1 Diversity and distribution of *Daphnia* across space and time in Danube Delta lakes explained by
2 food quality and abundance

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4 Ioana Enache^{1,2,3*}, Larisa Isabela Florescu¹, Mirela Moldoveanu¹, Maria Iasmina Moza^{1,2,3,4}, Laura
 5 Parpală¹, Cristina Sandu¹, Patrick Turko^{3,5}, Geta Rîșnoveanu², Piet Spaak³

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7 ¹ Institute of Biology Bucharest, Department of Ecology, Taxonomy and Nature Conservation,
 8 Romanian Academy, Bucharest, Romania;

9 ² University of Bucharest, Faculty of Biology, Department of Systems Ecology and Sustainable
 10 Development, Doctoral School in Ecology; Bucharest, Romania;

11 ³ Swiss Institute for Environmental Science and Technology (Eawag), Dübendorf, Switzerland;

12 ⁴ Present Address: Genetic Wildlife Monitoring Laboratory – Zarnesti, Foundation Conservation
 13 Carpathia, Soseaua Cristianului 12A, 500053, Brasov, Romania;

14 ⁵ Present Address: Department of Dermatology, University of Zürich Hospital, CH-8952 Zürich,
 15 Switzerland

16

17 * Corresponding author: ioana.enache_n@yahoo.ro

Abstract

Eutrophication of shallow lakes often triggers a series of cascading ecological effects. Among these are shifts in the zooplankton community structure due to phytoplankton changes, or shifts in the fish community reducing size-selective feeding of planktivorous fish. In such conditions larger zooplankton (e.g. *Daphnia*) can have a selective advantage. Re-oligotrophication can reverse such food web interactions. Europe's largest wetland system (the Danube Delta) went through a period of eutrophication and is now slowly recovering again. However, changes in the *Daphnia* populations triggered by eutrophication in this system have remained unstudied. We used different sampling strategies to screen 24 lakes (which differ in their ecological state) for the presence of *Daphnia* as well as for biotic and abiotic parameters potentially explaining *Daphnia* abundance. *Daphnia* densities were surprisingly low. We found *D. magna* ephippia in the sediment, but no *D. magna* in the water column. Microsatellite analyses on pelagic *Daphnia* populations confirmed the presence of the *Daphnia longispina* complex and provided evidence for significant hybridisation events. FluoroProbe data showed that *Daphnia* was mainly present in lakes with a higher phytoplankton production. Our study provides insights into the spatial and temporal distribution of *Daphnia* in a very dynamic wetland system.

Keywords: Sediment, FluoroProbe, ephippia, food abundance, Cladocera.

Introduction

The Danube river Delta (45° N, 29°20'E), Romania, is the second largest delta in Europe, and the largest compact reed bed wetland on Earth, covering a surface of 4455 km² (Gâstescu 2009), providing well-preserved habitat to many different species (Găldean and Ruști 2006; Oosterberg et al. 2000). It is a unique mosaic of habitats and one of the most complex and dynamic systems on the continent, governed by the hydrology and water quality of the main stream of the Danube (Tudorancea 2006).

The 479 shallow lakes of the Delta (maximum depth mostly under 3.5 m) that remained after the major post-1980s hydromorphological changes are distributed across an area of ca 23000 ha (Găldean and Ruști 2006; Oosterberg et al. 2000). They can be assigned to four lake complexes of decreasing age: from the apex to the Black Sea: Șontea-Furtuna (3705 ha), Matița-Merhei (5701 ha), Gorgova-Uzlina (6848 ha) and Roșu-Puiu (6519 ha) (Oosterberg et al. 2000; Panin 2003) (Fig. 1, Table 1). Except the Roșu-Puiu complex, which was formed approximately 7200-2000 years BP, the other older complexes were formed approximately 11700-7500 years BP (Panin 2003). They are interconnected by a rich network of natural and human-made canals mostly created during the last century, especially after 1930. It is this network of channels which support in different degrees the connectivity between and among lakes and the main Danube branches. Due to the high natural and anthropogenic dynamics of this complex system, the lakes and channel network has had an on-going spatial-temporal rearrangement – e.g. removal of floating islands, embankments to improve navigation and drainage patterns (Oosterberg et al. 2000).

Anthropogenic-induced eutrophication in the Danube Delta started around 1970 and the maximum peak was reached during 1987-1988 (Cristofor et al. 1993; Postolache 2006). To a great extent it was caused by the cumulative inflow of nutrients from the Danube River (Galatchi and Tudor 2006). Although after 1990 eutrophication decreased because of the socioeconomic changes in Eastern Europe, recovery of the Delta ecosystems was slow especially due to the internal supply of nutrients from the sediments (Rîșnoveanu et al. 2004).

Since almost a century ago, when the first zooplankton data about the Danube Delta was published (Spandl 1926), several taxonomic syntheses have been published for the pre-eutrophication period (Damian-Georgescu 1963; Negrea 1983; Rudescu 1960). Although many studies have been done since then (e.g. Oosterberg et al. 2000; Tudor et al. 2014; Zinevici and Parpală 2007), they were all very local and covered only short time periods, so that no detailed studies on keystone species are available. No detailed study on any zooplankton taxon in the Danube Delta lakes has been published so far.

