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Active and resting stages of zooplankton and its seasonal evolution in a hypersaline temporary pond of the Mediterranean coast (the "Vecchia Salina", SE Italy)*

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SUMMARY: The species composition of zooplankton and its variability were studied with an integrated water-sediment analysis for a period of two years in a hypersaline temporary pond in SE Italy. The basin was affected by extended drought during summer, and even during the wet period the sodium chloride salinity was never below 42.5‰. The zooplankton showed the presence of seasonal species (mainly Anostraca), together with not seasonal, but opportunistic ones (mainly Rotifera, and Ciliophora) with a shorter life cycle. Rotifers (mainly *Hexarthra fennica*, and *Proales similis*), and ciliates (*Fabrea salina*) attained 99-100% of total planktonic organisms in certain periods. Resting stages were extracted from the upper 6 cm of 14 sediment cores collected during a dry period (August 1998). A total of 24 resting morphotypes (cysts) were listed—more than double the number of the active organisms (11) found in the plankton over the two years. The seasonal succession of species was different in the two years studied. This fact, together with the richness of the cyst bank of the sediment, indicates that in each period the water column shows only a portion of the biodiversity, which the sediment contains unexpressed as resting stages. The study of cyst distribution (both horizontal and vertical) in sediments provided complementary suggestions to understand the space-time distribution of the plankton organisms. Laboratory tests showed that hatching of different cysts generally occurred in a wide range of salinity conditions, and was not synchronous. This allowed us to assume that even the cyst hatching rate could be an adaptation to highly variable extreme environments.

Key words: hypersaline environments, Mediterranean, zooplankton, resting stages, cyst.

RESUMEN: ESTADOS ACTIVOS Y DURMIENTES DEL ZOOPLANCTON Y SU EVOLUCIÓN ESTACIONAL EN UNA LAGUNA TEMPORAL HIPERSALINA DE LA COSTA MEDITERRÁNEA (LA "VECCHIA SALINA", ITALIA SE). – La composición específica del zooplancton y su variabilidad han sido estudiadas con un análisis integrado agua-sedimento, por un periodo de dos años, en una laguna temporal hipersalina en el SE de Italia. La cuenca se vio afectada por una prolongada sequía durante el verano e, incluso durante el periodo húmedo, la salinidad de cloruro sódico nunca fue inferior al 42.5‰. El zooplancton mostró la presencia de especies estacionales (principalmente Anostraca), junto con no estacionales, pero oportunistas (principalmente Rotifera y Ciliophora) con un ciclo de vida más corto. Los rotíferos (principalmente *Hexarthra fennica*, y *Proales similis*) y los ciliados (*Fabrea salina*) alcanzaron el 99-100% del total de organismos planctónicos en ciertos periodos. Los estados durmientes fueron extraídos de los 6 cm superiores de cores (testigos) de 14 cm recogidos durante un periodo seco (agosto 1998). Un total de 24 morfotipos durmientes (quistes) fueron catalogados, más del doble que el número de organismos activos (11) encontrados en los dos años. La sucesión estacional de especies fue diferente en los dos años estudiados. Este hecho, junto con la riqueza del banco de quistes del sedimento, atestigua que la columna de agua muestra en cada periodo sólo una fracción de la biodiversidad, que el sedimento contiene, sin expresarse, como estados durmientes. El estudio de la distribución de los quistes (tanto horizontal como vertical) en el sedimento aportó indicaciones complementarias para entender la distribución espacio-temporal de los organismos del plancton. Ensayos de laboratorio mostraron que la eclosión de los diferentes quistes generalmente ocurre en un amplio rango de condiciones de salinidad, y no fue sincrónico. Esto nos permitió suponer que incluso la tasa de eclosión de los quistes podría ser una adaptación a los muy variables ambientes extremos.

Palabras clave: ambientes hipersalinos, Mediterráneo, zooplancton, estados durmientes, quistes.

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INTRODUCTION

Physically-controlled fluctuating systems generally show a simplified assemblage of species. In these systems, therefore, an elemental taxonomic characterisation and a parallel description of the forcing physical variables are easier, and these descriptions are useful for the understanding and interpretation of the results (García *et al*., 1995).

Among these interesting systems are seasonal, temporary basins in which, according to the definition of Decksbach (1929), drastic changes in both the physical environment and community structure occur during each single year. Salt lakes are of particular interest because of their habitat homogeneity, discreteness, low taxonomic diversity and source of material for microecosystem studies (Vareschi, 1987). However, few studies have been devoted to the plankton community (Hammer, 1986). The ephemeral nature of the aquatic phase indeed excludes most fish and allows the large branchiopods to become some of the most successful and conspicuous inhabitants of temporary pools, where they bridge unfavourable (dry) periods by producing resting stages. As a consequence, temporary pools are sometimes depicted as so-called "enemy-free" spaces that give shelter to the vulnerable primitive large branchiopod crustaceans (Fryer, 1986). There is, however, mounting evidence that predators exist (turbellarians for Blaustein and Dumont, 1990; or diving coleoptera for Herwig and Schindler, 1996) and they have an important regulating effect on temporary pool communities (Spencer *et al*., 1999). Their presence, however, like that of all other plankters, is not easy to detect with standard sampling procedures, probably due to very fast presence-cycles (García *et al*., 1997).

The main feature of water species in temporary ponds is that the only biotic link between two successive hydrological cycles consists of the possibility of surviving harsh conditions (even drying out), mainly by producing resting stages. Many animals live in ephemeral habitats, and when the conditions become unsuitable for their life, they may enter into dormancy (see Ricci, 2001 for terminology). The resting eggs (dormant stages) produced by Rotifera Monogononta can withstand several stresses such as low temperatures and drying out (Gilbert, 1974). Resting stages generally lie on the bottom surface where they remain dormant until the next growing season, usually months later. The resumption of development is commonly influenced by a variety of factors, temperature, oxygen and light being the most common (Pourriot and Snell, 1983; Alexeev, 1990; Drinkwater and Clegg, 1991; Wallace, 1999). Hatching may be synchronous for a given group of eggs, or scattered over a wide time interval. Monogononta reduce the risk of unpredictable arrival of unsuitable conditions by maintaining a variable fraction of mictic individuals (which produce resting eggs) during the amictic phase (Ricci, 2001), and performing a non-synchronous hatching of resting eggs in order to maintain a pool of eggs which can hatch successively (the bet-hedging strategy, see Philippi and Seger, 1989).

