

## Ecology and distribution of living ostracod assemblages in a shallow endorheic lake: The example of Lake Trasimeno (Umbria, central Italy)

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### ABSTRACT

Ostracod assemblages from Lake Trasimeno (Umbria, central Italy), the largest endorheic lake in Italy, were investigated relating their species distribution and ecology to modern physical, chemical and biological parameters. Nineteen living species were collected in the lake (*Darwinula stevensoni*, *Candona (Candona) candida*, *Candona (Neglecandona) angulata*, *Fabaeformiscandona fabaeformis*, *Pseudocandona marchica*, *Cypria ophtalmica*, *Ilyocypris gibba*, *I. salebrosa*, *I. getica*, *Cypridopsis vidua*, *Eucypris virens*, *Trajancypris clavata*, *Herpetocypris helenae*, *Heterocypris salina*, *H. incongruens*, *Isocypris beauchampi*, *Cyprideis torosa*, *Limnocythere inopinata*, and *L. stationis*). All the identified species belong to the fresh-water Italian ostracod fauna but *Cyprideis torosa* is documented in an oligohaline athalassic lacustrine environment in Italy for the first time. The occurrence of *Ilyocypris salebrosa* represents the southernmost record in Italy and the westernmost in Eurasia. The recovery of *Limnocythere stationis* represents the westernmost record in Eurasia. The distribution of the different ostracods recovered in Lake Trasimeno is linked to the dominant physical and chemical parameters for each ecological niche. Physical and chemical data along with substratum type, grain-size and presence of aquatic macrophytes have been related to different ostracods using a multivariate analyses approach (NMDS, CCA, Spearman's rank correlation test). These results allow to differentiate several ecological niches within the lake and indicate that the main parameters affecting the ostracod assemblages are the aquatic macrophyte coverage, the Total Organic Carbon (TOC) and, to a lesser extent, temperature and type of substrate. *Cyprideis torosa* and *Candona (Neglecandona) angulata* have been recovered both in the distal part of the lake and in the lakeshore area. In both cases they are associated with scarce or absent aquatic macrophytes and low amounts of TOC. The alternate dominance of these two species in the distal deeper assemblages seems to be mainly linked with the bottom oxygen availability, being *C.(N.) angulata* dominant in the most oxygen-depleted sediments and *C. torosa* dominant in higher oxygen conditions. Along the lakeshore area they are often discovered together with other prevailing species, such as *Cypridopsis vidua* that is common in very shallow to shallow (20-140 cm) sites with high TOC content, abundant macrophytes and algae, and *Limnocythere inopinata*, which dominates slightly deeper areas (around 150-210 cm) where the previous species are almost absent. The Spearman's rank correlation test showed significant positive correlation between some ostracods and macrophyte species.

**Key words:** Non-marine ostracods; macrophytes; ecological indicators; multivariate analyses; Mediterranean area.

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### INTRODUCTION

Among benthic invertebrates, ostracods (tiny bivalve crustaceans) are particularly sensitive to environmental changes, being strongly dependent on several physical and chemical factors such as size, depth, energy level, and turbidity of the waterbody as well as temperature, dissolved oxygen content, water chemistry, food supply and substratum nature (including aquatic vegetation) (Rodríguez-Lazaro and Ruiz-Muñoz, 2012). The ability of several cypridoidean species to disperse passively in continental waters, either as adults and/or juveniles through survival in torpid stage and/or as eggs through diapause,

leads them to easily populate or re-populate aquatic ecosystems (Mesquita-Joanes *et al.*, 2012). Such characteristics make them a very useful tool for the reconstruction of environmental changes through time, since their calcite shells are easily fossilised and preserved in the sediments. The knowledge of the autoecology and synecology of ostracod species is particularly important when these organisms are used as proxies to investigate the present and past environmental vulnerability (Viehberg and Mesquita-Joanes, 2012).

Endorheic lakes, in particular, are known to be vulnerable ecosystems through time because their hydrological budget is mostly ruled by evaporation due to the absence

of a surficial drainage output. As a result, endorheic lakes are very sensitive to changes in air temperature and precipitation and thus they deserve special attention in the on-going debate about the possible effects of climate change and human impact on biodiversity and environmental preservation. Lake Trasimeno (Umbria, central Italy) is the largest endorheic basin in Italy. During the last 130 years the basin has been affected by climate changes, especially rainfall and air temperature variations, together with increasing human impact causing relevant lake level oscillations (Ludovisi and Gaino, 2010). These events caused periodical economic crises and the progressive depletion of the aquatic biocoenosis (particularly of the planktonic, macrobenthos, macrophyte and fish communities) (Martinelli, 2012). Such dramatic events are not uncommon in endorheic lakes, as documented, among others, by the historical changes undergone by the large lakes Chad, Aral and Neusiedl, located at different latitudes and under different climate conditions. Lake Chad (semi-arid Sahel region, Africa) was affected by a dramatic desiccation event driven by droughts occurred over the last 50 years, which caused its shrinkage from 25,000 km<sup>2</sup> to only 1350 km<sup>2</sup> (Helfert and Holz, 1985; Lauwaet *et al.*, 2012). Lake Aral (central Asian desert) underwent repeated lake level oscillations over the past 10–15 kyr, linked to natural climate changes, that culminated into the environmental disaster that is still affecting the lake since 1960's, due to the irresponsible deviation for irrigation purposes of its two main inflow rivers (Micklin, 2007; Shukla, 2015). The consequences of the Lake Aral shrinking affected dramatically not only the ecological balance of the region and the lake itself (high salinity increase of the waterbody from 10 to 35‰; Boomer *et al.*, 1996), but also the health of local population that now uses high saline water for irrigation (causing an important decrease of the agricultural output) and as drinking water (Ataniyazova, 2003). Lake Neusiedl (Austrian-Hungarian border), the largest shallow closed-basin in central Europe, completely dried up between 1866 and 1871 following more than ten temporary desiccation episodes since its formation 13,000 years ago (Soja *et al.*, 2013). The study on Lake Neusiedl ostracods has shown that past changes in the lake level induced the reduction of the *Phragmites* belt causing a decrease in the ostracod fauna biodiversity (Löffler, 1990) and economic damages to tourism and fishery activities (Soja *et al.*, 2013; Gallinaro *et al.*, 2014).

At present no data are available about Pleistocene changes in water level, despite Lake Trasimeno was supposed to have been settled during the Early Pleistocene (Gasperini *et al.*, 2010). The results of on-going investigations on these older sediments will be useful to disentangle the role of natural and human-induced climatic changes on the evolution of the lake system. The main aim of the present research is to investigate the different

living ostracod assemblages and their relation with the main physical, chemical and biological parameters, in order to use these results to reconstruct past environmental and climate variations and their impact on the lake. Additionally, these data improve the knowledge of the living ostracod fauna of Lake Trasimeno, up to now only investigated by von Hartmann (1964), as well as that of the still poorly known ostracod population of the Italian lakes.

