COMBINED EFFECTS OF DISSOLVED ORGANIC CARBON AND ZOOPLANKTON GRAZING ON PHYTOPLANKTON COMMUNITY

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ABSTRACT

COMBINED EFFECTS OF DISSOLVED ORGANIC CARBON AND ZOOPLANKTON GRAZING ON PHYTOPLANKTON COMMUNITY

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While global climate change has major impacts on freshwater ecosystems, a mechanistic understanding of these effects on food web dynamics is poorly understood. A key effect of climate change is increased allochthonous dissolved organic carbon (DOC) input to aquatic environments, which serves as an energy source for heterotrophic plankton and alters food web dynamics, and leads brownification. We aimed to link patterns and processes in a plankton ecosystem by comparing the bottom-up effects of DOC (i.e., recalcitrant and leaf leachate sources) to the top-down effects of zooplankton with contrasting grazing selectivity (*Daphnia* vs. *calanoid copepods*) on phytoplankton biomass and composition in laboratory and *in-situ* mesocosm grazing assays. We expected that DOC and zooplankton would reduce phytoplankton biomass; stronger herbivory by *Daphnia* than copepods; and stronger grazing on larger-sized phytoplankton. In the nutrient replete laboratory experiment, DOC reduced total phytoplankton biomass (especially smaller sized species). While grazers reduced total phytoplankton, they increased smaller phytoplankton and decreased larger phytoplankton biomass. Moreover, copepods

were stronger herbivores than *Daphnia*. In nutrient limited mesocosm experiments DOC increased phytoplankton biomass, though species-specific responses varied across treatments and time. Hence, labile DOC may reduce phytoplankton biomass in nutrient replete conditions, and increase phytoplankton during nutrient limitation. Mesozooplankton grazing is expected to reduce mostly larger sized phytoplankton (~20µm), with *calanoid copepods* having a stronger mass-specific effect than *Daphnia*. Results highlight the DOC quality's roles, nutrient and light limitation, microbial grazing, and meso-zooplankton traits as key regulators of phytoplankton community dynamics and plankton trophic interactions in a changing world.

Keywords: Phytoplankton, Food Web, Selectivity, DOC, Leaf-leachate

ÖZ

ÇÖZÜNMÜŞ ORGANİK KARBONUN VE ZOOPLANKTON OTLAMASININ FİTOPLANKTON KOMÜNİTELERİ ÜZERİNDEKİ ETKİLERİ

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Küresel ısınmasının tatlı su ekosistemleri üzerinde büyük etkileri olmasına rağmen, bu etkilerin besin zinciri üzerindeki etkileri tam olarak anlaşılamamıştır. İklim değişikliğinin önemli bir etkisi, heterotrofik plankton için bir enerji kaynağı olarak kullanılabilen, besin zinciri dengelerini değiştiren ve ışık sınırlamasına sebep olabilen çözünmüş allokton organik karbonun (DOC) sucul ekosistemlere girdisini arttırmasıdır. Laboratuvar ve mezokozom deneylerindeki amacımız; plankton ekosistemindeki örüntüler ve süreçler arasında ilişkileri, DOC'nin aşağıdan yukarıya etkilerini, zooplanktonun otlatma seçiciliğinin (*Daphnia* ve kalanoid kopepod) fitoplankton biyokütlesi üzerindeki yukarıdan aşağıya etkilerini karşılaştırarak açıklamaktır. Deneyler doğrultusunda; DOC ve zooplankton otlamasının fitoplankton biyokütlesini azaltması, *Daphnia* türlerinin kopepod türlerinden daha etkili herbivorlar olması ve büyük boyutlu fitoplankton türlerinin daha çok tüketilmesini beklenmiştir. Besinin yeterli miktarda bulunduğu laboratuvar deneyinin sonuçları, DOC'nin toplam fitoplankton biyokütlesini azalttığını ve özellikle küçük fitoplankton türlerinin biyokütlesinin azaldığını göstermiştir.

Zooplanktonlar daha küçük boyutlu fitoplanktonlarda artış ve daha büyük boyutlu fitoplanktonların biyokütlesinde azalma gibi türlere bağlı olarak etkiler gösterse de, toplam fitoplankton biyokütlesini azaltmıştır. Besinin sınırlı olduğu mezokozm deneyleri ise DOC'nin toplam fitoplankton biyokütlesini arttırdığını fakat DOC kaynaklarının etkisinin zamana ve fitoplankton türüne bağlı olarak değiştiği gözlenmiştir. Sonuçlarımız farklı DOC kaynaklarının fitoplankton üzerinde farklı etkiler yarattığı göstermiştir. Bu nedenle, DOC, yeterli besin bulunan koşullarda fitoplankton biyokütlesini azaltabilmekte ve sınırlı besin bulunan koşullarda fitoplankton biyokütlesini arttırabilmektedir. Mezozooplankton otlamasının, çoğunlukla daha büyük boyutlu fitoplanktonları (~20 µm) azaltması beklenirken, kalanoid kopepodların *Daphnia'dan* daha seçici otlayıcı olduğu gözlenmiştir. Sonuçlar, DOC kalitesinin, besin ve ışık sınırlamasının, mikrobiyal otlatmanın ve mesozooplankton özelliklerinin; fitoplankton komünitelerini ve planktonların trofik etkileşimlerini düzenlemede kilit rolü olduğunu vurgulamaktadır.

Anahtar Kelimeler: Fitoplankton, Besin Zinciri, Seçici Beslenme, Çözünmüş Organik Karbon, Yaprak Süzüntüsü

To My Parents, Ayşe and Mustafa Metin

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CHAPTER 1

INTRODUCTION

Freshwater ecosystems are connected to surrounding watersheds via flooding events from terrestrial ecosystems. Growing evidence shows that dissolved organic matter export from terrestrial ecosystems is increasing over the last decades (Cole et al., 2007; Battin et al., 2008; Tranvik et al., 2009; Lennon et. al., 2013). This increasing trend is associated with global changes including change in precipitation pattern (Parn & Mander, 2012; Lennon et al., 2013). Thus, through climate change, increased precipitation promotes flooding events all over the world. Lots of nutrient and particle flow to the lakes via floods, which are called as allochthonous dissolved organic matters as they come from outside the lake (Carpenter et al., 2005). One type of the allochthonous matter is dissolved organic carbon (i.e., DOC), is important component of aquatic ecosystems (Steinberg et al., 2008) and the global carbon cycle by providing carbon source for lake ecosystem. Despite this growing DOC issue, there are considerable uncertainties about how freshwater ecosystems, and especially how freshwater phytoplankton communities respond to increasing DOC input depending on phytoplankton species, DOC source quality and quantity (Hanson et al., 2011; Lennon et al.,2013).

DOC has various physical, chemical, and biological impacts on phytoplankton community (Lennon et al., 2013). First, DOC leads to brownification by increasing yellow-brown colour in lake (Granéli 2012, Hansson et al. 2013, Solomon et al. 2015; Lebret et al., 2018). This process decreases light transparency of water and

create light limitation, in other words, brownification promotes shade-tolerant phytoplankton species which are tolerant to low light availability (Granéli 2012, Hansson et al. 2013, Solomon et al. 2015; Urrutia-Cordero et al. 2017; Lebret et al., 2018). Moreover, DOC-rich runoff can provide nutrients to phytoplankton such as nitrogen and phosphorus in addition to carbon, resulting in a trade-off between light limitation and nutrient enrichment effects of DOC (Seekell et al., 2015). Even DOC has complex strong effects on phytoplankton community, research about DOC effects and effects' trade-off on phytoplankton community are scarce.

DOC can promote mixotrophic species in phytoplankton community since it supports bacterial biomass by providing carbon (Sanders 1991; Urrutia-Cordero et al. 2017). Mixotrophic species obtain nutrients by consuming bacteria (phagotrophy) or dissolved organic carbon while also having ability for photosynthesis, in other words, mixotrophic species can act as both autotrophs and heterotrophs (Hammer et al 2005). Thus, mixotrophic phytoplankton are photosynthetic organisms with the facultative ability to supplement their nutrition through the uptake of organic compounds (Riemann et al. 1995; Reynolds 2006; Lee 2008; Bottino et al. 2018). They can grow in the dark using organic compounds (heterotrophy). Together with nutrient enhancement, DOC gives competitive advantage to mixotrophic species as they are less sensitive to light limitation compared to obligate autotrophs (Bottino et al. 2018). Hence, in presence of DOC, mixotrophic species are expected to have an advantage and the phytoplankton community shifts to more mixotrophic species dominant (Znachor 2010).

Phytoplankton are the major primary producers in pelagic aquatic ecosystems, and they have a fundamental role in the flow of matter and energy through food webs (Shurin et al., 2006). There are two main energy transfer pathways in aquatic ecosystems depending on the basal producer, which may be either autotrophic phytoplankton, or heterotrophic bacteria (Azam et al., 1983; Legendre &

Rassoulzadegan, 1995, Jansson et al., 2007; Degerman et al., 2018). In general, types of paths regulate the energy transfer through food web. In autotrophic-based pathway, phytoplankton (as basal producer uptake inorganic compounds and make photosynthesis) are directly consumed by primary consumers like mesozooplankton, and subsequently to upper trophic levels such as fish (Brönmark & Hansson, 1998-2005). In contrast, in heterotrophic-based pathways (i.e., microbial loop), bacteria as basal producer uptake dissolved organic matters and nutrients and then they are consumed by protozoan micro-zooplankton (e.g., heterotrophic nanoflagellate (HNF) and ciliates) before reaching mesozooplankton and subsequent higher trophic levels such as fish (Hessen & Andersen, 1990; Brett et al., 2009; Degerman et al., 2018). Bacteria, phytoplankton, and ciliate interactions may also create cascades among the two main trophic pathways (autotrophic vs. heterotrophic basal production). For example, ciliates can consume on small phytoplankton (i.e., top-down) (Lischke et al., 2016) and mixotrophic phytoplankton can consume on bacteria (Hammer et al 2005). Moreover, phytoplankton and bacteria could compete for inorganic nutrient (i.e., bottom-up) (Azam et al., 1983). Besides, energy released from phytoplankton as dissolved organic matter return to main food chain by microbial loop since bacteria are the re-mineralizers, as bacteria recycle nutrients from organic to inorganic forms (Azam et al., 1983). Thus, while there are strong trophic links between bacteria, phytoplankton, and ciliates (Es & Meyer-Reil, 1982; Azam et al., 1983), our knowledge on the effects of increased DOC inputs on these trophic dynamics of freshwater phytoplankton biomass and species composition is scarce.

Phytoplankton growth is jointly controlled by nutrient availability, such as in resource-controlled bottom-up models, (Carpenter et al., 1985; Carpenter & Kitchell, 1993; Hehman et al., 2001), as well as by zooplankton grazers, such as predator-controlled top-down models that focus on the influence of predators on the structure and dynamics of prey species (Currie et al., 1999; Hehman et al., 2001). However, there is less research about the combined effect of (interplay between) both models

in general, and especially for understanding the effects of increased DOC inputs in light of different zooplankton communities. Research has generally focused on the effect of DOC on total phytoplankton or chlorophyll-a concentration (Carpenter et al., 1998; Stets and Cotner 2008). Hence, the combined effects of DOC (bottom-up) and zooplankton grazing (top-down) on phytoplankton communities is unknown.

In addition to DOC, mesozooplankton grazers are key regulators of phytoplankton biomass and composition. Cladocera (e.g., *Daphnia*) and Copepoda (e.g., copepod) taxa are the major component of freshwater mesozooplankton, and they create grazing pressure on phytoplankton depend on their grazing traits (Sommer et al., 2001). For example, copepods are selective feeders, they have an ability to individually handle and ingest prey particles according to size, taste or nutrient content (i.e., cellular chemical composition) (Cowles et al., 1988). Copepods prefer relatively larger (optimal prey to predator ratio is 1:18) (Frost 1972; Hanson et al., 1994) and nitrogen rich prey (Cowles et al., 1988) such as large phytoplankton species or ciliates. On the other hand, *Daphnia* is generalist feeder since they do not have a capability of choosing individual prey particles while ingesting (Brönmark and Hansson 1998, 2005). They can only completely inhibit eating /filtering in case of presence of only toxic prey condition (Lampert 1981). Even, *Daphnia* consumption range is large (filtered particle size for small Daphnia 1-24 μm and for large *Daphnia* 1-47μm), research shows that predator to optimal prey ratio of *Daphnia* is ~50:1 (Reynolds 1984; Brönmark and Hansson 1998,2005). Indicating that *Daphnia* create strong grazing pressure on small phytoplankton species compared to relatively large ones (Hanson et al., 1994,). Thus, copepod dominant communities are expected to result in phytoplankton communities dominated by smaller cells, while *Daphnia* dominant communities are expected to result in phytoplankton communities dominated by larger cells (Sommer et al 2001; Stibor et al., 2004). Besides, literature shows that link between copepod and phytoplankton is weaker compared to link between *Daphnia* and phytoplankton, in other words, *Daphnia* is a more efficient herbivore compared to copepods, which have stronger

links to ciliates (Lampert 1978, 1988; Sommer et al. 1986). However, the top-down effect of zooplankton with contrasting grazing traits on phytoplankton community is rarely studied (Ger et al. 2019).

In addition to such food web interactions, DOC effects also depend on its biochemical quality for bacteria and algal uptake (Cole et al., 2011; Kothawala et al., 2014; Tanentzap et al., 2017; Stadler et al., 2020). Indeed, the effects of DOC are regulated by both its quantity and quality. DOC can enter the food web via bacterial or phytoplankton uptake on its path to the higher trophic levels (Thurman,1985; Degerman et al., 2018). Allochthonous DOC is derived from surrounding terrestrial ecosystem and generally originating from plant tissue which includes cellulose and lignin compounds (Thurman, 1985). However, there are different types of DOC quality sources depend on their bioavailability (i.e., degradability). Depending on biochemistry, high bioavailable sources are considered as high-quality or labile while low bioavailable ones are considered as low-quality or recalcitrant. For example, DOC could be obtained from leaf leachates which contain more labile carbon and other nutrients (e.g., N and P), so it provides relatively high-quality, easily degradable, carbon source together with supportive nutrients to consumers (Sondergaard & Middelboe, 1995). On the contrary, DOC could be obtained via humic substances such as commercially available HuminFeed® (HuminTech GmbH, Grevenbroich, Germany) which is produced by oxidation of lignite, contains leonardite, a soft waxy, dark, mineral-like substance (Meinelt et al., 2007). It consists of low-quality (i.e., difficult to degrade) carbon for consumers (Moran & Hodson 1990; Tranvik, 1988), so HuminFeed® could be an example for recalcitrant DOC source (Lennon et al., 2013). Besides, HuminFeed® has a strong ability to absorb light due to its high chromophoric properties, which directly affect the light availability through the water column (Williamson et al., 2015; Minguez et al., 2020). Hence in terms of phytoplankton community, labile DOC sources could be more usable compared to recalcitrant DOC sources (Guillemette et al., 2016). However, to our knowledge, despite the importance of both DOC and zooplankton,

there is no study on how the quality of DOC (labile vs. recalcitrant) and zooplankton (generalist vs. selective grazing) jointly regulate phytoplankton communities.

Thus, we aimed to test the combined effects of DOC and zooplankton grazing traits on the biomass and community composition of phytoplankton in laboratory and *in-situ* grazing assays. In the laboratory experiment, we compared the bottom-up effect of DOC (i.e., mixed labile and recalcitrant sources) with the top down effect of either generalist or selectively grazing zooplankton. We hypothesized that DOC addition would 1) decrease the phytoplankton community biomass and effect size magnitude would be species specific; 2) increase the relative proportion of mixotrophic species; and that 3) zooplankton grazing would decrease total phytoplankton biomass; and 4) generalist grazing Daphnia would reduce phytoplankton more than selectively grazing copepods. For the in-situ mesocosm grazing assays, we compared the effects of DOC quality (i.e., labile, recalcitrant, mixed) and mesozooplankton grazing pressure on the phytoplankton community. We hypothesized that 1) DOC addition would decrease phytoplankton species biomass compared to control, 2) recalcitrant DOC would reduce phytoplankton biomass more than the labile source, 3) the magnitude of the DOC effect on phytoplankton would be phylum specific, 4) mesozooplankton grazing would decrease phytoplankton biomass and the effect magnitude from grazers will be phylum specific.

CHAPTER 2

MATERIAL AND METHOD

2.1 Experimental Design

The two different experiments were designed to study DOC and contrasting grazing effects on phytoplankton community. First experiment conducted in climate room conditions in Middle East Technical University (METU), Biological Sciences Department, Ankara, Turkey (39° 53' 28.99" N, 32° 47' 4.99" E). Secondly, *in-situ* mesocosm assays were designed parallel to long-term (36-days) mesocosm experiment in METU Mesocosm Systems which located in METU experimental lakes in Ankara, Turkey (998 m above sea level; 39° 52'13.18 "N, 32° 46'31.92 "E). The long-term mesocosm experiment was conducted mainly to understand the effects of different DOC sources on freshwater ecosystem. The system contained 16 mesocosm tanks (1.2 m height x 2.2 m width, volume 2480 L) on a floating platform and set-up included control (C), Humin Feed/ recalcitrant DOC (R, representing ~1.5 mg C L^{-1}), leaf leachate DOC (L, representing ~8 mg C L^{-1}, obtained from alder tree leaves), and a combination of recalcitrant and leaf leachate DOC (mixed, representing ~9.5 mg C L^{-1}) as treatments design with 4 replicates (Calderó-Pascual et al., 2021).

2.1.1 Laboratory Grazing Experiment

A laboratory experiment was conducted in the Department of Biological Sciences at Middle East Technical University (METU), Ankara, Turkey (39° 53' 28.99" N, 32° 47' 4.99" E) in order to quantify and distinguish the top-down effect of zooplankton with contrasting grazing traits (i.e., Daphnia vs. calanoid copepods) from the bottom-up effects of DOC on phytoplankton biomass, composition, and diversity. Hence, the experiment had a zooplankton and DOC treatment in a 3 x 2 factorial design (i.e., zooplankton: Daphnia or copepod or no grazer ; DOC: +DOC or –DOC), with 4 replicates each, and was carried out through 4 days from 17th to 21st of June 2019 (Table 2.1).

Table 2.1 Experimental design and labels of laboratory experiment (+ represented the addition of DOC and – represented the absence of DOC). Laboratory experiment was conducted through 4 days in 0.6 L glass jars with 4 replicates, which resulted in total 24 experimental units.

Treatment	No Grazer (grazer control)	*Daphnia*	Copepod
No DOC (no DOC control)	$-DOC_{No\ grazer}$	$-DOC_{Daphnia}$	$-DOC_{Copepod}$
DOC	$+DOC_{No\ grazer}$	$+DOC_{Daphnia}$	$+DOC_{Copepod}$

2.1.1.1 Obtaining Phytoplankton, Zooplankton, and DOC Sources for Grazing Experiments

Two different phytoplankton cultures were obtained from Norwegian Institute for Water Research (Norway, NIVA) culture collection to maintain zooplankton cultures and provide controlled prey in the laboratory experiment. The mixotrophic *Cryptomonas pyrenoidifera* (spheroid, mean cell dimensions 18.4 x 11.1 μm) and the autotroph *Chlamydomonas reinhardtii* (spheroid, mean cell dimensions 7.7 x 6.8 μm) species were grown in Wright's Cryptophyte (WC) medium, maintained in the

exponential 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 in a climate-controlled room (Figure 2.1).

