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TRANSYLVANIAN REVIEW OF SYSTEMATICAL AND ECOLOGICAL RESEARCH

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The Wetlands Diversity

Editors

Angela Curtean-Bănăduc, Doru Bănăduc & Ioan Sîrbu

Sibiu - Romania

2009

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"Lucian Blaga" University of Sibiu,
Faculty of Sciences,
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IN MEMORIAM

Petru Mihai Bănărescu

(1921 - 2009)

Petru Mihai Bănărescu, the greatest Romanian ichthyologist, was born on the 15th of September 1921 in Craiova, Romania. Prominent personality of science, Petru Mihai Bănărescu proved to be a brilliant student of the Faculty of Science, Department of Natural Science, Cluj and was awarded with the title of doctor (PhD) in natural sciences (1949) with a thesis on comparative anatomy on the encephalon of the teleostei fishes. However, due to the comunists political reasons, the ichthyologist was sent to prison for one year. Nonetheless, this unhappy event didn't interrupt his work and the biologist succeeded in finishing seven research papers. The year 1953 finds him working as a scientific researcher at the Institute of Fish Research in Bucharest, place where he receives the title of doctor docent in 1962. Later on, he worked within the Fauna Collective of the Romanian Academy. Since 1972, he becomes the head of the Animal Taxonomy Laboratory part of the Institute of Biology within the Romanian Academy.

Bănărescu has embraced the field of zoology and developed a special interest in ichthyology. His studies focused on the systematic of fresh water fish, the fish zoogeography and the revision of systematic within the Cyprinidae, as well as the Cobitidae fish families and their zoogeography. For his valuable scientific contribution, Bănărescu becomes in 1976 foreign member of the American Society of Ichthyology and Herpetology and in 1988 - honorific member of the European Society of Ichthyology. In 1991, the scientist was admitted as correspondence member of Romanian Academy and in 2000 became titular member of the highest cultural and scientific forum in Romania.

Due to his devoted work, Bănărescu was recognized national wide and worldwide as the greatest scientific authority concerning the Eurasian Cyprinidae and the taxonomy of Cobitidae - within this group he discovered new genres, subgenres, species and subspecies. In order to arrive to these results, the ichthyologist collected and studied, for long periods of time, fishes from the Romanian inner waters as well as international waters (Austria, Holland, Serbia, Greece, France, Korea, North America etc). The vast collection of fish is nowadays part of the National History Museum "Grigore Antipa", under the name "Petru Mihai Bănărescu Collection".

Further on, the scientist described, for the first time, two subgenres, a species and two subspecies of fish in Romania; as well as a new subfamily, 10 genres, 38 species and 26 subspecies of fish in China, Korea, Vietnam, Thailand, Myanmar, India, Pakistan, Afghanistan, Israel, Turkey and others.

Bănărescu enclosed his work in more than 300 books and scientific papers, published in some of the most prestigious professional journals in the world, namely: Zoogeography of fresh waters; General introduction to fishes: Acipenseriformes; Principles and problems of zoogeography; General distribution and dispersal of fresh water animals; Cyprindae; The freshwater fishes of Europe; Distribution and dispersal of freshwater animals in North America and Eurasia; Zoogeography of Fresh Water Fishes: Africa-Madagascar and Satellite Islands, South America, Central America and Caribbean Intermediary Areas, Australian.; Pisces - Osteichthyes, etc.

His great research work enriched national and world knowledge in ichthyology and zoogeography; therefore his international acknowledgments are completely justified.

As a person, all the people met him learned about what exquisite qualities you have to have to can be human! All of these persons obey their most sincere respect ...

The Editors

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Preface

In a global environment in which the climate changes are observed from few decades no more only through scientific studies but also through day by day life experiences of average people which feel and understand allready the presence of the medium and long-term significant change in the "average weather" all over the world, the most comon key words which reflect the general concern are: heating, desertification, rationalisation and surviwing.

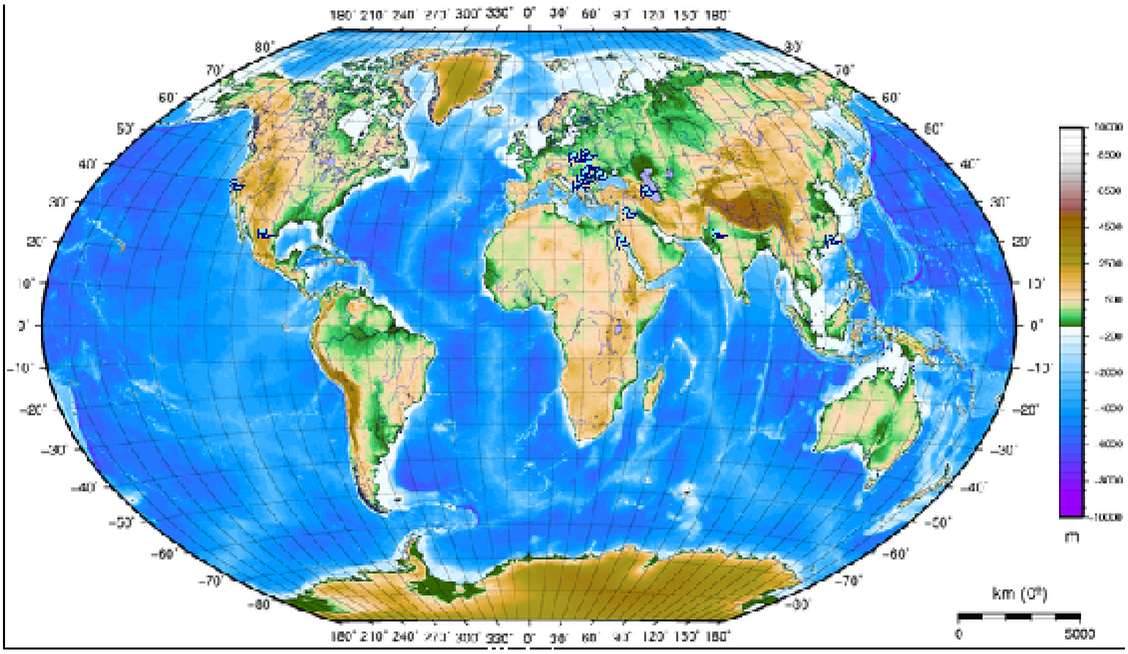
The causes, effects, trends and possibilities of human society to positively intervene to slow down this process or to adapt to it involve a huge variety of aproacess and efforts.

With the fact in mind that these aproaces and efforts shuld be based on genuine scientific understanding, the editors of the Transylvanian Review of Systematical and Ecological Research series launched a second annual volume dedicated to the wetlands, volumes resulted mainly as a result of the Aquatic Biodiversity International Conference, Sibiu/Romania 2009.

The therm wetland is used here in the acceptance of the Convention on Wetlands, signed in Ramsar, in 1971, for the conservation and wise use of wetlands and their resources. **Marine/Coastal Wetlands** - Permanent shallow marine waters in most cases less than six metres deep at low tide, includes sea bays and straits; Marine subtidal aquatic beds, includes kelp beds, sea-grass beds, tropical marine meadows; Coral reefs; Rocky marine shores, includes rocky offshore islands, sea cliffs; Sand, shingle or pebble shores, includes sand bars, spits and sandy islets, includes dune systems and humid dune slacks; Estuarine waters, permanent water of estuaries and estuarine systems of deltas; Intertidal mud, sand or salt flats; Intertidal marshes, includes salt marshes, salt meadows, saltings, raised salt marshes, includes tidal brackish and freshwater marshes; Intertidal forested wetlands, includes mangrove swamps, nipah swamps and tidal freshwater swamp forests; Coastal brackish/saline lagoons, brackish to saline lagoons with at least one relatively narrow connection to the sea; Coastal freshwater lagoons, includes freshwater delta lagoons; Karst and other subterranean hydrological systems, marine/coastal. **Inland Wetlands** - Permanent inland deltas; Permanent rivers/streams/creeks, includes waterfalls; Seasonal/intermittent/irregular rivers/streams/creeks; Permanent freshwater lakes (over eight ha), includes large oxbow lakes; Seasonal/intermittent freshwater lakes (over eight ha), includes floodplain lakes; Permanent saline/brackish/alkaline lakes; Seasonal/intermittent saline/brackish/alkaline lakes and flats; Permanent saline/brackish/alkaline marshes/pools; Seasonal/intermittent saline/brackish/alkaline marshes/pools; Permanent freshwater marshes/pools, ponds (below eight ha), marshes and swamps on inorganic soils, with emergent vegetation water-logged for at least most of the growing season; Seasonal/intermittent freshwater marshes/pools on inorganic soils, includes sloughs, potholes, seasonally flooded meadows, sedge marshes; Non-forested peatlands, includes shrub or open bogs, swamps, fens; Alpine wetlands, includes alpine meadows, temporary waters from snowmelt; Tundra wetlands, includes tundra pools, temporary waters from snowmelt; Shrub-dominated wetlands, shrub swamps, shrub-dominated freshwater marshes, shrub carr, alder thicket on inorganic soils; Freshwater, tree-dominated wetlands; includes freshwater swamp forests, seasonally flooded forests, wooded swamps on inorganic soils; Forested peatlands; peatswamp forests; Freshwater springs, oases; Geothermal wetlands; Karst and other subterranean hydrological systems, inland. **Human-made wetlands** - Aquaculture (e. g., fish/shrimp) ponds; Ponds; includes farm ponds, stock ponds, small tanks; (generally below eight ha); Irrigated land, includes irrigation channels and rice fields; Seasonally flooded agricultural land (including intensively managed or grazed wet meadow or pasture); Salt exploitation sites, salt pans, salines, etc.; Water storage areas, reservoirs/barrages/dams/impoundments (generally over eight ha); Excavations; gravel/brick/clay pits; borrow pits, mining pools; Wastewater treatment areas, sewage farms, settling ponds, oxidation basins, etc.; Canals and drainage channels, ditches; Karst and other subterranean hydrological systems, human-made.

The Editors of the ***Transylvanian Review of Systematical and Ecological Research*** started and continue this new annual series (***Wetlands Diversity***) as an international scientific debate platform for the wetlands conservation, and not to take in the last moment, some last heavenly "images" of a perishing wetlands world …

This second volume included variated researches from diverse wetlands around the world.



The subject areas ( ) for the published studies in this volume.

No doubt that this new data will develop knowledge and understanding of the ecological status of the wetlands and will continue to evolve.

Acknowledgements

The editors would like to express their sincere gratitude to the authors and the scientific reviewers whose work made the appearance of this volume possible.

The Editors

NAJAS SPP. GROWTH IN RELATION TO ENVIRONMENTAL FACTORS IN WADI ALLAQI (NASSER LAKE, EGYPT)

Hoda YACOUB *

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KEYWORDS: Egypt, Lake Nasser, Wadi Allaqi, *Najas* spp., human impacts, canonical correspondence analysis (CCA), fodder resources.

ABSTRACT

The study aims to evaluate the pattern of *Najas* spp. (aquatic plant) distribution along the shores of Allaqi, Lake Nasser, to identify the effect of physico-chemical variables on the growth and on the expansion of the plants. *Najas* spp. is vital for nomadic groups (Bedouins) living in the area, they harvest the plant out of lake to utilize it as fodder resource for their sheep and goats. Drought conditions that extended more than ten years increase the demands for the plants, in order to compensate the reduction of available grazing areas and the lack of grazing terrestrial plants. It was noticed that *Najas* growth declined in many areas in Allaqi due to anthropogenic and natural factors. *Najas* was exposed to negative conditions caused by human activities, including shores agriculture and fishing. The dense flocks of large migratory birds and the expansion of aquatic plants (other species of macrophytes and epiphytes) are additional factors that threat in many ways the *Najas* spp. presence. Environmental patterns were assessed over 17 abiotic parameters related to water (depth, temperature, pH, TDS, conductivity, dissolved oxygen, light transparency, phosphate, nitrate, nitrite, ammonium, sulphate) and hydrosoil (phosphate, nitrate, nitrite, ammonium, organic matter). Three field samplings were performed (May, July and September 2008) in the period when *Najas* (mainly *Najas horrida* which is dominant) starts and completes its life cycle; this period also represents the maximum human impact (harvesting time of *Najas* by Bedouins and the period of shores cultivation) covering a wide range of natural and anthropogenic conditions variations. The temporal and spatial variation of *Najas* spp. growth was obvious in Allaqi due to conditions related to: 1) variation in water and hydrosoil qualities caused mainly by human impacts, 2) variation in the nature and physical features of the shores, 3) mechanical damage from migratory birds and 4) competition of epiphytes and other macrophytes species for light, nutrients oxygen and space. CCA statistical technique was extremely useful in understanding the response of different *Najas* species growing in Allaqi (*Najas horrida*, *Najas marina* subsp. *armata* and *Najas minor*) to various conditions. It indicated that depth, TDS, water ammonium and hydrosoil phosphate are the key factors that control *Najas* growth in Allaqi, and this agreed with field observation, where *Najas* thrived and dominated in shallow sheltered areas with low nutrient contents. Remarkable reduction in *Najas* growth was recorded at cultivated shores, due to fertilizers reaching the water, and causing high ammonium contents. *Myriophyllum spicatum* known to be a sever competitor, able to grow in wide range of environmental conditions, and our study showed that *Myriophyllum* was more adapted to unfavourable conditions than *Najas*, suggesting the invasive ability of this species in the future.

RÉSUMÉ: Le développement des espèces de *Najas* en relation avec les facteurs environnementaux dans le Wadi Allaqi (Lac Nasser, Egypte).

L'étude vise l'évaluation des modèles de distribution de *Najas* spp. (plante aquatique) le long des rives d'Allaqi, Lac Nasser ainsi que l'identification des effets des paramètres physiques et chimiques sur la croissance et l'expansion de la plante. *Najas* spp. est une plante vitale pour les populations nomades (Bédouins) de la région, qui ramassent la plante du lac et l'utilisent en tant que fourrage pour leurs moutons et chèvres. Les conditions de sécheresse qui se sont maintenues sur plus de dix ans ont augmenté la demande pour cette plante appelée à compenser la réduction des pâturages disponibles et le manque des plantes terrestres disponibles. Nous avons remarqué le fait que la croissance de *Najas* a reculé dans plusieurs régions d'Allaqi à cause des facteurs naturels et anthropogéniques. *Najas* a été exposée à des conditions négatives à cause des activités humaines tel que la pèche et l'agriculture littorale. Les colonies de grands oiseaux migrateurs et l'expansion des plantes aquatiques (autres espèces de macrophytes et d'épiphytes) sont d'autres facteurs qui menacent de plusieurs façons la présence de *Najas* spp. Les modèles environnementaux ont été évalués sur la base de 17 paramètres abiotiques liés à l'eau (profondeur, température, pH, TDS, conductivité, oxygène dissout, transparence, phosphate, nitrate, nitrite, ammoniac, sulfate) et aux hydrosols (phosphate, nitrate, nitrite, ammoniac, matière organique). Trois campagnes de prélèvement ont été entreprises (mai, juillet et septembre 2008) pendant la période dans laquelle *Najas* (principalement *Najas horrida*, qui est dominante) commence et finit son cycle de vie, période également concernée par le maximum de l'impacte anthropique (la période de cueillette de *Najas* par les Bédouins et la période de culture des berges) couvrant une large palette de variations des conditions naturelles et non naturelles. La variation temporelle et spatiale de l'expansion de *Najas* spp. a été si visible à Allaqi à cause des conditions liées à: 1) des variations dans la qualité de l'eau et des hydrosols dues principalement à l'impacte anthropique; 2) des variations des caractéristiques naturelles et physiques des berges; 3) l'altération mécanique due aux oiseaux migratoires et 4) la compétition des espèces épiphytes et d'autres espèces macrophytes pour la lumière, les nutriments, l'oxygène et l'espace. La techniques statistique CAA a été particulièrement utile dans la compréhension des réponses des différentes espèces de *Najas* qui poussent à Allaqi (*Najas horrida*, *Najas marina* subsp. *armata* et *Najas minor*) à des différentes conditions. Cette analyse a indiqué le fait la profondeur, TDS, l'ammoniac de l'eau et le phosphate des hydrosols sont les facteurs clef qui contrôlent la croissance de *Najas* à Allaqi, fait confirmé par les observations du terrain indiquant le fait que *Najas* s'est bien développé et a dominé les régions abritées et peu profondes avec un faible contenu de nutriments. La réduction la plus importante des effectifs de *Najas* a été enregistrée pour les berges cultivées à cause des engrais mobilisés dans l'eau qui ont augmenté les concentrations d'ammoniac. *Myriophyllum spicatum*, connu en tant que compétiteur féroce, capable de pousser dans une large gamme de conditions, a été démontré par notre étude en tant que mieux adapté que *Najas* aux facteurs adverses, suggérant que cette espèce pourrait devenir invasive par la suite.

REZUMAT: Dezvoltarea subspeciilor de *Najas* în relație cu factori de mediu în Wadi Allaqi (Lacul Nasser, Egipt).

Studiul urmăreşte evaluarea modelelor de distribuție ale *Najas* spp., (plantă acvatică) în zona litorală Allaqi a Lacului Nasser, precum şi identificarea efectelor diferiților factori fizico-chimici asupra dezvoltării şi expansiunii acestei plante. *Najas* spp. este vitală pentru grupurile nomade din regiune (beduini), care o recoltează şi o folosesc pentru furajarea oilor şi caprelor. Seceta care a durat peste zece ani în zonă a făcut ca presiunea asupra acestei plante să crească pentru a compensa reducerea zonelor de păşunat disponibile şi lipsa plantelor terestre. S-a remarcat faptul că *Najas* nu a mai crescut în mai multe sectoare din Allaqi, datorită unor factori naturali şi antropogeni. *Najas* a fost expusă la condiţii de mediu negative datorită unor activităţi umane, precum pescuitul şi agricultura, practicate pe lângă maluri. Coloniile de păsări migratoare mari şi invazia plantelor acvatice (alte specii de macrofite şi epifite) sunt alţi factori care ameninţă în diferite feluri prezenţa *Najas* spp. Modelele ecologice au fost evaluate pe baza a 17 parametri abiotici ai apei (adâncime, temperatură, pH, TDS, conductivitate, oxigen dizolvat, transparenţă, fosfat, nitrat, nitrit, amoniac, sulfat) şi hidrosolurilor (fosfat, nitriat, nitrit, materie organică). Au fost efectuate trei campanii de teren (mai, iulie şi septembrie 2008) în perioada de început şi de final a ciclului de viaţă la *Najas* (în special la *Najas horrida*, specie dominantă), perioadă vizată şi de un maxim al activităţii antropice (perioada de recoltare a plantelor de *Najas* de către beduini şi de cultivare a malurilor), acoperind o paletă largă de variaţii ale condiţiilor naturale şi nu numai. Variaţia temporală şi spaţială a expansiunii *Najas* spp. a fost vizibilă la Allaqi din cauza condiţiilor de mediu legate de: 1) variaţii ale calităţii apei şi hidrosolurilor datorate în principal activităţilor antropice; 2) variaţii ale caracteristicilor naturale şi fizice ale malurilor; 3) deteriorarea mecanică datorată păsărilor migratoare şi 4) competiţia cu speciile epifite şi alte specii macrofite pentru lumină, nutrienţi, oxigen şi spaţiu. Tehnica statistică CAA a fost deosebit de utilă în înţelegerea răspunsurilor diferitelor specii de *Najas* prezente la Allaqi (*Najas horrida*, *Najas marina* subsp. *armata* şi *Najas minor*) la diferite condiţii de mediu. Această analiză indică faptul că adâncimea, TDS, amoniacul din apă şi fosfatul din hidrosoluri sunt factori cheie ce controlează dezvoltarea *Najas* la Allaqi, fapt confirmat şi de observaţiile din teren ce arătau că *Najas* a proliferat şi a dominat în zonele adăpostite şi puţin adânci cu un conţinut scăzut de nutrienţi. Reducerea cea mai importantă a creşterii plantelor de *Najas* a fost înregistrată în lungul malurilor cultivate datorită îngrăşămintelor dizolvate în apă care au dus la creşterea concentraţiei de amoniac. *Myriophyllum spicatum*, cunoscută ca fiind una dintre speciile cele mai competitive, capabilă să se dezvolte într-o gamă largă de condiţii de mediu, a fost găsită şi în cadrul studiului nostru ca fiind mai adaptată la condiţii vitrege decât *Najas*, sugerând posibilitatea invaziei viitoare a acestei specii în zona cercetată.

INTRODUCTION

It is well known that in aquatic ecosystems, water quality and consequent macrophytes growth are continuously affected by physical, chemical and biological process including nutrient availability, water flow, turbulence, solar radiation, water temperature, sediment, depth and epiphytic growth (Ham et al., 1981; Duarte et al., 1994; Wetzel, 2001). Previous studies gave more attention to variables that allow prediction of abundance and biomass of several species and on testing hypothesis regarding mechanisms that cause variations in these attributes (Bini et al., 1999; Lacoul and Freedman, 2006; Rolon and Maltchik, 2006). Such studies also provide the opportunity for species richness assessment of species richness in relation to environment in particular the factors that determine aquatic plant diversity (Murphy et al., 2003). In lacustrine environments, light was identified as the primary factor limiting macrophytes growth. Light intensity varied due to water clarity, plant self-shading and turbidity (Westlake, 1975) or due to shading through riparian vegetation (Dawson, 1978). Biotic factor such as competition and herbivores can also be considered as one of significant factors that affect macrophytic species distribution

(McCreary et al., 1983; Titus and Stephen, 1983; Rasch et al., 2004). Conversely, the macrophytes communities have their impacts on the aquatic habitats that influences the ecosystem functions by modifying for example, flow pattern (Sand-Jensen and Pedersen, 1999), sediment process (Clarke and Wharton, 2001), oxygen dynamics (Krik, 1994; Uehlinger et al., 2000) and nutrients cycles (Madsen and Cedergreen, 2002; Wilcock et al., 2004). The extent to which macrophytes could affect the aquatic system is by their interaction in physical, chemical and biological process depending mainly on biomass density, composition, and species morphology (Watson, 1987; Sand-Jensen and Mebus, 1996). The understanding of the mutual interaction between aquatic environment and macrophytes is essential particularly if the macrophytes community includes a rare or highly valued (environmentally and/or economically) species, for example, *Najas* spp. is vital for the nomadic groups (Bedouins) living at the shores of Lake Nasser. It is harvested out of the lake, dried and stored to be used latter in drought periods when other resources are limited. On the other hand, there is evidence to suggest that *Najas* can help to retain soil moisture for short periods before it rots down, so, ironically, it helps promoting the early growth of grass (Belal et al., 2009). Natural and anthropogenic impacts put *Najas* spp. under pressure and reduce in many ways its growth and productivity in shallow water areas in Allaqi. These circumstances threat one of Bedouins main fodder resources which they have been utilized for long time, so the central issue of present study is to evaluate the environmental conditions that positively and negatively impact the growth of *Najas* spp. This can be carried out through: determination of *Najas* spp. distribution in relation to other macrophytes species in Allaqi; determination of *Najas* spp. distribution in relation to physico-chemical variables of natural and anthropogenic origin.

Site description

The dam was built in 1902 and heightened twice in 1912 and 1934 to increase its storage capacity. Nevertheless, the stored water was not adequate for agricultural development and great amounts of flood water were released annually into the Mediterranean Sea. In 1959, the construction of a rocky-filled dam started on the River Nile, 17 km south of Aswan, 900 km from Cairo, which created one of the largest man-made lakes in Africa - the High Dam Lake.

The largest part of the lake lies in Egypt and is known as Lake Nasser, while the Sudanese part is called Lake Nuba. Lake Nasser extends between latitudes 22˚00´-23˚58´N and between longitudes 31˚19´-33˚19´E. The lake has many embayments, locally called khores. The total number of important khores is 85 (48 on the eastern side and 37 on the western). Some khores as Kalabsha, Tushka and Allaqi are wide, with a sandy bottom and a gently slope; others as Singari, El-Sabakha and Korosko are steep, relatively narrow, with a rocky bottom. Khore Allaqi is located in the Eastern side of Lake Nasser and considered one of the largest in the area. Wadi Allaqi extends about 250 km from the khore to Sudanese lands in the south. Wadi Allaqi was declared a conservation area in 1989 and has protected status within the Egyptian Environmental Affairs Agency (EEAA). Because of its arid environment and combination of two ecosystems (extreme arid desert and the shores of Lake Nasser; Figs. 1 and 2) inhabited by nomadic tribes, this area was designated as a biosphere reserve in 1993 within UNESCO Man and Biosphere Programme (MAB).

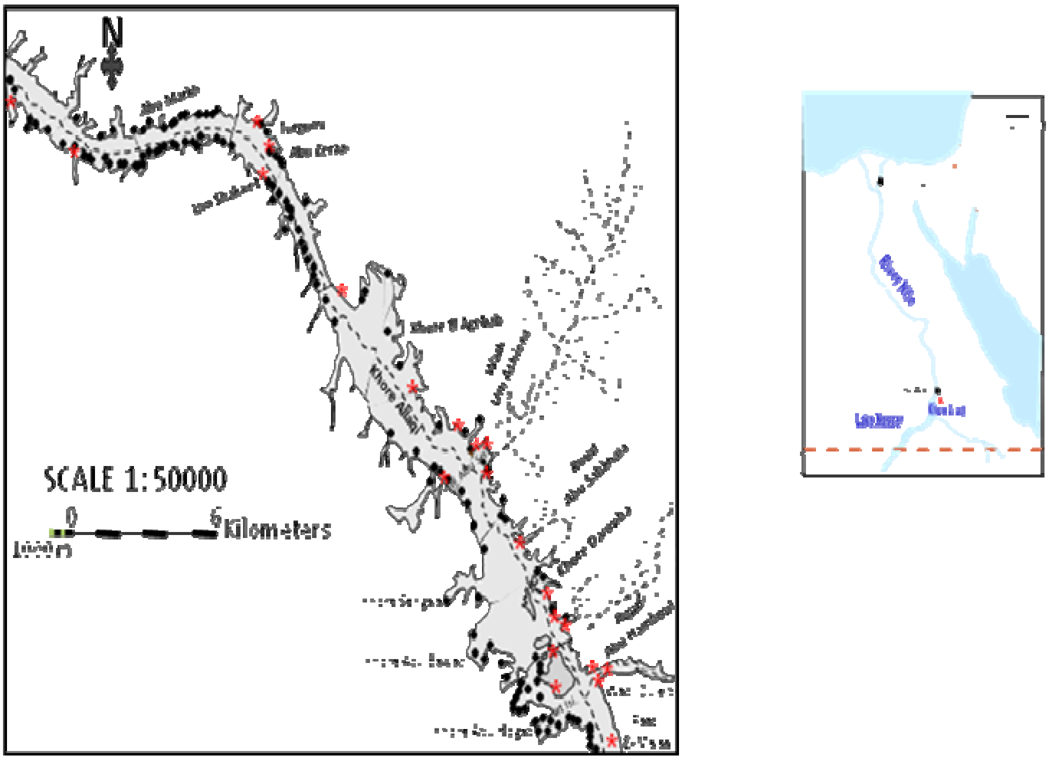

Figure 1: Map of khore Allaqi: survey sites (●) and selected sampling sites (*); the medallion shows the location on Nasser Lake.

MATERIALS AND METHODS

Field survey

158 sampling stations and 22 sampling sites analysed in detail were set up at both khore Allaqi shores (Fig. 1.), 84 sites on the eastern shore and 74 on the western one. The vegetation survey was carried out in three trips from May to September of 2008, when most of macrophytes species were sufficiently developed. Sites were selected randomly representing wide range of aquatic habitats. The location of each site was determined by a Garman GPS with 12 channels. Plant species were identified after Triest (1987, 1989) and Boulus (2005). All aquatic species were listed and the cover percentage for each species was estimated. The cover of macrophytes species growing in shallow water was calculated using an one square meter quadrate, while the cover of species growing in deeper water was estimated using a metallic grapnel, following the technique of Murphy et al. (1981). Specimens representing the collected aquatic plant species from khore Allaqi are kept in the Herbarium of Cairo University, Egypt.

Figure 2: Wadi Allaqi: an unique habitat between the water of Lake Nasser and the arid area.

Figure 3: *Najas horrida* (characterized by spiny margins) is the dominant species in Allaqi.

For monitoring, 22 sites were selected for detailed studies including floristic composition, limnology and hydrosoil chemical properties. The sites were selected according to the following criteria:

1. sites should be occupied by *Najas* spp.;
2. sites occupied by *Najas* spp. are in the neighbourhood of those clear of this plant;
3. sites show variations in *Najas* spp. cover, adjacent sites that show variation in *Najas* spp. growth, sites situated in different areas show similar cover;
4. sites with extensive growth and with low growth of *Najas;*
5. sites with and without human impact.

Samplings were focused on: *Najas* spp. life cycle, the period where local people (Bedouins) utilize the plant and agriculture cultivation cycles at the shores of Allaqi.

Floristic composition

Plants were identified, sorted according to species, dried and finally weighted.

Environmental Variables

Limnology

pH values and temperatures (°C) were determined using Misuraline model ML 1010. Depth (m) and transparency were measured using a Secchi disc. The Photosynthetic Active Radiation (PAR) represented by the light extinction coefficient (k) was calculated according to the formula given by Poole and Atkins (1929):

$$k = 1.7/D \text{ (where (D) is the depth of visibility in metres)}$$

Total dissolved salts (mg l^{-1}) and conductivity (µS/cm^{-1}) were measured with an electronic TDS meter (HANNA, model HI 99300). Dissolved oxygen (mg/l^{-1} and saturation percentage) were measured using the model HI 9146 by HANNA.

Soluble reactive phosphate was determined by the molybdate blue method (Allen et al., 1986). Nitrate was determined by means of chromic acid method (APHA, 1985). Sodium salicylate is added to an aliquot of filtered water samples. Nitrite was determined by the modified Griess-Ilosvary method (APHA, 1985). Ammonia was determined by Nessler's method, in which an alkaline solution of mercury chloride is used as a reagent for the colourimetric determination (APHA, 1985). Sulphate was determined by the turbidimetric method using NaCl/HCl - glycerol-ethanol reagent and barium chloride crystals (Sheen et al., 1935; Thomas and Cotton, 1954; Rossum and Villarruz, 1961).

Hydrosoil

The surface sediment was collected by a small hand held dredge in shallow waters, and by an Ekman grab in deeper waters. Five parameters were measured in hydrosoil samples suspended and soluble reactive phosphate, nitrite, nitrate, ammonia and organic matter content.

Hydrosoil samples were dried to remove the water; the soil aggregates were gently broken and sieved to separate the particles which were greater than 2 mm, because of their slight influence on the chemical properties. The sieved hydrosoil was added to extractant at 1:5 soil-water proportions. Two types of extractant were used depending on the type of element to be determined: i Olsen's reagent (0.5 M sodium bicarbonate at pH 8.5) for the determination of phosphate: soil was added to extractant at a 1:20 proportion and shaked for one hour; ii the remaining elements were extracted using distilled water a 1:5 soil-water dilution, shaked for one hour (Allen et al., 1986).

Suspended and soluble reactive phosphates were measured by the colorimetric molybdenum blue method using Olsen's reagent (Murphy and Riley, 1962). Nitrite was determined using the modified Griess-Ilosvary method (APHA, 1985), while nitrate was determined with the chromic acid method (APHA, 1985). Nessler's method was used to determine ammonia, an alkaline solution of mercury chloride being used as a reagent in the present of potassium iodide (APHA, 1985). Organic matter from hydrosoil was estimated by ignition at 500°C for six hours (Allen et al., 1986).

Data analysis

Data was depicted as two matrices: dry weight standing crops values x samples and environmental variables x samples. The data was normalized by logarithmation prior to analysis. Canonical Correspondence Analysis (CCA) of the two matrices was carried out using CANOCO for Windows version 4.0 (Ter Braak and Smilauer, 1998). Once the ordination of the data has been produced, it is possible to correlate the distribution of the sample on the major axes, with the environmental variables. CCA produces an ordination diagram, on which points represent the species and the sites, and vectors represent environmental variables.

RESULTS

Najas spp. in relation to macrophytes in Allaqi

The Allaqi's eastern shores are richer in aquatic macrophytes than the western ones. There eleven main areas were occupied by plants, opposed to only five in west. Also, the mean cover from the eastern shore ranged from 34 to 98%, while in west varied from 20 to 63.3% (Tab. 1).

Table 1: Floristic composition, number of species, and mean cover percentage in Allaqi.

Main Areas		sites	species	mean cover%
Eastern Shores	Ras El-Maiaa	1	1	98
	Wadi Quleib	3	4	96.6
	Roud Abu Hamboul	1	4	97
	Khore Darouba	1	2	60
	Roud Abu Sabkhaia	1	1	85
	Wadi Um Ashira	3	3	78.3
	Jebel Abu Seif	1	3	52
	Khore El Agebab	3	2	60
	Abu Zeraai	3	3	60
	Turgumi	4	3	51.2
	Abu Markh	5	1	34
Western Shores	Um Shakaiet	1	3	20
	Khore Sengaia	1	2	50
	Khore Abu Bawadi	3	2	63.3
	Khore Abu Hogab	2	2	52.2
	Um Fart Island	3	3	23.3

Although Ras El-Maia has extensive growth of macrophytes, recording 98% plant cover, it is one of the poor locations in plant diversity (one macrophyte species). Roud Abu Hamboul and Wadi Quleib are richer in plant species compared with Ras El-Maia, that they occupied by high number of species reached to four, with mean cover 97 and 96.6%, respectively. Although Roud Abu Sabkhaia is more than 30 km away from Ras El-Maia, it shows similar floristic structure related to number of species (one species), and cover percentage (85 and 98%, respectively). Three plant species were recorded in both Wadi Um Ashira and Jebel Abu Seif, with plant coverage of 78.3 and 52%, respectively. Khore Sengaia, khore Abu Bawadi and khore Abu Hogab show similarity in their plant diversity being occupied by two plant species and showing equivalent cover values: 50, 63.3 and 52.2%, respectively.

Three *Najas* sp. were identified in Allaqi; *Najas horrida* A. Br. Ex Magn., (Fig. 3), *Najas marina* subsp. *armata* (Lindb. f.) Horn and *Najas minor* All. The other macrophytes species were *Myriophyllum spicatum* L. and *Potamogeton schweinfurthii* A. Benn (Tab. 2).

Table 2: Cover percentage of different species of macrophytes including the total cover percentage of *Najas* (T. C%) from the main areas in Allaqi.

Main Areas		Plant species					
		N. horrida	*N. marina*	*N. minor*	*M. spicatum*	*P. schweinfurthii*	*T. C.% Najas*
Eastern Shores	Ras El-Maiaa	98.0	0.0	0.0	0.0	0.0	98.0
	Wadi Quleib	86.6	1.6	0.0	9.3	0.8	88.3
	Roud Abu Hamboul	90.0	1.0	0.0	5.0	5.0	91.0
	Khore Darouba	40.0	0.0	0.0	20.0	0.0	40.0
	Roud Abu Sabkhaia	85.0	0.0	0.0	0.0	0.0	85.0
	Wadi Um Ashira	61.7	0.0	0.0	13.3	3.3	61.7
	Jebel Abu Seif	20.0	60.0	0.0	20.0	0.0	80.0
	Khore El Agebab	50.0	0.0	10.0	0.0	0.0	50.0
	Abu Zeraai	40.0	6.6	0.0	0.0	13.3	46.6
	Turgumi	6.3	6.3	0.0	40.0	0.0	12.5
Western Shores	Abu Markh	0.0	0.0	0.0	34.0	0.0	0.0
	Um Shakaiet	30.0	0.0	0.0	20.0	10.0	30.0
	Khore Sengaia	30.0	0.0	0.0	20.0	0.0	30.0
	Khore Abu Bawadi	55.0	0.0	0.0	8.3	0.0	55.0
	Khore Abu Hogab	40.0	0.0	0.0	12.5	0.0	40.0
	Um Fart Island	19.3	0.0	0.0	3.3	0.6	19.3

Najas horrida is the most abundant plant species from the southern part, particularly at Ras El-Maiaa, Wadi Quleib and Roud Abu Hamboul (Fig. 4). Maximum percentage cover for *Najas horrida* (98, 86.6 and 90%, respectively) was recorded in these areas.

Najas horrida remarkably reduced towards the north, recording minimum cover in Turgumi (6.3%) and it disappeared in *Abu Markh* and in most sites in the north area of khore Allaqi. *Najas horrida* is growing in 101 sites recording 63.9% presence (Tab. 3).

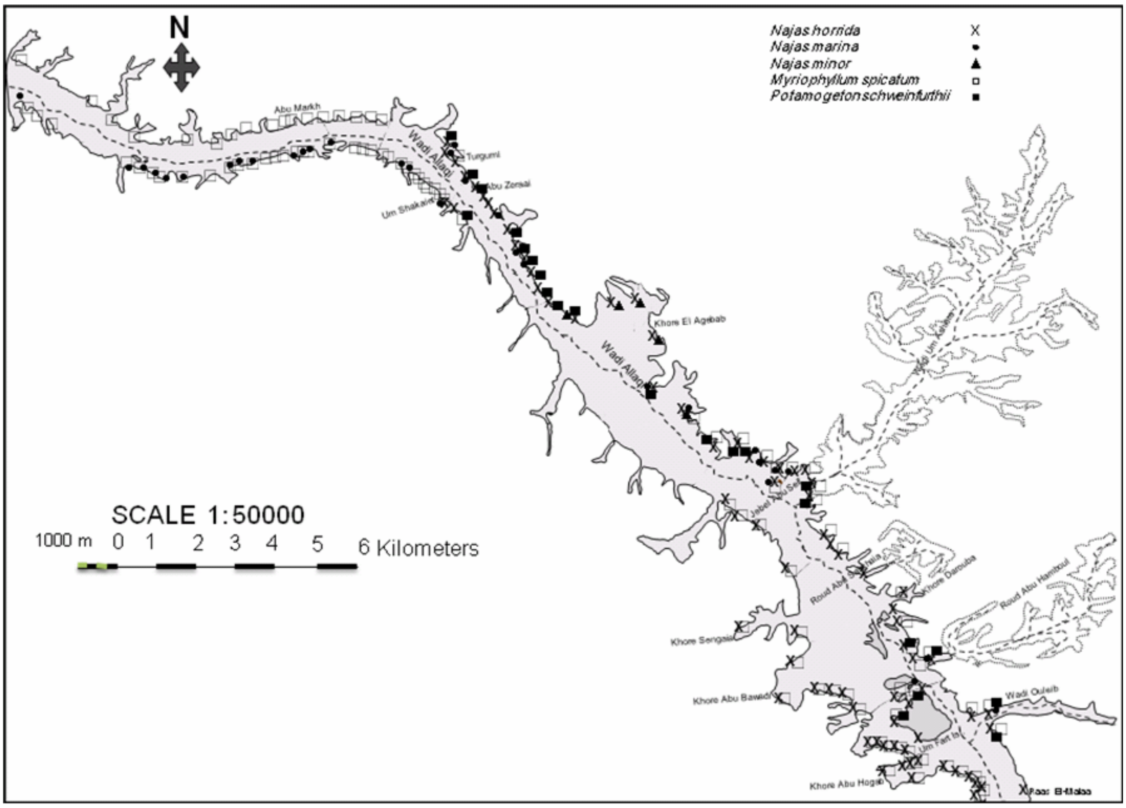

Figure 4: Distribution of macrophyte species in khore Allaqi.

Table 3: Presence and frequency of macrophytes species in Allaqi.

	Plant species	Presence (no. of stations)	Frequency%
1.	*N. horrida*	101	63.90
2.	*N. marina*	36	22.70
3.	*N. minor*	4	2.53
4.	*M. spicatum*	116	73.40
5.	*P. schweinfurthii*	29	18.35

Najas marina in Allaqi is less frequent than *Najas horrida* and dominates the northern areas of Allaqi (Fig. 4). *Najas marina* presence concentrates in Jebel Abu Seif where it is found in 36 stations (22.7% frequency). *Najas minor* was rare in Allaqi compared to other species growing in only four sites from khore El-Agebabe (2.53% frequency and 10% cover). Bedouins from Allaqi collect and utilize *Najas* spp. as a mixture, although they have experience to distinguish between the three species: they call *Najas horrida* - Shelbeika Abu Leif, *Najas marina* - Shelbeika Abu Shouk (spiny) and *Najas minor* - Shelbeika Naema (smooth).

It was essential to calculate the total cover percentage of *Najas* spp. in main areas in khore Allaqi (Tab. 2). Maximum *Najas* growth was on the southern part, including Ras El-Maiaa, Wadi Quleib and Roud abu Humboul with cover percentages of 98, 88.3 and 91%, respectively. Most areas in Allaqi show moderate growth of *Najas* (cover ranged between

40 - 61.7%), including khore Darouba, Um Ashira and khore Abu Hogaab. Very low growth of *Najas* was in Abu Markh (1%) and Turgumi (12.5%).

Myriophyllum spicatum is the most frequent species occupying 116 sites in Allaqi and recording a 73.4% frequency (Tab. 3). It forms pure stands in many areas in northern parts of Allaqi and on the shores of Abu Markh (Fig. 4). No *Myriophyllum spicatum* was found in middle Allaqi, nor in the area south of Turigumi to khore El Agebaba. Wadi Quleib, Roud Abu Humboul and Ras El-Maiaa were clear of *Myriophyllum spicatum*, being occupied by pure stands of *Najas horrida* (Fig. 4).

Potamogeton schweinfurthii is growing mainly on the eastern shores of Allaqi, the only western area being Um Shakaiet (10% cover percentage). It was found that *Potamogeton schweinfurthii* formes communities with *Najas horrida* and *Najas marina* in the area between khore El-Agebab and Abu Zeraai, and with *Myriophyllum spicatum* on the area between Um Ashira and Wadi Quleib (Fig. 4).

Aquatic macrophytes

The table 4 summarize the mean Dry Weight of Standing Crops (DWSC) and standard deviation of aquatic macrophytes in Allaqi, in order to represent its spatial variation, while table 5 shows the temporal variation in macrophytes species between the three samplings.

The spatial variation of aquatic macrophytes was more obvious than the temporal or the standard deviation (low in most sites). *Najas horrida* is the dominant plant species in Allaqi growing intensevely in Raas El-Maiaa, Qulieb 1 and in the area between Um Fart and small Islands, with mean DWSC of 45.1, 50.08 and 50.03 g/sample^{-1}, respectively. The mean DWSC reduces in other areas from the southern part of Allaqi including Roud Abu Hamboul and Roud Abu Sbkhaia, with 29.39 and 35.83 g/sample^{-1}, respectively. No *N. horrida* was found in most sites from the western shores of Allaqi, the only exception being the West Bank of khore Um Ashira, recording with 23.11 g/sample^{-1}. July was the best month for Najas horrida: the plant flourishes this month, recording its highest values of DWSC in all sites. It was noticed that Abu Zeiraa and Turgumi do not support the growth of *N. horrida*, particularly in September, DWSC dropping to 1.05 g/sample^{-1} in Abu Zeiraa and the plant disappearing from Turgumi.

Although *Myriophyllum spicatum* was the most frequent species in Allaqi (it occupies most of the selected sites), it showed relatively low growth (ranging from 3.72 to 22.04 g/sample) compared with *Najas horrida*. Maximum mean DWSC (22.04 g/sample) was at 50 m south of Jebel Abu Seif, while the minimum (3.72 g/sample) was in the area between Um Fart and small Island. It was noticed that the growth of the plant increased toward the north, recording relatively high DWSC (17.87 g/sample at 50 m north of Roud Abu Hamboul). *Myriophyllum spicatum* was the dominant species in only three sites: at 50 m north of Abu Hamboul, 50 m south of Jebel Abu Seif and at Turgumi, recording mean DWSC of 17.87, 22.04 and 11.26 g/sample, respectively. *M. spicatum* shows similarity with other species in the pattern of variation between the three sampling months. Maximum growth of *M. spicatum* was recorded in July while the plant DWSC remarkably reduces in May and September.

Potamogeton schweinfurtii showed low growh in Allaqi in relation to other plant species the plant occupying few sites with low DWSC. The highest mean DWSC (10.13 g/sample) was at 5 km north of Jebel Abu Seif, while the lowest (2.38 g/sample) was in the area between Um Fart and small Island. *Potamogeton schweinfurtii* was absent from more that 50% of selectes sites. The variation of plant growth between the three sampling months was not clear, showing slightly higher growth in July.

Table 4: The mean Dry Weight Standing Crops (DWSC, g/sample) and standard deviation of different macrophytes species from Allaqi.

		N. horrida		*N. marina*		*N. minor*		*M. spicatum*		*P. schweifurii*	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1.	Raas El-Maia	45.10	3.21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2.	Qulieb 1	50.08	4.20	0.00	0.00	0.00	0.00	4.49	0.99	3.06	0.52
3.	Qulieb 2	31.23	2.44	10.62	1.43	0.00	0.00	6.27	6.73	5.07	2.81
4.	Qulieb 3	17.44	3.92	0.00	0.00	0.00	0.00	7.20	0.64	0.00	0.00
5.	Um Fart Isi	8.51	1.43	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6.	between Um Fart and small Island	50.03	8.05	5.07	2.12	0.00	0.00	3.72	1.53	2.38	2.17
7.	Roud Abu Hamboul	29.39	2.54	6.84	0.44	0.00	0.00	3.75	0.93	5.67	2.83
8.	10 m north of Abu Hamboul	15.29	2.90	0.00	0.00	0.00	0.00	11.21	6.70	0.00	0.00
9.	50 m north of Abu Hamboul	5.08	0.59	0.00	0.00	0.00	0.00	17.87	5.94	0.00	0.00
10.	Roud Abu Sabkhaia	35.83	2.79	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11.	Khore Um Ashiera	19.71	7.55	0.00	0.00	0.00	0.00	17.84	2.41	8.26	2.29
12.	50 m South of Jebel Abu Seif	11.30	3.23	10.77	1.45	0.00	0.00	22.04	5.59	0.00	0.00
13.	Jebel Abu Seif	11.00	1.76	22.04	2.96	0.00	0.00	9.67	0.93	0.00	0.00
14.	100 m North of Jebel Abu Seif	0.00	0.00	13.77	1.21	0.00	0.00	0.00	0.00	0.00	0.00
15.	2 km North of Jebel Abu Seif	8.27	0.12	3.59	1.28	53.19	5.02	0.00	0.00	7.11	1.76
16.	5 km North of Jebel Abu Seif	21.63	3.62	0.00	0.00	32.88	5.21	0.00	0.00	10.13	4.02
17.	Abu Zeiraa	7.70	1.02	12.42	3.76	0.00	0.00	0.00	0.00	9.38	1.43
18.	Turgumi	5.08	1.60	5.45	3.34	0.00	0.00	11.26	2.08	0.00	0.00
19.	West Bank of Abu Markh 1	0.00	0.00	15.04	3.64	0.00	0.00	0.00	0.00	0.00	0.00
20.	West Bank of Abu Markh 2	0.00	0.00	33.37	7.07	0.00	0.00	11.82	1.14	0.00	0.00
21.	Um Shakaiet	18.80	4.90	0.00	0.00	0.00	0.00	12.73	2.85	8.60	1.88
22.	Khore Um Ashiera West Bank	23.11	4.81	0.00	0.00	0.00	0.00	9.90	3.56	9.19	3.50

Table 5a: The temporal variation in the dry weight standing crops of macrophytes species; M - May, J - July, S - September.

		N. horrida			*N. marina*		
		M	J	S	M	J	S
1.	Raas El-Maia	45.03	52.34	37.92	0	0	0
2.	Qulieb 1	50	58.32	41.92	0	0	0
3.	Qulieb 2	21.31	42.13	30.25	10.88	12.91	8.07
4.	Qulieb 3	13.25	21.01	18.05	0	0	0
5.	Um Fart Isi	8.04	14.15	3.33	0	0	0
6.	betweenUmFart and small Island	48.21	58.83	43.04	2	13.21	0
7.	Roud Abu Hamboul	27.04	38.86	22.28	2.83	15.43	2.26
8.	10 m north of Abu Hamboul	15.11	18.27	12.48	0	0	0
9.	50 m north of Abu Hamboul	0	8.93	6.31	0	0	0
10.	Roud Abu Sabkhaia	28.21	41.23	38.06	0	0	0
11.	Khore Um Ashiera	13.05	27.92	18.17	0	0	0
12.	50 m South of Jebel Abu Seif	10.01	14.98	8.92	8	19.21	5.11
13.	Jebel Abu Seif	10.27	13.01	9.73	22.05	25	19.08
14.	100 m North of Jebel Abu Seif	0	0	0	13	18.32	10
15.	2 km North of Jebel Abu Seif	10	8.9	5.91	2.77	5.06	2.94
16.	5 km North of Jebel Abu Seif	21.05	25.51	18.33	0	0	0
17.	Abu Zeiraa	10.02	12.08	1.05	13.42	15.58	8.27
18.	Turgumi	7.03	8.23	0	3.97	9.28	3.11
19.	West Bank of Abu Markh 1	0	0	0	15.91	18.17	11.05
20.	West Bank of Abu Markh 2	0	0	0	32	41.02	27.08
21.	Um Shakaiet	18.22	23.96	14.22	0	0	0
22.	KhoreUmAshiera West Bank	21.02	28.61	19.71	0	0	0

Table 5b: The temporal variation in the dry weight standing crops of macrophytes species; M - May, J - July, S - September.

		N. minor			*M. spicatum*			*P. schweinfurtii*		
		M	J	S	M	J	S	M	J	S
1.	Raas El-Maia	0	0	0	0	0	0	0	0	0
2.	Qulieb 1	0	0	0	2.08	7.83	3.55	2.72	3.66	2.79
3.	Qulieb 2	0	0	0	5.44	13.38	0	4.62	8.07	2.51
4.	Qulieb 3	0	0	0	8.52	10	3.08	0	0	0
5.	Um Fart Isi	0	0	0	0	0	0	0	0	0
6.	between Um Fart and small Island	0	0	0	2.89	5.48	2.78	4.25	2.88	0
7.	Roud Abu Hamboul	0	0	0	3.53	4.77	2.94	4	8.94	4.06
8.	10 m north of Abu Hamboul	0	0	0	5.47	18.58	9.59	0	0	0
9.	50 m north of Abu Hamboul	0	0	0	11.63	23.46	18.52	0	0	0
10.	Roud Abu Sabkhaia	0	0	0	0	0	0	0	0	0
11.	Khore Um Ashiera	0	0	0	17.49	20.41	15.62	5.95	10.52	8.32
12.	50 m South of Jebel Abu Seif	0	0	0	21	28.08	17.05	0	0	0
13.	Jebel Abu Seif	0	0	0	10.38	10	8.62	0	0	0
14.	100 m North of Jebel Abu Seif	0	0	0	0	0	0	0	0	0
15.	2 km North of Jebel Abu Seif	51.77	58.77	49.04	0	0	0	6.82	9	5.52
16.	5 km North of Jebel Abu Seif	31.94	38.49	28.2	0	0	0	10.42	14	5.97
17.	Abu Zeiraa	0	0	0	0	0	0	9.72	10.61	7.81
18.	Turgumi	0	0	0	10.31	13.65	9.83	0	0	0
19.	West Bank of Abu Markh 1	0	0	0	0	0	0	0	0	0
20.	West Bank of Abu Markh 2	0	0	0	11.92	15.91	7.64	0	0	0
21.	Um Shakaiet	0	0	0	14.82	13.88	9.49	8.08	7.04	10.69
22.	Khore Um Ashiera West Bank	0	0	0	7.53	14	8.18	9.82	12.33	5.41

Environmental variables

Limnology

The table 6 shows the mean values and the standard deviation of the 12 physico-chemical parameters of the water samples, and the table 7 summarizes the temporal variation of water characteristics for the three samplings.

The temporal and spatial variations in water depth were clear and highly related to lake water level. High depth was recorded in khore Um Ashiera, at 10 m north of Abu Hamboul and on the West bank of Abu Markh 2, with mean values of 4.64, 4.19 and 4.62 m. The shallow sites are Raas El-Maia, Qulieb 1, Qulieb 2 and the area 2 km North of Jebel Abu Seif, recording 1.58, 1.48, 1.35 and 1.41 m, respectively. Water depth showed a slight variation between July and May, while the variation was higher in September compared with other sampling months.

