

Mechanisms underlying recovery of zooplankton in Lake Orta after liming

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ABSTRACT

The goal of this study was to improve the understanding of the large-scale mechanisms underlying the recovery of the zooplankton of Lake Orta from historical contamination, following reduced input of ammonia and metals and the subsequent 1989/90 liming intervention. The industrial pollution had been severe and long-lasting (1929-1990). Zooplankton biodiversity has improved, but most of the new taxa appearing in our counts are rotifers, while many calanoids and the large cladoceran predators (Bythotrephes and Lepidodora) that are common in the nearby Lake Maggiore, were still absent from Lake Orta 17 years after liming. To aid understanding of the large-scale mechanisms controlling changes in annual richness, we assessed the annual persistence (P) of Crustacea and Rotifera taxa as an estimator of whether propagules that survived introduction, as result of the natural recolonization process, also thrived. We found that the rate of introduction of zooplankton colonists and their persistence in the water column of Lake Orta changed from 1971 to 2007. New rotifer taxa appeared in the lake after the mid-1980s, when discharge of toxic substances decreased, but their annual persistence was low ($P < 0.5$) until the turn of the century. The numerical values of rotifer and crustacean persistence in Lake Orta were unexpectedly high in 2001 and 2007 (0.55 and 0.72 for rotifers, 0.85 and 0.86 for crustacean, respectively), much higher than in limed lakes in Sudbury, Canada, and in adjacent Lake Maggiore. We hypothesize this could be related to the lack of Cladoceran predators and zooplanktivorous fish in the pelagic waters of Lake Orta.

Key words: Annual persistence; annual taxa richness; pollution; zooplankton; Lake Orta.

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INTRODUCTION

Lakes have been acidified by acid mine drainage, liquid industrial effluents, and by the long-range transport of air pollutants. While long-range transport of sulfur and nitrogen oxides was the dominant cause of lake acidification in eastern North America and Scandinavia (Henriksen *et al.*, 1988; Renberg *et al.*, 1990; Dillon *et al.*, 1987), Lake Orta, in northern Italy was acidified by decades of *in situ* oxidation of liquid ammonia waste from a textile plant. In addition, it was contaminated by heavy metals including copper, chromium, zinc, cadmium, and aluminum associated with metal plating industries in its watershed (Calderoni *et al.*, 1990; Calderoni and Tartari, 2001; Bonacina, 2001). Pollution of Lake Orta started in 1926 and within a few years damage to biota was so severe that its waters were deemed *sterile* (Monti, 1930). It was not until 1980 that the chemical improvement in the lake finally began when new regulations required substantial reductions in the discharge of pollutants. Recovery was then accelerated by a massive in-lake liming intervention in 1989-1990 (Calderoni *et al.*, 1990), which brought the water column pH back to circum-neutrality, inducing the precipitation of a large proportion of the dissolved metals from the water column into the sediments (Calderoni and Tartari, 2001). Zooplankton community

structure responded promptly to these improvements in water quality, increasing in diversity (Bonacina, 2001).

Given many successful efforts to reduce both acid deposition and liquid industrial effluents to receiving waters, the attention of aquatic ecologists has turned to what factors regulate the pace, extent and dynamics of recovery of previously damaged communities (Monteith *et al.*, 2005; Keller *et al.*, 2007). For lakes that were historically damaged by atmospheric acid inputs, both external (colonist delivery to the pelagic community from the metacommunity or egg bank) and internal (colonist survival and growth) factors may be important, and which are more important can vary among lake regions and over time (Gray and Arnott, 2012). In lake-rich regions such as the Canadian Shield, there are many colonist sources, and, for these lakes, internal processes appear to dominate, *i.e.* not the rate of arrival of colonists but their subsequent ability to survive and thrive (Yan *et al.*, 2016). We know less about the key drivers of recovery in historically acidified Lake Orta, which likely has fewer natural sources of colonists because it is not in a lake-rich region, at least in comparison with the Canadian Shield. It does, however, have a longer history of human development, and people do increase colonist delivery rates, sometimes by many fold (Hebert and Cristescu, 2002; Williamson, 1996). Because of these differences in the metacommunity

nity and in its development history, we might expect that the key mechanisms underlying recovery in Lake Orta may differ from those underlying recovery of atmospherically-acidified lakes on the Canadian or Scandinavian shields. Differences may also arise because of differences in the water quality trajectories in Lake Orta when compared with atmospherically-acidified lakes.

