Phytoplankton-zooplankton interactions in the context of biodiversity loss: a trait-based perspective

Inaugural-Dissertation
zur Erlangung des Doktorgrades
der Mathematisch-Naturwissenschaftlichen Fakultat
der Universitat zu Köln

vorgelegt von

Jessica Titocci
aus Rom, Italien

Köln, 2023

Berichtersatter:	PD Dr. Patrick Fink
	Prof. Dr. Eric von Elert
Tag der mündlichen Prüfung:	03.08.2023

A me stessa,
per averci creduto
e per non aver mollato.

Ai miei genitori e
ai miei piú cari amici
per esserci stati sempre e
per aver contribuito con tutto
il loro affetto ed il loro supporto
al raggiungimento
di questo mio obiettivo.

Remember! The actual outcome of your PhD is not your dissertation.

The outcome is YOU!

Table of contents

Abstract

Freshwater biodiversity is increasingly threatened by human activities and environmental changes, which have already caused severe declines in the range and abundance of many freshwater organisms. Since ecosystem functioning is highly dependent on biodiversity, both in terms of the distribution and abundance of organisms present in the ecosystem and, even more so, in terms of their functional characteristics, it has become increasingly urgent and necessary to investigate the impact that changes in biodiversity can have on the ecological functioning of aquatic systems. Although a large number of studies have already investigated and described the role of diversity and the consequences of diversity loss using taxonomic-based biodiversity metrics, this has resulted in an insufficient explanatory power, and the implementation of realistic loss scenarios and the identification of mechanisms underlying the relationship between biodiversity and ecosystem functioning remain challenging. In this context, the field of biodiversity-ecosystem functioning (BEF) studies is increasingly adopting a trait-based perspective. Monitoring functional traits and trait variability allows to determine the nature and strength of species interactions and the community responses and organisation to changes. By looking at their underlying mechanisms, we can better understand the role of biodiversity in maintaining multiple ecosystem functions and processes. Because phytoplankton are at the base of aquatic food webs and are of immense importance for global-scale processes such as oxygen and primary production, biodiversity loss at the level of primary producers has attracted particular interest among researchers, who aim to gain a better understanding of how phytoplankton species loss and functions are likely to affect trophic structure, community trait dynamics and ecosystem processes under future loss scenarios. By feeding on phytoplankton, herbivorous zooplankton is an important link in the transfer of energy, from basal resources to consumers higher in the food web. In these predator-prey interactions, effects go both ways, with phytoplankton taxonomic and functional diversity influencing and being influenced by zooplankton grazing. Thus, species and trait losses at the producer level may lead to shifts in phytoplankton composition and nutrient availability, which are thought to have cascading effects on herbivorous zooplankton abundances, composition and population dynamics, but it is not yet possible to predict the impact and direction of these changes, nor the consequences. By combining taxonomic and trait-based approaches with experimental manipulations of phytoplankton diversity, my goal was to examine how the loss of phytoplankton species and trait

diversity could affect phytoplankton-zooplankton interactions and their trait-related dynamics. Firstly, I investigated the role of phytoplankton trait diversity, examined in terms of biochemical characteristics (fatty acids) of algal food on the functional responses of the calanoid copepod *Eudiaptomus* sp., demonstrating how phytoplankton trait diversity is an important regulatory mechanism for the fitness, growth, reproduction, and survival of zooplankton and that key dietary contrasts, can shape adaptive evolution in consumers that seek to convert some missing dietary fatty acids through metabolic bioaccumulation and bioconversion mechanisms to maximize fitness and individual survival and growth. Secondly, I performed diversity manipulation experiments using size-fractionation and disturbance methods on natural phytoplankton assemblages to provide reliable simulations of phytoplankton-zooplankton trophic interactions and their consequences under changes or loss of phytoplankton trait-diversity. Specifically, I investigated how changes in phytoplankton morphological and biochemical trait diversity might affect different herbivorous zooplankton functional groups, cladocerans and calanoid copepods, represented by *Daphnia longispina* and *Eudiaptomus graciloides*, respectively. Alterations in phytoplankton morphological diversity revealed differences in phytoplankton morpho-functional traits and taxonomic composition in size-fractionated communities but did not significantly affect grazing of generalist unselective filter-feeders *Daphnia longispina* and selective feeders *Eudiaptomus graciloides*, in terms of grazing rates and size selectivity. On the contrary, alteration of phytoplankton functional diversity induced by the disturbance method allowed species losses and taxonomic shifts, resulting in the formation of distinct communities with different taxonomic and biochemical characteristics, which differentially affected the fitness, life history traits and lipid composition of both grazers, mainly depending on the differences in the grazers' feeding habits and their nutritional requirements.

Overall, combining taxonomy and trait-based approaches have provided a more comprehensive evaluation and understanding of ecological dynamics in phytoplankton-zooplankton interactions. Furthermore, the results obtained from the manipulation of the diversity of natural phytoplankton communities highlighted the importance of conducting similar experimental studies to deeply understand the mechanistic background as well as the potential impacts of biodiversity loss between phytoplankton and zooplankton, and thus more generally, in food web dynamics in aquatic ecosystems.

General introduction and aim of the study

Biodiversity

"The diversity of life forms, so numerous that we have yet to identify most of them, is the greatest wonder of this planet. The biosphere is an intricate tapestry of interwoven life forms"

(Wilson, 1988)

With these simple words, the famous entomologist Oscar Wilson, to whom we owe the rise of the term "biodiversity", praised and highlighted for the first time the immense role and potential of biological diversity on our planet. Surprisingly, the term was introduced more than 30 years ago for the necessity to qualify the impact of uncontrolled human activities on the natural environments and on the species that inhabit them, and thus, to describe the biodiversity crisis existing in the 1980s and to promote possible conservation measures. In a sense, biodiversity emerged from its own crisis as a valuable resource to be protected. Today, biodiversity has entered our lexicon and it is a central issue of scientific and political concern worldwide. Biodiversity is the foundation of our society, and its role is inestimable, although the idea of biodiversity has often changed over time and there is still a general lack of biodiversity awareness in public perception. Very often biodiversity is defined as the number of species present in a given environment, but this is extremely reductive (Cadotte *et al.*, 2011; Cernansky, 2017). Biodiversity is much more than just the number of species; it encompasses the compositional, structural, and functional diversity found at all levels of life organisation, from genes to ecosystems (Noss, 1990). On top of this, there is the array of intricate interactions occurring between organisms and the environment that strongly influence their presence or absence in ecological communities and thus determine the overall composition, functioning, and stability of ecosystems. Hence, biodiversity is "the complexity of living systems at all organization levels". It can be seen as a multi-dimensional concept encompassing genetic diversity, species diversity, ecosystem diversity and also functional diversity, with multi-faceted roles and values to maintain variability within and between them, and to support ecosystems functioning and services. The diversity of environments and living beings provides an incredible variety of resources that are fundamental to our survival. Perceiving and valuing biodiversity is essential to understand how humans interact with their surroundings and to develop meaningful policies and effective conservation management. Various evaluation techniques have been proposed and used to measure the value of biodiversity in terms of its

ecological, economic, and social aspects (Laurila-Pant *et al.*, 2015), although it remains complex and arbitrary to determine how people perceive and assign a value to biodiversity.

Biodiversity can be considered for its extrinsic value, defined in terms of human well-being, and related to the variety of products (wood, food, water, chemical organic products, genes, etc.) and ecosystem services provided through the maintenance of natural ecological processes (air quality maintenance, climate regulation, water quality, nutrient recycling, etc.), with their associated economic values. In addition, biodiversity can also be considered for its fully intrinsic value, regardless of its economic and instrumental value and other anthropogenic benefits (Nunes and van den Bergh, 2001; Justus *et al.*, 2009; Salles, 2011).

Regardless of the nature of its value, biodiversity is our wealth and immense heritage, and therefore monitoring, investigating, understanding, and preserving all aspects of biodiversity must be our primary goal (UN, 1992).

Linking biodiversity to ecosystem functioning toward trait-based approaches

One of the issues in biodiversity conservation concerns the approaches to measuring biodiversity. Efficient and reliable measurement methods for monitoring biodiversity changes and biodiversity loss are essential to track and to anticipate abrupt changes in ecosystem structure and functioning. However, due to its complexity, it can be extremely difficult to accurately quantify changes in the entirety of biodiversity and its inherent characteristics in response to different drivers of change (Navarro *et al.*, 2017). Traditionally, the species richness (number of species) and the species evenness, i.e. distribution of individuals among species (including evenness, equitability, and abundance) have served as the basis for quantifying biodiversity (Cadotte *et al.*, 2011). These biodiversity metrics provide valuable information about the structure of groups of organisms within ecosystems, however, they do not take into account the different functions that each species play in the ecosystem (Cardoso *et al.*, 2014). Taxonomic diversity metrics are widely used to assess the response of biological communities to environmental and anthropogenic changes, but they have a crucial limitation: they consider all species and individuals as equivalent (Magurran and McGill, 2010; Laureto *et al.*, 2015), ignoring their functional roles and how they affect ecosystem functioning (Naeem and Wright, 2003). Thus, conserving species richness alone is no longer

sufficient because the contributions of different species to ecosystem functions vary so much. It follows that the loss of some species may have far-reaching functional consequences and affect community structure and interactions differently than others and, therefore, taxonomic diversity alone may not capture all the key elements of biodiversity change in changing environments (Hillebrand *et al.*, 2018), providing an overly simplistic and reductive assessment. Furthermore, multiple species may perform similar, if not identical, functions in an ecosystem. This means that the removal of redundant species may not affect the functioning of an ecosystem, whereas if the removed species have unique functions, their loss may be very dramatic for ecosystem processes (Fetzer *et al.*, 2015). It must be considered, however, that some species can be functionally redundant in one environment and become pivotal in another. For all such reasons, ecologists have begun to focus on and emphasize the study of functional characteristics, or the "traits" of species. This has completely revolutionised the study of biodiversity, providing a more mechanistic link between species, communities, and multiple ecosystem functions. Traits are well-defined, measurable characteristics of organisms, usually measured at the individual level and used comparatively across species. They include morphological, physiological, or phenological characteristics, as well as functional features that strongly influence the growth, reproduction and survival of organisms, thus affecting their fitness and performance (McGill *et al.*, 2006; Violle *et al.*, 2007; Cadotte *et al.*, 2015). Functional traits have proven to be a highly versatile and sensitive approach to assess biological diversity, accounting for functional differences, and to better understand, and possibly generalise ecosystems functioning. In this context, the use of trait-based measures has become a powerful tool that allows a shift in perspective from a traditional taxonomy-oriented approach to an innovative function-focused approach (de Bello *et al.*, 2021). By moving away from the sole consideration of species identity, functional traits, and trait-based approaches have been increasingly used to understand ecosystem structure and functions and to track biodiversity loss and ecosystem re-assembly intensified by the global climate change (Kissling *et al.*, 2018). Functional diversity metrics, combined with taxonomic information, are useful indicators of the mechanisms driving community change and can be used as predictors of ecosystem functioning (Petchey and Gaston, 2006). In an effort to gain a clearer mechanistic understanding of the relationship between biodiversity and ecosystem functioning (BEF), trait-based approaches have been applied and are now firmly established in the empirical and theoretical ecological research (Flynn *et al.*, 2011; Cardinale *et al.*, 2012; Krause *et al.*, 2014). The shift in

perspective and the rise of functional trait approaches provided the scientists with the opportunity to move from "description to prediction" (Green *et al*., 2022) and to completely rebuild community ecology (McGill *et al*., 2006).

Biodiversity loss in freshwater ecosystems

Freshwater ecosystems are among the most diverse environments in the world (Strayer and Dudgeon, 2010). They cover a wide range of habitats, including ponds, lakes, reservoirs, streams and rivers, springs, wetlands, and estuaries. Freshwaters host a huge variety of microbial, plant, and animal communities that strongly interact with each other and with the environment, determining and influencing the composition, structure, diversity, and functioning of freshwater ecosystems. Thanks to their remarkable biodiversity, freshwater ecosystems play fundamental ecological roles and provide a wide range of valuable goods and economically important products and services for human societies (Covich *et al.*, 2004). They are central to our daily lives, providing water supply for all our livelihood activities. They are critical to the creation of employment, wealth, and livelihoods for many communities, supporting domestic, fishing, agricultural, and industrial activities, as well as being used for human recreation and tourism. They act as biological filters, facilitating the recycling of nutrients and water purification by breaking down pollutants. They recharge groundwater levels and store large amounts of rainwater and floodwater, increasing surface water reuse. They also play an important role in mitigating climate change, preventing and attenuating floods, and stabilizing and sequestering carbon dioxide.

When we consider that they cover only less than 1% of the whole Earth's surface, and when we examine all the provision of ecosystem services they support, we truly understand how fundamental and precious freshwater ecosystems are, and how important it is to manage them properly.

In recent times, these ecosystems have experienced the most dramatic biodiversity crisis mainly due to climate change and anthropogenic pressure such as overexploitation, water pollution, habitat destruction or degradation and invasion by exotic species (Dudgeon *et al.*, 2006). Such

anthropogenic threats make them extremely vulnerable to the loss of sensitive species and to an overall reduction in biological diversity.

"Bend the curve of freshwater biodiversity loss!" is the unanimous and urgent call of all scientists for the near future (Mace *et al.*, 2018; Tickner *et al.*, 2020), to protect freshwater ecosystems and all their associated ecosystem functions and services. While it is well known that ecosystem functioning can be strongly affected by changes in biodiversity (Covich *et al.*, 2004; Tilman *et al.*, 2014), the consequences of biodiversity loss are still largely unknown and there is still insufficient information available to make predictions about how different anthropogenic stresses will affect ecosystem functioning. Despite the multiple attempts to conserve and restore biodiversity, we still know too little about freshwater biodiversity, and current levels are far from adequate to conserve and sustainably manage it (Walpole *et al.*, 2009). Therefore, there is a growing need to better understand and investigate freshwater biodiversity and the relationship between biodiversity and ecosystem functioning in order to conserve their natural biodiversity and establish appropriate conservation measures for these ecosystems.

Phytoplankton and zooplankton: the base of freshwater food webs

Understanding the relationship between freshwater biodiversity and ecosystem functioning has been a core topic of ecological research in recent decades, with increasing attention paid to primary producers (Tilman *et al.*, 1997) and planktonic food webs dynamics (Litchman and Klausmeier, 2008; Litchman *et al.*, 2013). Some of the most important players in freshwater food web dynamics are phytoplankton and zooplankton organisms.

Phytoplankton includes more than 20 taxonomic classes of microalgae and protists, most of which are photosynthetic. By converting carbon dioxide and sunlight into energy through photosynthesis they produce organic matter that serves as a primary food source for other organisms, providing the fuel for the entire ecosystem to function. They are the first step in the system of energy transfer through aquatic food webs and represent the basis of all aquatic life webs. They play a crucial role in oxygen production and carbon sequestration, which determine the chemical composition of the global atmosphere and thus contribute to climatic modulation. The key role of phytoplankton in the functioning of aquatic ecosystems lies in their diversity. They come in a myriad of shapes, sizes, forms, and adaptations and express different biochemical functions, elemental requirements,

and trophic strategies, with which they determine the productivity of the entire aquatic food web and underpin the functioning of aquatic ecosystems (Falkowski *et al.*, 1998; Field *et al.*, 1998).

By feeding on phytoplankton, herbivorous zooplankton, in turn, constitute the crucial link in the energy transfer between the primary producers and higher trophic levels. The grazing behaviour of herbivorous zooplankton is one of the critical factors responsible for the variation in phytoplankton community composition (Kiørboe *et al.*, 2018) affecting phytoplankton survival and growth (Roelke and Spatharis, 2015) and thus, structuring planktonic food webs and maintaining a healthy balance in the ecosystem. In terms of biomass and productivity, the top-down control of phytoplankton in freshwater ecosystems is mainly regulated by representatives of the crustacean zooplankton, Cladocera and Copepoda.

In particular, cladocerans of the genus *Daphnia* are the dominant planktonic herbivores in most types of standing freshwater habitats such as lakes and reservoirs. They have simple life cycles and reproduce by cyclic parthenogenesis under normal conditions. However, they can switch their reproductive mode from parthenogenesis to sexual reproduction in response to certain unfavourable environmental and biological factors. They are non-selective filter feeders, able to feed on small, suspended particles in the water, that match the mesh size of their filtering apparatus.

Similar to cladocerans in freshwater environments, copepods are considered the prototype of zooplankton in the marine environment, where their diversity and contribution are the greatest. Nevertheless, copepods, and in particular, calanoid copepods also dominate freshwater habitats with the *Diaptomidae* family being the most species-rich and widespread calanoid family in inland waters (Boxshall and Defaye, 2008; Marrone *et al.*, 2017). They also play a major role in planktonic food webs, both as primary and secondary consumers or as food source for larval, juvenile, and adult fish of many species.

In freshwater environments, most studies have been carried out on cladocerans, rather than freshwater copepods. This bias reflects the ease of culturing and studying cladocerans, and especially *Daphnia* species, due to their feeding behaviour and nutritional requirements (Barnett *et al.*, 2007). Indeed, copepods differ from cladocerans in that they are able to actively capture and ingest individual suspended food particles (DeMott, 1986). They also have more complex life cycles (obligate sexuality, naupliar stages, copepodid stages, adult stage), slower somatic growth rates, and longer developmental and generational times, leading to difficulties in culturing and less

research on freshwater copepod species. Cladocerans and copepods also differ in the acquisition and regeneration of nutrients. Indeed zooplankton assemblages dominated by *Daphnia* have a differential retention of phosphorous in the biomass and a relatively high recycled of nitrogen (Sterner and Schulz, 1998), whereas calanoid copepods show the opposite trend, with a predominant retention of nitrogen in their body and differential recycling of phosphorous.

As a result of the differences in feeding modes, prey size range, nutrient acquisition and recycling, and life cycles, copepods and cladocerans affect the phytoplankton community to different extents (Sommer *et al.*, 2001) and, may therefore cause different effects on the food web.

Therefore, phytoplankton-zooplankton interactions are complex and dynamic, with both groups exerting important influences on each other. On the one hand, herbivory by generalist (ie. daphnids) or specialist (i.e. copepods) zooplankton grazers is an important factor influencing the abundance, structure, and composition of phytoplankton communities (Lampert *et al.*, 1986; Cyr and Pace, 1992). On the other hand, phytoplankton have evolved a variety of defence strategies, such as morphological, physiological, and behavioural strategies (i.e the production of spines, formation of cell clusters, colonial aggregates, production of mucilage and/or toxins) which in turn regulate the growth and survival, of zooplankton and influence the composition and dominance of the zooplankton (Pančić and Kiørboe, 2018; Lürling, 2021). Thus, the phytoplankton-zooplankton interface is the critical point where alterations, due to anthropogenic stressors and a decline in biodiversity, can potentially translate into changes in the entire food web and the health of the ecosystem as a whole.

Biodiversity loss and implications on phytoplankton-zooplankton trophic interactions

The study of phytoplankton diversity, and in particular the effects and consequences of changes and alterations in phytoplankton trait diversity, is therefore crucial for understanding the interplay between phytoplankton-zooplankton interactions for the functioning of aquatic ecosystems. Functional traits are promising eco-physiological traits for investigating and understanding these changes in phytoplankton community structure and how they are reflected in zooplankton grazing in response to climate change and biodiversity loss.

Despite the amount of knowledge gained to date, we are still in the early stages of understanding the interactions in freshwater planktonic food webs that take place under changing environmental conditions. The dynamics of food webs are strongly dependent on such functional traits of the organisms involved. Biodiversity loss can lead to pronounced changes in phytoplankton biomass (Boyce *et al.*, 2010; Lotze *et al.*, 2019) and taxonomic and functional composition (Graco-Roza *et al.*, 2021), altering the quantity and quality of phytoplankton as a food for consumers. However, there is still insufficient evidence on how biodiversity loss, and particularly the loss of functional trait diversity in primary producers, scales-up to whole ecosystems (Cardinale *et al.*, 2011).

To date, the majority of trait-based studies have focused on specific organisms within a single trophic level, or the ecosystem effects of a single trait (e.g. size, shape, body mass) and the relationships between traits and the environment despite the fact that in the context of biodiversity loss and global change, individuals may simultaneously vary in multiple traits and within different trophic levels. In particular, changes in phytoplankton-zooplankton species interactions in response to biodiversity loss remain understudied and experimental data are still scarce (Barnes *et al.*, 2018). A large number of experimental diversity manipulation studies have begun to test the effects of altered species richness and composition on ecosystem processes, particularly in grasslands (Zavaleta and Hulvey, 2004; Selmants *et al.*, 2012) and in marine benthos, where species manipulation is more feasible, by manually adding or removing species from communities (Bracken *et al.*, 2008; Bracken and Low, 2012). Studying the consequences of phytoplankton diversity loss for ecosystem functioning is also particularly important, however, manipulating phytoplankton communities is more complex. The main difficulty lies in the microscopic nature of phytoplankton, which makes the manipulation of phytoplankton richness and diversity in

laboratory or field experiments dealing with natural phytoplankton assemblages, particularly challenging. According to Hammerstein et al. (2017), dilution and disturbance methods can circumvent these difficulties, as they are two easily manageable tools for altering the diversity of natural algal communities. Gradual dilution of a natural community affects rare species, which are expected to be lost (Franklin *et al*., 2001; Giller *et al*., 2004) while experimental disturbance, intended as mechanical disturbance by mixing/shaking affects stress-sensitive species (Elmqvist *et al*., 2003; Gallagher *et al*., 2015). In addition to dilution and disturbance, other approaches have been already tested. For example, the filtration method can be used to remove large species while applying different environmental stressors i.e. heating can be used to affect sensitive species (Engel *et al*., 2017). All these methods can be used alone or in combination to simulate biodiversity loss, which is often associated with trait loss, and favour the creation of altered phytoplankton communities with distinct gradients in composition, richness, and diversity of species and associated functional traits.

One of the most interesting aspects of using such methods is to investigate how alterations/manipulations in phytoplankton diversity can lead to changes in phytoplankton nutritional quality, which in turn can influence the behaviour of herbivorous grazers, with significant effects on their feeding selectivity, reproduction, growth and survival, and ultimately result in directed and predictable shifts in the composition, biomass, abundance, and diversity of consumer communities. Thus, experimental manipulation of phytoplankton diversity can result in the loss of functional traits that directly determine changes in phytoplankton performance and feed back to zooplankton fitness. However, identifying and quantifying the traits that are relevant to phytoplankton-zooplankton interactions is not straightforward. Nevertheless, morphological and biochemical characteristics of phytoplankton are among the strongest driving forces shaping phytoplankton assemblages and influencing food quality and grazing susceptibility (Lehman, 1988). Traditionally, indeed, studies on phytoplankton food quality have focused on the relevance of morphological features of phytoplankton, specifically on the size and shape and their role in modulating zooplankton grazing. Phytoplankton cell size, for example, is considered a “master trait” (Litchman and Klausmeier, 2008) because of its well-known correlations with a wide range of physiological, demographic, behavioural, and predation-related traits (Brown *et al.*, 2004; Acevedo-Trejos *et al.*, 2016; Naselli-Flores *et al.*, 2007). It can strongly influence the strength and selectivity of grazing which is strictly dependent on the respective feeding mode, feeding

appendages, mode of ingestion, or food preferences of the herbivorous zooplankton grazers (Hansen *et al.*, 1994; Brose *et al.*, 2006; Kiørboe, 2011). Algae size classes also differ in their nutrient requirements and uptake kinetics (Litchman *et al.*, 2007). Therefore, alterations or shifts in phytoplankton size structure may determine the response of the phytoplankton community to nutrient availability, and feed back to the herbivorous zooplankton community. In addition to size, although less studied, the morphological trait "shape" is also an important morphological feature to consider when analysing phytoplankton assemblages, and their relationships with zooplankton (Naselli-Flores *et al.*, 2007; Ryabov *et al.*, 2021). Indeed, cell shape and geometry may determine the susceptibility of phytoplankton to certain grazers and, in some cases, represent an escape from predation (Lürling, 2021). Being size and shape the major determinants of phytoplankton edibility to herbivores, alteration of the morphological structure of the phytoplankton community can strongly affect zooplankton grazing, fitness, and population dynamics.

The quality of phytoplankton is also primarily determined by the taxon-specific biochemical composition they provide to consumers and zooplankton requirements (Müller-Navarra, 2008). These include fatty acids, amino acids, sterols, and vitamins, which can vary considerably between algal species and their physiology (Von Elert *et al.*, 2003; Lang *et al.*, 2011). Among them, fatty acids have received particular interest due to their key role in metabolism, representing the main metabolic fuel, storage, and transport of energy, essential components of all cell membranes (Müller-Navarra *et al.*, 2004; Ruess and Müller-Navarra, 2019). Their chemical structure is represented by a long hydrocarbon chain with a terminal carboxylic acid group. They vary in the number of carbons and the number and position of double bonds present in the fatty acid chain and can be classified into saturated (SAFAs), monounsaturated (MUFAs), and polyunsaturated fatty acids (PUFAs). Within the fatty acids, particular attention in nutritional ecology has focused on the class of PUFAs, where it is possible to distinguish polyunsaturated omega-3 and omega-6 fatty acids in terms of where the double bond is located. Omega-3 and omega-6-polyunsaturated FAs such as eicosapentaenoic acid (C20:5n-3; EPA) and docosahexaenoic acid (C22:6n-3; DHA), linolenic acid (C18:2n-6; LA), α-linolenic acid (C18:3n-3; ALA) and arachidonic acid (C20:4n-6; ARA) have been recognised as "essential fatty acids" (EFAs) due to their many physiological functions and large role in determining dietary adequacy as food for zooplankton physiology, growth, health, and reproduction (Müller-Navarra, 2008; Parrish, 2009; Taipale *et al.*, 2011; Ilić *et al.*, 2019). Their role is even more crucial because animals apparently lack the ability to

synthesise them de novo and must obtain them through diet (Kainz *et al.*, 2004; Brett, Müller-Navarra D.C., *et al.*, 2009). Thus, essential PUFAs are assumed to be a functional phytoplankton trait that may strongly affect the trophic transfer efficiency and dynamics between the primary producers and consumers. It is, therefore, necessary to investigate how biodiversity loss at the producer level, may alter phytoplankton biochemical traits, and in particular EFAs content to understand the larger framework of energy transfers between trophic levels in aquatic food webs, and predict possible effects under future scenarios.

Thesis objectives, approach and outline

Changes in phytoplankton functional diversity can lead to changes in phytoplankton structural and chemical composition, which may alter the trophic and energy transfer efficiency from phytoplankton to zooplankton, thereby influencing zooplankton fitness and affecting their growth, reproduction and secondary production, and ultimately changing the structure and function of aquatic food webs. My aim was therefore to investigate the potential impact of biodiversity loss on the dynamics of functional traits in phytoplankton-zooplankton trophic interactions.

In an attempt to better elucidate the mechanisms and forces that structure phytoplankton diversity, and to explain the "trait-trait" dynamics that occur in primary producer-herbivorous grazer interactions, I combined a trait-based approach with experimental manipulations of phytoplankton diversity.

The thesis consists of three chapters reporting the main results of laboratory grazing experiments of increasing complexity.

In **Chapter 1**, I investigated how phytoplankton diversity and changing biochemical characteristics of algal food can influence the reproductive responses and fitness of calanoid copepods. Specifically, I tested the role of phytoplankton food quality on the survival, development, growth, and reproduction of the freshwater calanoid copepod *Eudiaptomus* sp. by providing different monospecific and mixed diets of phytoplankton species that differed in fatty acid, and especially PUFAs content and composition, and following the functional responses throughout the life cycle of *Eudiaptomus* sp. Finally, I conducted a comparative study and estimated the fatty acids composition and content between *Eudiaptomus* sp. and their diets to test the extent to which copepods can modulate their own fatty acid profiles and contribute to the accumulation and regulation of their fatty acids composition depending on their respective diets. While the diet of cladocerans, especially daphnids, is well studied, less is known about freshwater calanoid copepods. This experiment, therefore, improved our understanding of the mechanisms involved in the feeding behaviour of *Eudiaptomus* sp. and, more generally, the feeding ecology of calanoid copepods and the tropho-dynamics between phytoplankton and calanoid copepods in aquatic ecosystems. In addition, it provided some interpretations of how possible adaptation strategies may be adopted by calanoid copepods under possible future scenarios of nutritional

deficits in food quality and changes in resource availability due to biodiversity loss in freshwater ecosystems.

In **Chapters 2** and **3** I investigated how changes in phytoplankton trait diversity might affect herbivorous zooplankton. To this end, I (i) carried out phytoplankton diversity manipulation experiments on natural phytoplankton assemblages and (ii) further investigated whether and how such diversity manipulations, designed to alter the diversity of phytoplankton functional groups, size classes, and nutritional value of diets (i.e. fatty acid composition) and food selection, affected *Eudiaptomus graciloides* and *Daphnia longispina*, representatives of specialist and generalist herbivorous crustacean zooplankton in lakes. By correlating several morphological and biochemical traits with the feeding behaviour, growth, and reproduction of the zooplankton organisms, I aimed to identify the parameters of the food that play a role in this interaction, and how the loss or modification of a particular trait or functional group from the phytoplankton community might mechanistically link and affect the morphological, physiological and life-history traits of the herbivorous zooplankton that feed on them.

Specifically, in **Chapter 2** I first simulated a loss of phytoplankton community traits using the filtration method to alter the size structure of a natural phytoplankton community, and second I studied phytoplankton dynamics and community reassembly after grazing by *Eudiaptomus* sp. and *D. longispina*. Knowing that cladocerans and copepods differ in feeding mode and have contrasting particle size preferences and feeding behaviour, I analysed how they grazed on phytoplankton communities of different size structures and the feedback response of the algal community in terms of functional diversity, composition, size, and shape distribution.

In **Chapter 3** I used the disturbance method as a tool to create diversity gradients within natural phytoplankton communities. By applying different intensities of mechanical disturbance to a natural phytoplankton community I induced shifts and alterations in the natural composition and structure of the primary producers, which mainly reflected in a loss of stress-sensitive species, accompanied by a loss of traits, and with the generation of "altered communities" with different composition, richness, taxonomic and trait diversity. I then provided the "altered and disturbed communities" as food sources for *Eudiaptomus graciloides* and *Daphnia longispina* and tested and evaluated the potential repercussions of these functional changes for herbivorous zooplankton grazers focusing mainly on monitoring morphological, physiological, and life-history responses.

As understanding biodiversity loss scenarios is challenging, I hope that this thesis will provide some new insights into the role of phytoplankton functional diversity and some evidence on how biodiversity loss and loss/change of specific traits may induce variation, adaptation, and reorganisation of communities and impact on consumer communities, food web dynamics, and ecosystem processes.

All chapters are based on individual research manuscripts. The first and the second chapters have been published in the Journal of Plankton Research and Microorganisms, respectively, while the third chapter is under review in the Hydrobiologia Journal.

Chapter 1

Food quality impacts on reproductive traits, development and fatty acid composition of the freshwater calanoid copepod Eudiaptomus **sp.**

This is the peer-reviewed version of the following article: Jessica Titocci & Patrick Fink, *Food quality impacts on reproductive traits, development and fatty acid composition of the freshwater calanoid copepod Eudiaptomus sp.*, Journal of Plankton Research, Volume 44, Issue 4, July/August 2022, Pages 528–541, which has been published in final form at https://doi.org/10.1093/plankt/fbac030

1.1 Abstract

The nutritional quality of phytoplankton is essential for the fitness of herbivorous zooplankton and efficient carbon fluxes in pelagic ecosystems. In freshwater lakes, cladocerans and calanoid copepods are the main pelagic herbivores in terms of both numbers and grazing impact. However, most studies focused on the easily cultivable cladocerans, while only a few studies addressed the impact of the diet on freshwater calanoid copepods due to their more complex life cycle. We here supplied five different phytoplankton diets to the freshwater calanoid copepod *Eudiaptomus* sp. to investigate their dietary quality for the copepods' fitness traits over the copepod's entire life cycle. While all tested diets supported comparable reproductive success in adults, egg production, hatching success and survival rate differed markedly between diets. In the offspring generation, diet affected developmental and reproductive periods, size at first reproduction and clutch size. *Eudiaptomus* body fatty acid composition only partially reflected their diet, indicating that the copepods are able to selectively accumulate and interconvert certain essential fatty acids. This capability may allow them to cope with nutritional deficiencies and may thus be interpreted as an ecological adaptation strategy to the fluctuating environmental conditions and resource availabilities in freshwater plankton.

1.2 Introduction

Herbivorous copepods play a major role in pelagic systems as grazers of phytoplankton and as a food source for higher trophic levels. Therefore, they are a key link in transferring essential dietary nutrients between primary producers and higher-level consumers. Most herbivorous copepods feed selectively (DeMott, 1986) and, based on their mechanical and chemical perception, they can use their "taste" and food quality as selection criteria to detect and ingest or reject prey. In this sense, the type of algal food, its size, concentration, biochemical composition and nutritional value are decisive phytoplankton traits determining the strength and selectivity of copepod grazing. Although is it true that a large body of knowledge on the impact of food availability and dietary quality for freshwater zooplankton has been already generated over the past two decades (Twombly *et al.*, 1998; Müller-Navarra *et al.*, 2000; Koussoroplis *et al.*, 2014) the role and impact of the biochemical composition of prey in determining feeding patterns and fitness of freshwater copepods remains much less investigated. The main reason is largely attributed to difficulties in copepod cultivation and laboratory handling, their obligate sexual reproduction and longer generation times compared to e.g. cladocerans. Zooplankton growth, reproduction and fitness strongly depend on the nutritional quality of their diet (Müller-Navarra *et al.*, 2000; Brett, Müller-Navarra D.C., *et al.*, 2009). Phytoplankton provides many important biochemical constituents to consumers, including fatty acids, amino acids, sterols, and vitamins, which can vary considerably according to the different algal species and their physiology (Von Elert *et al.*, 2003; Fink *et al.*, 2011; Lang *et al.*, 2011). Lipids, and in particular fatty acids play a big role in determining the adequacy of food for copepods' physiology, growth, health and reproduction (Ahlgren *et al.*, 1990; Koski *et al.*, 1998; Lacoste *et al.*, 2001). Within the fatty acids, particular attention has focused on the class of polyunsaturated fatty acids (PUFAs). Within PUFAs, the omega-3 and omega-6 PUFAs alpha-linolenic acid (C18:3n3, ALA), linoleic acid (C18:2n6, LIN), arachidonic acid (C20:4n6, ARA), eicosapentaenoic acid (C20:5n3, EPA), and docosahexaenoic acid (C22:6n3, DHA) are considered essential components for crustaceans' nutrition (Von Elert and Stampfl, 2000; Ilić *et al.*, 2019). They are required for the maintenance and integrity of cellular membranes and serve as precursors of hormones (Harrison, 1990; Fink and Windisch, 2019).

Generally, cryptophytes and diatoms are PUFA-rich (Ackman *et al.*, 1968; Volkman *et al.*, 1989), whereas green algae are typically characterized by their lack of long-chain PUFAs (Volkman *et al.*, 1989; Payne and Rippingale, 2000; Lacoste *et al.*, 2001). In cyanobacteria, PUFAs are typically

absent (Ahlgren *et al.*, 1990; Von Elert and Wolffrom, 2001). Moreover, omega-3 and omega-6 PUFAs cannot be synthesised *de novo* by many animals and are therefore considered essential components that must be derived from the diet (Pond *et al.*, 1996) or bioconverted from precursor fatty acids of the same omega-class (De Troch *et al.*, 2012; Boyen *et al.*, 2020). Even though copepods dominate the water column in marine environments, they are also present in all freshwater ecosystems, lakes and ponds where sometimes only one or few single species can represent large portions of the zooplankton biomass, dominating the whole zooplankton community (Pace, 1986). Moreover, copepods are also important food items in freshwater aquaculture, for larval, juvenile and adult fish of many species and play important roles in the food web and nutrient cycles (Frangoulis *et al.*, 2005). Investigations into how the diets' nutritional and biochemical composition of prey affects the survival, growth and reproduction of freshwater copepods are highly relevant and will promote a greater understanding of energy fluxes in freshwater aquatic ecosystems. To date, several comparative studies in FAs composition and content between zooplankton consumers and their diets have yielded contrasting results with respect to the diet dependence on copepod fatty acid content and composition (Hessen and Leu, 2006; Persson and Vrede, 2006). Calanoid copepods do not seem to have the necessary enzymes to produce high levels of PUFA, regardless of their levels in the diet (Sargent and Falk-Petersen, 1988; Bell *et al.*, 2007; Bell and Tocher, 2009). However, the view that all animals lack the ability to biosynthesize PUFA *de novo* has recently been challenged (Kabeya, Fonseca, David E.K. Ferrier, *et al.*, 2018; Boyen *et al.*, 2020; Kabeya *et al.*, 2021).

The aim of the present study was therefore to investigate the role of PUFAs in the dietary quality of phytoplankton for freshwater copepods and their fatty acid composition. Considering their key position in the pelagic food web and since investigations on obligately sexual freshwater copepods are still rare (De Meester *et al.*, 2002), we selected *Eudiaptomus* sp., one of the most abundant genera of freshwater calanoid copepods in Central European lakes, to investigate the nutritional value and dietary quality impact of four different phytoplankton species and their combination on the copepods. We conducted a laboratory feeding experiment to determine the effect of food quality on the fitness, reproduction, development, growth and survival of *Eudiaptomus* sp. using monospecific diets of two green algae (*Acutodesmus obliquus*, and *Chlamydomonas klinobasis*), a cryptophyte (*Cryptomonas* sp.), and a non-toxic cyanobacterium (*Synechococcus elongatus*), that differ in their PUFAs contents, as well as a mixed diet that combined the four taxa. We further

analyzed and compared the fatty acids profiles of the diets and consumers to test to what extent copepods can modulate their own fatty acid profiles and contribute to the accumulation and regulation of their fatty acid composition depending on each diet. This analysis of the importance and dependence of the nutritional quality of diet for copepods' survival, growth, reproduction and fitness will improve our understanding of the feeding ecology of freshwater calanoid copepods in particular, and contribute to the growing knowledge of trophic interactions and energy transfer between phytoplankton and zooplankton more generally.