Dormancy as a key phenomenon for successfully adapting to temporary habitats is a topic of interest in studies on the ecology of stressed environments, and it could be responsible for a total biodiversity in each system—generally higher than the one which is perceived by active stage presence. García *et al*. (1997) demonstrated that in a saline temporary lake of Southern Spain the plankton composition and seasonal succession varied from one year to another, according to the modification of hydrological conditions. These authors suggest extended samplings over time to obtain more precise information on species presence. In some cases a correlation between plankton abundance (during the flooded period) and cyst distribution on the bottom sediment has been proposed (Thiery, 1997) as a contribution to the understanding of space-time distribution of active stages in temporary ponds.

Here we propose a faunistic study which pays special attention to resting stages (their distribution and abundance in sediments) in order to better understand the total plankton biodiversity, and its variability, in a temporary salt-pond where a standard plankton collection (generally carried out for one-year periods) could underestimate the total biodiversity and the complexity of the related system.

MATERIALS AND METHODS

The *Vecchia Salina* at Torre Colimena (Gulf of Taranto, Ionian Sea, Mediterranean) (Fig. 1a-b) is a salt lake with a surface area that varies from 0.25 km^2 (during winter, the wet season) to only 10,000 m^2 (during summer, the dry season). This small basin has its major axis (about 950 m) parallel to the coastline, and is separated from the sea by a dune bar. In the past, a man-excavated channel breached the thin sandbar (and its rocky base). This direct

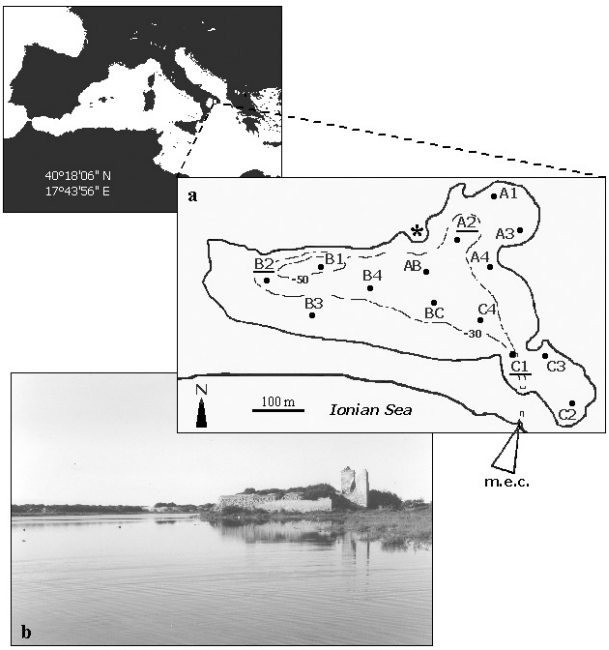

FIG. 1. – Location of Vecchia Salina, with a map (a) indicating the position of stations (A1, ... C4) where sediment was collected, and (b) a picture of ruins located at the position of the asterisk (*) on the map (a). Dotted lines indicate the depth (cm) at maximum flooded period. The zooplankton samples were collected at the deepest area (B1). The black arrow indicates the ancient man-excavated channel (m.e.c.) which connected the pond to the sea until the end of the 18th century. Geographic coordinates approximately refer to the centre of the pond (Station BC).

connection between the lake and the sea was closed in the middle of the 18th century (Lamusta and Nardone, 2000). Since that time the basin flood primarily depends on rainfall, but Montelucci and Parenzan (1967), and Parenzan (1983) reported the presence of weak freshwater springs on its north-western edge.

The basin has a triangular shape, and it was arbitrarily subdivided into three portions (A, B, C) (Fig. 1a). The zooplankton collection was limited to portion B, the deepest and the longest flooded one. Zooplankton sampling was carried out monthly from March 1998 to March 2000 in wet periods, for a total of 13 dates. On each sampling date, measurements of depth, temperature (by standard field thermometer), and conductivity (by a lab-conductimeter GLP31 CRISON) were carried out at Station B1. Salinity values were obtained by conductivity value conversion at 25°C ($y=3E\text{-}05x^3 - 0{,}0028x^2 + 0{,}8311x - 3{,}7719$; $r=0{,}99$ $p<0{,}001$ with x=conductivity in mS/cm, y=salinity in g/L). Zooplankton samples were collected with two plankton nets (mouth diameter, 25 cm; length, 65 cm; mesh size, 200 μm, and 50 μm) towed horizontally, equipped with a water-flow meter at the mouth. Between 325 and 1360 litres were filtered in each sample collection. When the water column was less than 30 cm deep, the collection was carried out by carefully towing the net with the circle mouth half submerged (a semicircular area of the mouth was considered in calculating the water volume filtered). Samples were fixed *in situ* with neutralised (pH = 7.3) 4% formalin solution. In the laboratory, aliquots of each sample were subdivided into the 6 wells (10 cc volume each) of 2 culture plates (total 120 cc) and analysed under an inverted microscope at 200x magnification, after sedimentation. Data are presented as number of individuals per m^3. When the same *taxon* was sampled with both plankton nets, the most abundant capture was considered.

Sediment samples were collected in August 1998. Fourteen sediment cores (8 cm diameter; 6 cm depth) were collected from the dried floor (Fig. 1b) by means of a core sampler (20 cm high; internal diameter, 8 cm). At three stations (A2, B2, C1), 12 cm length cores were collected. Sediment cores were stored at 4-6 °C in a refrigerator for two months. For the analysis, each sediment core was cut into 3 cm thick transverse layers. Each layer was ultrasonified and sieved at two mesh sizes (200 μm, and 45 μm). The sediment collected by both sieves was centrifuged at 1,090 g in a 1:1 sucrose-distilled water solution for 2.5 minutes. The supernatant derived from the centrifugation of the two sieve fractions was analysed to find resting stages. Resting stages were reported as number per 100 cm^{-3} of sediment. Non-parametric multidimensional scaling (nMDS) and cluster analysis were used to obtain bi-dimensional representations of resting stage assemblages in the sediment. Both analyses were performed by PRIMER 5 (Clarke and Warwick, 1994) on a Bray-Curtis similarity matrix based on untransformed data.