Here our goal is to improve the understanding of the large-scale mechanisms underlying the recovery of the zooplankton of Lake Orta from historical contamination following the 1989/90 liming (Bonacina, 2001). Our specific objectives were to determine: i) if the rate of introduction of zooplankton colonists or their persistence in the water column of Lake Orta have changed over time; ii) if changes in colonist introduction rates are correlated with anthropogenic or natural changes in the watershed; and iii) which changes in the lake's water quality best correlate with observed changes in annual persistence of zooplankton colonists in the recovering pelagic community. While the pattern of recovery of the zooplankton community of Lake Orta soon after liming has been described (Manca and Comoli, 1995; Bonacina, 2001; Signorini, 2005), to date possible zoogeographic vs ecotoxicological mechanisms that underpin this recovery have not been examined. We hypothesized: i) that the rate of introduction of colonists would have increased over time as settlement and use of the watershed increased; and ii) that the annual persistence of these colonists increased because of improvements in water quality, especially increases in pH and decreases in Cu levels after liming and changes in industrial practices. While changes in fish predation also appears to influence annual persistence of recovering zooplankton assemblages in Canada (Yan *et al.*, 2016), the continued absence of native, pelagic zooplanktivorous fish in Lake Orta (Volta *et al.*, 2016), and in pelagic, micro-invertebrate predators (Bonacina, 2001; Signorini, 2005) suggested to us that predators were likely as important as water quality changes as regulators of annual persistence.

METHODS

Lake Orta, the seventh largest Italian lake by volume, is a deep ($z_{\max}=143$ m, $\bar{z}=71$ m), warm, oligo-monomictic lake located in the Italian subalpine region. Details about pollution history and chemistry are described in Rogora *et al.* (2016).

Zooplankton samples were collected in the vicinity of a station corresponding to the maximum depth of the lake with a Clarke-Bumpus plankton sampler equipped with a 76 μm nylon net, which from previous experiment resulted to be representative of zooplankton population, both crustacean and rotifers (Monogononta; Ramoni, 1997). We sampled from a boat cruising at a low, constant speed (0.6 m s^{-1}), raising and lowering the net two times through 50 m in a sinusoidal trajectory and filtering at

least 1000 L of lake water (de Bernardi, 1984). The resulting integrated samples are little influenced by the occurrence of plankton swarms and represent the pelagic community, which is mainly restricted to the upper 50 m of the water column (Manca *et al.*, 2000). In all years, samples were immediately fixed in 90% denatured ethanol and then transferred into 10% formaldehyde. Using identical methods at all times, zooplankton were sampled monthly in 1971, 1980, 1981, 1986, 1988-1998 inclusive, 2001 and 2007. Zooplankton were identified to species wherever possible and counted at 63x magnification. At least 10% of the total sample volume was examined (Manca *et al.*, 1992).

Here we used the approach suggested by Yan *et al.* (2016) to calculate rates that colonists arrived, survived and thrived each year from the encountering of species new to our records in the lake, and from annual species persistence metrics. While Yan *et al.* (2016) compared lakes, here we mainly compared different blocks of times and differing groups of zooplankton (*i.e.*, rotifers vs groups of Crustacea). We examined the temporal dynamics of the accumulation of novel species (N), and the annual persistence of taxa (P), and their correlations with logical driver variables (*i.e.* chemical variables as determinants of P, and rotifer resting eggs abundances in sediment, human population and wind data from Maggiore as determinants of N, respectively reflecting *in situ* colonization potential, human assisted and natural dispersal vectors). We had no wind data from Lake Orta, but for reasons given in Sturman and Wanner (2001), we are confident that wind data from nearby Lake Maggiore can serve as an effective proxy for wind fields over Lake Orta.

Annual persistence of species (P) was calculated according to the following equation:

$$P=1-((ST-S)/ST)$$

where ST is the total number of taxa observed in a year and S is the average number of taxa observed in single samples. P, which varies between 0 and 1, represents the fraction of species that are present in each sample throughout the year. If P value is close to 0, the majority of taxa were found only once, while if it is close to 1 they populated most samples.

