# EFFECTS OF DISSOLVED ORGANIC CARBON (DOC) AND ZOOPLANKTON GRAZING PRESSURE ON BACTERIA AND CILIATES

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## ABSTRACT

### EFFECTS OF DISSOLVED ORGANIC CARBON (DOC) AND ZOOPLANKTON GRAZING PRESSURE ON BACTERIA AND CILIATES

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Global climate change results in extreme precipitation events that increase allochthonous organic matter (e.g., dissolved organic carbon, DOC) input into freshwater ecosystems via flooding. While DOC is a source of carbon and energy for heterotrophic organisms in freshwater ecosystems, its bottom-up effects on trophic interactions, and especially on the microbial food web, are poorly understood. Similarly, the top-down effect of contrasting zooplankton traits (i.e., generalist vs. selective) on DOC enriched food webs is also unknown. We compared both the bottom-up effects of DOC types (e.g., leaf-leachate DOC, HuminFeed®) and the top-down effects of zooplankton with contrasting grazing selectivity on the biomass and composition of microbial food webs (i.e., bacteria and ciliates) in a series of laboratory experiment and in-situ mesocosm grazing assays. We predicted that the total biomass of bacteria and ciliates were enhanced by DOC addition, especially with leaf leachate DOC, and reduced by zooplankton. Additionally, we expected that copepods would have higher grazing pressure on ciliates than Daphnia due to grazing mode. We found both DOC types and zooplankton had nonsignificant effect on bacteria biomass. DOC, especially leaf-leachate DOC, had a positive effect on ciliate biomass, while zooplankton had a negative effect. In general, the top-

down effect was in general stronger than the bottom-up effect and the strongest zooplankton effect was in the copepod – ciliate link. DOC had nonsignificant effect on the functional feeding groups of ciliates, and copepods reduced relative biomass of algivore and nonselective ciliates that was a novel proof of selectivity of copepods.

Keywords: Leaf-leachate, Humin Feed, Selectivity, Plankton, Microbial Food Web

# ÖZ

## ÇÖZÜNMÜŞ ORGANİK KARBONUN (DOC) VE ZOOPLANKTON OTLAMA BASKISININ BAKTERİ VE SİLİATLAR ÜZERİNDEKİ ETKİLERİ

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Küresel iklim değişikliği, sel yoluyla tatlısu ekosistemlerine allokton organik madde (örneğin, çözünmüş organik karbon, DOC) girişini artıran aşırı yağış olaylarıyla sonuçlanır. DOC, tatlısularda heterotrofik organizmalar için bir karbon ve enerji kaynağı olsa da aşağıdan yukarıya olan trofik etkileşimleri ve özellikle mikrobiyal besin ağı üzerindeki etkileri tam olarak anlaşılamamıştır. Benzer şekilde, zıt zooplankton özelliklerinin (yani seçici olmayana karşı seçici) DOC ile zenginleştirilmiş besin ağları üzerindeki yukarıdan aşağıya etkisi de bilinmemektedir. Hem DOC çeşitlerinin (yaprak sızıntı suyu, Humin Feed) aşağıdan yukarıya hem de zooplanktonun yukarıdan aşağıya etkilerini, bir dizi laboratuvar ve *in-situ* mezokozm grazing deneylerinde biyokütle ve mikrobiyal gıda ağlarının (yani bakteri ve siliatlar) bileşimi üzerindeki zıt otlama seçiciliği ile karşılaştırdık. Toplam bakteri ve siliat biyokütlesinin, DOC ilavesiyle ve özellikle yaprak sızıntı suyu ile arttığını ve zooplankton tarafından azaltıldığını tahmin ettik. Ek olarak, otlama modundan dolayı kopepodların siliatlar üzerinde *Daphnia*'dan daha yüksek otlama baskısına sahip olacağını tahmin edildi. Hem DOC türlerinin hem de zooplanktonun bakteri biyokütlesi üzerinde önemsiz bir etkiye sahip olduğunu bulduk. DOC, özellikle yaprak sızıntı suyunun, siliat biyokütlesi üzerinde olumlu

bir etkiye sahipken, zooplankton olumsuz bir etkiye sahipti. Genel olarak, yukarıdan aşağıya etki, aşağıdan yukarıya etkiden daha güçlüydü ve en güçlü zooplankton etkisi kopepod - siliat bağındaydı. Ayrıca DOC, siliatların fonksiyonel beslenme grupları üzerinde önemsiz bir etkiye sahipti ve kopepodlar, algivorların ve seçici olmayan siliatların nispi biyokütlesini azalttı; bu, kopepodların seçiciliğinin yeni bir kanıtıydı.

Anahtar Kelimeler: Yaprak Sızıntı Suyu, Humin Feed, Seçicilik, Plankton, Mikrobiyal Besin Ağı

To My Parents, Ayşe and Mevlüt Yetim

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# CHAPTER 1

# INTRODUCTION

One of the major consequences of global climate change during the last decades is extreme precipitation events (Tabari, 2020). These sudden and heavy rains cause flooding worldwide. Floods bring lots of allochthonous matter, including nutrients and particles, into lakes from the catchment area (Hongve et al., 2004; Carpenter et al., 2005; IPCC, 2007; Mellilo et al., 2014; IPCC, 2014; Weyhenmeyer et al., 2016). Dissolved organic carbon (DOC) is one crucial allochthonous matter for lake ecosystems because it provides either labile or recalcitrant carbon (Tranvik, 1988; Steinberg et al., 2008). Although research on DOC in the lakes has increased (Solomon et al., 2015; Degerman et al., 2018), there are still unknowns regarding how the freshwater ecosystems, particularly the microbial food web will adapt to increased different types (i.e., quality) of DOC input.

Bacteria have been considered decomposers and nutrient recyclers in the classical food web view (i.e., phytoplankton, zooplankton, fish). Still, after recognition of microbial food web (i.e., bacteria, heterotrophic nanoflagellates, and ciliates), it is seen that bacteria are also basal producers and competitors with phytoplankton for nutrients (Pomeroy, 1974; Azam et al., 1983; Caston et al., 2009). Moreover, the energy transfer through the food web depends on the primary producer (phytoplankton or bacteria). In the classical food web view (autotrophic pathway), primary consumers (i.e., zooplankton) directly consume phytoplankton. Then the energy flows to upper trophic levels such as planktivorous or piscivorous fish (Brönmark & Hansson, 2005). On the other hand, in the microbial food web (heterotrophic pathway), bacteria can be directly consumed by heterotrophic nanoflagellates (HNF) (Jurgens et al.,2000; Kisand et al., 2000; Sommer et al., 2003), by ciliates (Foissner & Berger, 1996), by *Daphnia* (Riemann, 1985;

Christoffersen et al., 1993; Jeppesen et al., 1992; Modenutti et al., 2003). Ciliates are top predators within the microbial food web (Sherr & Sherr, 2002; Calbet & Landry, 2004) and link the heterotrophic pathway to autotrophic pathway via consumption by mesozooplankton and higher trophic levels (Burns & Schallenberg, 2001; Calbet & Saiz, 2005; Brett et al., 2009). Moreover, interactions between phytoplankton, bacteria, and ciliates also link these two pathways. For instance, some phytoplankton (e.g., mixotrophs) are predators of bacteria (Sanders et al., 1989; Hammer et al., 2005), some ciliates (algivorous & non-selective) consume phytoplankton (Foissner & Berger, 1996), and bacteria can uptake nutrients released from phytoplankton (autochthonous nutrients) (Azam et al., 1983). Therefore, complex food web interactions are governed by bottom-up and top-down effects, which need to be well investigated to understand and predict the impact of DOC enrichment in freshwater ecosystems.

There is a growing interest in the ecological effects of increased DOC inputs in freshwater systems (Mellilo et al., 2014), especially on phytoplankton vs. bacterial production (Lebret et al., 2018). Both bottom-up elements (i.e., nutrients, light availability, etc.) (Seekell et al., 2015; Carpenter et al., 2005) and top-down effects (i.e., predation) (Cottingham et al., 2013; Carrick et al., 1991) control the dominance of phytoplankton or bacteria in the ecosystem. For example, as seen in Figure 1, before DOC addition, zooplankton mainly feeds on phytoplankton, the autotrophic pathway is observed (Degerman et al., 2018). On the other hand, after DOC addition, increased water color and nutrients enhance bacterial production, fueling the heterotrophic pathway (Hessen, 1985; Tranvik, 1988; Solomon et al., 2015; Degerman et al., 2018). When DOC is abundant in the aquatic ecosystems, bacteria can win the competition over phytoplankton since bacteria have a larger surface area: volume ratio than phytoplankton (Joint et al. 2002; Caston et al., 2009). Thus, bacteria and their primary consumers (i.e., heterotrophic nanoflagellates) have a critical role in transferring DOC to primary consumers (i.e., mesozooplankton) in the classic autotroph-grazer food web (Azam et al., 1983; Caston et al., 2009). Therefore, one of the expectations after DOC addition is a switch from the more efficient autotrophic pathway (i.e., high energy transfer from bottom to the top of

the food web) to the less efficient heterotrophic pathway (i.e., low energy transfer from bottom to the top of the food web, Degerman et al. 2018).

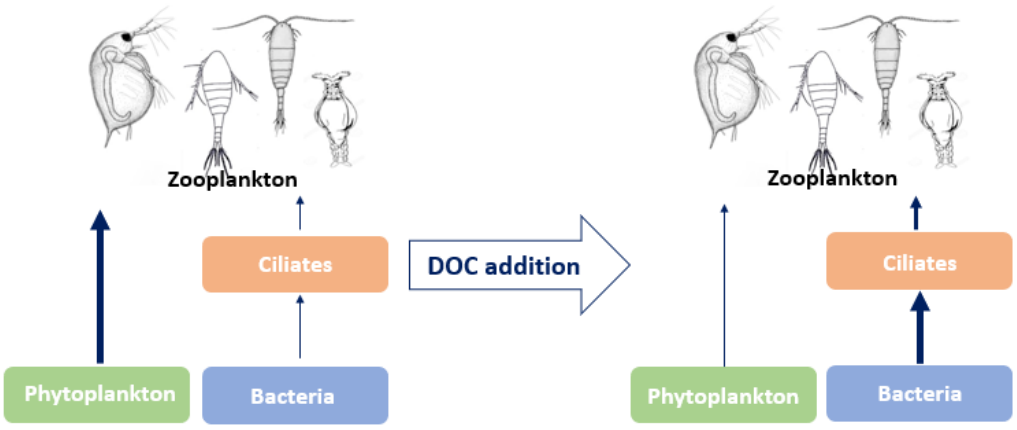


Figure 1: Autotrophic vs. heterotrophic pathway. It is adapted from Degerman et al., 2018.

Previous studies show that DOC enters the food web by bacterial or phytoplankton consumption (Graneli et al., 1999; Karlsson et al., 2007; McCallister & del Giorgio, 2008) and is transferred through higher trophic levels via autotrophic or microbial food web (Tranvik, 1992; Kritzberg et al., 2004; Berggren et al., 2010; Faithfull et al., 2012). To determine DOC effects on the food web, not only the quantity of DOC (Solomon et al., 2015) but also its quality is a crucial determinant for consumption by microbial food web (Catalán et al., 2013; Calderó-Pascual et al., submitted). The quality of DOC depends on the origin of the carbon source and its availability for uptake by bacteria. Leaf-leachate DOC (henceforth L) is an example of a labile carbon source with the most rapid turnover times (i.e., easy to degrade). Also, L contains other nutrients such as N and P (supports basal producers, Sondergaard & Middelboe, 1995). In contrast, examples of poor quality of DOC include recalcitrant carbon sources such as humic substances that are more resistant (i.e., difficult to degrade) to microbial decomposition (Moran & Hodson 1990; Tranvik, 1988). For example, HuminFeed® (henceforth HF; HuminTech GmbH, Grevenbroich, Germany), which is a commercially available humic substance (e.g., leonardite), can be isolated by lignite via alkaline extraction method (Meinelt et al., 2007). Furthermore, HF has high C: N and C: P ratios (McKnight & Aiken 1998) and high

chromophoric properties (i.e., absorb light intensely, Williamson et al., 2015; Minguez et al., 2020). Thus, the quality of HF is lower than the quality of L for consumers. Hence, the question arises of how the quality of DOC can influence the bottom-up effect on the food web, especially on the microbial food web.

In addition to DOC, zooplankton grazing traits like feeding selectivity can play a major role in regulating the structure and function of aquatic ecosystems (Burns & Schallenberg, 2001; Sommer & Sommer, 2003; Kiørboe et al., 2011; Ger et al., 2016). Selective grazers (i.e., calanoid copepods) can choose the type of food being taken (e.g., large, nutrient-rich, etc.) and also have a relatively high prey size ratio, as opposed to generalist grazers (i.e., *Daphnia*), which unselectively consume all captured prey within the edible prey size range (50:1, Burns & Gilbert, 1993; Hansen et al., 1994 & 1997; Wickham, 1995). Calanoid copepods can positively select ciliates over phytoplankton owing to ciliates usually being the optimal prey size range of copepods (18:1, Frost, 1972; Berggren et al., 1988; Hansen et al., 1994), compared to some small or large-sized phytoplankton (e.g., picoplankton, chain-forming diatoms) for consumption. Moreover, ciliates are more nutritious than phytoplankton in nitrogen content (Stoecker & Capuzzo, 1990). Thus, they are rich in proteins, amino acids, and polyunsaturated fatty acids (PUFA, Stoecker & Capuzzo, 1990). Finally, ciliates swim faster than phytoplankton, creating a strong detectable hydrodynamic signal, which acts as a cue for copepod grazing (Kiørboe & Visser, 1999). On the other hand, *Daphnia* consumes phytoplankton or ciliates randomly, based on food size (DeMott, 1986; Burns & Gilbert, 1993). Therefore, calanoid copepods can be expected to have a stronger link to ciliates and microbial food web when compared to *Daphnia* (Adrian & Schneider-Olt, 1999; Burns & Schallenberg, 2001). Moreover, calanoid copepods can change the relative abundance of ciliates, while *Daphnia* is not expected to have significant effects on the composition of ciliates (Burns & Gilbert, 1993; Wickham, 1995; Sommer & Sommer, 2006). Nonetheless, such zooplankton grazing traits on the microbial food web in freshwater ecosystems are not studied well, and even less so in regard to the effects of DOC enrichment.

There is little empirical evidence for DOC effects on bacteria and ciliates. First, DOC effects on the microbial food web components are less understood (Degerman et al., 2018). Second, how the bottom-up effects of DOC interact with the top-down effects of zooplankton and in this context, the effects of zooplankton with different grazing traits (selective vs. generalist) are essentially unknown (i.e., in the context of DOC and grazer interactions) (Brett et al., 2009; Cowles et al., 1988). Third, while global warming is known to increase DOC runoff, the effects of different DOC sources on the trophic interactions between zooplankton and the microbial food web are not understood (Guillemette et al., 2016). Because of these gaps, we are interested in evaluating the bottom-up (i.e., DOC) and top-down (i.e., mesozooplankton) effects on bacteria and ciliates.

For this purpose, we performed two experiments during a scenario of increased DOC via zooplankton grazing assays, and these experiments were parallel to a 36 days mesocosm experiment (Yıldız et al., prep.; Yalçın et al., prep.; Calderó-Pascual et al., submitted). First, the laboratory grazing experiment was performed to quantify the effect of DOC together with contrasting zooplankton grazing traits. The second was an *in-situ* mesocosm grazing assay to quantify the effects of DOC quality and mesozooplankton grazing on bacteria and ciliate biomass and composition. For the laboratory grazing experiment, hypotheses as follow; i) increase in DOC would increase the biomass of bacteria and ciliates (i.e., DOC would have a positive effect on bacteria and ciliate biomass), ii) both grazers (*Daphnia* and copepods) would decrease the biomass of bacteria and ciliate, and iii) the grazing pressure of copepods would be greater than *Daphnia* on ciliate biomass. For the *in-situ* mesocosm grazing assay, hypotheses were i) leaf leachate DOC would enhance bacteria and ciliate biomass compared to control (i.e., no DOC) and recalcitrant source, and ii) grazers reduce bacteria and ciliate biomass compared to no mesozooplankton control group.

# CHAPTER 2

# MATERIALS & METHODS

## 2.1 Experimental Design

### 2.1.1 Laboratory Grazing Experiment

A laboratory grazing experiment was conducted in the Department of Biological Sciences at Middle East Technical University (METU), Ankara, Turkey (39.89 ºN, 32.78 ºE). This experiment aimed to measure the bottom-up effects of dissolved organic carbon (i.e., DOC) and the top-down effects of zooplankton with contrasting grazing selectivity (i.e., generalist and selective-feeder) on the biomass and composition of microbial food web components (i.e., bacteria and ciliates) and phytoplankton. The experiment had a 2x3 factorial design with a DOC treatment (+DOC, -DOC) crossed with a zooplankton treatment (*Daphnia*, Copepod, no-grazer), with four replicates of each treatment from June 17 to June 21, 2019 (i.e., four days). The DOC treatment contained a mix of two types of dissolved organic carbon sources, which were a recalcitrant C source (i.e., HuminFeed®, R), and a labile C source (i.e., leaf-leachate DOC, L) (Table 1, Fig. 2). The zooplankton treatment included equal biomass of two different zooplankton functional groups with contrasting grazing selectivity (i.e., *Daphnia* as generalist grazers and calanoid copepods as selective grazers).

Table 1: The design of the laboratory experiment.

| | **No Grazer (i.e., grazer control)** | ***Daphnia*** | **Copepod** |
|---|---|---|---|
| **No DOC (i.e., DOC control)** | -$DOC_{No\ grazer}$ | -$DOC_{Daphnia}$ | -$DOC_{Copepod}$ |
| **Addition of DOC** | +$DOC_{No\ grazer}$ | +$DOC_{Daphnia}$ | +$DOC_{Copepod}$ |

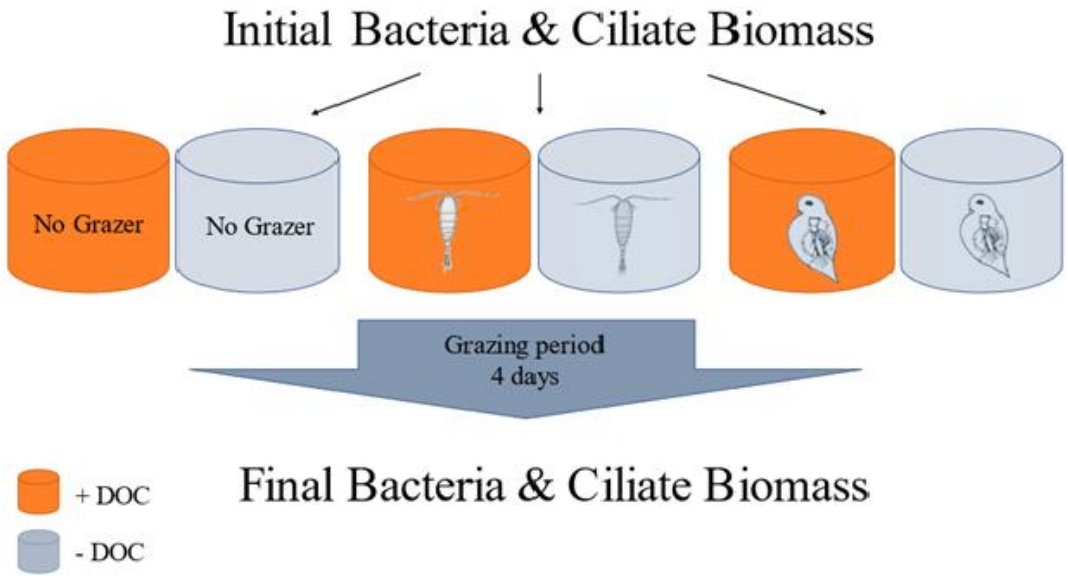


Figure 2: Basic experimental design of the laboratory experiment.

#### 2.1.1.1 Preparations Before Conducting the Laboratory Experiment

Before the laboratory experiment started, phytoplankton cultures were obtained from the Norwegian Institute for Water Research (NIVA) culture collection. Both the mixotrophic Cryptomonas pyrenoidifera and the autotroph Chlamydomonas reinhardtii were maintained in Wright's Cryptophyte (WC) medium at the exponential growth phase via semi-continuous batch cultures, under a 60 µm photon m-2 s-1 light intensity, 14:10 hour L:D cycle, and at 22 ± 1 °C (Fig. 3).

Figure 3: Phytoplankton culture set-up under a 60 µm photon $m^{-2}$ $s^{-1}$ light intensity, 14:10 hour L:D cycle, and at 22 ± 1 °C.

For the laboratory experiment, we would like to test the grazing interactions of the relevant zooplankton species and those dominated during the main mesocosm experiment. Accordingly, zooplankton cultures were created by using the dominant (i.e., biomass) two species of calanoid copepods and one species of Cladoceran collected by using 250 µm zooplankton net, from five different lakes nearby Ankara (i.e., their trophic states varied from oligotrophic to eutrophic). The calanoid copepod species used in the laboratory experiment were *Acanthodiaptomus denticornis* from Lake Yeniçağa, with a mean body length (i.e., prosome and urosome) of 1.39 ± 0.36 µm (SD, n = 22), and *Arctodiaptomus bacilliferus* from Lake Mogan, which had a mean length of 1.14 ± 0.32 µm (SD, n = 22). The Cladoceran species was *Daphnia magna,* also from Lake Mogan, which was medium-sized (~ 2-3 mm). Zooplankton cultures were maintained in GF/C filtered lake water and fed with an equivalent of 0.5 mg C $L^{-1}$ of a 1:1 biomass of the cultured phytoplankton described above every three days until the grazing experiment (Ger

et al., 2010). Animals were starved for 24 hours before the grazing experiment to minimize potential differences in prey ingestion due to variable gut fullness.

The +DOC and –DOC treatments were prepared in 10 liters buckets (for each) by mixing 45µm filtered lake (mesocosm) water with (for the +DOC treatment) or without (for the –DOC treatment) the two DOC sources. The buckets were spiked with nutrients (i.e., a final concentration equivalent to the final WC nutrient medium) to minimize nutrient limitation or any potential differences due to unaccounted nutrients added by the DOC solution during the grazing assay. The DOC mixture for the +DOC treatment was prepared with a final concentration of 10.91 ppm before starting the laboratory experiment. For the addition of HuminFeed® (7 ppm final concentration), 70 mL of 1 g/L stock solution was diluted in 10 liters of distilled water. The leaf-leachate DOC source was extracted from dry leaves of the locally abundant white poplar (*Populus alba*) by adding 60 g dry leaves per liter, in a total of 4 liters (240 g/4L) of distilled water, which was stored at 4 °C in the dark. After 72 hours of incubation, the concentration of the stock leaf-leachate DOC solution was measured as 342 ppm (Shimadzu TOC-L/CPN analyzer, Japan). For adding 3.91 ppm (final concentration) of leaf-leachate DOC, 114.3 mL of the stock DOC solution was filtered through 45 µm mesh before dilution in the 10 liters +DOC bucket (Fig. 4).

Figure 4: Preparation of the laboratory experiment mediums in the 10 liters of -DOC (left) and +DOC (right) buckets before being allocated to the individual experimental units.