Water temperature did not varied significantly due to location. In general, lower temperature was recorded in sites located south of Allaqi, characterized by shallow water and occupied by dense macrophytes growth, such as Raas El-Maia and Qulieb 1 (30.3 and 30.63°C, respectively). Maximum mean value of water temperature (32.73°C) was in Turgumi, while the minimum (30.3°C), in Raas El-Maia. In most selected sites, low variation of less than 1°C was detected between the samplings.

Water was alkaline, excepting the sites 100 m North of Jebel Abu Seif and West Bank of Abu Markh 1 where the mean pH values were 6.74 and 6.13, respectively. The most alkaline sites in Allaqi were Raas El-Maia, the area between Um Farat and small Island, and Qulieb 1, recording pH values of 10.18, 10.38 and 10.29, respectively. In most sites, pH values were higher in July than in other sampling months.

Total dissolved salts ranged between 115.67, at Raas El-Maia, and 153 mg/l, in Turgumi. There is no certain pattern of spatial variation, but in general it shows reverse variation from pH. Total dissolved salts showed lower values in July compared with other sampling times, especially in sites with intensive plant growth in south of Allaqi.

The variation in water conductivity was highly related to water dissolved salts. It was lower in Raas El-Maia, Qulieb 1 and in the area between Um Farat and small Island, recording 219.67, 222.67 and 222 µS/cm. July is the period with low conductivity for most sites.

Figure 5: Dense growth of epiphytes restricts the growth of *Najas* spp. in many areas in Allaqi.

Table 6a: The spatial variation of water parameters in selected sites in Allaqi (M - mean; SD - standard deviation).

		Depth (m)		T (˚C)		pH	
		M	SD	M	SD	M	SD
1.	Raas El-Maia	1.58	0.61	30.30	0.61	10.18	0.33
2.	Qulieb 1	1.48	0.42	30.63	0.64	10.29	0.92
3.	Qulieb 2	1.35	0.71	30.70	0.62	10.15	0.20
4.	Qulieb 3	2.80	0.65	30.77	0.68	8.67	0.43
5.	Um Fart Isi	3.05	0.76	31.00	0.80	9.17	0.39
6.	betweenUmFart and small Island	1.88	0.82	31.33	0.65	10.38	0.47
7.	Roud Abu Hamboul	1.80	0.68	31.27	0.64	9.17	0.34
8.	10 m north of Abu Hamboul	4.19	0.83	31.60	0.62	9.14	0.23
9.	50 m north of Abu Hamboul	3.89	0.80	31.90	0.61	9.50	0.47
10.	Roud Abu Sabkhaia	1.91	0.87	32.30	0.44	9.08	0.14
11.	Khore Um Ashiera	4.64	0.13	32.43	0.55	9.22	0.43
12.	50 m South of Jebel Abu Seif	3.94	0.81	32.10	0.62	8.50	0.60
13.	Jebel Abu Seif	4.05	0.91	31.73	1.10	9.07	0.04
14.	100 m North of Jebel Abu Seif	3.86	0.99	32.50	0.87	6.74	0.43
15.	2 km North of Jebel Abu Seif	1.41	0.58	32.40	0.72	8.70	0.37
16.	5 km North of Jebel Abu Seif	2.05	0.94	32.30	0.98	9.16	0.19
17.	Abu Zeiraa	3.06	0.94	32.33	1.04	9.12	0.12
18.	Turgumi	2.10	1.31	32.73	0.97	9.21	0.18
19.	West Bank of Abu Markh 1	2.46	1.88	32.53	1.10	8.16	1.77
20.	West Bank of Abu Markh 2	4.62	1.17	32.43	1.01	8.62	0.24
21.	Um Shakaiet	1.97	1.40	32.27	0.72	9.47	0.67
22.	KhoreUmAshiera West Bank	1.73	0.86	32.37	0.72	9.45	0.46

Table 6b: The spatial variation of water parameters in selected sites in Allaqi (M - mean; SD - standard deviation).

		TDS		Cond.		DO	
		M	SD	M	SD	M	SD
1.	Raas El-Maia	115.67	4.16	219.67	1.53	9.26	1.26
2.	Qulieb 1	117.33	8.62	222.67	3.21	7.41	2.57
3.	Qulieb 2	133.33	7.02	254.67	6.03	6.52	2.64
4.	Qulieb 3	136.00	5.57	262.67	3.51	6.83	2.19
5.	Um Fart Isi	134.67	5.86	250.67	11.15	6.42	1.28
6.	betweenUmFart and small Island	117.67	2.52	222.00	1.00	9.54	1.34
7.	Roud Abu Hamboul	131.00	5.29	246.00	14.42	7.25	2.03
8.	10 m north of Abu Hamboul	131.67	6.51	248.67	13.50	6.49	1.03
9.	50 m north of Abu Hamboul	133.00	7.21	252.33	8.33	6.10	1.93
10.	Roud Abu Sabkhaia	128.33	3.51	249.00	21.07	9.85	0.84
11.	Khore Um Ashiera	133.67	5.13	253.67	0.58	6.50	1.18
12.	50 m South of Jebel Abu Seif	138.67	8.02	257.33	11.02	9.33	1.05
13.	Jebel Abu Seif	140.67	4.04	263.00	16.70	6.48	1.25
14.	100 m North of Jebel Abu Seif	137.67	12.50	275.33	25.70	4.33	0.40
15.	2 km North of Jebel Abu Seif	124.33	4.16	224.67	2.08	9.37	0.95
16.	5 km North of Jebel Abu Seif	149.00	6.93	296.67	30.89	7.41	1.70
17.	Abu Zeiraa	145.67	7.77	283.33	33.08	7.17	1.05
18.	Turgumi	153.00	8.89	298.33	31.63	7.86	0.85
19.	West Bank of Abu Markh 1	134.00	8.19	255.33	5.13	7.40	1.38
20.	West Bank of Abu Markh 2	150.33	4.93	283.00	30.32	7.91	1.52
21.	Um Shakaiet	129.33	5.1	250.33	19.76	8.18	1.82
22.	KhoreUmAshiera West Bank	134.00	3.61	253.67	7.37	8.83	1.30

Table 6c: The spatial variation of water parameters in selected sites in Allaqi (M - mean; SD - standard deviation).

		PAR		PO_4		NO_3	
		M	SD	M	SD	M	SD
1.	Raas El-Maia	1.02	0.31	0.07	0.03	0.35	0.47
2.	Qulieb 1	0.91	0.13	0.10	0.04	0.41	0.25
3.	Qulieb 2	2.33	0.37	0.07	0.02	0.83	0.05
4.	Qulieb 3	1.32	0.36	0.12	0.04	0.84	0.50
5.	Um Fart Isi	5.15	0.58	0.04	0.00	0.70	0.10
6.	betweenUmFart and small Island	1.17	0.32	0.09	0.03	0.90	0.30
7.	Roud Abu Hamboul	2.39	0.27	0.10	0.04	0.70	0.17
8.	10 m north of Abu Hamboul	2.26	0.21	0.13	0.05	1.03	0.23
9.	50 m north of Abu Hamboul	4.14	0.15	0.04	0.02	1.20	0.10
10.	Roud Abu Sabkhaia	3.07	0.91	0.14	0.04	1.00	0.26
11.	Khore Um Ashiera	2.09	0.38	0.12	0.06	1.13	0.21
12.	50 m South of Jebel Abu Seif	3.53	0.41	0.07	0.01	1.00	0.46
13.	Jebel Abu Seif	4.91	0.41	0.06	0.01	1.00	0.35
14.	100 m North of Jebel Abu Seif	4.93	0.11	0.30	0.02	1.27	0.15
15.	2 km North of Jebel Abu Seif	1.97	0.15	0.19	0.06	1.07	0.23
16.	5 km North of Jebel Abu Seif	2.17	0.17	0.15	0.08	1.47	0.55
17.	Abu Zeiraa	2.65	0.31	0.13	0.09	1.60	1.22
18.	Turgumi	4.44	0.58	0.17	0.11	1.37	0.46
19.	West Bank of Abu Markh 1	3.14	0.12	0.17	0.02	1.23	0.71
20.	West Bank of Abu Markh 2	2.32	0.63	0.17	0.04	1.18	0.98
21.	Um Shakaiet	2.33	0.12	0.11	0.05	0.10	0.08
22.	KhoreUmAshiera West Bank	2.19	0.16	0.06	0.01	0.13	0.10

Table 6d: The spatial variation of water parameters in selected sites in Allaqi (M - mean; SD - standard deviation).

		NO_2		NH_4		SO_4	
		M	SD	M	SD	M	SD
1.	Raas El-Maia	0.01	0.01	0.00	0.00	4.33	1.53
2.	Qulieb 1	0.01	0.00	0.00	0.00	5.00	1.00
3.	Qulieb 2	0.01	0.00	0.00	0.00	5.33	3.51
4.	Qulieb 3	0.01	0.00	0.01	0.01	10.00	3.61
5.	Um Fart Isi	0.02	0.02	0.01	0.01	16.67	6.66
6.	between Um Fart and small Island	0.02	0.01	0.00	0.00	3.67	0.58
7.	Roud Abu Hamboul	0.02	0.02	0.02	0.01	13.67	3.21
8.	10 m north of Abu Hamboul	0.01	0.00	0.01	0.00	13.67	4.16
9.	50 m north of Abu Hamboul	0.01	0.00	0.01	0.01	15.67	8.96
10.	Roud Abu Sabkhaia	0.02	0.01	0.01	0.01	9.00	2.00
11.	Khore Um Ashiera	0.01	0.01	0.01	0.00	16.67	4.51
12.	50 m South of Jebel Abu Seif	0.02	0.01	0.01	0.01	20.33	1.53
13.	Jebel Abu Seif	0.02	0.02	0.01	0.00	22.00	1.73
14.	100 m North of Jebel Abu Seif	0.02	0.01	0.01	0.01	21.33	7.64
15.	2 km North of Jebel Abu Seif	0.02	0.02	0.01	0.01	6.33	3.79
16.	5 km North of Jebel Abu Seif	0.01	0.00	0.01	0.00	20.00	1.73
17.	Abu Zeiraa	0.01	0.00	0.11	0.08	23.67	5.86
18.	Turgumi	0.01	0.01	0.05	0.07	21.33	4.58
19.	West Bank of Abu Markh 1	0.01	0.01	0.01	0.01	12.67	6.35
20.	West Bank of Abu Markh 2	0.01	0.00	0.01	0.00	9.67	9.87
21.	Um Shakaiet	0.01	0.00	0.00	0.00	16.33	3.06
22.	Khore Um Ashiera West Bank	0.01	0.00	0.00	0.00	19.33	2.31

Table 7a: The temporal variation of water parameters in Allaqi; J - July, S - Setember, M - May.

	Locations	Depth (m)			T (ºC)			pH		
		J	S	M	J	S	M	J	S	M
1.	Raas El-Maia	1.13	2.27	1.33	30.00	29.30	31.00	10.56	9.99	9.99
2.	Qulieb 1	1.83	1.91	1.01	31.00	29.90	31.00	11.31	10.02	9.53
3.	Qulieb 2	0.85	2.17	1.04	30.90	30.00	31.20	10.38	10.06	10.00
4.	Qulieb 3	2.18	3.48	2.74	31.30	30.00	31.00	8.18	8.93	8.91
5.	Um Fart Isi	2.51	3.92	2.72	31.00	30.20	31.80	9.62	8.93	8.95
6.	between Um Fart and small Island	1.31	2.82	1.52	30.70	31.30	32.00	10.32	10.88	9.95
7.	Roud Abu Hamboul	1.07	2.41	1.93	31.00	30.80	32.00	9.56	8.97	8.98
8.	10 m north of Abu Hamboul	3.78	5.15	3.65	31.40	31.10	32.30	9.39	9.09	8.93
9.	50 m north of Abu Hamboul	3.38	4.81	3.47	31.50	31.60	32.60	9.41	9.08	10.01
10.	Roud Abu Sabkhaia	1.44	2.92	1.38	32.10	32.00	32.80	9.24	9.02	8.97
11.	Khore Um Ashiera	4.51	4.77	4.65	31.90	32.40	33.00	9.72	8.97	8.98
12.	50 m South of Jebel Abu Seif	3.35	4.87	3.61	31.90	31.60	32.80	9.20	8.14	8.17
13.	Jebel Abu Seif	3.35	5.07	3.72	31.00	31.20	33.00	9.10	9.07	9.03
14.	100 m North of Jebel Abu Seif	3.02	4.95	3.62	32.90	31.50	33.10	6.26	7.08	6.87
15.	2 km North of Jebel Abu Seif	1.14	2.08	1.02	32.20	31.80	33.20	9.05	8.32	8.73
16.	5 km North of Jebel Abu Seif	1.75	3.10	1.30	31.50	32.00	33.40	9.37	9.11	9.00
17.	Abu Zeiraa	2.24	4.08	2.87	31.50	32.00	33.50	9.25	9.11	9.01
18.	Turgumi	1.08	3.58	1.64	32.50	31.90	33.80	9.41	9.08	9.13
19.	West Bank of Abu Markh 1	1.18	4.61	1.58	32.00	31.80	33.80	9.35	6.13	9.00
20.	West Bank of Abu Markh 2	4.98	5.57	3.32	32.30	31.50	33.50	8.35	8.77	8.75
21.	Um Shakaiet	1.03	3.58	1.30	31.90	31.80	33.10	10.20	9.35	8.87
22.	Khore Um Ashiera West Bank	1.16	2.72	1.32	31.90	32.00	33.20	9.93	9.42	9.01

Table 7b: The temporal variation of water parameters in Allaqi; J - July, S - Setember, M - May.

Locations		TDS			Cond.			DO		
		J	S	M	J	S	M	J	S	M
1.	Raas El-Maia	111.00	117.00	119.00	218.00	221.00	220.00	10.12	9.85	7.81
2.	Qulieb 1	108.00	119.00	125.00	219.00	224.00	225.00	10.37	6.03	5.82
3.	Qulieb 2	126.00	134.00	140.00	249.00	254.00	261.00	9.45	5.80	4.32
4.	Qulieb 3	130.00	141.00	137.00	266.00	263.00	259.00	9.30	6.08	5.11
5.	Um Fart Isi	137.00	128.00	139.00	259.00	238.00	255.00	7.84	6.08	5.34
6.	UmFart - small Island	115.00	118.00	120.00	221.00	222.00	223.00	10.97	9.35	8.31
7.	Roud Abu Hamboul	129.00	137.00	127.00	230.00	258.00	250.00	9.16	7.48	5.12
8.	10 m north of Abu Hamboul	125.00	132.00	138.00	235.00	249.00	262.00	6.35	7.58	5.53
9.	50 m north of Abu Hamboul	125.00	139.00	135.00	243.00	259.00	255.00	8.04	6.08	4.18
10.	Roud Abu Sabkhaia	125.00	132.00	128.00	247.00	271.00	229.00	10.82	9.45	9.28
11.	Khore Um Ashiera	128.00	138.00	135.00	253.00	254.00	254.00	7.68	6.49	5.32
12.	50 m South of Jebel Abu Seif	138.00	131.00	147.00	258.00	246.00	268.00	9.83	10.03	8.12
13.	Jebel Abu Seif	145.00	137.00	140.00	281.00	248.00	260.00	5.79	7.92	5.72
14.	100 m North of Jebel Abu Seif	138.00	125.00	150.00	261.00	260.00	305.00	3.90	4.68	4.42
15.	2 km North of Jebel Abu Seif	129.00	121.00	123.00	223.00	227.00	224.00	9.82	10.01	8.28
16.	5 km North of Jebel Abu Seif	141.00	153.00	153.00	261.00	314.00	315.00	8.82	7.88	5.52
17.	Abu Zeiraa	137.00	152.00	148.00	249.00	315.00	286.00	7.42	8.07	6.01
18.	Turgumi	146.00	163.00	150.00	263.00	324.00	308.00	8.41	8.28	6.88
19.	West Bank of Abu Markh 1	125.00	136.00	141.00	254.00	251.00	261.00	8.82	7.32	6.07
20.	West Bank of Abu Markh 2	148.00	156.00	147.00	265.00	318.00	266.00	9.33	8.08	6.31
21.	Um Shakaiet	123.00	132.00	133.00	229.00	268.00	254.00	9.35	9.11	6.08
22.	Khore Um Ashiera WestBank	133.00	138.00	131.00	251.00	262.00	248.00	9.82	9.31	7.35

Table 7c: The temporal variation of water parameters in Allaqi; J - July, S - Setember, M - May.

	Locations	PAR			PO_4			NO_3		
		J	S	M	J	S	M	J	S	M
1.	Raas El-Maia	0.73	0.98	1.34	0.04	0.08	0.09	0.90	0.08	0.08
2.	Qulieb 1	0.77	1.02	0.93	0.06	0.14	0.11	0.50	0.60	0.12
3.	Qulieb 2	1.92	2.64	2.42	0.05	0.09	0.06	0.40	1.10	1.00
4.	Qulieb 3	0.92	1.62	1.42	0.07	0.13	0.15	0.90	1.30	0.31
5.	Um Fart Isi	4.73	5.82	4.91	0.04	0.04	0.04	0.70	0.60	0.80
6.	betweenUm Fart and small Island	0.81	1.43	1.27	0.07	0.08	0.12	0.60	0.90	1.20
7.	Roud Abu Hamboul	2.41	2.65	2.11	0.05	0.13	0.11	0.80	0.50	0.80
8.	10 m north of Abu Hamboul	2.03	2.44	2.30	0.07	0.15	0.17	1.30	0.90	0.90
9.	50 m north of Abu Hamboul	4.01	4.31	4.11	0.02	0.06	0.04	1.10	1.30	1.20
10.	Roud Abu Sabkhaia	3.51	2.02	3.68	0.11	0.13	0.18	0.90	0.80	1.30
11.	Khore Um Ashiera	1.78	2.52	1.98	0.09	0.18	0.08	1.30	1.20	0.90
12.	50 m South of Jebel Abu Seif	3.70	3.06	3.83	0.08	0.06	0.06	1.50	0.90	0.60
13.	Jebel Abu Seif	4.52	5.33	4.87	0.06	0.05	0.07	1.20	0.60	1.20
14.	100 m North of Jebel Abu Seif	4.81	5.01	4.97	0.32	0.29	0.29	1.10	1.30	1.40
15.	2 km North of Jebel Abu Seif	1.82	2.11	1.97	0.14	0.25	0.18	0.80	1.20	1.20
16.	5 km North of Jebel Abu Seif	2.33	2.18	2.00	0.17	0.21	0.06	1.50	2.00	0.90
17.	Abu Zeiraa	2.31	2.92	2.71	0.24	0.08	0.08	3.00	0.80	1.00
18.	Turgumi	4.53	4.98	3.82	0.29	0.09	0.12	1.90	1.10	1.10
19.	West Bank of Abu Markh 1	3.04	3.27	3.11	0.14	0.18	0.18	1.10	2.00	0.60
20.	West Bank of Abu Markh 2	2.54	2.81	1.61	0.13	0.18	0.21	2.10	1.30	0.15
21.	Um Shakaiet	2.21	2.45	2.33	0.08	0.17	0.09	0.03	0.08	0.18
22.	Khore Um Ashiera West Bank	2.01	2.31	2.25	0.05	0.07	0.06	0.05	0.10	0.24

Table 7d: The temporal variation of water parameters in Allaqi; J - July, S - Setember, M - May.

	Locations	NO_2			NH_4			SO_4		
		J	S	M	J	S	M	J	S	M
1.	Raas El-Maia	0.02	0.00	0.01	0.00	0.00	0.00	6.00	4.00	3.00
2.	Qulieb 1	0.01	0.01	0.01	0.00	0.00	0.00	6.00	5.00	4.00
3.	Qulieb 2	0.01	0.01	0.01	0.00	0.00	0.00	9.00	5.00	2.00
4.	Qulieb 3	0.01	0.01	0.01	0.01	0.01	0.02	14.00	9.00	7.00
5.	Um Fart Isi	0.01	0.04	0.01	0.01	0.01	0.02	20.00	21.00	9.00
6.	between Um Fart and small Island	0.01	0.03	0.01	0.00	0.00	0.00	4.00	4.00	3.00
7.	Roud Abu Hamboul	0.04	0.01	0.01	0.01	0.01	0.03	16.00	15.00	10.00
8.	10 m north of Abu Hamboul	0.01	0.01	0.01	0.01	0.01	0.01	17.00	15.00	9.00
9.	50 m north of Abu Hamboul	0.01	0.01	0.01	0.02	0.00	0.00	26.00	10.00	11.00
10.	Roud Abu Sabkhaia	0.03	0.01	0.01	0.01	0.01	0.02	7.00	11.00	9.00
11.	Khore Um Ashiera	0.01	0.02	0.01	0.01	0.01	0.01	21.00	17.00	12.00
12.	50 m South of Jebel Abu Seif	0.01	0.03	0.01	0.00	0.02	0.02	22.00	19.00	20.00
13.	Jebel Abu Seif	0.00	0.04	0.01	0.01	0.01	0.02	24.00	21.00	21.00
14.	100 m North of Jebel Abu Seif	0.01	0.01	0.03	0.02	0.01	0.00	28.00	23.00	13.00
15.	2 km North of Jebel Abu Seif	0.00	0.01	0.04	0.00	0.01	0.03	8.00	9.00	2.00
16.	5 km North of Jebel Abu Seif	0.00	0.01	0.01	0.01	0.01	0.01	21.00	18.00	21.00
17.	Abu Zeiraa	0.01	0.01	0.01	0.15	0.17	0.02	22.00	29.00	20.00
18.	Turgumi	0.01	0.02	0.01	0.01	0.13	0.02	19.00	27.00	18.00
19.	West Bank of Abu Markh 1	0.02	0.01	0.01	0.01	0.01	0.02	20.00	9.00	9.00
20.	West Bank of Abu Markh 2	0.01	0.01	0.01	0.01	0.01	0.02	21.00	3.00	5.00
21.	Um Shakaiet	0.00	0.01	0.01	0.00	0.00	0.01	17.00	13.00	19.00
22.	Khore Um Ashiera West Bank	0.00	0.01	0.01	0.00	0.00	0.01	22.00	18.00	18.00

Figure 6: Stem elongation is the adaptation feature in *Myriophyllum spicatum* for low light penetration.

Water dissolved oxygen showed clear spatial variation. Oxygen content ranged between 4.33 to 9.85 mg/l. The highest mean value was recorded in Roud Abu Sabkhaia and the lowest at 100 m North of Jebel Abu Seif. In general, water is more aerated in sites with dense growth of macrophytes as Raas El-Maia, area between Um Farat and small Island and Roud Abu Sabkhaia, recording 9.26, 9.54 and 9.85 mg/l, respectively. It was observed that site 100 m North of Jebel Abu Seif (which recorded lowest oxygen content, 4.33 mg/l) was occupied by damaged and decayed aquatic plants due to fishing (Tab. 11) boats impact. In most sites, the highest dissolved oxygen values were recorded in July, and the lowest was in May.

Light penetration, expressed as Photosynthetic Active Radiation (PAR), ranged between the minimum mean value of 0.90/m (high light condition) in Qulieb 1 and a maximum of 5.15/m, in Um Farat Island. In most sites, low variation in light penetration was observed between July and May.

Water phosphate showed low spatial variation with mean values varying between 0.04 and 0.3 mg/l. Minimum values were in Um Farat Island and at 50 m North of Abu Hamboul, while the maximum was at 100 m North of Jebel Abu Seif. It was noticed during the field work that the two sites of minimum phosphate content were characterized by high epiphytic growth, forming a layer on the macrophyte species. Clear difference in water phosphate was recorded between the three samples. High phosphate content was in July with few exceptions, such as Um Farat Island (no difference between the three samplings: 0.04 mg/l).

The content of nitrate do not show a certain variation pattern neither in spatial nor in time. Values of water nitrate varied between the lowest of 0.1 mg/l in Um Shakaiet and highest of 1.6 mg/l in Abu Zeiraa.

Najas marina growth was low compared with *N. horrida*, most sites from the eastern shores being free of the plant. The maximum mean DWSC was 33.37 g/sample, recorded at West bank of Abu Markh 2. Jebel Abu Seif and the surrounding area have relatively high growth of *N. marina*, up to 22.04 g/sample. *N. marina* growth concentrates mainly in northern Allaqi, the plant being completely absent from southern sites as Raas El-Maiaa, Qulieb 1, Qulieb 3, Um Fart Island, the area north of Roud Abu Hamboul and khore Um Ashira. Similar to *Najas horrida, Najas marina* grew more dense in July compared to the other sampling months. Maximum growth of the plant was at West Bank of Abu Markh 2, recording a mean DWSC of 41.02 g/sample (at the same site, in September, *Najas marina* biomass considerebly reduced to 27.08 g/sample). The area 2 km north of Jebel Abu Seif does not support *Najas marina* growth, the minimum mean DWSC from July (5.06 g/sample) being recorded here (the value drops to 2.94 g/sample in September and to 2.77 g/sample in May).

Although *Najas minor* was rare species in Allaqi (was recorded only in two sites), it intensively grew at 2 and 5 km north of Jebel Abu Seif, with mean DWSC 53.19 and 32.88 to g sample^{-1}, respectively. Peak growth of *Najas* minor was in July at 2 km north of Jebel Abu Seif with DWSC equal to 58.77 g sample^{-1}, the growth slightly reduced in May to 51.77 g sample^{-1} and to 49.04 g sample^{-1} in September.

Water nitrite concentrations showed little variation in their mean values. In general nitrite content was low, ranging between 0.01 and 0.02 mg/l. The highest value was in about of 20% of selected sites, while the lowest was in the rest sites. No temporal variation was observed between the sampling months.

Ammonia concentrations showed a much clearer spatial variation than both water nitrate and nitrite contents. Water ammonia content in ranged between the lowest of 0.01 mg/l in most sites and the highest of 0.11 mg/l in Abu Zeiraa. Low water ammonia was founded in sites occupied by dense macrophytes growth, such as Raas El-Maia, Qulieb 1, the area between Um Farat and small Island and Qulieb 2, with undetectable contents of ammonia. Water from the area with cultivated shores is characterized by higher ammonia content compared to other sites (Abu Zeiraa and Turgumi are recording 0.11 and 0.05 mg/l). The temporal variation was not as clear as the spatial one, but a significant observation was the higher content of ammonia from September at Abu Zeiraa and Turgumi compared with other two sampling months.

The sulphate content in the studied water varied between 3.67 mg/l in the area between Um Farat and small Island, and 22 mg/l in Jebel Abu Seif area. Similarly, as with ammonia, water sulphate was lower in sites with high growth of macrophytes as were Raas El-Maia, Qulieb 1, Qulieb 2 and the area between Um Farat and small Island, recording 4.33, 5, 5.33 and 3.67 mg/l, respectively. High sulphate concentrations were found in the studied sites with damaged and decayed macrophytes either due to dense growth of epiphytes or to shore cultivation as Jebel Abu Seif and Turgumi areas, recording 22 and 21.33 mg/l, respectively. In most sites, sulphate content of the water showed higher values in summer, in the month of July.

Hydrosoil

The table 8 represents mean and standard deviation values of hydrosoil measured parameters for the samples collected from Allaqi, while the table 9 shows the temporal variation in the chemical characteristics of the hydrosoil samples between the sampling months. The spatial variation indicates that most hydrosoil parameters, including phosphate, nitrate and ammonia are having lower values in the southern area of Allaqi, opposed to the sites from the north.

Phosphate content in hydrosoil ranged between the lowest of 0.02 mg/kg in the area between Um Farat and small Island, and the highest of 0.37 mg/kg at 100 m North of Jebel Abu Seif. Low concentrations were detected in southern sites, particularly those occupied by high growth of macrophytes as Raas El-Maia, the area between Um Farat and small Island and at 2 km North of Jebel Abu Seif, recording 0.05, 0.02 and 0.04 mg/kg, respectively. High hydrosoil phosphate was at 100 m North of Jebel Abu Seif and at the West bank of Abu Markh 1, with values of 0.37 and 0.3 mg/kg, respectively. The lowest hydrosoil phosphate content was in July, with values ranging from 0.01 to 0.59 mg/kg, while the highest were in most sites in September, particularly in Abu Zeiraa and Turgumi (0.5 and 0.43 mg/kg, respectively).

Hydrosoil nitrate did not show a certain pattern of variation between sites. Maximum nitrate content (2.43 mg/kg) was at 50 m North of Abu Hamboul, while the lowest contents were in Raas El-Maia, Qulieb 1 and at the West bank of khore Um Ashiera, with values 0.8, 1 and 0.967 mg/kg, respectively. Hydrosoil nitrate variation was not high between the sampling months.

Nitrite content in the hydrosoil was low. The nitrite values were between the lowest of 0.01 mg/kg in three sites: Raas El-Maia, Quleib 1 and in Qulieb 2, and the highest of 0.13 mg/kg at 2 and 5 km North of Jebel Abu Seif. In contrast to nitrate, the temporal variation of nitrite was clear, with lower values in July.

Hydrosoil ammonia was the only parameter (in both water and hydrosoil samples) with 0 values for about 30% of sites. It was noticed that most of the sites with no ammonia are located in the southern part of Allaqi. Low ammonia (0.03 mg/kg) was in the area between Um Farat and small Island, while the highest values were in Abu Zeiraa and Turgumi (0.4 and 0.42 mg/kg, respectively). The temporal variation of this element was not very clear.

The organic matter from the hydrosoil ranged between the highest value of 4.32% at the West bank of Abu Markh 2, while the lowest value of 0.36% was at the West bank of Abu Markh 1 area. High content of organic matter was also detected at 50 m distance of South of Jebel Abu Seif and at 10 m North of Abu Hamboul, recording 3.42 and 3.01%, respectively. Higher organic matter content was recorded in the month of July (between 0.47 and 4.32%).

Table 8a: Spatial variation of hydro soil sample (M - means, SD - standard deviation).

	Locations	PO_4		NO_3	
		M	SD	M	SD
1.	Raas El-Maia	0.05	0.03	0.80	0.30
2.	Qulieb 1	0.05	0.03	1.00	0.27
3.	Qulieb 2	0.05	0.02	1.30	0.70
4.	Qulieb 3	0.16	0.04	1.27	0.64
5.	Um Fart Isi	0.13	0.03	1.40	0.36
6.	UmFart - small Island	0.02	0.01	1.57	0.50
7.	Roud Abu Hamboul	0.27	0.15	2.13	0.67
8.	10 m north of Abu Hamboul	0.23	0.05	1.40	0.52
9.	50 m north of Abu Hamboul	0.17	0.09	2.43	1.31
10.	Roud Abu Sabkhaia	0.15	0.09	1.67	0.45
11.	Khore Um Ashiera	0.23	0.06	1.60	0.40
12.	50 m South of Jebel Abu Seif	0.12	0.12	1.40	0.62
13.	Jebel Abu Seif	0.18	0.06	1.43	0.49
14.	100 m North of Jebel Abu Seif	0.37	0.21	1.73	0.49
15.	2 km North of Jebel Abu Seif	0.04	0.01	1.24	0.22
16.	5 km North of Jebel Abu Seif	0.10	0.02	2.17	0.76
17.	Abu Zeiraa	0.11	0.02	1.97	1.07
18.	Turgumi	0.26	0.21	1.70	0.46
19.	West Bank of Abu Markh 1	0.30	0.12	1.70	0.44
20.	West Bank of Abu Markh 2	0.07	0.05	1.23	0.35
21.	Um Shakaiet	0.06	0.02	1.00	0.27
22.	Khore Um Ashiera West Bank	0.05	0.04	0.97	0.31

Table 8b: Spatial variation of hydro soil sample (M - means, SD - standard deviation).

	Locations	NO_2		NO_4		OM	
		M	SD	M	SD	M	SD
1.	Raas El-Maia	0.01	0.00	0.00	0.00	2.76	0.96
2.	Qulieb 1	0.01	0.01	0.00	0.00	2.33	1.12
3.	Qulieb 2	0.01	0.00	0.00	0.00	1.86	1.10
4.	Qulieb 3	0.02	0.02	0.16	0.02	0.60	0.31
5.	Um Fart Isi	0.06	0.04	0.12	0.06	0.74	0.25
6.	UmFart - small Island	0.02	0.02	0.03	0.05	0.72	0.14
7.	Roud Abu Hamboul	0.03	0.03	0.00	0.00	1.43	0.31
8.	10 m north of Abu Hamboul	0.05	0.05	0.15	0.07	3.01	0.29
9.	50 m north of Abu Hamboul	0.06	0.04	0.41	0.02	1.69	0.89
10.	Roud Abu Sabkhaia	0.05	0.04	0.00	0.00	1.43	0.63
11.	Khore Um Ashiera	0.10	0.08	0.24	0.07	2.89	0.04
12.	50 m South of Jebel Abu Seif	0.10	0.07	0.22	0.05	3.42	0.59
13.	Jebel Abu Seif	0.10	0.03	0.23	0.13	1.15	0.12
14.	100 m North of Jebel Abu Seif	0.11	0.05	0.39	0.06	2.45	1.55
15.	2 km North of Jebel Abu Seif	0.13	0.06	0.17	0.13	2.21	1.41
16.	5 km North of Jebel Abu Seif	0.13	0.02	0.19	0.10	1.07	0.13
17.	Abu Zeiraa	0.11	0.12	0.40	0.02	1.09	0.26
18.	Turgumi	0.07	0.01	0.42	0.04	0.72	0.83
19.	West Bank of Abu Markh 1	0.11	0.02	0.16	0.08	0.36	0.16
20.	West Bank of Abu Markh 2	0.03	0.03	0.09	0.02	3.44	0.54
21.	Um Shakaiet	0.02	0.01	0.00	0.00	2.29	0.16
22.	Khore Um Ashiera West Bank	0.02	0.02	0.00	0.00	1.40	0.47

Table 9a: The temporal variation in hydrosoil characteristics in selected sites in Allaqi.

	Locations	July				
		PO_4	NO_3	NO_2	NH_4	OM
1.	Raas El-Maia	0.03	2.50	0.01	0.00	2.43
2.	Qulieb 1	0.05	1.80	0.01	0.00	1.51
3.	Qulieb 2	0.03	2.10	0.01	0.00	2.63
4.	Qulieb 3	0.12	2.00	0.04	0.15	0.82
5.	Um Fart Isi	0.11	1.30	0.01	0.13	0.91
6.	between Um Fart and small Island	0.01	1.10	0.04	0.08	0.82
7.	Roud Abu Hamboul	0.13	1.40	0.06	0.00	1.65
8.	10 m north of Abu Hamboul	0.19	1.70	0.02	0.17	3.21
9.	50 m north of Abu Hamboul	0.11	3.90	0.00	0.42	4.32
10.	Roud Abu Sabkhaia	0.09	1.70	0.02	0.00	0.87
11.	Khore Um Ashiera	0.19	2.00	0.01	0.18	2.91
12.	50 m South of Jebel Abu Seif	0.04	1.20	0.02	0.21	2.83
13.	Jebel Abu Seif	0.13	1.10	0.11	0.38	1.23
14.	100 m North of Jebel Abu Seif	0.59	1.40	0.05	0.45	1.54
15.	2 km North of Jebel Abu Seif	0.04	1.30	0.07	0.31	1.21
16.	5 km North of Jebel Abu Seif	0.08	1.50	0.11	0.28	1.16
17.	Abu Zeiraa	0.29	0.80	0.24	0.37	1.27
18.	Turgumi	0.50	1.80	0.06	0.42	1.31
19.	West Bank of Abu Markh 1	0.43	1.90	0.11	0.25	0.47
20.	West Bank of Abu Markh 2	0.02	1.20	0.06	0.08	3.82
21.	Um Shakaiet	0.04	1.30	0.03	0.00	2.40
22.	Khore Um Ashiera West Bank	0.03	0.90	0.04	0.00	1.53

Table 9b: The temporal variation in hydrosoil characteristics in selected sites in Allaqi.

	Locations	September					May				
		PO_4	NO_3	NO_2	NH_4	OM	PO_4	NO_3	NO_2	NH_4	OM
1.	Raas El-Maia	0.08	1.10	0.01	0.00	2.32	0.03	0.80	0.01	0.00	1.08
2.	Qulieb 1	0.08	0.90	0.02	0.00	1.82	0.06	1.30	0.01	0.00	0.14
3.	Qulieb 2	0.04	0.80	0.01	0.00	2.73	0.03	1.00	0.01	0.00	1.08
4.	Qulieb 3	0.18	0.90	0.01	0.18	0.47	0.18	0.90	0.02	0.16	0.38
5.	Um Fart Isi	0.16	1.10	0.08	0.21	0.82	0.13	1.80	0.09	0.25	0.56
6.	between Um Fart and small Island	0.02	1.50	0.01	0.00	1.43	0.08	2.10	0.01	0.00	0.82
7.	Roud Abu Hamboul	0.42	2.30	0.03	0.00	1.08	0.25	2.70	0.01	0.00	1.21
8.	10 m north of Abu Hamboul	0.21	1.70	0.11	0.08	3.13	0.18	0.80	0.02	0.21	2.80
9.	50 m north of Abu Hamboul	0.28	2.00	0.08	0.41	3.72	0.13	1.40	0.02	0.39	3.06
10.	Roud Abu Sabkhaia	0.12	2.10	0.09	0.00	0.89	0.25	1.20	0.03	0.00	0.98
11.	Khore Um Ashiera	0.21	1.20	0.15	0.22	3.47	0.30	1.60	0.13	0.31	2.86
12.	50 m South of Jebel Abu Seif	0.06	0.90	0.13	0.18	2.35	0.25	2.10	0.14	0.28	3.91
13.	Jebel Abu Seif	0.24	1.20	0.06	0.16	0.82	0.17	2.00	0.07	0.15	1.46
14.	100 m North of Jebel Abu Seif	0.35	2.30	0.13	0.33	0.97	0.18	1.50	0.14	0.39	1.35
15.	2 km North of Jebel Abu Seif	0.05	2.50	0.18	0.08	1.24	0.04	1.80	0.15	0.11	1.21
16.	5 km North of Jebel Abu Seif	0.12	3.00	0.15	0.09	0.93	0.09	2.00	0.12	0.21	0.98
17.	Abu Zeiraa	0.13	2.90	0.03	0.41	1.24	0.11	2.20	0.05	0.41	0.91
18.	Turgumi	0.15	1.20	0.07	0.38	1.35	0.13	2.10	0.08	0.45	0.13
19.	West Bank of Abu Markh 1	0.28	2.00	0.09	0.12	0.52	0.19	1.20	0.12	0.11	0.24
20.	West Bank of Abu Markh 2	0.06	1.60	0.01	0.11	3.08	0.12	0.90	0.02	0.08	3.06
21.	Um Shakaiet	0.08	0.80	0.01	0.00	2.84	0.07	0.90	0.01	0.00	2.18
22.	Khore Um Ashiera West Bank	0.03	0.70	0.01	0.00	1.61	0.10	1.30	0.01	0.00	1.06

Vegetative-environmental variables relations

The relation between aquatic macrophytes species (including the three species of *Najas*) growing in Allaqi and environmental variables was tested using the Canonical Correspondence Analysis (CCA). The mean values of environmental variables for three sampling months (May, July and September) were applied in Canonical Correspondence Analysis since there was less temporal variation in comparison with the spatial one. To reduce any arching effect, water conductivity was excluded from the analysis because of its high correlation with total dissolved salts (r = 0.94); water and hydrosoil nitrite were also eliminated, being highly correlated with water and hydrosoil nitrate (r = 0.84 and 0.91, respectively).

The first two Axes in CCA model were significant and together explain 86.49% of variance. The Pearson correlation between species and environment are relatively high and significant for the two Axes (0.82 for Axis 1 and 0.69 for Axis 2). The ordination of environmental factors showed that successive eigenvalues of the four Axes (the analysis was carried out on all 22 sites) decreased from 0.693 in Axis 1 to 0.06 in Axis 4, suggesting a well structured data set (Tab. 10).

Table 10: The summary of CCA on plant communities and species including eigenvalues variance and correlations.

	Axis 1	Axis 2	Axis 3	Axis 4	Total
Eigenvalue	0.693	0.204	0.133	0.06	1.09
Variance explained %	42.58	41.49			84.07
Person correlation between species and environment	0.82	0.69	0.53	0.49	
Depth	0.73	0.14	0.08	0.05	
Water ammonia	0.62	0.64	0.13	0.03	
Hydrosoil organic matter	0.54	0.23	0.21	-0.05	
Water phosphate	-0.57	0.14	-0.25	0.04	

The high eigenvalue of 0.693 in Axis 1 indicates that most of the data were explained through this Axis and its influence was three times higher than Axis 2 influence. Axis 1 was correlated with water depth, water ammonia, hydrosoil organic matter and water phosphate, recording correlation values of 0.82, 0.73, 0.62, 0.54 and -0.57, respectively (Tab. 10). The species-environmental variables biplot (Fig. 7) shows the relations of the species and the environmental variables with the ordination axes. The ordination diagram of CCA displays sites, species and environmental variables. The position of species and sites (represented by points) in the ordination shows the variation in species composition over sites and their distribution over the gradient of environmental variable. The environmental variables are represented by arrows which points into the direction of maximum change across the diagram and their length is proportional to the rate of change in this direction. The CCA ordination diagram suggested that water depth, water ammonia and hydrosoil organic matters were the environmental variable that significantly affect the distribution of plant species in Allaqi. Shallow water with high light penetration is the favorable condition for *Najas horrida* growth. According to CCA diagram, *Najas horrida* was very sensitive to high nutrients contents in both water and hydrosoil particularly water phosphate, ammonia and nitrate. *Najas marina* was more tolerant to nutrients contents than *Najas horrida* but the well aerated water seemed to be essential factor for its growth. Ranking the three species of *Najas* according to their ability to survive in reverse conditions (represented by high nutrients, low under water light and low oxygen content) showed that *Najas horrida* is the most sensitive species, highly affected by un-normal conditions (mainly high total dissolved salts) followed by *Najas marina,* which is more adapted with such conditions and able to grow in deeper water than *Najas horrida*. *Najas minor* is the species most capable to flourish in unfavorable conditions, but deep water is the factor that may limit its development. *Myriophyllum spicatum* is the macrophytic species which shows maximum ability to survive in low light conditions and eutrophicated water (high concentrations of ammonia, nitrate and phosphate in both water and hydrosoil). In contrast, *Potamogeton schweinfurthii* was so sensitive to high nutrients contents.

Table 11: Fishing patterns in khore Allaqi (H. W. L. - high water level; L. W. L. - low water level).

<table>
<tr><th></th><th>J</th><th>F</th><th>M</th><th>A</th><th>M</th><th>J</th><th>J</th><th>A</th><th>S</th><th>O</th><th>N</th><th>D</th></tr>
<tr><td>Deep water fishing</td><td colspan="2">H. W. L.</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td colspan="3">H. W. L.</td></tr>
<tr><td>Shallow water fishing</td><td></td><td></td><td></td><td></td><td colspan="3">L. W. L.</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Low fishing activity</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td colspan="2">L. W. L.</td><td></td><td></td><td></td></tr>
<tr><td>No fishing (eggs laying period)</td><td></td><td></td><td colspan="2">H. W. L.</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
</table>

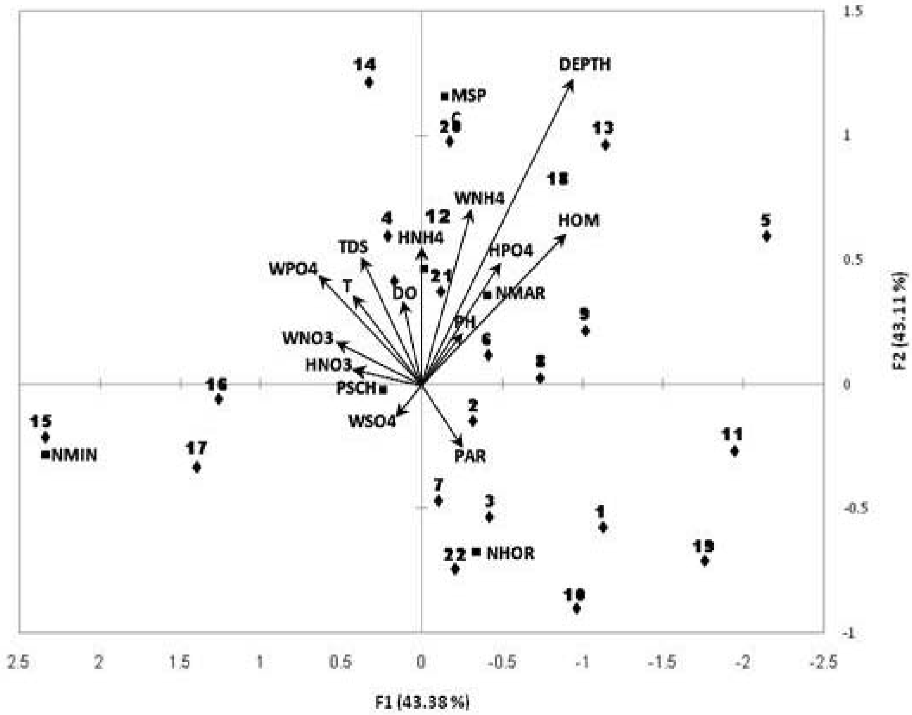

Figure 7: CCA biplot ordinates diagram of 14 environmental represented by arrows: PAR, Photosynthetic Active Radiation; HOM, Hydrosoil organic matter; pH, Water pH; HPO4, Hydrosoil phosphate; DEPTH, Water depth; WNH4, Water ammonia; HNH4, Hydrosoil ammonia; DO, Dissolved oxygen; TDS, Total Dissolved salts; T, Water temperature; WPO4, Water phosphate; WNH3, Water nitrate; HNO3, Hydrosoil nitrate and WSO4, Water sulphate. Five macrophytes species: NHOR, *Najas horrida*; NMAR, *Najas marina*; NMIN, *Najas minor*; MSP, *Myriophyllum spicatum* and PSCH; *Potamogeton schweinfurthii* and 22 sites represented by points arranged by numbers.

DISCUSSIONS

Plant density and species number in macrophytes communities in khore Allaqi were higher on the eastern shores compared with the western ones. The difference in macrophytes assemblage between the two shores is mainly associated with the natural and physical features of the shores. The small embayments distributed along the eastern shores form sheltered habitats and create favourable conditions for the growth of macrophytes (Duarte and Kalff, 1990; Gasith and Gafny, 1990; Gafny and Gasith, 1993). In contrast, most of the western shores are opened rocky bed areas with deep water, forming conditions that reversely affect the aquatic plants. The nature of the shores is not the only factor that causes the spatial variation of aquatic macrophytes, and the distribution pattern of *Najas* spp. in relation to the environmental conditions in area provides evidence to the role of other factors in this process. The spatial variation between *Najas* species was very clear in Allaqi, *Najas horrida* dominating the southern areas: Raas El-Maiaa, Wadi Quleib, Roud Abu Hamboul and Roud Sabkhaia. This could be because of good habitat conditions for *Najas horrida* including: shallow water (Stelzer et al., 2005; Torn et al., 2006), low water velocity (Birket et al., 2007) and low nutrients input (Horppila and Nurminer, 2005). *Najas marina* was found to be more resistant to reverse conditions created due to human impacts as shores cultivation and fishing including high nutrient inputs and low underwater light, this explaining its dominance in northern areas of khore Allaqi. These findings agree with Agami et al. (1980), who stated that *Najas marina* was not affected by light below as 60 µEm/1s (at water depths over 100 cm), explained by carbohydrate reserve usage for growth, as opposed to photosynthesis dependence. The light independent case may extend for months and, as soon as the reserves were depleted, the plant starts to lose weight and the present of light will be essential. *Najas minor* was previously identified in Lake Nasser (Ali et al., 1992) but it was first collected and identified in Allaqi, making this species a new record in the area. According to field results, *Najas minor* was detected only in Jebel Abu Seif at relatively high nitrate and ammonia contents.

The temporal variation between the sampling months was also obvious: macrophytes flourish in July and remarkably reduce in the other sampling months. The occasional floods disturbance occurred in September, destroying the vegetation succession and leading to decline of the aquatic macrophytes density and diversity, while in July the water conditions are more stable and favourable for plant development. White Pelican, Cormorants, White Stork and Flamingo, migratory water birds, are one of the causes of aquatic plants decline. The migratory birds are fairly common visitors to Allaqi, usually coming from eastern and southern Europe (Baha El-Din et al., 1989; Svensson and Grant, 1999), in dense flocks and resting at the shores of Allaqi from November till April. Although previous studies indicated the positive role of water birds on the aquatic habitat e.g. their role for dispersal of plants propagules (Green et al., 2002), rising water productivity (Little, 1979); water birds, particularly large ones (White Pelican and Flamingo), caused serious mechanical damage (by cutting during their movements, feeding and grazing) and dispersion of the bottom sediment, increasing the water turbidity. Our results support those of Agami and Waisel (1984), who found that *Najas marina* is eaten and exposed to damage by water birds, such as *Gallinala chloropus* Linnaeus 1758 and *Fulica atra* Linnaeus 1758, causing a serious reduction on plant growth, explaining minimum macrophytes from May density compared to other sampling months. On the other side, migratory birds may have a positive role that grazing of *Najas* enhance the seeds germination (following exposure to bird's digestive system) supporting the high coverage from July. Such results are not exclusive related to seeds of aquatic macrophytes, but also to the ones of terrestrial plants (Koller and Cohen, 1959).

Growth and distribution of *Najas* species in khore Allaqi are influenced by natural and anthropogenic environmental conditions. The natural ones include dense growth of epiphytes, invasion of other plants as *Myriophyllim spicatum*, and the impact of water birds. The study showed that the dense growth of epiphytes reduced the photosynthesis ability of the plants and caused a decline in macrophytes (Fig. 5). Its inhibition becomes severe in areas with turbid water and near the bottom, where the light intensity is already low. The shading effect was larger in shallow water, particularly when the epiphytes are present in large part of the water surface (McRoy and Goering, 1974; Mathiesen and Mathiesen, 1976; Sand-Jensen, 1977; Malderij, 2006). The detrimental role of epiphyton is not caused only by competition for light, but also for nutrients (Jones and Sayer, 2003) and by carbon dioxide at the surface of the leaves; the epiphytes may generate high day time pH and dissolved oxygen adjacent to the leaf surface, thereby exacerbating the inorganic carbon depletion and elevated dissolved oxygen values, adding to those already created by macrophytes through photosynthesis (Jones et al., 2000). Hilt and Gross (2008), show that *Najas marina* is among the ten most common macrophytes species in Germany that it is able to suppress the epiphytes and phytoplankton growth through production and release of allelopathically active substances, this result is in contradiction with our results. The inconsistent results may return to variation in the nature between the lakes in Germany and the Lake Nasser, which effect in return the epiphyte-macrophytes interaction mechanisms. The two areas may be varied in light availability, nutrients contents, water temperature, the species of macrophytes and epiphytes … etc., consequently the variation will take place in types and quantities of allelopathic substances and the sensitivity of algae toward these materials. In fact, the potential role of allelopathy by macrophytes on the algae in the field is still unclear and controversial (Forsberg et al., 1990; Van Donk and Van de Bund, 2002).

Although *Najas horrida* was the dominant species in Allaqi, *Myriophyllum spicatum* was the most frequent one, being detected in 73.4% of the surveyed sites. *Myriophyllum* dominates the sites from northern Allaqi, creeping towards to south. This seemed to be logic since *Myriophyllum* is known to adapt better on disturbed conditions (Adams et al., 1974; Chambers and Kalff, 1985; Scheffer et al., 1992) that characterizes those areas. There is an entrance on the north on khore Allaqi (Fig. 1), the area being characterized by deep water with high velocity, conditions restricting the development of most aquatic macrophytes species. The adaptation of macrophytes towards the depth and wave exposure is usually related to their morphological plasticity response, and, in such circumstances, *Myriophyllum* tends to produce taller plants, with fewer and longer stems, to stretch towards the water surface and take benefit of the light (Fig. 6). *Myriophyllum* followed the same reaction in shallow sheltered areas with dense growth of periphyton, to maximize its photosynthesis process (Tobiessen and Snow, 1984; Standard and Weisner, 2001). The anthropogenic impacts caused a significant variation in water conditions, limiting the growth of many species and providing the opportunity for *Myriophyllum spicatum* to compete with native species. This conclusion is in consistence with Agami and Waisel (1985, 2002), who found that the competitive relationship between *Myriophyllum spicatum* and *Najas marina* developed as a result of resources exploitation and environment deterioration, and the competition between the two species is usually in the benefit of *Myriophyllum*, causing the reduction of *Najas marina*. *Myriophyllum* showed a higher flexibility toward unfavourable conditions, making the plant more qualified to survive in such conditions than *Najas* spp. the leave forms and lacunae of *Myriophyllum spicatum* provide low resistance to oxygen diffusion between leaves and make the plant able to benefit of the low oxygen contents (Westlake, 1978; Laskov et al., 2006).

