunity structure of zooplankton in river networks: Roles of environmental and spatial factors. Ecological Indicators Elsevier Ltd 73: 96–104.

Zhao, K., L. Wang, C. Riseng, K. Wehrly, Y. Pan, K. Song, L. Da, W. Pang, Q. You, H. Tian, S. Liu, & Q. Wang, 2018. Factors determining zooplankton assemblage difference among a man-

made lake, connecting canals, and the water-origin river. Ecological Indicators Elsevier 84: 488–496.

Zimmermann-Timm, H., H. Holst, & H. Kausch, 2007. Spatial dynamics of rotifers in a large lowland river, the Elbe, Germany: How important are retentive shoreline habitats for the plankton community?. Hydrobiologia 593: 49–58.