The impact of climate change and brownification on primary and bacterial production

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Abstract

Climatic condition is what ultimately frames all ecosystems and is now undergoing dramatic change. IPCC models predict, in 100 years, a temperature increase between 2-5 ˚C and, as a consequence of warmer and wetter conditions, increased humic content in northern temperate freshwater systems. To study the impact of increasing temperature and humic content on shallow freshwaters a long-term outdoor mesocosm experiment was performed. Five different treatments were used, where temperature and water colour was gradually increased simultaneously to correspond to a future scenario of 100 years. This resulted in +5 ˚C and 250% increase in absorbance (as a proxy for humic content) at the highest treatment compared to the control. No consistent significant difference in either primary or bacterial production between the treatments was observed. There was a steady increase of PP during spring and a tendency in total cumulative PP, summarized for the whole experimental period, until intermediate treatment effect. Further, a tendency for increasing heterotrophy was found during June and July. The condition of fish was highest at intermediate treatment effect. Several factors can possibly control PP and BP, such as nutrient limitation and predation as well as different stable states. Since increasing heterotrophy has been suggested, due to climate change, it is of great importance to further investigate the question of how the basal production will be affected and how this shapes freshwater systems, considering both ecosystem and societal values.

Key words: Primary production, Bacterial production, Food web efficiency, Climate change, Global change, Autotrophy, Heterotrophy, Brownification, DOC, Temperature

Introduction

Climate change is one of the most challenging questions of our time and will have both direct and indirect effects on nature as well as society. Depending on present climate conditions the effects of climate change will differ between geographical locations. In a time frame of 100 years, predictions states a temperature increase of between 2 and 5 ˚C in northern temperate systems (Christensen et al. 2007). Along with increasing temperatures, the hydrological cycle will be affected with some areas experiencing increased average precipitation (Zhang et al. 2007). This will have severe effects on all ecosystems, terrestrial as aquatic.

Temperature

All trophic levels in the pelagic food web will be affected by increasing temperature through its impact on all vital rates. Several studies have shown that the effect on phytoplankton communities can be severe as well as complex (Behrenfeld et al. 2006, Lewandowska and Sommer 2010, Tadonleke 2010, Kosten et al. 2012, Lewandowska et al. 2012). According to Hansson et al. (2013) the effects of temperature is context dependent. In a two trophic system, i.e. phytoplankton and zooplankton, phytoplankton biomass decreased caused by heavy zooplankton grazing. In a three trophic system, i.e. phytoplankton, zooplankton and fish, the biomass of phytoplankton increased caused by fish predation on zooplankton that released phytoplankton from predation. Furthermore, it has been shown that cyanobacteria will be increasingly abundant in shallow lakes (Kosten et al. 2012). This will have implications on both the phytoplankton and zooplankton community as the light penetration decreases and the concentration of toxins produced by the cyanobacteria will increase (Hansson et al. 2007). Thus, an increased temperature will not only have direct, but also indirect effects on the phytoplankton community. Along with the autotrophic food web there is also the microbial food web, based on heterotrophic bacteria consuming dissolved organic carbon (DOC). There have been a convincing number of studies showing temperature-dependent bacterial production in both marine and freshwater systems (White et al. 1991, Staroscik and Smith 2004).

Dissolved organic carbon

Dissolved organic carbon (DOC) is composed by carbon, nitrogen, phosphorous, humic acids and other coloured substances (Jansson et al. 2000). Since the 1970's there has been observations of increasing water colour in large parts of the northern hemisphere, also known as brownification (Findlay 2005, Hruska et al. 2009, Kritzberg and Ekstrom 2012). DOC mainly affects lake ecosystems by changing two different abiotic factors, light availability and nutrient content (Brönmark and Hansson 2005). As the concentration of DOC increases, the light availability decreases and the carbon content increases. This will have implications for all trophic levels in aquatic ecosystems. In lakes and rivers, there are two major sources of DOC, allochthonous and autochthonous carbon. The allochthonous source originates from the surrounding catchment and is mainly transported by surface runoff to lakes and rivers. As a consequence of climate change, the allochthonous carbon is predicted to be increasingly important as precipitation and runoff increases (Meier 2006). Beside allochthonous derived carbon there is also carbon produced within aquatic systems, autochthonous carbon. The most important source of autochthonous carbon is primary production, and is a rest product excreted during photosynthesis (Cole 1982). Phytoplankton are negatively affected by an increase in DOC as light availability decreases and thereby restrict photosynthesis (Karlsson et al. 2009). Moreover, bacterial biomass are generally positively correlated with DOC (Tranvik 1988). In clear waters with low humic content many scientists have found a strong correlation between primary production (PP) and bacterial production (BP) (Cole 1982, Lovell and Konopka 1985). Hence, BP is positively affected by an increase in autochthonous carbon. Since terrestrial derived carbon probably has

undergone transformation and degradation along its way to the water body, this allochthonous source is thought to be less available for bacteria. Guillemette et al. (2013) was comparing the degradation dynamics of algal and terrestrial carbon by bacteria and found that autochthonous carbon was more readily degraded, as also suggested by Kritzberg et al. (2004).

Food web efficiency

There is a difference in the amount of trophic levels between the microbial food web and the traditional phytoplankton based food web. The energy in bacteria based production is transferred mainly via phagotrophic flagellates (Jansson et al. 1999). This means an extra trophic level in the transfer of energy along the food chain in heterotrophic compared to autotrophic systems. At every trophic level in the food web there is a loss of 70-90 % of the energy transferred (Sommer et al. 2002). Hence, an increase of DOC in aquatic ecosystems will therefore give lower food web efficiency (FWE) if the system changes from autotrophy to heterotrophy. In a study examining the ratio between bacterial and phytoplankton production (BP:PP ratio) and FWE it was shown that the productivity of mesozooplankton was lower in bacteria based compared to phytoplankton based production (Berglund 2007). On the contrary, a study conducted by Lefebure et al. (2013) did not reveal any difference in FWE comparing heterotrophic and autotrophic marine systems.