Daphnia is a non-selective filter-feeding zooplankter (Benzie 2005; Gliwicz 2004) that alternates between sexual and asexual phases. Their sexual reproduction phase begins when environmental conditions (e.g. food, temperature, photoperiod, presence of predators) worsen. Sexual reproduction is possible both intra- and interspecifically, producing complex hybrid systems (Wolf and Mort 1986; Xu et al. 2015). Sexually produced embryos undergo diapause allowing *Daphnia* to survive harsh periods (e.g. draught, winter conditions, high predation pressure, Zaffagnini 1988). The two sexual eggs are protected by a chitinous case called an ephippium, or resting egg (Lubbock 1857). Ephippia usually sink to the bottom of the lake, where they form an egg bank that can be sampled by taking sediment cores. Ephippia morphology is species specific and can be used to reconstruct past *Daphnia* species composition in lakes (Benzie 2005).

Daphnia are of central importance in many food webs, as they consume large quantities of a wide range of phytoplankton and bacteria, and are prey for fish and invertebrate predators (Carpenter and Kitchell 1993; Hambright et al. 2007). Their grazing capacity depends on body size: large-bodied species are more effective than small-bodied species (Gliwicz 2004; Kreutzer and Lampert 1999), but larger daphnids have a higher predation risk (Brooks and Dodson 1965). A consequence of these interactions can be a change in community structure from large-bodied to small-bodied *Daphnia* species or vice versa, depending on food supply and intensity and nature of predation pressure (Gliwicz 2004). *Daphnia* are thus subject to bottom-up population control, as food quality and quantity are important factors that control the evolution of life histories in *Daphnia*

(Lampert 2011). The optimum phytoplankton density is more easily assessed than the optimal food quality which is under debate (Lampert 1987; Masclaux et al. 2009). Important algal quality factors may include algal shape (e.g. colonies, spines or filaments), size, toxin production, resistance to digestion, stoichiometry, fatty acid and the amino acid composition (Lampert 1987; Lampert 2011). Food quantity can be practically assessed by chlorophyll *a* which accounts for 2% to 5% of the algal cell dry weight, and is easily measured and can be used as a proxy of algal abundance and lake productivity (Brönmark and Hansson 2010).

In addition to these bottom-up drivers, *Daphnia* are under strong top-down control by predators, which act as strong selection agents (Kerfoot and Sih 1987). Visually hunting fish prefer larger *Daphnia*, which have evolved many morphological defences (e.g. crests, spines, Larsson and Dodson 1993) and behavioural strategies (e.g. vertical migration, Ringelberg 1991) to reduce the risk of predation. Further, these strategies are plastic, allowing *Daphnia* to sense chemical cues excreted by predators (kairomones) and change their behaviour, life history and morphology according to the specific predation threat (see review by Lass and Spaak 2003).

Finally, *Daphnia* populations are shaped by the interactions between abiotic and biotic factors, either directly or, indirectly (Piepho 2010). Abiotic conditions in a lake (e.g. temperature, nutrients, pH, light) not only determine the phytoplankton community and so the food conditions for *Daphnia*. Transparency of the water, for example, determines how visible daphnids are to their predator. In general, unicellular green algae and cryptophytes are among the most preferred food by *Daphnia*, as revealed by numerous laboratory and field experiments. Examples of preferred food by *Daphnia* are the green alga *Acutodesmus* (formerly known as *Scenedesmus*), *Stichococcus*, diatom *Asterionella*, *Rhodomonas*, *Cryptomonas* and *Chlamydomonas* (Fink et al. 2011; Gliwicz 2004; Lampert 1987; Müller-Navarra and Lampert 1996).

Despite their central place in global aquatic food webs, information on *Daphnia* in the Danube delta is lacking. According to Török and Radu (2007), nine *Daphnia* species (*D. cristata*, *D. cucullata*, *D. curvirostris*, *D. galeata*, *D. longispina*, *D. magna*, *D. obtusa*, *D. pulex* and *D. pulicaria*)

have been reported in the literature about lotic and lentic Danube Delta water bodies. Unfortunately, this summary of 57 references merely lists every species of zooplankton that has been reported in the Delta since 1962, without reference to sampling strategy, year or season of detection, place of detection, or any other details. The underlying literature is primarily written in Romanian and published in difficult-to-obtain governmental reports and other grey literature, and not all may be fully reliable. However, all these species except *D. cristata* (which seems a misidentification) are reported to be present in Romania by Fauna Europaea (De Jong 2014).

This unexpected lack of information about *Daphnia* in the Danube Delta raises several questions concerning the structure and distribution of populations of this important zooplankton taxon. Therefore, we conducted a two year, multi-season, multi-sampling-strategy campaign, designed to detect the past and present presence of *Daphnia* and their population structure. To be able to relate these patterns to the biotic and abiotic conditions in the lakes, we concurrently measured several abiotic parameters, as well as the presence and abundance of the main phytoplankton groups using a multichannel in-situ fluorescence probe allowing quantification of basic phytoplankton groups (FluoroProbe®, bbe Moldaenke GmbH, Germany).