To help interpret the recent persistence patterns in Lake Orta, we calculated the persistence of zooplankton taxa in Lake Maggiore for years 2001 and 2007. Lake Maggiore is another subalpine lake that is close to Lake Orta and located in the same catchment basin. Unlike Lake Orta, it never acidified, and its zooplankton community was never decimated by acid or metal pollution. It thus can serve as a reasonable reference (Salmaso and Mosello, 2010). The zooplankton of Lake Maggiore was assessed in a identical manner to that of Lake Orta.

To determine the role of the internal rotifer egg bank we compared the cumulative curve of rotifer resting eggs extracted from a Lake Orta sediment core previous analysed (Piscia *et al.*, 2016) to the cumulative curve of annual new taxa. Between-sample differences in colonist taxa persistence, *P*, were analyzed by the parametric Scheffé test, while Spearman's rank correlation coefficient, *R*, was used to test the statistical significance between cumulative data of rotifer resting egg and cumulative data of new taxa in the pelagic column. All tests were run in Statistica v. 12.

RESULTS

The total number of taxa encountered each year (S_{total}) in Lake Orta began to increase in the mid-1980s and has been steadily increasing ever since (Fig. 1). Except for copepods, for which *S* was quite constant ($S_{copepods}=1-2$ taxa y^{-1}) during the entire period, the mean annual richness, *S*, of all other groups increased quite linearly. While the trends in *S* were positive for Cladocera, all Crustacea, and rotifers, the slopes did differ, at 0.15, 0.20 and 0.38 taxa y^{-1} , respectively. *S* started to increase before liming, likely as consequence of early water-quality improvement, but it certainly increased continually after liming.

The number of taxa of both rotifers and crustacean zooplankton increased by about 4 fold over the record, but the largest absolute number of new taxa detected in Lake Orta were rotifers. The cumulative number of rotifer taxa detected (*N*) increased from 17 in 1971 to 81 in 2007, but, for Crustacea, the overall increase was more modest (*i.e.*, from 7 in 1971 to 31 in 2007; Fig 2). Among crustaceans, the total increase of copepod species richness was very low (from 2 to 7); hence, it was colonizing Cladocera that dominated the crustacean time trend (Fig. 2). We can distinguish four phases in the species accumulation curve

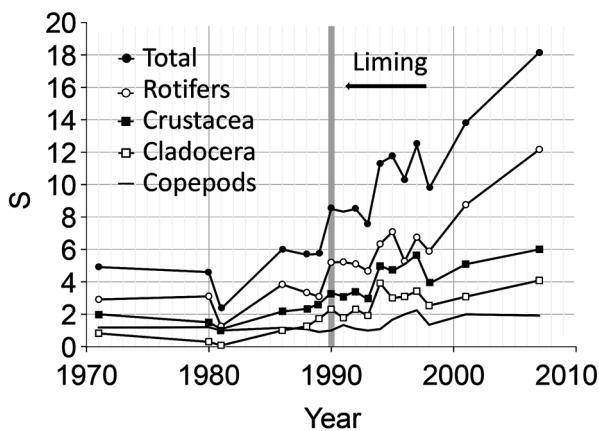


Fig. 1. Trend of average annual taxa richness (*S*) from 1971 to 2007 in Lake Orta. The gray bar indicates the liming period.

(*N*, Fig. 2). From 1971-1981 (Phase 1), *N* values for rotifer and crustaceans were quite stable, although only two years were sampled during this period. In Phase 1, chromium reached its maximum ($14 \mu g L^{-1}$), copper was constant but elevated (ca. $50 \mu g L^{-1}$) and pH was very acid (between 4.20 and 4.57; Fig. 2). From 1981-1989 (Phase 2), $N_{rotifer}$ almost doubled from 22 to 39, while few taxa of crustaceans (*Cyclops abyssorum*, *Eucyclops* sp., *Daphnia obtusa*, *Alona guttata*, *Bosmina* sp.) colonized the lake ($N_{crustacea}$ increased from 8 to 13). During these years chromium concentration declined (from 7 to $2 \mu g L^{-1}$), copper concentration was around $40 \mu g L^{-1}$; in addition, pH was still very acid (ca. 4) reaching its minimum of 3.89 at winter mixing in 1985. During Phase 3 (1990-2001), immediately after liming, N_{total} initially increased very rapidly until 1995, reflecting the arrival of new rotifer and new cladocera taxa, while after 1995 the increase was due almost totally to Cladocera. Pelagic waters during