Figure 2.1 Exponentially growing phytoplankton cultures used in the grazing experiment.

Two different zooplankton functional groups were selected for the laboratory experiment: filter-feeding, generalist cladocerans or current feeding, selective calanoid copepods. To obtain zooplankton cultures, two species of calanoid copepods and one species of Cladoceran were collected from lakes nearby Ankara. The calanoid copepod species used in the laboratory grazing experiment were *Acanthodiaptomus denticornis* (mean body length 1.39 ± 0.36 CI µm, n=22) from Lake Yeniçağa and *Arctodiaptomus bacilliferus* (mean body length 1.14 ± 0.32 CI µm, n=22) from Lake Mogan. The Cladoceran species was *Daphnia magna* (2-3 mm) also from Lake Mogan. These were the dominant zooplankton species at the time of collection and were from the same samples used to inoculate the main long-term mesocosm experiment. The GF/C filtered lake water was used as a medium for zooplankton cultures, fed with an equivalent of 0.5 mg C L^{-1} of a 1:1 (by biomass) mixture of the cultured phytoplankton species described above every three days. Zooplankton were starved for 24 hours before the grazing experiment to minimize potential differences in prey ingestion due to variable gut fullness.

The DOC effect was tested by adding a mixture of two different DOC sources with contrasting bioavailability for organism uptake. This ensured that DOC effects due to both uptake and light limitation would be accounted for. The labile DOC source was extracted from dry leaves of the white poplar tree (*Populus alba*) in distilled water (60 g L^{-1}), incubated at 4 °C in the dark for 72 hours, and subsequently filtered through a 45 μm mesh. This resulted in a solution of 342 mg C L^{-1} labile DOC (measured by Shimadzu TOC-L/CPN analyzer). The recalcitrant DOC solution was obtained from the commercially available HuminFeed® (HuminTech GmbH, Grevenbroich, Germany) and prepared by making a 1 g L^{-1} stock solution of HuminFeed®. Both solutions were then added to the grazing assay in the +DOC treatments (see below).

2.1.1.2 Experimental Setup

The suspensions with phytoplankton and contrasting DOC were prepared in two separate 10L buckets with or without DOC (i.e., +DOC and -DOC) such that the only difference among them was DOC. Buckets were filled with 45 μm filtered mesocosm water, which obtained by filtering METU experimental lake water through a 500 μm mesh. Buckets were spiked with nutrients (i.e., final concentration equivalent to Wright's Cryptophyte medium, Guillard et al., 1972), and subsequently bubbled with air for 30 minutes to homogenize contents and ensure sufficient oxygen before the start of the grazing assay (Figure 2). Nutrients were added to minimize any differences in nutrient limitation (due to potential nutrient addition with the labile DOC source) during the grazing assay. This ensures that observed phytoplankton responses were not driven by differences in nutrient limitation during the experiment. Before adding the nutrients, the +DOC bucket was spiked with 114.3 mL of the previously prepared labile DOC solution (which added a final concentration of 3.91 mg C L^{-1} DOC) and with 70 mL of the previously prepared 1 g L^{-1} stock solution of the recalcitrant DOC solution (which added a final concentration of 7 mg C L^{-1} DOC). To make comparison between laboratory and in-

situ mesocosm grazing assays, DOC concentrations were chosen close to long-term mesocosm experiment. Together, the total estimated DOC concentration at the start of the experiment was therefore 10.91 mg C L^{-1}. Following the nutrient addition, 62.9 mL of the *Cryptomonas pyrenoidifera* culture and 58.9 mL of *the Chlamydomonas reinhardtii* culture were added to both buckets to ensure a minimum of 0.5 mg C L^{-1} total phytoplankton concentration (~0.25 mg C L^{-1} of each species). The carbon equivalent biomass of cultured phytoplankton was determined by converting cell density to biovolume and subsequently applying the formula pgC $cell^{-1} = 0.1204\ (\mu m^3)^{1.051}$ (Rocha and Duncan, 1985).

Figure 2.2 The - DOC (left) and + DOC (right) buckets at the beginning of the experimental setup.

After homogenizing phytoplankton suspensions in the +DOC and -DOC buckets (via gentle mixing by a clean 1 L beaker), previously sterilized 0.6 L glass jars were filled with 0.55 L of either the +DOC (n = 12) or -DOC (n = 12) suspensions, resulting in 24 experimental units. Finally, an equal biomass of either *Daphnia* (n = 2 per jar) or calanoid copepods (n = 30, equal mix of the two copepod species above) were added to the jars designed as the grazer treatments, while the jars designed as no-grazer controls did not receive any zooplankton (Table 2.1). An equal grazer biomass enables a quantitative comparison of the mass-specific grazing effect between *Daphnia* vs. copepods on phytoplankton during the experiment (see below) (Ger et al., 2019). Grazing pressure was quantified by comparing the final prey biomass

concentration among jars that contained zooplankton (Daphnia or copepods) with jars that did not contain zooplankton (grazer controls).

The grazing experiment started once the zooplankton were added and lasted for four days which is the typical time necessary to observe the full effects of DOC addition on plankton food webs (Carrick et al., 1991; Burns & Schallenberg, 2001). The jars were maintained under identical conditions to the phytoplankton cultures (see above) and each jar was gently bubbled (~5 bubbles sec^{-1}) to keep phytoplankton prey in suspension and provide oxygen to zooplankton throughout the experiment. Samples for phytoplankton were taken at the start of the experiment (day 0), in the middle (day 2), and at the end (day 4). Sampling details are provided below. To maintain a comparable zooplankton biomass among treatments throughout the 4-days experiment, jars were checked twice a day for mortality and appearance of neonates, which were removed as soon as noticed. Given the small size of copepods, it was not possible to observe nauplii. Any dead zooplankton were removed and replaced as soon as noticed. At the end of the experiment (day 4), the zooplankton in each jar were filtered on a 100 μm mesh, transferred to a petri dish, checked for mortality (via motility and heartbeat under a dissecting microscope), rinsed with distilled water, placed in previously weighed tin capsules, dried at 60°C for 24 hours, and weighed to measure the final grazer biomass as dry weight. The mean biomass of copepods was 0.75 mg ± 0.10 CI per jar and the biomass of *Daphnia* was 0.88 mg ± 0.17 CI per jar.

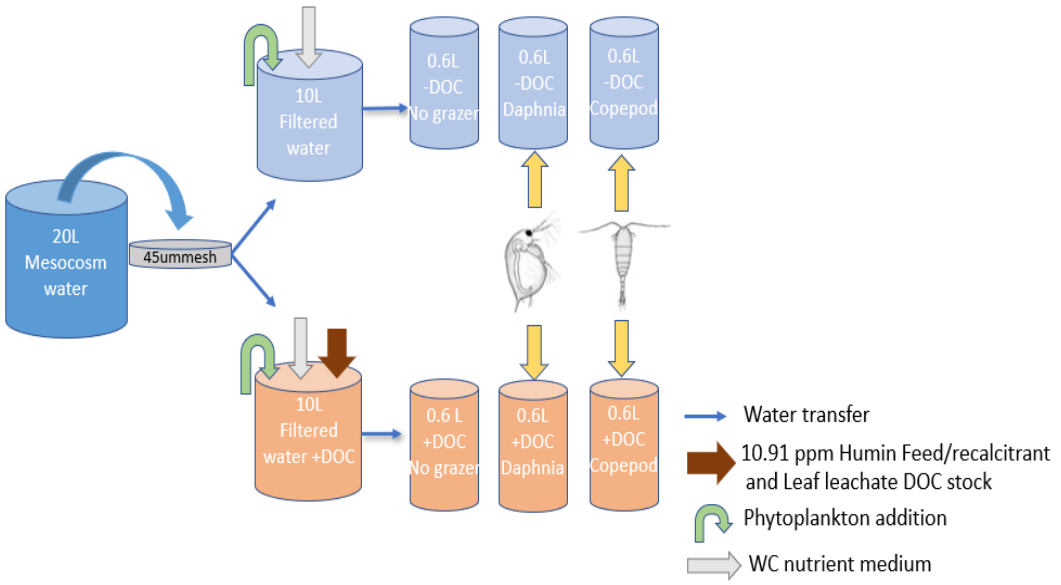


Figure 2.3 Preparation and experimental design of the laboratory grazing assay. The steps until the 0.6 L jars show preparation, while the experiment took place in the 0.6L, with four replicates for each treatment (i.e., + or – DOC crossed with no grazer control "-Z", *Daphnia*, and copepod).

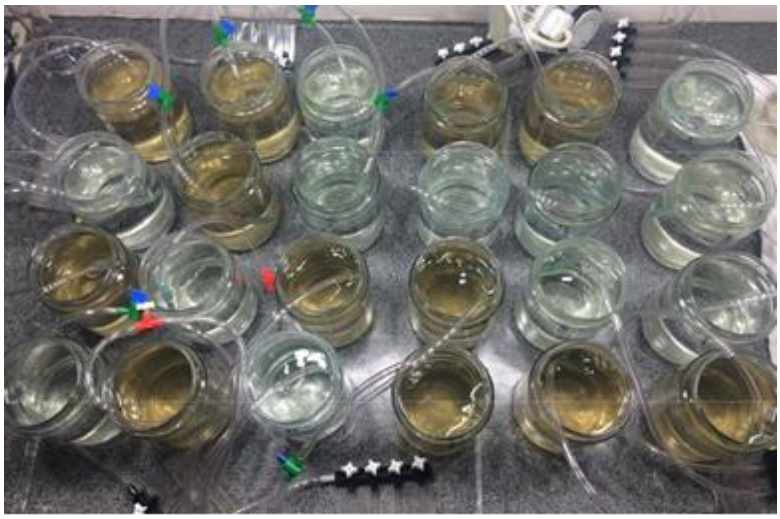


Figure 2.4 The laboratory experiment at the beginning of the experiment. Darker colored jars are those spiked with the DOC (i.e., the +DOC treatment).

2.1.2 *In-situ* Mesocosm Grazing Assays

24-h short-term grazing assays were designed to compare the top-down effect of the in-situ mesozooplankton community on phytoplankton biomass, composition, and diversity in each mesocosm tank during the long-term mesocosm experiment. The goal here was to compare how the *in-situ* grazer effects varied across mesocosm tanks that received different DOC sources (Figure 2.5). Grazing assays took place in paired 0.5 L opaque white plastic bottles placed in the surface layer of the mesocosm tanks. Grazing pressure was quantified by comparing the final prey biomass concentration among bottles that contained mesozooplankton, hereafter +Z, with bottles that did not contain zooplankton (grazer controls), hereafter -Z (Table 2.2). Hence, for a given assay, each mesocosm tank had two bottles (+Z and –Z). This was crossed with the four different DOC treatments of the main long-term mesocosm experiment, each with four replicates (Table 2.2), such that there was a total of 32 bottles in the 16 mesocosm tanks (Figure 2.5). The in-situ grazing assays were conducted one day (i.e., 21.06.20, first in-situ grazing assay) and four days (i.e., 24.06.20, second in-situ grazing assay) after the DOC pulse was added to main mesocosm tanks. This was done to quantify potential temporal changes in the top-down grazer effect following the DOC pulse.

2.1.2.1 Experimental Setup

Through the main long-term mesocosm experiment, the mesocosm tanks were sampled three times from surface to bottom by tube sampler then three samples were combined into 20 L buckets, which resulted in depth-integrated mixed mesocosm water for each tank in their specific buckets (Figure 2.6). For each assay, mesocosm water and plankton from each tank was obtained from mixed mesocosm water. From this, mesozooplankton was isolated by gently filtering 5 L of mixed mesocosm water

through a 200 μm mesh. The filtrate containing seston and microzooplankton (<200 μm) was used to fill the two 0.5L grazing bottles. Subsequently, the concentrated mesozooplankton from each mesocosm tank was transferred to one of the paired 0.5 L assay bottles designated as +Z, while the –Z bottle received no addition of mesozooplankton (Figure 2.5). Both bottles were topped off with the 200μm filtered mesocosm water and placed back into the respective tank. Thus, each mesocosm tank received two white plastic bottles (with and without mesozooplankton grazers) (Figure 2.5). After 24 hours, bottles were brought back to the laboratory in a cool box (<20 minutes transport time), and contents (i.e., phytoplankton, zooplankton) were preserved for further analysis.

Table 2.2 Experimental design and labels of both (one and four days after DOC pulse) in-situ mesocosm grazing assays with 4 replicates (C: no DOC Control, R: Recalcitrant DOC, L: Leaf leachate DOC, Mixed: Combined recalcitrant and Leaf leachate DOC source; treatments with grazers are noted with the subscript +Z, while no-grazer treatments are shown by '-Z').

	No Mesozooplankton	+ Mesozooplankton
No DOC Control	C_{-Z}	C_{+Z}
Leaf Leachate DOC	L_{-Z}	L_{+Z}
Humin Feed/Recalcitrant DOC	R_{-Z}	R_{+Z}
Leaf Leachate + Recalcitrant DOC (Mixed DOC)	Mixed $_{-Z}$	Mixed $_{+Z}$

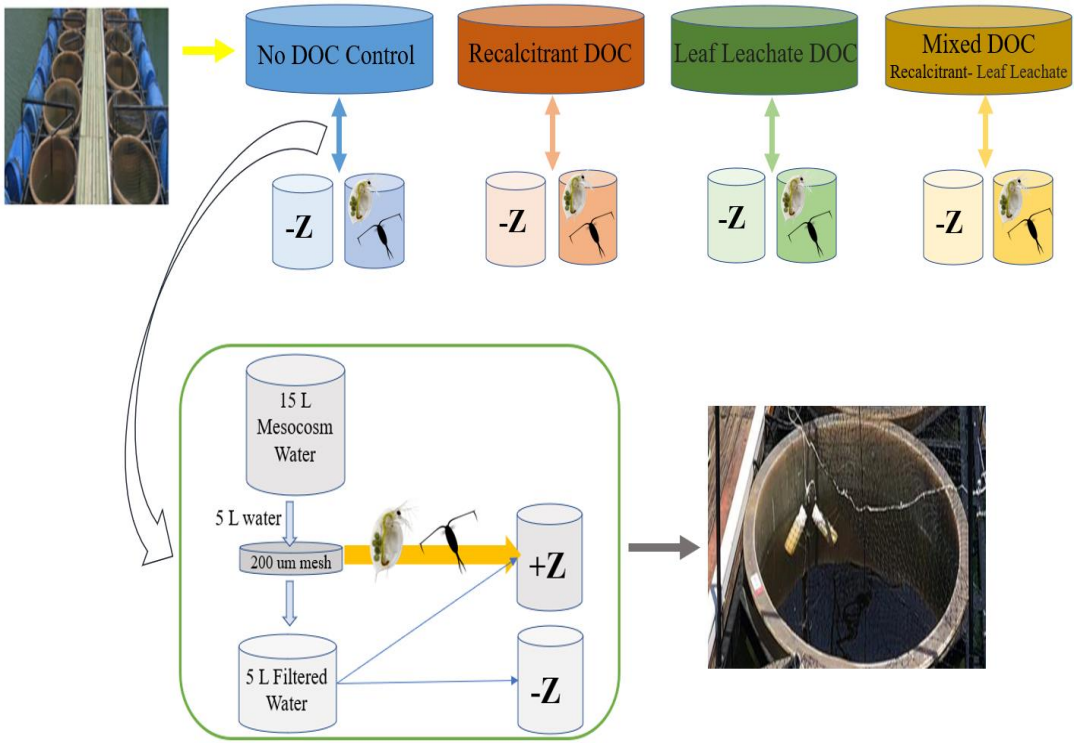


Figure 2.5 Experimental design of in-situ mesocosm experiments. The two 0.5 L white opaque bottles placed in mesocosm tanks throughout the in-situ mesocosm experiments (right).

Figure 2.6 Surface to bottom tube sampling process to obtain depth-integrated mixed mesocosm water from each tank.

2.2 Sampling and Analysis

In the laboratory experiment, phytoplankton was sampled at the beginning of the experiment (17.06.19, day 0) before adding zooplankton into the jars, in the middle (19.06.19, day 2), and at the end of the experiment (21.06.19, day 4). A total of 50 mL of the contents of each jar was sampled (via 10 mL pipettes with sterile tips), taking care to avoid zooplankton. In the mesocosm experiments, sampling was performed 24 hours after assay bottles were placed in main tanks. After filtering out mesozooplankton with a 200 µm mesh, 50mL of the mixed bottle contents was sampled for phytoplankton. Phytoplankton samples were placed into dark glass bottles previously filled with 1 mL acidic LUGOL's iodine solution, 2% final concentration (Sigma Aldrich) and stored in dark at room temperature until further analysis. Besides, mesozooplankton samples were fixed with acidic LUGOL's

iodine solution, 4% final concentration (Sigma Aldrich), and stored in dark bottles at room temperature.

Lugol preserved phytoplankton samples were counted via light microscopy, following the previously developed WISER project protocol (EU FP7, No.226273). Through the counting procedure, samples were placed in a sedimentation chamber. After sedimentation, at least 50 microscope fields were counted under 400X magnification of an inverted microscope (Leica DMI 4000B, Wetzlar, Germany), and photos of at least ten individuals of each species were taken with a digital camera (Leica DFC280, Wetzlar, Germany). The genus (or species) of observed phytoplankton taxa were identified to the lowest taxonomic degree. For each phytoplankton species, at least ten individuals' size were measured using the Leica image analysis program to calculate the biovolume and biomass of species. Biovolume ($\mu m^3 L^{-1}$) of species were calculated according to the geometric shape of species and converted to biomass ($\mu g L^{-1}$).

Zooplankton counting was performed by using Leica M125 (Wetzlar, Germany), stereomicroscope under 10x magnification and at most 25 individuals' mean body size were measured. The genus or species level identifications was performed (Scourfield & Harding, 1966; Harding & Smith, 1974). To calculate zooplankton's dry weight, the allometric relationship between weight and body size was used in order to calculate the zooplankton's dry weight (Dumont et al. 1975; Bottrell et al. 1976; Ruttner-Kolisko 1977; McCauley 1984). Mesozooplankton data obtained from long-term mesocosm sampling and as we used 200µm mesh during our in-situ mesocosm assays, only individuals' size larger than or equal to 200µm were considered. Thus, mesozooplankton data of in-situ mesocosm grazing assays were estimated from main long-term mesocosm experiment.

2.3 Statistical Analysis

The effect of zooplankton and DOC treatments on phytoplankton biomass (total and taxa specific) and diversity was evaluated by comparing differences relative to the respective controls (i.e., no-grazer or –DOC) either via Generalized Linear Models (GLM) or via effect size (log response ratios). Zooplankton type and presence, as well as DOC type and presence were independent variables in additive GLMs (e.g., biomass ~ zooplankton + DOC). As some data did not meet the normality and homogeneity of variance assumptions of ANOVA, GLM was preferred. All models were log(x+1) transformed to attain normally distributed data and homogeneous variance, which was controlled by Shapiro-Wilk and Levene's tests, respectively, as well as diagnostic plots. However, zooplankton biomass data from in-situ mesocosm assays, was not log(x+1) transformed as they were normally distributed. Students t-tests further evaluated pairwise differences among specific treatments (in between different samplings as well). 95% confidence level of the effect size calculations and p-values of statistical tests were used to show statistical significance. R Version 1.3.959 software was used for statistical analysis (R Core Team, 2020).

