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Modifying the functional diversity in the zooplankton assemblage of an oligotrophic lake differentially affects pelagic community structure and biomass

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ABSTRACT

Experimental ecology represents a fundamental tool to predict how a system reacts to the loss of functional groups in response to contingent disturbance.
Zooplankton, the heart of pelagic food webs, is characterized by short life cycles; therefore it can serve as a model system to test ecological theories.
In this study we performed a removal experiment to test the hypothesis that the absence of calanoid or cyclopoid copepods could have a different effect on plankton dynamics due to their distinct functional roles in freshwater ecosystems.
We selectively removed either Calanoida or Cyclopoida from the original zooplankton assemblage of the oligotrophic lake Brunnsee (Bavaria, Germany), then inspecting temporal changes in plankton community structure and biomass. Experimental communities were incubated in plastic enclosures, fed with cultured phytoplankton and analysed at four time steps over four weeks.
Our manipulations led to significant variations in environmental parameters (i.e. chlorophyll-a and microalgae concentration), in zooplankton community composition (dominance, diversity and equitability) and in normalized biomass spectra of zooplankton. The main driver of such changes was the small cladoceran *Ceriodaphnia reticulata*, the most abundant species at the final sampling in all the treatments. Calanoida facilitated the development of *C. reticulata*; Cyclopoida played a keystone role, indirectly regulating the abundance of this cladoceran through predation on Calanoida.
Our results offer a wider picture of the pelagic food web in oligotrophic freshwater environments and strongly support the view that ecological studies should rely on the measurement of both specific and functional diversity.

1. Introduction

Studies on food webs are progressing from the initial effort to map species interactions and to construct food webs as part of ecological communities, towards the use of functional biodiversity in studies on environmental change (de Ruiter et al., 2005). In ecology, functional groups are clusters in which one or more species influence an ecological component in a similar way, codifying for specific ecosystem services (Blondel, 2003). Functional diversity likely represents the main driver of ecosystem processes (Cadotte et al., 2011; Tilman et al., 1997); as a consequence, ecological studies should try to blend the "community ecology" approach with the "ecosystem ecology" one, with the aim to fill the gap between specific and functional diversity, respectively (Behl et al., 2011; Thompson et al., 2012; Walker, 1992). Food webs take into account several aspects in the study of ecological dynamics: species richness, community composition, flux of energy and material (Thomson et al., 2012). Food webs are natural systems to study species' ecological roles in ecosystem function. With current rates of biodiversity loss and anthropogenic perturbation of the environment, model systems allowing studying effects of species loss on ecosystem function are needed.

Zooplankton likely represents the heart of pelagic food webs: it affects the ecological dynamics occurring at the microbial scale and in the upper trophic levels (Burns and Schallenberg, 1998; Kiørboe, 1997; Zöllner et al., 2009), playing a fundamental role for many ecosystem processes (Sailley et al., 2015; Stibor et al., 2004). Studies of zooplankton dynamics represent a key issue for pelagic food webs and a functional diversity approach could be the key for a more comprehensive understanding of ecosystem dynamics (Pomerleau et al., 2015). Current understanding is that climate change has the potential to modify the functional diversity of zooplankton communities (van de Waal et al., 2010; Sommaruga, 2015). How will pelagic ecosystems respond to the loss of zooplankton species or functional groups? Removal experiments have been successfully used in terrestrial settings (e.g. Díaz et al., 2003) and are possibly a

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powerful tool to approach this complex question. Here, we propose the application of a removal experiment on zooplankton communities to address the role of the functional diversity in aquatic food webs.

Major contributors to zooplankton communities are 2814 copepod morphospecies, that dominate most of planktonic, benthic and groundwater assemblies (Boxshall and Defaye, 2008). Such ecological success is probably mediated by a high morphological plasticity that makes copepods able to adapt to different habitats and niches (Bron et al., 2011; Galassi et al., 2009; Kiørboe, 2011a). In general, zooplankton perceives food particles dispersed in a viscous environment through fluid disturbance (Kiørboe and Visser, 1999) or chemical stimuli (Heuschele and Selander, 2014) and their feeding strategies can be resumed in four main categories: (i) passive ambush feeding, (ii) active ambush feeding, (iii) feeding-current feeding and (iv) cruise feeding (Kiørboe, 2011b).

In freshwater ecosystems, calanoid and cyclopoid copepods can be clustered in two different functional groups, in relation to both feeding strategy and targeted preys (Barnett et al., 2007): cyclopoid copepods are known to be carnivorous ambush feeders (they predate on juvenile calanoids and cladocerans; Adrian, 1997; Gliwicz, 1994); calanoid copepods are selective grazers for phytoplankton (size between 15 and 60 μm, in freshwater systems; Sommer et al., 2003, 2001). In limnic ecosystems, copepods coexist with cladocerans that graze unselectively on small phytoplankton (size between 3 and 15 μm; Sommer et al., 2003, 2001). Compared to other herbivorous zooplankton, cladocerans have benefits from their rapid reproduction rate and optimal utilisation of resources at high food supplies. A typical life cycle of *Daphnia* at 20 °C lasts 7–8 days from egg to egg deposition. The life cycle of copepods is divided into six nauplius and six copepodit stages and lasts about 13–15 days (Allan, 1976; Hessen et al., 1986).

