Dispersal-mediated trophic interactions can generate apparent patterns of dispersal limitation in aquatic metacommunities

Dino Verreydt[1], Luc De Meester[2], Ellen Decaestecker[3], María-José Villena[4], Katleen Van Der Gucht[5], Pieter Vannormelingen[6], Wim Vyverman[7], Steven A.J. Declerck[8]

[1] Dino.Verreydt@bio.kuleuven.be; Laboratory of Aquatic Ecology and Evolutionary Biology, K. U. Leuven, Charles Deberiotstraat 32, B-3000 Leuven, Belgium.

[2] Luc.DeMeester@bio.kuleuven.be; Laboratory of Aquatic Ecology and Evolutionary Biology, K. U. Leuven, Charles Deberiotstraat 32, B-3000 Leuven, Belgium.

[3] Ellen.Decaestecker@kuleuven-kortrijk.be; Aquatic Biology, K. U. Leuven- Campus Kortrijk, E. Sabbelaan 53, B-8500 Kortrijk, Belgium.

[4] Mariajose.villena@ltlevante.com; Protistology & Aquatic Ecology, Ghent University, Krijgslaan 281-S8, B-9000 Ghent, Belgium. current address: Laboratorios Tecnológicos de Levante SL. C/ Leonardo da Vinci 4, Parque Tecnológico, 46980 Paterna, Valencia, Spain.

[5] Katleen.VanDerGucht@UGent.be; Protistology & Aquatic Ecology, Ghent University, Krijgslaan 281-S8, B-9000 Ghent, Belgium.

[6] Pieter.Vanormelingen@UGent.be; Protistology & Aquatic Ecology, Ghent University, Krijgslaan 281-S8, B-9000 Ghent, Belgium.

[7] Wim.Vyverman@UGent.be; Protistology & Aquatic Ecology, Ghent University, Krijgslaan 281-S8, B-9000 Ghent, Belgium.

[8] S.Declerck@nioo.knaw.nl; Netherlands Institute of Ecology (NIOO-KNAW), P.O. Box 50, 6700 AB Wageningen, The Netherlands.

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Corresponding author:

Dino Verreydt

Laboratory for Aquatic Ecology and Evolutionary Biology

Ch. Deberiotstraat 32

B-3000 Leuven

Belgium

tel: +32 16 373551

fax: +32 16 324575

e-mail: Dino.Verreydt@bio.kuleuven.be

Statement of authorship

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Abstract

Dispersal is a major organizing force in metacommunities, which may facilitate compositional responses of local communities to environmental change and affect ecosystem function. Organism groups differ widely in their dispersal abilities and their communities are therefore expected to have different adaptive abilities. In mesocosms, we studied the simultaneous compositional response of three plankton communities (zoo-, phyto- and bacterioplankton) to a primary productivity gradient and evaluated how this response was mediated by dispersal intensity. Dispersal enhanced responses in all three planktonic groups, which also affected ecosystem functioning. Yet, variation partitioning analyses indicated that responses in phytoplankton and bacterial communities were not only controlled by dispersal directly, but also indirectly through complex trophic interactions. Our results indicate that metacommunity patterns emerging from dispersal can cascade through the food web and generate patterns of apparent dispersal limitation in organisms at other trophic levels.

Introduction

Dispersal is a major structuring force in metacommunities, also affecting the composition, diversity and functioning of local communities (Loreau *et al.* 2003; Matthiessen & Hillebrand 2006; Duffy 2009; Hillebrand & Matthiessen 2009; Howeth & Leibold 2010). The qualitative and quantitative effects of dispersal are, however, difficult to predict and depend on many factors, such as the intensity of dispersal, the functional traits of the organisms, and the occurrence of complex ecological interactions. Dispersal can lead to increased local species richness because it allows new species to enter communities and compensate for local extinctions (Loreau & Mouquet 1999). Dispersal can also facilitate a rapid and efficient compositional response of local communities to changing environmental conditions through the process of species sorting. At the metacommunity level, this process can lead to enhanced differentiation among communities and an increase in beta diversity (Cottenie & De Meester 2004; Leibold & Norberg 2004). Conversely, dispersal can also increase the probability of invasions by superior competitors, predators or parasites potentially causing extinctions of local populations or entire metapopulations. In the case of mass effects, high dispersal rates may result in the presence of species in unsuitable habitat patches, and lead to a homogenization of metacommunities (Mouquet & Loreau 2003).

Lack of dispersal, on the other hand, may impede species to reach potentially suitable habitat patches (Loreau *et al.* 2003; Ozinga *et al.* 2005; McCauley 2006; Pärtel & Zobel 2007). At the local scale, this reduces the capacity of resident communities to track environmental change, which may have a profound impact on the performance of entire functional groups or trophic levels and as such affect ecosystem functioning. Although empirical evidence remains scarce, there are a limited number of recent experimental studies that have demonstrated such effects. Naeslund & Norberg (2006) found stronger responses of zooplankton communities to a change in basal productivity if the communities at the start of the experiment represented the entire regional

88 species pool instead of just local pools. They also found that the communities resulting from the
89 regional species pool treatment exerted stronger top-down control on phytoplankton than the
90 communities resulting from local species pools. Howeth & Leibold (2008, 2010) showed that
91 dispersal can affect ecosystem stability and dampen trophic cascade effects in plankton
92 communities that are subject to temporal fluctuations in the density of a top predator.

93 Metacommunity structure varies widely among organism groups (Beisner *et al.* 2006). One
94 potential reason for this is that dispersal capabilities of organisms vary greatly (Bohonak &
95 Jenkins 2003; Jenkins *et al.* 2007). Microbial organisms, for example, are supposed to have very
96 high dispersal rates (Finlay & Clarke 1999; Finlay 2002), in contrast to passively dispersing
97 macroscopic organisms of which dispersal rates tend to decrease with increasing body size
98 (Jenkins *et al.* 2007; Shurin *et al.* 2009). An additional level of complexity may be generated by
99 indirect mechanisms, such as dispersal-mediated trophic interactions. Indeed, the metacommunity
100 structure of a group of organisms that is strongly affected by trophic interactions can also reflect
101 patterns resulting from spatial dynamics at other trophic levels. In such case, an association
102 between connectivity patterns and the metacommunity structure of an organism group does not
103 unequivocally reflect dispersal limitation or mass effects (Staddon *et al.* 2010).