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## METHODS

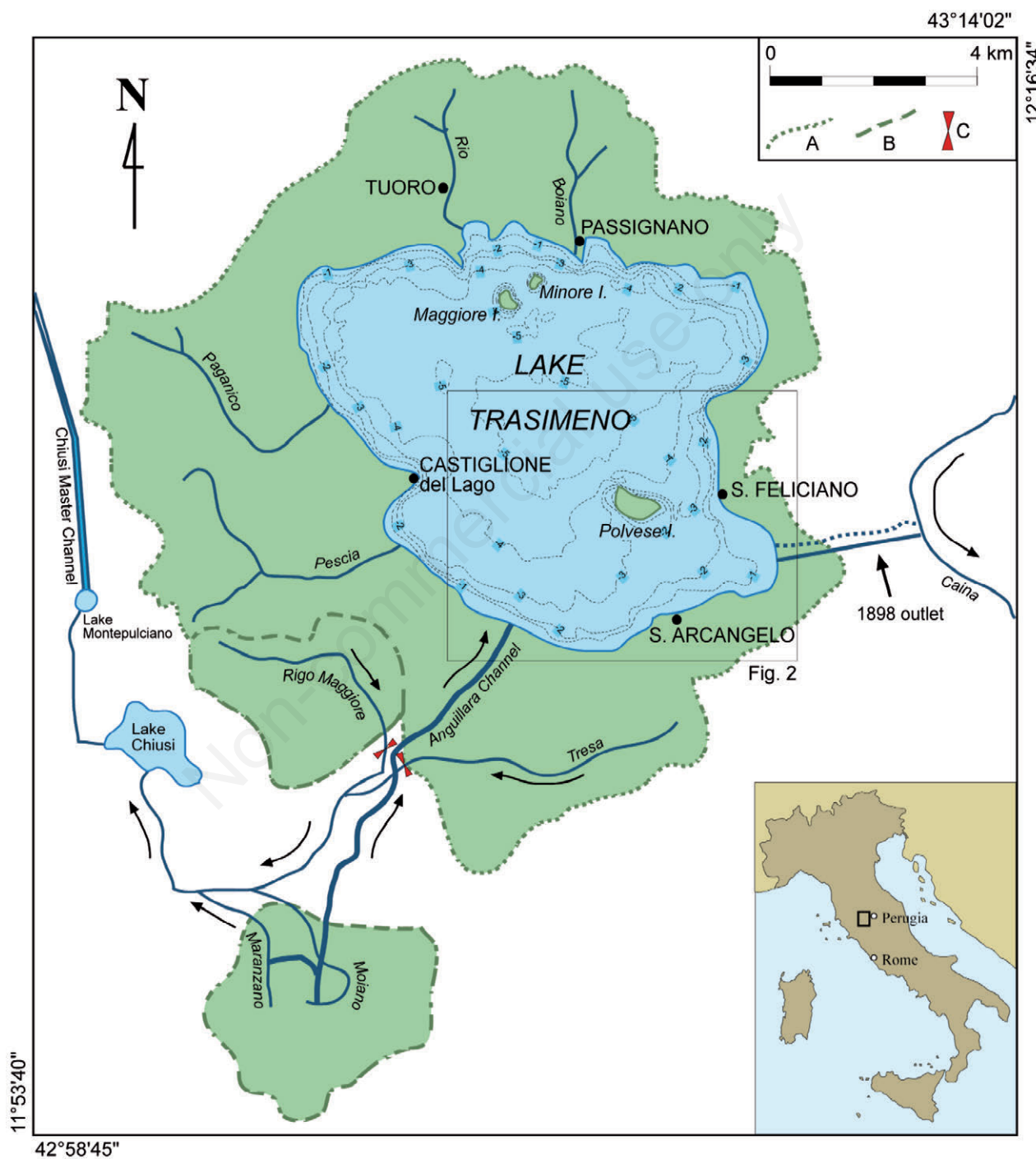
### Study area

Lake Trasimeno (Perugia, Umbria - 443°08'N; 12°06'E, lake bottom altitude 251.57 m asl) is the largest lacustrine system of central Italy and the fourth largest Italian lake in size (~120 km<sup>2</sup>). It is a shallow (average depth 4.7 m, maximum depth 6.3 m) endorheic lacustrine basin with few small tributaries. Since 1960, the Rigo Maggiore, Tresa, Moiana and Maranzano ditches were deviated into the artificial Anguillara Channel (Taticchi, 1992). This latter, together with the natural Paganico and Pescia ditches, represents the lake surficial inflows. No natural outflow exists, only an artificial channel built in 1898 near S. Feliciano village spills the Trasimeno waters into the Tiber River through the Caina creek (Fig. 1). There is no consensus concerning the groundwater input to the lake. Whilst Tiberi (1980) excludes any contribution, ARPA Umbria (2005) suggests that ~2.5% of the total water budget is coming from subsurface waters. Its closed condition makes the hydrological balance of Lake Trasimeno strongly affected by climate variations (precipitation vs evapotranspiration) (Ludovisi and Gaino, 2010; Dragoni *et al.*, 2012). Climate modulates strong lake level variations and at least three negative fluctuations were recorded during the last ninety years (Dragoni *et al.*, 2012). The last low stand occurred between 1988 and 2012, when the lake level was 1.87 m below the 'hydrometric zero' (257.33 m asl; refer to <http://www.biondiriccardo.it/LAGO/elaborations.htm>) (Dragoni *et al.*, 2012).

The shallow character of the lake allows a complete mixing of the water column throughout the year (polymictic lake), producing a continuous homogenization of the physical and chemical parameters such as temperature, conductivity, dissolved oxygen and pH along the water column. Lake Trasimeno waters are rather well oxygenated (surface DO varies between 5.8 and 12.6 mg L<sup>-1</sup>; bottom DO 6.6–12.1 mg L<sup>-1</sup>) with pH values ranging between 8 and 8.5 and summer maxima around 10, high Cl and Na contents (maximum values of 8.98 and 8.57 mg L<sup>-1</sup>, respectively) and mesotrophic to eutrophic conditions (Taticchi, 1992; Ludovisi and Gaino, 2010; Charavgis *et al.*, 2012). These parameters change seasonally and/or over longer periods: for example, in the last 40 years, conductivity has varied from around 750 in the 70's to 1700 µS cm<sup>-1</sup> at the

beginning of this century (Ludovisi and Gaino, 2010) corresponding to possible variations of TDS of around  $1 \text{ g L}^{-1}$  of magnitude. Chloride concentration has largely varied too, from  $3.63$  to  $8.98 \text{ m}^2 \text{ L}^{-1}$  as a result of lake level dropping (Ludovisi and Gaino, 2010).

Along the southern coast of the lake, the bottom sediments are mainly clay and silty-clay whereas sands with variably content of clay and silt dominate along the northern shores (Charavgis *et al.*, 2012). Mineralogical analyses show a great abundance of smectite (Charavgis *et al.*,



**Fig. 1.** Geographic location of the Trasimeno Lake. A, Natural catchment area; B, artificially-joined basins; C, sluice gates of the artificially-joined channels. Redrawn and modified from Fig. 4 (page 73) and Fig. 2 (page 125) in A. Martinelli (ed.), Tutela ambientale del Lago Trasimeno. Libri Arpa Umbria, Perugia, 2012 (with permission).

2012), a mineral that usually adsorbs heavy metals and organic matter (Charavgis *et al.*, 2012). According to Morgantini and Peruzzi (2012), the sediments have a content of organic matter (OM) up to 4%, mainly of algal origin as shown by the TOC/N<sub>tot</sub> ratio ranging between 4 and 8.

Recent studies on environmental conservation of Lake Trasimeno have shown that the lake is a relevant site for plant communities characterized by a good biodiversity level (Venanzoni *et al.*, 2006). Aquatic macrophytes cover around 25% of the lake surface, particularly in the coastal zones, while the central pelagic area is devoid of aquatic vegetation due to the water turbidity and increasing water depth preventing its development (Havens *et al.*, 2009). However, historical data indicate that the distribution of the hydrophyte and helophyte vegetation has greatly fluctuated depending on the lake level (Havens *et al.*, 2009). During the low lake level of the so-called 1944-1960 'crisis', the entire lake area was populated by aquatic macrophytes belonging to genera *Potamogeton*, *Myriophyllum* and *Chara*,

whilst riverbed communities dominated by *Phragmites australis* expanded in the lakeshore area. On the contrary, during the lake level high stand of the 1960s, the aquatic vegetation was reduced ~30% (Havens *et al.*, 2009) and riverbed communities were restricted to the emerged areas. Due to human impact and increased agricultural activities, a decrease in the macrophyte biodiversity was noticed between 1960s and 2007 (Charavgis *et al.*, 2012). Phytoplankton blooms mainly composed by dinoflagellates, diatoms and cyanobacteria, this latter group dominating since 1992, underlines the rather high trophic level of the lake (Havens *et al.*, 2009; Elia *et al.*, 2012).

### Sampling and data collection

During July 2014, a sampling campaign was carried out along the southern and south-eastern shores of Lake Trasimeno and on a N-S transect starting approximately from the middle of the lake (2.5 km north of Island

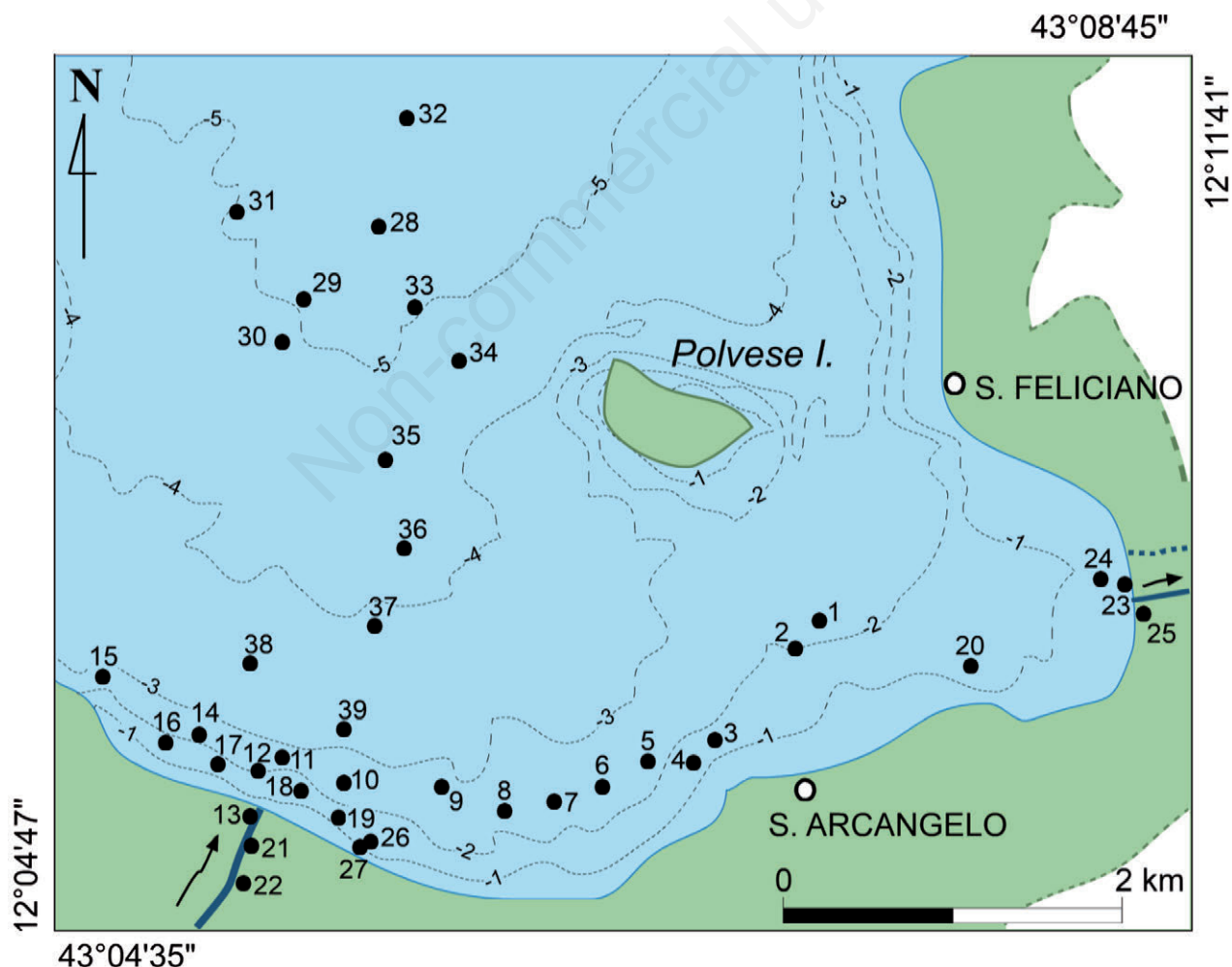


Fig. 2. Geographical location of sampling sites.

Polvese) towards the coast in front of the Anguillara Channel (Fig. 2). This area is of particular interest because it includes both the inflow and outflow channels, a relatively high anthropogenic pressure on the lakeshores (villages and cultivated fields and hills), and encloses the deepest part of the lake. In the field, the sample sites were roughly divided in two sets: sites deeper than 300 cm, distant from the lakeshore and characterised by few macrophytes, and sites shallower than 300 cm, apparently characterised by a quite variable range of bottom sediment and more or less abundant macrophytes.

Forty samples from the uppermost sediments (4 cm) were collected by scuba-diving using a rectangular hand-net (28 cm x 14 cm, 120 µm mesh size) (Tab. 1). One surface water sample was collected among free-floating macrophytes with a sampling bottle. In the field, samples were preserved in 75% ethanol and stained with Rose Bengal for 36-50 h, to differentiate living (or recently died) from sub-Recent ostracods (Walton, 1952; Bernhard *et al.*, 2006). They were washed in the laboratory with tap water through a 125 µm-mesh sieve, dried and observed under a stereomicroscope for ostracod identification on the base of the shell features of adult specimens. Gonzalez Mozo *et al.* (1996), Meisch (2000) and Fuhrmann (2012) were followed for taxonomic identification.