1.3 Methods

1.3.1 Phytoplankton cultures

Three cultures of eukaryotic algae, one culture of a prokaryotic cyanobacterium and a mixture of those four phytoplankton taxa were used as diet sources for *Eudiaptomus* sp. The chlorophytes *Chlamydomonas klinobasis* (strain 56, culture collection of the Limnological Institute of the University of Konstanz) and *Acutodesmus obliquus* (strain SAG 276-3a, culture collection of Algae at Göttingen University, SAG) and the cryptophyte *Cryptomonas* sp. (strain SAG 26.80) were all grown in semi-continuous 5 L batch cultures in either Cyano medium (Von Elert and Jüttner, 1997) for *C. klinobasis* and *Cryptomonas* sp., or Z/4 medium (Zehnder and Gorham, 1960) for *A. obliquus* by replacing 20 % of the medium every other day. Cultures were kept in a climate-controlled chamber at 20 °C with a PAR intensity of 120 µmol photons m^{-2} s^{-1}. The non-toxic cyanobacterium *Synechococcus elongatus* (strain SAG 89.79) was grown in a chemostat in Cyano medium at a dilution rate of 0.1 day^{-1} at 20 °C with a PAR intensity of 50 µmol photons m^{-2} s^{-1}. This ensured that all cultures were in the exponential growth phase when fed to the copepods.

1.3.2 Copepod cultures

Eudiaptomus sp. were isolated from lake Klostersee (Upper Bavaria, Germany) in July 2018 and then acclimated and cultured continuously under standardized conditions in the laboratory at the University of Cologne. Copepod cultures were maintained in aged (> 3 days) and aerated tap water in 0.5 L glass beakers in a climate chamber at 20 °C and 16:8 h light:dark cycle and fed *Cryptomonas* sp. *ad libitum*. Before starting the experiment, adult *Eudiaptomus* sp. were sexed using a dissecting microscope and placed individually into 100 mL aged tap water. All individuals

of each food treatment were fed every other day for two weeks with the designated diets to acclimatize them to the respective diet and to remove potential residual effects of previous diets under the conditions described above. In the acclimation phase and during the experiment, *Eudiaptomus* sp. were fed with the four different pure cultures, as well as a mixed diet (hereafter referred to as "Mix") that combined the four phytoplankton taxa in equal biomass (based on particulate organic carbon (POC L^{-1}), estimated based on the photometric light extinctions and culture-specific POC: extinction regressions. Given that light scatter in an algal suspension is proportional to the cell density over a certain range, we determined the light scatter (extinction) at 470 nm of a dilution series of each algal culture through a photometer. Moreover, for each of these dilutions, we determined the particulate organic carbon content per ml of algal suspension, yielding a linear calibration function that allowed a rapid estimate of the POC content of the respective algal cultures during the experiment.

1.3.3 Reproduction experiment (parental generation, F0)

After two weeks of pre-cultivation, 100 healthy females that had produced eggs during the acclimation period and 60 adult males were used for the experiment. Females and males (ratio 2:1) were placed together in glass beakers filled with 250 mL aged tap water in 5 replicates per food treatment (Figure 1). Each *Eudiaptomus* treatment received 1.5 mg POC L^{-1} of the respective diet, which is known to saturate copepod feeding (Kiørboe *et al.*, 1985). Size dimensions of at least 20 cells of all the used phytoplankton species were measured using an inverted microscope (Zeiss, 400x magnification, see Supplementary Table SI). Twice per day, all animals were checked visually and, if present, females with eggs were collected gently and incubated in separate jars until hatching. All eggs and hatched nauplii were counted under a dissecting microscope. Examination for eggs continued until no further hatching occurred. Several life history parameters were recorded. Reproductive success was calculated as the percentage of egg-carrying females per food treatment. Time for reproduction was determined as the time (in days) until eggs had formed in 100% of the reproducing females. Egg production was measured as the total number of eggs produced in every food treatment, as clutch size (CS), and the number of eggs produced per female per food treatment. Hatching success (HS) was determined after 24-48 h of incubation by counting the number of nauplii hatched from all eggs obtained.

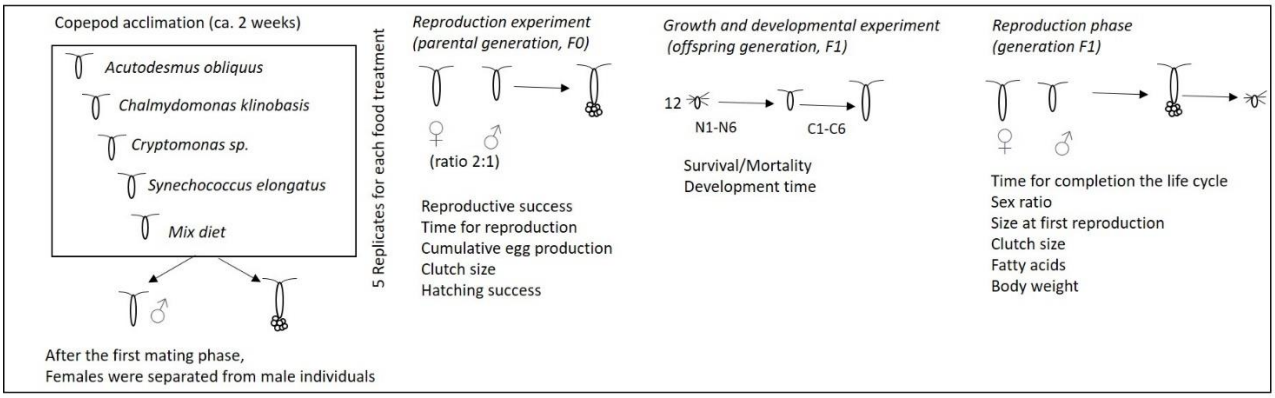

Figure1. Experimental design and phases. The design consisted of a first acclimation phase where a pool of adult copepods was fed with the respective diets for at least 2 weeks. In this case, after the first mating phase occurred, females were separated in different jars from males. The first mating phase served as indicator of healthy animals. After the hatching phase, 100 healthy females that had produced eggs during the acclimation period and 60 adult males were used for the reproduction experiment. In the second phase, the survival, growth and development of the nauplii were monitored until the completion of the lifecycle and the reproduction phase occurred again. At the end of the experiment adults were used for morphometric and fatty acids analyses.

1.3.4 Growth and developmental experiment (offspring generation, F1)

Twelve freshly hatched nauplii were carefully pipetted into 400 mL glass beakers containing the respective food suspension in five replicates per food treatment. In this sensitive phase, we avoided transferring the nauplii into fresh food medium every second day. Rather, we renewed 50% of the water every other day to minimize oxygen depletion (Uye and Fleminger, 1976). Once the first copepodite stage was reached (C1), the animals were transferred into fresh water with food suspension every second day. The developmental stage of individual copepods was checked by transferring carefully all experimental animals in Petri dishes and observing them under the stereomicroscope. Observations were made every second day until copepods reached maturity, during the regular transfer of the animals in new experimental jars, for the renewal of food and water. Development time was measured as the time (in days) occurring between the first naupliar stage N1 and the time when all living animals reached the adult and mature stages (C6). The time for completion of the life cycle was estimated as the time until the first reproduction occurred and females carrying eggs were visible. The prosome length of all egg-carrying females found in each food treatment was measured under a stereo microscope at 25x magnification for the estimation of size at first reproduction (SFR). At the end of the experiment, all surviving individuals were stored in pre-weighed aluminium boats and weighed on a Sartorius microbalance type CP2 P (accuracy

1 µg) for body mass determination. Clutch size was estimated as the number of eggs produced per egg-carrying female.

The net reproductive rate (R_0) was obtained by multiplying the proportion of females surviving to each age (l_x) by the average number of offspring produced at each age per female (m_x) and then adding the products from all the age groups according to the formula:

$$R_0 = \Sigma l_x m_x$$

The mean generation time (T) represents the rate at which the population can grow and is calculated as the average interval between the birth of an individual and the birth of its offspring according to the formula:

$$T = \Sigma x l_x m_x / R_0$$

The intrinsic rate of population growth (r_m) was estimated according to

$$r_m = \ln R_0 / T$$

1.3.5 Fatty acid analyses

Samples of all four phytoplankton species (approximately 1mg POC per sample) were filtered onto pre-combusted GF/F filters and stored in dichloromethane : methanol (2:1 v/v) at -20 °C until extraction. Adult *Eudiaptomus* sp. were collected at the end of the experiment and freeze-dried in pre-weighed aluminium boats. After drying and dry mass determination on the microbalance, they were transferred into glass tubes with 5 mL dichloromethane : methanol (2:1, v/v) each and extracted according to Windisch and Fink (Windisch and Fink, 2018). Fatty acid content and composition from algal and calanoid samples were analyzed via gas chromatography (GC). 1 µl of each sample was injected (splitless) into a 6890-N GC System (Agilent Technologies, Waldbronn, Germany) equipped with a flame ionization detector and a DB-225 (J&W Scientific) capillary column (length 30 m, internal diameter 0.25 mm, film thickness 0.25 µm). The GC conditions were as follows: injector and FID temperatures 220°C; initial oven temperature 60°C

for 1 min, followed by a 120°C /min temperature ramp to 180°C, then a ramp of 50°C/min to 200°C followed by 10.5 min at 200°C, followed by a ramp of 120°C /min to 220°C, which were held for 7.5 min. Helium gas was used as a carrier gas with a flow rate of 1.5 mL/min. FAMEs were identified by comparison of retention times and previously established calibration curves with reference compounds from a standard mixture and quantified via the internal standard. All analyses were done on triplicate samples for phytoplankton and five replicates for copepod samples.

1.3.6 Data analyses

All reproductive traits and life history parameters measured over the reproduction and growth experiment were checked for normal distribution with a Shapiro-Wilk's test and for homogeneity of variances with a Levene's test. Where these assumptions were met, one-way analyses of variance (ANOVA) followed by Tukey's post-hoc tests were performed. In cases of unequal sample sizes, a type III one-way ANOVA followed by Tukey's HSD was run instead. Multivariate permutation analysis (permANOVA) was used to analyze the fatty acids composition and content of the different algal diets and the calanoid copepod fed on them. A non-metric multidimensional scaling method (NMDS) based on Bray–Curtis similarity matrices was conducted with all fatty acids as variables using the vegan package in R. To assess the dissimilarity and to determine the main fatty acids contributing to differences between samples, similarity percentage analysis (SIMPER) was calculated. Differences in fatty acid classes and individual fatty acids detected with the multivariate analyses were tested with a one-way analysis of variance (ANOVA) with algal diets or calanoid copepods as independent variables. Further, we performed cluster analysis of individual fatty acids found in the experimental animals fed the different diets. For this, principal component analyses (PCA) of individual fatty acids were conducted using the factoextra package and nonsquared Euclidian distances. All the analyses were carried out using R (version 3.3.3).

1.4 Results

1.4.1 Reproduction experiment (parental generation, F0)

We observed no significant differences in the reproductive success expressed as percentage of egg-carrying females of *Eudiaptomus* sp. when fed with the different phytoplankton or the mix diet (ANOVA, $p > 0.05$, Table I). The percentage of females with eggs was higher than 85 % in all diets over the whole reproductive experiment, with the exception of *S. elongatus*, where the reproductive success was slightly below 80 %. The time needed for reproduction was quite consistent in all food treatments, with mean values (± S.D. of n = 5) ranging between 5.20 ± 1.64 (mix diet) and 6.00 ± 2.12 days (*Cryptomonas* sp.). High and significant variations were instead registered in the reproductive traits: egg production (cumulative and per female) and hatching success under the different algal diets (ANOVA, $p < 0.001$ and $p < 0.05$). Indeed, the highest egg production was observed in *Eudiaptomus* sp. females fed the mix diet (63.80 ± 13.22 eggs) with females carrying in average 13.23 ± 2.04 eggs. In contrast, *Eudiaptomus* fed with *S. elongatus* showed nearly three times lower egg production than on the mix diet (22.60 ± 8.17, Table I). The highest hatching success (HS) was measured in copepods fed with *Cryptomonas* sp. (95.22 ± 6.66 %) followed by the ones fed the mix diet (83.50 ± 23.38 %). Conversely, *Eudiaptomus* sp. fed with green algae showed the lowest HS, with values below 50 % on *C. klinobasis*. Despite the initial number of eggs produced, most of the eggs in this treatment were found either unhatched, empty or partially disintegrated (possibly unfertilized) after 24-48 h, and no further hatching was observed in these.

Table I. Reproductive traits of individuals of the parental generation (F0) of *Eudiaptomus* sp. (mean ± SD, n=5) fed with *Cryptomonas* sp., *C. klinobasis, A. obliquus, S. elongatus* and the mix diet (in equal amounts). The different letters indicate significant differences between the diets, (one-way ANOVA, followed by Tukey's HSD test, *p< 0.05, **p< 0.01,***p< 0.001).

Reproductive traits	*Cryptomonas sp.*	*C. klinobasis*	*A. obliquus*	*S. elongatus*	*Mix*	*P-value*
Egg-carrying females (%)	86 ± 21.91	92 ± 17.89	88 ± 17.89	79 ± 24.60	96 ± 8.94	0.687
Time for reproduction (days)	6 ± 2.12	5.8 ± 1.64	5.4 ± 1.82	5.4 ± 3.05	5.2 ± 1.64	0.974
Total egg production	51 ± 17.31 **ab**	37 ± 9.14 **bc**	31.40 ± 9.76 **bc**	22.60 ± 8.17 **c**	63.80 ± 13.22 **a**	0.0002 ***
Clutch size (eggs/ind^{-1})	12.73 ± 1.22 **a**	8.01 ± 0.96 **b**	7.35 ± 0.96 **b**	6.54 ± 2.19 **b**	13.23 ± 2.04 **a**	8.02e-07 ***
Hatching success (%)	95.22 ± 6.66 **a**	45.64 ± 37.60 **b**	56.03 ± 22.63 **ab**	63 ± 17.69 **ab**	83.50 ± 23.38 **ab**	0.022*

1.4.2 Growth and developmental experiment (offspring generation, F1)

The survival rates of *Eudiaptomus* sp. depended strongly on their diet (Figure 2a, b). Complete development of nauplii to adult copepods occurred on the Mix diet and on *Cryptomonas* sp., with survival rates above 80 %. In contrast, *Eudiaptomus* sp. fed with pure cultures of the chlorophyceans exhibited low survival rates with a pronounced drop in the survival curves during the naupliar stage (10-15 days). Naupliar mortality reached the maximum level with *A. obliquus* as food, where all individuals were found dead by day 17. *Eudiatptomus* fed *S. elongatus* exhibited only low (5 %) mortality, it increased to 45 % after 15 days when the animals were in their initial copepodite stage. Both *Cryptomonas* sp. and the Mix diet promoted a significantly faster development and growth towards maturity (ANOVA, $p < 0.001$, Figure 3 a and Supplementary Figure SI, for the time until reproduction). The development time in these cases was estimated in less than 20 days (Mix diet 17.8 ± 1.10 and *Cryptomonas* sp. 19.4 ± 0.89 days). All copepods were able to mate and reproduce a few days after they had reached the adult stage in these treatments (19.73 ± 0.71 days in Mix diet, and 21 ± 1.96 days for *Cryptomonas* sp., respectively, see Supplementary Figure SI). Moreover, egg production was significantly higher in these two diets compared to the chlorophyte diets (Table II). Egg numbers were significantly lower in copepods fed *S. elongatus*, or *C. klinobasis*, respectively (mean clutch size: 6.1±0.34 and 6.75±1.05 eggs respectively; Table II). This indicates healthy and fast developing individuals from the new generation under *Cryptomonas* sp. and the Mix diet. The suitability of the aforementioned diets was further confirmed by the animals' biomass and size at first reproduction (Table II). The prosome length and body mass were significantly larger in the females fed *Cryptomonas* sp. and the Mix diet (ANOVA, $p < 0.001$). Prolonged development was instead measured in copepods fed either *C. klinobasis* or *S. elongatus*. The copepod developmental time was 29 ± 0 days with the green algal diet and 30.5 ± 1.91 days with the cyanobacterial diet (Figure 3b). Moreover, the copepods' reproductive periods were extended on *C. klinobasis* and *S. elongatus* (averaging 35 and 37.5 days, respectively, see Supplementary Figure SI). Due to the high mortality rates under *C. klinobasis* and *S. elongatus* food treatments, only a few individuals were able to reach the reproductive maturity stage and mate. In this case, we were only able to check for egg-carrying females in two of the three replicates for *C. klinobasis* and four of five replicates in *S. elongatus*. The same applies to the estimates of biomass, size at first reproduction and clutch size of the adult

females (Table II), which were all significantly smaller compared to copepods fed *Cryptomonas* sp. and the Mix diet (ANOVA, $p < 0.001$, Table II).

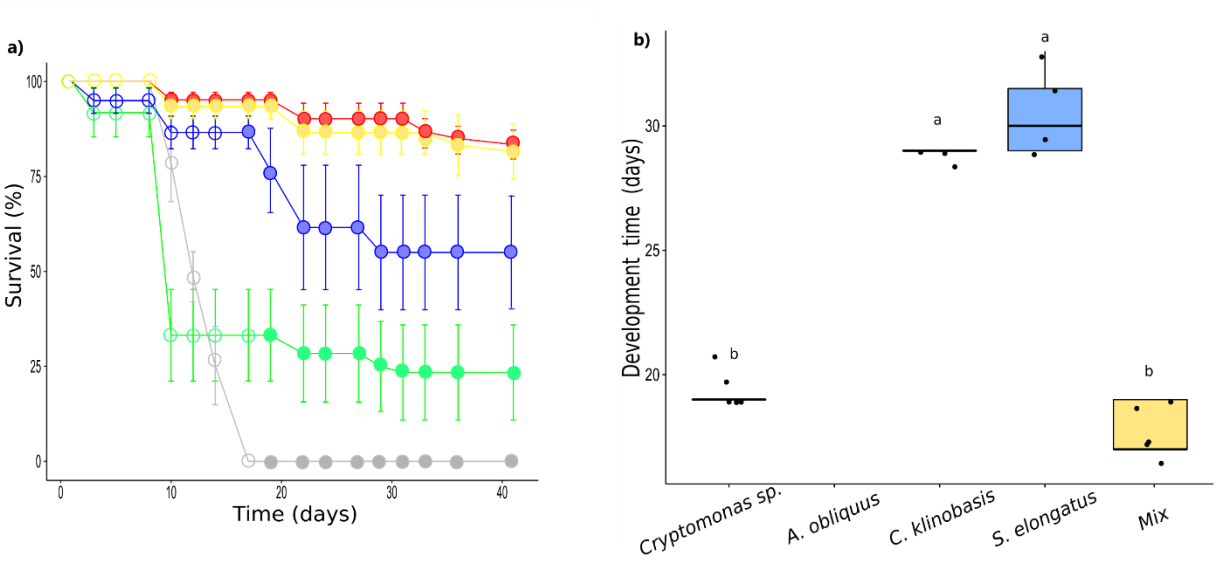

Figure 2 a) Survival curve of the offspring generation (F1) for the entire life cycle of *Eudiaptomus* sp. fed different diets: green *C.klinobasis*; grey *A.obliquus*; red *Cryptomonas* sp.; blue *S. elongatus*; yellow *mix diet*. Open and filled dots correspond to the naupliar and copepodite stages, respectively. **b)** Development time (in days) of the offspring generation (F1) in *Eudiaptomus* sp. needed to reach the adult stage (C6). Different letters indicate significant differences between treatments (one-way ANOVA, followed by Tukey's HSD test).

The sex ratio (♀/♂) of the *Eudiaptomus* sp. did not differ significantly between food treatments (ANOVA, p=0.383) and ranged from 0.81 ± 0.38 on the mix diet to 2.71 ± 2.33 in *S. elongatus* (data not shown). *Eudiaptomus* sp. fed *Cryptomonas* sp. had the highest net reproductive rate (31.57, Supplementary Table SII) followed by copepods fed the mix diet and *S. elongatus*. In contrast, *Eudiaptomus* sp. fed *C. klinobasis* showed the lowest net reproductive rates (5.66) and also a negative intrinsic growth rate (-1.60) indicating a population decline. Negative values in intrinsic growth rate were registered also on *S. elongatus* treatment (-0.40) as food source. For the generation time index, the minimum average interval between the birth of an individual and the birth of its offspring was found in the mix diet treatment (18.96 days, see Supplementary Table SII).

Table II. Life-history parameters in the offspring generation (F1) of *Eudiaptomus* sp. fed the different phytoplankton diets. Different letters indicate significant differences between treatments (one-way ANOVA, followed by Tukey's HSD test). N = 5 replicates were used in all treatments except for *C. klinobasis* n = 3 and *S. elongatus* n = 4. For calanoids fed with *A. obliquus* it was not possible to collect data about life-history parameters due to high mortality occurred in the first stages of the F1 generation and no occurrence of reproduction, therefore this treatment was omitted.

Life-history parameter	*Cryptomonas sp.*	*C. klinobasis*	*S. elongatus*	*Mix*	*P-value*
Size at First reproduction (µm)	973.28 ± 16.58 [a]	987.68 ± 35.14 [ab]	925.74 ± 54.71 [b]	881.95 ± 9.46 [a]	p<0.001 ***
Body weight (µg/ind)	18.78 ± 2.11 [a]	11.63 ± 1.64 [b]	9.65 ± 1.64 [b]	17.59 ± 1.24 [a]	p<0.001 ***
Clutch size (eggs/ind)	11.86 ± 1.03 [a]	6.75 ± 1.06 [b]	6.11 ± 0.34 [b]	12.13 ± 1.13 [a]	p<0.001 ***

1.4.3 Fatty acid analysis

Total fatty acid concentrations in the different diets ranged from 3.43 ± 0.29 µg mg POC^{-1} for *S. elongatus* to 78.96 ± 7.18 µg mg POC^{-1} for *A. obliquus* (see Supplementary Table SIII). The cyanobacterium *S. elongatus* contained predominantly saturated (SAFA) and monounsaturated (MUFA) fatty acids. The main constituents were palmitoleic acid (1.56 ± 0.15 µg mg POC^{-1}) and palmitic acid (1.23 ± 0.09 µg mg POC^{-1}). Polyunsaturated fatty acids (PUFA) were almost absent in *S. elongatus*, except for C18:3 n-3 (ALA), which was detected in trace amounts (0.21 ± 0.06 µg mg POC^{-1}). The two green algae investigated had similar fatty acid compositions but great differences in terms of absolute contents. All fatty acid classes (SAFA, MUFA and PUFA) were more abundant in *A. obliquus* than in *C. klinobasis*, with oleic acid (C18:1n9) being the main fatty acid in *A. obliquus* (33.96 ± 3.23 µg mg POC^{-1}). The major fatty acids of *Cryptomonas* sp. were C16:0 and polyunsaturated omega-3 fatty acids such as C18:4n3 and C20:5n3 (EPA) were the most abundant. Only low levels of monounsaturated fatty acids were identified in this alga. The Mix diet contained a comparable amount of SAFA, MUFA and PUFA. The concentration of saturated fatty acids was mainly due to the contents of palmitic (C16:0) and stearic (C18:0) acids, while the monounsaturated oleic (C18:1n9) and palmitoleic acid (C16:1n9) and polyunsaturated C18:3n3 (ALA), C18:4n3, and C18:2n6 (LIN) were the dominant unsaturated fatty acids. The fatty acid composition of *Eudiaptomus* sp. depended strongly on the animals' diet (Figure 3a and b, see also Supplementary Table SIV for detailed fatty acids data). Copepods consuming the green alga *C. klinobasis* showed a significantly higher amount of saturated (SAFA) and monounsaturated (MUFA) fatty acids compared to the other diets (Figure 3a). The amount of polyunsaturated omega-3 fatty acids was the highest and approximately comparable in all the calanoids. However, differences in the content and type of individual omega-3 fatty acids were recorded (see Supplementary Table SIV). Indeed, calanoid fed on *Cryptomonas* sp., were significantly reacher in C18:4n3 and EPA while ALA was the main dominant omega-3 PUFA recorded in the calanoid fed on *C.klinobasis* (33.05 ± 8.61 µg mg DW^{-1}), followed by DHA (6.66 ± 2.36 µg mg DW^{-1}). The lowest level of both omega-3 and omega-6 PUFAs were found instead in calanoid copepod fed the cyanobacteria *S. elongatus*, due to the insufficient presence of these fatty acids in the algal food source. At the contrary, the highest PUFA omega-6 content was recorded in calanoid-fed the Mix diet, due mainly to the high abundance of linoleic acid C18:2n6 recorded (5.46 ± 0.79 µg mg DW^{-1}, see Supplementary Table SIV for more details). Also, the PCA of the fatty acids composition of

Eudiaptomus sp. fed the different algal diets demonstrated a statistically clear distinction between the four different food treatments (Figure 3b, permANOVA, $p < 0.001$). Both components accounted for almost 60 % of the variation with PC1 that clearly separated fatty acids that predominate in *Eudiaptomus* sp. fed on *Cryptomonas* sp. C18:4n3, EPA and Mix diet C20:2n6, C20:1n9 and 20:3n3 (on the left) from C18:1n9c mostly abundant in calanoid fed on *C. klinobasis* and *S. elongatus* diet (on the right). PC2 accounted for a smaller percentage of variability (24.9%) with the the fatty acids C18:3n3, C18:2n6c, C:18:1n9t and C16:0 and showed a separation between calanoid fed on the Mix and green algal diets and the calanoid that had consumed *Cryptomonas* sp. and *S. elongatus*.

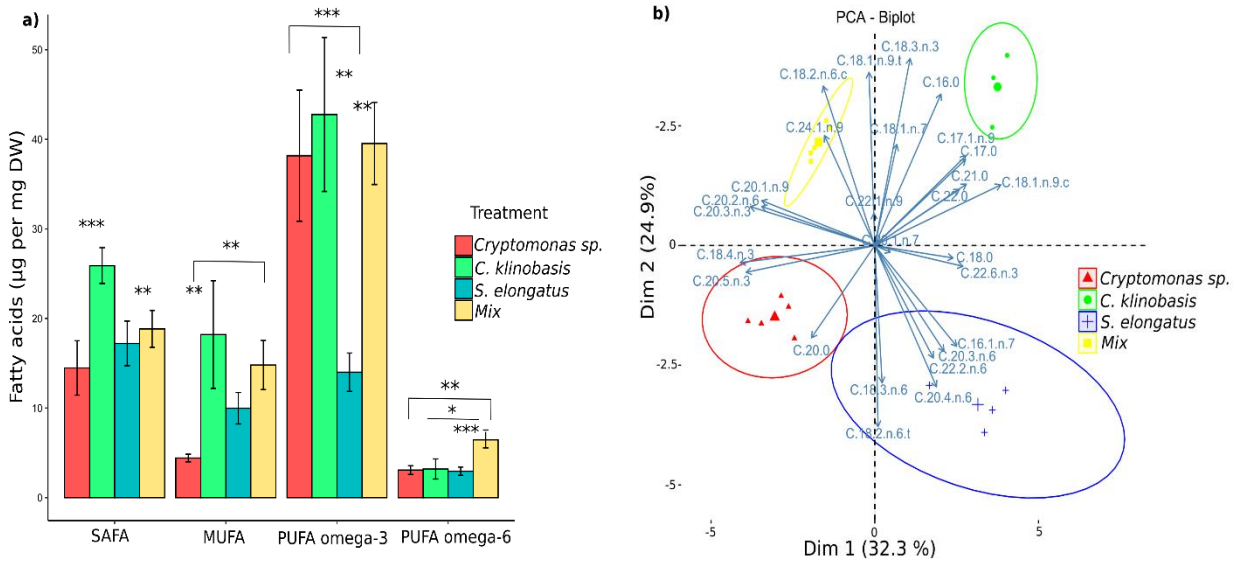

Figure 3 ***a)*** Mean (± SD) *Eudiaptomus sp*. biomass-specific content of SAFA, MUFA and PUFAs on the different diets and ***b)*** bi-plot of the principal component analysis based on the calanoids' biomass-specific fatty acid contents after consuming either pure phytoplankton (*Cryptomonas* sp. in red, n = 4, *C. klinobasis* in green, n = 3, and *S. elongatus* in blue, n = 4) or mixed diets (yellow, n = 4) in the experiment. For calanoid fed *A. obliquus* it was not possible to collect data about life-history parameters due to high mortality occurred in the first stages of the F1 generation, therefore this treatment was omitted. Asterisks indicate statistically different treatments. Ellipses represent different clusters according to permANOVA results.

The differences observed in the fatty acids from calanoids are clearly associated with divergence in the diets consumed (Figure 4). Copepods had a general relative decrease in SAFA and MUFA (all treatments) with respect to their diets and a relative increase in essential fatty acids (EFAs) (all treatments) except for LIN where the relative proportion was not significantly different between

the food source and the calanoid in *Cryptomonas* sp. (ANOVA, p = 0.413) and Mix diet treatments (ANOVA, p = 0.237) and significantly lower in calanoid fed on *C.klinobasis* (ANOVA, p < 0.01). On the contrary in the *Synechococcus* treatment, calanoid showed significantly higher proportion and accumulation of all EFAs in their body content. In addition, in all treatments, the relative abundance of DHA was significantly much higher in the calanoid concerning their diets (p<0.001 in *Cryptomonas* sp. and Mix diet and p < 0.01 in *C.klinobasis* and *S.elongatus*). Concomitantly, a generally lower relative amount in DHA with respect to EPA was observed in calanoid fed on *Cryptomonas* sp. and the Mix diet, while an opposite trend in the relative abundance of EPA and DHA was instead registered in *Eudiaptomus* fed the other two monoalgal diets. Finally, despite ARA was not detected in any diets, it was found, albeit in very low amounts, in all the calanoid treatments.

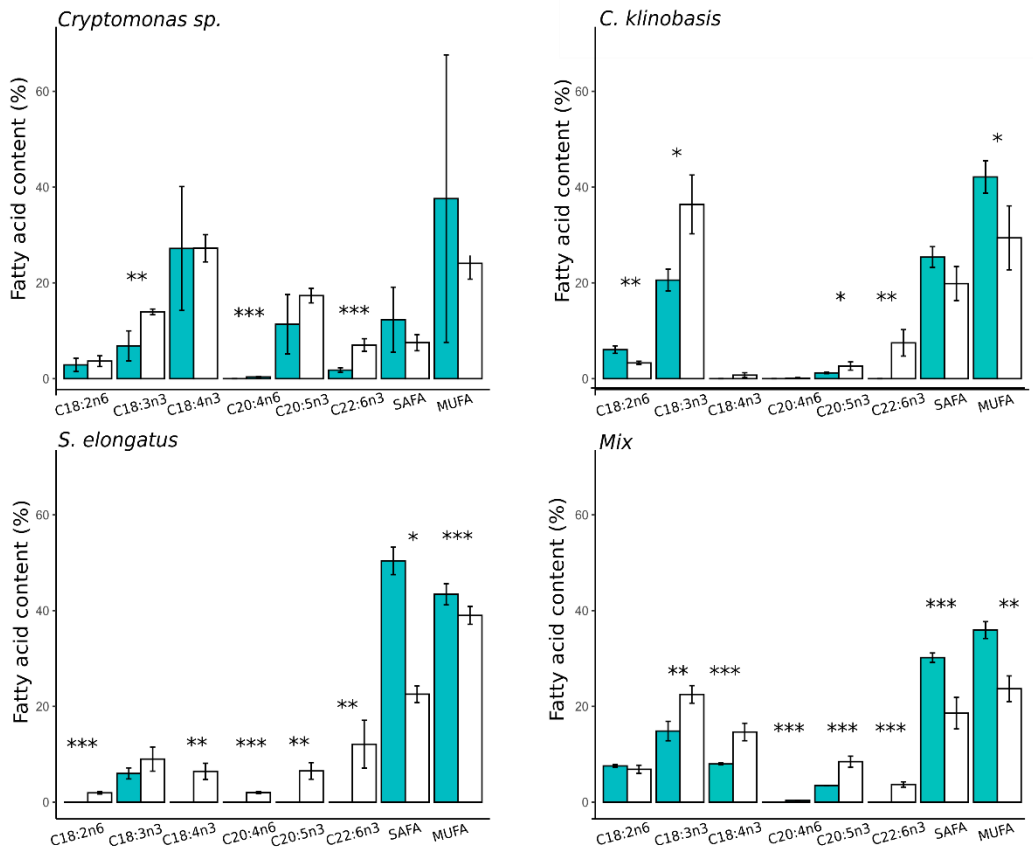

Figure 4. Mean relative fatty acid composition (%, ± SD) of *Eudiaptomus* sp. (white bars) versus their respective diets (light blue bars). Asterisks indicate statistically significant differences after permANOVA and SIMPER analysis (*p < 0.5; **p < 0.01; *** p < 0.001).

1.5 Discussion

1.5.1 Dietary impact on reproductive traits: egg production and hatching success (parental generation, F0)

We found clear differences in all the investigated reproductive and life history parameters of *Eudiaptomus* sp. when fed with different phytoplankton species. In particular, the cryptophyte *Cryptomonas* sp. and the Mix diet allowed for the highest egg production and naupliar hatching success over the reproductive phase of the experiment. This observation is similar to the known high food quality of *Cryptomonas* sp. for daphnids (Martin-Creuzburg, Von Elert, *et al.*, 2008; Windisch and Fink, 2018). The high quality of the Mix diet indicates that a higher dietary diversity can compensate for nutritional deficiencies of monospecific diets, as demonstrated previously in various consumer taxa (Milione and Zeng, 2007; Puello-Cruz *et al.*, 2009; Groendahl and Fink, 2016).

However, although demonstrated in several previous experimental (Jones and Flynn, 2005; Groendahl and Fink, 2016) and theoretical studies (Anderson and Pond, 2000), food mixtures do not always provide higher quality and better performances than monoculture diets. In our study, the fecundity and time needed for reproduction, as well as the egg production, the survival and development of *Eudiaptomus* sp. fed the Mix diet were comparably high as those of *Cryptomonas* sp. as a sole diet item. Our results thus indicate that a good monoalgal diet such as *Cryptomonas* sp. can be equivalent to a Mix diet and allow for multiple generations *Eudiaptomus* sp. with high survival and growth rates.

In contrast, we observed very low egg production and hatching success when *Eudiaptomus* sp. were fed with either the green algae *C. klinobasis*, *A. obliquus*, or the cyanobacterium *S. elongatus.* This could be related to biochemical deficiencies in these diets. In particular, many studies have shown that PUFAs and long-chain fatty acids are fundamental in oogenesis in crustaceans (Harrison, 1990; Ederington *et al.*, 1995; Payne and Rippingale, 2000; Broglio *et al.*, 2003). Specifically, DHA and EPA are both considered to be essential fatty acids for copepod reproduction (Støttrup and Jensen, 1990; Jónasdóttir, 1994). Our results corroborate this view, as the phytoplankton species used in our experiment differed markedly in their content and composition of specific fatty acids, especially long-chain PUFAs such as EPA and DHA which were present in very low amounts or not detectable in both green algae and *S. elongatus*

(Supplementary Table SIII). Therefore, our results are in agreement also with the findings by Jónasdóttir and Kiørboe (Jónasdóttir and Kiørboe, 1996) and Tang and Dam (Tang and Dam, 2001), where in most cases high egg production rates correspond to higher quality and more viable eggs.

1.5.2. Dietary impact on development and growth (offspring generation, F1)

Egg production *per se* is not a sufficient parameter to quantify fitness. It is important to also consider the viability of the produced eggs and the subsequent development and growth of the offspring (Ianora *et al.*, 1995; Miralto *et al.*, 1999). In this sense, both species of green algae were inadequate for the development of nauplii and copepodites, as these diets resulted in high mortality rates, in particular on *A. obliquus*. This might be explained by the morphological characteristics of the alga together with some biochemical deficiencies, as *A. obliquus* forms multi-celled coenobia that could adhere to swimming appendages and reduce the motility and feeding activities of the juvenile calanoids (Puello-Cruz *et al.*, 2009). Furthermore, *A. obliquus* can develop thick cell walls as a defense mechanism against grazing, which may result in a poor digestibility of this species and preclude its ingestion and utilization as a sole food source, especially for nauplii (Payne and Rippingale, 2000). Moreover, during the acclimation phase, any significant adult mortality in the copepods that were used as the parental generation to start the reproduction phase of the experiment was observed. This may indicate that the lack of long-chain PUFAs (such as EPA and DHA) in *A. obliquus* played a role in copepod reproduction and oogenesis while other prey characteristics such as size, shape, morphology and elemental composition (e.g., C:N:P stoichiometry) may pose issues for the development of early life stages of copepods.

Although the flagellated chlorophyte genus *Chlamydomonas* is frequently considered an adequate food source in cultures of freshwater zooplankton including calanoid and cyclopoid copepods (Hamburger and Boëtius, 1987; Soto and Hurlbert, 1991), our results do not support this. Already in the first reproductive phase of the experiment, *Eudiaptomus* hatching success was extremely low (<50%) in the *C. klinobasis* treatment, and post-hatching mortality in nauplii was high. This corresponds with the findings of Santer (Santer, 1994) and von Elert and Stampfl (Von Elert and Stampfl, 2000), who had demonstrated the nutritional inadequacy of the closely related species *C. reinhardii* for the development of *E. gracilis* nauplii. Despite this initial loss of experimental individuals, the few that managed to survive to the naupliar stage successfully moulted and reached

the adult stage, and were even observed to reproduce. However, the time needed to develop to the adult and mature stage needs to be considered. Very slow development was observed in copepods fed *C. klinobasis* with a naupliar stage that lasted almost 20 days on average. This suggests the low food quality of this species for nauplii. The ultimate reasons for the poor performance of *C. klinobasis* as a diet for *Eudiaptomus* sp. are still unclear.

When *Eudiaptomus* sp. were fed the cyanobacterium *S. elongatus*, they were able to complete ontogenesis and naupliar development, albeit at strongly increased mortality rates in the copepodite stages. This could be attributed to the cyanobacterium's small cell size. It is well known that different developmental stages have different food sizes and quality demands, with nauplii ingesting much smaller particles (Zánkai, 1991). On both the green algae and cyanobacterial diets, we observed high mortality rates, although with distinct nonlinear patterns over time. Mortality with *A. obliquus* as a dietary item was complete and occurred during the naupliar phase, likely related to an inability of juveniles to ingest this prey species. Not all nauplii died when fed *C. klinobasis*, and the surviving individuals even recovered after a few days. A *S. elongatus* diet led to a higher mortality rate during the early copepodite stages, instead young copepodites seemed unable to complete their life cycle. In this stage, many transformations of the body aspect and moults occur and the small size of the food items may have required extra energy investment in food uptake efforts to reach their nutritional demand. This indicates a strong relationship between the nutritional composition of the food and the specific nutritional and physiological needs of the copepods during their ontogeny and in different life stages. In addition, calanoids fed on a pure diet of *S. elongatus* exhibited much longer naupliar phases (approximately 15 days) with a longer time needed to reach adulthood and reproduction. Moreover, the mature females also showed the lowest body size and mass. This reflects a general poor nutritional value of cyanobacteria in terms of lipids (fatty acids and sterols) impacting on growth, fecundity and fitness of the consumers (Von Elert and Wolffrom, 2001; Von Elert et al., 2003; Martin-Creuzburg, Von Elert, et al., 2008) (see also Supplementary Table SIII).