The extensive agriculture from the shores of Abu-Zeiraa and Turgumi alter the soil chemistry due to chemicals (fertilizers and pesticides) that spreaded on the fields and found its way to water, causing nutrient enrichment. The study results indicated high levels of ammonia and sulphur (used in tomato fields to make the crops reddish) in the water at cultivated shores, limiting the growth of most species from Allaqi. Anderson and Kalf (1986) confirmed the present results, indicating the great productive potential in N-media of *Myriophyllum*; Laskov et al. (2006) and Ali and Sotan (2006) also indicated that *Myriophyllum* showed higher production in water with nitrogen compounds and high organic matter in sediment. CCA output confirmed that *Myriophyllum spicatum* can be an indicator for eutrophicated conditions represented by high concentrations of ammonia, nitrate and phosphate in both water and hydrosoil, while *Najas horrida* was very sensitive to high nutrients and can be an indicator for oligotrophic waters. Although *Najas marina* and *Najas minor* were more tolerant to such conditions compared with *Najas horrida*, their adaptability to deep and eutrophicated waters was lower than that of *Myriophyllum spicatum*.

Fishing is another form of human activity that creates a destructive impact on macrophytes from Allaqi. According to the records of Lake Nasser Development Authorty (LNDA) and Fishers NGO in Aswan, total number of boats in Allaqi is over 200 (133 motor boats and 78 rowing ones) a number considered high according to the size of khore Allaqi. Fishing in Allaqi has three patterns, based on season and associated conditions, such as water level and air temperature.

My study concerns with shallow water fishing (*Najas* spp. grew in shallow water) which extends from May to July. The results showed a low growth of *Najas* spp. in May due to boat impacts: direct damage from hulls and propellers and strong water movements. Boating also affects the aquatic habitat by elevated turbidity reducing light availability and uproots plants (Murphy and Eaton, 1983; Anthony and Downing, 2003; Eriksson et al., 2004). Fishers also manually harvest the plants in shallow waters, in order to make paths for their boats, preventing plant interlace with boats propellers; this activity puts in critical situation the *Najas* species in many areas from Allaqi.

Accordingly, one can suggest that Allaqi is exposed to constrains, mainly from anthropogenic disturbance, which caused a decline in density and diversity, not only to *Najas* spp., but also to other macrophytes species. The previous study of Ali et al. (1992) in Lake Nasser, indicated four plant species in Turgumi: *Najas marina*, *Najas horrida*, *Nitella hyalina* and *Zanichellia palustris*; in my study, small communities dominated by *Myriophyllum spicatum* were detected in Turgumi, and a very low growth of *Najas marina* and *Najas horrida*. The two other species, *Nitella hyalina* and *Zanichellia palustris* were not found in khore Allaqi. The field survey indicated that the Bedouins settlements areas (mainly Raas El-Maiaa, Roud Abu Hamboul and Quleib) showed high growth of *Najas* spp. (in some areas, the cover of *Najas* spp. reached to 98%), insuring that *Najas* harvesting by local communities for fodder does not impact the growth of plant, and that the Bedouins have the knowledge and experience to manage their resources.

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EFFECTS OF TEMPERATURE AND SALINITY ON THE LARVAE OF TWO SUBTIDAL NASSARIID GASTROPODS, *NASSARIUS SIQUIJORENSIS* AND *NASSARIUS CREMATUS* (GASTROPODA, NASSARIIDAE)

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ABSTRACT

Owing to degradation of seabed communities by environmental stresses and human perturbations, species which have specialized diets in the local marine benthic community are gradually replaced by opportunistic species of which a number of them are scavengers. This study investigated how two major environmental factors, temperature and salinity, affected the survival, behaviour and physiology of the larvae of two dominant scavenging gastropods, *Nassarius siquijorensis* and *Nassarius crematus*. Larval mortality at six temperatures (10, 15, 20, 25, 30 and 35ºC) and seven salinities (0, 5, 10, 15, 20, 25 and 30‰) were observed after 24 hours and 48 hours. Higher mortality was observed for both *Nassarius siquijorensis* and *Nassarius crematus* when the salinity was < 10‰ or temperature < 15ºC. Respiration rate, swimming velocity, and clearance rate of the larvae of *Nassarius siquijorensis* and *Nassarius crematus* were measured at combinations of three temperatures (20, 25, and 30ºC) and three salinities (20, 25, 30‰). Temperature, salinity and their interactions were significant in affecting oxygen consumption, clearance rate and swimming behaviour of both species. Significantly lower algal consumption and oxygen consumption rates were observed at lower salinities and temperatures, and the dispersal distance VSL was significantly higher at lower salinities. The results may provide insight into how the scavenging community in local waters is structured by natural stresses, and clues towards understanding similar communities in the subtropical region.

ZUSAMMENFASSUNG: Auswirkungen von Temperatur und Salzgehalt auf die im Subtidal lebenden Larven von *Nassarius siquijorensis* und *Nassarius crematus* (Gasteropoda, Nassaridae).

Infolge des Abbaus der Lebensgemeinschaften auf dem Meeresboden im subtidalen Bereich durch Umweltstress und menschliche Störungen, werden Arten mit spezialisierter Ernährungsweise in den marinen benthischen Gemeinschaften nach und nach durch Arten mit einem breiteren Ernährungsspektrum ersetzt, wobei es sich teils um Aasfresser handelt. Die vorliegende Untersuchung befasst sich mit der Frage, wie zwei wichtige Umweltfaktoren, Temperatur und Salzgehalt das Überleben, Verhalten und die Physiologie der Larven zweier dominanter Aasfresser-Schnecken *Nassarius siquijorensis* und *Nassarius crematus* beeinflussen. Dabei wurde die Sterberate der Larven bei sechs verschiedenen Temperaturen (10, 15, 20, 25, 30 und 35°C) und sieben Salinitätsstufen (0, 5, 10, 15, 20, 25 und 30‰) nach 24 und 48 Stunden untersucht. Höhere Mortalität wurde für beide Arten *Nassarius siquijorensis* und *N. crematus* bei einem Salzgehalt von < 10‰ oder einer Temperatur von < 15ºC festgestellt. Atmungsrate, Schwimmgeschwindigkeit sowie Filtrierrate der Larven von *Nassarius siquijorensis* und *N. crematus* wurde bei Kombinationen von drei Temperaturwerten (20, 25 und 30°C) und drei Salinitätswerten (20, 25, 30‰) gemessen. Temperatur, Salzgehalt und deren Wechselwirkung sind signifikant in ihrer Auswirkung auf Sauerstoffverbrauch, Filtrierrate und Schwimmverhalten beider Arten. Signifikant geringere Algenkonsum- und Sauerstoffverbrauchsraten wurden bei niedrigerem Salzgehalt und niedrigeren Temperaturen beobachtet. Die Dispersionsdistanz VSL war signifikant höher bei geringerem Salzgehalt. Die Ergebnisse gewähren einen Einblick darüber, wie die Aasfressergemeinschaft in lokalen Gewässern bei natürlichem Stress strukturiert ist und führen zu einem Verständnis ähnlicher Gemeinschaften in der subtropischen Region.

REZUMAT: Efectele temperaturii şi salinității asupra larvelor gastropodelor *Nassarius siquijorensis* şi *Nassarius crematus* (Gasteropoda, Nassaridae) din zona subtidală.

În urma degradării biocenozelor de pe fu1234ndul mării, în zona subtidală neinfluențată de maree prin stres de mediu şi influențe antropice, speciile cu nutriție specializată din comunitățile bentonice sunt treptat înlocuite prin specii oportuniste cu un spectru nutrițional mai larg, fiind vorba şi de unele specii necrofage. Prin investigațiile făcute se urmăreşte răspunsul la întrebarea: în ce măsură doi importanți factori de mediu, temperatura şi salinitatea influențează supraviețuirea, comportamentul şi fiziologia larvelor speciilor de gastropode necrofage, dominante, *Nassarius siquijorensis* şi *Nassarius crematus.* A fost cercetată rata de mortalitate a larvelor la şase temperaturi diferite (10, 15, 20, 25, 30 şi 35°C), precum şi la şapte valori de salinitate (0, 5, 10, 15, 20, 25 şi 30‰) după 24 şi 48 de ore. O rată de mortalitate mai ridicată a fost constatată pentru ambele specii *Nassarius siquijorensis* şi *N. crematus*, la un conținut de sare de < 10‰ sau o temperatură de < 15ºC. Rata de respirație, viteza de înot şi rata de filtrare a larvelor de *Nassarius siquijorensis* şi *N. crematus* a fost măsurată la combinații de trei valori de temperatură (20, 25 şi 30°C) şi trei valori de salinitate (20, 25, 30‰). Temperatura, conținutul în sare şi interacțiunea lor sunt semnificative în efectul lor asupra consumului de oxigen, a ratei de filtrare şi a comportamentului de înot al ambelor specii. Rate semnificativ mai mici ale consumului de alge şi de oxigen au fost observate la o salinitate mai scăzută şi temperaturi mai joase. Distanța de dispersie VSL a fost semnificativ mai ridicată la o salinitate mai joasă. Rezultatele permit o înțelegere a structurii comunităților de necrofage în ape locale, la stres natural, ducând şi la înțelegerea unor cenoze asemănătoare în condițiile regiunii subtropicale.

INTRODUCTION

Studies of the benthic community in Hong Kong were initiated in late 1970s, although most of these studies were confined to certain areas such as Tolo Harbour, Mirs Bay and southern waters. Results showed that the trophic structure of the benthic community dominated once by specialists is gradually replaced by generalists of which most of the members adopt a scavenging mode of life. The most representative members of these generalists are gastropod species from the family Nassariidae with seven species being recorded from local subtidal waters, including *Nassarius siquijorensis*, *Nassarius acuminatus*, *Nassarius crematus*, *Nassarius livescens*, *Nassarius succinctus*, *Nassarius pauperus* and *Nassarius reevenanus.* As degradation of seabed communities caused by environmental stress and human perturbations is wide-spread in the Asia-Pacific region, the scavenging community in local waters is facing many ecological problems over the period of their lives. In a most recent study in Hong Kong waters, only two dominant subtidal nassariid gastropods were found with *N. siquijorensis* being the most dominant one and occurred in all the study sites and at high abundance. In contrast, *N. crematus* was found only in southern waters and the abundance was low.

Temperature and salinity of seawater are two primary factors that affect the behaviour, physiology, morphology and life history traits of marine organisms. Active metabolism usually increases at higher temperatures within the normal physiological range of the animal. Salinity is regarded as an important factor limiting the penetration of species into estuaries. For euryhaline osmoconformers, respiration is independent of salinity fluctuations but it is increased in osmoregulators as additional energy is required for osmotic adjustment.

Investigating only the changes of a single factor (e. g. temperature or salinity) on metabolism may lead to erroneous conclusions about the importance of that factor on metabolism of organisms. Besides, metabolism also varies with the time the organisms were exposed to changes in these environmental variables. Cheung and Lam showed that changes in temperature and salinity as well as their interactions were found to be significant in affecting the respiration of *Nassarius festivus* both before and after acclimation. Among these factors, salinity has the greatest effect, and respiration was found to increase with salinity at all temperatures no matter the animals were acclimated or not. Following longer exposure to changed temperature and salinity conditions lasting days or weeks, the organisms may show adjustment of their rate functions which is referred to as acclimation for laboratory conditions. Like other biological rates, larval swimming speed changes as a function of temperature or salinity. Increase in temperature increases biochemical reaction rate and reduces water viscosity, hence increasing swimming speed of larvae.

This paper investigated the effects of temperature and salinity on the larvae of two subtidal nassariid gastropods, *Nassarius siquijorensis* and *Nassarius crematus*. The results may provide insight into how the scavenging community in local waters is structured by natural stresses, and clues towards understanding similar communities in the subtropical region.

MATERIAL AND METHODS

Collection and culturing of animals

In July and August, 2008, individuals of *N. siquijorensis* (shell length: 19 - 30 mm) were collected from Mirs Bay in the northeast of Hong Kong and from southern waters while *N. crematus* (shell length: 15 - 26 mm) were collected from southern waters of Hong Kong. In the laboratory, each species of gastropods were kept in separate fibre-glass tanks (500 l) equipped with a filtering system and air supply. The seawater was maintained at 24ºC by thermostatic heaters and at constant salinity (30‰). The water for keeping the animals was renewed every four days to avoid accumulation of metabolic wastes. Gastropods were fed with the shrimp *Metapenaeus ensis* once at every three days. Dead individuals were removed once encountered and water was changed.

Egg capsules laid on the sides of the aquaria were removed and reared in plastic aquaria containing well-aerated 0.2 um filtered seawater maintained at 24ºC and 30‰. Larvae hatched within 24 hrs were transferred with a wide bore pipette to a 1.3 l container and fed with the diatom *Thalassiosira pseudonana* Hasle and Heimdal (Guillard's clone 3H) at a concentration of 20 x 10^4 cells ml^{-1} everyday. Larvae were reared with filtered seawater at 24ºC and 30‰ with antibiotics added (50 ug ml^{-1} streptomycin sulphate and 50 ug ml^{-1} penicillin-g sodium cell). Food, water and antibiotics were replaced daily.

Experimental design

Temperature tolerance

Healthy larvae hatched for three days were chosen randomly and ten individuals were placed in a container with well-aerated seawater (30‰) kept at one of the six temperatures (10, 15, 20, 25, 30 and 35ºC) maintained by electrical heaters or chillers. Three containers were prepared for each temperature as replicates. Air stones were placed in the containers to improve water circulation so that an uniform temperature could be achieved. Larvae with ciliated movement of velum stopped 30 min after returning to normoxia were considered dead and removed once observed. Observation on mortality was performed twice at 24 hrs and 48 hrs and dead individuals were removed at 24 hrs.

Salinity tolerance

Healthy larvae hatched for three days were chosen randomly and 10 individuals were placed in a container with well-aerated seawater kept at one of the seven salinities (0, 5, 10, 15, 20, 25 and 30‰) and at ambient temperature (20ºC). Three containers were prepared for each salinity as replicates. The seawater was diluted by deionized water. Observation on mortality was performed twice at 24 hrs and 48 hrs and dead individuals were removed at 24 hrs.

Combined effects of temperature and salinity on clearance rate, respiration and swimming behavior

This study was a 3 x 3 factorial design with three levels of temperature (20, 25 and 30ºC) and three levels of salinity (20, 25 and 30‰). The temperature and salinity ranges chosen for the study were based on the results of temperature and salinity tolerance obtained in the first part of the experiment. Each experimental temperature (+ 0.5ºC) was maintained by an electric heater and each test of salinity was prepared by diluting seawater with deionised water with the aid of a refractometer.

Determination of algal consumption

Clearance rate (CR) of each individual was determined using the static method. Eighty larvae were maintained in a container with the filtered seawater containing monoculture of the unicellular alga *Thalassiosira pseudonana* Hasle and Heimdal (Guillard's clone 3H) at a predetermined concentration of 20 x 10^4 cells ml^{-1}. Three containers were prepared for each combination of temperature and salinity as replicates. The concentration of algae was measured at the beginning and at one hr intervals for four hrs. The average clearance rate of each larva was calculated according to Coughlan. All the containers were covered with dark cloth to limit the growth of algae.

Determination of respiration rate

Twenty healthy individuals were randomly chosen from each tank and put in a 1.65 ml sealed container, and the respiration rate was determined by measuring the amount of oxygen consumed in about 30 minutes using a stable optical oxygen instrument, model SOO-100. The decrease in oxygen level was < 30% of full saturation to prevent reduction in respiration due to reduced oxygen levels in the container. Corrections for control were determined by measuring oxygen changes in containers without animals inside. Each combination of temperature and salinity has three controls and three replicates.

Determination of swimming velocity

The swimming behaviour of all larvae after them were exposed to nine treatments of temperature and salinity for 48 hours was recorded and analysed using the CRISMAS sperm motility analysis system (Image House A/S, Denmark). Eight larvae from each treatment were transferred to separate covered Petri dishes. Swimming pathway of each larva was recorded using a CCD camera (JAI CV-M10RS) attached to a Carl-Zeiss Stemi SV11 microscope at 60 x magnification. Image of debris was filtered from the video analysis by GIPS object feature analysis provided by the CRISMAS system. Swimming pathway of each larva was recorded for 2.5 s and divided into 25 frames s^{-1} using the CRISMAS. Three motion parameters of individual larvae were assessed: (1) the curvilinear velocity ($\mu m.s^{-1}$) (VCL), the time average velocity of larva along its actual trajectory which can be used to assess the actual larval swimming velocity; (2) the straight line velocity ($\mu m.s^{-1}$) (VSL), the time average velocity of larva along a straight line between its first detected position and its last position. VSL can be used to assess dispersal distance of a larva since it indicates horizontal distance travelled by the larva; and (3) the average path velocity ($\mu m.s^{-1}$) (VAP), the distance was calculated by adding straight line values between every two frames.

RESULTS

Temperature tolerance

Both larvae of *N. siquijorensis* (one-way ANOVA, df = 5.12, F = 99.199, $P < 0.001$) and *N. crematus* (one-way ANOVA, df = 5.12, F = 122.643, $P < 0.001$) had highest mortalities at 10ºC after 48 hrs (Fig. 1a, b). The larval mortality of *N. siquijorensis* was significantly lower than *N. crematus* at 25ºC, 30ºC and 35ºC (t-test, $p < 0.05$).

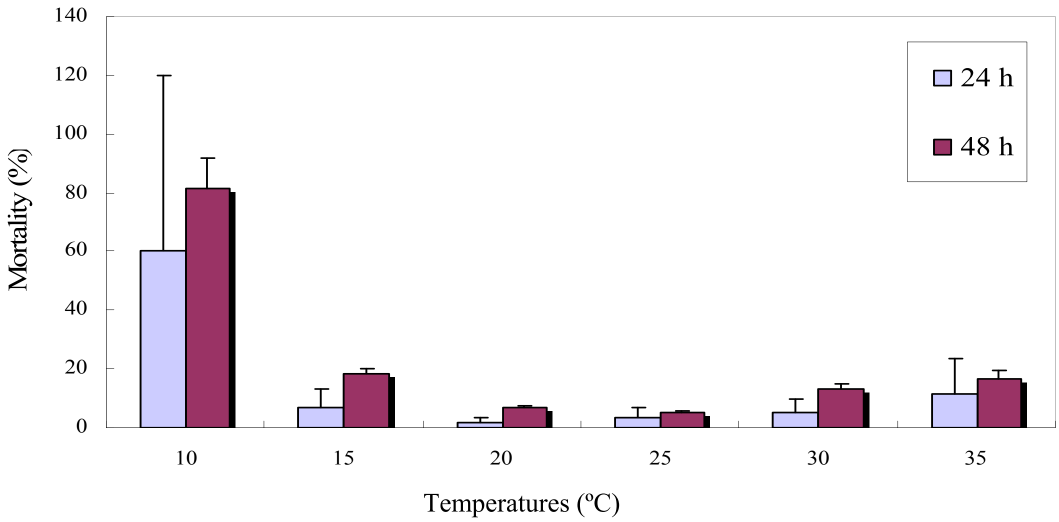

Figure 1a: Cumulative percentage mortality of the larvae after being exposed to six temperatures (10, 15, 20, 25, 30 and 35ºC) for 24 hrs and 48 hrs. (a) *N. siquijorensis* (mean + SD; n = 3).

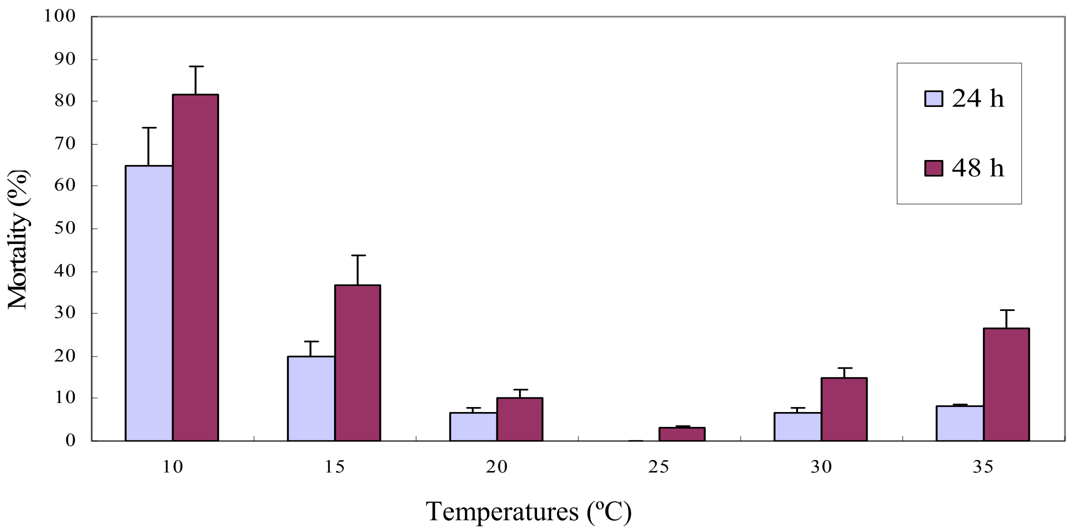

Figure 1b: Cumulative percentage mortality of the larvae after being exposed to six temperatures (10, 15, 20, 25, 30 and 35ºC) for 24 hrs and 48 hrs. (b) *N. crematus* (mean + SD; n = 3).

Salinity tolerance

Both *N. siquijorensis* (one-way ANOVA, df = 6.14, F = 642.475, P < 0.001) and *N. crematus* (one-way ANOVA, df = 6.14, F = 548.498, P < 0.001) had highest larval mortalities at 5‰ and 10‰ after 48 hrs (Fig. 2a, b). The larvae mortality of *N. siquijorensis* was significant lower than *N. crematus* at 10‰ (t-test, p < 0.05).

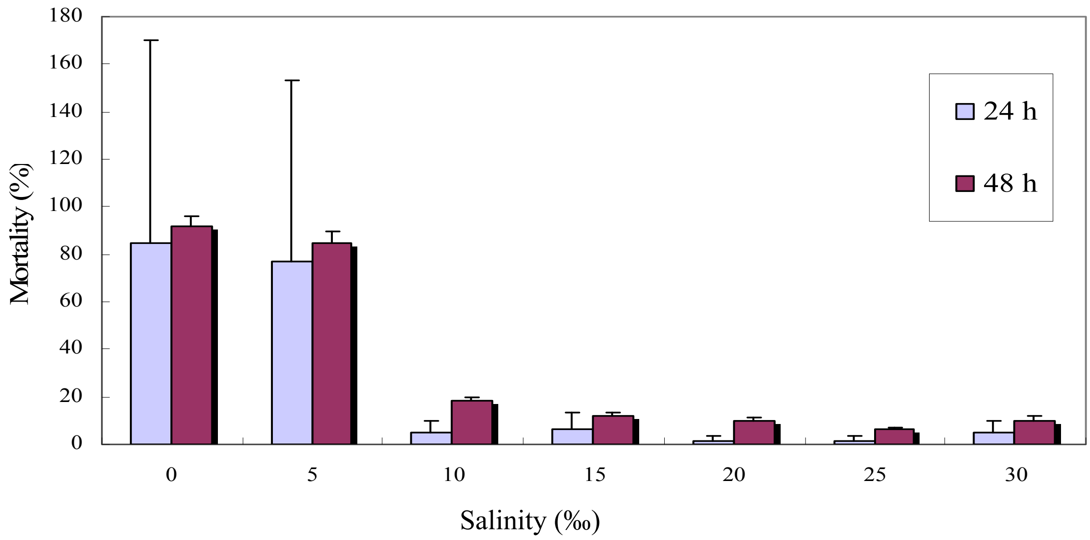

Figure 2a: Cumulative percentage mortality of the larvae after being exposed to six salinities (0, 5, 10, 15, 20, 25 and 30‰) for 24 hrs and 48 hrs. (a) *N. siquijorensis* (mean + SD; n = 3).

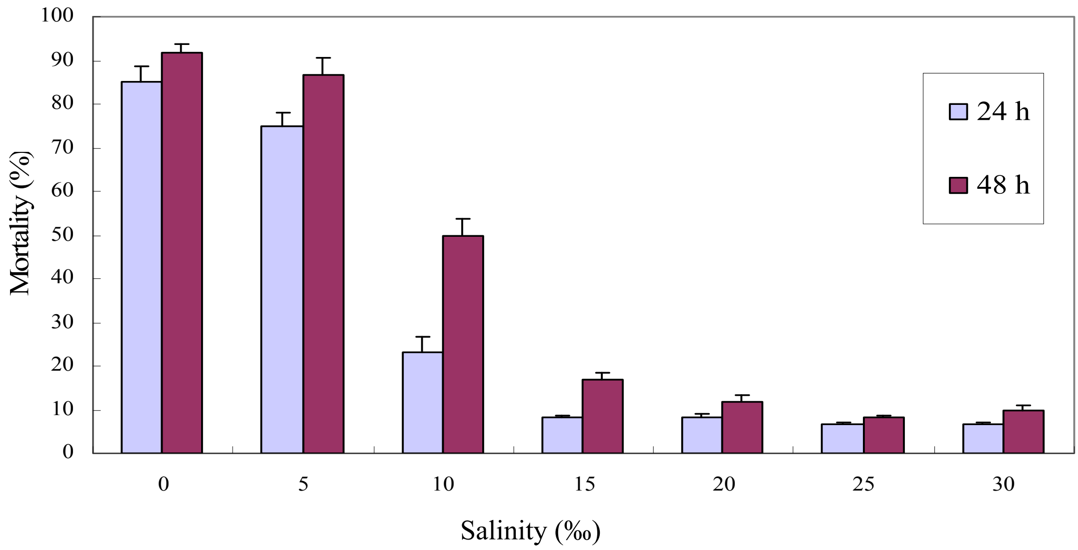

Figure 2b: Cumulative percentage mortality of the larvae after being exposed to six salinities (0, 5, 10, 15, 20, 25 and 30‰) for 24 hrs and 48 hrs. (b) *N. crematus* (mean + SD; n = 3).

Combined effects of temperature and salinity on larval clearance rate

The highest larval clearance rate was found at 30ºC and 30‰ for both *N. siquijorensis* and *N. crematus* (Fig. 3a, b). Results of two-way ANOVA showed that changes in temperature and salinity as well as their interactions were significant in affecting algal consumption rate of *N. siquijorensis*. The larval clearance rate increased significantly from 20ºC to 30ºC (20ºC < 25ºC < 30ºC) (Tab. 1). For *N. crematus*, only temperature has a significant effect on the clearance rate with the rate at 30ºC being higher than that at 20ºC. No significant differences were obtained between 20ºC and 25ºC and between 25ºC and 30ºC (Tab. 1).

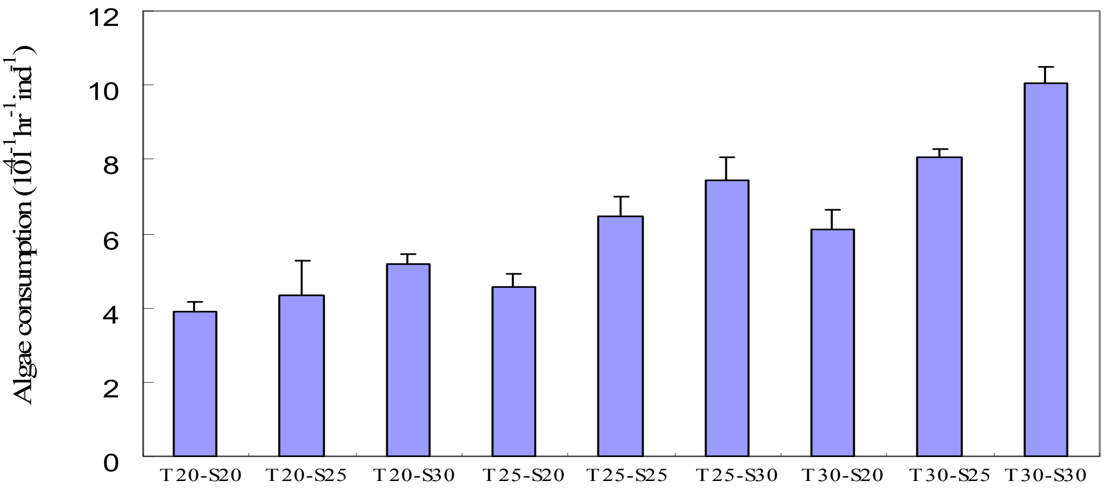

Figure 3a: Larval clearance rate at nine combinations of temperature (T: 20, 25 and 30ºC) and salinity (S: 20, 25 and 30‰) (mean + SD; n = 3) (a) *N. siquijorensis*.

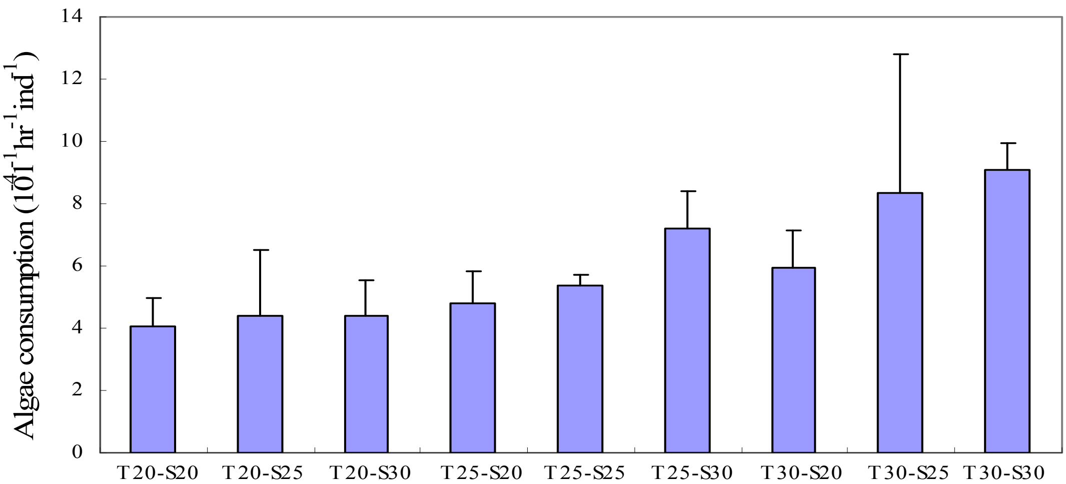

Figure 3b: Larval clearance rate at nine combinations of temperature (T: 20, 25 and 30ºC) and salinity (S: 20, 25 and 30‰) (mean + SD; n = 3); (b) *N. crematus.*

Combined effects of temperature and salinity on larval oxygen consumption

Respiration rate increased with salinity at all temperatures for both *N. siquijorensis* and *N. crematus* (Tab. 2). For *N. siquijorensis*, both temperature and salinity had significant effects on larval respiration rate. The respiration rate was significantly higher at 30ºC than at 20ºC and 25ºC. No significant difference in respiration rate was found between 20ºC and 25ºC (Fig. 4a).

For *N. crematus*, oxygen consumption rate was significantly higher at 30‰ than at 20‰ and 25‰, but no significant difference was found between 20‰ and 25‰. The effect of temperature on respiration rate was not significant for *N. crematus* (Fig. 4b).

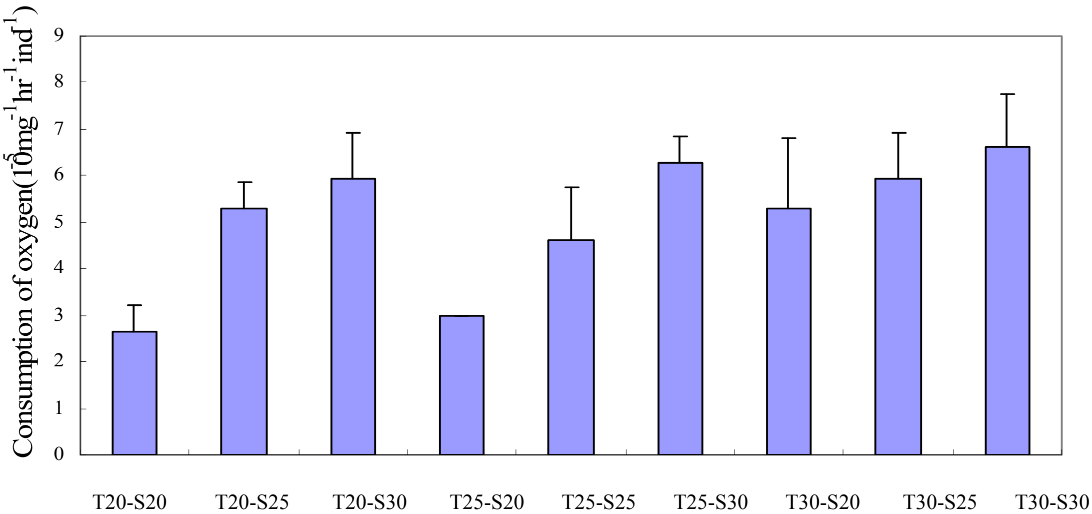

Figure 4a: Larval respiration rate at nine combinations of temperature (T: 20, 25 and 30ºC) and salinity (S: 20, 25 and 30‰) (mean + SD; n = 3) (a) *N. siquijorensis*.

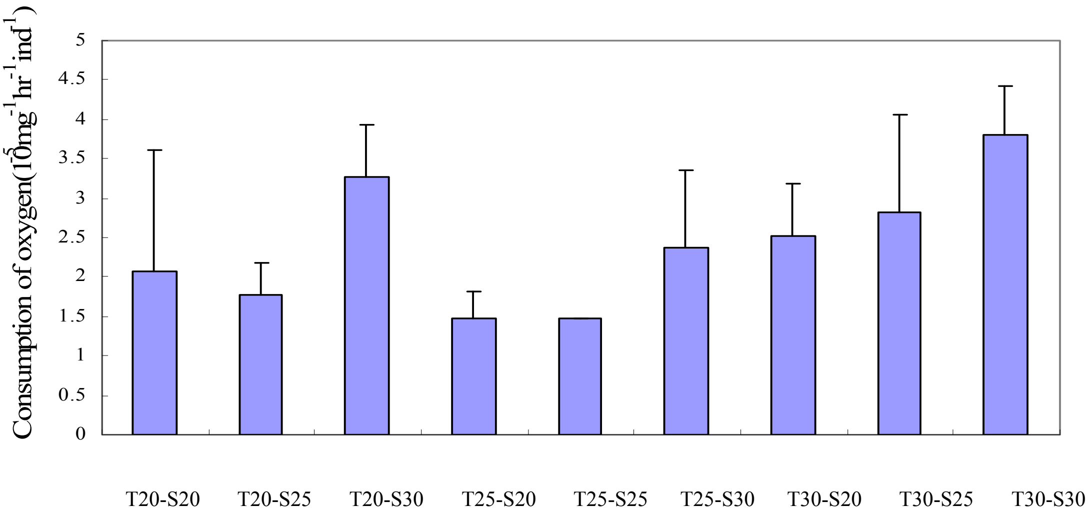

Figure 4b: Larval respiration rate at nine combinations of temperature (T: 20, 25 and 30ºC) and salinity (S: 20, 25 and 30‰) (mean + SD; n = 3) (b) *N. crematus*.

Table 1: Results of two-way ANOVA of larval clearance rate of (a) *N. siquijorensis* and (b) *N. crematus* under nine combinations of temperature and salinity.

Source of variation	Sum of squares	*df*	Mean square	*F*	Sig. of *F*
(a) *N. siquijorensis*					
Temperature	58.582	2	29.291	111.799	< 0.001
Salinity	32.848	2	16.424	62.687	< 0.001
Temperature x salinity	5.593	4	1.398	5.337	= 0.005
Error	4.716	18	0.262		
Total	1150.461	27			
(b) *N. crematus*					
Temperature	55.935	2	27.968	8.148	< 0.005
Salinity	17.309	2	8.655	2.522	0.108
Temperature x salinity	8.642	4	2.160	0.629	0.7648
Error	61.781	18	3.432		
Total	1100.393	27			

Table 2: Results of two-way ANOVA of larval oxygen consumption rate of (a) *N. siquijorensis* and (b) *N. crematus* at nine combinations of temperature and salinity.

Source of variation	Sum of squares	*df*	Mean square	F	Sig. of F
(a) *N. siquijorensis*					
Temperature	10.454	2	5.227	6.000	< 0.05
Salinity	32.017	2	16.008	18.375	< 0.001
Temperature x salinity	5.227	4	1.307	1.500	0.244
Error	15.682	18	0.871		
Total	754.677	27			
(b) *N. crematus*					
Temperature	4.484	2	2.242	3.183	0.053
Salinity	9.776	2	4.888	6.939	< 0.005
Temperature x salinity	1.617	4	0.404	0.574	0.683
Error	25.360	36	0.704		
Total	254.703	44			

Combined effects of temperature and salinity on larval swimming velocity

Results of two-way ANOVA showed that the effect of temperature and salinity on VCL (curvilinear velocity) was not significant for both *N. siquijorensis* and *N. crematus* (Fig. 5a, b; Tab. 3). Temperature, salinity and their interaction, however, were found to be significant in affecting VSL for both *N. siquijorensis* and *N. crematus* (Tab. 4).

For *N. siquijorensis*, VSL at 20ºC was significantly higher than at 30ºC, but no significant differences were found between 20ºC and 25ºC and between 25ºC and 30ºC. For *N. crematus*, VSL at 25ºC was significantly higher than at 20ºC and 30ºC, but no significant difference was found between 20ºC and 30ºC (Fig. 5b).

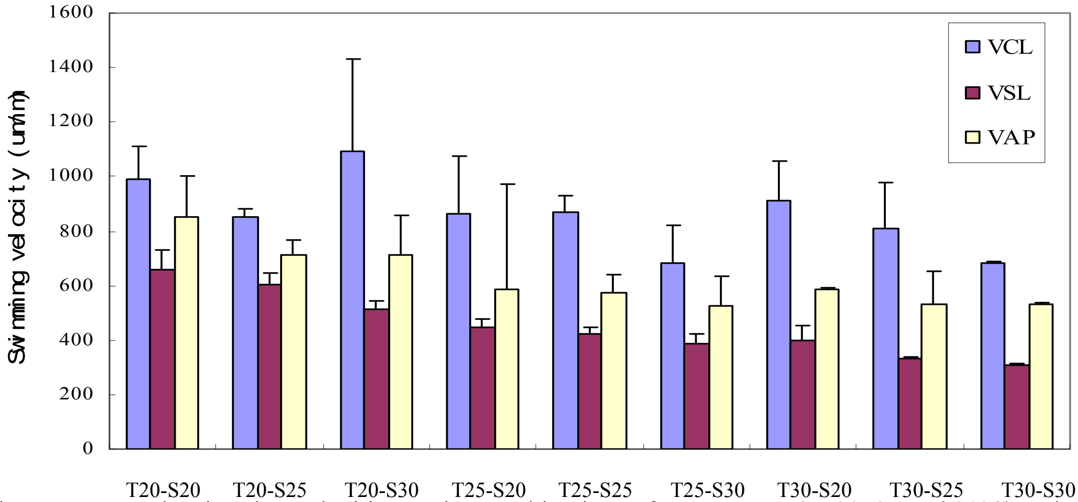

Figure 5a: Larval swimming velocities at nine combinations of temperature (T: 20, 25 and 30ºC) and salinity (S: 20, 25 and 30‰) (mean + SD; n = 3) (a) *N. siquijorensis*. VCL - curvilinear velocity (µm s^{-1}); VSL - straight line velocity (µm s^{-1}); VAP - average path velocity (µm s^{-1}).

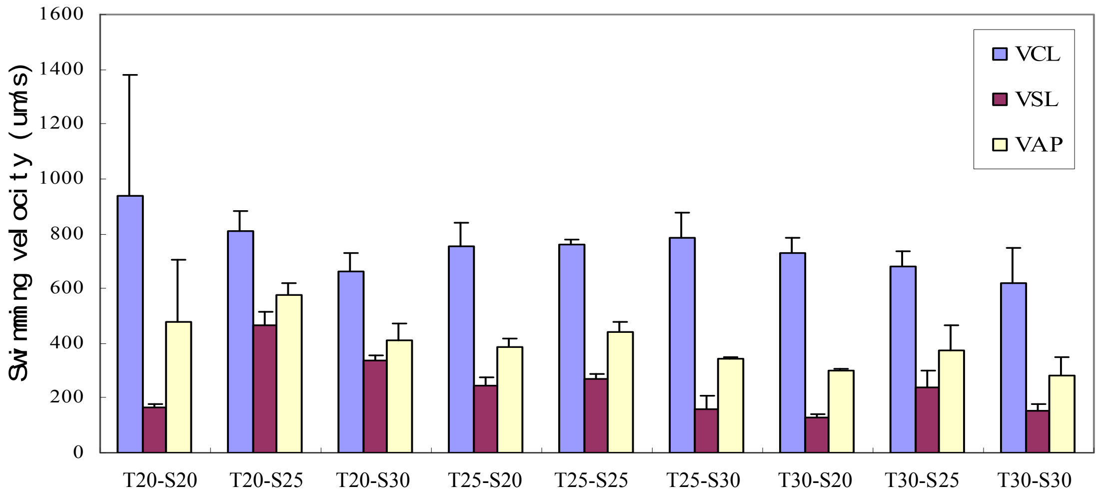

Figure 5b: Larval swimming velocities at nine combinations of temperature (T: 20, 25, and 30ºC) and salinity (S: 20, 25 and 30‰) (mean + SD; n = 3) (b) *N. crematus*. VCL - curvilinear velocity (µm s^{-1}); VSL - straight line velocity (µm s^{-1}); VAP - average path velocity (µm s^{-1}).

For both *N. siquijorensis* and *N. crematus*, VAP increased significantly at lower temperatures (Tab. 5) with values at 20ºC being significantly higher than at 30ºC, but no significant difference was found between 20ºC and 25ºC and between 25ºC and 30ºC for both *N. siquijorensis* and *N. crematus* (Fig. 5a, b).

For *N. crematus*, salinity also affected VAP with the values increased at lower salinities. VAP at 30ºC was significantly higher than at 20ºC and 25ºC, but no significant difference was found between 20ºC and 25ºC (Fig. 5b).

Table 3: Results of two-way ANOVA of VCL of *N. siquijorensis* and *N. crematus* at nine combinations of temperature and salinity.

Source of variation	Sum of squares	*df*	Mean square	F	Sig. of F
(a) *N. siquijorensis*					
Temperature	149883.093	2	74941.546	2.720	0.093
Salinity	25868.367	2	12934.183	0.469	0.633
Temperature x salinity	136114.601	4	34028.650	1.235	0.331
Error	495981.881	18	27554.549		
Total	2.191E7	27			
(b) VCL of *N. crematus*					
Temperature	89565.078	2	44782.539	1.586	0.232
Salinity	73873.234	2	36936.617	1.309	0.295
Temperature x salinity	72089.622	4	18022.405	0.638	0.642
Error	508104.882	18	28228.049		
Total	1.575E7	27			

Table 4: Results of two-way ANOVA of VSL of *N. siquijorensis* and *N. crematus* at nine combinations of temperature and salinity.

Source of variation	Sum of squares	*df*	Mean square	F	Sig. of F
(a) *N. siquijorensis*					
Temperature	277061.228	2	138530.614	91.429	< 0.001
Salinity	28171.129	2	14085.564	9.296	< 0.005
Temperature x salinity	19849.447	4	4962.362	3.275	< 0.05
Error	27273.199	18	1515.178		
Total	6106374.170	27			
(b) *N. crematus*					
Temperature	102095.498	2	51047.749	32.137	< 0.001
Salinity	103740.951	2	51870.576	32.665	< 0.001
Temperature x salinity	70262.925	4	17565.731	11.059	< 0.001
Error	28591.506	18	1588.417		
Total	1867432.723	27			

Table 5: Results of two-way ANOVA of VAP of *N. siquijorensis* and *N. crematus* at nine combinations of temperature and salinity.

Source of variation	Sum of squares	*df*	Mean square	F	Sig. of F
(a) *N. siquijorensis*					
Temperature	206769.032	2	103384.516	4.051	< 0.05
Salinity	122253.003	2	61126.502	2.395	0.120
Temperature x salinity	56030.871	4	14007.718	0.549	0.702
Error	459413.885	18	25522.994		
Total	1.236E7	27			
(b) *N. crematus*					
Temperature	149816.788	2	74908.394	9.710	< 0.001
Salinity	75988.735	2	37994.368	4.925	< 0.05
Temperature x salinity	3345.565	4	836.391	0.108	0.978
Error	138869.124	18	7714.951		
Total	4585800.623	27			

DISCUSSION

This study has demonstrated significant effects of temperature and salinity on survival, feeding, oxygen consumption and swimming velocity of the larvae of *N. siquijorensis* and *N. crematus.*

Both *N. siquijorensis* and *N. crematus* larvae showed higher mortalities at low temperatures and salinities, especially at 10ºC or at 5‰, with more than half of them died after 48 hrs. The larval mortality of *N. siquijorensis* under low temperatures and salinities was significantly lower than *N. crematus*, indicating that *N. siquijorensis* is more tolerant to these environmental stresses. This may help explain why *N. siquijorensis* was found throughout Hong Kong waters with salinity varying from 5‰ to 30‰ whereas *N. crematus* was restricted to southern waters where salinity is generally higher (25.7 to 34.4‰) (Environmental Protection Department, 2007).

The larval clearance rate increased significantly at higher temperatures for both *N. siquijorensis* and *N. crematus*. The rate was also higher at higher salinities for *N. siquijorensis*. Since active metabolism usually increases at higher temperatures within the normal physiological range of the animal, feeding activities should behave similarly. Increased ingestion was recorded as temperature increased resulting in higher growth throughout larval development of Pacific oyster larvae, *Crassostrea gigas*. Similar observations were reported upon by Beiras and Perez-Camacho on *Ruditapes decussatus* larvae.

Salinity has a major effect on respiration for both *N. siquijorensis* and *N. crematus.* Respiration decreased when salinity was reduced from 30‰ to 20‰ at each temperature. The oxygen consumption rate of the larvae of shore crab *Carcinus maenas* also decreased at low salinities, and the response suggested a very low or lack of osmoregulatory capacity according to the current understanding of the relationships between osmoregulation and energy metabolism.

The most of the gastropod larvae have a plankton period of life from weeks to months before settling as juveniles. Mollusc larvae use their velum to generate currents to avoid predation, optimize feeding and increase the dispersal distance. Typical swimming speed of marine invertebrate larvae ranges between the values of 1 and 10 mm s^{-1}. Swimming velocity of larvae can be influenced by temperature. The maximum swimming speed of a tropical marine fish *Amphiprion melanopus* at lower rearing temperatures resulted in poorer relative swimming capabilities. Swimming velocity of larvae can also be influenced by the salinity factor. Swimming speeds of oyster larvae *Crassostrea virginica* were determined in constant and increasing salinities, "Normal" non-directed swimming speeds of the oyster larvae *Crassostrea virginica* ranged from < 1 cm min^{-1} for early veligers to 5 cm min^{-1} for "eyed" veligers, with temperature being an important variable in this respect. When subjected to hourly salinity increases of 0.5‰, most larvae individuals swam upward or downward at approximately three times the above speeds.

In this study, temperature, salinity as well as their interaction had crucial effects on larval swimming behaviour for both *N. siquijorensis* and *N. crematus*, especially the dispersal distance (VSL). Although VCL (curvilinear velocity) did not change with temperature and salinity for both *N. siquijorensis* and *N. crematus*, VSL increased significantly at reduced temperatures and salinities. The larval swimming mode was observed less curvy and the dispersal distance increased at reduced temperature and salinity values. Such a change in the swimming pattern is possible to indicate escape response to stressful conditions. Moreover many laboratory studies have demonstrated the fact that many marine organisms are able to actively avoid water with lower dissolved oxygen levels. For example, the shrimp species *Metapenaeus ensis* specimens are able to escape from hypoxic areas to oxygenated water areas.

As a final conclusion, both *N. siqujorensis* and *N. crematus* species larvae had higher survival rates at higher temperature and salinity values. Clearance rate was also higher at higher temperature values whereas respiration rate was faster at higher salinity values. The results may help to explain the fact why *N. siqujorensis* was more abundant in the sourthen waters than in the Tolo Habour area while *N. crematus* was restricted to the southern waters, because the salinity of sourthern waters is generally higher and more constant. Besides, higher temperatures in summer and autumn may favour the survival of larvae which are hatched in these seasons. As shown in this study, the combined effects of temperature and salinity were significant on the physiology of *N. siqujorensis* and *N. crematus*. This highlights the importance of multifactor investigations in further understanding of the effects of marine environmental stressors on marine invertebrates.

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DETERMINATION OF OPTIMUM RANGE OF TEMPERATURE AND SALINITY IN HATCHING RATE OF *ARTEMIA URMIANA* (GÜNTHER, 1899)

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KEYWORDS: *Artemia*, brine shrimp, hatching, cyst, Lake Urmia.

ABSTRACT

Artemia (brine shrimp) is one of the most important live foods for aquatic animals and is essential for the larval stage of many species of fish. One of the usual ways of utilizing Artemia is to hatch the cyst and, if this hatching occurs at high percentage, production of Artemia and thus aquatic animals will be increased, and eventually human protein requirement will be provided. In this examination, in order to determine the optimum temperature and salinity conditions for hatching of *Artemia urmiana*, we have studied the effect of three levels of temperature (25, 30, 35°C) and three levels of salinity (25, 30, 35 ppm.), together with three time intervals (24, 26, 28 hours) after hydration on the percentage hatching of cyst. In total we had nine factors with 27 replicates (three replicates for every factor). The results indicate that the effect of salinity in this experiment does not produce any significant variance in all the factors ($p > 0.05$), but temperature is more effective on hatching and has significant variance in factors ($p < 0.05$). The highest percentage of hatching was observed at temperature of 30 C and salinity of 35 ppm., but in statistical results, the temperature of 30 C has high percentage of hatching at all salinity levels. The lowest percentage of hatching was observed at a temperature of 25°C and salinity of 25ppm but, statistically, it results that the temperature of 25 C has a low percentage of hatching in all salinity levels. Thus, temperature appeared to be more effective for hatching than salinity and percentage of hatching has shown to be increased by time, the highest percentage of hatching appearing 28 hours after hydration at 30 C and 35ppm.

RÉSUMÉ: La détermination du gradient optimal de température et de salinité pour le taux d'éclosion d'*Artemia urmiana* (Günther, 1899).

L'artémie est une des plus importantes sources de nutriments pour les animaux aquatiques, étant essentielle pour les états larvaires de la majorité des espèces de poissons. Une des utilisations les plus communes de l'artémie comprend l'éclosion des cystes et, au cas où cette éclosion se passe avec un pourcentage satisfaisant, la production d'artémies suivie par celles des animaux aquatiques s'agrandira et, finalement, la demande humaine de protéine sera couverte. Dans ce but, nous avons étudié l'effet de trois paliers de température (25, 30, 35°C) et de trois paliers de salinité (25, 30, 35‰) pour trois périodes de temps post hydratation (24, 26, 28 h) sur le pourcentage de cystes d'*Artemia urmiana* éclosés, afin de déterminer les conditions optimales de salinité et température pour la capacité d'éclosion du cyste d'*Artemia urmiana*. Au total, nous avons suivi 9 paramètres avec 27 répétitions (trois répétitions par paramètre). Pour cette expérience, les résultats indiquent le fait que l'effet de la salinité n'a pas une variance signifiante pour aucune combinaison de paramètres, (p > 0,05). Par contre, le taux d'éclosion était influencé par la température, qui a une variance signifiante dans les combinaisons de facteurs (p < 0,05). Le meilleur pourcentage d'éclosion a été observé pour une température de 30°C et une salinité de 35‰, mais les résultats statistiques montrent le fait que la température de 30°C présente un taux élevé d'éclosion pour toutes des paliers de salinité. Le plus bas taux d'éclosion a été enregistré pour la température de 25°C et la salinité de 25‰ mais les résultats statistiques indiquent le fait que la température de 25°C présente un faible taux d'éclosion pour tous les paliers de salinité. Ainsi la température semble avoir une incidence plus importance sur l'éclosion que la salinité et le taux d'éclosion a augmenté dans le temps, le plus élevé pourcentage d'éclosions apparaissant 28 h après hydratation à 30°C et 35‰.

