The impact of climate change on aquatic systems and phytoplankton communities

A quantitative study of the impacts of altering food-quality on microzooplankton growth rate

Linnéa Joandi

Department of Systems Ecology

Bachelor Degree Project
Marine Biology

Autumn 2012

Supervisor: Alfred Burian
Co-supervisor: Michael Tedengren

Stockholms universitet

The impact of climate change on aquatic systems and phytoplankton communities

A quantitative study of the impacts of altering food-quality on microzooplankton growth rate

Linnéa Joandi

Abstract

A global increase in atmospheric CO_2 and temperature is assumed to affect the marine ecosystems in numerous ways, e.g. by altering ocean circulation patterns and changing nutrient regimes. The changes are expected to impact heavily on both phytoplankton communities as well as the rest of the marine food-web. Based on previous experimental studies that have investigated the impacts of varied algae food-quality on zooplankton, this quantitative study hypothesizes that (i) the tested microzooplankton species *Brachionus plicatilis* (rotifer) and *Euplotes* sp. (ciliate) will show high population growth rates (*g*) when fed with *Nannochloropsis* sp. grown under nutrient replete conditions, (ii) that the species will show a population growth rate close to zero when fed with algae grown on phosphorous-deficient media and (iii) that microzooplankton will be negatively affected by the algae grown in nitrogen-deficient media. The study thus aims to investigate how changes in the balance of energy and several chemical elements in ecological interactions, *ecological stoichiometry*, affect the growth rates of algal grazers. The results show that food-independent factors had a large impact on growth rates and resulted in unexpected, deviating trends. However, as the growth rates for *B. plicatilis* fed with phosphorous-deficient algae were lower than those of *B. plicatilis* fed with nitrogen-deficient algae, there is some support for the hypotheses.

Keywords

Climate change, phytoplankton communities, microzooplankton, *B. Plicatilis*, *Euplotus* sp., P-limitation, N-limitation, nutrient replete conditions

Contents

Stockholms universitet
106 91 Stockholm
Telefon: 08–16 20 00
www.su.se

Stockholms universitet

1. Introduction

The impact of climate change on aquatic systems and phytoplankton communities

Climate change is affecting aquatic ecosystems in numerous ways. Two of the main impacts of climate change are (1) an elevated atmospheric CO_2 concentration and (2) an increase of atmospheric temperature. Increased atmospheric temperature result in higher sea surface temperatures (SST) (Anderson, 2005; Beardall & Stojkovic, 2006; Fu et al., 2007; Fu et al., 2008; Hutchins et al., 2007; Jochum et al., 2012; Paerl & Huisman, 2008). An increased SST will, in turn, result in shift of the global ocean productivity. This change is assumed to increase the atmospheric CO_2 concentration as the respiration of the food-web in the ocean increases (Behrenfeld, 2011).

It is important to understand the consequences of these two parameters as they can result in changed chemistry (Beardall & Stojkovic, 2006; Fu et al., 2007) and altered circulation (stratification) of the water layers (Pershing et al., 2004; Stenseth et al., 2004). Thus also to an altered circulation of the nutrients in the oceans (Beardall & Stojkovic, 2006; Behrenfeld, 2011; Behrenfeld et al., 2006; Doney, 2006; Falkowski et al., 1998; Falkowski & Oliver, 2007). This, in turn, will have an impact on the composition of the phytoplankton communities (Beardall & Stojkovic, 2006; Behrenfeld, 2011; Behrenfeld et al., 2006; Eppley, 1972; Fu et al., 2007; Falkowski et al., 1998).

The concomitant changes that take place in the structure of phytoplankton communities will result in (i) shifts in cell size (Atkinson et al., 2012; Eppley, 1972; Finkel, 2007), (ii) altered primary production (Behrenfeld, 2011; Behrenfeld et al., 2006; Danovaro et al., 2001) and (iii) changed biochemical composition of algae (their elemental *stoichiometry*) (Fu et al., 2007; Sterner & Elser, 2002). Implications of these changes will be transmitted to higher trophic levels (Beardall & Stojkovic: 2006; Hessen & Elser, 2005; Jochum et al., 2012; Sterner & Elser, 2002). Climate induced changes such as changed temperatures, alternation of nutrient concentrations and decreasing mixed-layer depth have therefore the potential to strongly alter the structure of aquatic food webs.

Climate change induced shifts in nutrient concentration of the oceans and phytoplankton growth conditions

While the coastal areas become more and more overenriched with nutrients (*eutrophicated*) (Paerl & Huisman, 2008), the situation for most of the open oceans is the opposite (Falkowski & Oliver, 2007; Finkel, 2007). An important key rule to consider is that the ratio between carbon (C), nitrogen (N) and phosphorous (P) of marine particulate matter (*seston*) reflects the C:N:P ratio of the surrounding water (Rothhaupt, 1995; Sterner, 1993; Sterner & Elser, 2002). So, if the environment of seston is P or N-limited (for example), the seston will be limited of the same nutrient(s) as well.

An increase in temperature will result in a decrease of the depth of the upper, mixing, strata of the ocean (Doney, 2006). Diatoms and other phytoplankton that sink quickly through the water column (Diehl, 2002) will, consequently, have a higher chance to "fall out" of the upper water strata. Once the carbon- and nutrient-rich organisms are lost from the surface layer, they cannot be recycled by other planktonic organisms such as bacteria and microzooplankton. This process will therefore additionally increase the nutrient limitation. Moreover, a lower depth of the mixing layer will not only contribute to lack of nutrients, but the time between mixing events in spring and autumn will increase as well. This will increase the duration of nutrient limitation.

