The role of terrestrial and phytoplankton-derived organic matter in planktonic food webs

Anja Wenzel
New ideas pass through three periods:
   It can't be done.
   It probably can be done, but it's not worth doing.
   I knew it was a good idea all along!

- Arthur C. Clarke
List of Papers

This thesis is a summary of the following papers:


*Papers I, II and IV are reprinted with kind permission from Blackwell Publishing Ltd. (I), NRC Research Press (II) and the Ecological Society of America (IV).*
Author Contributions

Paper I
AKB, MJ and TV had the idea for the study and all authors planned the experiments. AW raised cultures, conducted experiments and performed all biological and chemical analyses in the laboratory. AW analysed the data with statistical advice from TV. AW wrote the first draft of the manuscript and all authors contributed to the revision of the text.

Paper II
The initial idea for the study originated from TV and all authors planned the experiment. AW raised cultures, conducted experiments and performed all biological and chemical analyses in the laboratory. AW analysed the data with statistical advice from TV. AW wrote the first draft of the manuscript and all authors contributed to the revision of the text.

Paper III
AW had the idea for the study and planned the experiment together with AKB. AW raised cultures, conducted experiments and performed all biological and chemical analyses in the laboratory. AW analysed the data with statistical advice from TV. AW wrote the manuscript with comments from AKB, MJ and TV.

Paper IV
CF had the idea for the study and planned the experiment with AKB and TV. CF and AW conducted the mesocosm experiment in the field and analysed biological and chemical samples in the laboratory. CF performed statistical analyses and wrote the manuscript with comments from AW, AKB and TV.

Authors: AKB – Ann-Kristin Bergström, AW – Anja Wenzel, CF – Carolyn Faithfull, MJ – Mats Jansson, TV – Tobias Vrede
# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>List of Papers</td>
<td>iv</td>
</tr>
<tr>
<td>Author Contributions</td>
<td>v</td>
</tr>
<tr>
<td>Background</td>
<td>3</td>
</tr>
<tr>
<td>Autotrophic vs heterotrophic production</td>
<td>3</td>
</tr>
<tr>
<td>Terrestrial organic material in lakes</td>
<td>4</td>
</tr>
<tr>
<td>The role of food quantity</td>
<td>5</td>
</tr>
<tr>
<td>Stoichiometry and mineral nutrient limitation of the aquatic food web</td>
<td>6</td>
</tr>
<tr>
<td>The Light: Nutrient-Hypothesis</td>
<td>7</td>
</tr>
<tr>
<td>The importance of biochemicals</td>
<td>8</td>
</tr>
<tr>
<td>The quality of different food sources in planktonic food webs</td>
<td>10</td>
</tr>
<tr>
<td>Aims of the Thesis</td>
<td>11</td>
</tr>
<tr>
<td>Methods</td>
<td>12</td>
</tr>
<tr>
<td>Results</td>
<td>16</td>
</tr>
<tr>
<td>Paper I – <em>Daphnia</em> feeding on single and mixed <em>Pseudomonas</em> and <em>Rhodomonas</em> diets</td>
<td>16</td>
</tr>
<tr>
<td>Paper II – Peat layer t-POM exploitation by <em>Daphnia</em></td>
<td>16</td>
</tr>
<tr>
<td>Paper III – <em>Daphnia</em> performance along gradients of mixed <em>Rhodomonas</em>, <em>Pseudomonas</em> and t-POM</td>
<td>17</td>
</tr>
<tr>
<td>Paper IV – Testing the Light: Nutrient-Hypothesis in an unproductive boreal clear water lake</td>
<td>17</td>
</tr>
<tr>
<td>Discussion</td>
<td>18</td>
</tr>
<tr>
<td>Bacteria-vs phytoplankton-dominated zooplankton diets</td>
<td>18</td>
</tr>
<tr>
<td>Direct exploitation of peat layer t-POM by zooplankton</td>
<td>19</td>
</tr>
<tr>
<td>Comparing the nutritional quality of heterotrophic bacteria and t-POM</td>
<td>19</td>
</tr>
<tr>
<td>The nutritional availability of heterotrophic bacteria and t-POM at varying phytoplankton concentrations</td>
<td>20</td>
</tr>
<tr>
<td>Responses of the pelagic food web to changes in nutrient and light availability in an unproductive boreal clear water lake</td>
<td>20</td>
</tr>
<tr>
<td>Major conclusions</td>
<td>22</td>
</tr>
<tr>
<td>Future questions</td>
<td>23</td>
</tr>
<tr>
<td>How do heterotrophic bacteria support pelagic food webs?</td>
<td>23</td>
</tr>
<tr>
<td>Effects on zooplankton taxa other than <em>Daphnia</em></td>
<td>24</td>
</tr>
<tr>
<td>Zooplankton survival, growth and reproduction <em>in situ</em></td>
<td>24</td>
</tr>
<tr>
<td>References</td>
<td>25</td>
</tr>
<tr>
<td>Danke...</td>
<td>33</td>
</tr>
</tbody>
</table>
Background

The majority of lakes worldwide are small, shallow and situated between 40 and 70°N (Kalff 2002; Downing et al. 2006). Lakes and ponds cover ~9% of the surface area of Scandinavia with up to 70% of boreal and subarctic regions consisting of wetlands, lakes or rivers (Kalff 2002). Sweden has more than 100,000 lakes and 96% of them are also small (between 0.1 and 1 km²; Håkansson and Karlsson 1984). Many of these systems are nutrient-poor (unproductive). Wetlands in boreal regions often contain extensive organic peat layers. Peatlands are major reservoirs of terrestrial organic material (t-OM) that have sequestered up to 1/3 of the total global soil carbon in the past (Post et al. 1982). Therefore, peatlands may act as major sources of t-OM and it has been shown that terrestrial export of organic matter to aquatic systems is positively correlated with percent peatland cover of the catchment (Dillon and Molot 1997).

Inputs of t-OM into aquatic systems are expected to increase in the future due to a combination of different factors. Various climate change scenarios predict increases in precipitation and temperature in northern Europe, together with a higher frequency and magnitude of extreme weather events (Christensen et al. 2007). Warming will potentially lead to higher decomposition rates and increase the overall concentration of organic material in peatlands (Freeman et al. 2001). Higher rainfall and subsequent increases in discharge have been shown to increase the export of t-OM to aquatic systems in Sweden already during the 1970’s and 1980’s (Forsberg 1992). In addition, decreases in atmospheric sulphur deposition are implicated as a major driver of increased export of t-OM to lakes by reducing soil acidity and the ionic strength that binds OM in the soil (Monteith et al. 2007).

As a result, increased inputs of t-OM into lakes due to anthropogenic- and climate-driven changes may affect aquatic food webs by altering the balance between heterotrophic bacteria and phytoplankton biomass as well as overall productivity. This may have profound impacts on metazoan zooplankton populations and ecosystem functioning in general.