REZUMAT: Determinarea gradientului optim de temperatură şi de salinitate pentru rata de eclozare la *Artemia urmiana* (Günther, 1899).

Artemia este una din cele mai importante surse de hrană pentru viețuitoarele acvatice, fiind esențială pentru stadiile de alevin ale multor specii de peşti. Una din utilizările comune ale *Artemiei* implică eclozarea de chişti şi în cazul în care această eclozare are loc cu un procentaj bun, biomasa de *Artemia* şi, apoi, de alte viețuitoare acvatice va creşte şi, într-un final, cererea umană de proteină va fi acoperită. În acest articol, am studiat efectul a trei paliere de temperatură (25, 30, 35°C) şi trei paliere de salinitate (25, 30, 35‰) pentru trei perioade de timp (24, 26, 28 h) post hidratare asupra procentului de chişti eclozați de *Artemia urmiana* pentru a determina optimumul de salinitate şi temperatură pentru capacitatea de eclozare a chistului de *Artemia urmiana*. Am urmărit nouă factori cu 27 de repetări (trei repetări pentru fiecare factor). Rezultatele indică faptul că, în acest experiment, efectul salinității nu are o variantă semnificativă pentru nici o combinație de factori (p > 0,05) dar că rata de eclozare este mai influențată de temperatură, care are o variantă semnificativă în combinațiile de factori (p < 0,05). Procentul cel mai ridicat de eclozare s-a observat pentru o temperatură de 30°C şi o salinitate de 35‰, dar rezultatele statistice au arătat că la temperatura de 30°C este o rată mare de eclozare pentru toate palierele de salinitate. Cel mai scăzut procentaj de eclozare a fost observat pentru temperatura de 25°C şi salinitatea de 25‰, dar în statistici rezultă că la temperatura de 25°C este un procentaj scăzut de eclozare pentru toate palierele de salinitate. Astfel, temperatura pare să aibă o incidență mai mare asupra eclozării decât salinitatea, iar rata de eclozare a crescut în timp, cel mai ridicat procent de eclozări, apărând 28 de ore după hidratare la 30°C şi la 35‰.

INTRODUCTION

Agh (1997) reported that *Artemia urmiana* exists in the Lake Urmia on the Iranian territory (a lake with a salinity values range between 140 and 210 ppm.). At this time, cyst of *Artemia urmiana* species did not have a good quality (Tavassoli, 2000), so that the examination of certain factors on decrease of quality and determination of the optimum conditions for hatching may enable us to achieve high potential use of this valuable and enormous resource.

Exploitation of the *Artemia urmiana* cyst began during the last decade. Most researchers who have studied the cyst of *Artemia*, found an optimum range of the temperature of 25 - 30°C (Tavassoli, 2000; Thun and Starrett, 1987; Dana and Lenz, 1985), others stated a range of 15 - 30°C for hatching cyst of *Artemia urmiana*, with a range of 37 ppm. for salinity and 28°C for temperature (Agh, 1997), or 33 ppm. for salinity and 26°C for temperature (Agh, 1997); or for salinity they stated 29 ppm. (Dana and Lenz, 1985).

Hatching of cyst depends upon many factors, such as genetic and geographical differences, life condition of adults, method of harvest and post-harvest, etc., but the important factor is the effect of salinity and temperature. In this study we have assumed other factors and have just examined the effect of salinity and temperature on the hatching ability of the cyst of *Artemia urmiana.*

MATERIALS AND METHODS

This research was to examine the effect of various temperature levels (25, 30, 35°C) and salinities (25, 30, 35 ppm.) on the hatching ability of cyst of *Artemia urmiana* at three intervals after hydration (24, 26, 28 h), with a 3 × 3 factorial random design based on three replicates. Thus we had 27 replicates and required 27 zoak incubators.

This examination was tested at the fishery laboratory of IUT, Iran. We collected cyst from the surface of Lake Urmia with 120 M plankton net. Then we processed it by the diphase flotation method and dehydration (Dana et al., 1998). We created salinity with NaCl in the laboratory and regulated it with a reflectometer. We changed temperatures with an aquarium heater. The light intensity was 2000 luxe and air condition was 10 lit/min. In every zoak incubator we had 1 g/litre of cyst.

Sampling and counting methodology was according to the international standard for *Artemia* (Babu et al., 2001). Of course sampling was random and we sampled with MICROSAMPLER and counted with LOOP. To calculate the hatching percentage we used the formula: % H = N/N + U + E, where N = nauplia stage, U = umbrella stage, E = unhatched embryo.

For creating the effect of temperature on different salinities, we made up all zoaks to a favourable temperature (for example 30°C), and varied salinity (25, 30, 35ppm). On other days we did this for the remaining temperatures (25, 35°C). For establishing the effect of salinity we followed the above method. So we had nine factors (25°C and 25ppm), (30°C and 25ppm), (35°C and 25ppm), with 27 replicates.

For analysis of data we used the Fisher test (F-TEST) and SAS programme, for comparison of mean of factors we used the DUNCAN test; at the 0.05% and 0.02% probability level we used the MSTAT programme, and for drawing tables relating to the output of the SAS programme we used Microsoft Word and Excel.

RESULTS AND DISCUSSIONS

The results of hatching percentage are shown in the next page, in the table number 1.

Effect of temperature on hatching percentage

The results show that the maximum percentage of hatching at different times after hydration was related to the 30°C level and the minimum was at 25°C, and the difference between 25 and 35°C was not significant, but at 30°C it was significant, which means that by diminishing and raising the temperature from 30°C, hatching percentage will be decreased.

Effect of salinity on hatching percentage

The obtained results show the fact that the maximum percentage of hatching at 24 h period after hydration was related to 35ppm and at 26 and 28 h periods to 30 ppm. that at 24 h period was not significantly different, but at 26 and 28 h periods there was a significant difference; thus the maximum percentage of hatching was at 30 ppm. and the minimum was at 25ppm.

Table 1: Statistical analysis results of effect of different level of temperature and salinity on hatching cyst of *Artemia urmiana* at different intervals after hydration (28, 26, 24 h); *: significant difference at 5% level n. s.: no significant difference.

P - value			Degrees of freedom	Source of variation
28	26	24		
< 0.001*	< 0.001*	0.047*	8	Factor
< 0.001*	0.07 ns	0.51 ns	2	Salinity
0.001*	0.001*	0.01*	2	Temperature
0.0003*	0.002*	0.17*	4	Salinity × Temperature
			18	Error
			26	Total

Effect of different level of temperature and salinity on the studied species hatching percentage.

The obtained results show that at 24 and 26 h periods after hydration, the maximum percentage of hatching was at 30°C and 35ppm and the minimum percentage of hatching was at 25°C and 35ppm, but there was no significant difference between different salinity levels. At 28 h after hydration, the maximum percentage of hatching was at 30°C and 35ppm and the minimum was at 25°C and 25ppm, and there was no significant difference between different salinity levels.

The salinity does not establish a significant difference, contrary the temperature does, and thus temperature has more effect on hatching. With the progress of time, the hatching percentage increased. The maximum hatching percentage was at 28 h, 30°C and 35ppm.

A previous study emphasised an introduced species *Artemia urmiana* (Agh, 1997) and this species anatomy, and the research on the cyst was rather limited (Drihkwater and Crowe, 1991). But in the present different research we have studied the condition of the cyst too. Often previous researchers, with studies in this field of interest, studied just one parameter (Agh, 1997), but we have studied two parameters, together with their reciprocal effect.

The study presented here had a similar result to others cited here in the introduction section, and a rather small difference in the obtained results is probably due to the effect of the laboratory environment, genetic and geographical differences (Babu et al. 2001), life condition of adults (Fengqi et al. 2001), food and density (Dana et al. 1998), and method of harvest and post-harvest. In the present study we employed the optimum of other parameters, such as pH and density (Agh, 1997), and disregarded testing them.

So with this study we show that, with the implementation of optimum conditions and environmental control, we can achieve maximum hatching of the cyst of *Artemia urmiana* and make best use of this God-sent resource.

Because this study was done in the laboratory, we suggest that another future study in the natural environment may achieve a better result. With an increase of factors and replication we will have a complementary series, and suggest future study, with increased ranges of salinity and temperature. Maybe at the highest or lowest range of salinity and temperature we will have a more exact optimum for hatching cyst of *Artemia urmiana*.

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DISTRIBUTION OF DISSOLVED NUCLEIC ACIDS IN THE SOIL OF SOUTHERN JORDAN

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KEYWORDS: Jordan, dissolved nucleic acids, environment, microbial loop.

ABSTRACT

Dissolved nucleic acids (DNC) are ubiquitous components of the dissolved organic matter (DOM) pool of all oceanic, neritic, estuarine, and freshwater habitats studied to date, and may be also important for microbial growth, because of its enrichment in nitrogen and phosphorous, and as a source of nucleic acid precursors. Additionally, DNC could be genetically important, encoding for gene sequences with the potential to transform microbial populations. Transformation has been demonstrated for a variety of terrestrial bacteria and genetic exchange via transformation has been observed in soil. Under appropriate reaction conditions, dissolved DNA (D-DNA) and dissolved RNA (D-RNA) are efficiently extracted from samples. The purpose of the present study was to investigate DNA and RNA dynamics in soil of southern Jordan in order to provide quantitative estimates of DNC distribution and accumulation.

In order to understand the dynamics of DNA and RNA in soils, sampling was carried out at five different locations in southern Jordan, which is one of the driest places in the world. For nucleic acid determination, we sampled on each site the top 1cm slices of two parallel cores which were subsequently homogenized and deep frozen for later nucleic acid (DNA and RNA) analyses in the laboratory. Nucleic acid extraction and measurement were done by the procedures of Zachleder (1984) as applied by Danovaro et al., (1993), with a few modifications to enhance DNA extraction from the soil.

The results reveal two important facts. First: both DNA and RNA concentrations were extremely high and DNA concentration was significantly different between the sites studied. A similar trend has been noticed with RNA. These results confirm the large variability among different environments.

Secondly, our results are in agreement with the hypothesis that the microbial loop plays an important role in NA dynamics suggesting the presence of enzymatic activities decomposing organic materials and releasing DNA. Our results confirmed the hypothesis that DNA released from dry matter by decomposition in soil is rapidly degraded or adsorbed onto soil particles; the adsorption of DNA onto soil components retards DNA degradation and constitutes a major mechanism of DNA molecule persistence in soil.

DNA concentrations reported in this study were high when compared to the Mediterranean Sea indicating that organic matter accumulation is higher in continental environments. Also, RNA concentrations in the study areas were extremely high and comparable to those found in highly productive systems.

ZUSAMMENFASSUNG: Verteilung der gelösten Nucleinsäuren in Böden des südlichen Jordanien.

Gelöste Nucleinsäuren (DNC) sind allgegenwärtige Komponenten im Bestand aller gelösten organischen Stoffe (DOM), die bisher in Ozeanen, neritischen Zonen, Estuaren und Süßwasserhabitaten untersucht wurden. Sie könnten - bedingt durch ihre Anreicherung an Stickstoff und Phosphor - ebenfalls für mikrobielles Wachstum wichtig sein, aber auch als ein Baustein für Vorstufen der Nucleinsäure. Zusätzlich könnte DNC genetisch für das Kodieren von Gensequenzen von Bedeutung sein, mit dem Potential mikrobielle Populationen umzuwandeln. Solche Umwandlungen wurden für verschiedene terrestrische Bakterien nachgewiesen, wobei ein genetischer Austausch im Boden beobachtet wurde. Unter entsprechenden Reaktionsbedingungen wurden gelöste DNA (D-DNA) und gelöste RNA (D-RNA) effizient aus Proben extrahiert.

Ziel der vorliegenden Untersuchung war es, die Dynamik der DNS/DNA und RNA in Böden des südlichen Jordanien zu untersuchen, um quantitative Schätzungen über die Verbreitung und Akkumulation von DNC in Böden zu liefern.

Um die Dynamik von DNA und RNA in Böden zu verstehen, wurden an fünf verschiedenen Standorten in Südjordanien, einem der weltweit trockensten Gebiete der Erde, Proben entnommen. Zur Bestimmung der Nucleinsäure (DNS/DNA und RNS /RNA) wurden die obersten 1cm Schichten von zwei parallelen Bohrkernen homogenisiert und für spätere Analysen im Labor tiefgefroren. Die Extraktion sowie die Messungen wurden nach dem Verfahren von Zachleder (1984) durchgeführt, so wie es von Danovaro et al., (1993) angewendet wurde, mit einigen Änderungen, um die Extraktion von DNA aus dem Boden zu verbessern.

Die Ergebnisse zeigen zwei wichtige Fakten. Erstens: Die Konzentrationen von DNA und RNA waren extrem hoch und signifikant verschieden zwischen den untersuchten Stellen. Ein ähnlicher Trend wurde bei RNA verzeichnet. Die Ergebnisse bestätigen die bekannte große, standortabhängige Variabilität.

Zweitens sind unsere Ergebnisse in Übereinstimmung mit der Hypothese, die besagt, dass der mikrobielle Kreislauf eine wichtige Rolle in der NA Dynamik spielt. Dies legt die Präsenz enzymatischer Vorgänge nahe, die organisches Material zersetzen und DNA freigeben.

Unsere Ergebnisse bestätigen die Hypothese, dass die aus trockener, in Zersetzung befindlicher Substanz freigewordene DNA im Boden rasch abgebaut oder an Bodenpartikeln adsorbiert wird; die Adsorbtion von DNA an Bodenteilchen verzögert ihren Abbau und stellt dadurch einen Hauptmechanismus der Persistenz der DNA Moleküle im Boden dar.

Die gefundenen DNA Konzentrationen sind sehr hoch im Vergleich zum Mittelmeer, was zeigt, dass die Akkumulation organischer Substanz auf dem Kontinent höher ist. Ebenso war die Konzentration der RNA im Untersuchungsgebiet außergewöhnlich hoch und vergleichbar mit derjenigen in hochproduktiven Systemen.

REZUMAT: Distribuția acizilor nucleici dizolvați în soluri din sudul Iordaniei.

Acizii nucleici dizolvați (ADN) sunt componente omniprezente ale fondului materiei organice dizolvate (MOD) studiate până în prezent în oceane, zonele neritice, estuarine şi în habitatele de apă dulce şi ar putea fi o componentă importantă a MOD, pentru creştere microbiană, datorită îmbogățirii lor în azot şi fosfor, şi de asemenea ca o sursă a stadiilor precursoare ale acizilor nucleici. În plus ADN poate fi important din punct de vedere genetic, pentru codificarea secvențelor genetice cu potențialul de a transforma populații microbiene. Transformarea a fost demonstrată pentru multe bacterii terestre, observându-se în sol un schimb genetic prin transformare. În condiții de reacție corespunzătoare, ADN-ul dizolvat (D-ADN) şi ARN-ul dizolvat (D-ARN) au fost eficient extrase din probe.

Obiectivul acestui studiu a fost investigarea dinamicii ADN şi ARN din soluri, în sudul Iordaniei, pentru a oferi estimări cantitative a distribuţiei ADN şi a acumulării sale în soluri.

Pentru înțelegerea dinamicii ADN şi ARN în soluri, au fost prelevate probe în cinci situri diferite din sudul Iordaniei, aceasta fiind una dintre cele mai uscate regiuni de pe glob. Pentru determinarea acidului nucleic au fost luate probe din primul cm de la suprafaţă din două carote, acestea fiind, apoi, omogenizate şi congelate pentru analize ulterioare în laborator ale conţinutului în acizi nucleici (ADN şi ARN). Extragerea şi măsurătorile de acid nucleic au fost efectuate după procedura lui Zachleder (1984), aplicată de Danovaro et al., (1993), cu unele modificări pentru a înlesni extragerea din sol a ADN-ului.

Rezultatele scot în evidenţă două lucruri importante. În primul rând, atât concentraţiile ADN, cât şi ARN au fost extrem de ridicate şi semnificativ diferite între diversele situri din sudul Iordaniei. O tendinţă de dezvoltare similară a fost remarcată şi la ARN. Rezultatele confirmă ipoteza unei largi variaţii în diferite condiţii de mediu. În al doilea rând, rezultatele sunt în concordanţă cu ipoteza care indică faptul că linia microbiană joacă un rol important în dinamica acizilor nucleici (AN). Acest fapt sugerează prezenţa unor activităţi enzimatice, ce descompun materia organică, eliberând ADN. Rezultatele noastre confirmă ipoteza că ADN-ul, eliberat din materie uscată prin descompunere în sol, este rapid degradat sau adsorbit de particule de sol sau adsorpţia ADN-ului de către componentele din sol întârzie degradarea ADN-ului, constituind un mecanism major în persistenţa moleculelor de ADN în sol.

Concentraţia ADN raportată în acest studiu a fost ridicată în comparaţie cu datele din literatură, indicând faptul că acumularea materiei organice are o rată mai ridicată în mediul continental. De asemenea, concentraţiile de ARN au fost extrem de ridicate în zonele studiate şi comparabile cu cele găsite în sisteme foarte productive.

INTRODUCTION

Dissolved nucleic acid (DNC) may be an important component of the DOM (dissolved organic matter) for microbial growth, because of its enrichment in nitrogen and phosphorous, and also as a source of nucleic acid precursors. Additionally, DNC could be genetically important, encoding for gene sequences with the potential to transform microbial populations. Transformation has been demonstrated for a variety of terrestrial bacteria and genetic exchange via transformation has been observed in soil (Graham and Istock, 1978). Mechanisms of genetic exchange amongst natural populations of environmental bacteria are poorly understood, and transformation by extra cellular DNA in the marine environment has not been investigated to our knowledge.

The available information on soil nucleic acid concentrations demonstrated the presence of large amounts of DNA (about 0.5 g/100g of dry soil) also in deep-sea environments such as in the highly oligotrophic eastern Mediterranean Sea (Danovaro et al., 1999). Such DNA pools (converted to carbon equivalents) were 1.3 times higher than the total benthic biomass (Danovaro et al., 1999). Nucleic acid accumulation in the eastern Mediterranean Sea could be a basin scale anomaly due to an uncoupling between input and consumption (Danovaro et al., 1997) or a worldwide phenomenon in deep-sea sediments.

The purpose of the present study was to investigate DNA and RNA dynamics in soil of southern of Jordan in order to provide quantitative estimates of DNC distribution and accumulation in soils.

MATERIALS AND METHODS

Study area and sampling. Soil sampling was carried out at five different locations in the southern of the Jordan territory (Fig. 1). This studied area is one of the world's driest places. For nucleic acid determination, we sampled the top 1cm slices of two cores (2 cores sample for each location), which were subsequently homogenized and deep frozen for later analyses in the laboratory.

Figure 1: Sampling sites in southern Jordan.

Nucleic acid analysis. Before analysis, larger macroscopic organisms were removed from the samples. All materials used for nucleic acid analysis were carefully cleaned by soaking in 1 N NaOH-10% HCl-DDH$_2$O water to remove organic matter contamination and subsequently treated as described by Moran et al. (1993) to avoid nuclease contamination. All solutions were prepared with distilled water and then autoclaved. Amounts of DNA and RNA were determined by spectrophotometry. For the analysis internal standards of calf thymus DNA and baker's yeast RNA (5 to 10 mg) were added to replicate sub samples before extraction. The final yields of the internal standards of DNA and RNA were on average 70 and 81%. DNA and RNA concentrations in the soils were not corrected for percent recovery of the internal standards and were calculated from calibration curves of calf thymus DNA and baker's yeast RNA prepared according to analytical protocol. Data were normalized to soil dry weight after desiccation (60°C, constant weight).

Nucleic acid extraction and measurement were done according to the procedures of Zachleder (1984) as applied by Danovaro (Danovaro et al., 1993) with a few modifications to enhance DNA extraction from soil. Briefly, 1 g of soil (three replicates) was treated with 3.0 ml of 0.5 N perchloric acid, stirred for 3 min, and sonicated three times for 1 min (with intervals of 30 s). Nucleic acid extraction was carried out at 75°C for 30 min under continuous stirring. After centrifugation (3,000 rotations/min, 3g, 10 min), the absorbance of the total nucleic acid content (TNA) in the supernatant was measured at 260 nm. DNA absorbance was determined with a diphenylamine (2% in acetic acid) light-activated reaction (40 W, 12 h) at 598 nm and converted to concentration, using standard solutions of calf thymus DNA. DNA concentration was then reported as equivalent of absorbance at 260 nm in order to calculate by difference the absorbance due to RNA: $ABS_{RNA} = ABS_{TNA} - ABS_{DNA}$, where ABS_{RNA} is the absorbance of RNA, ABS_{TNA} is the absorbance of TNA, and ABS_{DNA} is the absorbance of DNA. RNA absorbance (260 nm) was then converted to concentration, using standard solutions of baker's yeast RNA. Since TNA absorbance at 260 nm might be affected by the interference of inorganic compounds, we used soil sub samples, previously treated in an oven (100°C, overnight) as blanks. Sensitivity of the method has been tested on DNA and RNA standards (accuracy of ± 2.0 µg) and appeared to be adequate for field investigations.

RESULTS AND DISCUSSION

DNA concentrations averaged 41.8 (median 35.2) µg/µl ranging from 8.7 to 105.2 µg/µl; RNA concentrations averaged 20.2 (median 17.2) µg/µl ranging from 4.2 to 50.7 µg/µl (Tab. 1).

Table 1: DNA and RNA concentrations, pH and conductivity at five different sites in southern Jordan.

Sampling stations	Mean concentration ± SD (µg/µl)			
	DNA	RNA	pH	Conductivity
Ma'an	105.2 ± 21.3	50.7 ± 21.3	8.65 ± 0.08	112.37 ± 23.53
Al Shoubk	35.7 ± 11.2	17.2 ± 4.8	8.38 ± 0.16	77.80 ± 2.69
Wadi Mousa	38.8 ± 5.4	18.7 ± 7.1	7.81 ± 0.06	707.00 ± 43.30
Al Tafila	20.75 ± 3.2	10.0 ± 0.8	9.01 ± 0.05	22.23 ± 0.78
Aeel	8.7 ± 1.8	4.2 ± 0.12	8.34 ± 0.11	115.03 ± 4.91

The distribution of dissolved nucleic acid (DNC) in the environments sampled appears in the figure 2.

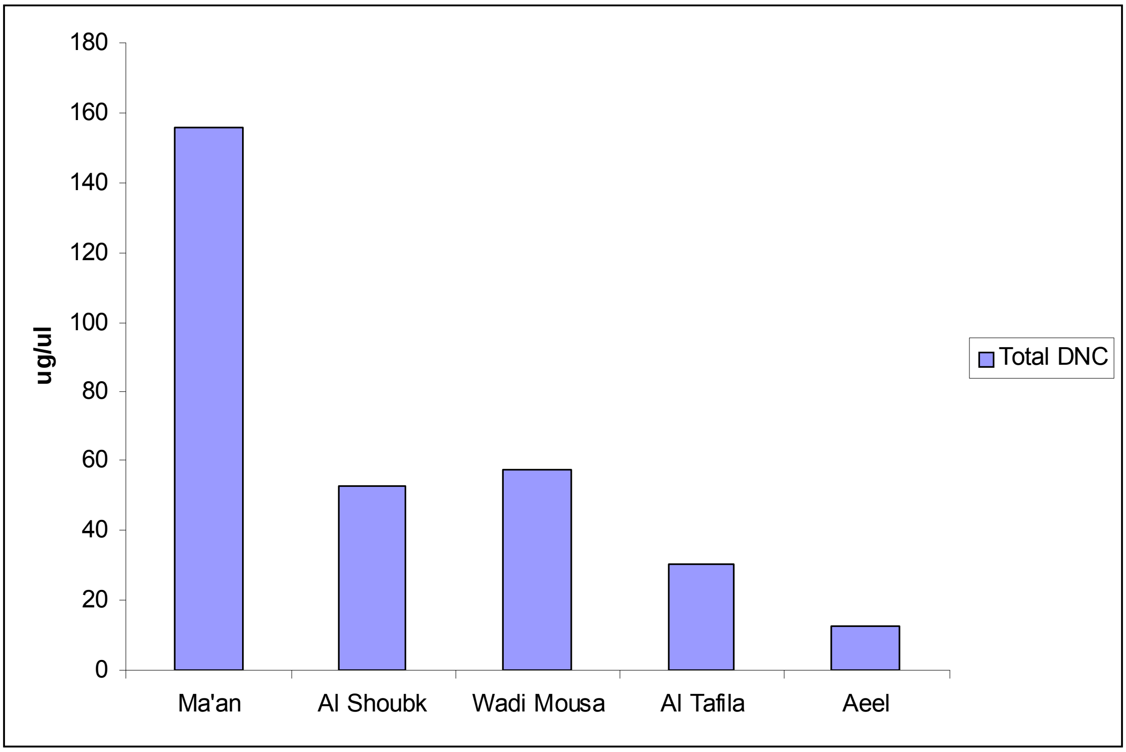

Figure 2: The concentration of dissolved nucleic acid (DNC) at the different studied sites (µg/µl).

The mean concentrations of the dissolved nucleic acid (DNC) in the soil samples were 155.9, 52.8, 57.5, 30.7 and 12.9 µg/µl at Ma'an, Al Shoubk, Wadi Mousa, Al Tafila and Aeel sites, respectively (Fig. 2). Following the same sequence of stations, the percentages of nucleic acid nitrogen (NC-ON%) were 57.1, 19.3, 21.1, 11.2 and 4.72, and of nucleic acid phosphorous (NC-OP%) 15.5, 5.2, 5.7, 3.1 and 1.2, respectively (Fig. 3).

The distribution of DNC found in this study follows a similar pattern characteristic of organic matter in soil as reported in Pote et al. (2007). We found a high concentration of total DNC in soil and observed a significant difference between the sampling sites. This suggests the presence of enzymatic activities decomposing organic materials (plant, animal, etc.) and releasing DNA. Our results confirmed the hypothesis that DNA released by decomposing dry matter in soil is rapidly degraded or adsorbed onto soil particles as well as the adsorption of DNA onto soil components retards DNA degradation and constitutes a major mechanism of DNA molecule persistence in soil (Trevors, 1996; Pote et al., 2005).

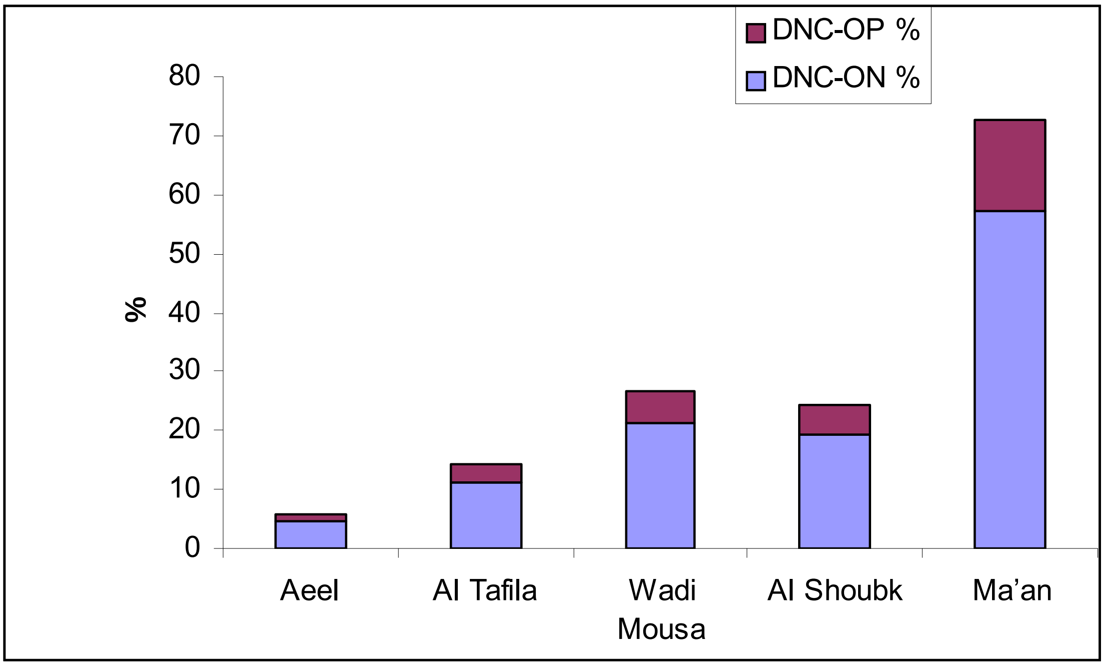

Figure 3: The percentage of nucleic acid nitrogen (NC-ON%) and the percentage of nucleic acid phosphorous (NC-OP%) at soil samples.

Previous studies have shown that nucleic acid concentrations in the sediments and soils might vary according to changes in environmental conditions and that higher sedimentary nucleic acid values are generally observed in highly productive systems (Danovaro et al., 1999). DNA concentrations reported in this study were high when compared to the Mediterranean Sea (Danovaro et al., 1999) indicating that organic matter accumulation is higher in continental environments. Also, RNA concentrations in the study areas were extremely high and comparable to those found in highly productive systems.

Earlier studies carried out on deep-sea sediments of the eastern Mediterranean Sea clearly demonstrated that DNA pools were largely unaccounted for by DNA associated with living biomass (detrital DNA represented about 90% of the total DNA pool (Danovaro et al., 1999). Accordingly, in our soil, bacterial DNA represented a large fraction of the total DNA pool. As far as RNA concentrations are concerned, studies of the oligotrophic eastern Mediterranean Sea indicated that bacteria alone accounted for about 26% of the total RNA pool (Danovaro et al., 1999); a similar conversion factor was noticed with bacterial RNA. This is not surprising since (i) bacteria contribute to a larger fraction of the total biomass in oligotrophic than in eutrophic systems (Danovaro et al., 1993; Danovaro et al., 1997) and (ii) detrital nucleic acids might represent a more important organic source to bacteria in food-limited than in food-rich environments (Pote et al., 2007). Data reported here are in agreement with these hypotheses, indicating that the microbial loop (reported here in terms of bacterial capability to recycle detrital organic compounds otherwise lost to the benthic food webs) might play a much more important role in nucleic acid dynamics.

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TERRESTRIAL SNAIL COMMUNITIES IN SOUTHERN TRANSYLVANIAN ALLUVIAL FORESTS (ROMANIA)

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ABSTRACT

The paper analyzes the terrestrial snail communities in six sampling points from Sibiu and Făgăraş Depressions (southern Transylvania) - Seviş, Tocile, Avrig, Ucea, Sâmbăta and Dridif.

A number of 51 species from 19 families were identified. The highest statistical density was 259 ind./m^2 - at Ucea, and the lowest 40 ind./m^2 - at Tocile all species confounded. Concerning the biomass, the values were between 35.53 g/m^2, at Avrig, and 16.2 g/m^2 at Seviş.

The more significant association between gastropod species was found between *Fruticicola fruticum* and *Perpolita hammonis*, *Perforatella bidentata* and *Perpolita hammonis*, *Perforatella bidentata* and *Valonia costata*, *Cochlicopa lubrica* and *Vitraea crystalina*, *Cochlicopa lubrica* and *Vallonia costata*, *Succinea oblonga* and *Vallonia costata*, *Perforatella bidentata* and *Vitrina pellucida.*

RÉSUMÉ: Les communautés de gastropodes terrestres, dans les forêts alluviaux du sud de la Transylvanie (Roumanie).

L'étude analyse les communautés de gastropodes terrestres dans six points de prélèvement situées dans le sud de la Transylvanie: Seviş, Tocile, Avrig, Ucea, Sâmbăta, Dridif, dans des habitats de forêts alluviales situés dans le bassin hydrographique de la rivière Olt. Dans chaque station ont été réalisés entre 40 et 100 prélèvements. 51 espèces de gastropodes terrestres ont été identifiées, la densité statistique enregistrée variant entre 259 ind./m^2 et 40 ind./m^2. Concernant la biomasse, elle varie entre 35,53 g/m^2 et 16,2 g/m^2.

Les plus significatives associations entre espèces de gastropodes terrestres ont étés trouvées entre *Fruticicola fruticum* et *Perpolita hammonis*, *Perforatella bidentata* et *Perpolita hammonis*, *Perforatella bidentata* et *Valonia costata*, *Cochlicopa lubrica* et *Vitraea crystalina*, *Cochlicopa lubrica* et *Vallonia costata*, *Succinea oblonga* et *Vallonia costata*, *Perforatella bidentata* et *Vitrina pellucida*.

REZUMAT: Comunități de gastropode terestre caracteristice pădurilor aluviale din sudul Transilvaniei (România).

Lucrarea prezintă o analiză a structurii comunităților de gastropode terestre, din şase puncte amplasate în două depresiuni din sudul Transilvaniei, Depresiunea Sibiului şi Depresiunea Făgăraşului - Seviş, Tocile, Avrig, Ucea, Sâmbăta şi Dridif.

În zona de referință, au fost identificate 51 de specii de gastropode terestre, aparținând la 9 familii, densitatea statistică înregistrată, variind între 259 ind./m^2 la Ucea şi 40 ind./m^2 la Tocile. În ceea ce priveşte biomasa, aceasta este cuprinsă între 35,53 g/m^2 la stația Avrig şi 16,2 g/m^2 la Seviş.

Cele mai semnificative asociații între speciile de gastropode terestre au fost găsite între *Fruticicola fruticum* şi *Perpolita hammonis*, *Perforatella bidentata* şi *Perpolita hammonis*, *Perforatella bidentata* şi *Valonia costata*, *Cochlicopa lubrica* şi *Vitraea crystalina*, *Cochlicopa lubrica* şi *Vallonia costata*, *Succinea oblonga* şi *Vallonia costata*, *Perforatella bidentata* şi *Vitrina pellucida*.

INTRODUCTION

Riparian zones are considered to be one of the most biologically diverse and ecologically complex habitat types. As ecotones between aquatic and terrestrial systems, alluvial forests are typically characterized by environmental gradients, and are exposed to frequent disturbance events, such as flooding and debris flows. In southern Transylvania, alluvial forests are one of the prioritary Natura 2000 habitats (91E0 habitat type). Many of these forests were drastically reduced, consecutive to land use and wood exploitation. Alluvial forests are mesohygrophylous vegetal associations witch abrite one of the richest terrestrial gastropod fauna due to the rich and diverse vegetation and humidity (Gheoca, 2007).

Little data are available concerning terrestrial gastropods from this area, most of them are historical data (Bielz, 1867; Kimakowicz, 1883-1884, 1890, 1894); still some records are mentioned by Grossu (1955, 1956, 1981, 1983, 1987, 1993). More recent papers (Gheoca and Popovici, 1999) discuss the terrestrial gastropods form the Olt River basin. All this are faunistic studies, with no concern in snail assemblages.

The present paper intends to analyze the structure of terrestrial gastropod communities of some alluvial forests in southern Transylvania. In the studied area (Fig. 1) the typical vegetal associations are: Salicetum albae fragilis, Salicetum triandrae, Aegopodio-Alnetum glutinosae with *Rubus caesius* and *Urtica dioica*. There were also observed transition zones between alluvial forests and antropophylous weeds, alluvial forest and orchard or alluvial forest and oak forest.

MATERIALS AND METHODS

The quantitative square samples of 0.0625 m^2 (25/25 cm) were collected between 1999 and 2002. A number of 80 samples were collected from each station. For the quantitative description of the gastropod communities, were used the values of the statistical density, of the relative abundance, of the frequency and of the Dzuba index.

The association analysis has been made using the mean quadratic-contingency index and the Cole interspecific association coefficient. These indices have been calculated for those pairs of species for which the χ^2 test indicated a significant association for a probability of 5% (χ^2 > 3, 89). The Fager cenotic affinity index was also used.

SYSTAT multivariate statistics program was also used for the evaluation of correlations and regression equations.

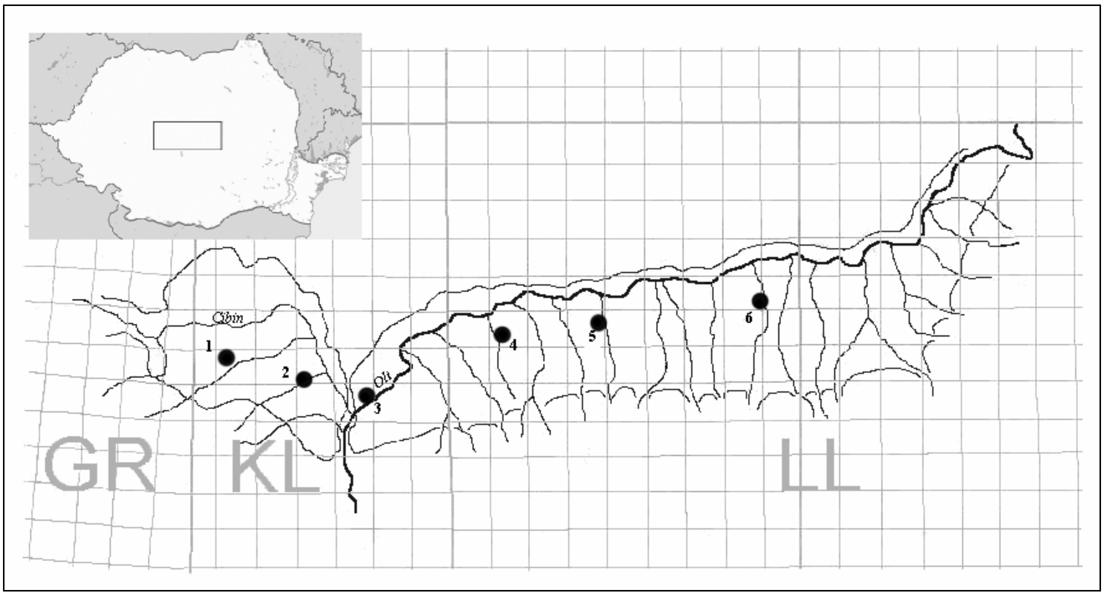

Figure 1: Location of sampling points in the area;
1 - Seviş, 2 - Tocile, 3 -Avrig, 4 -Ucea, 5 - Sâmbăta, 6 - Dridif.

RESULTS

A number of 51 terrestrial gastropod species (Tabs. 1a and 1b) were identified, four of them only as shells, probably transported by water. The statistical density values vary between 259 and 40 ind./m^2, while biomass values (Fig. 2) were between 35.53 g/m^2 and 16.2 g/m^2. High differences concerning statistical abundances are caused by the presence of Carychiidae, Cochlicopidae, Valoniidae, Gastrodontidae, Oxychilidae, with very small and abundant species.

In all sampling points except Tocile, the dominant species are *Vallonia costata* (A > 16%, F > 39%, W > 7) and *Cochlicopa lubrica* (A > 9%, F > 45%, W > 4). Other species have high amounts in different sampling points, being dependent of the local habitat conditions. The most important are *Zonitoides nitidus*, *Perpolita hammonis*, *Carychium tridentatum*, *Succinea oblonga*, *Perforatella bidentata* and *Fruticicola fruticum*.

The terrestrial gastropod community at Tocile sampling station has a different structure, due to a nearby oak forest, species characteristic to both habitats (alluvial and oak forest), as *Macrogastra latestriata*, *Cochlodina laminata*, *Fruticicola fruticum* and *Euomphalia strigella* being found here.

As for the distribution on families, the most abundant are the Valoniidae (23%), Cochlicopidae (11%), Succineidae (10%), Hygromiidae and Oxychilidae (9%) (Fig. 3).

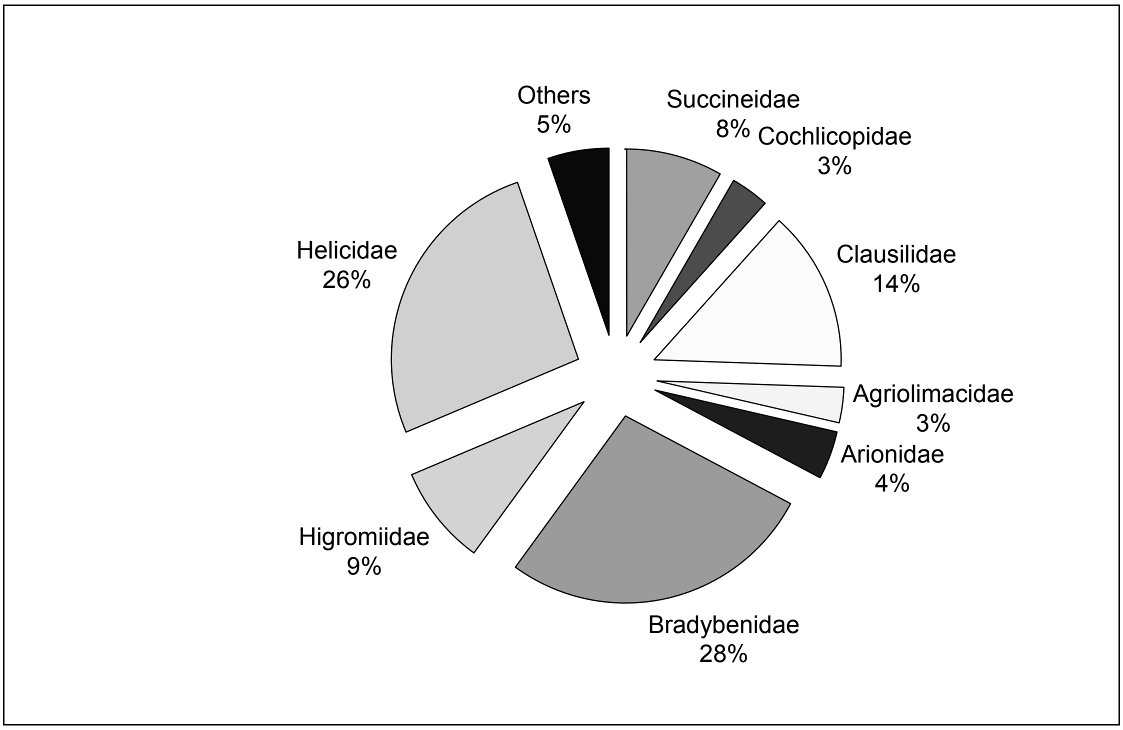

Figure 2: Contribution to the total biomass of different terrestrial gastropod families.

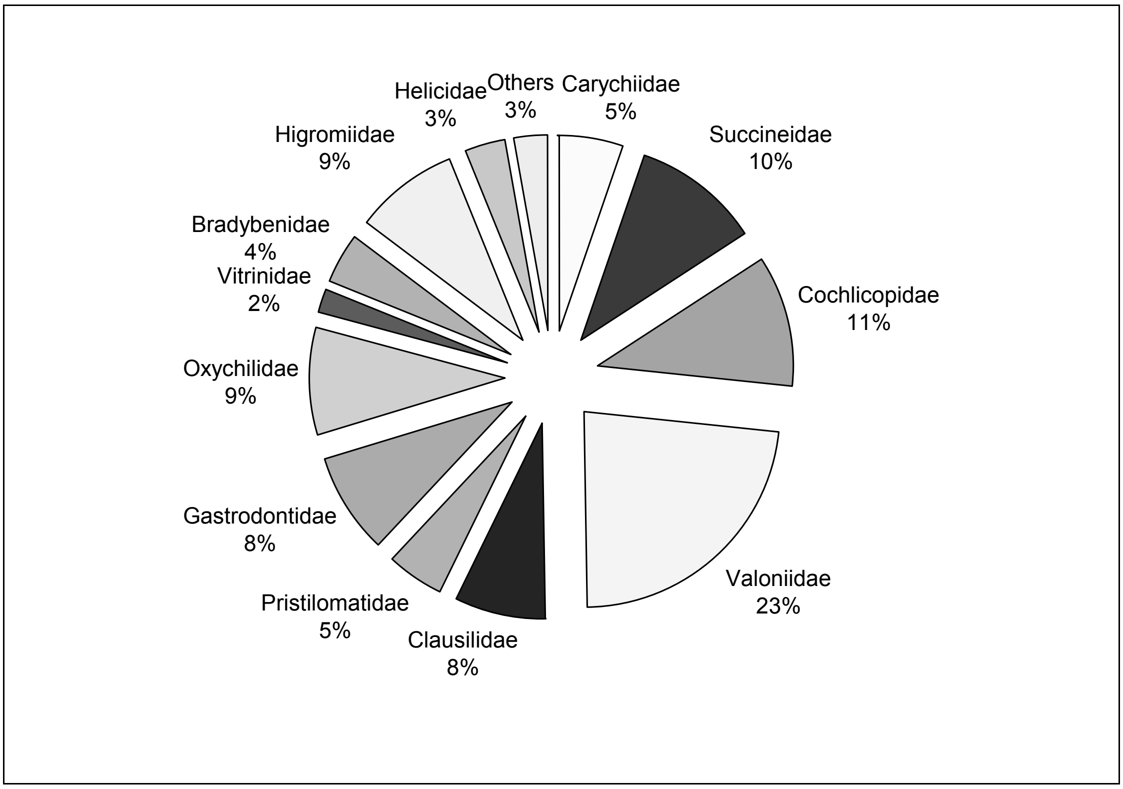

Figure 3: Relative abundance of terrestrial gastropod families.

Table 1a: List of terrestrial gastropods from alluvial forests in Southern Transylvania. (D_s - statistical density, A - relative abundance, F - frequency, W - ecological significance index, C* only shells).

Species	**Seviş**				**Avrig**				**Sâmbăta**			
	D	A%	F%	W	D	A%	F%	W	D	A%	F%	W
Carychium minimum Müller, 1774	-	-	-	-	-	-	-	-	3.50	2.73	6.25	0.17
Carychium tridendatum Risso, 1826	8	7.84	12	0.94	-	-	-	-	8	6.40	25	1.60
Succinea putris Linnaeus, 1758	4	3.92	29.41	1.15	9.3	6.07	42.1	2.55	4.20	3.28	12.5	0.41
Succinea oblonga Draparnaud, 1801	4	4.90	29.41	1.44	17.7	11.56	42.1	4.87	13	10.15	56.25	5.71
Cochlicopa lubrica Müller, 1774	14	13.72	47.05	6.45	21.5	14.05	52.63	7.39	12	9.37	43.75	4.10
Vallonia pulchella Müller, 1774	2	1.96	11.76	0.23	2.5	1.63	15.78	0.25	1.4	1.09	6.25	0.06
Vallonia costata Müller, 1774	20	19.6	39.21	7.68	32	21.17	70.58	14.19	32	25	68.75	17.18
Vallonia excentrica Sterki, 1893	-	-	-	-	6.6	4.31	29.4	1.26	-	-	-	-
Columella edentula Draparnaud, 1805	-	-	-	-	-	-	-	-	-	-	-	-
Truncatellina cylindrica Ferussac, 1807	-	-	-	-	1.10	0.71	5.88	4.22	-	-	-	-
Vertigo pusilla Müller, 1774	-	-	-	-	1.1	0.71	5.88	4.22	-	-	-	-
Vertigo pygmaea Draparnaud, 1801	C*	-	-	-	-	-	-	-	-	-	-	-
Vertigo angustior Jeffreys, 1830	-	-	-	-	-	-	-	-	-	-	-	-
Cochlodina laminata Montagu, 1803	3	2.94	23.52	0.9	-	-	-	-	-	-	-	-
Macrogastra latestriata Schmidt, 1856	-	-	-	-	-	-	-	-	-	-	-	-
Clausilia dubia Draparnaud, 1805	5	4.9	9.80	0.48	4.8	3.13	35.29	1.07	-	-	-	-

Table 1a: continued.

Species	**Seviş**				**Avrig**				**Sâmbăta**			
	D	A%	F%	W	D	A%	F%	W	D	A%	F%	W
Laciniaria plicata Draparnaud, 1801	-	-	-	-	3.2	2.09	23.52	0.49	-	-	-	-
Vestia elata Rossmaessler, 1836	2	1.96	5.88	0.11	-	-	-	-	-	-	-	-
Pseudalinda falax Rossmaessler, 1836	-	-	-	-	0.8	0.52	5.88	0.03	-	-	-	-
Bulgarica cana Held, 1836	-	-	-	-	-	-	-	-	-	-	-	-
Punctum pygmaeum Draparnaud, 1801	-	-	-	-	1	0.65	5.88	0.038	-	-	-	-
Discus ruderatus Hartmann, 1821	-	-	-	-	-	-	-	-	-	-	-	-
Vitrea contracta Westerlund, 1871	-	-	-	-	1	0.65	5.88	0.038	-	-	-	-
Vitrea cristallina Müller, 1774	5	4.90	35.29	1.72	6.3	4.11	23.52	0.96	1.2	0.78	6.25	0.04
Euconulus fulvus Müller, 1774	-	-	-	-	-	-	-	-	3.4	2.65	12.5	0.33
Zonitoides nitidus Müller, 1774	13	12.74	41.17	5.24	12	7.90	17.64	1.39	8	6.25	25	1.56
Oxychillus depressus Sterki, 1880	-	-	-	-	-	-	-	-	-	-	-	-
Aegopinella epidentostoma Fagot, 1879	-	-	-	-	-	-	-	-	-	-	-	-
Aegopinella minor Stabile, 1864	-	-	-	-	-	-	-	-	C*	-	-	-
Nesovitrea hammonis Strom, 1765	12	11.76	64.70	7.60	5	3.20	35.29	1.13	24	18.75	37.5	7.03
Vitrina pellucida Müller, 1774	4	3.92	29.41	1.15	-	-	-	-	5.5	4.29	6.25	0.26
Limax cinereoniger Wolff, 1803	-	-	-	-	-	-	-	-	-	-	-	-
Limax maximus Linnaeus, 1758	-	-	-	-	1.1	0.71	5.88	0.04	-	-	-	-

Table 1a: continued.

Species	**Seviş**				**Avrig**				**Sâmbăta**			
	D	A%	F%	W	D	A%	F%	W	D	A%	F%	W
Lehmania marginata Müller, 1774	-	-	-	-	-	-	-	-	-	-	-	-
Deroceras agreste Linnaeus, 1758	1	0.98	5.88	0.057	-	-	-	-	-	-	-	-
Deroceras reticulatum Müller, 1774	-	-	-	-	-	-	-	-	1.1	0.85	6.25	0.05
Arion subfuscus Draparnaud, 1805	-	-	-	-	4.5	2.94	17.64	0.52	-	-	-	-
Arion circumscriptus Johnston, 1828	-	-	-	-	1.2	0.78	5.88	0.04	-	-	-	-
Fruticicola fruticum Müller, 1774	2	1.96	70.58	1.38	3.4	2.22	88.23	1.96	3	2.34	81.25	1.9
Euomphalia strigella Draparnaud, 1801	0.11	0.10	11.76	0.01	-	-	-	-	0.2	0.15	12.5	0.019
Hygromia transsylvanica Westerlund, 1876	-	-	-	-	1	0.65	5.88	0.038	1	0.78	18.75	0.14
Pseudotrichia rubiginosa Rossmaessler, 1838	1	0.98	17.64	0.17	-	-	-	-	2	1.6	12.	0.19
Monachoides incarnates Müller, 1774	-	-	-	-	-	-	-	-	-	-	-	-
Monachoides vicinus Rossmaessler, 1842	-	-	-	-	-	-	-	-	C*	-	-	-
Perforatella bidentata Gmelin, 1791	-	-	-	-	12.4	8.10	23.52	1.90	4	3.12	12.5	0.39
Chilostoma banaticum Rossmaessler, 1838	0.16	0.15	11.76	0.017	-	-	-	-	0.1	0.07	12.5	0.08
Faustina faustina Rossmaessler, 1835	-	-	-	-	2.1	1.37	17.64	0.24	-	-	-	-
Cepaea vindobonensis Pfeiffer, 1828	0.24	0.23	17.64	0.04	0.2	0.13	17.64	0.023	-	-	-	-
Helix pomatia Linnaeus, 1758	0.48	0.47	29.41	0.138	0.32	0.20	17.64	0.036	0.2	0.15	18.5	0.02
Helix lutescens Rossmaessler, 1837	-	-	-	-	0.48	0.31	35.29	0.11	-	-	-	-

Table 1b: List of terrestrial gastropods from alluvial forests in Southern Transylvania. (D_s - statistical density, A - relative abundance, F - frequency, W - ecological significance index, C* only shells).