Another reason why nutrient deficiency occurs is that the nutrient exchange between different water layers is restricted by an enforced thermocline that arise due to warming of the oceans (Behrenfeld, 2011). In other words, diffusion of nutrients from P and N-rich bottom waters into warmer euphotic water layers becomes very limited due to an increased temperature gradient that enforces the thermocline. Therefore, water masses need to be moved physically so that nutrients, which have sunk out of upper strata and remineralized in bottom waters, can be reintroduced to nutrient cycles in the upper strata again (Figure 1.1).

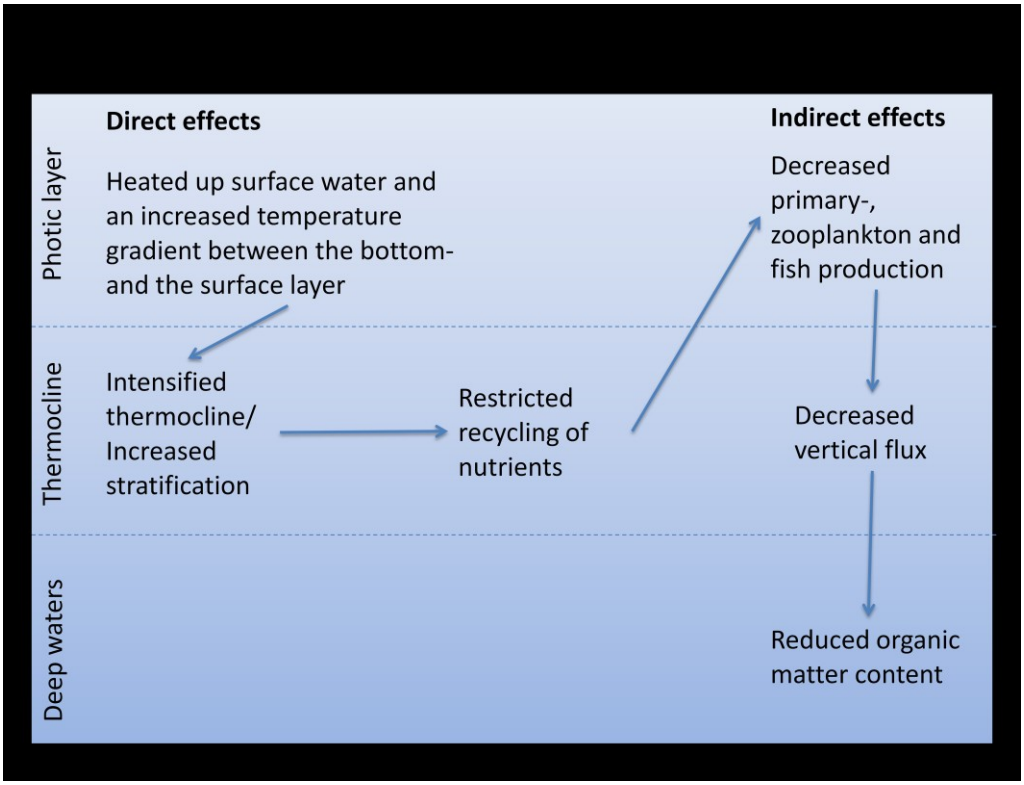

Figure 1.1 The direct- and indirect effects of climate-induced surface water warming.

In sum, the connection between the physical environmental changes and the biology of the oceans will affect the nutrients available for growth of phytoplankton through alternations of the surface layer´s temperature and stratification (Behrenfeld et al., 2006). But, it will also influence the rest of the pelagic food web and its stoichiometry (Danovaro et al., 2001;

Hessen & Elser, 2005; Sterner & Elser, 2002). Consequently, it becomes of great importance to better understand the stoichiometry of phyto- and zooplankton and how phytoplankton stoichiometry affects the microzooplankton.

Elemental stoichiometry in phyto- and zooplankton

There is raising evidence that both cell size (Atkinson et al., 2012; Eppley, 1972; Finkel, 2007) and the proportions of different elements that organisms are composed of (elemental *stoichiometry*) (Beardall & Stojkovic: 2006; Fu et al., 2007; Fu et al., 2008) are two ecophysiological traits that are affected by climate change and that determine growth and biomass of aquatic herbivores (Falkowski & Oliver, 2007; Sterner & Elser, 2002). This study will, however, focus only on the later effect.

The basic proportions of certain elements that an organism needs to exist, grow and/or reproduce optimally will determine its stoichiometry (Brown et al., 2004; Finkel, 2007; Valiela, 1995). The most accepted stoichiometric relationship for marine phytoplankton is an elemental ratio of 106C:16N:1P atoms called the *Redfield ratio* (Finkel et al., 2006; Sterner & Elser, 2002). However, variability of this elemental composition has been documented in both phytoplankton (e.g. Conde-Porcuna, 2000; Finkel et al., 2006; Meunier et al., 2011; Ramos-Rodríguez & Conde-Porcuna, 2003; Valiela, 1995) and zooplankton (e.g. Brown et al., 2004; Conde-Porcuna, 2000; Meunier et al., 2011; Sterner & Elser, 2002; Valiela, 1995). Phytoplankton show in general though a much higher variability in their C:N:P ratios than metazoan zooplankton. Heterotrophic protozoan are assumed to have an intermediate position in relation to the two previous mentioned.

These alternations depend on the organisms´ *stoichiometric plasticity,* their ability to alternate from their optimal somatic nutrient ratio (Meunier et al., 2011) as well as on environmental conditions (Brown et al., 2004; Conde-Porcuna, 2000; Finkel et al., 2006). Altering environmental conditions could, for example, be differences in bio-available growth-limiting nutrients such as nitrogen (N) and phosphorous (P) (Behrenfeld, 2011; Behrenfeld et al., 2006; Spencer et al., 2005; Valiela, 1995).