Implications

The potential ecological changes in aquatic ecosystems with climate change might have far reaching implications for the value of lakes and rivers. If heterotrophy will increase, as suggested by Moss (2010) and Yvon-Durocher et al. (2010), the role of aquatic systems in the global carbon cycle will change. Today many lakes and rivers are considered autotrophic and thus working as a sink for carbon, if heterotrophy increases these systems will instead function as a source of carbon dioxide (CO_2). The worlds buffering capacity against increasing levels of atmospheric CO_2 is therefore directly affected by lakes metabolic balance. In the IPCC models such negative biological feedbacks are not accounted for, which can lead to an underestimation of the predicted consequences (Moss 2010). Along with physical changes, there is also a social aspect of climate change. The possible ecological consequences will most likely also change our use of lakes and rivers. For instance, recreational angling does contribute to both local and national economies as well as life quality (Arlinghaus et al. 2002). These socio-ecological interactions will change with a change in fish composition and the possible reduction in fish production (Ficke et al. 2007).

To my knowledge, there has not been any study on the effects of climate change regarding simultaneous increased temperature and water colour on the primary and bacterial production and the balance between these in limnic systems.

Here I investigated the effect of temperature and brownification on the basal production (bacteria and phytoplankton) in a mesocosm experiment with a simultaneous increase of temperature and water colour along a gradient. I expected BP to increase with both temperature and water colour, thus observe the highest production at the highest treatment. PP was hypothesized to be highest at moderate treatment, where temperature would increase production at low treatment but at high treatment be suppressed by water colour. Further, I expected this to be reflected in the balance between BP and PP, where a gradual shift from autotrophy to heterotrophy would be seen along the treatment gradient.

Method

Experimental set-up

An outdoor mesocosm experiment was run between 3rd of April and 16th of October 2013. The experiment consisted of 24 insulated polyethylene enclosures of 400 L with approximately 1m in depth. All enclosures were filled with water from Lake Krankesjön, a mesotrophic clear water lake in southern Sweden. Temperature was increased

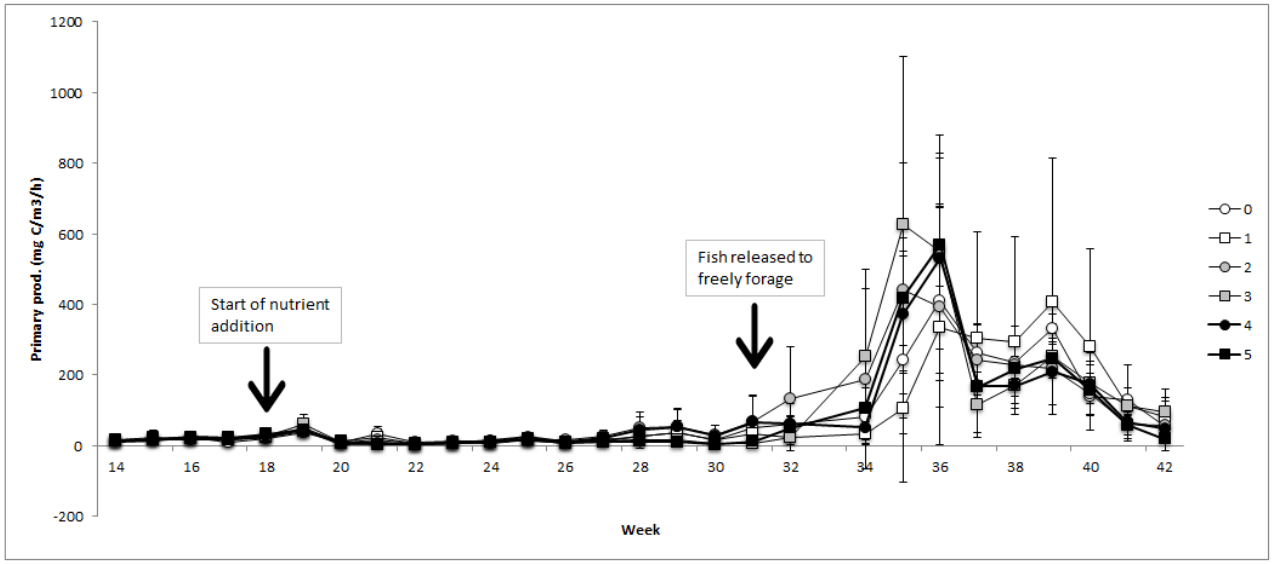

Fig 1. Effect of temperature and water colour on primary production from the 3rd of April until the 16th of October. The lines represent different treatments where the numbers correspond to the temperature increase in ˚C. For each ˚C the water colour was increased with 50%, resulting in a maximum of 5 ˚C and 250% increase relative to the control. The first arrow (1st of May) marks the start of nutrient addition that was done weekly. The second arrow (31st of July) marks the release of two Crucian carps (*Carassius carassius*) to freely forage in the whole water column, that had been kept in a restricted area of the enclosures. Each data point is a mean of four replicates with ±1 S.D.

by 1 to 5 ˚C with 1 ˚C increase between each treatment relative to the controls (fig. 2). The setup corresponds to a future development of temperature during the next 100 years according to IPCCs latest assessment report (IPCC 2007). A computerized system was adjusting the temperature relative to the average temperature in the controls every ten second. Simultaneously, the humic content (absorbance was used as a proxy) was increased with 50% per ˚C relative to the control. This resulted in a maximum of 5 ˚C and 250% in the highest treatment. All treatments were replicated four times. The level of brownification was determined by extrapolating historical absorbance data from several lakes in southern Sweden, to correspond to the future temperature changes (unpublished data, Hansson 2013). Sediment, collected in late March 2013 from Krankesjön, was added in 20x40x15cm boxes to all enclosures. Two juvenile (6-7 cm) Crucian carps (*Carassius carassius*) were kept in 20x30x50cm cages to allow a limited predatory pressure on the zooplankton community. Since primary production was low, the fishes were released to freely forage in the whole water column from the 31th of July throughout the study.

The system studied consisted of three trophic levels including phytoplankton and bacteria, zooplankton and zooplanktivorous fish.

Maintenance of experiment

To maintain the level of humic content in each treatment, the preparation HuminFeed® (containing 82% humic substances) was added every week to compensate for the loss in absorbance. From the 29th of May 1ml of commercial fertilizer (containing 5,1g N and 1,0g P per 100ml) was added weekly to each enclosure to avoid nutrient depletion. During the experimental period, water that evaporated was refilled with distilled water. Every week, 2L from each enclosure was

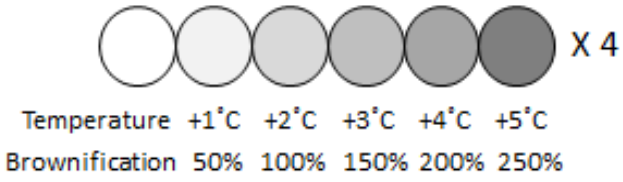

Fig. 2 Schematic illustration of the experimental design. A simultaneous gradual increase of brownification and temperature with 4 replicates resulted in 24 enclosures. Between each treatment there was an increase of 1 ˚C and 50% water colour.