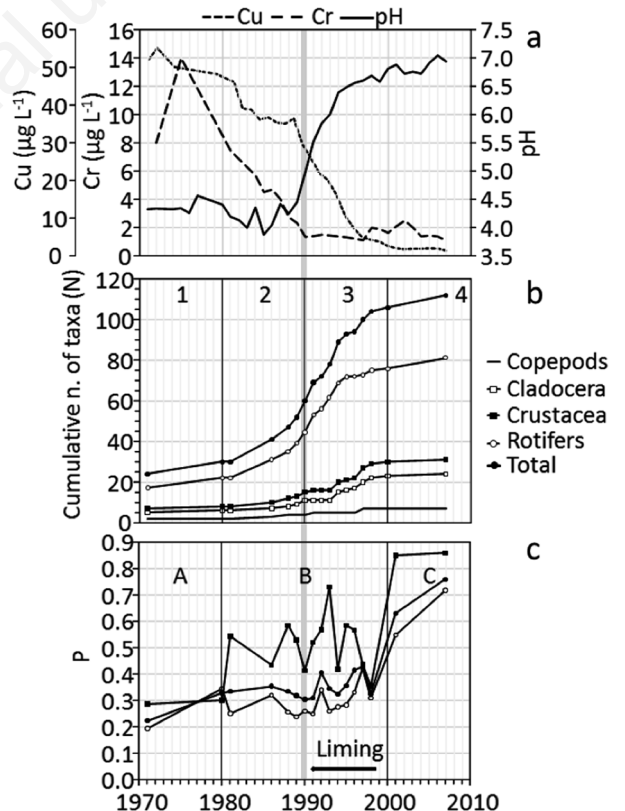


Fig. 2. a) pH and concentration of copper and chromium, calculated at winter overturn. b) Trend of cumulative number of new zooplankton taxa (*N*). c) Trend of annual persistence (*P*) of zooplankton organisms calculated for the main groups. All the data are referred to Lake Orta from 1971 to 2007. Numbers and letters highlight different phases and the gray bar indicates the liming period (see text for further explanation).

this period were characterized by a rapid increase of pH to values around neutrality, as a consequence of liming intervention (1989-1990), and by a very low concentration of metals, e.g. Cu declined from 30 to 2 $\mu\text{g L}^{-1}$ and Cr stabilized at 1-2 $\mu\text{g L}^{-1}$. Finally, from 2001 to 2007 (Phase 4), we detected only a slight additional increase in N_{rotifer} (from 76 to 81) and $N_{\text{crustacea}}$ (from 30 to 31), associated with the stabilization of good water quality in the lake, e.g., constant low levels of Cu and Cr, and circum-neutral pH (Fig 2). To detect the role of the rotifer egg bank we tested the correlation between the cumulative number of rotifer taxa (N_{rotifer}) and the cumulative curve of rotifer resting stages extracted from a sediment core (latter data from Piscia *et al.*, 2016), which resulted not statistically significant (Spearman's $r=0.31$, $P<0.05$).

Annual persistence (P) of taxa clearly increased over the period of observation, in 3 clearly discernable phases (Fig. 2). Annual persistence was lowest from 1971-1981, with both P_{rotifer} and $P_{\text{crustacea}}$ values below 0.3 (0.19 and 0.29, respectively). Thus, not only did few species appear in the lake, they did not persist for long in our collections, under the poor water quality conditions in the lake at that time (Fig. 2). From 1981-2001, annual persistence was very variable for crustacea, rising and falling between lows of 0.41 (1990) and highs of 0.73 (1993), while persistence of rotifer taxa was lower with a smaller range of values (0.24-0.43). This period was characterized by the reduction of metal concentration in pelagic waters and by liming intervention, which restored pH to neutrality. Interestingly, annual P fell during the liming intervention, a short-lived negative consequence of liming on zooplankton that has also occurred in lakes elsewhere (Yan *et al.*, 1996). Finally, from 2001-2007, P_{rotifer} and $P_{\text{crustacea}}$ increased reaching their highest values in 2007, *i.e.*, 0.71 and 0.86, respectively. During this recent period, few additional species appeared in the community (Fig. 2), but the resident species clearly had established populations that persisted for most of the year. In particular, both resident copepod species, 3 of 5 cladoceran species and 8 of 16 rotifer species were detected in every sample all year.