Treatment effect size values were calculated using log response ratios (ln R) by dividing the biomass of phytoplankton in the treatment with the respective control (Hillebrand & Gurevitch, 2016). This enables a quantitative comparison of treatment effects (i.e., DOC or zooplankton type) on selected response variables (i.e., total and taxa specific phytoplankton biomass) across the same scale. The calculations used values from the end (day 4) of the laboratory experiment (Table 2.3). The DOC and grazing effect size values were calculated by using formulas in tables 3 and 4 for laboratory and in-situ mesocosm assays, respectively. The confidence intervals of effect sizes were calculated to determine significance of treatment effects because effect size values that overlap with a zero value are not significant (Stibor et al., 2004).

Hence, for the lab experiment, the ‘treatment’ for calculating the DOC effect was the phytoplankton biomass in the jars that received DOC (i.e., +DOC), while the ‘control’ for calculating the DOC effect was the phytoplankton biomass in the jars that did not receive DOC (i.e., -DOC). Similarly, the ‘treatment’ for calculating the grazer effect was the phytoplankton biomass in the jars that received a zooplankton grazer (i.e., either *Daphnia* or copepod), while the ‘control’ for calculating the grazer effect was the phytoplankton biomass in the jars without any grazers (i.e., no-grazer). The designation of biomass values to calculate the effect size for the mesocosm grazing assays was similar (Table 2.4), such that the ‘treatment’ for calculating the DOC effect was the phytoplankton 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 was the phytoplankton biomass in the bottles that were in the mesocosm tanks that did not receive any DOC (i.e., -DOC). Similarly, the ‘treatment’ for grazer effect size was the phytoplankton biomass in the bottles that received the in-situ zooplankton grazer community, while the ‘control’ for calculating the grazer effect was the phytoplankton biomass in the jars without any grazers (i.e., no-grazer).

Table 2.3 The log ratio formula (ln R) used to calculate the effect size of either DOC or grazers on phytoplankton biomass in the laboratory experiment. The two effect size categories (i.e., DOC and Grazer) were calculated for specific treatments as shown below. Values in the formula represent the phytoplankton biomass within that given treatment at the end of the experiment (day4).

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

Table 2.4 The log ratio formula (ln R) used to calculate the effect size of either DOC or grazers on phytoplankton biomass in the 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 phytoplankton biomass within that given treatment at the end of the one-day grazing assay (see methods for details). Treatments with grazers are noted with the subscript +Z, while no-grazer treatments are shown by '-Z'. DOC treatments: R for recalcitrant, L for leaf leachate and Mixed for both recalcitrant and leaf leachate DOC sources.

	Treatment	ln R
DOC Effect Size	R with mesozooplankton(R_{+Z})	$\ln(R_{+Z}/C_{+Z})$
	R without mesozooplankton(R_{-Z})	$\ln(R_{-Z}/C_{-Z})$
	L with mesozooplankton(L_{+Z})	$\ln(L_{+Z}/C_{+Z})$
	L without mesozooplankton(L_{-Z})	$\ln(L_{-Z}/C_{-Z})$
	Mixed with mesozooplankton ($Mixed_{+Z}$)	$\ln(Mixed_{+Z}/C_{+Z})$
	Mixed without mesozooplankton ($Mixed_{-Z}$)	$\ln(Mixed_{-Z}/C_{-Z})$
Grazing Effect Size	DOC - (C)	$\ln(C_{+Z}/C_{-Z})$
	Recalcitrant DOC (R)	$\ln(R_{+Z}/R_{-Z})$
	Leaf Leachate DOC (L)	$\ln(L_{+Z}/L_{-Z})$
	Recalcitrant + Leaf Leachate DOC (Mixed)	$\ln(Mixed_{+Z}/Mixed_{-Z})$

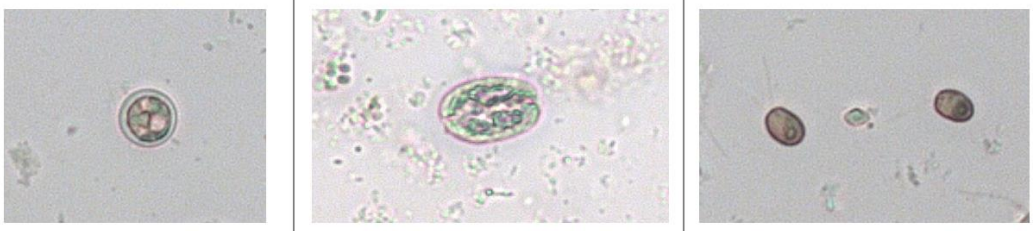
CHAPTER 3

RESULTS

3.1 Laboratory Grazing Experiment

3.1.1 Dominant Phytoplankton Taxa

The dominant species encountered in the lake water used in the laboratory experiment were the diatom *Cyclotella* spp., which, in addition to the two species added from laboratory cultures (i.e., *Cryptomonas pyrenoidifera* and *Chlamydomonas reinhardtii*) together comprised for more than 99% of the total phytoplankton biomass. In addition to these three taxa, the chlorophytes *Crucigenia tetrapedia*, and *Tetraëdron minimum* were also found, though at biomass <%1 of total. Examples of the phytoplankton species or taxa are depicted in Figure 3.1. At the beginning of the experiment (i.e., day 0), overall, 22% of total biomass comprised by *Cryptomonas*, 38% by *Cyclotella* and 40% by *Chlamydomonas*, while these values shifted to 47%, 37% and 12%, respectively, at the end of the experiment (i.e., day 4).



Cyclotella spp. | *Cryptomonas pyrenoidifera* | *Chlamydomonas reinhardtii*

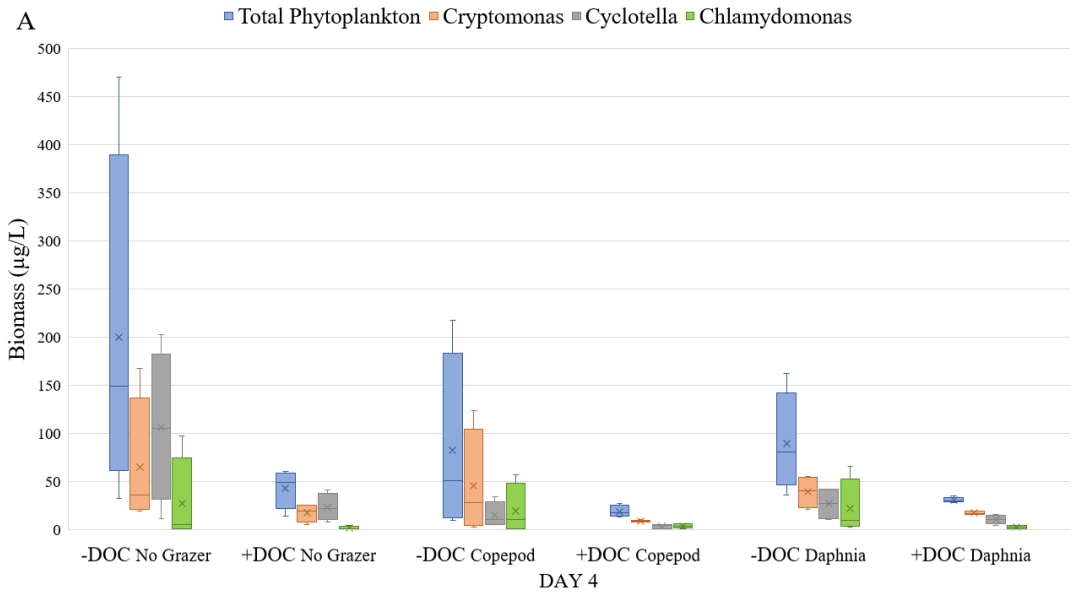
Figure 3.1 Examples of the dominant phytoplankton species observed in the 4 days long laboratory experiment.

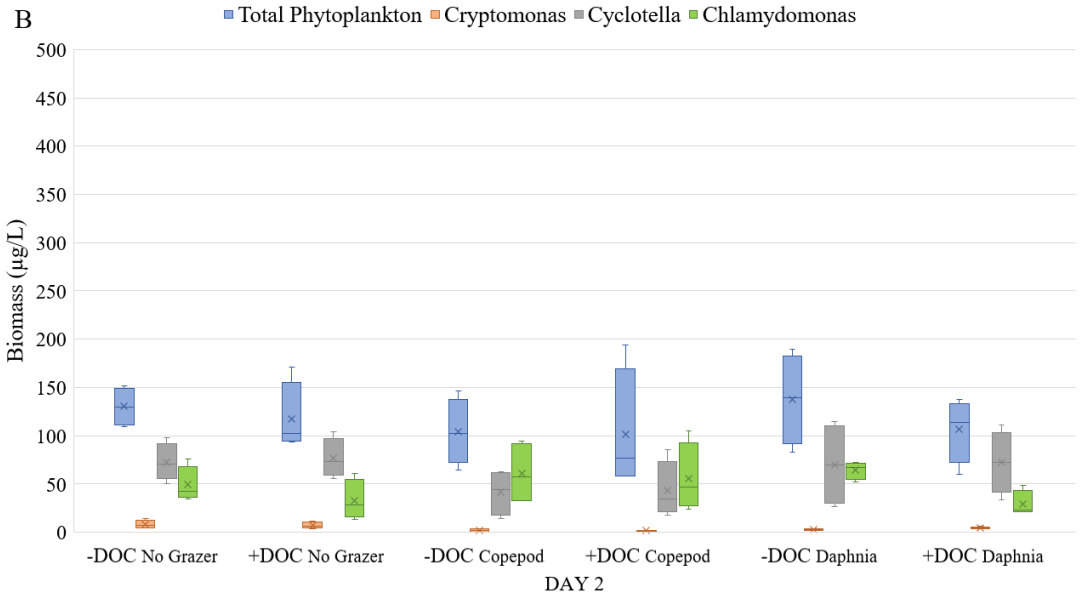
3.1.2 DOC and Grazer Impacts on Phytoplankton Biomass

There were significant differences in the phytoplankton biomass (total and species-specific biomass) between treatments and the respective controls due to both DOC and grazer effects by the end of the experiment (day 4). Specifically, treatments with DOC (+DOC) had significantly less (3-5x) total phytoplankton biomass compared to treatments without DOC (-DOC) (Figure 3.2 A). Similarly, in terms of prey-specific effects, the +DOC treatments had significantly less (by a factor of ~5) biomass of *Cryptomonas*, *Chlamydomonas* and *Cyclotella* compared to the -DOC treatments, regardless of presence or type of grazer (Figure 3.2 A, Table 3.1). Compared to the no grazer control, *Daphnia* treatments had a similar total phytoplankton biomass while copepods decreased total phytoplankton biomass (Figure 3.2 A, Table 3.1). In terms of prey specific responses, the biomass of *Cyclotella* was significantly reduced in the *Daphnia* and copepod treatments compared to no-grazer controls, though copepods significantly reduced *Cyclotella* biomass more than *Daphnia* (7x and 3x times, respectively) (Table 3.1). In contrast, compared to no-grazer controls, presence of grazers (copepod or *Daphnia*) did not significantly change the *Cryptomonas* and *Chlamydomonas* biomass (Table 3.1). Overall, total phytoplankton biomass was reduced by DOC and copepods, but not

Daphnia. Moreover, while DOC reduced the biomass of all phytoplankton species, copepods and *Daphnia* significantly reduced the biomass of *Cyclotella spp.* only.

On day 0, total and species-specific phytoplankton biomass were similar across all treatments except for *Cryptomonas* biomass in a single treatment (Figure 3.2 C). Specifically, there were about 1.5x more *Cryptomonas* biomass in the +DOC treatments with *Daphnia* compared to -DOC treatments with *Daphnia* (Table 5, t-test $p<0.05$). On day 2, the DOC treatment did not significantly change total phytoplankton biomass, though significant DOC and grazer impacts emerged for some of the phytoplankton species (Figure 3.2 B, Table 3.1). In terms of prey specific responses, +DOC treatments had significantly less *Chlamydomonas* biomass compared to the -DOC controls (by a factor of ~1.5), while the DOC did not change *Cryptomonas* and *Cyclotella*, regardless of presence and type of grazer. Both *Daphnia* and copepod treatments significantly reduced *Cryptomonas* biomass (~3.5x) compared to the no-grazer control, though copepods reduced the biomass of *Cryptomonas* more than *Daphnia* (Figure 3.2 B, Table 3.1). In addition, copepods significantly decreased *Cyclotella* biomass compared to the no grazer control, while *Daphnia* did not. Finally, neither *Daphnia* nor copepods lead to a significant change in *Chlamydomonas* biomass (Table 3.1). Hence, overall, significant differences between treatments (DOC or grazer) and controls (-DOC or no-grazer) increased from day 2 to day 4 (Table 3.1).


A
Total Phytoplankton
Cryptomonas
Cyclotella
Chlamydomonas
Biomass (µg/L)
500
450
400
350
300
250
200
150
100
50
0
-DOC No Grazer
+DOC No Grazer
-DOC Copepod
+DOC Copepod
-DOC Daphnia
+DOC Daphnia
DAY 4


B
Total Phytoplankton
Cryptomonas
Cyclotella
Chlamydomonas
Biomass (µg/L)
500
450
400
350
300
250
200
150
100
50
0
-DOC No Grazer
+DOC No Grazer
-DOC Copepod
+DOC Copepod
-DOC Daphnia
+DOC Daphnia
DAY 2

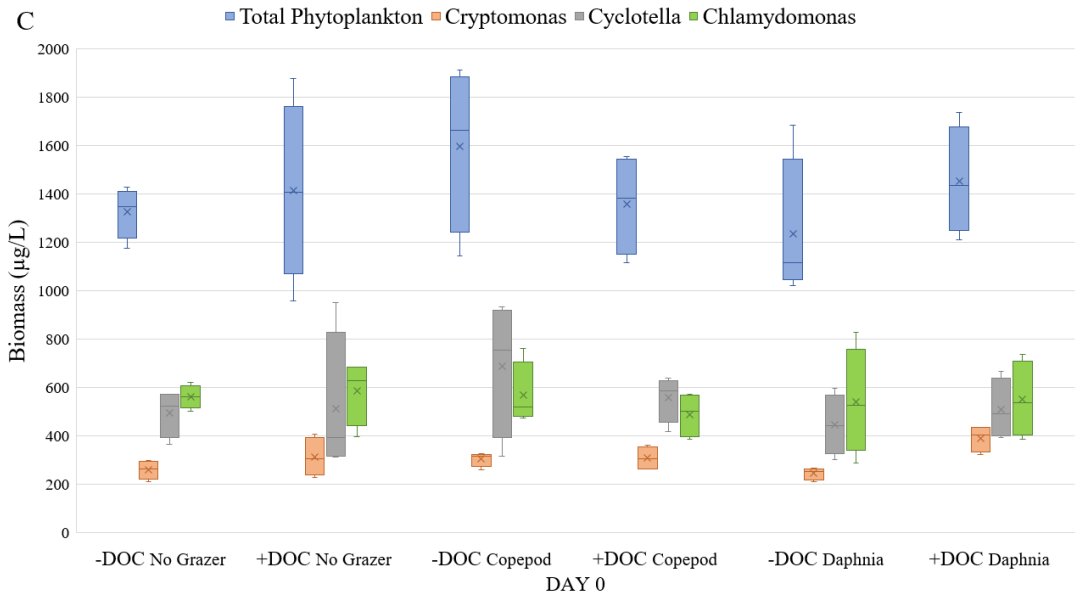


Figure 3.2 The median boxplot showing the total phytoplankton and species (*Cryptomonas pyrenoidifera*, *Cyclotella spp. Chlamydomonas reinhardtii*) biomass values (µg L^{-1}) of all treatments on day 4 (A), day 2 (B) and day 0 (C) (No grazer without DOC (-DOC), No grazer with DOC (+DOC), Copepod without DOC, Copepod with DOC, *Daphnia* without DOC, Daphnia with DOC). Bottom and upper lines(hinges) of the boxplots represent the 1st and 3rd quartiles.

Table 3.1 Generalized Linear Model (GLM) analysis (Biomass~DOC+grazer) results showing the effect of DOC and grazer (copepod or *Daphnia*) treatments on the biomass of total and species-specific phytoplankton (*Cryptomonas*, *Cyclotella*, *Chlamydomonas*) throughout the experiment (day 0; day 2; day 4) (i.e., effect of factor X1 on Y compared to factor X2).

Day	*Dependent Variable (Y)*	*Factor X1*	*Factor X2*	*Estimate*	*Standard Error*	*t value*	*Pr (>|t|)*
0	Total phytoplankton	+DOC	-DOC	0.02	0.08	0.25	0.8030
0	Total phytoplankton	Copepod	No grazer	0.07	0.10	0.75	0.4640
0	Total phytoplankton	*Daphnia*	No grazer	-0.02	0.10	-0.20	0.8450
0	Total phytoplankton	*Daphnia*	Copepod	-0.09	0.10	-0.94	0.3570
0	Cryptomonas	+DOC	-DOC	0.21	0.08	2.74	**0.0127 ****
0	Cryptomonas	Copepod	No grazer	0.08	0.09	0.89	0.3832
0	Cryptomonas	*Daphnia*	No grazer	0.10	0.09	1.04	0.3124
0	Cryptomonas	*Daphnia*	Copepod	0.01	0.09	0.14	0.8864
0	Cyclotella	+DOC	-DOC	-0.02	0.14	-0.13	0.8990
0	Cyclotella	Copepod	No grazer	0.22	0.17	1.29	0.2100
0	Cyclotella	*Daphnia*	No grazer	-0.02	0.17	-0.13	0.8960
0	Cyclotella	*Daphnia*	Copepod	-0.2	0.17	-1.43	0.1690
0	Chlamydomonas	+DOC	-DOC	-0.03	0.11	-0.25	0.8050
0	Chlamydomonas	Copepod	No grazer	-0.08	0.13	-0.66	0.5170
0	Chlamydomonas	*Daphnia*	No grazer	-0.09	0.13	-0.67	0.5080
0	Chlamydomonas	*Daphnia*	Copepod	-0.002	0.13	-0.01	0.9880
2	Total phytoplankton	+DOC	-DOC	-0.16	0.14	-1.15	0.2650

Table 3.1 (cont'd)

2	Total phytoplankton	Copepod	No grazer	-0.24	0.17	-1.42	0.1710
2	Total phytoplankton	*Daphnia*	No grazer	-0.05	0.17	-0.27	0.7910
2	Total phytoplankton	*Daphnia*	Copepod	0.20	0.17	1.15	0.262 0
2	Cryptomonas	+DOC	-DOC	0.13	0.16	0.84	0.4104
2	Cryptomonas	Copepod	No grazer	-1.14	0.20	-5.82	**1.08e-05*** **
2	Cryptomonas	*Daphnia*	No grazer	-0.58	0.20	-2.94	**0.0081 ****
2	Cryptomonas	*Daphnia*	Copepod	0.57	0.20	2.88	**0.0093 ****
2	Cyclotella	+DOC	-DOC	0.07	0.21	0.34	0.7377
2	Cyclotella	Copepod	No grazer	-0.69	0.25	-2.71	**0.0135 ****
2	Cyclotella	*Daphnia*	No grazer	-0.14	0.25	-0.57	0.5777
2	Cyclotella	*Daphnia*	Copepod	0.54	0.25	2.14	**0.0447 ***
2	Chlamydomonas	+DOC	-DOC	-0.48	0.20	-2.45	**0.0237 ***
2	Chlamydomonas	Copepod	No grazer	0.3273	0.24	1.36	0.1876
2	Chlamydomonas	*Daphnia*	No grazer	0.14	0.24	0.57	0.5770
2	Chlamydomonas	*Daphnia*	Copepod	-0.19	0.24	-0.80	0.4346
4	Total phytoplankton	+DOC	-DOC	-1.01	0.31	-3.19	**0.0046 ****
4	Total phytoplankton	Copepod	No grazer	-0.92	0.39	-2.36	**0.0282 ***
4	Total phytoplankton	*Daphnia*	No grazer	-0.37	0.39	-0.97	0.3445
4	Total phytoplankton	*Daphnia*	Copepod	0.54	0.39	1.40	0.1777
4	Cryptomonas	+DOC	-DOC	-0.85	0.32	-2.67	**0.0147 ****

Table 3.1 (cont'd)

4	Cryptomonas	Copepod	No grazer	-0.61	0.39	-1.56	0.1342
4	Cryptomonas	*Daphnia*	No grazer	-0.04	0.39	-0.09	0.9249
4	Cryptomonas	*Daphnia*	Copepod	0.57	0.39	1.47	0.1583
4	Cyclotella	+DOC	-DOC	-1.11	0.32	-3.48	**0.0024****
4	Cyclotella	Copepod	No grazer	-1.75	0.39	-4.49	**0.0002*****
4	Cyclotella	*Daphnia*	No grazer	-0.90	0.39	-2.31	**0.0314 ***
4	Cyclotella	*Daphnia*	Copepod	0.85	0.39	2.18	**0.0417 ***
4	Chlamydomonas	+DOC	-DOC	-1.12	0.47	-2.37	**0.0278 ***
4	Chlamydomonas	Copepod	No grazer	0.33	0.58	0.57	0.5775
4	Chlamydomonas	*Daphnia*	No grazer	0.39	0.58	0.66	0.5140
4	Chlamydomonas	*Daphnia*	Copepod	0.06	0.58	0.10	0.9228

3.1.3 Effects of The DOC and Grazer Treatments on Phytoplankton Biomass Using LRR (Effect Sizes)

Both DOC (i.e., bottom-up effect) and grazers (i.e., top-down effect) had significantly negative effect on total phytoplankton biomass, with mean values ranging from -1.5 to -0.4 (Figure 3.3). Although the mean effect of DOC was stronger in the no-grazer (-1.53± 0.52 CI) and copepod (-1.48 ± 0.59 CI) treatments when compared to the *Daphnia* treatment (-1.07 ± 0.29 CI), the effect of DOC was similar across grazer treatments due to overlapping CI values (Figure 3.3 A). In terms of grazer effects, the effects copepods (-0.89 ± 0.73 CI) and *Daphnia* were similar in the -DOC treatments (-0.80 ± 0.54 CI). Also, in +DOC treatments, effect size values of copepods (-0.83 ± 0.28 CI) and *Daphnia* (-0.34 ± 0.24 CI) were similar (Figure 3.3 B). Overall, the effect of DOC and grazer treatments on phytoplankton biomass were negative and similar in magnitude when measured via effect size (LRR).