We were further able to demonstrate that the different diets also impacted morphological traits such as body size and weight of the copepods, which also reflected in fecundity and clutch size. *Eudiaptomus* sp. females fed on *Cryptomonas* sp. or the Mix diet showed higher body mass and size at first reproduction, as well as significantly higher production of eggs than the individuals fed on *Chlamydomonas* and *Synechococcus*. This can be interpreted as a sign of good health and

physiological status and possibly be used as a proxy to reproduction success and fecundity. However, as demonstrated by Ali and colleagues (Ali *et al.*, 2009), small females may also be easier for a male to coerce and mate, even though large females may be the most attractive.

1.5.3. Comparison between dietary and consumer fatty acids and evidence of consumer modification of dietary fatty acids

In the case of *Cryptomonas* sp. and the Mix diet, the assimilation of fatty acids largely mirrored the composition of the respective diets. This suggests that diets rich in EPA, DHA, ALA and SDA are optimal for reproduction and growth in calanoids. However, in the Mix diet treatment, DHA was not detected in the diet, but a low relative amount (< 4 %) was found in the consumers. This can be explained by the fact that *Eudiaptomus* sp. is a selective filter feeder and a preferential grazing focus on the ingestion, assimilation and retention of a higher amount of cells of *Cryptomonas* sp. with respect to the other phytoplankton species may have occurred. An alternative but less likely option is that in this case, DHA may also have originated from bioconversion from dietary EPA (Hashimoto *et al.*, 2008; Boyen *et al.*, 2020).

In contrast to this, the fatty acids profiles of calanoid copepods fed *C. klinobasis* or *S. elongatus* showed marked differences between resource and consumers that exhibited an accumulation of EPA and DHA despite the lack or low presence of these essential fatty acids in the algal prey. This suggests that metabolic mechanisms of bioconversion and bioaccumulation and possible *de novo* synthesis of long-chain PUFAs were implemented from the animals and may have occurred in our experiment (De Troch *et al.*, 2012; Monroig *et al.*, 2013; Werbrouck *et al.*, 2017; Monroig and Kabeya, 2018; Boyen *et al.*, 2020). Although the general view is that crustacean zooplankton have limited capacity for the bioconversion of short-chain PUFA into long-chain PUFA (Castell *et al.*, 1972; Langdon and Waldock, 1981; Sargent *et al.*, 1999; Taipale *et al.*, 2011) and that the availability of essential fatty acids has to be derived from the diet (Ahlgren *et al.*, 1990), recent advances in genomic analyses indicated that the capability for *de novo* synthesis of PUFAs may be more widespread in the animal kingdom than previously assumed (Kabeya, Fonseca, David E.K. Ferrier, *et al.*, 2018; Kabeya *et al.*, 2021).

The presence of considerable amounts of DHA and EPA in calanoids fed diets devoid of these PUFAs support this view and highlight the hypothesis that the copepods' fatty acid composition

is strongly affected by the enzymatic reactions and transformations involved in the fatty acid metabolisms (Monroig and Kabeya, 2018; Nielsen *et al.*, 2019; Boyen *et al.*, 2020; Kabeya *et al.*, 2021). Moreover, the highly selective accumulation and retention of DHA and EPA in the calanoid body in all food treatments can be explained by the high eco-physiological role of those lipids for copepods life. DHA has been shown to be of particular importance for copepods' nervous system development (Brett, Müller-Navarra D.C., *et al.*, 2009) and for maintaining membrane homeoviscosity and fluidity (Farkas, 1979). Both DHA and EPA play crucial roles in determining reproductive success, somatic growth and development of copepods (Shields *et al.*, 1999; Arendt *et al.*, 2005; Persson and Vrede, 2006; Evjemo *et al.*, 2008; Jónasdóttir *et al.*, 2009; Taipale *et al.*, 2013). However, even though the general pattern was consistent across all treatments, is still interesting to note that in our experiment calanoids fed on *C. klinobasis* and *S. elongatus* showed a preferential accumulation of DHA relative to EPA and a higher ratio of DHA/EPA than calanoid fed on *Cryptomonas* sp. and the Mix diet where EPA was largely more present than DHA. This important difference in the DHA/EPA ratio between the calanoid fed with the "good" and "poor" dietary items may be linked to the higher proportion of EPA already occurring in the "good quality" diets. On the contrary, for the calanoid fed the "poor quality" food treatments, DHA may instead have originated and later on accumulated from bioconversion from EPA or ALA or through biosynthesis processes via fatty acid desaturation and elongation (Monroig *et al.*, 2013; Nielsen *et al.*, 2019; Lee *et al.*, 2020).

1.6 Conclusions

All tested phytoplankton diets supported comparable reproductive success in adult *Eudiaptomus* sp. However, several other reproductive traits such as egg production, hatching success as well as survival rate were strictly influenced by the diet organism in the F0 generation. In the offspring (F1) generation, even clearer impacts of dietary quality were observed, with substantial changes in naupliar survival, time for development and maturation effects. Thus, the dietary fatty acid composition plays overall an important role in regulating the reproductive traits, life cycle and strategy of *Eudiaptomus* sp. notwithstanding this, we found the copepods to be able to actively regulate long chain PUFAs composition independent of the diet's composition. Therefore, the commonly paraphrased principle "you are what you eat" applies only in part, as *Eudiaptomus* sp. was able to convert some missing dietary fatty acids to maximize fitness, as well as individual survival and growth. Because the ability of various species of freshwater copepods to synthesize and/or biochemically convert essential fatty acids is still unresolved and very controversial, we suggest further genomic investigations and more specific experiments to clarify the mechanisms involved, help to draw conclusions about the feeding ecology of calanoid copepods and improve our understanding on trophic interactions and dynamics between phytoplankton and calanoid copepods in aquatic ecosystems.

1.7 Supplementary Material

Table SI. Major features and total fatty acids composition of the five phytoplankton diets used for *Eudiaptomus* sp. in the present study.

Species name	*Class of Algae*	*Cell Size (µm)*	*Total fatty acids (µg/mg POC)*
Cryptomonas sp.	Cryptophyceae	19.3 ± 3.1	30.42 ± 20.78
Chlamydomonas klinobasis	Chlorophyceae	9.4 ± 2.4	78.98 ± 7.18
Acutodesmus obliquus	Chlorophyceae	12.7 ± 1.9	13.82 ± 2.01
Synechococcus elongatus	Cyanophyceae	2.7 ± 1.0	3.43 ± 0.29
Mix diet			26.53 ± 0.99

***Table SII*.** Population growth indices for *Eudiaptomus* sp. after consuming the different algal diets.

Population growth index	*Cryptomonas sp.*	*C. klinobasis*	*S. elongatus*	*Mix*
Ro	*31.57*	*5.66*	*19.48*	*28.84*
T	*23.69*	*28.03*	*28.95*	*18.96*
rm	*0.29*	*-1.6*	*-0.4*	*0.42*

Note: Ro represents net reproduction rate, T represents the generation time and rm is the intrinsic population growth rate.

Table SIII. Fatty acids composition (as µg FA mg POC-1) of the food sources used for *Eudiaptomus* sp. in the experiment.

Fatty acid	*Cryptomonas* sp.	*A. obliquus*	*C. klinobasis*	*S. elongatus*	*Mix*
C16:0	14.29 ± 19.90	22.34 ± 1.98	3.05 ± 0.22	1.23 ± 0.09	6.72 ± 0.16
C17:0	nd	0.84 ± 0.18	1.38 ± 0.84	nd	0.83 ± 0.25
C18:0	1.30 ± 0.64 ^{b}	2.75 ± 0.21 ^{a}	1.37 ± 0.05 ^{b}	0.07 ± 0.00 ^{c}	1.99 ± 0.26 ^{ab}
C21:0	nd	nd	nd	0.18 ± 0.09	nd
C22:0	nd	0.19 ± 0.03	nd	nd	nd
Σ SAFA	***15.59 ± 20.54***	***26.12 ± 2.23***	***5.81 ± 0.84***	***1.49 ± 0.14***	***9.54 ± 0.57***
C16:1n7	0.39 ± 0.06 ^{b}	1.70 ± 0.29 ^{a}	0.30 ± 0.01 ^{b}	1.56 ± 0.15 ^{a}	1.29 ± 0.05 ^{a}
C17:1n9	0.46 ± 0.04	0.47 ± 0.08	0.56 ± 0.04	nd	0.43 ± 0.13
C18:1n9t	0.45 ± 0.28 ^{c}	nd	1.33 ± 0.03 ^{b}	0.01 ± 0.00 ^{d}	5.24 ± 0.09 ^{a}
C18:1n9c	1.44 ± 0.13 ^{b}	33.96 ± 3.23 ^{a}	0.99 ± 0.05 ^{b}	0.07 ± 0.00 ^{b}	1.04 ± 0.07 ^{b}
C 20:1n9	nd	0.68 ± 0.13	nd	nd	nd
C20:1n7	0.02 ± 0.01	nd	nd	nd	nd
C22:1n9	0.06 ± 0.01	nd	0.30 ± 0.25	0.08 ± 0.01	nd
Σ MUFA	***2.81 ± 0.36 ^{c}***	***36.80 ± 3.48 ^{a}***	***3.49 ± 0.37 ^{c}***	***1.73 ± 0.15 ^{c}***	***8.00 ± 0.08 ^{b}***
C18:2n6c (LIN)	0.69 ± 0.04 ^{c}	7.87 ± 0.77 ^{a}	0.83 ± 0.03 ^{c}	nd	2.01 ± 0.05 ^{b}
C18:3n6	nd	0.46 ± 0.01	0.34 ± 0.08	nd	nd
C18:3n3 (ALA)	1.75 ± 0.57 ^{c}	5.63 ± 0.48 ^{a}	2.87 ± 0.68 ^{bc}	0.21 ± 0.06 ^{d}	3.95 ± 0.62 ^{b}
C18:4n3	6.48 ± 0.30 ^{a}	1.95 ± 0.27 ^{b}	nd	nd	2.12 ± 0.12 ^{b}
C20:3n3	nd	nd	0.32 ± 0.28	nd	nd
C20:5n3 (EPA)	2.61 ± 0.22 ^{a}	0.16 ± 0.00 ^{c}	0.16 ± 0.01 ^{c}	nd	0.92 ± 0.05 ^{b}
C22:2n6	nd	nd	nd	0.01 ± 0.01	nd
C22:6n3 (DHA)	0.49 ± 0.20	nd	nd	nd	nd
Σ PUFA	**12.02 ± 0.82 ^{b}**	**16.06 ± 1.48 ^{a}**	**4.52 ± 1.05 ^{d}**	**0.21 ± 0.06 ^{e}**	**9.00 ± 0.81 ^{c}**
Σ n3	**11.33 ± 0.78 ^{a}**	**7.73 ± 0.70 ^{b}**	**3.35 ± 0.96 ^{c}**	**0.21 ± 0.06 ^{d}**	**6.99 ± 0.77 ^{b}**
Σ n6	**0.69 ± 0.04 ^{cd}**	**8.33 ± 0.78 ^{a}**	**1.17 ± 0.11 ^{bc}**	**0.01 ± 0.01 ^{d}**	**2.01 ± 0.05 ^{b}**
Total lipids	**30.42 ± 20.78 ^{b}**	**78.98 ± 7.18 ^{a}**	**13.82 ± 2.01 ^{bc}**	**3.43 ± 0.29 ^{c}**	**26.53 ± 0.99 ^{bc}**

SAFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, n3 = polyunsaturated omega 3 fatty acids, n6 = poliunsaturated omega 6 fatty acids, nd = not detected. Different letters within a row represent a significant differencr among algal groups. The data represent the mean ± standard deviation

of three replicates. Different letters indicate significant differences between algal treatments (one-way ANOVA, followed by Tukey's post hoc test, $p \leq 0.05$).

Table SIV. Fatty acids composition as µg per mg DW^{-1} of *Eudiaptomus* sp. fed the respective phytoplankton culture.

Fatty acid	*Cryptomonas* sp.	*C. klinobasis*	*S. elongatus*	*Mix*
C16:0	8.47 ± 2.74 ^{c}	17.66 ± 2.22 ^{a}	9.78 ± 1.38 ^{bc}	12.80 ± 1.74 ^{b}
C17:0	1.36 ± 0.35 ^{b}	2.40 ± 0.38 ^{a}	1.87 ± 0.62 ^{ab}	1.73 ± 0.16 ^{ab}
C18:0	3.34 ± 0.57	4.54 ± 0.21	4.71 ± 1.73	3.88 ± 0.68
C 20:0	0.80 ± 0.44 ^{a}	nd	0.21 ±0.13 ^{b}	0.04 ± 0.03 ^{b}
C21:0	0.18 ± 0.18 ^{b}	0.72 ± 0.06 ^{a}	0.21 ± 0.03 ^{b}	0.03 ± 0.04 ^{b}
C22:0	0.32 ± 0.13	0.58 ± 0.04	0.44 ± 0.20	0.35 ± 0.08
Σ SAFA	***14.48 ± 3.03*** ^{b}	***25.89 ± 2.00*** ^{a}	***17.21 ± 2.50*** ^{b}	***18.83 ± 2.06*** ^{b}
C16:1n7	1.11 ± 0.37 ^{b}	1.26 ± 0.03 ^{ab}	1.93 ± 0.50 ^{a}	0.97 ± 0.10 ^{b}
C17:1n9	0.36 ± 0.06 ^{b}	6.54 ± 4.31 ^{a}	2.96 ± 1.93 ^{ab}	2.41 ± 1.42 ^{ab}
C18:1n9t	0.51 ± 0.09 ^{c}	4.82 ± 1.01 ^{b}	0.88 ± 0.19 ^{c}	7.66 ± 1.04 ^{a}
C18:1n9c	1.76 ± 0.29 ^{c}	4.92 ± 0.94 ^{a}	3.77 ± 0.89 ^{ab}	2.72 ± 0.35 ^{bc}
C18:1n7	0.23 ± 0.05	0.35 ± 0.06	0.27 ± 0.20	0.36 ± 0.07
C 20:1n9	0.26 ± 0.08 ^{a}	0.08 ± 0.13 ^{bc}	0.03 ± 0.06 ^{c}	0.24 ± 0.03 ^{ab}
C20:1n7	nd	nd	0.07 ± 0.10	0.07 ± 0.04
C22:1n9	0.19 ± 0.03	0.24 ± 0.42	0.08 ± 0.09	0.09 ± 0.01
C24:1n9	nd	nd	nd	0.28 ± 0.06
Σ MUFA	***4.42 ± 0.42*** ^{c}	***18.20 ± 6.01*** ^{a}	***9.99 ± 1.76*** ^{bc}	***14.80 ± 2.74*** ^{ab}
C18:2n6c (LIN)	2.13 ± 0.39 ^{b}	2.99 ± 0.78 ^{b}	0.87 ± 0.20 ^{c}	5.46 ± 0.79 ^{a}
C18:3n6	0.42 ± 0.13 ^{b}	0.07 ± 0.13 ^{c}	0.83 ± 0.18 ^{a}	0.51 ± 0.11 ^{b}
C18:3n3 (ALA)	7.10 ± 4.07 ^{c}	33.05 ± 8.61 ^{a}	2.86 ± 1.95 ^{c}	17.88 ± 1.39 ^{b}
C18:4n3	16.31 ± 2.53 ^{a}	0.71 ± 0.57 ^{c}	2.79 ± 0.65 ^{c}	11.70 ± 1.97 ^{b}
C20:2n6	0.23 ± 0.03 ^{a}	0.05 ± 0.08 ^{b}	nd	0.18 ± 0.02 ^{a}
C20:3n6	nd	nd	0.12 ± 0.10 ^{a}	0.02 ±0.03 ^{b}
C20:4n6	0.21 ± 0.02 ^{bc}	0.09 ± 0.15 ^{c}	0.87 ± 0.12 ^{a}	0.29 ± 0.04 ^{b}
C20:3n3	0.23 ± 0.15	nd	nd	0.26 ± 0.09
C20:5n3 (EPA)	10.35 ± 1.11 ^{a}	2.35 ± 0.62 ^{c}	2.89 ± 0.96 ^{c}	6.74 ± 1.17 ^{b}
C22:2n6	nd	nd	0.17 ± 0.12	nd
C22:6n3 (DHA)	4.17 ± 0.74 ^{ab}	6.66 ± 2.36 ^{a}	5.45 ± 2.55 ^{ab}	2.94 ± 0.52 ^{b}

Σ PUFA	**41.24 ± 7.12 [a]**	**45.97 ± 9.64 [a]**	**16.95 ± 2.54 [b]**	**45.98 ± 4.58 [a]**
Σ n3	**38.17 ± 7.33 [a]**	**42.77 ± 8.59 [a]**	**14.00 ± 2.14 [b]**	**39.53 ± 4.57 [a]**
Σ n6	**3.07 ± 0.49 [b]**	**3.20 ± 1.12 [b]**	**2.95 ± 0.46 [b]**	**6.45 ± 0.89 [a]**
Total lipids	**60.14 ± 8.75 [b]**	**90.07 ± 13.67 [a]**	**44.15 ± 6.08 [b]**	**79.62 ± 4.91 [a]**

SAFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, n3 = polyunsaturated omega 3 fatty acids, n6 = poliunsaturated omega 6 fatty acids, nd = not detected. Different letters within a row represent a significant differencr among algal groups. The data represent the mean ± standard deviation of five replicates. Different letters indicate significant differences among groups (one-way ANOVA, followed by Tukey's post hoc test, $p \leq 0.05$).

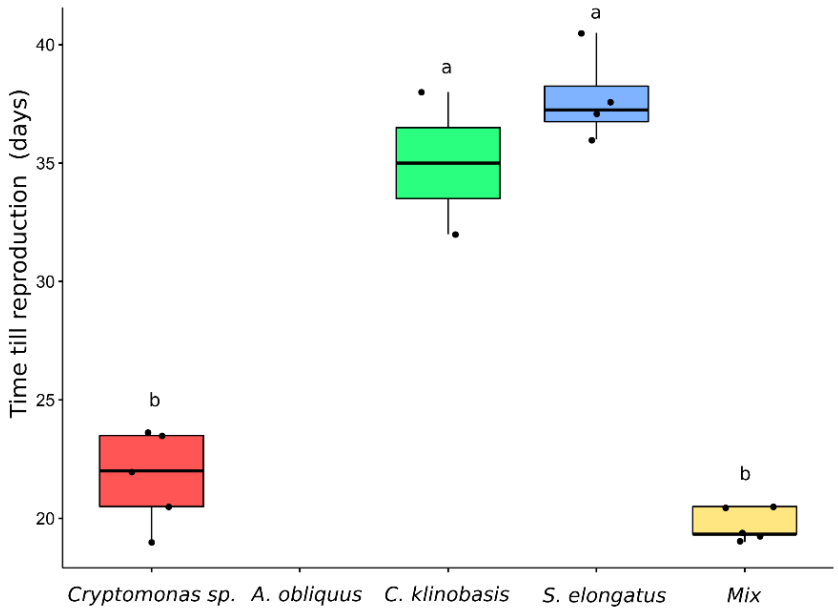

Figure SI. Time for completion life cycle (days) in the offspring generation (F1) of *Eudiaptomus* sp. fed the different phytoplankton diets.

Chapter 2

Morpho-functional traits reveal differences in size-fractionated phytoplankton communities but do not significantly affect zooplankton grazing.

This is the peer-reviewed version of the following article: Titocci J, Bon M, Fink P. *Morpho-Functional Traits Reveal Differences in Size Fractionated Phytoplankton Communities but Do Not Significantly Affect Zooplankton Grazing.* Microorganisms. 2022; 10(1):182 which has been published in final form at https://doi.org/10.3390/microorganisms10010182

2.1 Abstract

The recent emergence of approaches based on functional traits allows a more comprehensive evaluation of the role of functions and interactions within communities. As phytoplankton size and shape are the major determinants of its edibility to herbivores, alteration or loss of some morpho-functional phytoplankton traits should affect zooplankton grazing, fitness and population dynamics. Here, we investigated the response of altered phytoplankton morpho-functional trait distribution to grazing by zooplankton with contrasting food size preferences and feeding behaviours. To test this, we performed feeding trials in laboratory microcosms with size-fractionated freshwater phytoplankton (3 size classes, >30 µm; 5 - 30 µm and < 5 µm, obtained by filtration through a 5 µm and 30 µm mesh gauze, respectively) and two different consumer types: the cladoceran *Daphnia longispina*, (generalist unselective filter feeder) and the calanoid copepod *Eudiaptomus* sp. (selective feeder). We observed no significant changes in traits and composition between the controls and grazed phytoplankton communities. However, community composition and structure varied widely between the small and large size fractions, demonstrating the key role of size in structuring natural phytoplankton communities. Our findings also highlight the necessity to combine taxonomy and trait-based morpho-functional approaches when studying ecological dynamics in phytoplankton-zooplankton interactions.

2.2. Introduction

Phytoplankton forms the base of aquatic food webs and is extremely diverse. It is comprised of multiple photosynthetic organisms that vary vastly in size, shape, morphology, physiology, behaviour, functionality, and life history traits (Salmaso *et al.*, 2015; Martini *et al.*, 2021). Through photosynthesis, phytoplankton is responsible for producing up to half of the oxygen on Earth and is critical in supporting marine and freshwater food webs (Falkowski and Raven, 2007). Given its importance, phytoplankton has been studied for a very long time, mainly focusing on the identification and description of new species and their ecological role using a phenotype-based taxonomic approach. However, in recent years, trait-based approaches have gained popularity in ecological research (Weithoff, 2003; Litchman and Klausmeier, 2008; Borics *et al.*, 2012; Vallina *et al.*, 2017; Ye *et al.*, 2019), resulting in a more complete understanding of phytoplankton community structure and dynamics. Herbivorous crustacean zooplankton feeds on phytoplankton and thereby plays a key role in transferring energy from primary producers to the upper consumers in freshwater ecosystems. In this sense, the type of algal food, its size, shape, concentration, nutritional content and toxicity are decisive traits determining the strength and selectivity of zooplankton grazing (C.S. Reynolds, 1984; Helena Sipaúba-Tavares *et al.*, 2001; Zeng *et al.*, 2006; Ger *et al.*, 2016; Liu *et al.*, 2016).

The main representatives of crustacean herbivorous zooplankton in freshwater environments are Cladocera and calanoid Copepoda. They show distinct feeding modes and food size spectra for the selectivity of their prey (DeMott, 1986). Cladocera are unselective filter feeders: they use their sieve-like appendages to generate water currents from which particles exceeding the mesh size of the filter are retained for feeding (Brendelberger *et al.*, 1986). In contrast, calanoid Copepoda feed selectively and can use their "taste" and food quality as selection criteria (Bundy *et al.*, 1998). They have mechanical and chemical sensors in their antennae and can detect chemical composition (Huys, R., 1992; Ventelä *et al.*, 2002) and movements of the prey and actively capture it (Paffenhöfer *et al.*, 1982; Price *et al.*, 1983; Légier-Visser *et al.*, 1986; Paffenhöfer A., 1998; Alcaraz *et al.*, 1980; Landry, 1980).

As a defence mechanism against predation (grazing), phytoplankton can adopt several strategies like toxin production, chain formation, mucilage production, presence of spines, ability to survive gut passage and digestion (Naselli-Flores and Barone, 2011; Pančić and Kiørboe, 2018; Lürling,

2021). Between all these complex possible strategies that phytoplankton evolved in the arms race with the zooplankton (Smetacek, 2001), size and form selection are the strongest driving forces shaping phytoplankton assemblages (Morabito *et al.*, 2007) and can influence and as well be influenced by zooplankton grazing. Natural phytoplankton can be divided into several size classes: picoplankton (<2 μm), nanoplankton (2–20 μm), microplankton (20–200 μm), macroplankton (>2000 μm) with different ecological functions (Sieburth *et al.*, 1978; Beardall *et al.*, 2009).

Smaller cells have a much larger surface area/volume ratio (Lewis, 1976), can assimilate nutrients more efficiently (Lafond *et al.*, 1990), grow faster (Bruno *et al.*, 1983; Zafar, 1986) and have lower sinking rates (Waite *et al.*, 1992; Tremblay *et al.*, 1997) than larger cells. Grazers often consume small cells more readily than large cells which are often able to escape predation and dominate blooms. In particular, the food size selectivity of Cladocera and Copepoda strictly depends on their respective feeding modes and appendages: the lower limit for filterable cell size in Cladocera is determined by the mesh size of the filtration apparatus and ranges from 0.2–4.2 μm, while the upper size limit is determined by the width of frontal carapax gape of 20–30 μm (Lampert and Sommer, 2007). In calanoid copepods, the limiting factor is the opening width of the mandibles. In this case, the upper algal size limit can vary from 20 μm to >100 μm, depending on the specific copepod species. Thus, in freshwaters, cladocerans and copepods have contrasting effects: usually, the consumption of small phytoplankton cells by Cladocera (Von Rückert and Giani, 2008) and feeding of medium-size and large phytoplankton by copepods is observed.

However, not only the size of phytoplankton cells determines their susceptibility to particular grazers. Even though little is known, also the effect of the cell shape and geometry may regulate and affect the efficiency of the grazing with some shapes preferably eaten by herbivorous zooplankton. The complexity of phytoplankton forms and the coexistence of differently shaped organisms reflect the plasticity of phytoplankton populations in natural environments. It has been observed that the phytoplankton of intermediate volume display a wide variety of shapes, from oblate to extremely elongated forms, while cells of both large and small volumes are more compact and mostly spherical (Ryabov *et al.*, 2021). However, studies of morphological changes induced by the grazing pressure from natural environments are still very scarce (Böing *et al.*, 1998; Van Donk, 1997) and since natural communities are composed of different taxonomic groups with multiple cellular sizes and shapes, it is necessary to ascertain their role in the control of grazing

pressure to gain insight into the general trophic patterns at the basis of food webs observed in nature.

The main objective of this study was to investigate phytoplankton dynamics and community assembly after grazing of two different zooplankton taxa in a laboratory experiment, combining a classical taxonomic approach with a trait-based approach using morpho-functional groups (Kruk *et al.*, 2010). More specifically, we aimed to assess how grazing of herbivores with contrasting particle size preferences and feeding behaviour alters phytoplankton communities of different size structures and the response of the algal community in terms of composition, size and shape distribution.

The filter-feeding cladoceran *Daphnia longispina* and the calanoid copepods *Eudiaptomus* sp. are the dominant herbivorous zooplankters in the studied area. We selected them for our experiment due to their contrasting feeding modes, preferences and phytoplankton selectivity. The phytoplankton assemblage was derived from a natural freshwater community and fractionated into three size classes "small" (<5 µm), "intermediate" (5–30 µm) and "large" (>30 µm).

Assuming that *D. longispina* and *Eudiaptomus* sp. usually contribute to the reduction of phytoplankton abundance and biomass in a different way due to grazer-specific differences in feeding preference and selectivity, the following hypotheses were tested: (1) *D. longispina* will reduce mostly the small and intermediate phytoplankton size fractions (2) *Eudiaptomus* sp. will primarily reduce the intermediate to large size fractions.

Moreover, in terms of morpho-functional groups (MBFGs, (Kruk *et al.*, 2010)), based on relevant differences in relation to grazing behavior and selectivity of the two grazers and on previous findings by Colina et al. (Colina *et al.*, 2016) we expected (3) *D. longispina* to eat more organisms of medium size lacking specialized traits and medium size flagellates (MBFGs IV-V) because of the optimal size range and the absence of particular structures of the taxa belonging in these groups which might hinder manipulation (i.e., mucilage, spines, silica walls) and result easily to be filtered, ingested and cleared by cladocerans, but fewer organisms that produce mucilage (MBFG VII), or form long chains or filaments (MBFG III) that could clog their filtration apparatus (Sarnelle *et al.*, 2010) ; (4) *Eudiaptomus* sp. to be less affected and more able to feed on a greater diversity of phytoplankton morpho-functional groups, due to its feeding modes and its capability to select and manipulate the food (Blaxter *et al.*, 1998; Barnett and Beisner, 2007; Mauchline, 1998).

2.3. Materials and Methods

2.3.1. Sampling and Incubation Experiment

Seston samples were collected at the surface (0.5 m depth) of lake Fühlinger See, a complex of seven connected meso-eutrophic gravel pit lakes (total area 84-ha) close to the river Rhine in Cologne, Germany, in December 2018. Water samples were initially collected and filtered through a 100 µm mesh size to remove larger zooplankton. Subsequently, phytoplankton size fractionation was carried out by two consecutive filtrations through a 30 µm and 5 µm nylon mesh filters in order to obtain three phytoplankton size classes: larger than 30 µm, from 5 to 30 µm and smaller than 5 µm. Back in the laboratory, all fractions were placed in a climate chamber at 18 °C and a photon flux density of 100 µE s^{-1} m^{-2} PAR for 1 day for acclimation and then distributed evenly into 1 L polystyrene flasks.

Each of the experimental flasks with the respective size-fractionated phytoplankton community was populated with two different consumer types (in equal biomass) consisting of either 10 adult female calanoid copepod (*Eudiaptomus* sp.) or 10 four days old juvenile females of the cladoceran *Daphnia longispina*, the remaining flasks without grazers served as controls. We selected different life stages for each type of grazer to normalize their grazing pressure on the base of their biomass and to avoid the occurrence of reproductive events in *D. longispina* during the experiment. Every treatment consisted of five replicates and three size fractions. All the experimental flasks (feeding trials and controls) were incubated in dark conditions for 72 h. To quantify changes in phytoplankton due to the feeding of *Eudiaptomus sp*. and *D. longispina*, we estimated the abundances and biovolumes of the different size-fractionated phytoplankton communities in the control and grazed treatments at the end of each experiment. In addition, phytoplankton density and biovolume at the start of the experiment were estimated from the size-fractionated seston at the beginning of the experiment. This was used as a reference to show changes and temporal dynamics in control versus grazed treatments. At the end of the experiment, 100 mL of each sample were fixed with Lugol's iodine solution and counted with an inverted microscope (Utermöhl, 1958). A minimum of 400 cells were counted in two perpendicular transects at 400x magnification. Cell-specific volumes were calculated by determining an average cell size from 30 individual cells of each taxon and then multiplying by their respective cell counts (Rott, 1981). Where this was not possible, dimensions were taken from the literature in order to determine mean dimensions and

calculate a corresponding mean cell biovolume. The taxon richness, Shannon–Wiener diversity index (Shannon and Weaver, 1963) and Pielou's evenness index were calculated for each sample using phytoplankton abundance and biovolume values.

The specific grazing rates of *D. longispina* and *Eudiaptomus* sp. were calculated from algal concentrations in control and grazed flasks at the end of the incubation for all three distinct phytoplankton size classes (Bamstedt *et al.*, 2000) according to the equation:

$$G = (\ln Cc - \ln Cg) \times \frac{V}{t} \times N \quad (1)$$

where G is the grazing rate [mL individual^{-1} h^{-1}], C_c is the algal concentration in the control treatment, C_g is the algal concentration in the grazed treatments at the end of the experiment, V is the bottle volume [mL], t is the experimental duration [h] and N is the number of grazers.

2.3.2. Trait Analyses

In order to have a trait-based clustering of phytoplankton taxa, we classified the phytoplankton taxa according to their morpho-functional characteristics in seven morphology-based functional groups (MBFG) as described by Kruk et al. (2010). Group I includes all small organisms with a high surface-to-volume ratio, group II small flagellated organisms with a siliceous exoskeletal structure, group III is represented by large filaments (with aerotopes), group IV by organisms of medium size lacking specialized traits, group V is formed by unicellular flagellates of medium to large size, group VI consists of non-flagellated organisms with siliceous exoskeletons, and group VII is represented by organisms that form large mucilaginous colonies.

Information about morphological traits like biological form (unicellular, colonial/filaments, chains) and mucilage production as well as physiological traits like silica demand and N2 fixation processes and behavioral traits like the presence of flagella (motility) or aerotypes (buoyancy) were also investigated and included as binary traits (1-presence; 0-absence) to perform a trait-based cluster analysis.

We also determined the cell and individual form for each taxon as a morphological trait using eight simple geometric shapes: sphere, prolate spheroid, cylinder, ellipsoid, double cone, prism (on parallelogram base and on triangular base) and cone with half sphere according to (Ryabov *et al.*, 2021; Hillebrand *et al.*, 1999). All these morpho-functional characteristics were mainly extracted from the literature. Each taxon was assigned a value for each trait category: 1 for the presence and

0 for the absence of this characteristic. If no information was found for a taxon, 0 was assigned to all categories for this trait so that the taxon in question does not contribute to the analysis for this trait (Chevene *et al.*, 1994). The obtained taxa/trait matrix was multiplied by the taxa abundance matrix to obtain a matrix representing the abundance of each trait category for each sample.

2.3.3. Statistical Analysis

Differences in abundances, biovolumes, grazing rates and diversity indices measured over the grazing experiment were checked for normal distribution with a Shapiro–Wilk's test and for homogeneity of variances with a Levene's test. Where these assumptions were met, two-way analyses of variance (ANOVA) followed by Tukey's post-hoc tests were performed to determine the effect of treatments, size fractions and their combined effect. In cases of unequal sample sizes, a type III two-way ANOVA followed by Tukey's HSD was run instead. Multivariate permutation analysis (permANOVA) was used to analyze variations in the taxonomic and trait compositions and content of the different size fractionated algal communities at the initial condition and in the control and grazed treatments. Non-metric multidimensional scaling (NMDS) based on Bray–Curtis similarity matrices were conducted with all phytoplankton traits as variables using the vegan package in R (Oksanen *et al.*, 2014). To assess the dissimilarity and to determine the main taxonomic group and traits contributing to differences between samples, a similarity percentage analysis (SIMPER) was conducted. Differences detected with the multivariate analyses were subsequently tested with a one-way analysis of variance (ANOVA) with algal traits used as independent variables. Further, a Correspondence Analysis (CA) was used to evaluate the differences in the abundance of the taxonomic, morpho-functional groups and geometrical shapes in the different treatments and size fractions. CA was also used to visually identify the contribution of each trait category to the differences among the phytoplankton communities using the ggplot2 (Wickham, 2009) package, version 3.3.5 in R. All statistical analyses were performed using R version 3.3.3.

2.4. Results

2.4.1. Phytoplankton Abundance, Diversity and Grazing Rates

Phytoplankton abundance ranged from 1.36×10^5 to 5.04×10^6 cells L^{-1} with no significant differences between treatments in the three size-fractionated communities (ANOVA, $df = 3$, $F = 2.043$, $p = 0.126$, Figure 1a, for statistics, see Supplementary Tables S1 and S2).

However, size fractions showed significant differences with the smallest fraction (<5 µm) showing a significantly higher abundance in all treatments with respect to the intermediate (5–30 µm) and large (>30 µm) size fractions (ANOVA, $df = 2$, $F = 21.273$, $p < 0.001$, for more details, see Supplementary Tables S1, S2, S3, Figure 1a). In terms of biovolume, the opposite trend was observed, with the large phytoplankton size fraction having a significantly higher average biovolume than the 5-30 µm fraction (ANOVA, $df = 2$, $F = 5.194$, $p < 0.05$, see Supplementary Tables S1 and S2, S3). Grazing did not significantly affect total biovolume (ANOVA, $df = 3$, $F = 2.417$, $p = 0.0828$).

No significant differences in terms of the richness of taxa were found in the experiment between grazer type and phytoplankton size fractions (Supplementary Tables S1 and S2). However, Shannon–Wiener diversity changed throughout the experiment: abundance-based diversity (but not biovolume-based diversity) was highest at the start of the experiment (Figure 1b). The size fraction >30 µm exhibited a significantly higher abundance-based diversity than the other two size fractions, but a significantly lower diversity in terms of biovolume (see Supplementary Table S1 and S2, S3). The same results were observed also for Pielou's evenness index.

In general, grazing rates were always higher in *D. longispina* than in *Eudiaptomus* sp. (see Supplementary Table S1), and both grazers fed preferentially on the large and small phytoplankton size fractions. However, no statistical effect was observed in the size selectivity form *D. longispina* and *Eudiaptomus* sp. (ANOVA, $df = 2$, $F = 0.083$, $p = 0.921$). In a few cases, a positive response of total phytoplankton abundance to zooplankton grazing, and thus, negative grazing rates were observed, resulting in higher phytoplankton abundances in grazed versus control treatments.