Autotrophic vs heterotrophic production

Secondary production in lakes is based on autochthonous as well as allochthonous energy mobilization. While the classic concept of the pelagic food web regards phytoplankton (autochthonous) production as the dominant carbon source (Wetzel 2001), recent studies suggest that terrestrial (allochthonous) carbon can also be an important subsidy for higher trophic levels (e.g. zooplankton and fish), especially in unproductive lakes (Jones et al. 1998; Pace et al. 2004; Cole et al. 2011).
In the autotrophic pathway phytoplankton use dissolved inorganic carbon and inorganic nutrients (N, P) as well as light energy to produce biomass. Heterotrophic bacteria, on the other hand, use reduced carbon in the form of dissolved organic carbon (DOC) as an energy source and inorganic nutrients. This DOC can be of autochthonous (primary producer-derived) or allochthonous (imported terrestrial DOC) origin. Autochthonous DOC is the most important carbon source in marine systems and eutrophic lakes (Cole et al. 1988), while carbon of terrestrial origin supports a large share of bacterial production and respiration in unproductive lakes (Kritzberg et al. 2005). Even though bacteria prefer autochthonous DOC as a substrate (Kritzberg et al. 2004), and bacterial growth efficiency on allochthonous DOC is lower than on autochthonous DOC (Kamjunke et al. 2006), the low molecular weight fraction of terrestrial DOC can be readily used by bacteria (Berggren et al. 2010). This can lead to high bacterial biomass, especially in lakes with high DOC pools (Tranvik 1988; Kritzberg et al. 2004; Kritzberg et al. 2006). In unproductive clear water lakes with moderate inputs of DOC bacteria may also reach high concentrations (Karlsson et al. 2002) as they can outcompete phytoplankton for limiting nutrients (Vadstein et al. 1988).

When using autochthonous carbon, bacteria reintroduce phytoplankton-generated DOC into the food web (i.e. the microbial loop concept; Azam et al. 1983). However, when growing on allochthonous DOC, bacteria use energy from an external source (i.e. terrestrially-derived) and make it available to higher consumers (Fig. 1). Bacteria thereby become uncoupled from phytoplankton dynamics and can constitute an important basal resource in unproductive lakes (Tranvik 1988; Jones 1992; Jansson et al. 2007). The presence of high concentrations of coloured terrestrial material also decreases light availability in those systems which may affect overall pelagic primary producer biomass (Jones 1992). In addition, heterotrophic bacteria are superior competitors for limiting nutrients compared to phytoplankton (Vadstein et al. 1988). For those reasons, phytoplankton carbon rarely exceeds 100 µg L⁻¹ in unproductive lakes and the concentration is often lower (e.g. Jansson et al. 1999; Jansson et al. 2003), while bacterial biomass may be up to three times as high (Hessen et al. 1990; Hessen 1992).

**Terrestrial organic material in lakes**

In addition to the dissolved fraction, t-OM can enter lakes in the particulate form as well (t-POM; Cole et al. 2006). The magnitude of inputs of t-OM depends on different factors, e.g. catchment characteristics or season. Inputs of t-OM are generally high during spring flood and high flow events (Hope et al. 1994; Bergström and Jansson 2000; Jonsson et al. 2001) and the proportion of t-POM increases with discharge (Thurman 1985). Generally, the total organic carbon pool is dominated by the dissolved fraction and t-POM accounts for less than 10% of t-OM (Thurman 1985;
Ivarsson and Jansson 1994). However, as the total input of t-OM can be very high in many boreal unproductive lakes, the particulate fraction can nevertheless constitute a substantial portion, and may potentially contribute to zooplankton carbon nutrition. Concentrations of DOC in humic lakes can range from 20 to 50 mg L⁻¹ with mean values around 30 mgC L⁻¹ (Thurman 1985). Thus, the particulate fraction of OM can contain as much as 5 mgC L⁻¹ whereof phytoplankton and bacteria only account for a small portion (Jansson et al. 1999; Bergström et al. 2003).

It has been proposed that metazoan zooplankton can feed directly on t-POM (Hessen 1998; Pace et al. 2004; Cole et al. 2006). However, this conclusion has mostly been inferred from stable isotope studies and not from direct grazing experiments. As stable isotope analysis cannot distinguish between direct and indirect uptake of t-OM by zooplankton (i.e. via intermediate trophic levels such as heterotrophic bacteria and bacterivorous protozoa), the direct availability and nutritional value of t-POM have been poorly assessed and remain to be tested.

![Fig. 1: Simplified model of the planktonic food web in an unproductive lake with terrestrial subsidies (DOC/DOM: dissolved organic carbon/material; POM: particulate organic material; N: nitrogen; P: phosphorus)](image)

**The role of food quantity**

Growth rates and biomass development of metazoan zooplankton can be limited by food quality as well as food quantity. Whereas deficiencies in food quality only apply to one or few essential resources, food quantity limitation
concerns all necessary resources at the same time. However, food quantity has traditionally mainly been perceived as the intake of organic carbon (as a measure of energy). The potential of fluctuating food quantity to affect zooplankton population dynamics has been shown in several studies. The food threshold concentration has been defined as the quantity of food that is necessary for respiration to equal assimilation. Müller-Navarra and Lampert (1996) observed threshold concentrations of 0.016 mgC L⁻¹ for growth and reproduction of Daphnia populations in the field. Lower food quantities led to low egg numbers and population crashes even when high quality food was available. In laboratory experiments Gliwicz (1990) reported threshold food quantities between ~ 0.015 and 0.04 mgC L⁻¹, with increasing concentrations for smaller Daphnia species. Specific consequences of decreased food quantity may include delayed age at first reproduction (Giebelhausen and Lampert 2001) or decreased reproductive output (Vijverberg 1976).

In contrast to the food threshold concentration, the incipient limiting level (ILL) defines the food concentration above which ingestion rate is constant (Peters 1984). Below the ILL, ingestion rate increases with an increasing food concentration. For D. longispina (similar-sized as D. galeata), Lampert reported an ILL between 0.26 and 0.36 mgC L⁻¹ (1987).

One question that has not been fully answered is whether food quality and quantity limitation can interact. Some evidence suggests that food quality does not play a role at low food levels when energy limitation dominates (Sterner and Robinson 1994; Elser et al. 2003). Yet, other studies reported that food stoichiometry does affect grazer performance irrespective of food quantity (Boersma and Kreutzer 2002) and that the TER may depend on food quantity (Urabe and Watanabe 1992).

**Stoichiometry and mineral nutrient limitation of the aquatic food web**

The stoichiometric framework focuses on the ratios of the most important elements in food webs. Although Lotka (1925) did not use the term ‘stoichiometry’ explicitly, he already considered individual elements and their relative proportions in his work at the beginning of the last century. Since then the stoichiometric concept has been used frequently in aquatic studies to describe how the elemental composition of organisms influences ecosystem processes like secondary production or nutrient recycling (Sterner and Elser 2002).