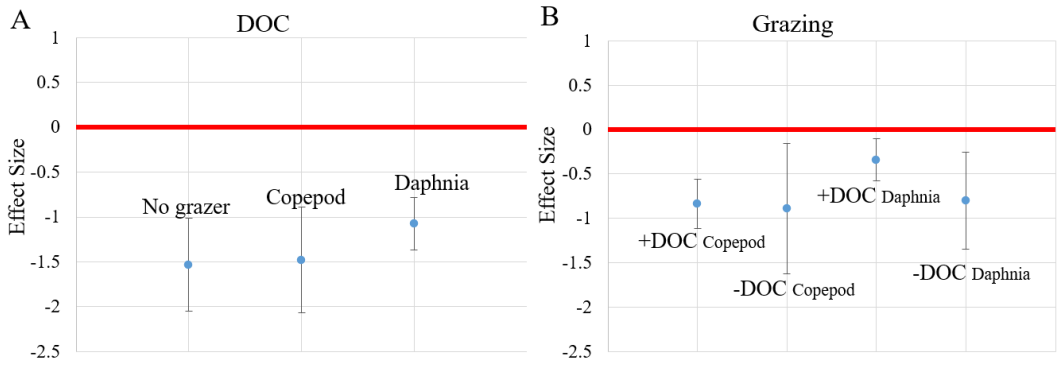


Figure 3.3 The mean effect size for (A) DOC and (B) zooplankton treatments on total phytoplankton biomass at the end of the laboratory grazing experiment (day 4). Error bars are 95% confidence intervals. The effect is significant if the confidence interval does not overlap zero (the red lines) (Hillebrand, 2016)

The effect size values of DOC was generally negative for all phytoplankton species, but its magnitude was species specific and varied significantly across different grazer treatments (Figure 3.4, 3.6). For example, the mean effect size of DOC for *Cryptomonas* and *Cyclotella* biomasses was about -1, regardless of presence and type of grazer. In contrast, the effect size of DOC for *Chlamydomonas* was strongest in the no-grazer treatments (-2.93 ± 1.03 CI), followed by the treatments with *Daphnia* (-2.16 ± 0.75 CI) and copepods (-1.65 ± 0.72 CI). Overall, across the phytoplankton species, DOC effect size was more negative for *Chlamydomonas* compared to *Cryptomonas* or *Cyclotella*.

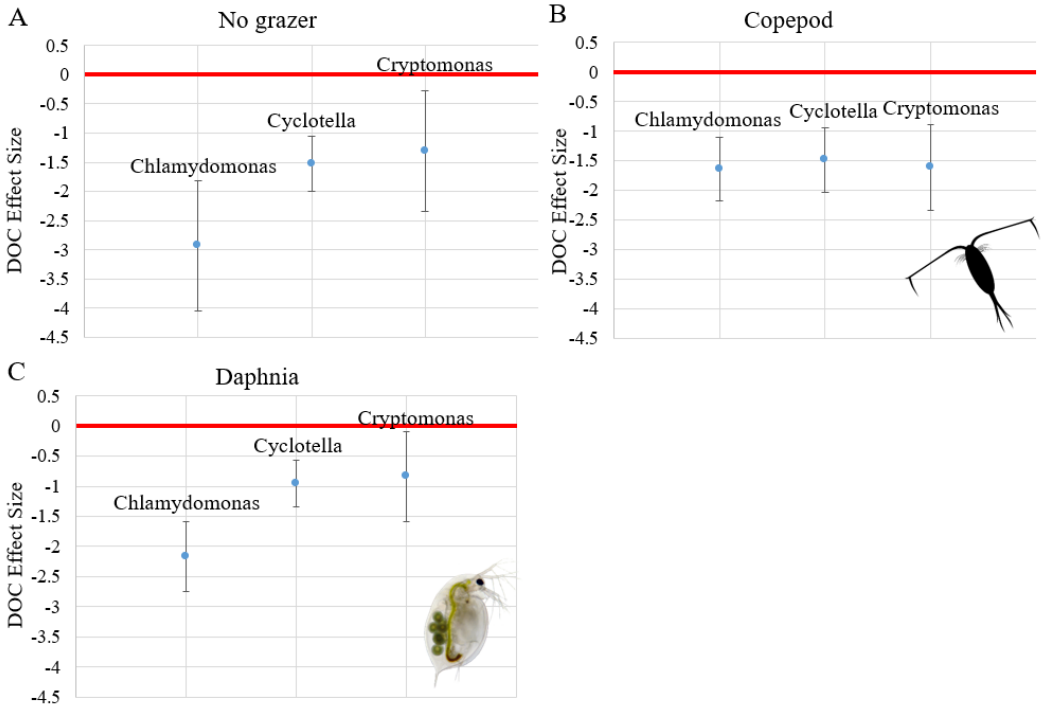


Figure 3.4 The mean effect size of DOC on species specific phytoplankton biomass (*Chlamydomonas reinhardtii*, *Cyclotella spp.*, and *Cryptomonas pyrenoidifera*) at the end of the laboratory grazing experiment (day 4) in the (A) no grazer (B) Copepod and (C) *Daphnia* treatments. Error bars are 95% confidence intervals. The effect of DOC is significant if the confidence interval does not overlap zero (the red lines) (Hillebrand, 2016).

While the top-down effect of grazers resulted in decreased in total phytoplankton biomass expressed as negative effect size values (Figure 3.3), the effect on the biomass of individual phytoplankton species resulted in variable negative and positive effect size values ranging from a mean of -2 to 1 (Figure 3.5). In general, the most negative effects were for *Cyclotella* (four treatments' average, -1.50 ± 0.47 CI) while the least negative (and sometimes positive) effects were for *Chlamydomonas* (four treatments' average, 0.25 ± 0.87 CI) (Figure 3.5). *Cyclotella* biomass was reduced by either copepod or *Daphnia* grazing as seen in negative effect sizes. Though with copepods it was the stronger (by a factor of ~2) effect size values

(average of +DOC and -DOC, -1.92 ± 0.5 CI) compared to *Daphnia* (average of +DOC and -DOC, -1.08 ± 0.42 CI) (Figure 3.5). For *Chlamydomonas* biomass, the effect size values of zooplankton grazing (either copepod or *Daphnia*) were either insignificant (-DOC treatment, -0.27 ± 1.08 CI average of copepod and *Daphnia*) or positive (+DOC treatment, 0.76 ± 0.66 CI average of copepod and *Daphnia*). For *Cryptomonas* biomass, the grazer effect size ranged from slightly negative (+DOC Copepod), to no effect (-DOC $_{\text{Copepod}}$, +DOC $_{\textit{Daphnia}}$ and -DOC $_{\textit{Daphnia}}$). Overall, the strongest negative effect was observed for *Cyclotella*, followed by *Cryptomonas*, and the negative grazer effects were the weakest (and even positive) for *Chlamydomonas* (Figure 3.6). Indeed, *Cyclotella* biomass was reduced >2x compared to the other phytoplankton species. Moreover, for *Chlamydomonas*, the effect of grazers contrasted among the +DOC vs. -DOC treatments such that the grazer effect was less negative with +DOC (Figure 3.5).

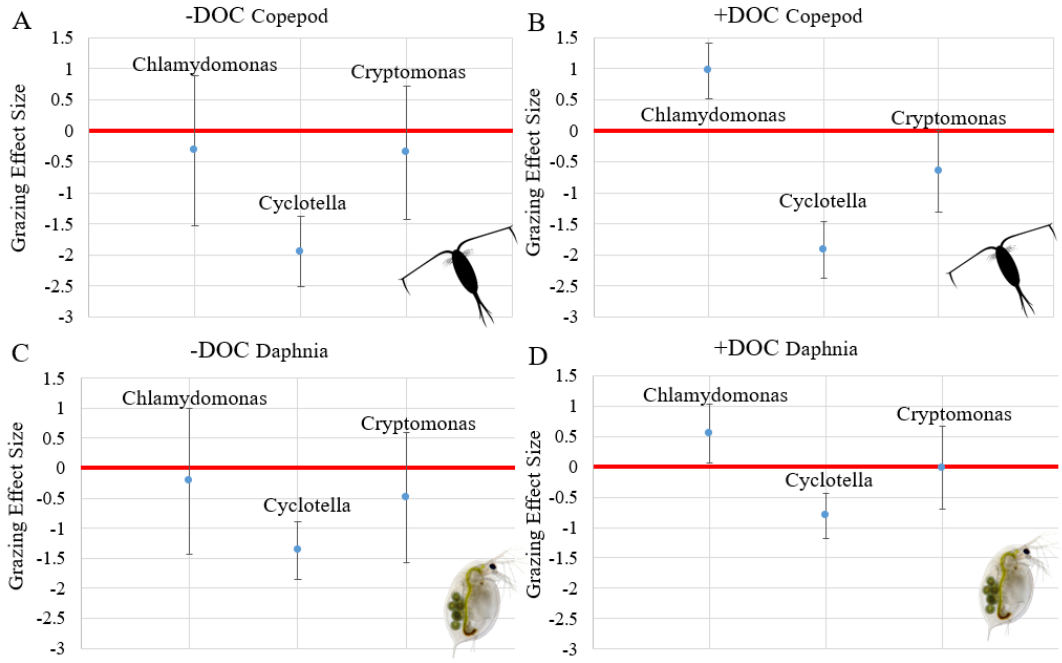


Figure 3.5 The grazing effect size of phytoplankton species (*Chlamydomonas reinhardtii, Cyclotella spp.*, and *Cryptomonas pyrenoidifera*) in day 4 (A) Copepod without DOC (i.e., -) (B) Copepod with DOC (i.e., +) (C) *Daphnia* without DOC (D)

Daphnia with DOC treatment. Error bars are 95% confidence intervals. The effect is significant if the confidence interval does not overlap zero (the red lines) (Hillebrand, 2016).

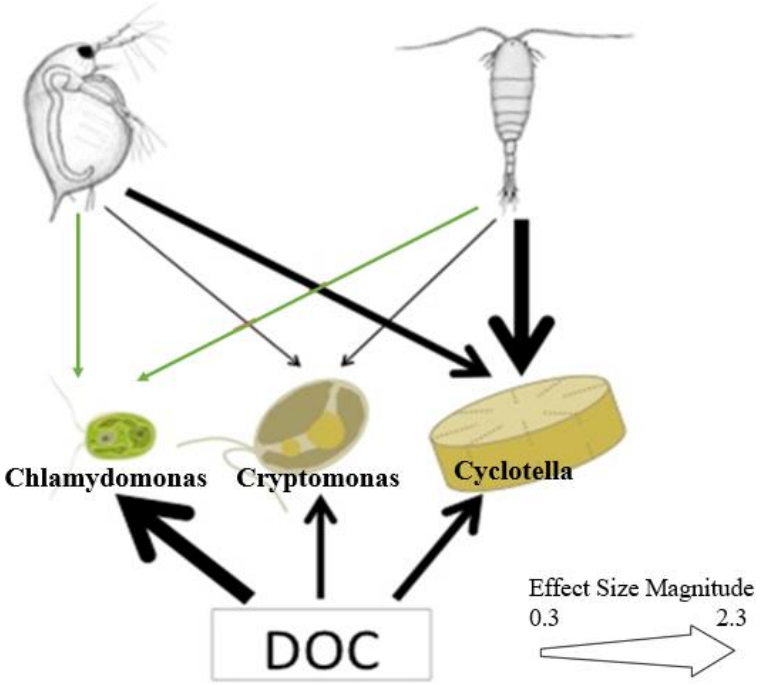


Figure 3.6 The relative mean effect size value of either DOC or zooplankton grazers (*Daphnia* or copepod) on the phytoplankton species in the experiment measured over 4 days. Arrow width is scaled to the absolute value of the mean effect size (mean of pooled copepod and *Daphnia* treatments for DOC effect, and pooled +DOC and -DOC treatments for the grazer effect). **Black** arrows indicated negative effect size values. **Green** arrows indicated positive mean effect size values. Arrows indicated positive and negative effects depending on the grazer (DOC effect in copepod vs. Daphnia treatments) or DOC (grazer effects in DOC vs. no DOC control) treatments.

3.1.4 Effect of Treatments of Phytoplankton Species Composition

The initial phytoplankton species composition (day 0) was dominated by *Chlamydomonas* (40 ± 6.68% CI) and *Cyclotella* (38 ± 7.56% CI) while *Cryptomonas* contributed relatively less (22 ± 4.86% CI) to the total biomass (Figure 3.7). Of the twelve total comparisons of composition between treatments, eleven of them showed that initial community composition was similar across all treatments ($p>0.05$). However, only one of them revealed significant difference, which was *Cryptomonas* biomass comparison between -DOC (20%) and +DOC (24%) ($p<0.05$), regardless of grazer. Thus, experiment started with same initial phytoplankton composition in each treatment (GLM; $p>0.05$).

The phytoplankton composition changed with time during the 4-day experiment (Figure 3.7). Overall, *Cryptomonas* increased from a mean of 22% to 47% of the total phytoplankton community over 4 days, except for the -DOC treatments with no-grazers and copepods, which did not change significantly over time (Table 3.2). Increase in copepod and *Daphnia* treatments was ~1.6x more than in no grazer controls (both for + and -DOC) (Table 3.2). In contrast, *Chlamydomonas* percent contribution significantly decreased across all treatments from 40% to 12% on average, though reduction in no-grazer controls was ~1.7x more than reduction in copepod and *Daphnia* treatments. Although the percent contribution of *Cyclotella* increased in no grazer controls (from 36% to 54%) and decreased in copepod (from 42% to 24%) and *Daphnia* (from 35% to 32%) treatments, only significant change was observed in copepod +DOC treatment from 41% to 17%. Overall, when compared across all treatments, the percent contribution of *Cryptomonas* significantly increased, while *Chlamydomonas* decreased, but change in *Cyclotella* was insignificant.

By the end of the experiment (day 4), DOC had no significant effect on the biomass contribution of phytoplankton species while the grazer effect was species-specific (Figure 3.7, Table 3.3). For instance, both grazers significantly increased the percent contribution of *Chlamydomonas* compared to no grazer control, regardless of DOC

treatment (Table 3.3). Moreover, *Daphnia* significantly increased the percent contribution of *Cryptomonas* compared to no grazer control (Table 3.3). In contrast, copepods significantly decreased the percent contribution of *Cyclotella* compared to no grazer control (Table 3.3). Overall, DOC effect on the percent phytoplankton biomass contribution was insignificant; however, both grazer increased *Chlamydomonas* and *Daphnia* increased *Cryptomonas* while copepods decreased *Cyclotella* % contribution.

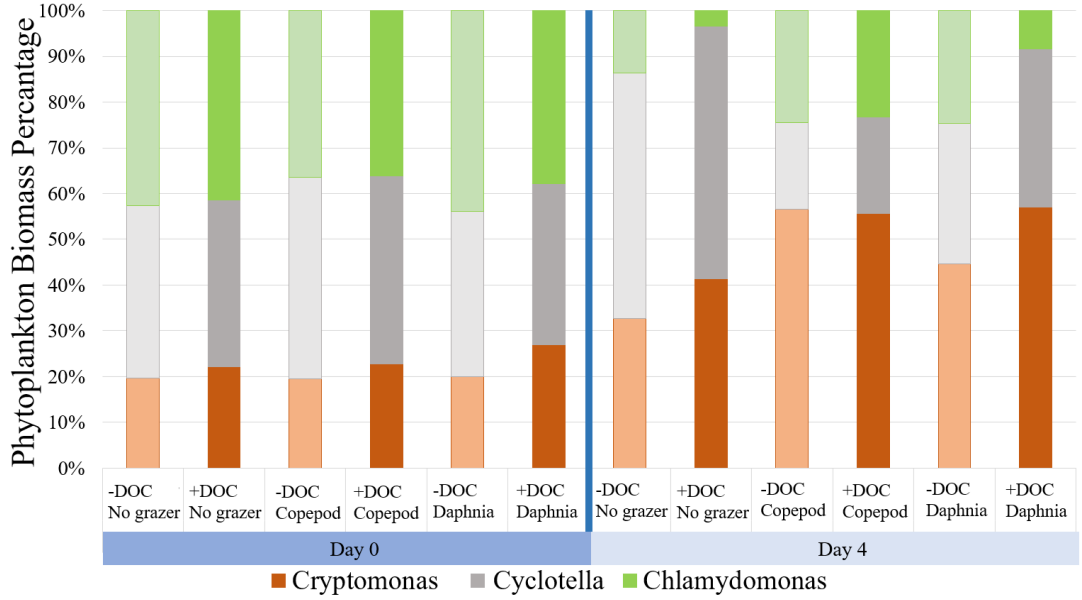


Figure 3.7 Percentage contribution of three phytoplankton species (*Cryptomonas pyrenoidifera, Cyclotella spp.* and *Chlamydomonas reinhardtii*) in terms of biomass for each treatment on day 0 and day 4.