The importance of microzooplankton in aquatic ecosystems

Microzooplankton are referred to as “key components of marine food webs” and are defined as heterotrophic and/or mixotrophic grazing organisms smaller than two hundred micrometers (<200 μm) (Calbet, 2008: 325). Microzooplankton can consume up to 75% of the daily phytoplankton production (Calbet & Landry, 2004). As main consumers of primary producers as well as valuable prey of mesozooplankton (Calbet, 2008; Calbet & Landry, 2004), they constitute an important link between primary producers and higher trophic levels (Calbet & Saiz, 2005; Gifford, 1991). Moreover, they are also a main

component of the *microbial loop* which is an indirect pathway between phyto- and zooplankton via bacteria, viruses and protozoa (Azam et al., 1983; Behrenfeld, 2011; Sherr & Sherr, 2002). Behrenfeld (2011) wrote that "almost all life in the oceans, from bacteria to blue whales, relies on phytoplankton activity, so the impact of ocean warming on these microscopic organisms [e.g. on their stoichiometry] can have repercussions for the entire ocean system" (Behrenfeld, 2011: 33). One party that is affected by altering stoichiometry in phytoplankton are the microzooplankton.

The influence of phytoplankton stoichiometry on microzooplankton-physiology and trophic interactions

Rothhaupt (1995) among others have shown that alternated elemental composition of algae will affect the microzooplankton growth rates in different ways (Conde-Porcuna, 2000; Doney, 2006; Ramos-Rodríguez & Conde-Porcuna, 2003; Rothhaupt, 1995; Valiela 1995). Most studies have concluded that of the two main growth-limiting nutrients, nitrogen and phosphorous (Behrenfeld, 2011; Behrenfeld et al., 2006; Doney, 2006; Valiela, 1995), it is P-limitations that restrict the growth rates the most (Conde-Porcuna, 2000; Ramos-Rodríguez & Conde-Porcuna, 2003; Rothhaupt, 1995). However, Ramos-Rodríguez & Conde-Porcuna (2003) show that the opposite holds for *Keratella cochlearis*. P-limitation in combination with low food-concentration levels resulted, in this case, in the highest growth rates of the rotifer, while the lowest growth rates were obtained when the algae were grown on nutrient-sufficient media. It can thus be concluded that it is not always P- or N-limited algae that have a growth limiting effect on (micro)zooplankton. It sometimes also might be the lack of other essential components.

In conclusion, little is known about the impacts of nutrient limited food on protozoa because microzooplankton ecology is generally a poorly investigated research-area. Our knowledge of the response of key species is scarce (Calbet, 2008: 325). Thus, further research is required.

Research aims

The aim of this study is to investigate the growth response of two different microzooplankton species (*Brachionus plicatilis* and *Euplotes* sp.) to algal food particles cultured under different nutrient conditions. This is of primary interest as nutrient availability is expected to further increase with climate change.

Several hypotheses that will be tested under controlled laboratory conditions have been established: (i) The two tested microzooplankton species will have high population growth rates when fed with algae grown under nutrient replete conditions. (ii) Microzooplankton will have a population growth rates close to zero when fed with algae grown in P-limited media. (iii) Microzooplankton will be negatively affected by algae grown in N-limited media, but not as severely as they would be of algae grown under P-limited conditions.

Material and Methodology

A small scale experiment was carried out in order to investigate how population densities of two different microzooplankton species from two taxonomic groups (*Brachionus plicatilis* (rotifer) and *Euplotes* sp. (ciliate)) were affected by algae (*Nannochloropsis* sp.) grown under different nutrient conditions. The algae were cultured in continuous flow-through systems (*chemostats*) to maintain stable nutrient conditions during the whole experiment (Valiela 1995).

Algae growth conditions

A strain of *Nannochloropsis* sp. was grown on three types of growth medium (a F/2 media) with nitrogen and phosphorous, adjusted to produce N-limiting (N:P ratio = 4), P-limiting (N:P ratio ≈ 200) and nutrient-sufficient (*replete*) conditions (N:P = 20). This was done in order to change the elemental composition of algae and to use this feature to alter the food-quality of microzooplankton (Ramos-Rodríguez & Conde-Porcuna, 2003).

Nutrient limited and non-nutrient limited algae were attained from chemostats where the algae cultures were kept constant by the *dilution rate*, the amount of water flowing through the chemostats. The dilution rate was 0.15 d^{-1} which means that15% of the total volume was exchanged every day. The temperature of the media was 18 °C and its salinity was 20 ‰. The total volume of one chemostats was 800 ml and the light-cycle was 12:12.

Experimental set up and data analysis

Algae concentrations in the experimental were kept constant by daily measures of the algae biomass of each replicate to quantify grazing losses and replacing losses with respective aliquots. The biomass of algae was measured by establishing a calibration curve between carbon content and photometric extinction. The photometric extinction, measured with a spectrometer at 750nm wavelengths, was used as an approximation for algae carbon content during the course of the experiment.

Microzooplankton were placed in 500 mL containers with a start food (algae) concentration of 1 mg carbon C/L. The algae culture densities were, thereafter, re-established to initial C concentration. This was done by first measuring the algae density in both the outflow of the chemostats, and the cultures themselves with a photospectrometer. Secondly, how much of the outflow that had to be re-added in order to get the initial volume in the algae cultures again was calculated. Additionally, the carbon contents of the replicates were summed up to get 1 mg C/L which was then fed to the microzooplankton.

During the whole experiment, the microzooplankton were fed once a day, at the same time as they were sampled. Thirty mL samples were taken from the zooplankton cultures each

day, and preserved in Bouins solution (5 mL/30 mL) for direct counts. Every experiment was set up in triplicates and maintained for 5 days/treatment (with exception for the nutrient replete experiment that lasted for 6 days).