#### 2.1.1.2 Performing the Laboratory Experiment

After full homogenization of DOC solution (via gentle mixing by a clean 1-liter beaker), an equivalent of 0.25 mg C $L^{-1}$ of *Cryptomonas pyrenoidifera* and *Chlamydomonas reinhardtii* were added to the buckets to reach the total concentration of 0.5 mg C $L^{-1}$ phytoplankton in addition to the natural phytoplankton community to minimize variability among jars. Subsequently, 12 previously acid-washed 0.6 L glass jars were filled with 0.55 L water from the +DOC bucket (i.e., +DOC treatment), while another 12 previously acid-washed 0.6 L glass jars were filled from the -DOC bucket (i.e., -DOC treatment), which resulted in 24 experimental units. All glass jars were slightly bubbled (~ 5 bubbles/s) to homogenize suspended prey and ensure adequate oxygen. Once the contents were homogenized (after about 5 minutes and gentle mixing by a plastic pipette tip), the initial (day 0) samples were taken for phytoplankton (50 mL) and bacteria (10 mL) from each replicate. Finally, the grazers were added as 2 medium-sized (2-3 mm) *Daphnia*/jar to the *Daphnia* treatments and 30 adult copepod/jar to the copepod treatments. The total biomass of 30 copepods was comparable to the total biomass 2 medium-sized *Daphnia* (see below), and therefore, each jar with grazers had similar zooplankton biomass. After the grazers' addition to grazer treatments, the four-day-long laboratory experiment started (Fig. 5). The experiment was maintained under identical conditions as the phytoplankton cultures (see above). The final grazer biomass (i.e., day 4) was measured and confirmed that each with grazer treatment had comparable biomass, with copepod treatments were a mean of 0.75 mg (± 0.10, n = 8) per jar, and *Daphnia* treatments a mean of 0.88 mg (± 0.17, n = 8) per jar regardless of DOC treatment. This enables a quantitative comparison of the mass-specific grazing effect between *Daphnia* vs. copepods on bacteria and ciliates during the experiment. In addition to the initial (day 0) samples, additional subsamples for phytoplankton (50 mL), ciliates (50 mL), and bacteria (10 mL) were gently (i.e., without damaging the mesozooplankton grazers) taken by using 10 mL pipettes with sterile tips in the middle (i.e., day 2) and at the end (i.e., day 4) of the experiment. Sampling details are provided below.

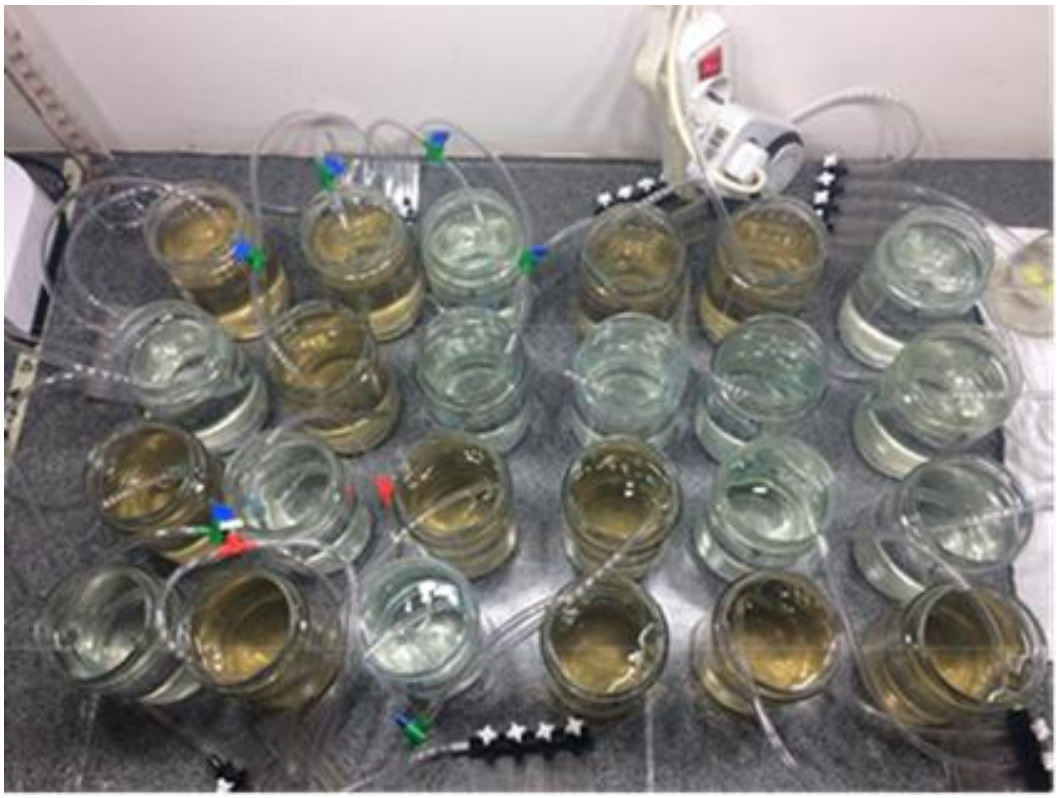

Figure 5: The laboratory experiment showing the random placement of treatment and control jars with the visible DOC addition. The experimental set-up was maintained under 60 µm photon $m^{-2}$ $s^{-1}$ light intensity, 14:10 hour L:D cycle, and at 22 ± 1 °C.

### 2.1.2 *In-situ* Mesocosm Grazing Assays

The *in-situ* mesocosm grazing assays were parallel to a 36 days mesocosm experiment, which was conducted in the Experimental Lake at Middle East Technical University (METU), Ankara, Turkey (39° 52' 13.18" N, 32° 46' 31.92" E). There were 16 cylindrical-shaped mesocosms with a diameter of 1.2 m and 2.2 m depth (volume 2480 L) on the floating mesocosm platform. A submersible pump filled each mesocosm with 500 µm filtered lake water. Plankton inoculation was performed by using zooplankton (250 µm zooplankton net) and phytoplankton sample mix from five local lakes to have a heterogeneous plankton community before nine days of the experiment started. The experimental setup was control (no DOC), leaf-leachate DOC (L, ~8 mg C $L^{-1}$) as a labile C source, HuminFeed® (R, ~1.5 mg C $L^{-1}$) as a recalcitrant C source, and a combination of leaf-leachate and

recalcitrant C sources (Mixed, ~9.5 mg C $L^{-1}$). We used leaves of alder tree (*Alnus sp.)* while preparing leaf-leachate C source. The main mesocosm experiment started with adding different DOC sources into mesocosms where they were randomly placed, respectively (Day 0 – June 20, 2019).

24-h short-term grazing assays were designed to quantify and compare the top-down effect of zooplankton on bacteria, ciliate, and phytoplankton biomass in each mesocosm tank and across the different DOC treatments during the long-term mesocosm experiment (see above). The main (i.e., long term) mesocosm experiment had a 4x4 factorial design (replicated four times) with four different DOC treatments (no DOC control (i.e., C), leaf-leachate DOC (i.e., L), recalcitrant DOC (i.e., R), and combination of leaf-leachate DOC and recalcitrant DOC (i.e., Mixed). Treatments of *in-situ* mesocosm grazing assays were identical to the main mesocosm experiment, crossed with two grazer treatments consisting of a no mesozooplankton control (i.e., no mesozooplankton present; -Z) and with grazer treatment (i.e., containing >200 µm mesozooplankton; +Z) (Table 2, Fig. 6). We performed two *in-situ* grazing assays to account for potential changes within the plankton community and microbial food web following the DOC addition. The first assay took place after one day of DOC addition (June 21, 2019), and the second assay took place four days after DOC addition (June 24, 2019) to the mesocosm system.

Table 2: The design of *in-situ* mesocosm grazing assays.

| | **Without Mesozooplankton** | **With Mesozooplankton** |
|---|---|---|
| **No DOC** | C | C Z+ |
| **Leaf-leachate DOC** | L | L Z+ |
| **Recalcitrant DOC** | R | R Z+ |
| **Leaf-leachate DOC & Recalcitrant DOC** | Mixed | Mixed Z+ |

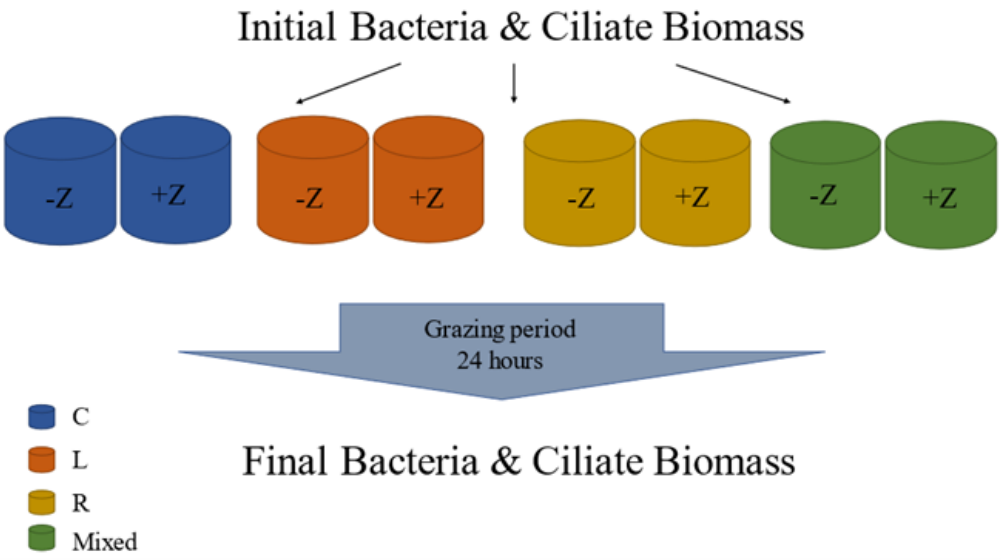


Figure 6: Basic experimental design of the *in-situ* mesocosm grazing assays.

To perform *in-situ* grazing assays, we used two white opaque 0.5 L plastic bottles, which were placed in each mesocosm (Fig. 7). By using a tube sampler, a 15 L water sample from each mesocosm was taken and filled into buckets of each mesocosm. For no mesozooplankton control (-Z), one of the bottles was filled with 200 µm filtered mesocosm water, which contained only seston and microzooplankton. For the addition of mesozooplankton (+Z) treatment, the second bottle was also filled with 200 µm filtered mesocosm water and concentrated mesozooplankton from each tank (contents remaining on 200µm mesh after filtering 5 L of mesocosm water) was added. The initial concentration of seston and zooplankton for both *in-situ* mesocosm experiments was assumed to be identical to those of the mesocosm at the time of sampling (>200 µm). Zooplankton samples of the main experiment were collected by filtering 5 L of mesocosm water through a 45 µm zooplankton net, and contents on the net were put in a 50 mL dark bottle. A day (24 h) later, the bottles were immediately brought back to the laboratory in a cool, dark box. Then, a 50 mL subsample for bacteria, a 100 mL subsample for ciliates, and a 50 mL subsample for phytoplankton analyses were taken after homogenizing the contents of each bottle. Sampling details are provided below.

Figure 7: The *in-situ* mesocosm grazing assay bottles in a mesocosm.

## 2.2 Sample Preservation and Counting

### 2.2.1 Bacteria

Subsamples for bacteria were fixed with glutaraldehyde (Sigma Aldrich) to a final concentration of 2% (v/v) and stored at -20 °C until enumeration. Bacteria samples were stained for 30 min with 4'6-diamidino-2-phenylindole (DAPI, Sigma Aldrich) at a final concentration of 10 µL DAPI $mL^{-1}$. For counting bacteria, 2 mL of subsamples were filtered by using 0.2 µm pore size black Nuclepore filters. The abundance of bacteria was determined by direct counting of cells under 1500X magnification of epifluorescence microscopy (Leica, DM 6000B, Wetzlar, Germany). For each sample, at least 400 bacteria cells from different fields were counted by using a 420 nm UV filter. Counted filters were stored at -20 °C. A factor of 0.22 pg C $\mu m^{-3}$ was used for conversion to carbon biomass for bacteria (Bratback & Dundas, 1984; Borsheim & Bratback, 1987).

### 2.2.2 Ciliate

Subsamples for ciliates were fixed with acidic Lugol's iodine (Sigma Aldrich) to a final concentration of 4% (v/v)) and stored at room temperature at dark until enumeration. Utermöhl's (1958) counting procedure was performed by using

sedimentation chambers. Ciliates were counted under 630X magnification of an inverted microscope (Leica DMI 4000B, Wetzlar, Germany), and taken photos of at least 30 individuals of one species with a digital camera (Leica DFC280, Wetzlar, Germany). For each sample, at least 300 ciliate cells or the entire chamber were counted. The genus or species of ciliates were identified according to Foissner, Berger & Schaumburg (1999). The length and width dimensions of animals were measured by ImageJ software. Biovolumes of ciliates were calculated based on an appropriate geometric shape (Sun & Liu, 2003). A factor of 0.14 pg C $\mu m^{-3}$ was used to convert biovolume to carbon biomass for ciliates (Putt & Stoecker, 1989).

Ciliates were classified into four functional feeding groups based on their food preference (i.e., main food). The algivore and bacterivore groups are assumed to specialize in ingesting mostly algae or bacteria, respectively. The predator group was supposed to feed on other heterotrophic protozoa, mostly small ciliates, while the nonselective group was assumed to feed on algae and bacteria (Foissner & Berger, 1996).

### 2.2.3 Zooplankton

Subsamples for zooplankton were fixed with acidic Lugol's iodine (Sigma Aldrich) to a final concentration of 4% (v/v)) and stored at room temperature at dark until enumeration. Zooplankton was counted under 10X magnification of a stereomicroscope (Leica M125, Wetzlar, Germany), and taken photos of at most 25 individuals. The genus or species of zooplankton were identified according to Scourfield & Harding (1966) and Harding & Smith (1974). The dry weight of the zooplankton was calculated based on the allometric relationship between weight and body size (Dumont et al., 1975; Bottrell et al., 1976; Ruttner-Kolisko, 1977; McCauley, 1984; Yıldız et al., prep.).

## 2.3 Statistical Analysis

### 2.3.1 Bacteria and Ciliate Biomass Analysis

In both experiments, additive generalized linear models (i.e., GLM, Biomass ~ DOC + grazer) were used to test if there was a difference between treatments of both DOC and grazers on the biomass of bacteria and ciliates relative to controls (i.e., -DOC or –Z, respectively) through the glm function in statistical package R Version 1.3.959 software (R Core Team, 2020). In the laboratory experiment, paired t-tests were used to compare changes in biomass over time among the treatments via the t.test function in the stats package.

Data from the laboratory experiment were log (x+1) transformed, while data of *in-situ* mesocosm experiment were log (x) transformed to attain the assumptions of linear models (i.e., normal distribution). Zooplankton data of the in-situ mesocosm experiments were normal, and variances were homogenous, so the data did not require any transformation. The normality of distribution and homogeneity of variance was tested with a Shapiro-Wilk test and Levene's test through shapiro.test function of the stats package and leveneTest of the car package, respectively, and with diagnostic plots.

### 2.3.2 Effect Size Analysis of Bacteria and Ciliate Biomass

The effect size metric was used to compare the normalized effect of a given treatment on the biomass of bacteria or ciliates when compared to the control group, which enables a quantitative comparison of the treatment effects (e.g., DOC vs. grazer effects) on a given response variable (i.e., bacteria and ciliate biomass) across the same effect scale. Log response ratios (ln R) and sampling variances were calculated to show the relative increase or decrease of the response variable in the treatment compared to its control group by dividing the treatment biomass with the respective control (Hillebrand & Gurevitch, 2016). For the laboratory experiment, the calculations were performed by using biomass of bacteria or ciliates to observe

the effect of DOC or grazing treatments, respectively, using biomass data from the end (day 4) of the laboratory experiment (Table 3). Hence, for the laboratory experiment, the 'treatment' for calculating the DOC effect was the bacteria or ciliate biomasses in the jars that received DOC (i.e., +DOC), while the 'control' for calculating the DOC effect is the bacteria or ciliate biomass in the jars that did not receive DOC (i.e., -DOC). Similarly, the 'treatment' for calculating the grazer effect was the bacteria or ciliate biomasses in the jars that received a zooplankton grazer (i.e., either *Daphnia* or copepod), while the 'control' for calculating the grazer effect is the bacteria or ciliate biomass in the jars without any grazers (i.e., no-grazer). The designation of biomass to estimate the effect size for the mesocosm grazing assays was similar (Table 4), such that the 'treatment' for grazer effect size is the bacteria or ciliate biomass in the bottles that received the *in-situ* mesozooplankton grazer community (+Z), while the 'control' for calculating the grazer effect is the bacteria or ciliate biomass in the jars without any grazers (i.e., -Z). Similarly, the 'treatment' for calculating the DOC effect is the bacteria or ciliate biomass in the bottles that were in the mesocosm tanks receiving one of the three DOC sources (i.e., R, L, Mixed), while the 'control' for calculating the DOC effect is the phytoplankton biomass in the bottles that were in the mesocosm tanks that did not receive any DOC (i.e., -DOC). The specific formula used to calculate the effect size values are shown in Table 3 (laboratory experiment) and Table 4 (*in-situ* grazing assay). The two effect size categories (i.e., DOC and Grazer) were calculated for specific treatments as shown below. Values in the formula represent the bacteria or ciliate biomass within that given treatment at the end of the experiment (day4).

Table 3: The log-ratio formula (ln R) used to calculate the effect size of either DOC or grazers on bacterial or ciliate biomasses in the laboratory experiment.

| | **Treatment** | **ln R** |
|---|---|---|
| **DOC Effect Size** | No grazer | ln ($+DOC_{no\ grazer}/-DOC_{no\ grazer}$) |
| | Copepod | ln ($+DOC_{Copepod}/-DOC_{Copepod}$) |
| | *Daphnia* | ln ($+DOC_{Daphnia}/-DOC_{Daphnia}$) |
| **Grazing Effect Size** | +DOC Copepod | ln ($+DOC_{Copepod} / +DOC_{no\ grazer}$) |
| | -DOC Copepod | ln ($-DOC_{Copepod}/-DOC_{no\ grazer}$) |
| | +DOC *Daphnia* | ln ($+DOC_{Daphnia}/ +DOC_{no\ grazer}$) |
| | -DOC *Daphnia* | ln ($-DOC_{Daphnia} / -DOC_{no\ grazer}$) |

Table 4: The log-ratio formula (ln R) used to calculate the effect size of either DOC or grazers on bacteria or ciliate biomasses in the *in-situ* grazing assays.

| | **Treatment** | **ln R** |
|---|---|---|
| **Grazing Effect** | No DOC control (C) | $\ln(C_{Z+}/C)$ |
| | Recalcitrant DOC (R) | $\ln(R_{Z+}/ R)$ |
| | Leaf-leachate DOC (L) | $\ln(L_{Z+} / L)$ |
| | Mixed | $\ln(Mixed_{Z+} / Mixed)$ |
| **DOC Effect** | R Z+ | $\ln(R_{Z+} / C_{Z+})$ |
| | R | ln(R / C) |
| | L Z+ | $\ln(L_{Z+} / C_{Z+})$ |
| | L | ln(L / C) |
| | Mixed Z+ | $\ln(Mixed_{Z+} / C_{Z+})$ |
| | Mixed | ln(Mixed / C) |

### 2.3.3 Analysis of Functional Feeding Groups of Ciliates

In the laboratory experiment, biomass data of functional feeding groups of ciliates were log (x) transformed for normality. An additive generalized linear model (i.e., GLM, Biomass ~ DOC + grazer, Biomass% ~ DOC + grazer) was used to test if there was a difference between treatments of both DOC and grazers on biomass and relative biomass of ciliate functional feeding groups on each sampling day. The change in relative biomass over time was analyzed by Mann Whitney Wilcoxon test through wilcox.test in R. In the *in-situ* mesocosm assay, biomass data of functional feeding groups of ciliates were log (x) transformed for normality. The additive generalized linear model (i.e., GLM, Biomass ~ DOC + grazer, Biomass% ~ DOC + grazer) was used to analyze the biomass and relative biomass of feeding groups of total ciliates with respect to the presence of mesozooplankton and the type of DOC in each experiment. The effect size of functional feeding groups in the laboratory experiment was calculated using the mean biomass values of each functional feeding group (i.e., algivore, bacterivore, nonselective, predator) according to the formula shown in Table 3 or Table 4, respectively.

# CHAPTER 3

# RESULTS

## 3.1 Laboratory Grazing Experiment

### 3.1.1 Effects of DOC and Zooplankton Treatments on Bacteria and Ciliate Biomass

#### 3.1.1.1 Bacteria

According to general linear model (GLM) analysis, there was no significant effect of either the bottom-up (i.e., DOC) or the top-down (i.e., grazer) on bacteria biomass throughout the experiment (Table 5). Neither the copepods nor the *Daphnia* changed the biomass of bacteria. At the beginning of the experiment (i.e., day 0), the mean bacteria biomass values were 45.5 ± 11.3 and 35.6 ± 7.2 µg CI (n=12) for +DOC and -DOC treatments (i.e., with and without DOC), respectively (Fig. 8A). After two days (i.e., day 2), the mean biomass of bacteria in -DOC treatments were doubled, and the value was 69.3 ± 14.6 µg CI (n=12), and in +DOC treatment, it also increased to 65.9 ± 17.4 µg CI (n=12) (Fig. 8B). At the end of the experiment (i.e., day 4), in both +DOC and -DOC treatments, the mean biomass of bacteria reduced to 51.0 ± 8.0 and 53.5 ± 11.6 µg CI (n=12), respectively (Fig. 8C).

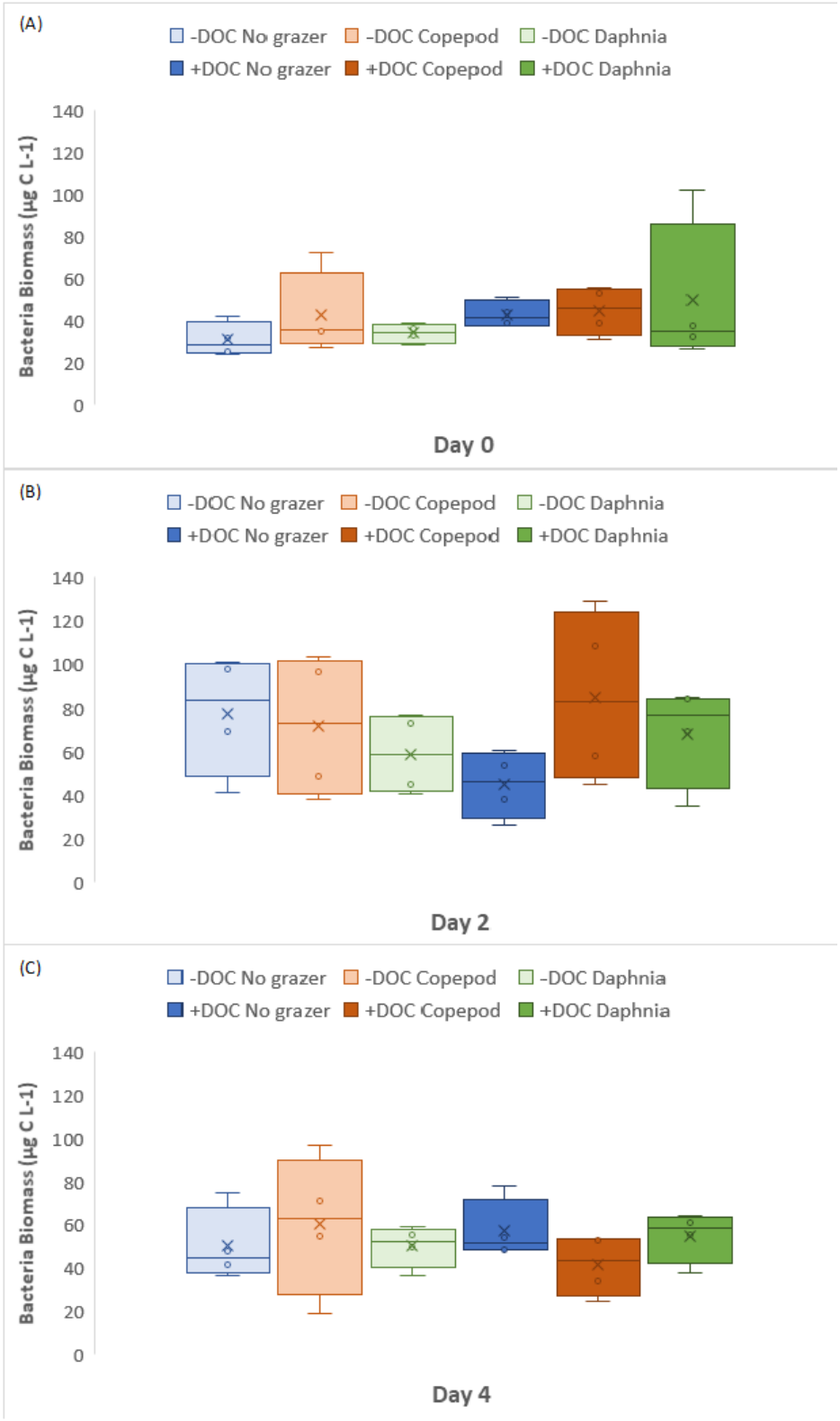


Figure 8: Boxplots showing the median biomass (µg C/ L) of bacteria across the grazer and DOC treatments in (A) Day 0, (B) Day 2, (C) Day 4.