Table 3.2 Two sample t test results of species % biomass (*Cryptomonas pyrenoidifera, Cyclotella spp.* and *Chlamydomonas reinhardtii*) contribution change in each treatment between day 0 and day 4.

Species	*Grazer Treatment*	*DOC Treatment*	*df*	*Mean Day 0*	*Mean Day 4*	*t value*	*p value*
Cryptomonas	No grazer	-	6	19.80	36.04	-1.75	0.1300
Cryptomonas	Copepod	-	6	19.99	47.26	-3.57	0.1170
Cryptomonas	Daphnia	-	6	20.56	48.88	-3.24	**0.0178***
Cryptomonas	No grazer	+	6	22.50	41.51	-2.66	**0.0376***
Cryptomonas	Copepod	+	6	22.67	50.71	-5.13	**0.0021****
Cryptomonas	Daphnia	+	6	27.50	56.32	-5.52	**0.0015***
Cyclotella	No grazer	-	6	37.22	55.16	-1.76	0.1284
Cyclotella	Copepod	-	6	41.62	31.13	0.89	0.4089
Cyclotella	Daphnia	-	6	36.36	31.51	0.54	0.6083
Cyclotella	No grazer	+	6	35.01	53.24	-2.12	0.0777
Cyclotella	Copepod	+	6	41.09	17.29	3.51	**0.0127****
Cyclotella	Daphnia	+	6	34.93	33.34	0.22	0.8307
Chlamydomonas	No grazer	-	6	42.51	7.42	7.45	**0.0003*****
Chlamydomonas	Copepod	-	6	36.21	15.35	3.12	**0.0206***
Chlamydomonas	Daphnia	-	6	42.82	17.75	2.68	**0.0366***
Chlamydomonas	No grazer	+	6	42.27	2.75	8.99	**0.0001*****
Chlamydomonas	Copepod	+	6	35.96	19.80	3.38	**0.0149***
Chlamydomonas	Daphnia	+	6	37.37	8.21	6.54	**0.0006*****

Table 3.3 Generalize Linear Model (GLM) analysis results showing the effect of DOC and grazer (copepod or *Daphnia*) treatments on percent contribution of phytoplankton species biomasses (*Cryptomonas pyrenoidifera*, *Cyclotella spp.* and *Chlamydomonas reinhardtii*) on day 4. (i.e., effect of factor X1 on Y compared to factor X2).

Dependent Variable (Y)	*Factor X1*	*Factor X2*	*Estimate*	*Standard Error*	*T value*	*Pr(>|t|)*
Cryptomonas	+DOC	-DOC	0.15	0.13	1.14	0.2660
Cryptomonas	Copepod	No grazer	0.27	0.16	1.66	0.1119
Cryptomonas	*Daphnia*	No grazer	0.34	0.16	2.09	**0.0494***
Cryptomonas	*Daphnia*	Copepod	0.07	0.16	0.43	0.6730
Cyclotella	+DOC	-DOC	-0.29	0.31	-0.95	0.3520
Cyclotella	Copepod	No grazer	-1.12	0.37	-2.98	**0.0075****
Cyclotella	*Daphnia*	No grazer	-0.55	0.37	-1.46	0.1589
Cyclotella	*Daphnia*	Copepod	0.57	0.37	1.51	0.1460
Chlamydomonas	+DOC	-DOC	-0.28	0.31	-0.92	0.3668
Chlamydomonas	Copepod	No grazer	1.27	0.38	3.37	**0.0030****
Chlamydomonas	*Daphnia*	No grazer	0.90	0.38	2.38	**0.0272***
Chlamydomonas	*Daphnia*	Copepod	-0.37	0.38	-0.99	0.3357

3.2 Mesocosm Grazing Assays

3.2.1 Impact of Different DOC Sources and Grazing on Phytoplankton Biomass *In-Situ* Mesocosm Assays

In mesocosm grazing assays, the main phytoplankton genus and species that were encountered and grouped into phylum, that were included *Chlorella spp., Crucigenia tetrapedia, Nephrochlamys sp., Scenedesmus sp., Selenastrum sp., Tetraedron minimum, Dinobryon sp., Cyclotella sp.,* and *Pseudoanabeana sp.*.

In the first in-situ grazing assay, the effects of different DOC sources (bottom-up) on phytoplankton biomass were treatment and species specific. Total phytoplankton biomass significantly increased (~2x) in the experimental bottles with mixed DOC when compared to the no DOC control (-DOC), regardless of the presence of grazers. The total phytoplankton biomass with the other two DOC sources (R or L) was similar to the no DOC control (-DOC) bottles. Thus, the only DOC treatment that increased the total phytoplankton biomass was mixed DOC (Figure 3.8, Table 3.4). None of the DOC treatments had a significant effect on *Chlorophyta* spp. when compared to no DOC control (Table 3.4), but among the treatments, *Chlorophyta* biomass significantly increased in L-DOC treatment compared to R-DOC and mixed DOC treatments (Table 3.5). In contrast, *Chrysophyta* spp. biomass significantly increased in mixed DOC treatment (~3-6x) relative to no DOC control (as well as relative to the L and R treatments). Moreover, *Bacillariophyta* biomass significantly increased in R-DOC (~1.4x) and mixed (~1.8x) DOC treatments compared to no DOC control. Hence, among the different DOC sources, mixed DOC increased biomasses of total phytoplankton, *Chrysophyta* and *Bacillariophyta* the most. Notably, none of the DOC sources had a significant effect on *Chlorophyta* compared to no DOC control in first mesocosm assay.

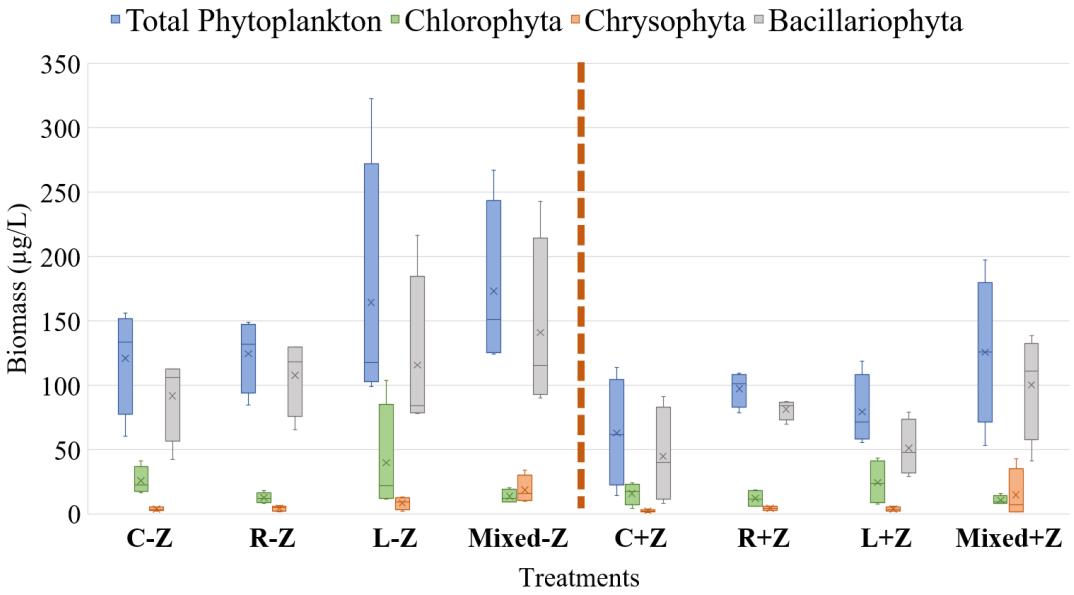


Figure 3.8 The median boxplots of phytoplankton biomass in first *in-situ* grazing assay. The colors represents the total phytoplankton (blue), *Chlorophyta spp.* (green), *Chrysophyta spp.* (orange) and *Bacillariophyta spp.* (grey) biomasses (µg L-1) in treatments (C-Z: no DOC Control, C+Z: no DOC control with mesozooplankton, R-Z: Recalcitrant DOC, R+Z: Recalcitrant DOC with mesozooplankton, L-Z: Leaf leachate DOC, L+Z: Leaf leachate DOC with mesozooplankton, Mixed-Z: Combined recalcitrant and Leaf leachate DOC source, Mixed+Z: Combined recalcitrant and Leaf leachate DOC source with mesozooplankton). Bottom and upper lines (hinges) of the boxplots represent the 1st and 3rd quartiles in first *in-situ* grazing assay.

Table 3.4 General Linear Model (GLM) analysis (biomass ~ DOC + grazer) results showing the effect of the DOC and mesozooplankton grazer treatments on total phytoplankton and species biomasses (*Chlorophyta spp.*, *Chrysophyta spp.*, and *Bacillariophyta spp.*) compared to the respective controls in the first *in-situ* grazing assay (C: no DOC Control, R: Recalcitrant DOC, L: Leaf leachate DOC, Mixed: Combined recalcitrant and leaf leachate DOC source) (i.e., effect of factor X1 on Y compared to factor X2).

Dependent Variable(Y)	*Factor X1*	*Factor X2*	*Estimate*	*Standard Error*	*T value*	*P value*
Total phytoplankton	R	C	0.36	0.23	1.55	0.1337
Total phytoplankton	Mixed	C	0.59	0.23	2.53	**0.0174***
Total phytoplankton	L	C	0.33	0.23	1.42	0.1669
Total phytoplankton	+Z	-Z	-0.51	0.17	-3.11	**0.0044****
Chlorophyta	R	C	-0.44	0.28	-1.56	0.1300
Chlorophyta	Mixed	C	-0.43	0.28	-1.51	0.1430
Chlorophyta	L	C	0.22	0.28	0.77	0.4470
Chlorophyta	+Z	-Z	-0.30	0.20	-1.52	0.139
Chrysophyta	R	C	0.26	0.31	0.86	0.3995
Chrysophyta	Mixed	C	1.16	0.31	3.78	**0.0008*****
Chrysophyta	L	C	0.45	0.31	1.47	0.1538
Chrysophyta	+Z	-Z	-0.42	0.22	-1.93	0.0641
Bacillariophyta	R	C	0.56	0.26	2.11	**0.0443***
Bacillariophyta	Mixed	C	0.73	0.26	2.76	**0.0103****
Bacillariophyta	L	C	0.30	0.26	1.13	0.2692
Bacillariophyta	+Z	-Z	-0.59	0.19	-3.13	**0.0042****

Table 3.5 General Linear Model (GLM) analysis (biomass ~ DOC) results showing the effect of the different type of DOC treatments on total phytoplankton and species biomasses (*Chlorophyta spp., Chrysophyta spp.,* and *Bacillariophyta spp.)* compared among different treatments in the first *in-situ* grazing assay (C: no DOC Control, R: Recalcitrant DOC, L: Leaf leachate DOC, Mixed: Combined recalcitrant and Leaf leachate DOC source) (i.e., effect of factor X1 on Y compared to factor X2).

Dependent Variable(Y)	*Factor X1*	*Factor X2*	*Estimate*	*Standard Error*	*T value*	*P value*
Total phytoplankton	L	R	-0.03	0.23	-0.13	0.9007
Total phytoplankton	Mixed	L	0.26	0.23	1.11	0.2758
Total phytoplankton	Mixed	R	0.23	0.23	0.99	0.3328
Chlorophyta	L	R	0.66	0.28	2.33	**0.0273***
Chlorophyta	Mixed	L	-0.64	0.28	-2.28	**0.0306***
Chlorophyta	Mixed	R	0.01	0.28	0.05	0.9591
Chrysophyta	L	R	0.19	0.31	0.61	0.5462
Chrysophyta	Mixed	L	0.71	0.31	2.31	**0.0287***
Chrysophyta	Mixed	R	0.90	0.31	2.92	**0.0069****
Bacillariophyta	L	R	-0.26	0.26	-0.98	0.3349
Bacillariophyta	Mixed	L	0.43	0.26	1.63	0.1151
Bacillariophyta	Mixed	R	0.17	0.26	0.65	0.5237

In the second *in-situ* grazing assay, the effect of different types of DOC sources (bottom-up) on phytoplankton was also treatment and species specific. Total phytoplankton biomass significantly increased (~1.7x) in the bottles with leaf leachate (L) DOC when compared to the no DOC control (-DOC) bottles, regardless of the presence of grazers (Figure 3.9, Table 3.6). The total phytoplankton biomass in the bottles with the other two DOC sources (R or mixed) was similar to the no DOC control bottles (Figure 3.9, Table 3.6, 3.7). Similarly, the only DOC treatment that increased *Chlorophyta* biomass (> 2x) was L-DOC. Compared among the different +DOC treatments (R, L, Mixed), *Chlorophyta* biomass increased in L-DOC and mixed-DOC treatments compared to R-DOC treatment, though the increase was more in L-DOC (>4x) relative to mixed DOC treatment (~2x). In contrast, there were no significant effects of any of the DOC sources on *Chrysophyta* and *Bacillariophyta* biomass. Overall, in the second *in-situ* grazing assay, total phytoplankton biomass increased in the tanks with leaf leachate DOC when compared to the no DOC control tanks (as well as other treatments). Moreover, the positive effect of the leaf leachate DOC on phytoplankton species was limited to *Chlorophyta* only.

In both of the *in-situ* grazing assays (i.e., one and four days after DOC pulse), mesozooplankton significantly reduced the total phytoplankton biomass, regardless of the DOC treatments (Figure 3.8, 3.9, Table 3.4, 3.6). Mesozooplankton grazing had phylum specific impacts in both assays such that they significantly reduced *Bacillariophyta* (~2x) but not *Chlorophyta* and *Chrysophyta* (Figure 3.8, 3.9, Table 3.4,3.6).

Taken together, either mixed (1st assay) or L- DOC (2nd assay) increased the total phytoplankton biomass, and mesozooplankton reduced total phytoplankton biomass (but only due to grazing on *Bacillariophyta spp.*) in both assays. Hence, recalcitrant (R)-DOC had no effect on phytoplankton biomass in neither of the assays. Similarly, grazers had no effect on *Chlorophyta* and *Chrysophyta* in neither of the assays.

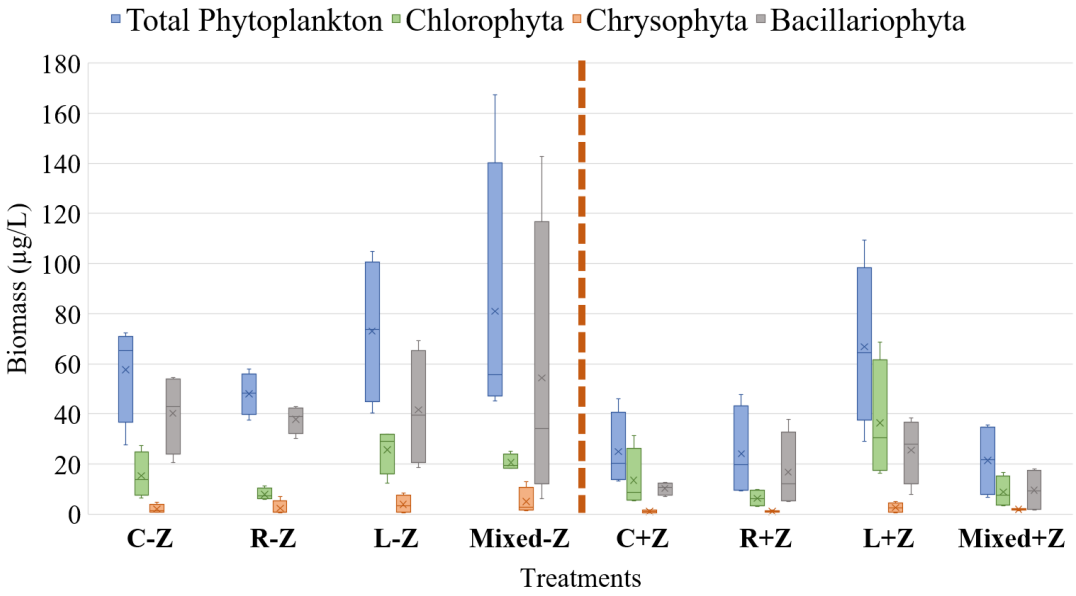


Figure 3.9 The median boxplots of phytoplankton biomass in second *in-situ* grazing assay. The colors represents the total phytoplankton (blue), *Chlorophyta spp.* (green), *Chrysophyta spp.* (orange) and *Bacillariophyta spp.* (grey) biomasses (µg L-1) in treatments (C-Z: no DOC Control, C+Z: no DOC control with mesozooplankton, R-Z: Recalcitrant DOC, R+Z: Recalcitrant DOC with mesozooplankton, L-Z: Leaf leachate DOC, L+Z: Leaf leachate DOC with mesozooplankton, Mixed-Z: Combined recalcitrant and Leaf leachate DOC source, Mixed +Z: Combined recalcitrant and Leaf leachate DOC source with mesozooplankton). Bottom and upper lines (hinges) of the boxplots represent the 1st and 3rd quartiles in second *in-situ* grazing assay.

Table 3.6 General Linear Model (GLM) analysis (Biomass ~ DOC + grazer) results showing the effect of DOC and mesozooplankton grazer on total phytoplankton and species biomasses (*Chlorophyta spp., Chrysophyta spp.,* and *Bacillariophyta spp*.) compared to respective controls (no DOC control or mesozooplankton control) in second *in-situ* grazing assay (C: no DOC Control, R: Recalcitrant DOC, L: Leaf leachate DOC, Mixed: Combined recalcitrant and Leaf leachate DOC source) (i.e., effect of factor X1 on Y compared to factor X2).

Dependent Variable(Y)	*Factor X1*	*Factor X2*	*Estimate*	*Standard Error*	*T value*	*P value*
Total phytoplankton	R	C	-0.13	0.29	-0.45	0.6538
Total phytoplankton	Mixed	C	0.01	0.29	0.02	0.9846
Total phytoplankton	L	C	0.60	0.29	2.09	**0.0465 ***
				0.20	-3.93	**0.0005***
Total phytoplankton	+Z	-Z	-0.80			**
Chlorophyta	R	C	-0.53	0.27	-1.93	0.0645
Chlorophyta	Mixed	C	0.04	0.27	0.14	0.8874
				0.27	2.88	**0.0077**
Chlorophyta	L	C	0.79			**
Chlorophyta	+Z	-Z	-0.29	0.19	-1.52	0.1399
Chrysophyta	R	C	-0.04	0.27	-0.13	0.8950
Chrysophyta	Mixed	C	0.43	0.27	1.56	0.1310
Chrysophyta	L	C	0.38	0.27	1.39	0.1760
Chrysophyta	+Z	-Z	-0.29	0.19	-1.52	0.1400
Bacillariophyta	R	C	0.09	0.36	0.26	0.7949
Bacillariophyta	Mixed	C	-0.25	0.36	-0.70	0.4911
Bacillariophyta	L	C	0.36	0.36	0.99	0.3273
				0.25	-4.18	**0.0003***
Bacillariophyta	+Z	-Z	-1.06			**

Table 3.7 General Linear Model (GLM) analysis (Biomass~DOC results showing the effect of the different type of DOC treatments on total phytoplankton and species biomasses (*Chlorophyta spp.*, *Chrysophyta spp.*, and *Bacillariophyta spp*.) in second *in-situ* mesocosm assays (C: no DOC Control, R: Recalcitrant DOC, L: Leaf leachate DOC, Mixed: Combined recalcitrant and Leaf leachate DOC source) (i.e., effect of factor X1 on Y compared to factor X2).