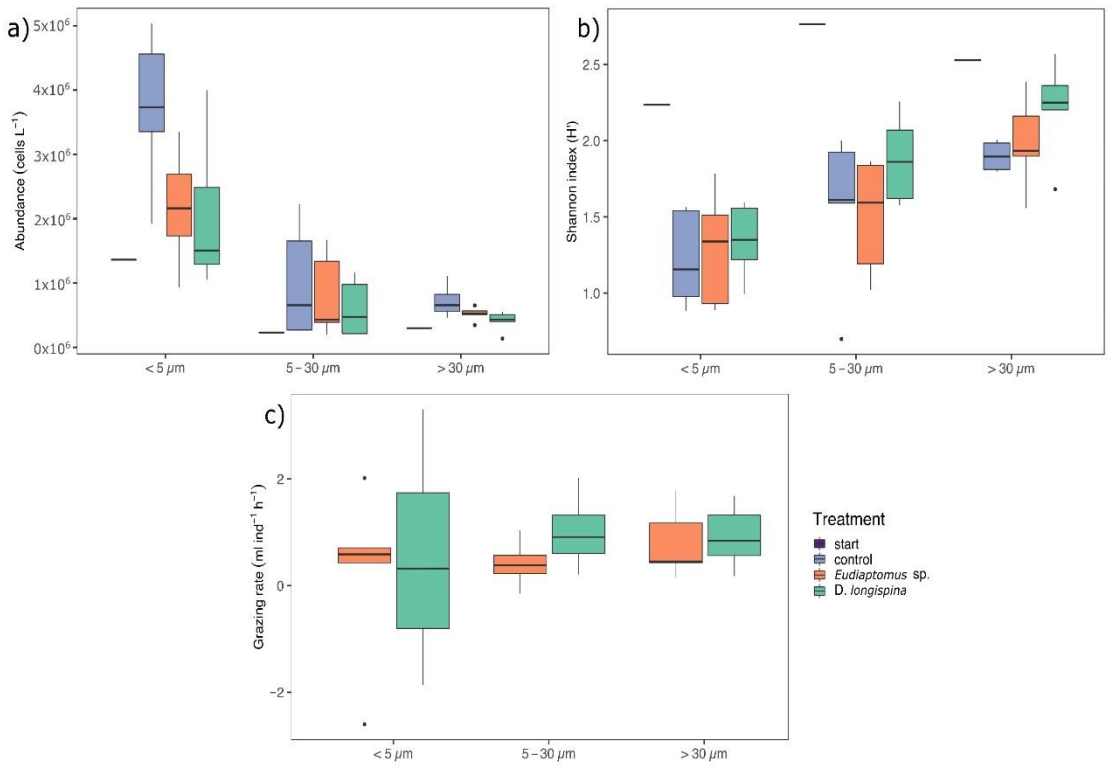

Figure 1. Box-plot showing (**a**) median absolute abundance of phytoplankton, (**b**) Shannon diversity index calculated on abundances (**c**) median grazing rates. In the first two plots values are shown at the starting point of the experiment (purple, start, n = 1) and in the three size-fractions <5 µm, 5–30 µm and >30 µm in the control (light purple, n = 5 except in >30 µm where n = 4) and grazed treatments (orange for *Eudiaptomus* sp. and green for *D. longispina*, n = 5). In the last plot, grazing rates are shown only between the two grazers type (n = 5 in all treatments, except in >30 µm size fraction where n = 4). Horizontal bars indicate the median, and the upper and lower edges of the box denote the 25 and 75 percentile, respectively. Points indicate outliers.

2.4.2. Phytoplankton Trait Analyses

A total of 60 algal taxa were identified in the samples. These belonged to seven major taxonomical groups: Bacillariophyceae (13), Chlorophyceae (23), Cryptophyceae (2), Chrysophyceae (5), Cyanophyceae/cyanobacteria (9), Dinophyceae (4), and Zygnematophyceae (4). Significant differences were found in taxonomic composition in all the size fractions (permANOVA, $df = 2$, $F = 19.89$, $p < 0.001$) and between treatments (permANOVA, $df = 3$, $F = 2.37$, $p < 0.05$). Cyanobacteria were the dominant taxonomic group in the small size fraction (<5 µm), contributing between 65.06–92.48 % to the total phytoplankton abundance in all the treatments (Figure 2a). They were mainly represented by colonial forms like *Aphanocapsa* sp., *Microcystis*, *Synechococcus* and filamentous ones like *Anabaenopsis* sp., *Planktothrix agardhii* and *Limnothrix*

redekei. Chlorophyceae were mostly co-dominant with cyanobacteria, contributing 38.29 to 54.48 % to the total abundance in both the intermediate (5–30 µm) and large (>30 µm) size fractions. The most abundant genera were *Chlamydomonas*, *Chlorella*, *Chloroccocales* and groups of flagellate algae. In the intermediate size fraction, the phytoplankton community grazed by *Eudiaptomus* sp. showed a significantly higher content of Chlorophyceae (mainly due to groups of flagellate green algae) compared to the *D. longispina* treatment and a significantly lower abundance in respect to the control treatment (permANOVA, $p < 0.05$). In contrast, Chrysophyceae were significantly more abundant in *D. longispina* in the large size fraction than in the calanoid treatment (permANOVA, $p < 0.05$) but no significant differences were detected between grazed and control treatments. The starting phytoplankton community composition varied widely within size classes and in comparison, with the other treatments showing a more heterogeneous taxonomic composition. In the starting treatment, a higher proportion of Bacillariophyceae (mainly represented by the genera *Asterionella, Navicula, Fragilaria, Eunotia, Cyclotella*), Chrysophyceae (with *Dynobrion* sp. as the most abundant taxon) and Zygnematophyceae (mainly represented by *Closterium* sp.) were observed. Other taxonomic groups like Cryptophyceae and Dinophyceae made little or no contribution to the total phytoplankton abundance in all the samples.

When focusing on morpho-functional groups (Kruk *et al.*, 2010), most cyanobacterial taxa found in the experiment belonged to group III (large filaments with aerotopes) and VII (large mucilaginous colonies), most of the taxa of Chlorophyceae clustered in groups IV(medium-sized organisms lacking specialized traits as *Monoraphidium*, *Coelastrum*, *Scenedesmus*, etc.) and V (unicellular flagellates of medium to large size as *Carteria*, *Chlamydomonas*, etc.) and all Bacillariophyceae belonged to group VI (non-flagellated organisms with siliceous exoskeletons). All seven MBFG were detected in all samples but in different proportions (Figure 2b). Group III, followed by group I were dominant in the small size (<5 µm) phytoplankton communities. In the intermediate-size communities group V was the most abundant, followed by groups I and VII. Group V was also highly represented in the large fraction, followed by groups III, I and VII in similar proportions. At the start of the experiment, the phytoplankton community showed more diverse morpho-functional traits in all size fractions, with a high abundance of group VI in the intermediate and large fractions. There were significant differences in phytoplankton morpho-functional-based assemblages among the different size fractions (permANOVA, $df = 2$, $F = 2.62$ $p < 0.001$), however, no significant differences were found between the different grazed treatments

and the control (permANOVA, *df* = 3, F = 1.65, *p* = 0.103) while the starting conditions were significantly different respect to the experimental ones (permANOVA, $p < 0.05$ for the pairwise comparisons start vs control, start vs *D. longispina* and start vs *Eudiaptomus* sp. treatments).

All eight shapes were present in all the size fractions and in each treatment but in different proportions (Figure 2c). In the small-sized fraction cylinder (65–68 %) and sphere (30–32 %) were the most abundant shapes in all the treatments and a significantly higher abundance of organisms with a double conical shape were detected at the starting conditions (permANOVA, $p < 0.05$). Intermediate-size organisms were highly dominated by spherical shapes (90–95 %) in the control and grazed treatments while the initial intermediate-size phytoplankton community showed a significantly lower abundance of spherical organisms and a significantly higher proportion of organisms belonging to cylindrical, prismatic and conical shapes than the rest of the samples (permANOVA, $p < 0.05$). Sphere (38%) and cylinder (29%) resulted in the most representative forms also in large-sized organisms. However, in the large fraction, the form ellipsoid was recorded as significantly more abundant in control than in grazed treatments (permANOVA, $p < 0.05$).

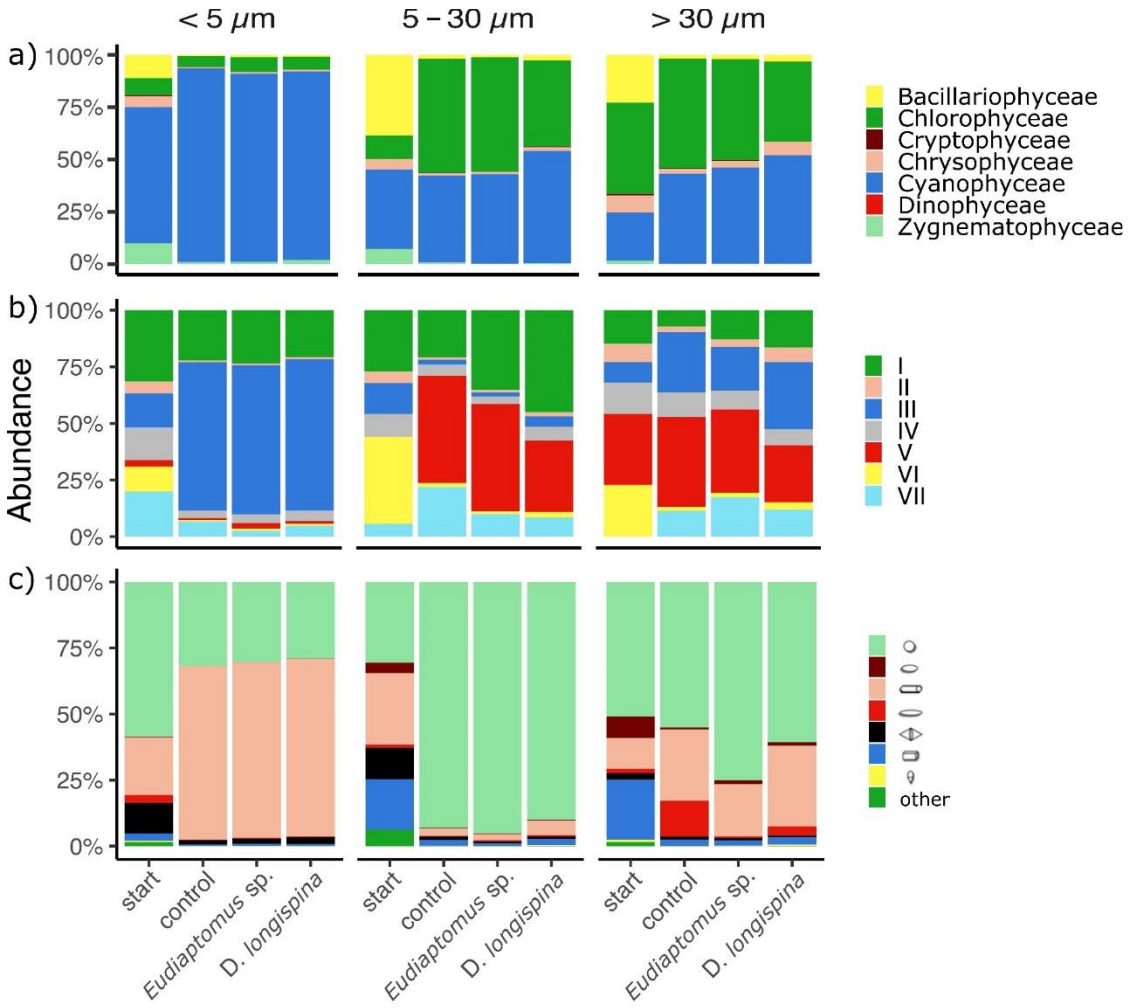

Figure 2. Mean relative abundance (%) of phytoplankton (**a**) taxonomic groups (**b**) morpho-functional groups and (**c**) shapes for each of the three size fractions and the starting condition, the control and grazed treatments. Morpho-functional groups are: (I) small organisms with high surface/volume (II) small flagellated organisms with siliceous exoskeletal structure (III) large filaments (with aerotopes), (IV) organisms of medium size lacking specialized traits (V) unicellular flagellates of medium to large size (VI) non-flagellated organisms with siliceous exoskeletons and (VII) large mucilaginous colonies. In 2c, the eight dominant shapes are: spheres (light green), prolate spheroids (brown), cylinders (pink), ellipsoids (red), double cones (black), prisms (blue), cones with half sphere (yellow) and others (green) among the three size classes and between the starting conditions and the control and grazed treatments.

The correspondence analysis (CA) also revealed that phytoplankton composition and structure variability along the experiment was mainly driven by size (Figure 3a). Axis 1 discriminated small size fraction communities from large and intermediate ones, while axis 2 separated mostly the starting phytoplankton communities from the experimental ones. Grazing by either *Eudiaptomus*

sp. or *D. longispina* did not appear as a key factor structuring the phytoplankton community, as all the size clusters overlapped in most experimental units.

Moreover, a taxonomic, functional and shape variation was recorded according to phytoplankton size structure (Figure 3b). Part of the intermediate size fractions of the control and *Eudiaptomus* treatments were clearly represented by Chlorophyceae and Dinophyceae that comprised unicellular flagellates organisms of medium to large size as morpho-type V organized in spherical and ellipsoidal shapes. A more heterogeneous composition in terms of taxonomic, morpho-functional and shape groups was instead detected in the rest of the intermediate and large-size fractions. In this case, siliceous organisms (groups VI-II) belonging to the classes of Bacillariophyceae and Chrysophyceae, organisms of medium size lacking specialized traits (group IV) and large mucilaginous colonies-forming organisms (group VII) were the main representatives, showing high variability in forms with flattened, conical and prismatic shapes. In contrast, small-size fractions were mainly dominated by filamentous cyanobacteria (group III) with cylindrical shapes.

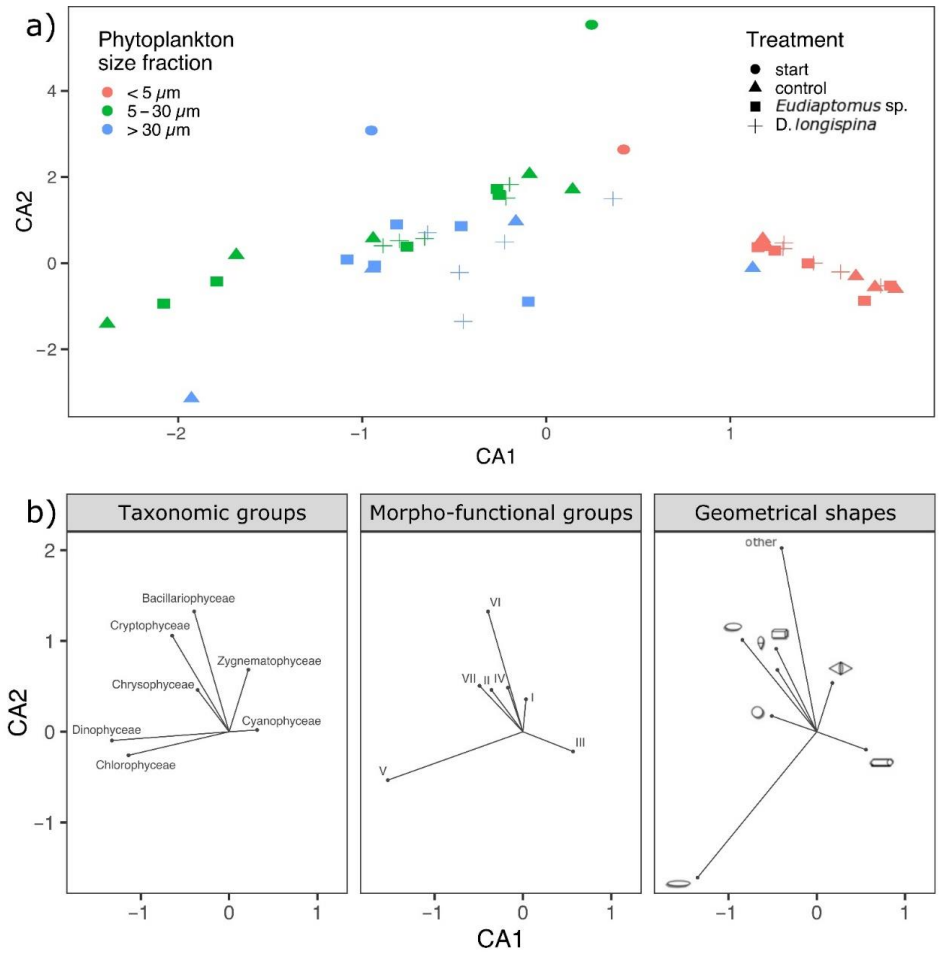

Figure 3. Ordination plot resulting from a correspondence analysis (CA), (**a**) main biplot with the mean phytoplankton trait abundance composition according to treatments and size fractions represented by different symbols and colors respectively, (**b**) plots for taxonomic, morpho-functional and geometrical shape based groups with relative vectors.

Further analyses of the phytoplankton traits confirmed that the largest differences between communities were based on sizes. Indeed, all statistical differences were observed among phytoplankton size fractions and not between the grazed treatments and the control (Table 1). The small fraction significantly differed from the large and intermediate fractions, showing a community structure mainly characterized by organisms without flagella, mainly forming chains or long filaments with low sinking properties and without displaying a large amount of mucilage production (Supplementary Table S4). In contrast to this, the intermediate and large fractions were mostly dominated by unicellular organisms, most often motile cells that may be able to produce mucilage. However, in terms of trait abundance, the intermediate size fraction showed a

significantly higher proportion of the previously mentioned traits than the large size fraction (Supplementary Table S4).

Table 1. Results of permANOVA analysis conducted on phytoplankton traits abundance. Variances among traits were analyzed in the different treatments (starting condition, control and grazing treatments), in all phytoplankton size fractions (<5 µm, 5–30 µm and >30 µm). Asterisks indicate statistically significant effects.

Mucilage Presence/Absence	**Df**	**Sum of Squares**	**Mean Square**	**F value**	**R^2**	***p*-value**
Treatment	3	0.363	0.121	1.436	0.055	0.219
Fraction	2	2.889	1.444	17.115	0.439	0.001***
Treatment x Fraction	6	0.371	0.061	0.734	0.056	0.752
Residuals	35	2.954	0.084		0.449	
Total	46	6.58			1	
Flagella Presence/Absence	**Df**	**Sum of Squares**	**Mean Square**	**F value**	**R^2**	***p*-value**
Treatment	3	0.382	0.127	1.42	0.048	0.199
Fraction	2	3.88	1.94	21.61	0.495	0.001***
Treatment x Fraction	6	0.419	0.069	0.779	0.053	0.716
Residuals	35	3.141	0.089		0.401	
Total	46	7.823			1	
Aerotopes Presence/Absence	**Df**	**Sum of Squares**	**Mean Square**	**F value**	**R^2**	***p*-value**
Treatment	3	0.328	0.109	1.285	0.046	0.263
Fraction	2	3.291	1.645	19.32	0.468	0.001***
Treatment x Fraction	6	0.421	0.07	0.824	0.059	0.624
Residuals	35	2.981	0.085		0.424	
Total	46	7.022			1	
Unicellularity/Coloniality	**Df**	**Sum of Squares**	**Mean Square**	**F value**	**R^2**	***p*-value**
Treatment	3	0.331	0.11	1.339	0.049	0.229
Fraction	2	3.165	1.582	19.186	0.469	0.001***
Treatment x Fraction	6	0.351	0.058	0.71	0.052	0.749
Residuals	35	2.887	0.082		0.428	
Total	46	6.736				

2.5. Discussion and conclusions

2.5.1. Grazing Phase and Food Selectivity

While we had expected grazing by cladocerans and calanoid to be very different in terms of selectivity, feeding behaviour and quality and quantity of food items ingested, we found no significant differences in terms of grazing rates and size selectivity between *D. longispina* and *Eudiaptomus* sp. This is in clear contrast with previous studies that had reported Cladocera and Copepoda differ considerably in their nutritional demands and feeding modes, which should, in turn, affect phytoplankton community size structure in natural environments (Sommer and Sommer, 2006). While it has been generally accepted that cladocerans, including *D. longispina*, prefer smaller algal cells over larger ones (DeMott, 1986; Lampert and Sommer, 2007), copepods tend to feed on medium-sized to larger food particles (Sommer and Sommer, 2006). However, our data did not corroborate this observation. Even though the mean grazing rates of *D. longispina* appeared to be higher than those of *Eudiaptomus* sp. in our study, both reduced the same phytoplankton size fractions (>30 µm and <5 µm) preferentially, without any significant differences in their selectivity. Even though we observed a reduction of mean total abundance and biovolume across all grazed treatment and size fractions, no clear differences between grazed and ungrazed phytoplankton were observed in terms of abundance and morpho-functional traits.

In terms of groups based on morpho-functional traits, we observed a particular decrease in organisms of medium size lacking specialized traits and medium-sized flagellates (MBFGs IV-V) when grazed by *D. longispina*. This is in accordance with previous findings (Kruk, 2015). Mucilage-producing taxa (MBFG VII) were also reduced in the small size fraction when grazed by the cladoceran. *Eudiaptomus* sp. diminished, in particular, MBFG IV, accompanied by a reduction of MBFGs III and VII in the intermediate and small fractions. The decrease of organisms belonging to the MBFG III and VII in the small fractions from both grazers highlighted the possibility that even though these groups are generally considered not palatable because they can clog the filtration apparatus and they generally reflect poor food quality; they should not be deemed totally resistant to grazing. Nevertheless, also in this case the differences observed between grazed and control treatments were not significantly statistically proven.

Knowing that the phytoplankton communities in the experimental flasks showed a lower diversity and in general a quite different composition than the natural settings (starting condition) we

believed that some form of competition among phytoplankton could have occurred during the experiment. In particular, some organisms or taxa could have been favoured over others to adapt and survive to the laboratory conditions. In this sense, the insignificant difference between control and grazed treatments might be attributed partially to the composition of the natural phytoplankton community itself. Indeed, according to our results, we found a high prevalence of Cyanobacteria in all the size fractions, mainly represented by filamentous organisms and mucilaginous colonies that might have interfered with the grazing of both grazers, posing difficulties with their filter-type feeding behaviour.

Finally, variability between replicates of the same treatment was probably another main factor masking patterns of grazing and size selectivity in our data. This highlights the large intrinsic variability of natural plankton communities and the need for deep replication in experimental studies to smooth out the variance between replicates, improve the measurement of variation in the treatments and provide stronger statistical support.

2.5.2. Phytoplankton Size-Fractionated Composition and Structure

The taxonomy-based approach identified patterns explaining the changes in size distribution: Cyanobacteria dominated the small fraction with colonial and long filamentous taxa, whereas Chlorophyceae and Cyanobacteria were present in the intermediate and large size classes in similar abundance. The unexpected dominance of long filamentous cyanobacteria in the small size fraction was caused by the chosen gauze filtration method. Size fractionation is made on the linear dimensions of the algal cells and not on the basis of their volumes (Harbison and McAlister, 1980). Thus, it does not always separate properly based on cell size. Indeed, many larger and thin filamentous organisms passed through small filters (<5 µm) not on the basis of their length dimension, but for instance according to their elongated shapes and their narrow width. Moreover, cell or colony breakage during the filtration process may result in another source of error, with a certain portion of large particles passing through small filters and accounting for small size classes of organisms (Mullin, 1965) Although size fractionation techniques are frequently used in phytoplankton research (Runge and Ohman, 1982; Cermen˜o *et al.*, 2005; Marañón *et al.*, 2012; Sin *et al.*, 2000; McCarthy *et al.*, 1974; Durbin *et al.*, 1975; Bruno *et al.*, 1983) the accuracy and validity of portioning phytoplankton assemblages through filtration remain debated (Durbin *et al.*,

1975). We thus confirmed that fractionation by filtration has a rather low level of absolute cell size resolution (Sommer *et al.*, 2017), and hence recommend that it should be used with care, especially in grazing experiments where natural phytoplankton communities are dominated by thin filaments (Runge and Ohman, 1982).

In terms of taxon richness, no differences were found across size fractions. However, the fraction <5 µm exhibited the lowest Shannon diversity and evenness due to the high dominance of a few cyanobacterial taxa. Another interesting aspect was the higher diversity in terms of taxonomic composition in the phytoplankton community sampled at the beginning of the experiment (start treatment). This loss of diversity between the start and the experimental samples might be attributed to the laboratory conditions that selected Cyanophyceae and Chlorophyceae over Bacillariophyceae, Crysophyceae and Zygnematophyceae which decreased rapidly within the first few days of the experiment. This rapid change in the community structure should be also taken into account once performing lab experiments with the manipulation of natural assemblages.

By contrast, differences in size community structure were expressed more in detail using morpho-functional traits (Kruk *et al.*, 2010). In our experiment, the phytoplankton communities exhibited a clear differentiation between the small, intermediate and large fractions. Small fractions were mainly characterized by organisms without flagella that form chains or long filaments with high sinking velocities and without larger amounts of mucilage production. In contrast to this, the intermediate and large fractions were dominated by unicellular motile and mucilage-producing organisms.

Regarding the shape distribution, no clear distinction between size fractions could be observed for the occurrence of spherical and cylindrical forms in each size class. Nevertheless, including the analysis of the geometric shapes in phytoplankton-zooplankton studies may be a useful tool to better understand the grazing dynamics and the forms and structure used as an adaptive strategy to avoid predation and enhance resistance by phytoplankton taxa.

The concept of 'functional redundancy' with species showing similar ecological roles, using a trait-based approach and grouping taxa based on their morphological, structural and/or physiological and behavioural features represents a good instrument to summarize the diversity and ecological roles of the taxa by simplifying the complexity and variability of natural ecosystems. However, species composition varies a lot between ecosystems and according to environmental conditions and anthropogenic pressures and knowing the identity and the

taxonomical details of a specimen is fundamental for studying biodiversity, changes and conservation of the ecosystems. Knowing the taxonomic details of communities is also the first step to get an idea of the traits associated and to help in understanding and organizing the diversity and the structure of the natural communities. Thus, it is of primary importance to not exclude a taxonomic perspective from phytoplankton and zooplankton studies. Using only one or the other approach could be reductive and lead to generalizations and misleading interpretations of ecological processes (Litchman and Klausmeier, 2008). In this sense, we recommend the use of a combined taxonomic and trait-based approach to improve the understanding of phytoplankton and zooplankton interactions and to help to describe them with a broader perspective on phytoplankton community assembly and its changes under grazing pressure by herbivorous zooplankton.

2.6 Supplementary Material

Supplementary Table S1. Total abundance (cell L^{-1}), total biovolume ($\mu m^3 L^{-1}$), grazing rates (ml $ind^{-1} h^{-1}$) and diversity indices: richness (S), Shannon-Wiener (H') and Pielou's evenness (J') for each size fraction. Values are mean ± standard deviation, n = 5 in all treatments except for the start treatment where n = 1.

Fraction <5 µm	***Start***	***Control***	***Eudiaptomus* sp.**	***D. longispina***
Total Abundance (cell L^{-1})	$1.36x10^6$	$3.59x10^6 \pm 1.46x10^6$	$2.17x10^6 \pm 9.20 x10^5$	$2.47x10^6 \pm 2.06 x10^6$
Total Biovolume (cell L^{-1})	$6.62x10^8$	$3.16x10^8 \pm 1.08x10^8$	$2.15x10^8 \pm 7.34 x10^7$	$2.97x10^8 \pm 1.55x10^8$
Grazing rate (ml $ind^{-1} h^{-1}$)	-	-	0.66 ± 0.67	0.92 ± 0.60
Richness (S)	25	25.60 ± 2.88	22.6 ± 1.34	21.8 ± 1.10
Shannon index Abundance (H'a)	2.18	1.21 ± 0.31	1.27 ± 0.38	1.33 ± 0.24
Shannon index Biovolume (H'b)	1.54	1.66 ± 0.15	1.57 ± 0.25	1.40 ± 0.16
Evenness index Abundance (J'a)	0.68	0.37 ± 0.08	0.41 ± 0.12	0.43 ± 0.08
Evenness index Biovolume (J'b)	0.48	0.51 ± 0.04	0.50 ± 0.08	0.46 ± 0.06
Fraction 5-30 µm	***Start***	***Control***	***Eudiaptomus* sp.**	***D. longispina***
Total Abundance (cell L^{-1})	$2.28 x10^5$	$1.01x10^6 \pm 8.85x10^5$	$8.03x10^5 \pm 6.54x10^5$	$6.07x10^5 \pm 4.42x10^5$
Total Biovolume (cell L^{-1})	$2.38 x10^8$	$2.85x10^8 \pm 1.36x10^8$	$1.53x10^8 \pm 6.99x10^7$	$2.15x10^8 \pm 1.19x10^8$
Grazing rate (ml $ind^{-1} h^{-1}$)	-	-	0.23 ± 1.70	0.54 ± 2.04
Richness (S)	21	25.80 ± 4.02	23.2 ± 3.27	27.2 ± 2.77
Shannon index Abundance (H'a)	2.73	1.55 ± 0.52	1.47 ± 0.36	1.86 ± 0.29
Shannon index Biovolume (H'b)	1.86	1.37 ± 0.66	1.41 ± 0.40	1.29 ± 0.37
Evenness index Abundance (J'a)	0.9	0.48 ± 0.15	0.47 ± 0.11	0.56 ± 0.09
Evenness index Biovolume (J'b)	0.61	0.42 ± 0.20	0.45 ± 0.12	0.39 ± 0.12
Fraction >30 µm	***Start***	***Control***	***Eudiaptomus* sp.**	***D. longispina***
Total Abundance (cell L^{-1})	$2.97 x 10^5$	$7.20 x 10^5 \pm 2.79 x10^5$	$5.18 x10^5 \pm 1.12x10^5$	$4.04x10^5 \pm 1.62x10^5$
Total Biovolume (cell L^{-1})	$3.61 x 10^8$	$5.02 x10^8 \pm 4.06 x10^8$	$2.96x10^8 \pm 2.32x10^7$	$4.31x10^8 \pm 1.46x10^8$
Grazing rate (ml $ind^{-1} h^{-1}$)	-	-	0.41 ± 0.48	1.01 ± 0.77
Richness (S)	24	28.50 ± 3.42	24 ± 5.29	24 ± 4.995
Shannon index Abundance (H'a)	2.53	1.90 ± 0.11	1.99 ± 0.31	2.17 ± 0.31
Shannon index Biovolume (H'b)	1.99	1.24 ± 0.71	1.18 ± 0.36	0.72 ± 0.37
Evenness index Abundance (J'a)	0.8	0.57 ± 0.02	0.63 ± 0.06	0.69 ± 0.09
Evenness index Biovolume (J'b)	0.63	0.37 ± 0.21	0.37 ± 0.09	0.22 ± 0.10

Supplementary Table S2. Results of the two-way ANOVA of total abundance, biovolume, grazing rates and diversity indices for the different treatments (Start, Control, *D. longispina* and *Eudiaptomus* sp.), and the phytoplankton size fractions (< 5µm, 5-30 µm and > 30 µm) and their interactions. Bold values represent statistically significant results.

Total Abundance	Df	Sum of Squares	Mean Square	F value	p-value
Treatment	3	6.09E+12	2.03E+12	2.043	0.126
Fraction	2	4.23E+13	2.03E+12	21.273	**8.99e-07 *** **
Treatment x Fraction	6	3.07E+12	5.12E+11	0.515	0.793
Residuals	35	3.48E+13	9.94E+11		
Total Biovolume	Df	Sum of Squares	Mean Square	F value	p-value
Treatment	3	1.86E+17	6.20E+16	2.417	0.0828
Fraction	2	2.66E+17	1.33E+17	5.194	**0.0106***
Treatment x Fraction	6	1.19E+17	1.98E+16	0.772	0.597
Residuals	35	8.97E+17	2.56E+16		
Grazing rate	Df	Sum of Squares	Mean Square	F value	p-value
Treatment	1	0.75	0.7497	0.486	0.493
Fraction	2	1.17	0.5849	0.379	0.689
Treatment x Fraction	2	0.25	0.1274	0.083	0.921
Residuals	22	33.96	1.5439		
Richness	Df	Sum of Squares	Mean Square	F value	p-value
Treatment	3	83.1	27.68	2.261	0.0985
Fraction	2	33.9	16.97	1.386	0.2634
Treatment x Fraction	6	75.9	12.65	1.033	0.4208
Residuals	35	428.6	12.25		
Shannon index Abundance	Df	Sum of Squares	Mean Square	F value	p-value
Treatment	3	2.575	0.8583	7.646	**0.000466*** **
Fraction	2	4.051	2.0256	18.044	**4.12e-06 *** **
Treatment x Fraction	6	0.331	0.0551	0.491	0.810545
Residuals	35	3.929	0.1123		

Shannon index Biovolume	Df	Sum of Squares	Mean Square	F value	p-value
Treatment	3	1.38	0.4599	2.687	0.0614
Fraction	2	1.544	0.772	4.51	**0.0181***
Treatment x Fraction	6	0.72	0.12	0.701	0.6505
Residuals	35	5.991	0.1712		
Evenness index Abundance	Df	Sum OF Squares	Mean Square	F value	p-value
Treatment	3	0.2881	0.096	10.211	**5.65e-05 *** **
Fraction	2	0.3654	0.1827	19.426	**2.11e-06 *** **
Treatment x Fraction	6	0.0353	0.0059	0.626	0.708
Residuals	35	0.3292	0.0094		
Evenness index Biovolume	Df	Sum OF Squares	Mean Square	F value	p-value
Treatment	3	0.1391	0.04637	3.157	**0.03677***
Fraction	2	0.1782	0.08909	6.066	**0.00547****
Treatment x Fraction	6	0.0725	0.01209	0.823	0.55971
Residuals	35	0.5141	0.01469		

Supplementary Table S3. Results of the post hoc comparison tests of total abundance, biovolume, Shannon and Evenness indices among fractions and treatments, derived from two-way ANOVA analysis. Bold type indicates significant results.

Post-hoc test

Total Abundance by Fraction	**p-value**
5-30 µm vs <5 µm	**<0.001**
>30 µm vs <5 µm	**<0.001**
>30 µm vs 5-30 µm	0.816
Total Biovolume by Fraction	**p-value**
5-30 µm vs <5 µm	0.333
>30 µm vs <5 µm	0.183
>30 µm vs 5-30 µm	**<0.05**
Shannon index Abundance by Treatment	**p-value**
Control vs Start	**<0.001**
D. longispina vs Start	**<0.05**
Eudiaptomus sp. vs Start	**<0.001**
D. longispina vs Control	0.18
Eudiaptomus sp. vs Control	0.981
Eudiaptomus sp. vs *D. longispina*	0.324
Shannon index Abundance by Fraction	
5-30 µm vs <5 µm	**<0.001**
>30 µm vs <5 µm	**<0.001**
>30 µm vs 5-30 µm	**<0.05**
Shannon index Biovolume by Fraction	**p-value**
5-30 µm vs <5 µm	0.543
>30 µm vs <5 µm	**<0.05**
>30 µm vs 5-30 µm	0.147
Evenness index Abundance by Treatment	**p-value**
Control vs Start	**<0.001**
D. longispina vs Start	**<0.001**
Eudiaptomus sp. vs Start	**<0.001**
D. longispina vs Control	0.057
Eudiaptomus sp. vs Control	0.772
Eudiaptomus sp. vs *D. longispina*	0.336
Evenness index Abundance by Fraction	
5-30 µm vs <5 µm	**<0.05**
>30 µm vs <5 µm	**<0.001**
>30 µm vs 5-30 µm	**<0.05**
Evenness index Biovolume by Treatment	**p-value**
Control vs Start	0.323
D. longispina vs Start	<0.05

Eudiaptomus sp. vs Start	0.327
D. longispina vs Control	0.287
Eudiaptomus sp. vs Control	0.999
Eudiaptomus sp. vs *D. longispina*	0.259
Evenness index Biovolume by Fraction	
5-30 µm vs <5 µm	0.377
>30 µm vs <5 µm	**<0.001**
>30 µm vs 5-30 µm	**<0.05**

Supplementary Table S4. Traits abundance composition (cell L^{-1}). Values are mean ± standard deviation, n = 5 in all treatments except for the start treatment where n = 1 and the control treatment of the fraction > 30 µm where n = 4.

Fraction <5 µm	***Start***	***Control***	***Eudiaptomus* sp.**	***D. longispina***
Mucillage presence	2.70×10^5	$2.91 \times 10^5 \pm 3.03 \times 10^5$	$8.46 \times 10^4 \pm 5.46 \times 10^4$	$1.32 \times 10^5 \pm 6.06 \times 10^4$
Mucillage absence	1.09×10^6	$3.43 \times 10^6 \pm 1.32 \times 10^6$	$2.09 \times 10^6 \pm 9.02 \times 10^5$	$1.93 \times 10^6 \pm 1.19 \times 10^6$
Flagella presence	8.23×10^4	$1.00 \times 10^5 \pm 4.97 \times 10^4$	$8.99 \times 10^4 \pm 4.08 \times 10^4$	$7.21 \times 10^4 \pm 2.40 \times 10^4$
Flagella absence	1.28×10^6	$3.62 \times 10^6 \pm 1.17 \times 10^6$	$2.08 \times 10^6 \pm 9.37 \times 10^5$	$1.99 \times 10^6 \pm 1.20 \times 10^6$
Aerotopes presence	4.76×10^5	$2.60 \times 10^6 \pm 1.05 \times 10^6$	$1.45 \times 10^6 \pm 7.71 \times 10^5$	$1.43 \times 10^6 \pm 1.09 \times 10^6$
Aerotopes absence	8.86×10^5	$1.12 \times 10^6 \pm 4.53 \times 10^5$	$7.19 \times 10^5 \pm 3.43 \times 10^5$	$6.41 \times 10^5 \pm 1.34 \times 10^5$
Unicellular	8.80×10^5	$9.76 \times 10^5 \pm 4.33 \times 10^5$	$6.43 \times 10^5 \pm 3.17 \times 10^5$	$5.43 \times 10^5 \pm 1.70 \times 10^5$
Filaments/colonies	8.91×10^5	$3.51 \times 10^6 \pm 1.18 \times 10^6$	$2.00 \times 10^6 \pm 9.42 \times 10^5$	$1.90 \times 10^6 \pm 1.19 \times 10^6$
Fraction 5-30 µm	***Start***	***Control***	***Eudiaptomus* sp.**	***D. longispina***
Mucillage presence	1.29×10^4	$2.39 \times 10^5 \pm 2.92 \times 10^5$	$1.11 \times 10^5 \pm 1.00 \times 10^5$	$7.95 \times 10^4 \pm 3.44 \times 10^4$
Mucillage absence	2.15×10^5	$7.72 \times 10^5 \pm 7.10 \times 10^5$	$6.92 \times 10^5 \pm 6.22 \times 10^5$	$5.27 \times 10^5 \pm 4.27 \times 10^5$
Flagella presence	9.00×10^3	$5.03 \times 10^5 \pm 7.77 \times 10^5$	$4.16 \times 10^5 \pm 4.30 \times 10^5$	$2.25 \times 10^5 \pm 2.24 \times 10^5$
Flagella absence	2.19×10^5	$5.08 \times 10^5 \pm 5.80 \times 10^5$	$3.88 \times 10^5 \pm 3.41 \times 10^5$	$3.81 \times 10^5 \pm 2.30 \times 10^5$
Aerotopes presence	3.09×10^4	$1.55 \times 10^5 \pm 1.86 \times 10^5$	$4.01 \times 10^4 \pm 6.79 \times 10^4$	$2.75 \times 10^4 \pm 9.73 \times 10^3$
Aerotopes absence	1.97×10^5	$8.56 \times 10^5 \pm 7.83 \times 10^5$	$7.63 \times 10^5 \pm 6.23 \times 10^5$	$5.79 \times 10^5 \pm 4.38 \times 10^5$
Unicellular	1.54×10^5	$7.33 \times 10^5 \pm 7.04 \times 10^5$	$6.70 \times 10^5 \pm 6.16 \times 10^5$	$4.88 \times 10^5 \pm 4.21 \times 10^5$
Filaments/colonies	1.29×10^5	$4.70 \times 10^5 \pm 5.31 \times 10^5$	$3.84 \times 10^5 \pm 3.68 \times 10^5$	$3.64 \times 10^5 \pm 2.37 \times 10^5$
Fraction >30 µm	***Start***	***Control***	***Eudiaptomus* sp.**	***D. longispina***
Mucillage presence	nd	$1.00 \times 10^5 \pm 6.14 \times 10^4$	$1.06 \times 10^5 \pm 6.44 \times 10^4$	$5.96 \times 10^4 \pm 4.83 \times 10^4$
Mucillage absence	2.97×10^5	$6.20 \times 10^5 \pm 2.39 \times 10^5$	$4.12 \times 10^5 \pm 1.36 \times 10^5$	$3.45 \times 10^5 \pm 1.47 \times 10^5$
Flagella presence	1.17×10^5	$3.13 \times 10^5 \pm 3.18 \times 10^5$	$2.22 \times 10^5 \pm 8.38 \times 10^4$	$1.29 \times 10^5 \pm 8.18 \times 10^4$
Flagella absence	1.79×10^5	$4.07 \times 10^5 \pm 7.62 \times 10^4$	$2.96 \times 10^5 \pm 5.00 \times 10^4$	$2.76 \times 10^5 \pm 1.48 \times 10^5$
Aerotopes presence	2.57×10^4	$2.23 \times 10^5 \pm 1.08 \times 10^5$	$1.37 \times 10^5 \pm 1.04 \times 10^5$	$1.35 \times 10^5 \pm 7.26 \times 10^4$
Aerotopes absence	2.71×10^5	$4.97 \times 10^5 \pm 2.58 \times 10^5$	$3.81 \times 10^5 \pm 1.47 \times 10^5$	$2.70 \times 10^5 \pm 9.70 \times 10^4$
Unicellular	1.91×10^5	$4.13 \times 10^5 \pm 2.60 \times 10^5$	$2.98 \times 10^5 \pm 1.32 \times 10^5$	$2.11 \times 10^5 \pm 8.74 \times 10^4$
Filaments/colonies	1.47×10^5	$3.44 \times 10^5 \pm 1.01 \times 10^5$	$2.68 \times 10^5 \pm 5.97 \times 10^4$	$2.47 \times 10^5 \pm 1.43 \times 10^5$

Chapter 3

Disturbance alters phytoplankton functional traits and consequently drives changes in zooplankton life history traits and lipid composition.