The most abundant resting stages (3 for the fraction > 200 μm; 7 for the fraction 45-200 μm) were used in hatch experiments in the laboratory. Sets of 20 resting stages (cysts) of each morphotype were stored in 3 cc wells raised with 2 cc of original water (conductivity, 90.5 mS/cm) filtered at 0.45 μm. To avoid bacterial growth, in each well, 20 μl of an antibiotic mix (streptomycin/penicillin 1:1) was added. Resting stages were submitted to different storage conditions in thermostatic rooms (1. an "equinox" simulation, with 13 °C and 12L:12D photoperiod; 2. an "early June" simulation, with 23 °C and 14L:10D photoperiod) at five different conductivity values (90.5; 49.4; 31.8; 15.5; freshwater)

TABLE 1. – Variation of water physico-chemical parameters in the Vecchia Salina, from March 1998 to March 2000. Salinity (sodium chloride) values were obtained by the conversion of the conductivity values.

	1998 Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	1999 Jan	Feb	Mar	Apr	May-Oct	Nov	Dec	2000 Jan	Feb	Mar
Conductivity (mS/cm)	60	63.6	76.2	95.3	191.7	-	157.4	146.5	128.8	70.7	60.6	70	71.1	78.8	-	68,9	70,6	70	75,6	72,3
Equivalent Salinity (g/L)	42.5	45.5	56.6	76	264	-	174.6	152.2	120.9	51.6	43	51	52	59	-	50	51,5	51	56	53
Temperature (°C)	8.4	17.8	19.5	30.5	31.5	-	21.5	15	14	7.3	11	9.3	16.5	17	-	10,3	9,5	9	10,5	11,5
Depth (cm)	55.5	49.5	40	34	22	-	19.5	21.5	22	42	43.5	51	41	35	-	55	50	52,5	44	60

TABLE 2. – Abundance variation of the planktonic assemblage (11 taxa, alphabetic order) in the Vecchia Salina (Station B1) from March 1998 to March 2000, expressed as number of individuals per 1 m^3 of water. Data are absent from columns representing dry periods.

	1998 Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	1999 Jan	Feb	Mar	Apr	May-Oct	Nov	Dec	2000 Jan	Feb	Mar
Acarina	700	819	1075	637	0	-	0	0	0	0	0	0	0	301	-	0	0	0	0	0
Artemia parthenogenetica Bowen and Sterling, 1978	0	0	0	0	0	-	0	0	0	832	1171	237	31	0	-	227	574	747	0	0
Branchinella spinosa Milne-Edwards, 1840	36	39	14	0	0	-	0	0	0	0	0	0	30	220	-	6	9	16	109	182
Chironomidae *larvae*	105	534	430	0	0	-	0	0	0	20	11	6	337	0	-	0	0	120	0	21
Fabrea salina Henneguy, 1890	0	0	70606	446027	45666	-	8650	38106	610533	5937	0	15649	32205	88717	-	5460	3150	0	0	76
Hexarthra fennica (Levander) Neal, 1951	385	5643	26981	0	0	-	0	0	0	688	4795	151679	1095241	2366	-	643440	596260	70710	0	0
Nematoda	875	1228	1462	6880	1038	-	1038	1165	0	98	140	556	542	10	-	560	211	450	57	13
Nitocra sp.	630	765	4300	765	0	-	0	166	0	70	24	890	14	0	-	0	0	210	57	14
Potamonectes cerisyi Aubé, 1836	0	0	6	0	0	-	0	0	0	0	0	3	9	20	-	2	2	3	0	4
Proales similis de Beauchamp, 1908	1855	1388	0	3852	2322	-	0	0	0	38	210	926	339	11786	-	1890	1750	0	779	84
Turbellaria	0	0	817	1656	5160	-	0	0	0	0	0	0	339	93	-	1330	610	630	247	9
Total	4586	10416	105691	459817	54186	-	9688	39437	610533	7683	6351	169940	1129087	103513	-	652915	602566	72886	1249	403

obtained by diluting the original water with freshwater. Hatching plates were checked daily for the presence of active stages, which were counted and removed for identification.

RESULTS

Table 1 shows values of the considered abiotic features in the sampling area. The water depth (at Station B1) reached its maximum (60 cm) in March, 2000; the conductivity and the temperature reached their maximum values (191.7 mS/cm, and 31.5°C respectively) in July, 1998. Minimum values for these parameters (60.0 mS/cm, and 8.4°C respectively) were obtained in March, 1998. In 1998 only in August was it impossible to collect zooplankton; this impossibility (less than 19 cm of water column, in portion B) was more extended in time in 1999 (from May to October).

Table 2 shows the abundance variations in the planktonic assemblages. The zooplankton was composed of 11 taxa, with Rotifera, Ciliophora, and Anostraca as the most abundant. Four peaks of abundance were registered over the two year period,

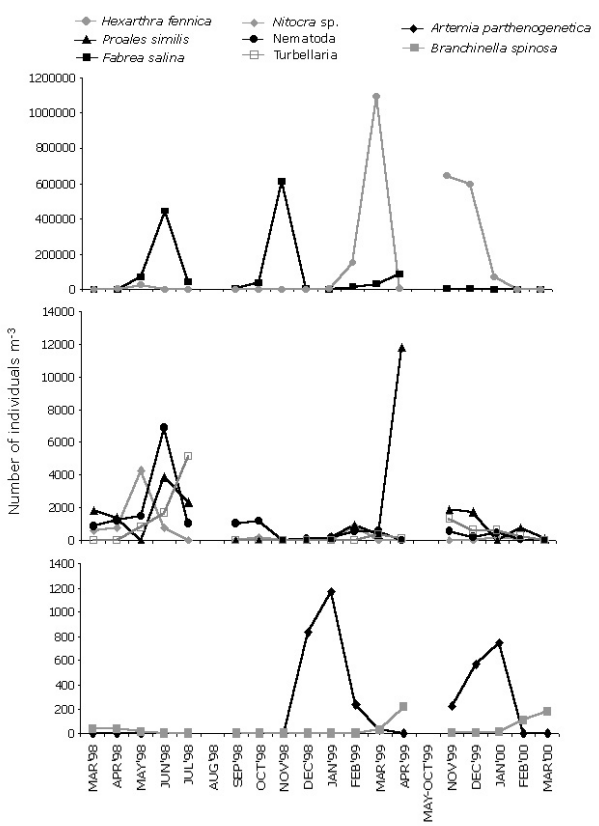

FIG. 2. – Abundance fluctuations of the main taxa of the Vecchia Salina zooplankton from March 1998 to March 2000.

