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Plankton changes as critical processes for restoration plans of lakes Kastoria and Koronia

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Abstract: During the past decades efforts have been made through restoration measures to reverse eutrophication and improve water quality in lakes. In this study, phytoplankton was studied in two different water bodies under restoration in Greece; Lake Kastoria and the temporary brackish water body Koronia. In Koronia, zooplankton structure was also examined. The study was based on phytoplankton structure and dynamics in order to identify targets for ecological restoration. Diverse changes in plankton community were seen suggesting that restoration targets should be set including both biodiversity and target species. Sixteen years after sewage diversion in Lake Kastoria phytoplankton species diversity was indicative of community restoration. However, the long persistence of the toxic *Microcystis aeruginosa* was indifferent to sewage diversion. In the temporary brackish water body Koronia, phytoplankton abrupt changes were observed. However, species diversity remained poor with the local species pool shared by first settlers, invaders and ‘seed bank’ species. ‘Seed bank’ species both of phytoplankton and zooplankton were abundant during the whole study period. These species contributed to the ability of the temporary water body to supply recruits supporting the alternative species sequences assembled each year. Monitoring of plankton species richness, composition, blooms of toxic, invading and sediment ‘seed bank’ species should become a target in itself by the management authorities for ecological restoration.

Key words: ecological restoration targets, biodiversity, phytoplankton-zooplankton species pool, sediment ‘seed bank’ species, invasion

1. INTRODUCTION

Freshwaters are essential resources for life. However, in most parts of the world freshwaters have been degraded by human activities. In Europe, according to the Water Framework Directive (WFD) (2000/60/EC) (European Parliament, Council, 2000) all surface waters should achieve good ecological water quality by 2015. In Greece, degradation of water quality through anthropogenic eutrophication is evident in most lakes by its symptoms such as phytoplankton blooms and particularly toxic cyanobacterial blooms and scums (Vardaka et al, 2005). Phytoplankton is a key biological element for ecological water quality assessment in lakes and reservoirs. Under eutrophic-hypertrophic conditions excessive phytoplankton accumulated biomass is mostly made up by large-sized and/or toxic phytoplankton organisms and represents the “leftovers” rather than the food for most zooplankton (Moustaka-Gouni et al, 2006). Thus, in such highly eutrophic conditions the grazing pressure by zooplankton, i.e. the top-down control on phytoplankton, can have low impact leading to poor energy flow through the food-web (Lampert and Sommer, 1997).

In the Mediterranean lakes, permanent and temporary ponds the prolonged growth seasons due to higher irradiance and temperature, droughts and slow water renewal promote long-lasting cyanobacterial blooms (Vardaka et al, 2005; Naselli-Flores and Barone, 2012). Also, strong trophic cascading effects leading to the lack of large-bodied zooplankton grazers such as *Daphnia* and high sensitivity to climate factors (Beglioglou et al, 2007), make restoration of Mediterranean lakes more

challenging. However, to the best of our knowledge, only few papers are available on long-term monitoring of plankton and particularly in temporary water bodies (Naselli-Flores and Barone, 2012). This can be associated with difficulties in identifying the planktic compartment from the benthic and metaphytic ones in these very shallow ecosystems (< 2 m maximum depth). Benthic and metaphytic filamentous algae and cyanobacteria, competitors to phytoplankton, originate from the sediment and rise to surface water forming blooms and scums. On the other hand, truly planktic species having life-cycle stages such as resting cysts and akinets pass a part of their life in the sediment (Michaloudi et al, 2009).

These life-history traits contribute to maintenance of species diversity and the ability of the system to supply recruits and support established species (Palmer et al, 1997). The knowledge on species pools is significant for community restoration (Zobel et al, 1998). In many lakes, restoration through nutrient control aims at a significant lowering of phytoplankton biomass, the total property of phytoplankton. The response of phytoplankton biomass to nutrient reduction has a lag-phase (Romo et al, 2005). This is also true for the phytoplankton community structure (diversity, composition, dominance) which resists for years. As a result insufficient restoration for the first decade has been observed in Mediterranean lakes (Romo et al, 2005). Examining different levels of phytoplankton organization can provide important information on how the community assembles during restoration (Rojo, 2004) bridging the gap between theory and practice in restoration. Unexpected outcomes of restoration are usually due to the focus on restoring the abiotic factors ignoring biotic ones and the feedback between abiotic and biotic changes (Suding et al, 2004).