Species	**Ucea**				**Dridif**				**Tocile**			
	D	A%	F%	W	D	A%	F%	W	D	A%	F%	W
Carychium minimum Müller, 1774	-	-	-	-	8	4.14	14.28	0.59	-	-	-	-
Carychium tridendatum Risso, 1826	11	7.92	33.33	2.64	-	-	-	-	1.66	2.74	72.9	1.99
Succinea putris Linnaeus, 1758	4.6	2.22	44.44	0.98	8	4.14	14.28	0.59	1.5	2.47	27.08	0.66
Succinea oblonga Draparnaud, 1801	7.5	3.71	-	-	-	-	-	-	2.25	3.71	20.83	0.77
Cochlicopa lubrica Müller, 1774	18.5	9.15	83.33	7.62	25	12.95	61.90	8.01	3.33	5.49	77.08	4.23
Vallonia pulchella Müller, 1774	3.9	1.98	27.77	0.55	13	6.73	38.09	2.56	2.5	4.12	68.75	2.83
Vallonia costata Müller, 1774	33	16.58	83.33	13.81	52	26.92	57.14	15.39	2.82	4.67	72.91	3.40
Vallonia excentrica Sterki, 1893	-	-	-	-	-	-	-	-	-	-	-	-
Columella edentula Draparnaud, 1805	19.5	0.99	11.11	0.11	-	-	-	-	0.32	0.54	8.33	0.045
Truncatellina cylindrica Ferussac, 1807	1.1	0.49	11.11	0.05	-	-	-	-	-	-	-	-
Vertigo pusilla Müller, 1774	C*	-	-	-	C*	-	-	-	-	-	-	-
Vertigo pygmaea Draparnaud, 1801	2.5	1.23	22.22	0.27	C*	-	-	-	-	-	-	-
Vertigo angustior Jeffreys, 1830	C*	-	-	-	-	-	-	-	-	-	-	-
Cochlodina laminata Montagu, 1803	-	-	-	-	-	-	-	-	5.85	9.82	83.33	8.18
Macrogastra latestriata Schmidt, 1856	-	-	-	-	-	-	-	-	6.32	10.44	81.25	8.48
Clausilia dubia Draparnaud, 1805	1	0.24	5.55	0.013	-	-	-	-	2.86	4.74	60.41	2.86
Laciniaria plicata Draparnaud, 1801	-	-	-	-	-	-	-	-	-	-	-	-

Table 1b: continued.

Species	**Ucea**				**Dridif**				**Tocile**			
	D	A%	F%	W	D	A%	F%	W	D	A%	F%	W
Vestia elata Rossmaessler, 1836	-	-	-	-	-	-	-	-	2.28	3.78	41.66	1.57
Pseudalinda falax Rossmaessler, 1836	-	-	-	-	-	-	-	-	-	-	-	-
Bulgarica cana Held, 1836	7	1.73	16.16	0.28	-	-	-	-	-	-	-	-
Punctum pygmaeum Draparnaud, 1801	-	-	-	-	5	2.59	9.52	0.24	0.23	0.75	12.5	0.09
Discus ruderatus Hartmann, 1821	-	-	-	-	2.40	1.24	4.76	0.05	-	-	-	-
Vitrea contracta Westerlund, 1871	-	-	-	-	-	-	-	-	-	-	-	-
Vitrea cristallina Müller, 1774	25	12.37	72.22	8.93	9	4.66	42.85	1.99	-	-	-	-
Euconulus fulvus Müller, 1774	4	0.99	16.66	0.16	3.6	1.85	9.52	0.17	0.18	0.61	16.66	0.10
Zonitoides nitidus Müller, 1774	13	3.21	33.33	1.07	17	8.80	28.57	2.51	2.97	9.82	33.33	3.27
Oxychillus depressus Sterki, 1880	-	-	-	-	-	-	-	-	0.29	0.96	18.75	0.18
Aegopinella epidentostoma Fagot, 1879	-	-	-	-	-	-	-	-	0.39	1.30	22.91	0.29
Aegopinella minor Stabile, 1864	-	-	-	-	-	-	-	-	0.1	034	8.33	0.02
Nesovitrea hammonis Strom, 1765	20	9.9	61.11	6.05	12	6.21	14.28	0.88	-	-	-	-
Vitrina pellucida Müller, 1774	3	0.74	16.66	0.12	5.2	2.69	4.76	0.12	0.31	1.03	22.91	0.07
Limax cinereoniger Wolff, 1803	-	-	-	-	-	-	-	-	0.125	0.41	12.5	0.05
Limax maximus Linnaeus, 1758	-	-	-	-	-	-	-	-	-	-	-	-

Table 1b: continued.

Species	**Ucea**				**Dridif**				**Tocile**			
	D	A%	F%	W	D	A%	F%	W	D	A%	F%	W
Lehmania marginata Müller, 1774	-	-	-	-	-	-	-	-	0.06	0.21	6.25	0.013
Deroceras agreste Linnaeus, 1758	1	0.24	5.55	0.013	-	-	-	-	-	-	-	-
Deroceras reticulatum Müller, 1774	0	0.24	5.55	0.013	-	-	-	-	0.12	0.41	12.5	0.05
Arion subfuscus Draparnaud, 1805	-	-	-	-	-	-	-	-	0.1	0.34	10.41	0.03
Arion circumscriptus Johnston, 1828	-	-	-	-	-	-	-	-	0.06	0.20	6.25	0.012
Fruticicola fruticum Müller, 1774	8	1.98	66.66	1.32	10	5.18	85.71	4.44	3.22	10.65	85.54	9.09
Euomphalia strigella Draparnaud, 1801	0.24	0.05	11.11	0.005	C*	-	-	-	2.68	8.86	79.16	5.59
Hygromia transsylvanica Westerlund, 1876	1	0.24	11.11	0.026	-	-	-	-	0.54	1.78	27.08	0.48
Pseudotrichia rubiginosa Rossmaessler, 1838	3	0.74	16.66	0.12	3.3	1.7	9.52	0.16	-	-	-	-
Monachoides incarnates Müller, 1774	C*	-	-	-	-	-	-	-	-	-	-	-
Monachoides vicinus Rossmaessler, 1842	-	-	-	-	-	-	-	-	-	-	-	-
Perforatella bidentata Gmelin, 1791	47	24.25	66.66	16.16	-	-	-	-	-	-	-	-
Chilostoma banaticum Rossmaessler, 1838	0.32	0.078	5.55	0.004	-	-	-	-	1	3.29	72.9	2.39
Faustina faustina Rossmaessler, 1835	-	-	-	-	3.5	1.81	33.33	0.60	-	-	-	-
Cepaea vindobonensis Pfeiffer, 1828	0.1	0.02	11.11	0.002	0.24	0.12	9.52	001	0.85	2.81	43.75	1.23
Helix pomatia Linnaeus, 1758	0.2	0.04	10	0.004	0.48	0.24	33.33	0.08	1.08	3.98	68.75	2.73
Helix lutescens Rossmaessler, 1837	0.56	0.13	55	0.07	4.2	2.19	19.04	0.41	-	-	-	-

As biomass, the most represented species are the Bradibenidae (*Fruticicola fruticum*), the Helicidae (*Helix pomatia*, *Helix lutescens*, *Faustina faustina*, *Chilostoma banaticum* and *Cepaea vindobonensis*), the Clausilidae (*Clausilia dubia*, *Cochlodina laminata*), and the Hygromiidae (*Perforatella bidentata*, *Euomphalia strigella*, *Pseudotrichia rubiginosa* and *Hygromia transsylvanica*).

The analyze of contingence tables between the most frequent 17 species based onCole's coefficient, shows significant positive associations for a 5% probability level, between the following species: *Fruticicola fruticum* and *Perpolita hammonis* (χ^2 = 4.974; CCM = 0.236; C = 0.556 ± 0.187), *Perforatella bidentata* and *Perpolita hammonis* (χ^2 = 7.537; CCM = 0.287; C = 0.598 ± 0.187), *Perforatella bidentata* and *Valonia costata* (χ^2 = 5.288; CCM = 0.234; C = 1 ± 0.187), *Cochlicopa lubrica* and *Vitraea crystalina* (χ^2 = 5.888; CCM = 0.256; C = 0.486 ± 0.187), *Cochlicopa lubrica* and *Vallonia costata* (χ^2 = 3.937; CCM = 0.212; C = 0.249 ± 0.187), *Succinea oblonga* and *Vallonia costata* (χ^2 = 7.379; CCM = 0.287; C = 0.553 ± 0.187), *Perforatella bidentata* and *Vitrina pellucida* (χ^2 = 4.246; CCM = 0.233; C = 0.393 ± 0.187).

The affinity degree between terrestrial gastropod species is represented as a cluster tree (Fig. 4), based on Fager affinity index (Tab. 2).

The more significant association were found for *Fruticicola fruticum* and *Perpolita hammonis*, *Perforatella bidentata* and *Perpolita hammonis*, *Perforatella bidentata*, and *Valonia costata*, *Cochlicopa lubrica* and *Vitraea crystalina*, *Cochlicopa lubrica* and *Vallonia costata*, *Succinea oblonga* and *Vallonia costata*, *Perforatella bidentata* and *Vitrina pellucida*.

The most important affinity was found between *Cochlicopa lubrica* and *Vallonia costata*, and between these two species and *Fruticicola fruticum*.

Table 2: Fager affinity index for terrestrial gastropod species. The line marks the species pair with non significant index values. Species codification: 1 - *Succinea oblonga*, 2 - *Succinea putris*, 3 - *Cochlicopa lubrica*, 4 - *Vallonia costata*, 5 - *Vitrea crystalina*, 6 - *Zonitoides nitidus*, 7 - *Perpolita hammonis*, 8 - *Vitrina pellucida*, 9 - *Fruticicola fruticum*, 10 - *Euomphalia strigella*, 11 - *Pseudotrichia rubiginosa*, 12 - *Perforatella bidentata*, 13 - *Drobacia banatica*, 14 - *Faustina faustina*, 15 - *Cepaea vindobonensis*, 16 - *Helix pomatia*, 17 - *Helix lutescens.*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	1																
2	0.138	1															
3	-	0.324	1														
4	0.652	0.325	0.734	1													
5	0.387	-	0.571	0.535	1												
6	0.294	0.339	-	-	0.258	1											
7	-	-	-	-	0.291	0.037	1										
8	0.238	0.182	-	0.182	0.167	0.048	0.229	1									
9	-	-	0.59	0.667	-	0.361	0.554	0.094	1								
10	0.1	0.192	0.145	0.063	0.176	0.2	-	-	0.065	1							
11	0.19	-	-	0.091	-	0.091	0.171	-	0.063	-	1						
12	0.213	0.263	-	-	0.244	0.213	-	-	0.145	-	0.222	1					
13	-	0.071	-	0.033	0.129	0.108	-	0.222	0.068	0.222	-	-	1				
14	0.14	0.059	0.207	0.149	0.162	0.047	-	-	-	-	0.118	-	-	1			
15	0.089	-	0.053	-	0.051	0.063	0.053	-	0.09	0.118	-	-	-	-	1		
16	0.227	0.171	0.237	0.176	0.158	0.273	0.054	-	0.182	0.125	0.111	-	-	-	0.286	1	
17	0.133	0.222	0.133	0.203	0.154	0.178	0.158	-	-	0.118	-	-	-	0.2	0.091	0.182	1

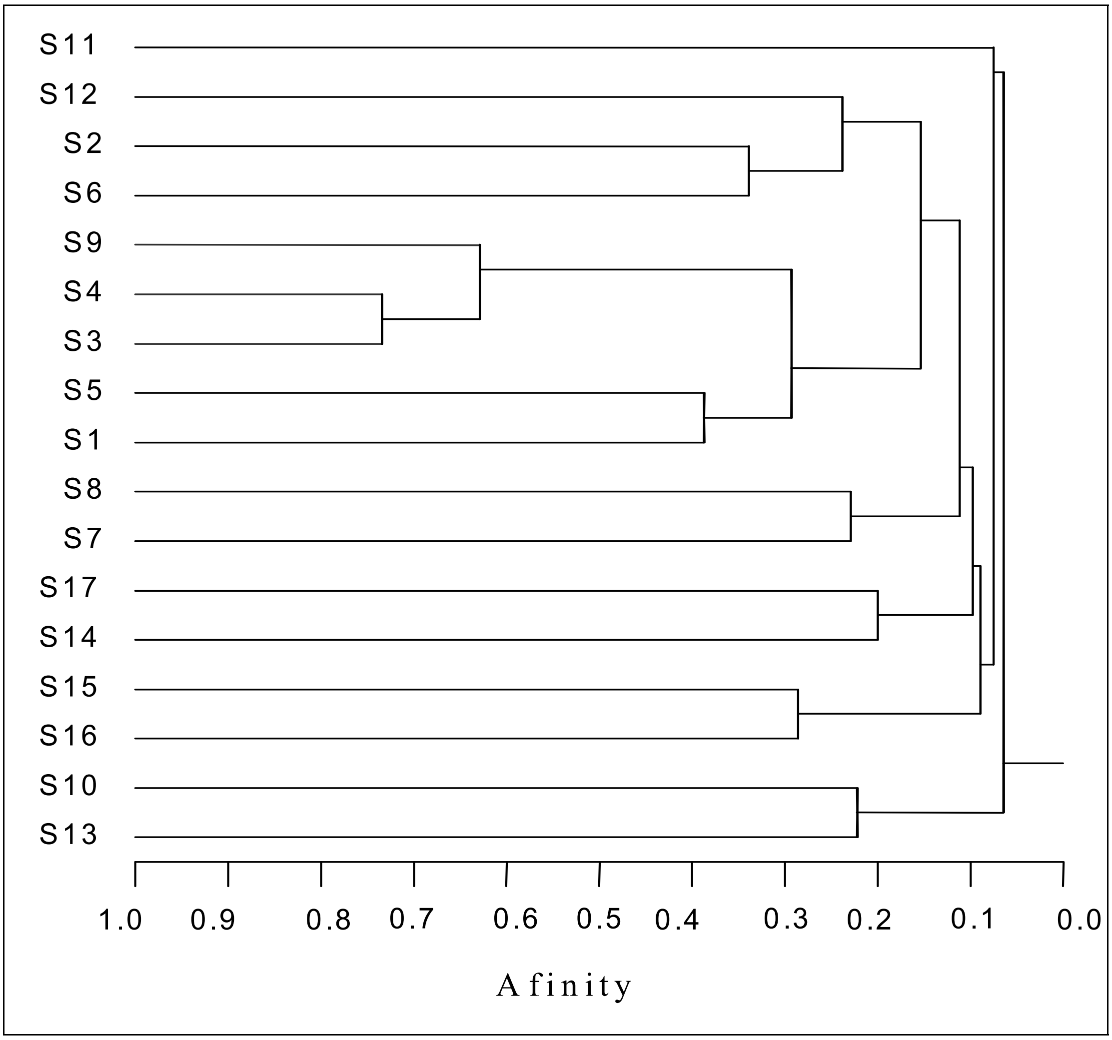

Figure 4: Cluster tree using Fager affinity index values.
The same species codification was used as in the table 2.

CONCLUSIONS

Of the 51 total identified terrestrial gastropod species only five were present in all the six analyzed alluvial forests: *Succinea putris*, *Cochlicopa lubrica*, *Vallonia pulchella*, *Vallonia costata* and *Helix pomatia*.

Finally it must be underlined the fact that the relatively large variability of snail assemblages is a consequence of the habitat heterogeneity. The most different snail assemblage composition and the highest diversity (29 species) was found in the Tocile area, caused by the vicinity of an oak forest, witch maintains humidity and the diversity of herbaceous vegetation. The presence of species like *Clausilia dubia*, *Cochlodina laminate*, *Macrogastra latestriata*, *Vestia elata*, *Oxichillus depresus*, *Aegopinella epidentosoma* and *Limax cinereoniger* is a prove of forest influence on gastropod community in this specific sampling station.

The lowest diversity of this study was found in Dridif area (18 species), Sâmbăta area (20 species) and Seviş area (21 species), as the consequence of a disturbed habitat (extension of agricultural practices and also riverside pollution by waste storage). In these points high frequencies were found for the ubiquist species like *Succinea putris* and *Fruticicola fruticum*.

The most significant associations were found between *Fruticicola fruticum* and *Perpolita hammonis*, *Perforatella bidentata* and *Perpolita hammonis*, *Perforatella bidentata*, and *Valonia costata*, *Cochlicopa lubrica* and *Vitraea crystalina*, *Cochlicopa lubrica* and *Vallonia costata*, *Succinea oblonga* and *Vallonia costata*, *Perforatella bidentata* and *Vitrina pellucida*.

The riparian zones as alluvial forests often comprise a heterogeneous mosaic of microhabitats, where species may be distinctive (Sabo et al., 2005) and biological diversity is assumed to be higher than in the surrounding forests (Gregory et al., 1991; Naiman and Decamps, 1997; Sabo et al., 2005). Terrestrial gastropod communities are depending on this heterogeneity, and they are intensely affected by disturbances that affect habitat diversity.

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OCCURRENCE OF PLANKTONIC ROTIFERA IN THAR DESERT (SINDH, PAKISTAN)

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KEYWORDS: Pakistan, Thar Desert, Nagarparkar, Zooplankton, Rotifer fauna.

ABSTRACT

Thar Desert is located in South East corner of the province of Sindh, between latitude 24°-26°N and longitude 69°-71°E. The whole area comprises sand dunes and sandy flat areas. The Nagarparkar area is distinguished by the presence of Karoonjhar hills, a granite area about 1000 feet high. It is an arid area where aquatic resources are meager. Average rainfall range in Thar is 5 - 15 inches/year. During the rainy season, the water from the hills flows down to the ravines and some quantity is retained in shallow depressions. Across the hill streams of Karoonjhar, few dams are constructed for the storage of water. Apart from these resources the people of Thar use subterranean water obtained from deep wells.

The rain water is retained for a couple of months where aquatic vegetation and planktonic communities establish their populations. Planktonic rotifer community of Nagarparkar area was studied for the first time during October 2008. Plankton and water samples were collected from 16 different stations. Most of them were temporary and retained water for 3 - 6 months. The water bodies included ponds, small dams and wells.

A total of 36 rotifer species have been identified. Major genera recorded were *Brachionus* (13 species), *Lecane* (five species), *Keratella* (three species), *Monostyla, Mytilina* and *Platyias* (two species each). Among the above rotifer species, 11 are recorded for the first time from Pakistan, *Brachionus caudatus aculeatus* Hauer being the most abundant. All the *Lecane* species occurring in Thar Desert are also recorded for the first time from Pakistan and hence are new record.

Water temperature ranged from 24.8 to 35.5°C while dissolved oxygen varied from 3.9 to 8.8 mg/l. Conductivity and salinity range was 141.1 - 40700 µS/cm and 0.1 - 26.1 ppm., respectively. TDS and pH ranged in between 66 - 26048 mg/l and 7.4 - 8.6, respectively.

The distribution of rotifer species in relation to water quality showed that *Brachionus caudatus aculeatus* was most abundant and a common species of small ponds where salinity was very low. On the contrary *Lecane candida* occurred only in saline water bodies. The other *Lecane* species however, occurred in soft water bodies with low salinity.

The documentation of 11 newly recorded rotifer species is an addition to the rotifer fauna of Pakistan.

RÉSUMÉ: Occurrence des rotifères planctoniques dans le Désert de Thar (Sindh, Pakistan).

Le Désert de Thar est situé dans le coin sud-ouest de la province Sindh, entre 24°-26°N et 69° - 71°E. Toute la région est composée de dunes de sable et des plateaux sablonneux. La région de Nagarparkar est mise à part par la présence des collines de granite de Karoonjhar, aux environs de 300 m altitude. Ceci est une région aride où les ressources en eau sont spartiates. À Thar, la quantité moyenne de précipitations se situe entre 127 et 381 mm/année. Pendant la saison des pluies, l'eau des collines s'écoule dans les ravins et une petite quantité est retenue dans les dépressions peu profondes. Dans la région collinaire de Karoonjhar il y a peu de barrages construits pour le stockage de l'eau de pluie. À part ces ressources la population de Thar utilise les eaux souterraines obtenues des puits profonds.

L'eau de pluie est retenue pour quelques mois dans des bassins où la végétation aquatique et les communautés planctoniques établissent leurs populations. La communauté des rotifères planctoniques de la zone de Nagarparkar a été étudiée pour la première fois durant le mois d'Octobre 2008. À cette occasion, des échantillons d'eau et de plancton ont été collectés de 16 stations différentes. Pour la plupart, il s'agit de l'eau stockée pour 3 - 6 mois et de l'eau des bassins temporaires. Les corps d'eau échantillonnés ont compris des étangs, des petits réservoirs et des puits.

On a identifié un total de 36 espèces de rotifères. Les genres principaux ont été *Brachionus* (13 espèces), *Lecane* (5 espèces), *Keratella* (3 espèces), *Monostyla*, *Mytilina* et *Platyias* (chaque genre avec deux espèces). Parmi les espèces identifiées, 11 espèces ont été enregistrées pour la première fois au Pakistan, la plus abondante étant *Brachionus caudatus aculeatus* Hauer. Toutes les espèces du genre *Lecane* trouvées dans le désert de Thar ont été aussi enregistrées pour la première fois au Pakistan et sont, donc, des espèces nouvelles pour le pays.

La température de l'eau a été comprise entre 24.8 et 35.5°C et l'oxygène dissout a été situé entre 3.9 et 8.8 mg/l. La conductivité et la salinité ont eu des valeurs entre 141.1 - 40700 µS/cm et respectivement 0.1 - 26.1‰. Le TDS et le pH ont pris des valeurs entre 66 - 26048 mg/l et respectivement 7.4 - 8.6.

La distribution des espèces de rotifères en corrélation avec la qualité de l'eau a montré que *Brachionus caudatus aculeatus* a été l'espèce la plus abondante et commune dans les petits étangs avec une salinité très basse. Par contre, *Lecane candida* a été trouvée uniquement dans des eaux à salinité importante. Les autres espèces du genre *Lecane* ont été retrouvées dans de l'eau douce.

La documentation des 11 nouvelles espèces de rotifères retrouvées constitue une addition à la faune des rotifères de Pakistan.

REZUMAT: Ocurența rotiferelor planctonice în deşertul Thar (Sindh, Pakistan).

Deşertul Thar este situat în extremul sud-vest al provinciei Sindh, între 24° - 26°N şi 69° - 71°E. Întreaga regiune este formată din dune de nisip şi platouri nisipoase. Regiunea Nagarparkar se distinge prin prezența colinelor de granit din Karoonjhar, de circa 300 m altitudine. În această regiune aridă, resursele de apă sunt rare. În Thar, cantitatea medie de precipitații se situează între 127 şi 381 mm/an. În timpul sezonului umed, apa din regiunea colinară se scurge pe ravene, iar o anumită cantitate este reținută în depresiuni de mică adâncime. În regiunea colinară Karoonjhar, se găsesc puține baraje amenajate pentru stocarea apei de ploaie. Cu excepția acestor resurse, populația din Thar utilizează apa subterană prin intermediul unor puțuri de mare adâncime.

În apa de ploaie, reținută pentru câteva luni în bazine, se instalează vegetație acvatică şi comunități planctonice. Comunitatea de rotifere planctonice din zona Nagarparkar a fost studiată pentru prima oară în octombrie 2008. Cu această ocazie, din 16 stații s-au colectat eşantioane de apă şi plancton. În cele mai multe cazuri, a fost vorba de apă stocată pentru trei până la şase luni şi apă din bălți temporare. Eşantioanele au fost prelevate din bălți, lacuri de baraj de mici dimensiuni şi puțuri.

Au fost identificate 36 de specii de rotifere. Genurile principale au fost *Brachionus* (13 specii), *Lecane* (5 specii), *Keratella* (trei specii), *Monostyla*, *Mytilina* şi *Platyias* (fiecare cu câte două specii). Dintre speciile identificate, 11 specii au fost găsite pentru prima dată în Pakistan, cea mai abundentă fiind *Brachionus caudatus aculeatus* Hauer. Toate speciile genului *Lecane* găsite în deşertul Thar au fost găsite pentru prima oară în Pakistan, constituind o premieră în acest sens.

Temperatura apei a fost situată între 24,8 şi 35,5°C, iar oxigenul dizolvat a fost situat între 3,9 şi 8,8 mg/l. Conductivitatea şi salinitatea au luat valori între 141,1 - 40700 µS/cm şi respectiv 0,1 - 26,1‰. STD şi pH au luat valori între 66 - 26048 mg/l şi respectiv 7,4 - 8,6.

Distribuția speciilor de rotifere, în funcție de calitatea apei, a arătat că *Brachionus caudatus aculeatus* a fost specia cea mai abundentă şi mai des întâlnită în apele dulci de mică adâncime. Din contră, *Lecane candida* a fost găsită numai în ape sărate. Celelalte specii ale genului *Lecane* au fost găsite numai în ape dulci.

Semnalarea celor 11 specii noi de rotifere găsite constituie o completare a faunei rotiferelor din Pakistan.

INTRODUCTION

Thar Desert is the largest desert of Pakistan. It is located in Southeast corner of the province of Sindh at a latitude of 24° - 26°N and a longitude of 69° - 71°E. The whole area mostly consists of barren tracts of sand dunes covered with thorny bushes. Thar Desert is part of greater Rajputana Desert which extends into the Indian state of Rajasthan. The Nagarparker area is distinguished by the presence of Karoonjhar hills, a high area of granite rocks. These hills are spread over about 20 kilometres in length and attain a height of about 1000 feet.

The Thar area has a tropical desert climate. The months of April, May and June are the hottest ones during the day. The average maximum and minimum temperatures during hot period remain 41°C to 24°C, respectively. December, January and February are comparatively the coldest months with average maximum and minimum temperatures 28°C to 9°C, respectively. Rainfall varies from year to year. Most of the rain falls in the monsoon months between June and September whereas the winter rains are insignificant. Average rainfall in Thar area is about 5 - 15 inches\year. During rainy season water from hills flows down to the ravines and some quantity is retained in shallow depressions, called “Tarai”. Few dams have been constructed across the hill streams of Karoonjhar, which also store water during rainy season. Apart from these sources, the people of Thar use subterranean water obtained from deep wells. Some of these wells contain saline water.

Literature on rotifer fauna of Pakistan is very meager. Only few references are available so far, those include, Akhtar and Ali (1976), Baloch (2000), Mahar et al. (2000) and Baloch and Soomro (2004). As no scientific study is available on the quality of water and planktonic fauna of Thar Desert therefore, present study was planned and conducted during October 2008.

MATERIALS AND METHODS

Qualitative samples of rotifers were collected using a fine mesh (55 µm) plankton net. These samples were obtained from 16 different water bodies (Tab. 1) including depressions or ponds, hill stream dams and one sample each from a spring and a well. All samples were preserved in 5% formalin on the spot and were brought to the laboratory for detailed study.

Water samples were analyzed in the field for temperature, dissolved oxygen (DO) and pH using a portable meter (pH/Oxi 340i/WTW), conductivity, total dissolved solids (TDS) and salinity were determined using a portable meter (Cond 330i/WTW). Rotifer species were carefully observed under binocular Microscope (Nikon Eclipse E200, Japan) and identifications were made following standard methods with the aid of keys and illustrations given by Ruttner-Kolisko (1974), Mizuno and Takahashi (1991) and Battish (1992).

Table 1: Physico-chemical parameters of different water bodies in Thar Desert.

Name and type of water body		Temperature	(DO)	Conductivity	Salinity	TDS	pH
		(°C)	(mg/l)	(µs/cm)	(ppm.)	(mg/l)	
1.	Anchlesar Spring	33.9	5.1	1600	0.8	779	8.0
2.	Eisai Dam	31	5	368	0.2	173	7.7
3.	Markhar Pond	32	5.2	284	0.1	135	7.8
4.	Lorlai Pond	31	8.2	250	0.1	120	7.4
5.	Chitrasar Pond	29.8	5.6	269	0.1	127	8.4
6.	Nikharjo Pond	34.8	8.5	309	0.1	147	7.5
7.	Barh Talau Pond	32.8	7.6	176.4	0.1	83	8.6
8.	Nariasar Dam	35.5	6.7	141.1	0.1	66	7.6
9.	Ghantiari Dam	28.2	5.2	1882	0.9	913	7.5
10.	Kuara Pond	31.8	7	147.8	0.1	70	7.4
11.	Bhodesar Pond	33.1	5.4	173	0.1	81	8.0
12.	Mor Dam	32.4	8.8	640	0.3	302	8.0
13.	High School Well	30.6	3.9	2760	1.4	1370	7.6
14.	Wadhrai Mori Pond	24.8	5.4	40700	26.1	26048	7.8
15.	Sangha Pond	27	4.8	153	0.1	72	7.6
16.	Ranasar Pond	32	6.5	178	0.1	85	7.8

RESULTS AND DISCUSSIONS

The water quality of most of the ponds was good and potable. However, some dams, a well and a spring were slightly brackish (Tab. 1). A small pond (station 14) that receives seawater through a channel from Rann of Kuchchh, showed a high salinity value of 26.1 ppm.

A total of 36 rotifer species belonging to 15 genera were recorded (Fig. 1; Tab. 2). The *Brachionus* genus was dominant (13 taxa) followed by *Lecane* (five species) and *Keratella* (three species). There are eleven rotifer species which are recorded for the first time from Pakistan (Tab. 2). The Brachionids, *B. caudatus* var *aculeatus* was most dominant species and occurred in most of the soft water bodies. The other species like *B. forficula* f. *reducta*, *B. forficula* f. *angularis*, *B. quadridentatus* and *B. calyciflorus* f. *amphiceros* were also commonly present. Whereas, *B. plicatilis*, *B. leydigi* var. *rotandus*, *B. urceolaris*, *B. calyciflorus* f. *dorcas* and *B. rubens* were uncommon and restricted to single water body. *B. plicatilis* was restricted to high salinity only (Tab. 2).

There occurred five species of *Lecane* in which *L. aculeata*, *L. hastata*, *L. leontina* and *L. ungulata* were found in soft water bodies while *L. candida* was seen in saline waters only. *L. leontina* was more common than other *Lecane* species. All the occurring *Lecane* species are recorded for the first time from Pakistan. The other newly recorded rotifers were *B. budapestinensis*, *B. quadridentatus*, *Scaridium longicaudum* and *Tetramastix opoliensis*. These species showed rare presence.

Rotifer genera like *Lecane* and *Brachionus* are zoogeographical considered as tropic-centred (thermophil) while *Cephalodella* and *Synchaeta* are cold water species (Segers, 2001). The dominance of the genus *Brachionus* is in agreement with the previous studies undertaken in Pakistan (Baloch and Soomro, 2004; Baloch et al., 2008) where genus *Brachionus* was dominant followed by *Lecane* or *Keratella*. In riverine system however, *Keratella* was found dominant (Baloch, 2000). *Asplanchna priodonta* occurred in all types of water bodies, but contributed with very low populations.

The *Brachionus* was found as dominant genus in tropical areas like in Malaysia (Fernando and Zankai, 1981) and West Africa (Green, 1972; Fafioye and Omoyinmi, 2006). On the other hand, *Lecane* genus dominated in tropical areas in India (Sharma, 2009), Thailand (Sanoamuang, 1998; Savatenalinton and Segers, 2005) and Guatemala and Belize (Garcia-Morales and Elias-Guterrez, 2007), where genus *Lecane* was always dominant and *Brachionus* never dominated. In subtropical Argentina also *Lecane* genus was found dominant (Paggi, 2001).

Rotifers are considered highly adaptive to a wide range of freshwater conditions (Hutchinson, 1967). The dominance of *Lecane* with paucity of *Brachionus* is probably due to special characteristics of water bodies as in Loktak Lake, where *Brachionus* paucity was attributed to acidic water bodies (Sharma, 2009). In temperate lakes *Brachionus* did not appear and *Lecane* rarely occurred. On the contrary *Conochilus* and *Ploesoma* were commonly found (Baloch et al., 1998a, 1998b). *Conochilus* were strongly associated with low trophic state whereas *Brachionus* with high trophic state (Duggan et al., 2001).

The literature on rotifer fauna of Pakistan is very meager hence the documentation of eleven newly recorded rotifer species from Thar Desert is attributed to the paucity of literature on Rotifera.

Table 2: List of rotifer species occurring at different stations in Thar Desert; P - pond, D - dam, S/W - spring or weel, + - presence.

Rotifera	P	D	S/W	Location
Asplanchna priodonta Gosse	+	+	+	1, 8
Brachionus angularis Gosse	+	+		2, 16
B. budapestinensis Dady		+		12
B. calyciflorus f. *dorcas* (Gosse)		+		2
B. calyciflorus f. *amphiceros* (Ehrenberg)	+	+		2, 12
Brachionus caudatus var. *aculeatus* Hauer	+			4, 5, 6, 7, 16
Brachionus caudatus (Barrois and Daday)	+			5, 7
Brachionus forficula f. *angularis* (Sudzuki)		+		12
Brachionus forficula f. *reducta* (Grese)	+	+		10, 12
Brachionus leydigi var. *rotandus* (Rousselet)	+			2
Brachionus plicatils (O. F. Muller)	+			14
Brachionus quadridentatus Hermamm	+			5
Brachionus rubens Ehrenberg	+			5
Brachionus urceolaris O. F. Muller	+			10
Euchlanis dilatata Ehrenberg	+	+		4, 7, 10
Filinia longiseta (Ehrenberg)	+	+		1, 5
Hexarthra mira Hudson	+	+		2, 3, 8, 9
Keratella cochlearis (Gosse)	+			3
Keratella quadrata var. *heimalis* Carlin		+		2
Keratella valga (Ehrenberg)	+	+		4, 6, 7, 8
Lecane aculeata (Jakubski)		+		10
Lecane candida Harring and Myers	+	+	+	9, 13, 14
Lecane hastata (Murray)	+			10
Lecane leontina (Turner)	+	+		2, 8, 5
Lecane ungulata (Gosse)	+			16
Monostyla bulla Gosse	+	+		2, 3
Monostyla furcata Murray	+			10
Mytilina ventralis (Ehrenberg)	+			10
Mytilina ventralis var. *brevispina* Ehrenberg	+			10
Platyias patulus (O. F. Muller)	+			6
Platyias quadricornis var. *brevispinus* Daday	+			12
Polyarthra trigla	+	+		2, 5, 12
Scaridium longicaudum (O. F. Muller)	+			10
Tetramastix opoliensis Zacharias	+			8
Tetsudinella patina (Hermann)	+	+		13
Trichocerca capucina (Wiezeuski and Zachaias)	+			10

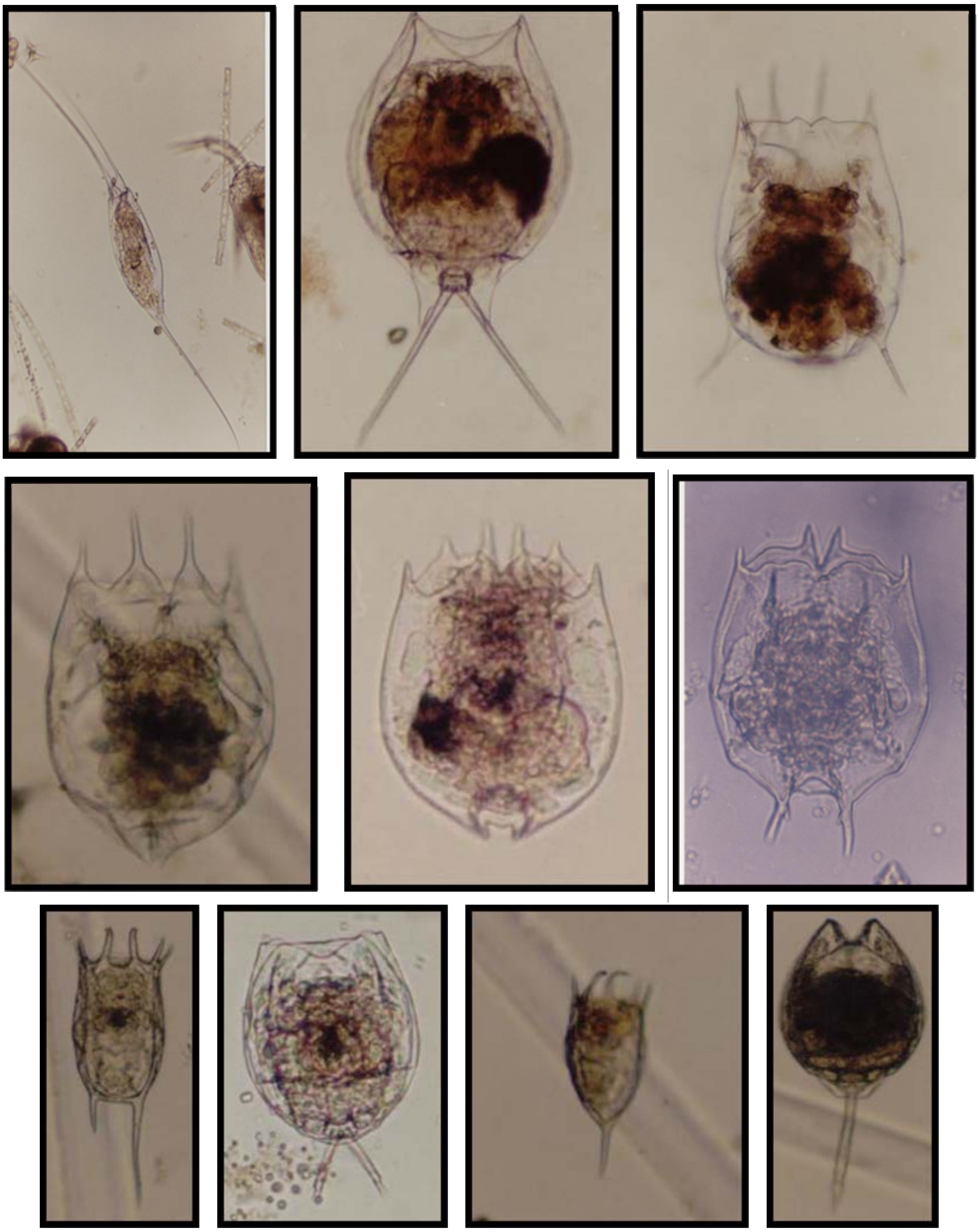

Figure 1: Some rotifer species occurring in the Thar Desert:
Top from left, *Tetramastix opoliensis*, *Lecane leontina*, *B. calyciflorus* f. *amphiceros*.
Centre from left, *Brachionus calyciflorus* f. *dorcas*, *Brachionus rubens*, *B. caudatus* var. *aculeatus*.
Bottom from left, *Keratella valga*, *Lecane candida*, *Keratella cochlearis*, *Monostyla bulla.*

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THE MARAMUREŞ MOUNTAINS NATURE PARK (ROMANIA) MAYFLY (INSECTA, EPHEMEROPTERA) COMMUNITIES DIVERSITY ANALYSE

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KEYWORDS: Romanian Carpathians, Maramureş Mountains, Vişeu River basin, Ţâşla River, Vaser River, Ruscova River, Ephemeroptera larvae communities.

ABSTRACT

This study presents the description of the structure and diversity analyse of Ephemeroptera larvae communities of the Vişeu River basin. The paper is based on quantitative benthic macroinvertebrates and mayflies qualitative samples, sampled in 2007 (June-September), in 24 sampling stations.

In the reference zone 24 mayfly species were identified, belonging to 12 genera and six families, representing 33.33% of the Romanian Ephemeroptera fauna.

The mayfly larvae communities present the highest diversity on the Ruscova River, on the lower Vaser River and on the Vişeu River - upper cours (Vişeuţ Stream), downstream the confluence with Ruscova River and in the Vişeu Gorge. In these lotic sectors the aquatic habitats are in good state, close to the natural one and the anthropogenic impact in this area is not significant. These areas should to be managed for the aquatic biodiversity conservation.

The mayflay communities present the lowest diversity in the Ţâşla Stream two km upstream the confluence with Bălăsâna and in the Vişeu River 50 m upstream the Moisei locality and one km downstream Vişeu de Jos locality. In these sectors ecological restoration measurements should be implemented for the lotic habitats, these being degraded due to the pollution, respectively due to the substratum exploitation.

ZUSAMMENFASSUNG: Diversitätsanalyse der Eintagsfliegen-Gemeinschaften (Insecta, Ephemeroptera) des Naturparks Munţii Maramureşului/Maramuresch - Gebirge (Rumänien).

Die Arbeit umfasst eine Beschreibung der Struktur der Larvengemeinschaften von Eintagsfliegenarten aus dem Einzugsgebiet des Vişeu-Flusses und die Analyse der Diversität dieser Gemeinschaften. Die in der Arbeit vorgestellten Ergebnisse beruhen auf der Auswertung von quantitativen Zoobenthos- und qualitativen Ephemeropterenproben, die 2007 (Juni-September) an 24 Probestellen entnommen wurden.

Im Referenzgebiet wurden 24 Ephemeropterenarten festgestellt, die zu zwölf Gattungen und sex Familien gehören und 33,33% der in Rumänien bekannten Ephemeropterenarten ausmachen.

Die Gemeinschaften der Ephemeropterenlarven haben eine hohe Diversität im Ruscova-Fluss, im Unterlauf des Vaser-Flusses sowie im Oberlauf des Vişeu (Vişeuţ-Bach), unterhalb der Einmündung des Ruscova-Flusses und in der Vişeu-Klamm. In diesen Abschnitten sind die Gewässerhabitate in einem guten, naturnahen Zustand, wobei der menschliche Einfluss sehr gering ist. Daher muss hier das Management im Sinne der Erhaltung der aquatischen Biodiversität durchgeführt werden.

Die geringste Biodiversität an Ephemeropteren wurde im Ţâşla-Fluss zwei km oberhalb des Zusammenflusses mit dem Bălăsâna-Bach und im Vişeu 50 m oberhalb von Moisei und ein km unterstrom der Ortschaft Vişeu de Jos/Unterwischau festgestellt. In diesen Abschnitten sind Maßnahmen zur Wiederherstellung der lotischen Habitate erforderlich, da sie sich durch die Verschmutzung, bzw. durch Substratabbau in einem schlechten Zustand befinden.

REZUMAT: Analiza diversităţii comunităţilor de efemeroptere (Insecta, Ephemeroptera) din Parcul Natural Munţii Maramureşului (România).

Lucrarea prezintă descrierea structurii comunităţilor larvelor de efemeroptere din bazinul hidrografic Vişeu şi analiza diversităţii acestor comunităţi. Datele prezentate în lucrare se bazează pe probe cantitative de bentos şi calitative de efemeroptere, colectate în anul 2007 (iunie - septembrie) din 24 staţii de prelevare.

În zona de referinţă, au fost identificate 24 specii de efemeroptere, din 12 genuri şi şase familii, acestea reprezintă 33,33% dintre speciile de efemeroptere semnalate în România.

Comunităţile larvelor de efemeroptere prezintă o diversitate mare în râul Ruscova, în cursul inferior al Vaserului şi în Vişeu - cursul superior (pârâul Vişeuţ), aval de confluenţa cu Ruscova şi în Cheile Vişeului. În aceste sectoare de râu, habitatele acvatice prezintă o stare bună, apropiată de cea naturală, iar impactul antropic este nesemnificativ. Aceste zone trebuie gestionate în sensul conservării biodiversităţii acvatice.

Cea mai mică diversitate a efemeropterelor se înregistrează în Ţâşla, la doi km amonte de confluenţa cu Bălăsâna şi în Vişeu la 50 m amonte de localitatea Moisei şi la un km aval de localitatea Vişeu de Jos. În aceste sectoare, se impun măsuri de redresare a habitatelor lotice, acestea fiind degradate datorită poluării, respectiv exploatării substratului.

INTRODUCTION

This study presents the description of the structure and diversity analyse of Ephemeroptera larvae communities of the Vişeu River basin.

The information resulted from this study will be useful for the Maramureş Mountains Nature Park management plan attainment.

Vişeu River is a second order tributary of Danube localized in the north of Romania. The most part of the Vişeu River basin was included in the Maramureş Mountains Nature Park.

Vişeu River has its sources in the Rodna Mountains, 80 km river length, 1,606 km^2 basin surface and a multiannual average flow at the confluence with Tisa River of 30.7 m^3/s. Some of the most important tributaries of Vişeu River are (from upstream to downstream): Ţâşla River (20 km length, 106 km^2 drain surface), Vaser River (42 km length, 422 km^2 drain surface, 9 m^3/s multiannual average flow at the confluence with Vişeu River) and Ruscova River (39 km length, 435 km^2 drain surface**,** 11 m^3/s multiannual average flow at the confluence with Vişeu River) (Roşu, 1980; Badea et al., 1983; Posea et al., 1982).

Actual hydrobiological research in this area are few, in this respect we have to mention the study concerning the benthic macroinvertebrates and fish along Vişeu River, realised by Staicu, Bănăduc and Găldean (1998). Until the present, coenological studies regarding the mayfly larvae of the Vişeu River basin were not made.

MATERIAL AND METHODS

This paper is based on quantitative benthic macroinvertebrates and mayflies qualitative samples, sampled in 2007 (June-September), at 24 sampling stations (Fig. 1).

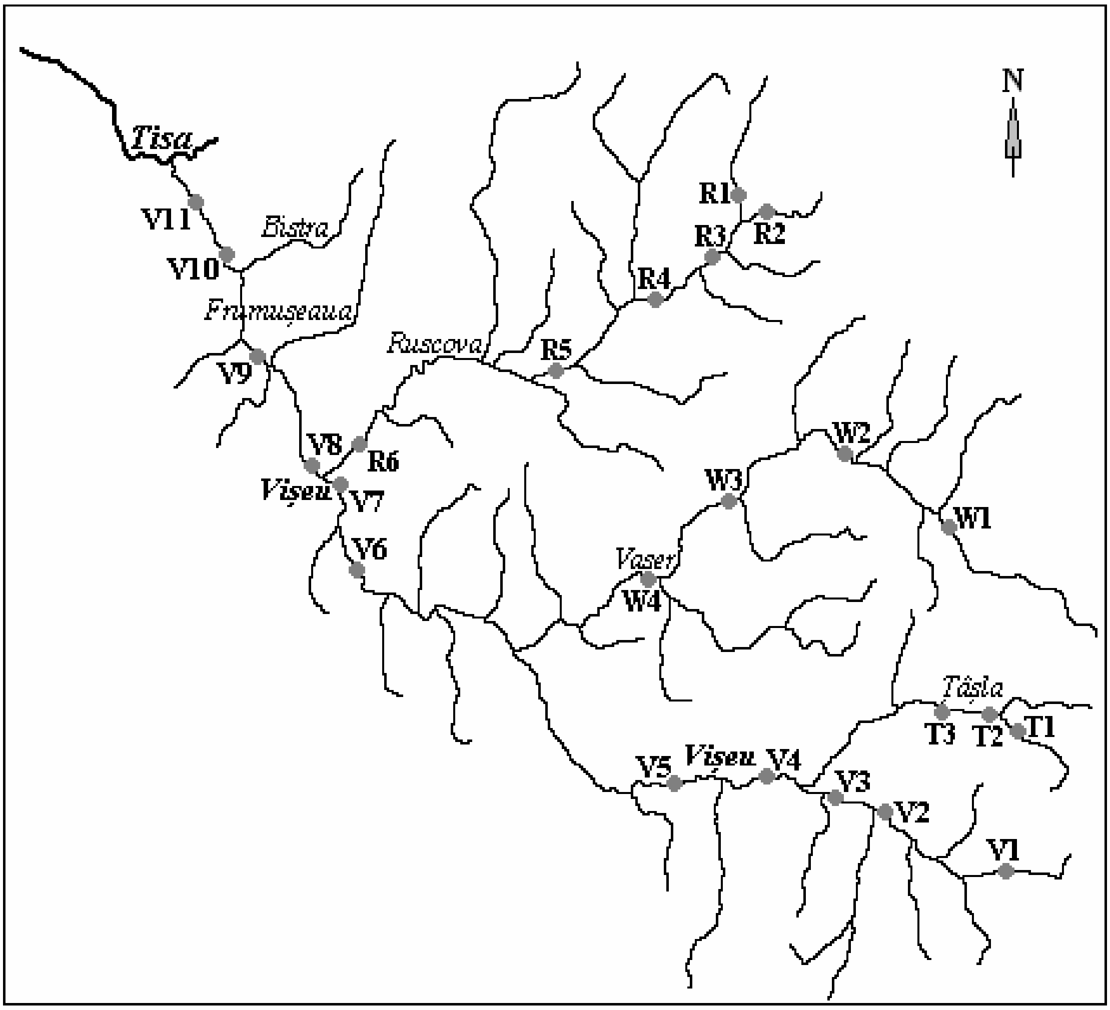

Figure 1: The Vişeu River basin sampling stations (V1 - V11, Vişeu Basin; T1 - T3, Ţâşla Basin; W1 - W4, Vaser Basin; R1 - R6, Ruscova Basin) layout.

The sampling stations were chosen according to the valley morphology, the confluence with the main tributaries and the human impact types and degrees on the river sectors - hydro-technical works, pollution sources, and over exploitation of the riverbed mineral resource and exploitation of riverine lands, in order to highlight the Ephemeroptera species diversity, and also the variation of the benthic macroinvertebrate communities structure. At each site were sampled quantitative samples from five points, in order to highlight the micro-habitats specific diversity. In the study period 260 quantitative benthic macroinvertebrates samples were sampled and analysed. The benthic macroinvertebrates quantitative samples were carried out with an 887 cm^2 surface Surber Sampler, with a 250 µm mesh net. The sampled biological material was fixed in 4% formaldehyde solution at which $NaHCO_3$ was added.

The analysed biological material included 4,160 Ephemeroptera larvae in life cycle periods which allowed their identification to the species level.

For the quantitative structure description of the mayfly communities we have used the relative abundance (A%) and the statistical density (Ds). For the mayfly communities' diversity quantifying, the heterogeneity index Simpson (Gomoiu and Skolka, 2001) were determined, based on the quantitative samples.

To analyse and quantify the association degree among species, the average square contingency coefficient (CCM) values and the Cole interspecific association coefficient were determined; to test which of the species are statistically significantly associated the χ^2 test was used for the probability level of 5% ($\chi^2 > 3.89$) (Krebs, 1989).

RESULTS AND DISCUSSION

24 mayfly species were found, belonging to 12 genera and six families.

The identified mayfly species list of the Maramureş Mountains Nature Park, with the specific sampling sites (V1 - V11, T1 - T3, W1 - W4, R1 - R6 - sampling stations):

Fam. Leptophlebiidae

Leptophlebia marginata (Linnaeus 1767) - W4, R6
Habrophlebia fusca (Curtis 1834) - R6
Habroleptoides modesta (Hagen 1864) - V3

Fam. Ephemerellidae

Serratella ignita (Poda 1761) - V3, V8, V9, V11, W4, R5, R6
Ephemerella notata (Eaton 1887) - R4, R6

Fam. Caenidae

Caenis robusta Eaton 1884 - V7
Caenis horaria (Linnaeus 1758) - R6
Caenis macrura Stephens 1835 - V8

Fam. Baëtidae

Baëtis rhodani (Pictet 1843) - V1, V2, V3, V4, V6, V7, V9, V10, T1, T2, T3 W3, W4, R1, R2, R3, R4, R5, R6
Baëtis vernus Curtis 1834 - V1, V2, V3, V4, V5, V11, T2, T3, W2, W3, R1, R2, R3, R4
Baëtis alpinus (Pictet 1843) - V2, W1, W2, R5
Baëtis lutheri Müller-Liebenau 1967 - W4, R6
Baëtis fuscatus (Linnaeus 1761) - V11

Fam. Siphlonuridae

Siphlonurus aestivalis Studemann, Tomka, Landolt 1992 - V7

Fam. Heptageniidae

Heptagenia flava Rostock 1878 - Frumuşeaua River (only in qualitative samples)
Ecdyonurus venosus (Fabricius 1775) - V1, V8, W4, R1, R5, R6
Ecdyonurus alpinus Hefti, Tomka and Zurwerra 1987 - V1
Ecdyonurus dispar (Curtis 1843) - V7, V8, R4, R6
Ecdyonurus picteti (Meyer-Dür 1864) - R1, R2
Ecdyonurus helveticus (Eaton 1877) - R4
Ecdyonurus torrentis (Kimmins 1942) - R6
Epeorus sylvicola (Pictet 1865) - R5
Rhithrogena semicolorata (Curtis 1834) - V2, V3, V4, V8, V10, V11, T2, W1, W2, W3, W4, R1, R2, R3, R4, R5, R6
Rhithrogena picteti Sowa 1971 - V1, V2, W1, R1, R2

In the reference zone, the mayflies had the highest species diversity (11 species) in the Ruscova River 50 m upstream the confluence with Vişeu River (R6) (Tab. 1).