during which abundance values were two-three orders of magnitude higher than those of preceding and successive samples. The first peak of abundance (459,817 ind. m^{-3}) was recorded in June 1998, at the end of the first wet cycle. The ciliate *Fabrea salina* was the main species responsible for this peak, with undetermined Nematoda and the rotifer *Proales similis* as minor components (Fig. 2). *F. salina* was the only active zooplankter in November 1998, when the second peak of abundance (610,533 ind. m^{-3}) occurred. From December 1998 to February 1999 the community shifted from the dominance of *F. salina* (in December 1998) to that of *Hexarthra fennica* (in February 1999), with the anostracan *Artemia parthenogenetica* as the second most abundant species, both in December 1998 and in January 1999. In March 1999, the third peak of abundance (the absolute maximum, 1,129,087 ind. m^{-3}) was recorded, mainly due to *H. fennica*. Early developmental stages of the anostracan *Branchinella spinosa* (30 ind. m^{-3}) were present too. During April 1999 *A. parthenogenetica* disappeared, while the *B. spinosa* population grew (220 ind. m^{-3}). In this period, we observed the predation by *Potamonectes cerisyii* larvae (Coleoptera, Dytiscidae) on *B. spinosa* adults (particularly gravid females). The April 1999 sample was characterised by the evident reduction of *H. fennica* (2,366 ind. m^{-3}) in favour of *F. salina* (88,717 ind. m^{-3}) and *Proales similis* (11,786 ind. m^{-3}). During the last wet phase, *H. fennica* dominated the fourth peak of abundance (652,915 ind. m^{-3}), which lasted for two months (November-December 1999). In January 2000 the maximum *A. parthenogenetica* population (747 ind. m^{-3}) was recorded.

During the whole investigation period, we collected typical benthic organisms in the plankton, mainly represented by nematodes, turbellarians, harpacticoid copepods and acari.

Table 3 shows the mean abundance of 24 morphotypes of resting stages found in bottom sediments from 14 sampling stations, which represented the whole basin (see Fig. 1). Only 7 of the morphotypes were assigned to a *taxon*. The highest densities of resting stages were recorded in the central zone of the basin (Stations A2, AB, B1, B2, B4, BC, C1, C4), where the total abundance exceeded 1,000 cysts 100 cm^{-3} in the first 6 cm from the surface. Station B4 was the richest one (average of 1,850 cysts 100 cm^{-3}). Low cyst abundance occurred in the peripheral zone of the basin, with the minimum at Station A4 (average of 209 cysts 100 cm^{-3}). As rep-

TABLE 3. – Distribution of resting stages (24 morphotypes) in sediment cores from 14 stations (A1, ... C4) in the Vecchia Salina. Each sediment core has been subdivided into 2 portions (0-3 and 3-6 cm from the surface). A2, B2, and C1 cores were subdivided into 4 portions (0-3, 3-6, 6-9, 9-12 cm from the surface). Resting stage abundances are indicated as number per 100 cm^3 of sediment.

Resting stages morphotypes	A1 0-3	A1 3-6	A2 0-3	A2 3-6	A2 6-9	A2 9-12	A3 0-3	A3 3-6	A4 0-3	A4 3-6	AB 0-3	AB 3-6	B1 0-3	B1 3-6	B2 0-3	B2 3-6	B2 6-9	B2 9-12	B3 0-3	B3 3-6	B4 0-3	B4 3-6	BC 0-3	BC 3-6	C1 0-3	C1 3-6	C1 6-9	C1 9-12	C2 0-3	C2 3-6	C3 0-3	C3 3-6	C4 0-3	C4 3-6
A. parthenogenetica cyst	91	70	235	245	336	497	78	150	63	17	56	82	175	139	256	91	77	147	53	159	36	56	27	6	161	132	158	91	86	286	63	120	91	336
B. spinosa cyst	245	182	549	595	371	259	330	165	126	50	1071	309	371	233	1050	560	413	252	280	154	448	465	233	56	763	491	315	172	235	78	175	183	791	301
Aloninae ephippium	42	28	67	0	0	0	22	15	35	0	7	0	0	0	0	0	0	0	0	0	0	45	0	0	0	0	0	0	0	0	0	0	0	0
Fabrea salina cyst	0	0	16	0	0	0	0	0	0	0	21	0	193	0	554	0	0	0	3	0	140	0	42	28	140	0	0	0	76	0	59	0	62	0
Hexarthra fennica r. e.	35	28	52	4	24	38	29	10	29	0	76	17	174	31	155	49	105	50	62	6	77	49	97	62	98	0	0	0	59	0	137	31	221	6
Ptygura sp. r. e.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	17	21	0	17	0	14	0	0	0	35	0	8	11	3	0	6	0	11	0
Staphylinidae r. e.	140	7	370	0	0	0	140	0	0	0	55	157	658	145	145	14	0	0	45	0	1211	78	0	0	896	0	0	0	252	0	252	0	168	0
Cyst type 1	63	63	42	7	17	38	25	66	8	13	29	7	0	10	8	17	0	0	6	14	28	35	185	263	14	0	0	0	6	6	6	8	8	0
Cyst type 2	91	0	0	4	0	0	42	0	4	0	0	0	0	0	14	28	13	8	0	0	0	0	223	22	24	0	0	0	45	0	39	0	17	0
Cyst type 3	63	84	58	98	98	105	8	59	13	34	29	63	8	24	21	122	350	290	84	67	220	357	76	168	21	63	45	50	0	20	34	81	132	48
Cyst type 4	0	0	0	7	10	29	0	0	4	0	0	10	11	7	37	115	133	101	31	31	105	129	542	67	10	28	48	11	6	0	8	31	22	11
Cyst type 5	0	0	31	25	59	42	8	24	0	4	0	17	11	10	0	17	21	8	11	48	24	42	0	174	10	14	0	11	0	0	0	8	3	0
Cyst type 6	0	0	0	0	0	0	13	0	4	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	36	0	3
Cyst type 7	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	3	0	0	0	0	3	14	0	0	0	0	0	0	0	0	0	0	0	0
Cyst type 8	0	0	0	0	3	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cyst type 9	0	0	5	4	0	0	0	0	0	0	4	24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	6	17	0	8	0
Cyst type 10	42	0	31	10	7	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cyst type 11	0	14	31	10	7	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cyst type 12	0	0	52	31	38	8	0	0	0	0	0	3	0	0	31	0	0	46	6	0	7	10	0	0	0	24	0	0	6	0	6	0	8	0
Cyst type 13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cyst type 14	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cyst type 15	0	0	0	18	17	34	21	7	0	0	0	0	0	0	21	0	0	29	0	0	0	0	0	0	0	14	6	0	0	0	0	0	8	0
Cyst type 16	0	0	0	0	0	0	0	10	0	0	0	0	0	10	21	0	0	0	0	0	10	7	0	0	3	10	6	8	17	11	14	17	14	3
Cyst type 17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	20	6
TOTAL	812	476	1518	1056	980	1058	716	506	286	131	1348	692	1601	609	2268	1050	1148	936	606	479	2323	1287	1425	846	2165	776	586	354	799	407	816	535	1584	714