Direct counts were made to calculate the daily densities of the microzooplankton. This was done by using an inverted microscope, following the Utermöhl protocol (1958). Thereafter were daily growth rates calculated. This was done according to the following formula:

$$g = (\ln(C_n) - \ln(C_{n-1}))/t,$$

where *g* is the growth rate, (C_n) is the daily concentration of microzooplankton, (C_{n-1}) is the concentration from microzooplankton from the day before, and *t* is the time between (C_n) and (C_{n-1}).

Results

Results for *B. plicatilis*

The population density of *B. plicatilis* steadily declined when the rotifer was fed with algae grown in nutrient replete media (NR-treatment). It declined, on the average, with 32% per day (Figure 1). The average starting population density was 11834 individuals L^{-1}.

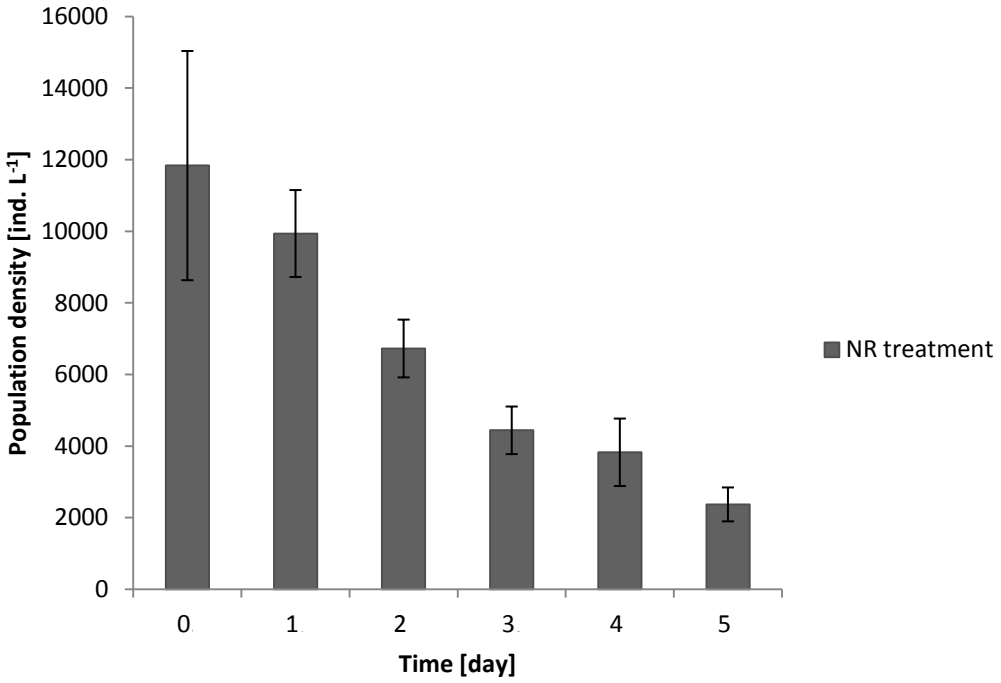

Figure 1. The population densities per day for *B. plicatilis* fed with algae grown in nutrient replete (NR) media.

A decline of the frequency of individuals can also be seen in the experimental treatment where *B. plicatilis* has been fed with algae grown in P-limited media (P-limited treatment); see day 1, 2 and 3 (Figure 2). However, a moderate increase of individuals can be seen the last three days of the experiment (day 3, 4 and 5). P-treated *B. plicatilis* had, on average, an initial population density of 3330 individuals L^{-1}.

The population density of *B. plicatilis* fed with algae grown in N-limited media (N-limited treatment) first declined (see day 1, 2 and 3), but rebound and increased again from day 3 onwards (see Figure 2). The average starting density of the population was 2317 individuals L^{-1}.

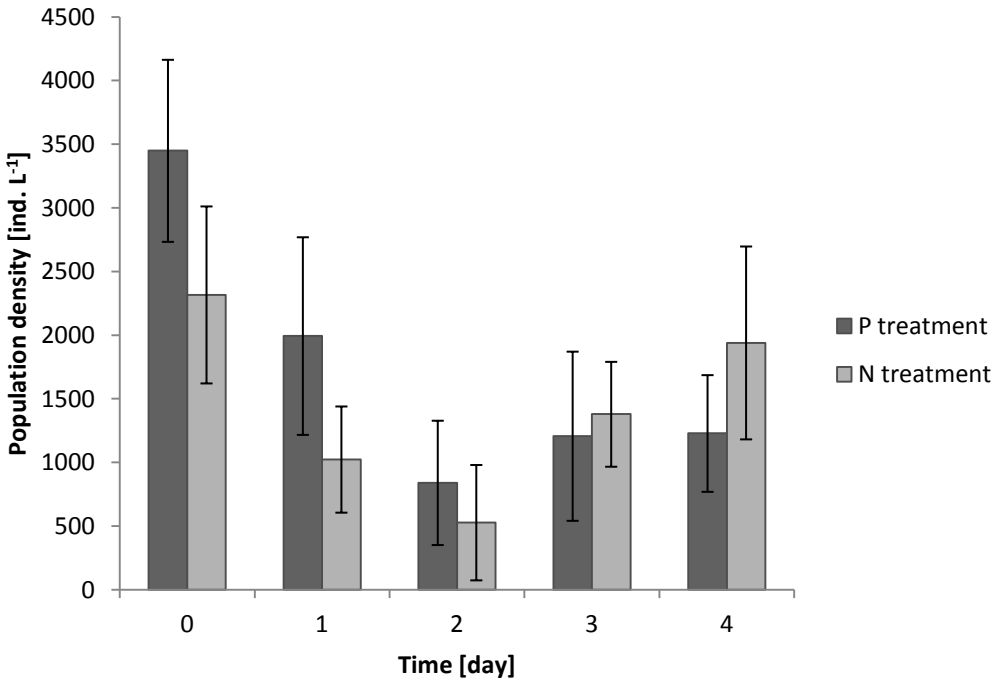

Figure 2. The population densities per day for *B. plicatilis* fed with algae grown in P-, respectively, N-limited media.

