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Spring rotifer community structure in the Alcantara River (Sicily, Italy), using different mesh size nets: relation to environmental factors

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Abstract – The present study focus on some aspects of zooplankton structure in the Alcantara River (Sicily, Italy), in relation to environmental factors. Zooplankton samplings were performed in spring in four sites, located from up- to downstream along the river course. Four low-flow velocity station points were chosen along a transversal river section in each site. Samples were taken from all station points in the four sites by two different mesh sizes (55 and 100 μm) rectangular nets. Rotifer abundances were an order of magnitude higher in 55 μm mesh size samples than in 100 μm mesh size ones. The two communities also resulted significantly different (ANOSIM test, $\rho = 0.212$; $P = 0.1\%$). Generally, low abundances (from 3470 ± 5133 to 422 ± 474 ind.m^{-3}) were explained by low chlorophyll *a* concentration and the high-flow regime of this river. Rotifer dominated the zooplankton community. Cladocerans, copepods and nauplii occurred with considerably lower abundances than rotifers. However, the relative contributions of these taxa to total abundances depended on the mesh sizes used. *Euchlanis* and *Adineta* genera exhibited the highest abundances in the rotifer assemblage. Conductivity alone or in association with temperature and dissolved oxygen was the most important environmental factor affecting rotifer community distribution. *Cephalodella* sp., *Lepadella* sp. and *Trichotria pocillum* showed a high positive relation to pico-plankton, showing this fraction as a possible rotifer food item. This paper demonstrated a higher efficiency of the finest net to characterize riverine zooplankton community that increases from up to downstream in terms of abundances and diversity.

Key words: Zooplankton / net selection / abundance and diversity / freshwater ecosystem

Introduction

In aquatic system food chain, zooplankton represents a fundamental component since it is not only the main link of the energy flow between primary producers and consumers at higher trophic levels (Calbet, 2001). Also, it is involved in nutrient recycling, since it modulates nitrogen and phosphorus concentrations through their excretion processes (Horne and Goldman, 1994). Recently, the main part of studies, carried out on freshwater ecosystems, focused on micro- and macroinvertebrate community structures in lentic systems (Fernández Aláez, *et al*., 1999; Boix *et al*., 2001a, 2001b; Boix and Sala, 2002; Beklioglu *et al*., 2003; Romo *et al*., 2004; Álvarez-Cobelas *et al*., 2005; Martinoy *et al*., 2006; Boix *et al*., 2008). Despite the latest increasing interest on large river ecosystems, our knowledge of the riverine zooplankton remains fragmentary. Relatively little early researches were conducted on phyto and zooplankton compared to several studies on macroinvertebrates, fishes and freshwater mussels (Thorp *et al*., 1994; Welker and Walz, 1998; Jack and Thorp, 2002). This lack of research could be a consequence of the impression that rivers are not suitable environments for zooplankton component. This resulted in an underestimation of the essential role of zooplankton in trophic dynamic of rivers (Lair, 2006). On the other hand, studies on zooplankton community may be useful in predicting long-term changes in aquatic ecosystems, since they are very sensitive to environmental variations (Branco *et al*., 2000; Pereira *et al*., 2002). Its spatial and temporal patterns are of crucial importance for understanding ecosystem functioning because it can affect its

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processes and stability, and it can constitute a quickly response to major shifts in environmental conditions (Suikkanen *et al.*, 2007; Zhou *et al.*, 2009). In fact, changes in zooplankton abundance and diversity provide important indications of environmental alteration or disturbance (Chalkia *et al.*, 2012).

In rivers, zooplankton assemblage is often dominated by rotifers, bosminids and juvenile copepods throughout the year, with a negligible occurrence of large-body cladocerans and calanoid populations (Kobayashi, 1997; Viroux, 1997; Welker and Walz, 1998; Špoljar *et al.*, 2007, 2012a, 2012b; Zimmermann-Timm *et al.*, 2007). Rotifers, often the main component in freshwater ecosystems, play a key role in the pelagic food chain; they are a fundamental link between phytoplankton and secondary consumers, since they act as vector in the matter and energy transfer from bacteria and small detrital particles, not consumed by other planktonic organisms at higher food chain levels (Conde *et al.*, 2004). Their importance in aquatic food webs (Porter, 1995; Jurgens *et al.*, 1999; Miracle *et al.*, 2007) is explained also at the higher trophic levels, as prey items for fishes (Bass *et al.*, 1997; Chick and Van Den Avyle, 1999; Sampson *et al.*, 2008). This group has been far less studied relative to crustacean zooplankton (Chick *et al.*, 2010). Chick *et al.* (2010) explained this tendency to overlook rotifers by methodological roots that caused an underestimation of rotifer abundances. Net mesh size variations have a significant effect on the organism capture and prey selection and thus the choice of the mesh size depends on the taxa to be sampled and it varies mainly in relation to the size of the organisms and their ability to avoid the sampler. It follows that individuals in the lower range of mesozooplankton have been largely ignored in many studies based on standard sampling with 200–330 μm plankton nets (Evans and Sell, 1985; Paffenhöfer, 1998; Hwang *et al.*, 2007; Pitois *et al.*, 2009). Overall, the 200 μm mesh nets capture $<10\%$ of the metazooplankton community in terms of numbers, while the biomass is underestimated by one-third and the secondary production by two-thirds. In oligotrophic offshore regions, for example, the 63 μm mesh nets have been shown to capture one order of magnitude more individuals than the 200 μm nets (Hopcroft *et al.*, 1998; Gallienne and Robins, 2001). Furthermore, owing to their small size and permeable integument (Nogrady *et al.*, 1993), rotifers are particularly susceptible to physical and chemical variations in their environment, especially temperature, oxygen concentration, light intensity and pH (Hoffman, 1977). Among abiotic factors the flow regime is surely the most important one since stream flow generally reduces zooplankton density (Pace *et al.*, 1992; Jack and Thorp, 2002). In contrast, rivers may resemble lentic systems during the extended periods of low flow and may develop "lentic" zooplankton communities especially in large lowland rivers (Shiel and Walker, 1984) or in their backwaters with very slow water flow. For example, in Danube River, high zooplankton abundances were detected within slow flowing inshore habitats (Reckendorfer *et al.*, 1999; Baranyi *et al.*, 2002), a phenomenon that supported the "inshore retention concept" (Schiemer *et al.*, 2001).