resented in Figure 3, the cluster analysis and the relative non-parametric multi-dimensional scaling (nMDS) ordination segregated two main groups of stations: the "peripheral group" and the "central group", with Station BC and A4 well separated from all the others.

Cyst abundance varied considerably according to the species. Only those of the two anostracan species were present in all the cores and all the sediment layers (Table 3). They were also the most abundant, together with the Staphylinidae eggs, and the Cyst type 3. The number of cysts of *Branchinella spinosa* ranged between 88 (Station A4) and 690 (Station AB) 100 cm^{-3}, while they were between 16.5 (Station BC) and 328.25 (Station A2) 100 cm^{-3} for *Artemia parthenogenetica* (for these two species, see Moscatello *et al*., 2002). Figure 4 shows the abundance distribution of cysts at the 14 sampling stations. *A. parthenogenetica* cysts were less abundant in the sediment of the central sampling stations (BC, B4, AB) (see Table 3). Cladocera (Aloninae) ephippia were found almost exclusively in portion A stations. The opposite pattern was noted for *Ptygura* sp., whose resting eggs were not numerous, but they were absent from portion A of the basin. Cysts of *F. salina* were practically absent from the peripheral stations, and were accumulated particularly in the deepest area of the pond (B1, B2, B4). In the case of *H. fennica*, resting eggs were more or less regularly distributed within the pond along the axis B2-C3.

As regards the vertical distribution of the cysts, generally the upper sediment layers were richer in cyst content than the lower ones (as shown in Table 3), but "type 3" (the fourth most abundant cyst) was evidently less abundant in surface layers than deeper ones in 12/14 of the collected cores.

Laboratory experiments under controlled conditions let us follow the

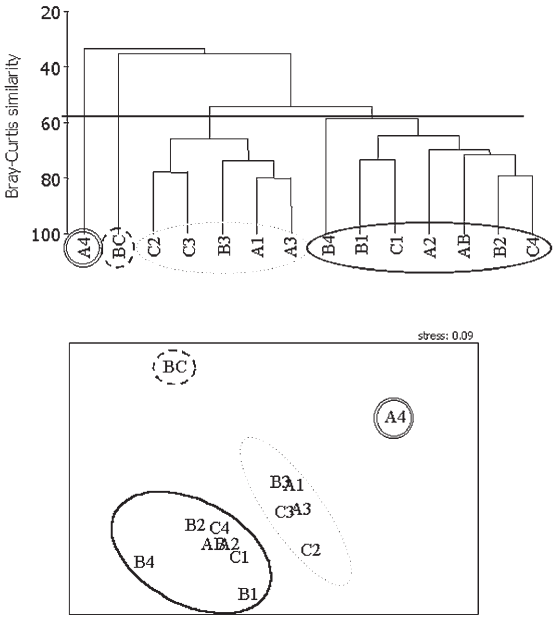

FIG. 3. – a) Dendrogram using group-average linking on Bray-Curtis species abundance from untransformed abundance data; the 4 groups defined at arbitrary similarity level of 57% are indicated. b) bi-dimensional nMDS of the species abundance's similarity matrix as in Fig. 3a. The 4 groupings from the cluster analysis are indicated (stress = 0.09). A1, ... C4 are the sampling stations of sediments (August 1998), as shown in Fig. 1.

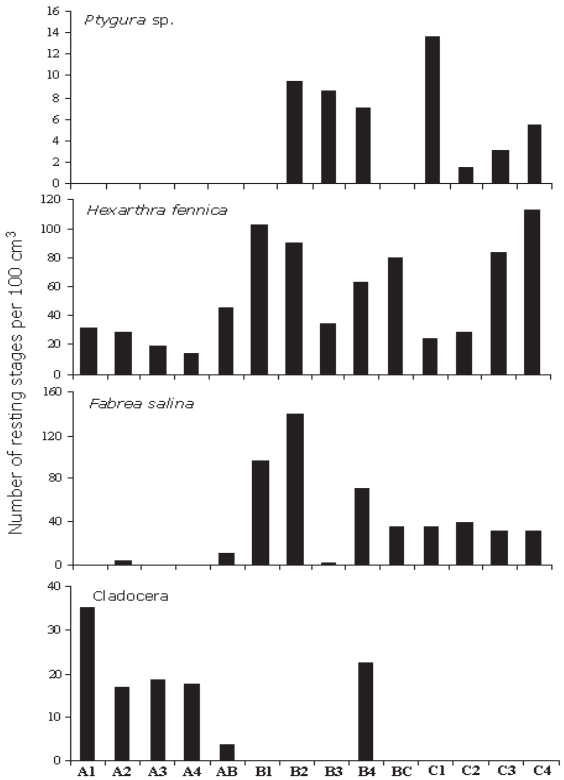

FIG. 4. – Abundance of cysts in sediments of Vecchia Salina from 14 sampling stations (A1, ... C4) visited in August 1998.