Table 5: General Linear Model (GLM) analysis results of total bacteria biomass in relation to the treatments (DOC or grazer) relative to respective controls throughout the experiment. (Biomass ~DOC + Grazer)

| Day | Dependent Variable (y) | Independent Variable/ Factor (x) | Estimate | Standard Deviation | t val. | P val. |
|---|---|---|---|---|---|---|
| 0 | Bacteria | DOC | 0.21715 | 0.13372 | 1.624 | 0.120 |
| 0 | Bacteria | Copepod | 0.14611 | 0.16378 | 0.892 | 0.383 |
| 0 | Bacteria | Daphnia | 0.05955 | 0.16378 | 0.364 | 0.720 |
| 2 | Bacteria | DOC | -0.07963 | 0.17741 | -0.449 | 0.658 |
| 2 | Bacteria | Copepod | 0.24226 | 0.21728 | 1.115 | 0.278 |
| 2 | Bacteria | Daphnia | 0.07583 | 0.21728 | 0.349 | 0.731 |
| 4 | Bacteria | DOC | -0.01342 | 0.15229 | -0.088 | 0.931 |
| 4 | Bacteria | Copepod | -0.14158 | 0.18652 | -0.759 | 0.457 |
| 4 | Bacteria | Daphnia | -0.01300 | 0.18652 | -0.070 | 0.945 |

#### 3.1.1.2 Ciliates

There was a significant negative effect of zooplankton grazers on ciliate biomass, but no significant effect of DOC (Table 6). Moreover, grazer effects were observed mainly on the last day (day 4) of the experiments, with ciliate biomass reduced in both *Daphnia* and copepod treatments compared to the no-grazer control. The ciliate biomass in the copepod treatment was lower than the no-grazer at the start of the experiment (day 0) and also on day 2 (Table 6). However, this was due to a single treatment (i.e., $-DOC_{Copepod}$), which started with lower ciliate biomass compared to the other copepod treatments (Table 8).

At the beginning of the experiment (i.e., day 0), the mean biomass of ciliates in -DOC and +DOC treatments were 3.82 ± 0.07 and 4.6 ± 0.26 µg CI (n=12), respectively (Fig. 9A). The initial ciliate biomass was similar across all treatments, except for the $-DOC_{Copepod}$ treatment, which had about 30% less ciliate biomass compared to the no-grazer ($t = -4.2047$, $p = 0.005657$) and *Daphnia* ($t = -4.6838$, p

= 0.00339) treatments (Fig. 9A). There was no significant change in the total mean ciliate biomass in the first two days of the experiment for any of the treatments (Table 7, Fig. 9). In contrast, between days 2-4, ciliate biomass increased by 7-8 fold in the no-grazer controls and by 3-4 fold in the treatments with *Daphnia* (Table 7, Fig. 9). Yet, during the same time, ciliate biomass in the treatments with copepods (-DOC or +DOC) remained similar to the initial conditions (Table 7, Fig. 9). Hence, by the end of the experiment (i.e., day 4), both copepods and *Daphnia* had a significant negative effect on ciliate biomass (in both +DOC and -DOC treatments), though copepods reduced ciliate biomass by 16-18 fold, while *Daphnia* by a factor of 2-3-fold compared to no-grazer controls (Table 6, Fig. 9C). Taken together, while both zooplankton treatments significantly reduced ciliate biomass, the effect of copepods was stronger than that of *Daphnia* (Table 6, Fig. 9). Indeed, ciliate biomass was significantly higher by a factor of 6-10 times in the *Daphnia* treatments compared to those with copepods (Table 6).

In contrast to the top-down effect of grazers, DOC addition had no significant effect on ciliate biomass throughout the experiment, regardless of the presence or type of grazer (Table 6). Indeed, ciliate biomass was similar among the +DOC and -DOC treatments for any given day. Overall, grazers reduced ciliate biomass (with copepods having a stronger effect than *Daphnia*), while DOC had no effect on ciliate biomass Table 6, Fig. 9).

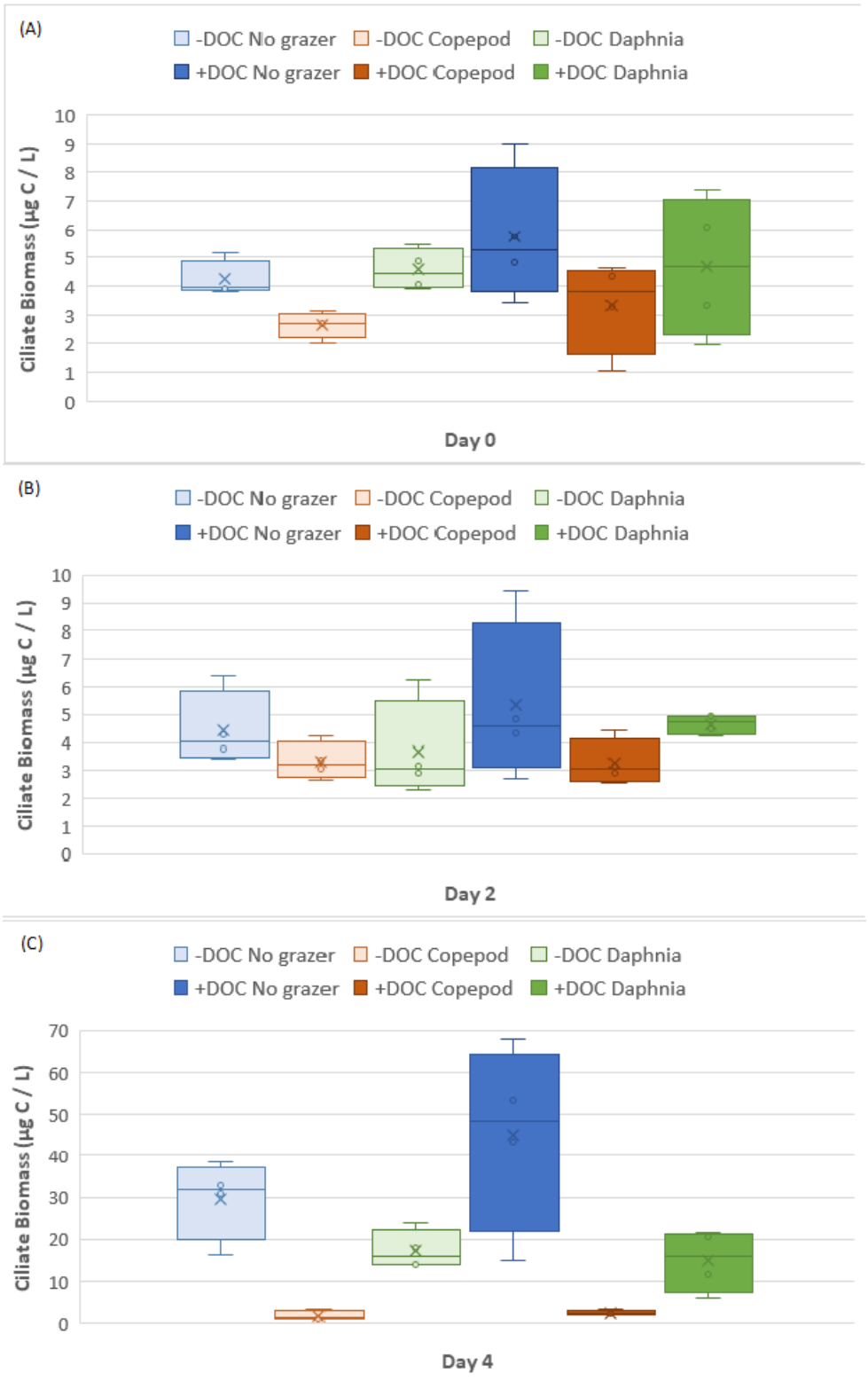


Figure 9: Boxplots showing the median biomass (µg C/ L) of ciliates across the DOC and grazer treatments in (A) Day 0, (B) Day 2, and (C) Day 4. Note the different y-axis scales among graphs.

Table 6: General Linear Model (GLM) analysis results of total ciliate biomass in relation to the treatments (DOC or grazer) relative to respective controls throughout the experiment. (Biomass ~DOC + Grazer)

| **Day** | **Dependent Variable (y)** | **Independent Variable/ Factor (x)** | **Estimate** | **Standard Deviation** | **t val.** | **P val.** |
|---|---|---|---|---|---|---|
| 0 | Ciliate | DOC | 0.09016 | 0.12488 | 0.722 | 0.478 |
| 0 | Ciliate | Copepod | -0.41625 | 0.15295 | -2.722 | **0.013 *** |
| 0 | Ciliate | Daphnia | -0.07207 | 0.15295 | -0.471 | 0.642 |
| 2 | Ciliate | DOC | 0.1040 | 0.1042 | 0.999 | 0.330 |
| 2 | Ciliate | Copepod | -0.2756 | 0.1276 | -2.160 | **0.043 *** |
| 2 | Ciliate | Daphnia | -0.1107 | 0.1276 | -0.868 | 0.396 |
| 4 | Ciliate | DOC | 0.1185 | 0.1728 | 0.686 | 0.500 |
| 4 | Ciliate | Copepod | -2.4366 | 0.2117 | -11.511 | **2.83e-10 *** ** |
| 4 | Ciliate | Daphnia | -0.7608 | 0.2117 | -3.594 | **0.001 ** ** |

Table 7: Paired t-test results for comparison of ciliate biomass change in time in the laboratory experiment.

| **DOC** | **Grazer treatment** | **Day X** | **Day Y** | **Mean of differences** | **t-value** | **df** | **p-value** |
|---|---|---|---|---|---|---|---|
| Yes | No grazer | 4 | 2 | 1.924648 | 10.621 | 3 | **0.001783** |
| Yes | Copepod | 4 | 2 | -0.2057324 | -3.1094 | 3 | 0.05291 |
| Yes | Daphnia | 4 | 2 | 0.9312579 | 3.7811 | 3 | **0.03242** |
| Yes | No grazer | 2 | 0 | -0.0936995 | -0.5434 | 3 | 0.6246 |
| Yes | Copepod | 2 | 0 | 0.04436971 | 0.18102 | 3 | 0.8679 |
| Yes | Daphnia | 2 | 0 | 0.07247267 | 0.29742 | 3 | 0.7855 |
| No | No grazer | 4 | 2 | 1.701653 | 10.99 | 3 | **0.001613** |
| No | Copepod | 4 | 2 | -0.4899652 | -2.3133 | 3 | 0.1037 |
| No | Daphnia | 4 | 2 | 1.394928 | 20.129 | 3 | **0.000268** |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| No | No grazer | 2 | 0 | 0.02726417 | 0.40446 | 3 | 0.713 |
| No | Copepod | 2 | 0 | 0.1704849 | 1.2201 | 3 | 0.3096 |
| No | Daphnia | 2 | 0 | -0.2162055 | -1.8791 | 3 | 0.1568 |

Table 8: Two-sample t-test results for equality of means of ciliate biomass in grazer treatments in the presence and absence of DOC. [*D0 Daphnia ciliate data was not homogenous, Welch test was run.]

| **Day** | **DOC** | **Grazer X** | **Grazer Y** | **Mean of X** | **Mean of Y** | **t-value** | **df** | **p-value** |
|---|---|---|---|---|---|---|---|---|
| 4 | Yes | Copepod | NoGrazer | 1.233 | 3.696 | -7.36 | 6 | **0.0003214** |
| 4 | Yes | Daphnia | NoGrazer | 2.662 | 3.696 | -2.42 | 6 | 0.05142 |
| 4 | Yes | Copepod | Daphnia | 1.233 | 2.662 | -4.92 | 6 | **0.002655** |
| 4 | No | Copepod | NoGrazer | 0.968 | 3.377 | -9.39 | 6 | **8.282e-05** |
| 4 | No | Daphnia | NoGrazer | 2.890 | 3.377 | -2.27 | 6 | 0.06342 |
| 4 | No | Copepod | Daphnia | 0.968 | 2.890 | -8.86 | 6 | **0.0001149** |
| 2 | Yes | Copepod | NoGrazer | 1.438 | 1.772 | -1.42 | 6 | 0.2039 |
| 2 | Yes | Daphnia | NoGrazer | 1.731 | 1.772 | -0.18 | 6 | 0.8571 |
| 2 | Yes | Copepod | Daphnia | 1.438 | 1.731 | -3.00 | 6 | **0.02388** |
| 2 | No | Copepod | NoGrazer | 1.458 | 1.676 | -1.59 | 6 | 0.1626 |
| 2 | No | Daphnia | NoGrazer | 1.495 | 1.676 | -0.88 | 6 | 0.4102 |
| 2 | No | Copepod | Daphnia | 1.458 | 1.495 | -0.20 | 6 | 0.8473 |
| 0 | Yes | Copepod | NoGrazer | 1.394 | 1.865 | -1.63 | 6 | 0.1524 |
| 0 | Yes | Daphnia | NoGrazer | 1.658 | 1.865 | -0.71 | 5.42 | 0.5049 |
| 0 | Yes | Copepod | Daphnia | 1.394 | 1.658 | -0.79 | 5.99 | 0.4558 |
| 0 | No | Copepod | NoGrazer | 1.287 | 1.649 | -4.20 | 6 | **0.005657** |
| 0 | No | Daphnia | NoGrazer | 1.711 | 1.649 | 0.74 | 5.92 | 0.4854 |
| 0 | No | Copepod | Daphnia | 1.287 | 1.711 | -4.68 | 5.99 | **0.00339** |

### 3.1.2 Effect Size of DOC and Zooplankton Treatments on Bacteria and Ciliate Biomasses

The effect size of the treatments (DOC and grazers) on bacterial biomass was either positive or negative, with mean effect size values ranging from -0.4 to 0.2 depending on the treatment. Specifically, while the mean effect size of DOC on bacterial biomass was positive (~0.2) in the *Daphnia* and no-grazer treatments, it was negative (~-0.4) in the copepod treatments (Fig. 10A). The mean effect size of grazer treatments on bacterial biomass also varied among copepods and *Daphnia* and a given grazer treatment depending on the presence of DOC. Specifically, the effect size of *Daphnia* was similar to zero (i.e., no effect) regardless of the DOC treatment (Figure 10B). In contrast, the effect size of copepods on bacterial biomass was either positive (-DOC treatment) or negative (+DOC treatment), with similar absolute values (Fig. 10B). Overall, the top-down effect size of copepods and bottom-up effect size of DOC on bacterial biomass was variable but similar in terms of absolute values (<0.5), while the top-down effect of *Daphnia* had an effect size value similar to zero.

The effect size of DOC on ciliate biomass was positive (~0.2-0.4) in the no-grazer and copepod treatments but negative (~-0.2) in the *Daphnia* treatments (Fig. 10C). In contrast, the effect size of both grazers (i.e., *Daphnia* and copepod) on ciliate biomass was negative, though there were differences among their effects. Specifically, the mean effect size value was about -2.8 (± 0.2 CI) in the copepod treatments (regardless of DOC); while it was about -1.1 (± 0.25 CI) in the *Daphnia* treatment with DOC, and about -0.5 (± 0.14 CI) in the *Daphnia* treatment DOC. Hence, the top-down effect size of copepods was > 2x stronger than *Daphnia* in the +DOC treatments and > 5x stronger compared to the -DOC treatments. Overall, the top-down effect of both grazers on ciliate biomass was stronger (i.e., effect size values between -0.5 and -2.8) compared to the bottom-up effect of DOC (effect size values between -0.2 and +0.5) when measured via effect size (Fig. 10D).

The effect size schema summarized the average effects of DOC and grazer on bacteria and ciliates on the last day (day 4) of the laboratory experiment,

respectively (Fig. 11). The blue color indicates the positive effect, while the red color shows a negative effect (Fig. 11). DOC has been placed to the bottom to show the bottom-up effect, and *Daphnia* and copepod have been placed to the top to show the top-down effect. The thickness of arrows depends on how strong the effect is (i.e., thick arrows indicate a stronger effect) (Fig. 11). The effects were calculated with respect to the control group (i.e., DOC effect relative to no DOC control; *Daphnia* effect relative to no grazer control). On day 4, both DOC and grazers had a negative effect on bacteria, and these effects were weaker compared to effects on ciliates. On the other hand, DOC had a positive effect on ciliate biomass, while both *Daphnia* and copepods had a stronger negative effect. The strongest negative effect was copepod grazing on ciliates (Fig. 11).

Taken together, the effect of DOC addition on bacteria and ciliate biomass was relatively weak (mean absolute values <0.5) and variable in terms of direction (positive or negative). Similarly, the top-down effect of zooplankton on bacteria biomass was also relatively weak (mean absolute values <0.4) and also variable in terms of direction (positive or negative). In contrast, the top-down effect of zooplankton on ciliate biomass was stronger (mean absolute values between 0.5 – 2.8), always negative, and the strongest effect size values observed for treatments with copepods (mean value -2.8), followed by treatments with *Daphnia* (mean value -1.1 (+DOC), -0.5 (-DOC)). Hence, top-down grazer effects on bacteria and ciliate biomass were significantly stronger than the bottom-up effects of DOC addition.

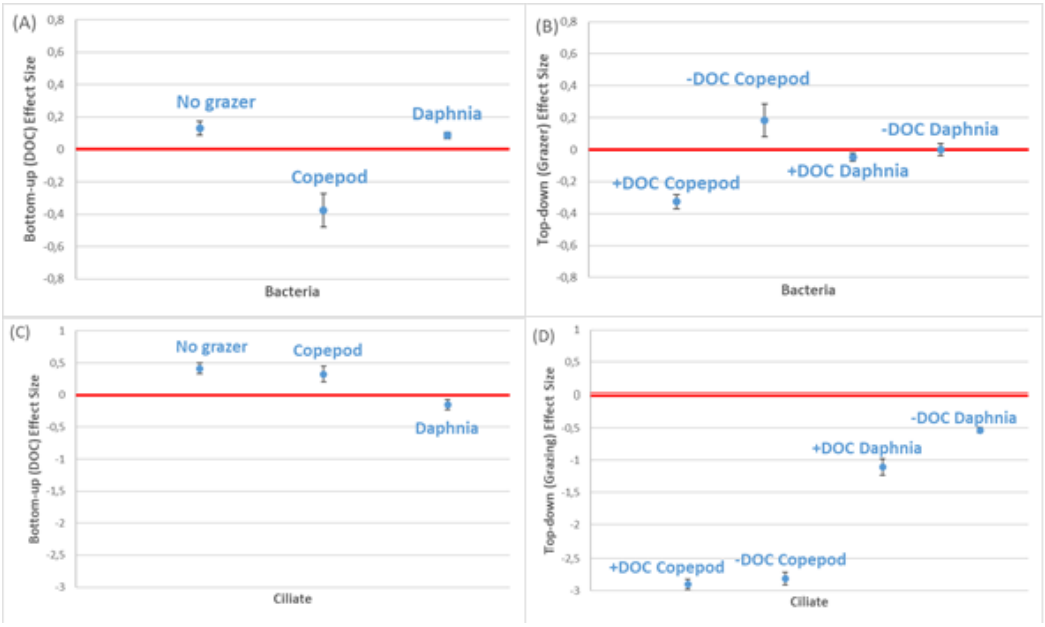


Figure 10: The mean effect size (log ratio) of (A) DOC on bacteria biomass, (B) grazer on bacteria biomass, (C) DOC on ciliate biomass, and (D) grazer on ciliate biomass in day 4 (end of the experiment). Error bars are 95% confidence intervals. The effect of DOC/ grazers is significant if the confidence interval does not overlap zero (the red lines).

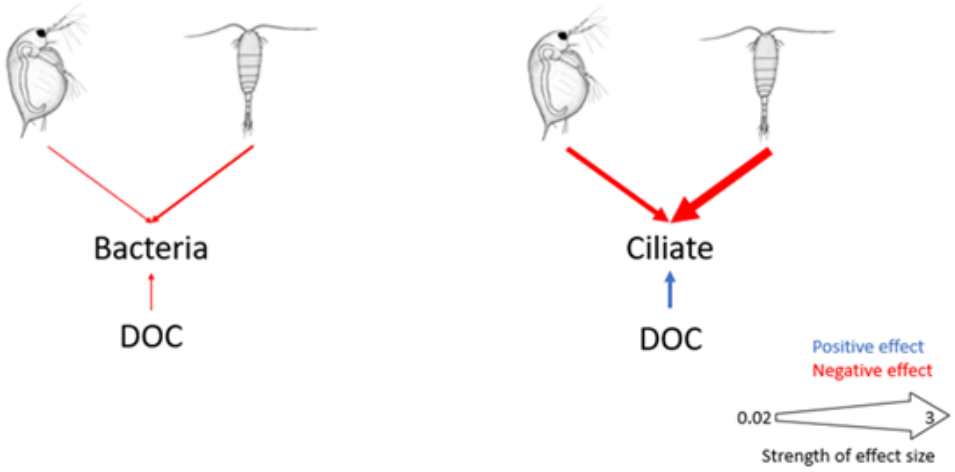


Figure 11: Effect size schema for biomass of bacteria and ciliates at the end of the laboratory experiment.

### 3.1.3 Effect of DOC and Zooplankton Treatments on Ciliate Functional Feeding Groups

#### 3.1.3.1 Biomass and Relative Biomass of Ciliate Functional Feeding Groups

At the start of the experiment, bacterivores dominated the relative and absolute ciliate biomass (~60% of total), followed by nonselective (~ 20% of total), followed by algivores and predators across all treatments (Fig. 12). This pattern continued on the second day as well (Fig 12). By the end of the experiment, however, nonselective ciliates dominated (>50% of total biomass) the treatments with no grazers and *Daphnia* regardless of DOC (Table 9), while bacterivores continued to dominate in the copepod treatments (Table 9). Hence, in the treatments with no grazers and *Daphnia*, the ciliate functional group dominance switched from bacterivores to nonselectives during the experiment. In contrast, the copepod treatments (-DOC and +DOC) had higher relative biomass of bacterivores and lower relative biomass of the algivore and nonselective ciliates, while *Daphnia* treatments had no significant difference on the relative dominance of functional feeding groups compared to no grazer controls on day 4 (Table 9). Overall, DOC had no significant effect on the relative biomass of ciliate functional groups during the experiment and hence, there were no bottom-up effects of DOC on ciliate composition. (Table 9). Moreover, copepods reduced the relative biomass of algivores and non-selective ciliates when compared to the no-grazer and *Daphnia* controls.