104 With this study, we wanted to explore the direct and indirect effects of dispersal on the
105 composition and functioning of communities representing different trophic levels within a
106 metacommunity context. We used freshwater plankton in mesocosms as a model system and
107 performed an experiment combining varying degrees of dispersal with an important environmental
108 gradient (i.e. a gradient in primary productivity) according to an orthogonal design. The main
109 objectives of our study were to (1) study the extent to which dispersal can mediate the response of
110 plankton organisms to a change in basal productivity, (2) compare these responses among three
111 functional groups of plankton organisms that differ widely in body size, life strategy and expected

dispersal rates (i.e. zoo-, phyto- and bacterioplankton), (3) evaluate the consequences of dispersal on food web structure and a crucial ecosystem function (i.e. zooplankton grazing on phytoplankton), and (4) evaluate whether the response of the functional groups to the dispersal gradient is caused directly by dispersal itself or rather indirectly by changes in trophic interactions that are caused by dispersal-mediated community shifts at other trophic levels. In the absence of positive size-selective predators, grazing by large-bodied cladoceran zooplankton can strongly affect community composition of phytoplankton and bacterioplankton (Jürgens 1994; Lampert 2006). We therefore expect that dispersal-enhanced differentiation of zooplankton communities along a productivity gradient may generate patterns of community differentiation at lower trophic levels that appear to be caused by dispersal limitation but in fact are generated by indirect dispersal-mediated trophic interactions.

Material and methods

Experimental design

Using mesocosms (n = 96), we studied the simultaneous interactive effects of nutrient addition and dispersal on communities of planktonic bacteria, phytoplankton and zooplankton. For this, we first collected plankton from 16 lakes (LAKE ID) representing a broad gradient in trophic state and limnological characteristics. The plankton of each of these lakes was used to inoculate six mesocosms per lake (6 x 16 = 96 mesocosms) at the start of the experiment (see Appendix S1 in Supporting Information). In each of these sets of six mesocosms, we created two levels of nutrient addition and three levels of dispersal intensity, so that the entire experimental set-up accorded to a cross factorial randomized block design (with LAKE ID as blocks). With the dispersal treatment, we tried to achieve a broad range of dispersal intensities among our experimental containers, ranging from no dispersal to strong dispersal. In each block, the no dispersal (NDISP) and low dispersal (LDISP) mesocosms were inoculated with plankton originating from one single lake. In contrast, the high dispersal (HDISP) mesocosms were initially inoculated with a plankton mixture from all 16 lakes (see below for details). During the entire experiment, we tried to prevent any input of organisms from other mesocosms into the NDISP-mesocosms. The communities in these mesocosms thus consisted solely of species collected from one individual lake, although some airborne exchange of phytoplankton and bacteria could probably not be entirely excluded. For the other two dispersal levels, we achieved dispersal by manually exchanging water among mesocosms. For this, we collected water from all mesocosms of the respective dispersal level (n=32) and redistributed the pooled volume in equal parts over the same mesocosms again. In this way, a level of low dispersal (LDISP) was achieved through the exchange of 40 mL, whereas the highest dispersal (HDISP) was generated by exchanging 2L volumes. We initially applied the dispersal treatment on a weekly basis but switched to a biweekly treatment from day 59 on until

148 the end of the experiment. The NDISP-treatment was meant to represent a metacommunity
149 without dispersal among habitat patches. The LDISP-treatment was designed to represent a
150 situation where locally abundant species can disperse in low numbers among habitat patches, with
151 sporadic exchange of locally rare species. The HDISP-treatment represents metacommunities with
152 relatively high exchange rates among local communities, where each species has historically had
153 the occasion to enter each habitat patch, but where current dispersal rates are not high enough to
154 cause mass-effects (Michels *et al.* 2001; Howeth & Leibold 2008).
155 At day 1 of the experiment (23 May 2006), we filled plastic containers (volume: 200L) with a
156 mixture of 120 L distilled water and 60 L tap water. The nutrient treatments were established on
157 days 4 and 5 through addition of phosphate (KH_2PO_4) and nitrogen ($NaNO_3$). Initial nutrient
158 additions were equivalent to 1000µg P L^{-1} and 16000 µg N L^{-1} in the high nutrient (HNUT)
159 containers and 10µg P L^{-1} and 160 µg N L^{-1} in the low nutrient (LNUT) containers. Because
160 earlier mesocosm experiments have indicated that nutrient gradients can decline with time , we
161 continued with a weekly addition of a tenth of these concentrations throughout the experiment.
162 We added phyto- and bacterioplankton to the containers on the 5^{th} day of the experiment. For
163 this, we collected a 30 L volume of lake water and filtered it twice (mesh sizes: 100 µm and 50
164 µm, respectively) to remove zooplankton. NDISP and LDISP mesocosms were all inoculated with
165 an inoculum originating from one individual lake, whereas the inoculum of HDISP mesocosms
166 consisted of a mixture of all 16 lakes (each experimental container received an equal amount of
167 chlorophyll a and for the HDISP mesocosms there was an equal representation of lakes in terms of
168 chlorophyll a). From the moment we observed a consistent difference in phytoplankton biomass
169 between LNUT and HNUT mesocosms (day 32), we inoculated the zooplankton. Total
170 zooplankton biomass was the same in all inocula. Similar as with the phytoplankton inoculation,
171 we inoculated NDISP and LDISP mesocosms with inocula from individual lakes, whereas HDISP

containers received an inoculum for 80 % consisting of the respective lake and for 20 % consisting of a mixture of all 16 lakes. Throughout the experiment, mesocosms were covered by mosquito netting to prevent contamination by macro-invertebrates. The experiment was ended at day 87.