Results for *Euplotes* sp.

The numbers of individuals for *Euplotes* sp. fed with algae grown under nutrient replete (NR) conditions varied from day to day (Figure 3). The population density first decreases one day and then increases the next day. This trend can be seen throughout the course of the experiment. The NR-treated *Euplotes* sp. had, on average, an initial population density of 138137 individuals L^{-1}.

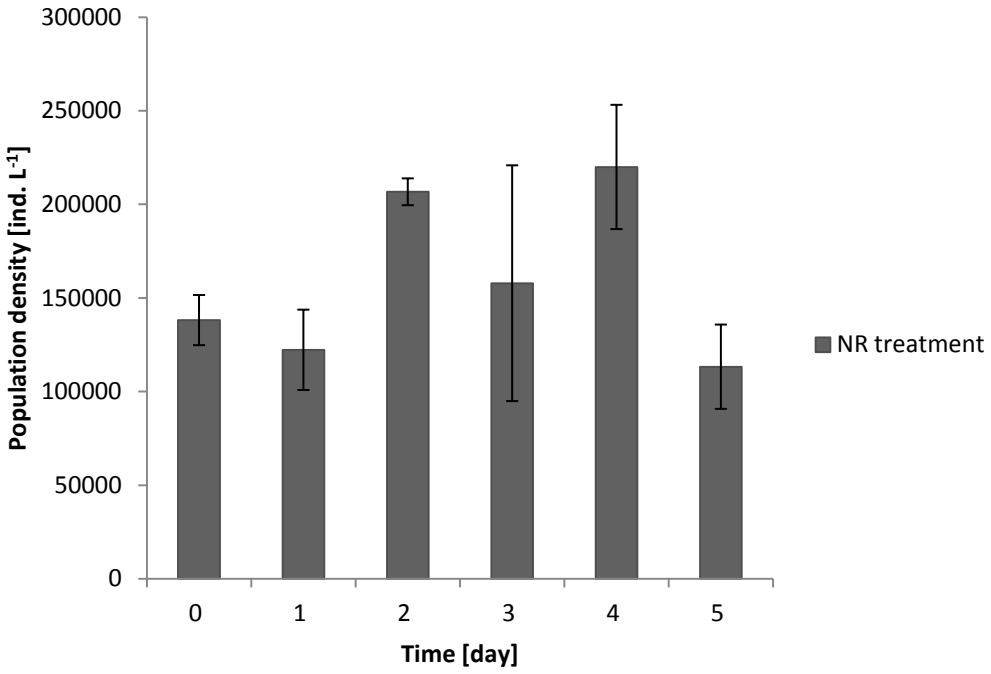

Figure 3. The population densities per day for *Euplotes* sp. fed with algae grown in nutrient replete (NR) media.

The population density for *Euplotes* sp. fed with algae grown in low-P media was, on average, 38200 individuals. However, the starting population density was 32977 individuals L^{-1}. The population density declined from day 0 to day 1, and then started to increase again on day 2. *Euplotes* sp. increased drastically with 72% from day 2 to day 3, but was most abundant on the fifth day (day 4).

The initial population density for *Euplotes* sp. fed with algae grown in N-limited media was, on average, 29544 individuals $L^{-1.}$ The population density showed a moderate decrease during the first and second day. On day three, the trend was inverted again and ciliate populations increase for the remaining days (Figure 4). *Euplotes* sp. was most abundant during the last two days.

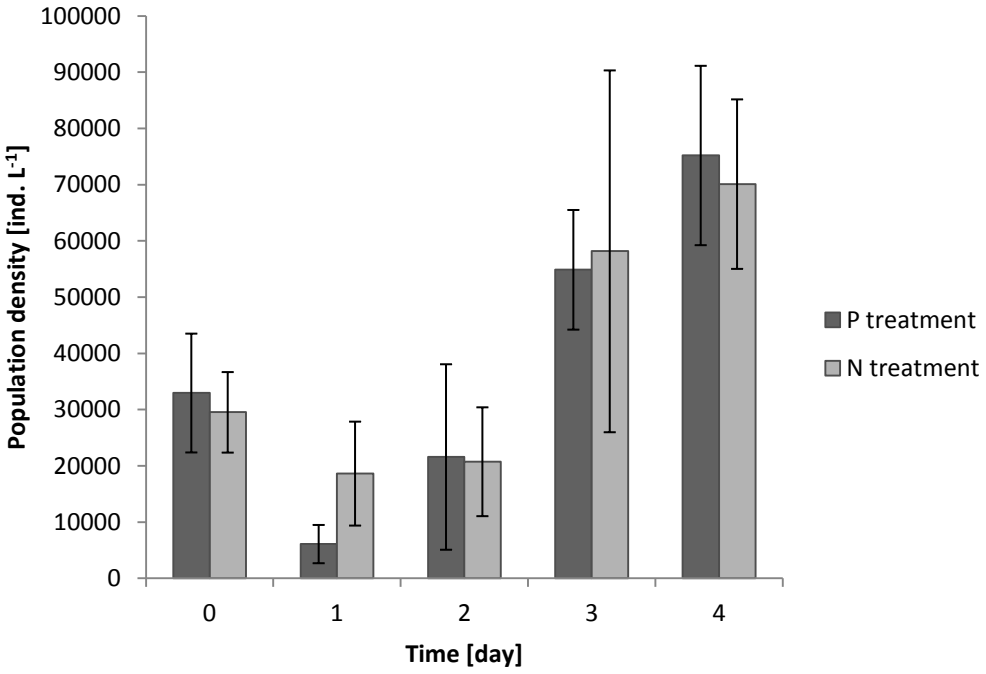

Figure 4. The population densities per day for *Euplotes* sp. fed with P-limited, respectively, N-limited algae.