The character of a watershed, including the topography, hydrology and biota, is the product of three quasi-independent processes or features: geology, climate and tectonic settings (Mount, 2010). In Sicily, volcanism is concentrated at the junction of the Siracusa escarpment and the thrust belt in the Mount Etna area. Pre-Etnan basaltic volcanism began at about 5 Ma and was later superceded by the trachyandesites and trachytes of Etna itself (Behncke, 2001). The Alcantara River has the peculiar features of volcano origin basin, resulted from repeated eruptions of Volcano Etna in the prehistoric and protohistoric epochs. Its bed was affected by the invasion of imposing flows coming from the middle-low north slope of Etna. This flows reaching the ancient bed of the river, must at different times have obstructed or modified the course, pushing it toward the buttresses of the Nebrodi, in the mountain stretch, and of the Peloritani, in the valley stretch. Incandescent fluid and fuming lavas, which were first channeled and then slowly cooled along the watercourse, allowed gorges edged by prismatic morphologies.

The main purpose of the present study was to analyze riverine plankton community structure with particular emphasis to the dominant group of rotifers from the Alcantara River (Sicily, Italy), along the entire river course. Furthermore, we aimed to measure the most efficient sampling method between two mesh size nets, and to investigate relation between environmental parameters and zooplankton/rotifer community/structure.

Materials and methods

Study area

The Alcantara River is placed in the eastern part of Sicily (Italy) and it is the second most important river in Sicily after Simeto River. It rises on the south side of Monti Nebrodi, at an altitude of 1250 m above sea level (a.s.l.), and its mouth in the Ionian Sea at Capo Schiso in Giardini-Naxos (Fig. 1). The river is 52 km long, with a catchment area of ~ 570 km^2 an average discharge of 60 m^3.s^{-1}. Over the millennia, the river flowing and naturally eroding Etna basalt, created in different stretch remarkable gorges and ravines, the so called "*Gole dell'Alcantara*". This area represents one of the wettest habitats in Sicily and Alcantara River is one of the little perennial water streams. The waters of this river are used to electrical energy production, irrigation and drinking supply. The main sources of contamination are runoff from agriculture, waste and urban runoff, industrial types such as citrus processing and paper mills.

Four section areas, indicated as sites (S) were chosen to cover the entire river course in accordance to their accessibility. The first site ("Giardini", S1: 37°48'29.01"N, 15°15'15.25"E; 10 m a.s.l., 25 cm mean depth) was placed at the river estuary, where the waters of the river mixed

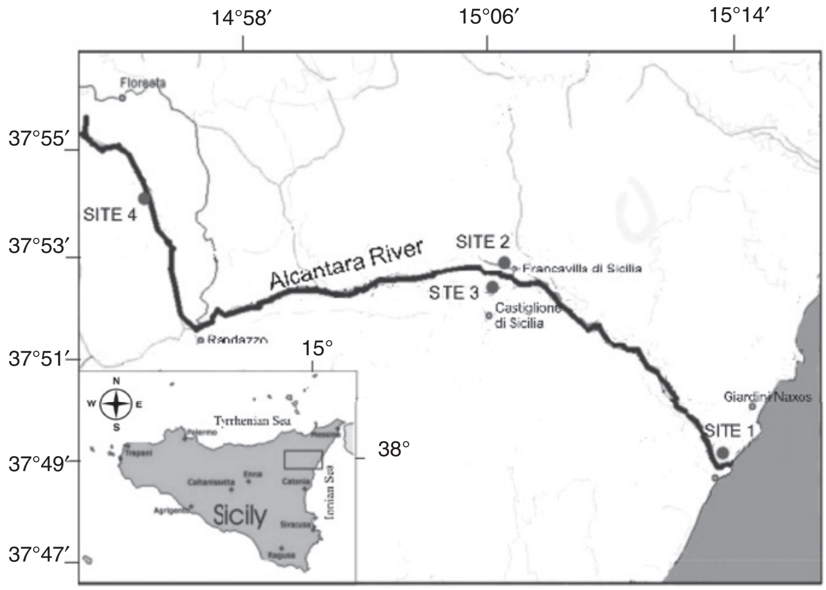

Fig. 1. Study area: four zooplankton sampling sites in the Alcantara River.

into the Ionian Sea; the second site ("Francavilla", S2: 37°53'31.22"N, 15°8'16.18"E, 320 m a.s.l., 60 cm mean depth), in this section, the Alcantara in its incessant erosive action operated for millennia on the lava flows, has created a series of small round-shaped lakes known as "Gurne"; the third ("Castiglione", S3: 37°53'21.22"N, 15°6'20.73"E, 430 m a.s.l.; 20 cm mean depth) is the central basin of the river, where the rapid flow of water forms the so-called "*Gole di Castiglione*" in which the result of slow cooling of lava flow produced unusual hexagonal basalt prisms; and the forth one ("Randazzo", S4: 37°56'59.3"N; 14°55'16.3"E, 1062 m a.s.l.; 30 cm mean depth), located at the upstream, with turbulent water flow, along about 11 km path with an average slope of 3.2%.

The average precipitation did not show wide oscillations during sampling occasions, exhibiting a very restricted range from 0.7 to 0.9 mm (History/Weatherunderground accessed by http://www.wunderground.com/history/airport/LICC/2010/4/3/MonthlyHistory.html?req_city = NA&req_state = NA&req_statename = NA).

Zooplankton samplings and environmental measures

In the framework of a large inter-disciplinary project "Ecological water quality assessment of the Alcantara, James and Guadalfeo rivers using bioindicators", in Alcantara River, sampling was conducted over a 4-day period between 18 and 24 April 2010, in four sites located along the river course (Fig. 1). In each site, four sampling points were selected across the transversal river section, regarding to the depth and the flow velocity. Zooplankton samples were collected by two rectangular mouth plankton nets, with an area of 0.1 m^2 but fitted with two meshes of 55 and 100 μm. Volume of filtered water was estimated by a flowmeter (Hydro-Bios Kiel) mounted on each plankton net. Sampling nets were kept below water surface, for 10–15 min to filter a final mean water volume of 1.2 and 1.7 m^3, for 55 and 100 μm nets, respectively. Contact with the bottom was avoided keeping the net 3–5 cm above the bed as far as possible. Zooplankton samples were preserved by a buffered 4% formaldehyde and river water solution. Organisms were counted and identified to the highest possible taxonomic level (genus or species) under a stereoscopic microscope (Leica, Wild M10, 50 × magnification) according to Dussart (1969) for copepods and Margaritora (1983) for cladocerans. Rotifer species identification was performed under an optical microscope (Zeiss, Axioskop 10 × /20, 40 × magnification), according to Braioni and Gelmini (1983).