hatching of *F. salina*, *H. fennica*, and *Ptygura* sp. cysts (Fig. 5). No hatching was obtained from *B. spinosa*, type 1, type 5, type 17, and type 18 cysts. We observed no hatching for all three species in freshwater conditions. All cysts of *H. fennica* hatched at conductivity of 49.4 mS/cm both in early June (temperature, 23°C; photoperiod, 14L:10D) and in equinox (13°C ; 12L:12D) simulations, after 7 and 4 days of incubation respectively. In *F. salina*, maximum hatching rate (100%) was obtained at conductivity of 95 mS/cm (after 3 days incubation) in June conditions (23°C; 14L:10D). But 100% hatching also occurred in equinox conditions (13°C; 12L:12D), on the 7th day of incubation. In *Ptygura* sp., the hatching peak (90%) occurred at a conductivity of 31.8 mS/cm in June conditions after 15 days. The species showed a lower hatching rate but hatched at a wider range of conductivity (from 15.5 to 49.4 mS/cm) in equinox conditions.

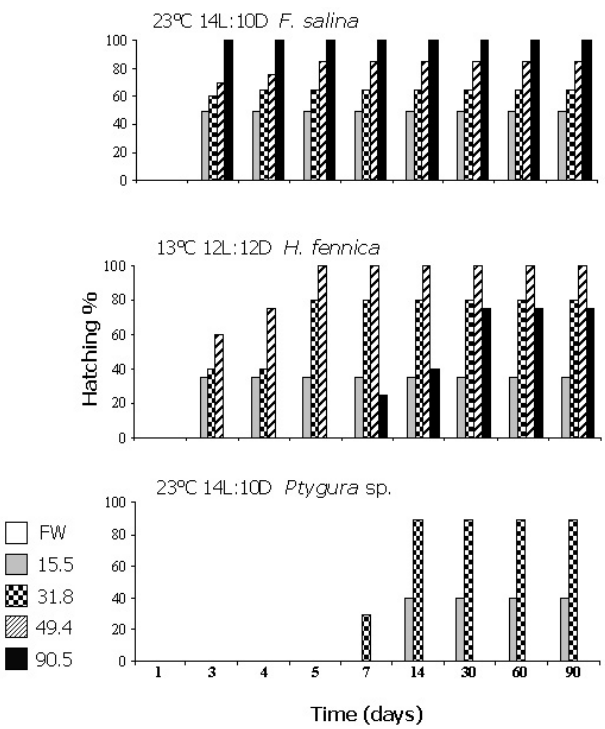

FIG. 5. – a) Hatching pattern of *Fabrea salina* cysts submitted to different conductivity conditions in lab-simulated June (temperature, 23°C; photoperiod, 14L:10D). b) Hatching pattern of *Hexarthra fennica* resting eggs submitted to different conductivity conditions in lab-simulated equinox (temperature, 13°C; photoperiod, 12L:12D). c) Hatching pattern of *Ptygura* sp. resting eggs submitted to different conductivity conditions in lab-simulated June. Values in the legend are expressed as mS/cm (Conductivity); FW is freshwater.

DISCUSSION

As regards abiotic conditions, the recorded values of water conductivity (never under 60 mS/cm, corresponding to 42.5‰ of Sodium-Chloride salinity) showed how the system was hypersaline even in

