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GENUARIO BELMONTE[1], SALVATORE MOSCATELLO[1],
E. A. BATOGOVA[2], T. PAVLOVSKAYA[2], NICKOLAY V. SHADRIN[2],
LARISA F. LITVINCHUK[3]

[1] Laboratorio di Zoogeografia e Fauna, DiSTeBA,
University of the Salento, Ecotekne, 73100 Lecce, Italy
[2] Institute of Biology of Southern Seas, Nakhimov avenue, 99011 Sevastopol, Ukraine
[3] University of Saint Petersburg, Russia

FAUNA OF HYPERSALINE LAKES OF THE CRIMEA (UKRAINE)

SUMMARY

Saline water bodies are critic, often extreme habitats with a small number of species. The small number of species can, however, be organized in different assemblages typical of the salt category dissolved into the water. For the first time a wide faunal study has been conducted (from 2002 to 2006) on hypersaline lakes of the Crimea peninsula (Ukraine) allowing us to recognize a total of 40 categories of zooplankton from 10 lakes in representation of the whole lake habitat of the peninsula. The only lake with water evidently influenced by the sea hosted the highest number of categories (20). The lowest number of categories (5) has been found in the lake which showed the highest salinity concentration. Apart the lake conditioned from sea water, other two categories of lakes were recognized: thalassohaline and atalassohaline on the basis of the species composition.

RIASSUNTO

I laghi salati sono ambienti critici, spesso estremi, per l'esistenza della vita ed ospitano generalmente un basso numero di specie. Il tipo di specie presenti, comunque, può risultare condizionato dalla tipologia dei sali disciolti. Nonostante la abbondante presenza di laghi salati nella penisola di Crimea, è questa la prima volta che è stato affrontato (con campionamenti condotti dal 2002 al 2006) uno studio completo sulla fauna ospitata. Lo studio ha consentito di riconoscere 40 categorie di organismi zooplanctonici da un campione di 10 laghi scelti a rappresentare l'intero territorio. L'unico lago influenzato direttamente dal mare era quello col maggior numero di categorie (20). Il lago con il minor numero di categorie (5) era anche quello che

presentava i valori più elevati di salinità. È stata notata una differenza nella qualità delle categorie presenti, tra laghi a salinità di origine marina (talassoalini) e laghi a salinità di diversa origine (atalassolaini).

INTRODUCTION

The biodiversity of hypersaline environments is generally lower than that of other types of water habitats (Wharton, 2002). In fact, vertebrates generally cannot support high salt concentrations (Zhao *et al*., 2005; Boix *et al*., 2008). The lowering of the species number is evident even in the same environment when this experiences an increase of salt concentration. The biodiversity diminution in the Aral Sea, subjected to a drastic diminution of the water volume, has been proposed as a consequence of the rise of salinity in the last decades, (Aladin, 1995).

As a consequence of the low biodiversity, generally a short trophic chain is typical of hyperhaline lakes, with single species in each of not more than 2-3 ecological roles (Wharton, 2002). The absence of complex ecological networks and the short trophic chains lead to the periodical outbursts of monospecific populations which face limits just in their same numbers (Fig. 1).