In this mesocosm experiment we selectively removed two distinct functional groups, i.e. Calanoida or Cyclopoida, from the original zooplankton community of an oligotrophic freshwater system in order to inspect consequent changes in the community structure and biomass. To our knowledge this is one of the first attempts of a selective modification in the occurrence of calanoid and cyclopoid copepods. Presumably, the absence of Calanoida would favour the growth of bigger microalgae, then hindering the development of smaller phytoplankton fractions. The lacking of Cyclopoida, by contrast, is expected to favour the abundance of both Calanoida and Cladocera (both suitable preys), that could then lead to a fast decrease of large and small microalgae.

Selective removal of Calanoida and Cyclopoida are also expected to affect significantly the biomass size spectra of our experimental communities. Normalized biomass size spectra (NBSS) represent a useful tool to characterize the plankton community structure and the carbon flows through the pelagic food web (Platt and Denman, 1997; San Martin et al., 2006; Tarling et al., 2012). Taxonomic groups, and hence the intrinsic morphotypes and functional groups, contribute to different sections of the NBSS (e.g. Marcolin et al., 2015). Variation in the biomass spectrum can demonstrate whether the removal of different functional groups has an effect on size dependent processes "up the food chain" (for example the foraging field for juvenile fishes) and has the potential to show how sensitive the system is to perturbation (Petchey and Belgrano, 2010).

At present, aquatic systems are challenged by human impacts (e.g. climate change, chemical pollution, anthropogenic disturbances). This experiment presents a wider picture of the food web in freshwater oligotrophic systems involving micro- (20–200 μm) and meso-plankton (0.2–2 mm) and could offer a measure of the consequences deriving from contingent alterations in the occurrence of zooplankton functional groups in these habitats due to environmental change.

2. Materials and methods

2.1. Experimental set-up

Zooplankton was collected from the groundwater fed oligotrophic lake Brunnsee (Bavaria, Germany) in the evening through repeated 10 m hauls with a 60 μm plankton net, diluted with deep well groundwater in 30 l plastic containers and analysed in laboratory the day after. A full description of the features of the lake is available in Sebastian et al. (2012).

In the laboratory, zooplankton was concentrated with an 80 μm mesh and then anesthetized (1:1000 ml dilution of MS-222; Streble and Krauter, 1981); Copepoda were selectively removed under a stereomicroscope with plastic pipettes so that two treatments plus the control were realized: "No Calanoida" (i.e. the original community without Calanoida) and "No Cyclopoida" (i.e. the original community without Cyclopoida). While the predatory insect larvae *Chaoborus* were removed, nauplii and cladocerans remained unchanged; rotifers were not observed. For each of the two treatments and the control, seven replicates were prepared and maintained in beakers (total of 21) with well water until the start of the experiment. One replicate beaker of each treatment was conserved for the t0 sampling (i.e. immediately after the manipulations of the original community) in order to verify the actual modifications of the original zooplankton assembly. While at t0 only one replicate was available, for the other time steps of each treatment two replicates were designed. The employment of two replicates has already been successfully applied during small enclosure experiments inspecting plankton communities (Sommer et al., 2003).

The remaining beakers with 300 zooplankton specimens for each treatment were then released into 18 plastic enclosures (Tricoron foil, diameter of 21 cm and a length of 115 cm, containing approximately 40 l of groundwater from a deep well) which had been deployed the day before in an outdoor experimental pool. A weight was connected to the bottom of each enclosure and a white fabric mesh (1 mm mesh size) was applied at the top; this mesh hindered the arrival of aerial predators and allowed for air exchanges without modifying the light intensity. Despite these precautions, two replicate enclosures (one at t1 and the other at t2) of treatment "No Calanoida" resulted highly affected by Libella larvae that clearly dominated the community; therefore these enclosures were discarded from further statistical analyses. The other plankton communities were not affected by insect predators.

After the acclimatization period of at least 24 h, zooplankton was fed with phytoplankton adding to each enclosure 3 $\mu g\ l^{-1}$ of chlorophyll-a (chl-a). The food contained a mixture of an equal number of three green algae (i.e. *Chlamydomonas reinhardtii*, *Monoraphidium minutum* and *Scenendesmus* sp.), two diatoms (i.e. *Fragilaria crotonensis* and *Navicula pelliculosa*) and the dinoflagellate *Peridinium* sp., previously cultivated in the laboratory in WC medium (Schlösser, 1994), with 90 μmol photons $m^{-2}\ s^{-2}$. These cultured phytoplankton groups were chosen in order to have a controlled environment at the beginning of the experiment. In addition these algae are commonly observed in the original system, where they represent an edible food resource for zooplankton.

2.2. Sampling and analysis of the plankton community

The experiment lasted for four weeks, during which the samplings were conducted at three time steps: after one week (t1), two weeks (t2) and four weeks (t3). Four weeks represent a reasonable duration for mesocosm experiments in order to detect contingent treatment effects without artificial bias in the enclosures (Haupt et al., 2012). While the samples after one and two weeks (t1 and t2, respectively) could offer a more progressive perspective of the immediate changes, we decided to take the subsequent sample at the fourth week (t3) in order to have the possibility to observe zooplankton population growth and a consequent shift in our communities. Two replicate enclosures from each treatment were sacrificed at each of the three time steps.

Twice a week, a water sample was taken for chlorophyll-a from all the enclosures that had not yet been sacrificed and then analysed with GAT System. Temperature and dissolved oxygen were measured with WTW Oxi 330 Oxymeter at the top and at the bottom of four fixed enclosures selected randomly at the beginning. Samples for ammonium analysis were stored at 4 °C in the dark. NH_4 concentration was determined by