Sampling and sample analysis

We measured chlorophyll a on a weekly basis with a fluorometer (Trilogy Laboratory Fluorometer, Turner Designs). Near the end of the experiment, we sampled the zoo-, phyto-, and bacterioplankton communities. At day 77, we sampled phytoplankton with a 250 mL jar approximately 10 cm below the water surface. The phytoplankton samples were preserved with a mixture of Lugol's solution, formaldehyde and sodium thiosulfate (Sherr & Sherr 1993) and counted using an inverted microscope to the genus level. *Desmodesmus* was a dominant phytoplankton taxon in some treatments of the experiment. *Desmodesmus* colony size has been shown to be a defense against zooplankton grazing and may therefore serve as an indicator for the prevailing zooplankton grazing regime (Vanormelingen *et al.* 2009). We therefore characterized the size distribution of *Desmodesmus* by counting the number of cells per colony in each sample. At day 79, we sampled the bacterioplankton. Samples were filtered over a 0.22 µm filter and stored at -80°C for later analysis with denaturing gradient gel electrophoresis (DGGE). DGGE analysis followed Van der Gucht et al. (2007); details are given in Appendix S2 in Supporting Information. In short, DNA was extracted directly using the bead-beating method concomitant with phenol extraction and ethanol precipitation and purified on a Wizard column. A small rDNA fragment was amplified with primers specific to the domain Bacteria (357F-GC-clamp and 518R). PCR products were analyzed on a 35 to 70% denaturant DGGE gel, and DGGE gels were stained with Sybr Gold solution. The 96 samples were analyzed on 12 parallel DGGE-gels, which were aligned with Bionumerics 5.10 (Applied Maths BVBA, Kortrijk, Belgium) using three standard

196 lanes (known mixtures of DNA from 9 clones from a clone library) on each gel. A matrix was
197 compiled based upon the relative contribution of individual bands to the total band signal in each
198 lane, with bands corresponding to OTU's (Operational Taxonomic Units). Zooplankton samples
199 were taken at day 86 and 87 of the experiment. Two samples were taken in each container with a
200 Schindler Patalas (volume: 12 L; mesh size: 30 µm) and preserved with acid lugol solution. One
201 sample was used to measure zooplankton dry weight. These samples were weighed after drying at
202 100°C during 24h. The other sample was used for the assessment of species composition and
203 population densities. A minimum of 300 individuals were counted. Taxa were identified to species
204 level for cladocerans using Flössner (2000); for copepods we made a distinction between
205 cyclopoids and calanoids.
206
207 *Grazing experiment*
208 Zooplankton grazing is widely acknowledged as a key characteristic of the aquatic food web that
209 determines patterns of energy and material flows and underwater light climate, and may mediate
210 regime shifts in ponds and shallow lakes (Scheffer 1998). To evaluate its importance as potential
211 driving force underlying phyto- and bacterioplankton community responses to the experimental
212 treatments, we performed assays to assess zooplankton grazing pressure on phytoplankton at day
213 81. In each mesocosm, we incubated 2 bottles (250 mL) with mesocosm water, one with ambient
214 zooplankton densities and one without zooplankton (water filtered over 64 µm mesh). The bottles
215 were incubated at the bottom of the mesocosms and gently shaken twice a day in order to keep the
216 phytoplankton in suspension. At day 1 and day 11 of the experiment, we measured the chlorophyll
217 a concentrations with a fluorometer. Per bottle, we calculated the change in chlorophyll a using the
218 formula: $(\ln[\text{chl a}]_{t11} - \ln[\text{chl a}]_{t0})/\text{time}$ and used the difference between the treatments as a measure
219 of zooplankton grazing pressure.

Data analysis

We applied mixed model ANOVA to evaluate the impact of our experimental treatments on chlorophyll a concentration, *Desmodesmus* colony size, total zooplankton biomass (dry weight) and *in situ* zooplankton grazing pressure. In these analyses, we specified LAKE ID as a random factor. For chlorophyll a, we analyzed the time weighted averages so as to give more weight to data that are collected later during the experiment. These averages were calculated for each mesocosm by multiplying each chlorophyll *a* value with the time that had passed since the start of the experiment (expressed in numbers of days). The sum of these values was then divided by the total duration of the experiment. For *Desmodesmus* colony size, we analyzed the weighted average of cell number. Significant effects where explored with Tukey HSD post hoc tests.

We tested the effects of the experimental treatments on the composition of zooplankton, phytoplankton and bacterioplankton communities with redundancy analysis (RDA). In these analyses, we followed a two-step approach. First, we evaluated the overall effects of the experimental treatments and their potential interactions on each of the communities separately (Lepš & Šmilauer 2003). Second, we performed variation partitioning analyses (Peres-Neto *et al.* 2006) on more elaborate RDA-models to explore the extent to which indirect trophic interactions can explain apparent dispersal effects in phytoplankton and bacterioplankton. We constructed RDA models for these groups in each of the nutrient addition levels separately with the dispersal treatment and the biomass of the principal zooplankton grazer, *Daphnia magna*, as explanatory variables. We also included summary variables of phytoplankton community composition as explanatory variables in the RDA model of bacterioplankton because phytoplankton composition can be a determining factor for bacteria and may itself be directly affected by dispersal or indirectly by dispersal mediated zooplankton grazing. With variation partitioning, we assessed the

unique contribution of the dispersal treatment (conditional effect) as well as its degree of collinearity with *Daphnia magna* density (in the phyto- and bacterioplankton models) and phytoplankton community composition (in the bacterioplankton models). The summary variables for phytoplankton community composition were extracted prior to the RDA analyses through principal components analysis of the phytoplankton data (i.e. the four sample scores vectors with the highest eigen values; prior analyses indicated that these four vectors all had a unique and significant contribution to variation in the bacterioplankton community). All community data were Hellinger transformed prior to analysis (Legendre & Gallagher 2001). Associations of *Daphnia magna* densities with *in situ* measured grazing pressure and the weighted average of *Desmodesmus* colony size were tested with Spearman rank correlation. All statistical analyses were performed in R (v2.10.1, R Development Core Team 2008), using the rda and varpart functions of the vegan library (Peres-Neto *et al.* 2006; Oksanen *et al.* 2010). Adjusted R^2 values were calculated on residuals after partialling out the effect of LAKE ID. The significance of model components was tested through 9999 random permutations.