Contemporaneously to all samplings, environmental parameters (dissolved oxygen, temperature, pH and conductivity) were measured by a multiparameter probe (YSI 6600 V2-type Multiparameter Water Quality Probe). For chlorophyll *a* (chl *a*), size-fractioned pigment analysis (microphytoplankton (> 10 μm), nanophytoplankton (10–2.0 μm) and picophytoplankton (2.0–0.5 μm)) and nutrients (orthophosphates (PO_4^{3-}), ammonia (NH_4^+), nitrites (NO_2^-, NO_3^-) and nitrates (NO_4^-)), water samples were collected from an only designated point at each site, and processed according methods described by Bergamasco *et al*. (2010).

Statistical analyses

Rotifer diversity was evaluated by the application of different diversity indices: Shannon and Wiener index (*H*': Shannon and Weaver, 1963), species richness (*d*: Margalef, 1958) and Pielou's evenness (*J*': Pielou, 1969). To study rotifer spatial distribution, cluster analysis

Table 1. Physicochemical parameters: temperature (°C), ODO ($mg.L^{-1}$), pH, conductivity (SpCond, $\mu S.cm^{-1}$), depth (m) and flow velocity ($m.s^{-1}$) in all the stations at each site.

	1(S1)	2(S1)	3(S1)	4(S1)	1(S2)	2(S2)	3(S2)	4(S2)	1(S3)	2(S3)	3(S3)	4(S3)	1(S4)	2(S4)	3(S4)	4(S4)
Temp	15.39	–	–	–	–	13.63	14.40	14.63	15.65	15.60	15.74	16.46	9.13	9.45	9.99	11.48
ODO	10.59	–	–	–	–	10.56	10.51	10.76	11.80	11.62	11.48	11.52	11.28	11.15	11.17	10.98
pH	8.86	–	–	–	–	8.01	8.27	8.24	8.67	8.69	8.78	8.80	8.82	8.86	8.97	8.97
Cond	850	–	–	–	–	800	830	830	630	630	630	630	370	380	360	370
Depth	0.40	–	–	–	–	1.40	0.20	1.30	0.50	0.70	0.35	0.40	0.35	0.80	0.40	0.50
Flow	1.40	–	–	–	–	0.50	–	0.35	0.40	0.10	0.40	0.30	0.20	0.25	0.40	–

Table 2. Two-way ANOVA for testing differences in spatial patterns of environmental parameters.

	Sumsqrs	Df	Meansqr	*F*	*P*
Temperature					
Stations	9.33	11	0.85	1.12	0.39
Sites	20.41	2	10.20	13.47	$\mathbf{1.5\times10^{-4}}$
Error	16.67	22	0.76		
Total	46.41	35			
DO					
Stations	9.69	11	0.88	1.19	0.35
Sites	22.17	2	11.09	15.03	$\mathbf{8.0\times10^{-4}}$
Error	16.23	22	0.74		
Total	48.09	35			
pH					
Stations	9.69	11	0.88	1.19	0.35
Sites	24.81	2	12.40	16.82	$\mathbf{4.0\times10^{-4}}$
Error	16.23	22	0.74		
Total	50.72	35			
Spcond					
Stations	9.27	11	0.84	1.11	0.40
Sites	51.24	2	25.62	33.79	$\mathbf{2.0\times10^{-7}}$
Error	16.68	22	0.76		
Total	77.18	35			

was run on abundances previously square root transformed and organized in the BRAY-CURTIS similarity matrix (Bray and Curtis, 1957). SIMPROF test, performed on the clusters, identified groups with significantly homogenous community structures. BIOENV option was used to relate physical and chemical parameters to biota. Differences among Sites/Stations were evaluated by ANOSIM test, grouping samples according to factor "site", "station" or factor "mesh size" to estimate differences between samples collected by the two nets. Primer Beta 6 software (Clarke and Warwick, 2004) was used for all of the above-mentioned analyses. Total chl *a* with each of its fractions and nutrients were related to zooplankton abundances by Spearman's correlation. These analyses were run on those zooplankton group abundance values sampled with the highest efficiency (55 µm mesh size net abundances for rotifers, cladocerans and copepod nauplii, instead those in 100 µm mesh size net samples for adult copepods). Differences in the catches of the most important taxa between 55 and 100 µm mesh sizes were evaluated by one-way ANOVA. Two-way ANOVA was applied to test the significance of the differences in the environmental parameters at all stations in each site and their interactions. All these tests were performed by SPSS 17.0 version.

Results

Environmental factors

In general, lower temperatures expectedly were detected upstream (min 9.13 °C, 1(S4): sampling station point (site)) and higher downstream (max 16.46 °C, 4(S3)). Dissolved oxygen concentrations varied from a maximum of 11.8 $mg.L^{-1}$ at 1(S3) to a minimum of 10.5 $mg.L^{-1}$ at 3(S2). The pH (Table 1) was mainly basic (range: 8.01–8.87). Conductivity (Table 1) ranged from 850 and 360 $\mu S.cm^{-1}$. More significant oscillations of environmental factors were estimated among sites than among stations at each site (Table 2).

Chlorophyll *a* and nutrients showed a rather similar values at the upstream sites (S4 and S3), with slightly increasing values moving downstream (S1, Table 3) with no significant differences among site ($F = 1.29$; df = 3; $P = 0.26$). The size structure of phytoplankton community was characterized by the dominance of the microfraction (Table 3). Among nutrients, concentrations ranged from 0.01 to 0.09 $mg.L^{-1}$ for ammonia; 0.01 to 0.02 $mg.L^{-1}$ for nitrites; 0.44 to 2.26 $mg.L^{-1}$ for nitrates; and 0.04 to 0.54 $mg.L^{-1}$ for orthophosphates (Table 3).

Table 3. Total chlorophyll *a*, percentage of each chlorophyll fraction (> 10, > 2 and 2–0.5 μm) and nutrient concentration at every sites in Alcantara River. These parameters were measured once a time in each site at an only station.