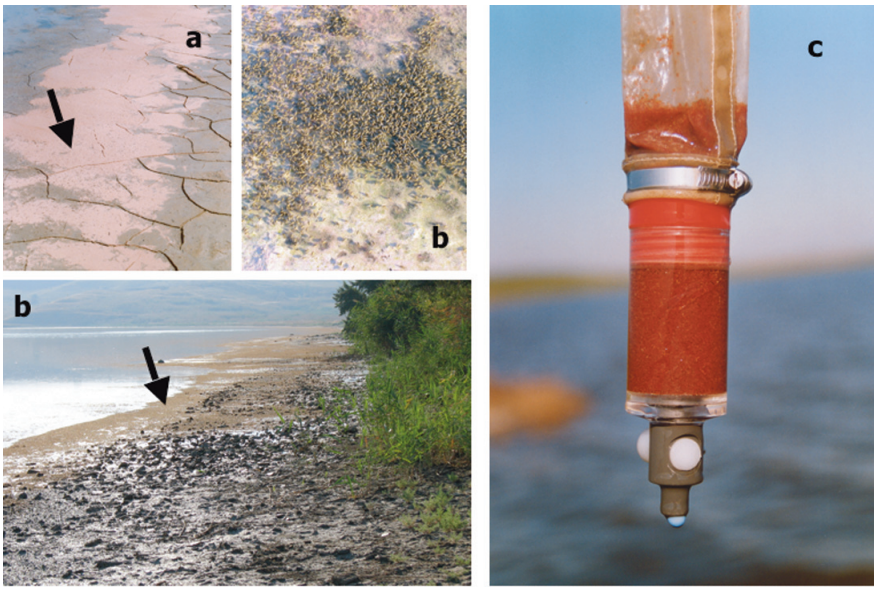

Fig. 1 Examples of crowding populations of just one species in salt lakes. a) pink coloration of a lake shoreline due to *Artemia* cysts accumulated by wind, Koyashskoe lake. b) brown coloration of shoreline (also in detail) due to a massive presence of the dipteran *Ephydra*, Tobechicskoe lake. c) red water collected with a plankton net, due to the massive presence of the copepod *Arctodiaptomus salinus*, Kirkoyashskoe lake.

Multicellular, eterotrophic organisms are absent at salinity values > 300 ‰. At lower salinity they are represented by Crustacea Anostraca (genus *Artemia*) or Copepoda (genera *Cleptocamptus*, *Arctodiaptomus*, *Halicyclops*), which feed on bacteria and microalgae, and by predaceous insects (Rhyncota, Diptera, and Coleoptera).

Generally the salt composition of water has its responsibility in the qualitative composition of the metazoan community. According to Derry *et al*. (2003) *Artemia* shrimps adapt to hypersaline waters (> 50 ‰), and Copepoda and Cladocera adapt to waters with SO_4^- salt content, to avoid competition from each other, and to escape predation by fishes which suffer both conditions.

Cyanophyta (Procariota), Calanoida (Crustacea), and Notonectidae (Insecta, Rhyncota) are generally reported as typical of those waters whose salinity derives from CO_3 and SO_4 salts. These waters are named "athalassic" because their salinity is not of marine salt composition.

The number of *taxa* grows as the salt concentration diminishes (see, for example, Alonso, 1990; Zhao *et al*., 2005), and typically insects are less important in marine salt composition lakes (the "thalassic" salt lakes) (Boix *et al*., 2008). The community composition and dynamics are complicated by the habitat persistence and predation (Williams, 1998; Herbst, 2001). As a consequence, the few species living in such extreme conditions (the so called haloextremophylous) are not easily recordable. Extremophylous species are generally adapted to a wide range of environmental variability, but the preference for narrower ranges, different for each species, could be present and responsible of different dominance situations in the community according with seasons. In short, extremophylous are probably generalists obliged to stay at extreme conditions within their life box (*sensu* Wharton, 2002) to avoid the competition existing where conditions are not extreme. Strong variations in salinity concentration of salt lakes can be realised in relatively short times, at every important rain episode, so determining a prompt environmental shift which could favour a different species composition of the community.

The variability of the environment, at the extreme, leads also to a complete disappearing of living organisms in those environments which arrive even to suffer a periodic dehydration. As a consequence of this extreme environmental variability any information regarding the biological composition of hypersaline lakes should be conducted in different periods of the year and, as suggested by Garcia *et al*. (1997), even in different years to obtain results as complete as possible about the presence of species. In fact, species evolved the possibility to rest in a "non living" status under extremely harsh conditions, and wait for the return of the suitable situation. The possibility to resist to adverse conditions could be extended for many years in the case of

some species. A recent study (Moscatello and Belmonte, 2004) confirmed that in a salt lake the biodiversity evaluated from cyst assemblage in the sediments quite doubled what has been found in the water column after a repeated plankton collection lasted one year. In addition, even the different community assemblage recorded in the same environment at distance of years could be explained with the existence of a multi-annual variability of the active biological community sustained by the existence of a cyst bank in the sediments (Moscatello and Belmonte, 2009).

The aim of the present study is to describe the zooplankton in the hypersaline environments of the Crimea (South Ukraine). The Crimea is an elective site for studies on salt lakes due to their abundance, their limited extension (maximum 25 km^2 per site, if the large coastal Sasik-Sivash is not considered) and its geographic position in the middle of the Paleartic Biogeographic Region. In the Crimea many hypersaline lakes have marine origin (they are thalassohaline) and many are of continental origin (they are athalassohaline). The size and other characteristics of lakes vary broadly. This makes the lakes remarkably diverse in physicochemical characteristics. Features vary seasonally and interannual differences are aspected, too.