The aim of the paper is to: (1) quantify the abundance of *Daphnia* species in the water column, (2) assess the taxonomic structure of *Daphnia* communities based on genetic analyses, (3) assess the presence of *Daphnia* ephippia in lake sediments and reconstruct the distribution and presence of *Daphnia* in Danube Delta shallow lakes now and in the past and (4) assess the relative and shared effects of food availability and quality as well as abiotic conditions like depth, pH, turbidity, oxygen and conductivity in shaping *Daphnia* distribution and abundance across the Delta. Based on the reported eutrophic state of the Danube Delta lakes we hypothesized a strong positive correlation between food availability and *Daphnia* density as well as a correlation with food quality, based on FluoroProbe data.

Materials and Methods

Sampling sites

Our sampling strategy was designed to cover lakes representative for the four lake complexes of the Danube Delta. We sampled a total of 24 lakes which were distributed over all four complexes in accordance to the number and accessibility of lakes in each complex (Fig. 1, Table 1). Limnological parameters of these lakes are given in Table 1. Except where noted these 24 lakes were included in all of the sampling efforts mentioned below. Samples were taken from the middle of each lake. Sampling was done in May, July and September 2013 and May, July and October 2014 (for details see Table S1).

Quantitative zooplankton sampling to assess *Daphnia* abundances

Quantitative samples were obtained using a 5 L Patalas-Schindler trap. Ten samples (total 50 L) taken from near-bottom, the middle-layer and the surface layer were filtered through a 65 µm mesh size net and preserved in a 4% formaldehyde solution until taxonomic identification. Samples were screened for *Daphnia* which were identified to species level whenever possible according to Negrea (1983) using a Kollwitz chamber and a Zeiss inverted microscope with 10x - 40x magnification.

Qualitative zooplankton sampling for *Daphnia* genetic analysis

Since the density of *Daphnia* was low in most lakes we used an 18 cm diameter plankton net with a 95 µm mesh size attached to a pole, and swept through a 1m arc approximately 50 times while the boat was drifting. In this way we filtered over 1000 L of water for *Daphnia* genetic analyses, on all sampling dates for all lakes. The filtered zooplankton was preserved in pure ethanol and refrigerated until analysis. Since *Daphnia* densities were extremely low in 2013 in most lakes, only individuals from May and October 2014 (lakes Roşu, Roşuleţ, Tătaru, Puiu and Mândra, belonging to Roşu-Puiu complex) were used for genetic analyses.

Genotyping using microsatellite markers

Since we knew from morphological analyses that the lakes were inhabited by the *D. longispina* complex, we used microsatellites to investigate which species and possible hybrids were present. This species complex comprises *D. galeata*, *D. cucullata* and *D. longispina* (previous *D. hyalina*) that all are reported to hybridize with each other (Wolf and Mort 1986). Preserved *Daphnia* individuals were soaked for at least 2 hours in 1x TE buffer to remove any residual ethanol. DNA was then extracted using the HotSHOT protocol (Montero-Pau and Gomez 2008). We amplified a panel of 9 microsatellite markers (DaB10/14, Dp512, SwiD1, SwiD10, SwiD12, SwiD14, SwiD4, and SwiD5; Brede et al. 2006) in a multiplex protocol and analysed them on an ABI 3130 XL sequencer (Applied Biosystems). The lengths of these fragments were inferred using STRand software (Toonen and Hughes 2001) version 2.4.59 (<http://www.vgl.ucdavis.edu/STRand>) and binned into integer alleles using the R package MsatAllele.

To identify species, the binned data was combined with our panel of reference genotypes made up of 57 *D. galeata*, 30 *D. longispina*, 31 *D. cucullata* and 49 *D. galeata x longispina* hybrids drawn from lakes covering much of western Europe, North and South of the Alps (Möst 2013). We supplemented these data by producing 50 random hybrids of *D. cucullata* with each of the other parental species, using the R function “hybridize” from the R package “adegenet” (Jombart 2008). This combined data set was clustered using Discriminant Analysis of Principal Components (DAPC) in R using the package “adegenet”. As our reference genetic data set contains 3 species and 3 classes of hybrids, we set K to 6. The discriminant functions derived from the training data were then applied to the field-collected *Daphnia*. Only in the Roşu-Puiu complex *Daphnia* densities were high enough to do genetic analysis. Danube Delta *Daphnia* were considered to be assigned to a species if their posterior probability of cluster membership was greater than 0.9. In the rare case that membership of an individual was approximately equally likely between two hybrid classes, or between a hybrid and parental species, we considered that individual to be an unassigned hybrid class.