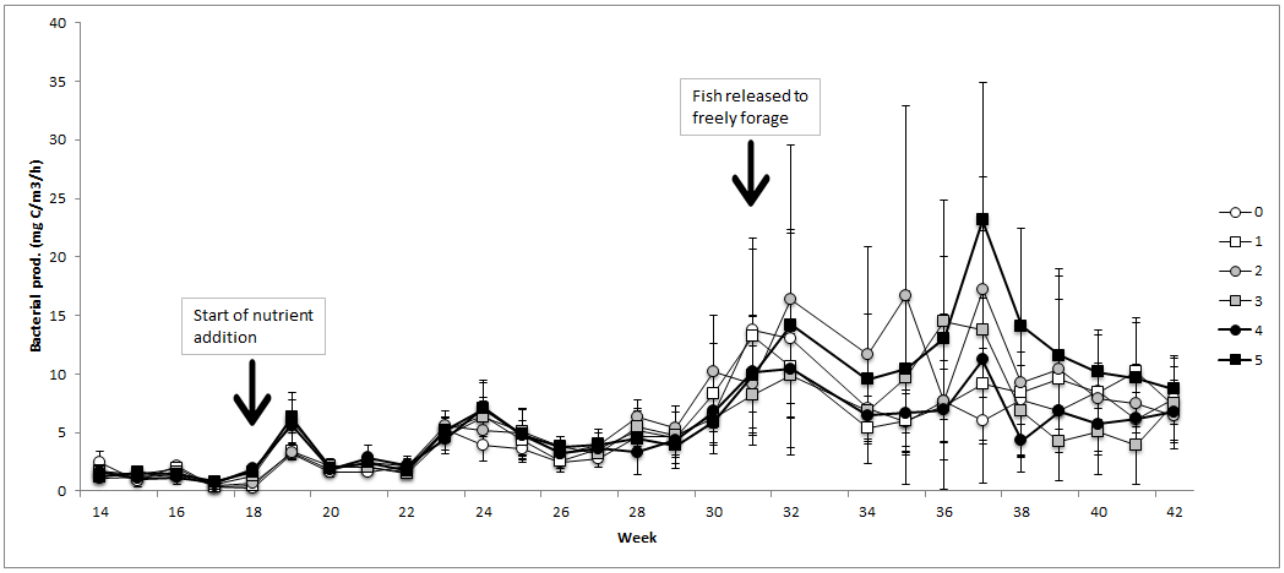

Fig. 3 Effect of temperature and water colour on bacterial production from the 3rd of April until the 16th of October. The lines represent different treatments where the numbers correspond to the temperature increase in °C. For each °C the water colour was increased with 50%, resulting in a maximum of 5 °C and 250% increase relative to the control. The first arrow (1st of May) marks the start of nutrient addition that was done weekly. The second arrow (31st of July) marks the release of two Crucian carps (*Carassius carassius*) to freely forage in the whole water column, that had been kept in a restricted area of the enclosures. Each data point is a mean of four replicates with ± 1 S.D.

mixed in a container and redistributed to mimic in- and outflow. To prevent algae growth on the walls scrubbing was performed once every week. For establishment of a phytoplankton dominated system macrophytes growing from the sediment was cut biweekly.

Chlorophyll-a analysis

Analyses of chlorophyll-a was taken twice a week and measured with an Algae Lab Analyser (ALA) (bbe moldaenke®) through fluorescence pattern of the pigment excitation. The values given by the ALA contains a systematic error, and therefore had to be corrected. This was done by creating a relation between the ALA and values measured according to Jespersen and Christoffersen (1987), where 50ml of water was filtered through a GF/C filter (Whatman, 25mm) and then extracted with ethanol. The analyses were done with a Shimadzu UV-2600 spectrophometer. This resulted in the equation y=1,397x+4,8267 with an R^2=0,9234 which was used to correct the values given by the ALA.

Primary production

Net primary production was measured with the carbon-14 method first described by Steemann-Nielson (1952). When the amount of CO_2 is known and a tracer amount of $^{14}CO_2$ is added it is possible to measure and calculate the proportional carbon assimilation of the phytoplankton. Samples were taken at a depth of approximately 20cm into the water column and transferred to 100ml glass bottles. Each sample was incubated with 50µl of $NaH^{14}CO_3$ (specific activity 40-60mCi/mmol; 1.48-2.22GBq/mmol) and incorporated at 50cm depth weekly for 4 h during noon. After incubation all bottles were taken to the lab where a subsample of 10-50ml, depending on productivity, of the incubated water was filtrated through a 0.45µm cellulose nitrate membrane filter. The filters were placed in scintillation vials and soaked with 500µl of 0.1M HCl overnight to evaporate the excess ^{14}C. Thereafter each scintillation vial was filled with 10 ml of scintillation cocktail (Ultima gold®) and shaken roughly. After at least 12 h, ^{14}C DPM (disintegration per minute) was measured in a Beckman LS 6500 scintillator. Following Wetzel and Likens (1995), the obtained activity was used to calculate the productivity rates. For