The mayflay assemblage present the lowest species diversity (one species) in the Ţâşla Stream, two km upstream the confluence with Bălăsâna (T1) - sector degraded due to the pollution coming from the local mining exploitations and in the Vişeu River 50 m upstream the Moisei locality (V5) and one km downstream Vişeu de Jos (V6) - sectors degraded due to the impact generated by the riverine localities Borşa, Vişeul de Sus and Vişeul de Jos (Tab. 1).

The Ephemeroptera larvae communities with the highest heterogeneity (according to the Simpson Index) are present in the Ruscova River, in the lower Vaser River and in the Vişeu River - upper course (Vişeuţ Stream - V1, V2), downstream confluence with Ruscova River (V8) and in the Vişeu Gorge (V11).

The mayfly species with the widest distribution in the Vişeu River basin are *Baëtis rhodani* (present in 19 of the 24 studied lotic sectors) and *Rhithrogena semicolorata* (present in 17 of the 24 studied lotic sectors). The species with the most restricted distributions are *Habrophlebia fusca*, *Caenis horaria*, *Ecdyonurus torrentis*, *Ecdyonurus picteti*, *Ecdyonurus alpinus*, *Habroleptoides modesta*, *Caenis robusta*, *Siphlonurus aestivalis* and *Baëtis fuscatus* sampled only in the one of the 24 studied lotic sectors.

The numerical weight of the mayfly larvae in the benthic macroinvertebrate comunities vary, between the reference area with 93.24% in the Vişeu River 50 m upstream the Moisei locality (V5) and 7.14% in upper course of Vaser River (W2) (Tab. 1).

Table 1: The structure of mayfly communities present in the 24 lotic sectors analysed in the Maramureş Nature Park and the numerical weight of this systematic group in the benthic macroinvertebrate communities (P - Ephemeroptera numerical weight in the benthic macroinvertebrate communities structure, Ds - Ephemeroptera average density, A% - relative abundance of each species).

Sampling station	P (%)	Ds (number of individuals/m^2)	Inverted Simpson index (1-l)	The specific structure of the ephemeroptera larvae community	A (%)
V1	18.54	54.17	0.791	*Baëtis rhodani*	22.22
				Baëtis vernus	16.67
				Ecdyonurus venosus	38.89
				Ecdyonurus alpinus	11.11
				Rhithrogena picteti	11.11
V2	48.98	135.29	0.714	*Baëtis rhodani*	40.63
				Baëtis vernus	34.38
				Baëtis alpinus	12.5
				Rhithrogena semicolorata	9.37
				Rhithrogena picteti	3.12
V3	48.00	135.28	0.558	*Habroleptoides modesta*	3.17
				Serratella ignita	1.59
				Baëtis rhodani	57.14
				Baëtis vernus	3.18
				Rhithrogena semicolorata	34.92
V4	69.01	552.42	0.669	*Baëtis rhodani*	27.42
				Baëtis vernus	40.32
				Rhithrogena semicolorata	32.26

Table 1: continued.

Sampling station	P (%)	Ds (number of individuals/m^2)	Inverted Simpson index (1-l)	The specific structure of the ephemeroptera larvae community	A (%)
V5	93.24	129.65	0	*Baëtis vernus*	100
V6	25.55	129.65	0	*Baëtis rhodani*	100
V7	50.92	625.70	0.582	*Caenis robusta* *Baëtis rhodani* *Siphlonurus aestivalis* *Ecdyonurus dispar*	17.39 47.83 13.04 21.74
V8	18.95	163.47	0.784	*Serratella ignita* *Caenis macrura* *Ecdyonurus venosus* *Ecdyonurus dispar* *Rhitrogena semicolorata*	40 15 10 15 20
V9	24	67.64	0.441	*Serratella ignita* *Baëtis rhodani*	29.41 70.59
V10	40	56.37	0.467	*Baëtis rhodani* *Rhithrogena semicolorata*	70 30
V11	22.43	135.29	0.724	*Serratella ignita* *Baëtis vernus* *Baëtis fuscatus* *Rhithrogena semicolorata*	18.52 22.22 44.45 14.81
T1	12.56	5.67	0	*Baëtis rhodani*	100
T2	25.0	50.73	0.467	*Baëtis rhodani* *Baëtis vernus* *Rhithrogena semicolorata*	70.83 20.83 8.34
T3	13.79	22.55	0.429	*Baëtis rhodani* *Baëtis vernus*	75 25
W1	37.5	33.82	0.628	*Rhithrogena semicolorata* *Rhithrogena picteti* *Baëtis alpinus*	39.13 47.83 13.04
W2	7.14	11.27	0.668	*Baëtis vernus* *Baëtis alpinus* *Rhithrogena semicolorata*	46.16 26.92 26.92
W3	41.82	129.65	0.706	*Baëtis rhodani* *Baëtis vernus* *Ecdyonurus venosus* *Rhithrogena semicolorata*	33.33 36.67 3.33 26.67
W4	57.90	248.03	0.834	*Leptophlebia marginata* *Serratella ignita* *Baëtis rhodani* *Baëtis lutheri* *Ecdyonurus venosus* *Rhithrogena semicolorata*	16.67 9.52 21.43 14.29 26.19 11.9

Table 1: continued.

Sampling station	P (%)	Ds (number of individuals/m²)	Inverted Simpson index (1-l)	The specific structure of the ephemeroptera larvae community	A (%)
R1	53.34	45.10	0.733	*Baëtis rhodani*	5.71
				Baëtis vernus	14.29
				Ecdyonurus venosus	11.43
				Ecdyonurus picteti	2.86
				Rhithrogena semicolorata	42.86
				Rhithrogena picteti	22.85
R2	39.37	428.4	0.757	*Baëtis rhodani*	23.81
				Baëtis vernus	42.86
				Ecdyonurus picteti	9.52
				Rhithrogena semicolorata	14.29
				Rhithrogena picteti	9.52
R3	60.14	484.78	0.532	*Baëtis rhodani*	65.38
				Baëtis vernus	19.24
				Rhithrogena semicolorata	15.38
R4	50.0	473.51	0.787	*Ephemerella notata*	8.33
				Baëtis rhodani	36.11
				Baëtis vernus	13.89
				Ecdyonurus dispar	11.11
				Ecdyonurus helveticus	5.56
				Rhithrogena semicolorata	25
R5	33.87	236.75	0.695	*Serratella ignita*	9.09
				Baëtis rhodani	27.27
				Baëtis alpinus	9.09
				Ecdyonurus venosus	2.27
				Epeorus sylvicola	4.55
				Rhithrogena semicolorata	47.73
R6	41.48	411.50	0.907	*Leptophlebia marginata*	8.33
				Habrophlebia fusca	5
				Serratella ignita	11.67
				Ephemerella notata	6.67
				Caenis horaria	5
				Baëtis rhodani	18.33
				Baëtis lutheri	6.67
				Ecdyonurus dispar	13.33
				Ecdyonurus venosus	10
				Ecdyonurus torrentis	5
				Rhithrogena semicolorata	10

Analysing the similarity of the mayfly larvae communities in the 24 sampled lotic sectors, on the basis of the species relative abundance (Tab. 1), allows these communities to be grouped in 11 classes (Fig. 2).

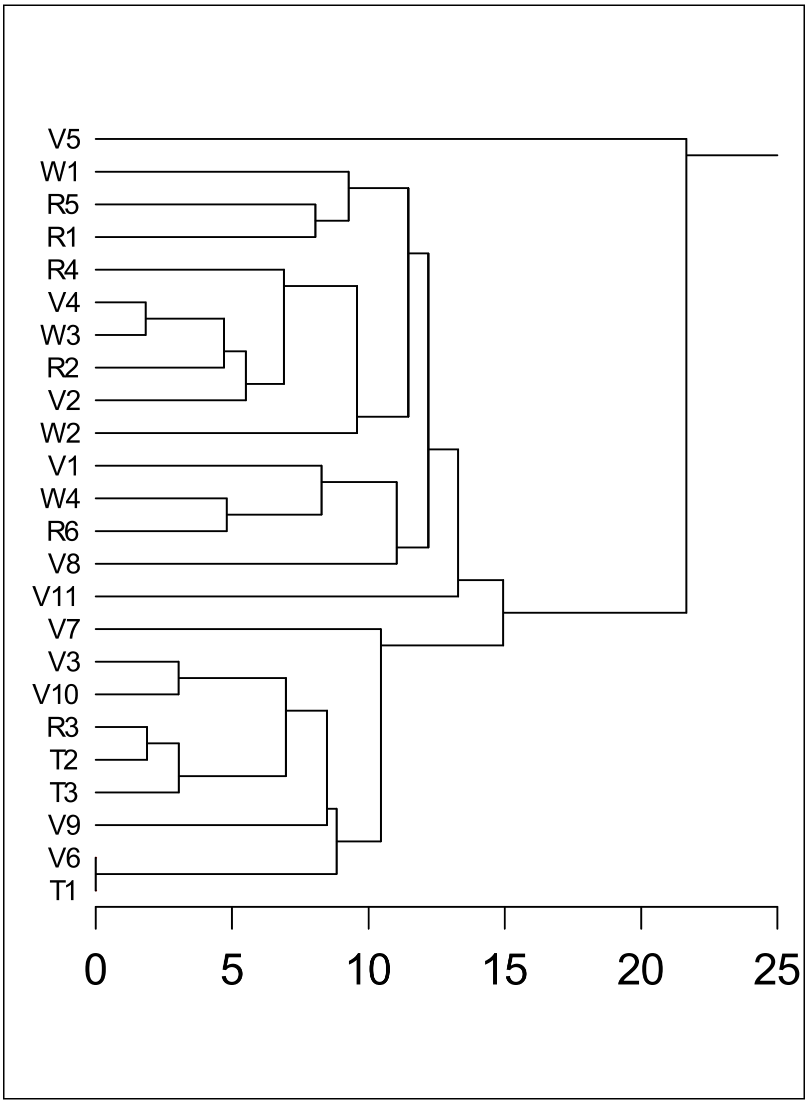

Figure 2: Tree diagram based of the mayfly species relative abundance (A%), of the 24 analysed lotic sectors (Euclidian distances, V1 - V11, T1 - T3, W1 - W4, R1 - R6; sampling stations).

The present analysis of the contingency tables in the cases of the total of 24 mayfly species identified in the Vişeu River basin, taken as pairs, based on the Cole interspecific association coefficient (C) and on the average square contingency coefficient (CCM), indicate significant positive associations, for a significance level of 5%, among the species: *Baëtis rhodani* and *Baëtis vernus* (χ^2 = 4.863, CCM = 0.512, C = 0.890 ± 0.323), *Baëtis rhodani* and *Rhithrogena semicolorata* (χ^2 = 5.780, CCM = 0.444, C = 0.538 ± 0.192), *Ecdyonurus venosus and Baëtis rhodani* (χ^2 = 3.956, CCM = 0.372, C = 0.583 ± 0.267).

CONCLUSIONS

The studied Ephemeroptera group fauna of the Vişeu River basin presents a relative high species diversity. In the studied area a total of 24 mayfly species belonging to 12 genera and six families were identified, representing 33.33% of the Romanian Ephemeroptera fauna.

The mayfly larvae communities present the highest diversity in the Ruscova River, also in the lower Vaser River and in the Vişeu River - its upper course (Vişeuț Stream - V1, V2), downstream the confluence with Ruscova River (V8) and in the Vişeu Gorge (V11). In all these studied lotic sectors the aquatic habitats are considered as being in a good state, close to the natural one and the anthropogenic impact in this areas is not a significant one. These areas should be managed for the aquatic biodiversity conservation.

The mayfly assemblage present the lowest diversity in the Țâşla Stream two km upstream the confluence with Bălăsâna Stream (T1) and in the Vişeu River 50 m upstream the Moisei locality (V5) and one km downstream Vişeu de Jos locality (V6). In all these sectors ecological rehabilitation measurements are needed for the lotic habitats, these being degraded due to pollution, respectively due to the river courses substratum exploitation.

ACKNOWLEDGEMENTS

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MULTIDISCIPLINARY EVALUATION OF THE FUNCTION AND IMPORTANCE OF THE SMALL WATER RESERVOIRS: THE BIODIVERSITY ASPECT

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KEYWORDS: Slovakia, Carpathians, water reservoirs, biodiversity, phytobentos, macrophytes, benthic and pelagic invertebrates, mollusks, fish, birds.

ABSTRACT

Small water reservoirs are the very important landscape elements for effective water management. Although these man-made artificial biotopes change the proportion of lotic and lentic habitats and thus influence the species community structure, them secondarily offer a broader scale of microhabitats and, in general, can significantly influence the fauna’s sustainable development. The evaluation of the function and importance of small water reservoirs in the biodiversity development on all levels is still unappreciated. In this case, preliminary results of the study on six small water reservoirs in West Slovakia are presented.

The research has covered the major species groups (phytobentos, macrophytes, benthic and pelagic invertebrates, mollusks, fish and birds). As the first step, we are focusing on the description of the biodiversity patterns within the particular water reservoirs followed by the analyses of species links to the environmental variables using the multidimensional methods (neural networks, ordination methods and generalized linear methods) as the second step. The third step covers the compilation of obtained results and evaluation of the function and importance of the small water reservoirs.

The major benefits of this study are as follows: (i) significant improvement of the knowledge on the biodiversity of aquatic ecosystems influenced by small water reservoirs, (ii) implementation of the innovative methods of the multidisciplinary ecological research, (iii) support for sustainable development of the biodiversity in artificial biotopes, development of the multidisciplinary network of researchers and experts from the applied sphere, (iv) effective application of outputs in the ecological management oriented to the sustainable development of the artificial aquatic ecosystems in combination with their primary use and implementation of the results gained at the international scale.

RÉSUMÉ: Evaluation multidisciplinaire de la fonction et de l'importance des étangs. L'aspect de la biodiversité.

Les petits corps d'eau sont des éléments de paysage très importants pour la gestion efficace de l'eau. Même si ces biotopes artificiels changent les proportions des habitats lotiques et lentiques, elles offrent également une échelle plus large de microhabitats et, généralement, elles peuvent influencer de manière signifiante le développement durable de la faune. L'évaluation de la fonction et de l'importance des étangs dans le développement de la biodiversité à tous niveaux est encore sous appréciée. Dans ce cas, nous présentons les résultats préliminaires d'une étude effectuée sur 6 étangs anthropiques de l'Ouest de la Slovakie.

La recherche a compris les groupes spécifiques majeurs (phytobentos, macrophytes, invertébrés benthiques et pélagiques, mollusques, poissons et oiseaux). En première étape, nous nous sommes concentrés sur la description des modèles de biodiversité de ces étangs suivie, en deuxième étape, par l'analyse des relations entre les espèces et les variables de l'environnement en utilisant des méthodes multidimensionnelles (des réseaux neuronaux, des méthodes d'ordination et des méthodes linéaires généralisées); la troisième étape comprend la compilation des résultats obtenus et l'évaluation de la fonction et de l'importance des étangs.

Les bénéfices majeures de cette étude sont les suivantes: (i) amélioration significative de la connaissance de la biodiversité des écosystèmes aquatiques sous l'influence des étangs, (ii) l'implémentation des méthodes nouvelles dans la recherche écologique multidisciplinaire, (iii) un support pour le développement durable de la biodiversité dans les biotopes artificiels, le développement des réseaux multidisciplinaires des chercheurs et d'experts de la sphère appliquée, (iv) l'application effective des résultats dans la gestion écologique orientée vers le développement durable des écosystèmes aquatiques artificielles combinée à leur utilisation primaire et l'implémentation à l'échelle internationale des résultats obtenus.

REZUMAT: Evaluare multidisciplinară a funcţiei şi importanţei iazurilor: aspectul biodiversităţii.

Iazurile şi heleşteele sunt elemente peisagistice foarte importante pentru gestiunea eficientă a apei. Deşi aceste biotopuri artificiale modifică proporţiile habitatelor lotice şi lentice şi influenţează astfel structura comunităţilor biologice, ele oferă secundar o gamă mai largă de microhabitate, în general, pot influenţa de manieră semnificativă dezvoltarea durabilă a faunei. Evaluarea funcţiei şi importanţei iazurilor în dezvoltarea biodiversităţii la toate nivelurile este încă neglijată. În acest caz, sunt prezentate rezultatele preliminare ale unui studiu pe şase iazuri mici, din vestul Slovaciei.

Cercetările au acoperit grupele majore de specii (fitobentos, macrofite, nevertebrate bentice şi pelagice, moluşte, peşti şi păsări). Într-o primă etapă, ne-am concentrat pe descrierea modelelor de biodiversitate, aplicate la iazurile studiate, apoi am efectuat analiza relaţiilor interspecifice cu ajutorul metodelor multidimensionale (reţele neuronale, metode de ordonare şi metode liniare generalizate) care au constituit a doua etapă a studiului. A treia etapă a acoperit compilarea datelor obţinute şi evaluarea funcţiei şi importanţei iazurilor.

Beneficiile principale ale studiului sunt următoarele: (i) ameliorarea semnificativă a cunoaşterii biodiversităţii ecosistemelor acvatice sub influenţa iazurilor, (ii) implementarea de metode noi, inovatoare în cercetarea ecologică multidisciplinară, (iii) suport pentru dezvoltarea durabilă a biodiversităţii în biotopurile artificiale, dezvoltarea de reţele multidisciplinare de cercetători şi experţi din sfera aplicată, (iv) aplicarea efectivă a rezultatelor gestiunii ecologice, orientate spre dezvoltare durabilă a ecosistemelor acvatice artificiale, în paralel cu utilizarea lor primară şi implementarea rezultatelor obţinute la scară internaţională.

INTRODUCTION

Small water reservoirs

The artificial small water reservoirs were built on small streams in the first place for the purpose of their potential for irrigation and as flood protection. But also their contribution to the nature protection through the water self-purification is not negligible (Ertl, 1960; Illyová and Štefková, 1995). They serve as stability element of the landscape (Fulín et al., 1995).

Small water reservoirs are very important landscape elements for effective water management. Although these man-made artificial ponds change the proportion of lotic and lenitic habitats and thus influence the species community structure, secondarily offer a broader scale of microhabitats and, in general, can significantly influence the fauna sustainable development. The evaluation of the function and also of the importance of the small water reservoirs on the biodiversity development on all levels is still unappreciated.

Ertl (1960) and Vranovský (1985) dealt with the limnology of small reservoirs and fish ponds of the region Záhorie (west part of Slovakia). Works of Timková and Hudec (1997), Hudec and Hucko (2002) deal with zooplankton of small water reservoirs of east Slovakia.

The aim of the project SAV-FM-EHP-2008-03-04 is to identify the function of small water reservoirs in the system of aquatic biotopes of the Slovakia in respect to preservation and development of water biota diversity. The results should enable to model the development of the biodiversity for the purpose of reservoir management with the respect of ecological value of the reservoirs. Management should involve the primary purposes of reservoirs together with effective protection of the nature. This paper brings first results of limnology of selected six reservoirs of the west Slovakia.

MATERIALS AND METHODS

Studied area

Six small water reservoirs (south-west Slovakia) and their inlets and outlets are localised on the both sides of the Small Carpathians. They differ in respect to the land use and anthropic pressure. Three reservoirs (Doľany, Suchá nad Parnou and Dolné Dubové) belong to river Váh Basin. Intensive agricultural land use is characteristic for them, leading to runoff from adjacent agricultural land and settlements, reflected in chemical agents content (Tab. 1).

Other three reservoirs (Kuchyňa, Lozorno and Vý점vrat) belong to river Morava Basin, lies at the higher altitude and are not directly under human pressure. Inlets catchments lie within the protected area. Also chemical parameters suggest different trophic status of reservoirs of these two geographical areas (Tab. 1).

Table 1: Basic characteristics of water in all studied reservoirs.

	Morava River basin			Váh River basin		
	Lozorno	Kuchyňa	Vývrat	Dol'any	Suchá n/P.	D. Dubové
altitude	219 m	260 m	237 m	195 m	179 m	191 m
pH	8.44	8.38	8.45	8.11	8.22	8.27
BSK	2.2	2.7	3.6	10	6.1	6.1
N contect	0.78	1.59	0.85	2.67	1.32	2.28
P content	0.04	0.05	0.06	0.25	0.17	0.11
Chlorophyll a (μg.l-1)	4.1	10.8	26.7	180.3	87.8	39.1

All samples were taken in September and October 2008.

Zooplankton

Quantitative and qualitative samples of zooplankton were taken from medial and littoral zone of the lakes. Taking, fixing and processing was done according Hrbáček et al. (1972). For quantitative assessment abundance ($N.L.^{-1}$) and biomass ($g.m.^{-3}$) was set.

Macrozoobenthos

Benthic invertebrates were sampled using kick-net (mesh size 500 μm) with retractable frame 25 x 25 cm for quantitative sampling. Samples were fixed in the field with 96% alcohol and sorted out in laboratory. Most invertebrate groups were determined to the species level.

Fishes

Fish species occurring in reservoir inlets and outlets were sampled using electrofishing and data on fish occurrence in reservoirs were obtained from the statistics of local anglers clubs and supplemented by own sampling using electrofishing or beach seining.

Chemical parameters

Water temperature, dissolved oxygen, pH and conductivity were measured directly in the field using multimeter. Total N (TN), NH_4^+, (NO_3^-), total P (TP), PO_4^{3-}, and BOD_5 were assessed according Slovak technical norms (STN), chlorophyll concentration was set according standardizes method (ISO 10260:1992).

Statistical analysis

For the assessment of the main environmental gradients and the relationship of sampling sites the principal component analysis (PCA) was used. Species data were transformed log (x + 1). Chemical parameters were used as supplementary variables for the interpretation of the main environmental gradient in the ordination - BSK_5, N- NH_4^+, N- NO_3^-, P-PO_4, TN and TP. Those parameters with non normal distribution were transformed. BSK5 and TN were log10 transformed; N-NO_3^- and P-PO_4 were square root transformed; and TP were log e transformed. Ordination analysis was done using program CANOCO (Ter Braak and Šmilauer, 1998).

Analysis of similarity (ANOSIM, Clarke, 1988, 1993) was used to test the qualitative differences among groups of samples identified by PCA. R value represents the measure of the group's similarity. Valued above 75 indicated that groups are well separated, values 0.5 - 0.7 indicate that there is some overlapping among groups and by values below 0.25 the groups are not different (Reid and Thoms, 2008). Qualitative samples of all invertebrate groups were used.

RESULTS AND DISCUSSIONS

Zooplankton

Species composition: the 23 species of Rotatoria, 25 of Cladocera and six species of Copepoda were found in reservoirs of river Váh Watershed. *Bosmina longirostris* (97%) dominated from cladocerans and *Thermocyclops oithonoides* (82%) form copepods. The 14 species of Rotatoria, 34 species of Cladocera and 11 of Copepoda were found in reservoirs of river Morava Watershed. The most abundant were *Bosmina (E.) coregoni* and *Daphnia cucullata*.

The big differences in biomass of zooplankton were found between the two groups of ponds (Fig. 1). In the reservoirs (Doľany, Dolné Dubové and Suchá nad Parnou) with the high trophy (Tab. 1) the biomass of zooplankton was high. The rotifers predominated and contributed up to 80% of the total zooplankton biomass. The high dominance of rotifers and small average size of cladocerans, mainly *Bosmina longirostris*, indicated the fish predation in reservoirs. On the other hands in the ponds (Kuchyňa, Lozorno and Vý
vrat) with low trophy, the low quantity of zooplankton and chlorophyll a was found. As for water quality, based on zooplankton structure, chemical parameters and phytoplankton concentration are the better beta-mesosaprobic and oligosaprobic degree. From the aspect of cladocerans diversity (34 species) these reservoirs are reaching and should be protected.

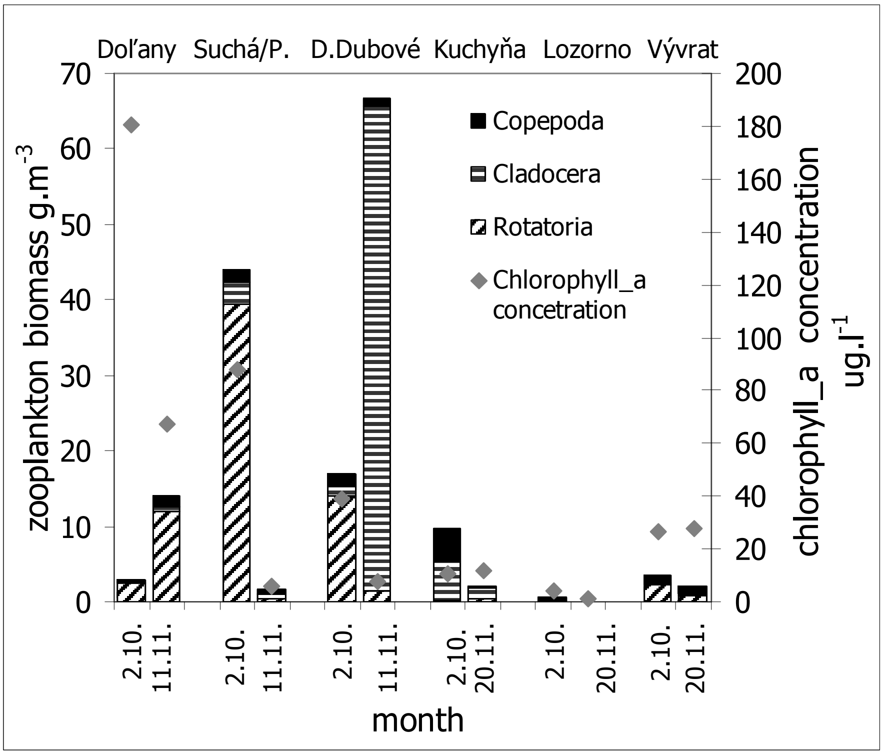

Figure 1: Zooplankton biomass (Copepoda, Cladocera and Rotatoria) and chlorophyll a concentration in studied reservoirs.

Macrozoobenthos

Quality and quantity is studied in 18 localities (littoral, inlet and outlet of every reservoir). Based on preliminary results from two autumn sampling quite high biodiversity were found. From more than 270 taxa, 43% was found only on one locality. In all inlets and outlets *Gammarus* sp. dominated - in outlet of reservoir Suchá n/P. and Výyvrat together with *Hydropsyche angustipennis*; and in inlet of Lozorno reservoir together with *Limnius* sp. larvae. Density of littoral fauna was more-less balanced, or various species dominated (in Suchá n/P. *Caenis luctuosa*, in Lozorno *Dreissena polymorpha*, in Výyvrat *Endochironomus tendens* (Fig. 2).

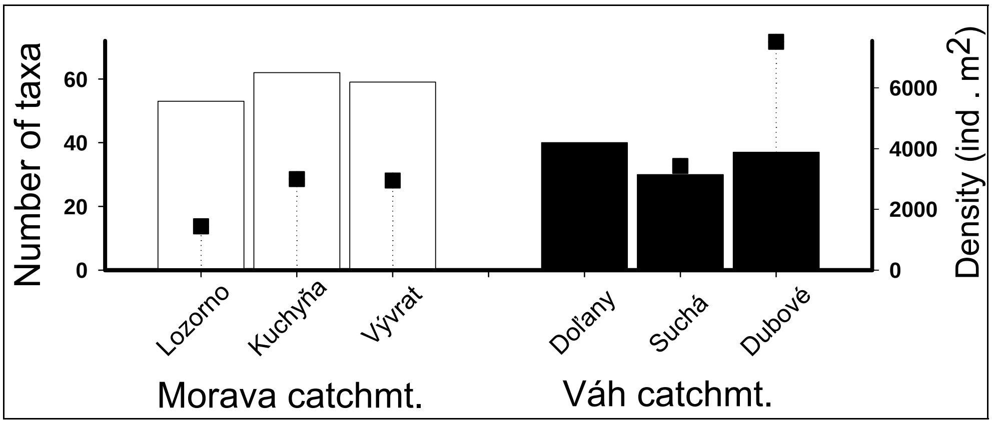

Figure 2: Number of taxa and density on individual sites (littoral, inlet, and outlet).

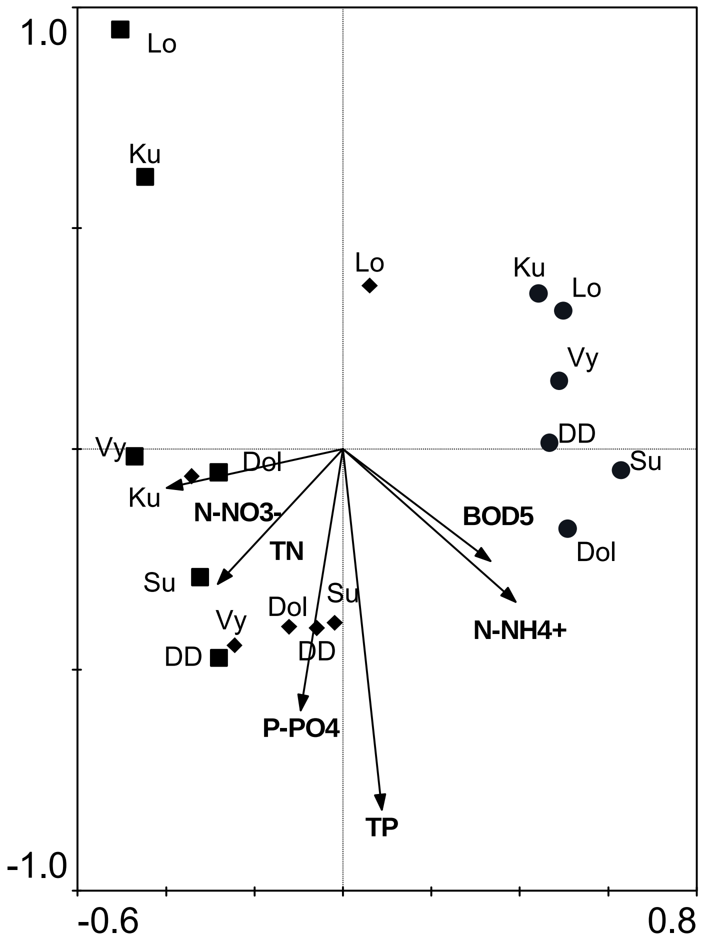

Figure 3: PCA Diagram with environmental parameters as supporting explanatory variables;
■ - inlet, ♦ - outlet, ● - littoral;
Dol - Doľany, DD - Dolné Dubové, Su - Suchá nad Parnou, Ku - Kuchyňa, Lo - Lozorno, Vy - Vývrat.

PCA

First and second axis explained 18.5% and 10.8% of all variability. The first axis can be explained as flow gradient - littoral samples separated in the right part of the diagram. Total P is correlated with the second axis. Along this gradient sampling sites ordered according their „natural status“. In the upper part sites with natural character separated - Lozorno, Výyvrat a Kuchyňa belonging to the Morava Watershed. Sites with the lowest proportion of chemical parameters are isolated - inlets of Lozorno and Kuchyňa. Dolné Dubové, Doľany a Suchá nad Parnou belonging to river Váh Watershed separated in the lower part of the diagram. This separation is kept within the littoral samples and also within the inlets/outlets samples (Fig. 3).

These results were further analysed using ANOSIM (Tab. 2).

1. Littoral assemblages: ANOSIM results suggest the existence of significant differences between assemblages of Morava and Váh watersheds (R = 0.54). For the littoral assemblages of reservoirs in Morava Watershed higher abundances of following taxa: *Endochironomus tendens*, *Microtendipes* gr. *pedellus*, *Dreissena polymorpha*, *Polypedilum pedestre*. For Váh catchment *Helobdella stagnalis*, *Caenis luctuosa*, *Micronecta* sp., *Cladotanytarsus* gr. *mancus*, *Glyptotendipes pallens*, *Dicrotendipes modestus*, *Psammoryctides barbatus*.

The presence of different habitats in littoral zone play important role in assemblages composition. Diversity of different substrata such as coarse and fine substrata, macrophytes and detritus support different taxa (Weatherhead and James, 2001).

2. Inlets and outlets: Some differences can be observed between stream of both catchments (R = 0.39). At the same time inlets of Morava catchments confirmed their well preserved natural condition and probable negative influence of reservoirs on the outlets (R = 0.44). Several taxa were found only in these inlets, mainly from Ephemeroptera and Plecoptera - *Ephemera danica*, representatives of Nemouridae, Leuctridae, Perlidae and Perlodidae.

3. Inlets and outlets of Váh Watershed could not be separated (R = 0.24), probable their fauna was negatively influenced by anthropic pressure of this whole area.

Negative effect of water reservoirs on outlets bottom fauna consist in increased erosion leading to the changes in macro and microzoobenthos abundance and taxonomic composition (Krno et al., 1993). Specific changes depend on local environmental conditions (Kubíček, 1994). Here the effect of water reservoirs resulted in changes in chemical parameters - indicators of BOD_5 and $N\text{-}NH_4^+$ increased in outlets, while $N\text{-}NO_3^-$, TN, $P\text{-}PO_4$, TP and $N\text{-}NO_3^-$ tent to decrease or did not change. The changes were more evident in sites of Morava Basin probable due to more intense human pressure.

Table 2: Results of ANOSIM (R and P values), comparing littorals, inlets and outlets of reservoirs between the two studied catchments.

		P	R
littoral (Morava Watershed)	littoral (Váh Watershed)	0.05	0.54
inlets (Morava Watershed)	inlets (Váh Watershed)	0.05	0.44
outlets (Morava Watershed)	inlets (Váh Watershed)	0.15	0.24
inlets, outlets (Morava Watershed)	inlets, outlets (Váh Watershed)	< 0.01	0.39

Fish

Altogether, 28 fish species were recorded in the study area. In reservoirs, eight species occurred in the frequency of 100%, but bream (*Abramis brama*), grass carp (*Ctenopharyngodon idella*), common carp (*Cyprinus carpio*), pike (*Esox lucius*), pikeperch (*Sander lucioperca*) and wells (*Silurus glanis*) are stocked by anglers and perch (*Perca fluviatilis*) and non-native Prussian carp (*Carassius gibelio)* were probably released unintentionally. On the other hand, the frequency of brown trout (*Salmo trutta*) reached 50% in reservoirs inlets and the frequency of roach reached 83% in reservoirs outlets. Selected reservoirs can be divided in several ways. Doľany is used for intensive fish production where non-native topmounth gudgeon (*Pseudorasbora parva*) dominated in the reservoir and outlet. Two other reservoirs in Váh Watershed as well as Vývrat in Morava Watershed are characterised by common cyprinid and percid species. Coldwater inlets and outlets of the remaining two reservoirs in Morava Watershed have salmonid character, while the reservoirs are dominated by common percid and cyprinid species. In general, fish species diversity there is influenced by fish stocking, where mainly common carp (*Cyprinus carpio*) is stocked.

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FLOODPLAIN FORESTS ALONG THE LOWER DANUBE

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KEYWORDS: Danube, floodplain forests, black and white poplar gallery forests, poplar-elm forests, elm-oak forests relict habitat types.

ABSTRACT

The present paper presents the results of the studies conducted in the floodplain forests along the Lower Danube. Emphasis was prevalently put on near-natural black poplar and white poplar forests, poplar-elm forests as well as the few remaining oak-elm floodplain forests that have been studied on various sections along the Romanian-Bulgarian Danube. They are allocated to the Salici-Populetum, Fraxino-Ulmetum and Ulmo-Quercetum roboris associations. Floodplain forests are threatened all over Europe and are listed in Appendix I of the Directive on the Conservation of natural habitats and of wild fauna and flora under 91E0 *Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (Alno-Padion, Alnion incanae, Salicion albae), presented more on smaller water courses, 91F0 Riparian mixed forests of *Quercus robur*, *Ulmus laevis* and *Ulmus minor*, *Fraxinus excelsior* or *Fraxinus angustifolia*, 92A0 *Salix alba* and *Populus alba* galleries. Further studied communities are tamarisk bushes that are listed under 92D0 Southern riparian galleries and thickets (Nerio-Tamaricetea and Securinegion tinctoriae), occurring frequently together with the White poplar gallery forest. The curtains of wild wine (*Vitis sylvestris*) and on the eastern section of the Lower Danube also of the silkvine *Periploca graeca* and *Cynanchum acutum* give them a tropical touch.

ZUSAMMENFASSUNG: Die Auwälder entlang der unteren Donau.

In vorliegender Arbeit werden die Ergebnisse der Untersuchungen in Auenwäldern entlang der Unteren Donau vorgestellt. Dabei geht es vorwiegend um die naturnahen Schwarz-Pappel und Silberpappel-Wälder, um die Pappel-Ulmenwälder sowie um die Eichen-Ulmen-Auenwälder. Diese wurden in mehreren Abschnitten entlang der rumänisch-bulgarischen Donaustrecke untersucht. Sie sind den Assoziationen Salici-Populetum, Fraxino-Ulmetum und Ulmo-Quercetum roboris zugeordnet. Die europaweit gefährdeten Auenwälder sind in Anhang I der FFH Richtlinie unter 91 E0 *Auenwälder mit *Alnus glutinosa* und *Fraxinus excelsior* (Alno-Padion, Alnion incanae, Salicion albae), die mehr an kleineren Gewässerläufen vorkommen, 91 F0 Eichen-Ulmen-Eschen - Auenwälder (*Quercus robur*, *Ulmus laevis*, *Ulmus minor*, *Fraxinus excelsior* oder *Fraxinus angustifolia*), 92D0 Galeriewälder mit *Salix alba* und Po*pulus alba* eingegliedert. Hinzu kommen Tamariskenbüsche, die zu Lebensraumtyp 92D0 Südliche Galeriewälder und Tamariskengebüsche (Nerio-Tamaricetea und Securinegion tinctoriae) gehören. Die Schleier von Wilder Weinrebe (*Vitis sylvestris*) und im östlichen Abschnitt der Unteren Donau auch Griechischer Liane (*Periploca graeca*) sowie zum Teil *Cynanchum acutum* verleihen den Auenwäldern einen tropisch anmutenden Charakter.

REZUMAT: Pădurile de luncă, de-a lungul Dunării inferioare.

Lucrarea prezintă rezultatele cercetărilor efectuate în pădurile de luncă de-a lungul Dunării inferioare. Studiul se referă, în primul rând, la păduri cvasi naturale de plop negru şi plop alb, păduri de tranziție de plop alb, plop negru şi ulm, precum şi la suprafețele mici rămase din pădurile de stejar şi ulm, care au fost studiate în diferite locuri ale Dunării inferioare, în sectorul de frontieră între Bulgaria şi România. Fitocenozele, cercetate, au fost încadrate în asociațiile Salici-Populetum, Fraxino-Ulmetum şi Ulmo-Quercetum roboris. Pădurile de luncă, periclitate la scară europeană, sunt listate în anexa I a Directivei FFH sub numerele 91E0* Păduri aluviale cu *Alnus glutinosa* şi *Fraxinus excelsior* (Alno-Padion, Alnion incanae, Salicion albae) prezente mai mult pe râurile interioare, 91F0 Păduri mixte ripariene cu *Quercus robur*, *Ulmus laevis* şi *Ulmus minor*, *Fraxinus excelsior* sau *Fraxinus angustifolia* de-a lungul râurilor şi fluviilor, 92A0 Galerii cu *Salix alba* şi *Populus alba*. Deseori în strânsă relație cu galeriile de plop alb, apar tufărişuri de cătină roşie *Tamarix ramossisima* cuprinse în tipul de habitat 92D0, galerii ripariene şi tufărişuri sudice (Nerio-Tamaricetea şi Segurinetea). Perdelele de viță sălbatică în sectorul estic al Dunării inferioare şi de liană grecească (*Periploca graeca*) şi *Cynanchum acutum* oferă acestor păduri o nuanță tropicală.

INTRODUCTION

Man's intervention on European rivers, and thus on the Danube River as well, that took place in the nearer and farer past resulted in a dramatic reduction of floodplain forests. On the one hand the surfaces became smaller, on the other hand the remaining floodplain forests have been dramatically altered as for their structure and species composition as a result of interventions for sylvicultural purposes. From an ecological point of view the remainders of near-natural floodplain forests are thus of very great importance. There are on the one hand the softwood floodplain forests, on the other hand there are those of the hardwood floodplain and those showing transition characteristics. Depending on their ecological significance they attract special attention as from a point of view of nature conservation. Accordant to their importance the floodplain forests have been classified as habitats of pan-European importance and as such recorded in Appendix I of the Directive on the Conservation of natural habitats and of wild fauna and flora 92/43/CEE.

Climatic and phytogeographic aspects of floodplain vegetation along the Danube River.

The Danube River and its floodplains shows a considerable West-Southeast extension and thus its life communities and vegetation composition is not only defined by the ecologically determining factors such as the hydrological and morphological dynamics, but also by the continentally gradients of the climate. This becomes obvious from the comparison of the floodplain vegetation occurring along the varying sections of the Danube River. Whereas the influence of the sub continental-central European climate prevails on the Upper Danube (abundant summer precipitations and moderately cold winters), the pannonian lowlands already show a continental influence (moderately poor winter precipitations and relatively frequent years with dry late summers). In the southern regions the central European-submediterranean climate affects the vegetation of this area. In the easternmost Danube region, the large floodplain on the Lower Danube, as well as in the Danube Delta, the impact of a typically continental climate becomes apparent (summer aridity, frequent frosty winters with little snow) (see also Walter and Lieth, 1964; Horvat, Glavac and Ellenberg, 1974; Schneider, 2003).

The influence of these above mentioned factors on the azonal floodplain vegetation results in a very concise classification of the Danube floodplains. This classification differentiates, with a growing continentally in the eastward direction, by the occurrence of section-specific species. These influences reflect increasingly in the species composition of hardwood floodplain forests and may be anticipated from the occurrence of geographic differential species. The sometimes still gallery-like softwood floodplain stands, however, are more azonal.

The alpine-prealpine Upper Danube (in Bade-Wurttemberg/Germany) is characterized by the occurrence of alpine species in the river-attending vegetation that is frequently reduced to a small fringe. The central European moderately continental upper Danube area is mainly characterized by European respectively central European species of the colline level. Very characteristic of the whole Upper Danube's pre-alpine area are also numerous alpine plants that have been washed ashore in the lowlands by the Danube's tributaries. These are partly species of dynamic pioneer stands with large grain sized sediments, among others *Salix elaeagnos*, German Tamarisk (*Myricaria germanica*) and grey alder (*Alnus incana*). Moreover, the hardwood floodplain forests comprise a number of alpine calcareous beech forests species (Schneider, 2003). The pannonian section is much more continental and is characterized by the occurrence of pontic-pannonian and continental but also submediterranean, thermophilic species. These do already occur partly in the Danube floodplains east of Vienna (Margl, 1971; Schneider, 2003b). In the Southern area of the pannonian lowlands thermophilic species such as Black bryonia (*Tamus communis*), Butcher's broom (*Ruscus aculeatus*) and *Carpesium abrotanoides* point out a submediterranean-illyric influence (Pócs, 1991). This influence also proceeds in the breach valley area of the Iron Gate („Clisura Dunării"), where a submediterranean-illyric characterized rock vegetation of Orno-Cotinetalia dominates. This thermophilic, submediterranean but also Balkan-Moesian characterized rock vegetation occurs over long stretches along the right bank of the Lower Danube, where the hills of the Bulgarian chalkstone table are steeply sloping down to the river.

Downstream the Iron Gate up to around the mouth of the Olt River in the Danube ensues a submediterranean and increasingly Balkan-Moesian section (Borza and Boşcaiu, 1965; Călinescu, 1969; Horvat, Glavac and Ellenberg, 1974). *Fraxinus angustifolia danubialis* (Popescu, Sanda and Fişteag, 1997), Moraine Ash (*Fraxinus holotricha*) and the oak *Quercus pedunculiflora* occur here. Summer Snowflake (*Leucojum aestivum*) and Wilde Vine (*Vitis sylvestris*), being characteristic also for the Pannonian stretch of the Danubian floodplains are also characteristic of this section. The transition to the Pontic-Danubian section is smooth and characterized by the occurrence of species such as Moraine Ash (*Fraxinus holotricha*), Greek Liane (*Periploca graeca*), Wild Asparagus (*Asparagus tenuifolius*, *A. pseudoscaber*), *Cynanchum acutum* and Liquorice root (*Glycyrhiza echinata*). On pioneer stands one may find the Tamarisk (*Tamarix ramosissima*), indicating also lightly halophilous stands, that escorts the Danube up to its mouth in the Black Sea (Simon and Dihoru, 1963) and also occurs frequently on the lower courses of its tributaries such as e. g. in the Olt River mouth, Ialomiţa, Buzău and Siret (Schneider, 1991; Schneider, 2003; Schneider et al., 2005; Simon and Dihoru, 1963; Schneider and Dihoru, 2004 mscr.). Further pontic species may be found in the Danube-Delta, but Irano-Turanian and eastern mediterranean elements do occur all the same.

MATERIALS AND METHODS

In view of the classification of Natura 2000 areas a gathering of biodiversity data, i. e. floodplain-specific species and habitats as well as an ecological evaluation of habitat types was carried out within the frame of a PHARE-financed project on the Lower Danube between river kilometers 838 and 375 Călăraşi/Silistra, on the basis of the Appendixes of the Directive on the Conservation of natural habitats and of wild fauna and flora. During the 2004 vegetation period both the Danube River's and the islands' floodplain forests have been studied and samples have been taken by means of the Braun-Blanquet seven-step abundance-dominance scale (1964). Wherever possible samples of 20 m x 20 m have been taken along transects so as to record the sequence according to the various floodplain levels as well. The emphasis was put on the area of Cama-Dinu upstream Giurgiu 524-500, samples having been taken on further representative Danube River sections all the same. Further studies have been conducted in 2006 near Pietroşani, river kilometer 530. The studies realized within the frame of the restoration project polder Călăraşi-Răul (2002) have been included all the same.

Vegetation data have been recorded and evaluated according to the vegetation layers herb layer, shrub layer between 1 - 3 m and between 3 - 5 m, and both a lower and a higher tree layer. Subsequent to this, the samples have been gathered in phytocoenological tables and classified according to their affiliation to various associations. Last but not least they were also allocated to the habitat types of the Directive on the Conservation of natural habitats and of wild fauna and flora, given that special attention is drawn to the Danube River especially with regard to the Natura 2000 network.

RESULTS AND DISCUSSIONS

In the recent floodplain transect reaching from the lowest vegetation levels around mean water level to the highest levels, the vegetation distribution corresponds to the hydrological dynamics and the dynamics of sediments with varying grain sizes. Flood depth, duration, moment and frequency play an important role at this (Schneider 1991, 2003b). This vegetation distribution which is conditioned by the river dynamics and grain sizes applies to the whole Danube River. It implies the formation of characteristic plant communities that occur in various densities as a result of the intervention of man. Some communities have almost completely disappeared in consequence of the Danube River development, so e. g. in the case of the *Myricaria germanica* community (Tab. 1), others however occur abundantly to very abundantly and in site-characteristic stands.

On the Lower Danube the crucial flood level for the alignment of the forest communities is measured in hydrogrades. In the relevant Romanian scientific literature 1HG corresponds to the tenth part of the difference between lowest and highest water level. When considering the flood levels according to hydrogrades, in the historical situation of the Lower Danube floodplains there is no indication of any shrub vegetation below hydrograde 3 (below mean water level). Between HG 3 - 6 white willow settlements have been recorded on the lower spots and tamarisks in the more elevated ones. Between HG 6 - 9 willow coppices occurred in more elevated areas and pure ash woods bordered the depressions together with alder (*Alnus glutinosa*). Within the hydrograde band width the swampy alder areas in the depressions are limited to hydrogrades 7 - 8. Between 8 - 9 the shrub vegetation consists in a conglomerate composed of oak, elm and poplar (Stoiculescu, 2008; Popescu-Zeletin, 1967). On sandy soils the white poplar occurs as a pioneer species.

Table 1: Floodplain forest and bush communities along the Danube River; x = low, xx = medium, xxx = site typical composition and relatively good represented.

Repartition of communities of soft- and hardwood floodplain forests along the different Danube stretches	Upper Danube	Middle Danube	Lower Danube	Danube Delta
Bush vegetation				
Salici-Myricarietum	x			
Salix incana-Hippophae rhamnoides	xx			
Salicetum purpureae	xxx	xxx	xx	
Tamaricetum ramosissimae			xx	xxx
Hippophae-Elaeagnus angustifolia				xxx
Salicetum triandrae	xxx	xxx	xxx	xxx
Softwood floodplain forests				
Alnetum incanae	xxx			
Alnus incana-Salix alba	xxx			
Salix alba-Populus nigra-Alnus incana (Szigetköz, H)		x		
Salici albae-Populetum nigrae		xxx	xxx	xxx
Salix alba-Populus alba			xxx	xx
Populetum nigrae-albae		xxx	xxx	
Salicetum albae (incl. Salicetum albae-fragilis)	xx	xxx	xxx	xxx
Transition type from softwood to hardwood forest				
Alnus incana-Fraxinus excelsior	xxx			
Salix alba-Ulmus laevis			xx	
Populus alba, Populus nigra, Ulmus laevis			xxx	
Hardwood floodplain forests				
Querco-Ulmetum/Fraxino-Ulmetum	xxx	xxx	xx	
Galio-Carpinetum	xxx			
Fraxino pannonicae-Ulmetum		xxx		
Leucojo-Fraxinetum angustifoliae		xxx	x	
Genisto elatae-Quercetum robori		xxx		
Asparago-Quercetum pedunculiflorae			xx	xxx
Fraxinetum pallisae				xxx

Even though there were man-induced changes and near-natural floodplain forests have been transformed into hybrid poplar and other forest cultures, a vegetation distribution along ecological gradients becomes distinctly apparent, especially in those areas that were less exposed to human interference, as is the case most of all for a number of islands. It has to be stated here that the most elevated floodplain areas are sometimes situated directly adjacent to the shore, where the Danube River inundates the whole area at the moment of the floods and where continuous sediment deposits entailed the formation of natural levees. These have been taken over by hardwood floodplain forests that developed in the course of the years.

Considering the historic situation, i. e. the time when the broad Lower Danube floodplains had not yet been cut off from the river, drained and transformed for agriculture, becomes apparent that given the former floodplain extension and the small gradient, larger swamp areas with extended rush and reed stands must have existed with floodplain forests and alder woods around depressions. Now large areas of softwood stands with a predominance of White willow occur as simply structured gallery forests in the recent floodplain of the Lower Danube (Schneider, 2003a, 2003b). They are characteristic of this river section with low slopes and fine-grained sediments.

White willow, White poplar and Black poplar forests.

White willow softwood forests (Salicetum albae) occur in two different variants, the flood duration being clearly visible in the species composition of the herbaceous layer (Doniță, Dihoru and Bîndiu, 1966; Schneider, 2003a). In those places where the flood duration is of about six months the softwood forest is poor in species and structure, with a tree-layer composed only by White willow (*Salix alba*) and presenting a very poor shrub layer. The herbaceous layer is mainly composed of moisture indicators that are well adapted to fluctuating water levels, e. g. *Rorippa amphibia*, *Polygonum hydropiper*, *P. lapathifolium* and *Senecio paludosus.* In places where the flood duration does not exceed four months the structure of the softwood forest is different. In these places one may observe the White willow but also Black poplar (*Populus nigra*), White poplar (*Populus alba*) and locally Grey poplar (*Populus canescens*) on alluvial soils with a high proportion of sand. *Aristolochia clematis*, a characteristic indicator of sandy soils occurs frequently in the herbaceous layer.