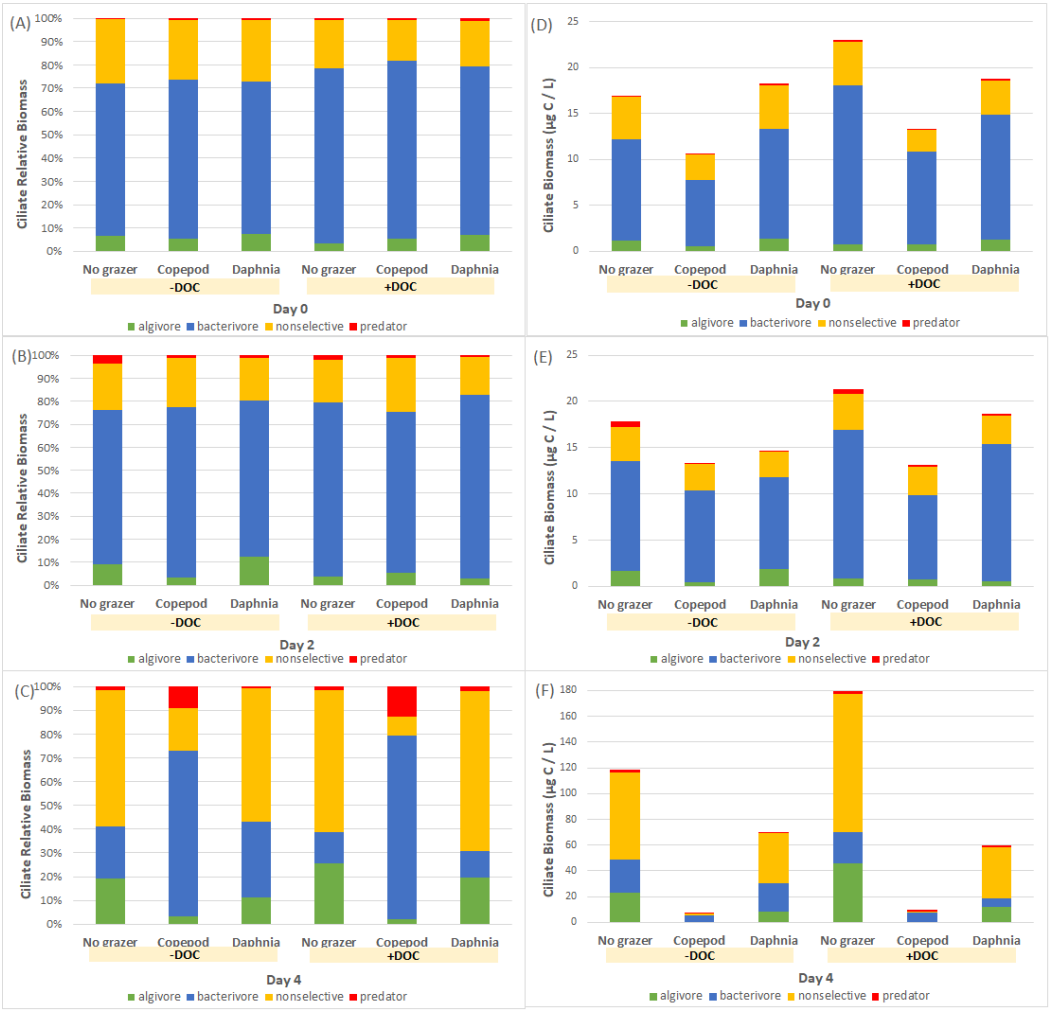


Figure 12: The relative biomass of ciliate functional feeding groups in (A) Day 0, (B) Day 2, and (C) Day 4; the total ciliate biomass (µg C/ L) graphs of functional feeding groups of ciliates (D) Day 0, (E) Day 2, and (F) Day 4.

Table 9: General Linear Model (GLM) analysis results of relative biomass of ciliate functional feeding groups depend on DOC or grazer throughout the experiment. (Biomass% ~DOC + Grazer)

| **Day** | **Dependent Variable (y)** | **Independent Variable/ Factor (x)** | **Estimate** | **Standard Deviation** | **t val.** | **P val.** |
|---|---|---|---|---|---|---|
| **4** | Algivore | DOC | 0.1688 | 0.4007 | 0.421 | 0.6786 |
| **4** | Algivore | Copepod | -1.6210 | 0.5042 | -3.215 | **0.0048** |
| **4** | Algivore | Daphnia | -0.2975 | 0.4627 | -0.643 | 0.5284 |
| **4** | Bacterivore | DOC | -0.3436 | 0.3058 | -1.124 | 0.274486 |
| **4** | Bacterivore | Copepod | 1.7516 | 0.3746 | 4.677 | **0.000145** |
| **4** | Bacterivore | Daphnia | 0.3710 | 0.3746 | 0.991 | 0.333693 |
| **4** | Nonselective | DOC | -0.5457 | 0.4099 | -1.331 | 0.198055 |
| **4** | Nonselective | Copepod | -2.0859 | 0.5020 | -4.156 | **0.000489** |
| **4** | Nonselective | Daphnia | -0.0234 | 0.5020 | -0.047 | 0.963278 |
| **4** | Predator | DOC | 0.2743 | 0.4391 | 0.625 | 0.54010 |
| **4** | Predator | Copepod | 2.2708 | 0.5304 | 4.281 | 0.00045 |
| **4** | Predator | Daphnia | 0.3626 | 0.5469 | 0.663 | 0.51573 |
| **2** | Algivore | DOC | -0.2834 | 0.2793 | -1.015 | 0.322 |
| **2** | Algivore | Copepod | -0.1069 | 0.3421 | -0.312 | 0.758 |
| **2** | Algivore | Daphnia | -0.3122 | 0.3421 | -0.913 | 0.372 |
| **2** | Bacterivore | DOC | 0.05543 | 0.0435 | 1.275 | 0.217 |
| **2** | Bacterivore | Copepod | -0.00093 | 0.0532 | -0.017 | 0.986 |
| **2** | Bacterivore | Daphnia | 0.051672 | 0.0532 | 0.971 | 0.343 |
| **2** | Nonselective | DOC | -0.08215 | 0.1210 | -0.679 | 0.505 |
| **2** | Nonselective | Copepod | 0.11341 | 0.1482 | 0.765 | 0.453 |
| **2** | Nonselective | Daphnia | -0.15374 | 0.1482 | -1.037 | 0.312 |
| **2** | Predator | DOC | -0.01956 | 0.3178 | -0.062 | 0.95157 |
| **2** | Predator | Copepod | -1.04322 | 0.3799 | -2.746 | **0.01283** |
| **2** | Predator | Daphnia | -1.17737 | 0.3938 | -2.989 | **0.00753** |
| **0** | Algivore | DOC | -0.2645 | 0.2993 | -0.884 | 0.3874 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **0** | Algivore | Copepod | 0.4575 | 0.3666 | 1.248 | 0.2265 |
| **0** | Algivore | Daphnia | 0.6459 | 0.3666 | 1.762 | 0.0934 |
| **0** | Bacterivore | DOC | 0.1242 | 0.0621 | 2.000 | 0.0592 |
| **0** | Bacterivore | Copepod | 0.0106 | 0.0760 | 0.140 | 0.8904 |
| **0** | Bacterivore | Daphnia | -0.0229 | 0.0760 | -0.301 | 0.7668 |
| **0** | Nonselective | DOC | -0.3526 | 0.1315 | -2.682 | **0.0143** |
| **0** | Nonselective | Copepod | 0.0056 | 0.1610 | 0.035 | 0.9725 |
| **0** | Nonselective | Daphnia | -0.0310 | 0.1610 | -0.192 | 0.8493 |
| **0** | Predator | DOC | 0.0584 | 0.4923 | 0.119 | 0.908 |
| **0** | Predator | Copepod | -0.0483 | 0.6432 | -0.075 | 0.942 |
| **0** | Predator | Daphnia | 0.3012 | 0.6432 | 0.468 | 0.651 |

#### 3.1.3.2 Effect Size Values of DOC and Zooplankton Treatments on the Biomass of Ciliate Functional Feeding Groups

The effect size of DOC on the biomass of ciliate functional feeding groups was insignificant, except for predatory ciliates, which increased with DOC but only in the presence of copepod and *Daphnia* (Fig. 13G). In contrast, the effect size of grazers (i.e., *Daphnia* and copepod) on the biomass of ciliate functional feeding groups was generally significant and negative, though there were differences among grazers. Specifically, the mean effect size of copepods on the biomass of algivore and nonselective ciliates was about -4 ± 0.5 CI (regardless of DOC). While the mean effect size of *Daphnia* on algivore biomass was about -1.2 ± 0.6 CI in the -DOC treatment, -0.82 ± 0.73 CI in the +DOC treatment. On the nonselective biomass, the mean effect size was -1 ± 0.5 CI in the +$DOC_{Daphnia}$ treatment and -0.56 ± 0.48 CI -$DOC_{Daphnia}$ treatment. Moreover, the effect size of copepods on bacterivore biomass was negative and similar among treatments with (-1.3 ± 0.6 CI) or without DOC (-1.9 ± 0.7 CI). While the effect size of *Daphnia* on bacterivores was significant in +DOC treatment (-1.2 ± 0.7 CI), and nonsignificant in -DOC treatment (-0.42 ± 0.68). On the predator biomass, both grazers (i.e., copepod and *Daphnia*) had a negative effect size with absolute value -1 ± 0.4 CI regardless of DOC. Therefore,

the top-down effect size of copepods was 4x stronger than *Daphnia* treatments (-DOC and +DOC) on the biomass of both algivore and nonselective ciliates. Overall, the top-down effect of both grazers on ciliate biomass was stronger and more negative (i.e., effect size values between -0.6 and -4.5) compared to the bottom-up effect of DOC (effect size values between -1.1 and +0.7) (Fig. 13).

The effect size schema summarized the average effects of DOC and grazer on the functional feeding groups of ciliates on the last day (day 4) of the laboratory experiment, respectively (Fig. 14). The blue color indicates the positive effect, while the red color shows a negative effect (Fig. 14). DOC has been placed to the bottom to show the bottom-up effect, and *Daphnia* and copepod have been placed to the top to show the top-down effect. The thickness of arrows depends on how strong the effect is (i.e., thick arrows show a stronger effect) (Fig. 14). The effects were calculated with respect to the control group (i.e., DOC effect relative to no DOC control of one functional feeding group). The bottom-up effect was weaker than the top-down effect on the ciliate functional feeding group. DOC had a contrasting effect on different feeding groups. DOC affected both algivores and predators positively, while bacterivore and nonselective ciliates were affected negatively. Besides, both *Daphnia* and copepods affected ciliate composition negatively but with different magnitudes. As seen in Figure 14, the negative effect of *Daphnia* was similar through each functional feeding group. On the other hand, the negative effect of copepods was stronger than the negative *Daphnia* effect on the ciliate composition. Also, copepods had a stronger effect on ciliate functional feeding groups in order of nonselectives, algivores, bacterivores, and predators.

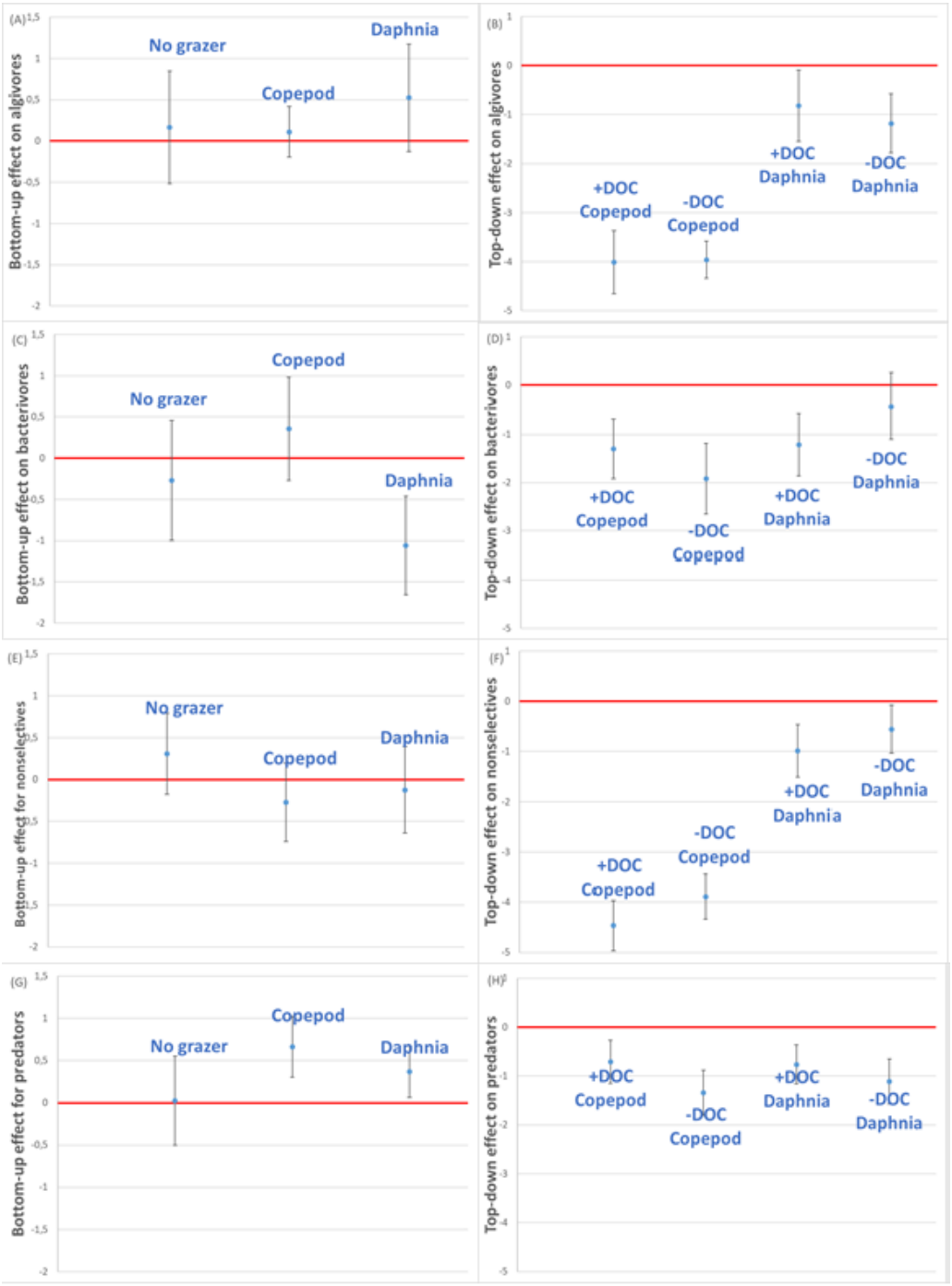


Figure 13: The effect size (loge ratio) of biomass of ciliate functional feeding groups on (A, B) algivores, (C, D) bacterivores, (E, F) nonselectives, and (G, H) predators on day 4 (end of the experiment). The left graphs show the bottom-up (i.e., DOC) effect, while the right graphs represent the top-down (i.e., grazing) effect size. Error bars are 95% confidence intervals. The effect of DOC/grazers is significant if the confidence interval does not overlap zero (the red lines).

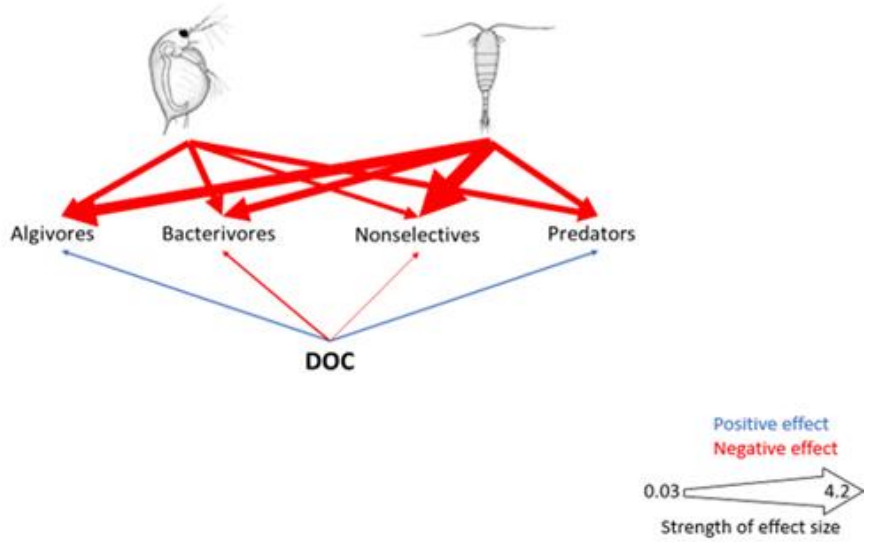


Figure 14: Effect size schema for functional feeding groups of ciliates at the end of the laboratory experiment (day 4).

## 3.2 *In-situ* Mesocosm Grazing Assays

### 3.2.1 Effects of DOC Types and Mesozooplankton Grazing on Bacteria and Ciliate Biomass

#### 3.2.1.1 Bacteria

In the first grazing assay, the total bacteria biomass significantly decreased in treatments with mesozooplankton (+Z) compared to without mesozooplankton (-Z), while neither of the DOC sources had a significant effect (Table 10, Fig. 15A). The mean biomass of bacteria in the no mesozooplankton treatments (-Z) was 56 ± 18 CI (n=16), and 30 ± 7 CI (n=16) µg C $L^{-1}$ in treatments with mesozooplankton (+Z). In contrast, the mean bacteria biomass in the second grazing assay was not affected by mesozooplankton (Table 10). Moreover, in the second assay, bacteria biomass was significantly lower in L and mixed DOC treatments compared to the C (no DOC control) regardless of mesozooplankton presence (Table 10, Fig. 15B). The R treatment, however, had no effect on bacterial biomass compared to the C (Table 10).

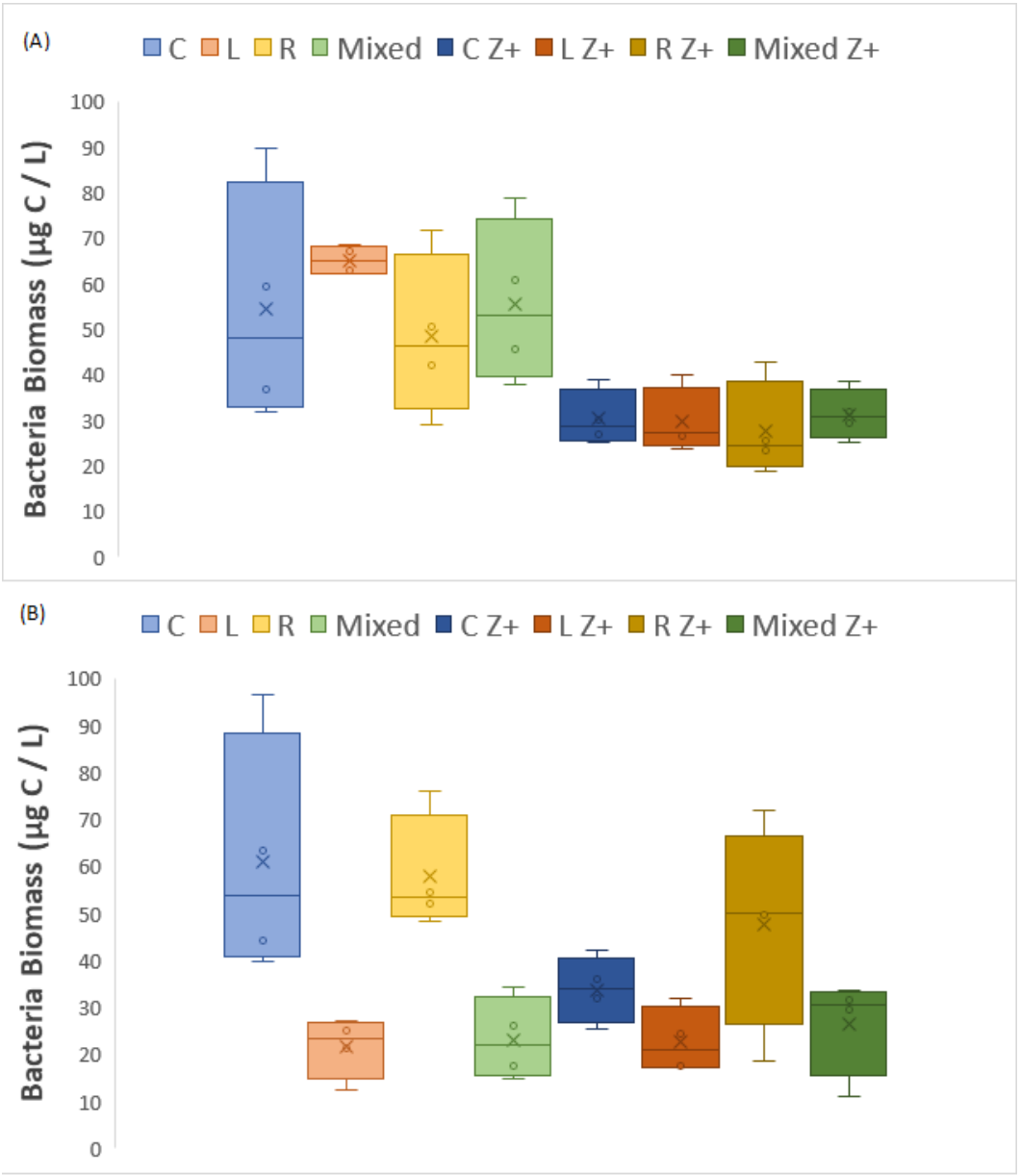


Figure 15: Boxplots showing the median biomass (µg C/ L) of bacteria with DOC and mesozooplankton treatments in (A) first assay (after one day of the DOC addition), (B) second assay (after four days of DOC addition). Bottom and upper lines (hinges) of the boxplots represent the 1st and 3rd quartiles.

Table 10: General Linear Model (GLM) analysis results of total bacteria biomass with respect to the presence of mesozooplankton (+Z) and the type of DOC in the first (i.e., one day after the DOC addition) and second assay (i.e., four days after the DOC addition).

| **Assay** | **Dependent variable (y)** | **Independent variable (x)** | **Estimate** | **SD** | **t val.** | **P val.** |
|---|---|---|---|---|---|---|
| 1 | Bacteria | Mesozooplankton | -0.60895 | 0.102 | -5.962 | **2.34e-06** |
| 1 | Bacteria | L | 0.11810 | 0.144 | 0.818 | 0.421 |
| 1 | Bacteria | R | -0.10639 | 0.144 | -0.736 | 0.468 |
| 1 | Bacteria | Mixed | 0.05089 | 0.144 | 0.352 | 0.727 |
| 2 | Bacteria | Mesozooplankton | -0.1658 | 0.139 | -1.187 | 0.24567 |
| 2 | Bacteria | L | -0.7139 | 0.197 | -3.613 | **0.00122** |
| 2 | Bacteria | R | 0.1247 | 0.197 | 0.631 | 0.53345 |
| 2 | Bacteria | Mixed | -0.6373 | 0.197 | -3.225 | **0.00329** |

#### 3.2.1.2 Ciliates

The effect of DOC and mesozooplankton treatments were similar among the two assays. Specifically, in both assays, mesozooplankton reduced ciliate biomass across all DOC treatments (Figure 16, Table 11). Also, in both assays, the ciliate biomass in the L treatments was significantly higher compared to the C (no DOC control), regardless of the mesozooplankton presence, while the other DOC types used here had no effect on ciliate biomass (Fig 16, Table 11).