Annual persistence values in Lake Maggiore were comparable to those of crustaceans in Lake Orta during years 2001 and 2007. In fact, persistence of all crustaceans was as high (0.76 in 2001 and 0.74 in 2007) in Lake Maggiore as in Lake Orta (0.85 in 2001 and 0.86 in 2007). In contrast, persistence of rotifers in Lake Maggiore was much lower at 0.16 in 2001 and 0.12 in 2007 than in Lake Orta, where P for rotifers was 0.55 in 2001 and 0.71 in 2007. Statistical analysis (Scheffé test; Tab. 1) revealed that persistence of copepods has not changed during the different phases, while significant differences were observed for the other groups. In particular, persistence of cladocera, crustacea and rotifers has changed significantly between periods A and B (Tab. 1; Fig. 2), while the differ-

ence was statistically significant only for total Crustacea and Rotifera between periods B and C.

DISCUSSION

The recolonization of an historically disturbed habitat is dependent upon both the rates of colonist arrival and by their ability to survive and persist in that habitat (Yan *et al.*, 2016). In Lake Orta the quantity of colonists seems to be not a limiting factor as assessed by the increase of the average number of taxa per sample and the constant detection of new taxa (Figs. 1 and 2). In contrast, annual persistence of taxa was low and without significant changes until the end of 1990's, when persistence increased dramatically reaching the highest values. This result could be partly explained by chemical conditions. Concentrations of copper and chromium decreased steadily during our period of study, but concentrations were high enough to be toxic until the liming intervention (1989-1990; Fig. 2; Calderoni and Tartari, 2001). The tolerance to toxicants can vary greatly between zooplankton species and among clones within species (Stuhlbacher *et al.*, 1992; Soares *et al.*, 1992; Barata *et al.*, 2002), but many studies confirm that the metal levels in Lake Orta were high enough to be toxic in the earlier part of our record. For example, Arnold *et al.* (2011) reported a 48-h LC50 for Cu was 13.4 $\mu\text{g L}^{-1}$ for rotifer *Brachionus plicatilis*, while Bossuyt and Jansen (2005) reported a value of 22.6 $\mu\text{g L}^{-1}$ for *D. galeata* inhabiting Lake Leuven, confirming the toxicity of Lake Orta waters. Moreover, previous ecotoxicological research (Ponti *et al.*, 2010), conducted on *Daphnia galeata* from Lake Orta, showed that despite their high tolerance to copper (48-h EC50=67.2 $\mu\text{g L}^{-1}$), these daphniids were already negatively affected by short exposures to a sub-lethal concentration of 6.9 $\mu\text{g L}^{-1}$ of Cu. Similar results were found by Coniglio and Baudo (1989) for *Daphnia obtusa* exposed to Cr from Lake Orta. While the *D. obtusa* that have populated the lake since 1986 (Bonacina, 2001) are quite tolerant to chromium (48h-LC50=61 $\mu\text{g L}^{-1}$), short-term exposures to Cr at comparable concentrations in the lake reduce animal demographic metrics (Coniglio and Baudo, 1989). In addition Cu and Cr were not the only metals present at elevated levels in the lake in those years (1971-

Tab.1. Comparison between the 3 phases (Scheffé Test) identified in persistence curves (P) for different groups.

	A/B	A/C	B/C
P_{cop}	0.699	0.225	0.334
P_{clad}	0.043	0.001**	0.011
P_{cru}	0.025	0.000**	0.001**
P_{rot}	0.900	0.000**	0.000**
P_{tot}	0.177	0.000**	0.000**

1990). Fe, Ni, Mn and Al were also a concern (Calderoni and Tartari, 2001) and the lake was also very acid. The negative effect of acidity on zooplankton is well documented in both field and laboratory studies (Keller *et al.*, 1990; Havens *et al.*, 1993). Many species cannot tolerate pH values lower than 5.5 and in that period the pH of Lake Orta was 4–4.5 at winter overturn. Finally, additive or perhaps synergistic negative effects of all of these factors are certainly possible.