Dependent Variable(Y)	*Factor X1*	*Factor X2*	*Estimate*	*Standard Error*	*T value*	*P value*
Total phytoplankton	L	R	0.73	0.29	2.54	**0.0171***
Total phytoplankton	Mixed	L	-0.60	0.29	-2.07	**0.0484***
Total phytoplankton	Mixed	R	0.14	0.29	0.47	0.6400
Chlorophyta	L	R	1.32	0.27	4.81	**5x10-05 *** **
Chlorophyta	Mixed	L	-0.75	0.27	-2.74	**0.0107** **
Chlorophyta	Mixed	R	0.57	0.27	2.07	**0.0481***
Chrysophyta	L	R	0.42	0.27	1.52	0.1393
Chrysophyta	Mixed	L	0.04	0.27	0.16	0.8700
Chrysophyta	Mixed	R	0.46	0.27	1.69	0.1028
Bacillariophyta	L	R	0.26	0.36	0.73	0.4686
Bacillariophyta	Mixed	L	-0.61	0.36	-1.70	0.1015
Bacillariophyta	Mixed	R	-0.34	0.36	-0.96	0.3453

3.2.1.1 Zooplankton Biomass

In mesocosm assays, all treatments were dominated with *Cladoceran* species (*Bosmina, Ceriodaphnia, Daphnia, Diaphanosoma, Polyphemus* and *Chydorus)* which found 1.5 to 2.5 times more compared to *Copepoda* species (*Calanoid* and *Cyclopoid*). Specifically, *Cladoceran* community was dominated by *Ceriodaphnia* and *Daphnia* species while *Copepoda* community was dominated by *Calanoid* copepods. However, at the day of first mesocosm experiment, total zooplankton, particularly zooplankton groups (*Cladoceran* and *Copepoda*) biomasses were similar between all treatments ($p>0.05$) with some exceptions (Table 13). For example, the R-DOC treatment (406.28±322.37 CI) significantly had higher total zooplankton biomass than L-DOC treatment (223.71±95.90 CI) ($p<0.05$). Specifically, R-DOC (161.90±50.91 CI) had significantly higher *Copepoda* biomass than mixed DOC treatment (51.47±61.78 CI) ($p<0.05$).

Table 3.8 Total mean estimated zooplankton biomass (µg $5L^{-1}$) concentrated in the first grazing assay bottles, and confidence interval (C: no DOC control, R: Recalcitrant DOC, L: Leaf leachate DOC, Mixed: Combination of recalcitrant and leaf leachate DOC)

	Mean Biomass (µg $5L^{-1}$)		
	Cladocera	*Copepoda*	*Total*
C	*181.04 ± 236.51 CI*	*68.45 ±86.81 CI*	*249.49 ±322.37 CI*
R	*244.38 ±117.98 CI*	*161.90 ±50.91 CI*	*406.28 ±105.91 CI*
L	*153.88 ±134.16 CI*	*69.83 ±94.03 CI*	*223.71 ±95.90 CI*
Mixed	*249.03 ±139.92 CI*	*51.47 ±61.78 CI*	*300.50 ±201.39 CI*

3.2.2 Effect Size of The Different Types of DOC and Mesozooplankton Grazing on Phytoplankton Biomass

3.2.2.1 Assessment of The Impacts of Different DOC (Bottom-Up) Sources Using LRR (Effect Size)

In the first grazing assay, the effect of the different DOC sources on total phytoplankton biomass was positive or insignificant (Figure 3.10 A). In general, recalcitrant (R) DOC containing treatments (R+Z, Mixed +Z, Mixed-Z) had positive effect sizes, except for the R-DOC treatment with no-grazers (R-Z), while the effect sizes on total phytoplankton biomass for the L-DOC treatments were insignificant (Figure 3.10 A). In the second grazing assay, only the L-DOC treatment with zooplankton (L+Z) had significant positive effect size values on total phytoplankton biomass (Figure 3.10 B). Hence, the effect of different DOC sources varied with time (Figure 3.14 A, B).

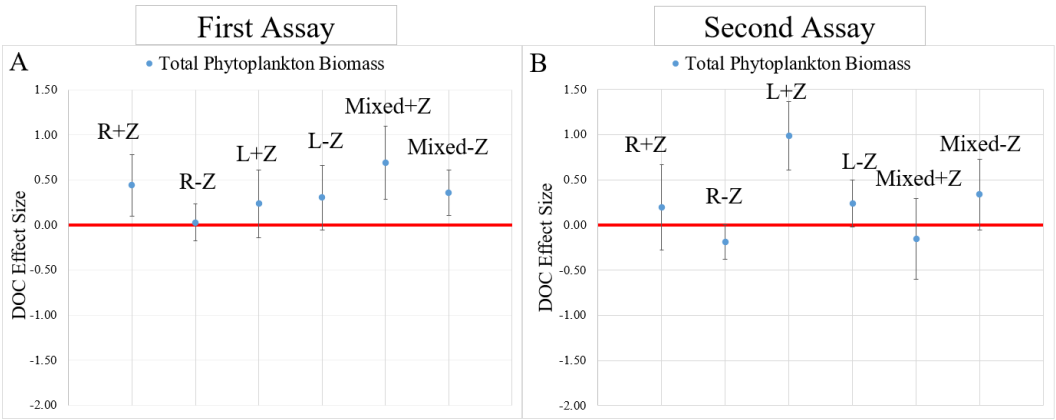


Figure 3.10 The effect size of the different DOC treatments on total phytoplankton biomass in experiment carried out (A) first and (B) second *in-situ* grazing assays (C-Z: no DOC Control, C+Z: no DOC control with mesozooplankton, R-Z: Recalcitrant DOC, R+Z: Recalcitrant DOC with mesozooplankton, L-Z: Leaf leachate DOC,

L+Z: Leaf leachate DOC with mesozooplankton, Mixed-Z: Combined recalcitrant and Leaf leachate DOC source, Mixed +Z: Combined recalcitrant and Leaf leachate DOC source with mesozooplankton). Error bars are 95% confidence intervals. The effect of DOC/grazing is significant if the confidence interval does not overlap zero (the red lines).

In first grazing assay, effect size values of the different DOC sources on *Chlorophyta* biomass were negative in the R-Z, Mixed +Z and Mixed -Z treatments. None of the other treatment effect size values were different than zero statistically. Thus, DOC effects on *Chlorophyta* biomass were limited and any significant values showed a negative effect (Figure 3.11 A). In the second grazing assay, effect size values of the different DOC sources for *Chlorophyta* biomass were positive in treatments with L (0.75±0.45 CI average + and -Z), negative with R (-0.96±0.42 CI average + and -Z), and insignificant with mixed DOC treatments, regardless of the presence of grazers (Figure 3.11 B). Overall, treatments containing recalcitrant DOC (i.e., R, mixed) decreased *Chlorophyta* biomass in both grazing assays, while leaf leachate DOC increased *Chlorophyta* biomass, but only in the second grazing assay (Figure 3.14 C, D).

In first grazing assay, effect size values of the different DOC sources on *Chrysophyta* biomass were generally positive, except in the R-Z treatment. The positive effect size values for DOC were higher in the mixed DOC treatments when compared to R-DOC or L-DOC, regardless of the presence of grazers (i.e., Mixed-Z (1.85±0.35 CI) and Mixed+Z (1.64±0.69 CI)) (Figure 3.11 C). In the second grazing assay, effect size values of the DOC sources for *Chrysophyta* biomass were positive in the L+Z, Mixed+Z, and Mixed-Z treatments, negative in the R+Z treatment, and insignificant in the R-Z, and L-Z treatments (Figure 3.11 D). Overall, mixed DOC treatments had a clear positive effect on *Chrysophyta* biomass in both grazing assays, while the R-DOC treatments had negative effects in second grazing assay (Figure 3.14 C, D).

In first grazing assay, effect size values of the different DOC sources for *Bacillariophyta* biomass were either positive (i.e., R+Z, Mixed+Z, and Mixed-Z) or insignificant (Figure 3.11 F). In the second grazing assay, effect size values of the DOC sources for *Bacillariophyta* biomass were also either positive (R+Z and L+Z) or insignificant (Figure 3.11 G). Notably, the effect size value for the R-DOC and L-DOC treatments were significantly higher in the presence of mesozooplankton when compared to the respective no grazer (-Z) treatments (Figure 3.11 G). Overall, all DOC treatments had a positive effect on *Bacillariophyta* biomass, though this varied between the individual assays and the presence of mesozooplankton (Figure 3.14 C, D).

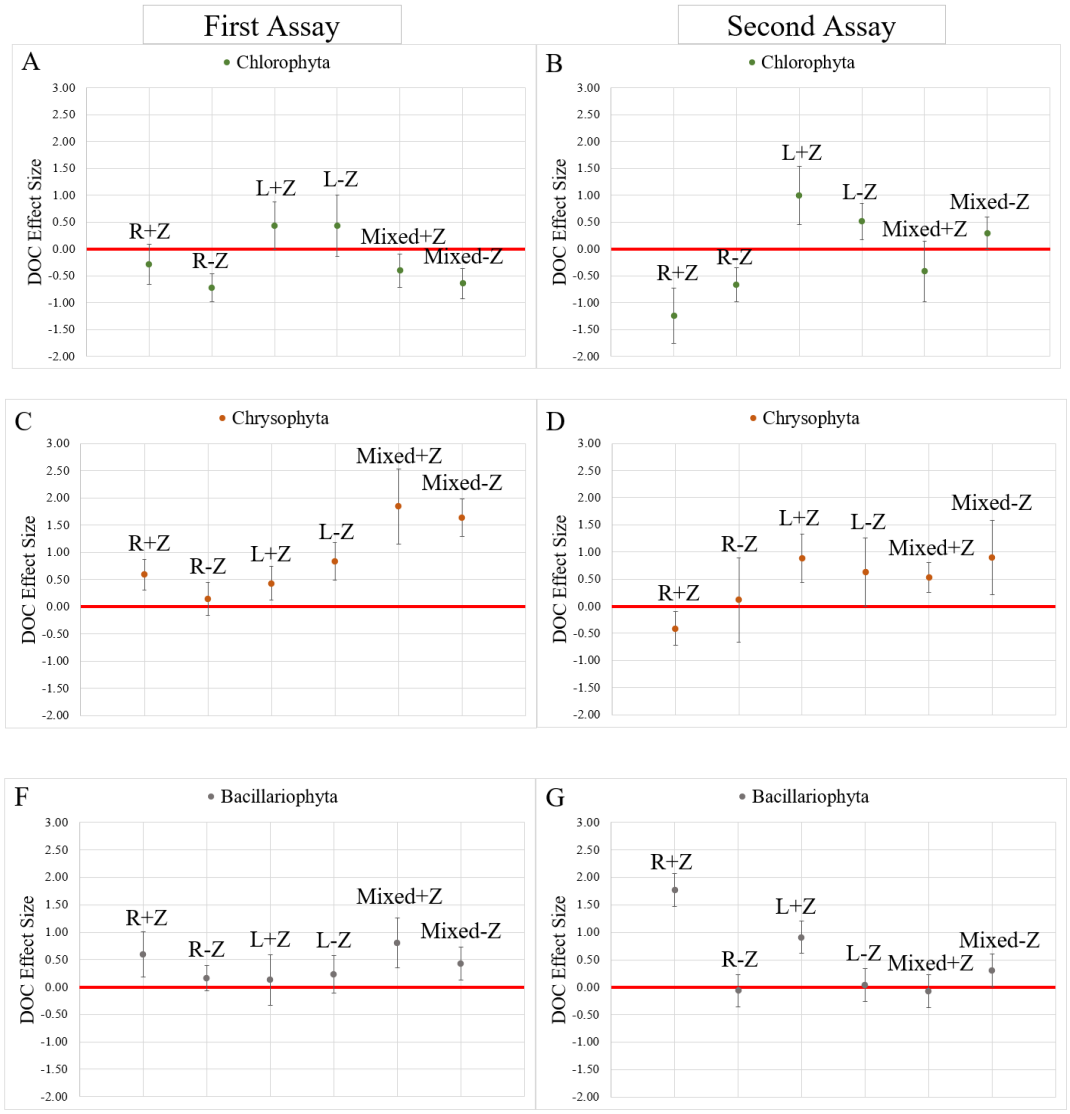


Figure 3.11 The effect size of the different DOC treatments on (A, B) *Chlorophyta spp.*, (C, D) *Chrysophyta spp.*, and (F, G) *Bacillariophyta spp*. biomasses in first and second *in-situ* grazing assays (C-Z: no DOC Control, C+Z: no DOC control with mesozooplankton, R-Z: Recalcitrant DOC, R+Z: Recalcitrant DOC with mesozooplankton, L-Z: Leaf leachate DOC, L+Z: Leaf leachate DOC with mesozooplankton, Mixed-Z: Combined recalcitrant and Leaf leachate DOC source, Mixed +Z: Combined recalcitrant and Leaf leachate DOC source with

mesozooplankton). Error bars are 95% confidence intervals. The effect of DOC is significant if the confidence interval does not overlap zero (the red lines).

3.2.2.2 Assessment of Impact of Grazing (Top-Down) Using LRR (Effect Size)

In the first *in-situ* grazing assay, mesozooplankton decreased total phytoplankton biomass in all DOC treatments (average effect size value about -0.5) (Figure 3.12 A). In the second grazing assay, the effect was similar to the first assay except for the L-DOC treatment (Figure 3.12 B). The effect size values were similar to those observed in the first assay, though the grazing effect was strongest in the mixed DOC treatment (-1.33±0.49 CI, Figure 3.12 B). Overall, mesozooplankton grazing decreased total phytoplankton biomass regardless of DOC treatments in both assays, except the L-DOC treatment in the second assay (Figure 3.14 A, B).

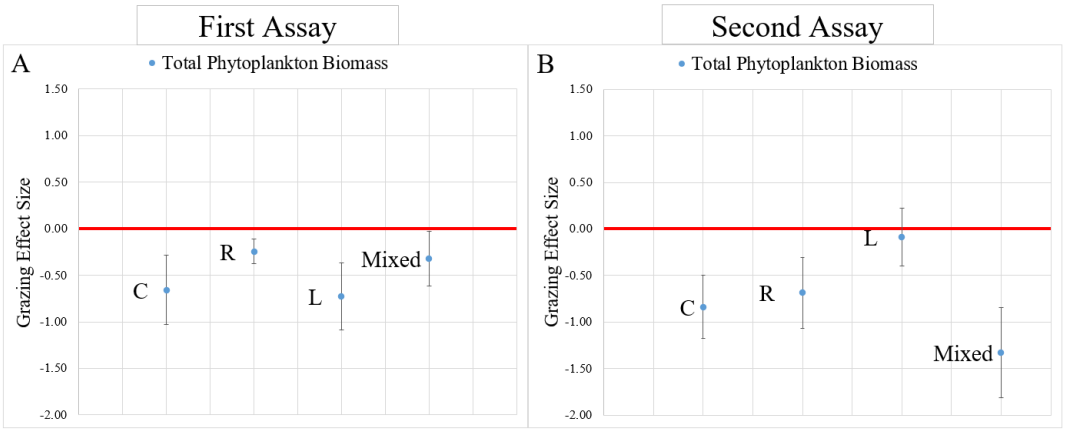


Figure 3.12 The mesozooplankton grazing effect size on total phytoplankton biomass in the first (A) and second (B) *in-situ* grazing assay (effect of grazer in; C: no DOC Control, R: Recalcitrant DOC, L: Leaf Leachate DOC, Mixed: Combination of recalcitrant and leaf leachate DOC sources treatments). Error bars are 95%

confidence intervals. The effect of mesozooplankton grazing is significant if the confidence interval does not overlap zero (red line).

In both of the *in-situ* assays, mesozooplankton grazing effect on *Chlorophyta* biomass was insignificant in almost all DOC treatments (Figure 3.13 A, B), with significant negative effects observed in the C treatment (first assay, -0.49 ±0.33 CI) and the mixed DOC treatment (second assay, -0.84±0.35 CI). In contrast, grazing effect size values were generally negative for *Chrysophyta* biomass. Specifically, in the first assay, effect size values for *Chrysophyta* biomass were negative in the C (-0.44±0.29 CI) and L-DOC treatments (-0.85±0.36 CI) (Figure 3.13 C). In second mesocosm assay, effect size values for *Chrysophyta* biomass were negative in all DOC treatments except L-DOC, which was insignificant (Figure 3.13 D). Finally, grazing effect size values were negative for *Bacillariophyta* biomass in all DOC treatments and in both grazing assays (Figure 3.13 F, G). Overall, grazers had the strongest negative effect on *Bacillariophyta*, followed by DOC specific effects on *Chrysophyta*, and the least effect on *Chlorophyta* (Figure 3.14 C, D).

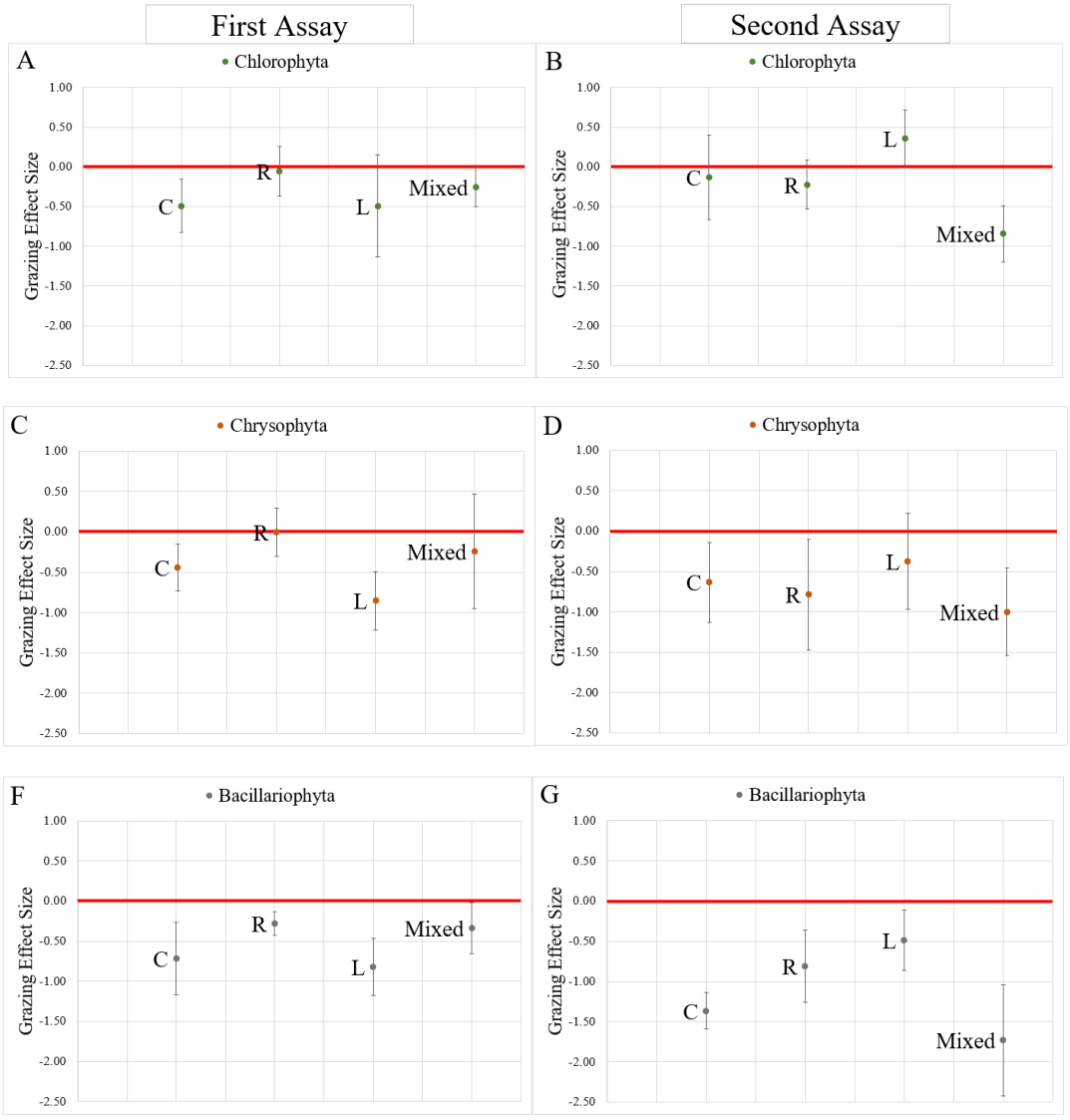


Figure 3.13 The effect size of mesozooplankton on (A, B) *Chlorophyta spp.*, (C, D) *Chrysophyta spp.* (F, G) *Bacillariophyta spp*. biomass in first and second *in-situ* grazing assays (effect of grazer in; C: no DOC Control, R: Recalcitrant DOC, L: Leaf Leachate DOC, Mixed: Combination of recalcitrant and leaf leachate DOC sources treatments). Error bars are 95% confidence intervals. The effect of mesozooplankton grazing is significant if the confidence interval does not overlap zero (the red lines).