Results

Zooplankton

There was a significant nutrient x dispersal treatment interaction effect on zooplankton biomass (dry weight) (Table 1). Zooplankton biomass was higher in the containers with high than low nutrient addition, but only at high levels of dispersal (Figure 1A). Similarly, the RDA analyses indicated significant main effects and an interaction effect of the nutrient addition and dispersal treatments on zooplankton community composition (Table 2, Figure 2A). Separate analyses for each of the dispersal levels showed that the response strength of the zooplankton community to the nutrient addition treatment increased with increasing dispersal, explaining 9, 20 and 59 % of the zooplankton community variation in the no, low and high dispersal treatment, respectively (Table 3). Most species responded negatively to high nutrient addition (Figure 2A). The nutrient by dispersal interaction could almost entirely be attributed to the specific response of the large cladoceran *Daphnia magna.* The absolute and relative abundance of this species was especially high in high-nutrient cattle tanks subjected to high dispersal (see Appendix S3). Zooplankton species richness was negatively affected by nutrient addition and positively by dispersal (see Appendix S4).

Phytoplankton

High nutrient addition resulted in a strong increase of chlorophyll a concentrations throughout the course of the experiment (Figure 1B; Table 1). According to RDA analysis, the nutrient addition and dispersal treatments affected phytoplankton community composition and there was also an interaction between both factors (Figure 2B; Table 2). Overall, nutrient addition resulted in a strong increase in the contribution of *Desmodesmus* species, while containers with low nutrient addition tended to be mainly characterized by a variety of other phytoplankton taxa (Figure 2B).

Analyses for each of the dispersal treatments separately showed an increase of the impact of nutrient addition with increasing dispersal intensity (13, 11 and 22 % of the total community variation explained by nutrient addition in the no, low and high dispersal treatment, respectively) (Table 3). When dispersal was tested separately for each of the nutrient addition levels, there was only a significant effect in containers with high nutrient addition (see Table S1). In containers with high nutrient addition, colony size of the *Desmodesmus* morphs decreased with dispersal (Figure 1C): large colonial morphs were most abundant in the absence of dispersal, whereas unicellular morphs were mainly associated with high levels of dispersal. This was also confirmed by an ANOVA on the weighted average of colony cell number ($F(2,30) = 64.28$, $p < 0.001$). Phytoplankton richness was positively affected by dispersal but only at low nutrient addition levels (see Appendix S4).

Bacterioplankton

Nutrient addition and dispersal treatments affected the DGGE profiles of the bacterioplankton communities (Table 2; Figure 2C). The compositional response to the nutrient addition increased with increasing dispersal (nutrient x dispersal interaction): the contribution of the nutrient addition to the community variation increased from 9, over 19 to 22 % in the no, low and high dispersal treatment, respectively (Table 3). Dispersal was equally important under low and high nutrient addition (see Table S1). The number of OTUs was significantly higher under high than low nutrient addition levels, but only in the high dispersal treatment (see Appendix S4).

Indirect dispersal-mediated intertrophic interactions

Zooplankton grazing rates on the phytoplankton community, as measured by the in situ grazing experiments, increased with dispersal intensity but only at high nutrient levels (Figure 1D, Table

306 1). Overall, grazing pressure was positively correlated with *D. magna* density (Spearman rank
307 correlation: n = 96, r_s = 0.477, p < 0.001). This correlation was especially strong in the mesocosms
308 with high nutrient levels (n = 48, r_s = 0.704, p < 0.001), but insignificant in containers with low
309 levels of nutrients (n = 48, r_s = 0.0688, p = 0.642).

310 Phytoplankton community composition was significantly associated with the population
311 density of *D. magna* and dispersal intensity but only under high nutrient conditions (Figure 3;
312 Table S2). Under these conditions, the dispersal treatment and *D. magna* jointly explained
313 approximately 35% of the total phytoplankton community variation. A large fraction of the
314 variation explained by *D. magna* proved highly collinear with dispersal (15%), whereas the
315 conditional effect of *D. magna* was relatively small (4%). At high nutrient concentrations,
316 weighted average *Desmodesmus* colony size was negatively correlated to *D. magna* density (n =
317 48, r_s = -0.546, p <0.001; Figure S1). We detected very similar patterns when using the estimated
318 zooplankton grazing rate and zooplankton dry biomass as explanatory variables in these analyses
319 (results not shown).

320 The dispersal gradient, *D. magna* density and phytoplankton community composition jointly
321 explained 17% of the compositional variation in the bacterioplankton communities under high
322 nutrient conditions (Figure 3; Table S2). The marginal effects of each of the three categories of
323 variables were highly significant (explaining 12, 7 and 14% of bacterial community variation,
324 respectively). Variation partitioning showed that relatively large fractions of community variation
325 were explained by joint effects, mainly of all three variable categories together (5%) or effects
326 shared by the dispersal treatment and phytoplankton community composition (6%). In contrast, the
327 conditional effects of the explanatory variables were relatively low and statistically insignificant.
328 The effect of *D. magna* was almost entirely collinear with dispersal and phytoplankton community

329 composition. Under low nutrient conditions, dispersal contributed most to bacterioplankton
330 community variation (11%).
331

Discussion

We observed highly significant interaction effects between the dispersal and nutrient addition treatments for the zoo-, phyto- and bacterioplankton communities. In each of these functional groups, the interaction effects reflected increased strength in community compositional shifts to nutrient addition with increasing rates of dispersal. These results show that the degree of connectivity among habitat patches within a metacommunity can profoundly affect the composition of the constituent communities by facilitating species sorting. In addition, the results of the variation partitioning analyses indicate that responses observed for specific communities (*in casu* phytoplankton and bacterioplankton) are not uniquely caused by the physical exchange of members of those communities but also indirectly by changed trophic interactions that result from the impact of dispersal at other trophic levels (*in casu* zooplankton).