With regard to the chemical composition of the water there are two types of Crimean salt lakes of marine origin (Kurnakov *et al.*, 1936). One type has water containing Calcium, Magnesium, or Sodium sulphates in addition to the corresponding chlorates; the major water inflow is from the sea. The second type has water containing sulphuric acid ions sufficient to generate sulphuric Calcium salt; these lakes receive water supply from terrestrial and underground sources. The Crimea hosts also a group of continental hypersaline lakes (*koli*, in the local dialect; athalassohaline, according to the terminology above reported) concentrated in the Kerch peninsula (Eastern Crimea) in calderas of ancient mud volcanoes (Gubanov and Klyukin, 1979). These are shallow and relatively small lakes, whose waters are poor in chlorates and rich in sulphates with a Na_2SO_4 / $MgSO_4$ rate varying from 0.32 to 2.48 (Kurnakov *et al*., 1936; Shadrin and Naidanova, 2002). A number of seasonal variations in ion ratios (e.g., Ca and SO_2 ions in the water of lake Khersonesskoe; Table 4) is probably related to biotic components, such as the overabundance of Calcium-shelled ostracods, anoxic photosynthesis, chemosynthesis (Shadrin, 2008).

Notwithstanding the abundance of salt lakes, either athalassohaline or thalassohaline, a complete study of the hypersaline biota in the Crimea has never been organized after the first attempt of Kulagin (1888). To tell the truth such an ancient study has been completely disregarded due to the fact that many species names he reported for fairy shrimps were successively ignored.

MATERIALS AND METHODS

A group of 10 lakes have been selected as a preliminary representation of the hypersaline habitats present in the Crimea (116, according to FEDCHENKO, 1870) (Fig. 2; Tab. 1). The 10 selected lakes were repeatedly visited, in different seasons of a 5 years period (2002-2006), to obtain data as reliable as possible.

Fig. 2 Crimea map with the indication of salt lakes. Numbers indicate the lakes considered in the present study: 1, Khersonesskoe; 2, Bakalskoe; 3, Achi; 4, Marphovskoe; 5, Koyashskoe; 6, Kirkoyashskoe; 7, Shimakhanskoe; 8, Tobechikskoe; 9, Feodosijskoe; 10, Aktashskoe.

Investigation recorded some abiotic parameters (pH, Conductivity, Salinity, Temperature) from the lakes (but not 2 of them). Lakes have been initially grouped in 6 coastal and 4 inland, simply according their geographic location in the Crimea Peninsula. The small lake Kersonesskoye has been repeatedly (monthly) sampled over a solar year (2005-2006). In presence of ion analysis of the water, an attempt to individuate indicators among organisms has been proposed.

Zooplankton samples were collected in different periods of each year (at least 2 seasons), and in different years (2002, 2004, 2005, 2006), by using a plankton net with 200 µm mesh size, and a mouth diameter of 28 cm. It has not been possible to establish the volume of filtered water during periods when the water column was so shallow to impede a complete immersion of the net mouth without the risk to collect also mud. For this reason it was not possible to report quantitative data. Samples were immediately fixed with a solution of formalin and alcohol at a final concentration of 10%. Aliquots of each well mixed sample were analysed under an inverted microscope for the identification of *taxa*. The organisms were listed according to "categories" not always corresponding to species. In fact, in many cases larval-juvenile stages cannot be identified as well as adults.

Table 1. Limnological features of the Crimean hypersaline lakes considered in the present faunal study (2002-2006).

Lake	**Coordinates**		**Region**	**Sal. (‰) range**	**Depth max (m)**
Khersoneskoye	44°35′12″ N	33°24′00″ E	Kherson	35-164	0,5
Bakalskoye	45°44′35″ N	33°10′00″ E	Steregushchee	25-110	0,7
Adzji	45°16′53″ N	35°36′24″ E	Kertch	116-245	0,3
Marphovskoye	45°12′13″ N	36°06′05″ E	Kertch	95-480	0,35
Kojashskoye	45°02′06″ N	36°12′06″ E	Kertch	184-340	0,5
Kirkojashskoye	45°04′24″ N	36°13′13″ E	Kertch	17-360	1,55
Shimakhanskoye	45°06′14″ N	36°15′03″ E	Kertch	22-360	1,25
Tobetchikskoye	45°11′16″ N	36°19′33″ E	Kertch	57-290	1,45
Feodosijskoye	45°05′58″ N	35°26′58″ E	Feodosiya	50-265	0,5
Aktashskoye	45°09′51″ N	35°24′06″ E	Azov	30-116	0,6

RESULTS AND DISCUSSION

Table 1 shows the abiotic characterization of the 10 studied lakes. All the lakes froze during winter, and show the highest temperature values during summer. Salinity, accordingly with temperature, showed a wide range of values, varying between 16 and 480 ‰. pH ranged from 7.3 to 11.00.