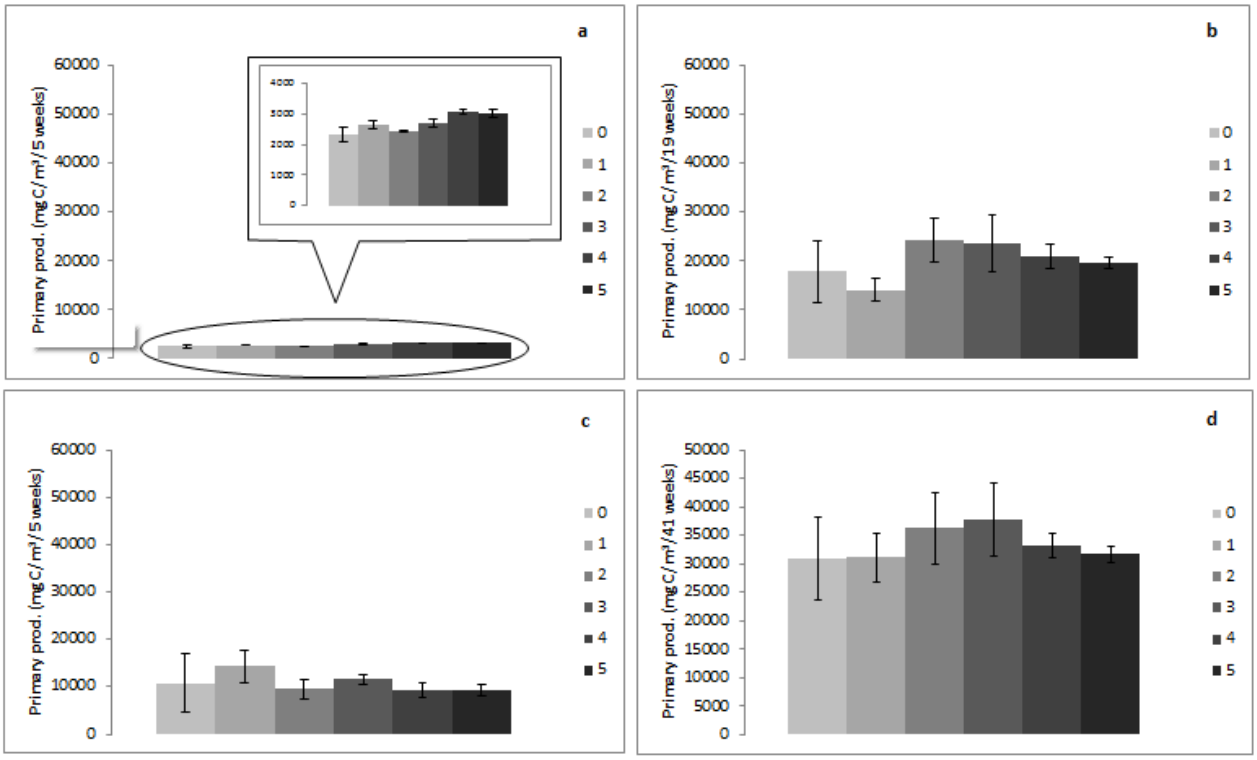

Fig. 4 The effect of temperature and water colour on the primary production during spring (a), summer (b), autumn (c) and the whole experimental period (d) for each treatment. For each °C the water colour was increased with 50%, resulting in a maximum of 5 °C and 250% increase relative to the control. Each bar is a mean of four replicates with ±1 S.E.

calculation of primary production the carbon content of the water is needed. This was obtained from alkalinity, pH and temperature measurements, which was done with a Mettler toledo titration excellence. To expand the obtained production to daily values of primary production each day's sun hours was divided into five equal periods. The incubation was performed during the second and third period, which is assumed to correspond to 60% of the total daily production. Hence, the obtained values were compensated by adding 40% extra production.

To ensure that the variation in alkalinity would not be affected by the addition of huminfeed as brownifier, a small test was performed. The test revealed that the alkalinity was stable and not affected by temperature, time or HuminFeed. During photosynthesis the alkalinity of the water changed. Thereby it was concluded that alkalinity would be sampled at the same day as primary production and stored cool and dark to the next day for alkalinity measurements.

Bacterial production

Bacterial production was measured by the ^{3}H-leucine incorporation method first described by Kirchman et al. (1985), and later modified by Smith and Azam (1992). Duplicate samples of 1.5ml were incubated in darkness in situ for 2 h in 2.0ml Axygen micro tubes during midday. To each sample 20µl of diluted ^{3}H-leucine (specific activity 13.7C_i/mmole) was added resulting in a final concentration of 95nM. After samples were terminated with 79µl of 100% TCA (final concentration of 5%), they were vortexed, centrifuged at 16 000g for 10 minutes and rinsed of supernatant. The samples were then cleaned by adding 1.7ml 5% TCA and 1.7 ml of 80% EtOH. For each cleaning step the samples were vortexed, centrifuged and aspirated of supernatant. 0.5ml of scintillation cocktail (Ultima gold) was added to each sample and analysed for ^{3}H activity in a Beckman LS 6500 scintillator. The analysed