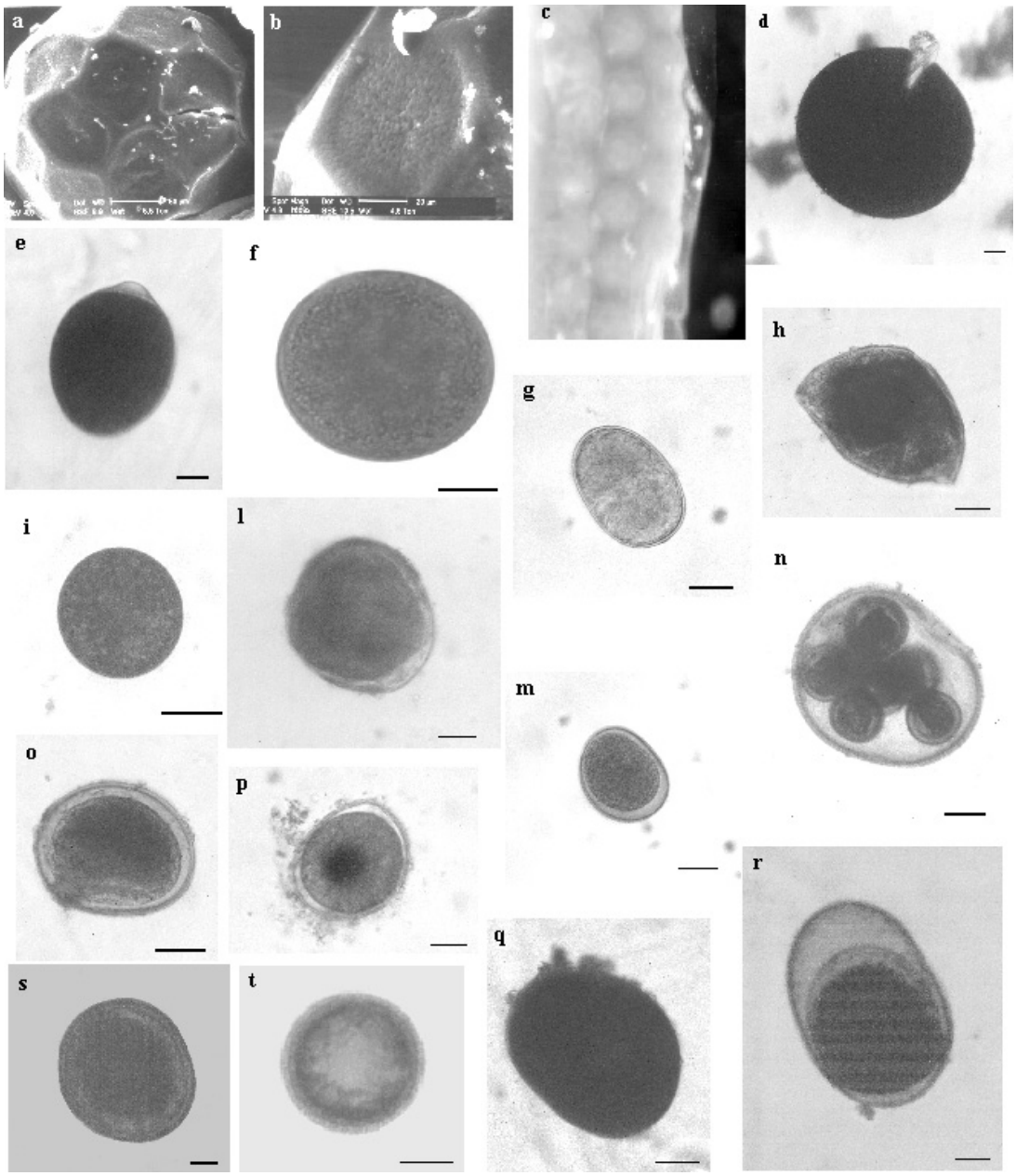

FIG. 6. – Resting stages of plankton organisms collected in the Vecchia Salina sediments. a-b), SEM of *Branchinella spinosa* cyst; c) *B. spinosa* cyst (ovisac); d) *Artemia parthenogenetica* cyst; e) *Fabrea salina* cyst; f) *Hexarthra fennica* resting egg; g) *Ptygura* sp. resting egg; h) Cyst type 1; i) Cyst type 2; l) Cyst type 3; m) Cyst type 4; n) Cyst type 8; o) Cyst type 9; p) Cyst type 10; q) Cyst type 12; r) Cyst type 15; s) Cyst type 16; t) Cyst type 17. Scale bar: 30 μm.

its maximum flooding condition. The dry season was of different length in the two studied years, being of one and six months respectively. This fact probably greatly affected the different zooplankton calendar of the two flooded periods.

The two anostracan species could be classified as seasonal: a previous study (Moscatello *et al.*, 2002) showed that they adopt a different strategy (in terms of reproductive cycle, cyst production and cyst hatching) to optimise their time of residence in the system, also avoiding possible competition between stages of overlapping size.

The abundance of zooplankton in the Vecchia Salina pulses with "peaks and troughs" during the year. We noted that peaks of abundance did not occur in corresponding periods of the two considered years, and that the responsible organisms were different. In fact the protistan *Fabrea salina* was responsible for two abundance peaks, in June and November 1998, but it was scarce in 1999, when the

rotifer *Hexarthra fennica* was responsible for peaks in March and November (and it was scarce or even absent in the same periods of the preceding year). This also suggests that the analysis of the water column content of such an environment, even after two years, could have not given us the correct information about its biodiversity content. The sudden dominance of one or a few species is a common phenomenon causing the appearance of time-localised abundance maxima. Vecchia Salina usually shows a very low species diversity (in terms of active stages), but high abundance values (up to ~ 1,200,000 ind. m^{-3} in March 1999). This was due to opportunistic and fugitive species (as ciliates and rotifers can be considered) of small size adopting an *r*-reproductive strategy to face the extreme variability of the system.

During the flooding phase, typically seasonal species of larger size, such as *A. parthenogenetica*, *B. spinosa* and *P. cerisyi*, succeed each other. We surmise (according to Moscatello *et al.*, 2002) that the presence of competitors could be possible on the basis of different hatching and reproductive strategies that might determine temporal fluctuations in recruitment.

As regards the faunistic composition of the basin, we noted how the study of cyst presence and distribution in bottom sediments was a good indicator of the biodiversity potential of Vecchia Salina. The study of the zooplankton, whose composition cannot be considered to be clarified even after two years of sample collection, received additional data and information. The number of cyst morphotypes (24) collected on a single date heavily exceeded the number of species (11) found in the plankton on 13 sampling dates over two years. However, we cannot consider the cysts found as entirely belonging to the "zooplankton". In one case the hatching tests revealed the presence of a benthic rotifer (*Ptygura* sp.) never found in the water column, and also Staphylinidae eggs, one of the most abundant morphotypes, were laid by rove beetles which dug tunnels in the sediment during dry periods (our sediment cores were typically crossed by these tunnels). Also many of the zooplankton species here listed are "unconventional" because they do not belong to the plankton (e.g. Nematoda, Acarina, Harpacticoida, Turbellaria, and Coleoptera). The plankton assemblage here recorded remains well below that which has been found into the sediments, and the advantage of investigating biodiversity by means of sediment study is confirmed. The only obstacle to the use of such an approach is the actual scant knowledge about cyst identification. In particular, among the most abundant, undetermined cysts (types 1, 2, 3, or 4; see Fig. 6) we were not able to identify that of the rotifer *Proales similis*, one of the most abundant plankters, also because we did not obtain hatches to attribute one cyst type to this species. Furthermore, the study of cyst distribution in the sediments could be useful to understand the environmental preferences of plankton species. Cysts found all over the bottom are probably those of species which prefer winter conditions (and lower salinity values); while those of species which prefer summer conditions (and higher salinity values), when the pond was greatly reduced, were found at the central stations of the basin. The vertical partitioning of resting stages should also reflect the plankton dynamics and/or the sedimentation trend in a defined area (Anderson *et al.*, 1987), and the bottom "seed bank" should contain the total resting stages of subsequent plankton assemblages proceeding from different sedimentation periods (Lampert, 1995). The analysis of the vertical distribution of resting stages in sediments generally reported the upper layers as the richest ones. This is generally due to the fact that the superficial sediments are those of more recent sedimentation, and therefore subjected to lower deterioration processes. However, in some cases (e.g. for the cyst type 4 and 9) the cyst presence in sediments grew with depth, suggesting that in the past there could be different scenarios in the plankton community structure and/or relative abundance.

Ricci (2001) proposed to use the term "biodiversity bank" to extend the concept of seed or egg banks. Dormancy is of survival value when its duration spans unfavourable periods in the natural environment. Thus, its greatest significance is that it carries the population over to the next suitable condition. The dormant forms constitute a biodiversity bank, as they survive through environmental disasters, preserving species diversity, and provide a reliable colonisation source when conditions improve. The hatching pattern and the newly hatched survival, affected by environmental conditions, influences the species structure of the plankton community. In this case dormancy promotes species coexistence by means of resource partitioning and spatial or temporal separation of the competitors.