This is the version of the following article: Jessica Titocci & Patrick Fink, *Disturbance alters phytoplankton functional traits and consequently drives changes in zooplankton life history traits and lipid composition.*, which was submitted in date 29/03/2023 in this form at Hydrobiologia Journal, for the Topical collection *Functional ecology of aquatic organisms.*

3.1 Abstract

In light of the current biodiversity crisis that affects in particular freshwater ecosystems, it becomes crucial to understand the effects of functional diversity loss on phytoplankton-zooplankton interactions in freshwater food webs. Here, we simulated the loss of phytoplankton trait diversity by applying different intensities of mechanical disturbance to a natural phytoplankton community in a laboratory experiment. Different disturbance regimes clearly affected the trait distribution and functional diversity of these phytoplankton communities. In the experiment's second phase, these altered communities were provided as a food source to the herbivorous zooplankton grazers *Daphnia longispina* and *Eudiaptomus graciloides* and their life history traits and lipid compositions were investigated. Both zooplankton fitness and reproductive success were affected differently, depending on the grazers' feeding modes. Phytoplankton fatty acid composition was generally reflected in the consumers' tissue. Nevertheless, some selective PUFAs accumulation occurred and mismatches in some fatty acids suggested a possible enzymatic modification of dietary fatty acids adopted to face biochemical deficiencies of the diets. Overall, this study highlights how a loss of specific traits in the resource community could impact consumer communities and infer how these altered community traits may affect food web dynamics.

3.2 Introduction

Freshwater habitats are experiencing and suffering a dramatic biodiversity crisis. Nevertheless, the consequences of biodiversity loss remain still largely unknown and insufficient information is yet available to make predictions on the future status and trends of freshwater biodiversity, ecosystem functioning and services. It is hence crucial to investigate the impact of diversity loss on freshwater aquatic organisms and all the potential cascading and feedback effects on aquatic food webs.

Phytoplankton, being composed of thousands of species, each with a variety of different functional traits, represents a treasure trove of biodiversity. It forms the base of aquatic food webs and through photosynthesis, it is responsible for roughly half of the global primary production (Falkowski, 1994). It also contributes to nutrient cycling and the regulation and maintenance of all higher trophic levels in aquatic ecosystems. Phytoplankton provides important biochemical constituents to consumers such as carbohydrates, fatty acids, amino acids, sterols and vitamins (Peltomaa *et al.*, 2017). Among them, a particular focus has been on essential fatty acids (EFAs) which play an important role in zooplankton development, health and reproduction. EFA must be obtained from the zooplankton's diet because many animals cannot synthesize them de novo (Pond *et al.*, 1996; Bell *et al.*, 2007), although new findings are questioning this long-standing belief (Kabeya, Fonseca, David E.K. Ferrier, *et al.*, 2018; Boyen *et al.*, 2022). Phytoplankton biochemical traits are highly diverse and depend on both the respective algal species and their physiological condition (Fink *et al.*, 2011; Lang *et al.*, 2011). As a consequence, a loss or change in the nutritional value of phytoplankton will impact the nutritional quality of zooplankton and consequently the production and transfer of essential biomolecules through the food web in general (Müller-Navarra *et al.*, 2004; Lau *et al.*, 2021). Phytoplankton functional trait diversity is, therefore, the key determinant of the fitness of planktonic consumers, influencing the overall functioning of pelagic ecosystems (Irwin and Finkel, 2017). In light of biodiversity loss, it should be of primary concern and importance to understand the relationships and mechanisms between the variability in phytoplankton functional diversity and its effects on zooplankton communities, food web dynamics, and ecosystem processes.

To achieve this goal, experimental research needs to directly manipulate the diversity of taxa and traits as a strategy to identify and interpret the effects that changing biodiversity has on planktonic communities functioning and aquatic ecosystems (Litchman *et al.*, 2007; Irwin and Finkel, 2017; Engel *et al.*, 2017; Gerhard *et al.*, 2021). For example, phytoplankton density, biomass,

composition and diversity can be directly manipulated by the addition or removal of species or assembling communities with different taxonomic or functional compositions and diversities. However, as phytoplankton is composed of microscopic organisms, these controlled changes become particularly challenging in laboratory or field experiments dealing with natural phytoplankton assemblages.

According to Hammerstein et al. (2017), gradients of hydrodynamic disturbance can be used as an easily manageable tool to alter the diversity of natural algal communities. There, mechanical disturbance as mixing or shaking of natural phytoplankton communities affects particularly stress-sensitive species (Elmqvist *et al.*, 2003; Gallagher *et al.*, 2015), generating communities with different composition, richness, taxonomic and trait diversity.

We here used this disturbance method to simulate the loss of phytoplankton functional trait diversity by applying different intensities of mechanical disturbance to a natural lake phytoplankton community. Subsequently, we provided the altered phytoplankton communities as a food source to *Daphnia longispina* and *Eudiaptomus graciloides*, here selected for their distinct feeding modes and diet selectivity and as representatives of the Cladocera and calanoid Copepoda, the two main taxonomical and functional widespread zooplankton groups in freshwater environments.

The aim of the experiment was to investigate how changes in phytoplankton community composition and trait diversity may affect the development, fitness and reproductive success of herbivorous zooplankton feeding on them. Moreover, we investigated how *D. longispina* and *E. graciloides* respond to changes in phytoplankton nutritional quality, analyzing and comparing their fatty acid profiles with the lipid profiles of the altered phytoplankton communities.

Although the two grazers are quite similar in size and occupy the same freshwater habitats, they exhibit distinct and specific differences in diet preferences, selectivity, reproductive strategies and life cycles. *Daphnia longispina* are opportunistic filter feeders that cannot select food particles individually but only food items that have appropriate size to pass the filtering appendages will be retained (Geller and Müller, 1981; Gophen and Geller, 1984). In contrast, *Eudiaptomus graciloides* feed selectively via mechanical and chemical sensors in their mouthparts, on the antennae and body surface that help them to detect the chemical composition and movements of the prey (DeMott, 1986; Heuschele and Selander, 2014).

Considering this, we thus expect that, in general, the disturbance method will modify phytoplankton trait composition and diversity and this will strongly modulate and influence their palatability and accessibility for zooplankton grazing. Specifically, altered phytoplankton communities

(i) will impact the nutritional quality of zooplankton in a different way in *D. longispina* and *E. graciloides* due to their different feeding strategies

(ii) will result in a variety of life-history trait effects/responses in both grazers, which may include variation in developmental time, body size and fecundity

(iii) will primarily penalize daphnids, which, being unable to perceive phytoplankton nutritional quality will require extra energy investment and efforts in food uptake through filtration to satisfy their nutritional and physiological demands

(iv) will mainly favour copepods, which, being able to select their food particles individually, will actively choose the food items which most closely match their dietary needs.

This experiment thus aids our understanding of how the loss of functional traits in the resource community could impact consumer traits and to clarify to what extent dietary dependency occurs in *D. longispina* and *E. graciloides*. Furthermore, it tests whether consumers may have evolved adaptation strategies to fulfil their physiological requirements in response to variation or reduction of resource quality due to biodiversity loss, thereby modifying the energy transfers to higher trophic levels.

3.3 Materials and Methods

3.3.1 Phase I: Plankton sampling and disturbance method

Natural seston samples were collected on June 2020 at the surface of lake Fühlinger See, a recreational lake and former gravel pit located north of the city of Cologne in Western Germany (51°01'21.0"N 6°55'48.1"E). Plankton was filtered *in situ* through a 150 µm mesh to remove large zooplankton. Upon transportation to the laboratory, the samples were placed in a climate chamber at 20 ± 0.5 °C (similar to the June surface water temperature of the lake) and under 100 µE s^{-1} m^{-2} PAR for 1 day for acclimation, before evenly distributing them into fifteen 1 L borosilicate glass Erlenmeyer flasks.

The first phase of the experiment consisted of the manipulation of the phytoplankton community by applying three different disturbance intensity levels: no disturbance, intermediate disturbance and high disturbance (replicated five times each) to create phytoplankton trait diversity loss and community changes. Experimental flasks were continuously subjected to mixing using a laboratory stirrer with different intensities. The "undisturbed" treatment was not mixed, phytoplankton subjected to the "intermediate disturbance" was gently mixed on a horizontal magnetic laboratory shaker with a speed set on 70 rounds min^{-1}, while the shaker for the "high disturbance" treatment was set to 135 rounds min^{-1}. Every 4 days, 25 vol. % of each sample was removed under a sterile atmosphere and replaced by fresh growth medium (1/5 of WC medium, Guillard & Lorenzen, 1972, diluted with ultrapure water). Those subsamples were subsequently analyzed for phytoplankton biomass, pigments, fatty acids and community composition analyses (see below).

3.3.2 Phase II: Feeding experiment and zooplankton life history trait analysis

In the second phase of the experiment, the phytoplankton communities altered via different disturbance treatments were subsequently used as food sources for the calanoid copepod *Eudiaptomus graciloides* (25 newly hatched nauplii per replicate) and the cladoceran *Daphnia longispina* (15 neonates per replicate, in order to equalize grazer biomass between species). The animals were placed into fifteen (3 treatments, n = 5) replicate glass jars with 200 mL aged, membrane-filtered (pore size: 0.45 µm) tap water each and fed one of the three differently disturbed algal communities every second day equivalent to approx. 50.000 phytoplankton cells

ml^{-1}. All the disturbed algal communities were filtered through a 30 µm gauze before being supplied to zooplankton. During the experiment, individual body mass (µg ind^{-1}), body size at first reproduction (SFR, µm), mass-specific somatic growth rates (MSGR, mg day^{-1}), size-specific somatic growth rates (SSGR, mm day^{-1}), time at first reproduction (days) and clutch size (CS, as the total number of eggs produced in every food treatment and individual number of eggs per female per food treatment) were recorded as life-history traits for both zooplankton species (for details see Titocci & Fink, 2022). The carapace length for *D. longispina* and the prosome length of all *E. graciloides* egg carrying females were measured under a stereomicroscope (Axioskop 40, Carl Zeiss Microscopy, Germany) and analyzed through the image-processing application Image J for the calculation of the SFR. The SSGR was determined as the increase in body length from the beginning of the experiment till maturity. The MSGR was calculated according to the formula:

$$[(\ln(W_t) - \ln(W_0))] \times t^{-1}$$

where W_0 is the initial dry weight of neonates/nauplii, W_t is the weight of the individuals after reaching maturity, divided by the time to first reproduction (Lampert and Trubetskova, 1996).

3.3.3 Analytical methods

Biomass determination

Algal samples were filtered and collected on pre-combusted GF/F filters, dried at 60 °C for 24 hours and then weighed on a microbalance (Sartorius CP2 P, accuracy 1 µg) for biomass determination. All surviving adult individuals of *D. longispina* and *E. graciloides* at the end of phase II were placed in aluminum boats and then freeze-dried, before determining their weight with the microbalance. Subsequently, the dried animals were placed in glass tubes with 5 ml dichloromethane/methanol (2:1, v/v) and stored at -20 °C for subsequent fatty acids extraction.

Fatty acid analysis

Fatty acids were extracted from phytoplankton and zooplankton and converted to fatty acid methyl esters (FAMEs) according to Windisch and Fink (2018). The FAME composition of the samples was analyzed using a gas chromatograph (6890N GC System, Agilent Technologies, Waldbronn, Germany) equipped with a DB-225 capillary column (30 m, 0.25 mm i.d., 0.25 µm filmthickness;

J&W Scientific, Folsom, CA, USA, 1 µl splitless injection), He as carrier gas and a flame ionization detector. The GC oven temperature program was as follows: initial 60 °C for 1 minute, then heated at 120 °C/min to 180 °C, held for 2 minutes, then heated at 50 °C/min to 220 °C, constant for 13 minutes and finally heated at 120 °C/min to 220 °C, where temperature was held for 10 minutes. Individual sample fatty acids were identified based on the retention times of FAME standards (Sigma-Aldrich) and their quantification was performed with internal standards (C19:0, C23:0) and previously established calibration functions for each FAME (Couturier *et al.*, 2020).

Pigment analysis

Samples for pigment analysis were filtered and collected on pre-combusted GF/F filters and stored dark in aluminium foil at -20 °C until further analysis. Pigments were extracted using acetone at 4 °C overnight in darkness. 50-100 µL of internal standard (trans-β-apo-8'-carotenal, Sigma-Aldrich). After extraction, each sample was centrifuged, and 1 mL of the supernatant was stored at -20 °C prior to injection into the HPLC column within 72 h after the extraction. Pigments were analyzed using the HPLC System (Prominence HPLC system; Shimadzu Co., Kyoto, Japan) with a binary pump LC-20AB, auto-sampler SIL-A20C, column oven CTO-10AC set at 40°C, diode array detector (PDA) SPD-M20A and a reverse phase Spherisorb ODS2 column (stationary octadecyl-phase C18) with the dimensions 250 mm x 4.6 mm, particle size: 5 µm. The pigments were separated with a method modified after (Garrido and Zapata, 1993). The solvents used were methanol: 1 M ammonium acetate: acetonitrile (50:20:30, v/v, Solvent A) and acetonitrile: ethyl acetate (50:50, v/v, Solvent B). The gradient system used was as follows: 0 min: A: 90%, B: 10%; 2 min: A: 90%, B: 10%; 26 min: A: 40%, B: 60%; 28 min: A: 10%, B: 90%; 30 min: A: 10%, B: 90%. The composition of the solvents was returned to initial conditions over a 1 min gradient, followed by 2 min of system re-equilibration before the next sample was injected. The flow rate was 1 ml min^{-1}. Absorbance was recorded in the PDA from 350 to 700 nm. Pigments were identified by the retention times and the absorption spectra, which were obtained from previous measurements of the pure pigment standards. Peak areas were integrated at 436 nm and corrected for the internal standard.

Phytoplankton identification

Phytoplankton samples were fixed in Lugol's iodine solution and counted according to Utermöhl (1958). For taxonomic identification, the whole bottom area of the chamber was checked first at low magnification (100x-200x) for large forms. Small species were next counted on pairs of two perpendicular diametrical transects at higher magnification (630x). At least 400 cells were identified and counted to species level (where possible) and 20-50 filaments or colonies per species were measured and converted to single cell counts. Biovolumes were estimated using geometric shapes and equations (Hillebrand *et al.*, 1999) and expressed in $\mu m^3 L^{-1}$ as absolute and relative biomass. The phytoplankton species were classified in major taxonomical groups and according to their morpho-functional characteristics were arranged in 7 morphology-based functional groups (MBFG) as described by Kruk et al. (2010). In the MBFG system, Group I includes all small organisms with a high surface-to-volume ratio, group II small flagellated organisms with a siliceous exoskeletal structure, group III is represented by large filaments (with aerotopes), group IV by organisms of medium size lacking specialized traits, group V is formed by unicellular flagellates of medium to large size, group VI consist of non-flagellated organisms with siliceous exoskeletons, and group VII is represented by organisms that form large mucilaginous colonies. Shannon diversity (H') was calculated from phytoplankton MBFGs, pigments and fatty acids with n = 3 each.

3.3.4 Data analysis

All data were analyzed using R (version 3.3.3). Biomass data, Shannon indices and the zooplankton life-history traits were checked for normal distribution with a Shapiro-Wilk's test and for homogeneity of variances with a Levene's test. One-way and two-way ANOVA and Tukey's post-hoc tests were used to analyze and describe their changes in the different disturbance treatments and time. Permutational multivariate analysis of variance (PERMANOVA, (Anderson, 2006) was used to test whether differences in phytoplankton community composition, fatty acids and pigments composition were statistically significant among the disturbed treatments and over time. Moreover, permutational multivariate analysis of variance was used also to investigate

variations in the fatty acid profile of *D. longispina* and *E. graciloides* raised by feeding the three disturbed phytoplankton communities. Non-metric multidimensional scaling (NMDS; package vegan) was used to ordinate zooplankton fatty acids based on Bray-Curtis dissimilarities. Similarity percentages (SIMPER; package vegan) were used to identify and quantify the contribution of individual and classes of fatty acids to the overall Bray-Curtis dissimilarities within treatments and grazers. Finally, correlation analysis between phytoplankton and zooplankton fatty acids was computed using R stats's cor.test function. In calculating correlation coefficients, dietary fatty acids and consumer's fatty acids were used in absolute values, expressed on a dry weight basis. Pearson correlation coefficients were used when data were normally-distributed while correlations were assessed using Spearman's correlation coefficient when variables were non-normally distributed. For all statistical analyses, p-values below 0.05 were used to indicate the statistical significance. Graphics were generated using ggplot2 v3.3.3 (https://ggplot2.tidyverse.org) and dplyr v1.0.3 (https://CRAN.R-project.org/package=dplyr) in R.

3.4 Results

3.4.1 Phase I: Phytoplankton trait alteration by disturbance

Morpho-functional groups

The natural phytoplankton community was initially dominated by *Dinobryon sp*., which had the highest biomass (73%) followed by unicellular flagellates of medium to large size belonging to morpho-functional group V and mainly represented by dinophytes and chlorophytes, 20% (Figure 1a, Supplementary Table 1a). After one week of disturbance, a significant change in biomass composition was observed between the starting community and all the experimental treatments, (Permanova Time, df = 1, F = 41.001, $p < 0.001$) while no significant differences between disturbance treatments were observed (Permanova Treatments, df = 2, F = 2.364, p = 0.057). This was seen in the replacement of *Dinobryon sp*. by several taxa of diatoms (MBFG VI) and organisms of medium size lacking of specialized traits (MBFG IV) in all treatments and probably caused by the transfer of a field community to laboratory conditions (different medium, light, mixing). After three and five weeks of differential disturbance, a clear shift in phytoplankton morpho-functional groups and diversity emerged between treatments (Figure 1a). Reduced or absent turbulent mixing appeared to facilitate the potential of cyanobacteria to dominate the phytoplankton community in the undisturbed and intermediately disturbed treatments, shifting the species composition of phytoplankton communities in favour of buoyant cyanobacteria (MBFG III, 61% and 47%, respectively, Supplementary Table 1a) such as *Anabaenopsis sp*., *Limnothrix sp*., *Oscillatoria sp*., *Planktothrix agardhii* and organisms that form large mucilaginous colonies (MBFG VII, 12% and 3%, respectively, Supplementary Table 1a). On the contrary, the intense turbulence in the highly disturbed treatment promoted the rapid decline of filamentous cyanobacteria and facilitated the increase of organisms of medium size lacking specialized traits, mainly represented by different taxa of green algae (MBFG IV) and the spread of pennate diatoms (MBFG VI) which alone, contributes in this treatment to the 75% of the total biomass in week 5 (Supplementary Table 1a). At the end of the experiment, the highest phytoplankton density was found in the undisturbed community (1.21×10^9 cells L^{-1}, Supplementary Table 2), while the highly disturbed treatment registered the highest biomass (1.48×10^{11} μm^3 L^{-1}, Supplementary Table 2). In terms of diversity, a significant reduction in species richness and functional diversity was observed over time and between treatments (Supplementary Table 2, 3). In particular, the

phytoplankton community that received no and intermediate levels of disturbance exhibited a general decrease of 6 - 10% of the taxa richness and the lowest functional diversity, which was registered in weeks 3 and 5 (Figure 1 a, Supplementary Table 2, 3).

Biochemical traits

The above-mentioned changes in phytoplankton community composition and diversity were also reflected in changes in their biochemical traits depending on disturbance (Figure 1 b, c). Indeed, the highest fatty acid diversity was registered after the first week of manipulation, both in the highly disturbed and undisturbed treatment (H' = 2.37 and H' = 2.33 respectively, Supplementary Table 2, 3). In all treatments, a significant reduction of fatty acid diversity was registered over time (PermANOVA Time, df = 2, F = 34.063, $p < 0.001$, Supplementary Table 3). The highest total content of fatty acids was found in the highly disturbed community (95.7 µg per mg DW in week 3 and 80.47 µg per mg DW in week 5 Supplementary Table 2, 3) followed by the intermediate treatment which had a peak of 73.59 µg per mg DW in week 3. The undisturbed phytoplankton community showed a lower total FA content compared to the other treatments throughout the whole experiment (Supplementary Table 2, 3). Even though significant differences in fatty acid class composition and content were registered between the starting community and the altered communities (permANOVA Time, df = 2, F = 8.339 $p < 0.001$), no significant differences in fatty acid class composition were found between treatments after the first week of manipulation (permANOVA Treatment, df = 2, F = 2.08, p = 0.106) (Figure 1 b, Supplementary Table 1b). Nevertheless, changes in fatty acid composition were evident through the later phases of the experiment. Specifically, a significant decrease of polyunsaturated n-3 fatty acids was recorded in the undisturbed treatment (from week 1 to week 5, SIMPER analysis, $p < 0.001$), caused mainly by a dramatic drop in C20:5 n-3 (EPA), C18:4 n-3, C22:6 n-3 (DHA) and C18 fatty acids. A constant increase of monounsaturated fatty acids (MUFA) was found in the highly disturbed treatment (SIMPER analysis, $p < 0.01$), mainly due to the contribution of single fatty acids such as C16:1 n-9 and C18:1 n-9.

After excluding Chl *a*, pheophytin and beta-carotene, which were the most abundant pigments and most widely occurring in all classes of algae, our pigments analyses demonstrated how the composition and content of accessory and marker pigments strongly reflected the phytoplankton

taxonomic composition and subsequent variation during the disturbance experiment (Figure 1c, Supplementary Table 1c). Significant differences in the concentrations of pigments were found between treatments and time (permANOVA Treatment, df = 3, F = 7.17, $p < 0.001$; permANOVA Time, df = 3, F = 11.36, $p < 0.001$). Specifically, the starting phytoplankton community showed the highest pigment diversity (H' = 2.26) with 15 of 17 pigments detected in quite similar proportions, reflecting a more diverse and heterogeneous phytoplankton taxonomical composition. After one week of disturbance, no significant changes were detected in pigment composition between treatments. However, pigment diversity showed a gradual decrease with the lowest values reached in week 3 in the intermediate and undisturbed treatments (H' = 0.79, H' = 0.97, respectively, see Supplementary Table 2). Alloxanthin (3.2%), a typical cryptomonad pigment, was initially present but almost disappeared in the later weeks. Moreover, the relative abundance of Chlorophyll c, a marker pigment for chrysophytes, was quite high in the starting phytoplankton community (5.6%), but gradually decreased over time in the other treatments corresponding to the replacement chrysophyte taxa detected through microscopic observations. Chlorophyte marker pigments (Violaxanthin, Neoxanthin, Lutein, Chlorophyll b) showed an increase in their relative abundances in all the treatments over time, matching the increasing occurrence of green algae in the later phase of the experiment. Moreover, Zeaxanthin and Echinenone, marker pigments of cyanobacteria, reached relatively high concentrations in the undisturbed and intermediate disturbed treatments (4.8% and 4.7%), but extremely low abundances in the high disturbed treatment (0.3%) at the end of the disturbance experiment. The opposite trend was observed for Fucoxanthin, the characteristic pigment of diatoms, which contributed almost 10.2% in the highly disturbed treatment in week 5. Other diagnostic pigments for phytoplankton groups such as Peridinin, Diadinoxanthin and Diatoxanthin had relatively low concentrations, with mean contributions < 1% (Supplementary Table 1c).

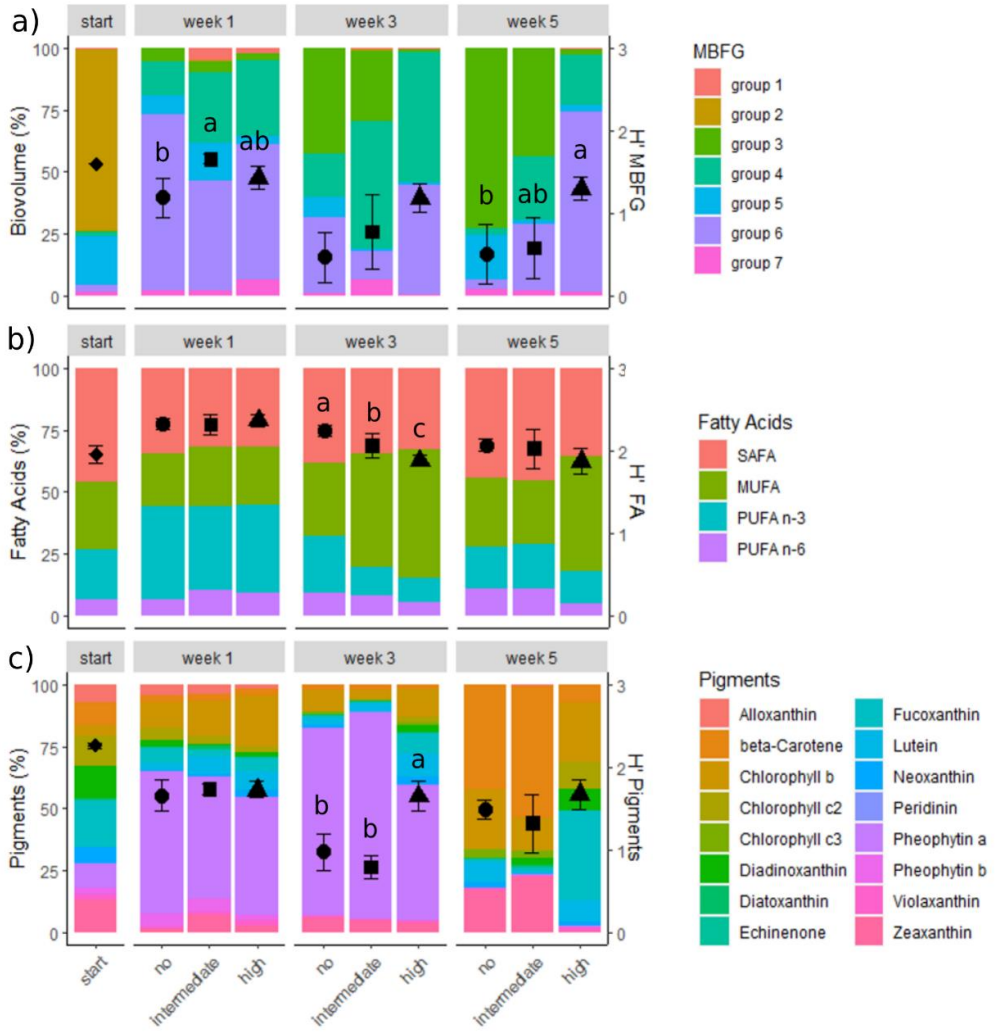

Figure 1. Distribution of phytoplankton morpho-functional and biochemical traits before the manipulation (start) and throughout the course of the disturbance experiment after 1, 3 and 5 weeks of disturbance application. All the results are mean values (n = 3 for biovolume, n = 5 for fatty acids and pigments). **a)** Phytoplankton community structure was expressed as relative biomass (% of total biovolume) of each morphologically based functional group (MBFG from I to VII, Kruk et al.,2010, top row) followed by **b)** relative abundances of fatty acids and **c)** pigments in the undisturbed, intermediate and highly disturbed phytoplankton communities. Shannon diversity was calculated from MBFG groups, fatty acids and pigment at the beginning of the experiment (filled diamonds, start) and for the phytoplankton community subjected to no (filled circles), intermediate (filled squares) and high (filled triangles) disturbance during weeks 1, 3 and 5 of the experiment, respectively. H' values are mean ± standard deviation (n = 3 for functional diversity and n = 5 for fatty acid and pigment diversity).

3.4.2 Phase II: Dietary impact on zooplankton

Life-history traits

The two herbivorous grazers *D. longispina* and *E. graciloides* showed a different responses in growth, development and reproduction once fed the disturbance-altered phytoplankton communities (Table 1). In *D. longispina*, only the trait clutch size showed significant variation between food treatments. The total number of eggs produced per individual at the first brood, and consequently the potential number of offspring available for the future generations was significantly reduced when *D. longispina* was fed with the intermediate and undisturbed phytoplankton diets compared to those fed with the highly disturbed phytoplankton diet (one-way ANOVA; $df = 2$, $F = 4.554$, $p < 0.05$). *E. graciloides* on the other hand was strongly affected by the disturbance-altered phytoplankton communities. Several life-history traits such as clutch size (total number of eggs), body mass and age at first reproduction (one-way ANOVA, $p < 0.05$ in all previously mentioned traits). *E. graciloides* grown on the highly disturbed phytoplankton community were bigger in terms of body mass at maturation, showed higher size-specific growth rates and needed less time to become mature and reproduce for the first time, with respect to the others. Moreover, *E. graciloides* fed the highly disturbed phytoplankton community showed the highest total egg production (one-way ANOVA, $df = 2$, $F = 6.24$, $p < 0.05$) while the animals fed the intermediate disturbed treatment exhibited high variability in the reproduction phase, with two out of five replicates without egg-carrying females. At the end of the experiment, the sex ratio (♀/♂) was on average more than 1, indicating a predominance of females in favour of males in all treatments.

Table 1. Reproductive traits and life history parameters of individuals of *D. longispina* and *E. graciloides* (mean ± SD, n = 5) fed with undisturbed, intermediately and highly disturbed phytoplankton diets.

Life-history parameters	*D. longispina*			*E. graciloides*		
	No disturbance	Intermediate Disturbance	High Disturbance	No disturbance	Intermediate Disturbance	High Disturbance
Mass specific growth rate (mg day^{-1})	0.22 ± 0.04	0.19 ± 0.05	0.23 ± 0.04	0.035 ± 0.010	0.033 ± 0.004	0.045 ± 0.004
Size-specific growth rate (mm day^{-1})	0.08 ± 0.04	0.09 ± 0.01	0.09 ± 0.02	0.032 ± 0.005	0.032 ± 0.001	0.032 ± 0.002
Body weight (µg ind^{-1})	13.32 ± 2.40	11.22 ± 3.29	14.15 ±3.74	**8.97 ± 3.30 ^{ab}**	**7.76 ± 1.32 ^{b}**	**12.10 ± 2.16 ^{a}**
Size at first reproduction (µm)	1162.42 ± 41.11	1106.18 ± 92.44	1147.53 ± 122.98	986.13 ± 19.23	954.60 ± 65.09	963.37 ±11.40
Age at first reproduction (days)	7.60 ± 0.55	7.60 ± 0.45	7.80 ± 0.55	**30.44 ± 3.05 ^{a}**	**28.00 ± 3.68 ^{ab}**	**27.62 ± 3.15 ^{b}**
Total egg production	13.8 ± 4.76	13.80 ± 2.28	18.80 ± 4.67	**26.80 ± 19.87 ^{b}**	**20.60 ± 20.88 ^{b}**	**69.2 ±29.17 ^{a}**
Clutch size (eggs ind^{-1})	**1.95 ± 0.52 ^{ab}**	**1.91 ± 0.19 ^{b}**	**2.62 ± 0.46 ^{a}**	7.29 ± 1.35	7.22 ± 0.96	**69.2 ±29.17 ^{a}**

Bold font and different letters indicate significant differences between experimental diets as determined by one-way ANOVA with Tukey's comparison test ($p < 0.05$).

Fatty acid composition

Twenty-seven fatty acids, from C16:0 to C24:1 n-9 were identified and compared among the two grazers fed the different food treatments. A peak, always present of uncertain identity found between C22:6 n-3 and the internal standard C23:0 was omitted from the calculations (Tables 2, 3). Two-dimensional non-metric multidimensional scaling (NMDS) ordination plots revealed a significant and clear difference in fatty acid profiles between the two grazer taxa and the dietary treatments (Figure 2). Three distinct and separate clusters could be identified for *E. graciloides* fed the different algal diets, showing that the copepods' fatty acid profiles were different in each diet treatment, while they overlapped in *D. longispina.* The permANOVA did not indicate significant differences in *D. longispina* fatty acid body content and class composition (permANOVA, df = 2, F = 0.91 $p < 0.46$, Table 2). For *E. graciloides* however, significant variations in the fatty acid content were observed (permANOVA, df = 2, F = 6.72 $p < 0.001$, Figure 2). For example, the total FA content (per dry weight) was significantly higher in the calanoids fed the highly disturbed community (51.40 ± 12.83 ng per µg DW) compared to calanoids from the other treatments, which showed approx. half of the total lipid content (29.78 ± 7.04 ng per µg DW intermediate and 27.27 ± 15.43 ng per µg DW in undisturbed treatments, respectively). Moreover, except for saturated fatty acids (SAFA) where no differences were observed between treatments, the calanoids fed the highly disturbed phytoplankton community were significantly richer in n-3 and n-6 PUFA, as well as in monounsaturated fatty acids (Table 3). Copepods fed intermediately and undisturbed phytoplankton communities showed high content of SAFA (Figure 2). When we compared the fatty acid contents between the two grazer taxa, we observed significant differences between them (permANOVA, $p < 0.001$). In general, the total lipid content was higher in daphnids than in copepods and a different selective accumulation of certain fatty acids was observed (Table 2, 3). Specifically, respect to *E. graciloides*, *D. longispina* tended to accumulate the double content of EPA and its precursor stearidonic acid (SDA) once fed with the no and intermediate disturbed phytoplankton communities, while no significant differences in those fatty acids content were observed in both grazers fed the high disturbed phytoplankton community. ARA was significantly more present in daphnids than copepods (all treatments) while an opposite trend was registered for DHA, which was accumulated in almost four times higher amounts in the body content of *E. graciloides* fed the three disturbed phytoplankton communities compared to *D. longispina*.

In order to identify the FAs that have the most potential to serve as dietary trophic markers for cladocera and copepods, we compared their fatty acid composition to that of their respective diets (Figure 3). The fatty acid composition of the highly disturbed phytoplankton community was strongly correlated with that of both grazers. The correlation between the fatty acid composition of *E. graciloides* and their diet was strongest for SDA (C18:4 n-3, $p < 0.05$, $r^2 = 0.83$), EPA (C20:5 n-3, $p < 0.01$, $r^2 = 0.93$) and C22:1 n-9 (erucic acid, $p < 0.05$, $r^2 = 0.80$). We found a significantly positive correlations between *D. longispina* and their diet for ALA (α-linolenic acid, C18:3 n-3 $p < 0.05$, $r^2 = 0.76$) and a significantly negative correlation with erucic acid ($p < 0.05$, $r^2 = 0.80$). The fatty acid composition of *E. graciloides* matched that of their diets much more closely than for *D. longispina*. This was particularly evident in the highly disturbed treatment, where except for C20:0, all fatty acids from C18:2 n-6 to C22:6 n-3 in *E. graciloides* showed strong positive correlations ($r^2 > 0.5$) with the lipid composition of the respective diet, while for *D. longispina*, correlation coefficients were on average lower in all treatments. In the undisturbed treatment, *D. longispina* showed a high positive correlation with two monounsaturated fatty acids: C22:1 n-9 and C16:1 n-7, and a negative correlation with ALA and DHA, while *E. graciloides* showed a significant positive correlation in C18:1 n-9c ($p < 0.05$, $r^2 = 0.80$), followed by C20:3 n-3 and DHA even though the slope of the relationship was not significant. Moreover, in some cases, a mismatch between the nutritional content of the diets and the biochemical composition of the consumers were found. In fact, although in low concentration, a general presence of C18:1 n-9t, C20:1 n-7, C21:0 and C20:4 n-6 has been observed in *D. longispina* body content despite the absence of those fatty acids in their diets. For *E. graciloides* this result was even more pronounced. Indeed, nine further fatty acids (C18:1 n-9t, C20:0, C21:0, C20:1 n-7, C20:2 n-6, C20:3 n-5, C20:3 n-6, C20:4 n-6 and C24:1) were recorded in *E. graciloides* despite their absence in their respective phytoplankton diets.