Producers such as phytoplankton and bacterioplankton differ in their elemental composition. While most phytoplankton are characterized by a high C:P ratio (usually Redfield C:N:P 106:16:1 is assumed), bacteria often have a higher phosphorus content (C:N:P 50:10:1), leading to lower C:P ratios compared to algae (Sterner and Elser 2002). Bacteria are usually considered strictly homeostatic, i.e. they exhibit a fixed elemental...
composition (Thingstad 1987). Some phytoplankton, especially mixotrophic flagellates, may have a less flexible stoichiometry with comparably low C:nutrient ratios (Vrede 1998; Katechakis et al. 2005). Most algal taxa however, can be heavily affected by environmental conditions like light and nutrient availability, and thus algal stoichiometry may vary considerably (the resulting relationships have been postulated in the Light:Nutrient-Hypothesis, see below).

Herbivorous zooplankton, on the other hand, are less flexible in their elemental composition, normally only displaying a narrow range of C:nutrient ratios within species (Andersen and Hessen 1991; Elser 2000; Elser et al. 2000), even though some studies suggest a certain degree of flexibility in cladocerans (e.g. DeMott et al. 1998; Plath and Boersma 2001). If the C:nutrient ratio of the food deviates substantially from the C:nutrient of the consumer those will be either carbon-limited (for C:nutrientfood \(<<\) C:nutrientconsumer) or nutrient-limited (for C:nutrientfood \(>>\) C:nutrientconsumer). Mineral requirements may differ substantially between taxa. The threshold elemental ratio concept (TER) has been introduced to define the ratio above which mineral nutrient limitation occurs. Owing to their low body C:P ratio, cladocerans are more prone to P-limitation while copepods have a higher body C:P and therefore are more sensitive to low N-environments (Hessen and Lyche 1991). A TER_{C:P} \approx 200-300 has been reported for Daphnia (Brett et al. 2000; Sterner and Elser 2002), i.e. when food C:P < 200 daphnids will be C-limited while C:P > 300 characterizes P-limited diets. In contrast to Daphnia, diaptomid copepods may be able to tolerate C:P ratios > 300 without being nutrient-limited (Hessen and Lyche 1991). N-limitation in calanoid copepods occurs when N:P < 30 while TER_{N:P} \approx 12-18 has been reported for cladocerans (Andersen and Hessen 1991; Hessen and Lyche 1991).

The Light:Nutrient-Hypothesis

Many algal species are stoichiometrically flexible and the relationships between light, nutrient availability and phytoplankton elemental composition have been predicted theoretically in the Light:Nutrient-Hypothesis (LNH; Sterner et al. 1997). The LNH states that, in a system with high light:nutrient ratios, phytoplankton will have an increased maximum specific growth rate due to the high amount of solar energy available. At the same time, the low nutrient concentration will limit growth, leading to low relative growth rates (the percentage of maximum growth that is actually achieved) and high algal C:nutrient ratios. The opposite is expected in low light:nutrient environments. Here, the low light leads to a decreased maximum specific growth rate with low nutrient demands that are well met by the high amount of nutrients available. Thus, high relative growth and low C:nutrient ratios of phytoplankton are predicted. Zooplankton stoichiometry
is usually less flexible with comparably low body C:nutrient ratios (Andersen and Hessen 1991). Therefore, a mismatch may exist between producer and grazer composition under high light and low nutrient conditions when algal quality is low due to high C:nutrient ratios. Accordingly, food quality is expected to be higher under low light and high nutrient scenarios when algal C:nutrient is low.

Although the predictions of the LNH have been confirmed in several laboratory and mesocosm studies (Hessen et al. 2002; Urabe et al. 2002; Hall et al. 2004), they may not be applicable to all aquatic systems. While most of those studies used obligate phototrophs, unproductive lakes with high inputs of terrestrial material often have a high abundance of mixotrophs (Bergström et al. 2003) whose stoichiometry is usually much less flexible than that of obligate photoautotrophs under fluctuating environmental conditions (Katechakis et al. 2005). Mixotrophs can either be predominantly autotrophic when light or nutrients are not limiting or switch to heterotrophy under low light and/or low nutrient conditions (Sibbald and Albright 1991; Raven 1997). When concentrations of dissolved nutrients decrease, mixotrophs can use bacteria – characterized by their high P-content – as a P-source (Caron et al. 1990; Jansson et al. 1996). Hence, mixotrophs often constitute a high quality food for consumers in nutrient-poor environments as well as when light conditions are unfavourable. Under these conditions mixotrophs often dominate phytoplankton communities (Riemann et al. 1995; Bergström et al. 2003). It remains to be tested whether the predictions of the LNH are valid in unproductive systems.

The importance of biochemicals

In addition to mineral nutrient limitation (i.e. N and P), biochemical deficiencies can severely affect zooplankton performance. The majority of studies investigating biochemical limitation have focused on fatty acids but sterols have recently gained increasing attention as well.

**FATTY ACIDS.** Many fatty acids are essential for zooplankton and cannot be synthesized by them in sufficient quantities, i.e. they have to be taken up through the diet. In general, fatty acids play crucial roles in regulating cell membrane properties and are precursors of important animal hormones (Brett and Müller-Navarra 1997). It has been shown that especially polyunsaturated fatty acids (PUFA; fatty acids with ≥ 2 double bonds) are critical for growth, survival and reproduction of metazoan zooplankton. Linoleic acid (LA, 18:2ω6), α-linolenic acid (α-LA, 18:3ω3), arachidonic acid (ARA, 20:4ω6), eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3) are considered to be the biologically most important fatty acids (Sargent et al. 1995; Brett and Müller-Navarra 1997). LA and α-LA are mainly plant-derived and can be synthesized *de novo* by few animals. However, some 18-C PUFA (in particular LA, α-LA and also 18:4ω3) may be
elongated and desaturated by many animals to yield longer C-chain PUFA (Wallis et al. 2002). For example, *Daphnia galeata* is capable of converting α-LA (and DHA) into EPA (von Elert 2002). Yet, conversion from ω3 to ω6 PUFAs and vice versa is not possible in animals (Leonard et al. 2004). Especially EPA and ARA play crucial roles for reproduction, e.g. it has been shown that *Daphnia* eggs contain more EPA than somatic tissue (Becker and Boersma 2005; Wacker and Martin-Creuzburg 2007). Further, ARA availability can affect reproductive success (Martin-Creuzburg et al. 2010).

Planktonic organisms differ in their fatty acid composition. Bacteria mainly contain saturated and monounsaturated fatty acids (SAFA and MUFA respectively), and low amounts of PUFA (McCallister et al. 2006; Bec et al. 2010). Cyanobacteria may also contain a few essential PUFA (Ahlgren et al. 1992). Yet, many phytoplankton taxa such as cryptophytes and diatoms are characterized by high amounts of PUFA (Ahlgren et al. 1992; Bec et al. 2003a). Cryptophytes can be especially abundant in humic lakes (Isaksson et al. 1999; Sarvala et al. 1999; Drakare et al. 2002), serving as a source of high quality fatty acids in those systems. Specific fatty acid requirements of zooplankton differ between taxa. While cladocerans are characterized by their high EPA-content (12 – 23% of total fatty acids), they only contain 0.9 – 2.1% DHA (Persson and Vrede 2006). A different pattern can be seen in copepods with a lower EPA-content (7 – 11%) but much higher DHA levels (14 - 21%; Persson and Vrede 2006).