The present work constitutes a study of long-term plankton changes in two different water bodies under restoration. Our aim was to identify targets for ecological restoration. The studied water bodies were shallow polymictic and eutrophic lakes, 30 years ago. Nowadays, Koronia being severely impacted by human activities mostly due to water overexploitation has become a temporary, very shallow, brackish water body. Due to its temporary character, the study on Koronia focuses on available plankton species pool in water and sediment and the phytoplankton life-histories in the community assembly.

2. METHODS

2.1 Study site

Lake Kastoria is an urban, shallow (maximum depth 8 m, average depth 4 m) polymictic lake with a surface area of 29 km² located in the northwestern part of Greece (40°30'N, 21°18'N). The lake had been receiving sewage effluents of the town of Kastoria for decades until 1994. Another human impact in the lake is fish stock management with the introduction of cyprinids (Beklioglu et al, 2007). Heavy and toxic cyanobacterial blooms have a long history in the lake (Vardaka et al, 2005; Moustaka-Gouni et al, 2006). Phytoplankton community assembly during restoration after sewage diversion from Lake Kastoria has been the subject of our recent work in this urban lake.

Lake Koronia is located in northern Greece (40° 40' 58''N, 23° 09' 33''E). The lake used to be the fourth largest lake in Greece occupying an area of 46.2 km² and having a maximum depth of 8 m in the 1970's. Over the past 20 years a massive decrease in lake volume occurred, with dramatic decrease in surface area and maximum depth due to unsustainable water resource management. In August 1995 (surface area 30 km², maximum depth 1 m) a kill of all fish occurred. In the summer of 2002 the lake dried up and early in 2003 water started accumulating again in the temporary lake. During the period from 2003 onwards water depth was < 2 m and the water body dried up again in autumn 2009. In the meantime within the framework of a restoration Master Plan which is under implementation, a ditch connecting Koronia with the neighbouring Lake Volvi and its diverted torrents resulted in water supply to Koronia with a first flooding in February 2010.

2.2 Phytoplankton data

Phytoplankton data were obtained in different periods for the studied freshwaters. In Lake Kastoria data were collected in the years 1994-1996, 1999, 2003-2005, 2007, 2010-2011. In Koronia data were collected in years 2003-2007 and 2009-2011. The phytoplankton samples were collected from the surface water layer (0-1 m) with a Niskin-type sampler. Samples were collected with different frequencies in different periods (from weekly to seasonally) in the studied freshwaters. Water samples were collected from inshore stations in Lake Kastoria. In Koronia samples were taken from a central offshore and two inshore stations during 2003-2004. Afterwards, we collected inshore water samples. This sampling protocol allowed us to study life-histories of plankton and available species pool at the water-sediment interface. In 2011 extended mats of metaphytic algae contributed in patchy phytoplankton distribution. During this year the samples with the lowest metaphytic biomass were examined. Live and preserved samples were examined using an inverted microscope (Nikon SE 2000). Phytoplankton individuals were identified to species level on the basis of their morphology using taxonomic keys and papers. In addition, for dominant species and species known to produce toxins molecular analysis was performed (e.g. Genitsaris et al. 2009; Moustaka-Gouni et al. 2009; Kormas et al. 2011). Phytoplankton counts were performed using Utermöhl's sedimentation method. For biomass estimation, the dimensions of 30 individuals (cells, colonies or filaments) of each species were measured using tools of the Nikon DS-L1 camera. Mean cell or filament volume estimates were calculated using appropriate geometric formulae. Phytoplankton biovolume was estimated according to the Hillebrand et al. (1999) method. In the present study the samples (based on weekly to bi-monthly frequency) were pooled in seasonal data to uniform the data sets.