The data in Fig. 2 represent conditions at winter overturn, a useful reference for long-term comparisons, but water quality varies with depth and season in Lake Orta. Zooplankton mainly occupy the top 50 m of the lake (Manca *et al.*, 2000). In the euphotic zone (0–20m), pH increases to circum-neutral levels in the summer in response to phytoplankton photosynthesis (Rogora *et al.*, 2016), allowing the establishment of chemical conditions favourable to the growth of zooplankton organisms. Persistence was also low after liming intervention, until the end of 1990s when pH was neutral and concentration of metals abated; therefore, during this period chemical conditions cannot explain our results. Certainly biological recovery occurs gradually and typically lags behind chemical recovery (Keller *et al.*, 2002; Frost *et al.*, 1998; Keller and Yan, 1991; Locke *et al.*, 1994).

The total persistence curve mainly reflected the persistence of rotifers, which had by far the highest number of taxa in Lake Orta (Figs. 1 and 2). Cumulative new taxa and the average annual richness both suggest that biological recovery was not limited by the arrival of propagules, as observed in Sudbury lakes (Yan *et al.*, 2016); in fact, we observed an increase of richness and a constant detection of new taxa since 1981. Cladocera and rotifers, and also some copepods, produce resting stages which can survive in sediments for decades (Hairston *et al.*, 1995), where they constitute a genetic reserve of potential colonists (Hairston, 1996). Rotifer resting eggs extracted from a Lake Orta sediment cores confirm the presence of an egg bank (Piscia *et al.*, 2012; Piscia *et al.*, 2016). Both the composition (*i.e.*, morphotypes) and the abundance of this internal reserve were related to the different phases of recovery in the lake. In addition, we found many open or empty cases of *Brachionus calyciflorus* resting eggs, evidence of attempts at recolonization of the lake. The hatching of these resting stages likely has contributed to the process of recolonization and could have affected the cumulative curve of new rotifer taxa, but statistical analysis did not support our hypothesis. In fact, we did not find a significant correlation between the cumulative curve of new rotifer taxa and the cumulative curve of new morphotypes of eggs in the sediment record. This result is easily explained. The attribution of morphotypes to rotifer species was not possible, and some of them were likely produced by benthic or littoral species which do not con-

tribute to the pelagic population. Moreover, the abundance of organisms derived by the hatch of resting stages buried in Lake Orta sediments could be lower than our threshold of detection of pelagic taxa, or simply we could have missed them because they persisted in the water column for too short period of time. Moreover, while the cumulative curve for living rotifers tracked data from single years, data of the cumulative curve of resting eggs was from single layers of sediment core, which integrated at least 2 or 3 years.

Resting stages can be transported to a lake from within or beyond its catchment by migrating animals, rain and wind, all of which can contribute to the process of recolonization (Cáceres and Soluk, 2002; Incagnone *et al.*, 2014). We cannot quantify the birds or insects vector, but we do not believe that increases in wind-induced dispersal account for the rising rate of species accumulation, because average wind speeds in the region decreased from the 1970s to 2005 (Ambrosetti *et al.*, 2006). We also do not believe that human induced dispersal would have substantially increased over the last 40 years because the population increase until the mid-seventies has actually been fairly modest (Tonolli *et al.*, 1975)

Total persistence was quite variable in the middle period (period B, Fig. 2), and it was reduced by liming. This also happened in Sudbury lakes (Yan *et al.*, 1996), presumably because for species and clones that are adapted to acidity, rapid pH rise associated with liming, or the rapid precipitation and flocculation of metals associated with rising pH may actually be stressful.

After the sprinkling of carbonate, the waters of the lake were milky and this could negatively affect zooplankton organisms, or perhaps metal flocculation stripped them from the water column (Manca M., *personal communication*).