While Rose Bengal staining is widely adopted in foraminifer studies, the method is not as commonly used by ostracodologists and sometimes its results have been questioned (Browers *et al.*, 2000). Our results in the ostracode samples of Lake Trasimeno show that the staining is not reliable to discriminate between living and sub-Recent specimens. In the present study, we have considered as living (or recently died) specimens, stained or not stained, those with preserved appendages or setae on the shell surface. On this basis, the density of each species (expressed by individuals per 1 dm<sup>2</sup>) was counted after identification.

All macrophytes were listed at each ostracod sampling station and their coverage was further estimated, as percentages, in the field. Different collection methods were used: floating masses of filamentous macroalgae were collected by means of a 25 µm-mesh plankton net; epilithic algae by scraping submerged stones; vascular plants by direct observation *in situ* or collecting samples using a grappling iron. All macroalgae were fixed in formalin (4% was the approximate final concentration). Taxonomical determination and nomenclature were based on specific algae (John *et al.*, 2002) and vascular plants literature (Pignatti, 1982; Conti *et al.*, 2005).

During the sampling, physical and chemical parameters of the lake water such as temperature (T, °C), dissolved oxygen content (DO, mg L<sup>-1</sup>), conductivity (C, µS cm<sup>-1</sup>), Total Dissolved Solids (TDS, g L<sup>-1</sup>) and pH were measured, using a Hydrolab Minisonde 4a multi-param-

eter instrument. Water depth (D, cm) was measured with a graduated rope. The concentration of major anions, cations and alkalinity (HCO<sub>3</sub><sup>1-</sup> + CO<sub>3</sub><sup>2-</sup>) were measured on two water samples collected near the bottom at sites 1 and 28, following standard methods by LabService s.r.l. (Anguillara Sabazia, RM, Italy).

A visual estimation of the grain size and other components, such as plant remains and mollusc shell fragments, was made to get qualitative analyses of the substrate. The presence of abundant plant detritus (from few millimeters to few centimeters in size), persistent even after a H<sub>2</sub>O<sub>2</sub> treatment, prevented us to perform standard grain size analyses based on the weight of the different size fractions. Laser grain size analyses particularly adapted for the finest fraction were not performed due to the abundance of particles greater than 0.7 mm.

Rock-Eval Pyrolysis has been used for characterizing the amount and nature of the organic matter of the bottom sediment. This method provides a good estimation of total organic carbon (TOC) and its quality based on the hydrogen and oxygen indices (Tissot and Welte, 1984; Peters, 1986; Meyers and Lallies-Vergès, 1999). It was initially used to study the hydrocarbon potential of petroleum source rocks (Espitalié *et al.*, 1985) and nowadays it is also used in limnogeological studies (Ariztegui *et al.*, 2001; Steinmann *et al.*, 2003). A small amount of lake bottom sediments (ca. 0.45 - 0.55 mg) was pyrolysed (starts isothermally at 300°C and after heated to 650°C) and completely oxidized (starts isothermally at 400°C and heated up to 850°C). Following the alternative Rock-Eval method of Steinmann *et al.* (2003) for organic matter from recent lake sediments, four peaks were measured during the organic carbon decomposition: S1 (during the 300°C isotherm), S2a (between 300-400°C, peaking at ca. 365°C), S2b (above 400°C, peaking at ca. 465°C) and S3 (CO<sub>2</sub> released above 400°C). The total amount of residual and pyrolysed organic carbon corresponds to TOC. The S2/TOC ratio or Hydrogen index (HI) and the S3/TOC or Oxygen index (OI) represent the H/C and the O/C ratios of the organic matter, respectively (Espitalié *et al.*, 1985). These two parameters allow separating different sources of organic matter (Ariztegui *et al.*, 2001; Steinmann *et al.*, 2003).

### Statistical analyses

The collected data were processed through multivariate analyses using different algorithms: Non Metric Dimensional Scaling (NMDS), Canonical Correspondence Analysis (CCA), and the Spearman's rank correlation test using the PAST software, ver. 2.17b (Hammer *et al.*, 2001). Three matrices have been used to perform multivariate analyses, using as respondents the sample sites and as variables the density (percentage of individuals dm<sup>-2</sup>) of each taxa in each sample, including a minimum 2% density. In the first matrix the physical and chemical pa-

**Tab. 1.** Physical and chemical water parameters and bottom features at sampling sites.

Sample	Latitude N	Longitude E	D (cm)	T (°C)	pH	C (µs/cm)	DO% (mg/l)	DO (mg/l)	TDS (g/l)	Bottom grain-size	Notes
PAN 1	43°05'20.1"	12°09'04.2"	240	25.33	7.95	1353	63.1	4.88	0.87	CA	In front of the S. Arcangelo ichtyogenic centre; no bottom vegetation
PAN 2	43°05'19.6"	12°08'53.3"	206	25.33	7.95	1353	63.1	4.88	0.87	SS	Vegetated bottom
PAN 3	43°05'15.4"	12°08'31.8"	173	25.33	7.95	1353	63.1	4.88	0.87	SSg	Vegetated bottom
PAN 4	43°05'10.8"	12°08'31.2"	140	25.09	7.87	1362	64.5	5.20	0.87	SSg	<i>Phragmites</i> fragments
PAN 5	43°05'12.2"	12°08'16.2"	230	25.1	7.76	1340	35.1	2.69	0.87	FSvd	Vegetated bottom
PAN 6	43°05'04.8"	12°08'00.1"	250	24.98	8.30	1360	56.3	4.78	0.87	FSS	Vegetated bottom
PAN 7	43°04'54.5"	12°07'27.2"	250	25.06	8.30	1359	60.5	4.75	0.87	SSg	Vegetated bottom
PAN 8	43°04'53.7"	12°07'02.6"	240	25.09	7.38	1319	45.2	3.87	0.84	FSS	No bottom vegetation
PAN 9	43°05'03.0"	12°06'32.0"	270	25.01	8.30	1357	67.8	4.91	0.87	FSS	Vegetated bottom
PAN 10	43°05'11.8"	12°05'58.5"	240	25.15	8.29	1362	55.2	4.67	0.87	SS	No bottom vegetation
PAN 11	43°05'20.1"	12°05'46.6"	240	25	8.21	1362	57.1	4.60	0.87	FSG	In front of the Anguillara channel; no bottom vegetation
PAN 12	43°05'20.1	12°05'46.6"	200	25	8.21	1362	57.1	4.60	0.87	FSG	In front of the Anguillara channel; vegetated bottom
PAN 13	43°05'11.9"	12°05'36.9"	200	24.40	7.58	1245	30.9	2.90	0.81	FSvd	Pond on the left bank of the Anguillara channel
PAN 14	43°05'24.9"	12°05'25.4"	210	24.95	8.43	1361	56.0	4.63	0.87	SS	No bottom vegetation
PAN 15	43°5'35.0"	12°05'6.5"	250	24.8	8.15	1359	57	4.56	0.87	FSS	No bottom vegetation
PAN 16	43°05'24.3"	12°05'21.3"	180	24.94	8.17	1365	53.1	4.34	0.87	CA	No bottom vegetation
PAN 17	43°05'18.6"	12°05'33.8"	160	25	8.05	1364	59	4.65	0.87	SS	No bottom vegetation
PAN 18	40°05'10.9"	12°05'48.0"	150	25.14	8.10	1364	63	4.90	0.87	SSg	No bottom vegetation
PAN 19	43°05'04.9"	12°06'04.7"	120	24.20	8.15	1389	58.6	4.93	0.89	SSg	No bottom vegetation
PAN 20	43°05'28"	12°09'52.9"	130	23.72	7.91	1370	58.8	4.91	0.88	FSG	In front of passo serpaio dock; vegetated bottom
PAN 21	43°05'07.7	12°05'32.2"	20	23.62	7.31	1123	55.8	4.88	0.72	FSvd	Along the right artificial bank of the Anguillara channel; nearby edge with <i>Phragmitetum australis</i>
PAN 22	43°05'06.3"	12°05'31.8"	20	22.88	6.97	1338	43.3	3.68	0.86	FSvd	Small channel on the right of the Anguillara channel; nearby edge with <i>Phragmitetum australis</i>
PAN 23 sur	43°06'23.3"	12°11'15.9"	superficial sample	22.26	6.87	1406	44.3	3.37	0.90		Surface sample among free floating macrophytes along the artificial outflow channel (Oasi la Valle)
PAN 23 bot	43°06'23.3"	12°11'15.9"	80	22.26	6.87	1406	40	3.37	0.90	FSvd	Bottom sample along the artificial outflow channel (Oasi la Valle)
PAN 24	43°06'18.4"	12°11'02.9"	50	24.20	7.46	1356	33.9	2.79	0.87	FSvd	Nearby the dock of Oasi la Valle
PAN 25	43°06'16.2"	12°11'15.0"	20	25.19	7.41	1442	60	4.60	0.92	FSvd	Small pond at the entrance of Oasi la Valle; <i>Phragmitetum australis</i> and hygrophilic macrophytes
PAN 26	43°05'01.3"	12°06'08.1"	60	24	7.27	1397	52	4.28	0.89	FSvd	Panicale harbour; <i>Phragmitetum australis</i>

*To be continued on next page*

Tab. 1. Continued from previous page.