Phytoplankton species exhibiting abundance of $1,000 \text{ cells mL}^{-1}$ were considered to be abundant. The abundant species were assigned into three groups of species of similar introduction/recruitment traits for establishment in plankton of Koronia. The group of "first settlers" comprises species observed as pioneers in primary succession after flooding. The group of "invaders" comprises species known to grow in brackish waters excluding "first settlers". The third group consists of "seed bank" species observed to recruit from the sediment, excluding "invaders". Phytoplankton abundant species were assigned into two groups of species of similar size, the nanoplankton with a maximum dimension $< 20 \mu\text{m}$ and the netplankton with dimension $> 20 \mu\text{m}$.

2.3 Zooplankton data

In Koronia zooplankton sampling in 2003-2004 was carried out at the deepest point of the lake on a monthly basis, provided that access to the lake was possible. A 2 L Niskin sampler (50 cm height) was used for collecting at least 30 L of water that were filtered at each sampling time through a filter with a mesh size of $50 \mu\text{m}$. Afterwards (2005-2007, 2009-2010), samples were collected inshore filtering a volume of 10 L of lake water through a filter with a mesh size of $50 \mu\text{m}$. The samples were preserved in 4% formalin and were examined using a light microscope (Leitz Laborlux S). In addition, every time samples were collected with vertical and horizontal hauls using plankton nets (50 and $100 \mu\text{m}$ mesh size) that were examined live.

3. RESULTS

3.1 Phytoplankton long-term changes

The long-term changes of the phytoplankton species richness, total phytoplankton biomass, percentage contribution of cyanobacterial biomass to the total, percentage contribution of phytoplankton size groups biomass to the total and *Microcystis aeruginosa* biomass are presented

for the two studied water bodies in Figure 1. The figure representing a synthesis to show the trends followed by the same variables was considered much more informative for comparison than absolute values.

In Lake Kastoria, phytoplankton species richness showed a significant increase ten years after sewage diversion. Phytoplankton total biomass exhibiting extremely high levels ($> 500 \text{ mg L}^{-1}$) in summer-autumn of the years 1994-1996, showed a considerable decrease over the years after the sewage diversion. However, high levels ($> 50 \text{ mg L}^{-1}$) were measured during 2010-2011. In contrast to considerable changes both in species richness and total phytoplankton biomass, cyanobacterial percentage contribution to total phytoplankton biomass did not change, remaining high. Similarly, the large-sized phytoplankton dominated despite considerable changes in species composition and biomass. On a species-based analysis, the biomass changes of the toxic cyanobacterium *Microcystis aeruginosa* showed a similar trend to that of the total phytoplankton and cyanobacterial biomass.

In Koronia, the phytoplankton species richness (< 50 species in total) remained poor for many years (2003-2011). Phytoplankton total biomass in summer-autumn exhibited extremely high levels ($> 500 \text{ mg L}^{-1}$ in 2007) with a minimum ($< 50 \text{ mg L}^{-1}$) in 2003. Cyanobacterial percentage contribution to total phytoplankton biomass remained under 50% except for the years 2007 and 2010. Large-sized phytoplankton dominance coincided with the highest cyanobacterial dominance in 2007 and 2010, although they also dominated in 2006 and 2011. *Microcystis aeruginosa* biomass changes showed a similar trend to that of the cyanobacterial percentage contribution to the total biomass (Figure 1).