**STEROLS.** Cholesterol, the most important animal sterol (Teshima and Kanazawa 1971), is a precursor of many bioactive molecules, e.g. ecdysteroids that are necessary for the moulting process, and cannot be synthesized *de novo* by most crustaceans (Goad 1981). Both quality and quantity of sterol composition of phytoplankton vary considerably among taxa with possible differences even between strains (Volkman 2003) and in general cholesterol is not present in sufficient amounts in algae to fulfil metazoan zooplankton needs (Nes and McKean 1977). However, crustaceans possess the ability to convert many different phytosterols into cholesterol (Teshima 1971). In contrast to phytoplankton, heterotrophic bacteria and cyanobacteria contain very few or no sterols (Volkman 2003) and there is experimental evidence of the negative effects of this deficiency on zooplankton performance. When feeding on mixtures of eukaryotes and cyanobacteria, *Daphnia* needed at least 20 to 50% eukaryotic algae in the diet to compensate for the low sterol content of cyanobacteria (von Elert 2002; von Elert et al. 2003). Supplementation experiments with heterotrophic bacteria have demonstrated that sterols are also primarily limiting for *Daphnia* growth on this food source (Martin-Creuzburg et al. 2011).

Often, zooplankton food organisms may lack both sterols and essential polyunsaturated fatty acids (e.g. heterotrophic bacteria and cyanobacteria
are both characterized by very low levels of those biochemicals or lack them entirely), leading to co-limitation by those two important groups of biochemicals.

**The quality of different food sources in planktonic food webs**

Metazoan zooplankton diets in unproductive lakes are often composed of a variety of food components (e.g. phytoplankton, heterotrophic bacteria, protozoa and particulate terrestrial material; see Fig. 1) that differ in their nutritional value. The proportions of those components may be highly variable among different lake types and can also fluctuate throughout the seasons. These differences and fluctuations may have pronounced effects on zooplankton population dynamics as many taxa are characterized by different nutritional requirements during different life stages (Sterner and Schulz 1998).

Even though some phytoplankton (in particular chlorophytes) are very susceptible to P-limitation, algae are generally perceived as a high quality food for metazoan zooplankton. Most phytoplankton species contain fatty acids and sterols that are essential for zooplankton. One exception are biochemically insufficient cyanobacteria which can be important components of the phytoplankton community in eutrophic systems but usually play a minor role in unproductive lakes (DeMott and Müller-Navarra 1997).

Due to their low C:P ratio (Vadstein 2000), bacteria match the P-requirements of cladocerans better than most phytoplankton. Hence, from a stoichiometric perspective, they should constitute a high quality food. On the other hand, bacterial cells are small (usually 0.2 – 1 µm) and may thus be ingested less efficiently than larger phytoplankton cells (around 5-20 µm; Geller and Müller 1981). Some taxa such as copepods cannot feed directly on bacteria at all. In addition, heterotrophic bacteria lack essential fatty acids and sterols (Zelles 1999; Volkman 2003).

Terrestrial particulate inputs into lakes can occur in a variety of forms but generally the material is highly recalcitrant and has been degraded in the catchment before being exported to the aquatic system (Wetzel 1984). The nutritional quality of some terrestrially-derived materials has been tested experimentally in grazing studies. Ground leaves were a poor quality food for metazoan zooplankton owing to their high C:nutrient ratios and lack of essential biochemicals (Wehr et al. 1998; Brett et al. 2009). Also, direct grazing on pollen, which may be an important subsidy in smaller lakes, did not enhance zooplankton performance due to its inedible size (Graham et al. 2006) or low digestibility (Masclaux et al. 2011). Yet, adding microorganisms as an intermediate trophic level led to increased zooplankton growth.
(Masclaux et al. 2011), implying that this group of organisms exhibits the ability of trophic upgrading of the low quality pollen.

In northern boreal lakes t-POM often originates from soils in forested catchments that are dominated by pine and spruce with high percentages of land coverage by *Sphagnum*-rich mires and peatlands. So far, no one has tested the food quality of this peat layer t-POM and it remains an open question whether it can fulfil the nutritional requirements of zooplankton.

**Aims of the Thesis**

The objective of my thesis was to investigate how different food components affect metazoan zooplankton survival, growth and reproduction. For the laboratory studies (Paper I-III), I focused on systems that receive high amounts of terrestrial subsidies where zooplankton diets consist of mixtures of phytoplankton, heterotrophic bacteria and terrestrial particulate organic material (t-POM). A mesocosm experiment in an unproductive clear water lake investigated zooplankton responses to changes in food quality in a system with moderate terrestrial inputs (Paper IV).

The aim of Part I was to assess how quality and quantity of different food components affect zooplankton performance. The laboratory experiments used *Rhodomonas*, *Pseudomonas*, *Daphnia* and peat layer t-POM as a model organisms and materials (Fig. 2).

- **Paper I**: Investigation of the effects of different ratios of heterotrophic bacteria and phytoplankton on *Daphnia* survival, growth and reproduction at non-limiting food concentrations.
- **Paper II**: Examination of the bioavailability and nutritional value of t-POM from peat layers for *Daphnia* over a food concentration gradient.
- **Paper III**: Assessment of the effects of increased dietary amounts of heterotrophic bacteria or peat layer t-POM on *Daphnia* performance at varying phytoplankton food concentrations.

The purpose of Part II was the examination of food quality and quantity in a broader context by testing the Light:Nutrient-Hypothesis in a mesocosm experiment in an unproductive boreal clear water lake. In contrast to Part I, this study was performed with a natural plankton community.

- **Paper IV**: Study of the effects of changes in organic carbon, inorganic nutrients and light on zooplankton dynamics in a boreal clear water lake.
Methods

Paper I-III are based on studies conducted under controlled conditions in the laboratory.

Culture conditions were the same for all experiments. Phytoplankton (the cryptophyte *Rhodomonas lacustris*, originating from the culture collection of the Norwegian Institute for Water Research) and heterotrophic bacteria (*Pseudomonas* sp., originally isolated from lakes in the Örebro region in southern Sweden) were cultured separately in 4 L chemostats in modified 3xL16 medium (Lindström 1991), adjusted to pH ~8, with 100 µL L⁻¹ Wright’s solution, vitamins and animal trace elements as in COMBO medium (Kilham et al. 1998). *Rhodomonas* chemostats were run in a 16:8h light:dark cycle at an irradiance of c. 60 µmol m⁻² s⁻¹. *Pseudomonas* cultures were kept in the dark with an additional 40 mgC L⁻¹ in the form of glucose. Chemostats were run at 18-20°C with a dilution rate of c. 0.1 day⁻¹ and gently mixed with a magnetic stirrer for 10 s every 10 min. For each experiment, *Rhodomonas* and *Pseudomonas* cultures were diluted to the desired concentrations with P-free 3xL16.