Ephippia sampling

In September 2013, one sediment core was retrieved from 20 of the 24 lakes (see Table 1) using a piston corer (6 cm diameter). The top 20 cm of the integrated sample was isolated on site and transported in the dark on ice to the laboratory where it was stored in dark and cool (4°C) conditions until further analysis. This sediment represents approximately 30 years of deposition, assuming a sedimentation rate around 0.7 cm per year (Cremer et al. 2004). Each core was sieved through a 1500 µm mesh to remove large plant fragments, and then washed through a 250 µm sieve to retain all *Daphnia* ephippia. In October 2014, this coring was repeated (for all 24 lakes), but the sediment cores were processed in two parts because first results of 2013 showed that different *Daphnia* species were present in the lake. The first 2 cm was taken to represent the present or recent past (last 3 years), while the sediments from 10 – 20 cm depth were taken as representative of a period of 15 to 30 years ago. After sieving, retrieved *Daphnia* ephippia were sorted into two categories: *D. magna* and small-bodied *Daphnia* (which we considered as belonging to the *D. longispina* complex) ephippia (Vandekerckhove et al. 2004). Because the sieved material of the 10 to 20 cm sediment sample contained a lot of small particles searching for ephippia was restricted up to 30 min to obtain presence/absence data for both ephippia types for all lakes in a standardized way. The top samples (0-2 cm depth) were analysed entirely.

Abiotic parameters and in situ quantification of main algae groups

At each sampling date and location, concurrent measurements of conductivity (µS/cm), pH, oxygen saturation (%) and temperature were conducted using a WTW340i multi-parameter meter. Furthermore depth (m) and Secchi transparency (m) were recorded and the ratio transparency/depth was calculated as an index of water turbidity ranging from 1 (the water column is transparent till the bottom of the lake) to 0 (total lack of transparency).

A submersible fluorometer (FluoroProbe II, bbe-Moldaenke, Kiel, Germany) was used to assess the total phytoplankton biomass (reported as µg of Chl *a* per litre of water). The device allows

the discrimination of four algal groups including (i) 'green' algae (Chlorophyta and Euglenophyta) rich in chlorophyll *a* and *b*, (ii) 'brown' xanthophyll-containing algae (Bacillariophyta, Chrysophyta and Dinophyta), (iii) 'blue' phycocyanin-rich algae (Cyanophyta) and (iv) 'red' phycoerythrin-rich algae (Cryptophyta). Their biomass assessment was done by submerging the probe slowly in the water column while the sensors did one measurement each second. The mean value was used for further analysis and considered as the integrated value for the water column (for details see Catherine et al. 2012).

Statistical analysis

To address the effect of potentially explanatory variables (i.e. the chlorophyll *a* concentrations as a proxy for the biomass of the different phytoplankton groups and the measured abiotic variables) on *Daphnia* abundances, Redundancy Analysis (RDA) (Jyrkänkallio-Mikkola et al. 2017; Legendre and Legendre 2012) was applied based on data sets for all sampled lakes and for the Roşu-Puiu complex separately. The "ordistep" function (with 200 permutations) from the R package "vegan" was used (Oksanen et al. 2007; R Core Team 2013). This function creates a backwards selection model for the ordination objects created by RDA. To measure the explanatory strength of the model, adjusted coefficient of determination R^2 and *p* values were generated. Afterwards, variation partitioning was performed using the R-package "vegan", function "varpart", aiming to assess the relative and the shared effects of (1) abiotic variables and (2) the biomass of main phytoplankton groups, on *Daphnia* abundance. Additionally, a PCA was done to analyse in more detail how the different abiotic as well as the algal groups influenced *Daphnia* density. For this we used Statistica (StatSoft 2011).

Results

Daphnia spatial and temporal occurrence in the water column

During the entire research period, only individuals from the *D. longispina* complex were found in the water column of the studied lakes. In either qualitative or quantitative samples, *Daphnia* were found in 10 out of 24 lakes: Roşu, Roşuleţ, Tătaru, Puiu, Mândra, Erenciuc, Uzlina, Isac, Bogdaproste and Rădăcinoasele (Fig. 1, Table 1). No *Daphnia* were found in the lakes of the oldest complex (Şontea-Furtuna). In the two southern, youngest, complexes, *Daphnia* was detected in 8 out of the 10 sampled lakes. The highest occurrence was recorded in the Roşu-Puiu complex either in spring or autumn (Fig. 1, Table 2). Very low abundances of *Daphnia* spp. in the water column were recorded in the Isac-Gorgova complex, in lakes Isac and Uzlina (2 and respectively 3 individuals) at only one sampling occasion (September 2013), and only in the qualitative samples. The qualitative samples from the two northern complexes did not reveal the presence of *Daphnia* in the water column at any moment of sampling, while the quantitative samples revealed its presence in lakes Rădăcinoasele (September 2013) and Bogdaproste (May 2014) – Table 2. In July 2013 and 2014 no *Daphnia* was found in the water column of any of the sampled lakes.

Daphnia species identity in the Roşu-Puiu lake complex

Since *Daphnia* were only present in the samples from May and October 2014 of a limited number of lakes, our genetic data is too restricted to compare lakes or lake complexes. Therefore, it was used to determine the taxonomic status of the sampled *Daphnia* within the *D. longispina* complex. Discriminant Analysis of Principal Components (Fig. 2) shows that in May the analysed individuals clustered around *D. galeata* x *D. cucullata* hybrids and pure *D. longispina* whereas in October they clustered around the *D. cucullata* standard clones and *D. longispina* x *D. cucullata* hybrids. These proved consistent with the taxonomic identification (Table 2).

Distribution of Daphnia ephippia in the sediment

Daphnia ehippia were detected in the surface sediment samples of 18 out of 24 surveyed lakes. However, deeper sediment samples (10-20 cm depth) revealed their occurrence in all lakes except Cuibul cu Lebede (Complex Gorgova-Uzlina). The analysis of the integrated samples showed the presence of ehippia in 16 out of 20 sampled lakes (Fig. 1).