SITE	Total chl *a* (mg m^{-3})	Chl*a* % > 10	Chl*a* % > 2	Chl*a* % 2–0.5	PO_4^{3-}, (mg.L^{-1})	NH_4^+ (mg.L^{-1})	NO_2^- (mg.L^{-1})	NO_3^- (mg.L^{-1})	N:P
1	6.02	87.90	2.20	9.90	0.38	0.09	0.02	2.14	9.83
2	2.29	42.40	19.30	38.30	0.54	0.02	0.01	1.42	4.27
3	1.73	58.50	11.30	30.20	0.25	0.04	0.02	2.26	14.68
4	1.75	82.60	8.60	8.80	0.05	0.01	0.01	0.44	15.76

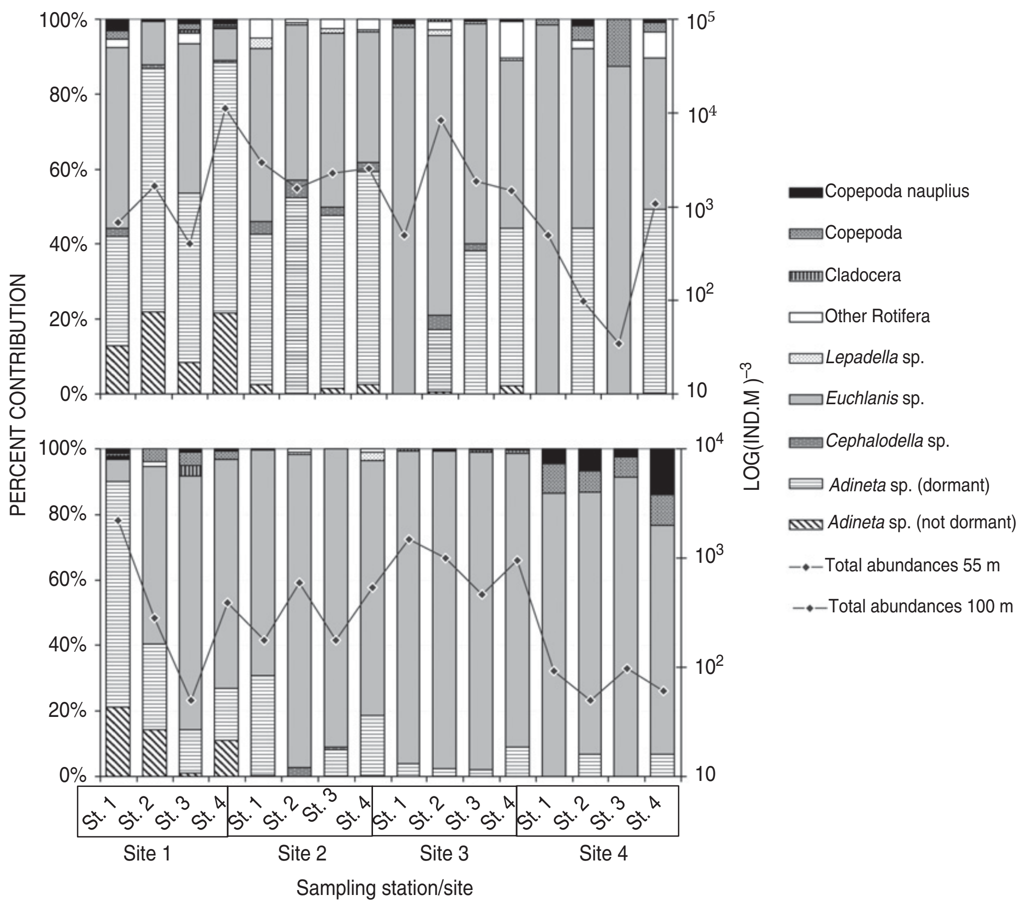

Fig. 2. Percent contribution of each taxon to total abundances in samples collected by 55 and 100 μm mesh size nets.

Zooplankton assemblage

55 μm mesh size net zooplankton abundances and distribution

Zooplankton community of the Alcantara River exhibited mean total abundances varying from numerous population of 3470 ± 5133 ind.m^{-3} at S1 to a small population of 422 ± 474 ind.m^{-3} at S4 (Fig. 2). Abundance peak occurred at station 4(S1), with a maximum of 11 126 ind.m^{-3}, and the minimum found at station 3(S4), with 34 ind.m^{-3}.

Zooplankton assemblage was almost totally represented by rotifers (98.9%). The remaining fraction was constituted by copepod nauplii (0.51%) and adult copepods (0.46%). Within rotifers, 11 taxa were recognized. Rotifers attained peak abundances of 909 ind.m^{-3} at the station 4(S1). Low densities were recorded at all stations (S4) where the minimum (2.5 ind.m^{-3}) was detected at the station 3. Bdelloid and Monogonont rotifers constituted respectively the 54 and 46% of the total rotifer community.

The most abundant rotifer genera were *Adineta* and *Euchlanis* with densities of 1236 ± 2355 and 935 ± 1461 ind.m^{-3}, correspondingly. The highest abundances (7403 and 2422 ind.m^{-3}, dormant and not dormant, respectively) of the genus *Adineta* were detected at station 4(S1). *Euchlanis* was the only genus occurring at all stations, in every sites, and overwhelming at the station 2(S3), with maximum abundance of 6215 ind.m^{-3}. The other rotifers that followed, in rank of abundance, the former two genera were: *Cephalodella* sp., that peaked in abundance

(308 ind.m^{-3}) at station 2(S3); *Lepadella* sp. with peak in abundance (128 ind.m^{-3}) at station 2 (S3); and *Trichotria pocillum* with an abundance maximum (109 ind.m^{-3}) at station 1(S2). All these three species were totally absent in S4. The only rotifer species occurring in S4, with the exception of the two above-cited dominant genera, was *Notholca squamula* that exhibited the highest density (71 ind.m^{-3}) at station 4, in this site. On average, Shannon index and species richness showed an increasing trend from up – to downstream (mean range: H', 0.43–1.06; d, 0.25–0.90), whereas an almost opposite pattern was observed for Pielou's Evenness (mean range: J', 0.64–0.51). Shannon index (Fig. 3) attained the maximum (1.2) at station 1(S2) and the minimum (0.8) at station 2(S4); species richness ranged from 1.0 at station 3(S2) to the minimum at station 2(S4). Pielou's evenness varied between 0.7 at station 2(S4) and 0.4 at station 2(S3), with higher mean values in S4.

Cladocerans, the 0.04% of zooplankton assemblage, showed the highest density at station 4(S1) and the lowest one at station 3(S1) with 12 and 5 ind.m^{-3}, respectively. Copepod attained the peak in abundance at station 2(S3) with 51 ind.m^{-3}, whereas the lowest abundance (2 ind.m^{-3}) at station 2(S1). Copepod nauplii were more abundant (153 ind.m^{-3}) at station 4(S1), whereas their lowest values (2 ind.m^{-3}) were recorded at station 2(S4).