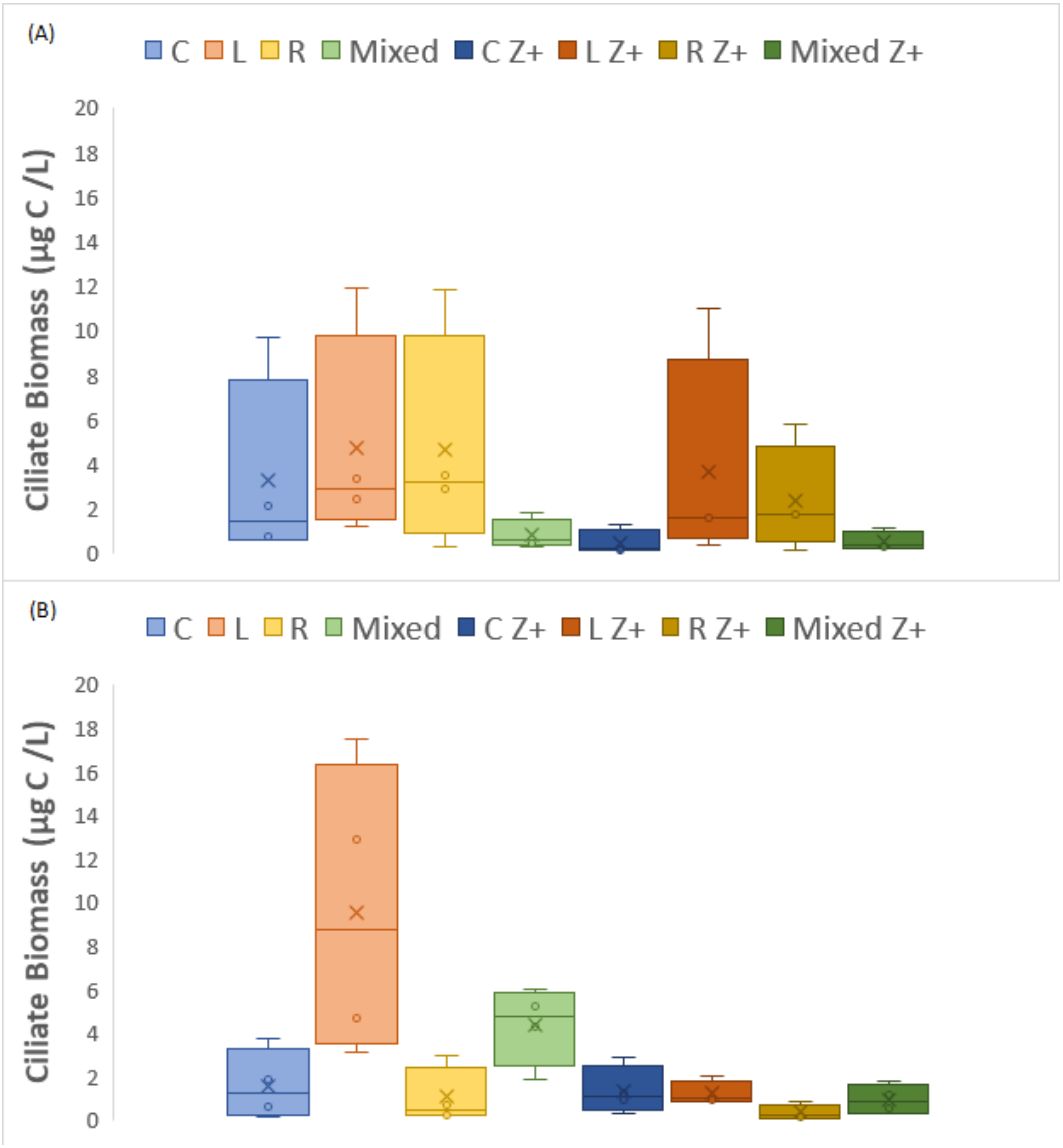


Figure 16: Boxplots showing the median biomass (µg C/ L) of total ciliate with DOC and mesozooplankton treatments in (A) first assay (after one day of the DOC addition), (B) second assay (after four days of DOC addition). Bottom and upper lines(hinges) of the boxplots represent the 1st and 3rd quartiles.

Table 11: General Linear Model (GLM) analysis results of total ciliate biomass with respect to the presence of mesozooplankton and the type of DOC in both assay 1 (i.e., one day after the DOC addition) and assay 2 (i.e., four days after the DOC addition) (Biomass ~ DOC + grazer).

| **Assay** | **Dependent variable (y)** | **Independent variable (x)** | **Estimate** | **SD** | **t val.** | **P val.** |
|---|---|---|---|---|---|---|
| 1 | Ciliate | Mesozooplankton | -0.87992 | 0.42305 | -2.08 | **0.0472** |
| 1 | Ciliate | L | 1.28631 | 0.59829 | 2.15 | **0.0407** |
| 1 | Ciliate | R | 0.92032 | 0.59829 | 1.53 | 0.1356 |
| 1 | Ciliate | Mixed | -0.26035 | 0.59829 | -0.43 | 0.6669 |
| 2 | Ciliate | Mesozooplankton | -1.1278 | 0.3738 | -3.01 | **0.00551** |
| 2 | Ciliate | L | 1.1873 | 0.5286 | 2.24 | **0.03310** |
| 2 | Ciliate | R | -0.9867 | 0.5286 | - 1.86 | 0.07288 |
| 2 | Ciliate | Mixed | 0.6466 | 0.5286 | 1.22 | 0.23182 |

#### 3.2.1.3 Zooplankton Biomass (First Assay Only)

The total mesozooplankton biomass in the treatments was 249.5 ± 322.4 CI for no DOC control, 406.3 ± 105.9 CI for recalcitrant DOC, 200.4 ± 95.9 CI for leaf-leachate DOC, and 300.5 ± 201.4 CI µg C $L^{-1}$ for mixed DOC (Fig. 17). According to GLM results, there was no significant difference in treatments with respect to C (no DOC control). In general, mean values of cladocerans were higher than copepods, which indicated that cladocerans dominated zooplankton in the *in-situ* mesocosm grazing assays (Fig. 17). In addition to GLM, independent t-test results showed that only Copepoda was significantly higher in R (recalcitrant DOC) treatment than mixed DOC treatment ($t = 2.704$, $p = 0.03668$).

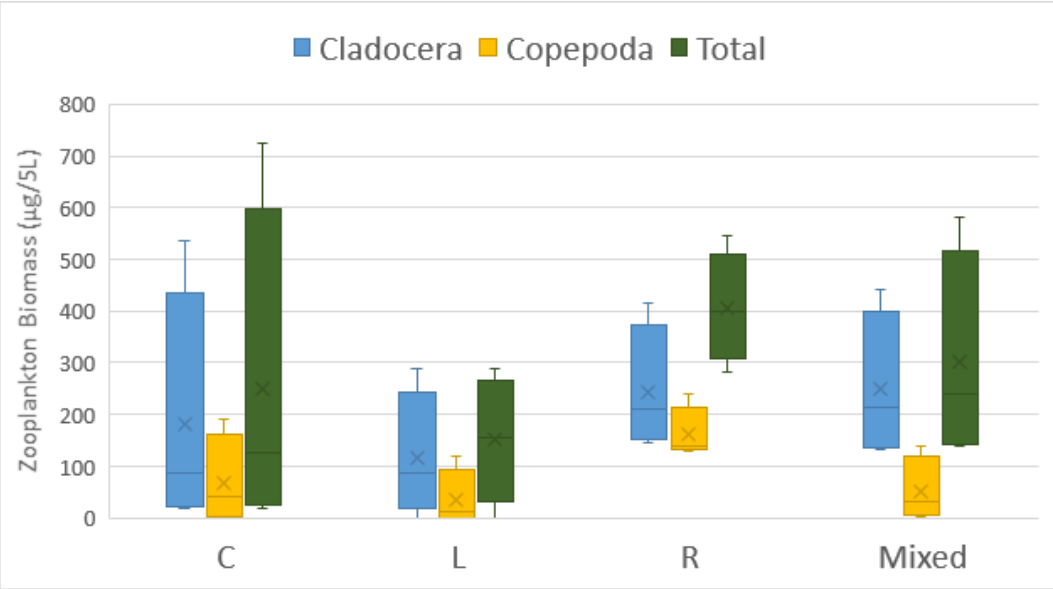


Figure 17: Box plots showing the median biomass (µg C/ 5L) of total zooplankton in the *in-situ* mesocosm grazing assays.

### 3.2.2 Effect Sizes Values of DOC Types and Mesozooplankton Grazing on Bacteria and Ciliate Biomass

#### 3.2.2.1 Bacteria

One day after the DOC addition, DOC (i.e., bottom-up) had a significant negative effect on bacteria in mixed DOC with mesozooplankton treatment and had a significant positive effect in L without mesozooplankton treatment with absolute value $< 0.5$ (Fig 18A). On the other hand, four days after the DOC addition, the effect of DOC was significantly negative in L and mixed DOC treatments regardless of the presence of mesozooplankton, while it was significantly positive in R (recalcitrant DOC) with mesozooplankton treatment. The negative effect of DOC in both L and mixed DOC with mesozooplankton treatments was $< 0.5$, while in L and mixed DOC without mesozooplankton treatments the effect size was about ~ -1 (Fig 18C). In general, the effect of DOC was inhibited by mesozooplankton after one day of the DOC addition; in contrast, mesozooplankton catalyzed the DOC effect after four days of the DOC addition.

The top-down (i.e., grazing) effect on bacteria in all treatments was significantly negative, with absolute values from 0.5 to 0.8 one day after the DOC addition (Fig.18B). Four days after the DOC addition, the top-down (i.e., grazing) effect on bacteria was significantly negative in both C (no DOC control) and R (recalcitrant DOC) treatments. The negative effect in C was three times stronger than the negative effect in R treatment (Fig 18D).

The effect size schema summarized the average effects of DOC types and mesozooplankton on bacteria in both *in-situ* mesocosm grazing assays, respectively (Fig. 19). The blue color indicates the positive effect, while the red color shows a negative effect (Fig. 19). DOC types have been placed to the bottom to show the bottom-up effect, and mesozooplankton have been placed to the top to show the top-down effect. The thickness of arrows depends on how strong the effect is (i.e., thick arrows show a stronger effect) (Fig. 19). The effects were calculated with respect to the control group (i.e., mesozooplankton effect relative to no mesozooplankton control). On bacteria biomass, both mesozooplankton and mixed DOC had a negative effect in both assays. R had a negative effect in the first grazing assay, then a positive effect on bacteria biomass in the second grazing assay. In contrast, in the first grazing assay, L had a positive effect, and in the second grazing assay, L had a negative effect on bacteria biomass. Moreover, all effects on bacteria biomass were relatively weak (i.e., changing between 0.07-0.7, Fig. 19).

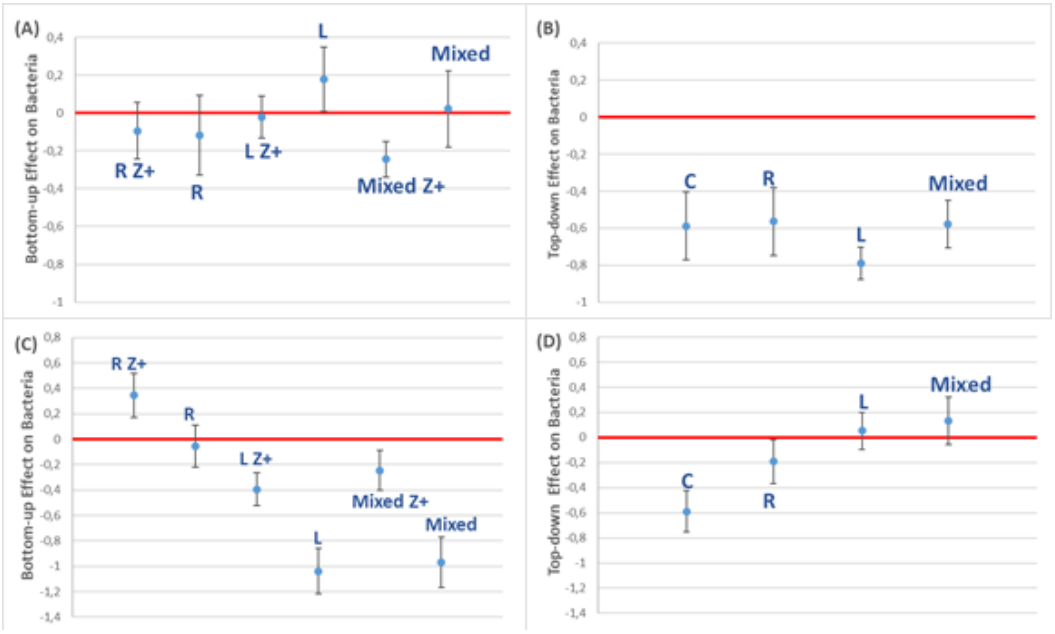


Figure 18: The effect size ($\log_e$ ratio) on bacteria biomass of (A, C) DOC (i.e., bottom-up), (B, D) grazing (i.e., top-down). The graphs in the first row show the effect size in the first *in-situ* grazing assay (after one day of the DOC addition), while second-row graphs represent the effect size in the second *in-situ* grazing assay (after four days of the DOC addition). The scale of graphs differs between assays. Error bars are 95% confidence intervals. The effect of DOC/grazing is significant if the confidence interval does not overlap zero (the red lines).

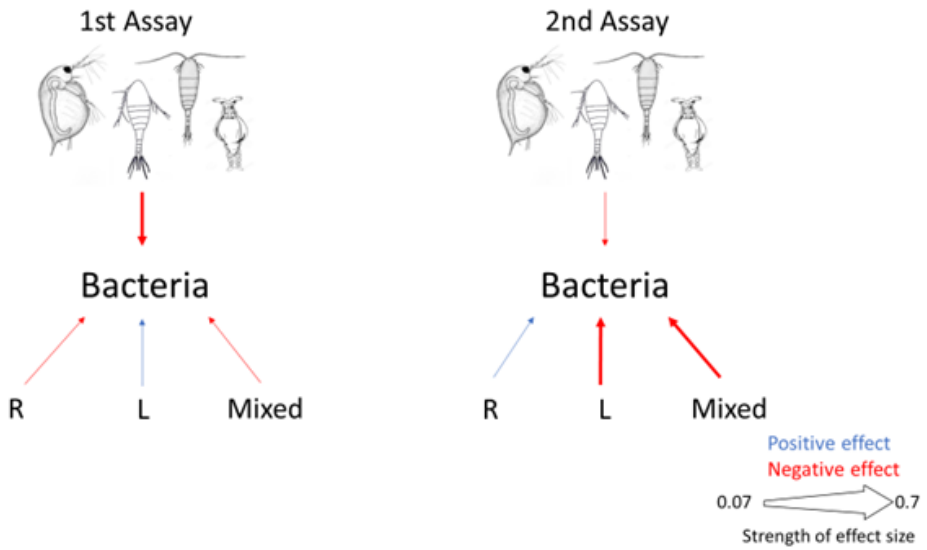


Figure 19: Effect size schema for bacteria biomass in the *in-situ* mesocosm grazing assays.

#### 3.2.2.2 Ciliates

One day after the DOC addition, the bottom-up (i.e., DOC) effect on ciliates was significantly positive in both R and L with mesozooplankton treatments, while it was significantly negative in mixed DOC without mesozooplankton treatment (Fig. 20A). Four days after the DOC addition, DOC effect on ciliates was significantly positive in both L and mixed DOC without mesozooplankton treatments, whereas DOC had a significant negative effect on ciliates in R with mesozooplankton treatments (Fig 20C). In general, the effect of DOC was catalyzed by mesozooplankton after one day of the DOC addition; in contrast, mesozooplankton inhibited the DOC effect after four days of the DOC addition.

The effect size schema summarized the average effects of DOC types and mesozooplankton on ciliates in both *in-situ* mesocosm grazing assays, respectively (Fig. 21). The blue color indicates the positive effect, while the red color shows a negative effect (Fig. 21). DOC types have been placed to the bottom to show the bottom-up effect, and mesozooplankton have been placed to the top to show the top-down effect. The thickness of arrows depends on how strong the effect is (i.e., thick arrows show a stronger effect) (Fig. 21). The effects were calculated with respect to the control group (i.e., mesozooplankton effect relative to no mesozooplankton control). In the first grazing assay, the bottom-up effect of R and L was positive on ciliated biomass, while the effect of mixed DOC was negative. On the other hand, in the second grazing assay, the bottom-up effect of R and mixed DOC on ciliates reversed, and the effect of mixed DOC was positive, the effect of R was negative. L had still a positive effect on ciliate biomass in the second grazing assay, but its magnitude was lower compared to the first grazing assay. The top-down effect of mesozooplankton was negative in both grazing assays, the negative effect was stronger in the second assay than the first assay (Fig. 21).

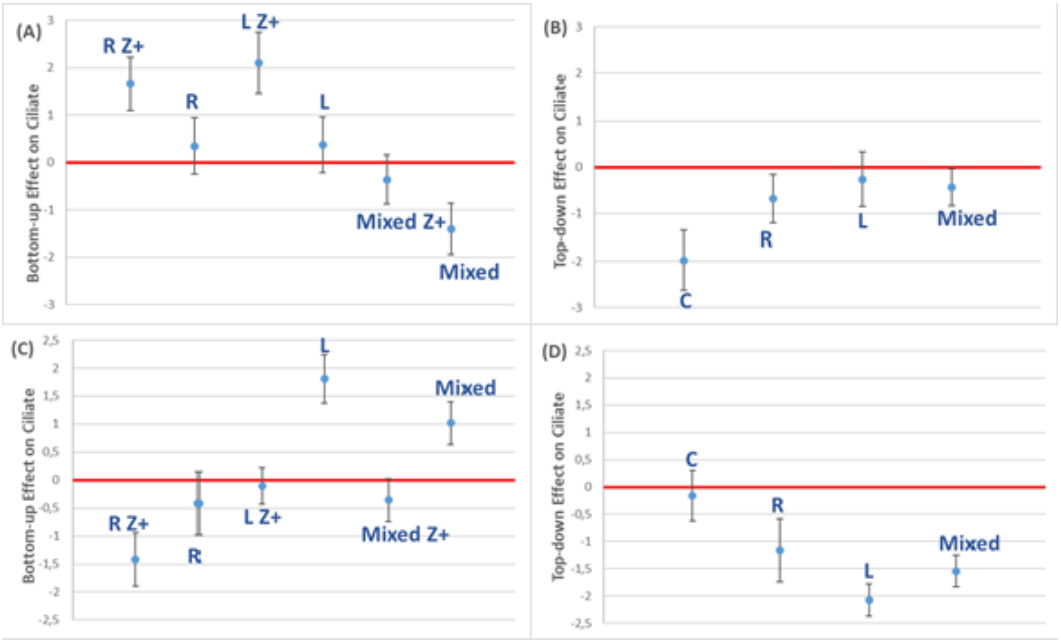


Figure 20: The effect size (loge ratio) on ciliate biomass of (A, C) DOC (i.e., bottom-up), (B, D) grazing (i.e., top-down). The graphs in the first row show the effect size in the first *in-situ* grazing assay (after one day of the DOC addition), while second-row graphs represent the effect size in the second *in-situ* grazing assay (after four days of the DOC addition). The scale of graphs differs between assays. Error bars are 95% confidence intervals. The effect of DOC/grazing is significant if the confidence interval does not overlap zero (the red lines).

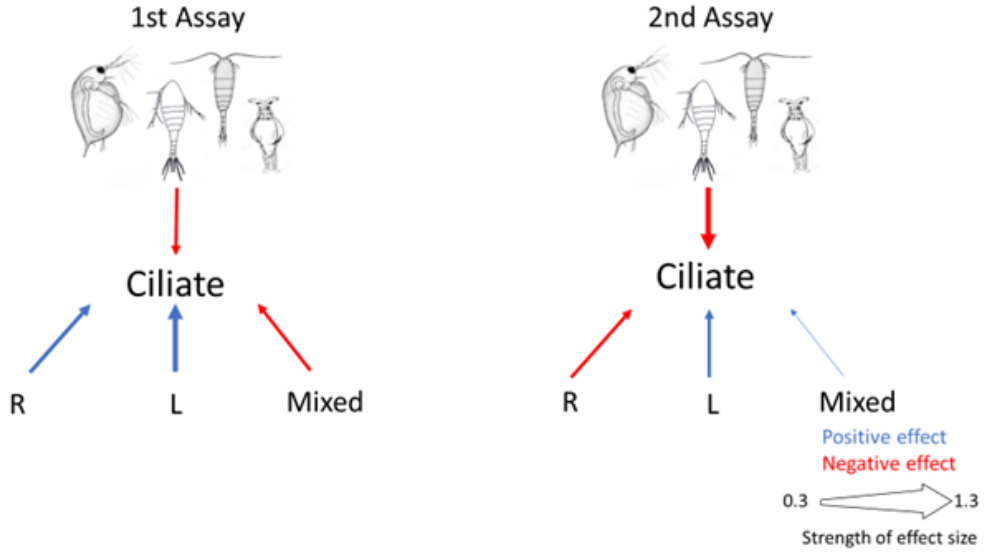


Figure 21: Effect size schema for biomass of ciliates in the *in-situ* mesocosm grazing assays.

### 3.2.3 Effects of DOC Types and Mesozooplankton Grazing on Ciliate Functional Feeding Groups

#### 3.2.3.1 Biomass and Relative Biomass of Ciliate Functional Feeding Groups

In the first assay, the composition of feeding groups of ciliates was similar, and nonselective ciliates were dominated the relative and absolute ciliate biomass more than 75% in all treatments regardless of the presence of mesozooplankton (Fig 22A). The relative biomass of the algivore group was higher in L and mixed DOC treatments in both the presence and absence of the mesozooplankton compared to C and R treatments. Moreover, the predator group had the least percent value as <1% (Fig. 22A). According to GLM analysis of functional feeding groups' biomass, algivore group biomass was significantly higher in L treatment regardless of the presence of mesozooplankton with respect to C (Table 12). Also, mesozooplankton significantly reduced algivores relative to no mesozooplankton treatments (Table 12). Additionally, the relative biomass of bacterivore group biomass in L treatment was significantly lower than C (Table 13).

In the assay four days after the DOC addition, the nonselective ciliates were still dominated the relative and absolute ciliate biomass more than 60% in all treatments. The biomass of the bacterivore group was significantly higher in both mixed DOC treatments with and without mesozooplankton (Table 14, Fig. 22D). Relative biomass of bacterivores was significantly higher in the L and mixed DOC treatments regardless of mesozooplankton, and also in the mesozooplankton treatments relative to without mesozooplankton treatments (Table 15, Fig. 22B). In contrast, the relative biomass of the nonselective group was significantly lower in mixed DOC treatments. Additionally, the relative biomass of the predator group with mesozooplankton treatments was significantly higher than without mesozooplankton treatments (Table 15). Relative to the assay one day after the DOC addition, the relative biomass of the bacterivore group increased in L and mixed DOC treatments regardless of mesozooplankton, and the relative biomass of the

algivore group decreased in all treatments in the assay four days after the DOC addition (Fig. 22B).

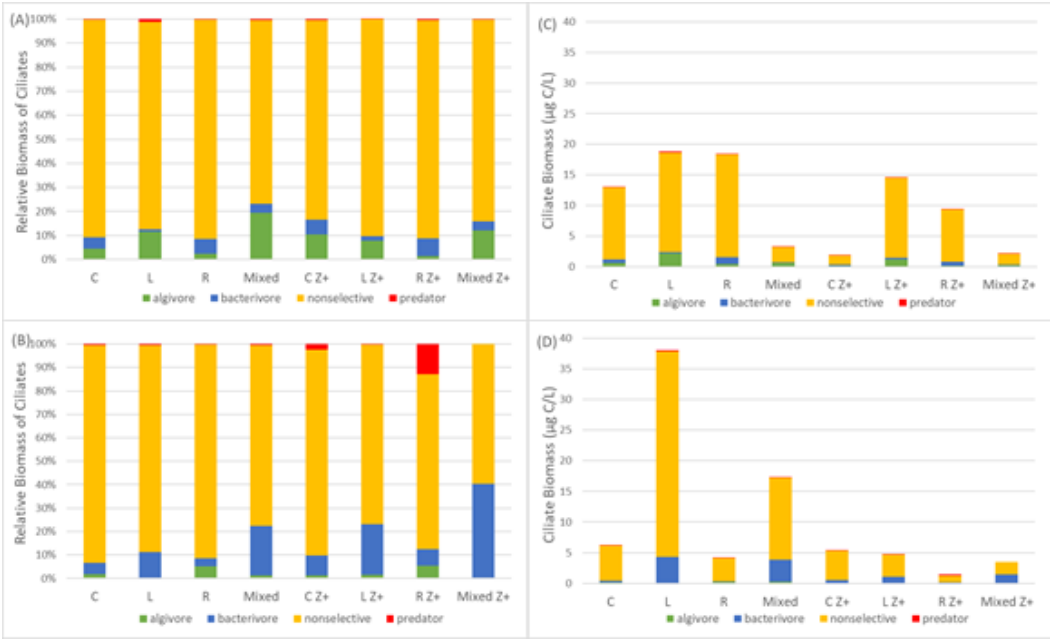


Figure 22: The relative biomass of ciliate functional feeding groups in the assay (A) after one day of DOC addition, (B) after four days of DOC addition; the biomass (µg C/ L) of ciliate functional feeding groups in the assay (C) after one day of DOC addition, (D) after four days of DOC addition with DOC and mesozooplankton treatments.