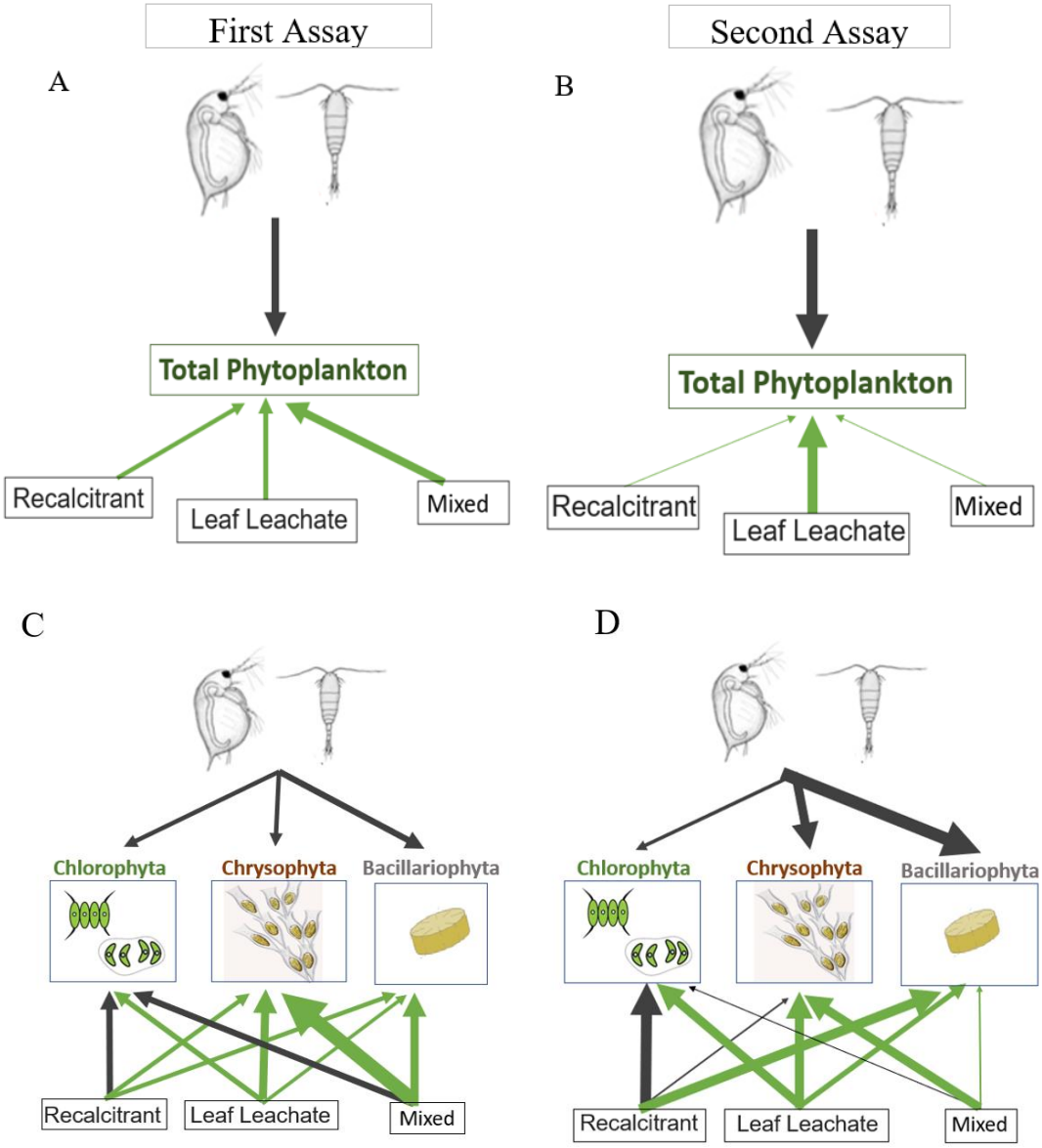


Figure 3.14 Summary figure showing the relative mean effect size value of DOC type (bottom up effect) and mesozooplankton grazers (top-down effect) on the total phytoplankton (A, B) and biomass of the dominant phytoplankton taxa (*Chlorophyta*, *Chrysophyta* and *Bacillariophyta*) in the (C, D) first and second *in-situ* grazing assays. Arrow width is scaled to the absolute value of the mean effect

size (mean of pooled grazer treatment for DOC effect, and pooled DOC treatments for the grazer effect). Arrows indicated positive (green) and negative effects (**black**).

3.2.3 Effect of Treatments of Phytoplankton Species Composition

Phytoplankton composition was dominated by *Bacillariophyta* (76±9.58%), *Chlorophyta* (18±9.04%) and least by Chrysophyta (6±3.95%) species at the end of the first *in-situ* grazing assay (Figure 3.15). The phytoplankton community composition varied across DOC treatments but did not vary by the presence of grazers (Table 3.9). Although all treatments were dominated by *Bacillariophyta*, the percent contribution of *Chlorophyta* and *Chrysophyta* biomass were different depending on the DOC type. For example, R-DOC and mixed DOC decreased percent contribution of *Chlorophyta* compared to the C and L-DOC treatment (Table 3.9, 3.10). The mixed DOC treatment significantly increased percent contribution of *Chrysophyta* compared to C and R-DOC treatments. Finally, the R-DOC treatment increased the percent *Bacillariophyta* biomass contribution compared to the C and L-DOC treatments. Similarly, the % contribution of *Bacillariophyta* was higher in the mixed DOC treatment compared to the L-DOC. Overall, in R-DOC treatments, part of *Chlorophyta* biomass contribution was replaced with *Bacillariophyta*, while *Chrysophyta* biomass contribution increased in mixed DOC, compared to the no DOC control (C).

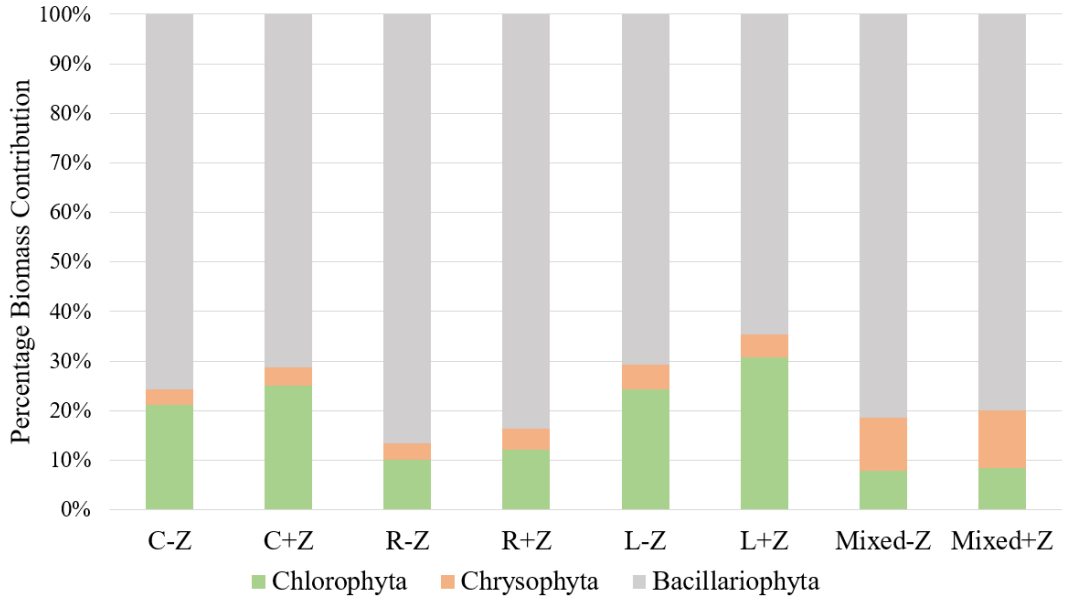


Figure 3.15 Percentage contribution of three phytoplankton phylum (*Chlorophyta*, *Chrysophyta* and *Bacillariophyta*) in terms of biomass for each treatment on the first *in-situ* grazing assay (C-Z: no DOC Control, C+Z: no DOC control with mesozooplankton, R-Z: Recalcitrant DOC, R+Z: Recalcitrant DOC with mesozooplankton, L-Z: Leaf leachate DOC, L+Z: Leaf leachate DOC with mesozooplankton, Mixed-Z: Combined recalcitrant and Leaf leachate DOC source, Mixed +Z: Combined recalcitrant and Leaf leachate DOC source with mesozooplankton).

Table 3.9 Generalize Linear Model (GLM) analysis results showing the effect of types of DOC and grazer treatments on percent contribution of phytoplankton species/phylum biomasses (*Chlorophyta, Chrysophyta* and *Bacillariophyta*) compared to respective controls in first *in-situ* grazing assay (C: no DOC Control, R: Recalcitrant DOC, L: Leaf Leachate DOC, Mixed: Combination of recalcitrant and leaf leachate DOC sources treatments, +Z: with mesozooplankton, -Z: without mesozooplankton) (i.e., effect of factor X1 on Y compared to factor X2).

Dependent Variable(Y)	*Factor X1*	*Factor X2*	*Estimate*	*Standard Error*	*T value*	*P value*
Chlorophyta	R	C	-14.19	5.10	-2.78	**0.0097****
Chlorophyta	Mixed	C	-16.33	5.10	-3.20	**0.0035 ****
Chlorophyta	L	C	-0.82	5.10	-0.16	0.8728
Chlorophyta	+Z	-Z	4.65	3.60	1.29	0.2075
Chrysophyta	R	C	-0.65	2.37	-0.28	0.7855
Chrysophyta	Mixed	C	6.22	2.37	2.62	**0.0141 ****
Chrysophyta	L	C	1.79	2.37	0.75	0.4567
Chrysophyta	+Z	-Z	-0.33	1.68	-0.19	0.8447
Bacillariophyta	R	C	14.84	5.30	2.80	**0.0093****
Bacillariophyta	Mixed	C	10.04	5.30	1.90	0.0687
Bacillariophyta	L	C	-1.44	5.30	-0.27	0.7879
Bacillariophyta	+Z	-Z	-4.30	3.75	-1.15	0.2609

Table 3.10 Generalize Linear Model (GLM) analysis results showing the effect of types of DOC treatments on percent contribution of phytoplankton species/phylum biomasses (*Chlorophyta*, *Chrysophyta* and *Bacillariophyta*) in the first *in-situ* grazing assay. (C: no DOC Control, R: Recalcitrant DOC, L: Leaf Leachate DOC, Mixed: Combination of recalcitrant and leaf leachate DOC sources treatments) (i.e., effect of factor X1 on Y compared to factor X2).

Dependent Variable(Y)	*Factor X1*	*Factor X2*	*Estimate*	*Standard Error*	*T value*	*P value*
Chlorophyta	Mixed	R	-2.14	5.10	-0.42	0.6781
Chlorophyta	L	R	13.37	5.10	2.62	**0.0142 ***
Chlorophyta	Mixed	L	-15.50	5.10	-3.04	**0.0052 ****
Chrysophyta	Mixed	R	6.87	2.37	2.89	**0.0073****
Chrysophyta	L	R	2.44	2.37	1.03	0.3121
Chrysophyta	Mixed	L	4.43	2.37	1.87	0.0725
Bacillariophyta	Mixed	R	-4.80	5.30	-0.90	0.3730
Bacillariophyta	L	R	-16.28	5.30	-3.07	**0.0048****
Bacillariophyta	Mixed	L	11.48	5.30	2.17	**0.0399 ***

At the end of the second *in-situ* grazing assay, phytoplankton composition was still dominated by *Bacillariophyta* (56±13.43%), followed by *Chlorophyta* (36±11.04%), and least by *Chrysophyta* (7±5.76%) (Figure 3.16). The phytoplankton community composition varied across DOC treatments, and in contrast to the first assay, was also affected by the presence of grazers (Table 3.11, 3.12). Specifically, R-DOC significantly decreased the percent contribution of *Chlorophyta* biomass compared to C, L-DOC and mixed DOC treatments (Table 3.11, 3.12). Moreover, mixed DOC significantly increased the percent contribution of *Chrysophyta* biomass compared to the no DOC control. Notably, none of the DOC treatments had a significant effect on the % contribution of *Bacillariophyta* when compared to the no DOC control

(Table 3.11). When compared among the DOC treatments only (i.e., excluding the no DOC control), however, the R-DOC treatment significantly increased the % contribution of *Bacillariophyta* compared to the mixed DOC and L-DOC treatments. On the other hand, mesozooplankton grazing increased the *Chlorophyta* biomass contribution while decreasing the *Bacillariophyta* percent biomass contribution significantly, while having no effect on the relative biomass of *Chrysophyta* (Table 3.11).

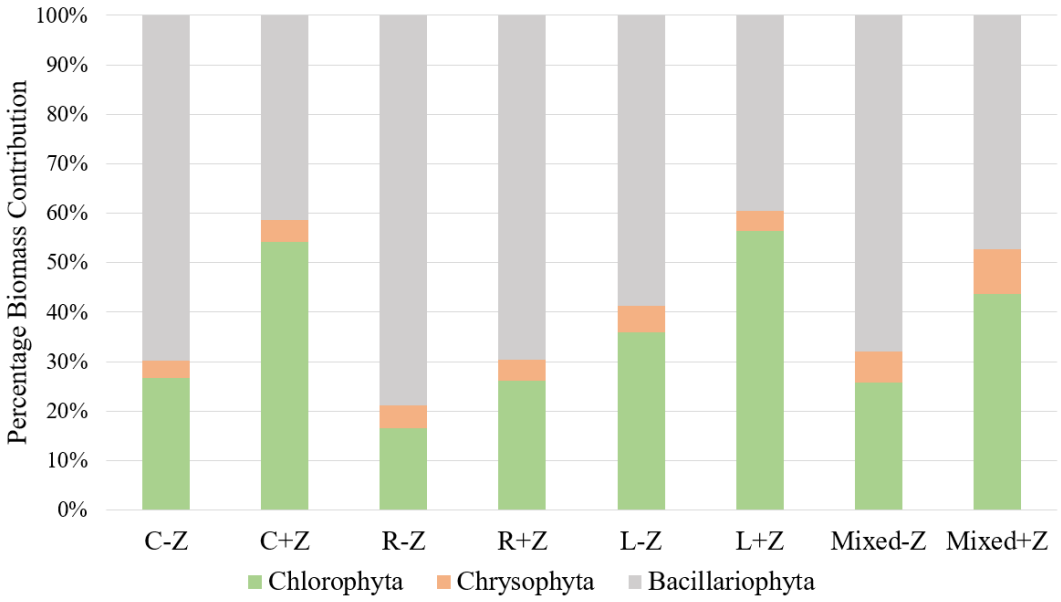


Figure 3.16 Percentage contribution of three dominant phytoplankton phyla biomass (*Chlorophyta*, *Chrysophyta* and *Bacillariophyta*) for each treatment in the second *in-situ* grazing assay (C-Z: no DOC Control, C+Z: no DOC control with mesozooplankton, R-Z: Recalcitrant DOC, R+Z: Recalcitrant DOC with mesozooplankton, L-Z: Leaf leachate DOC, L+Z: Leaf leachate DOC with mesozooplankton, Mixed-Z: Combined recalcitrant and Leaf leachate DOC source, Mixed +Z: Combined recalcitrant and Leaf leachate DOC source with mesozooplankton).

Table 3.11 Generalized Linear Model (GLM) analysis results showing the effect of types of DOC and grazer treatments on percent contribution of phytoplankton species/phylum biomasses (*Chlorophyta*, *Chrysophyta* and *Bacillariophyta*) compared to respective controls on the second *in-situ* grazing assay (C: no DOC Control, R: Recalcitrant DOC, L: Leaf Leachate DOC, Mixed: Combination of recalcitrant and leaf leachate DOC sources treatments , +Z : with mesozooplankton , -Z : without mesozooplankton) (i.e., effect of factor X1 on Y compared to factor X2).

Dependent Variable(Y)	*Factor X1*	*Factor X2*	*Estimate*	*Standard Error*	*T value*	*P value*
Chlorophyta	R	C	-13.91	5.71	-2.43	**0.0218***
Chlorophyta	Mixed	C	1.14	5.71	0.20	0.8435
Chlorophyta	L	C	7.74	5.71	1.36	0.1865
Chlorophyta	+Z	-Z	15.33	4.04	3.79	**0.0008*****
Chrysophyta	R	C	1.09	3.27	0.33	0.7424
Chrysophyta	Mixed	C	7.27	3.27	2.22	**0.0349***
Chrysophyta	L	C	2.24	3.27	0.68	0.5000
Chrysophyta	+Z	-Z	0.94	2.31	0.41	0.6883
Bacillariophyta	R	C	12.95	7.12	1.82	0.0801
Bacillariophyta	Mixed	C	-11.80	7.12	-1.66	0.1091
Bacillariophyta	L	C	-12.99	7.12	-1.82	0.0792
Bacillariophyta	+Z	-Z	-17.92	5.04	-3.56	**0.0014*****

Table 3.12 Generalized Linear Model (GLM) analysis results showing the effect of types of DOC treatments on percent contribution of phytoplankton species/phylum biomasses (*Chlorophyta*, *Chrysophyta* and *Bacillariophyta*) in second *in-situ* grazing assay (C: no DOC Control, R: Recalcitrant DOC, L: Leaf Leachate DOC, Mixed: Combination of recalcitrant and leaf leachate DOC sources treatments). (i.e., effect of factor X1 on Y compared to factor X2).