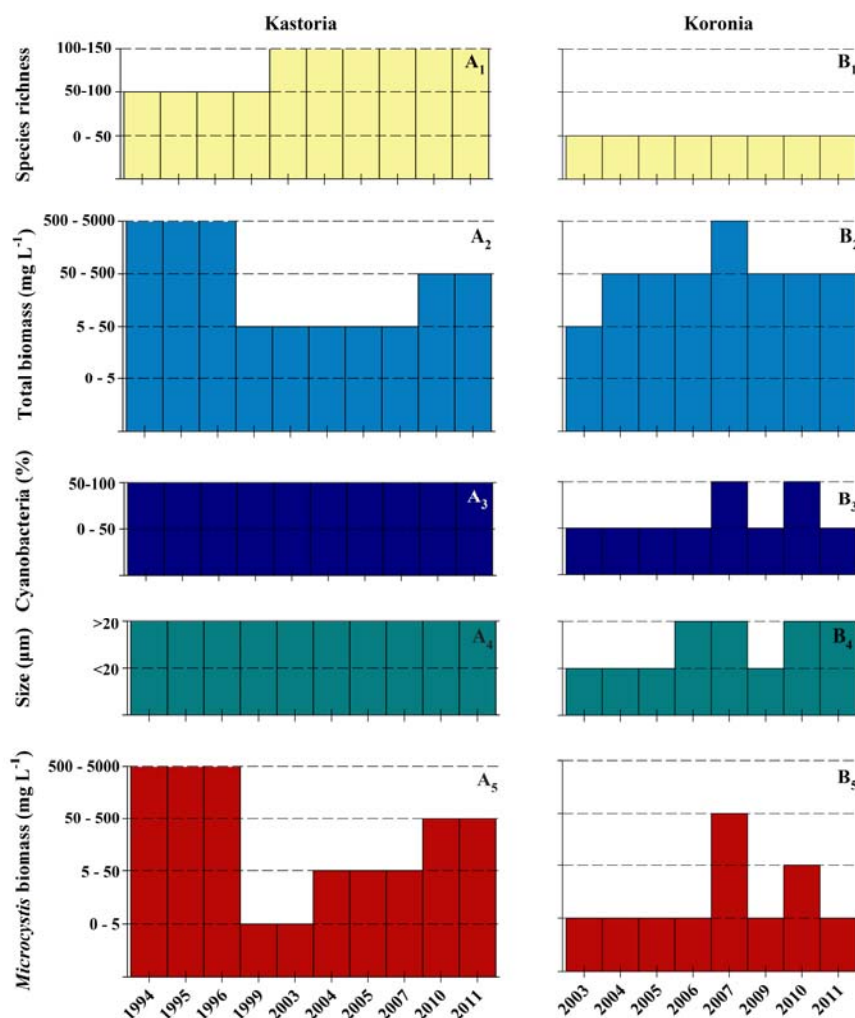


Figure 1. Long-term changes of the phytoplankton species richness (A_1 and B_1), total phytoplankton biomass (A_2 and B_2), percentage contribution of cyanobacterial biomass to the total (A_3 and B_3), percentage contribution of phytoplankton size groups biomass to the total (A_4 and B_4), and *Microcystis aeruginosa* biomass (A_5 and B_5) for Lake Kastoria and Koronia.

3.2 First settlers, invaders and sediment “seed bank” species in the plankton

In Koronia a phytoplankton species pool of less than 50 species was identified during 2003-2011. The abundant species were assigned in three categories: the first settlers (3 species), the invaders (6) and the sediment “seed bank” species (5). The first settlers, the chlorophyta *Koliella* cf. *longiseta* in 2003 and *Ankyra juday* in 2011 (Figure 2) and the prasinophyte *Pyramimonas* sp. in 2010 exceeded abundance of 10,000 cells mL⁻¹.

The invaders (Figure 2) were: the haptophyte *Prymnesium parvum* exceeding 100,000 cells mL⁻¹ in 2004, the cyanobacteria *Arthrospira fusiformis* and *Anabaenopsis arnoldii* exceeding 100,000 cells mL⁻¹ in 2007, *Nodularia spumigena* with abundance reaching 1,000 cells mL⁻¹ in 2009, the prasinophyte *Tetraselmis* cf. *suicica* exceeding 10,000 cells mL⁻¹ in 2009 and the diatom *Amphiprora alata* reaching 1,000 cells mL⁻¹ in 2010. This year, the metaphytic *Spirogyra* sp. was abundant in spring-early summer together with few filaments of *Oedogonium/Cladophora* that proliferated in 2011, forming extended algal mats. During the whole study period (2003-2011), the sediment “seed bank” abundant species were the chlorophytes *Oocystis* sp. exceeding 10,000 cells mL⁻¹ and *Pediastrum boryanum* exceeding 10,000 cells mL⁻¹, the cyanobacteria of the genus *Microcystis* (Figure 2) and *Anabaena* exceeding 100,000 cells mL⁻¹ and the euglenophyte *Euglena* sp. exceeding 10,000 cells / mL⁻¹.