Persistence of total crustaceans was highest after 1981, while rotifer persistence was lowest at this time. This result was likely attributable in part to the lower annual average richness for crustacea compared with rotifers ($S_{\text{crustacea}}=1.5\text{--}6$ taxa, $S_{\text{rotifer}}=3.1\text{--}12.2$ taxa), and by a less marked seasonality of crustacean taxa, most of which were present the entire year. The high persistence of crustaceans may also be related to the absence of pelagic zooplanktivorous fish in Lake Orta (*i.e.*, whitefish and shad; Volta *et al.*, 2016). If this is the explanation for the high persistence, then the planned introduction of pelagic fish from 2016 to 2020 could modify the seasonality and thus the annual persistence of crustacean zooplankton in the lake.

High persistence certainly contributes to high average species richness, but it is not always a sign of ecological recovery. Annual persistence of rotifers (P_{rotifer}) was very low (2001=0.16 and 2007=0.12) in Lake Maggiore compared to Lake Orta. The simplest explanation for this difference is the presence in Lake Maggiore of the two pelagic macroinvertebrate zooplanktivores *Leptodora* and

Bythotrephes, which have been shown to regulate the abundance of rotifers and small crustaceans (de Bernardi *et al.*, 1987; Manca and Comoli, 1995), reducing their population size below our detection limits on occasion and thus lowering their annual persistence. These two predators are still absent from Lake Orta, allowing unharvested prey populations to persist much of the year. Before industrial pollution, the zooplanktonic populations of Lake Orta and Lake Maggiore were similar (Salmaso and Mosello, 2010). Pioneering limnological studies reported that zooplankton were very abundant and diverse in Lake Orta, the community including *Daphnia* spp., cyclopoids, diaptomids, *Leptodora* sp., *Sida* sp., *Bosmina* spp., *Diaphanosoma brachyurum*, *Asplanchna priodonta*, *Kellicottia longispina*, *Keratella cochlearis* and *Polyarthra platyptera* (Monti, 1930). Zooplankton disappeared in the water column after the beginning of industrial pollution and *Leptodora*, diaptomids and *Sida* remain absent to this day. *Sida crystallina* was also present in Lake Maggiore until 1967 (Manca and Comoli, 1995) when the lake was undergoing rapid eutrophication. Diaptomids are very important in Lake Maggiore, but they disappeared from Lake Orta after the pollution, while they have recolonized historically acidified Sudbury lakes (Locke *et al.*, 1994; Arnott *et al.*, 2001), including limed lakes (Yan *et al.*, 2004). Their lack of recovery in Lake Orta might be linked to their lack of production of resting stages, and to the fact that Lake Orta waters flow into Lake Maggiore, while recovering Sudbury lakes are surrounded by many lakes which can provide colonists.

CONCLUSIONS

In this study we analyzed recent zooplankton data from Lake Orta in order to understand broad-scale drivers of their recovery after 80 years of acid and metal contamination and a restorative liming intervention. Following Yan *et al.* (2016), we used the annual persistence of zooplankton species as one parameter to estimate the mechanism underpinning zooplankton recovery. Contrary to Middle and Clearwater lakes in Sudbury (Yan *et al.*, 2016), the only other lakes where this metric has been used, annual persistence has changed over time in Lake Orta, suggesting that different processes regulate recovery of richness in these case studies. The lack of pelagic fish predators (Volta *et al.*, 2016) in Lake Orta could provide the explanation. Planktivorous fish populations increased after liming in Sudbury lakes (Yan *et al.*, 2016). In contrast in Lake Orta, not only have pelagic species of zooplanktivorous fish not yet returned, but the large native predatory cladocerans, *Bythotrephes* and *Leptodora*, which are common in the nearby Lake Maggiore, have not yet returned to Lake Orta. In particular, we hypothesize that the unusually high annual persistence of rotifers in Lake Orta in comparison to Lake Maggiore is related

to the absence of the top-down control from cladoceran predators. Should they reinvade Lake Orta, we suspect that a more typical seasonality of rotifers may reoccur.

In summary, our examination of three zooplankton richness metrics results suggests that the recovery of the zooplankton community of Lake Orta was not limited by the arrival of new colonists but that the bottleneck was the ability of organisms to survive and thrive in the lake, confirming that annual persistence and taxa accumulation parameters can help provide understanding of mechanisms underlying the recovery of zooplankton. When compared with Lake Maggiore, our results also suggest that not all increases in annual persistence suggest recovery, rather if much higher than reference systems they may suggest a transient state of recovery brought on by unusually low predation pressure in the absence of both planktivorous fish and predatory Cladocera.

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