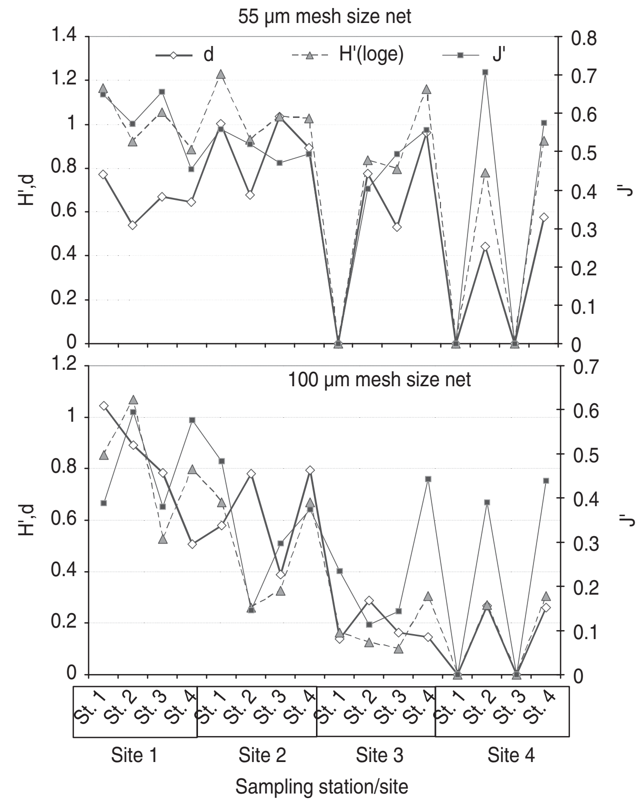

Fig. 3. 55 and 100 µm mesh size net rotifer diversity trends at every station in each site.

100 µm mesh size net zooplankton abundances and distribution

Zooplankton abundances were a magnitude order lower than those in 55 µm samples. Mean abundances in every site ranged from a maximum of 978 ± 418 ind.m^{-3}, in S3 to a minimum 75 ± 24 ind.m^{-3} in S4. The maximum (2182 ind.m^{-3}) occurred at station 1(S3) and the minimum (49 ind.m^{-3}) at station 2(S4), (Fig. 2). With regard to zooplankton composition, rotifers constituted the 98.2% of the whole zooplankton communities, copepods represented the 1.1%, copepod nauplii the 0.6% and cladocerans the 0.1%. Within the rotifer taxon, different relative contributions to total abundances of the two subclasses (Bdelloidea and Monogononta), were estimated with respect to 55 µm mesh size net samples, with percentages of 29.9 and 70.1%, respectively.

The most important genera were *Euchlanis* and *Adineta*. The former was widespread along all the river course, occurring with the highest abundance of 1421.6 ind.m^{-3} at station 1(S3). The latter attained peaks abundances (1494 and 457 ind.m^{-3}, dormant and not dormant, respectively) at station 1(S1), and was completely absent in S3 and S4. The third rotifer in terms of abundance was *Cephalodella* sp. that peaked at the station 2(S2) with abundance of 17 ind.m^{-3}. This species occurred only in this station and in stations 1(S1) and 2(S2). It was followed by *Lepadella* sp. with abundance maxima of 15 ind.m^{-3}, at station 4(S2), but occurring with lower densities only in other two stations (1(S1) and 2(S2)). Rotifer diversity (Fig. 3) showed a clearer decreasing pattern from the estuary to headwater (with the exception of some stations), but indices showed lower values than those evaluated for 55 µm mesh size net one. They varied on average from 0.13 and 0.14, respectively, d and H', to 0.81 for both indices. Pielou's evenness did not exhibit a so clear trend, ranging on average from 0.23 at S3 to 0.48 at S1. Shannon index was maximum (1.1) at station 2(S1) and minimum (0.1) at station 3(S3). This index slightly increased at some stations in S4. Species richness showed the highest value (1.0) at station 1(S1) and the lowest one (0.14) at station 1(S3). Evenness index exhibited the peak (0.6) at station 2(S1) and the minimum at station 2(S3).

Copepods, nauplii and adults, and cladocerans, showed the highest abundances at station 1 (S1) (29.0, 18.0 and 3.8 ind.m^{-3}, respectively). Copepods and cladocerans exhibited the lowest densities at stations 2 and 3 (1.6 and 0.9 ind.m^{-3}, respectively), both in S3, whereas copepod nauplii at station 3(S1) (0.5 ind.m^{-3}).

Zooplankton community structure

Significant differences between 55 and 100 µm mesh sizes in zooplankton communities were estimated by Anosim test (Global R: 0.212; $P = 0.1\%$). The significance of the differences evaluated for the main part of rotifer species are shown in Table 4. No significant differences were evaluated for the other taxa (Table 4).

Cluster analysis performed on abundances data of 55 µm mesh size (Fig. 4) identified two sample groups at

Table 4. One-way ANOVA for testing differences in the catches of the most important taxa between 55 and 100 μm mesh size nets.

df 1 (within groups) df 30 (between groups)	*F*	*P*
Adineta sp.(not_dormant)	**4.73**	**0.04**
Adineta sp. (dormant)	**7.57**	**0.01**
Cephalodella sp.	**11.32**	**0.00**
Euchlanis sp.	3.72	0.06
Keratella hiemalis	0.05	0.83
Lecane sp.	**4.59**	**0.04**
Lepadella sp.	**7.17**	**0.01**
Monommata sp.		
Notholca squamula	**5.89**	**0.02**
Trichotria pocillum	**7.28**	**0.01**
Trichotria tetractis		
Cladocera	0.01	0.94
Copepoda	0.00	0.94
Copepoda nauplius	1.01	0.32

43% of similarity: the largest one included mainly samples from S1, S2 and S3; Simprof test, applied to clusters, distinguished two community structures in this group, at 5% of significance level (gray bars in Fig. 4): the former occurred at stations 1, 2 and 3(S1), and the latter present at all stations in S2 and stations 3 and 4(S3). The other cluster included samples from three stations in S4 and station 1(S3). Pairwise comparison estimated more significant differences between S1 and S2 ($R = 0.50$; $P = 2.9\%$) and between S2 and S4 ($R = 0.68$; $P = 2.9\%$) groups of samples.