D. magna ehippia occurred in the sediment of 8 lakes, distributed across all four lake-complexes. The analyses of the integrated sediment samples (2013) revealed its presence in four lakes: Uzlina, Rădăcinoasele, La Amiază, Trei Iezere. In the deeper sections of the 2014 samples (10-20 cm depth), the *D. magna* ehippia were present in four more lakes (Roşuleţ, Erenciuc, Bogdaproste, Rădăcinos) but these were absent in the top layers (0-2 cm depth) (Fig 1).

Abiotic parameters and phytoplankton quality and quantity

Out of seven abiotic parameters (data in Table S1), the backwards selection method in RDA returned temperature and the turbidity index as highly significant factors (both $p < 0.005$, R^2 adjusted = 0.15) explaining *Daphnia* density. The turbidity index was positively correlated with *Daphnia* density, whereas the temperature correlated negatively. Both turbidity index and temperature correlated with the first RDA axis which explains 15.84% of the total variance in the *Daphnia* densities.

Chlorophyll concentrations, used as a measure of lake productivity and to estimate the food quality and quantity for *Daphnia*, showed significant differences among lake complexes, seasons and algal groups (Fig. 3). The highest algal biomass was reached in lakes belonging to Roşu-Puiu complex and this proved consistent over the time. In the lakes of the Şontea-Furtuna complex, amounts of chlorophyll were very low as was also indicated by the high transparency in those lakes. Generally, cyanobacteria and cryptophytes tended to peak in mid-summer (July). In the Roşu-Puiu complex green algae had their maximum in September-October. In a Spearman's Rank-Order Correlation between *Daphnia* presence/absence and the amount of chlorophyll in the four algal groups we found significant correlations with green algal biomass ($r_s = 0.259$; $p = 0.0026$) as well as cryptophyte algal biomass ($r_s = 0.240$; $p = 0.0054$). In an RDA only the green algae biomass was returned as highly

significant ($p = 0.002$, R^2 adjusted = 0.16) in determining the *Daphnia* abundance while the cryptophytes biomass was returned as marginally significant ($p=0.070$). Both variables correlated with the first RDA axis which explains 17.3% of the variance in the *Daphnia* abundance.

The variance partitioning revealed that the abiotic and chlorophyll variables collectively explained 20% of the total variation in *Daphnia* density. The unique effects of abiotic variables (temperature and turbidity index) and phytoplankton biomass (green algae and cryptophytes) accounted for 4% and 6%, respectively of the total variation. The shared effect of all significant variables accounted for the largest amount of variation (10%) in *Daphnia* densities.

Since the highest *Daphnia* densities were found in the Roşu-Puiu lake complex we repeated our analysis for this complex. The backwards selection method in RDA returned again temperature as being negative and highly significant ($p = 0.005$) whereas the green algae biomass ($p = 0.06$) and depth ($p = 0.08$) were returned as having positive and marginal significance in shaping *Daphnia* distribution and abundance across the complex. In this case, temperature and depth explained 15 % of the variation in *Daphnia* abundance, whereas the phytoplankton biomass explained only 4%. The shared effects of significant variables accounted for about 2%. The value of residuals remained high (79%).

PCA (Fig. 4) confirmed the findings above. The first principal component (Eigenvalue of 3.32) explained 30.18% of the variation, PCA 2 17.92% and PCA 3 13.52 %. From the figure it is clear that *Daphnia* density correlates negatively with transparency and temperature and positively with chlorophyll of four algal groups as well as with turbidity.

Discussion

Daphnia spatial and temporal occurrence in the water column

In the past, Danube Delta zooplankton community structure and its dynamics have been inconsistently studied at a spatial and temporal scales, and hence, there are no reliable data on

Daphnia distribution in the Danube Delta shallow lakes. All earlier studies taken together found a wide distribution of *Daphnia* in the Delta, as well as a large species richness (9 species – including synonymies – reviewed by Török and Radu 2007). However, it is not sure which part of these results are due to synonyms or maybe miss qualifications. In the current study we could identify only species and hybrids from the *Daphnia longispina* complex (Fig. 2) in the water column of Danube Delta lakes. We found, *D. longispina* and *D. cucullata* in both study years, mostly in lakes from the Roşu-Puiu complex. There was no clear pattern of presence either in May or autumn. Remarkably, ephippia of *Daphnia* were found in lakes in which no *Daphnia* were found in the water column. We even frequently found resting stages of *D. magna* in older sediments, a *Daphnia* species never found in our pelagic samples. Despite the high hydrological connectivity of lakes and lake complexes in the Danube Delta (Oosterberg et al. 2000) we still expect structural heterogeneity for the zooplankton community due to lake-specific biotic and abiotic factors that can regulate the community composition (Cottenie et al. 2003).