Table 12: General Linear Model (GLM) analysis results of biomass of ciliate functional feeding groups depend on DOC or grazer in the first assay (after one day of DOC addition). (Biomass ~DOC + Grazer)

| **Dependent Variable (y)** | **Independent Variable/ Factor (x)** | **Estimate** | **Standard Error** | **t - value** | **p-value** |
|---|---|---|---|---|---|
| Algivore | R | -0.3034 | 0.1635 | -1.856 | 0.161 |
| Algivore | Mixed | 0.1788 | 0.1635 | 1.094 | 0.354 |
| Algivore | L | 1.5595 | 0.1635 | 9.540 | **0.002** |
| Algivore | Mesozooplankton | -0.9524 | 0.1156 | -8.240 | **0.004** |
| Bacterivore | R | 1.2295 | 0.6063 | 2.028 | 0.136 |
| Bacterivore | Mixed | -0.9727 | 0.6063 | -1.604 | 0.207 |
| Bacterivore | L | -0.0991 | 0.6063 | -0.163 | 0.881 |
| Bacterivore | Mesozooplankton | -0.5455 | 0.4287 | -1.272 | 0.293 |
| Nonselective | R | 1.0505 | 0.6052 | 1.736 | 0.181 |
| Nonselective | Mixed | -0.7003 | 0.6052 | -1.157 | 0.331 |
| Nonselective | L | 1.2515 | 0.6052 | 2.068 | 0.131 |
| Nonselective | Mesozooplankton | -0.8292 | 0.4279 | -1.938 | 0.148 |
| Predator | R | 1.3675 | 0.8191 | 1.670 | 0.194 |
| Predator | Mixed | -0.6333 | 0.8191 | -0.773 | 0.496 |
| Predator | L | 1.2761 | 0.8191 | 1.558 | 0.217 |
| Predator | Mesozooplankton | -1.2184 | 0.5792 | -2.104 | 0.126 |

Table 13: General Linear Model (GLM) analysis results of relative biomass of ciliate functional feeding groups depend on DOC or grazer in the first assay (after one day of DOC addition). (%Biomass ~DOC + Grazer)

| **Dependent Variable (y)** | **Independent Variable/ Factor (x)** | **Estimate** | **Standard Error** | **t - value** | **p-value** |
|---|---|---|---|---|---|
| Algivore | R | -1.3059 | 0.4405 | -2.964 | 0.059 |
| Algivore | Mixed | 0.8001 | 0.4405 | 1.816 | 0.167 |
| Algivore | L | 0.3255 | 0.4405 | 0.739 | 0.514 |
| Algivore | Mesozooplankton | -0.1131 | 0.3115 | -0.363 | 0.741 |
| Bacterivore | R | 0.2270 | 0.1825 | 1.243 | 0.302 |
| Bacterivore | Mixed | -0.3514 | 0.1825 | -1.925 | 0.150 |
| Bacterivore | L | -1.3331 | 0.1825 | -7.303 | **0.005** |
| Bacterivore | Mesozooplankton | 0.2938 | 0.1291 | 2.276 | 0.107 |
| Nonselective | R | 0.0480 | 0.0554 | 0.867 | 0.450 |
| Nonselective | Mixed | -0.0790 | 0.0554 | -1.427 | 0.249 |
| Nonselective | L | 0.0175 | 0.0554 | 0.315 | 0.773 |
| Nonselective | Mesozooplankton | 0.0102 | 0.0391 | 0.259 | 0.812 |
| Predator | R | 0.365 | 1.209 | 0.302 | 0.782 |
| Predator | Mixed | -0.012 | 1.209 | -0.010 | 0.993 |
| Predator | L | 0.042 | 1.209 | 0.035 | 0.974 |
| Predator | Mesozooplankton | -0.379 | 0.855 | -0.444 | 0.687 |

Table 14: General Linear Model (GLM) analysis results of biomass of ciliate functional feeding groups depend on DOC or grazer in the second assay (after four days of DOC addition). (Biomass ~DOC + Grazer)

| **Dependent Variable (y)** | **Independent Variable/ Factor (x)** | **Estimate** | **Standard Error** | **t - value** | **p-value** |
|---|---|---|---|---|---|
| Algivore | R | 0.371 | 0.888 | 0.418 | 0.704 |
| Algivore | Mixed | -0.474 | 0.888 | -0.534 | 0.630 |
| Algivore | L | -0.083 | 0.888 | -0.094 | 0.931 |
| Algivore | Mesozooplankton | -1.123 | 0.628 | -1.790 | 0.172 |
| Bacterivore | R | -1.142 | 0.556 | -2.017 | 0.137 |
| Bacterivore | Mixed | 1.807 | 0.556 | 3.192 | **0.047** |
| Bacterivore | L | 1.711 | 0.556 | 9.022 | 0.057 |
| Bacterivore | Mesozooplankton | -0.563 | 0.400 | -1.407 | 0.254 |
| Nonselective | R | -0.958 | 0.632 | -1.515 | 0.227 |
| Nonselective | Mixed | 0.008 | 0.632 | 0.012 | 0.991 |
| Nonselective | L | 0.731 | 0.632 | 1.157 | 0.331 |
| Nonselective | Mesozooplankton | -1.381 | 0.447 | -3.090 | 0.054 |
| Predator | R | -0.981 | 0.651 | -1.507 | 0.271 |
| Predator | Mixed | 1.196 | 0.651 | 1.838 | 0.207 |
| Predator | L | 1.604 | 0.651 | 2.465 | 0.133 |
| Predator | Mesozooplankton | 0.349 | 0.412 | 0.847 | 0.486 |

Table 15: General Linear Model (GLM) analysis results of relative biomass of ciliate functional feeding groups depend on DOC or grazer in the second assay (after four days of DOC addition). (%Biomass ~DOC + Grazer)

| **Dependent Variable (y)** | **Independent Variable/ Factor (x)** | **Estimate** | **Standard Error** | **t - value** | **p-value** |
|---|---|---|---|---|---|
| Algivore | R | 1.296 | 0.981 | 1.321 | 0.278 |
| Algivore | Mixed | -0.797 | 0.981 | -0.812 | 0.476 |
| Algivore | L | -0.927 | 0.981 | -0.944 | 0.415 |
| Algivore | Mesozooplankton | 0.108 | 0.694 | 0.155 | 0.886 |
| Bacterivore | R | -0.216 | 0.103 | -2.106 | 0.126 |
| Bacterivore | Mixed | 1.485 | 0.103 | 14.454 | **0.0007** |
| Bacterivore | L | 0.867 | 0.103 | 8.443 | **0.0035** |
| Bacterivore | Mesozooplankton | 0.668 | 0.073 | 9.193 | **0.0027** |
| Nonselective | R | -0.032 | 0.083 | -0.388 | 0.724 |
| Nonselective | Mixed | -0.315 | 0.083 | -3.789 | **0.032** |
| Nonselective | L | -0.113 | 0.083 | -1.354 | 0.269 |
| Nonselective | Mesozooplankton | -0.150 | 0.059 | -2.550 | 0.084 |
| Predator | R | -0.779 | 0.503 | -1.550 | 0.261 |
| Predator | Mixed | 0.149 | 0.503 | 0.296 | 0.795 |
| Predator | L | 0.036 | 0.503 | 0.072 | 0.949 |
| Predator | Mesozooplankton | 1.942 | 0.318 | 6.109 | **0.026** |

#### 3.2.3.2 Effect Size for Biomass of Ciliate Functional Feeding Groups

##### 3.2.3.2.1 The Bottom-up Effect Size on Biomass of Ciliate Functional Feeding Groups

One day after the DOC addition, the effect size of DOC varied by DOC type and the presence of mesozooplankton. The DOC effect on the algivore biomass was

significantly positive in L treatments with and without mesozooplankton (Fig 23A). On the bacterivore biomass, DOC had a significant positive effect in both R and L with mesozooplankton treatments, while it had a negative effect in both L and mixed DOC without mesozooplankton treatments (Fig 23C). Nonselective group biomass was affected significantly positive by DOC in both R and L with mesozooplankton treatments; on the other hand, DOC effect on the nonselective group was significantly negative in mixed DOC without mesozooplankton treatment (Fig 23E). The DOC effect on the predator group biomass was significantly positive in both R treatments with and without mesozooplankton and also in L without mesozooplankton treatment, while it was significantly negative in both L and mixed DOC with mesozooplankton treatments (Fig. 23G). Generally, mesozooplankton catalyze the bottom-up effect on bacterivore and nonselective ciliates, and reduce it on predator ciliates while did not influence the DOC effect on algivores.

Four days after the DOC addition, the effect size of DOC varied by DOC type and the presence of mesozooplankton. The DOC effect on the algivore biomass was significantly positive in the R with and without mesozooplankton treatments and also in the mixed DOC without mesozooplankton treatment; on the other hand, DOC effect on algivore biomass was significantly negative in mixed DOC with mesozooplankton treatment (Fig 23B). On the bacterivore biomass, DOC had a significant positive effect in L without mesozooplankton treatment and both mixed DOC with and without mesozooplankton treatments (Fig 23D). Nonselective ciliate biomass was affected significantly positive by DOC only in L without mesozooplankton treatment (Fig 23F). The DOC effect on the predator biomass was significantly negative in the L, R, and mixed DOC without mesozooplankton treatments (Fig. 23H). Overall, the presence of mesozooplankton inhibited the DOC effect on algivore, bacterivore, and nonselective ciliates; in contrast, it slightly enhanced the bottom-up effect on predator ciliates.

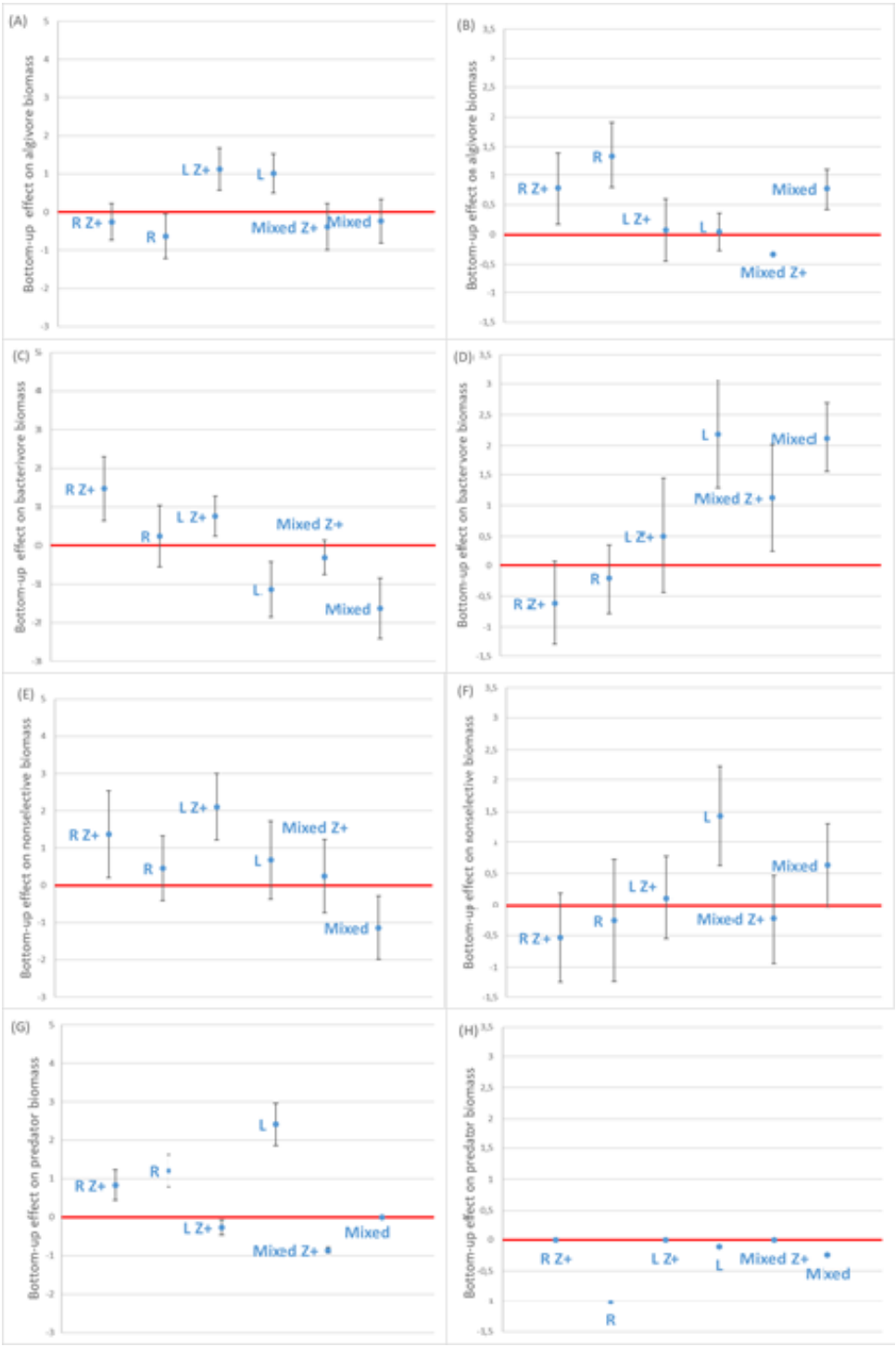


Figure 23: The bottom-up (i.e., DOC) effect size ($\log_e$ ratio) of biomass of ciliate functional feeding groups on (A, B) algivores, (C, D) bacterivores, (E, F) nonselective, and (G, H) predators. The graphs in the first column show the first assay one day after the DOC addition, while the second-column graphs represent the second assay four days after the DOC addition. Error bars are 95% confidence intervals. The effect of DOC/grazers is significant if the confidence interval does not overlap zero (the red lines).

#### 3.2.3.2.2 The Top-down Effect Size on Biomass of Ciliate Functional Feeding Groups

The top-down (i.e., grazing) effect on functional feeding groups was mostly negative, and the magnitudes were varied by DOC type in the assay one day after the DOC addition. The grazing effect was significantly negative on both algivore and predator biomass in C (no DOC control), L, and mixed DOC treatments; on both bacterivore and nonselective biomass in only C (Fig. 24A, 24C, 24E & 24G). The highest negative effect of grazing was on the predator biomass in the L treatment, with the absolute value $-2.9 \pm 0.6$ CI.

The top-down (i.e., grazing) effect on functional feeding groups was mostly negative on algivore, bacterivore, and nonselective groups and positive on the predator group, and the magnitudes were varied by DOC type four days after the DOC addition. The grazing effect was significantly negative on the algivore biomass in L, R, and mixed DOC treatments; on both bacterivore and nonselective biomass in L and mixed DOC treatments (Fig. 24B, 24D & 24F). The significant positive effect of grazing on the predator biomass was in both R and mixed DOC treatments (Fig. 24H).

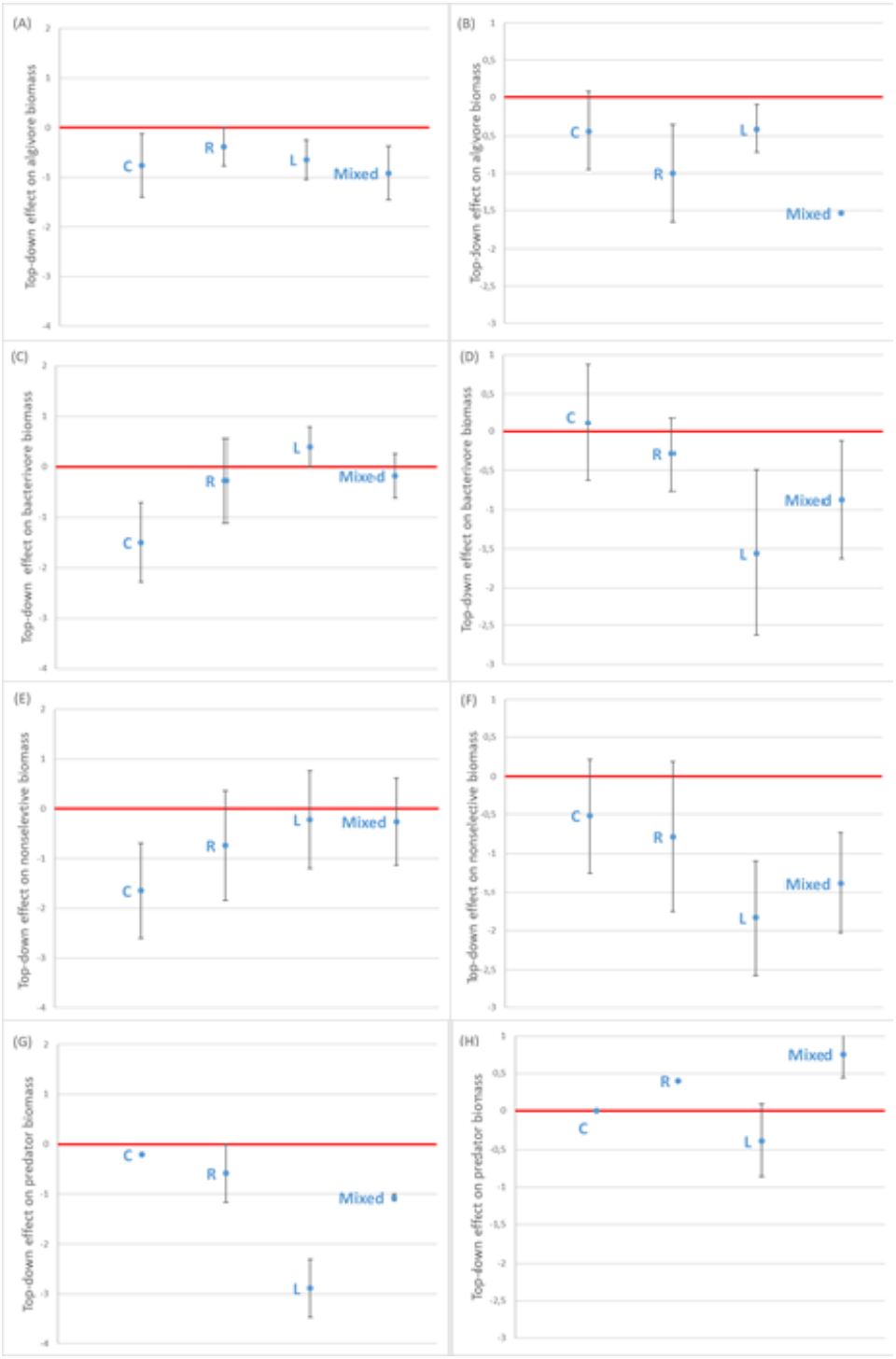


Figure 24: The top-down (i.e., grazing) effect size (loge ratio) of biomass of ciliate functional feeding groups on (A, B) algivores, (C, D) bacterivores, (E, F) nonselective, and (G, H) predators. The graphs in the first column show the first assay one day after the DOC, while second-column graphs represent the second assay four days after the DOC addition. Error bars are 95% confidence intervals. The effect of DOC/grazers is significant if the confidence interval does not overlap zero (the red line).

The effect size schema summarized the average effects of DOC types and mesozooplankton on functional feeding groups of ciliates in both *in-situ* mesocosm grazing assays, respectively (Fig. 25). The blue color indicates the positive effect, while the red color shows a negative effect. DOC types have been placed to the bottom to show the bottom-up effect, and mesozooplankton have been placed to the top to show the top-down effect. The thickness of arrows depends on how strong the effect is (i.e., thick arrows show a stronger effect). The effects were calculated with respect to the control group (i.e., mesozooplankton effect relative to no mesozooplankton control). In the first grazing assay, R had a positive effect with similar magnitude on ciliate functional feeding groups except for algivores (algivores were affected negatively by R). Similarly, L had a positive effect, and its strength was getting stronger in order on algivores, nonselectives, and predators; but only bacterivores were affected negatively by L. In contrast to R and L, mixed DOC had a negative effect on algivores, bacterivores, and nonselectives. The bottom-up effect of mixed DOC on predator ciliates was nonsignificant (i.e., no effect). The top-down effect of mesozooplankton was negative on all ciliate functional feeding groups in the first grazing assay. The strongest negative effect was on predator ciliates and followed by nonselectives, algivores, and bacterivores. In the second grazing assay, the bottom-up effect of R reversed. R affected only algivore ciliates positively, while other functional feeding groups were affected negatively by R. The positive effect of L was remained on algivores and nonselectives, but the strength of the effect was weaker compared to the first grazing assay. Additionally, L also affected bacterivores positively in the second grazing assay, while predators were affected negatively by L. Moreover, mixed DOC had a positive effect on ciliate functional feeding groups except predator ciliates. The top-down effect of mesozooplankton was negative on algivores, bacterivores and nonselectives, but

predators were affected positively by mesozooplankton in the second grazing assay (Fig. 25).

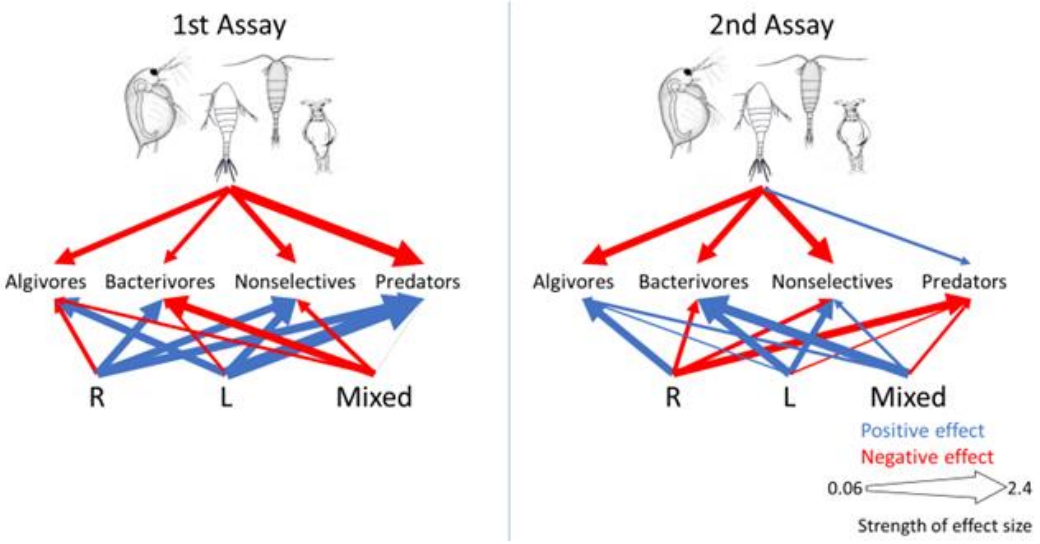


Figure 25: Effect size schema for ciliate functional feeding groups in the *in-situ* mesocosm grazing assays.