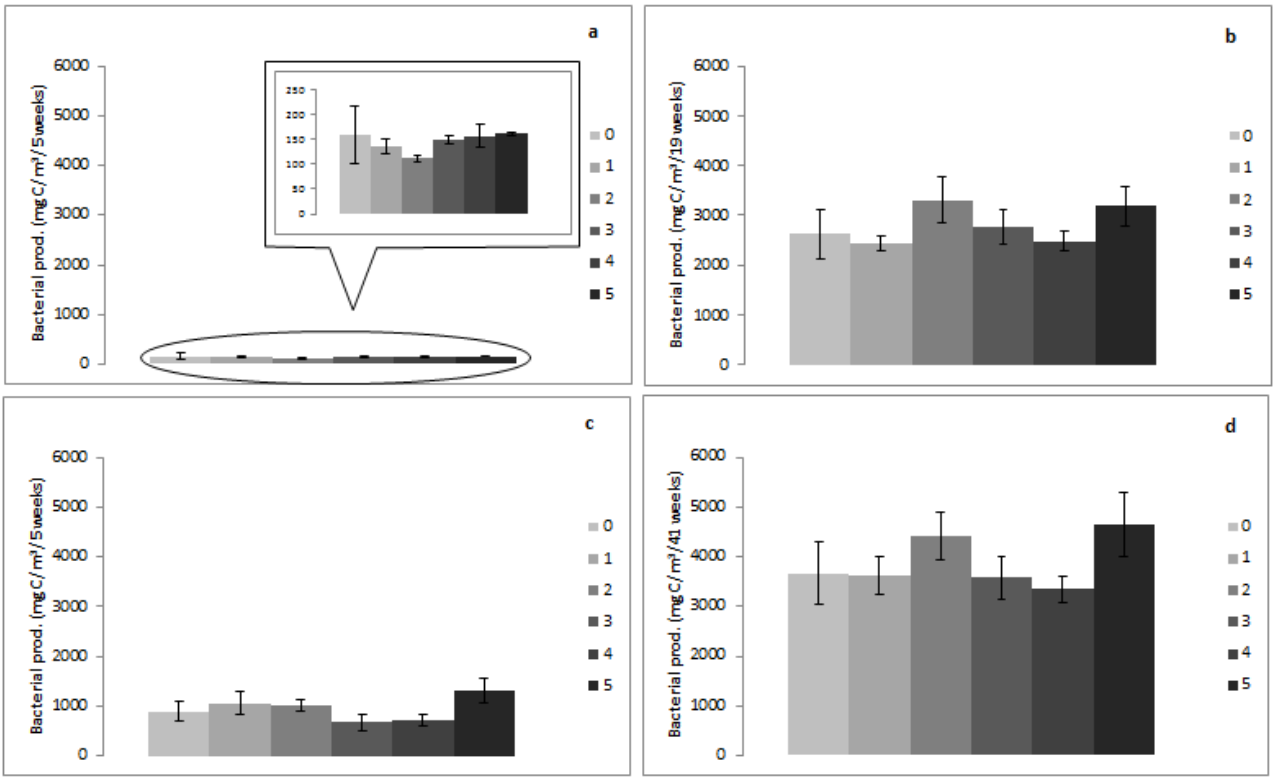

Fig. 5 The effect of temperature and water colour on the primary production during spring (a), summer (b), autumn (c) and the whole experimental period (d) for each treatment. For each °C the water colour was increased with 50%, resulting in a maximum of 5 °C and 250% increase relative to the control. Each bar is a mean of four replicates with ±1 S.E.

DPM was converted into carbon units according to Simon & Azam (1989) and extrapolated to 24-h day.

A test to evaluate the bioavailability of huminfeed was carried out with 5 different concentrations during three days in room temperature. The test revealed a steady increase of bacterial production with increasing concentration of huminfeed. Hence, it was concluded that huminfeed is available as an alternative source for bacteria.

BCD/NPP

Bacterial carbon demand (BCD) was obtained from the formula BCD=(BP/BGE)-BP, were BCD=Bacterial Carbon Demand, BP=Bacterial Production and BGE=Bacterial Growth Efficiency. In a review by del Giorgio and Cole (1998) evaluating several values of BGE in natural aquatic systems it was found that BGE was close to 0.37 in Danish lakes. Hence, due to the geographical proximity and bedrock similarity to Denmark, BGE was assumed to be 0.37 in this study. It should be noted that BGE, since it was beyond the scope of this study to measure directly, was assumed to be equal in all treatments.

FWE

Food web efficiency (FWE) is calculated as $FWE=Fish_p/(PP+BP)$, where the total body carbon content of the fish is known. Here, FWE was instead defined as the change in fulton condition factor of the fishes in each enclosure. Fulton condition factor is calculated by $F=(100*M)L^{-3}$, where M=weight and L=length. This should work as a proxy for FWE, since a high FWE would give a high F and vice versa. Two fishes from different enclosures and treatments died during the experimental period. Since the condition of fish is dependent on the supply of food, these enclosures were excluded from further analyses.

Seasonal division

Spring, summer and autumn was defined using the standardized method used by the Swedish meteorological and hydrological

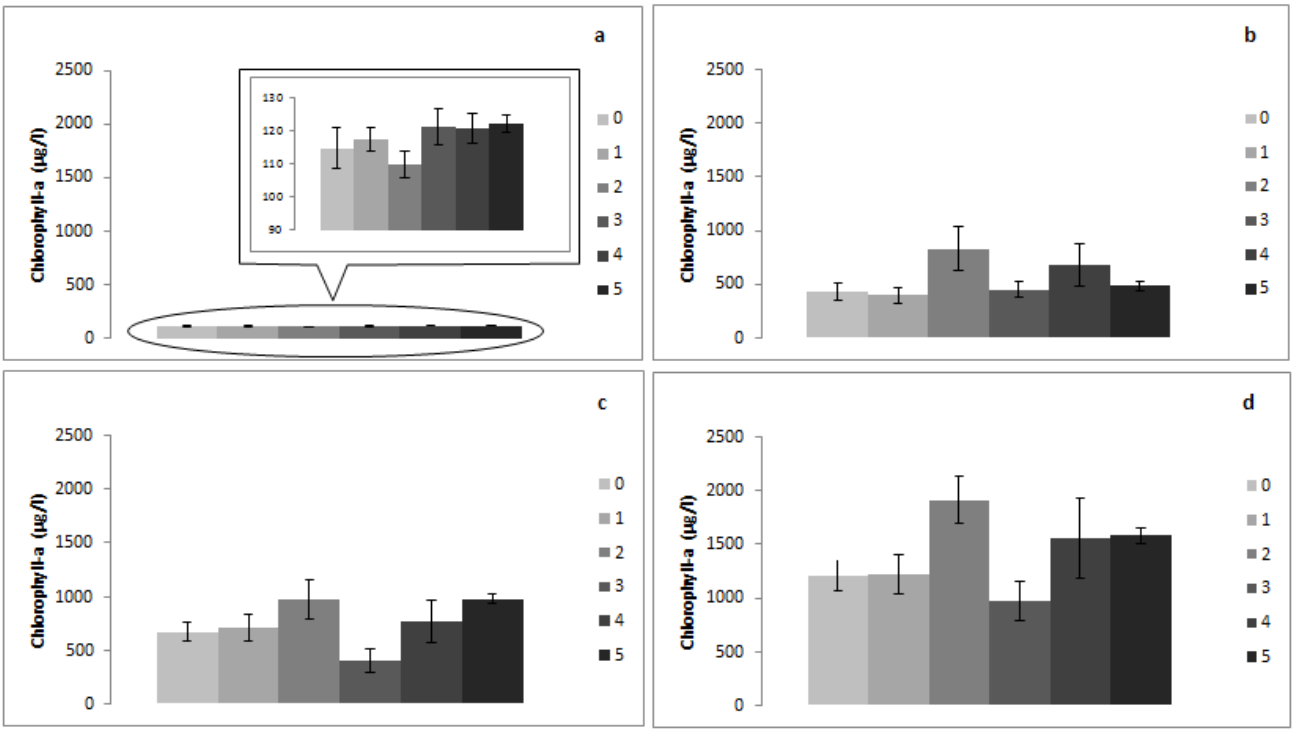

Fig. 6 The effect of temperature and water colour on the primary production during spring (a), summer (b), autumn (c) and the whole experimental period (d) for each treatment. For each ˚C the water colour was increased with 50%, resulting in a maximum of 5 ˚C and 250% increase relative to the control. Each bar is a mean of four replicates with ±1 S.E.

institution (SMHI). Spring was defined by increasing temperature that is between 0 and 10 ˚C, summer by an average temperature above 10 ˚C and autumn by a decreasing temperature that is between 0 and 10 ˚C. Thereby spring includes 5 weeks, summer 19 weeks and autumn 5 weeks.