Densities of *Daphnia* in the sampled lakes were very low (Table 2). In many lakes no *Daphnia* were found, despite filtering large amounts of water with a plankton net. This cannot be explained by the availability of food alone. In July we found no *Daphnia* in any of our sampled lakes, but phytoplankton abundances, as measured with the FluoroProbe, were not substantially different in July compared to spring and autumn (Fig. 3) indicating that other factors play a role in *Daphnia* abundance too. Our PCA showed a negative correlation between *Daphnia* density and transparency and a positive correlation with turbidity (Fig. 4). Other filter-feeding zooplankton groups were more abundant (Fig. S1), especially copepods and rotifers, showing that they might have a competitive advantage over *Daphnia*. These species could be better in avoiding or escape from predatory fish (Carpenter et al. 1985; Jensen et al. 1994) or have other food requirements like a larger range of edible particles (DeMott 1986; Ger et al. 2014; Tillmanns et al. 2008).

Distribution of *Daphnia* ephippia in the sediment

We found two types of *Daphnia* resting eggs in the sediments: large ones (*D. magna*) and smaller ones. Based on our molecular analysis of *Daphnia* in the water column we assume that the smaller ephippia are from the *D. longispina* complex, however we cannot totally exclude other species of the subgenus *Daphnia* (e.g. *D. pulicaria*). The sampling of resting egg showed, in contrast to the samples of the water column, the recent presence of *Daphnia* in most lakes. In surface sediment (1 – 3 years old) we found *Daphnia* of the *D. longispina* complex in 18 lakes, compared to 9 in the water column. Nevertheless, we did not find a frequent presence of ephippia in the surface sediments except for lakes from the Roşu-Puiu complex (qualitative assessment, data not shown here), which is in accordance with the undetectable abundances of live *Daphnia* in the water column of the lakes of the other three complexes.

Although we did not find *D. magna* in the water column, we found their ephippia in 8 lakes (in 4 lakes at depth lower than 10 cm: approximately 15 years ago (assuming a sedimentation rate of 0.7 cm / year); and in the integrated samples in another 4 lakes, Fig. 1). Obviously some factors made (parts of) the lakes in this period more suitable habitats for *D. magna*. The littoral reed beds might have been a place where *D. magna* could survive, since daphnids are less visible to predators when being between macrophytes (Lauridsen and Buenk 1996). It can also be that *D. magna* thrived in parts of the lake that almost dried up and therefore were less accessible for fish. Our data did not allow relating the relative abundance of each of the two *Daphnia* ephippia groups with other environmental factors such as fish abundance or water level fluctuations.

Genetic structure of *Daphnia* communities in Roşu-Puiu lake complex

Our genetic analyses confirmed that the *Daphnia* in all the studied lakes belong to the *D. longispina* species complex. The *Daphnia* community composition changed from *D. galeata* x *D. cucullata* and *D. longispina* in May to *D. cucullata* and *D. longispina* x *D. cucullata* in October (Fig. 2). No pure *D. galeata* was found.

The three *Daphnia* species from the *D. longispina* complex (*D. galeata*, *D. cucullata*, and *D. longispina*) are known to be adapted to different types of habitats and all hybridize with each other (Wolf and Mort 1986). In general one can say that *D. longispina* is adapted to more oligotrophic conditions (Spaak et al. 2012). *D. cucullata* seems to be adapted to eutrophic lakes with high fish predation (Brzeziński et al. 2012; Spaak and Hoekstra 1995; Spaak and Hoekstra 1997), whereas *D. galeata* invaded lakes during eutrophication (Brede et al. 2009a; Karabanov et al. 2018; Rellstab et al. 2011a). In October almost all genotyped *Daphnia* was *D. cucullata*. Fish data for the Danube Delta lakes is scattered and not available in a quantitative way (see Table S2) which makes it impossible to use fish abundance as an explanatory factor in our statistical analysis. However, the hypothesis that fish predation is an important selective factor on *Daphnia* in the Danube Delta lakes is partly supported by our data. Not only the presence of the small bodied *D. cucullata* in autumn when fish predation might be higher, but also the absence of *D. magna* might be explained by intensive fish predation. Also *Daphnia* was more abundant in less transparent waters (Fig. 4) in which they are expected to be less visible for visual hunting predator like fish.

It has been shown for several pre-alpine lakes, using genotyped ephippia collected from sediment cores, that *D. galeata* could invade eutrophic lakes (Brede et al. 2009b; Rellstab et al. 2011b; Seidendorf et al. 2007). The Danube Delta lakes seem not to follow this pattern. Only from one lake, (Gorgova, Monchamp et al. 2017) the *Daphnia* population was reconstructed from the sediment. Also in this lake recent sediments are dominated by *D. cucullata* and their hybrids, but no clear species shift related to eutrophication could be observed, suggesting that *D. cucullata* adapted to the Danube Delta lake conditions as it did in other European reservoirs (Seda et al. 2007). This makes it likely that other factors, e.g. predation by fish or food availability determine taxon success in Danube Delta lakes.

Resource availability as factor explaining *Daphnia* occurrence?

The abundance of *Daphnia* in Danube Delta lakes was negatively correlated with temperature (seasonal change) and with water transparency. During our sampling in summer with the highest water temperatures no *Daphnia* were found. This was also the month with the highest abundance of cyanobacteria (Fig. 3), suggesting suboptimal food conditions. Summer cyanobacteria dominance was reported for some other Danube Delta lakes (Török 2005; Török 2011) in the past. Other studies reported high presence of filamentous and poor nutritional (sometimes even toxic) cyanobacteria explaining low zooplankton densities (Moldoveanu and Florescu 2013; Török 2005).