Here and there along the Lower Danube one may also find gallery-like forests that are characterized by Black Poplar (*Populus nigra*) and White poplar (*Populus alba*) in varying proportions (Tabs. 2, 3 and 4). A smaller share of Black Poplar (*Populus nigra*) is specified on the Bulgarian Danube. (Zanov, 1992). On sandier floodplain soils the White poplar also forms pure stands or occurs together with a small number of White willows, i. e. stands that are rather comparable to the Mediterranean *Salix alba* and *Populus alba* gallery forests as for their forest structure (Tab. 4, column 1, 2 and 7). They are listed in Appendix I of the Directive on the conservation of natural habitats and of wild fauna and flora under 92A0 *Salix alba* and *Populus alba* galleries (Fig. 1). Characteristic of the white poplar gallery forests just as of poplar-elm forests is the Mediterranean-submediterranean *Aristolochia clematitis* (Ciocârlan, 2000, p. 140). The tamarisk (*Tamarix ramosissima*) occurs in some spots as well (Tab. 5). It constitutes the very characteristic plant community of Calamgrosti-Tamaricetum ramosissimae (Simon and Dihoru, 1963) which is listed in Appenix I of the Directive on the conservation of natural habitats and of wild fauna and flora, habitat type 92D0 Southern riparian galleries and tickets (Nerio-Tamaricetea). Pioneer Tamarisk bush communities under the name Tamaricetum Pallasii has been mentioned in the past from Bulgarian islands (Stojanov, 1948). Both habitat types are frequently closely interwoven and occur in the south-east of Romania, on the Lower Danube in the Pontic region (Toniuc, Negulici and Boşcaiu, 1981; Doniță et al., 2005). The Lower Danube's white poplar gallery forests connect the Mediterranean and Central European river-attendant forests.

Figure 1: *Populus alba* gallery forest on the Danube Island Cama (Giurgiu County, Romania).

Poplar and elm forests.

Intermediary stages between softwood and hardwood floodplain forests do mainly occur on the islands. In willow-poplar and pure poplar stands small-leaved ash (*Fraxinus angustifolia*) and elm (*Ulmus laevis* and *U. minor*) settle in some places and do thus show the transition characteristics from softwood to hardwood floodplain forests (Tabs. 2, 3, 4 and 6), the hardwood floodplain characteristics being already clearly apparent. Stands of the small-leaved ash do merely occur in a few places (e. g. on the Lower Jiu in the Zăval Forest), whereas the smooth-leaved elm is well represented and regenerates well (Fig. 2). In these forests the herbaceous layer is characterised during spring time by *Leucojum aestivum*, in some spots also by *Ranunculus ficaria*. The stands of these forests stand out for their abundance-dominance and high frequency of elms. They shelter nitrogen indicators such as *Parietaria officinalis*, *Galium aparine*, *Urtica dioica*, *Anthriscus cerefolium* ssp. *trichosperma* and others in their herbaceous layer that point out the nutrient abundance of the periodically flooded recent floodplain. The species mentioned occur both in the poplar and elm transition forests and in the oak-elm forests, backing up, along with further common species, the close relation between these two. This relation allows the pooling of these two in the habitat type 91 F0 Riparian mixed forests of *Quercus robur*, *Ulmus laevis* and *Ulmus minor*, *Fraxinus excelsior* or *Fraxinus angustifolia*.

Figure 2: Natural regeneration of elm (*Ulmus laevis*) on Cama Island (Giurgiu County, Romania).

Due to their lowland river conditions with extensive reed-abundant wetlands, natural hardwood floodplain forests merely occur on high natural river levees, the so called ‘grinduri de mal’, and are thus less expanded as compared to softwood forests. In his vegetation map of Romania for the Lower Danube, Enculescu (1914) notes “forests with softwood forest species (willows and poplars), lakes, reeds, levees, floating reed carpets “plaur” and forest enclaves composed of “*Fraxinus* with *Periploca graeca*”. It may be retained from this description that hardwood floodplain forests were not very expanded. This fact is stated also for some Bulgarian Danube islands (Stojanov, 1948). Moreover, with only few exceptions the hardwood floodplain forests disappeared as a consequence of man-induced changes in the course of the centuries or decades. Only few patches of near-natural hardwood floodplain forests (Querco-Ulmetum) have been left over (Schneider et al., 2005; Stoiculescu, 2008; Atlas LDGC, 2009 in printing; in the figure 3). In some areas one may merely find single trees or groups of trees, mainly oaks that give evidence (“martori”) of the former hardwood floodplain forests. They may be found for instance downstream the mouth of Sâiu (Oltu Mic), along the Danube River near the village of Năvodari (Teaca), in the Cama Dinu area, on the river bank between km 510 - 524, near Greaca, downstream of the town Giurgiu, near to the mouth of Argeş near Olteniţa, etc. on the Bulgarian Vardim island, as well as relicts on Belene island /Bulgaria (Bulgarian Ministry of Agriculture and forests, 2001). The famous hardwood floodplain forests in the dune areas of Letea and Caraorman in the Danube Delta are very similar to the hardwood floodplain forests of the Lower Danube but nevertheless they present very specific characteristics. They have yet not been considered in this paper.


Figure 3: Hardwood floodplain forest on Danube River at the km 574 (2004).

Along the Romanian river stretches of the Lower Danube River, hardwood floodplain forests composed of Balkan oak (*Quercus pedunculiflora*), Common oak (*Quercus robur*), elm (*Ulmus laevis*) and locally small-leaved ash (*Fraxinus angustifolia*) (Tab. 7). In these forests one may also find the Hairy ash (*Fraxinus pallisae*). The Greek liane (*Periploca graeca*), the Swallow wort (*Cynanchum acutum*), the Traveller's joy (*Clematis vitalba*), Hop (*Humulus lupulus*) as well as the Wilde wine (*Vitis sylvestris*) which locally forms thick curtains adding a tropical character to the Lower Danube forests (Fig. 4). *Periploca graeca* occurs in the floodplain forests from downstream Giurgiu and reaches a high frequency (V) in some spots of the floodplain forests (Tab. 6). A high frequency of *Asparagus pseudoscaber* in the hardwood oak-elm floodplain forests resulted in the description of the plant community Asparago-Quercetum which comprises both the forests of the Lower Danube and the forests of the Danube Delta (Popescu, Sanda and Fişteag, 1997; Sanda, Öllerer and Burescu, 2008).

Figure 4: Curtains of Wilde wine on the Danube River bank near the river km 508.

As regards the structure of these forests it has to be noted the fact that the high tree layer is well developed in only a few places, i. e. in all those spots where the remaining oak trees and smooth-leaved elms make up the larger part of the forest stands. The tree layer below ten m is represented by a few oaks, smooth-leaved elms and black poplars. Even the mulberry *Morus alba* occurs frequently in these floodplain forests and may be found both in the more elevated parts of the softwood floodplain and in the hardwood floodplain forests. The Green Ash (*Fraxinus pennsylvanica*) occurs frequently in many spots of the floodplain forests and seems to supplant the domestic ash. In the studied shrub layer one may find smooth-leaved elm, mulberry, dogwood and most of all the problematic neophyte *Amorpha fruticosa*, which spreads departing from the poplar cultures in all those places, where the natural floodplain forest structure can be considered as disturbed.

Table 2: Transition forest between softwood and hardwood floodplain forest with *Salix alba*, *Populus nigra* and *Ulmus laevis*; **Anthriscus cerefolium* ssp. *trichosperma* and *Leucojum aestivum* are characteristic for the spring aspect of the herbaceous layer and develops before the trees are covered with foliage. In the time when samples have been taken, these plants where in a withered, yellow and dying state.

Number of sampling	1	2	3	4	5	6	7	8	9	
Covering of tree crowns, shrubs and lianas	0.7	0.7	0.8	0.9	0.8	0.6	0.5	0.9	0.8	
										F
1. Tree layer										
Ulmus laevis	4	4	4	4	4	3	+	.	2	V
Ulmus minor	.	.	.	.	.	+	.	1	.	II
Fraxinus angustifolia	.	.	+	.	.	.	.	.	.	I
Populus nigra	.	+	.	.	.	.	.	+	+	II
Salix alba	.	+	+	+	2	2	3	3	3	V
Morus alba	.	.	+	+	.	+	.	2	+	III
1. Shrub layer (3 - 5 m)										
Ulmus laevis	+	.	.	.	+	+	+	+	.	III
Cornus sanguinea	.	.	+	.	+	+	.	.	.	II
Fraxinus pennsylvanica	.	.	.	+	.	.	.	+	.	II
Morus alba	.	.	+	.	.	.	.	.	+	II
2. Shrub layer (1 - 3 m)										
Amorpha fruticosa	+	+	+	+	.	2	+	.	3	IV
Ulmus laevis	+	.	.	.	+	.	.	.	.	II
Fraxinus pennsylvanica	.	.	.	2	.	+	.	.	+	II
Cornus sanguinea	.	.	+	.	+	.	.	.	.	II
Regeneration										
Cornus sanguinea	.	.	.	.	+	+	.	.	.	II
Amorpha fruticosa	.	.	+	+	.	.	.	.	.	II
Liana and liana-like species										
Vitis sylvestris	+	+	+	3	+	+	.	4	3	V
Humulus lupulus	.	.	3	.	+	.	+	+	.	III
Rubus caesius	+	+	+	+	+	+	1	.	2	V
Cucubalus baccifer	+	+	+	.	.	.	.	2	.	III
Herbaceous layer*										
Aristolochia clematitis	2	2	+	1	+	2	.	.	+	IV
Leucojum aestivum	1	+	.	.	+	.	+	.	+	III
Anthriscus cerefolium trichosperma	.	2	3	4	+	.	.	+	.	III
Parietaria officinalis	.	.	.	3	.	.	.	.	.	I
Galium aparine	.	3	2	+	3	3	+	3	.	IV
Alliaria petiolata	+	1	.	.	+	.	.	.	.	II
Chelidonium majus	.	+	+		.	+	.	.	+	III
Urtica dioica	+	.	+	+	+	+	.	+	.	IV
Glecoma hederacea	2	+	+	.	+	+	+	+	+	V
Aster lanceolatus	+	.	+	.	+	.	3	3	2	IV
Malachium aquaticum	.	.	.	.	+	.	.	+	.	II
Stellaria media	.	+	+	.	.	.	.	.	.	II

Number of sampling	1	2	3	4	5	6	7	8	9	
Covering of tree crowns, shrubs and lianes	0.7	0.7	0.8	0.9	0.8	0.6	0.5	0.9	0.8	
										F
Rumex conglomeratus	.	.	+		.	.	.	+	.	II
Lysimachia nummularia	+	.	.	.	+	.	+	.	.	II
Bidens tripartita	.	.	+	.	.	+	.	.	.	II
Lythrum salicaria	.	+	+	.	.	.	+	.	.	II
Symphytum officinale	+	+	.	.	.	.	+	.	.	II
Carex acutiformis	1	.	.	.	.	.	+	.	.	II
Agrostis stolonifera	.	.	.	.	.	.	.	+	+	II
Lycopus europaeus	+	.	+	.	.	.	.	.	+	II

Species noted with + in one sampling area: 1: *Galium verum*, *Phalaris arundinacea*; 2: *Fraxinus angustifolia* (Shrub layer 1), *Taraxacum officinale*; 3: *Ulmus laevis* (regeneration), *Fraxinus angustifolia* (regeneration), *Fraxinus penssylvanica* (regeneration), *Crataegus monogyna* (regeneration), *Prunus spinosa* (regeneration), *Ranunculus repens*; 4: *Abutilon theophrasti*, *Brachypodium sylvaticum*, *Lactuca tatarica*; 5: *Populus alba* (shrub layer 1), *Crataegus monogyna* (shrub layer 2), *Sambucus nigra* (shrub layer 2), *Asparagus tenuifolius*; 7: *Iris pseudacorus*, *Oenanthe aquatica*, *Phragmites australis*, *Potentilla reptans*; 8: *Ulmus minor* (shrub layer 2), *Calystegia sepium*, *Elytrigia repens*, *Poa palustris*, *Vicia sepium*; 9: *Fraxinus excelsior* (Shrub layer 1), *Althaea officinalis*.

Place and data of sampling: 1: mouth of Oltu Mic into the Danube, 24.05.2004; 2: Danube island Cama /Giurgiu County, 1.06.2004; 3: Danube island Cama/ Giurgiu County, southern part, 27.05.2004; 4: Danube island Cama/ Giurgiu County, near to the forestry house, 27.07.2004; 5: Danube island Gâsca, Teleorman County, near to the mouth of Vedea River, 25.05.2004; 6: Danube islet Păsărica /Giurgiu County, 25.05.2004; 7: Danube River bank near to Păsărica islet, river km 520, 25.05.2004; 8: Danube river bank, downstream Păsarica islet, 25.05.2004; 9: Danube island Slobozia /Giurgiu County, 28.05.2004.

Table 2: Transition forest from softwood forest to hardwood floodplain forest with *Populus nigra*, *Populus alba* and *Ulmus laevis*; **Anthriscus cerefolium* ssp. *trichosperma* and *Leucojum aestivum* are species characteristic for the spring aspect of the herbaceous layer and develops before the trees are covered with foliage.

Serial number	1	2	3	4	5	6	7	8	9	10	
Covering of tree crowns, shrubs and lianas	0.8	0.6	0.8	0.5	0.6	0.7	0.9	0.7	0.8	0.7	
											F
Tree layer											
Populus nigra	2	2	4	3	3	4	2	2	2	3	V
Populus alba	.	.	.	.	.	.	2	+	.	.	I
Salix alba	.	.	.	+	+	.	.	.	.	+	II
Ulmus laevis	+	.	.	+	+	3	3	3	4	3	IV
Morus alba	.	2	2	+	.	+	.	.	.	+	III
Fraxinus excelsior	.	.	.	.	.	.	.	+	.	.	I
1. Shrub layer (3-5 m)											
Populus nigra	3	.	.	+	.	.	.	+	.	.	II
Cornus sanguinea	2	.	.	.	.	.	.	.	.	+	II

Serial number	1	2	3	4	5	6	7	8	9	10	
Covering of tree crowns, lianas	0.8	0.6	0.8	0.5	0.6	0.7	0.9	0.7	0.8	0.7	
											F
Morus alba	.	+	+	.	.	+	.	.	.	.	II
Ulmus laevis	.	.	.	.	+	.	.	+	.	.	I
Fraxinus excelsior	.	.	+	.	.	.	.	.	.	.	I
2. Shrub layer (1-3 m)											
Ulmus laevis	.	.	.	.	.	+	+	.	.	.	I
Quercus robur	.	.	.	.	.	+	.	.	.	.	I
Amorpha fruticosa	.	.	+	+	3	2	+	+	.	+	IV
Morus alba	.	+	.	.	.	+	+	+	.	.	II
Cornus sanguinea	.	+	+	.	+	.	+	.	+	.	III
Fraxinus pennsylvanica	.	.	+	.	.	.	+	.	.	.	I
Regeneration											
Ulmus laevis	.	.	.	.	+	.	+	.	+	.	II
Cornus sanguinea	.	.	.	.	.	.	+	.	.	+	I
Fraxinus pennsylvanica	.	+	.	.	+	.	.	.	+	.	II
Amorpha fruticosa	+	+	1	.	.	.	.	.	+	.	II
Liana and liana-like species											
Vitis sylvestris	.	+	.	.	+	.	3	.	+	+	III
Humulus lupulus	+	.	.	+	+	.	+	+	+	+	IV
Rubus caesius	+	+	.	+	+	+	+	1	+	.	IV
Cucubalus baccifer	.	1	2	.	.	.	+	+	+	+	III
Herbaceous layer											
Aristolochia clematitis	+	1	+	+	+	+	3	1	+	+	V
*Leucojum aestivum**	.	.	.	.	.	.	+	.	.	.	I
*Anthriscus ceref. trichosperma**	+	3	4	.	.	.	3	2	2	3	IV
Galium aparine	.	2	1	3	+	3	+	.	3	3	IV
Urtica dioica	+	+	+	.	+	2	.	+	.	+	IV
Parietaria officinalis	.	2	+	.	.	.	+	+	.	1	III
Alliaria petiolata	.	+	.	+	2	.	+	.	.	.	II
Chelidonium majus	+	+	1	.	.	.	.	.	.	.	II
Glecoma hederacea	.	.	.	+	+	+	.	.	.	.	II
Bromus racemosus	+	+	.	.	.	.	+	+	.	.	II
Asparagus tenuifolius	.	+	.	.	.	.	.	+	.	.	I
Marrubium vulgare	.	.	.	.	.	+	+	.	.	.	I
Arctium lappa	.	.	+	.	.	+	.	.	.	.	I
Elytrigia repens	.	.	.	.	.	.	.	1	.	.	I

Species noted with + in one sampling area: 1: *Bidens frondosa*, *Cyperus fuscus*; 2: *Morus alba* (regeneration), *Cynanchum acutum* (liana and liana-like species), *Armoracia macrocarpa*, *Dactylis glomerata*, *Physocaulis nodosus*; 4: *Solanum dulcamara* (liana and liana-like species), *Agrostis stolonifera*; 5: *Aster lanceolatus*, *Lycopus exaltatus*, *Taraxacum officinale*; 6: *Ulmus minor* (shrub layer 2), *Calystegia sepium* (liana and liana-like species), *Bidens tripartita*, *Iris pseudacorus*, *Lolium perenne*, *Symphytum officinale*; 7: *Populus alba* (regeneration), *Asparagus pseudoscaber*, *Brachypodium sylvaticum*; 8: *Robinia pseudacacia* (shrub layer 2), *Leonurus cardiaca*, *Poa pratensis*; 10: *Viburnum opulus* (regeneration).

Place and data of sampling. 1: Danube River bank near river-km 524, upstream Păsărica islet /Giurgiu County, 27.07.2004; 2: Danube River bank at river-km. 506, Giurgiu County, 1.06.2004; 3: Danube River bank at river km 506, 1.06.2004; 4: Danube island Cama, southern part, 27.05.2004; 5: Danube River bank at river-km 511, 29.05.2004; 6: Danube River bank at Saica, river-km 518, 28.05.2004; 7: Danube island, Cama /Giurgiu County, river-km 509, 27.07.2009; 8: Danube island Berzina Mica, 25.05.2004; 9: Danube island Cama, 29.05.2004; 10: Danube island Cama, 29.05.2004.

Contrary to the common belief in the region that the oak may no longer regenerate in the Lower Danube's recent floodplains, well developed oak regenerations have been observed on many sites. The reasons backing up this statement are an altered hydrological regime as a result of hydropower dam constructions at the Iron Gate as well as the restriction of the recent floodplain and higher water levels resulting from this in the area situated between Danube banks and dyke. Of course, in years with long-lasting high water levels this oak regeneration may not take place. It may however develop if floods are moderate over a number of successive years and it may grow high enough to exceed the critical area. The restoration of near-natural oak-elm floodplain forests as did occur on smaller surfaces is by no means to be excluded.

Depending on the microrelief, the herbaceous layer is composed of species with various ecological needs as for humidity, ranging from more or less wet to dry. Among the species of the more arid sites is *Aristolochia clematitis*, reaching a high frequency in transition stands all the same. Very remarkable is the high frequency of species occurring on nutrient-rich sites such as *Parietaria officinalis*, *Galium aparine*, *Urtica dioica*, the annual species *Anthriscus cerefolium* ssp. *Trichosperma* that may be found in spring as dense, blanket-like covering, *Alliaria officinalis* etc. *Aristolochia clematitis* occur as distinct fringes of tall herbaceous vegetation along the forest edges, species of the floodplain meadow such as *Clematis integrifolia*, *Veronica longifolia*, *Scutellaria galericulata*, *Viola elatior*, *Galium rubioides*, *Carex praecox* may be found here as well. These hardwood oak/ash/elm forests locally shelter old relict Black poplars, i. e. remnants of the natural Lower Danube floodplain forests, and therefore require special attention from a nature conservation point of view.

Table 3: Transition forest from softwood forest to hardwood floodplain forest with *Populus nigra*, *Populus alba* and *Ulmus laevis*; **Anthriscus cerefolium* ssp. *trichosperma* and *Leucojum aestivum* are species characteristic for the spring aspect of the herbaceous layer and develops before the trees are covered with foliage.

Serial number	1	2	3	4	5	6	7	8	9	10	
Covering of tree crowns, shrubs and lianas	0.8	0.6	0.8	0.5	0.6	0.7	0.9	0.7	0.8	0.7	
											F
Tree layer											
Populus nigra	2	2	4	3	3	4	2	2	2	3	V
Populus alba	.	.	.	.	.	.	2	+	.	.	I
Salix alba	.	.	.	+	+	.	.	.	.	+	II
Ulmus laevis	+	.	.	+	+	3	3	3	4	3	IV
Morus alba	.	2	2	+	.	+	.	.	.	+	III
Fraxinus excelsior	.	.	.	.	.	.	.	+	.	.	I
1. Shrub layer (3 - 5 m)											
Populus nigra	3	.	.	+	.	.	.	+	.	.	II
Cornus sanguinea	2	.	.	.	.	.	.	.	.	+	II
Morus alba	.	+	+	.	.	+	.	.	.	.	II

Ulmus laevis	.	.	.	.	+	.	.	+	.	.	I
Fraxinus excelsior	.	.	+	.	.	.	.	.	.	.	I
2. Shrub layer (1 - 3 m)											
Ulmus laevis	.	.	.	.	.	+	+	.	.	.	I
Quercus robur	.	.	.	.	.	+	.	.	.	.	I
Amorpha fruticosa	.	.	+	+	3	2	+	+	.	+	IV
Morus alba	.	+	.	.	.	+	+	+	.	.	II
Cornus sanguinea	.	+	+	.	+	.	+	.	+	.	III
Fraxinus pennsylvanica	.	.	+	.	.	.	+	.	.	.	I
Regeneration											
Ulmus laevis	.	.	.	.	+	.	+	.	+	.	II
Cornus sanguinea	.	.	.	.	.	.	+	.	.	+	I
Fraxinus pennsylvanica	.	+	.	.	+	.	.	.	+	.	II
Amorpha fruticosa	+	+	1	.	.	.	.	.	+	.	II
Liana and liana-like species											
Vitis sylvestris	.	+	.	.	+	.	3	.	+	+	III
Humulus lupulus	+	.	.	+	+	.	+	+	+	+	IV
Rubus caesius	+	+	.	+	+	+	+	1	+	.	IV
Cucubalus baccifer	.	1	2	.	.	.	+	+	+	+	III
Herbaceous layer											
Aristolochia clematitis	+	1	+	+	+	+	3	1	+	+	V
*Leucojum aestivum**	.	.	.	.	.	.	+	.	.	.	I
*Anthriscus ceref. trichosperma**	+	3	4	.	.	.	3	2	2	3	IV
Galium aparine	.	2	1	3	+	3	+	.	3	3	IV
Urtica dioica	+	+	+	.	+	2	.	+	.	+	IV
Parietaria officinalis	.	2	+	.	.	.	+	+	.	1	III
Alliaria petiolata	.	+	.	+	2	.	+	.	.	.	II
Chelidonium majus	+	+	1	.	.	.	.	.	.	.	II
Glecoma hederacea	.	.	.	+	+	+	.	.	.	.	II
Bromus racemosus	+	+	.	.	.	.	+	+	.	.	II
Asparagus tenuifolius	.	+	.	.	.	.	.	+	.	.	I
Marrubium vulgare	.	.	.	.	.	+	+	.	.	.	I
Arctium lappa	.	.	+	.	.	+	.	.	.	.	I
Elytrigia repens	.	.	.	.	.	.	.	1	.	.	I

Place and data of sampling: 1: Danube River bank near river-km 524, upstream Păsărica islet/Giurgiu County, 27.07.2004; 2: Danube River bank at river km 506, Giurgiu County, 1.06.2004; 3: Danube River bank at river km 506, 1.06.2004; 4: Danube island Cama, southern part, 27.05.2004; 5: Danube River bank at river km 511, 29.05.2004; 6: Danube River bank at Saica, river km 518, 28.05.2004; 7: Danube island, Cama /Giurgiu County, river-km 509, 27.07.2009; 8: Danube island Berzina Mica, 25.05.2004; 9: Danube island Cama, 29.05.2004; 10: Danube island Cama, 29.05.2004.

1: *Bidens frondosa*, *Cyperus fuscus*; 2: *Morus alba* (regeneration), *Cynanchum acutum* (liana and liana-like species), *Armoracia macrocarpa*, *Dactylis glomerata*, *Physocaulis nodosus*; 4: *Solanum dulcamara* (liana and liana-like species), *Agrostis stolonifera*; 5: *Aster lanceolatus*, *Lycopus exaltatus*, *Taraxacum officinale*; 6: *Ulmus minor* (shrub layer 2), *Calystegia sepium* (liana and liana-like species), *Bidens tripartita*, *Iris pseudacorus*, *Lolium perenne*, *Symphytum officinale*; 7: *Populus alba* (regeneration), *Asparagus pseudoscaber*, *Brachypodium sylvaticum*; 8: *Robinia pseudacacia* (shrub layer 2), *Leonurus cardiaca*, *Poa pratensis*; 10: *Viburnum opulus* (regeneration).

Table 4: Transition forest between softwood and hardwood floodplain forest with *Populus alba*, *Populus nigra* and *Ulmus laevis*. **Anthriscus cerefolium* ssp. *trichosperma* and *Leucojum aestivum* are species characteristic for the spring aspect of the herbaceous layer and develops before the trees are covered with foliage.

Serial number	1	2	3	4	5	6	7	
Covering degree of tree crowns	0.6	0.8	0.7	0.7	0.8	0.7	0.5	
								F
1. Tree layer (10 - 15 m)								
Populus alba	3	4	3	3	4	3	1	V
Ulmus laevis	.	+	3	3	2	4	.	IV
Populus nigra	.	.	.	.	.	.	1	I
Populus canescens	+	.	+	.	.	.	+	III
Quercus robur	.	.	.	.	.	+	.	I
2. Tree layer (5 - 10 m)								
Populus alba	2	.	+	+	.	1	3	IV
Ulmus minor	.	2	.	.	.	.	.	I
Morus alba	.	.	.	+	.	+	.	II
1. Shrub layer (3 - 5 m)								
Morus alba	+	.	.	.	+	.	+	III
Ulmus laevis	.	.	+	.	+	+	.	III
Salix alba	.	+	.	.	.	.	+	II
2. Shrub layer (1 - 3 m)								
Populus alba	.	+	.	.	.	.	+	II
Amorpha fruticosa	.	.	.	+	+	2	1	III
Cornus sanguinea	.	+	.	.	.	+	.	II
Fraxinus pennsylvanica	.	.	.	.	+	+	.	II
Regeneration								
Populus alba	1	+	+	+	.	.	.	III
Quercus robur	.	.	.	.	+	.	+	II
Ulmus laevis	.	.	.	+	+	+	+	III
Amorpha fruticosa	.	.	.	.	.	2	.	I
Liana and liana-like species								
Vitis sylvestris	.	.	4	+	+	+	.	III
Humulus lupulus	.	.	+	+	.	3	+	III
Rubus caesius	+	.	+	+	.	.	.	III
Cucubalus baccifer	.	.	.	+	.	+	.	II
Herbaceous layer								
Aristolochia clematitis	.	2	+	2	2	+	+	IV
*Leucojum aestivum**	.	.	.	.	.	1	+	II
Parietaria officinalis	.	.	.	2	+	+	.	III
*Anthriscus cerefolium ssp. trichosperma**	+	+	.	3	3	.	.	III
Galium aparine	+	.	+	+	+	2	.	IV
Alliaria petiolata	.	.	.	2	+	.	.	III
Urtica dioica	+	+	.	+	+	+	+	V
Chelidonium majus	.	.	.	+	1	+	.	III
Geranium columbinum	+	.	.	+	+	.	+	III
Glecoma hederacea	.	.	.	.	.	+	+	II
Lysimachia nummularia	.	.	.	.	.	+	2	II

Serial number	1	2	3	4	5	6	7	
Covering degree of tree crowns	0.6	0.8	0.7	0.7	0.8	0.7	0.5	
								F
Arctium lappa	.	+	+	+	+	+	.	IV
Aster lanceolatus	.	.	2	.	.	+	3	III
Rumex conglomeratus	.	+	.	+	+	+	.	III
Bromus mollis	+	+	.	.	.	.	.	II
Plantago major	.	+	.	.	.	+	+	III
Poa pratensis	2	+	.	.	.	+	+	III
Marrubium vulgare	+	+	.	.	.	+	.	II
Leonurus cardiaca	+	+	+	.	.	.	.	III
Xanthium italicum	+	+	.	.	.	.	.	II
Bromus racemosus	3	.	.	.	.	.	.	I
Artemisia vulgaris	.	+	.	.	+	+	.	II
Carex hirta	+	+	.	.	.	.	.	II
Althaea officinalis	+	.	+	.	.	+	.	II
Stellaria media	.	.	.	.	+	+	.	II
Hordeum murinum	+	.	.	.	.	.	+	II

Species noted with + in one sampling area. 1: *Salix alba* (second tree layer), *Crataegus monogyna* (shrub layer 1), *Bromus sterilis*, *Calamagrostis epigeois*, *Calystegia sepium*, *Tragopogon dubius*; 2: *Populus alba* (shrub layer 1), *Evonymus europaea* (regeneration), *Artemisia annua*, *Cerastium caespitosum*, *Chrysanthemum vulgare*, *Convolvulus arvensis*, *Lamium purpureum*, *Lepidium ruderale*, *Matricaria inodora*, *Medicago lupulina*, *Papaver dubium*, *Polygonum aviculare*, *Plantago major*, *Poa angustifolia*, *Poa trivialis*, *Rumex crispus*, *Verbena officinalis*; 3: *Cornus sanguinea* (shrub layer 1), *Fraxinus angustifolia* (shrub layer 1), *Gleditschia triacanthos* (shrub layer 1), *Ulmus minor* (shrub layer 1), *Senecio vernalis*, *Lithospermum arvense*; 4: *Cornus sanguinea* (regeneration), *Cucubalus baccifer*, *Lythrum salicaria*, *Rorippa sylvestris*; 5: *Fraxinus pennsylvanica* (shrub layer 2), *Gleditschia triacanthos* (shrub layer 2), *Morus alba* (shrub layer 2), *Prunus spinosa* (shrub layer 2), *Quercus robur* (regeneration), *Atriplex hastata*, *Chenopodium album*; 6: *Fraxinus pennsylvanica* (regeneration), *Astragalus pseudoscaber*, *Bidens tripartita*, *Taraxacum officinale*, *Lactuca serriola*, *Solanum nigrum*; 7: *Fraxinus excelsior* (shrub layer 1), *Populus nigra* (shrub layer 2), *Sambucus nigra* (shrub layer 2).

Place and data of sampling. 1: Mouth of Olt River into the Danube, 24.05.2004; 2: Mouth of Jiu River into the Danube, left river bank, 22.05.2004; 3: Danube Island Cioara, 24.05.2004; 4: Danube Island Cama, 29.05.2004; 5: Danube Island Cama, 29.05.2004; 6: river km 524, upstream Păsărica islet, 27.07.2004; 7: Danube Island Berzina Mică, 25.05.2004.

Place and data of sampling. 1: island in the Olt river on the mouth into the Danube, 24.05.2004; 2: island on the mouth of Olt River into the Danube, 24.05.2004; 3: river bank of the Olt River on the mouth into the Danube; 4: Danube island Calnovăț, downstream part of the island, 25.05.2004; 5: Danube island Calnovăț, upstream the mouth of Olt River, 25.05.2004.

Table 5: Tamarix ramosissima community (pioneer setteling).

Number of sampling	1	2	3	4	5	
Covering degree in %	50	45	60	60	85	
						F
Tamarix ramosissima	3	3	2	3	4	V
Equisetum palustre fluviatile	1	.	.	+	.	II
Xanthium strumarium	+	+	.	+	+	IV
Agrostis stolonifera	+	+	.	+	2	IV
Mentha pulegium	1	+	3	1	+	V
Elytrigia repens	+		1	2	1	IV
Veronica peregrina	+	+	+	.	.	III
Rorippa sylvestris	.	+	.	+	.	II
Alopecurus aequalis	.	+	.	.	.	I
Plantago intermedia	.	.	+	.	.	I
Potentilla reptans	.	.	+	.	+	II
Polygonum aviculare	.	.	.	+	.	I
Rorippa amphibia	.	.	.	+	.	I
Althaea officinalis	.	.	.	+	.	I

Table 6: Querco-Ulmetum floodplain forest.

Number of sampling	1	2	3	4	5	6	7	8	9	10	11	12	
	0.9	0.8	0.8	0.7	0.8	0.8	0.8	0.8	0.5	0.7	0.7	0.6	
													F
1. Tree layer (12-20 m)													
Quercus robur	3	3	1	3	2	2	.	.	3	2	2	.	IV
Quercus pedunculiflora	+	+	.	.	.	.	2	2	.	.	.	3	III
Fraxinus angustifolia	.	.	.	.	.	.	.	.	.	.	.	2	I
2. Tree layer (7-12 m)													
Ulmus laevis	.	1	4	3	4	4	4	2	.	.	.	.	III
Populus nigra	3	3	2	.	.	.	.	1	.	.	2	.	III
Populus alba	.	.	.	+	.	.	1	3	1	2	4	+	III
Populus canescens	.	.	.	.	.	.	.	+	.	.	.	+	I
Morus alba	2	.	.	.	+	.	+	.	+	3	.	.	III
Ailanthus altissima	.	.	.	.	.	.	.	.	.	.	.	+	I
3. Tree layer (5-7 m)													
Ulmus laevis	.	.	.	+	.	.	+	+	1	.	+	+	III
Crataegus monogyna	+	.	.	.	.	.	.	.	.	.	.	.	I
Morus alba	+	+	.	.	.	.	+	.	.	.	.	.	II
1. Shrub layer (3-5 m)													
Crataegus monogyna	+	.	.	.	.	.	.	.	.	.	.	+	I
Ulmus laevis	.	.	+	+	.	.	.	+	+	.	.	.	II
Morus alba	+	+	+	+	.	.	+	.	.	.	+	+	III
Cornus sanguinea	.	.	.	+	.	.	.	.	.	+	.	.	I
2. Shrub layer (1-3 m)													
Amorpha fruticosa	1	+	+	+	+	3	.	1	.	2	+	+	V
Ulmus laevis	+	.	.	.	.	+	.	+	+	.	+	.	II
Regeneration													

Number of sampling	1	2	3	4	5	6	7	8	9	10	11	12	
	0.9	0.8	0.8	0.7	0.8	0.8	0.8	0.8	0.5	0.7	0.7	0.6	
													F
Quercus robur	.	+	+	.	2	+	.	.	+	2	+	.	III
Quercus pedunculiflora	+	+	.	.	.	.	+	.	.	.	.	+	II
Ulmus laevis	+	.	+	.	+	+	.	+	+	.	+	+	IV
Amorpha fruticosa	.	.	+	.	.	.	+	.	.	.	+	.	II
Liana and liana-like sp.													
Vitis sylvestris	.	.	.	+	.	+	.	.	.	.	.	.	I
Humulus lupulus	+	.	.	.	.	.	.	.	.	3	.	.	I
Cynanchum acutum	.	+	.	.	.	.	.	.	.	.	.	.	I
Rubus caesius	.	+	+	+	.	+	+	.	+	.	+	.	III
Cucubalus baccifer	.	.	.	.	.	+	.	.	.	2	.	.	I
Herbaceous layer													
Recognition species													
Asparagus pseudoscaber	+	+	+	+	+	+	1	1	.	.	+	.	IV
Leucojum aestivum	.	+	.	.	.	.	.	.	.	.	+	.	I
Aristolochia clematitis	1	+	2	+	+	+	+	1	.	+	+	1	V
Other species													
Asparagus tenuifolius	.	.	+	+	.	.	.	.	.	.	.	+	I
Elymus repens	+	.	.	.	+	.	.	+	+	.	+	.	III
Clematis integrifolia	.	.	+	.	.	.	.	.	.	.	+	.	I
Carex praecox	.	.	+	.	.	+	.	.	.	.	+	.	II
Symphytum officinale	.	+	.	.	+	+	.	.	.	.	.	.	II
Galium aparine	3	+	3	+	2	+	.	.	.	.	2	.	III
Urtica dioica	+	+	+	+	+	.	.	.	+	+	+	+	IV
Anthriscus trichosperma	.	.	+	+	.	2	.	.	.	.	+	3	III
Cannabis sativa	.	+	+	+	+	.	.	.	.	+	.	+	III
Leonurus cardiaca	.	+	.	+	.	+	.	.	+	+	.	.	III
Arctium lappa	+	+	+	+	.	.	.	.	.	.	+	.	III
Chelidonium majus	.	.	.	+	.	.	.	.	.	1	.	.	I
Glecoma hederacea	.	.	.	.	.	.	.	.	.	.	2	+	I
Bromus racemosus	.	.	.	+	.	.	.	.	.	.	.	+	I
Malachium aquaticum	+	.	.	.	.	.	.	.	.	.	.	+	I
Atriplex hastata	.	.	.	.	.	+	.	.	.	.	.	+	I
Stellaria media	.	.	+	.	.	.	.	.	.	.	.	+	I
Aster lanceolatus	.	.	.	+	.	.	.	.	+	.	.	.	I
Rumex conglomeratus	+	.	.	+	.	.	.	.	.	.	.	.	I
Lysimachia nummularia	+	.	.	.	.	+	.	.	.	.	+	.	II
Poa trivialis	.	.	.	+	.	+	.	.	+	.	.	.	II
Lactuca serriola	+	.	.	.	+	.	.	.	.	+	.	.	II
Bidens tripartita	.	.	.	.	.	.	.	+	.	+	.	.	I
Vicia sepium	.	.	.	.	.	+	.	.	.	.	+	.	I
Bidens frondosa	.	+	.	.	.	.	.	.	+	.	.	.	I

Species noted with + in one sampling area. 1: *Crataegus monogyna* (tree/shrubs layer 3 - 5 m), *Poa pratensis*, *Carex hirta*; 2: *Cynanchum acutum*; 3: *Alopecurus pratensis*, *Cirsium arvense*, *Sonchus arvensis*; 4: *Fraxinus pennsylvanica*, *Sambucus nigra* (shrub layer 1), *Populus alba* (regeneration), *Ranunculus repens*, *Lapsana communis*; 5: *Carex acutiformis*; 6: *Crataegus monogyna* (shrub layer 2), *Crataegus monogyna* (regeneration); 7: *Fraxinus pennsylvanica*, *Morus alba* (tree/shrubs layer 3 - 5 m); 8: *Cornus sanguinea*, *Morus alba* (shrub layer 2), *Cornus sanguinea* (regeneration), *Brachypodium sylvaticum*; 9: *Calystegia sepium*, *Plantago major*, *Erigeron annuus*, *Prunella vulgaris*, *Sambucus ebulus*, *Scrophularia scopoli*; 10: *Ailanthus altissima* (tree layer 2), *Ailanthus altissima* (tree/shrubs layer 3 - 5 m), *Populus alba* (shrub layer 2), *Circaea lutetiana*; 11: *Populus alba* (tree/shrubs layer 3 - 5 m), *Morus alba* (regeneration), *Iris pseudacorus*; 12: *Fraxinus angustifolia* (tree/shrubs layer 3 - 5 m), *Hordeum murinum*, *Conium maculatum*, *Polygonum aviculare*.

Place and data of sampling. 1: km 518, Saica, Vedea community, Giurgiu County, 28.05.2004; 2: river km 521, river bank near islet Păsărica /Cetățuia, Giurgiu County, 27.07.2004; 3: Protected Area Cama-Dinu, near river km. 519, on the foot of dyke, 4.06.2004; 4: Năvodari, Teaca Forest, on river km 574, 25.05.2004; 5: river bank on river km 518, Giurgiu County, 4.06.2004; 6: river bank on river km 518, Giurgiu County, 4.06.2004; 7, 8: Pietroşani, Teleorman County, river km 530, 30.07.2006; 9: near to the flood protection dyke, on river km 521, Protected Area Cama-Dinu, 27.07.2004; 10: near to the flood protection dyke on river km 523, 27.07.2004; 11: Protected area Cama-Dinu, near river km 517, 4.06.2004; 12: near the mouth of Oltu Mic (Sâiu), 25.5.2004.

Table 7: Transition forest of poplar species and elm on Călăraşi islands area.

Serial number	1	2	3	4	5	6	7	8	
Covering degree of tree crowns	0.6	0.7	0.5	0.7	0.8	0.4	0.6	0.9	
									F
1. Tree layer (> 15 m)									
Populus nigra	.	2	.	3	.	.	2	.	II
Populus alba	.	.	.	2	3	.	1	.	II
Fraxinus excelsior	.	.	.	+	.	+	2	.	II
Populus canadensis	+	.	.	.	1	.	.	3	II
2. Tree layer (15 m)									
Salix alba	2	2	1	+	.	+	+	.	IV
Ulmus minor	.	.	.	2	3	3	2	.	III
Morus alba	+	+	+	.	+	.	+	.	IV
3. Tree layer (5 - 8 m)									
Ulmus minor	3	3	3	.	.	.	.	2	III
Morus alba	.	.	.	.	.	.	.	3	I
1. Shrub layer (2 - 5 m)									
Ulmus minor	.	1	.	.	.	.	.	.	I
Amorpha fruticosa	1	1	2	+	+	+	+	+	V
2. Shrub layer (1.5 - 2 m)									
Ulmus minor	2	.	.	.	.	+	1	.	II
Fraxinus pennsylvanica	.	+	+	+	+	+	+	.	IV
Crataegus monogyna	.	.	.	.	.	.	+	+	II
Rosa canina	.	.	.	.	.	.	.	+	I
Amorpha fruticosa	.	.	.	.	.	.	+	.	I

Serial number	1	2	3	4	5	6	7	8	
Covering degree of tree crowns	0.6	0.7	0.5	0.7	0.8	0.4	0.6	0.9	
									F
3. Shrub layer (0.80 - 1.00 m)									
Ulmus minor	1	.	.	.	.	+	+	.	II
Populus alba	.	.	.	.	+	.	.	.	I
Forest regeneration									
Ulmus minor	.	.	.	.	+	+	+	+	III
Morus alba	.	.	.	.	.	+	.	.	I
Fraxinus excelsior	.	.	.	.	.	+	+	.	II
Crataegus monogyna	.	.	.	.	.	+	.	+	II
Liane and liane-like species									
Periploca graeca	+	+	+	+	+	+	+	+	V
Cynanchum acutum	.	.	.	+	.	.	+	+	II
Asparagus pseudoscaber	.	.	.	+	.	.	.	.	I
Rubus caesius	3	.	.	.	+	2	2	+	IV
Calystegia sepium	+	.	.	.	+	.	+	+	III
Solanum dulcamara	+	+	.	.	.	.	.	.	II
Herbaceous layer									
Aristolochia clematitis	1	2	2	+	+	1	+	2	V
Elymus repens	2	+	.	+	.	+	+	2	IV
Lythrum salicaria	+	.	.	.	.	+	.	.	II
Agrostis stolonifera	+	+	.	.	.	.	.	.	II
Lycopus europaeus	+	+	.	.	.	.	.	.	II
Stachys palustris	+	+	.	.	.	.	.	.	II
Symphytum officinale	+	.	.	.	+	+	+	.	III
Althaea officinalis	.	.	.	.	.	.	+	+	II
Potentilla reptans	+	.	+	+	.	.	.	.	II
Ranunculus repens	.	+	+	+	.	.	+	.	III
Veronica longifolia	.	+	+	.	.	.	.	.	II
Rumex conglomeratus	.	.	+	+	.	.	.	.	II
Rumex limosus	+	.	.	.	.	.	+	.	II
Bidens tripartita	+	+	2	+	+	+	+	+	V
Galium aparine	+	.	+	.	+	+	+	+	IV
Glecoma hederacea	2	3	2	+	2	2	.	2	IV
Aster lanceolatus	+	+	+	3	.	+	3	.	IV
Urtica dioica	+	.	+	.	2	.	.	+	III
Chenopodium album	+	.	.	.	.	+	.	+	II
Arctium lappa	.	.	.	.	+	+	.	+	II
Lactuca tatarica	.	+	.	+	.	.	.	.	II
Carex hirta	.	.	.	+	.	+	.	.	II
Lactuca serriola	.	.	.	+	.	+	.	.	II
Fagopyrum convolvulus	.	.	.	.	+	.	.	+	II
Cannabis sativa	.	.	.	.	+	+	.	.	II
Bidens cernua	.	.	.	.	.	+	+	.	II
Conyza canadensis	.	.	.	.	.	+	.	+	II

Species noted with + in one sample. 1: *Cirsium arvense*, *Bromus sterilis*; 2: *Polygonum hydropiper*, *Veronica longifolia*; 5: *Populus alba* (shrub layer 3); 6: *Morus alba* (regeneration); 7: *Marrubium vulgare*, *Amorpha fruticosa* (shrub layer 2); 8: *Crataegus monogyna*, *Rosa canina* (shrub layer 2), *Xanthium strumarium*.

Place and data of sampling. 1: River bank in the southern part of Călăraşi-Răul island, on river-km 356, 25.06.2002; 2, 3, 4: eastern part of Călăraşi-Răul island, 27.06.2002; 5: River bank in the southern part of Călăraşi-Răul island, near river-km 356, 28.06.02; 6: between river bank of the southern part of Călăraşi-Răul island and the flood protection dyke; 7: Ostrov /island Păcuiu lui Soare, 29.06.2002; 8: Călăraşi-Răul island, near to river-km 356, old flood channel, 29.06.02.

CONCLUSIONS

Due to its hydrological and morphological dynamics and the formation of new sediment banks on the Lower Danube, in particular the growth of already existing islands and the formation of new ones, one may observe many processes on the Danube islands: all evolution processes of pioneer stages in willow stands, the natural regeneration of White and Black Poplar, the formation of gallery-like softwood forests and first settlings of hardwood forests, particularly with elm species, in more elevated places. The White willow and Black poplar forests on some small Romanian and Bulgarian islands that emerged on natural habitats without human interference are actual pristine forests, that, together with the pioneer vegetation of ephemerous species in the river bed at low water levels (below the mean water level) reach the highest degree of naturalness (Schneider, 2003a, 2003b). Their development and survival depend on the water level dynamics, on erosion and accretion. They stand at the beginning of a whole series of developments and are the prerequisite for a natural development of floodplain forests. As they have considerably decreased all over Europe, they deserve special attention from the point of view of nature conservation.

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SPREADING AND ECOLOGY OF *MANAYUNKIA CASPICA* ANNENKOVA 1928 (POLYCHAETA) IN THE SERBIAN DANUBE STRETCH

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KEYWORDS: Danube, Serbia, Polychaeta, *Manayunkia caspica*, Ponto-Caspian relict, spreading, ecology, meio- and macrozoobenthic communities.

ABSTRACT

Detailed limnological investigations of the Serbian Danube stretch at eight standard locations in a 261 km long sector of the Danube (from 1112 to 851 river km) were performed from April 2002 to June 2009. Samples were collected seasonally (April, June, September and November). A Ponto-Caspian element, *Manayunkia caspica* Annenkova, 1929 was found for the first time in the Serbian Danube stretch in November 2005. After that the species has been repeatedly found at the town of Kladovo, in the reservoir Iron Gate II - Djerdap II - 934 r-km, mainly at the depth of 5 - 11 m. *Manayunkia caspica* has been repeatedly found only in Kladovo sampling site in every season over the period from November 2005 to June 2009. The most abundant populations of *Manayunkia caspica* were recorded in November 2007 (7.696 individuals per square meter, calculated biomass 645.2 mg/m^2, calculated individual biomass values ranged from 0.020 - 0.122 mg) in habitats with gravel and sand substrate. The data on Danube water physical and chemical characteristics as well as those relating to its saprobiological status are presented. Findings of *M. caspica* in the Serbian part of the Danube prove that this Ponto-Caspian relict, as invasive species, shifted the limit of its distribution upstream in the Danube, which is the main corridor for its spreading from the east/south east (Bulgaria, Romania) to Central Europe.

ZUSAMMENFASSUNG: Verbreitung und Ökologie von *Manayunkia caspica* Annenkova 1928 (Polychaeta) im serbischen Abschnitt der Donau.

Im serbischen, 261 km langen Donauabschnitt (Strom-km 1112 bis 851), wurden von April 2002 bis Juni 2009 an acht Probestellen umfassende limnologische Untersuchungen durchgeführt. Die Proben wurden jährlich, jeweils Saison gebunden im April, Juni, September und November entnommen. Dabei wurde im Jahr 2005 zum erstenmal in der Serbischen Donau ein pontisch-kaspisches Element, *Manayunkia caspica* Annenkova 1928, festgestellt. Danach konnte die Art wiederholtemale nahe der Stadt Kladovo, im Bereich des Stausees Eisernes Tor II - Djerdap II - Strom-km 934, vorwiegend in einer Tiefe von 5 - 11 m nachgewiesen werden. *Manayunkia caspica* wurde nur an der Probestelle in Kladovo über die Jahre hin zum Zeitpunkt jeder Probenahme von November 2005 - Juni 2009 festgestellt. Die höchste Abundanz der Populationen (7.696 Individuen pro Quadratmeter mit einer errechneten Biomasse von 645,2 mg/m^2 und einem Biomassenwert von 0,020 - 0,122 mg pro individuum) wurde im November 2007 in Habitaten mit Kies- und Sandsubstrat verzeichnet. Angaben zu den physikalischen und chemischen Parametern, ebenso auch jene betreffend ihren saprobiologischen Zustand, wurden ebenfalls erfasst. Die Untersuchungen von *Manayunkia caspica* in der serbischen Donau belegen, dass dieses pontisch-kaspische Relikt, als eine invasive Art, seine Verbreitungsgrenze in der Donau stromaufwärts verschiebt, wobei diese den Hauptkorridor für die Verbreitung von Osten bzw. Südosten nach Zentraleuropa darstellt.

REZUMAT: Răspândirea şi ecologia la *Manayunkia caspica* Annenkova, 1928 (Polichaeta) în sectorul sârbesc al Dunării.

Au fost realizate investigaţii limnologice, detaliate asupra sectorului sârbesc al Dunării în opt locaţii standard, într-un sector lung de 261 km (de la km 1112 la km 851), din aprilie 2002 până în iunie 2009. Probele au fost prelevate sezonier (aprilie, iunie, septembrie şi noiembrie). Un element Ponto-Caspic, *Manayunkia caspica* Annenkova, 1929 a fost găsit pentru prima dată în sectorul sârbesc al Dunării în noiembrie 2005. După aceasta, specia a fost găsită în repetate rânduri în oraşul Kladovo, în acumularea Porţile de Fier II - Djerdap II - km 934, în principal la adâncimea de 5 - 11 m. *Manayunkia caspica* a fost, în mod repetat găsită, în fiecare sezon de-a lungul perioadei noiembrie 2005 - iunie 2009, doar în zona de prelevare Kladovo. Cele mai abundente populaţii de *Manayunkia caspica* au fost înregistrate în noiembrie 2007 (7.696 indivizi/m^2, biomasa calculată 645,2 mg/m^2, biomasa individuală, calculată a variat între 0,020 - 0,122 mg) şi au fost găsite în habitate cu substrat de pietriş şi nisip. Sunt prezentate datele referitoare la caracteristicile fizice şi chimice ale Dunării, ca şi cele legate de starea saprobiologică. Găsirea lui *M. caspica* în sectorul sârbesc al Dunării dovedeşte faptul că acest relict Ponto-Caspic, ca specie invazivă, şi-a modificat limita de distribuţie înspre amontele Dunării, care este principalul coridor pentru răspândirea ei din est, respectiv sud-est (Bulgaria, România) spre Europa Centrală.

INTRODUCTION

The Danube River is the second-largest European river, with a length of 2.857 km and a drainage area of about 817.000 km^2, and it is the only European big river that flows from the south-east to west. On its way from the Black Forest to the Black Sea, it links 10 states, and drains a total of 18 countries via its tributaries connecting Central with Eastern Europe.

In Serbia, the Danube River is a typical large lowland river with a continuous macrobiotope of characteristic potamobenthic communities. With its major tributaries (Tisa, Sava, Tamis/Timiş, Morava, Pek and Timok), Serbian sector of the Danube, the length of which in Serbia is 587.4 km, is a significant natural resource. It is divided into the Upper part - riverine zone, Middle part - flow through reservoir and Lower part - reservoirs Iron Gate I and II (Martinovic-Vitanovic and Kalafatic 2002a; Martinovic-Vitanovic et al., 2006).