Statistics

Statistical analyses to look for differences between treatments were performed in IBM SPSS Statistics 20, where a one-way ANOVA was used. The treatments were separately tested against each other. All statistics presented here passed the assumption of equal variances tested with Levene´s test.

Results

Overall there was no difference in primary production between the treatments (fig. 1). A seasonal variation could be seen in production where spring had a lower production than summer. Autumn had a higher production than spring, but less than during summer (fig. 1). When dividing the cumulative production by season, there was an increasing trend for primary production in spring (fig. 3). This was also confirmed with the control being statistically significant different from treatment 4 (P=0.029) and 5 (P=0.049). During summer there was a tendency for a bell-shaped relation, with the highest PP in treatment 2 and 3, in autumn there was a decreasing trend (fig. 4). Note that the production was higher both in the summer and autumn compared to the spring. The cumulative PP for the whole experimental period was bell-shaped where treatments 0, 1, 4 and 5 had a lower production than 2 and 3.

No difference was found in bacterial production between treatments (fig. 3). From the beginning of the experiment until autumn BP was steadily increasing, but decreased during autumn. The peak in BP occurred during late summer. There was no difference in BP during any of the seasons (fig. 5).

No difference was found in chl-a content between the treatments during any of the seasons (fig. 6). Spring had the lowest chlorophyll concentration whereas summer

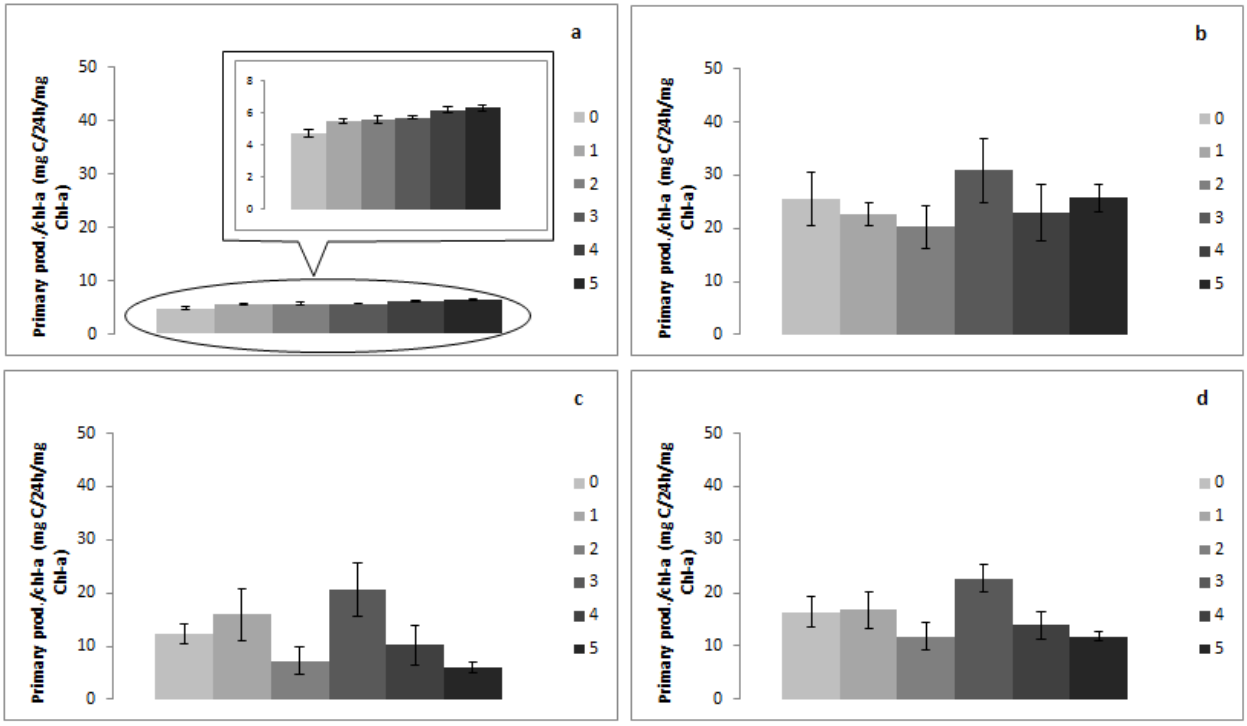

Fig. 7 The effect of temperature and water colour on the primary production during spring (a), summer (b), autumn (c) and the whole experimental period (d) for each treatment. For each °C the water colour was increased with 50%, resulting in a maximum of 5 °C and 250% increase relative to the control. Each bar is a mean of four replicates with ±1 S.E.

and autumn had similar concentrations across treatments. When dividing PP with chl-a there was an increasing production/chl-a with treatment during the spring (fig. 7). This was confirmed by the control being significantly different from 2 (P=0.045), 3 (P=0.026), 4 (P=0.01) and 5 (P=0.000). During summer and autumn there was no difference between treatments. Comparing chl-a and production/chl-a there was opposite patterns in treatment 2 and 3. The total chl-a was highest in treatment 2 and lowest in 3 (fig. 5), whereas production/chl-a was lowest in treatment 2 and highest in 3 (fig. 7).

The BCD/NPP ratio was low during spring, increasing during summer and reached its peak in June and July (fig. 8). During autumn the ratio was low again. All treatments reached a ratio above 1 during June and July. There was a tendency for a greater ratio with increasing water colour and temperature.

The condition of the fish in the different treatments differs from each other (fig. 9). The treatments with the highest fish condition were 2, 3 and 4. Treatment 1 and 5 contained the lowest fish condition whereas the control was higher than 1 and 5 but less than 2, 3 and 4, revealing a bell-shaped relation to temperature and water colour.

Discussion

The direct effect of increasing temperature on phytoplankton and bacteria is an increase in metabolism, which in turn increase rates of production. Humic content has a positive effect on BP whereas it has a negative effect on PP through light attenuation. Since none of these effects were seen in this experiment, other factors have likely also been affecting production.