Sample	Latitude N	Longitude E	D (cm)	T (°C)	pH	C (µs/cm)	DO%	DO (mg/l)	TDS (g/l)	Bottom grain-size	Notes
PAN 27	43°05'01.6"	12°06'07.9"	30	24.21	7.37	1387	53.5	4.52	0.89	FSvd	Pond at the Panicale harbour; <i>Phragmites australis</i>
PAN 28	43°07'51.6"	12°06'13.4"	510	23.68	7.72	1231	45.8	3.55	0.80	CA	No bottom vegetation
PAN 29	43°07'37.2"	12°05'46.8"	500	23.65	8.31	1351	90.1	6.80	0.87	CA	No bottom vegetation
PAN 30	43°07'29.9"	12°05'41.0"	490	23.66	8.33	1348	81.7	7.15	0.86	SS	No bottom vegetation
PAN 31	43°08'00.0"	12°05'31.4"	500	23.63	8.27	1349	60.8	5.28	0.86	SS	No bottom vegetation
PAN 32	43°08'43.1"	12°06'24.0"	520	23.77	8.28	1347	66.7	5.46	0.86	SS	No bottom vegetation
PAN 33	43°07'35.5"	12°06'24.4"	500	23.74	8.35	1352	62.9	5.17	0.86	CA	No bottom vegetation
PAN 34	43°07'02.2"	12°06'47.5"	480	23.58	8.49	1343	75.9	6.5	0.86	FSs	No bottom vegetation; abundant <i>Dreissena</i> shells
PAN 35	43°06'38.3"	12°06'37.2"	450	23.66	8.48	1349	65.9	5.45	0.86	SS	No bottom vegetation
PAN 36	43°06'16.7"	12°06'20.4"	430	23.51	8.66	1351	70	5.88	0.87	SS	No bottom vegetation
PAN 37	43°05'58.3"	12°06'00.3"	390	23.41	8.35	1353	70.1	5.42	0.87	SS	No bottom vegetation
PAN 38	43°05'45.2"	12°05'33.0"	350	23.16	8.15	1359	57.4	4.99	0.87	SS	No bottom vegetation
PAN 39	43°05'27.4"	12°05'56.0"	310	22.98	8.5	1360	54	4.39	0.87	FSvd	No bottom vegetation

*D*, water depth (cm); *T*, bottom water temperature (°C); *pH*, bottom water pH; *C*, bottom water conductivity (µs cm<sup>-1</sup>); *DO*%, percentage of dissolved oxygen in bottom water; *DO*, dissolved oxygen in bottom water (mg L<sup>-1</sup>); *TDS*, total dissolved solutes (mg L<sup>-1</sup>); *CS*, clayey silts with minute plant remains and mollusc shell fragments; *SS*, silty sands with vegetal remains and mollusc shell fragments or juvenile specimens; *SSg*, silty sands with vegetal remains, mollusc shell fragments or juvenile specimens and gravels; *FSvd*, fine sands with plant detritus and few mollusc shells; *FSs*, fine sands with abundant mollusc shells and scarce plant detritus; *FSg*, fine sands with mollusc shells, vegetal detritus and gravels.

rameters measured at each sampling station (depth, TDS, T, DO, pH, TOC), bottom grain size (GR) and macrophytes (mac) were added to the variables. This matrix was used to run the NMDS using Euclidean distance in Q-mode (samples) and the Canonical Correspondence Analysis (CCA) in R-mode (species). The variables of the second matrix data include the ostracod densities and the frequencies of each identified macrophyte species. The variables of the third matrix data are the ostracod densities and the different type of substratum. The Correlation test using Spearman's rank index was applied to these matrices, to identify a relation among the different data sets.

RESULTS

Physical and chemical parameters of the water

During summer 2014 and for the first time since 1988, Lake Trasimeno held a water level above the hydrometric zero. Indeed, the lake level was +12 cm, while in the previous years it was -41 cm (2013) and -133 cm (2012) with a maximum drawdown of -142 cm recorded in 2008 (data from Club Velico Trasimeno, <http://www.clubvelicotrasimeno.it/livellolago.aspx>). This high lake level was linked to particularly high rainfall conditions which affected the Lake Trasimeno hydrographic basin during 2014, corresponding to a annual rainfall of 940 mm (Perugia Province, unpublished data) in comparison with the mean annual rainfall of 700-800 mm/year for the 1963-2014 period (Valigi et al., 2016).

The physical and chemical parameters measured at each sampling site are reported in Tab. 1. Bottom water temperatures display small variations, being the differences between the distal (deepest) area and the lakeshore zone not greater than 3°C, ranging from 22 to 25°C. Conductivity and TDS are rather homogeneous, 1123 to 1442 µs cm<sup>-1</sup>, and 0.7 to 0.9 g L<sup>-1</sup>, respectively; pH values are more variable, from 6.9 to 8.7, as well as the dissolved oxygen content at the bottom that varies from 2.69 to 7.15 mg L<sup>-1</sup>. According to the results reported in Tab. 2 the water of Lake Trasimeno can be defined as chlorine, sulphate-calcium and magnesium-rich. Because of the present high stand conditions of the lake, the measured chlorine concentration is lower in comparison with the values of the last twenty years (5.78 vs 6.06-8.98 m<sup>2</sup> L<sup>-1</sup>, respectively), although the conductivity values are only

Tab. 2. Water chemical analyses (m<sup>2</sup> L).

Samples	Cl <sup>-</sup>	Alk	SO <sub>4</sub> <sup>-</sup>	Ca <sup>++</sup>	Mg <sup>++</sup>	K <sup>+</sup>	Na <sup>+</sup>
PAN 1	5.78	1.88	0.94	0.6	2.96	0.13	0.81
PAN 28	6.52	1.79	0.92	1.42	1.86	0.18	0.84

Alk., total alkalinity.

slightly lower (1231-1442 vs 1440  $\mu\text{S cm}^{-1}$  during 2005) (Ludovisi and Gaino, 2010).

### Bottom sediment features

Sediment texture along with the amount and type of organic matter (OM) have been investigated in all sampling sites. They contain fine sediments (clays, silts, fine sands) with some coarser elements such as plant detritus, shells (or shell fragments) and gravels. Six different kind of substrates were distinguished based on visual observations of the bottom sediments: CS, clayey silts with few plant remains and mollusc shell fragments; SS, silty sands with vegetal remains and mollusc shell fragments or juvenile specimens; SSg, silty sands with vegetal remains, mollusc shell fragments or juvenile specimens and gravels; FSvd, - fine sands with vegetal detritus and few mollusc shells; FSs, fine sands with abundant mollusc shells and scarce plant detritus; FSg, fine sands with mollusc shells, vegetal detritus and gravels (Tab. 1).

CS and SS substrates are mainly located in the center of the lake, in the deeper zone, except for samples 10, 14, and 17; substrates SSg and FSg are typical of lakeshore areas; FSvd is found in vegetated lakeshore zones; and FSs is recognizable mainly around depths of 240-270 cm except for the deep site 34 (480 cm). TOC ranges from 0.36 to 10.21% of sample weight, but most of the samples varied between 1.90 to 2.40%. OI ranges between 127 and 329  $\text{mg CO}_2 \text{ g}^{-1}$  and HI between 144 and 744  $\text{mg Hc g}^{-1}$ . Using a Van Krevelen-type plot it is possible to separate different types of organic matter and therefore their sources (Fig. 3a). Three types of organic matter are identified (Meyers and Lallies-Vergès, 1999): Type I (micro-

bial biomass or waxy coatings of land plants), Type II (algae) and Type III (woody plant matter). HI decreases whereas OI increases from Type I to III. As shown in Figs. 3a and 3b, almost all samples from Lake Trasimeno are grouped around Type II and Type III, *i.e.*, the organic carbon present in the bottom sediments derives from both algae and terrestrial plants. Some exceptional samples, PAN 14 and PAN 9, show relatively higher values indicating higher algal content and plotting close to the Type I field.

### Ostracod assemblages

Thirty-eight out of the forty-one collected samples contained living ostracod assemblages. The composition and density of the ostracod coenoses recorded during our campaign corresponds to high-stand conditions (+ 12 cm above the hydrometric zero). On the whole, nineteen species were recognized (Figs. 4 and 5). *Darwinula stevensoni* (Brady and Robertson, 1870), *Candona (Candona) candida* (O.F. Müller, 1776), *Candona (Neglecandona) angulata* G.W. Müller, 1900, *Fabaeformiscandona fabaeformis* (Fischer, 1850), *Pseudocandona marchica* (Hartwig, 1899), *Cyprina ophthalmica* (Jurine, 1820), *Ilyocypris gibba* (Ramdohr, 1808), *I. salebrosa* Stepanaitys, 1959, *I. getica* Masi, 1905, *Cypridopsis vidua* (O.F. Müller, 1776), *Eucypris virens* (Jurine, 1820), *Trajancypris clavata* (Baird, 1838), *Herpetocypris helenae* G.W. Müller, 1908, *Heterocypris salina* (Brady, 1868), *Heterocypris incongruens* (Ramdohr, 1808), *Isocypris beauchampi* (Paris, 1920), *Cyprideis torosa* (Jones, 1850), *Limnocythere inopinata* (Baird, 1843), and *Limnocythere stationis* Vavra, 1891 were represented by living individuals. Moreover, only loose valves of *Potamo-*