Table 2. Fatty acid composition and concentration (ng per µg dry weight) of *D. longispina* fed the experimental diets: no, intermediate and highly disturbed phytoplankton communities. Values expressed as means and standard deviations, with n = 5.

Fatty acid	No Disturbance	Intermediate Disturbance	High Disturbance
C16:0	9.43 ± 2.27	9.73 ± 3.32	8.83 ± 1.83
C17:0	0.89 ± 0.38	0.90 ± 0.26	0.78 ± 0.28
C18:0	3.54 ± 0.90	3.06 ± 0.80	2.29 ± 0.50
C 20:0	0.02 ± 0.05	nd	nd
C21:0	0.28 ± 0.10	0.25 ± 0.04	0.17 ± 0.06
C22:0	0.08 ± 0.07	0.05 ± 0.02	0.14 ± 0.15
Σ SAFA	**14.24 ± 3.36**	**13.99 ± 4.23**	**12.21 ± 2.24**
C16:1 n-7	4.85 ± 2.29	4.03 ± 1.29	4.97 ± 1.36
C17:1 n-9	0.41 ± 0.23	0.29 ± 0.10	0.24 ± 0.17
C18:1 n-9t	3.63 ± 1.37	3.54 ± 1.18	4.54 ± 1.30
C18:1 n-9c	4.67 ± 1.10	4.85 ± 0.93	3.19 ± 1.82
C18:1 n-7	0.16 ± 0.08	0.16 ± 0.05	0.09 ± 0.02
C20:1 n-7	0.01 ± 0.02	nd	0.01 ± 0.01
C22:1 n-9	0.12 ± 0.04	0.08 ± 0.10	0.03 ± 0.02
Σ MUFA	**13.85 ± 4.65**	**12.96 ± 2.97**	**13.07 ± 3.50**
C18:2 n-6c (LIN)	1.49 ± 0.49	1.92 ± 0.46	1.88 ± 0.49
C18:3 n-6	0.47 ± 0.22	0.51 ± 0.16	0.47 ± 0.12
C18:3 n-3 (ALA)	4.17 ± 2.77	5.99 ± 1.23	5.03 ± 1.19
C18:4 n-3	9.03 ± 1.16	11.03 ± 2.74	7.96 ± 2.11
C20:4 n-6 (ARA)	1.61 ± 0.36 [ab]	1.71 ± 0.24 [a]	1.15 ± 0.26 [b]
C20:3 n-3	0.02 ± 0.04	nd	nd
C20:5 n-3 (EPA)	5.37 ± 0.87	6.57 ± 1.37	6.82 ± 1.47
C22:2 n-6	0.07 ± 0.15	0.01 ± 0.02	0.03 ± 0.06
C22:6 n-3 (DHA)	0.25 ± 0.21	0.17 ± 0.10	0.42 ± 0.32
Σ PUFA	**22.47 ± 4.42**	**27.92 ± 4.69**	**23.75 ± 3.54**
Σ n-3	**18.85 ± 3.79**	**23.76 ± 4.40**	**20.23 ± 3.19**
Σ n-6	**3.63 ± 0.86**	**4.16 ± 0.58**	**3.52 ± 0.68**
Total lipid	**50.57 ± 10.55**	**54.86 ± 7.39**	**49.03 ± 8.29**

Table 3. Fatty acid composition and concentration (ng per µg dry weight) of *E. graciloides* fed the experimental diets: no, intermediate and highly disturbed phytoplankton communities. Values expressed as means and standard deviations, with n = 5.

Fatty acid	No Disturbance	Intermediate Disturbance	High Disturbance
C16:0	8.37 ± 7.67	5.73 ± 1.02	9.64 ± 2.77
C17:0	0.64 ± 0.47	0.74 ± 0.17	0.94 ± 0.17
C18:0	5.18 ± 4.63	2.81 ± 0.65	1.95 ± 1.24
C19:0	0.39 ± 0.55	0.09 ± 0.13	nd
C 20:0	0.12 ± 0.25	0.02 ± 0.03	0.02 ± 0.02
C21:0	0.10 ± 0.10	0.08 ± 0.07	0.10 ± 0.07
C22:0	0.35 ± 0.30	0.21 ± 0.08	0.29 ± 0.09
C 24:0	0.34 ± 0.64	0.11 ± 0.09	0.23 ± 0.33
Σ SAFA	**15.49 ± 3.97**	**9.78 ± 1.80**	**13.17 ± 3.90**
C16:1 n-7	1.08 ± 0.54 [b]	1.28 ± 0.46 [b]	5.30 ± 2.00 [a]
C17:1 n-9	0.22 ± 0.04	0.38 ± 0.18	0.76 ± 0.60
C18:1 n-9t	nd	0.09 ± 0.20	0.43 ± 0.97
C18:1 n-9c	0.47 ± 0.97 [b]	0.62 ± 0.30 [b]	5.69 ± 4.24 [a]
C18:1 n-7	0.87 ± 0.42	1.12 ± 0.28	1.34 ± 0.13
C 20:1 n-9	nd	0.06 ± 0.06 [b]	0.24 ± 0.14 [a]
C20:1 n-7	0.01 ± 0.02	0.02 ± 0.02	0.03 ± 0.02
C22:1 n-9	0.74 ± 0.72	0.57 ± 0.16	0.41 ± 0.11
C 24:1 n-9	0.01 ± 0.02 [b]	0.04 ± 0.04 [b]	0.29 ± 0.14 [a]
Σ MUFA	**3.40 ± 1.40 [b]**	**4.18 ± 0.77 [b]**	**14.49 ± 6.07 [a]**
C 18:2 n-6 t	nd	0.02 ± 0.04	0.06 ± 0.05
C18:2 n-6c (LIN)	0.44 ± 0.23 [b]	1.05 ± 0.38 [b]	2.51 ± 0.94 [a]
C18:3 n-6	0.13 ± 0.11	0.19 ± 0.07	0.26 ± 0.04
C18:3 n-3 (ALA)	2.46 ± 1.47 [b]	3.67 ± 1.30 [b]	6.98 ± 1.55 [a]
C18:4 n-3	1.58 ± 1.05 [b]	4.36 ± 2.13 [a]	5.96 ± 1.46 [a]
C 20:2 n-6	0.01 ± 0.02 [b]	0.06 ± 0.04 [a]	0.10 ± 0.02 [a]
C20:4 n-6 (ARA)	0.17 ± 0.10	0.30 ± 0.14	0.20 ± 0.05
C20:3 n-3	nd	0.03 ± 0.06	0.02 ± 0.03

C20:5 n-3 (EPA)	1.78 ± 0.84 [b]	3.58 ± 1.18 [ab]	4.90 ± 1.16 [a]
C22:6 n-3 (DHA)	1.81 ± 1.49	2.56 ± 0.76	2.75 ± 0.78
Σ PUFA	**8.39 ± 2.02**	**15.81 ± 5.37**	**23.74 ± 4.32**
Σ n-3	**7.63 ± 1.64 [c]**	**14.20 ± 4.86 [b]**	**20.61 ± 3.73 [a]**
Σ n-6	**0.76 ± 0.41 [b]**	**1.62 ± 0.55 [b]**	**3.13 ± 0.98 [a]**
Total lipid	27.27 ± 15.43 [a]	**29.78 ± 7.04 [b]**	**51.40 ± 12.83 [a]**

SAFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; n-3, polyunsaturated fatty acid n-3, n-6, polyunsaturated fatty acid n-6, nd, not detected. The values in bold indicates the sum of SAFA, MUFA, PUFA, PUFA n-3 and PUFA n-6. a,b, values denote significant differences ($p < 0.05$) between different algal diet treatments.

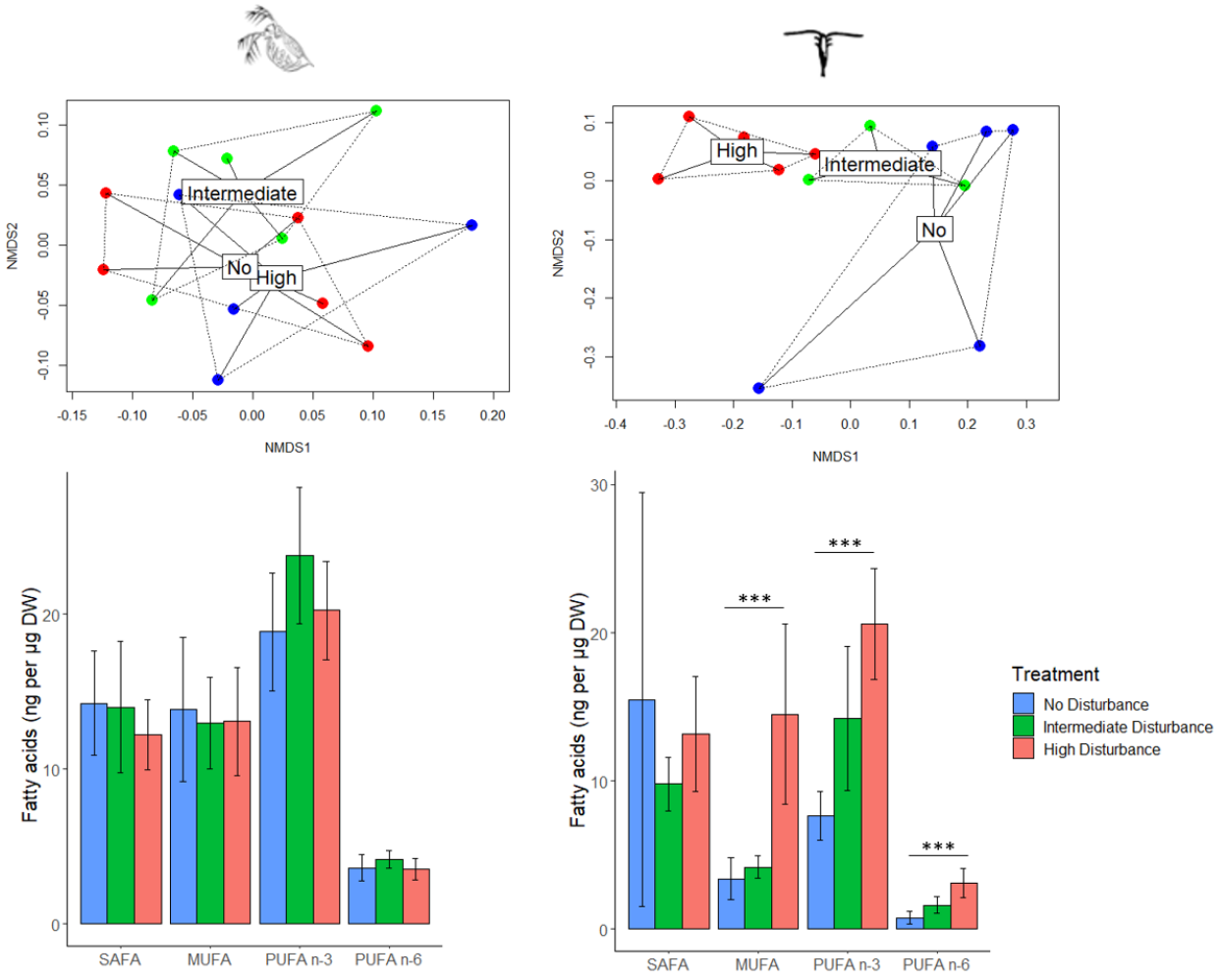

Figure 2. Non-metric multidimensional scaling (NMDS, top panels) based on Bray-Curtis dissimilarities for the fatty acid (FA) profile for *D. longispina* (left) and *E. graciloides* (right) fed the experimental diets. Below, absolute abundances (ng FA/µg dry weight, mean ± SD in each FA class, n = 5 per treatment) of

SAFA, MUFA, n-3 and n-6 PUFA from adult *D. longispina* and *E. graciloides* fed the undisturbed (light blue) and intermediate (green) and highly (light red) disturbed phytoplankton communities. Asterisks denote significant differences ($p < 0.05$) between different diets.

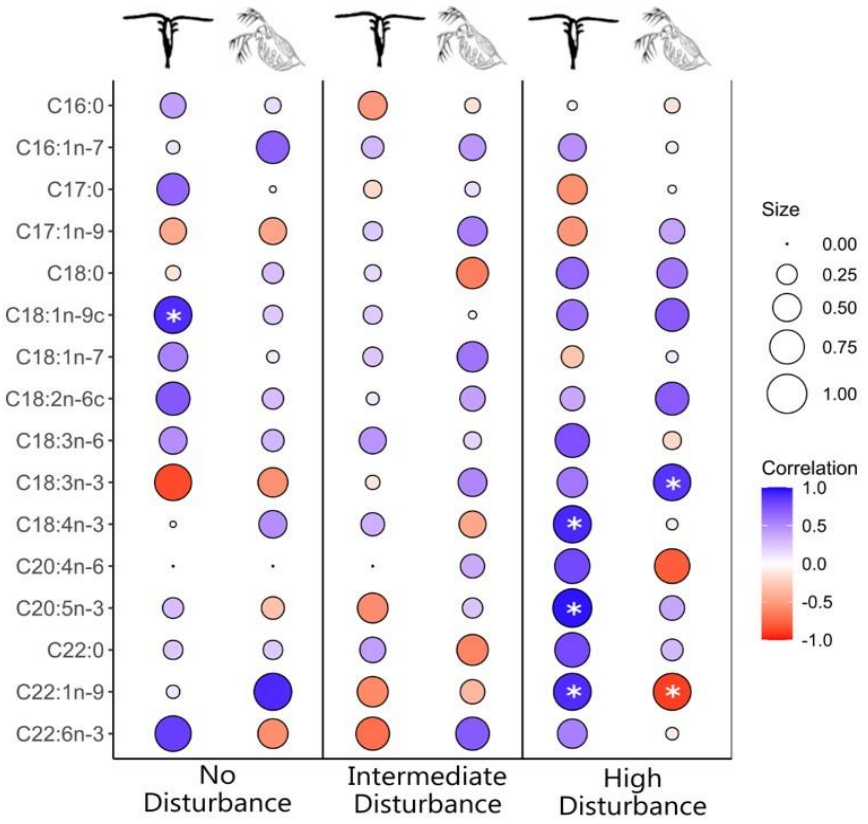

Figure 3. Correlations between phytoplankton and zooplankton fatty acid (FA) content and composition. Correlogram between fatty acid content of *D. longispina* and *E. graciloides* with their respective dietary FA from the undisturbed, intermediately and highly disturbed phytoplankton communities. The color intensity and size of the point is proportional to the absolute value of the Pearson/Spearman correlation coefficient, the stronger the correlation (i.e., the closer to −1 or 1), the darker and larger the points. The color legend shows a negative correlation (red color) when the two variables varied in opposite directions, and a positive correlation (blue color) when the two variables varied in the same direction. Significant correlations ($p < 0.05$) are indicated by a white asterisk.

3.5 Discussion

3.5.1 Phase I: Phytoplankton trait alteration by disturbance

In our experiment, alterations in the physical environment, induced by the application of different disturbance intensities, resulted in profound shifts in the composition and structure of a natural phytoplankton community. These responses depended on the responses of the single taxa and the extent to which their tolerances to mixing conditions contributed to subsequent variations in dominance in their respective populations. Despite receiving the same environmental conditions in terms of light, temperature and dissolved nutrients, the capacity to accrue biomass varied significantly across taxa. Phytoplankton trait dynamics differed depending on the hydrodynamic mixing regime. In particular, diatoms and motile algae of medium size lacking specialized traits consistently dominated under sustained mixing, while buoyant species and large mucilaginous colonies built the majority of communities at low disturbance levels. These responses resembled those that occur in natural freshwater environments, where seasonal phytoplankton succession is basically controlled by the physical environment and water circulation (movement) represents one of the main factors in conditioning the availability of light and nutrient resources for planktonic organisms (Margalef, 1978). Hydrodynamics plays a crucial role in phytoplankton succession, where stronger hydrodynamic forces typically shift phytoplankton communities towards large species (cells, colonies or long filaments) and low surface area:volume ratio (Colin S. Reynolds, 1984). In our experiment, high turbulence strongly influenced and re-structured phytoplankton composition favouring taxa with increased size and cell volume, which resulted in high community biomass, without a numerical increase. In the highly disturbed treatment, three-quarters of the algal biomass was represented by medium to large-sized pennate diatoms. This corresponds to previous findings that turbulent environments typically favour larger phytoplankton species (Arin *et al.*, 2002; Rokkan Iversen *et al.*, 2010; Lepistö and Rosenström, 1998). Under low disturbance conditions, the strategies of cell division and chain elongation as well as cell adhesion and mucilage formation appeared to dominate. In the undisturbed and intermediately disturbed communities, we observed the highest densities and a predominance of colonial and filamentous cyanobacteria. Indeed, mucilaginous sheaths coupled with the presence of gas vacuoles appear to be beneficial traits for organisms living in calm or intermediate disturbed water, allowing them to enhance buoyancy and floating movements for better utilization of light and nutrients (Fogg and Walsby, 1971; Reynolds and Walsby, 1975). Moreover, the high occurrence of nitrogen-fixing

cyanobacteria like *Pseudoanabaena, Chroococcus, Oscillatoria*, more frequently found in the undisturbed treatment as layers of thin mats attached to flask substrates, confirmed their ecological adaptation to slow-moving waters (Marcarelli *et al.*, 2008).

On the contrary, other species like *Dinobryon sp.* declined rapidly within the first week of the experiment. This was probably not due to mechanical stress induced by the hydrodynamic disturbance, as this occurred in all treatments irrespective of the disturbance treatment. In this case, unfavourable laboratory conditions such as light and cultivation medium may have not met the nutritional requirements of this species. *Dinobryon*, like many chrysophytes, are mixotrophs, a characteristic particularly favourable under nutrient-poor conditions. Thus, the enriched nutrient conditions supplied in our experiment may have put *Dinobryon* at a competitive disadvantage in relation to other taxa, disfavoring their growth and survival (Sandgren, 1988).

Overall, in our experiment, hydrodynamic stress strongly influenced changes in species dominance, composition and traits diversity, with the high disturbed community showing in general the highest values of richness and functional diversity. A similar pattern emerged for the biochemical trait of the phytoplankton's pigments. Specifically, a clear match between the pigment and the taxonomic composition was particularly evident in the latest phase of the experiment when Zeaxanthin and Echinenone, marker pigments of cyanobacteria, reached relatively high concentrations in the undisturbed and intermediate disturbed treatments and Fucoxanthin, the characteristic pigment of diatoms contributed greatly in the highly disturbed treatment. The similar changing dynamics observed clearly demonstrated how the classical taxonomic identification together with the use of pigments as biomarkers are both valid and robust methods for phytoplankton functional determination and community dynamics investigation (Mackey *et al.*, 1996; Schlüter *et al.*, 2000; Irigoien *et al.*, 2004; Ilić *et al.*, 2023).

The highest total content of FA was registered in the highly (week 3 and week 5) and intermediately (week 3) disturbed communities. Those exhibited more than double the FA content compared to the starting community and the undisturbed treatment. This suggests that intermediate and high levels of mixing contributed to enhancing the chemical composition and nutritional efficient in terms of fatty acids content in these treatments. Although, in our understanding, there are no pieces of evidence that hydrodynamic disturbance may enhance lipid productivity, from our result we can suppose that stress conditions, and specifically, mixing may have favoured more suitable conditions of circulation and exchange of the nutrients in the cultures respect to the

unmixed treatment, implying a possible acceleration in phytoplankton metabolism with consequent increase of activation of biochemical pathways for fatty acids synthesis and storage. This finds some correspondence with several studies which reported the synergistic effect of several stress conditions on the improvement of lipid productivity in selected algal species (Kwak *et al.*, 2016; Ho *et al.*, 2014; Singh *et al.*, 2015).

Even though no significant differences in fatty acid class composition were found between treatments after the first week of manipulation, significant changes in FA classes' proportion were registered over time. In particular, a constant relative increase of MUFA was found during the late phase of the experiment, especially in the highly disturbed treatment. This was mainly due to the contribution of a few single peaks of monounsaturated fatty acids such as C16:1 n-7, C16:1 n-9 and C18:1 n-9, commonly recognized as diatom and flagellate lipid biomarkers (Reuss and Poulsen 2002; Taipale et al., 2013). This corresponds to the observed dominance of diatoms (75% of total biomass at the end of the experiment) and contributed to the lowest fatty acid diversity in this treatment. In contrast to this, n-3 PUFA decreased significantly in the undisturbed treatment. This can be explained by the gradual increase of cyanobacteria and chlorophytes in this treatment. Indeed, these two phytoplankton groups are known to be poor producers of PUFAs and are commonly classified as non-EPA and non-DHA-synthesizers (Ahlgren *et al.*, 1990; Taipale *et al.*, 2013; Jónasdóttir, 2019).

In general, alteration of species distribution and dominance due to the disturbance levels have been strongly reflected in variation in phytoplankton traits composition and dynamics along the experiment highlighting, even more, the importance of investigating not only taxonomic but also functional diversity of competing species in order to understand the effects of biodiversity and even more of biodiversity loss across trophic levels (Duffy *et al.*, 2007).

3.5.2 Phase II: Dietary impact on zooplankton

In the second phase of the experiment, the disturbance-altered phytoplankton communities had distinct impacts on functional traits and fitness of the two herbivores *D. longispina* and *E. graciloides*, mainly as a consequence of their feeding strategies.

As expected, phytoplankton community changes strongly modulated the algal palatability and accessibility for *E. graciloides*. Thanks to mechanical and chemical sensors, *E. graciloides* is able to perceive the chemical composition and movements of its prey (DeMott, 1986). They actively select and choose food items which most closely match their metabolic requirements in the altered phytoplankton communities. In particular, due to their selective feeding mode, copepods have resulted exceptionally well adapted to optimize their nutrition in the highly disturbed community, which provided them with the highest fitness. In fact, under a mainly diatom-dominated diet, the copepods exhibited higher body mass, and a higher occurrence of ovigerous females in a shorter period of time that were subsequently able to produce a higher number of eggs compared to copepods in the other food treatments. Moreover, after analyzing the biochemical composition of *E. graciloides*, almost the double total fatty acid content was found in individuals fed the highly disturbed community than in individuals fed and grown on the undisturbed and intermediate disturbed communities as their diet.

In particular, the dominance of diatoms accounted for the high amount of PUFA n-3, PUFA n-6 and the elevated amounts of C16:1 n-7, a commonly recognized diatom biomarker, found in the body of calanoids fed with the highly disturbed treatment. This confirmed the ability of *E. graciloides* to select the most nutritional diet item from a mixed phytoplankton community. Furthermore, this supports the general view that diatom-dominated communities promote high biomass, growth and reproduction in calanoid copepods (Legendre, 1990; Kleppel, 1993) and that PUFAs and long-chain fatty acids are fundamental constituents for oogenesis in crustaceans in general (Payne and Rippingale, 2000; Broglio *et al.*, 2003) and for copepod reproduction in particular (Støttrup and Jensen, 1990; Jónasdóttir, 1994). Moreover, in our experiment, the highly disturbed community was also the one with the highest functional diversity compared to the other diets with lower taxonomic richness and biodiversity, supporting the idea that dietary diversity increases the probability of obtaining a nutritionally complete dietary mixture for herbivores (Groendahl and Fink, 2016).

In contrast, calanoids fed the undisturbed and intermediate disturbed community showed lower body weight, longer time needed for maturation and reproduction and approximately half of the total lipid content and higher accumulation of saturated fatty acids in their body. Since most animals cannot (efficiently) synthesize PUFAs *de novo*, the availability of PUFA is largely dependent on the phytoplankton community composition (Strandberg *et al.*, 2015). The undisturbed and intermediate-disturbed diets were mainly composed of green algae and cyanobacteria. These phytoplankton groups are typically characterized by their lack of long-chain PUFAs (Ahlgren *et al.*, 1990; Brown *et al.*, 1997; Von Elert and Wolffrom, 2001; Lacoste *et al.*, 2001). This nutritional deficiency may thus have caused a strongly negative impact on copepod reproduction (Von Elert and Stampfl, 2000). In these treatments, due to poorer quality and availability of palatable resources, *E. graciloides* couldn't maximize all components of fitness (growth, development and reproduction) simultaneously. In this trade-off, they apparently invested more in survival and growth, rather than in reproduction. This was further confirmed by the lowest total egg production registered in these treatments and with the finding of egg-carrying females in only two out of five replicates in the intermediate disturbed treatment. Moreover, our results corroborate those of previous experiments where *Eudiaptomus* sp. exhibited low egg production, hatching success and survival rates when fed monoalgal diets of *Chlamidomonas klinobasis*, *Acutodesmus obliquus* and *Synechococcus elongatus* as representatives of green algae and cyanobacteria (Titocci and Fink, 2022).

In contrast to *E. graciloides*, the disturbance-dependent alteration of the phytoplankton community did neither significantly affect life-history traits, nor FA composition of *D. longispina*. This can probably be explained by the different feeding strategies of the two grazers. Although the two crustacean zooplankton grazers are quite similar in size and therefore they can be quite equivalent in terms of grazing pressure and biomass, they exhibit completely distinct feeding modes, selectivity and diet preferences. *D. longispina* are unselective filter feeders, and thus unable to exercise a specific impact based on the phytoplankton organisms' nutritional quality, but only as a function of numeric cell density (Barnett *et al.*, 2007; Kiørboe, 2011). As a consequence, unselective filter-feeding may be a disadvantageous feeding strategy in a scenario of phytoplankton diversity loss.

Consumers' fatty acid composition generally reflects the FA composition of their diets (Iverson, 2009). However, the fatty acid composition of *E. graciloides* matched that of their diets much

more closely than the composition of *D. longispina*. This was particularly evident in the highly disturbed treatment, suggesting once more how selective feeding favours a preferential direct assimilation of more nutritious cells within higher food quality diets. Moreover, our FA analyses showed that in general, zooplankton had a significantly higher proportion of essential fatty acids than phytoplankton, suggesting preferential retention of EFAs in both grazers (Kainz *et al.*, 2004; Brett, Müller-Navarra, *et al.*, 2009), despite some differences in PUFA accumulations between the two taxa.

D. longispina accumulated twice as much EPA and its precursor SDA when fed with low PUFA communities. This corroborates the generally acknowledged key role of EPA for cladoceran fitness (e.g. Von Elert, 2002; Abrusán et al., 2007; Windisch & Fink, 2018) and that FA internal transformation, mobilization and bioconversion mechanisms when essential fatty acids availability was limiting (Bell and Tocher, 2009; Twining *et al.*, 2021) have occurred. Moreover, as previously reported (Persson and Vrede, 2006; Smyntek *et al.*, 2008; Brett, Kainz, *et al.*, 2009), ARA was significantly more present in *D. longispina* than *E. graciloides* (all treatments) while the opposite trend was registered for DHA, which was accumulated in almost four times higher amount in the body content of *E. graciloides* fed the three disturbed phytoplankton communities compared to *D. longispina*. This is in agreement with previous findings showing that copepods have the ability to synthesize or regulate DHA (Ravet *et al.*, 2010; Kabeya *et al.*, 2021) and confirm the eco-physiological role of docosahexaenoic acid for copepod growth and reproduction (Chen *et al.*, 2012; Deschutter *et al.*, 2019; Kainz *et al.*, 2004; Brett, Müller-Navarra D.C., *et al.*, 2009). The consumer-specific PUFA demands may have prompted transformations in consumers' fatty acids metabolism to actively regulate and/or convert some missing dietary fatty acids and to compensate for the low availability and abundance of PUFAs in order to face the altered biochemical composition of the diets and maximize consumers' fitness. Even though it is generally believed that the ability of crustacean zooplankton to bioconvert and modify fatty acids is scarce (Sargent and Falk-Petersen, 1988; Bell *et al.*, 2007) the presence in our experiment, although in low concentration, of several fatty acids in both grazers' tissues despite the absence in their respective phytoplankton diets, support even more the hypothesis that *D. longispina* and *E. graciloides* have modified fatty acids via internal transformation, such as selective FA retention and bioconversion, by elongating and/or desaturating shorter chained fatty acid precursors into PUFAs (Bell and Tocher, 2009; Boyen *et al.*, 2022) during the experiment. Findings in support of this hypothesis

are still very controversial, and our understanding of selective PUFAs accumulation, allocation and retention in freshwater zooplankton is still limited. In general, several studies revealed a mismatch between FA composition of zooplankton and its food (Desvillettes *et al.*, 1997; Hessen and Leu, 2006; Persson and Vrede, 2006; Smyntek *et al.*, 2008; Taipale *et al.*, 2011; Brett, Müller-Navarra D.C., *et al.*, 2009) and recent advances in genomic analyses have proved the occurrence of some desaturases enzyme in a plethora of invertebrates, which would enable them to biosynthesize PUFAs *de novo* (Monroig and Kabeya, 2018), corroborating the idea that PUFAs synthesis and conversion may be more widespread in the animal kingdom than previously assumed (Kabeya, Fonseca, David E. K. Ferrier, *et al.*, 2018; Nielsen *et al.*, 2019; Boyen *et al.*, 2020, 2022; Kabeya *et al.*, 2021; Twining *et al.*, 2021). Thus, phytoplankton fatty acid composition plays overall an important role in regulating the life-history traits of herbivorous zooplankton, however, biochemical deficiencies of the food may imply the potential endogenous PUFA synthesis and bioconversion independently of the diet's composition, with consumers facing higher metabolic costs, and potentially associated reduced fitness. Thus, it becomes of primary importance to understand the ability and the extent of consumers to modify the dietary fatty acids (Galloway and Budge, 2020; Jardine *et al.*, 2020).

In our experiment, the key nutritional contrasts in the altered phytoplankton communities generated by the disturbance method have influenced and driven some behavioural and/or metabolic adaptation in consumers, demonstrating that a very plastic response in lipid transfer from phytoplankton to zooplankton may occur in freshwater environments. In the prevalent patterns of biodiversity loss and environmental changes, this may have large-scale implications for food web dynamics in aquatic environments.

3.6 Conclusions

Our disturbance method was able to alter for the taxonomic and trait composition of a natural phytoplankton community. It is thus a useful tool for the simulation of biodiversity loss and in particular the loss of stress-sensitive species. The profound shifts in phytoplankton composition and structure subsequently modulated the fitness and lipid composition of higher trophic levels represented by the two grazers *D. longispina* and *E. graciloides* via their distinct feeding modes and diet selectivities. The fatty acid composition of the phytoplankton diets and zooplankton consumers matched only partially. This suggests that consumers actively transform dietary fatty acids to adjust the dietary PUFA composition to their physiological needs under unfavourable conditions. Overall, this study highlights how a loss of functional traits in the resource community could impact consumer traits and eventually may lead to community re-organization and ecological adaptation in trophic dynamics under environmental change and biodiversity loss.

3.7 Supplementary Material

Supplementary Table 1a) Relative biovolume expressed as percentage of each phytoplankton morpho-functional based group at the beginning of the experiment and after one, three and five weeks of disturbance manipulation per treatment (no, intermediate and high). Values are mean ± standard deviation, n = 3 in all treatments except for the start treatment where n = 1.

Time	Treatment	group I	group II	group III	group IV	group V	group VI	group VII
Start	Start	1.69	73.49	0.76	1.22	20.04	2.72	1.29
Week 1	No disturbance	0.32± 0.37	-	4.81±1.32	13.96±5.01	7.51±5.04	71.75±10.97	1.66±0.71
	Intermediate disturbance	5.19±1.12	0.18±0.16	4.66±0.75	28.60±14.31	14.83±5.00	44.43±11.73	2.11±1.05
	High disturbance	2.26±0.85	0.57± 0.47	3.09±2.00	34.39±12.92	3.20±1.45	50.89±12.33	5.60±3.89
Week 3	No disturbance	0.16±0.12	-	50.16±22.25	14.70±9.52	8.39±2.40	25.69±18.39	0.90±0.57
	Intermediate disturbance	0.83±0.50	0.17±0.29	25.49±29.60	54.10±32.06	1.29±1.73	11.70±1.15	6.42±2.21
	High disturbance	0.69±0.28	-	1.00±0.62	51.84±18.91	1.57±0.92	44.80±18.24	0.09±0.16
Week 5	No disturbance	0.04±0.03	-	60.83±28.02	2.74±0.14	11.73±12.40	20.91±34.92	3.75±3.04
	Intermediate disturbance	0.12±0.05	-	47.56±37.31	21.07±17.71	2.29±2.12	25.35±33.66	3.60±5.80
	High disturbance	0.87±0.36	0.02±0.04	1.71±2.75	17.71±17.45	2.76±1.97	75.84±22.69	1.09±0.92

Supplementary Table 1b) Relative fatty acids content expressed in percentage measured at the beginning of the experiment and after one, three and five weeks of disturbance manipulation per treatment (no, intermediate and high disturbance). Values are mean ± standard deviation, n = 5 in all treatments except for the start treatment where n = 1.

Time	Treatment	SAFA	MUFA	PUFA n-3	PUFA n-6
Start	Start	46.46 ± 6.91	26.70 ± 5.76	20.54 ± 5.52	6.30 ± 1.25
Week 1	No disturbance	34.80 ± 4.62	21.66 ± 3.16	37.30 ± 7.65	6.23 ± 1.82
	Intermediate disturbance	31.48 ± 11.27	23.58 ± 8.28	34.54 ± 8.56	10.40 ± 1.56
	High disturbance	31.71 ± 4.16	23.77 ± 7.73	35.24 ± 2.15	9.28 ± 1.51
Week 3	No disturbance	38.41 ± 3.14	30.38 ± 4.46	22.39 ± 2.53	8.82 ± 0.65
	Intermediate disturbance	35.92 ± 4.95	43.08 ± 8.86	12.61 ± 3.80	8.39 ± 1.93
	High disturbance	32.72 ± 1.07	52.28 ± 1.03	9.75 ± 1.13	5.25 ± 0.93
Week 5	No disturbance	46.39 ± 9.94	27.70 ± 5.81	15.39 ± 9.02	10.52 ± 2.25
	Intermediate disturbance	44.99 ± 13.45	25.53 ± 8.16	18.31 ± 9.60	11.18 ± 4.58
	High disturbance	36.05 ± 6.87	46.40 ± 4.09	12.70 ± 2.92	4.85 ± 0.48

Supplementary Table 1c) Relative pigments abundance expressed as percentage of each phytoplankton pigment measured at the beginning of the experiment and after one, three and five weeks of disturbance manipulation per treatment (no, intermediate and high disturbance). Values are mean ± standard deviation, n = 5 in all treatments except for the start treatment where n = 1.

Pigments	Start	Week 1			Week 3			Week 5		
	Start	No disturbance	Intermediate disturbance	High disturbance	No disturbance	Intermediate disturbance	High disturbance	No disturbance	Intermediate disturbance	High disturbance
Chlorophyll c3	-	-	-	-	0.78±0.39	0.16±0.14	1.37±0.27	2.86±3.15	0.66±1.47	0.12±0.28
Chlorophyll c2	12.43±2.22	5.06±1.37	3.16±1.13	2.03±0.52	0.17±0.16	0.14±0.20	2.28±1.57	0.60±1.04	0.53±0.53	10.39±2.47
Peridinin	-	-	-	-	-	0.48±1.07	-	-	-	-
Fucoxanthin	19.10±4.26	7.55±3.03	2.46±0.50	5.68±1.43	0.82±0.74	0.75±0.59	9.34±4.75	1.14±1.86	1.88±2.12	31.58±15.58
Violaxanthin	2.41±1.86	0.93±0.45	0.81±0.30	2.38±0.32	0.52±0.28	0.38±0.44	1.33±0.59	0.96±1.12	0.43±0.96	1.52±1.51
Neoxanthin	6.07±0.70	0.58±0.30	1.25±0.49	2.87±0.46	0.92±0.58	0.38±0.10	3.57±0.92	1.64±1.14	0.42±0.94	2.06±2.03
Diadinoxanthin	12.85±2.84	3.95±2.11	0.70±0.33	1.50±1.50	0.21±0.16	0.34±0.22	3.10±1.63	0.09±0.19	0.62±1.39	9.03±5.06
Alloxanthin	7.14±0.83	4.50±1.50	4.36±3.06	1.55±0.28	0.09±0.09	0.04±0.09	0.35±0.34	0.56±0.96	0.10±0.23	-
Diatoxanthin	0.55±0.75	-	1.30±2.91	0.65±1.46	0.38±0.16	0.07±0.07	0.15±0.14	0.52±0.82	0.25±0.57	-
Lutein	0.30±0.68	2.69±0.86	7.14±1.05	7.49±1.67	2.97±1.62	3.50±1.50	10.74±2.75	8.33±1.93	5.09±3.38	9.67±5.27
Zeaxanthin	12.87±1.61	1.38±0.41	7.23±2.66	2.42±1.39	5.99±0.40	4.68±0.76	2.98±1.31	16.31±4.95	14.06±9.87	0.61±1.37
Chlorophyll b	4.25±0.57	11.29±3.20	14.32±1.67	20.73±4.16	8.78±4.14	4.96±3.08	12.71±3.06	24.40±8.01	25.57±10.68	26.43±12.93
Echinenone	0.60±0.25	0.12±0.26	-	0.16±0.18	0.20±0.17	0.29±0.17	-	0.06±0.12	0.33±0.60	0.19±0.44
Pheophytin b	2.67±0.65	5.51±1.50	5.55±1.49	2.25±0.36	0.24±0.31	-	-	-	-	-
Pheophytin a	9.95±0.59	53.76±8.93	49.28±1.94	47.22±5.13	75.92±7.18	81.89±4.74	50.80±8.95	-	3.39±5.83	0.19±0.42
beta-Carotene	8.79±5.13	2.69±1.10	2.44±0.63	3.07±0.99	2.01±0.66	1.94±0.50	1.28±0.21	42.54±7.20	46.67±11.56	8.20±3.44

Supplementary Table 2. Absolute values of density, biomass, fatty acids and pigments and diversity community indices of phytoplankton measured at the beginning of the experiment and after one, three and five weeks of disturbance manipulation per treatment (no, intermediate and high disturbance). Values are mean ± standard deviation, n = 5 in all treatments except for the start treatment where n = 1.