When comparing population densities for microzooplankton treated with N and P-limited algae, it is seen that the same decrease- and increase pattern applies to both *Euplotes* sp. and *B. Plicatilis*. Moreover, it is noticeable that the starting population densities for microzooplankton fed with algae grown in NR-media are much higher than the once fed with P- and N-limited algea.

Daily growth rates (g) for the populations of each species, and each treatment, were calculated according to the growth-formula given in the previous section. For example, *day 2* in Table 1 below refers to the growth rate one get if *g* is equivalent to $(ln(C_n) - ln(C_{n-1}))/t$, if $ln(C_n)$ represents the calculated values of the second day of the experiment, and $ln(C_{n-1})$ the values of the first day. Negative g-values indicate declining growth rates with the previous 24 hours.

Table 1: Daily growth rates of *Euplotes* sp. and *B. plicatilis* expressed in percent.

	B. plicatilis	***Euplotes* sp.**
NR treatment, day 1	-17	-12
NR treatment, day 2	-39	53
NR treatment, day 3	-42	-27
NR treatment, day 4	-15	33
NR treatment, day 5	-48	-66
P treatment, day 1	-80	-168
P treatment, day 2	-86	126
P treatment, day 3	36	93
P treatment, day 4	2	32
N treatment, day 1	-82	-46
N treatment, day 2	-66	11
N treatment, day 3	96	103
N treatment, day 4	34	19

Discussion

Based on former experimental studies that have investigated the impacts of different food-quality of algae on zooplankton (Conde-Porcuna, 2000; Ramos-Rodríguez & Conde-Porcuna, 2003; Rothhaupt, 1995), it was hypothesized for this study that (a) a high population growth rate would be the outcome for *Euplotes* sp. and *B. plicatilis* when fed with nutrient replete algae, (b) that P-limited food would result in population growth rates close to zero for these microzooplankton, and that (c) N-limitation would affect population growth rate of these organisms negatively. Similar results could thus be expected for this study. The results showed, however, deviating patterns.

Discussion about the results for microzooplankton fed with algae grown under nutrient replete conditions

The first hypothesis stated that the tested microzooplankton species fed with algae grown under nutrient replete conditions (NR-treatment) would have a high population growth rate. However, a steady decrease could be observed for the *B. plicatilis* population throughout the experiment (see Figure 1).

This declining trend could be due to factors such as: (1) *Starvation*. However, this factor was excluded as enough food was left after each day when initial algae concentration had been re-established; (2) *density dependent self-regulatory mechanisms* such as the production of resting eggs. This is not either a likely explanation since (a) a much higher rotifer-concentration were obtained in stock cultures than in the samples taken for counting, (b) stock cultures showed high growth rates and since (c) no resting eggs were formed there; (3) *dissolved oxygen depletion* (Hall & Threlkeld, 1973). This can be excluded, since intensive bubbling took place throughout the experiment and because low oxygen values would not have resulted in a slow decrease of population growth, but to a quick mass death of rotifers; (4) *bad food-quality*. As already mentioned, the rotifer stock cultures exclude this possibility as they showed positive population growth rates when kept with the same nutrient replete algae as the samples; (5) *pH concentration* that will affect the rate at which these organisms gather food and therefore also affect their population growth rates (Hall & Threlkeld, 1973). (7) The presence of *virus*(es) that has been proven to, occasionally, result in reduction of microzooplankton populations (Garza & Suttle, 1995) or (8) other *environmental factors* such as the transfer into plastic experimental containers (hereafter referred to as *the bottle effect*). It can, however, be concluded that no matter what the decline was caused by, it was not food-quality related and thus could the first hypothesis not be tested.

The number of individuals for *Euplotes* sp. on the other hand varied from day to day throughout the experiment (Figure 3). The population growth rate decreased one day and then increased the next day. The trend could, in this case, possibly be a result of density

dependent factors such as the number of individuals in relation to the amount of food obtained throughout the experiment, and not as a result of the food quality itself.

In more detail, as the population peaked at approximately 210.000 individuals, the food obtained (1 mg carbon (C)/L) seemed to not be enough anymore in order for the population to continue to grow. Consequently, the frequency of individuals reduced. Once they reached a certain lower-limit, which in this case seemed to be within the scope of approximately 110.000-160.000 individuals, the population seems to reach a kind of "steady-state" where the amount of food now was enough to keep the individuals alive. This state seemed to make them start to reproduce again. They seemed to keep reproducing until they, once again, reach the same upper limit as before (approximately 210.000 individuals). In sum, the irregular trend(s) seen for *Euplotes* sp. fed with nutrient replete algae could be explained by the fact that 1 mg carbon was only enough for a number of organisms (a range of 110.000-210.000 individuals), but not for more than that (Mitra, 2006).

To address the first hypothesis concerning the nutrient replete conditions, it can be stated that the hypothesis cannot be falsified due to external factors that led to unexpected results in both the case of *B. plicatilis* and *Euplotes* sp.

Discussion about the results for microzooplankton fed with N- and P-limited algae

When trends of N and P-limited treatment of *B. plicatilis* are compared, it can be seen that the population densities declined the first three days but then increased the last two days of the experiment. The decrease was more drastic for the microzooplankton fed with algae grown under P-limited conditions (P-limited treatment) than for the organisms fed with algae grown on N-limited media (N-limited treatment). Accordingly also the increase of *B. plicatilis* was more moderate than for the one fed with N-limited algae.

The same development could also be observed for *Euplotes* sp. Namely, that the density of the population first decreased and rebounded afterwards. However, while the decrease (in this case) took place the first two days, the decrease for *B. plicatilis* took place the first three days. The decline was also, just as for *B. plicatilis*, more drastic for the microzooplankton fed with P-limited algae than for organisms fed with N-limited algae.