Cluster analysis performed on abundances data of 100 μm mesh size net (Fig. 4) identified two sample groups at 50% of similarity: the former included all stations in S4 and the station 3(S1), whereas the latter the all other samples, with exception of the station 1(S1). No significant homogenity in community structures among sites was estimated by Simprof test. In 100 μm mesh size net community, the pair of sample groups that showed significant differences were: S1 and S3 ($R = 0.448$; $P = 2.9\%$), S2 and S3 ($R = 0.521$; $P = 2.9\%$), S2 and S4 ($R = 0.844$; $P = 2.9\%$), S3 and S4 ($R = 1$; $P = 2.9\%$).

Correlation between zooplankton structure and environmental factors

Two-way ANOVA estimated non-significant differences of environmental parameters among study stations and sites (Table 5). The physical factor better explaining zooplankton spatial distribution was conductivity with a Spearman's coefficient (ρ) of 0.41 ($P = 0.05$) evaluated by BIO-ENV analysis. The best result of $\rho = 0.51$ was obtained by a combination of three parameters (temperature, oxygen concentration and conductivity).

More specifically, some zooplankton groups and rotifer species were significantly correlated to physico-chemical parameters (Table 6): flow velocity significantly affected spatial distribution of the rotifer species *Platyas quadricornis* and copepod nauplii; *Adineta* sp., *Cephalodella* sp. and *T. pocillum* were positively related to conductivity as well copepods. *Cephalodella* sp., *Euchlanis* sp. and *Lepadella* sp. were negatively affected by pH, whereas copepods were positively related to this parameter. *Trichotria pocillum* was the only rotifer species positively related to temperature. *Cephalodella* sp., *Lepadella* sp. and *T. pocillum* showed a high positive relation to picoplankton. Adult copepods were positively related to microphytoplankton and inversely to nanofraction (Table 7).

Discussion

The only data existing in literature on the Alcantara River zooplankton structure were provided by Pantò *et al.* (2007). However, this study is spatially restricted to the only estuary, where the current flow is very high, and limited by the too coarse mesh size used (200 μm). Therefore, the present research provides the first and more complete data on the zooplankton community in relation to environmental conditions along the entire Alcantara River.

1. Conductivity values oscillated in the normal range reported for freshwater environments (rivers and lakes) from up to downstream (Horne and Goldman, 1994). Higher, typically basic, pH was recorded in the Alcantara River with respect to other similar ecosystems, such as River Kars (Özbay and Altindag, 2009) and River Yamuna (Arora and Mehra, 2003).
2. Orthophosphate concentration was higher in the Alcantara River than in Kielstau catchment (Wu *et al.*, 2011) in the same season and almost an order of magnitude higher than that in Manahadi River, Chhattisgarh, India, throughout the year (Das and Panda, 2010). Instead, nitrates showed lower values in our study area than in Kielstau catchment in spring (Wu *et al.*, 2011), and the Manahadi River throughout the year (Das and Panda, 2010), but much higher than in the Kars River in the same season (Özbay and Altindag, 2009). In Alcantara River, high nutrient (ammonia and orthophosphate) concentrations were related to surface runoffs, containing domestic wastes and inputs from fertilizers applied to farmlands, as in the Kars River in some seasons (Özbay and Altindag, 2009).
3. The mean spring value of chl *a* in Alcantara River is much lower than the annual mean in the Kielstau River (35.8 mg.L^{-1}; Wu *et al.*, 2011) and many other European rivers, such as the Ebro (Spain) (20–45 mg.L^{-1} in the 1990s) (Sabater *et al.*, 2008) and Rhine (Germany) (21–30 mg.L^{-1} since 1992) (Friedrich and Pohlmann, 2009), and one order of magnitude lower than that from such rivers in Hungary (740 mg.L^{-1}; Kiss *et al.*, 1994), Greece (740 mg.L^{-1}; Montesanto *et al.*, 2000) or Estonia ($\sim$740 mg.L^{-1}; Piirsoo *et al.*, 2008). However, chl *a* concentration in Alcantara River is similar to that of some other rivers in Hungary (Istvánovics and Honti,

Table 5. One-way ANOVA for testing differences in physicochemical parameters among stations and sites. Significant values are highlighted in bold.

	Sumsqrs	df	Meansqr	*F*	*P*
Station	0.01	3	0.003	0.018	0.9968
Site	3.21	3	1.071	5.464	**0.0018**
Station × site	3.52	9	0.391	1.995	0.0505
Within	15.70	80	0.196		
Total	22.40	95			