Time	Treatment	Species Richness	Total Density (cells L^{-1})	Total Biomass ($\mu m^3 L^{-1}$)	H' functional	Total FA (µg per mg DW)	H' Fatty Acids	Total Pigments (ng per mg DW)	H' Pigments
Start	Start	28	$1.25x10^6$	$1.04x10^9$	1.6	31.29±6.22	1.96±0.11	950.55±262.09	2.26±0.03
Week 1	No disturbance	31.33±2.31	$1.35x10^8±9.11x10^7$	$3.70x10^{10}±2.36x10^{10}$	1.20±0.21	38.22±12.11	2.33±0.06	286.17±193.05	1.66±0.19
	Intermediate disturbance	29.33±2.52	$1.67x10^8±1.26x10^7$	$1.81x10^{10}±2.62x10^9$	1.65±0.05	25.50±6.51	2.32±0.12	225.13±48.27	1.73±0.06
	High disturbance	28±3.46	$1.54x10^8±1.17x10^7$	$2.11x10^{10}±5.81x10^9$	1.43±0.11	29.59±6.37	2.37±0.07	269.99±188.09	1.73±0.10
Week 3	No disturbance	27.67±4.73	$5.54x10^8±5.15x10^7$	$2.52x10^{10}±1.29x10^{10}$	0.46±0.25	24.56±9.18	2.25±0.06	208.47±52.39	0.97±0.22
	Intermediate disturbance	22.67±0.58	$1.27x10^8±5.64x10^7$	$1.36x10^{10}±8.22x10^9$	0.77±0.37	73.59±26.35	2.06±0.14	203.33±75.29	0.79±0.14
	High disturbance	22.33±3.79	$3.98x10^8±1.86x10^7$	$1.48x10^{11}±8.86x10^{10}$	1.18±0.15	95.68±16.52	1.88±0.06	73.07±44.84	1.65±0.18
Week 5	No disturbance	23.33±4.16	$1.21x10^9±1.07x10^8$	$3.43x10^{10}±7.86x10^9$	0.50±0.30	23.22±14.17	2.07±0.07	192.63±81.49	1.49±0.12
	Intermediate disturbance	22.67±2.08	$1.96x10^8±1.28x10^8$	$8.58x10^9±3.63x10^9$	0.58±0.31	25.74±19.64	2.02±0.24	235.79±386.58	1.32±0.35
	High disturbance	25±3.00	$3.29x10^8±2.58x10^8$	$1.16x10^{11}±4.12x10^{10}$	1.30±0.12	80.47±33.04	1.87±0.16	78.017±40.37	1.68±0.18

Supplementary Table 3. Statistical analysis of variance (ANOVA).

Species Richness	Df	Sum Sq	Mean Sq	F value	p-value
Treatment	3	40.68	13.56	1.336	0.29386
Time	2	190.3	95.15	9.376	0.00162**
Treatment x Time	4	43.04	10.76	1.06	0.40461
Residuals	18	182.67	10.15		
Functional diversity (Shannon Abundance)	Df	Sum Sq	Mean Sq	F value	p-value
Treatment	3	17.34	5.78	118.55	<2e-16 ***
Time	2	16.504	8.252	169.24	<2e-16 ***
Treatment x Time	4	5.144	1.286	26.37	<2e-16 ***
Residuals	200	9.751	0.049		
Total Fatty Acids	Df	Sum Sq	Mean Sq	F value	p-value
Treatment	3	13451	4484	14.956	1.12e-06 ***
Time	2	8642	4321	14.414	1.93e-05 ***
Treatment x Time	4	11698	2924	9.755	1.33e-05 ***
Residuals	40	11992	300		
Fatty acids diversity	Df	Sum Sq	Mean Sq	F value	p-value
Treatment	3	0.3622	0.1207	8.082	0.000251 ***
Time	2	1.0179	0.5089	34.063	2.3e-09 ***
Treatment x Time	4	0.2203	0.0551	3.686	0.012022 *
Residuals	18	0.5977	0.0149		
Total Pigments	Df	Sum Sq	Mean Sq	F value	p-value
Treatment	3	2639341	879780	31.796	1.12e-10 ***
Time	2	97489	48744	1.762	0.185
Treatment x Time	4	54854	13713	0.496	0.739
Residuals	40	1106776	27669		

Pigments diversity	Df	Sum Sq	Mean Sq	F value	p-value
Treatment	3	4.342	1.4474	45.19	6.46e-13 ***
Time	2	2.46	1.23	38.402	4.92e-10 ***
Treatment x Time	4	1.045	0.2613	8.157	6.58e-05 ***
Residuals	40	1.281	0.032		

Df-Degree of freedom; Sum Sq-Sum of squares; Adj Mean Sq-Mean squares; P-Value-Probability; F-Value-Test statistics

Concluding remarks and perspectives

As the specific results have already been discussed in the previous chapters, I would like to highlight here the main conclusions that can be drawn from the results of the different experiments and make some concluding remarks on the challenges and future prospects of using trait-based approaches and experimental diversity manipulations to study and assess the impact of biodiversity loss on aquatic ecosystem functions and food web dynamics.

- Phytoplankton trait diversity, examined in terms of PUFAs availability, strictly determined the quality of the food on which zooplankton organisms are highly dependent and that the dietary quality constraints may be an important regulatory mechanism for zooplankton fitness, growth, reproduction and survival (Chapter 1 and 3).

- Diversity manipulation experiments using the "size fractionation" and "disturbance" methods resulted to be efficient tools for simulating more realistic species and trait loss scenarios in phytoplankton diversity studies (Chapters 1 and 2).

- Changes in phytoplankton morphological diversity induced by the "size fractionation method" revealed differences in phytoplankton morpho-functional traits and taxonomic composition in size-fractionated phytoplankton communities, but did not significantly affect zooplankton grazing by the cladoceran *Daphnia longispina* (generalist unselective filter feeder) and the calanoid copepod *Eudiaptomus* sp. (selective feeder) in terms of grazing rates and size selectivity (Chapter 2).

- The alteration of phytoplankton functional diversity, induced by the disturbance method, led to species losses and taxonomic shifts, resulting in distinct communities with different taxonomic and functional characteristics which affected the fitness and lipid composition of *Daphnia longispina* and *Eudiaptomus graciloides* differently, mainly depending on the grazers' feeding habits and their nutritional requirements. (Chapter 3).

- *Daphnia longispina*, unable to perceive the nutritional quality of phytoplankton, did not show significant changes in either life history trait responses or fatty acid composition under "good" and "poor" food scenarios (Chapter 3).

- *Eudiaptomus graciloides* actively selected the most suitable prey based on the nutritional value. In the "good" food scenario, represented by high total fatty acid content and greater amounts of

essential fatty acids, the fatty acid composition of calanoid copepods largely reflected the composition of the respective high-quality prey, which in turn had a positive effect on copepod fitness. Under food quality constraints, the responses of life history traits in *E. graciloides* showed an overall lower performance and some trade-off mechanisms between survival, growth and reproduction (Chapter 3).

- Nutritional diversity of resources affected the adaptive trait-responses of consumer fatty acid metabolism, with *D. longispina* and *E. graciloides* showing selective and differential accumulation of essential fatty acids. *D. longispina* accumulated preferentially EPA and its precursor SDA and ARA, while *E. graciloides* preferentially accumulated DHA (Chapter 3).

- Mismatches between the fatty acid composition of zooplankton and its food have occurred, suggesting that the specific demands for PUFAs in the consumers may have resulted in possible enzymatic modifications and bioconversion mechanisms in both grazer's fatty acid metabolisms to face biochemical deficiencies of the diets (Chapter 3).

Overall, the studies presented in this dissertation contribute to our understanding of the role of phytoplankton functional diversity and provide some evidence on how biodiversity loss and loss/change of specific traits in the resource community may induce variation, adaptation and reorganisation of communities and impact on consumer communities, food web dynamics and ecosystem processes. However, the interpretation of mechanism results from biodiversity manipulation experiments can be critically dependent on the experimental design and approaches used (Allison, 1999). Therefore, addressing these issues in a more rigorous framework is necessary and highly relevant for substantial progress in future BEF research.

As there is no single concept of diversity and little agreement on the most appropriate measure to use, my research has mainly applied the recent and innovative functional trait-based approach in combination with the more descriptive and traditional species-based approach. The use of functional traits of species currently appears to be one of the most promising ways to study and understand diversity effects in ecosystems and to predict community responses to change (McGill *et al.*, 2006; Violle *et al.*, 2007), and there is an emerging trend to use these approaches to determine phytoplankton and zooplankton responses to environmental perturbations induced by

intensified anthropogenic activities and global change (Baker *et al.*, 2016; Thomas *et al.*, 2016; Van de Waal and Litchman, 2020; Bishop *et al.*, 2022). However, differences between species and their interactions can also be predicted by their evolutionary history as estimated by traditional taxonomic classification, so the use of functional traits should not exclude the presence and identity of taxa (Weiss and Ray, 2019). Although the correct naming and identification of species can often require more research, time and expertise, knowing the taxonomic details of a specimen is crucial for the study of biodiversity and conservation. In this study, functional traits combined with the species-based approach have provided a unifying measure to investigate the diversity of natural phytoplankton assemblages and to assess the consequences of phytoplankton diversity loss for ecosystem functioning and organisation. Traits for phytoplankton and zooplankton have been fairly well studied and described (Litchman and Klausmeier, 2008; Litchman *et al.*, 2013), but a limitation of trait-based approaches is that the traits, and therefore the associated process of interest to be studied, must be identified *a priori*. Up until now, the *'a priori'* choice of traits has resulted in phytoplankton and zooplankton research mainly focusing on some specific and easily measurable characteristics of these organisms, such as morphological traits (e.g. size, shape, body mass), with less emphasis on behavioural or physiological traits, which may require more analysis and more specific equipment to obtain accurate and reliable trait data. In addition, researchers tend to focus on understanding the ecosystem effects of a single trait and thus a single biological function, even though in the context of biodiversity loss and global change, individuals may simultaneously vary in multiple traits and thus affect multiple processes, oversimplifying the role of species and underestimating the impact of species loss (Vaughn, 2010). For example, in phytoplankton-zooplankton trophic dynamics, several functional traits of both zooplankton (e.g. food preferences, feeding modes, grazing rates, functional response) and phytoplankton (e.g. palatability, quality as food, size, shape, fatty acids) are interdependent and alteration of trait diversity may determine the occurrence of independent or interactive feedback mechanisms in plankton communities, which can in turn influence ecological processes. Thus, a single-trait approach may oversimplify the interpretation of these ecological aspects. Therefore, there is a need to make the multi-trait approach a promising way and to incorporate trophic complexity in order to understand the effects of biodiversity and biodiversity loss across trophic levels (Duffy *et al.*, 2007).

In this dissertation, the role of phytoplankton diversity and the consequences of its loss for herbivorous zooplankton, and more generally for food web dynamics, was investigated using a multi-trait approach, focusing on several key phytoplankton and zooplankton traits and clustering phytoplankton and zooplankton species into functional groups (Hulot *et al.*, 2000; Hubbell, 2006; Litchman and Klausmeier, 2008; Kruk *et al.*, 2010)Kruk, 2010). Monitoring and measuring multiple traits (i.e. morphological, behavioural, physiological, and life history traits) helped us to better describe and capture the complexity of ecological interactions between phytoplankton and zooplankton.

In my laboratory experiments, I have shown that it is possible to manipulate the diversity of natural phytoplankton communities using size fractionation and disturbance methods. Both techniques successfully altered phytoplankton community and trait diversity and established diversity gradients where the experimental "altered communities" were significantly different to the starting conditions, due to species losses and taxonomic shifts that have occurred. However, in some cases, some shifts could have been induced not directly by the manipulation method used but more by laboratory conditions that might have favoured some taxa over others. This aspect should be taken into account once performing laboratory experiments with the manipulation of natural assemblages.

Moreover, it has to be considered that trait analyses and trait-related dynamics investigated in laboratory conditions may differ from the actual behavior and response of species in nature. Thus, may well reduce the predictive power of trait-based experiments performed on microcosm controlled scales and may lead to misleading conclusions. Indeed, taxa cultured for generations in the laboratory may result in less plastic and be over-adapted to standardised laboratory conditions, and this aspect may influence their physiological or behavioural responses. In order to get a deeper understanding into the identification of the mechanisms generating the relationship between declining diversity and impacts on the functioning of food web dynamics and to avoid possibly limited interpretations, the best would be to investigate diversity loss by scaling up from small scale laboratory diversity manipulation experiments to larger mesocosm studies in the field to ascertain (in a short- and long-term) mechanisms influencing planktonic community trait distribution and dynamics, in setups that closely resemble natural conditions. Moreover, because biodiversity may have the capacity to buffer ecosystem responses to perturbations (McCann, 2000), also the longer-term effects of biodiversity need to be studied to avoid reliance on transient

dynamics (Tsai *et al.*, 2014). However, this is experimentally challenging. In this regard, there is an immense amount of long-term trait data that have been collected in the last decades that could help us unveil the ecosystem functioning in response to diverse perturbations. However, currently it remains extremely challenging for researchers to utilize them for monitoring community responses to environmental impacts, ecosystem changes and biodiversity loss. The lack of common guidelines for acquiring, organizing and describing trait data result in profound inconsistencies in the terminologies used and their meanings, hampering trait data aggregation and integration. Concerning this matter, the Open Traits Network (OTN) was created as a "decentralised alliance of international researchers and institutions focused on collaborative integration and standardisation of the exponentially increasing availability of trait data" and embracing the use of Open Science (Gallagher *et al.*, 2019). This will largely improve the findability, accessibility, interoperability and reusability (FAIR) of trait-based data and advance trait-based approaches in BEF research by promoting further innovation, especially in mechanistically linking organismal-level traits to ecosystem-level functioning. In the current scenario of profound global biodiversity loss, information on future and historical trends in biodiversity is critical to developing meaningful policy and effective conservation management. The adoption of Open Science and FAIR principles and the ongoing research in a trait-based context, that combine multiple traits across trophic levels are promising way for a more efficient and realistic assessment of biodiversity loss impact and consequences in phytoplankton-zooplankton interface and will surely enable exciting advancements in aquatic community ecology in the years to come.

General references

Abrusán, G. *et al.* (2007) Biochemical limitation of resting egg production in Daphnia. *Limnol. Oceanogr.*, **52**, 1724–1728.

Acevedo-Trejos, E. *et al.* (2016) PhytoSFDM version 1.0.0: Phytoplankton Size and Functional Diversity Model. *Geosci. Model Dev.*, **9**, 4071–4085.

Ackman, R. G. *et al.* (1968) Marine Phytoplankter Fatty Acids. *J. Fish. Res. Board Canada*, **25**, 1603–1620.

Ahlgren, G. *et al.* (1990) Lipid composition and food quality of some freshwater phytoplankton for cladoceran zooplankters. *J. Plankton Res.*, **12**, 809–818.

Alcaraz, M. *et al.* (1980) Catching the algae: A first account of visual observations on filter-feeding calanoids. *In: Kerfoot, W.C (e.d) Evolution and ecology of zooplankton communities. University press of New England. Hanover*. pp. 241–248.

Ali, A. K. *et al.* (2009) Morphological correlates of mating frequency and clutch size in wild caught female Eudiaptomus graciloides (Copepoda: Calanoida). *J. Plankton Res.*, **31**, 389–397.

Allison, G. W. (1999) The Implications of Experimental Design for Biodiversity Manipulations. *Am. Nat.*, **153**, 26–45.

Anderson, M. J. (2006) Distance-Based Tests for Homogeneity of Multivariate Dispersions. *Biometrics*, **62**, 245–253.

Anderson, T. R. and Pond, D. W. (2000) Stoichiometric theory extended to micronutrients: Comparison of the roles of essential fatty acids, carbon, and nitrogen in the nutrition of marine copepods. *Limnol. Oceanogr.*, **45**, 1162–1167.

Arendt, K. E. *et al.* (2005) Effects of dietary fatty acids on the reproductive success of the calanoid copepod Temora longicornis. *Mar. Biol.*, **146**, 513–530.

Arin, L. *et al.* (2002) Combined effects of nutrients and small-scale turbulence in a microcosm experiment. I. Dynamics and size distribution of osmotrophic plankton. *Aquat. Microb. Ecol.*, **29**, 51–61.

Baker, K. G. *et al.* (2016) Thermal performance curves of functional traits aid understanding of thermally induced changes in diatom-mediated biogeochemical fluxes. *Front. Mar. Sci.*, **3**, 44.

Bamstedt, U. *et al.* (2000) Feeding. *ICES Zooplankton Methodology Manual*. Academic Press, San Diego, pp. 297–399.

Barnes, A. D. *et al.* (2018) Energy Flux: The Link between Multitrophic Biodiversity and Ecosystem Functioning. *Trends Ecol. Evol.*, **33**, 186–197.

Barnett, A. and Beisner, B. E. (2007) Zooplankton biodiversity and lake trophic state: Explanations invoking resource abundance and distribution. *Ecology*, **88**, 1675–1686.

Barnett, A. J. *et al.* (2007) Functional diversity of crustacean zooplankton communities: towards a trait-based classification. *Freshw. Biol.*, **52**, 796–813.

Beardall, J. *et al.* (2009) Allometry and stoichiometry of unicellular, colonial and multicellular phytoplankton. *New Phytol.*, **181**, 295–309.

Bell, M. V. *et al.* (2007) Application of liposome and stable isotope tracer techniques to study polyunsaturated fatty acid biosynthesis in marine zooplankton. *J. Plankton Res.*, **29**, 417–422.

Bell, M. V. and Tocher, D. R. (2009) Biosynthesis of polyunsaturated fatty acids in aquatic ecosystems: general pathways and new directions. *Lipids in Aquatic Ecosystems*. Springer New York, New York, NY, pp. 211–236.

de Bello, F. *et al.* (2021) Functional trait effects on ecosystem stability: assembling the jigsaw puzzle. *Trends Ecol. Evol.*, **36**, 822–836.

Bishop, I. W. *et al.* (2022) Thermal trait variation may buffer Southern Ocean phytoplankton from anthropogenic warming. *Glob. Chang. Biol.*, **28**, 5755–5767.

Blaxter, J. H. S. *et al.* (1998) *The Biology of Calanoid Copepods: The Biology of Calanoid Copepods*.

Böing, W. J. *et al.* (1998) Phytoplankton responses to grazing by Daphnia galeata in the biomanipulated Bautzen reservoir. *Hydrobiologia*, **389**, 101–114.

Borics, G. *et al.* (2012) Functional groups of phytoplankton shaping diversity of shallow lake ecosystems. *Hydrobiologia*, **698**, 251–262.

Boxshall, G. A. and Defaye, D. (2008) Global diversity of copepods (Crustacea: Copepoda) in freshwater. *Hydrobiologia*, **595**, 195–207.

Boyce, D. G. *et al.* (2010) Global phytoplankton decline over the past century. *Nature*, **466**, 591–596.

Boyen, J. *et al.* (2020) Fatty acid bioconversion in harpacticoid copepods in a changing environment: a transcriptomic approach. *Philos. Trans. R. Soc. B*, **375**.

Boyen, J. *et al.* (2022) Functional characterization reveals a diverse array of metazoan fatty acid biosynthesis genes. *Mol. Ecol.*, **32**, 970–982.

Bracken, M. E. S. *et al.* (2008) Functional consequences of realistic biodiversity changes in a marine ecosystem. *Proc. Natl. Acad. Sci.*, **105**, 924–928.

Bracken, M. E. S. and Low, N. H. N. (2012) Realistic losses of rare species disproportionately impact higher trophic levels. *Ecol. Lett.*, **15**, 461–467.

Brendelberger, H. *et al.* (1986) Daphnia's filters are not solid walls. *undefined*.

Brett, M. T., Müller-Navarra D.C., *et al.* (2009) *Lipids in Aquatic Ecosystems*. Kainz, M. *et al.* (eds). Springer New York, New York, NY.

Brett, M. T., Kainz, M. J., *et al.* (2009) Phytoplankton, not allochthonous carbon, sustains herbivorous zooplankton production. *Proc. Natl. Acad. Sci.*, **106**, 21197–21201.

Broglio, E. *et al.* (2003) Effect of heterotrophic versus autotrophic food on feeding and reproduction of the calanoid copepod Acartia tonsa: Relationship with prey fatty acid composition. *Aquat. Microb. Ecol.*, **31**, 267–278.

Brose, U. *et al.* (2006) Consumer-resource body-size relationships in natural food webs. *Ecology*, **87**, 2411–2417.

Brown, J. H. *et al.* (2004) Toward a metabolic theory of ecology. *Ecology*, **85**, 1771–1789.

Brown, M. R. *et al.* (1997) Nutritional properties of microalgae for mariculture. *Aquaculture*, **151**, 315–331.

Bruno, S. F. *et al.* (1983) Primary productivity and phytoplankton size fraction dominance in a temperate North Atlantic estuary. *Estuaries*, **6**, 200–211.

Bundy, M. H. *et al.* (1998) Perception of inert particles by calanoid copepods: Behavioral observations and a numerical model. *J. Plankton Res.*, **20**, 2129–2152.

Cadotte, M. W. *et al.* (2011) Beyond species: Functional diversity and the maintenance of ecological processes and services. *J. Appl. Ecol.*, **48**, 1079–1087.

Cadotte, M. W. *et al.* (2015) Predicting communities from functional traits. *Trends Ecol. Evol.*, **30**, 510–511.

Cadotte, M. W. *et al.* (2009) Using Phylogenetic, Functional and Trait Diversity to Understand Patterns of Plant Community Productivity. *PLoS One*, **4**, e5695.

Cardinale, B. J. *et al.* (2012) Biodiversity loss and its impact on humanity. *Nature*, **486**, 59–67.

Cardinale, B. J. *et al.* (2011) The functional role of producer diversity in ecosystems. *Am. J. Bot.*, **98**, 572–592.

Cardoso, P. *et al.* (2014) A new frontier in biodiversity inventory: a proposal for estimators of phylogenetic and functional diversity. *Methods Ecol. Evol.*, **5**, 452–461.

Castell, J. D. *et al.* (1972) Essential fatty acids in the diet of rainbow trout (Salmo gairdneri): growth, feed conversion and some gross deficiency symptoms. *J. Nutr.*, **102**, 77–85.

Cermen˜o, P. *et al.* (2005) Size dependence of coastal phytoplankton photosynthesis under vertical mixing conditions. *J. Plankton Res.*, **27**, 473–483.

Cernansky, R. (2017) Biodiversity moves beyond counting species. *Nature*, **546**, 22–24.

Chen, M. *et al.* (2012) Effects of dietary essential fatty acids on reproduction rates of a subtropical calanoid copepod, Acartia erythraea. *Mar. Ecol. Prog. Ser.*, **455**, 95–110.

Chevene, F. *et al.* (1994) A fuzzy coding approach for the analysis of long-term ecological data. *Freshw. Biol.*, **31**, 295–309.

Colina, M. *et al.* (2016) A trait-based approach to summarize zooplankton–phytoplankton interactions in freshwaters. *Hydrobiologia*, **767**, 221–233.

Couturier, L. I. E. *et al.* (2020) State of art and best practices for fatty acid analysis in aquatic sciences. *ICES J. Mar. Sci.*, **77**, 2375–2395.

Covich, A. P. *et al.* (2004) The role of biodiversity in the functioning of freshwater and marine benthic ecosystems. *Bioscience*, **54**, 767–775.

Cyr, H. and Pace, M. L. (1992) Grazing by zooplankton and its relationship to community structure. *Can. J. Fish. Aquat. Sci.*, **49**, 1455–1465.

DeMott, W. R. (1986) The role of taste in food selection by freshwater zooplankton. *Oecologia*, **69**, 334–340.

Deschutter, Y. *et al.* (2019) Seasonal and spatial fatty acid profiling of the calanoid copepods Temora longicornis and Acartia clausi linked to environmental stressors in the North Sea. *Mar. Environ. Res.*, **144**, 92–101.

Desvillettes, C. *et al.* (1997) Use of fatty acids for the assessment of zooplankton grazing on bacteria, protozoans and microalgae. *Freshw. Biol.*, **38**, 629–637.

Van Donk, E. (1997) Defenses in phytoplankton against grazing induced by nutrient limitation, UV-B stress and infochemicals. *Aquat. Ecol.*, **31**, 53–58.

Dudgeon, D. *et al.* (2006) Freshwater biodiversity: importance, threats, status and conservation challenges. *Biol. Rev.*, **81**, 163.

Duffy, J. E. *et al.* (2007) The functional role of biodiversity in ecosystems: Incorporating trophic complexity. *Ecol. Lett.*, **10**, 522–538.

Durbin, E. G. *et al.* (1975) Seasonal studies on the relative importance of different size fractions of phytoplankton in Narragansett Bay (USA). *Mar. Biol. 1975 323*, **32**, 271–287.

Ederington, M. C. *et al.* (1995) Trophic transfer of fatty acids, sterols, and a triterpenoid alcohol between bacteria, a ciliate, and the copepod Acartia tonsa. *Limnol. Oceanogr.*, **40**, 860–867.

von Elert, E. (2002) Determination of limiting polyunsaturated fatty acids in Daphnia galeata using a new method to enrich food algae with single fatty acids. *Limnol. Oceanogr.*, **47**, 1764–1773.

Von Elert, E. *et al.* (2003) Absence of sterols constrains carbon transfer between cyanobacteria and a freshwater herbivore (Daphnia galeata). *Proc. R. Soc. B Biol. Sci.*, **270**, 1209–1214.

Von Elert, E. and Jüttner, F. (1997) Phosphorus limitation and not light controls the extracellular release of allelopathic compounds by Trichormus doliolum (Cyanobacteria). *Limnol. Oceanogr.*, **42**, 1796–1802.

Von Elert, E. and Stampfl, P. (2000) Food quality for Eudiaptomus gracilis: The importance of particular highly unsaturated fatty acids. *Freshw. Biol.*, **45**, 189–200.

Von Elert, E. and Wolffrom, T. (2001) Supplementation of cyanobacterial food with polyunsaturated fatty acids does not improve growth of Daphnia. *Limnol. Oceanogr.*, **46**, 1552–1558.

Elmqvist, T. *et al.* (2003) Response diversity, ecosystem change, and resilience. *Front. Ecol. Environ.*, **1**, 488–494.

Engel, F. G. *et al.* (2017) Manipulation of Non-random Species Loss in Natural Phytoplankton:

Qualitative and Quantitative Evaluation of Different Approaches. *Front. Mar. Sci.*, **4**, 1–12.

Evjemo, J. O. *et al.* (2008) Effect of essential dietary fatty acids on egg production and hatching success of the marine copepod Temora longicornis. *J. Exp. Mar. Bio. Ecol.*, **365**, 31–37.

Falkowski, P. G. *et al.* (1998) Biogeochemical Controls and Feedbacks on Ocean Primary Production. *Science (80-.).*, **281**, 200–206.

Falkowski, P. G. (1994) The role of phytoplankton photosynthesis in global biogeochemical cycles. *Photosynth. Res.*, **39**, 235–258.

Falkowski, P. G. and Raven, J. A. (2007) *Aquatic Photosynthesis*. Princeton University Press.

Farkas, T. (1979) Adaptation of fatty acid compositions to temperature-a study on planktonic crustaceans. *Comp. Biochem. Physiol. -- Part B Biochem.*, **64**, 71–76.

Fetzer, I. *et al.* (2015) The extent of functional redundancy changes as species' roles shift in different environments. *Proc. Natl. Acad. Sci.*, **112**, 14888–14893.

Field, C. B. *et al.* (1998) Primary Production of the Biosphere: Integrating Terrestrial and Oceanic Components. *Science (80-.).*, **281**, 237–240.

Fink, P. *et al.* (2011) Dietary Essential Amino Acids Affect the Reproduction of the Keystone Herbivore Daphnia pulex. *PLoS One*, **6**, 28498.

Fink, P. and Windisch, H. S. (2019) The essential omega-3 fatty acid EPA affects expression of genes involved in the metabolism of omega-6-derived eicosanoids in Daphnia magna. *Hydrobiologia*, **846**, 5–16.

Flynn, D. F. B. *et al.* (2011) Functional and phylogenetic diversity as predictors of biodiversity–ecosystem-function relationships. *Ecology*, **92**, 1573–1581.

Fogg, G. E. and Walsby, A. E. (1971) Buoyancy regulation and the growth of planktonic blue-green algae. *Int. Vereinigung für Theor. und Angew. Limnol. Mitteilungen*, **19**, 182–188.

Frangoulis, C. *et al.* (2005) Comparison of Marine Copepod Outfluxes: Nature, Rate, Fate and Role in the Carbon and Nitrogen Cycles. *Advances in Marine Biology*. pp. 253–309.

Franklin, R. B. *et al.* (2001) Impact of dilution on microbial community structure and functional potential: Comparison of numerical simulations and batch culture experiments. *Appl. Environ. Microbiol.*, **67**, 702–712.

Gallagher, A. J. *et al.* (2015) Evolutionary theory as a tool for predicting extinction risk. *Trends Ecol. Evol.*, **30**, 61–65.

Gallagher, R. V *et al.* (2019) The open traits network: Using open science principles to accelerate trait-based science across the tree of life. *Nat. Ecol. Evol.*

Galloway, A. W. E. and Budge, S. M. (2020) The critical importance of experimentation in biomarker-based trophic ecology. *Philos. Trans. R. Soc. B Biol. Sci.*, **375**.

Garrido, J. L. and Zapata, M. (1993) High performance liquid chromatographic separation of polar and non-polar chlorophyll pigments from algae using a wide pore polymeric octadecylsilica column. *J. High Resolut. Chromatogr.*, **16**, 229–233.

Geller, W. and Müller, H. (1981) The filtration apparatus of Cladocera: Filter mesh-sizes and their implications on food selectivity. *Oecologia*, **49**, 316–321.

Ger, K. A. *et al.* (2016) The interaction between cyanobacteria and zooplankton in a more eutrophic world. *Harmful Algae*, **54**, 128–144.

Gerhard, M. *et al.* (2021) Nonrandom species loss in phytoplankton communities and its effect on ecosystem functioning. *Limnol. Ocean.*, **66**, 779–792.

Giller, P. S. *et al.* (2004) Biodiversity effects on ecosystem functioning: emerging issues and their experimental test in aquatic environments. *Oikos*, **104**, 423–436.

Gophen, M. and Geller, W. (1984) Filter mesh size and food particle uptake by Daphnia. *Oecologia*, **64**, 408–412.

Graco-Roza, C. *et al.* (2021) Functional rather than taxonomic diversity reveals changes in the phytoplankton community of a large dammed river. *Ecol. Indic.*, **121**, 107048.

Green, S. J. *et al.* (2022) Trait-based approaches to global change ecology: moving from description to prediction. *Proc. R. Soc. B Biol. Sci.*, **289**.

Groendahl, S. and Fink, P. (2016) The Effect of Diet Mixing on a Nonselective Herbivore. *PLoS One*, **11**, e0158924.

Guillard, R. R. L. and Lorenzen, C. J. (1972) YELLOW-GREEN ALGAE WITH CHLOROPHYLLIDE C 1, 2. *J. Phycol.*, **8**, 10–14.

Hamburger, K. and Boëtius, F. (1987) Ontogeny of growth, respiration and feeding rate of the freshwater calanoid copepod Eudiaptomus graciloides. *J. Plankton Res.*, **9**, 589–606.

Hammerstein, S. K. *et al.* (2017) Directed diversity manipulations within natural phytoplankton communities. *Limnol. Oceanogr. Methods*, **15**, 653–662.

Hansen, B. *et al.* (1994) The size ratio between planktonic predators and their prey. *Limnol. Oceanogr.*, **39**, 395–403.

Harbison, G. R. and McAlister, V. L. (1980) Fact and artifact in copepod feeding experiments1. *Limnol. Oceanogr.*, **25**, 971–981.

Harrison, K. E. (1990) The role nutrition in maturation, reproduction and embryonic development of decapod crustaccens: a review. *J. Shellfish Res.*, **9**, 1–28.

Hashimoto, K. *et al.* (2008) The repertoire of desaturases and elongases reveals fatty acid variations in 56 eukaryotic genomes. *J. Lipid Res.*, **49**, 183–191.

Helena Sipaúba-Tavares, L. *et al.* (2001) Effects of food quality on growth and biochemical composition of a calanoid copepod, Argyrodiaptomus furcatus, and its importance as a natural food source for larvae of two tropical fishes. *Hydrobiologia*, **453**–**454**, 393–401.

Hessen, D. O. and Leu, E. (2006) Trophic transfer and trophic modification of fatty acids in high Arctic lakes. *Freshw. Biol.*, **51**, 1987–1998.

Heuschele, J. and Selander, E. (2014) The chemical ecology of copepods. *J. Plankton Res.*, **36**, 895–913.

Hillebrand, H. *et al.* (2018) Biodiversity change is uncoupled from species richness trends: Consequences for conservation and monitoring. *J. Appl. Ecol.*, **55**, 169–184.

Hillebrand, H. *et al.* (1999) Biovolume calculation for pelagic and benthic microalgae. *J. Phycol.*, **35**, 403–424.

Ho, S.-H. *et al.* (2014) Optimizing biodiesel production in marine Chlamydomonassp. JSC4 through metabolic profiling and an innovative salinity-gradient strategy. *Biotechnol. Biofuels*, **7**, 97.

Hubbell, S. P. (2006) Neutral theory and the evolution of ecological equivalence. *Ecology*, **87**, 1387–1398.

Hulot, F. D. *et al.* (2000) Functional diversity governs ecosystem response to nutrient enrichment. *Nature*, **405**, 340–344.

Huys, R., and G. A. B. 1991 (1992) Copepod evolution. *J. Crustac. Biol.*, **12**, 731–734.

Ianora, A. *et al.* (1995) A comparative study of the inhibitory effect of diatoms on the reproductive biology of the copepod Temora stylifera. *Mar. Biol.*, **121**, 533–539.

Ilić, M. *et al.* (2019a) Equal relevance of omega-3 and omega-6 polyunsaturated fatty acids for the fitness of Daphnia spp. *Limnol. Oceanogr.*, **64**, 2512–2525.

Ilić, M. *et al.* (2019b) Equal relevance of omega-3 and omega-6 polyunsaturated fatty acids for the fitness of Daphnia spp. *Limnol. Oceanogr.*, **64**, 2512–2525.

Ilić, M. *et al.* (2023) Pigment and fluorescence proxies to estimate functional diversity of phytoplankton communities. *Fundam. Appl. Limnol.*

Irigoien, X. *et al.* (2004) Using HPLC pigment analysis to investigate phytoplankton taxonomy: The importance of knowing your species. *Helgol. Mar. Res.*, **58**, 77–82.

Irwin, A. J. and Finkel, Z. V. (2017) Phytoplankton functional types: A trait perspective. *bioRxiv*.

Iverson, S. J. (2009) Tracing aquatic food webs using fatty acids: from qualitative indicators to quantitative determination. *Lipids in Aquatic Ecosystems*. Springer New York, New York, NY, pp. 281–308.

Jardine, T. D. *et al.* (2020) Unlocking the power of fatty acids as dietary tracers and metabolic signals in fishes and aquatic invertebrates. *Philos. Trans. R. Soc. B Biol. Sci.*, **375**, 20190639.

Jónasdóttir, S. (2019) Fatty Acid Profiles and Production in Marine Phytoplankton. *Mar. Drugs*, **17**, 151.

Jónasdóttir, S. H. *et al.* (2009) Assessing the role of food quality in the production and hatching of Temora longicornis eggs. *Mar. Ecol. Prog. Ser.*, **382**, 139–150.

Jónasdóttir, S. H. (1994) Effects of food quality on the reproductive success of Acartia tonsa and Acartia hudsonica: laboratory observations. *Mar. Biol.*, **121**, 67–81.

Jónasdóttir, S. H. and Kiørboe, T. (1996) Copepod recruitment and food composition: Do diatoms affect hatching success? *Mar. Biol.*, **125**, 743–750.

Jones, R. H. and Flynn, K. J. (2005) Nutritional Status and Diet Composition Affect the Value of Diatoms as Copepod Prey. *Science (80-.).*, **307**, 1457–1459.

Justus, J. *et al.* (2009) Buying into conservation: intrinsic versus instrumental value. *Trends Ecol. Evol.*, **24**, 187–191.

Kabeya, N. *et al.* (2021) A complete enzymatic capacity for biosynthesis of docosahexaenoic acid (DHA, 22 : 6n–3) exists in the marine Harpacticoida copepod Tigriopus californicus. *Open Biol.*, **11**, rsob.200402.

Kabeya, N., Fonseca, M. M., Ferrier, David E.K., *et al.* (2018) Genes for de novo biosynthesis of omega-3 polyunsaturated fatty acids are widespread in animals. *Sci. Adv.*, **4**, 1–9.

Kabeya, N., Fonseca, M. M., Ferrier, David E. K., *et al.* (2018) Genes for de novo biosynthesis of omega-3 polyunsaturated fatty acids are widespread in animals. *Sci. Adv.*, **4**.

Kainz, M. *et al.* (2004) Essential fatty acids in the planktonic food web and their ecological role for higher trophic levels. *Limnol. Oceanogr.*, **49**, 1784–1793.

Kiørboe, T. *et al.* (2018) Adaptive feeding behavior and functional responses in zooplankton. *Limnol. Oceanogr.*, **63**, 308–321.

Kiørboe, T. (2011) How zooplankton feed: Mechanisms, traits and trade-offs. *Biol. Rev.*, **86**, 311–339.

Kiørboe, T. *et al.* (1985) In situ feeding rates of plantonic copepods: A comparison of four methods. *J. Exp. Mar. Bio. Ecol.*, **88**, 67–81.

Kissling, W. D. *et al.* (2018) Towards global data products of Essential Biodiversity Variables on species traits. *Nat. Ecol. Evol.*, **2**, 1531–1540.

Kleppel, G. (1993) On the diets of calanoid copepods. *Mar. Ecol. Prog. Ser.*, **99**, 183–195.

Koski, M. *et al.* (1998) Effect of food quality on rate of growth and development of the pelagic copepod Pseudocalanus elongatus (Copepoda, Calanoida). *Mar. Ecol. Prog. Ser.*, **170**, 169–187.

Koussoroplis, A. M. *et al.* (2014) Famine and feast in a common freshwater calanoid: Effects of diet and temperature on fatty acid dynamics of Eudiaptomus gracilis. *Limnol. Oceanogr.*, **59**, 947–958.

Krause, S. *et al.* (2014) Trait-based approaches for understanding microbial biodiversity and ecosystem functioning. *Front. Microbiol.*, **5**.

Kruk, C. *et al.* (2010) A morphological classification capturing functional variation in phytoplankton. *Freshw. Biol.*, **55**, 614–627.