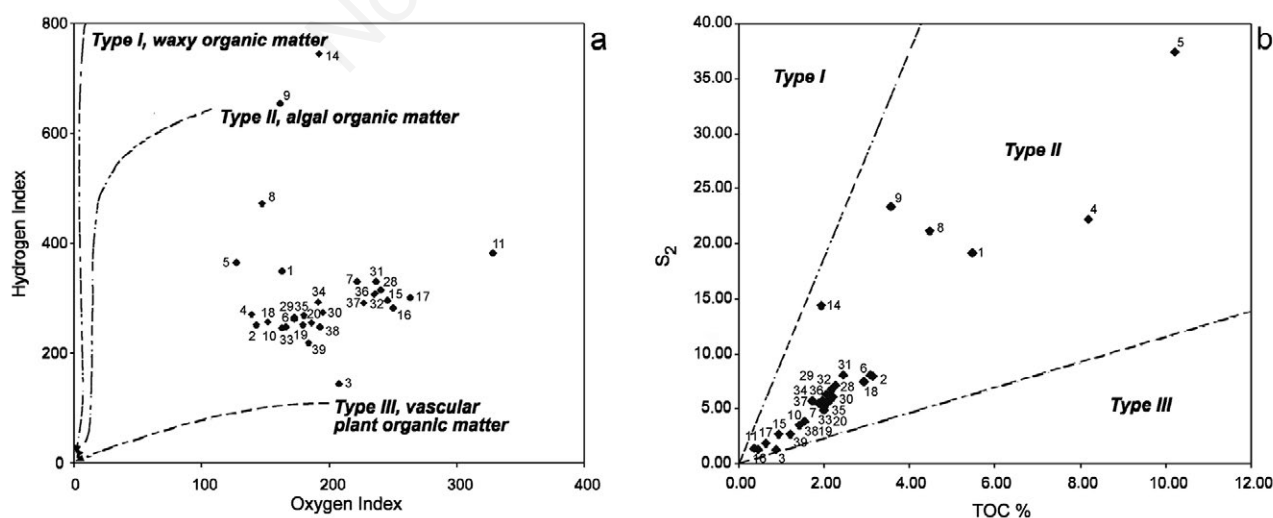
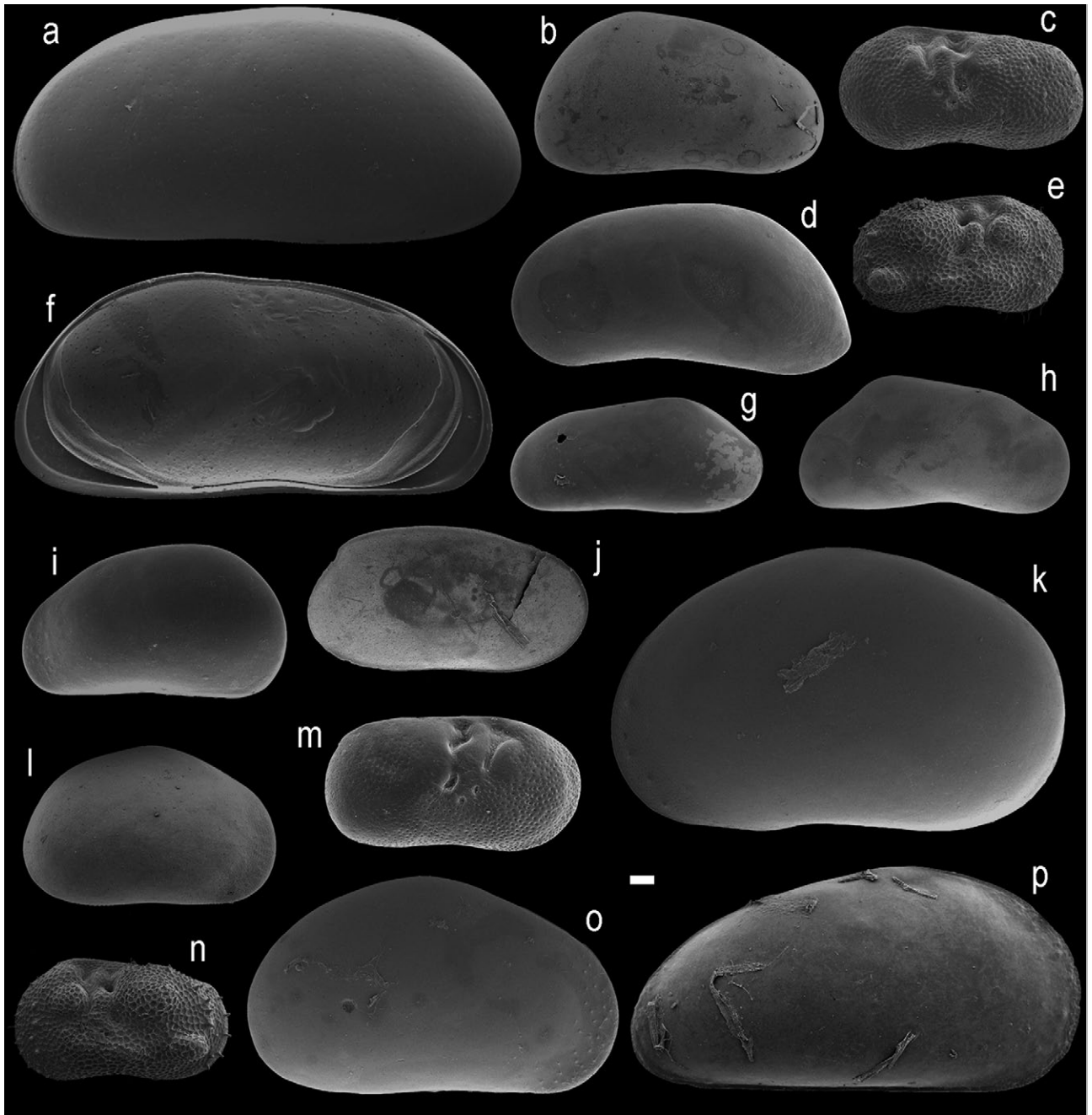


Fig. 3. a) Van Krevelen-type diagram for Lake Trasimeno sedimentary organic matter; dots represent samples and dashed lines trace the maturity paths for organic matter types. b) S<sub>2</sub> vs TOC diagram. Dashed lines define the boundaries of organic matter types.



*cypris variegata* (Brady and Norman, 1889) and *Fabaeformiscandona harmsworthi* (Scott, 1899) were recovered, thus these species are not considered as living in the lake.

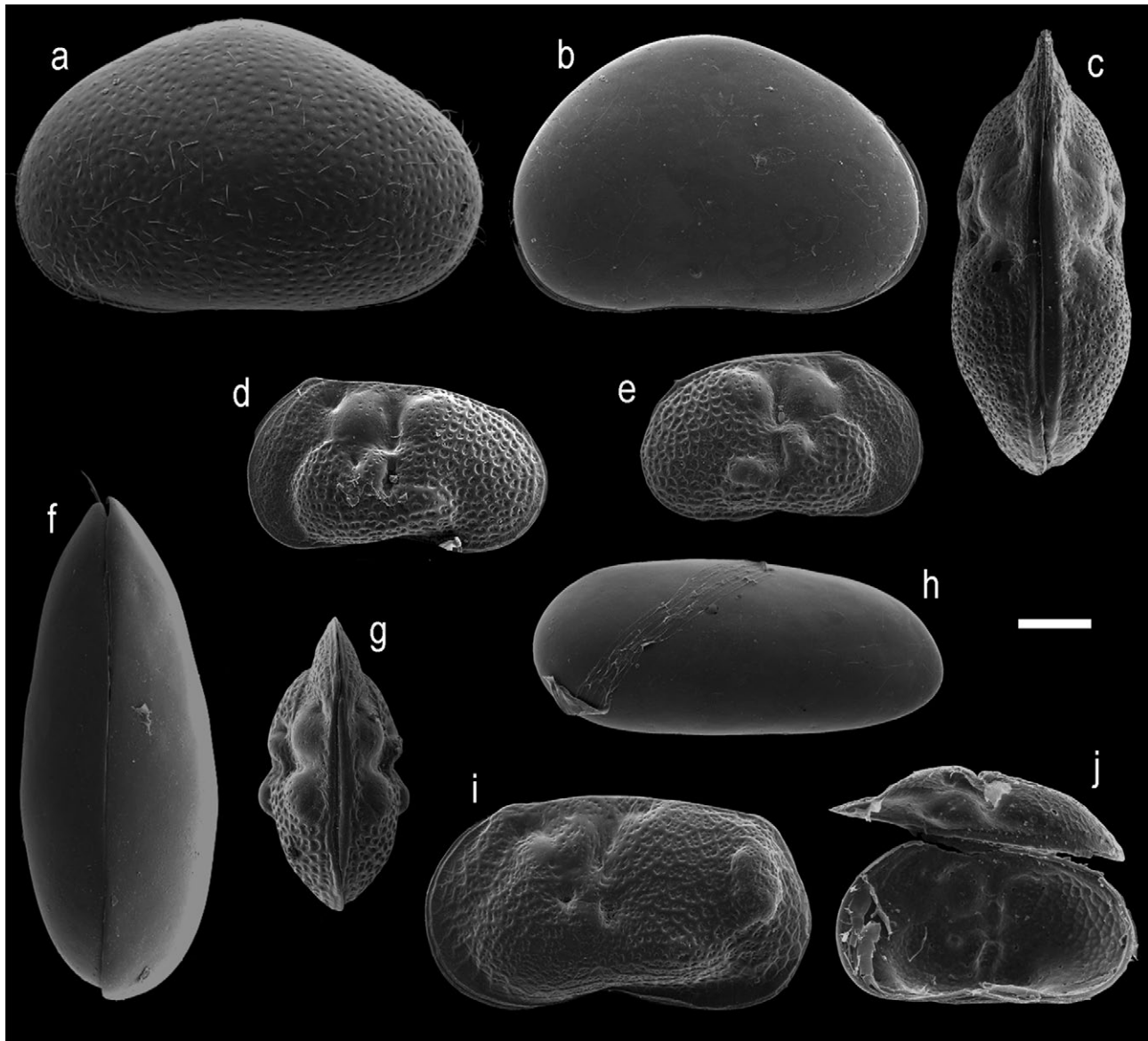
Several carapaces and valves of *H. helenae* were collected in the lake. The valve outline is slightly different from the typical outline of the species illustrated by Gon-



**Fig. 4.** SEM photos of the living ostracods from Lake Trasimeno. a. *Herpetocypris helenae*, female LV, sample PAN 11; b. *Candona (Candona) candida*, female RV, sample PAN 23bot; c. *Ilyocypris gibba*, female LV, sample PAN 6; d. *Candona (Neglecandona) angulata*, female LV, sample PAN 38; e. *Ilyocypris salebrosa*, female RV, sample PAN 11; f. *Herpetocypris helenae*, LV, inner view, sample PAN 11; g. *Fabaeformiscandona fabaeformis*, female LV, sample PAN 3; h. *Fabaeformiscandona harmsworthi*, male (?) RV, sample PAN 24; i. *Pseudocandona marchica*, female LV, sample PAN 39; j. *Isocypris beauchampi*, female RV, sample 35, slightly damaged anteriorly and posteriorly; k. *Eucypris virens*, female LV, sample PAN 39; l. *Heterocypris salina*, female RV, sample PAN 11; m. *Ilyocypris getica*, female RV, sample PAN 9; n. *Ilyocypris salebrosa*, female LV, sample PAN 11; o. *Herpetocypris incongruens*, female RV, sample PAN 11; p. *Trajancypris clavata*, female RV, sample PAN 11. LV, left valve; RV, right valve; C, carapace. White bar corresponds to 0.1 mm.

zalez Mozo *et al.* (1996) and Fuhrmann (2012), with the dorsal margin slightly inclined forward rather than horizontal. Conversely, the features visible on the inner margin are comparable. Masi (1909), describing the valves of '*Cypria intermedia* var. *latialis*' from the surroundings of Rome and Sicily, remarked that the dorsal border was faintly inclined forwards. Masi's variety is considered synonym of *H. helenae* by Pieri *et al.* (2015). Thus, we consider this slightly different feature to fall within the morphological variability of *H. helenae*.