# CHAPTER 4

# DISCUSSION

The laboratory experiment was conducted to determine the effects of DOC and different grazing modes (i.e., generalist vs. selective grazing) on microbial food web components such as bacteria and ciliates. In the laboratory experiment, our key findings were: (i) The effect of DOC or grazers on bacteria was nonsignificant, (ii) DOC increased ciliate biomass and zooplankton reduced it, (iii) The top-down effect on ciliate biomass was stronger than the bottom-up effect, (iv) The top-down effect of copepods on ciliate biomass was stronger than the top-down effect of *Daphnia*.

The hypothesis that DOC would enhance bacteria biomass was not supported by our results. Neither was the hypothesis that zooplankton would reduce bacteria. In addition, effect size results were weak and negative for bacteria. These results might be explained by that both bottom-up and top-down effects might neutralize each other. In other words, either mesozooplankton could have a strong grazing pressure on bacteria biomass enough to cancel out the DOC effect, or DOC might enhance bacteria biomass enough to eliminate the top-down effect. Moreover, taking samples on day 2 might have been late to observe DOC effect on bacteria because bacteria response to allochthonous DOC within a day (Azam et al., 1983; Catalan et al., 2013) and got higher biomass before samples were taken (day 2 and day 4). Such direct and indirect top-down interactions, together with the 2–4-day sampling, may have obscured any DOC effects on bacteria in this experiment. To clarify this point, taking bacteria samples within 24 hours and sampling heterotrophic nanoflagellates (HNF) are suggested for future research.

*Daphnia*, as a generalist grazer, might have key roles in controlling bacterial biomass by direct grazing on bacteria (Riemann, 1985; Christoffersen et al., 1993; Jeppesen et al., 1992; Modenutti et al., 2003). At the same time, *Daphnia* may have indirect positive effects on bacteria by grazing on ciliates (e.g., bacterivore and

nonselective ciliates) and heterotrophic nanoflagellates (HNF), which are themselves protozoan consumers of bacteria (i.e., cascading trophic interactions) (Fenchel 1982; Riemann, 1985; Sanders & Porter, 1988; Markosova & Jezek, 1993; Jürgens, 1994; Jürgens & Stolpe, 1995; Gasol et al., 1995; Foissner & Berger, 1996). On the other hand, due to low grazing pressure of ciliates on HNF in the presence of copepods (selective grazers), lower bacteria biomass might be explained by the control of bacteria by HNF over bacteria (Jurgens et al.,2000; Kisand & Zingel, 2000; Simek et al., 2000; Modunetti et al., 2003; Sommer et al., 2003). Additionally, we were unable to check both HNF and rotifer biomass. HNF is known as highly impact grazers on bacteria (Fenchel 1982; Pernthaler et al., 1998; Sanders et al., 2000; Callieri et al., 2002), and rotifers can control bacteria biomass (Starkweather et al., 1979; Bogdan et al., 1980; Boon & Shiel, 1990). Overall, lower bacteria biomass could be explained by direct grazing of HNF, ciliates, rotifers, and *Daphnia*, and by an indirect effect of *Daphnia* and copepods as changing the top-down control.

In the +DOC treatments, we expected an increase in bacteria biomass and a decrease in phytoplankton biomass. Although the hypothesis for phytoplankton was accepted (Metin, 2021), the hypothesis for bacteria was rejected due to an insignificant change in bacteria biomass. For this reason, we were unable to say that bacteria had an advantage over phytoplankton due to their small size and large surface-to-volume ratio for nutrient absorption (Azam et al., 1983).

Our findings indicated that DOC increased ciliates while mesozooplankton reduced them and therefore supported our hypotheses. Moreover, the top-down effect of copepods on ciliates was stronger than the top-down effect of *Daphnia*. Additionally, effect size results were parallel to the GLM results. Overall, grazer traits (selectivity) regulated ciliate biomass more than DOC in the laboratory experiment.

The fact that mesozooplankton reduced ciliate biomass significantly influences the positive effect of DOC on ciliates. Due to high grazing pressure on ciliates, we could not see the DOC effect so clearly in the copepod and *Daphnia* treatments. We can directly compare copepod and *Daphnia* grazing pressure even though their grazing

mode is different. We added 2 medium size Daphnia or 30 copepods to have the same final zooplankton biomass.

At the beginning of the experiment (day 0), GLM results showed that ciliate biomass in copepod treatments was significantly lower. Further t-test analysis showed that this significance was seen only in -$DOC_{Copepod}$ treatment. Because initial samples were taken before the addition of grazers to the glass jars, it was not caused by copepod grazing, the reason was a slight random difference between replicates (See Materials & Methods). In the middle of the experiment (day 2), according to GLM analysis, both DOC and *Daphnia* had no significant effect on ciliates on day 2. Moreover, a paired t-test showed no significant ciliate biomass change in time (day 0 – day 2) in either DOC treatment throughout the experiment. Additionally, when we check the mean values of -$DOC_{Copepod}$ and -$DOC_{No\ grazer}$, the difference between them decreased on day 2 and then increased significantly on day 4. For these reasons, we can say that the grazing pressure of copepods kept ciliate biomass constant in -$DOC_{Copepod}$ treatment throughout the laboratory experiment.

At the end of the experiment (day 4), GLM results showed that both copepods and *Daphnia* reduced ciliate biomass significantly negative, while DOC had no significant effect on ciliates. Previous studies showed that the effect of DOC could be observable at the ciliate level in 4 days period (Carrick et al., 1991; Burns & Schallenberg, 2001). In the laboratory experiment, DOC effect was observable in no grazer control, ciliate biomass on day 4 increased significantly with respect to day 2. On the other hand, ciliate biomass was significantly decreased in both copepod and *Daphnia* treatments; for this reason, we were unable to see DOC effect in copepod and *Daphnia* treatments. In other words, the top-down effect was stronger than the bottom-up effect on ciliates in copepod and *Daphnia* treatments. All ciliate species in the laboratory grazing experiment were less than 45 μm in length because we filtered lake (mesocosm) water through a 45 μm plankton net (See Materials & Methods). Optimal predator vs. prey size values for copepods is 18:1, and for cladocerans 50:1 (Hansen & Bjornsen, 1994), and all ciliate species were in the optimal grazing range of copepods and *Daphnia*. Moreover, previous stoichiometry studies showed that for freshwater phytoplankton, the range of

nutrients was 0.2-20 mmol P mol$^{-}$ C and 0.014-0.180 mol N mol$^{-}$ C (Sommer, 1988, 1991; Rodriguez et al., 2016). On the other hand, nutrient values for ciliates were higher than phytoplankton values (Stoecker & Capuzzo 1990; Golz et al., 2015). Additionally, copepods, as selective grazers, prefer to consume larger and nutritious prey, so they reduce biomass of ciliates more than *Daphnia* (Burns & Gilbert, 1993; Wiackowski et al., 1994; Wickham, 1998; Calbet & Saiz, 2005). In the laboratory experiment, ciliates were larger than phytoplankton (Metin, 2021). Hence, copepods consumed ciliates approximately three times higher than *Daphnia*.

That copepods but not *Daphnia* changed the relative biomass of ciliates functional groups (i.e., composition) highlights the effect of zooplankton selectivity on microbial prey communities exposed to copepods in the laboratory experiment. Our results also suggest that calanoid copepods may be expected to reduce the relative abundance of algivore and nonselective ciliates while enhancing the dominance of bacterivores. These results are proof that copepods are selective feeders, and so they can change the composition of ciliates. The selection between ciliate functional feeding groups could not be caused by the size of ciliates because there was no significant size difference between ciliates. Copepods might select ciliates based on their taste (stoichiometric values), but little is known about the stoichiometry of different ciliates species, and this issue is open for further investigations.

The *in-situ* mesocosm grazing assays were done to observe the effects of different DOC types and mesozooplankton grazing on microbial food web components such as bacteria and ciliates. In the *in-situ* mesocosm grazing assays, our key findings according to general linear model (GLM) analysis were: (i) Mesozooplankton significantly reduced bacteria in the first grazing assay, (ii) L (leaf-leachate DOC) and mixed DOC (leaf-leachate & recalcitrant DOC) had a significant negative effect on bacteria biomass in the second grazing assay, (iii) While the bottom-up effect on bacteria biomass was stronger in the second assay, the top-down effect got weaker, (iv) L increased ciliate biomass, and mesozooplankton reduced ciliates in both grazing assays, (v) While the top-down effect on ciliate biomass was stronger in the second assay, the bottom-up effect got weaker.

Although our hypothesis was that leaf leachate DOC (L) would enhance bacteria biomass, there was no significant DOC effect in the first grazing assay (after a day of DOC addition) according to GLM results. Even though bacteria respond to allochthonous DOC within a day (Azam et al., 1983; Catalan et al., 2013), this response could be masked by the strong negative top-down effect of mesozooplankton after a day of DOC addition (Carrick et al., 1991; Burns & Schallenberg, 2001). However, the bottom-up effect size results showed that L had a significant positive effect on bacteria, while L had a significant negative effect on phytoplankton (Metin, 2021). In the first grazing assay, bacteria had an advantage over phytoplankton since bacteria have a large surface-to-volume ratio for nutrient uptake compared to phytoplankton (Azam et al., 1983). In contrast, mesozooplankton significantly reduced bacteria biomass, which supported our hypothesis. Furthermore, the top-down effect size results for bacteria were parallel to GLM results in both grazing assays. The mesozooplankton community was more diverse than the laboratory experiment and was dominated by Cladocera in all treatments (Yıldız et al., 2021). Cladocera, especially *Daphnia,* has a both direct and indirect top-down effect on bacteria (Hessen & Andersen, 1990; Vaque & Pace, 1992; Cottingham et al., 2013), while Copepoda has a strong negative indirect effect by releasing HNF (the effective predator of bacteria) from the ciliate grazing (Jurgens et al.,2000; Kisand & Zingel, 2000; Simek et al., 2000; Modunetti et al., 2003; Sommer et al., 2003). In addition, in the second grazing assay (after four days of DOC addition), mesozooplankton had a negative but insignificant effect on bacteria biomass, such that our hypothesis was rejected for the second *in-situ* assay. In contrast to our hypothesis that L enhances biomass of bacteria because leaf-leachate DOC contains a higher amount of nutrients that support bacterial growth (Cottingham et al., 2013), we found that bacteria biomass was reduced in both L and mixed DOC with respect to C (no DOC control) in the second assay. Bottom-up effect size results supported this significant negative effect of L on bacteria. This may be caused by ciliate grazing on bacteria (Jürgens, 1994; Foissner & Berger, 1996) since there was a significant increase in ciliate biomass in L and mixed DOC treatments in the second assay. Moreover, L had a significant positive effect on phytoplankton (Metin, 2021). This might be explained by bacteria made DOC more

available for phytoplankton uptake (Sanders & Porter, 1988), or changes in cascading trophic interactions such as increasing grazing pressure on ciliates had a more negative effect on bacteria while a positive effect on phytoplankton (Jürgens, 1994; Jürgens & Stolpe, 1995; Gasol et al., 1995; Sommer & Sommer, 2006). Additionally, R (recalcitrant DOC) followed C (no DOC control) pattern, likely because the recalcitrant carbon source is resistant to bacterial decomposition (Tranvik, 1988; McKnight & Aiken, 1998; Solomon et al., 2015). Overall, both bottom-up and top-down effects varied significantly in magnitude and direction among the two *in-situ* grazing assays.

In both assays, our hypotheses for ciliates that L increases ciliate biomass while mesozooplankton reduce ciliates was supported by our findings. Moreover, effect size results were similar to GLM results. In the first grazing assay, the positive effect of DOC on ciliates in L treatment was not caused by leaf-leachate DOC because the utilization of DOC by ciliates takes four days (Carrick et al., 1991; Burns & Schallenberg, 2001). Instead, the reason of the significant difference in L treatment was caused by one of the replicate tanks. Furthermore, the top-down effect of mesozooplankton on ciliates was the strongest in C Z+ treatment relative to other mesozooplankton treatments, although total zooplankton biomass was the highest in R Z+ treatment in the main mesocosm experiment. This might be caused by sampling error while preparing mesozooplankton treatments in the *in-situ* grazing assays. In other words, the water column sample was taken randomly from each tank, and maybe so grazing assay bottles had different amounts of mesozooplankton relative to the zooplankton samples of each mesocosm. To clarify this, it is suggested that zooplankton samples would be taken in further assays. In the second grazing assay, as expected L significantly increased ciliate biomass since DOC effect could be observable at ciliate level after four days of DOC addition (Carrick et al., 1991; Burns & Schallenberg, 2001) and L contains additional nutrients (i.e., P and N) that also supported ciliate biomass (Sanders & Porter, 1988; Cottingham et al., 2013; Faithfull et al., 2015). Moreover, mesozooplankton reduced ciliates significantly in the second grazing assay, and the strength of the negative mesozooplankton effect on ciliates was bigger compared to the first grazing assay.

The strongest mesozooplankton effect was observed in the L Z+ treatment. Although total zooplankton biomass was the lowest in L Z+ treatment compared to other treatments and control, ciliate biomass was the highest in L treatment. In other words, there was more available food (i.e., ciliates) for mesozooplankton for consumption in L treatment. Overall, both bottom-up and top-down effects varied considerably among the two assays for ciliates, which suggests that the cascading effects of DOC and mesozooplankton likely vary over relatively short time periods (i.e., days), with important implications on biomass.

In both grazing assays, the most dominant functional feeding group was nonselective ciliates. In the first assay, the second dominant group was algivores, but in the second assay, the second dominant group was bacterivores. The presence of mesozooplankton had a significant positive effect on the relative biomass of bacterivore and predator ciliates in the second assay. This might be explained by copepods that could consume more algivores and nonselectives, as we observed in the laboratory grazing experiment. In the *in-situ* grazing assays, the size of nonselective ciliates was relatively larger than other groups, so that mesozooplankton might have a negative effect on nonselective ciliates because of their size (Berggreen et al. 1988; Burns & Gilbert, 1993; Hansen et al., 1994). On the other hand, the size of algivore ciliates was relatively smaller than other groups, so the negative effect of mesozooplankton on algivores might not be explained by the size, but might be explained by their taste (i.e., nutritious values, DeMott, 1986). However, growing literature is limited about stoichiometry of ciliate functional feeding groups, and further research on the stoichiometry of different ciliate groups/species is suggested. Moreover, both R and mixed DOC had the opposite effect in the first and second grazing assays. As mixed DOC contained more nutrients coming from leaf-leachate relative to the R treatments, these nutrients could enhance ciliate biomass in the second assay (Sanders & Porter, 1988; Cottingham et al., 2013; Faithfull et al., 2015). Overall, these results showed that both effects of different DOC types and mesozooplankton on ciliate community composition were highly variable over time.

In both laboratory experiment and *in-situ* grazing assays, we found similar DOC effects on both bacteria and ciliates. Specifically, mixed DOC affected bacteria biomass was insignificant and negative in the laboratory experiment and the first *in-situ* grazing assay. On the other hand, mixed DOC had a negative effect on ciliates in the first *in-situ* mesocosm assay and had a positive effect after four days of DOC addition to jars (i.e., laboratory experiment) or mesocosms (the second *in-situ* grazing assay). Interestingly, the biomass of bacteria was similar (~50 µg C/L) in both laboratory experiment and *in-situ* assays, while ciliate biomass was about 10 times higher in the laboratory experiment (~30 µg C/L) compared to *in-situ* mesocosm grazing assays (~3 µg C/L). Several reasons could explain this huge difference in ciliate biomass. First, we should remind that the grazing period of the laboratory experiment was four days, while in the *in-situ* grazing assays, it was 24 hours, and the experiments were repeated twice, after one day and four days of DOC pulse. Second, in the laboratory experiment, the DOC concentration was about 11 mg C/ L (R: 7 mg C/L & L: 4 mg C/L) and prepared by using leaves of the poplar tree, while in the *in-situ* grazing assays, the concentration of mixed DOC was about 9.5 mg C/ L (L: 8 mg C/L & R: 1.5 mg C/L) and prepared by using alder tree leaves. Third, the composition of phytoplankton, ciliates, and zooplankton was different in laboratory experiment and *in-situ* assays. Specifically, in the laboratory experiment, Cladocerans (i.e., *Daphnia*) biomass was equal to Copepoda, but in the *in-situ* assays, Cladocerans were dominant in the zooplankton composition. In addition, in the laboratory experiment, zooplankton biomass was the same throughout the experiment, while it was changing between treatments in the *in-situ* assays. Fourth, potential differences in the light levels among laboratory experiment vs. *in-situ* mesocosm grazing assays might have caused the difference in results. Bacteria biomass was directly inhibited by UV light (Lindell et al., 1996) and indirectly enhanced by solar radiation because it increases the lability of DOC via phototransformation of recalcitrant DOC to labile DOC (Kieber et al., 1989; Lindell et al., 1995; Lindell et al., 1996). Ciliates were affected negatively by light since it affects the ingestion rate of ciliates negatively (Chen & Chang, 1999), or UV light might cause mutation in swimming behavior that makes ciliates vulnerable to predators (Kammerlander et al., 2018). Finally, nutrient availability differed

between the laboratory experiment and *in-situ* grazing assays. Specifically, the laboratory experiment was performed in a nutrient-rich medium, while *in-situ* mesocosm assays were C (no DOC control) and R treatments had a severe P limitation (Calderó-Pascual et al., submitted).

GLM results differed from the effect size (log response ratio) results in some occasions for some treatments. Although both GLM analysis and effect size could use to identify the treatment effect compared to the control group, we used both together to discuss our results because effect size analysis enabled us to compare the top-down vs. bottom-up effects across the same units (Hillebrand & Gurevitch, 2016) and their calculations were different. In the GLM analysis, both treatment and control groups were merged. For example, in the laboratory experiment, the DOC effect was calculated as the comparison of the addition of +DOC groups ($+DOC_{no\ grazer}$, $+DOC_{Copepod,}$ and $+DOC_{Daphnia}$) relative to the addition -DOC groups ($-DOC_{no\ grazer}$, $-DOC_{Copepod}$ and $-DOC_{Daphnia}$) together. On the other hand, effect size compared treatment and control groups more specifically. For example, DOC effect in no grazer control group was calculated as ln ($+DOC_{no\ grazer}$ / - $DOC_{no\ grazer}$), or DOC effect in copepod treatment as ln ($+DOC_{Copepod}$ / $-DOC_{Copepod}$) (See Materials & Methods).

# CHAPTER 5

# CONCLUSION

As global climate change causes changing precipitation events, lots of allochthonous matter are transported from the catchment area to lakes via flooding. However, in-lake processes after allochthonous matter input are still uncertain. This study highlights the effects of a major allochthonous matter, dissolved organic carbon (DOC) input, and grazing pressure of zooplankton with different selectivity on the microbial food web. In addition, how the different DOC types affect the planktonic food web in the presence/absence of mesozooplankton is determined. Our study showed that direct effects on bacteria are likely short-lived and masked by indirect trophic interactions. On the other hand, DOC, especially leaf-leachate DOC (L), positively affected ciliate biomass, while grazers had a negative effect. Specifically, copepods had a higher grazing pressure on ciliates than *Daphnia*. Moreover, copepods consumed more algivore and nonselective ciliates that was novel proof of copepods' selectivity. The copepod-ciliate link seems critical in connecting the microbial and classical food web. Overall, mesozooplankton traits regulate ciliate biomass more than DOC. Sampling bacteria within a day, genetic analyses of the bacteria community, and HNF sampling are suggested for further microbial food web studies. Further stoichiometry analyses of the ciliate community composition of these experiments will provide more detailed information about the selectivity of copepods.

## REFERENCES


Adrian, R.. (1999). Top-down effects of crustacean zooplankton on pelagic microorganisms in a mesotrophic lake. Journal of Plankton Research, 21(11), 2175–2190. https://doi.org/10.1093/plankt/21.11.2175

Azam, F., Fenchel, T., Field, J., Gray, J., Meyer-Reil, L., & Thingstad, F.. (1983). The Ecological Role of Water-Column Microbes in the Sea. Marine Ecology Progress Series, 10, 257–263. https://doi.org/10.3354/meps010257

Berggreen, U., Hansen, B., & Kiørboe, T.. (1988). Food size spectra, ingestion and growth of the copepodAcartia tonsa during development: Implications for determination of copepod production. Marine Biology, 99(3), 341–352. https://doi.org/10.1007/bf02112126

Berggren, M., Ström, L., Laudon, H., Karlsson, J., Jonsson, A., Giesler, R., Bergström, A.-K., & Jansson, M.. (2010). Lake secondary production fueled by rapid transfer of low molecular weight organic carbon from terrestrial sources to aquatic consumers. Ecology Letters, 13(7), 870–880. https://doi.org/10.1111/j.1461-0248.2010.01483.x

Bogdan, K. G., Gilbert, J. J., & Starkweather, P. L.. (1980). In situ clearance rates of planktonic rotifers. Hydrobiologia, 73(1-3), 73–77. https://doi.org/10.1007/bf00019428

Boon PI, Shiel RJ (1990) Grazing on Bacteria by Zooplankton in Australian Billabongs. Marine and Freshwater Research 41, 247-257. https://doi.org/10.1071/MF9900247

Borsheim, K., & Bratbak, G.. (1987). Cell volume to cell carbon conversion factors for a bacterivorous Monas sp. enriched from seawater. Marine Ecology Progress Series, 36, 171–175. https://doi.org/10.3354/meps036171

Bottrell, H.H. & Duncan, A. & Gliwicz, Z. & Grygierek, E. & Herzig, A. & Hilbricht-Ilkowska, A. & Kurasawa, H. & Larsson, Petter & Weglenska, T..

(1976). Review of some problems in zooplankton production studies. Norwegian Journal of Zoology. 21. 477-483.

Bratbak, G., & Dundas, I.. (1984). Bacterial dry matter content and biomass estimations. Applied and Environmental Microbiology, 48(4), 755–757. https://doi.org/10.1128/aem.48.4.755-757.1984

Brett, M. T., Kainz, M. J., Taipale, S. J., & Seshan, H.. (2009). Phytoplankton, not allochthonous carbon, sustains herbivorous zooplankton production. Proceedings of the National Academy of Sciences, 106(50), 21197–21201. https://doi.org/10.1073/pnas.0904129106

Brönmark, C., & Hansson, L-A. (2005). The biology of lakes and ponds (2nd edition). (Biology of habitats). Oxford University Press.

Burns, C.W., Gilbert, J.J. (1993) Predation on ciliates by freshwater calanoid copepods: rates of predation and relative vulnerability of prey. Freshwat. Biol. 30, 377–393. https://doi.org/10.1111/j.1365-2427.1993.tb00822.x

Burns, C. W., & Schallenberg, M.. (2001). Calanoid copepods versus cladocerans: Consumer effects on protozoa in lakes of different trophic status. Limnology and Oceanography, 46(6), 1558–1565. https://doi.org/10.4319/lo.2001.46.6.1558

Calbet, A., & Landry, M. R.. (2004). Phytoplankton growth, microzooplankton grazing, and carbon cycling in marine systems. Limnology and Oceanography, 49(1), 51–57. https://doi.org/10.4319/lo.2004.49.1.0051

Calbet, A., & Saiz, E.. (2005). The ciliate-copepod link in marine ecosystems. Aquatic Microbial Ecology, 38, 157–167. https://doi.org/10.3354/ame038157

Calderó-Pascual, M., Yıldız, D., Yalçın, G., Metin, M., Yetim, S., Fiorentin, C., Andersen, M.R., Jennings, E., Jeppesen, E., Ger, K.A., Beklioğlu, M., McCarthy, V.. (2021) The importance of allochthonous organic matter quality when investigating pulse disturbance events in freshwater lakes: a mesocosm experiment. Manuscript submitted for publication.