Kruk, C. (2015) *phytoplankton communities in relation to their environment Morphology Captures Function in Phytoplankton A Large-Scale Analysis of Phytoplankton Communities in Relation to their Environment Carla Kruk.*

Kwak, H. S. *et al.* (2016) Synergistic effect of multiple stress conditions for improving microalgal lipid production. *Algal Res.*, **19**, 215–224.

Lacoste, A. *et al.* (2001) New evidence of the copepod maternal food effects on reproduction. *J. Exp. Mar. Bio. Ecol.*, **259**, 85–107.

Lafond, M. *et al.* (1990) Biomass and photosynthesis of size-fractionated phytoplankton in Canadian Shield lakes. *Hydrobiologia*, **196**, 25–38.

Lampert, W. *et al.* (1986) Phytoplankton control by grazing zooplankton: A study on the spring clear-water phase1. *Limnol. Oceanogr.*, **31**, 478–490.

Lampert, W. and Sommer, U. (2007) *Limnoecology The Ecology of Lakes and Streams*. 2nd ed. Oxford University Press.

Lampert, W. and Trubetskova, I. (1996) Juvenile Growth Rate as a Measure of Fitness in Daphnia. *Funct. Ecol.*, **10**, 631.

Landry, M. R. (1980) Detection of prey by Calanus pacificus : Implications of the first antennae. *Limnol. Oceanogr.*, **25**, 545–549.

Lang, I. *et al.* (2011) Fatty acid profiles and their distribution patterns in microalgae: a comprehensive analysis of more than 2000 strains from the SAG culture collection. *BMC Plant Biol.*, **11**, 124.

Langdon, C. J. and Waldock, M. J. (1981) The effect of algal and artificial diets on the growth and fatty acid composition of crassostrea gigas spat. *J. Mar. Biol. Assoc. United Kingdom*, **61**, 431–448.

Lau, D. C. P. *et al.* (2021) Lowered nutritional quality of plankton caused by global environmental changes. *Glob. Chang. Biol.*, **27**, 6294–6306.

Laureto, L. M. O. *et al.* (2015) Functional diversity: an overview of its history and applicability. *Nat. Conserv.*, **13**, 112–116.

Laurila-Pant, M. *et al.* (2015) How to value biodiversity in environmental management? *Ecol. Indic.*, **55**, 1–11.

Lee, M. C. *et al.* (2020) An improved genome assembly and annotation of the Antarctic copepod Tigriopus kingsejongensis and comparison of fatty acid metabolism between T. kingsejongensis and the temperate copepod T. japonicus. *Comp. Biochem. Physiol. - Part D Genomics Proteomics*, **35**, 100703.

Legendre, L. (1990) The significance of microalgal blooms for fisheries and for the export of particulate organic carbon in oceans. *J. Plankton Res.*, **12**, 681–699.

Légier-Visser, M. F. *et al.* (1986) Mechanoreception in calanoid copepods. *Mar. Biol.*, **90**, 529–535.

Lehman, J. T. (1988) Ecological principles affecting community structure and secondary production by zooplankton in marine and freshwater environments1. *Limnol. Oceanogr.*, **33**, 931–945.

Lepistö, L. and Rosenström, U. (1998) The most typical phytoplankton taxa in four types of boreal lakes. *Hydrobiologia*, **369**, 89–97.

Lewis, W. M. (1976) Surface/Volume Ratio: Implications for Phytoplankton Morphology. *Science (80-.).*, **192**, 885–887.

Litchman, E. *et al.* (2007) The role of functional traits and trade-offs in structuring phytoplankton communities: scaling from cellular to ecosystem level. *Ecol. Lett.*, **10**, 1170–1181.

Litchman, E. *et al.* (2013) Trait-based approaches to zooplankton communities. *J. Plankton Res.*, **35**, 473–484.

Litchman, E. and Klausmeier, C. A. (2008) Trait-Based Community Ecology of Phytoplankton. *Annu. Rev. Ecol. Evol. Syst.*, **39**, 615–639.

Liu, X. *et al.* (2016) Responses of Phytoplankton Communities to Environmental Variability in the East China Sea. *Ecosystems*, **19**, 832–849.

Lotze, H. K. *et al.* (2019) Global ensemble projections reveal trophic amplification of ocean biomass declines with climate change. *Proc. Natl. Acad. Sci.*, **116**, 12907–12912.

Lürling, M. (2021) Grazing resistance in phytoplankton. *Hydrobiologia*, **848**, 237–249.

Mace, G. M. *et al.* (2018) Aiming higher to bend the curve of biodiversity loss. *Nat. Sustain.*, **1**, 448–451.

Mackey, M. D. *et al.* (1996) CHEMTAX - A program for estimating class abundances from chemical markers: Application to HPLC measurements of phytoplankton. *Mar. Ecol. Prog. Ser.*, **144**, 265–283.

Magurran, A. E. and McGill, B. J. (2010) *Biological diversity: frontiers in measurement and assessment*. OUP Oxford.

Marañón, E. *et al.* (2012) Temperature, resources, and phytoplankton size structure in the ocean. *Limnol. Oceanogr.*, **57**, 1266–1278.

Marcarelli, A. M. *et al.* (2008) Is in-stream N2 fixation an important N source for benthic communities and stream ecosystems? *J. North Am. Benthol. Soc.*, **27**, 186–211.

Margalef, R. (1978) Life-forms of phytoplankton as survival alternatives in an unstable environment. *Ocean. Acta*, **1**, 493–509.

Marrone, F. *et al.* (2017) Diversity patterns and biogeography of Diaptomidae (Copepoda, Calanoida) in the Western Palearctic. *Hydrobiologia*, **800**, 45–60.

Martin-Creuzburg, D., von Elert, E., *et al.* (2008) Nutritional constraints at the cyanobacteria-Daphnia magna interface: The role of sterols. *Limnol. Oceanogr.*, **53**, 456–468.

Martin-Creuzburg, D., Von Elert, E., *et al.* (2008) Nutritional constraints at the cyanobacteria-Daphnia magna interface: The role of sterols. *Limnol. Oceanogr.*, **53**, 456–468.

Martini, S. *et al.* (2021) Functional trait-based approaches as a common framework for aquatic ecologists. *Limnol. Oceanogr.*, **66**, 965–994.

Mauchline, J. (1998) *The biology of calanoid copepods*. Academic Press, San Diego ;

McCann, K. S. (2000) The diversity–stability debate. *Nature*, **405**, 228–233.

McCarthy, J. J. *et al.* (1974) Significance of nanoplankton in the Chesapeake Bay estuary and problems associated with the measurement of nanoplankton productivity. *Mar. Biol. 1974 241*, **24**, 7–16.

McGill, B. *et al.* (2006) Rebuilding community ecology from functional traits. *Trends Ecol. Evol.*, **21**, 178–185.

De Meester, L. *et al.* (2002) The Monopolization Hypothesis and the dispersal–gene flow paradox in aquatic organisms. *Acta Oecologica*, **23**, 121–135.

Milione, M. and Zeng, C. (2007) The effects of algal diets on population growth and egg hatching success of the tropical calanoid copepod, Acartia sinjiensis. *Aquaculture*, **273**, 656–664.

Miralto, A. *et al.* (1999) The insidious effect of diatoms on copepod reproduction. *Nature*, **402**, 173–176.

Monroig, Ó. *et al.* (2013) Biosynthesis of polyunsaturated fatty acids in marine invertebrates: Recent advances in molecular mechanisms. *Mar. Drugs*, **11**, 3998–4018.

Monroig, Ó. and Kabeya, N. (2018) Desaturases and elongases involved in polyunsaturated fatty acid biosynthesis in aquatic invertebrates: a comprehensive review. *Fish. Sci.*, **84**, 911–928.

Morabito, G. *et al.* (2007) Seasonal morphological plasticity of phytoplankton in Lago Maggiore (N. Italy). *Hydrobiologia*, **578**, 47–57.

Müller-Navarra, D. C. *et al.* (2000) A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. *Nature*, **403**, 74–77.

Müller-Navarra, D. C. (2008) Food Web Paradigms: The Biochemical View on Trophic Interactions. *Int. Rev. Hydrobiol.*, **93**, 489–505.

Müller-Navarra, D. C. *et al.* (2004) Unsaturated fatty acid content in seston and tropho-dynamic coupling in lakes. *Nature*, **427**, 69–72.

Mullin, M. M. (1965) Size fractionation of particulate organic carbon in the surface of the Western Indian Ocean. *Limnol. Oceanogr.*, **10**, 459–462.

Naeem, S. and Wright, J. P. (2003) Disentangling biodiversity effects on ecosystem functioning: deriving solutions to a seemingly insurmountable problem. *Ecol. Lett.*, **6**, 567–579.

Naselli-Flores, L. *et al.* (2007) Shape and size in phytoplankton ecology: do they matter? *Hydrobiologia*, **578**, 157–161.

Naselli-Flores, L. and Barone, R. (2011) Invited review Fight on plankton! Or, phytoplankton shape and size as adaptive tools to get ahead in the struggle for life. *Cryptogam. Algol.*, **32**, 157–204.

Navarro, L. M. *et al.* (2017) Monitoring biodiversity change through effective global coordination. *Curr. Opin. Environ. Sustain.*, **29**, 158–169.

Nielsen, B. L. H. *et al.* (2019) n-3 PUFA biosynthesis by the copepod Apocyclops royi

determined by fatty acid profile and gene expression analysis. *Biol. Open*, **8**.

Noss, R. F. (1990) Indicators for Monitoring Biodiversity: A Hierarchical Approach. *Conserv. Biol.*, **4**, 355–364.

Nunes, P. A. L. . and van den Bergh, J. C. J. . (2001) Economic valuation of biodiversity: sense or nonsense? *Ecol. Econ.*, **39**, 203–222.

Oksanen, A. J. *et al.* (2014) Vegan: Community Ecology Package.

Pace, M. L. (1986) An empirical analysis of zooplankton community size structure across lake trophic gradients1. *Limnol. Oceanogr.*, **31**, 45–55.

Paffenhöfer, G.-A. and -A., G. (1998) On the relation of structure, perception and activity in marine planktonic copepods. *JMS*, **15**, 457–473.

Paffenhöfer, G. A. *et al.* (1982) Suspension-feeding by herbivorous calanoid copepods: A cinematographic study. *Mar. Biol.*, **67**, 193–199.

Pančić, M. and Kiørboe, T. (2018) Phytoplankton defence mechanisms: traits and trade-offs. *Biol. Rev.*, **93**, 1269–1303.

Parrish, C. C. (2009) Essential fatty acids in aquatic food webs. *Lipids Aquat. Ecosyst.*, **9780387893662**, 309–326.

Payne, M. F. and Rippingale, R. J. (2000) Evaluation of diets for culture of the calanoid copepod Gladioferens imparipes. *Aquaculture*, **187**, 85–96.

Peltomaa, E. T. *et al.* (2017) The Importance of Phytoplankton Biomolecule Availability for Secondary Production. *Front. Ecol. Evol.*, **5**, 1–12.

Persson, J. and Vrede, T. (2006) Polyunsaturated fatty acids in zooplankton: Variation due to taxonomy and trophic position. *Freshw. Biol.*, **51**, 887–900.

Petchey, O. L. *et al.* (2004) How do different measures of functional diversity perform? *Ecology*, **85**, 847–857.

Petchey, O. L. and Gaston, K. J. (2006) Functional diversity: back to basics and looking forward. *Ecol. Lett.*, **9**, 741–758.

Pond, D. *et al.* (1996) Environmental and nutritional factors determining seasonal variability in the fecundity and egg viability of Calanus helgolandicus in coastal waters off Plymouth, UK. *Mar. Ecol. Prog. Ser.*, **143**, 45–63.

Price, H. J. *et al.* (1983) Modes of cell capture in calanoid copepods. *Limnol. Oceanogr.*, **28**, 116–123.

Puello-Cruz, A. C. *et al.* (2009) Culture of the calanoid copepod Pseudodiaptomus euryhalinus (Johnson 1939) with different microalgal diets. *Aquaculture*, **290**, 317–319.

Ravet, J. L. *et al.* (2010) The effects of seston lipids on zooplankton fatty acid composition in Lake Washington, Washington, USA. *Ecology*, **91**, 180–190.

Reuss, N. and Poulsen, K. (2002) Evaluation of fatty acids as biomarkers for a natural plankton

community . A field study of a spring bloom and a post-bloom period off West Greenland. 423–434.

Reynolds, C.S. (1984) Phytoplankton periodicity : the interactions of form , function and environmental variability. *Freshw. Biol.*, **14**, 111–142.

Reynolds, Colin S. (1984) The ecology of freshwater phytoplankton. 384.

Reynolds, C. S. and Walsby, A. E. (1975) Water-blooms. *Biol. Rev.*, **50**, 437–481.

Roelke, D. L. and Spatharis, S. (2015) Phytoplankton Succession in Recurrently Fluctuating Environments. *PLoS One*, **10**, e0121392.

Rokkan Iversen, K. *et al.* (2010) Effects of small-scale turbulence on lower trophic levels under different nutrient conditions. *J. Plankton Res.*, **32**, 197–208.

Rott, E. (1981) Some results from phytoplankton counting intercalibrations. *Schweizerische Zeitschrift für Hydrol.*, **43**, 34–62.

Von Rückert, G. and Giani, A. (2008) Biological interactions in the plankton community of a tropical eutrophic reservoir: Is the phytoplankton controlled by zooplankton? *J. Plankton Res.*, **30**, 1157–1168.

Ruess, L. and Müller-Navarra, D. C. (2019) Essential Biomolecules in Food Webs. *Front. Ecol. Evol.*, **7**, 1–18.

Runge, J. A. and Ohman, M. D. (1982) Size fractionation of phytoplankton as an estimate of food available to herbivores1. *Limnol. Oceanogr.*, **27**, 570–576.

Ryabov, A. *et al.* (2021) Shape matters: the relationship between cell geometry and diversity in phytoplankton. *Ecol. Lett.*, **24**, 847–861.

Salles, J.-M. (2011) Valuing biodiversity and ecosystem services: Why put economic values on Nature? *C. R. Biol.*, **334**, 469–482.

Salmaso, N. *et al.* (2015) Functional classifications and their application in phytoplankton ecology. *Freshw. Biol.*, **60**, 603–619.

Sandgren, C. D. (1988) The ecology of chrysophyte flagellates: their growth and perennation strategies as freshwater phytoplankton. *Growth Reprod. Strateg. Freshw. Phytoplankt.*, 9–194.

Santer, B. (1994) Influences of food type and concentration on the development of Eudiaptomus gracilis and implications for interactions between calanoid and cyclopoid copepods. *Arch. für Hydrobiol. Hydrobiol.*, **131**, 141–159.

Sargent, J. R. *et al.* (1999) Lipid nutrition of marine fish during early development: Current status and future directions. *Aquaculture*, **179**, 217–229.

Sargent, J. R. and Falk-Petersen, S. (1988) The lipid biochemistry of calanoid copepods. *Hydrobiologia*, **167**–**168**, 101–114.

Sarnelle, O. *et al.* (2010) Effects of cyanobacteria on fitness components of the herbivore Daphnia. *J. Plankton Res.*, **32**, 471–477.

Schlüter, L. *et al.* (2000) The use of phytoplankton pigments for identifying and quantifying phytoplankton groups in coastal areas: Testing the influence of light and nutrients on pigment/chlorophyll a ratios. *Mar. Ecol. Prog. Ser.*, **192**, 49–63.

Selmants, P. C. *et al.* (2012) Realistic plant species losses reduce invasion resistance in a California serpentine grassland. *J. Ecol.*, **100**, 723–731.

Shannon, C. E. and Weaver, W. W. (1963) *The mathematical theory of communications.* University of Illinois Press, Urbana.

Shields, R. J. *et al.* (1999) Natural copepods are superior to enriched Artemia nauplii as feed for halibut larvae (Hippoglossus hippoglossus) in terms of survival, pigmentation and retinal morphology: Relation to dietary essential fatty acids. *J. Nutr.*, **129**, 1186–1194.

Sieburth, J. M. N. *et al.* (1978) Pelagic ecosystem structure: Heterotrophic compartments of the plankton and their relationship to plankton size fractions 1. *Limnol. Oceanogr.*, **23**, 1256–1263.

Sin, Y. *et al.* (2000) Seasonal variations of size-fractionated phytoplankton along the salinity gradient in the York River estuary, Virginia (USA). *J. Plankton Res.*, **22**, 1945–1960.

Singh, P. *et al.* (2015) Investigation of combined effect of nitrogen, phosphorus and iron on lipid productivity of microalgae Ankistrodesmus falcatus KJ671624 using response surface methodology. *Biochem. Eng. J.*, **94**, 22–29.

Smetacek, V. (2001) A watery arms race. *Nat. 2001 4116839*, **411**, 745–745.

Smyntek, P. M. *et al.* (2008) Taxonomic differences in the essential fatty acid composition of groups of freshwater zooplankton relate to reproductive demands and generation time. *Freshw. Biol.*, **53**, 1768–1782.

Sommer, U. *et al.* (2017) Benefits, costs and taxonomic distribution of marine phytoplankton body size. *J. Plankton Res.*, **39**, 494–508.

Sommer, U. *et al.* (2001) Complementary impact of copepods and cladocerans on phytoplankton. *Ecol. Lett.*, **4**, 545–550.

Sommer, U. and Sommer, F. (2006) Cladocerans versus copepods: the cause of contrasting top–down controls on freshwater and marine phytoplankton. *Oecologia*, **147**, 183–194.

Soto, D. and Hurlbert, S. H. (1991) Short term experiments on calanoid-cyclopoid-phytoplankton interactions. *Hydrobiologia*, **215**, 83–110.

Sterner, R. W. and Schulz, K. L. (1998) Zooplankton nutrition: Recent progress and a reality check. *Aquat. Ecol.*, **32**, 261–279.

Støttrup, J. G. and Jensen, J. (1990) Influence of algal diet on feeding and egg-production of the calanoid copepod Acartia tonsa Dana. *J. Exp. Mar. Bio. Ecol.*, **141**, 87–105.

Strandberg, U. *et al.* (2015) Inferring phytoplankton community composition with a fatty acid mixing model. *Ecosphere*, **6**.

Strayer, D. L. and Dudgeon, D. (2010) Freshwater biodiversity conservation: recent progress and

future challenges. *J. North Am. Benthol. Soc.*, **29**, 344–358.

Taipale, S. J. *et al.* (2011) Diet-switching experiments show rapid accumulation and preferential retention of highly unsaturated fatty acids in Daphnia. *Oikos*, **120**, 1674–1682.

Taipale, S. J. *et al.* (2013) Fatty acid composition as biomarkers of freshwater microalgae: Analysis of 37 strains of microalgae in 22 genera and in seven classes. *Aquat. Microb. Ecol.*, **71**, 165–178.

Tang, K. W. and Dam, H. G. (2001) Phytoplankton inhibition of copepod egg hatching: Test of an exudate hypothesis. *Mar. Ecol. Prog. Ser.*, **209**, 197–202.

Thomas, M. K. *et al.* (2016) Environment and evolutionary history determine the global biogeography of phytoplankton temperature traits. *Glob. Ecol. Biogeogr.*, **25**, 75–86.

Tickner, D. *et al.* (2020) Bending the Curve of Global Freshwater Biodiversity Loss: An Emergency Recovery Plan. *Bioscience*, **70**, 330–342.

Tilman, D. *et al.* (2014) Biodiversity and ecosystem functioning. *Annu. Rev. Ecol. Evol. Syst.*, **45**, 471–493.

Tilman, D. *et al.* (1997) The Influence of Functional Diversity and Composition on Ecosystem Processes. *Science (80-.).*, **277**, 1300–1302.

Titocci, J. and Fink, P. (2022) Food quality impacts on reproductive traits, development and fatty acid composition of the freshwater calanoid copepod Eudiaptomus sp. *J. Plankton Res.*, **44**, 528–541.

Tremblay, J. É. *et al.* (1997) Estimation of f-ratios in oceans based on phytoplankton size structure. *Limnol. Oceanogr.*, **42**, 595–601.

De Troch, M. *et al.* (2012) Bioconversion of fatty acids at the basis of marine food webs: Insights from a compound-specific stable isotope analysis. *Mar. Ecol. Prog. Ser.*, **465**, 53–67.

Tsai, C.-H. *et al.* (2014) Phytoplankton functional group dynamics explain species abundance distribution in a directionally changing environment. *Ecology*, **95**, 3335–3343.

Twining, C. W. *et al.* (2021) The evolutionary ecology of fatty-acid variation: Implications for consumer adaptation and diversification. *Ecol. Lett.*, **24**, 1709–1731.

Twombly, S. *et al.* (1998) Life History Consequences of Food Quality in the Freshwater Copepod Boeckella triarticulata. *Ecology*, **79**, 1711.

UN, I. R. . (1992) Convention on biological diversity. *Treaty Collect.*

Utermöhl, H. (1958) Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. *SIL Commun. 1953-1996*, **9**, 1–38.

Uye, S. and Fleminger, A. (1976) Effects of various environmental factors on egg development of several species of Acartia in Southern California. *Mar. Biol.*, **38**, 253–262.

Vallina, S. M. *et al.* (2017) Phytoplankton functional diversity increases ecosystem productivity and stability. *Ecol. Modell.*, **361**, 184–196.

Vaughn, C. C. (2010) Biodiversity losses and ecosystem function in freshwaters: emerging conclusions and research directions. *Bioscience*, **60**, 25–35.

Ventelä, A. M. *et al.* (2002) The effect of small zooplankton on the microbial loop and edible algae during a cyanobacterial bloom. *Freshw. Biol.*, **47**, 1807–1819.

Violle, C. *et al.* (2007) Let the concept of trait be functional! *Oikos*, **116**, 882–892.

Volkman, J. K. *et al.* (1989) Fatty acid and lipid composition of 10 species of microalgae used in mariculture. *J. Exp. Mar. Bio. Ecol.*, **128**, 219–240.

Van de Waal, D. B. and Litchman, E. (2020) Multiple global change stressor effects on phytoplankton nutrient acquisition in a future ocean. *Philos. Trans. R. Soc. B*, **375**, 20190706.

Waite, A. M. *et al.* (1992) Does energy control the sinking rates of marine diatoms? *Limnol. Oceanogr.*, **37**, 468–477.

Walpole, M. *et al.* (2009) Ecology. Tracking progress toward the 2010 biodiversity target and beyond. *Science*, **325**, 1503–1504.

Weiss, K. C. B. and Ray, C. A. (2019) Unifying functional trait approaches to understand the assemblage of ecological communities: synthesizing taxonomic divides. *Ecography (Cop.).*, **42**, 2012–2020.

Weithoff, G. (2003) The concepts of 'plant functional types' and 'functional diversity' in lake phytoplankton - a new understanding of phytoplankton ecology? *Freshw. Biol.*, **48**, 1669–1675.

Werbrouck, E. *et al.* (2017) Fatty acid recovery after starvation: insights into the fatty acid conversion capabilities of a benthic copepod (Copepoda, Harpacticoida). *Mar. Biol.*, **164**, 1–15.

Wickham, H. (2009) *ggplot2 Elegant Graphics for Data Analysis*. second edi. Springer-Verlag.

Wilson, E. O. (1988) Biodiversity.

Windisch, H. S. and Fink, P. (2018) The molecular basis of essential fatty acid limitation in Daphnia magna: A transcriptomic approach. *Mol. Ecol.*, **27**, 871–885.

Ye, L. *et al.* (2019) Functional diversity promotes phytoplankton resource use efficiency. *J. Ecol.*, **107**, 2353–2363.

Zafar, A. R. (1986) Seasonality of phytoplankton in some South Indian lakes. *Hydrobiologia*, **138**, 177–187.

Zánkai, N. P. (1991) Feeding of nauplius stages of Eudiaptomus gracilis on mixed plastic beads. *J. Plankton Res.*, **13**, 437–453.

Zavaleta, E. S. and Hulvey, K. B. (2004) Realistic Species Losses Disproportionately Reduce Grassland Resistance to Biological Invaders. *Science (80-.).*, **306**, 1175–1177.

Zehnder, A. and Gorham, P. R. (1960) Factors influencing the growth of Microcystis aeruginosa. *Can. J. Microbiol.*, **6**, 645–660.

Zeng, H. *et al.* (2006) Distribution of phytoplankton in the Three-Gorge Reservoir during rainy and dry seasons. *Sci. Total Environ.*, **367**, 999–1009.

Record of achievement and publications

CHAPTER 1: Food quality impacts on reproductive traits, development and fatty acid composition of the freshwater calanoid copepod *Eudiaptomus* sp.

Titocci, J., & Fink, P. (2022). Journal of Plankton Research, 44(4), 528-541.

The experiment described in this chapter was exclusively performed by me or under my direct supervision. Patrick Fink was involved in the design of the experiment, and has critically read and commented on the manuscript.

CHAPTER 2: Morpho-functional traits reveal differences in size fractionated phytoplankton communities but do not significantly affect zooplankton grazing.

Titocci, J., Bon, M., & Fink, P. (2022). Microorganisms, 10(1), 182.

The experiment described in this chapter was conceived and performed by me or under my direct supervision. Patrick Fink was involved in the design of the experiment. Melanie Bonn was involved in the data analysis. The first draft of the manuscript was written by me and Patrick Fink and Melanie Bonn have critically discussed the results, commented and edited the manuscript.

CHAPTER 3: Disturbance alters phytoplankton functional traits and consequently drives changes in zooplankton life history traits and lipid composition

Titocci J., & Fink, P. Hydrobiologia Journal, under review.

All authors contributed to the study conception and design of the experiment described in this chapter. Material preparation, data collection and analysis were exclusively performed by me or under my direct supervision. The first draft of the manuscript was written by me and Patrick Fink have critically read and commented on the manuscript.

Erklärung zur Dissertation
gemäß der Promotionsordnung vom 12. März 2020

“Hiermit versichere ich an Eides statt, dass ich die vorliegende Dissertation selbstständig und ohne die Benutzung anderer als der angegebenen Hilfsmittel und Literatur angefertigt habe. Alle Stellen, die wörtlich oder sinngemäß aus veröffentlichten und nicht veröffentlichten Werken dem Wortlaut oder dem Sinn nach entnommen wurden, sind als solche kenntlich gemacht. Ich versichere an Eides statt, dass diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegen hat; dass sie - abgesehen von unten angegebenen Teilpublikationen und eingebundenen Artikeln und Manuskripten - noch nicht veröffentlicht worden ist sowie, dass ich eine Veröffentlichung der Dissertation vor Abschluss der Promotion nicht ohne Genehmigung des Promotionsausschusses vornehmen werde. Die Bestimmungen dieser Ordnung sind mir bekannt. Darüber hinaus erkläre ich hiermit, dass ich die Ordnung zur Sicherung guter wissenschaftlicher Praxis und zum Umgang mit wissenschaftlichem Fehlverhalten der Universität zu Köln gelesen und sie bei der Durchführung der Dissertation zugrundeliegenden Arbeiten und der schriftlich verfassten Dissertation beachtet habe und verpflichte mich hiermit, die dort genannten Vorgaben bei allen wissenschaftlichen Tätigkeiten zu beachten und umzusetzen. Ich versichere, dass die eingereichte elektronische Fassung der eingereichten Druckfassung vollständig entspricht."

Teilpublikationen:

[1] Titocci, J., & Fink, P. (2022). *Food quality impacts on reproductive traits, development and fatty acid composition of the freshwater calanoid copepod Eudiaptomus sp*. Journal of Plankton Research, 44(4), 528-541. https://doi.org/10.1093/plankt/fbac030

[2] Titocci, J., Bon, M., & Fink, P. (2022). *Morpho-functional traits reveal differences in size fractionated phytoplankton communities but do not significantly affect zooplankton grazing.* Microorganisms, 10(1), 182. https://doi.org/10.3390/microorganisms10010182

[3] Titocci J., & Fink, P. *Disturbance alters phytoplankton functional traits and consequently drives changes in zooplankton life history traits and lipid composition.* Hydrobiologia Journal, *under review.*

[1] Chapter 1
[2] Chapter 2
[3] Chapter 3

Jessica Titocci

Lecce, 2nd of May 2023

Declaration for the doctoral thesis
According to the doctoral regulations published on 12th March 2020

Non-official English translation of the “Erklärung zur Dissertation”

"I hereby declare that I have completed the present dissertation independently and without the use of any aids or literature other than those referred to. All passages that have been taken, either literally or in sense, from published and unpublished works, are marked as such. I declare that this dissertation has not been submitted to any other faculty or university; that - apart from the partial publications and included articles and manuscripts listed below - it has not yet been published, and that I will not publish the dissertation before completing my doctorate without the permission of the PhD Committee. I am aware of the terms of the doctoral regulations. In addition, I hereby declare that I am aware of the “Regulations for Safeguarding Good Scientific Practice and Dealing with Scientific Misconduct” of the University of Cologne, and that I have observed them during the work on the thesis project and the written doctoral thesis. I hereby commit myself to observe and implement the guidelines mentioned there in all scientific activities. I assure that the submitted electronic version is identical to the submitted printed version".

Partial publications of the thesis:

[1] Titocci, J., & Fink, P. (2022). *Food quality impacts on reproductive traits, development and fatty acid composition of the freshwater calanoid copepod Eudiaptomus sp*. Journal of Plankton Research, 44(4), 528-541. https://doi.org/10.1093/plankt/fbac030

[2] Titocci, J., Bon, M., & Fink, P. (2022). *Morpho-functional traits reveal differences in size fractionated phytoplankton communities but do not significantly affect zooplankton grazing.* Microorganisms, 10(1), 182. https://doi.org/10.3390/microorganisms10010182

[3] Titocci J., & Fink, P. *Disturbance alters phytoplankton functional traits and consequently drives changes in zooplankton life history traits and lipid composition.* Hydrobiologia Journal, *under review.*

[1] Chapter 1
[2] Chapter 2
[3] Chapter 3

Jessica Titocci

Lecce, 2nd of May 2023

Acknowledgements

First and foremost, I would like to thank my supervisor PD Dr. Patrick Fink for giving me the opportunity to do my PhD under his supervision. I feel that I have learnt so much thanks to your help, support and understanding over the past few years. You have shared your expertise, sincere guidance and many precious points in our discussions and I hope I have managed to address some of them during my research and experimental studies.

I would also like to thank the members of my thesis committee, Prof. Dr. Eric von Elert, Prof. Dr. Patrick Grunert and Dr. Anja Scherwaß, for kindly accepting to be the second examiner of my thesis, the chair of the committee and the minute taker during my examination, at very short notice and despite the approaching summer holidays. Thank you for generously sharing your knowledge, expertise and feedback. In particular, I would also like to thank Prof. Dr. Eric von Elert for his kind acceptance into his Aquatic Chemical Ecology group and for his support throughout my time as a doctoral student.

I would also like to thank Prof. Dr. Herwig Stibor, Dr. Maria Stockenreiter, Luna Benitez Requena, Angelika and Achim. Although some time has passed, I cherish beautiful memories of the great time I spent at the Seeon Limnological Station and I will always be grateful for all the experience I gained during the field mesocosm experiments and the many things I learned from all of you. I have not forgotten all the efforts and hard work we have done and I hope to publish the manuscript soon to share the interesting results we have achieved together.

From the Cologne side, I would also like to thank Katja, Thomas and Sascha for always being so kind and open to all my questions and for helping me with all my "technical and not only" problems that I had in my daily laboratory routine. My thanks also go to Carlos and Kathrine, although we did not spend much time together, you also supported me personally and professionally during my time at the University of Cologne. Thank you so much also to all my students Laura, Alexandra, Alissa, Dominik and Katalin for your hard work, help and perseverance during the field and lab experiments and the endless samples we analysed.

Getting through my PhD took more than academic support. Words cannot express my gratitude to all my doctoral colleagues Chris, Meike, Jacky, Sandra, Bubu, Stephi, Alessandra and Maja for listening to me and sometimes having to tolerate me with all my doubts, my fears and anxieties, my loneliness and homesickness over the past few years. I must express my deepest gratitude for your friendship and for your constant encouragement, help, feedback sessions, professional and moral support. You have all influenced and inspired me.

For all the memorable days I spent away from the office, I have to thank all my colleagues, but especially le mie belle Stephi and Rike. Thank you for always being there, for making my life in Germany easier! The help you gave me was invaluable and you have been a second family to me! I have shared so many wonderful moments with all of you, which I miss so much now that we are far apart, that I almost want to go back to Germany…or…better yet, I hope to see each other soon in the warm and sunny Italy! ☺

I am also extremely grateful to Luca, my partner, who has tolerated and supported me through the last phase of this venture, and I am also indebted to all my new colleagues, especially Andrea, Cristina and Martina who, directly or indirectly, have lent a hand in these recent times.

Most importantly, none of this would have been possible without "my rocks", my family and friends. Their belief in me has kept my spirits up and my motivation high throughout this process. Thank you for your unwavering support and constant encouragement throughout my years of study and the process of researching and writing this thesis. This achievement would not have been possible without you.

To be honest, it has been a real adventure, full of highs and lows, and there have been countless times when I felt I would not make it, especially after my return to Italy, starting (again) a new career and a new life in Lecce. That is why, at the very end, I would like to dedicate this work to myself, in the hope that I have learnt to believe in myself a little more and with the aim to be always determined to go through life and achieve all the next goals that come my way.

Curriculum Vitae
Jessica Titocci

Nationality: Italian **Place and date of Birth:** Rome, 02/12/1991

Address: Via Egidio Milinanni 7, 73100 Lecce (Italy)

Mobile number: (+39) 3493564826

E-mail address: titoccij@gmail.com

EDUCATION

PhD Freshwater Ecology
University of Cologne, Helmholtz-Zentrum für Umweltforschung–UFZ, Magdeburg, Germany [01/05/2018 —]
DynaTrait project: "*Trait- related feedback dynamics in plankton communities*"

Master degree in Marine Bio-ecology (LM-6)
University of Cagliari, Cagliari [01/11/2013 – 22/07/2015]
score of 110/110 cum laude
Master thesis: *Analysis of macrozoobenthic community and applicability of ecological quality index in transitional waters. (the case of St. Gilla lagoon).*

Bachelor degree in Natural Sciences (L-32)
University of Sassari, Sassari [01/09/2010 – 22/10/2013]
score of 110/110 cum laude
Bachelor thesis: *Role of intraspecific genetic diversity in Skeletonema marinoi*

High School Degree
Scientific High school Aristotele, Rome [01/09/2005 – 30/07/2010]
Score of 100/100

PROFESSIONAL EXPERIENCE

Research fellow

University of Salento, Lecce [01/09/2022 – today]
LifeWatch Plus program: Harmonization, integration, and analysis of data and metadata on phytoplankton morpho-functional traits following FAIR and Open Science principles.

Research fellow
CNR IRET- Research Institute on Terrestrial Ecosystems, Lecce [01/09/2021 – 31/08/2022]
LifeWatch Plus program: Harmonization, integration, and analysis of data and metadata on phytoplankton morpho-functional traits following FAIR and Open Science principles.

Research assistant
Open University, Anton Dohrn Zoological Station, Naples [01/10/2016 – 30/06/2017]
Study of diatom-bacteria interaction in the context of oxylipin production

Internship, Erasmus Plus fellow
University of Murcia, Spain [01/03/2015 – 30/06/2015]
Evaluation of the ecological quality status (EcoQ) of transitional water using macrozoobenthos as bioindicator.

Internship, Erasmus Traineeship fellow
Finnish Environmental Institute (SYKE); Tvärminne Zoological Station, Helsinki, Finland [01/06/2013 – 30/09/2013]
Analysis of phytoplankton and study of the role of intraspecific genetic diversity in *Skeletonema marinoi*.

CONFERENCES

SFE² GfÖ EEF Joint meeting, International Conference on Ecological Sciences, Ecology and Evolution: New perspectives and societal challenges.
Metz, France
[21/11/2022 – 25/11/2022]
"Trait-based approaches: e-science tools and new perspectives" (oral presentation)

OPEN TRAITS NETWORK Virtual Meeting
[31/08/2022 – 01/09/2022]
"Introducing a collaborative initiative for harmonized trait-related semantic resources" (oral presentation)

International Conference on Copepoda (e-ICOC)
[25/07/2022 – 30/07/2022]
"Food quality impacts on reproductive traits, development and fatty acid composition of the freshwater calanoid copepod *Eudiaptomus sp.*" (oral presentation)

DynaTrait Annual Meeting
Potsdam, Germany [14/09/2020 – 17/09/2020]
"Loss of functional traits in freshwater ecosystems: what are the impacts on phytoplankton-zooplankton dynamics?" (oral presentation);
"Zooplankton grazing affects a phytoplankton functional trait (fatty acids composition) in pelagic mesocosms" (poster)

DynaTrait Annual Meeting
Potsdam, Germany [16/09/2019 – 20/09/2019]
"Influence of cell size and elemental stoichiometry on phytoplankton-zooplankton dynamics" (poster)

ASLO Aquatic Sciences Meeting
Puerto Rico, Caraibi, USA [24/02/2019 – 01/03/2019]
"Loss of functional traits in phytoplankton-zooplankton dynamics from a lake mesocosm experiment" (oral presentation)

DynaTrait Annual Meeting
Potsdam, Germany [08/10/2018 – 11/10/2018]
"Trait- related feedback dynamics in natural plankton communities" (poster)

PUBLICATIONS

1. **Titocci, J**., Fink, P. (2022) Food quality impacts on reproductive traits, development and fatty acid composition of the freshwater calanoid copepod Eudiaptomus sp., Journal of Plankton Research, Volume 44, Issue 4, July/August 2022, Pages 528–541. https://doi.org/10.1093/plankt/fbac033

2. **Titocci J.,** Bon M., Fink P. Morpho-Functional Traits Reveal Differences in Size Fractionated Phytoplankton Communities but Do Not Significantly Affect Zooplankton Grazing. Microorganisms. 2022 Jan 14;10 (1):182. DOI: 10.3390/microorganisms10010182

3. Bastianini M., F. Riminucci, M. Pansera, A. Coluccelli, R. Casotti, E. Dal Passo, L. Dametto, M. Van Dijk, E. Russo, **J. Titocci**, J. Pazzaglia, S. Virgili (2017). Rapporto sulle attività biologiche, oceanografiche, geologiche e di manutenzione della stazione Boa E1 svolte durante la campagna INTERNOS17 (14-21 marzo 2017) con N/O Minerva Uno nel Mare Adriatico centro-settentrionale. Rapporto Tecnico CNR-ISMAR N° 146, 2017, pp. 1-37.