Usually in spring European lakes are dominated by diatoms, providing a good food source for grazers like *Daphnia*, followed by a clear water phase (Sommer et al. 1986). Török (2008) detected the same pattern for Danube Delta lakes of shifting communities from diatoms in spring towards cyanobacteria starting with June. This does not seem to be the case for the currently analysed time frame and lakes where diatoms never dominated (Fig. 3). That food presence and abundance might be an important factor to explain *Daphnia* presence is also shown from the RDA that showed that only green algae, as measured with the FluoroProbe, correlated significantly and positively with the *Daphnia* abundance.

Our results show that the productivity among the lake complexes in the Danube Delta is different (Fig. 3). With the FluoroProbe the highest chlorophyll densities were measured in the Roşu-Puiu complex, suggesting that this is the most productive complex with the most food to sustain a *Daphnia* population. Food conditions in the other complexes seem to be suboptimal for *Daphnia*.

Planktivorous fish impact on *Daphnia* community structure

The top-down control by visually-feeding planktivorous fish (Pijanowska and Stolpe 1996) can reduce *Daphnia* populations to untraceable levels. High catch of fish per unit of effort often coincides with low zooplankton and *Daphnia* biomass (Jeppesen et al. 2000). Hülsmann (2003) showed in a literature review (34 studies) that *Daphnia* generally tends to decline during mid-summer, mainly because of top-down control.

In the Danube Delta zooplanktivorous fish like *Abramis brama*, *Blicca bjoerkna*, *Scardinius erythrophthalmus*, *Rutilus rutilus*, and invasive *Carassius gibelio* might exert a major predation pressure for *Daphnia*. Năvodaru et al. (2000) and Năstase and Năvodaru (2010) predicted an increasing dominance of these species in the fish communities, meaning a potentially increasing impact on *Daphnia* and other zooplankton. Unfortunately the literature about fish composition and abundance in the various lakes and complexes in the Danube Delta is very scattered. In Table S2 we list all papers published during the last 4 decades we could find about fish presence and abundance in the Danube Delta. Unfortunately, based on these papers it is hard to extract specific and clear conclusions concerning the predation pressure of the fish community on zooplankton in general and on *Daphnia* in particular either in time (including the moments of sampling of the present study) or space in Danube Delta lakes.

Synthesis and Conclusion

Daphnia proved to be rare in zooplankton samples collected during spring, summer and autumn in the lakes that we studied. Our integrative method to assess *Daphnia* presence indicates that *Daphnia* may play a less important role in the food web of Danube Delta lakes than in some other European shallow lakes. Based on FluoroProbe data we conclude that food quantity and quality explains at least partially the observed *Daphnia* abundance. Fish predation might be an important factor to explain the low densities of *Daphnia* in Danube Delta lakes but unfortunately lake based quantitative fish data is lacking. A more extensive project would be needed to understand the complex and dynamic lake ecosystems in the Danube Delta.

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Figure legends

Figure 1

The Danube Delta with the 24 sampled lakes (for lake numbers and names see Table 1). The map indicates in which lakes *Daphnia* was found as active stage (pelagic samples) and as ehippia (sediment samples). The occurrence of ehippia of small bodied *Daphnia* (*D. longispina* complex) and *D. magna* in the upper (0-2 cm) and lower sediment (10-20 cm) is indicated with different symbols. A black circle surrounds the information on a specific lake. Lakes are grouped in lake complexes: Şontea-Furtuna (22-25), Matiţa-Merhei (11-20), Isac-Gorgova (7-10) and Roşu-Puiu (1-6). (Map source Google maps)

Figure 2

Discriminant Analysis of Principal Components showing the species assignment for 5 of the studied lakes (Mândra, Puiu, Roşu, Roşuleţ and Tătaru) in May and October 2014. Small dots coloured in bright green (*D. galeata*), purple (*D. longispina*), blue (*D. galeata* x *D. longispina*) and yellow (*D. cucullata*) represent the standard clones. The small pink dots represent in silico *D. longispina* x *D. cucullata* hybrids. Larger dots (May samples) and triangles (October samples) represent the analysed individuals.

Figure 3

Mean chlorophyll values ($\mu\text{g} \cdot \text{L}^{-1}$) as determined with the FluoroProbe for four phytoplankton groups ('green' algae (Chlorophyta and Euglenophyta) rich in chlorophyll a and b, (ii) 'brown' xanthophylls containing algae (Bacillariophyta, Chrysophyta and Dinophyta), (iii) 'blue' phycocyanin-rich algae (Cyanophyta) and (iv) 'red' phycoerythrin-rich algae (Cryptophyta) for the four Danube Delta lake complexes in 2013 and 2014 for May, July and September / October. Error bars indicate 95% confidence limits, negative Y-axis is included to visualize complete error bars. ANOVA showed significant differences among lake complexes ($F(3,484) = 27.129$, $p < 0.001$), dates ($F(2,484) = 8.112$, $p < 0.001$) and phytoplankton groups ($F(3,484) = 4.357$, $p < 0.01$).