Primary production

Primary production over time did not reveal any clear differences between the different treatments (fig. 1). As hypothesized, temperature was predicted to have a positive effect on production until light attenuation was too high and would suppress photosynthesis. In our long-term outdoor mesocosm experiment this pattern was not

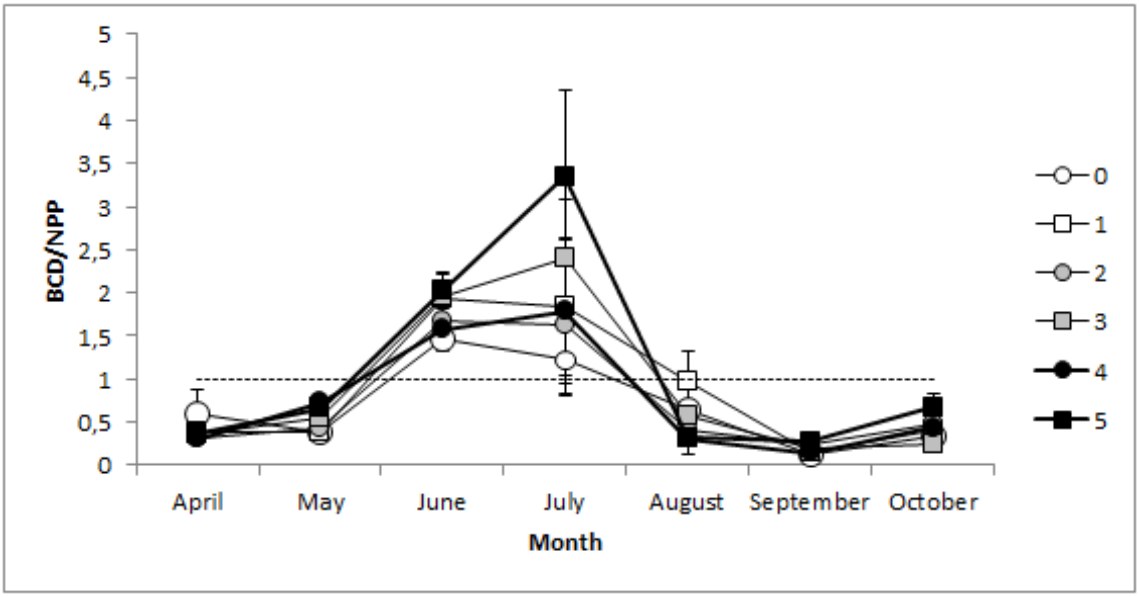

Fig. 8 Effect of temperature and water colour on BCD(Bacterial Carbon Demand)/NPP(Net Primary Production) from April to October. For each °C the water colour was increased with 50%, resulting in a maximum of 5 °C and 250% increase relative to the control. The dashed line marks the threshold between heterotrophy and autotrophy, a value >1 indicates heterotrophy and a value <1 indicates autotrophy. Each point is a mean of four replicates with ±1 S.E.

very clear (fig. 1). Even if tendencies could be seen in the cumulative PP (fig. 4) it was expected that the effects of temperature and water colour would be greater than was found. The PP during the beginning of the experiment, compared to late summer and autumn, was remarkably low. It has long been known that zooplankton can control phytoplankton biomass and production through heavy grazing (Lampert et al. 1986). The nutrient concentration was at the same time low during this period (unpublished data) which also could have restricted the production. Additionally, the clear water phase might have been strongly stabilized by macrophyte growth. The treatment effect might therefore be reflected in macrophyte production rather than PP. Since macrophyte growth is dependent on light availability, the depth is a crucial factor influencing the competitiveness of macrophytes. Our experimental setup did reflect shallow lake conditions rather than deep-water lakes. In non-shallow lakes the competition between macrophytes and phytoplankton most probably would show a different relation. Here, zooplankton predation, low nutrient levels and competition are the most likely explanations for the low PP.

The two crucian carps that were kept in cages in each enclosure were meant to express a limited predatory pressure on the zooplankton community. On the 31th of July the fishes were released to freely forage on the zooplankton which was followed by a rapid increase in PP in august. Hence, it can be hypothesized that the PP was suppressed by zooplankton grazing during spring and early summer. This is further supported by the condition of the fishes that first increased during the end of the experiment (unpublished data). This is in accordance with the theory of trophic cascades first described by Carpenter et al. (1985). The release of the planktivorous fish increased the predatory pressure of the zooplankton community which released phytoplankton from grazing.

The cumulative primary production during spring was increasing with temperature and water colour (fig. 4). This suggests that temperature is more important than light availability during spring. Further, if coupled with production/chl-a it can be seen that each phytoplankton produces more the warmer and browner it gets (fig. 7). Knowing that phytoplankton acclimatize to decreased light availability by increasing chlorophyll a

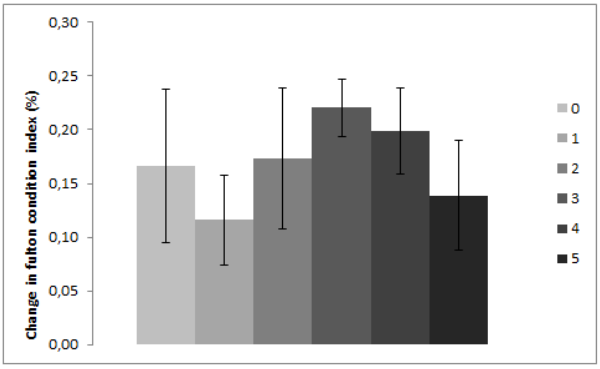

Fig. 9 Change, in terms of percentages, in fish condition during the period of free foraging (from the 31st of July) in different treatments. For each ˚C the water colour was increased with 50%, resulting in a maximum of 5 ˚C and 250% increase relative to the control. Each bar is a mean of four replicates with ±1 S.E.

concentration per unit (Longhurst and Harrison 1989), the increased metabolism is therefore probably the explanation for the increase in PP and not biomass. During summer and autumn the variation within each treatment is covering all possible differences. Hence, it seems that the increase in water colour and temperature does not have any effect on PP during these periods.

The total cumulative PP during the study had its maximum in treatment 2 and 3, even if not significantly different from the other treatments (fig. 4). This is in accordance to what was hypothesized. At minor increase of temperature and water colour, PP increases because of higher metabolism. When water colour becomes too high, the assumed increase in metabolism does not compensate for the loss of light availability. Hence, PP increases with temperature and water colour until light availability becomes the limiting factor.