Figure 5 gives information on the hatching rates of a number of resting stages and, despite variation between species, the rates were often close to 100% after a few days. Apparently, the resting eggs viability

was not significantly affected by increasing duration of dormancy. In fact the hatching of the oldest resting stages (because they were taken from the deepest sediment layers of the lake) was also close to 100%.

The wide hatching pattern in tested species is thought to be an obligatory adaptation to the variable temporary habitat. Hatching tests suggested that planktonic species inhabiting Vecchia Salina dislike freshwater conditions. Species-specific differences in requirements for development and cyst hatching may also, to some extent, explain year-to-year discontinuous occurrence patterns in planktonic assemblages. Hatching has been found to be often highly variable, even within the same batch of cysts, and is thought to be an adaptation to ensure the persistence of populations in a variable and unpredictable environment (for example when newly hatched individuals could be suppressed by sudden variations in abiotic conditions).

Dissimilar requirements for optimal hatching in a mixed propagule-pool may contribute to changes in population density and species composition, depending on prevailing conditions (Brendonck, 1996). Survival in unstable environments with a high probability of total reproductive failure needs a fast hatching response to favourable conditions, a spread of risk among the progeny by the hatching of a fraction of the propagule bank that correlates with chances for successful reproduction, and the ability of propagules to survive for many generations in the soil. Thus, considerable variation in hatching behaviour may be expected in order to spread the risk of habitat uncertainty.

Hatching experiments, together with closer field study, will hopefully help us to understand the hatching patterns, timing and interaction of species adapted to extreme habitats like Vecchia Salina.

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REFERENCES

Alexeev, V.R. – 1990. Diapause in Crustacea. Ecological and physiological aspects. (in Russian) Nauk, Moscow, pp. 144.

Anderson, D.M., C.D. Taylor and E.V. Armbrust. – 1987. The effects of darkness and anaerobiosis on dynoflagellate cyst germination. *Limnol. Oceanogr.*, 32: 340-351.

Blaustein, L. and H.J. Dumont. – 1990. Typhloplanid flatworms (*Mesostoma* and related genera): mechanisms of predation and evidence that they structure aquatic invertebrates communities. *Hydrobiologia*, 198: 61-77.

Brendonck, L. – 1996. Diapause, quiescence, hatching requirements: what we can learn from large freshwater branchiopods (Crustacea: Branchiopoda: Anostraca, Notostraca, Conchostraca). *Hydrobiologia*, 320: 85-97.

Clarke, K.R. and R.M. Warwick. – 1994. Change in marine communities: an approach to Statistical Analysis and Interpretation. Natural Environment Research Council, UK: p. 144.

Decksbach, N.K. – 1929. Zur klassifizierung der Gewasser vom astatischen Typus. *Arch. Hydrobiol.*, 20: 399-406.

Drinkwater, L.E. and J.S. Clegg. – 1991. Experimental biology of cyst diapause. *In:* R.A. Browne, P. Sorgeloos and C.N.A. Trotman (eds.), *Artemia Biology*. CNR Press, USA: 93-117.

Fryer, G. – 1986. Enemy free space: a new name for an ancient ecological concept. *Biol. J. linn. Soc.*, 27: 287-292.

García, C.M., E. Echevarría and F.X. Niell. – 1995. Size structure of plankton in a temporary saline inland lake. *J. Plankton Res.*, 17(9): 1803-1817.

García, C.M., R. García-Ruiz, M. Rendón, F.X. Niell and J. Lucena. – 1997. Hydrological cycle and interannual variability of the aquatic community in a temporary saline lake (Fuente de Piedra, Southern Spain). *Hydrobiologia*, 345: 131-141.

Gilbert, J.J. – 1974. Dormancy in rotifers. *Trans. Am. Microsc. Soc.*, 93: 490-513.

Hammer, U.T. – 1986 Saline lake ecosystems of the world. W. Junk Publishers, Dordrecht: p. 616.

Herwig, B.R. and D.E. Schindler. – 1996. Effects of aquatic insect predators on zooplankton in fishless ponds. *Hydrobiologia*, 324: 141-147.

Lampert, W. – 1995. Egg bank investment. *Nature*, 377: 479.

Lamusta, S. and D. Nardone. – 2000. Tra sole e sale - la flora della salina dei "Monaci di Bevagna" sullo Ionio tarantino. Ed. Amici della "A. De Leo", Brindisi: 11-82.

Montelucci, G. and P. Parenzan. – 1967. Primo contributo allo studio botanico della costa neretina. *Thalassia Salentina*, 2: 42-107.

Moscatello, S., G. Belmonte and G. Mura. – 2002. The co-occurrence of *Artemia parthenogenetica* and *Branchinella spinosa* (Branchiopoda: Anostraca) in a saline pond of South Eastern Italy. *Hydrobiologia*, 486: 201-206.

Parenzan, P. – 1983. Puglia Marittima. Ed. Congedo, Vol. 1: 29-34.

Philippi, T. and J. Seger. – 1989. Hedging one's evolutionary bets, revisited. *Trends Ecol. Evol.*, 4(2): 41-44.

Pourriot, R. and T.W. Snell. – 1983. Resting eggs in rotifers. *Hydrobiologia*, 104: 213-224.

Ricci, C. – 2001. Dormancy patterns in rotifers. *Hydrobiologia*, 446/447: 1-11.

Spencer, M., L. Blaustein, S.S. Schwartz and J.E. Cohen. – 1999. Species richness and the proportion of predatory animal species in temporary freshwater pools: relationships with habitat size and permanence. *Ecology Letters*, 2: 157-166.

Thiéry, A. – 1997. Horizontal distribution and abundance of cysts of several large branchiopods in temporary pool and ditch sediments. *Hydrobiologia*, 359: 177-189.

Vareschi, E. – 1987. Saline lake ecosystems. *In:* E.D. Schultz and H. Zwölfer (eds.), *Potentials and Limitations of Ecosystem Analysis*, pp. 139-150. Springer Verlag, Berlin.

Wallace, R.L. – 1999. Rotifera. *In:* Encyclopedia of Reproduction. Vol. 4. Academic Press.

Scient. ed.: M. Alcaraz