Figure 4

PCA of seven abiotic factors as well as the chlorophyll concentrations determined by the FluoroProbe for four phytoplankton groups explaining the density of *Daphnia* in the studied Danube Delta lakes. The 3 plotted PCA axes explain together 61.6% of the total variance.

Table legends

Table 1. General overview of the lakes presented in this study, lakes are sorted on lake complex from east to west, the western lakes being the oldest and most upstream in the Delta.

Lake	Lake ID	Lake complex	N (DDM)	E (DDM)	S (ha)	Shore length (km)	Mean Depth	Number of samples 2013	Number of samples 2014	Sediment core Sept 2013	Sediment core Oct 2014
Roșu	01	Roșu-Puiu	45°03.777'	029° 34.934'	1445.57	21.20	2.98	3	3	1	1
Roșuleț	02	Roșu-Puiu	45°04.257'	029° 36.028'	428.39	21.81	2.17	3	3	1	1
Tătaru	03	Roșu-Puiu	45°02.224'	029° 36.721'	30.45	4.93	1.38	2	3	1	1
Mândra	04	Roșu-Puiu	45°02.395'	029° 30.817'			1.93	3	3	1	1
Puiu	05	Roșu-Puiu	45°02.906'	029° 28.355'	851.34	14.79	3.12	3	3	1	1
Erenciuc	06	Roșu-Puiu	45°008.41'	029° 24.597'			2.59	3	3	1	1
Gorgostel	07	Isac-Gorgova	45°03.426'	029° 19.748'	65.16	6.43	2.00	3	3	1	1
Uzlina	08	Isac-Gorgova	45°05.664'	029° 16.270'	451.25	12.86	1.52	3	3	1	1
Isac	09	Isac-Gorgova	45°06.433'	029° 16.884'	1109.43	17.81	2.12	3	3	1	1
Cuibul cu Lebede	10	Isac-Gorgova	45°08.086'	029° 20.477'	200.22	8.34	1.53	3	3	1	1
Bogdaproste	11	Matița-Merhei	45°13.621'	029° 21.256'	418.48	11.84	1.51	3	3	1	1
La Amiază	12	Matița-Merhei	45°13.636'	029° 19.899'	90.84	7.69	1.24	3	3	1	1
Trei Iezere	13	Matița-Merhei	45°14.209'	029° 19.443'	628.01	19.54	1.71	3	3	1	1
Matița	14	Matița-Merhei	45°17.588'	029°21.807'	684.10	11.98	2.48	3	3	1	1
Babina	15	Matița-Merhei	45°18.325'	029° 20.859'	473.53	12.16	2.03	3	3	1	1
Rădăcinoasele	16	Matița-Merhei	45°18.945'	029° 21.287'	244.40	11.48	1.57	3	3	1	1
Dracului	17	Matița-Merhei	45°19.306'	029° 21.303'	161.87	6.48	1.72	3	3	1	1
Merhei	18	Matița-Merhei	45°19.020'	029° 26.868'	1270.36	38.45	1.68	3	3	1	1
Merhei Mic	19	Matița-Merhei	45°19.746'	029° 28.677'	182.80	7.64	1.33	3	3	1	1
Lung	20	Matița-Merhei	45°18.123'	029° 26.217'	46.99	5.82	1.28	3	3	1	1
Furtuna	22	Șontea-Furtuna	45°13.052'	029° 06.853'	939.36	15.05	1.99	1	3	0	1
Băclăneștii Mari	23	Șontea-Furtuna	45°14.582'	029° 08.030'	277.76	12.74	1.85	1	3	0	1
Ligheanca	24	Șontea-Furtuna	45°12.475'	029° 12.107'	45.52	3.82	0.98	1	3	0	1
Rădăcinos	25	Șontea-Furtuna	45°13.469'	029° 05.598'	35.03	4.37	1.83	1	3	0	1

Table 2. Abundance (number of individuals per litre) of *Daphnia* in the quantitative samples, according to the microscopic observations, from the 24 Danube Delta lakes, mentioned in Table 1. Lakes L01-L05 belong to the Roşu-Puiu complex, lake L11 and L16 to Matîta-Merhei. In the other lakes *Daphnia* densities were below the detection limit (0.02 per litre). All *Daphnia* not being *D. cucullata* are grouped in the “*D. longispina* complex” column.

Lake ID	Lake	Year	Month	<i>D. cucullata</i> (ind./L)	<i>D. longispina</i> complex (ind./L)
L01	Roşu	2013	5	0.32	1.16
L01	Roşu	2014	5	0.66	0.66
L01	Roşu	2014	10	7.48	
L02	Roşuleţ	2013	5	0.17	
L02	Roşuleţ	2014	10	1.68	
L03	Tătaru	2014	5	0.36	
L03	Tătaru	2014	10	0.25	
L04	Mândra	2013	5	0.34	
L04	Mândra	2014	10	0.4	
L05	Puiu	2013	5	0.26	0.52
L05	Puiu	2013	9	3.96	
L05	Puiu	2014	10	0.96	
L11	Bogdaproste	2014	5		0.07
L16	Rădăcinoasele	2013	9	1.52	