It can be observed for both *Euplotus* sp. and for *B. plicatilis* that the population densities decreased within the first couple of days. Once they had stabilized, however, the populations started to increase again (Figure 2 and Figure 4). While the *Euplotes* sp. population already increased after the second day, the negative trend for *B. plicatilis* did not shift until after the third day. Noticeable is that all the different trends of population densities followed the same decrease-/increase "pattern", no matter whether they were compared within the species itself or with each other (between the species).

The population densities for all treatments decreased until they reached a certain level from which they started to increase again. This could be explained by the fact that the species were initially affected by food-quality independent factors (e.g. the bottle effect) and that they, because of this reason, only showed food-quality dependent growth rates the last couple of days. *Euplotus* sp. did, however, increase both sooner and showed higher growth rates (measured in %) than *B. plicatilis* did, especially in the P-limited treatment.

As phytoplankton in general show a much higher variability in their C:N:P ratios than metazoan zooplankton do (they have a higher plasticity in their elemental composition), and heterotrophic protozoan are assumed to be somewhere in between these; one possible explanation for the sooner increase and the higher growth rates of *Euplotes* sp. could be that it is closer related to algae than *B. plicatilis* is; and that *Euplotes* sp., therefore, is more flexible in its stoichiometric plasticity than the rotifer. This could explain why it managed to cope better with nutrient-limitations and could thus be considered to be more "tolerant" than *B. plicatilis*.

One could even postulate that the steadily declining *B. plicatilis* population, which was fed with NP-treated algae, might have started to show positive growth rates if the duration of the experiment would have been longer. This assumes, however, that the population would manage to adapt with time. Nonetheless, further investigation needs to be done before these matters can be settled.

Conclusion

While food seems to have been a key factor that affected the population growth rates of *Euplotes* sp. fed with NR-treated algae; a likely explanation for the steady decline of *B. plicatilis* grown under nutrient replete conditions involves food independent factors instead. The results for N- and P-limited treatments indicate that there is some support for the second and third hypotheses if the initial decreases are primarily ascribed to food-quality independent factors (e.g. the bottle effect) and if the increases are considered to be responses to differences in food-quality instead.

The first hypothesis for *B. plicatilis* and *Euplotes* sp. cannot be falsified nor confirmed since other factors than food-quality seem to have influenced the outcomes. The second hypothesis, which stated that microzooplankton would have a population growth rates close to zero when fed with P-limited algae, as well as the third hypothesis concerning microzooplankton being affected negatively by N-limited food (but not as severely as they would be of P-limitation) gained some support as the growth rates for *B. plicatilis* fed with P-limited algae were lower than the once of the N-limited treatment.

In conclusion, even though the hypotheses seem to be supported to some extent, other factors besides the food-quality seem to have played a crucial role in the outcome of the

experiment. In order to investigate the effects of the food-quality on microzooplankton, I recommend repeating this experiment after an identification and elimination of the factor causing the initial population decline in all treatments.

References

Anderson, R. T. (2005). Plankton functional type modeling: running before we can walk? *Journal of Plankton Research, 27*(11), 1073-1081.

Atkinson, D., Ciotti, J. B., & Montages, S. J. D. (2003). Protists decrease in size linearly with temperature: ca. 2.5% °C-1. *Proceeding of the Royal Society Biology 270*(1522), 2605-2611.

Azam, F., Fenchel, T., Field, Jg., Gray, Js., Meyerreil, La., & Thingstad, F. (1983). The ecological role of water-column microbes in the sea. *Marine Ecology Progress Series 10*, 257–263.

Beardall, J., & Stojcovik, S. (2006). Microalgae under global environmental change: Implications for growth and productivity, populations and trophic flow. *ScienceAsia 32*(1), 1-10.

Behrenfeld, J. M., O'Malley, T. R., Siegel, A. D., McClain, R. C., Sarmiento, L. J., Feldman, C. G., Milligan, J. A., Falkowski, G. P., Letelier, M. R., & Boss, S. E. (2006). Climate-driven change in contemporary ocean productivity. *Nature, 444*, 752 -755.

Behrenfeld, M. (2011). Uncertain future for ocean algae. *Nature 1*, 33-34.

Brown, H. J., Gillooly, F. J., Allen, P. A., Savage, M. V., & West, B. G. (2004). Towards a metabolic theory of ecology. *Ecology 85*(7), 1771-1789.

Calbet, A. & Landry, R. M. (2004). Phytoplankton growth, microzooplankton grazing, and carbon cycling in marine systems. *Limnology and Oceanography, 49*(1), 51-57.

Calbet, A. (2008). The trophic roles of microzooplankton in marine systems. *ICES Journal of Marine Science 65*(3), 325-331.

Calbet, A., & Saiz, E. (2005). The ciliate–copepod link in marine ecosystems. *Aquatic Microbial Ecology, 38*, 157–167.

Conde-Porcuna, M. J. (2000). Relative importance of competition with Daphnia (cladocera) and nutrient limitation on Anuareopsis (rotifera) population dynamics in a laboratory study. *Freshwater Biology 44*, 423-430.

Danovaro, R., Dell'Anno, A., Fabiano, M., Pusceddu, A., & Tselepides, A. (2001). Deep-sea ecosystem response to climate changes: the eastern Mediterranean case study. *TRENDS in Ecology & Evolution 16*, (9), 505-510.

Diehl, S., Berger, S., Ptacnik, R., & Wild, A. (2002). Light, and nutrients in a gradient of mixing depths: Field experiments. *Ecology 83*(2), 399-411.

Doney, C. S. (2006). Plankton in a warmer world. *Nature, 444*, 695-696.

Eppley, W. R. (1972).Temperature and phytoplankton growth in sea. *Fishery Bulletin 70*(4), 1063-1085.