The chemical analysis carried out on some lakes helped us to associate Metazoa groups to lakes according their typology (athalassohaline, thalassohaline, or marine invaded ones).

The athalassohaline lakes were characterized and dominated by Calanoida Diaptomidae and Insecta Notonectidae. The dominant species was *Arctodiaptomus salinus* (Fig. 3) a copepod typical of salt lakes. Its populations showed generations with adults very different in size, often co-existing. This problem induced a misunderstanding identification of one of the different morphotypes as a different species (Litvinchuck *et al.*, 2007).

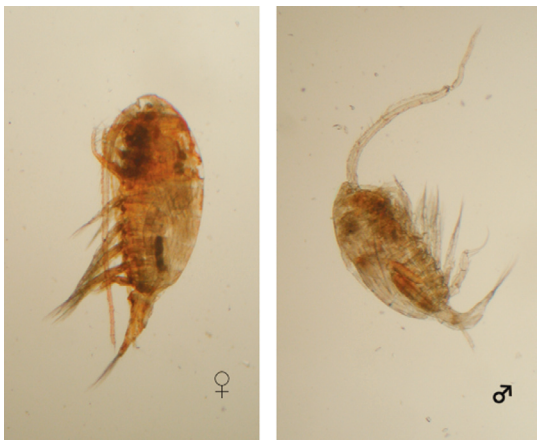

Fig. 3. *Arctodiaptomus salinus* from Kirkoyashskoe lake. Left, female; Right, male.

Bakalshskoe lake hosted *Artemia* species in the past (2000-2003, in Shadrin *et al.*, 1999), but it was recently invaded by the sea and now its salinity and its fauna are typically conditioned by the sea. This lake presented exclusive categories such as Mysidacea, Bivalvia veliger, Cirripedia nauplii, and Calanoida Acartiidae, which are typical of the coastal marine environment of the Crimea (Shadrin *et al.*, 1999), and are not shared by the other hypersaline lakes considered in the present study.

In thalassohaline salt lakes, Calanoida are not common, and Anostraca of the genus *Artemia* were often very abundant. The *Artemia* cysts have been found everywhere, but this has been attributed to the high dispersal power

they have, being they transported by the wind. However the presence of cysts has not been considered as sufficient to attribute the presence of the species in all the lakes. In fact, only in thalassohaline salt lakes an adult population of brine shrimps has been easily found (at least once). In athalassohaline situations the species could survive, but only episodically (it has been observed in Shimahanskoye and Adzji, but out of the period studied. data not published).

The most of thalassohaline lakes hosted an asexual form of *Artemia* (*A. parthenogenetica*), but in the Aktashskoye lake the bisexual *A. salina*, and in the Koyashskoye lake the bisexual *A. urmiana* have been found. The last species was never been reported outside of its original site (lake Urmia, Iran) before the present survey. As a consequence, the record of *A. urmiana* from Koyashskoye lake is the first record of the species in Europe (Abatzopoulos *et al*., 2009) (Fig. 4). Even if the lake Urmia is situated at 1280 m above the sea level, it has been classified as thalassohaline in consequence of its salt quality content (Eimanifar and Mohebbi, 2007).

Fig. 4. *Artemia urmiana* from lake Koyashskoe. Up, male; down, female.

As it regards the fauna composition, a total of 40 categories of organisms were found (see Tab. 2). The zooplankton assemblages showed many benthic components particularly evident during highest salinity periods (corresponding to the summer).

Table 2. Fauna list of organisms found in 10 hyperhaline lakes in the Crimea Peninsula. BAK, Bakalskoe; FEO, Feodosijskoe; KHER, Khersonesskoe; KOY, Koyashskoe; KIRK, Kirkoyashskoe; SHIM, Shimahanskoe; TOB, Tobechickskoe; MARP, Marphovskoe; ADZ, Adzji; AKT, Aktashskoe. White column, athalassohaline lakes; grey columns, thalassohaline lakes;. The column of total presences indicates the distribution grade of species. The row of total categories indicates the species richness per each lake.