Bacterial production

There was no difference in bacterial production between the treatments (fig. 3). The simultaneous increase in temperature and water colour did not have any effect on BP. With time there was a continuous increase in BP, from spring until autumn. The most obvious change with season is temperature, which positively affect BP (White et al. 1991). However, if temperature would increase BP this would have revealed a gradual increase in BP along our treatment gradient, which was not observed. Since nutrients were added on a weekly basis from the 1th of May the increase in BP over time may have been a response to nutrients. However, the variation in BP was not explained by either total phosphorous (TP), total nitrogen (TN) nor by TN/TP ratio (unpublished data). In this experiment, an alternative source of allochthonous carbon was added as a treatment factor. This was predicted to increase BP, since several authors have reported a positive correlation between BP and allochthonous carbon (Tranvik 1988, Hessen 1992, Kritzberg et al. 2004). Still no effect on BP was observed. BP has been reported often coupled with PP and chlorophyll concentration (Cole 1982), in this experiment no such clear relation was found (unpublished data). Many other factors are also affecting BP other than nutrients and the extracellular release of DOC by phytoplankton that could disrupt the relation between nutrients and BP, such as predation. Both ciliates and heterotrophic nanoflagellates (HNF) predate on bacteria (Pernthaler 2005) which could affect the relationship between BP and nutrients. This relates to the discussion on what ultimately controls population growth, bottom-up or top-down processes. It has been suggested that BP is limited by predation (top-down) in oligotrophic systems and nutrient limited in eutrophic systems (bottom-up) (Pernthaler 2005). Since no correlation was found with nutrient levels in this experiment it would be interesting to estimate the effect from predation, and the interaction between these and BP to reach a satisfying explanation. However, this was beyond the scope of this study.

The total cumulative BP was highest in treatment 2 and 5 (fig. 5). A possible explanation for the high BP in treatment 2 could be found in the coupling between BP and chl-a. As has been reported by Cole (1982) BP often increases with PP, because of the extracellular release of organic compounds by phytoplankton. In our

experiment, the high BP in treatment 2 is therefore possibly explained by the high PP. Treatment 5 did not show the same high PP as 2 but still a high BP. Instead, the humic content in treatment 5 was remarkably higher than in 2, which might have been used as an alternative carbon source by the bacteria, as well as temperature.

BCD/NPP ratio

The BCD/NPP ratio was similar across all treatments (fig. 8). During the beginning of the experimental period the ratio was close to 0 and increasing above 1 during June and July and then falls close to 0 again during late summer and autumn. A BCD/NPP ratio of more than 1 indicates that the processed carbon by bacteria is greater than that by primary production and is considered a proxy for heterotrophy (Jansson et al. 2000). As a consequence of climate change and increasing humic content in freshwaters, scientists have been warning for increasing heterotrophy (Moss 2010, Yvon-Durocher et al. 2010). A heterotrophic system is a source for CO_2 emissions and thereby works as a negative feedback mechanism for climate change. During June and July treatment 3 and 5 had a higher BCD/NPP ratio compared to the other treatments (fig. 8). This suggests that net heterotrophy might increase in the future. Since such biological feedback mechanisms are not accounted for by IPCC models they might underestimate the consequences predicted (Moss 2010). Further, this might have implications for the reconstruction of wetlands. Wetlands are constructed as a measure against eutrophication and to increase biodiversity (Brönmark and Hansson 2005). If freshwater systems will be increasingly net heterotrophic the reconstruction of wetlands might deteriorate the situation. It has also been suggested that overall biodiversity will change dramatically with climate change (Sala et al. 2000). On one hand biodiversity is positively affected by wetlands, but on the other hand indirect effects might decrease the overall biodiversity. Policy and decision makers are here brought to a dilemma, and will have to deal with prioritization of different questions and issues.

Primary production/chl-a suppressed by chl-a

The treatment that had the highest total chl-a concentration was an increase of 2 ˚C and 100% water colour (fig. 7). The highest PP/chl-a was not observed in the same treatment but in the treatment with the lowest chl-a concentration (fig. 6). Hence, PP/chl-a seems to be suppressed by a high chl-a content, probably because of light attenuation. In a future climate change scenario with increasing temperature and water colour it is therefore important to understand the relationship between chl-a concentrations, PP/chl-a and humic content and how this affects the dynamic of lake ecosystems.

FWE

The effect of temperature and water colour on the condition of the two free living crucian carps was as expected (fig. 9). The highest condition was found in intermediate treatment effect. As been described before, the production and condition of fish, among other factors, is dependent on the supply of food (Ficke et al. 2007), in this case zooplankton. Further, zooplankton abundance is dependent on phytoplankton, independently if heterotrophic or autotrophic. Since no trend was found in BP between treatments (fig. 5), but in PP (fig. 4), a possible driver for fish condition in this experiment might have been production by autotrophic plankton. As described by Hansson et al. (2013) the energy mobilized in production is revealed every second trophic level of the food chain. During the period of free foraging fish, the energy mobilization should therefore be shown in primary production and fish production whereas zooplankton abundance would be low. An indication of fish production could therefore be sampled by production in phytoplankton in three-trophic systems. In this experiment, fish production and primary production is following similar patterns and hence supports the findings by Hansson et al. (2013).

Conclusion

To summarize, the effects of the simultaneous increase in temperature and water colour on primary and bacterial production was almost

negligible in this experiment. There was a marginal increase in PP with treatment during spring that indicates that temperature is more important than light availability during this period. Further there was also a trend for a bell shaped relation in the total PP, with the highest PP at intermediate treatment. A likely explanation for this is that temperature has a positive effect until light availability becomes limiting. Several factors have been discussed as explanations to these patterns, including nutrients, predation, trophic cascades and different stable states.

No clear difference in the BCD/NPP ratio was found, except that there was a trend in June and July for increasing heterotrophy with treatment. Since IPCC models does not account for this biological feedback (Moss 2010) it is crucial to investigate if freshwaters will act as a negative feedback mechanism. Further, increasing heterotrophy and stable total production will lead to lower FWE. Here, fish production was probably driven by PP since BP was stable across treatments. This will have consequences for the recreational value of lakes and river, for example through the decreased production of fish. To clearly evaluate and understand the outcome of this experiment more research about the different factors, some of them discussed here, affecting BP and PP is needed.

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