For zooplankton, we observed enhanced compositional responses to the productivity gradient already at low exchange rates. A shift from no to low dispersal (i.e. from no to the weekly exchange of 0.02% of container volumes) more than doubled the community response strength to the nutrient addition treatment (see Table 3). This response was still considerably enhanced upon further increase in connectivity, as the nutrient addition treatment contributed to a total of 59% of the variation in zooplankton community composition at maximal dispersal rates. These dispersal-mediated responses also affected ecosystem functioning: zooplankton grazing rates measured *in situ* were higher at high than low nutrient conditions, but only at the highest levels of dispersal. This effect mainly seemed to result from the response of one single zooplankton key stone species, i.e. *Daphnia magna*. Although most zooplankton species responded negatively to nutrient addition, *D. magna* performed very well under high nutrient conditions. *D. magna* was detected in only a limited number of experimental communities in the absence of dispersal. With increasing dispersal rates, the species expanded within the metacommunity and became dominant in

containers with high nutrient levels. The species was so influential that the response of its populations to the experimental treatments constituted most of the response patterns of total zooplankton community biomass. Consequently, zooplankton grazing rates also correlated strongly with *D. magna* population density. In conclusion, our results demonstrate the potential importance of dispersal-related spatial dynamics for ecosystem functioning, in agreement with predictions of theoretical metacommunity models (Loreau *et al.* 2003; Gonzalez & Loreau 2009).

We observed effects of the dispersal treatment on the compositional response of the phyto- and bacterioplankton communities to nutrient enrichment. Although such effects can be considered evidence for dispersal limitation, our results suggest that they may also have resulted from indirect effects, such as dispersal-mediated changes in trophic interactions. Indeed, as discussed above, increased dispersal rates resulted in a strong increase of zooplankton grazing pressure at high nutrient levels. Variables related to this gradient (i.e. *D. magna* population density, zooplankton biomass, grazing rates) explained a substantial part of the variation in phytoplankton community composition at high nutrient levels and most (79%) of this explained variation was collinear with the dispersal treatment. The quality of the shifts in phytoplankton characteristics also strongly suggests grazing intensity as a strong community structuring factor. Along with a gradient of increasing dispersal intensity and increasing population densities of *D. magna*, average *Desmodesmus* colony cell number decreased and large colonial and spined *Desmodesmus* morphs were replaced by spineless, unicellular or smaller colonial morphs. Given that spine and colony formation are well-known defenses against zooplankton grazing, this response appears contra-intuitive. However, it can be well understood in the light of a trade-off between grazing resistance and grazing tolerance (Agrawal 1998; Chase *et al.* 2000). While colony formation may indeed decrease the vulnerability of phytoplankton cells to grazing by small and intermediate sized zooplankton, such defense is largely ineffective against grazing by zooplankton with a very wide

food particle size range such as *D. magna* (Matveev *et al.* 2000; Mayeli *et al.* 2004; Sarnelle 2005). The phytoplankton community composition shift observed by us in response to the dispersal and grazing gradients thus represents a shift towards smaller colonies and single cells that may be more vulnerable to predation but that are better able to compensate for mortality losses through faster population growth (Agrawal 1998). Our results thus suggest that the dispersal treatment affected the phytoplankton community in an indirect way through an intensification of top-down control by zooplankton. *D. magna* thrived under high nutrient addition, and release from dispersal limitation allowed this species to successfully spread through the metacommunity, developing dense populations in containers with high nutrients, which then resulted in major quantitative and qualitative changes in the phytoplankton communities compared to mesocosms where no dispersal was applied. It should be noted, however, that our statistical analysis suggests that such indirect dispersal induced trophic interactions can only account for about half of the phytoplankton community variation that was caused by the dispersal treatment, which indicates that phytoplankton communities were also directly affected by the dispersal treatment.

Similar to our observations for the phytoplankton communities, effects of the dispersal treatment on bacterial communities were not only due to increased exchange rates of bacteria among mesocosms, but may also have been shaped by several indirect mechanisms. According to our variation partitioning results, a large part (65 %) of the total explained bacterioplankton variation was explained by variation among phytoplankton communities that was also collinear with the dispersal treatment. Phytoplankton community composition can affect bacterioplankton composition through competition for nutrients (Cherif & Loreau 2007; Daufresne *et al.* 2008) or via the composition of DOC that it excretes (Giroldo *et al.* 2007). Dispersal may thus have affected the composition of bacteria indirectly by its direct and indirect effects on the composition of phytoplankton communities. An important subfraction of the variation in bacterial community

composition was also collinear with *D. magna*. Zooplankton can affect bacterioplankton directly through selective grazing (Zollner *et al.* 2003; Hambright *et al.* 2007) or indirectly by structuring bacterivore communities (e.g. ciliates, flagellates; Jürgens 1994; Jürgens & Stolpe 1995; Lampert 2006). The strong collinearity in a large fraction of the variation in zooplankton composition, phytoplankton composition and dispersal suggests that the dispersal treatment affected an important part of the bacterioplankton variation via a cascade of effects: increased dispersal rates affected zooplankton community composition and grazing rates, which induced changes in the phytoplankton community composition, which then affected bacterioplankton community composition.

While our analysis suggests that there is a direct effect of dispersal limitation on phytoplankton species composition in our experiment, we have no unequivocal evidence for dispersal limitation of bacteria under high nutrient conditions, because the effects of the dispersal treatment were strongly reduced and became insignificant when *D. magna* and phytoplankton community composition were accounted for. There were, however, indications for dispersal limitation of bacteria at low nutrient levels. Dispersal limitation is generally assumed to be of minor importance in determining the composition of micro-organisms, because taxa are supposed to be omnipresent, especially at small spatial scales (Finlay & Clarke 1999; Finlay 2002). Our data, nevertheless, also suggest that dispersal limitation in micro-organisms can matter at the spatial and temporal scales of our experiment. Indeed, we found indications for enhanced species sorting with increasing dispersal rates by physical exchange of medium and organisms for phytoplankton and bacteria after correction for indirect effects caused by dispersal limitation of zooplankton. This suggests that not all taxa were present in the original inocula or at least not in sufficient densities to have an impact during the course of our experiment. The fact that our results indicate more direct dispersal limitation effects in the low than in the high nutrient treatment may reflect that existent

communities can intrinsically more easily adapt to high than to low nutrient conditions, or alternatively may reflect that nutrient rich habitats are more abundant in the study region so that these tend to be more omnipresent than species that are pre-adapted to low nutrient conditions.