LAKES	BAK	FEO	KHER	KOY	KIRK	SHIM	TOB	MARP	ADZ	AKT	total
ORGANISMS n. SAMPLES	3	3	15	6	6	6	6	4	2	1	pres.
Foraminifera undetermined	x										1
Infusoria undetermined			x		x			x			3
Turbellaria sp. 1	x		x		x	x		x	x		6
Turbellaria sp. 2						x					1
Nematoda undetermined	x	x	x			x					4
Polychaeta larvae	x		x								2
Bivalvia veligers	x										1
Gastropoda veligers	x										1
Brachionus plicatilis	x	x			x	x	x				5
Brachionus nilsoni						x					1
Hexarthra oxyurus		x			x	x	x	x			5
Keratella sp.					x						1
Phallocryptus spinosa cysts		x							x	x	2
Artemia cysts	x	x	x	x	x	x	x	x	x	x	9
Artemia parthenogenetica juveniles	x	x	x	x			x	x	x	x	7
Artemia parthenogenetica adults	x		x	x			x	x			4
Artemia juveniles				x							1
Artemia urmiana adults				x							1
Daphnia ulomskyi					x						1
Moina salina	x	x	x		x	x	x		x		7
Cladocera efippia						x					1
Mesopodopsis slabberi	x										1
Ostracoda sp. 1	x	x	x		x		x	x			6
Ostracoda sp. 2		x									1
Cirripedia nauplii	x								x	x	3
Cletocamptus retrogressus		x	x		x	x	x	x	x		7
Harpacticoida sp. 2			x								1
Harpacticoida sp. 3	x	x	x								3
Longipedia nauplii	x										1
Diaptomidae nauplii and copepodids		x			x	x	x	x			5
Arctodiaptomus salinus		x			x	x		x			4
Acartiidae nauplii and copepodids	x										1
Acartia tonsa adults	x										1
Centropagidae undetermined									x		1
Cyclopoida undetermined.					x			x			2
Amphipoda undetermined.			x								1
Corophiidae undetermined	x		x		x						3
Hydracarina undetermined.										x	1
Chironomidae larvae undetermined.	x		x		x			x	x		5
Coleoptera larvae undetermined			x		x		x				3
Total categories	**20**	**13**	**16**	**5**	**16**	**12**	**10**	**12**	**9**	**5**	

The marine invaded lake (Bakalskoye) was the most biodiverse (20 categories), and the less saline of the all considered lakes. The fauna in 2002 (before the sea invasion) was represented by only 7 categories, dominated by *Artemia* shrimps (Anostraca) and *Moina salina* (Cladocera). The fauna of the lake after the sea invasion comprised 20 categories with the extreme reduction (and even disappearing) of the formerly dominant species.

Due to the possibility of hypersaline organisms to rest, our opinion is that the disappeared species are simply resting in the sediments, waiting for the restoration of suitable conditions. The most saline of the studied lakes (Marfovskoye, continental, and Koyashskoye, marine derived) were the poorest in terms of metazoan categories. Only *Artemia* populations survive to extremely high salinity values, but in Marfovskoye lake (the most distant from the sea of all the considered lakes) also some *Arctodiatomus* (Calanoida) have been found, confirming the link among this species and the probable non marine origin of the lake.

In general the number of categories per lake resulted inversely correlated with the maximum water salinity (Fig. 5), and even in the same lake the species richness decreases with the increase of the water salinity (occurring generally at the end of the Summer).

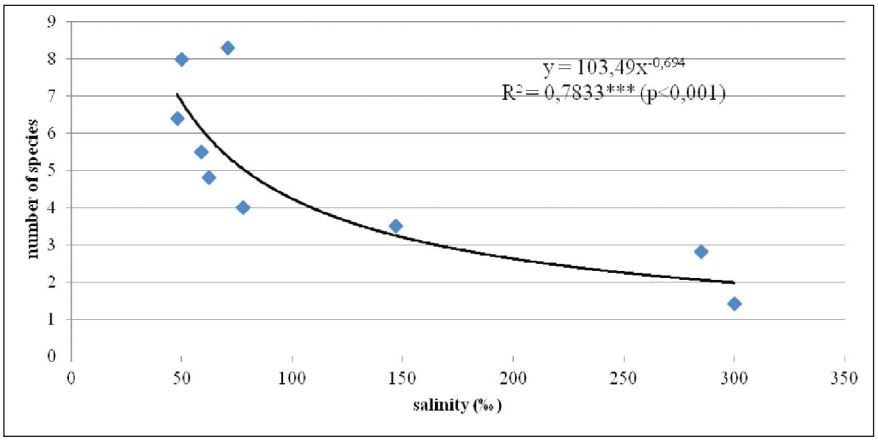

Fig. 5 Non-linear correlation among salinity (average from multiple measurements, according the number of visits for each lake) and species number in the water column of 10 lakes studied in the Crimea. Bakalshskoe has not been considered.