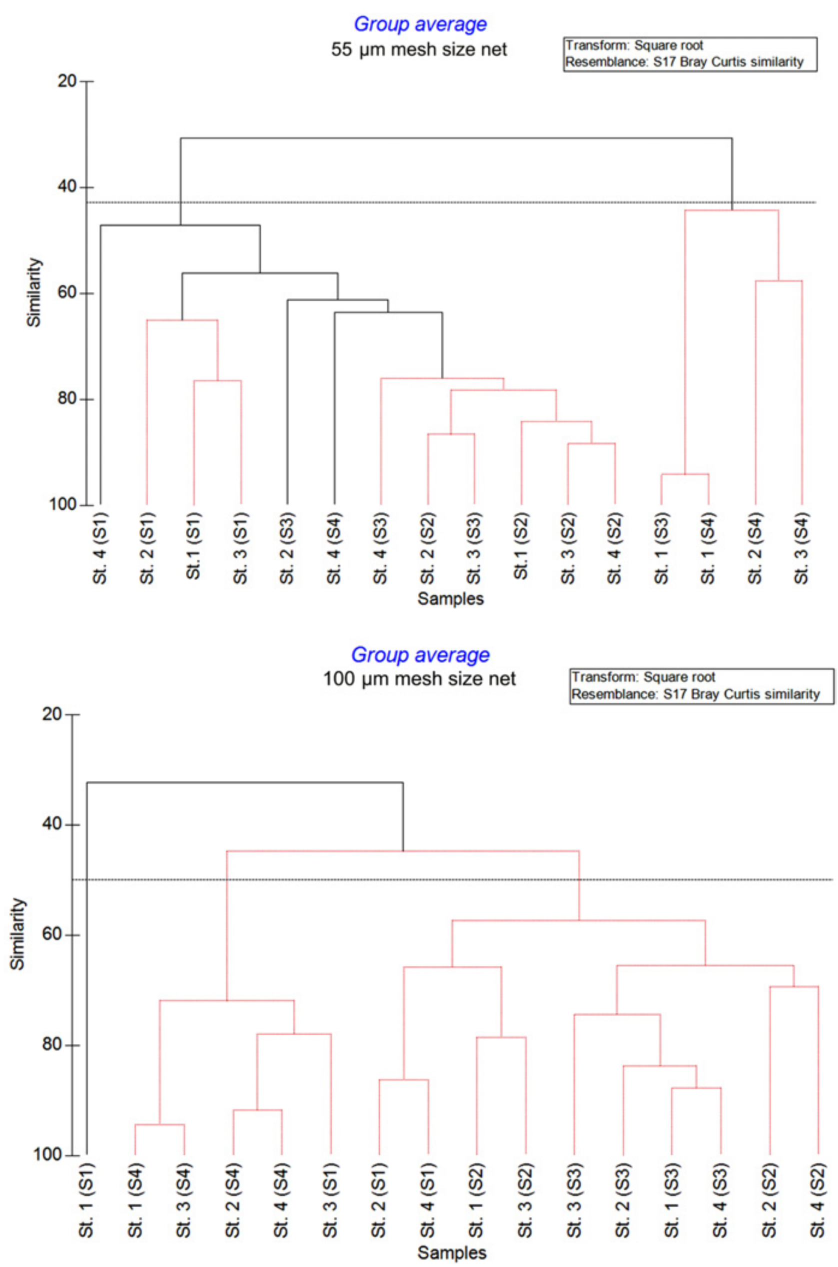

Fig. 4. Cluster analysis performed on Bray–Curtis similarity matrices of previously square root transformed 55 and 100 µm mesh size net abundances.

2011) and to Grabia and Brodnia Rivers from central Poland (~ 5 mg.L^{-1}; Sumorok *et al.*, 2009). These differences may be related to the water residence time, a key parameter that controls the biogeochemical and biological activities of aquatic ecosystems (Soballe and Kimmel, 1987; Rueda *et al.*, 2006). Reynolds'

Table 6. Spearman's ρ coefficients of zooplankton taxa and rotifer species relationship to physical and chemical factors.

	Depth (m)	Flow ($m.s^{-1}$)	Temperature (°C)	ODO ($mg.L^{-1}$)	pH	SpCond ($\mu S.cm^{-1}$)
Adineta sp.	0.07	0.54	0.34	– 0.29	– 0.18	0.68*
Cephalodella sp.	0.38	0.15	0.27	– 0.32	– 0.72**	0.72**
Euchlanis sp.	0.24	– 0.12	0.55	0.14	– 0.69*	0.43
Lecane sp.	0.39	– 0.36	0.32	– 0.02	– 0.29	0.14
Lepadella sp.	0.46	– 0.18	0.40	– 0.15	– 0.73**	0.55
Monommata sp.	0.73**	– 0.23	0.41	0.10	– 0.23	0.16
Notholca squamula	– 0.14	– 0.29	0.10	– 0.02	– 0.02	0.07
P. quadricornis	0.65**	0.73**	0.13	– 0.31	0.22	0.48
Trichotria pocillum	0.46	– 0.18	0.64*	– 0.02	– 0.49	0.63*
Trichotria tetractis	– 0.18	–	– 0.04	– 0.48	– 0.31	0.31
Cladocerans	– 0.19	0.49	0.44	0.14	0.06	0.41
Copepods	– 0.36	0.31	0.15	0.42	0.60*	– 0.34
Copepod nauplius	– 0.40	0.71**	0.47	0.18	0.39	0.03

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).
Correlations were calculated on 55 µm mesh size net abundances for rotifers, cladocerans and copepod nauplii, instead on those in 200 µm mesh size net samples for adult copepods).

Table 7. Spearman's ρ coefficients of zooplankton taxa and rotifer species relationship to trophic factors with all chlorophyll fractions and nutrients.

	Chl a ($mg.m^{-3}$)	> 10 micro	> 2 nano	2–0.5 pico	PO_4^{3-}	NH_4^+	NO_2^-	NO_3^-
Adineta sp.	0.80	0.20	– 0.20	0.40	0.80	0.80	0.80	0.40
Cephalodella sp.	0.00	– 0.80	0.80	1.00**	0.80	0.20	0.20	0.40
Euchlanis sp.	– 0.40	– 0.60	0.60	0.80	0.40	0.40	0.40	0.80
Keratella hiemalis	0.78	0.78	– 0.78	– 0.26	0.26	0.78	0.78	0.26
Lecane sp.	– 0.80	– 0.80	0.80	0.60	0.00	– 0.20	– 0.20	0.40
Lepadella sp.	0.00	– 0.80	0.80	1.00**	0.80	0.20	0.20	0.40
Monommata sp.	– 0.21	– 0.95	0.95	0.95	0.63	– 0.11	– 0.11	0.21
Notholca squamula	– 0.80	– 0.20	0.20	– 0.40	– 0.80	– 0.80	– 0.80	– 0.40
P. quadricornis	0.74	– 0.32	0.32	0.63	0.95	0.21	0.21	– 0.11
Trichotria pocillum	0.00	– 0.80	0.80	1.00**	0.80	0.20	0.20	0.40
Trichotria tetractis	0.26	– 0.78	0.78	0.78	0.78	– 0.26	– 0.26	– 0.26
Cladocerans	0.78	0.78	– 0.78	– 0.26	0.26	0.78	0.78	0.26
Copepods	0.40	1.00**	– 1.00**	– 0.80	– 0.40	0.40	0.40	0.00
Copepod nauplius	0.20	0.80	– 0.80	– 0.40	– 0.20	0.80	0.80	0.60

** Correlation is significant at the 0.01 level (2-tailed).
Correlations were calculated on 55 µm mesh size net abundances for rotifers, cladocerans and copepod nauplii, instead on those in 200 µm mesh size net samples for adult copepods).

observation that the river must be "low or slow" for phytoplankton to reach considerable biomass, is probably also true for zooplankton (Reynolds, 1988).

Higher abundances were recorded in the 55 µm mesh size samples, demonstrating an higher efficiency in collecting rotifers, the main component of the Alcantara River zooplankton, with respect to 100 µm mesh size net. This confirmed that differences in sampling methods, especially those in the mesh size of the nets used, preclude detailed comparison of zooplankton densities. Moreover, different mesh size nets result in different ratios between zooplankton groups, as evident from the data of the present study, with no significant effect on the less abundant groups but a significant underestimate of the almost all dominant rotifers species and a different ratio between the overwhelming two rotifer subclasses. More remarkable differences in densities were estimated when entire zooplankton and rotifer communities were sampled by 63 and 20 µm mesh size nets, respectively with two or three magnitude order abundance variations (Chick *et al.*, 2010).