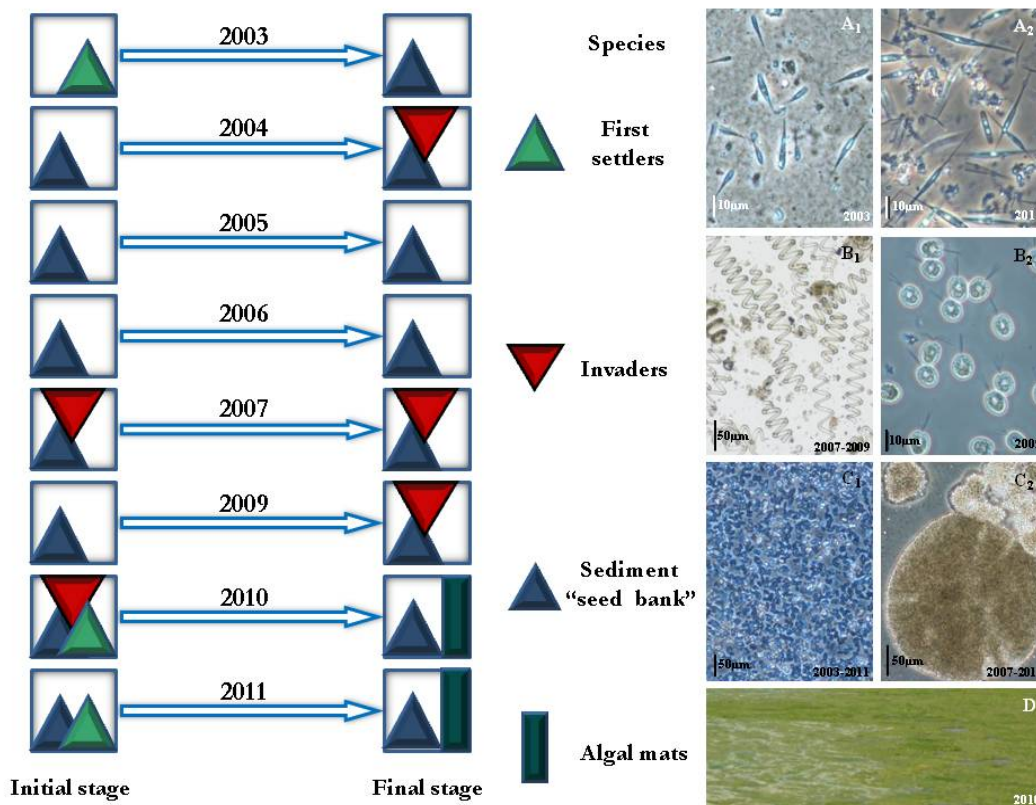


Figure 2. A conceptual view of phytoplankton species sequence of first settlers (light micrographs of *Koliella* cf. *longiseta* - A₁ and *Ankyra juday* - A₂), invaders (light micrographs of *Arthrospira fusiformis* - B₁ and *Tetraselmis* cf. *suicica* - B₂), and sediment “seed bank” species (light micrographs of *Oocystis* sp. - C₁ and *Microcystis* sp. - C₂), and algal mats (D) as alternative yearly pathways from the initial stage (high inflows or floods in winter) to final stage (minimum water depth in early autumn) from 2003 to 2011 in Koronia.

3.3 Zooplankton

A total of 25 zooplankton species were identified (15 rotifers, 7 cladocerans, 3 copepoda) throughout the study years (Table 1). Rotifers were mainly dominated by representatives of the

genus *Brachionus*; *Daphnia magna* was the dominant cladoceran, and *Acanthocyclops robustus* the dominant copepod. At any one time more than 80% of the zooplankton community was comprised by maximum 3 species, while the lake could be characterized as a monoculture depending on the species present, e.g. *Notholca salina*, *Brachionus plicatilis* species complex, *Daphnia magna*. During 2003 and 2010, years following a dry phase, the zooplankton community was characterized by high percentage (53 and 38%, respectively) of species not previously recorded in the lake (Michaloudi and Kostecka, 2004). In both cases the first to arrive were the rotifers *Notholca salina* and *Hexarthra polyodonta* followed by *Daphnia* species and the developmental stages (nauplii and copepodites) of the copepod.