Falkowski, G. P., & Oliver, J. M. (2007). Mix and match: how climate selects phytoplankton. *Nature Reviews Microbiology 5*, 813-819.

Falkowski, G. P., Barber, T. R., & Smetacek, V. (1998). Biochemical controls and feedbacks on ocean primary production. *Science 281*(200), 200-206.

Finkel, V. Z. (2007). Does phytoplankton cell size matter? The evolution of modern marine food webs. In P. Falowski & A. Knoll (Eds.), *Evolution of primary producers in the sea* (pp. 333-350).

Finkel, V. Z., Quigg, A., Raven, A. J., Reinfelder, R. J., Schofield, E. O., & Falkowski, G. P. (2006). Irradiance and the elemental stoichiometry of marine phytoplankton. *Limnology and Oceanography 51*(6), 2690-2701.

Fu, F-X., Fu a, Zhang, Y., Warner, E. M., Feng Y., Sun, J., & Hutchins, A. D. (2008). A comparison of future increased CO_2 and temperature effects on sympatric Heterosigma akashiwo and Prorocentrum minimum. *Harmful Algae, 7*(1), 76-90.

Fu, F-X., Warner, E. M., Zhang, Y., Feng, Y., & Hutchins, A. D. (2007). Effects of increased temperature and CO_2 on photosynthesis, growth, and elemental ratios in marine Synechococcus and Proclorococcus (cyanobakteria). *Journal of Phycology*, *43*(3), 485-496.

Garza, R. D., & Suttle, A. C. (1995). Large double-stranded DNA viruses which cause the lysis of a marine heterotrophic nanoflagellate (Bodo sp.) occur in natural marine viral communities. *Aquatic Microbial Biology 9*, 203-210.

Gifford, D. J. (1991). The protozoan–metazoan trophic link in pelagic ecosystems. *Journal of Protozoology 38*, 81–86.

Hall, J. D., & Threlkeld, T. S. (1976). The size-efficiency hypothesis and the size structure of zooplankton communities. *Annual Review of Ecology and Systematics 7*, 177-208.

Hessen, O. D., & Elser J. J. (2005). Elements of ecology and evolution. *Oikos 109*, 3-5.

Hutchins, A. D., Fu, F-X., Zhang, Y., Warner, E. M., Feng, Y., Portune, K., & Bernhardt, W. P. (2007). CO_2 control of Trichodesmium N_2 fixation, photosynthesis, growth rate, and elemental ratios: Implications for past, present, and future ocean biogeochemistry. *Limnology and Oceabography, 52*(4), 1293-1304.

Jochum, M., Schneider, D. F., Crowe, P. T., Brose, U., & O'Gorman, J. E. (2012). Climate-

induced changes in bottom-up and top-down processes independently alter a marine ecosystem. *Philosophical Transactions of the Royal Society B 367*, 2962-2970.

Meunier, L. C., Haafke, J., Oppermann, B., Malzahn, A. M., & Boersma, M. (2012). Dynamic stoichiometric response to food quality fluctuations in the heterotrophic dinoflagellate Oxyrrhis marina. *Marine Biology 159*(10), 2241-2248.

Meunier, L. C., Hantzsche, M. F., Cunha-Dupont, Ö. A., Haafke, J., Oppermann, B., Malzahn, A. M., & Boersma, M. (2011). Intraspecific selectivity, compensatory feeding and flexible homeostasis in the fagotrophic flagellate Oxyrrhis marina: three ways to handle food quality fluctuations. *Hydrobiologia 680*, 53-62.

Mitra, A. (2006). A multi-nutrient model for the description of stoichiometric modulation of predation in meso- and microzooplankton. *Journal of Plankton Research 28*(6), 597-611.

Paerl, W. H. & Huisman, J. (2008). Blooms like it hot. *Science 320*(57), 57-58.

Pershing, J. A., Greene, H. C., Planque, B., & Fromentin, J-M. (2004). The influence of climate variability on North Atlantic zooplankton populations. In N. C. Stenseth & G. Ottersen (Eds.), *Marine ecosystems and climate variation: the North Atlantic: a comparative perspective (pp.59-69).* New York: Oxford University Press.

Ramos-Rodríguez, E. & Conde-Porcuna, M. J. (2003). Nutrient limitation on a planktonic rotifer: life history consequences and starvation resistance. *Limnology and Oceanography 48*(2), 933-938.

Rothhaupt, O. K. (1995). Algal nutrient limitation affects rotifer growth rate but not ingestion rate. *Limnology and Oceanography 40*(7), 1201-1208.

Sherr, E. B. & Sherr, B. F. (2002). Significance of predation by protists in aquatic microbial food webs. *Antonie van Leeuwenhoek 81*, 293–308.

Spencer, R. H., Smith, H. V., Lytle, A. D., & Leibold, A. M. (2005). Constraints on primary producer N:P supply ratio gradients. *Ecology 86*(7), 1894-1904. Stenseth, N.C. (Eds.) (2004). *Marine ecosystems and climate variation: the North Atlantic: a comparative perspective*. New York: Oxford University Press.

Sterner W. R. (1993). Daphnia growth on varying quality of Scenedesmus: Mineral limitation of zooplankton. *Ecology 74*(8), 2351-2360. Sterner, W. R. & Elser J. J. (2002). *Ecological Stoichiometry*. Princeton: Princeton Univ. Press.

Utermöhl, H. (1958). Zur Vervollkommnung der quantitative Phytoplankton-Methodik. *Mitt.Int.Ver.Limnol*. 9: 1-38.

Valiela, I. (1995). *Marine Ecological Processes*. (2 ed). New York: Springer.

Stockholms universitet
106 91 Stockholm
Telefon: 08–16 20 00
www.su.se

Stockholms universitet