The lake Khersonesskoye has been visited 15 times and it has been considered as a paradigm of the hypersaline water fauna of the Crimea. The lake appeared dominated, periodically, by Harpacticoida (mainly *Cletocamptus retrogressus*), Ostracoda, and Nematoda. Anostracans (*Artemia parthenogenetica,* but also *A. salina,* in 2002) appeared only episodically. This *Artemia*

rarity is here interpreted as the consequence of low salinity level of the sampled site (year average, 47,5 ‰) and not with the of the lake appurtenance to the non "marine derived" group.

CONCLUSIONS

A general framework can be proposed for the Crimea salt lakes on the basis of the study carried out. The species assemblages were different according to the lake considered; this notwithstanding thalassohaline salt lakes resulted roughly distinguishable from the athalassohaline ones due to some faunal indicators. The communities of continental (athalassohaline) salt lakes were dominated by Calanoida, whereas the coastal ones (thalassohaline) were typically dominated by *Artemia* fairy shrimps. This datum was confirmed even in the Feodosijskoe lake which, during the flooding period (i.e., with a water level higher than that of the sea, about 0.5 m) showed swarming populations of *Arctodiaptomus salinus*. In other periods, however, when the continental water input is depressed, water from the sea introduces and the possibility of a mixed (and alternate) faunal presence is not to be excluded.

Whatever is their origin, the species of Feodosijskoe lake are typical of hyperhaline waters and they could not be found in adjacent compartments (fresh or marine water).

When this has been the case (as in Bakalshskoe lake) the lake has been classified as "invaded" by another environment (the sea, in that case).

Artemia species are widely distributed in all the lakes (even if not everywhere dominating) probably due to the high dispersal ability of their cysts. These, in fact, do not sink to the lake bottom when they are produced (as other resting eggs do in the same environment) but they float on the surface of the water, so exposing themselves to winds and accumulating along lake shorelines, to be carried in the air when the lakes reduce their water volumes.

Some lakes were considered typically continental although the presence of *Artemia* cysts was recorded, and of juveniles born from them, because adults were never observed there, during the four years of the present study. This suggested that the environment should not be considered as suitable to sustain an entire life cycle (i.e., an entire population) of that species. Those *Artemia* populations could not be considered as autochthonous but simply supplied propagules coming from "thalassohaline" salt lakes by wind transport.

The ancient paper of Kulagin (1888) already reported three different species of *Artemia* from salt habitats of the Crimea (Fig. 6). Apart *A. salina*, we are not able to establish a correspondence between his *A. arietina* - *A. M.*

Mulhausinii and *A. parthenogenetica - A. urmiana* found in the present study. These last two species have been described after the Kulagin's publication, but the Authors of these descriptions did not refer to his report. However the things went, an interesting datum is that the Crimea is unique, as regarding the *Artemia* spp presence, due to the fact that in a relatively small territory, salt lakes host at least three different *Artemia* species. *Artemia* shrimps of bisexual and parthenogenetic populations have been found co-existing in 2 out of 84 sites in Countries of the former Soviet Union (reported in Mura and Nagorskaya, 2005). Both these two cases were reported from the Crimea (Sasik Sivash, Saki), but in any case the bisexual population has been recorded as *A. salina*.

Fig. 6 The original organisms indicated by Kulagin as Artemia arietina. Museum of the Marine Biology Station of Karadag (the Crimea).

Origin of the genus *Artemia* was probably in coastal salt lakes of the ancient Tethys Ocean. Crimea can be considered, also on the basis of the present results, a remnant of that origin centre of the *Artemia* biodiversity.

A final consideration is that a sharp separation between benthos and plankton was not possible in such hypersaline lakes (benthos samples contained plankton species, and *vice versa*). A general trend was observed with the

growth of benthic category numbers in the plankton as directly proportional to the water salinity (which varied according the period of the year). This was probably due to the parallel growth of water density which allows a better suspension of organisms in the water column. But we cannot ignore that the growth of the salinity is accompanied by a reduction of the water column, this obliging the collection of samples hauling the net closer to the bottom.

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