Table 1. Zooplankton taxa identified in water samples from Koronia collected during the study period.

	2003	2004-2009	2010
<i>Brachionus angularis</i>	*	+	-
<i>Brachionus calyciflorus</i>	+	+	+
<i>Brachionus dimidiatus</i>	+	+	-
<i>Brachionus diversicornis</i>	-	+	-
<i>Brachionus plicatilis</i> species complex	+	+	+
<i>Brachionus rubens</i>	-	+	-
<i>Brachionus urceolaris</i>	+	+	-
<i>Cephalodella catellina</i>	-	*	+
<i>Colurella adriatica</i>	-	-	*
<i>Filinia longiseta</i>	+	-	-
<i>Hexarthra polyodonta</i>	*	+	+
<i>Keratella cochlearis</i>	-	-	-
<i>Keratella quadrata</i>	*	+	-
<i>Lecane lamellata</i>	-	-	*
<i>Lecane luna</i>	-	-	-
<i>Notholca salina</i>	*	+	+
<i>Trichocerca pusilla</i>	-	*	-
<i>Alona</i> cf. <i>salina</i>	-	-	*
<i>Daphnia magna</i>	+	+	+
<i>Daphnia obtusa</i>	-	-	*
<i>Daphnia</i> cf. <i>curvirostris</i>	-	-	*
<i>Diaphanosoma</i> sp.	*	+	-
<i>Macrothrix</i> sp.	*	-	+
<i>Moina brachiata</i>	-	+	-
<i>Acanthocyclops robustus</i>	+	+	+
<i>Cyclops</i> sp.	*	-	-
<i>Diacyclops</i> sp.	*	-	-

+/- indicate present/absent taxa; * indicate present species not being previously recorded as compared to previous years and previous studies (Michaloudi and Kostecka, 2004)

4. DISCUSSION

Efforts for restoring system function must take into account the role of individual species, particularly if some species play a key role, or entire assemblages (Palmer et al, 1997). In our study, both water bodies, Lake Kastoria and the temporary brackish Koronia showed different changes of phytoplankton from key role species to entire assemblages and community properties. These changes indicated self-organization of phytoplankton community during restoration. In Lake Kastoria, a significant decrease of total phytoplankton biomass and cyanobacterial biomass was evident ten years after sewage diversion in 1994. Still cyanobacterial dominance persisted because of the persistent dominance of the toxic species *Microcystis aeruginosa*. An increase of its biomass the last years (2010-2011) could be associated with phosphorus release from the phosphorus rich sediment (data not shown) and cyprinids introduction (Beglioglou et al, 2007). However, the significant increase in species diversity after sewage diversion was indicative of community

restoration (see Zobel et al, 1998). Biodiversity (e.g. species richness, abundance) seems a good target and overall indicator for evaluating the success of ecological restoration (Baker et al, 2000). On the basis of species level the long persistence of the toxic *Microcystis aeruginosa* was indifferent to sewage diversion. This species has been observed to tolerate unsuitable conditions both by using water-sediment interface for resting and nutrient uptake and remaining in vegetative growth under ice (Katsiapi et al. 2011). High microcystin concentrations and heavy hepatotoxic cyanobacterial blooms have been reported in Lake Kastoria (Cook et al. 2004). The presence of hepatotoxic *Microcystis* blooms in the lake is known to pose a potential hazard to human health (Gkelis et al. 2005). *Microcystis* bloom samples collected in 2007 and 2010-2011 were found positive for the *mcyA* gene (data not shown). It follows that in Lake Kastoria monitoring of phytoplankton species richness, composition, total and cyanobacterial biomass and blooms of toxic species should become a target in itself by the management authorities for ecological restoration. Furthermore, this monitoring can be used as tool in assessing health risks.