Peat layer t-POM originated from the riparian zone of the northern boreal forest stream Degerbäcken at 63°59.26’N, 20°22.19’E. The catchment upstream of the sampling point consists of 53% coniferous forest (spruce and pine), 13% coniferous forest on wetland, 22% open wetland (peat bogs), 7% mixed forest (spruce, pine, birch, and aspen), 3% clear-cut forest, and 2% other. The total catchment area is 8.0 km². The material was collected from peat layers next to the stream that are subject to freeze-thaw cycles in situ with peat particles being flushed downstream and into lakes during high flow events such as snow melt. The material was stored in a refrigerator until the experiments. The detritus was suspended in deionised water, sieved through a coarse mesh net to remove large particles and freeze-dried. Small batches of the dry material were ground with a sediment grinder, suspended in P-free 3xL16, filtered through a 35 µm mesh size nylon net to exclude particles outside the edible size range of *Daphnia* and diluted to the desired concentration with P-free 3xL16.

For all laboratory studies a clone of *Daphnia galeata* isolated from mesotrophic lake Nydalasjön (63°49’N, 20°20’E) was grown in 3xL16 and fed unlimited *Rhodomonas* prior to the experiments. Neonates from the third clutch or later that had hatched within 24 h were used in all experiments.
Fig. 2: Experimental organisms: (A) Daphnia galeata, (B) Rhodomonas lacustris, and peat layer terrestrial organic material: (C) microscopic picture, (D) collection site. (Photos: A-C: A. Wenzel; D: T. Vrede)

Paper I – Daphnia feeding on single and mixed Pseudomonas and Rhodomonas diets

This study assessed how different food ratios of the heterotrophic bacterium Pseudomonas sp. and the cryptophyte Rhodomonas lacustris affect D. galeata performance.

A 13 day growth/reproduction experiment was conducted, using five different Pseudomonas:Rhodomonas ratios (in terms of carbon content) at a non-limiting food concentration (1 mgC L⁻¹): 100:0, 80:20, 50:50, 20:80 and 0:100. Survival, growth rate and reproductive output of D. galeata were measured and the fatty acid composition of the two food types was analysed. In a second experiment, Pseudomonas and Rhodomonas were labeled with either ¹⁴C or ³²P. Specific ingestion, assimilation and net incorporation rates of C and P by D. galeata were quantified through feeding experiments over different time periods (minutes to hours). This combined design of short- and long-term experiments allowed an assessment of short-term elemental fluxes as well as investment in somatic growth and reproduction over an ecologically relevant time scale.

Paper II – Peat layer t-POM exploitation by Daphnia

In this study the bioavailability and dietary value of peat layer t-POM for Daphnia nutrition was tested.

First, the effects of different t-POM:Rhodomonas ratios in the food on survival, growth and reproduction of D. galeata were assessed. Daphnids were fed seven different ratios of t-POM and Rhodomonas (in terms of C content: 100:0, 90:10, 80:20, 65:35, 50:50, 20:80 and 0:100) at a non-
limiting food concentration (1 mgC L⁻¹). For the second experiment *D. galeata* was grown on three different food types over a food concentration gradient from low (0.01 mgC L⁻¹) to high (1.28 mgC L⁻¹) food quantity. The food contained either pure *Rhodomonas*, a 50:50 mixture of t-POM and *Rhodomonas* or a 50:50 mix of *Pseudomonas* and *Rhodomonas*. In both experiments, survival, growth rate and reproduction of individuals were quantified after 10 days.

**Paper III – *Daphnia* performance along gradients of mixed *Rhodomonas*, *Pseudomonas* and t-POM**

This study examined the effects of increasing amounts of low quality food (heterotrophic bacteria or peat layer t-POM) on *Daphnia* performance when high quality food (phytoplankton) was present at different, constant concentrations. It was tested in particular whether the ability of *Daphnia* to use low quality food depends on the amount of high quality food available and *vice versa*.

*Daphnia* were fed three different concentrations of *Rhodomonas* (0.22, 0.37 and 0.55 mgC L⁻¹) that were below, similar to or above the incipient limiting level for growth. Increasing amounts of *Pseudomonas* or t-POM were added to the diet (0.15 – 2.4 mgC L⁻¹). Survival, growth and reproduction of *D. galeata* were then quantified again after 10 days.

**Paper IV** describes the results of a three week mesocosm experiment conducted in an unproductive boreal clear water lake, Lake Aborrtjärn (64°29’N, 19°26’E).

The treatments comprised of carbon (in the form of glucose), nitrogen and phosphorus additions as well as light manipulation by shading in a full factorial design in 32 mesocosms. The mesocosms consisted of clear plastic bags that were open to the atmosphere (depth 2000 mm, diameter 850 mm, volume 1.13 m³; Fig. 3) that were filled with lake water filtered through 100 µm mesh net and fastened to wooden rafts. For the low light treatments shade cloth was attached to half of the mesocosms that covered the top and the sides down to 1 m. Glucose, Na₂HPO₄ and NH₄NO₃ were added every three days in the following concentrations: 221 µgC L⁻¹, 7.86 µgN L⁻¹ and 0.714 µgP L⁻¹ giving total additions of 1550 µgC L⁻¹, 55 µgN L⁻¹ and 5 µgP L⁻¹ (C:N:P 249:18:1 in CNP addition treatments). During the first half of the experiment metazoan zooplankton was excluded from the mesocosms to investigate the effects of treatments on phytoplankton alone. Then, zooplankton was added to assess impacts on the whole plankton community.
Paper IV – Testing the Light:Nutrient-Hypothesis in an unproductive boreal clear water lake

This study tested the applicability of the Light:Nutrient-Hypothesis (LNH) in an unproductive boreal clear water lake by manipulating carbon, nutrients (nitrogen and phosphorus) and light availability.

The experiment ran for three weeks and zooplankton were excluded from the mesocosms during the first 10 days and added for the remaining time (11 days). Mesocosms were sampled three times: initial sampling (1st June), second sampling before zooplankton addition (9th June) and final sampling after zooplankton addition (22nd June). Specifically, changes in bacterial and phytoplankton production and biomass, C:nutrient stoichiometry of edible seston and zooplankton biomass in response to treatment additions were investigated to test the predictions of the LNH.

Fig. 3: (A) Shaded and (B) unshaded mesocosms. (Photos: A. Wenzel)
Results

Paper I – Daphnia feeding on single and mixed Pseudomonas and Rhodomonas diets

Increasing proportions of the heterotrophic bacterium Pseudomonas in the food had strong negative impacts on Daphnia survival, growth and reproduction. All individuals died on pure bacterial diets. Survival was possible when ~20% of the diet consisted of phytoplankton (Rhodomonas) but somatic growth in this treatment was poor compared to higher Rhodomonas proportions in the food and daphnids did not reproduce. At least 50% algal food was necessary for egg production and hatching of viable offspring.

Carbon:phosphorus stoichiometry of Rhodomonas and Pseudomonas was comparable (53 ± 8 and 74 ± 13 respectively) and below the TER of Daphnia (>200; Sterner and Elser 2002). Even though daphnids ingested small Pseudomonas cells less efficiently than larger Rhodomonas cells, carbon and phosphorus were incorporated with equal or even higher efficiency from Pseudomonas than from Rhodomonas. In contrast to the similar mineral composition, both food sources contained different amounts of fatty acids. Rhodomonas had a six-fold higher total fatty acid content and a much larger fraction of essential PUFA than Pseudomonas. Further, Pseudomonas did not contain any biologically important ω3 PUFA compared to 60% of total fatty acids being ω3 PUFA in Rhodomonas.