Dependent Variable(Y)	*Factor X1*	*Factor X2*	*Estimate*	*Standard Error*	*T value*	*P value*
Chlorophyta	Mixed	R	15.05	5.71	2.63	**0.0138****
Chlorophyta	L	R	21.66	5.71	3.79	**0.0008*****
Chlorophyta	Mixed	L	-6.60	5.71	-1.16	0.2577
Chrysophyta	Mixed	R	6.18	3.27	1.89	0.0696
Chrysophyta	L	R	1.15	3.27	0.35	0.7278
Chrysophyta	Mixed	L	5.03	3.27	1.54	0.1360
Bacillariophyta	Mixed	R	-24.75	7.12	-3.48	**0.0017****
Bacillariophyta	L	R	-25.94	7.12	-3.64	**0.0011*****
Bacillariophyta	Mixed	L	1.19	7.12	0.17	0.8686

CHAPTER 4

DISCUSSION

4.1 Laboratory Experiment

The aim of the laboratory experiment was investigating the DOC and zooplankton grazing effects on phytoplankton community. As expected, DOC decreased total phytoplankton biomass, and the largest decrease was observed in the smallest species, *Chlamydomonas*, was followed by *Cyclotella* spp. and the mixotrophic *Cryptomonas*. Zooplankton grazing also decreased total phytoplankton biomass, which was largely by a reduction in *Cyclotella* biomass. Besides, DOC changed the phytoplankton community composition to favor mixotrophs from the beginning to the end of the experiment. Contrary to expectation, however, copepods were significantly stronger grazers than *Daphnia*.

4.1.1 DOC and Indirect Grazing Effects

By the end of experiment, DOC significantly decreased total phytoplankton biomass and the presence of grazers did not affect magnitude of negative effect of DOC. In previous studies, reduction in phytoplankton biomass was attributed to browning effect of DOC, where DOC created light limitation (Kankaala et al., 2010; Lebret et al., 2018). While light limitation may have explained these results, however, effect the small volume and depth of the experimental jars (0.6 L) limits the degree of light limitation in our setup. Thus, there may be other mechanisms that explain the negative effect of DOC on phytoplankton observed here (Figure 4). For example, DOC may increase the biomass of micro-grazers' growth (i.e., ciliates for our design) through enhancing heterotrophic pathway (i.e., increasing bacterial biomass by proving a labile carbon source) (Wehr et al., 1998) in fact, Yetim et al. in prep.

demonstrated that ciliate biomass increased and thus micro-grazers grazing pressure on phytoplankton biomass increased. This indirect effect of DOC on phytoplankton via enhancing micro-grazers is poorly understood, and our results indicate that potentially strong micro-grazers grazing (i.e., ciliates), may be an key regulator of DOC effects on phytoplankton biomass. Thus, even without any brownification effect, DOC could decrease total phytoplankton biomass most likely via increase ciliate grazing on phytoplankton in the DOC treatments.

The magnitude of negative DOC effect was species-specific. For example, the most indirect negative effect of DOC was observed on *Chlamydomonas*, which might be related with light limitation and its size. As in our experimental design *Chlamydomonas* was the most photosynthetic species, light limitation of DOC most likely to limit its growth by decreasing available light for photosynthesis (Jannson 1986; Lischke et al., 2015; Kanayama et al., 2020). Besides, since they were the smallest species, they were likely under stronger micro-grazing pressure (Hansen et al., 1994; Kanayama et al., 2020)) (Gall et al., 2017; Lischke et al. ,2015). Specifically, DOC effect on *Chlamydomonas* was the strongest in no grazer control, where there was no top-down control on ciliates to release phytoplankton from ciliate grazing. On the other hand, the least negative indirect DOC effect on *Chlamydomonas* was observed in the copepod treatments, might suggesting that copepod could consume ciliates, so ciliate grazing on *Chlamydomonas* was likely suppressed via top-down control (Wickham and Gilbert, 1993; Wickham, 1998; Stibor et al., 2004; Schnetzer et al., 2005; Lischke et al. 2015; Kunzmann et al., 2019). *Daphnia* also lead to the same effect but not as strong as copepods, this might indicate that copepods were more effective controller over ciliate (Burns et al., 2001; Stibor et al., 2004). According to literature, first preference of ciliate would be *Chlamydomonas*, likely resulted in least ciliate grazing effect on *Cryptomonas* and *Cyclotella* due to their larger size (Lischke et al., 2015; Kanayama et al., 2020). Also, as *Cyclotella* species were better adapted to low light conditions, they were not probably too much affected from light limitation caused by DOC (Reynolds et. al., 1988; Ismael, 2003). Moreover, *Cryptomonas* were mixotrophic species so even if

light limitation decrease their growth, possible consumption on bacteria might support their growth (Porter 1988; Sanders et. al., 1988; Caron et. al., 1992). Overall, DOC negatively affected phytoplankton species biomass likely by light limitation and increasing micro-grazing on them, that could be regulated by top-down control over micro-grazers, the most probable micro-grazing was on smallest species, *Chlamydomonas*. However, some other feature of phytoplankton species might decrease negative affect of DOC which should be studied for future studies.

Phytoplankton species percentage biomass contribution changed through the 4-day long experiment to higher contribution of mixotrophic *Cryptomonas*, especially in with DOC treatments, likely by ingesting nutrients and DOC supported bacteria (advantage over obligate autotrophs) (Ismael, 2003; Urrutia-Cordero et.al., 2017; Kanayama et al., 2020).

4.1.2 Grazing Effect

Copepods significantly decreased total phytoplankton biomass, but not *Daphnia*, indicating a strong role of contrasting grazing traits in plankton food webs. This also highlights a *Daphnia* bias in the literature. In the literature, *Daphnia* is considered as the most efficient grazer (Lampert 1978, 1988; Sommer et al. 1986). *Daphnia* are generally dominant species in temperate freshwater ecosystems, and so they are studied more compared to freshwater copepods (Sommer et al., 2001; Shurin et al., 2002). In our study, stronger copepod herbivory may be a result of selective ingestion of larger and higher nutrient quality phytoplankton prey like diatoms, and less ingestion of smaller autotrophic or bacterial prey that *Daphnia* is expected to graze on (Hansen 1997; Stibor et al., 2004; Calbet et al., 2005). Copepods have a larger optimal predator:prey size ratio (18:1) compared to *Daphnia* (50:1) (Hansen et al., 1994), and actively select larger particles compared to *Daphnia* (Frost 1972; Gliwicz 1980; Geller & MuÈller 1981; Kleppel 1993; Sommer et al. 2000, 2001). Thus, our study highlighted that copepods could be stronger herbivores than *Daphnia* in freshwater plankton ecosystem and that future studies on the top-down effect of

zooplankton should account for differences in zooplankton grazing traits such as selectivity.

Specifically, *Cyclotella* was the most preferred prey likely due to its non-motile feature and highest abundance, which might increase prey-predator encounter (Reynolds 2006; Carbone et al., 2010). However, being selective feeder made copepods stronger grazer on nutrient rich and large *Cyclotella* compared to generalist *Daphnia* (Cowles et al., 1988; Schnetzer et al., 2005). This was also suggested that both quality and density-dependent grazing on phytoplankton species together with effects of species abilities (DeMott 1995). Additionally, effect size results indicated positive copepod grazing effect on *Chlamydomonas* in presence of DOC, which might suggest that copepod decreased the micro-grazing pressure on *Chlamydomonas* by feeding on ciliates. Also, copepod grazing on *Chlamydomonas* might decreased as copepods likely to prefer DOC promoted ciliates. Moreover, as *Daphnia* were generalist feeders, they did not affect the percent community composition of phytoplankton. On the other hand, selective feeder copepods decreased *Cyclotella* by mostly feeding on them and indirectly increased *Chlamydomonas* by decreasing micro-grazing pressure on them. Overall, throughout the experiment, copepods were stronger grazers and changed the community composition of phytoplankton while generalist *Daphnia* did not have a significant effect on community composition.

Thus, our laboratory experiment showed that DOC had stronger affect on phytoplankton community compared to grazing affect.

4.2 *In-situ* Mesocosm Grazing Assays

In-situ mesocosm grazing assays examined the effects of different DOC sources and mesozooplankton grazing on phytoplankton community. In contrast to expectation, DOC either had a positive or no effect on phytoplankton biomass. Specifically, mixed DOC increased total phytoplankton biomass and *Chrysophyta* and *Bacillariophyta* biomass, while recalcitrant DOC only increased *Bacillariophyta* biomass in the first assay. In second in-situ grazing assay, leaf leachate DOC increased total phytoplankton biomass, specifically increased *Chlorophyta* biomass, indicating the positive effect of nutrient addition. On the other hand, in both assays mesozooplankton decreased total phytoplankton biomass, particularly, *Bacillariophyta* biomass. Overall, results highlighted how DOC did not have the expected negative effect, and how DOC quality, including macronutrient content, is a key factor regulating its effects. Moreover, results show the taxa specific effects of different DOC sources.

4.2.1 DOC and Indirect Grazing Effects

In terms of DOC effect, there is a trade-off between light and nutrient availability (Seekel 2015), that increasing DOC provides nutrient while it decreases light transparency of water column. Moreover, phytoplankton biomass generally peaks at intermediate levels where they obtain nutrient provided by DOC and have sufficient light to perform photosynthesis (Cottingham et al., 2013). Thus, negative effect of brownification (i.e., light limitation) could compensate with coming nutrient until some threshold which is specific to lake and species (Ask et al., 2009).

In the first *in-situ* grazing assay mixed DOC increased total phytoplankton biomass, suggesting that leaf leachate DOC provided carbon and nutrients (i.e., P and N) to both phytoplankton and likely to bacteria which could promote mixotrophic phytoplankton species (Porter 1988; Kankaala et al., 2010; Cottingham et al., 2013;

Faithfull et al., 2015; Calderó-Pascual et al., 2021). Specifically, mixotrophic *Chrysophyta* biomass increased in mixed DOC treatment, likely by using mixotrophic abilities; feeding on DOC supported bacteria or directly on DOC particles (Porter 1988; Sanders et. al., 1988; Caron et. al., 1992). In contrast, the effect size results showed that *Chlorophyta* decreased in mixed and recalcitrant DOC treatment, likely due to light limitation and ciliate grazing, as they are highly sensitive to grazing by micro-zooplankton (Jannson 1986; Hansen et al., 1994; Stibor et al., 2004; Lischke et al., 2015; Kanayama et al., 2020). However, obligate autotrophs *Bacillariophyta* (i.e., diatom) significantly increased in mixed and recalcitrant DOC treatments since their low-light adapted nature likely make them less vulnerable for brownification (Reynolds et. al., 1988; Ismael, 2003). Moreover, recent reviews revealed the higher silicification respond to DOC addition and CO_2 increase, which could also positively affected *Bacillariophyta* in these treatments (Znachor et al., 2010; Bach et al., 2019). Besides, considering that *Chlorophyta* species require high light availability (due to their buoyancy) and nutrient than *Bacillariophyta* (Reynolds et. al., 1988; Bottino et. al., 2018), so *Bacillariophyta* might increase their biomass by using disadvantage of *Chlorophyta* in mixed and recalcitrant DOC treatments.

In the second mesocosm assay, leaf leachate DOC increased total phytoplankton biomass, likely by providing labile nutrients and bacteria (Kankaala et al., 2010; Cottingham et al., 2013; Faithfull et al., 2015, Calderó-Pascual et al., 2021). Yet, according to effect size results, only leaf leachate DOC with zooplankton treatment had significantly positive effect on total phytoplankton biomass, specifically, the presence of grazer enhanced the positive DOC effect. In this case, grazers in leaf leachate treatments could consume on micro-grazers or bacteria, as DOC supports their biomass (Hessen, 1985; Tranvik, 1988; Solomon et al., 2015; Degerman et al., 2018). However, mesozooplankton in no DOC control treatments were expected to decrease phytoplankton biomass more since there were not expected a bacteria or ciliate boost. Thus, grazers in leaf leachate treatments might consume more on micro-grazers and bacteria than phytoplankton, which results in a clear positive DOC

effect on phytoplankton biomass by additional labile nutrients in presence of grazers (Calderó-Pascual et al., 2021). In other word, leaf leachate DOC likely to promote heterotrophic pathway rather than autotrophic pathway, so meso-grazers in leaf leachate treatments did not decrease phytoplankton biomass as much as they decreased them in no DOC controls.

In the second mesocosm assay, in species specific response, positive leaf leachate DOC effect on *Chlorophyta* likely due to addition of extra labile nutrients (Reynolds et. al., 1988; Bottino et. al., 2018). In addition, negative recalcitrant DOC effect on *Chlorophyta* likely by decreasing light attenuation. On the other hand, in DOC effect size results, recalcitrant and leaf leachate DOC effect on *Bacillariophyta* were *significantly* positive only in presence of mesozooplankton since both DOC sources likely to promote heterotrophic pathway resulting in suppressed grazing on phytoplankton. In the recalcitrant DOC treatment, increase in *Bacillariophyta* might be related with decrease in *Chlorophyta* due to competition between them for both capturing the light and nutrient (Urrutia-Cordero et al. 2017). In *Chrysophyta* effect size results, likely indicated that the main positive effect on *Chrysophyta* was coming from leaf leachate DOC via fueling nutrient or promoting bacteria but effect of recalcitrant DOC, light limitation, was not effective for *Chrysophyta* due to mixotrophy (Sanders et. al., 1988; Caron et. al., 1992).

Overall, our results revealed that DOC effects depended on DOC quality and were specific across phytoplankton species. Thus, effects in nature are likely dependent on the specific phytoplankton community structure at the same time on the DOC input as well as the quality of the specific DOC source. Accounting for these differences will therefore benefit efforts at predicting plankton responses to DOC in nature.

4.2.2 Grazing Effect

In both grazing assays, regardless of DOC sources, mesozooplankton (i.e., *Cladocerans* and copepods) significantly decreased total phytoplankton biomass, particularly *Bacillariophyta* by strong top-down control. Since *in-situ* experimental bottles with zooplankton treatment were dominated by generalist *Cladocerans*, it is likely that they consumed the most abundant species in bottles. Thus, *Bacillariophyta's* non-motile feature as they cannot escape from predator and their relatively higher abundance could increase their prey-predator encounter and so the consumption on them (Reynolds et. al., 1988). Moreover, their large size and higher nutrient quality could make them more vulnerable to selective feeder copepod compared to other species (Cowles et al., 1988). Grazing effect on other species were weaker likely due to their low relative abundance and motile ability. Besides, having larger size and forming colonies as a defense strategy might protect *Chrysophyta* from grazing pressure (Lürling, 2021). Overall, strongest grazing pressure observed on *Bacillariophyta* likely due to their higher relative abundance, non-motile ability, and nutrient quality.

In first grazing assay, grazing effect size results for *Chrysophyta* were only significantly negative in no DOC control and leaf leachate DOC treatments. This might suggest that for grazers, handling with recalcitrant DOC particles may lead to loss of time rather than grazing on phytoplankton (Frost, 1972; DeMott, 1995). Grazers negatively affected *Chlorophyta* in only no DOC control treatment. Specifically, in no DOC control, *Cladocerans* could consume bacteria, so in presence of mesozooplankton, prey (i.e., bacteria) for ciliates might decrease and ciliate likely to consume more on *Chlorophyta*. In with DOC treatments, this is not the case since DOC expected to provide additional support for bacteria biomass.

In second grazing assay, only significant difference between DOC treatments was observed between leaf leachate and mixed DOC treatment, where grazers more negatively affected total phytoplankton, *Chlorophyta* and *Bacillariophyta* biomasses in mixed DOC. This might be related with having least total mesozooplankton biomass in leaf leachate treatment. Moreover, leaf leachate DOC could promote mesozooplankton grazing on ciliate and bacteria rather than phytoplankton. Specifically, effect size results revealed that grazers negatively affected *Chrysophyta* in all treatments, in other words, grazing effect on *Chrysophyta* did not depend on presence or sources of DOC since being motile and relatively larger size compared to other species determined grazing rate here.

4.3 General Discussion

Why was the mixed DOC effect on phytoplankton biomass in laboratory experiment negative, while it was positive in mesocosm assays? The main difference between experiments was nutrient limitation as *in-situ* mesocosm assays were nutrient limited, but laboratory experiment was not since we added nutrients with WC medium (Calderó-Pascual et al., 2021). Another potential explanation is the different DOC concentrations and sources. In mesocosm, total DOC concentration was lower than 10 mg C L^{-1} (R: ~1.5, L: ~8 , Mixed ~9.5 mg C L^{-1}) but in laboratory concentration was 10.91 mg C L^{-1}. In the literature, previous studies showed that DOC concentration above 10 mg C L^{-1} generally decreased phytoplankton by light limitation, while below 7 mg C L^{-1} increased their biomass by fueling nutrients (Feuchtmayr et al., 2019). Also, leaf leachate DOC sources were different between laboratory (poplar tree) and mesocosm experiments (alder tree), so their nutrient release or proportion of labile carbon content could be different. Thus, these differences might create different results.

Bacteria, phytoplankton, ciliate, and zooplankton communities of both experiments were not identical. Specifically, in laboratory experiment copepods and *Daphnia* were equal in biomass; however, in mesocosm assays, it was *Cladocerans* dominant. Besides, in laboratory experiment, biomass of zooplankton in all treatments and replicates was stable, but in mesocosm, different DOC treatments had different zooplankton biomass. Also, laboratory experimental jars contain almost 27 times more zooplankton biomass compared to mesocosm grazing assays bottles. Finally, experimental durations were also different, 4-day long laboratory experiment and 1-day in-situ mesocosm assays. Thus, these differences created the distinguishable responses to DOC and grazing effects.

Why GLM results were different than the effect size results? Both statistical methods were used to identify differences due to DOC or grazing effect; however, in some points effect size results revealed some magnified results which were insignificant in GLM results. The answer was difference in calculations of both methods, in other word, both has different variances. While calculated significancy of treatment effects, GLM pooled all data; for example, to calculate DOC effect, GLM pooled +DOC and -DOC results regardless of grazer treatments and compare them (pooled data of $+DOC_{No\ grazer}$, $+DOC_{Copepod}$, and $+DOC_{Daphnia}$ and same for -DOC), thus differences in different grazer treatments were disappeared while looking at DOC effect (Hastie and Pregibon 2017). In contrary, the effect size results calculated the effects magnitude and significancy by comparing specific treatment with its control (e.g., $+DOC_{No\ grazer}$ vs. $-DOC_{No\ grazer}$), so it directly provided DOC and grazer specific results (Hillabrand et al., 2016). Thus, the GLM results gave us an overview of the DOC and grazer treatments effects, whereas the effect size provided more closer look to each specific treatments effects.

CHAPTER 5

CONCLUSION

Our results contribute to a growing understanding to effects of DOC and different grazing traits on freshwater phytoplankton ecosystems. The impacts vary depend on type of DOC, time, species community and grazing traits. Specifically, we showed that DOC might decrease phytoplankton via browning or related with ciliate grazing, while it could also increase phytoplankton biomass via fueling nutrient and bacteria for mixotrophs. On the other hand, presence of grazer decreased phytoplankton biomass, in particular copepod were stronger grazers than Daphnia, especially for larger phytoplankton species. In addition, top-down and bottom-up effect could neutralize or stimulate each other depend on DOC or grazer type and these interactions are critical to understand results of DOC effect via climate change, flood event. Our study provides an insight for these issues; however, in the future role of other micro-grazers (e.g., HNF or rotifers) and stoichiometric impact of DOC on these organisms needed to be studied and clarified. Both DOC quality and the grazer community matters. DOC effects or grazer effects are not universal and depend on DOC-grazer-prey interactions. Predicting DOC effects would thus benefit from understanding these underlying mechanisms.

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