The results of our study have important implications for the interpretation of metacommunity patterns observed in the field. An increasing number of studies (Cottenie 2005; Beisner *et al.* 2006; Van der Gucht *et al.* 2007; Pandit *et al.* 2009; Declerck *et al.* 2011) have tried to link patterns of community variation with existing metacommunity paradigms, by partitioning community variation into spatial and environmental components, using direct gradient ordination techniques. Our results show that spatial structures observed in such studies on natural metacommunities may not only be caused by dispersal limitation or mass effects, but may also emerge as the result of strong ecological interactions with other groups of organisms, which are themselves impacted by dispersal limitation or mass effects. A spatial signal can emerge from an insufficient sampling of relevant variation in environmental conditions (Langenheder & Ragnarsson 2007). If variation in the environment is not measured and spatially structured, it will result in a spatial signal. Our results indicate that species composition at other trophic levels is an important environmental factor to be taken into account when determining the response of a given group of organisms to environmental and spatial factors. Failing to incorporate this information may introduce a significant spatial signal, even when the focal group of organisms is not dispersal limited and mainly the subject of species sorting.

Conclusions

Our study provides evidence that increased dispersal rates within a metacommunity can strongly mediate the compositional response of zoo-, phyto- and bacterioplankton communities to a gradient in primary productivity, and that this strong response can be largely generated by the

response of a limited number of keystone species at higher trophic levels (such as *Daphnia magna* in our experiment) that are dispersal limited and strongly affect the intensity of trophic interactions. We show that key-stone species mediated impacts cause major changes in ecosystem functioning (grazing pressure) and lead to apparent dispersal effects at lower trophic levels (phytoplankton and bacterioplankton). Our results suggest that these indirect effects of metacommunity structure may strongly impact species composition in local communities and even ecosystem functioning, and should be taken into consideration in metacommunity analyses.

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585 **Supporting Information**

586 **The following Supporting Information is available for this article:**

587 **Appendix S1** *Supplementary information on the composition of zooplankton and phytoplankton*
588 *communities in the source ponds.*

589 **Appendix S2** *Supplementary information on the DGGE analysis of the bacterioplankton*
590 *communities.*

591 **Appendix S3** *Supplementary information on the zooplankton and phytoplankton species responses*
592 *to the experimental treatments.*

593 **Appendix S4** *Supplementary information on the* e*ffects of the experimental treatments on the*
594 *taxon richness of zooplankton, phytoplankton and bacterioplankton.*

595 **Table S1** *RDA analysis per nutrient addition level.*

596 **Table S2** *Variation partitioning results quantifying the marginal and conditional effects on the*
597 *phyto- and bacterioplankton communities.*

598 **Table S3** *Abbreviations of phytoplankton and zooplankton species in the PCA biplot*

599 **Figure S1** *Averages* Desmodesmus *colony cell number along the* Daphnia magna *density gradient.*

600

601 Additional Supporting Information may be found in the online version of this article.

602

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Table 1. ANOVA results testing for the effect of nutrient addition (NUT) and dispersal (DISP) treatments and their interaction on zooplankton dry weight, phytoplankton chlorophyll a and grazing rate of the zooplankton on the phytoplankton. Grazing rates were measured by experiments *in situ*. LAKE ID refers to the origin of inoculation samples and was specified as a random block factor in the models.

	d.f.	SS	MS	*F*	*P*-value
Zooplankton dry weight					
LAKE ID	15	22.30	1.49	0.91	0.585
NUT	1	11.02	11.02	7.66	0.014
DISP	2	18.91	9.46	5.51	0.0092
NUT x DISP	2	17.88	8.94	5.9	0.0069
Phytoplankton chlorophyll a					
LAKE ID	15	0.50	0.034	1.39	0.280
NUT	1	13.52	13.52	641	< 0.001
DISP	2	0.06	0.030	1.90	0.167
NUT x DISP	2	0.08	0.039	3.07	0.0611
Zooplankton grazing rate					
LAKE ID	15	0.035	0.0021	2.52	0.281
NUT	1	0.017	0.017	14.89	0.0016
DISP	2	0.026	0.013	8.34	0.0013
NUT x DISP	2	0.013	0.0065	3.44	0.0451

Table 2. RDA results, testing for the effect of nutrient addition (NUT) and dispersal (DISP) treatments and their interaction on the variation in the zoo-, phyto-, and bacterioplankton community composition in the experimental containers. LAKE ID refers to the origin of inoculation samples and was specified as a random block factor in the models.

Variable	Covariables	R^2	*F*	*P*-value
Zooplankton				
NUT	DISP	0.19	26.64	< 0.001
	LAKE ID			
DISP	NUT	0.079	5.64	< 0.001
	LAKE ID			
NUT x DISP	NUT	0.057	4.43	< 0.001
	DISP			
	LAKE ID			
Phytoplankon				
NUT	DISP	0.091	10.29	< 0.001
	LAKE ID			
DISP	NUT	0.061	3.41	0.002
	LAKE ID			
NUT x DISP	NUT	0.052	3.066	0.002
	DISP			
	LAKE ID			
Bacterioplankton				
NUT	DISP	0.11	11.72	< 0.001

		LAKE ID			
DISP	NUT	0.05	2.73	< 0.001	
	LAKE ID				
NUT x DISP	NUT	0.046	2.65	< 0.001	
	DISP				
	LAKE ID				

Table 3. RDA-analysis results, testing for the effect of nutrient addition (NUT) within each of the dispersal treatments for zoo-, phyto-, and bacterioplankton communities in the experimental containers. In all these analyses, we specified LAKE ID as covariable to adjust for differences among lake origin of inoculation samples.