Paper II – Peat layer t-POM exploitation by Daphnia

Diets consisting solely of peat layer t-POM could not sustain Daphnia populations even though the material was successfully ingested. When feeding on variable mixtures of t-POM and Rhodomonas, daphnids survived when ~10% of the food consisted of Rhodomonas while at least 20% was necessary for the production of viable offspring. The time of first reproduction decreased with a higher Rhodomonas proportion in the food but we found no difference in total reproductive output between treatments. When comparing pure Rhodomonas and 1:1 mixtures of Pseudomonas or t-POM and Rhodomonas, daphnids performed best on the former. Survival, growth and reproduction increased with increasing food quantity on all three food types and were higher on Pseudomonas compared to t-POM mixtures. There were no significant interactions between food quality and quantity in any of the treatments.

C:P was similar for Rhodomonas and Pseudomonas (84 ± 15 and 74 ± 9 respectively, average for both experiments), but much higher for t-POM (714 ± 353). Further, fatty acid composition differed substantially between Rhodomonas, Pseudomonas and t-POM. Total fatty acid content was 20
times higher in *Rhodomonas* compared to t-POM. Also, t-POM only contained SAFA and MUFA but no PUFA.

**Paper III – *Daphnia* performance along gradients of mixed *Rhodomonas*, *Pseudomonas* and t-POM**

When feeding on three different concentrations of *Rhodomonas* supplemented with different amounts of *Pseudomonas* or t-POM, *Daphnia* responses varied depending on the amount of phytoplankton present. When *Rhodomonas* was available in non-limiting concentrations (0.37 and 0.55 mgC L⁻¹), the addition of increasing amounts of *Pseudomonas* or t-POM led to decreased *Daphnia* performance. Differences between *Pseudomonas*- and t-POM-supplemented diets were higher for survival and population growth than for somatic growth or reproduction. When *Rhodomonas* quantity was limiting (0.22 mgC L⁻¹), intermediate *Pseudomonas* additions led to increased *Daphnia* performance. No such beneficial effect was seen for the addition of t-POM to limiting concentrations of *Rhodomonas*, rather daphnids went extinct in all but the lowest t-POM addition treatment.

**Paper IV – Testing the Light:Nutrient-Hypothesis in an unproductive boreal clear water lake**

Food quantity in general (measured as particulate organic carbon) increased from the initial to the second sampling day when zooplankton was excluded and decreased during the second half of the experiment in the presence of zooplankton. The decreased light availability in shaded treatments did not lead to lower primary production and phytoplankton biomass and there were no significant changes in seston C:nutrient stoichiometry under these conditions. However, phytoplankton taxonomic composition changed with gymnoids and chrysophytes dominating in shaded treatments. As expected, C:N and C:P decreased with nutrient additions during the course of the experiment. Further, N- and P-additions had positive effects on primary and bacterial production respectively and bacterial production was lower in shaded mesocosms. Nutrient additions did not affect zooplankton biomass, suggesting that zooplankton were not nutrient-limited. Seston C:nutrient ratios below zooplankton TERs in all treatments support this conclusion. However, contrary to the predictions of the LNH, zooplankton biomass decreased in low light treatments.
Discussion

Bacteria- vs phytoplankton-dominated zooplankton diets

Unproductive lakes with high inputs of terrestrial material are often characterized by their high bacterial biomass (Tranvik 1988) and low abundance of phytoplankton (Jansson et al. 1999). **Paper I** showed that pure bacterial diets cannot sustain *Daphnia* populations and that overall *Daphnia* performance is suppressed when the diet contains a high percentage of heterotrophic bacteria. Similar results were reported by Iwabuchi and Urabe (2010) who fed *Daphnia* with pure and mixed bacterial and algal diets and found that daphnids grew better on mixtures and pure algal diets compared to feeding on bacteria only. The results from the labeling experiment in **Paper I** showed that the inability of daphnids to survive, grow and reproduce on a pure bacterial diet was not caused by mineral limitation or low assimilation of bacterial carbon and phosphorus. Daphnids incorporated carbon and phosphorus from *Pseudomonas* with equal or even higher efficiency than from *Rhodomonas*. In line with this finding, high assimilation efficiencies of the cyanobacterium *Synechococcus* have been found previously (Lampert 1977a; Lampert 1977b) and DeMott (1998) concluded that cyanobacteria can fulfil cladoceran P-demands in mixed diets with P-limited phytoplankton. Nevertheless, similar to heterotrophic bacteria, the biochemical composition and quality of cyanobacteria is rather poor with essential fatty acids and sterols lacking in cyanobacteria-dominated diets (von Elert et al. 2003; Martin-Creuzburg et al. 2008).

As bacteria can provide high amounts of phosphorus to *Daphnia* nutrition (Hessen et al. 1989; Hessen and Andersen 1990; **Paper I**), we attribute the differences in performance on phytoplankton- and bacteria-dominated diets to the lack of essential biochemicals in heterotrophic bacteria. Fatty acid analysis showed that *Pseudomonas* contained no essential ω3 PUFA (**Paper I**), which is a general characteristic of heterotrophic bacteria (Zelles 1999). It has also been reported earlier that bacteria lack essential sterols (Martin-Creuzburg et al. 2011).

Our results suggest that the performance of *Daphnia* and potentially other filter-feeding cladocerans in aquatic systems can depend on the relationship between prokaryotes (cyanobacteria or heterotrophic bacteria) that may provide necessary mineral nutrients, and eukaryotes (phytoplankton) which support zooplankton with minerals as well as essential biochemicals (**Paper I**).
Direct exploitation of peat layer t-POM by zooplankton

Results of stable isotope studies have suggested that zooplankton can feed directly on t-POM (Pace et al. 2004; Cole et al. 2006). However, this finding has rarely been tested explicitly in grazing experiments. We fed Daphnia with t-POM originating from boreal peat layers and found that its quality was even worse than that of other terrestrial materials that have been tested earlier, e.g. pollen or ground leaves (Wehr et al. 1998; Brett et al. 2009; Masclaux et al. 2011; Paper II). As t-POM had a much higher C:P than Rhodomonas, mineral limitation of high body-P organisms such as Daphnia on t-POM-dominated diets is likely. Further, the C-component of t-POM may be difficult to digest for metazoan zooplankton that lack carbohydrases (Goedkoop et al. 2007) and the assimilation efficiency of detritus in general is usually low (~20%; Hessen 1998). In addition, we found that t-POM is devoid of essential fatty acids and the material quite likely also lacks sterols (Paper II).

These results suggest that the direct exploitation of peat layer t-POM by zooplankton is of minor importance in boreal lakes due to mineral and biochemical deficiencies as well as low general digestibility of the terrestrial material (Paper II). We propose that high terrestrial stable isotope signals in metazoan zooplankton (e.g. Jones et al. 1998; Karlsson et al. 2003) rather derive from grazing on heterotrophic bacteria that use t-DOM than from the direct consumption of t-POM. Some zooplankton (e.g. cladocerans) may be able to directly ingest bacteria while others may consume bacteria indirectly via feeding on bacterivorous protozoans (Sommer and Sommer 2006).