Overall, low zooplankton abundances from the Alcantara River are probably consistent with the low chl *a*. The abundance found in 55 µm mesh size net community are similar to those recorded in that collected by 35 µm mesh size one from the Aliakmon River (Greece) in spring (Zarfdjian *et al.*, 2000). According to this study, maximum densities are lower than those of large rivers which often exceed 10^6 $ind.m^{-3}$ (Klimowicz, 1981; Ferrari *et al.*, 1989; Van Dijk and Van Zanten, 1995; Chick *et al.*, 2010). Alcantara River with only 60 $m^3.s^{-1}$ of water discharge, as well as rivers Aliakmon (Zarfdjian *et al.*, 2000) and Illinois (Brown *et al.*, 1989), has a lower water discharge and so a more reduced water volume than other large rivers (*e.g.,* the river Rhine 1000–800 $m^3.s^{-1}$, Van Dijk and Van Zanten, 1995). This limits the habitat where the animal can develop. The spatial abundance pattern of zooplankton densities showed higher values at the down – than upstream in agreement with the unidirectional flux in the rivers, implying a clear downstream transport of river zooplankton organisms, reported by many authors (*e.g*., Tafe, 1990; Conley and Turner, 1991; Kobayashi *et al*., 1998). However, relatively high abundances occurred also at the intermediate part of the river course. Differently from the above-cited literature, freshwater species are not displaced by saline ones at the river mouth of the Alcantara River, during the study period, probably because conductivity remains in a freshwater range, representing a salinity barrier to marine species. This is also confirmed by the absence of brackish water genera, such as *Synchaeta* (Hartmut *et al*., 1990; Dolan and Gallegos, 1991, 1992; Lopes, 1994; Gaughan and Potter, 1995; Holst *et al*., 1998). The abundance pattern found in Alcantara River is consistent with groups separated by cluster analysis, in which it may be distinguished an upstream community, characterized by low densities; an intermediate and a downstream communities with increasing abundance patterns, that reached the maximum at the river mouth. Diversity followed a similar trend even with slightly higher values occurring in the small round-shaped lakes "*Gurne*" (Site 2). This confirms earliest river concepts such as the River Continuum Concept (Vannote *et al*., 1980) a downstream directed plankton development, observed in many larger rivers (De Ruyter van Steveninck *et al*., 1992; Meister, 1994; Viroux, 1997, 2002; Kim and Joo, 2000). High river flow regime is probably the reason of the low abundance of cladocerans and copepods with lower adaptation level to different habitat types, than rotifers. Eriksson (2002) explained that the difference in resistance to hydraulic stress results from body shape. Namely, the author concluded that compact-bodied taxa, such as cladocerans, are more resistant to turbulent forces than copepods. These results support the fact that copepods avoid fast flowing water and prefer standing waters (Lair and Reyes-Marchant, 1997).

Rotifer dominance can be attributed to their *r*-strategy. In fact, they are small, opportunistic organisms, with short life cycles and wide tolerance to a variety of environmental factors (Green, 1972; Robertson and Hardy, 1984; Neves *et al*., 2003); furthermore, they have morphological adaptations in their foot, acting as an anchor and preventing displacement, adhesive glands, which serve to adhere firmly to a surface (Ricci and Balsamo, 2000). This predominance of rotifers was reported in other rivers, such as in Rhine (Van Dijk and Van Zanten, 1995) and Aliakmon in Greece (Zarfdjian *et al*., 2000). Furthermore, according to Watson (1974), freshwater ecosystems, such as temperate lakes and rivers, strongly and adversely affected by seasonal variability, are often characterized by the absence of zooplankton organisms in some periods. The adjustment for the irregular events is by colonization of organisms with a reproductive *r*-strategy type, short life cycle and low turnover times, such as rotifers that would be very advantageous.

Adineta dormancy presents a type called anhydrobiosis, a particular form of latency, caused by the loss of water by evaporation, which subjects the animal to experience various metabolic adjustments, as the protective chemical synthesis (Keilin, 1959; Crowe, 1971; Ricci, 1998) to withstand unfavorable periods. The anhydrobiosis form of *Adineta* genus was the most abundant one at some of the mouth stations in both the two mesh size net samples; this is consistent with the highest nutrient concentration with respect to other sites. However, no significant correlations were found between zooplankton groups and nutrients with exception of particular chl *a* fractions: for example, copepods were positively affected by microphytoplankton, whereas two species of rotifers: *Cephalodella* sp. and *Lepadella* sp. exhibited high significant relation to picoplankton, demonstrating that rotifers and copepods pray on different phytoplankton sizes. Some freshwater rotifers can utilize algal picoplankton (Caron *et al*., 1985; Stockner, 1987), and because of their ubiquity and rapid grazing rates, it is likely that rotifers may be the major grazer of picoplankton, particularly in oligotrophic lakes. Conversely, Özbay and Altindag (2009) reported a strong positive relationship between nutrients and zooplankton abundances. In the present study, physical factor best explaining zooplankton abundance spatial pattern was conductivity alone or in association with temperature and dissolved oxygen. Conductivity was found to enhance zooplankton growth and abundance (Hujare, 2005; Jafari *et al*., 2011), as demonstrated by the high correlation coefficients resulted for some rotifer species. Hoffman (1977) suggested that temperature and oxygen are the main but not the only determinative factors influencing rotifer abundance distribution as confirmed by not significant correlation found between rotifer species and these parameters, with the only exception of *Tricotria pocillum*, positively related to temperature. Alkaline pH was also found to favor zooplankton development in the river (Jafari *et al*., 2011), but our analysis revealed an inverse relationship between pH and some rotifer species, such as those belonging to *Cephalodella*, *Euchlanis* and *Lepadella* genera. Some species of these genera were found occurring with high abundances at pH values lower than 7 (Bērziņš and Pejler, 1987). However, a positive correlation was found between this parameter and copepods. Thus, there are different data on the relationship between rotifers and environmental factors that have to be improved by more detailed study.

Conclusions

This study represented a more comprehensive investigation of zooplankton community of volcanic headwater stream. Zooplankton assemblage was more affected by conductivity alone and in combination with temperature and oxygen concentration. We were able to confirm the importance of rotifer community in river ecosystem by using a finer mesh size net, resulted more efficient to reliably depict the zooplankton community than a coarser one. Lastly, but not less importantly, the present work identified a potential indicator of eutrophication as the dormant/not dormant form ratio of the *Adineta* genus. However, more detailed studies are necessary throughout the year.

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