Except 10 samples in which only loose valves or very few carapaces were recovered (samples 1, 13, 14, 16, 19, 20, 21, 21bis, 22, 27), all the other samples displayed abundant carapaces and valves (from 50 to 500 specimens including instars). *C. torosa*, *C. vidua*, *C. (N.) angulata*, and *L. inopinata* are the most abundant species. They are represented by several adult carapaces (271, 87, 78, and 53, respectively) and hundreds of loose valves. Except for the asexual *C. vidua*, *L. inopinata* is represented only by female specimens whereas the other two species were rep-



**Fig. 5.** SEM photos of the living ostracods from Lake Trasimeno. a. *Cypridopsis vidua*, female LV, sample PAN 13; b. female *Cypria ophthalmica*, RV, sample PAN 23bot; c. *Limnocythere inopinata*, female C, dorsal view, sample PAN 2; d. *Limnocythere stationis*, female LV, sample PAN 5; e. female *Limnocythere stationis*, RV, sample PAN 5; f. *Darwinula stevensoni*, female C, dorsal view, sample PAN 3; g. *Limnocythere stationis*, female C, dorsal view, PAN 5; h. *Darwinula stevensoni*, female RV, sample PAN 6; i. *Limnocythere inopinata*, female LV, sample PAN 10; j. *Limnocythere stationis*, open female C with appendage remains, sample PAN 6. Abbreviations as in Fig. 6. White bar corresponds to 0.1 mm.

resented both by males and females with the following sex female/male ratios (calculated on the recovered carapaces): *C. torosa* 6:5 and *C. (N.) angulata* 3:1.

These dominant species have been recovered in the bottom samples of the lake with densities reaching maximum values of 25.0 (*C. torosa*), 12.2 (*C. vidua*), 6.3 (*C. (N.) angulata*), and 36.8 (*L. inopinata*) individuals  $\text{dm}^{-2}$ .

The remaining species occur only in few samples. Carapaces, adult loose valves and juveniles were recovered except for *I. beauchampi*, *T. clavata* and *E. virens* that were represented by only 1-3 valves.

### Macrophytes assemblages

Macrophytes were identified in twenty-one out of the forty-one sampling stations. Species richness ranges from one to nine different taxa. The absence of macrophytes is in correspondence with the deepest stations in the central zone of the lake and with turbid waters. A total of 17 macrophyte taxa (ten flowering plants, seven algae) were recorded, among which the most common were *Phragmites australis* (Cavanilles) Trinius ex Steudel, *Najas marina* Linnaeus, *Ceratophyllum demersum* Linnaeus, *Potamogeton perfoliatus* Linnaeus and *Vallisneria spiralis* Linnaeus. The first three species were also the most abundant locally. Communities characterized by *P. australis*

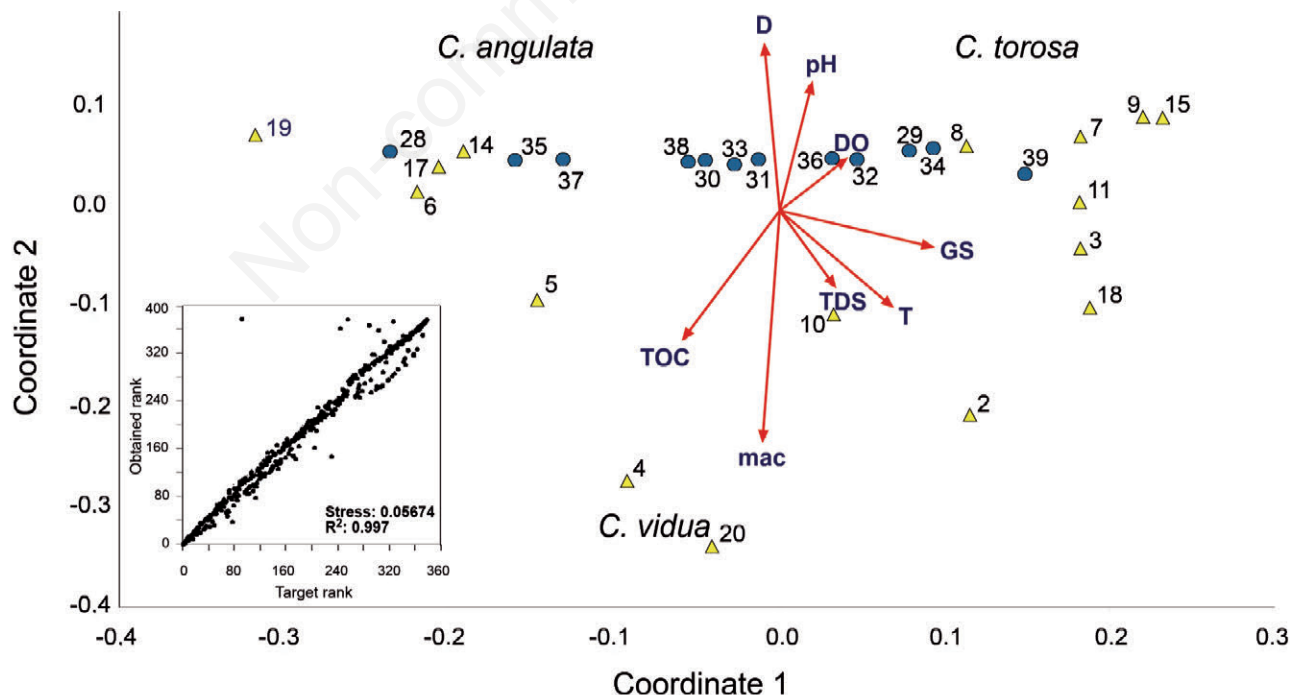
were prevalent along and strictly close to the banks, while those dominated by *N. marina* or *C. demersum* were the more widespread in the lake.

Ostracods were found in all sampling stations with aquatic vegetation. They were either in the substrate among the vegetation or, less often, attached to it.

### Ostracod communities, macrophytes and substrate

The NMDS plot (Fig. 6) related to the first matrix, shows the distribution of the sampling sites depending on their physical and chemical, sedimentological and biological parameters. The dots in the Shepard plot appear strongly aligned; the very low value of stress (0.056) and the consequent very high nonlinear fit ( $R^2=0.997$ ) confirm the rejection of the null model ( $R^2=0$ ), where all the observations 'fall in the same point'.

The sample distribution in the plot illustrates the different ecological niches recognized in the study area. Samples characterised by depths below 300 cm are all grouped in the upper portion of the diagram. They share pH values between 7.7 and 8.7, absence of macrophytes, fine substrate, and ostracod assemblages (*C. (N.) angulata*-*C. torosa* assemblage) and display a rather wide range of DO values (3.55-6.80  $\text{mg L}^{-1}$ ). Conversely, samples with depth above 300 cm are more scattered in the



**Fig. 6.** Non-Metric Multidimensional Scaling (NMDS) ordination plot for sampling sites (circle, deep samples; triangles, lakeshore samples), abiotic parameters (D, water depth; DO, dissolved oxygen in bottom water; GS, bottom grain size; pH, bottom water pH; T, bottom water temperature; TDS, total dissolved solutes; TOC, total organic carbon), and macrophytes (mac) (Euclidean similarity measure). The Shepard plot is shown on the left lower corner. The dominant species of each sample group are also reported in the plot.

diagram, showing a plurality of niches. Some samples located in the upper part of the plot (6,14,17,19 on the left and 7, 8, 9, 11, 15 on the right) are devoid or contain scarce macrophytes. They are separated in two different groups, due to the different dominance of ostracod species, with *C. (N.) angulata* and *C. torosa* prevailing in the groups on the left and on the right, respectively. Other shallow samples, in the lower part of the plot, are strongly influenced by TOC and macrophytes abundance (5, 4 and 20), with samples 4 and 20 characterized by the dominance of *C. vidua* and sample 5 by *C. (N.) angulata*. Additional parameters influencing this distribution are oxygen depletion and substrate features that enclose silty sands, plant detritus, shells and gravels.

The Canonical Correspondence Analysis plot (Fig. 7), also related to the first matrix, shows the relationship between physical and chemical parameters and ostracod species. Axis 1 represents 61.22% of the total variance. A Monte Carlo permutation test (999 permutations) allowed for rejecting ( $P < 0.01$ ) the null hypothesis of non-significant gradients on the first axis (ter Braak and Verdonschot, 1995). The first axis separates the species on the base of the macrophytes occurrence (regression coefficient 0.78) and TOC (regression coefficient 0.50) and, to a lesser extent, depth (regression coefficient -0.48) and pH (-0.41) gradients. *C. vidua*, *D. stevensoni* and *C. oph-*

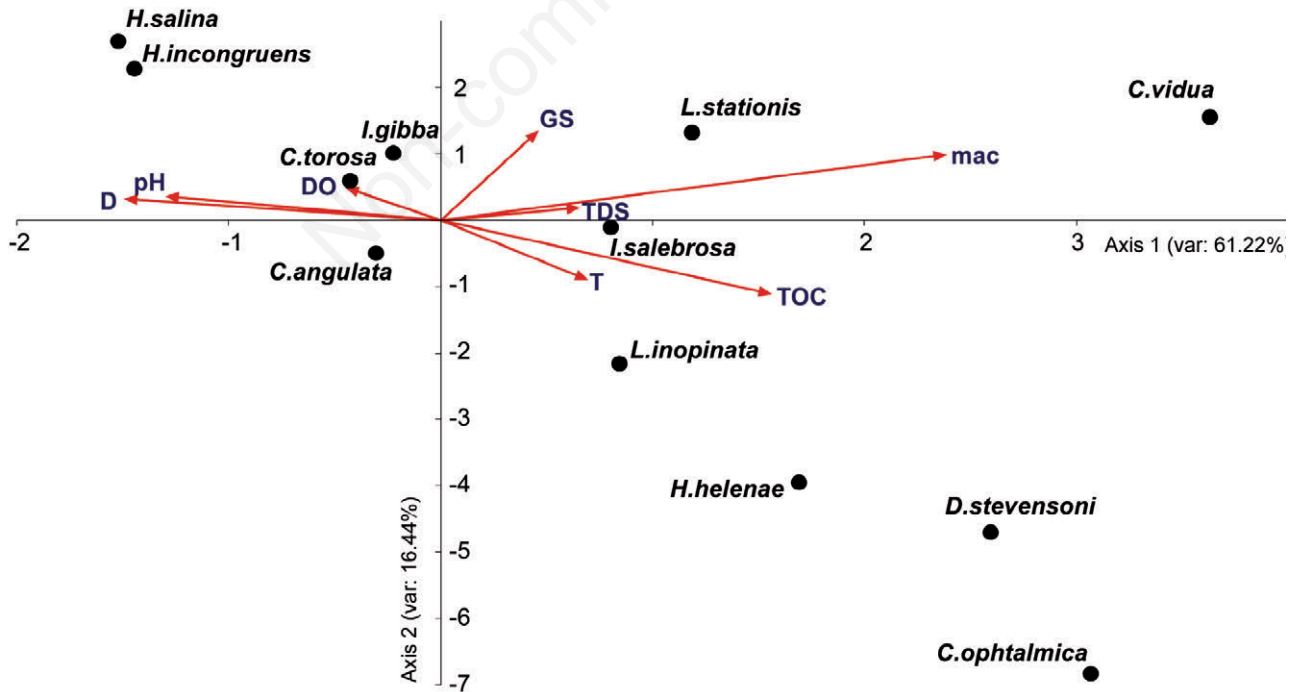
*talmica* correlate positively with macrophytes and TOC whereas they correlate negatively with depth and pH. *H. helenae*, *H. salina* and *H. incongruens* display a strong correlation to depth and pH.