Comparing the nutritional quality of heterotrophic bacteria and t-POM

Paper I and II showed that daphnids feeding on different mixtures of either Pseudomonas or t-POM and Rhodomonas at non-limiting food concentrations had similar responses of survival, growth and reproduction. These findings suggest a comparable nutritional value of Pseudomonas and t-POM. However, when feeding on 1:1 mixtures of Pseudomonas or t-POM and Rhodomonas as well as pure Rhodomonas over a food quantity gradient, daphnid performance differed between these treatments (Paper II). As growth rates were constant above the same food quantity (~0.2 mgC L⁻¹) for all three food types, we assign the differences in Daphnia performance to differences in food quality. While daphnids performed best on pure Rhodomonas, mixtures with Pseudomonas were a better quality food than mixtures with t-POM. When feeding on mixtures of Rhodomonas and t-POM a higher total food quantity (i.e. a higher quantity of Rhodomonas) was necessary to reach maximum performance than on the other two diets.

Our results imply that t-POM does not directly support metazoan zooplankton diets. Instead, heterotrophic bacteria as well as protozoans are
necessary links between terrestrial OM and metazoan biomass as they upgrade the refractory material in terms of nutritional quality. However, substantial proportions of high quality phytoplankton are still necessary to provide essential biochemicals in systems with high terrestrial inputs (Paper I, II). The importance of the microbial food web for zooplankton nutrition has also been shown in Paper IV when decreased bacterial production was correlated with lower zooplankton biomass.

The nutritional availability of heterotrophic bacteria and t-POM at varying phytoplankton concentrations

In lakes with high inputs of terrestrial material zooplankton are exposed to diets containing variable amounts of phytoplankton, heterotrophic bacteria and t-POM. As the food quality of these components may differ substantially, the dilution of high quality phytoplankton with low quality bacteria or t-POM may affect zooplankton performance and population dynamics. This hypothesis was confirmed in Paper III. In this study, increasing additions of Pseudomonas or t-POM to non-limiting concentrations of Rhodomonas led to decreased Daphnia performance. Further, we found clear differences between the effects of Pseudomonas and t-POM additions at low Rhodomonas proportions, indicating that the quality of these two food sources differs (Paper III). As nutritional requirements of organisms may shift during ontogeny (Sterner and Schulz 1998), susceptibility of juveniles or adults to food limitation may be variable, especially under fluctuating dietary conditions. In agreement with our results (Paper III) it has been shown that Daphnia survival is often more sensitive to mineral nutrient (P) limitation (Urabe and Sterner 2001) while the production of eggs and juveniles requires essential biochemicals (Becker and Boersma 2003).

Our findings suggest that the use of low quality food by zooplankton is affected by the availability of high quality food. When the quantity of high quality food is limiting, metazoan zooplankton diets may be nutritionally supported by low quality food. However, this beneficial effect depends on the type of the low quality food and high quality phytoplankton has always to be present in sufficient quantities as a complimentary food source that fulfils biochemical requirements of zooplankton (Paper I, II, III).

Responses of the pelagic food web to changes in nutrient and light availability in an unproductive boreal clear water lake

Many phytoplankton species are stoichiometrically flexible and their elemental composition heavily depends on environmental conditions such as nutrient and light availability. The arising relationships have been postulated in the Light:Nutrient-Hypothesis (LNH; Sterner et al. 1997). When manipulating C, N, P and light in mesocosms in an unproductive boreal clear
water lake, we could not confirm most of the predictions of the LNH. We suggest two different possible causes for the mismatch between the theoretical predictions and the actual observations in our mesocosms.

First, the microbial food web might have been a weaker link between basal producers and metazoan zooplankton under low light scenarios compared to ambient conditions. We found that decreased zooplankton biomass was correlated with lower bacterial production. As few zooplankton taxa can feed on bacteria directly and the nutritional quality of pure bacterial diets is low (Paper I), intermediate trophic levels such as bacterivorous protozoa are a necessary link between bacteria and zooplankton that may potentially upgrade low quality food. A negative correlation between total ciliates and bacterial biomass suggests the existence of this pathway in the mesocosms (Paper IV). Paper III showed that intermediate concentrations of heterotrophic bacteria may support zooplankton populations when phytoplankton concentrations are low as in our unproductive study system (50 – 150 µgC L⁻¹), so the low abundance of heterotrophic bacteria may have been one reason for the decline in zooplankton biomass.

Secondly, a shift in phytoplankton community composition towards less edible taxa may have been another cause for the decreased zooplankton biomass under low light conditions. We observed a dominance of chrysophytes (in particular Dinobryon) and gymnoids in the shaded mesocosms. Both taxa are potentially mixotrophic. Dinobryon often forms large colonies as a defence against grazers and we found a negative correlation between gymnoid and zooplankton biomass (Paper IV). Further, some mixotrophs may be able to produce toxins under low light scenarios or when feeding on bacteria (Katechakis et al. 2005). Mixotrophic grazing on heterotrophic bacteria in low light scenarios may have been a reason for the decrease in bacterial production. The high abundance of mixotrophs may also explain why we did not see a change in seston C:nutrient ratios in the low light treatments. In contrast to most obligate phototrophic phytoplankton, mixotrophs are stoichiometrically less flexible and their elemental composition is less susceptible to environmental changes.

Our findings suggest that the LNH is not generally applicable to all aquatic systems. Contrary to the theoretical predictions, zooplankton biomass decreased in low light environments. We attribute this mismatch to a shift in the phytoplankton community towards less edible species when light availability was low. Further, we suggest that a weakening of the microbial food web may have been another reason of the decreased zooplankton abundance (Paper IV).
Major conclusions

- Pure bacterial diets cannot sustain Daphnia populations due to biochemical deficiencies (i.e. essential fatty acids and sterols). (Paper I)

- Daphnia can incorporate carbon and phosphorus from phytoplankton and heterotrophic bacteria with similar efficiencies. (Paper I)

- Terrestrial particulate organic carbon (t-POM) from northern boreal peat layers is not a sufficient food source for Daphnia as it lacks essential mineral nutrients as well as biochemicals and may be generally hard to digest for metazoan zooplankton. (Paper II)

- The nutritional quality of northern boreal peat layer t-POM is worse than that of heterotrophic bacteria. High terrestrial stable isotope signals in metazoan zooplankton are most likely derived from terrestrial material that is channeled to higher trophic levels via the microbial food web but not from direct metazoan feeding on t-POM. (Paper II, III)

- Zooplankton performance decreases when non-limiting concentrations of high quality phytoplankton are diluted with increasing concentrations of low quality heterotrophic bacteria or peat layer t-POM. (Paper III)

- The amount of phytoplankton available is critical for zooplankton performance. Low quality food may be an important supplement to zooplankton diets when phytoplankton quantity is limiting. However, this beneficial effect depends on the type of low quality food. While we found beneficial effects of the addition of Pseudomonas, t-POM did not support metazoan zooplankton nutrition. (Paper III)

- The Light:Nutrient-Hypothesis is not applicable to unproductive boreal clear water systems where the phytoplankton community is dominated by mixotrophic organisms. Here, low light levels can negatively affect zooplankton biomass dynamics by shifting algal composition towards less edible taxa and decreasing the importance of the microbial loop. (Paper IV)
Future questions

How do heterotrophic bacteria support pelagic food webs?