In the temporary Koronia, on the basis of phytoplankton total abundance and biomass, abrupt changes were in agreement with the temporary nature of the heavily polluted and artificially brackish water Koronia (Michaloudi et al, 2009). On the basis of species, *Microcystis aeruginosa*, assigned to the sediment “seed bank” species, was tolerant to harsh conditions for years (salinity up to 25 psu, complete drought and vigorous flooding re-suspending sediment). The species was observed alive as shrunken-flat colonies in dry sediment, recruited in the lake water through sediment re-suspension during flooding in February 2010 (establishing high population in summer-autumn. For Koronia, except for its species supply through recruitment of “seed bank” the colonization and invasion processes were also important. The poor available species pool made by all species, the first settlers, invaders and sediment “seed bank” species in association with the changeable and harsh environment could not facilitate a sustainable recovery in the near future. An availability of a large phytoplankton species pool could facilitate lake response in a similar way that high diversity facilitates grassland recovery from drought (Carpenter and Cottingham, 1997). The very low species diversity and disturbed local plankton communities increased invasibility by the known toxic phytoplankters *Prymnesium parvum*, *Arthrospira fusiformis*, *Anabaenopsis arnoldii* and *Nodularia spumigena*. Blooms of the established invaders, *P. parvum* in 2004, and *A. fusiformis*, *A. arnoldii* in 2007 coincided with mass kill of birds and fish (Moustaka-Gouni et al, 2007; Michaloudi et al, 2009). In 2010, increase of *N. spumigena*, the first cyanobacterium reported in the scientific literature by Francis (1878) poisoning animals in the Australian Lake Alexandrina, was disrupted by sharp decrease in salinity from 11 to 2 psu within two weeks of vigorous flooding in February 2010. Concerning the pioneers in colonization of Koronia, the successfully established high populations (*Koliella*, *Ankyra* and *Pyramimonas* species) all r-strategists (e.g. Lampert and Sommer, 1997) were replaced rapidly by sediment “seed bank” species (e.g. *Oocystis* and *Euglena* species). These winners have been observed dominating in heavily polluted environments such as wastewater treatment plants or sewage stabilization ponds (e.g. Alvarez-Cobelas and Jacobsen, 1992).

For zooplankton the community following dry periods was open to new arrivals, as seen by the increased percentage of species not previously recorded, through dispersal and water connection (Havel and Shurin, 2004). When the community is not saturated it is open to colonization. Nevertheless, the recovery of zooplankton is facilitated by the bank of diapausing eggs in the sediment. Species emerging from the lakes’ sediment depending on the water quality and species interactions will be able or not to establish successful populations (De Meester et al, 2002; Brendonck and De Meester, 2003). The species that finally dominated the zooplankton community were those that came from the lakes’ own egg bank (Michaloudi et al, 2012).

Assembly of plankton communities on the basis of species group sequence of the established species pool (first settlers, invaders and sediment “seed bank” species) showed alternative pathways. These pathways had obvious similarities in the years of similar hydro-morphological features experiencing a dry phase to a lesser or greater extent. The critical impact of the sediment supply to established species as final winners in succession was evident each year. The ‘seed bank’

from the sediment plays role for phytoplankton community development and succession as well as the egg bank for the recovery of the zooplankton community on flooding of a temporary wetland after a drying event (Brock et al, 2003). The cyclical nature of the dry phase and its predictability in natural temporary ponds allows temporary water species to be adapted to water loss (Naselli-Flores and Barone, 2012).

In conclusion, as we have dealt with plankton community assembly of different water bodies under restoration, our study suggests that restoration targets for both water bodies should be set including biodiversity and target species by themselves. In particular, Koronia's phytoplankton species richness, composition, blooms of toxic, invading and sediment "seed bank" species winners and available species pool monitoring should become a target in itself by the management authorities for its restoration. This work shows some prerequisites regarding setting targets for managing ecological restoration of lakes and temporary water bodies under a Mediterranean climate.

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