		R^2	*F*	*P*-value
Zooplankton				
	No Dispersal	0.085	3.64	0.008
	Low Dispersal	0.20	8.90	< 0.001
	High Dispersal	0.59	44.008	< 0.001
Phytoplankton				
	No Dispersal	0.13	4.82	< 0.001
	Low Dispersal	0.11	3.55	0.017
	High Dispersal	0.22	8.033	< 0.001
Bacterioplankton				
	No Dispersal	0.086	2.86	< 0.001
	Low Dispersal	0.19	7.73	< 0.001
	High Dispersal	0.22	7.83	< 0.001

680 **Figure legends**

681

682 Figure 1: Zooplankton biomass (A), phytoplankton chlorophyll a (B), *Desmodesmus* colony size
683 (C) and *in situ* measured zooplankton grazing pressure (D) for each of the multifactorial
684 combinations of experimental treatments. Chlorophyll a data represent time weighted averages;
685 *Desmodemus* colony size is expressed as the mean colony cell number weighted by the relative
686 abundance of colony size classes (only data given for high nutrient levels); the percentages in (C)
687 indicate the contribution of *Desmodesmus* to total phytoplankton biomass. White triangles and
688 black circles indicate containers of the low and high nutrient addition, respectively. Error bars
689 denote twice the standard error of the mean. Characters indicate significant post hoc differences
690 ($p<0.05$).

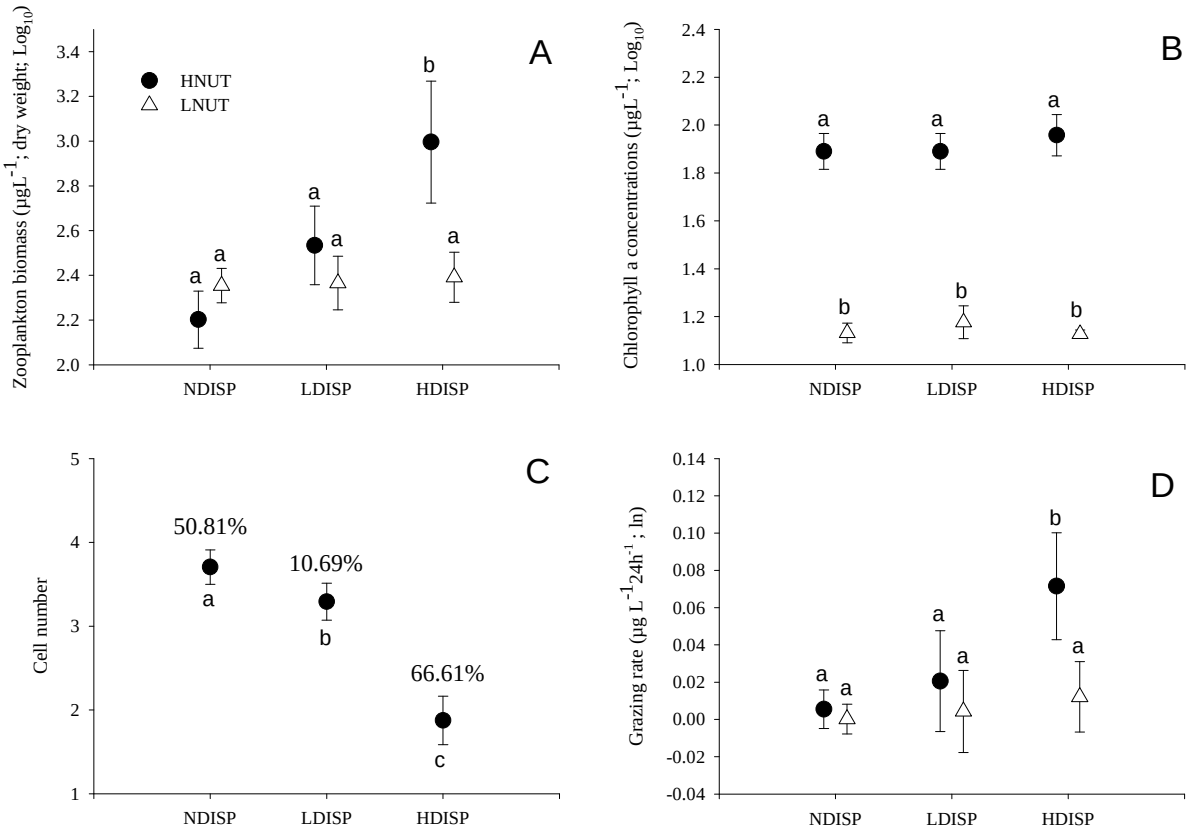
691

692 Figure 2: Biplot of principal component analysis representing the response of zooplankton (A),
693 phytoplankton (B), and bacterioplankton (C) community composition to the experimental
694 treatments. Centroids (filled circles) indicate the average location of communities belonging to the
695 same multifactorial treatment combinations of nutrient addition (HNUT = high nutrient content,
696 LNUT = low nutrient content) and dispersal (NDISP = no dispersal, LDISP = low dispersal, and
697 HDISP = high dispersal). For phyto- and bacterioplankton only species are shown for which a
698 minimum of 5% of the variation can be explained by the treatments. In (B), *D.magna* is plotted as
699 a supplementary variable; abbreviations of species are given in supplementary Table S3. In (C),
700 each OTU is labeled by a code in which the number is referring to a specific band location on the
701 DGGE-gel. Data were Hellinger transformed prior to analysis.

702

Figure 3: Venn diagrams presenting the results of variation partitioning analyses performed on the phytoplankton and bacterial community data at low and high nutrient levels, separately. For the phytoplankton communities, the diagrams represent the unique and shared contributions of the dispersal treatment and *Daphnia magna* population densities. For the bacterial communities, the diagrams represent the unique and shared contributions of the dispersal treatment, *Daphnia magna* densities and phytoplankton community composition. Figures outside the diagrams represent the R^2 of the marginal effects of the factors tested. Figures within the diagrams represent the R^2 of the conditional effects. R^2-values express the percentage of total variance explained. Asterisks denote significance level: *: P <0.05; **: P <0.01; ***: P <0.001; ^{NS}: not significant.