Even though Paper I clearly showed that heterotrophic bacteria alone are an insufficient food source for Daphnia due to biochemical deficiencies, they nevertheless contribute to metazoan zooplankton nutrition. Bacterial biomass can be very high in unproductive lakes and the results of Paper III give some indication that intermediate amounts of bacteria can stimulate zooplankton performance when phytoplankton quantity is limiting. Paper IV also showed that a weakening of the microbial food web through decreases in bacterial production may negatively affect zooplankton biomass. Studies on cyanobacteria (which lack essential sterols but may contain some PUFA) suggest that some protozoans such as ciliates or flagellates possess the ability to upgrade low quality food for higher trophic levels in terms of biochemical quality (Bec et al. 2006). However, this effect has so far only been described for algivorous protozoans. On the other hand, strictly bacterivorous protozoans are generally a poor quality food for zooplankton, suggesting that the biochemical composition of heterotrophic bacteria is so poor that it cannot be upgraded by protozoans (Mohr and Adrian 2002; Martin-Creuzburg et al. 2006). It has been shown that the biochemical composition of obligate heterotrophs resembles the fatty acid profile of their food. Studies in marine (Ederington et al. 1995; Harvey et al. 1997) as well as freshwater habitats (Bec et al. 2003b; Boéchat and Adrian 2005) showed pronounced differences in the fatty acid composition of algivorous and bacterivorous ciliates and flagellates. It has been suggested that fatty acids originating from low quality algae can be elongated and desaturated to yield essential PUFA (Veloza et al. 2006) but no such mechanism has been described for bacteria-derived fatty acids yet.

Commonly, mixotrophs are considered a high quality food for zooplankton but several studies have recently found that the nutritional value of mixotrophs also depends on their feeding mode. When grown autotrophically, mixotrophically or heterotrophically, the PUFA concentration in a freshwater flagellate (Boéchat et al. 2007) and in a chlorophyte (Poerschmann et al. 2004) decreased from autotrophic over mixotrophic to heterotrophic feeding mode. These findings imply that mixotrophs are not a high quality food per se but that their nutritional value depends on the growing conditions they experience. However, under natural conditions mixotrophs can probably be expected to grow autotrophically to a certain degree which may explain their generally high food quality.

Given these findings, it would be interesting to investigate in more detail through which pathways heterotrophic bacteria can support metazoan zooplankton diets. Further studies on obligate heterotrophic protozoans as
well as on the role of mixotrophs in unproductive systems may help to elucidate some more details of the relationships between organisms in the microbial food web.

**Effects on zooplankton taxa other than *Daphnia***

The grazing experiments in *Paper I-III* have all studied the effects of different food types on one group of zooplankton, *Daphnia*. Especially in unproductive lakes other taxa may exist as well or even be more abundant, e.g. copepods dominated the zooplankton community in *Paper IV* with *Daphnia* being almost completely absent. Selectively feeding copepods are likely to occupy different trophic positions in the pelagic food web than cladocerans due to their lower body P-content and their inability to graze directly on small food particles such as bacteria. Therefore, additional grazing experiments with other metazoan zooplankton taxa are necessary to assess the role of food quality in unproductive lakes.

**Zooplankton survival, growth and reproduction *in situ***

As all the grazing experiments in *Paper I-III* have been conducted in the laboratory, it would be interesting to test whether the observed effects can be corroborated under natural conditions. A field survey in lakes with different amounts of t-OM inputs could compare *Daphnia* sizes and egg numbers to indicate whether lakes with higher bacterial biomass and t-POM inputs have fewer and smaller individuals and whether these individuals suffer a fitness consequence in the form of reproductive success. To make results comparable between different lakes, laboratory-grown individuals of the same age should be incubated in the field and survival, growth and reproduction quantified over time. As mortality of big ovigerous females due to predation may be much higher than that of small individuals, incubations should be conducted in cages or mesocosms that exclude planktivorous fish.

**Acknowledgements**

I would like to thank Kenyon B. Mobley, Ann-Kristin Bergström and Tobias Vrede for valuable comments on this thesis summary.
References


Danke...

... Anki and Tobias for giving me the chance to come to Umeå and discover the world’s most important organism :-) Thanks for all the discussions, comments on manuscripts and positive input over the last 4.5 years. I definitely learned a lot! Many thanks also to you, Mats, for all your good ideas and valuable comments on the manuscripts, I’m glad you always took the time to be involved in my work.

... Carola and Anders for answering many questions about work in the lab and all your practical help in the beginning.

... Carolyn for letting me escape from my climate room and getting out into the real world. But do we REALLY have to pump water from one lake into another now? ;-) ... Bernadette for showing me how to produce fatty acid beads even though I never ended up working with them – maybe in the next life...

... Kathrin für Film- und Kochabende, Mittagsschwimmen im IKSU, Konzertbesuche, Teekränzchen im Büro und ein jederzeit offenes Ohr für das alltägliche Auf & Ab des Doktorandendaseins. Alte, ist ganz schön leer geworden hier auf dem Korridor, seit Du weg bist!

... Helena, Owen, Johanna and Folmer for making me eat a lot of good food within and outside of work. I will miss Peasoup & Pancakes, BBQ's, visits to the Library Café, feasts at the Indian restaurant... (Why are most of my memories of you guys somehow related to food?!)

... many other people that made life outside of EMG fun and less cold and dark during the long winters. Thanks Andre, Arne, Birte, Carolyn, Christoph, Erik, Gustaf, Jenny, Karin, Magnus, Martin, Melanie, Mårten, Patricia, Peter, Tim, Toni and Wojciech (to name just a few, sorry to those I did not mention) for Friday pubs, birthday parties, conference trips, game evenings, lunch company, BBQ's, international dinners, trips to IKEA, New Year’s celebrations etc.

... Cordu, Friedi, Jessi und Steffy, meine Oldenburger Mädels. Auch wenn Ihr mittlerweile um die Erde verstreut seid, haben wir es doch immer irgendwie geschafft, uns ab & zu mal zu treffen. Ich hoffe, das bleibt auch in der Zukunft so – wann gibt’s den nächsten Cocktail im Patio???

... meinen Eltern für all die Unterstützung und ungezählte (Spekulatius-) Survival-Pakete in den letzten Jahren. Im Tintenklecks wird man sich langweilen in Zukunft... Leider kann ich immer noch nicht die Frage beantworten, was man als Mariner Umweltwissenschaftler eigentlich mal macht – immerhin hab ich’s bis nach Nordschweden geschafft!

... Kenyon for becoming a part of my life and putting up with me over the last three years :) Looking forward to meet K. in April! ♥