The Spearman's rank values with  $P < 0.001$  show that *P. australis* is the macrophyte species with the highest correlation with ostracods displaying a moderate positive correlation with *H. helenae* ( $\rho = 0.51121$ ) and *C. vidua* ( $\rho = 0.55081$ ) and a moderate negative correlation with *C. torosa* ( $\rho = -0.5423$ ). Moderate positive correlations are also observed between *C. demersum* and *L. inopinata* ( $\rho = 0.50615$ ). Finally, a very strong positive correlation ( $\rho = 1$ ) has been identified between *Potamogeton natans* Linnaeus and *L. stationis*.

For the substratum type, the Spearman's rank values with  $P < 0.001$  show that *H. salina* strongly correlates with fine sands containing plant detritus, shells and gravels ( $\rho = 0.66009$ ).

**DISCUSSION**

Living ostracod assemblages have been seldom investigated in Italian lakes. Some information on ostracods are reported for Lake Maggiore (Fox, 1965), Ragogna (Colizza *et al.*, 1987), Mantova (Melis *et al.*, 1995, 1996; Salvi



**Fig. 7.** Canonical Correspondence Analysis (CCA) ordination plot for ostracods, abiotic parameters (D, water depth; DO, dissolved oxygen in bottom water; GS, bottom grain size; pH, bottom water pH; T, bottom water temperature; TDS, total dissolved solids; TOC, total organic carbon), and macrophytes (mac) in the space defined by the first two canonical axes (77.66% eigenvalue).

and Degrassi, 1995), Bracciano (Mastrantuono, 1995), and Martignano (Mastrantuono and Mancinelli, 2003). Additionally, Pieri *et al.* (2015) reported ostracods from some small mountain lakes in Piedmont, the Dolomites (northern Italy) and the central Apennines (Abruzzo Region). However, only few of these studies analyse the presence of ostracods in their environment, linking their assemblages with the dominant physical, chemical and biological conditions. Moreover, the relationship between ostracods, macrophytes and the substrate is poorly investigated in Italian freshwater systems (Mazzini *et al.*, 2014b) despite the definition of such links could be extremely useful to identify former ecological niches and habitat (Matsuda *et al.*, 2015).

Little is known about the ostracods of Lake Trasimeno. Von Hartmann (1964) pointed out the presence of living *C. torosa* and, more generally, the occurrence of ostracods has been signaled in several articles dealing with the diet of fishes inhabiting the lake and crucial for the local economy (Mantilacci *et al.*, 1990). Meisch (1985) recovered few valves of *P. variegata* from an irrigation channel near Lake Trasimeno.

The 19 species collected among the living meiobenthos of Lake Trasimeno are widely recorded in other Italian freshwater or brackish sites (Pieri *et al.*, 2015) but three of them (*C. torosa*, *I. salebrosa*, *L. stationis*) deserve an in-depth discussion.

*C. torosa* is widespread in Eurasia and Africa (Wouters, 2002) in marine-derived brackish waters of marginal environments or water bodies influenced by the sea. However, Van Harten (1990) defined the species as anomalohaline due to its ability to adapt also to athalassic waters. In fact, records of this species in athalassic waters were reported by Klie (1938) from Germany (Gruber See, Bavaria; Trammer See, Plön), and thermal waters in Iceland, while Mezquita *et al.* (1999) recorded it in freshwater-oligohaline springs in eastern Spain. The identification of *C. torosa* in Lake Trasimeno further confirms this statement. Neale (1988) and Griffiths and Holmes (2000) reported a very wide salinity range 0.4–150 g L<sup>-1</sup> for *C. torosa*, with specimens displaying rather large size and developing noded shells below the osmoregulation threshold of 8 g L<sup>-1</sup> (Van Harten, 1996, 2000; Keyser and Aladin, 2004; Keyser, 2005; Boomer and Frenzel, 2011; Frenzel *et al.*, 2012). As already observed by von Hartmann (1964), in our samples *C. torosa* shells from Lake Trasimeno are mostly noded (69%), but on some valves nodes are barely visible or totally absent. Following Sandberg's notation (1964), in our specimens we typically observed well-developed nodes 1, 2 and 3, whereas nodes 2a, 5 and 6 are often visible, and nodes 4 and 7 are very rarely present (Fig. 8). Nodes are also present on instars, starting from the A-3 moult. Concerning the size, on average the specimens of Lake Trasimeno are slightly larger

than those reported by Decima (1964) for a marginal marine population of *C. torosa* recovered at Forte dei Marmi (Tuscany, Italy) (average length of the female right valve 1.03 mm vs 0.92 mm and average length of the male left valve 1.17 mm vs 1.00 mm, respectively).

*I. salebrosa* in Lake Trasimeno is the southernmost appearance of this species in Italy. Mazzini *et al.* (2014b) and Pieri *et al.* (2015) reported it only from northern Italy (Lombardia and Emilia-Romagna regions). More generally, Italy is the Eurasian westernmost country in which this species occurs. Up to now it has been described from lakes Biwa and Okinawa in Japan (Schornikov, 2004; Smith *et al.*, 2011), lakes Bosten and Taihu in China (Mischke, 2001; Mischke and Schudack, 2001, shells only; Yu *et al.*, 2005), in Korea (Lee *et al.*, 2000), in India as *Ilyocypris shawneetownensis* (Bhatia and Singh, 1971; Mischke, 2001), and in Thrace Turkey (Özuluğ, 2005). Smith *et al.* (2011) consider *Pelocypris alatobulbosa* Delorme, 1970 from Canada synonym of *I. salebrosa* and suspects its presence also in Serbia, quoted by Karan-Žnidaršič and Petrov (2007) under the name *Ilyocypris decipiens*.

Living specimens of *L. stationis* were previously found in Italy only at Cà Nuova rice-field (Lombardia, northern Italy; Moroni and McKenzie, 2007). Because this species has not been previously reported in the Italian fossil record, these authors considered it as part of the 'foreign guest' contingent that was introduced by man in Italy with the trial strains of seed rice or other cereals coming from abroad and/or in dust associated with trade goods. However, recently *L. stationis* has been recovered in the fossil record of the Panicarola borehole (south Lake Trasimeno onshore), possibly aged Early-Middle Pleistocene (Marchegiano, *personal communication*) and from Holocene boreholes drilled in Gorgo Basso and Lake Preola (Trapani, Sicily) (Curry *et al.*, 2013) thus, although rare, this species must be considered part of the Italian ostracod fauna. Outside Italy, the species has been reported in Eurasia, particularly in France (Paris, 1920), Austria (Löffler, 1971), Germany (Wohlgemuth, 1914; Fuhrmann and Pietrzeniuk, 1990; Fuhrmann, 2012), Macedonia (Petkovski, 1964), Finland (Meisch, 2000), Russia (Schornikov, 2004), Turkey (Altınışıl, 2001), Israel [Mischke *et al.*, 2012 (shells only)], Jordania [Mischke *et al.*, 2012 (shells only)], Yemen [Mohammed *et al.* (2014) (shells only)], Sudan (Martens, 1984), China (Schornikov, 2004), Japan (Smith and Janz, 2009), Thailand (Savate-nalinton, 2014), and Korea (Lee *et al.*, 2000).

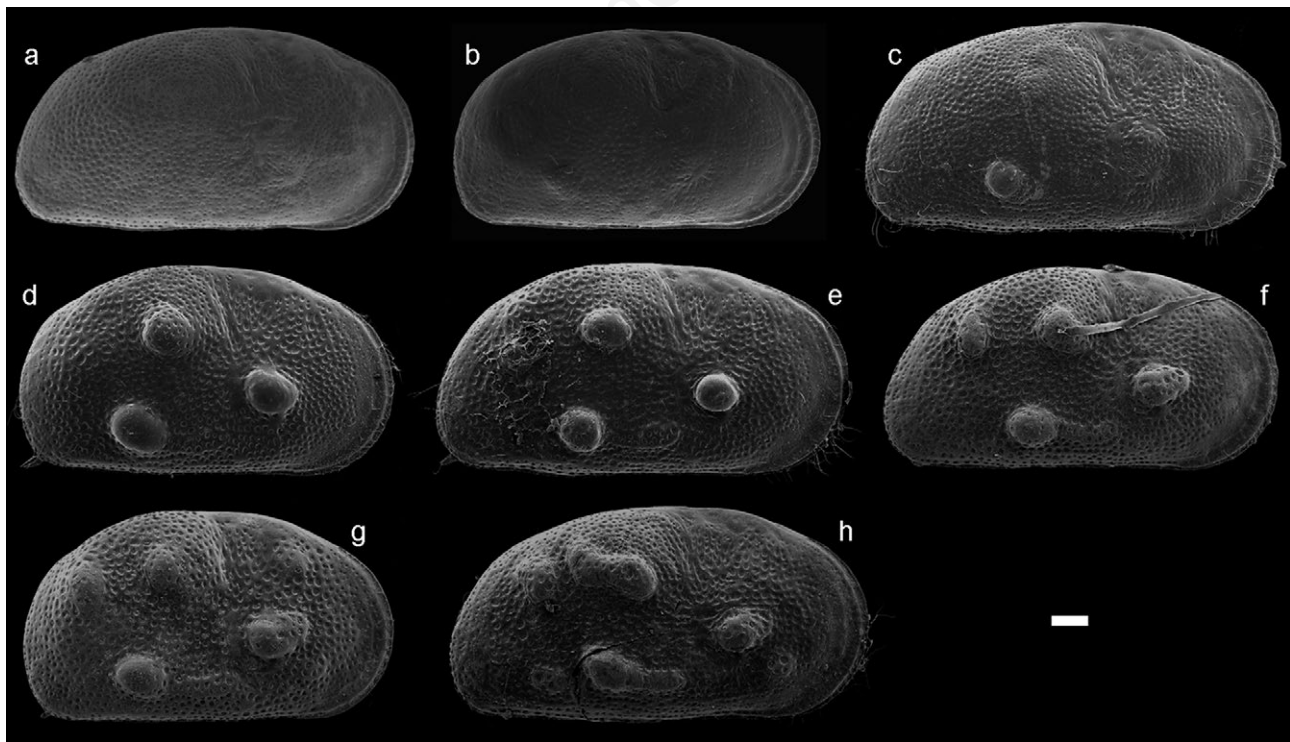
*C. torosa* and *C. (N.) angulata* are the most frequent species in the lake. These halophilic species are mainly found in marginal marine environments (Meisch, 2000; Fuhrmann, 2012). Their frequency and abundance in Lake Trasimeno could be explained by the chemistry of the lake waters which are rich in chlorine and sodium (Charavgis *et al.*, 2012). According to Ludovisi and Gaino (2010) this

peculiar chemical composition is due to the geology of the catchment area and the watershed flowing in the lake that is rich in these ions as a consequence of weathering processes or wastewater discharges. The groundwater hydrogeochemistry of the Trasimeno area shows that the aquifers linked to alluvial and Plio-Pleistocene sediments are mainly alkaline-earth bicarbonate or chloride alkaline sulphate in nature. Analogously, the deep aquifers settled in Miocene turbidites are enriched in chloride and alkaline-earth bicarbonate due to the dissolution of chlorides or the incongruent dissolution of aluminosilicates (plagioclase and K-feldspar) (ARPA Umbria, 2005). Tiberi (1980) excluded a significant exchange between groundwater and lake waters but ARPA Umbria (2005) estimated a groundwater contribution of ca. 2.43-3.64 Mm<sup>3</sup> year<sup>-1</sup>. The latter would represent around 2.5% of the total water supplied to the water body by rainfall and surficial runoff and would be enough to drive the chemical composition of the lake.