714 Figure 1.



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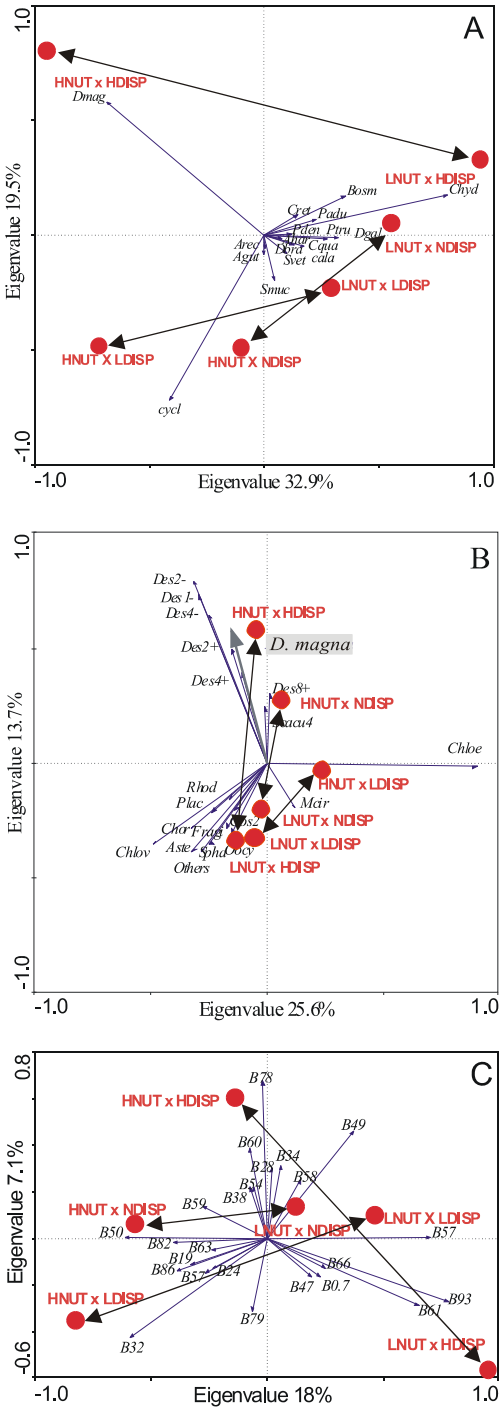
719 Figure 2.



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722 Figure 3.

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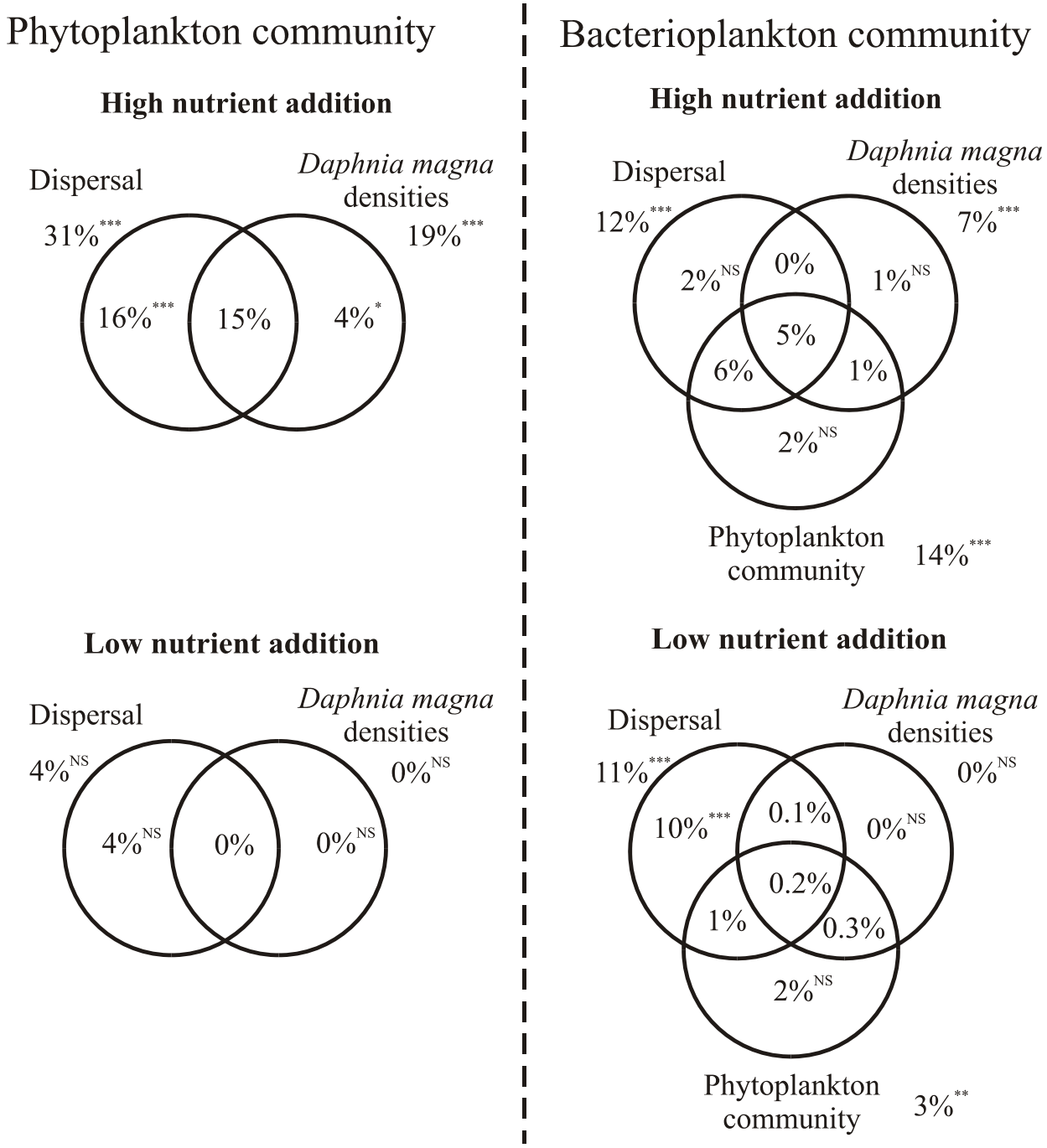

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