Callieri, C.. (2002). Grazing by ciliates and heterotrophic nanoflagellates on picocyanobacteria in Lago Maggiore, Italy. Journal of Plankton Research, 24(8), 785–796. https://doi.org/10.1093/plankt/24.8.785

Carpenter, S.R., Cole, J.J., Pace, M.L., Van De Bogert, M., Bade, D.L., Bastviken, D., Gille, C.M., Hodgson, J.R., Kitchell, J.F., Kritzberg, E.S., (2005) Ecosystem Subsidies: Terrestrial Support of Aquatic Food Webs From 13C Addition to Contrasting Lakes. Ecology 86, 2737–2750.. https://doi.org/10.1890/04-1282

Carrick, H. J., Fahnenstiel, G. L., Stoermer, E. F., & Wetzel, R. G.. (1991). The importance of zooplankton-protozoan trophic couplings in Lake Michigan. Limnology and Oceanography, 36(7), 1335–1345. https://doi.org/10.4319/lo.1991.36.7.1335

Caston, C. B., Nowlin, W. H., Gaulke, A., & Vanni, M. J.. (2009). The relative importance of heterotrophic bacteria to pelagic ecosystem dynamics varies with reservoir trophic state. Limnology and Oceanography, 54(6), 2143–2156. https://doi.org/10.4319/lo.2009.54.6.2143

Catalán, N., Obrador, B., Felip, M., & Pretus, J. L.. (2013). Higher reactivity of allochthonous vs. autochthonous DOC sources in a shallow lake. Aquatic Sciences, 75(4), 581–593. https://doi.org/10.1007/s00027-013-0302-y

Chen, K., Chang, J.. (1999). Short communication. Influence of light intensity on the ingestion rate of a marine ciliate, Lohmanniella sp. Journal of Plankton Research, 21(9), 1791–1798. https://doi.org/10.1093/plankt/21.9.1791

Christoffersen, K., Riemann, B., Klysner, A., & Søndergaard, M.. (1993). Potential role of fish predation and natural populations of zooplankton in structuring a plankton community in eutrophic lake water. Limnology and Oceanography, 38(3), 561–573. https://doi.org/10.4319/lo.1993.38.3.0561

Cottingham, K. L., & Narayan, L.. (2013). Subsidy quantity and recipient community structure mediate plankton responses to autumn leaf drop. Ecosphere, 4(7), art89. https://doi.org/10.1890/es13-00128.1

Cowles, T. J., Olson, R. J., & Chisholm, S. W.. (1988). Food selection by copepods: discrimination on the basis of food quality. Marine Biology, 100(1), 41–49. https://doi.org/10.1007/bf00392953

Degerman, R., Lefébure, R., Byström, P., Båmstedt, U., Larsson, S., & Andersson, A.. (2018). Food web interactions determine energy transfer efficiency and top consumer responses to inputs of dissolved organic carbon. Hydrobiologia, 805(1), 131–146. https://doi.org/10.1007/s10750-017-3298-9

Demott, W. R.. (1986). The role of taste in food selection by freshwater zooplankton. Oecologia, 69(3), 334–340. https://doi.org/10.1007/bf00377053

Dumont, H. J., Van De Velde, I., & Dumont, S.. (1975). The dry weight estimate of biomass in a selection of Cladocera, Copepoda and Rotifera from the plankton, periphyton and benthos of continental waters. Oecologia, 19(1), 75–97. https://doi.org/10.1007/bf00377592

Faithfull, C. L., Huss, M., Vrede, T., & Bergström, A.-K.. (2011). Bottom-up carbon subsidies and top-down predation pressure interact to affect aquatic food web structure. Oikos, 120(2), 311–320. https://doi.org/10.1111/j.1600-0706.2010.18683.x

Faithfull, C., M. Huss, T. Vrede, J. Karlsson & A.-K. Bergstro¨m, (2012) Transfer of bacterial production based on labile carbon to higher trophic levels in an oligotrophic pelagic system. Canadian Journal of Fisheries and Aquatic Sciences 69: 85–93. https://doi.org/10.1139/f2011-142

Fenchel, T.. (1982). Ecology of Heterotrophic Microflagellates. IV Quantitative Occurrence and Importance as Bacterial Consumers. Marine Ecology Progress Series, 9, 35–42. https://doi.org/10.3354/meps009035

Foissner W., Berger H. & Schaumburg J. (1999) Identification and ecology of limnetic plankton ciliates. Informationsber Bayer Landesamt Wasserwirtsch 3/99, 1- 793.

Foissner, W., & Berger, H. (1996). A user-friendly guide to the ciliates (Protozoa, Ciliophora) commonly used by hydrobiologists as bioindicators in rivers, lakes, and waste waters, with notes on their ecology. Freshwater Biology, 35, 375-482. https://doi.org/10.1111/j.1365-2427.1996.tb01775.x

Frost, B. W.. (1972). EFFECTS OF SIZE AND CONCENTRATION OF FOOD PARTICLES ON THE FEEDING BEHAVIOR OF THE MARINE PLANKTONIC COPEPOD CALANUS PACIFICUS 1. Limnology and Oceanography, 17(6), 805–815. https://doi.org/10.4319/lo.1972.17.6.0805

Gasol, J.M, A.M. Simons & J. Kalff. (1995) Patterns in the top-down versus bottom-up regulation of heterotrophic nanoflagellates in temperate lakes. J. Plankton Res. 17: 1879-1903. https://doi.org/10.1093/plankt/17.10.1879

Ger, K. A., Urrutia-Cordero, P., Frost, P. C., Hansson, L. A., Sarnelle, O., Wilson, A. E., & Lürling, M. (2016). The interaction between cyanobacteria and zooplankton in a more eutrophic world. Harmful algae, 54, 128–144. https://doi.org/10.1016/j.hal.2015.12.005

Ger, K. A., Teh, S. J., Baxa, D. V., Lesmeister, S., & Goldman, C. R.. (2010). The effects of dietary Microcystis aeruginosa and microcystin on the copepods of the upper San Francisco Estuary. Freshwater Biology, 55(7), 1548–1559. https://doi.org/10.1111/j.1365-2427.2009.02367.x

Golz, A.-L., Burian, A., & Winder, M.. (2015). Stoichiometric regulation in micro- and mesozooplankton. Journal of Plankton Research, 37(2), 293–305. https://doi.org/10.1093/plankt/fbu109

Granéli, E., Carlsson, P., & Legrand, C.. (1999). None. Aquatic Ecology, 33(1), 17–27. https://doi.org/10.1023/a:1009925515059

Guillemette, F., Leigh Mccallister, S., & Del Giorgio, P. A.. (2016). Selective consumption and metabolic allocation of terrestrial and algal carbon determine allochthony in lake bacteria. The ISME Journal, 10(6), 1373–1382. https://doi.org/10.1038/ismej.2015.215

Hammer, A. C., & Pitchford, J. W.. (2005). The role of mixotrophy in plankton bloom dynamics, and the consequences for productivity. ICES Journal of Marine Science, 62(5), 833–840. https://doi.org/10.1016/j.icesjms.2005.03.001

Hansen, B., Bjornsen, P. K., & Hansen, P. J.. (1994). The size ratio between planktonic predators and their prey. Limnology and Oceanography, 39(2), 395–403. https://doi.org/10.4319/lo.1994.39.2.0395

Hansen, P. J., Bjørnsen, P. K., & Hansen, B. W.. (1997). Zooplankton grazing and growth: Scaling within the 2-2,-µm body size range. Limnology and Oceanography, 42(4), 687–704. https://doi.org/10.4319/lo.1997.42.4.0687

Harding, J.P. & Smith, W.A. (1974) A Key to the British Freshwater Cyclopoid and Calanoids. 2nd Edition, Freshwater Biological Association, UK, Scientific Publication No. 18, 54 p.

Hessen, D. O.. (1992). Dissolved organic carbon in a humic lake: effects on bacterial production and respiration. Hydrobiologia, 229(1), 115–123. https://doi.org/10.1007/bf00006995

Hillebrand, H., & Gurevitch, J. (2016). Meta-Analysis and Systematic Reviews in Ecology. In: eLS. John Wiley & Sons, Ltd: Chichester. https://doi.org/10.1002/9780470015902.a0003272.pub2

Hongve, D., Riise, G., & Kristiansen, J. F.. (2004). Increased colour and organic acid concentrations in Norwegian forest lakes and drinking water? a result of increased precipitation?. Aquatic Sciences, 66(2), 231–238. https://doi.org/10.1007/s00027-004-0708-7

IPCC, 2007. Summary for policymakers. In Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel in Climate Change (eds S. Solomon, D. Qin, M. Manning et al.), Cambridge University Press, Cambridge: 1–18.

IPCC, 2014. In: Core Writing Team, Pachauri, RK, Meyer LA (Ed.), Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and

III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC, Geneva, Switzerland.

Jeppesen, E., Sortkjær, O., Søndergaard, M., & Erlandsen, M. (1992). Impact of a trophic cascade on heterotrophic bacterioplankton production in two shallow fish-manipulated lakes. Archiv für Hydrobiologie. Beiheft, 37, 219-231.

Joint, I., Henriksen, P., Fonnes, G., Bourne, D., Thingstad, T., & Riemann, B.. (2002). Competition for inorganic nutrients between phytoplankton and bacterioplankton in nutrient manipulated mesocosms. Aquatic Microbial Ecology, 29, 145–159. https://doi.org/10.3354/ame029145

Jurgens, K. & Jeppesen, E., (2000) The impact of metazooplankton on the structure of the microbial food web in a shallow, hypertrophic lake. Journal of Plankton Research, 22(6), 1047–1070. https://doi.org/10.1093/plankt/22.6.1047

Jürgens, K., & Stolpe, G.. (1995). Seasonal dynamics of crustacean zooplankton, heterotrophic nanoflagellates and bacte-ria in a shallow, eutrophic lake. Freshw. Biol.33:27–38. https://doi.org/10.1111/j.1365-2427.1995.tb00383.x

Jurgens, K., Arndt, H., Rothhaupt, K.O., (1994) Zooplankton-mediated changes of bacterial community structure. Microbial Ecology, 27(1). https://doi.org/10.1007/bf00170112

Kammerlander, B., Tartarotti, B., & Sonntag, B.. (2018). The Impact of UV Radiation onParameciumPopulations from Alpine Lakes. Journal of Eukaryotic Microbiology, 65(2), 250–254. https://doi.org/10.1111/jeu.12463

Karlsson, J., Jansson, M., & Jonsson, A.. (2007). Respiration of allochthonous organic carbon in unproductive forest lakes determined by the Keeling plot method. Limnology and Oceanography, 52(2), 603–608. https://doi.org/10.4319/lo.2007.52.2.0603

Kieber, David & McDaniel, Julie & Mopper, Kenneth. (1989). Photochemical Source of Biological Substrates in Sea Water: Implications for Carbon Cycling. Nature. 341. 637-639. https://doi.org/10.1038/341637a0

Kiørboe, T.. (2011). How zooplankton feed: mechanisms, traits and trade-offs. Biological Reviews, 86(2), 311–339. https://doi.org/10.1111/j.1469-185x.2010.00148.x

Kiørboe, T., & Visser, A.. (1999). Predator and prey perception in copepods due to hydromechanical signals. Marine Ecology Progress Series, 179, 81–95. https://doi.org/10.3354/meps179081

Kisand, V., & Zingel, P.. (2000). Dominance of ciliate grazing on bacteria during spring in a shallow eutrophic lake. Aquatic Microbial Ecology, 22, 135–142. https://doi.org/10.3354/ame022135

Kritzberg, E. S., Cole, J. J., Pace, M. L., Granéli, W., & Bade, D. L.. (2004). Autochthonous versus allochthonous carbon sources of bacteria: Results from whole-lake 13 C addition experiments. Limnology and Oceanography, 49(2), 588–596. https://doi.org/10.4319/lo.2004.49.2.0588

Lebret, K., Langenheder, S., Colinas, N., Östman, Ö., & Lindström, E.. (2018). Increased water colour affects freshwater plankton communities in a mesocosm study. Aquatic Microbial Ecology, 81(1), 1–17. https://doi.org/10.3354/ame01858

Leigh Mccallister, S., & Del Giorgio, P. A.. (2008). Direct measurement of the d13C signature of carbon respired by bacteria in lakes: Linkages to potential carbon sources, ecosystem baseline metabolism, and CO2 fluxes. Limnology and Oceanography, 53(4), 1204–1216. https://doi.org/10.4319/lo.2008.53.4.1204

Lindell, M. J., Granéli, W., & Tranvik, L. J.. (1995). Enhanced bacterial growth in response to photochemical transformation of dissolved organic matter. Limnology and Oceanography, 40(1), 195–199. https://doi.org/10.4319/lo.1995.40.1.0195

Lindell, M., Granéli, H., & Tranvik, L.. (1996). Effects of sunlight on bacterial growth in lakes of different humic content. Aquatic Microbial Ecology, 11, 135–141. https://doi.org/10.3354/ame011135

Markošová, R., & Ježzek, J.. (1993). Bacterioplankton interactions with Daphnia and algae in experimental enclosures. Hydrobiologia, 264(2), 85–99. https://doi.org/10.1007/bf00014096

McCauley, E. (1984). The estimation of the abundance and biomass of zooplankton in samples. In: Downing JA, Rigler FH, eds. A Manual on Methods for the Assessment of Secondary Productivity in Freshwaters. Oxford: Blackwell Scientific Publications, 228–65.

McKnight D.M., Aiken G.R. (1998) Sources and Age of Aquatic Humus. In: Hessen D.O., Tranvik L.J. (eds) Aquatic Humic Substances. Ecological Studies (Analysis and Synthesis), vol 133. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-662-03736-2_2

Meinelt, T., Paul, A., Phan, T. M., Zwirnmann, E., Krüger, A., Wienke, A., & Steinberg, C. E. W.. (2007). Reduction in vegetative growth of the water mold Saprolegnia parasitica (Coker) by humic substance of different qualities. Aquatic Toxicology, 83(2), 93–103. https://doi.org/10.1016/j.aquatox.2007.03.013

Melillo, Jerry M., Terese (T.C.) Richmond, and Gary W. Yohe, Eds.. (2014) Climate Change Impacts in the United States: The Third National Climate Assessment. U.S. Global Change Research Program, 841 pp. doi:10.7930/J0Z31WJ2.

Metin, M. (2021). Combined bottom-up effects of DOC and top-down effects of zooplankton with contrasting traits on phytoplankton biomass and composition [Unpublished master's thesis]. Middle East Technical University.

Minguez, L., Sperfeld, E., Berger, S. A., Nejstgaard, J. C., & Gessner, M. O.. (2020). Changes in food characteristics reveal indirect effects of lake

browning on zooplankton performance. Limnology and Oceanography, 65(5), 1028–1040. https://doi.org/10.1002/lno.11367

Modenutti, B., Queimaliños, C., Balseiro, E., Reissig, M.. (2003) Impact of different zooplankton structures on the microbial food web of a South Andean oligotrophic lake. Acta Oecologica 24, S289–S298.. doi:10.1016/s1146-609x(03)00030-4

Moran, M. A., & Hodson, R. E.. (1990). Bacterial production on humic and nonhumic components of dissolved organic carbon. Limnology and Oceanography, 35(8), 1744–1756. https://doi.org/10.4319/lo.1990.35.8.1744

Pernthaler, J., Glockner, F. O., Unterholzner, S., Alfreider, A., Psenner, R., & Amann, R. (1998). Seasonal community and population dynamics of pelagic bacteria and archaea in a high mountain lake. Applied and environmental microbiology, 64(11), 4299–4306. https://doi.org/10.1128/AEM.64.11.4299-4306.1998

Pomeroy, L. R.. (1974). The Ocean's Food Web, A Changing Paradigm. Bioscience, 24(9), 499–504. https://doi.org/10.2307/1296885

Sanders R.W., Porter K.G. (1988) Phagotrophic Phytoflagellates. In: Marshall K.C. (eds) Advances in Microbial Ecology. Advances in Microbial Ecology, vol 10. Springer, Boston, MA. https://doi.org/10.1007/978-1-4684-5409-3_5

Putt, M., & Stoecker, D. K.. (1989). An experimentally determined carbon : volume ratio for marine “oligotrichous” ciliates from estuarine and coastal waters. Limnology and Oceanography, 34(6), 1097–1103. https://doi.org/10.4319/lo.1989.34.6.1097

R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Ramos Rodríguez, E., Pérez-Martínez, C., & Conde-Porcuna, J. M.. (2016). Strict stoichiometric homeostasis of Cryptomonas pyrenoidifera (Cryptophyceae)

in relation to N:P supply ratios. Journal of Limnology. https://doi.org/10.4081/jlimnol.2016.1487

Riemann, B.. (1985). Potential Importance of Fish Predation and Zooplankton Grazing on Natural Populations of Freshwater Bacteria. Applied and Environmental Microbiology, 50(2), 187–193. https://doi.org/10.1128/aem.50.2.187-193.1985

Ruttner-Kolisko, A. (1977). Suggestion for biomass calculation of planktonic rotifers. Archiv für Hydrobiologie 8: 71-76

Sanders, R., Berninger, U., Lim, E., Kemp, P., & Caron, D.. (2000). Heterotrophic and mixotrophic nanoplankton predation on picoplankton in the Sargasso Sea and on Georges Bank. Marine Ecology Progress Series, 192, 103–118. https://doi.org/10.3354/meps192103

Sanders, R.W., Porter, K.G., Bennett, S.J., and DeBiase, AE. (1989) Seasonal patterns of bacterivory by flagellates, ciliates, rotifers, and cladocerans in a freshwater planktonic community. Limnol. Oceanogr., 34, 673–687.

Scourfield, J. D. Harding, P. J. (1966). A Key to the British Species of freshwater Cladocera. Freshwater Biological Association Scientific Publication. No.5, 1966

Seekell, D. A., Lapierre, J.-F., Ask, J., Bergström, A.-K., Deininger, A., Rodríguez, P., & Karlsson, J.. (2015). The influence of dissolved organic carbon on primary production in northern lakes. Limnology and Oceanography, 60(4), 1276–1285. https://doi.org/10.1002/lno.10096

Sherr, E. B., & Sherr, B. F.. (2002). None. Antonie Van Leeuwenhoek, 81(1), 293–308. https://doi.org/10.1023/a:1020591307260

Simek, K., Jürgens, K., Nedoma, J., Comerma, M., & Armengol, J.. (2000). Ecological role and bacterial grazing of Halteria spp.: small freshwater oligotrichs as dominant pelagic ciliate bacterivores. Aquatic Microbial Ecology, 22, 43–56. https://doi.org/10.3354/ame022043

Solomon, C. T., Jones, S. E., Weidel, B. C., Buffam, I., Fork, M. L., Karlsson, J., Larsen, S., Lennon, J. T., Read, J. S., Sadro, S., & Saros, J. E.. (2015). Ecosystem Consequences of Changing Inputs of Terrestrial Dissolved Organic Matter to Lakes: Current Knowledge and Future Challenges. Ecosystems, 18(3), 376–389. https://doi.org/10.1007/s10021-015-9848-y

Sommer, F., Santer, B., Jamieson, C., Hansen, T., & Sommer, U.. (2003). Daphnia population growth but not moulting is a substantial phosphorus drain for phytoplankton. Freshwater Biology, 48(1), 67–74. https://doi.org/10.1046/j.1365-2427.2003.00971.x

Sommer, U. (1988) Does nutrient competition among phytoplankton occur in-situ? SIL Proceedings, 1922-2010, 23:2, 707-712, https://doi.org/10.1080/03680770.1987.11899697

Sommer, U. (1991) The application of the droop-model of nutrient limitation to natural phytoplankton, SIL Proceedings, 1922-2010, 24:2, 791-794, https://doi.org/10.1080/03680770.1989.11898851

Sommer, U., & Sommer, F.. (2006). Cladocerans versus copepods: the cause of contrasting top–down controls on freshwater and marine phytoplankton. Oecologia, 147(2), 183–194. https://doi.org/10.1007/s00442-005-0320-0

Søndergaard, M., & Middelboe, M.. (1995). A cross-system analysis of labile dissolved organic carbon. Marine Ecology Progress Series, 118, 283–294. https://doi.org/10.3354/meps118283

Starkweather, P. L., Gilbert, J. J., & Frost, T. M.. (1979). Bacterial feeding by the rotifer Brachionus calyciflorus: Clearance and ingestion rates, behavior and population dynamics. Oecologia, 44(1), 26–30. https://doi.org/10.1007/bf00346392

Steinberg, C. E.W, Stürzenbaum, S. R, & Menzel, R. (2008). Genes and environment -- Striking the fine balance between sophisticated biomonitoring and true functional environmental genomics. Science of the

total environment, 400, 142-161. https://doi.org/10.1016/j.scitotenv.2008.07.023

Stoecker, D. K., & Capuzzo, J. M.. (1990). Predation on Protozoa: its importance to zooplankton. Journal of Plankton Research, 12(5), 891–908. https://doi.org/10.1093/plankt/12.5.891

Sun, J. & Liu, D.. (2003). Geometric models for calculating cell biovolume and surface area for phytoplankton. Journal of Plankton Research, 25(11), 1331–1346. https://doi.org/10.1093/plankt/fbg096

Tabari, H.. (2020). Climate change impact on flood and extreme precipitation increases with water availability. Scientific Reports, 10(1). https://doi.org/10.1038/s41598-020-70816-2

Tranvik, L. J.. (1992). Allochthonous dissolved organic matter as an energy source for pelagic bacteria and the concept of the microbial loop. Hydrobiologia, 229(1), 107–114. https://doi.org/10.1007/bf00006994

Tranvik, L. J.. (1988). Availability of dissolved organic carbon for planktonic bacteria in oligotrophic lakes of differing humic content. Microbial Ecology, 16(3), 311–322. https://doi.org/10.1007/bf02011702

Utermöhl H. (1958) Zur Vervollkommnung der quantitativen PhytoplanktonMethodik. Mitteilungen der Internationale Vereinigung für Limnlogie, 9, 1-38.

Vaqué D & Pace LM. (1992) Grazing on bacteria by flagellate and cladocerans in lakes of contrasting food-web structure. Journal of Plankton Research. 14, 307–321. https://doi.org/10.1093/plankt/14.2.307

Weyhenmeyer, G. A., Müller, R. A., Norman, M., & Tranvik, L. J.. (2016). Sensitivity of freshwaters to browning in response to future climate change. Climatic Change, 134(1-2), 225–239. https://doi.org/10.1007/s10584-015-1514-z

Wiackowski, K., Brett, M. T., & Goldman, C. R.. (1994). Differential effects of zooplankton species on ciliate community structure. Limnology and Oceanography, 39(3), 486–492. https://doi.org/10.4319/lo.1994.39.3.0486

Wickham, S. A.. (1998). The direct and indirect impact of Daphnia and Cyclops on a freshwater microbial food web. Journal of Plankton Research, 20(4), 739–755. https://doi.org/10.1093/plankt/20.4.739

Williamson, C. E., Overholt, E. P., Pilla, R. M., Leach, T. H., Brentrup, J. A., Knoll, L. B., Mette, E. M., & Moeller, R. E.. (2015). Ecological consequences of long-term browning in lakes. Scientific Reports, 5(1), 18666. https://doi.org/10.1038/srep18666

Yalçın, G., Yıldız, D., Metin, M., Yetim, S., Calderó-Pascual, M., Karakaya, N., Ger, K.A., Jeppesen, E., Beklioğlu, M.. (2021) Response of microbial loop to different quality of dissolved organic carbon (DOC) pulse disturbance. Manuscript in preparation.

Yıldız, D., Calderó-Pascual, M., Yalçın, G., Metin, M., Yetim, S., Filiz, N., Kavak, P., Ger, K.A., McCarthy, V., Jeppesen, E., Beklioğlu, M.. (2021) Effects of DOC quality on zooplankton community structure: a mesocosm experiment. Manuscript in preparation.