*C. torosa* and *C. (N.) angulata* have been recovered both in the distal part of the lake and in the lakeshore area. As shown in the NMDS and CCA plots (Figs. 6 and 7) in both cases these species are associated with scarce or absent macrophytes and low TOC values. The alternate

dominance of these two species in the distal deeper assemblages seems to be linked mainly to bottom water oxygen availability: *C. (N.) angulata* dominates in the most oxygen-depleted sediments (3.55-5.45 mg L<sup>-1</sup>) while *C. torosa* becomes dominant with higher oxygen values (5.46-6.80 mg L<sup>-1</sup>). The former is rather surprising although the tolerance range for dissolved oxygen of *C. (N.) angulata* is not known (Ruiz *et al.*, 2013). Indeed, previous work shows that this species generally occurs in oxygenated waters from 9.20 to 22.8 mg L<sup>-1</sup> (Martin *et al.*, 1993; Akdemir, 2008; Özüluğ, 2012; Altınışıl, 2014). On the contrary, *C. torosa* is known for its ability to survive also in depleted oxygen environments until hypoxia (Jahn *et al.*, 1996; Mesquita-Joanes *et al.*, 2012).

Although *C. torosa* and *C. (N.) angulata* sometimes are the dominant species also in samples from shallow lakeshore areas, they are often accompanied by other species such as *C. vidua* that become frequent in very shallow to shallow (20-140 cm) sites with high TOC, macrophytes and algae (samples in the low left quarter of NMDS plot, Fig. 6). It is known that *C. vidua* is a phytophilic species that prefers abundant plant coverage containing macrophytes as well as algae (Characeae) (Meisch, 2000). In Lake Trasimeno this species correlates strongly with the



**Fig. 8.** SEM photos of *Cyprideis torosa* from Lake Trasimeno. a. Male RV, un-noded; b. Female RV, un-noded; c. male RV, node 2; d. female RV, nodes 1, 2, 3; e. male RV, nodes 1, 2, 2a, 3; f. male RV, nodes 1, 2, 2a, 3, 6; g. female RV, nodes 1, 2, 2a, 3, 4, 6; h. male RV, nodes 1, 2, 2a, 3, 5, 6, 7. Abbreviations as in Fig. 6. White bar corresponds to 0.1 mm.

presence of vegetation (Figs. 6 and 7) and we found only this species (mainly instars) among the roots of free-floating plants as *S. polyrrhiza*, *L. minor* and *L. trisulca*, while, at the bottom of the same site, adults of this species are accompanied by *C. ophthalmica* and *C. (C.) candida*. The same has been recognized by Kiss (2007) at Lake Fehér (Hungary). It is particularly interesting to confirm the strict relation between some species and free-floating macrophytes, already recognized by Kiss (2007) and Mazzini *et al.* (2014a). *L. inopinata* dominates areas with low presence of *C. torosa* and *C. (N.) angulata*, slightly deeper (around 150-210 cm) (samples in the low right quarter of the NMDS plot, Fig. 6). It seems to prefer less vegetated bottoms. Despite the ability of this species to adapt to several environmental conditions (Meisch, 2000), in Lake Trasimeno it shows a moderate correlation to T (Fig. 7) suggesting that it could be a polythermophilic species.

The rest of the ostracod species occur in few samples and represents a subordinate component of the ostracod assemblage, except for *H. helenae*, *I. salebrosa*, and *H. incongruens* that could be locally dominant. The first species represents up to 67% of the ostracod fauna in a very shallow (20 cm) lakeshore pond densely populated by *P. australis*. It is accompanied by *I. gibba* (20%) and *I. salebrosa* (13%). *I. salebrosa* reaches 57% of the ostracod community (accompanied by *H. salina* and *H. incongruens*) in a 200 cm deep pond lateral to the inflow of the Anguillara Channel, rich in algae (pennate and centric diatoms, *Cladophora glomerata* Linnaeus, *Oedogonium* sp. and *Spyrogyra* sp.) and with moderate *P. australis* coverage. *H. incongruens* reaches 25% of an assemblage dominated by *C. vidua* (75%) along the cemented banks of the Anguillara Channel, nearby its inflow in the lake, in very shallow conditions (20 cm) and rather coarse bottom with macrophyte allochthonous remains and pebbles. The ecology of *H. helenae* and *H. incongruens* is rather well known (Meisch, 2000; Fuhrmann, 2012) and their presence in the described environments does not record any additional autoecological data. Conversely, *I. salebrosa* ecology is relatively unknown. In Lake Biwa (Japan) it has been collected at water depths ranging from 5.5 to 16 m (Smith *et al.*, 2011), while in Turkish lakes in the Thracia region occurs at rather high temperatures (24.6-30°C), pH (8.0-8.8) and, at least at Lake Gala, it was found with *V. spiralis* and *Myrophyllum spicatum* Linnaeus (Özuluğ, 2005). In Lake Trasimeno *I. salebrosa* has been collected at shallower depths (0.2 to 2.5 m), slightly lower pH (7.6-8.3), and comparable temperatures (24.4-25.3°C). Furthermore, it seems that it tolerates rather low oxygen content (DO 2.9-5.2 mg L<sup>-1</sup>), and oligohaline waters (TDS 0.80-0.87 g L<sup>-1</sup>). Only in one sample it is associated with *P. australis* and in another samples with abundant diatoms.

The results of the multivariate analyses indicate that the central and southern areas of Lake Trasimeno enclose

several ecological niches. The main parameters affecting the ostracod assemblages are the macrophyte coverage, TOC and, to a lesser extent, depth and pH.

The changes in macrophyte covers is clearly influenced by sediment characteristics, as well as hydrodynamism and underwater irradiance (Van Duin *et al.*, 2001). In Trasimeno, the deepest part of the lake has a dominant clay substrate and a complete absence of macrophytes, they colonize lakeshore areas encompassing coarser sediments. *Phragmites australis* communities dominate the southwestern coast, nearby the Anguillara Channel, while aquatic macrophytes (*Potamogeton* spp., *V. spiralis*, *Najas* spp. and *C. demersum*) are frequent along the southeastern coast.

The TOC values obtained by Rock-Eval Pyrolysis mirrors the macrophyte distribution, being higher along the coast (except for the most western sector) and decreasing towards the distal part of the lake. This is in agreement with the TOC distribution previously reported by Morgantini and Peruzzi (2012) indicating the highest TOC values in the southeastern shoreline and the lowest contents in the southwestern area. Intermediate values (around 2%) were recorded in the central (deepest) part of the lake. The differences recorded on the TOC distribution could be linked to multiple, complex processes, also occurring outside the lake. For instance, land use changes can affect the input of nutrients in the inflowing rivers and thus can lead to changes in lake productivity that subsequently alter the TOC burial in the sediment (Sifeddine *et al.*, 2011). The linkage between macrophyte distribution and TOC is also shown by the HI and OI that clearly suggest a mixed source of allochthonous (plant material such as vascular plants) and autochthonous organic matter (algal type such as phytoplankton). Based on TOC/N<sub>tot</sub> ratios, Morgantini and Peruzzi (2012) ascribed an algal origin to this sedimentary organic matter. Most likely, these differences in interpretation are due to the use of different analytical methods. As suggested by Meyers (1997) TOC/N<sub>tot</sub> ratios can be affected by the presence of clay-rich sediments ('matrix effect') that adsorb ammonium ions (NH<sub>4</sub><sup>+</sup>) causing a decrease of this ratio. The latter is coherent with the high OI values obtained by Rock-Eval and explain the mixed character of the OM as shown in the Van Krevelen plot of Fig. 3.

## CONCLUSIONS

The living ostracod fauna of the shallow endorheic Lake Trasimeno has been investigated for the first time. Within the 19 species identified, several assemblages have been recognized, related to different ecological niches distinguished on the basis of the physical and chemical water parameters as well as different type of substratum and macrophytes coverage, confirming once

more the strong ties between ostracods and their host environment. Although no endemic taxa have been identified in the lake, the occurrence of the three species *C. torosa*, *I. salebroso*, and *L. stationis* addresses several issues related to biodiversity and (palaeo)-limnology. In particular, the occurrence of *C. torosa*, living in an inland lake with low salinity waters, highlights the need of a cautious palaeoenvironmental interpretation when this species occurs in older records. A careful observation of the shell morphology must be carried out especially when this is the dominant species. The occurrence of *I. salebroso* expands the distribution area of the species to central Italy, with consequences on its meaning within the Mutual Ostracod Temperature Range applications (Horne, 2007). *L. stationis* has been considered for a long time an alien species, disseminated by passive transport in the rice fields of Northern Italy. As a matter of fact, the occurrence of fossil representatives in Umbria and Sicily and the occurrence of this species living in present Lake Trasimeno, could tell a completely different story, linked to loss of habitats and lack of specific studies more than to freshwater invasions as previously thought.

The knowledge of the present-day ostracod population of Lake Trasimeno represents a further *tessera* in the mosaic of the distribution of ostracods in Italian inland waters. It provides a starting point to evaluate both past and present climate- and anthropogenically-induced changes of this fragile environment.

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