Complex limnological investigations of the Serbian Danube stretch started in the middle of the 20th century (Nedeljkovic, 1967, 1979; Jankovic, 1975, 1978; Jankovic and Jovicic, 1994; Martinovic-Vitanovic and Kalafatic, 1990, 1995, 2002a, 2002b; Martinovic-Vitanovic et al., 1999a, 1999b, 2004, 2006, 2007, 2008).

Detailed and extensive limnological investigations of the Danube in the Serbian sector of the river have been performed ever since 2002. During these investigations, in November 2005, a Ponto-Caspian element, the polychaete *Manayunkia caspica* Annenkova, 1929 was found for the first time in Kladovo sampling site - 934 r-km in Iron Gate II reservoir.

According to Glasby and Timm (2008), less than 2% of all Polychaeta (Annelida) live in freshwater (168 species), being most diverse in Paleartic. One of the main areas of Polichaeta diversity in Palaearctic is the Ponto-Caspian Basin. During the past 200 years, the dispersal of Ponto-Caspian aquatic invertebrates, including polichaets, outside their historic geographical range was mainly caused by the construction of canals between once separated biogeographic regions and by unintentional introduction of vessels ballast water (Ketelaars, 2004). This has clearly been human-mediated. One of four main migration corridors is a south western corridor, connecting the Danube with the Rhine and neighbouring basins (Ketelaars, 2004).

There are really few data regarding records of a Ponto-Caspian element, *Manayunkia caspica*, in the European freshwaters (Annenkova, 1930; Zenkevitch, 1963; Russev et al., 1993, 1998; Arvanitidis et al., 2002; Grigorovich et al., 2002; Popescu-Marinescu, 2008; Glasby and Timm, 2008; Fauchald, 2009).

A subspecies *Manayunkia caspica danubialis* Băcescu in Dumitrescu, 1957 is registered in the Taxon List of the World Register of Marine Species (WoRMS) (Fauchald, 2009).

The first records of *Manayunkia caspica* (Polychaeta, Sabellidae) was at Dubova (970 r-km), in Romanian sector of the Danube in 1943 (Băcescu, 1944 after Popescu-Marinescu, 2008) long before the Iron Gate I (Djerdap I) dam construction in 1972. Spreading of this species distribution range has been prevented due to Iron Gate I - Djerdap I (and Iron Gate II - Djerdap II) dam construction and consequences of its construction that resulted in habitat changes (Nedeljkovic, 1967, 1979; Jankovic, 1975, 1978; Grigorovich et al., 2002; Brezeanu and Cioboiu, 2006).

The purpose of the present eight year study (from 2002 to 2009) was to provide an insight into the state of the qualitative and quantitative composition of Danube benthocenoses at Kladovo site as well as evidence that *Manayunkia caspica*, a Ponto-Caspian relict and invasive species, shifted the limit of its distribution through the Danube River corridor up to 934 r-km. Judging by the literature data, this finding is the most upstream record and the most recent one.

MATERIALS, METHODS AND STUDY AREA

According to Martinovic-Vitanovic and Kalafatic (2002a) and Martinovic-Vitanovic et al. (2006) the Serbian Danube stretch is divided into three parts:

I - Upper and Lower part – riverine zone I (upstream river part) and riverine zone II (downstream river part):
from r-km 1425 (Batina-Bezdan) to r-km 1250 (Novi Sad) and
from r-km 863.4 (Iron Gate II dam) to r-km 845.6 (the mouth of Timok River into the Danube);

II - Middle part – transitional zone (flow-through reservoir):
from r-km 1216 (Slankamen) to r-km 1072 (Ram) - where the impact of the impoundment of the course is evident; and

III - Lower part – lacustrine zone (lake part - reservoirs: Iron Gate I and II):
from r-km 1072 (Ram) to dam Iron Gate I (Djerdap I) at r-km 942.9 and
from r-km 942.9 to 863.4 dam Iron Gate II (Djerdap II), respectively.

Extensive limnological investigations of the Danube River were conducted in the period from April 2002 to June 2009 at eight standard sites in a 261 km long sector of the Danube (Fig. 1) situated along its course as follows:

Sampling site 1 - in flow-through reservoir part of the Danube;

Sampling sites from 2 to 5 - in Iron Gate I - Djerdap I reservoir;

Sites 6 and 7 - in Iron Gate II - Djerdap II reservoir;

Site 8 - in the riverine part of the Danube downstream Iron Gate II dam.

Samples, deriving from sites 1 to 8, were assigned temporary codes in situ and their code was then changed to permanent reference numbers in the laboratory and in the Collection of Limnological Material (where they are stored) of the Institute of Biological Research, in the Hydrobiological Department.

Figure 1: Map of the Danube stretch in Serbia (from 1433 to 845.6 km) Sector from 1112 to 851 river km and location of eight standard sampling sites (1 - 8) including Kladovo site (6); 1 - Smederevo - 1112 river km; 2 - Ram after the Nera River mouth into the Danube - 1072 river km + 400 m; 3 - Veliko Gradiste - 1059 river km; 4 - Donji Milanovac - 991 river km; 5 - Tekija - 956 river km; 6 - Kladovo - 934 river km; 7 - Kusjak - 864 river km; 8 - Radujevac - 851 river km; I - dam Iron Gate I (Djerdap I) at river km 942.9; II - dam Iron Gate II (Djerdap II) at river km 863.4.

The dam Iron Gate I (Djerdap I) was built in 1972 and the dam Iron Gate II (Djerdap II) in 1984.

Kladovo sampling site (GPS position - 44°37’02.7’’N 022°36’34.8’’E) is within the zone that is affected by the Djerdap I (and Djerdap II) hydropower plants operations, where forced oscillations of the water level and sudden changes in the course velocity are prevailing. Namely, it is the zone where the hydrologic and hydrographic parameters of the course have been extremely altered.

In our study, *Manayunkia caspica* was found for the first time in November 2005 solely in Kladovo site and ever since it has been a permanent member of the benthocenoses. Samples were collected at three points of the transversal profile: L - left - centre of the riverbed; M - middle point; R - right bank of the Danube. In total, 44 samples were gathered: 19 quantitative and 25 qualitative samples.

Samples were collected seasonally (April, June, September and November).

Limnological studies were performed using standard methods and techniques (APHA-AWWA-WEF, 1995).

Quantitative samples of sediment with benthic organisms were taken with Van-Veen type of grab (270 cm^2 grab area). The samples were gathered from water depth varying between 5 and 11 m.

Material for the analysis of meio- and macroinvertebrates came from the different bottom facies. Substrate classification was performed by visual evaluation in situ, and in the laboratory (using stereo zoom microscope with binocular magnifier-magnification 5-50x, Krüss, Germany), based on the diameter of sediment particles (Wentworth, 1922) and according to national classification after Martinovic-Vitanovic and Kalafatic (Martinovic-Vitanovic and Kalafatic, 1995; Lacusic et al., in Lakusic, ed., 2005).

Physical and chemical water analysis was performed in situ using standard methods and techniques APHA-AWWA-WEF (1995). The following parameters were analyzed: water flow, temperature, pH, oxygen concentration and conductivity. Concentration of dissolved oxygen, percentage of O_2 saturation, pH and conductivity were measured with Waterproof Multi-Parameter Field Meter (WTW, Germany).

Aquatic invertebrates in each sample were separated from the sediment by washing and sieving (200 µm sieve). All samples were fixed in situ with 4% formaldehyde and then transported to the laboratory for further processing. A stereo zoom microscope with binocular magnifier (magnification 5-50x), Krüss, Germany, and microscope (10 x 10, and 10 x 40), Opton, Germany were used for sorting and identification of organisms. Appropriate keys were used for determining bottom fauna representatives either up to the species level or to the lowest possible taxonomic level (Brinkhurst and Jamieson, 1971; Timm, 1999; Wiederholm, 1983; Lellak, 1980; Croft, 1986 and Annenkova, 1930).

Appearance frequencies (F = 0 - 1) of taxa and groups recorded at Kladovo sampling site were calculated as $\mathbf{F = m \cdot M^{-1}}$, where **m** stands for the number of samples, in which the particular species was found, and **M** stands for the total number of samples.

Density of the bottom fauna populations at sampling sites was defined by counting individuals in quantitative samples and calculated per unit area of the bottom surface (APHA-AWWA-WEF, 1995). Absolute abundance of *Manayunkia caspica* is given as the number of individuals per square meter (no. ind. m^{-2}) and/or as the number of individuals in the sample whereas their participation in the total abundance of benthocenosis at sampling sites (transversal profile with three points) and in qualitative samples is given as a percentage.

Wet biomass of individuals was measured on an analytical digital balance with accuracy of 0.1 mg (Chyo JK-200, Chyo Balance Corp., Tokyo, Japan).

Saprobic index (S), calculated according to Pantle and Buck (1955) and saprobity levels classification system, taken over from Moog et al. (2000), on the basis of saprobity system after Moog (1995, 2002), and Sladecek (1973), were used to estimate water quality at each point of the Kladovo transversal profile in samples where *M. caspica* was found.

For the calculation of saprobic index (S) the following formula was used:

$$\mathbf{S = \Sigma sh/\Sigma h};$$

where **s** stands for saprobic valence i. e. species degree of saprobity (after Moog ed., 1995, 2002), and **h** for abundance (rare = 1, frequent = 3, abundant = 5).

The following scheme shows the classification of the Saprobic Index (S), water quality classes and saprobity levels:

Saprobic Index	Water quality class	Saprobity level
under 1.25	I	oligosaprobic
1.25 to 1.75	I to II	oligo- to beta-mesosaprobic
1.76 to 2.25	II	beta-mesosaprobic
2.26 to 2.75	II-III	beta-meso- to alpha-mesosaprobic
2.76 to 3.25	III	alpha-mesosaprobic
3.26 to 3.75	III-IV	alpha-meso- to polysaprobic
over 3.75	IV	polysaprobic

RESULTS

From November 2005 to June 2009 44 samples in all (19 quantitative and 25 qualitative samples) were collected at Kladovo site. *Manayunkia caspica* was found in 68.2% of samples (30 samples - seven quantitative and 23 qualitative). Only in September 2008 *Manayunkia* couldn't be found because due to unfavourable hydrometorological conditions (wind and big waves) sampling was impossible. It was established that over a five-year period, *Manayunkia* has never been recorded in June in benthic cenosis i. e. in samples taken close to the right bank neither was it found in samples taken in April 2008, September 2007 and November 2005, 2007 and 2008. Thus, out of 14 samples in all taken close to the right bank *Manayunkia* was found in five samples only - 35.7%. Out of 15 samples in all, taken from the mid-course (L) *Manayunkia* was recorded in 12 samples (80%), and out of 15 samples in all taken at the middle point of transversal profile (M) *Manayunkia* was present in 13, i. e. even in 86.7% of samples.

The 30 samples analysed derived mainly from the following types of bottom facies, classified according to Wentworth (1922), shown in the table 1: cobbles and pebbles in most cases with coarse and medium sized sand (26 - 86.7%). Mollusc's shells are found in substrate in 8 out of 30 samples (26.7%) with coarse and medium sized particles. Only few samples i. e. samples deriving mainly from the right bank of the Danube contained traces of fine settled particles, and sometimes with detritus (4 - 13.3% and 2 - 6.7%, respectively).

Table 1: Sampling site at Kladovo - 934 km of the Danube's course and region of the river with depth (m) from which samples come and the description of the type of substrates (according to Wentworth, 1922); Transversal profile: L - left - centre of the riverbed; M - middle (medium point) of the transversal profile; R - right bank of the Danube; Substrate: c (cobble) - 64 - 256 mm, p (pebble) - 4 - 64 mm, csd (coarse sand) - 500 µm-1 mm, msd (medium sand) - 250 - 500 µm, fsd (fine sand) - 125 - 250 µm, shz (shelly zone), d (detritus), ssp (settled suspended particles).

Month	April			June		
Profile	L	M	R	L	M	R
Year	Depth (m) / Substrate	Depth (m) / Substrate	Depth (m) / Substrate	Depth (m) / Substrate	Depth (m) / Substrate	Depth (m) / Substrate
2005						
2006		8.5 p, msd	8.5 c, msd	8.5 msd	9.5 c, msd	
2007	7.0 p, msd	11.0 shz, p, msd	6.5 fsd, ssp	8.0 c, csd	8.0 p, msd	
2008	9.0 c, msd	9.0 shz, c, msd		7.7 p, msd	8.0 p, msd	
2009	10.0 p, csd	10.0 p, fsd	5.0 shz, msd, d, ssp	8.5 p, msd	7.0 p, csd	

Month	September			November		
Profile	L	M	R	L	M	R
Year	Depth (m) / Substrate	Depth (m) / Substrate	Depth (m) / Substrate	Depth (m) / Substrate	Depth (m) / Substrate	Depth (m) / Substrate
2005					8.0 shz, msd, ssp	
2006	7.0 p, msd	7.5 shz , p, msd	1.5 p, msd, d, ssp	8.0 c, msd	9.0 shz, p, msd	6.0 shz, p, msd, ssp
2007	9.0 p, msd, ssp	9.0 p, msd		7.0 p, msd	9.0 shz, p, msd	
2008				7.0 p, csd		
2009						

Results of the analysis of physical and chemical water characteristics 0.5 m above the bottom (at narrow depth interval, 7.0 - 10.0 m, through the years) in the middle of the Danube course (L) at Kladovo over a five year period are shown in the table 2**.** Maximal value of water flow (v_{max}) was between 0.724 m/s (September) and 1.354 m/s (April), mean value (v_{mean}) ranged from 0.465 m/s (September) to 0.944 m/s (April). Water temperature varied widely being from 7.6°C in November to 25.2°C in June. pH varied from 7.24 to 8.09, showing neutral to weakly alkaline reaction. Conductivity at 20°C was between 229 and 437 µS/cm (June and November, respectively). Dissolved oxygen concentration was between 4.9 mg/l in June and 11.7 mg/l in April. Oxygen saturation ranged from 60% to 109% (June and April, respectively).

Table 2: Physical and chemical Danube River water characteristics 0.5 m above the bottom (site in the middle of the Danube's course - (Left) in the transversal profile) at Kladovo in the years 2005 - 2009.

Month	IV	VI	IX	XI
Water flow - $v_{max.}$ (m/s)	1.354	1.117	0.724	1.069
Water flow - v_{mean} (m/s)	0.944	0.845	0.465	0.723
Depth (m)	7.0-10.0	8.0-8.5	7.0-9.0	7.0-8.0
Temperature (ºC)	10.4-15.9	19.8-25.2	19.8-23.8	7.6-13.5
pH	7.45-7.94	7.24-7.68	7.43-7.82	7.68-8.09
Conductivity (µS/cm 20ºC)	328-395	229-419	321-385	382-437
DO (mg/l)	8.0-11.7	4.9-8.4	5.3-7.7	8.4-10.7
O_2 saturation (%)	82-109	60-103	62-85	77-88

The meio- and macrozoobenthic community in Kladovo site was composed of representatives of 13 faunistic groups totalling 54 taxa: Polychaeta (two taxa), Turbellaria (three taxa), Oligochaeta (22 taxa), Amphipoda (two taxa), Isopoda (one taxon), Hydrozoa (one taxon), Gastropoda (four taxa), Bivalvia (three taxa), Chironomidae (12 taxa), whereas representatives of Nematoda, Spongia, Hydracarina and Bryozoa were not determined to the lower taxonomic levels. The qualitative bottom fauna composition in Kladovo site is shown in the table 3 as a list of all taxa with their occurrence frequencies (F) for the period from November 2005 to June 2009.

The highest occurrence frequencies in descending order have the faunistic groups Polychaeta and Oligochaeta (F = 1), Bivalvia (F = 0.97), Amphipoda (F = 0.93), Isopoda (F = 0.90), Nematoda (F = 0.80), Gastropoda (F = 0.73), and Chironomidae (F = 0.50), while other groups have lower occurrence frequencies being in the range from 0.03 - 0.23.

In addition to *Manayunkia caspica* (F = 1), the highest occurrence frequencies in descending order have the species *Corophium curvispinum* (F = 0.97), *Jaera sarsi* (F = 0.90), *Hypania invalida* (F = 0.83), *Dreissena polymorpha* (F = 0.80), and *Theodoxus fluviatilis* (F = 0.70).

Minimal number of taxa (3) was found in April 2007 in the sample collected at the right bank, and maximal number of taxa (21) was also recorded in April, but in 2009 in the sample gathered in the middle of the transversal profile.

The number of species recorded in the samples collected at the three points of transversal profile was 7 - 15 in the centre of the riverbed (L - left), 9 - 21 at the middle point (M), and 3 - 16 on the right Danube bank (R). Percentage of the species in the benthocenoses, at the three mentioned points of the transversal profile of the total of 54 taxa found at Kladovo over a five year period of investigations was: 40%, 43.3%, and 16.7%, respectively.

Quantitative analysis of the samples collected at Kladovo shows that total abundance of benthic organisms varied between 666 ind./m^2 (R) in April 2007 and 21,571 ind./m^2 (M) in November 2007.

Population density of *M. caspica* at Kladovo sampling site is given in the table 4 and is presented by seasons per each year of a five year period from 2005 - 2009**.**

Table 3a: Composition of the Danube's bottom fauna and frequencies of taxa (F = 0 - 1) at sampling site Kladovo, Iron Gate II reservoir, from November 2005 to June 2009.

Year	2005	2006											2007				
Month	XI	IV		VI		IX			XI			IV			VI	
Taxa / Profile	M	M	R	L	M	L	M	R	L	M	R	L	M	R	L	M
Nematoda	+		+	+		+	+	+	+	+	+	+	+			+
Manayunkia caspica Annennkova, 1929	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hypania invalida (Grube, 1860)	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+
Dendrocoelum lacteum (Müller, 1774)																
Polycelis tenuis Ijima, 1884																
Planaria torva (Müller, 1774)	+															
Aulodrilus pluriseta (Piguet, 1906)																
Tubifex tubifex (Müller, 1774)								+	+		+					+
Limnodrilus hoffmeisteri Claparede 1862					+	+	+	+							+	+
Limnodrilus udekemianus Claparede, 1862																
Limnodrilus claparedeanus Ratzel, 1868				+			+	+			+					
Potamothrix hammoniensis Michaelsen, 1901											+					
Potamothrix bavaricus (Oschmann, 1913)					+											
Vejdovskyella comata (Vejdovsky, 1884)																
Enchytraeus albidus Henle, 1837																
Spirosperma ferox Eisen, 1879	+		+								+					
Spirosperma velutinus (Grube, 1860)	+															
Branchiura sowerbyi Beddard, 1892	+						+	+								
Psammoryctides albicola (Michaelsen, 1901)																
Nais barbata Müller, 1774																
Nais pseudobtusa Piguet, 1906								+								
Enchytronia parva Nielsen & Christensen, 1959		+														
Propappus volki Michaelsen, 1916																
Fridericia sp.																
Isochaetides michaelseni (Lastockin, 1936)																
Uncinais uncinata (Ørsted, 1842)																
Stylaria lacustris (Linnaeus, 1767)								+								
Einseniella tetraedra (Savigny, 1826)											+					+
Oligochaeta fragments				+				+								

Table 3b: Composition of the Danube’s bottom fauna and frequencies of taxa (F = 0 - 1) at sampling site Kladovo, Iron Gate II reservoir, from November 2005 to June 2009.

Year	2008					2009					F (0 - 1)
Month	IV		VI		XI	IV			VI		
Taxa / Profile	L	M	L	M	L	L	M	R	L	M	
Nematoda	+	+		+	+	+	+	+	+	+	0.80
Manayunkia caspica Annennkova, 1929	+	+	+	+	+	+	+	+	+	+	1
Hypania invalida (Grube, 1860)		+	+	+	+		+		+	+	0.83
Dendrocoelum lacteum (Müller, 1774)				+							0.03
Polycelis tenuis Ijima, 1884											0.03
Planaria torva (Müller, 1774)											0.03
Aulodrilus pluriseta (Piguet, 1906)						+					0.03
Tubifex tubifex (Müller, 1774)		+	+	+	+	+	+	+			0.47
Limnodrilus hoffmeisteri Claparede 1862		+	+		+		+	+	+		0.47
Limnodrilus udekemianus Claparede, 1862					+			+			0.07
Limnodrilus claparedeanus Ratzel, 1868	+						+	+			0.23
Potamothrix hammoniensis (Michaelsen, 1901)	+	+	+	+				+			0.27
Potamothrix bavaricus (Oschmann, 1913)											0.03
Vejdovskyella comata (Vejdovsky, 1884)								+			0.03
Enchytraeus albidus Henle, 1837								+	+		0.07
Spirosperma ferox Eisen, 1879							+				0.17
Spirosperma velutinus (Grube, 1860)											0.03
Branchiura sowerbyi Beddard, 1892					+		+				0.23
Psammoryctides albicola (Michaelsen, 1901)		+					+				0.10
Nais barbata Müller, 1774							+				0.03
Nais pseudobtusa Piguet, 1906	+	+									0.10
Enchytronia parva Nielsen & Christensen, 1959							+				0.07
Propappus volki Michaelsen, 1916					+						0.03
Fridericia sp.				+	+						0.07
Isochaetides michaelseni (Lastockin, 1936)				+							0.03
Uncinais uncinata (Ørsted, 1842)	+										0.03
Stylaria lacustris (Linnaeus, 1767)	+										0.07
Einseniella tetraedra (Savigny, 1826)											0.07
Oligochaeta fragments					+	+	+	+	+		

Table 3c: Composition of the Danube's bottom fauna and frequencies of taxa (F = 0 - 1) at sampling site Kladovo, Iron Gate II reservoir, from November 2005 to June 2009; Sampling Site - Transversal profile: L - left - centre of the riverbed; M - middle of the transversal profile; R - right bank of the Danube.

Year	2005	2006										2007		
Month	XI	IV		VI		IX			XI			IV		
Taxa / Profile	M	M	R	L	M	L	M	R	L	M	R	L	M	R
Corophium curvispinum Sars, 1895	+	+	+	+	+	+	+	+	+	+	+	+	+	
Gammarus sp.	+	+	+	+	+	+	+	+		+	+	+	+	
Jaera sarsi Valkanov, 1936	+	+	+	+	+	+	+		+	+	+	+	+	
Hydra sp.														
Bryozoa											+			
Spongia						+								
Hydracarina	+													
Theodoxus fluviatilis (Linnaeus, 1758)	+	+	+	+	+	+	+			+	+	+	+	
Theodoxus danubialis (Pfeiffer, 1828)									+		+			
Lithoglyphus naticoides (Pfeiffer, 1828)						+								
Bithynia tentaculata (Linnaeus, 1758)													+	
Dreissena polymorpha (Pallas, 1771)	+	+	+			+	+	+	+	+	+	+	+	
Corbicula fluminalis (Müller, 1774)	+	+	+	+	+	+	+		+			+		
Corbicula fluminea (Müller, 1774)										+	+		+	+
Cricotopus bicinctus (Meigen, 1818)								+						
Cricotopus sylvestris (Fabricius, 1794)								+	+					
Polypedilum scalaenum (Schrank, 1803)								+						
Polypedilum albicorne (Meigen, 1838)														
Harnishia fuscimana Kieffer, 1921														
Dicrotendipes nervosus (Staeger, 1839)														
Virgatanytarsus arduennensis (Goetghebuer)														
Procladius Skuse														
Cladotanytarsus mancus (Walker, 1856)														
Nanocladius rectinervis (Kieffer, 1911)														
Demicryptochironomus vulneratus (Zetterstedt)									+					
Paratanytarsus sp.				+										
Nuber of taxa by site Σ = 54	14	9	10	11	9	12	12	16	11	9	16	9	10	3

Table 3d: Composition of the Danube's bottom fauna and frequencies of taxa (F = 0 - 1) at sampling site Kladovo, Iron Gate II reservoir, from November 2005 to June 2009; Sampling Site - Transversal profile: L - left - centre of the riverbed; M - middle of the transversal profile; R - right bank of the Danube.

Year	2007						2008					2009					F(0-1)
Month	VI		IX		XI		IV		VI		XI	IV			VI		
Taxa / Profile	L	M	L		L	M	L	M	L	M	L	L	M	R	L	M	
C. curvispinum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0.97
Gammarus sp.		+		+		+	+	+	+	+			+		+	+	0.73
J. sarsi	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	0.90
Hydra sp.			+	+	+					+						+	0.17
Bryozoa		+	+						+	+					+	+	0.23
Spongia																	0.03
Hydracarina			+	+									+		+		0.17
T. fluviatilis	+	+	+	+	+	+	+				+		+			+	0.70
T. danubialis	+					+											0.13
L. naticoides																	0.03
B. tentaculata																	0.03
D. polymorpha		+		+	+	+	+	+	+	+	+	+	+		+	+	0.80
C. fluminalis		+	+			+		+		+		+	+		+		0.57
C. fluminea		+	+	+	+	+			+		+		+				0.40
C. bicinctus							+					+					0.10
C. sylvestris																	0.07
P. scalaenum						+								+			0.10
P. albicorne														+			0.03
H. fuscimana														+			0.03
D. nervosus				+				+						+			0.10
V. arduennensis													+				0.03
Procladius														+			0.03
C. mancus									+								0.03
N. rectinervis	+														+		0.07
D. vulneratus																	0.03
Paratanytarsus sp.																	0.03
Taxa no. by site Σ = 54	7	14	13	12	11	18	13	14	12	15	15	10	21	16	14	10	

Table 4: Population density of *Manayunkia caspica* Annenkova, 1929 as number of individuals per square meter and its relative abundance in benthic communities at sampling site Kladovo, from the year 2005 to 2009; Transversal Profile: L - left - centre of the riverbed; M - middle (medium point) of the transversal profile; R - right bank of the Danube.

Month	April											
Profile	L				M				R			
	in/ m^2	%	in/ sa	%	in/ m^2	%	in/ sa	%	ind/ m^2	%	in/ sa	%
2005												
2006							45	45.45			229	50.33
2007			221	63.69			366	77.05	518	77.78		
2008	4551	82.55					196	70.5				
2009			11	29.73			1036	85.13	1036	20.9		

Month	June							
Profile	L				M			
	in/ m^2	%	in/ sa	%	in/ m^2	%	in/ sa	%
2005								
2006			18	23.38			16	41.03
2007			95	70.37			366	83.18
2008			110	68.32			117	53.18
2009			728	91.69			10	13.89

Month	September											
Profile	L				M				R			
	in/ m^2	%	in/ sa	%	in/ m^2	%	in/ sa	%	in/ m^2	%	in/ sa	%
2005												
2006			273	71.09			384	88.07	37	0.57		
2007			122	85.31	1924	23.64						
2008												

Month	November											
Profile	L				M				R			
	in/ m^2	%	in/ sa	%	in/ m^2	%	in/ sa	%	in/ m^2	%	in/ sa	%
2005							2237	91.23				
2006			62	54.39			17	14.53			111	66.07
2007			102	68.92	7696	35.68						
2008	5291	73.71										

Total abundance of *M. caspica*, calculated per square meter, was 37 ind./m^2, in September 2006 (R) and 7,696 ind./m^2 in November 2007 (M). Relative abundance ranged from 0.6% in September 2006 (R) to 91.7% in June 2009 (L), being 91.2% in November 2005 (M).

In 83.3% (25 quantitative and qualitative samples) of all gathered and analysed samples (30), *M. caspica* was dominant species, and in two samples it was subdominant species in zoobenthic community at Kladovo sampling site. In 2005 *M. caspica* was found for the first time in November as dominant species (91.2%) in meio- and macrozoobenthic community in the middle of the transversal profile, on sandy substrate with great number of empty Molluscs shells (shelly zone), used as a shelter by this species. In 2006 percentage participation of *M. caspica* in the bottom fauna ranged from 0.6% (September, R - on hard-bottom facies with fine settled particles and detritus) to 88.1% (September, M - on sandy substrate with gravel and great number of empty Molluscs shells). In 2007 minimum participation of this species was 23.6% (September, M - on gravel and sand) and maximum 85.3% was also in September (L - gravel and sand). *Manayunkia caspica* was dominant species in bottom fauna in the whole of 2008 with percentage participation between 53.2% (June, M - gravel and sand) and 82.5% (April, L - coarse gravel and sand), whereas in 2009 its percentage participation ranged from 13.9% (June, M - gravel and sand) to 91.7% (June, L - gravel and sand).

The fact that only one individual per sample (0.6%) was found in September 2006 (R) demonstrates that its presence is substrate dependent. Namely, only at that time *M. caspica* was found on hard-bottom substrate with few fine settled particles and detritus. In all other samples substrate was composed of coarse and medium-sized sand, pebble and cobble, where *M. caspica* was almost always dominant species in bottom fauna.

In 16.7% samples *Manayunkia caspica* was found in riparian zone (R), which is in correlation with substrate composition and water flow. In the riparian zone, water flow is lower and consequently the sedimentation of fine suspended particles is higher than in the centre of the Danube course. However in the middle zone (points L and M of the transversal profile), where water flow is higher and substrate is mainly of gravel and sand, proportion of findings of *M. caspica* at the points L and M is 40% and 43.3%, respectively.

The average mass of individuals during the whole period of investigation (Tab. 5), was 0.069 mg, minimal individual mass was 0.020 mg (June 2009, M), maximal individual mass was 0.122 mg (April 2009, M). In quantitative samples biomass of *Manayunkia caspica* ranged between 3.7 mg m^{-2} (September 2006, R) and 654.2 mg m^{-2} (November 2007, M).

Apart from *Manayunkia caspica*, a Ponto-Caspian relict, other Ponto-Caspian seven species, were recorded at Kladovo: *Hypania invalida*, *Isochaetides michaelseni*, *Corophium curvispinum*, *Jaera sarsi*, *Theodoxus danubialis*, *Lithoglyphus naticoides* and *Dreissena polymorpha*, five of them having the status of invasive species. Species new to the Danube invertebrate fauna, denoted as introduced species - Neozoa, are: *Corbicula fluminalis*, *C. fluminea*, and *Theodoxus fluviatilis* (Tabs. 3 and 6).

The highest occurrence frequencies in descending order, in addition to *Manayunkia caspica* F = 1, have four Ponto-Caspian species: *Corophium curvispinum* F = 0.97, *Jaera sarsi* F = 0.90, *Hypania invalida* F = 0.83 and *Dreissena polymorpha* F = 0.80; and the lowest have *Theodoxus danubialis* F = 0.13, *Lithoglyphus naticoides* and *Isochaetides michaelseni* F = 0.03 each. Neozoa have also high occurrence frequencies: *Theodoxus fluviatilis* F = 0.70, *Corbicula fluminalis* F = 0.57 and *C. fluminea* F = 0.40 (Tabs 3 and 6).

Calculated values of Saprobic Index (according to Pantle and Buck, 1955) of bottom fauna communities (only samples containing *M. caspica*) at Kladovo in the Iron Gate II reservoir (r-km 934) in the period from November 2005 to June 2009 are given in the table 7. According to this index, the saprobic status of the Danube locality in the Iron Gate II reservoir, estimated on the basis of evidenced faunal groups and recorded bottom fauna bioindicators, corresponds to mesosaprobic conditions, being in the range from beta-meso- to beta-alpha-mesosaprobity. Calculated values of Saprobic Index (S) varied from 1.95 (M, in November 2006) to 2.75 (R, in September 2006), being the extreme values for the whole research period.

Table 5: Biomass of *Manayunkia caspica* at sampling site Kladovo, Danube - Iron Gate II reservoir, from November 2005 to June 2009; Biomass (mg/m²) - measured value of Manayunkia specimens' biomass in sample (calculated per square meter) Manayunkia individual's mass (mg) - biomass calculated value per individual. Transversal profile: L - left - centre of the riverbed; M - middle (medium) point of the transversal profile; R - right bank of the Danube.

Season	April					
Profile	L		M		R	
Year	Biomass (mg/m²)	Individual's mass (mg)	Biomass (mg/m²)	Individual's mass (mg)	Biomass (mg/m²)	Individual's mass (mg)
2005						
2006				0.030		0.070
2007		0.060		0.099	15.5	0.030
2008	295.8	0.065		0.117		
2009		0.030		0.122	82.9	0.080

Season	June					
Profile	L		M		R	
Year	Biomass (mg/m²)	Individual's mass (mg)	Biomass (mg/m²)	Individual's mass (mg)	Biomass (mg/m²)	Individual's mass (mg)
2005						
2006		0.039		0.037		
2007		0.024		0.059		
2008		0.077		0.079		
2009		0.062		0.020		

Season	September					
Profile	L		M		R	
Year	Biomass (mg/m²)	Individual's mass (mg)	Biomass (mg/m²)	Individual's mass (mg)	Biomass (mg/m²)	Individual's mass (mg)
2005						
2006		0.041		0.018	3.7	0.100
2007		0.046	92.4	0.048		
2008						
2009						

Season	November					
Profile	L		M		R	
Year	Biomass (mg/m²)	Individual's mass (mg)	Biomass (mg/m²)	Individual's mass (mg)	Biomass (mg/m²)	Individual's mass (mg)
2005				0.054		
2006		0.036		0.046		0.076
2007		0.112	654.2	0.085		
2008	185.2	0.035				
2009						

Table 6: Ponto-Caspian relicts and introduced species of Danube's bottom fauna at sampling site Kladovo in the years 2005 - 2009; (*) - invasive species.

Month	IV				VI				IX		XI			
Year	2006	2007	2008	2009	2006	2007	2008	2009	2006	2007	2005	2006	2007	2008
Ponto-Caspian species														
**Manayunkia caspica* Anennkova, 1929	+	+	+	+	+	+	+	+	+	+	+	+	+	+
**Hypania invalida* (Grube, 1860)	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Isochaetides michaelseni (Lastockin, 1937)							+							
Theodoxus danubialis (C. Pfeiffer, 1828)						+						+	+	
**Lithoglyphus naticoides* C. Pfeiffer, 1828									+					
**Dreissena polymorpha* (Pallas, 1771)	+	+	+	+		+	+	+	+	+	+	+	+	+
Jaera sarsi Valkanov, 1936	+	+	+	+	+	+	+	+	+	+	+	+	+	+
**Corophium curvispinum* Sars, 1895	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neozoa (introduced species)														
Corbicula fluminalis (Muller, 1774)	+	+	+	+	+	+	+	+	+	+	+	+	+	
Corbicula fluminea (Muller, 1774)		+		+		+	+			+		+	+	+
Theodoxus fluviatilis Linnaeus, 1758	+	+	+	+	+	+		+	+	+	+	+	+	+

Table 7: Values of the Saprobic index S (Pantle and Buck, 1955) based on saprobiological analysis of the benthic communities of the River Danube at Kladovo (r-km 934) - Iron Gate II reservoir, Serbia in the years 2005 - 2009.

Month	April			June		September			November		
Profile	L	M	R	L	M	L	M	R	L	M	R
Year											
2005										2.00	
2006		2.09	2.01	2.24	2.41	2.11	2.21	2.75	2.28	1.95	2.25
2007	2.10	2.02	2.30	2.11	2.48	2.22	2.00		2.46	2.75	
2008	2.13	2.50		2.39	2.37				2.32		
2009	2.53	2.29	2.71	2.30	2.00						

The whole five year period of investigation (from November 2005 to June 2009) was characterized by small site-specific variations of saprobic levels recorded at L - left side of transversal profile - riverbed centre, M - middle point and R - right bank of the Danube at Kladovo locality, being in the equal relatively wide range from beta-meso- to beta-alpha-mesosaprobity (class II to II-III): 2.10-2.53; 1.95-2.75 and 2.01-2.75, respectively.

Saprobic index values varied seasonally: from 2.01 to 2.71 in April; from 2.00 to 2.48 in June; from 2.00 to 2.75 in September; and from 1.95 to 2.75 in November. It is evident that seasonal variations of saprobic index values were small whereby the smaller difference between maximum and minimum values was in June, the greatest being in November.

Saprobiological analysis shows that in the period between November 2005 and June 2009, out of 54 identified bottom fauna species 41 were bioindicators with individual saprobic values, whereby 17 were beta-mesosaprobic indicators with saprobic index from 2.0 - 2.3, nine were beta-meso- and beta-alpha-mesosaprobic indicators with individual saprobic values from 2.4 to 2.8. Ten and five bioindicators had saprobic valences in the lower and upper range of saprobity, being from 0.8 to 1.9 (oligo- and beta-mesosaprobic indicators) and from 2.9 to 3.6 (alpha-meso- and poly-alpha-mesosaprobic indicators), respectively. The majority of recorded bioindicators, which were most frequent as well as most abundant, indicated mesosaprobic conditions. Five species: *Manayunkia caspica*, *Polycelis tenuis*, *Enchytronia parva*, *Corbicula fluminalis* and *C. fluminea* haven't individual saprobic indicator values.

At Kladovo sampling site water quality of samples in which *M. caspica* was found over the period from 2005 - 2009 was assessed on the basis of saprobiological analysis of benthic communities (Pantle and Buck, 1955; Moog et al., 2000) and ranged from class II (50% of samples) and class II - III (another 50% of samples).

Manayunkia caspica does not have saprobic valence (Sladecek, 1973). This polychaete species has been shown to have the highest percentage in the total abundance of meio- and macrozoobenthos in samples water quality of which is class II - III (Tabs. 4 and 7). Out of 30 samples in all in which *M. caspica* was found 50% belong to class II, in 12 samples *M. caspica* was the most abundant species in benthocenoses with participation of 23.4% (June 2006, L), and from 45.4% (April 2006, M) to 91.2% (November 2005, M). In two samples this polychaeta was subdominant component. Water quality of 33% of samples is of class II-III, with Saprobic (S) index values ranging from 2.25 - 2.50 and *M. caspica* was dominant quantitativelly in all ten samples with participation between 41% (June 2006, M) and 91.7% (June 2009, L). Water quality of other 17% of samples was also of class II - III with Saprobic index values range from 2.50 - 2.75, whereby the participation of *M. caspica* in benthocenoses total abundance was lower. In four samples, relative abundance of this polychaeta ranged from 0.6% (September 2006, R) to 35.7% (November 2007, M), while only in April 2008 (M) *M. caspica* significantly participated in bottom fauna total abundance (70.5%).

DISCUSSION

The review of Glasby and Timm (2008), dealing with Polychaeta (Annelida) living in freshwater included 168 species, 70 genera and 24 families, representing all of the major polychaete clades. These 168 freshwater species account for less than 2% of all Polychaeta, out of which the most diverse in the Palaearctic region are 67 species representing 32 genera. Most of them occur not too far from the sea (euryhaline species). Within the Palaearctic region the main areas of diversity are Lake Baikal and the Ponto-Caspian region in particular. Most freshwater polychaets from the family Sabellidae (22 species in total) belong to subfamily Fabriciinae, *Manayunkia caspica* being one of them. The Ponto-Caspian sabellids (*Manayunkia* spp.) are one of several unrelated clades that occur isolated from related taxa and from their main centre of distribution. Arvanitidis et al. (2002) includes *M. caspica* (Annenkova, 1929) into a group of five exclusively endemic species in the Ponto-Caspian

Basins. They studied the mechanisms of migration and extinction rates of endemic species as the critical zoogeographic category in providing information on the evolutionary history of the taxon in the region. The Ponto-Caspian region is characterized by very high level of endemism (Ketelaars, 2004). The euryhalinity of the Ponto-Caspian biota makes them ideally pre-adapted to invade and survive in new environments. An important corridor for the Ponto-Caspian fauna to reach Western Europe is undoubtedly the Main-Danube Canal, which directly connects the Rhine basin with that of the Danube.

During the past 200 years, the dispersal of Ponto-Caspian aquatic invertebrates including polichaets, outside their historic geographical range has been mainly caused by the construction of canals between previously separated biogeographic regions and by unintentional introduction via vessels ballast water (Ketelaars, 2004). One of four main migration corridors among major canals, linking large rivers in Europe, distinguished by Ketelaars (2004), in his comprehensive book chapter on range extensions of Ponto-Caspian aquatic invertebrates in continental Europe, is a south western corridor, connecting the River Danube with the Rhine and neighbouring basins.

According to Grigorovich et al. (2002) *M. caspica* has “nonindigenous species status” denoted as “probable”, and its entry vector is hydrotechnical construction. The authors classified the species according to their residence or invasion status. Category “probable” includes species for which indirect evidence exists that species’ spread beyond its historic range (Ponto-Caspian region) was mediated by human activities, including habitat modification.

In the period from 2002 - 2009, within detailed and extensive limnological studies on the entire, 261 km long Danube sector surveyed, which included eight standard localities in the flow-through reservoir and reservoirs Djerdap I and II as well as the river stretch downstream from the dam, *Manayunkia caspica*, one out of the three hitherto recorded species of the Danube polychaetes, was found in November 2005 for the first time. Later it was found frequently on each of four occasions a year, solely in Kladovo locality in Djerdap II reservoir at 934 km river course, at about nine km downstream from the dam Iron Gate I (Djerdap I).

Manayunkia caspica was recorded for the first time in 1943 in the Cazane (Kazan in Serbian) zone, which is the narrowest part of the Danube in Djerdap Gorge - Iron Gate, in Romania in Dubova - 970 r-km, on rocky facies covered with *Corophium* cushions, at 35 m water depth (Băcescu, 1944 in Popescu-Marinescu, 2008), at the site, the characteristics of which highly differed from those prevailing currently on the same site in the Iron Gate I reservoir, due to the Danube damming in 1970s and the consequent reservoir formation which resulted in remarkable changes in this Danube sector, and possibly in species disappearance.

However, after 1943 during the detailed benthic surveys (carried in 1958 and in the period from 1966 - 1968 before the Lake Iron Gate I formation), and after Iron Gate I dam construction in Romanian stretch of the Danube the presence of *Manayunkia caspica* has never been discovered again on this, as it has been shown, the most upstream location of its record (Brezeanu and Cioboiu, 2006; Popescu-Marinescu, 2008).

Popescu-Marinescu (2008) in her review article, dealing with spreading and ecology of *M. caspica*, reported that she repeatedly found it in the Danube zoobenthos ever since 1943 along almost entire Romanian stretch. The most recent and most upstream species record ever was recorded in 2002 in the Iron Gate II Lake, on the left riparian and navigable zone at 928 r-km at the mouth of the Topolnița unto the Danube.

In Danube stretch from 928 to 926 r-km there is an isle Simijan, formed by the deposit of the tributary Topolnița (Topolnica in Serbian). Given that the Danube bed between the left bank and the isle is narrowed, water current in the navigable zone is strong.

Nowadays, this species spreading is correlated with hydrotechnical works (Grigorovich, et al., 2002). Thus, species spreading through the Danube has been prevented by Iron Gate I and II - Djerdap I and II dams construction which resulted in habitat changes (Nedeljkovic, 1967, 1979; Jankovic, 1975, 1978; Grigorovich et al., 2002; Brezeanu and Cioboiu, 2006).

Negative consequences of the damming are complex and numerous. From hydrological point of view, negative consequences included water level rise and water velocity decrease, which intensified mineral and organic suspensions deposition induced by the slow water velocity due to which reservoir bottom was covered with silt, especially in the gulfs and along the flat shallow areas (Jankovic, 1978; Nedeljkovic, 1979; Brezeanu and Cioboiu, 2006).

Hydrological circumstances and morphological characteristics at Kladovo (934 km) in Iron Gate II - Djerdap II reservoir were suitable for *Manayunkia* presence. In our studies, water flow average maximal value (v_{max}) was 1.121 m/s/year, and average mean value (v_{mean}) was 0.773 m/s/year. However, Nedeljkovic (1979) reported that before Iron Gate I (Djerdap I) dam construction the Danube water flow in Djerdap gorge ranged from 2.4 to 4.9 m/s, substrate being rock and gravel (pebbles and cobbles). Based on Brezeanu and Cioboiu (2006) the Danube damming induced the decreased water velocity from 3 - 5 m/s - as it has been registered along the sectors characterized by a steeper slope (mainly between 991 and 1018 r-km) - to 1 m/s, whereas within certain areas, e. g. in the gulfs and along flat areas, it was almost zero. Popescu-Marinescu (2008) showed that in 2002 *M. caspica* was well developed at about 1 m/s water flow in the Lake Iron Gate II (928 r-km) on the left riparian and navigable zone.

In our studies, at Kladovo, generally, *M. caspica* prefers habitat with hard substrate that mainly contains gravel (cobbles and pebbles) and coarse medium-sized sand (Wentworth, 1922). Mollusc's shells are found in substrate samples with coarse and medium sized particles. In the centre of the Danube course (point L), and in the middle zone of the transversal profile (point M), where water flow is higher and substrate is mainly formed of gravel and sand, proportion of findings on points L and M of *M. caspica* was 40% and 43.3%. In 16.7% samples *M. caspica* was found in riparian zone (R), and that is in correlation with substrate composition and water flow. In the riparian zone, water flow is lower and sedimentation of fine suspended particles is high (Jankovic, 1978). The samples deriving mainly from the right bank of the Danube have traces of fine settled particles, and sometimes some detritus, but these are only a few, and in these samples *Manayunkia* is the rarest. This observation is based on visual analysis of substrate particle size in the field and in the laboratory by using stereo zoom microscope with binocular magnifier. According to Verdonschot (1999), the comparison of characteristics of field and laboratory substrate/habitat parameters are needed. Thus, the comparison of the respective results that we obtained in our studies showed that the particle classes observed in the field were refined by laboratory analyses.

Based on Martinovic-Vitanovic and Kalafatic habitat classification (Martinovic-Vitanovic and Kalafatic, 1995; Lakusic, ed., 2005), at Kladovo, in uniform habitats, benthic communities are predominant on hard bottom. Bottom facies belong to hard bottom type (code 2.1.3), with prevailing granulation coded as: 2.1.3.2 - from 1 - 10 cm and 2.1.3.3 - from 10 - 100 cm. 0.1 - 1 mm particles are always present on the substrate combined with larger fractions, so that generally the substrate at Kladovo may be classified into a mixed type (code 2.1.4), whereby prevailing granulation particles are those that belong to facies type on which benthic communities of hard bottom develop (> than 1 cm - 100 cm - fractions 2.1.3.2 and 2.1.3.3) with admixture of sand fractions (code 2.1.2.3 - from 0.1 mm - 1 mm).

From hydrobiological point of view, negative consequences of dams led to significant modifications of the bottom fauna. Given that the rock, sand, clay and their mixtures gone, a large number of organisms, which used to inhabit these substrates, has disappeared as well, and the pelophylic biocoenosis is the most largely spread. The meio- and macrozoobenthic community at Kladovo site were represented by 54 taxa from 13 faunistic groups. Benthic community was qualitatively dominated by Oligochaeta, which was the most diversified group represented by 22 species. Subdominant Chironomidae were represented by 12 taxa. Gastropoda and Bivalvia followed by four and three taxa, respectively. In addition to *M. caspica*, one of the three registered polychaet species in the Danube, *Hypania invalida* was recorded at Kladovo site (with high occurrence frequency). Brezeanu and Cioboiu (2006) showed that the benthic community in Romanian Danube stretch has a completely new spatial and taxonomic structure. Out of the 493 taxa, determined before damming, 353 disappeared during the first years after the reservoir(s) formation. Only about 90 taxa have been identified, currently.

In addition to *M. caspica* (F = 1), the most frequent were *Corophium curvispinum* (F = 0.97), *Jaera sarsi* (F = 0.90), *Hypania invalida* (F = 0.83), *Dreissena polymorpha* (F = 0.80), and *Theodoxus fluviatilis* (F = 0.70). Results of Nedeljkovic (1967, 1979), Jankovic (1975, 1978) and Popescu-Marinescu (2008), obtained before and in the first ten years after Iron Gate I dam construction (first stage), as well as those of Brezeanu and Cioboiu (2006), Martinovic-Vitanovic and Kalafatic (1995, 2002a) and Martinovic-Vitanovic et al. (2006), showed that in first stages, after Iron Gate I and II dams construction rheophilic organisms disappeared totally or partially from benthocenoses, and the new limnophilic organisms appeared, so that currently, the structure of the zoobenthos is characteristic to the limnic-rheophilic ecosystem.

Analysis of physical and chemical water characteristics, 0.5 m above bottom, and substrate composition show that conditions prevailing in Kladovo locality are suitable for *Manayunkia caspica* survival. During the whole period of the study water flow was around 1 m/s, dissolved oxygen concentration ranged from 4.9 to 11.7 mg/l, and oxygen saturation varied between 60 and 109%. This is in accordance with Popescu-Marinescu (2008) findings, who showed that *M. caspica* prefers the stony and coarse sand facies, and develops well at about 1 m/s water flow and is the most oxyphilic among the three Ponto-Caspian polychaetes in the Danube, another two being *Hypania invalida* and *H. kowalewskii*.

In our studies *M. caspica* total abundance, calculated per m^2 reached maxim value of 7,696 ind./m^2 in November 2007 (M) relative abundance being 35.7%, and biomass 654.2 mg/m^2. These results are consistent with those of Popescu-Marinescu (2008) who shows that *M. caspica* usually form dense populations consisting of hundreds or thousands of individuals per m^2. However, owing to their small sizes the biomass is low. In June 2002, at 928 r-km (Topolnița) *M. caspica* reached the maximum numerical density as compared to other benthic coenoses within the whole Lake, being 24,125 ind./m^2 which accounts for 52.2% of total zoobenthic organisms developed on a stony-sandy facies in the navigable zone, at 11 m depth. Respective biomass of 1,492.50 mg/m^2 constituted only 0.09% of the zoobenthic biomass.

Nedeljković (1979) observed that between 1960 - 1965, before the construction of Iron Gate I (Djerdap I) dam, from 861 to 1040 r-km (lake part, current Iron Gate I reservoir) average values of zoobenthic abundance and biomass were very low (70 - 130 ind./m^2 and 0.2 - 0.66 g/m^2, respectively). In the fist stage after the Danube dam construction, due to banks silting and silt sedimentation, benthic fauna abundance and biomass were increased both in the flow-through reservoir and in the lake part. Some ten years after dam construction in the lake part population abundance and macrozoobenthic biomass reached on average of 35,000 ind./m^2 and 50.0 g/m^2 respectively, which manly resulted from dam construction.

Brezeanu and Cioboiu (2006) emphasised that the loss in biodiversity did not involve a reduction in density and biomass. On the contrary, an increase in the abundance and biomass of the benthic invertebrates between 1971 and 1980 was in a reverse trend of the number of taxa. The dominant groups are Chironomidae, Oligochaeta and Corophydae, with the development burst of the mussel species *Dreissena polymorpha* and *Sphaerium rivicolum*. Densities (the number of individuals per square meter - 30,000 ind./m^2) and the biomass (2,500 g/m^2) of these two species are ten times higher than those registered before damming.

Penetration of European continental waters by invasive and introduced species, originating mainly from Asia occurred some 30 years ago (Nedeljkovic, 1979; Lyashenko et al., 2004; Ojaveer et al., 2002) and this trend was observed also by us (Martinovic-Vitanovic and Kalafatic, 2002a and 2002b; Martinovic-Vitanovic et al., 1999b, 2004, 2006, 2007, 2008).

According to Tittizer (1997), Russev et al. (1993, 1998) and Ojaveer et al. (2002), out of eight Ponto-Caspian relicts, recorded during the period of investigation at Kladovo site, five species - *Manayunkia caspica*, *Hypania invalida*, *Corophium curvispinum*, *Lithoglyphus naticoides* and *Dreissena polymorpha* are **invasive species** in the Danube limnofauna. New species to the Danube invertebrate fauna, denoted as **introduced species - Neozoa,** are: *Corbicula fluminalis*, *C. fluminea*, and *Theodoxus fluviatilis*.

In addition to *M. caspica* a Ponto-Caspian relict, invasive species, in this study proved to shift the limit of its distribution up to 934 r-km in the Danube, according to Nedeljkovic (1979), another Polychaeta, *Hypania invalida*, has expanded its range from the Iron Gate I dam to the mouth of the Morava into the Danube in conjunction with abundant populations of the amphipod *Corophium robustum*. In our recent studies (Martinovic-Vitanovic and Kalafatic, 2002a; Martinovic-Vitanovic et al., 2004, 2006, 2008) we observed that since that time *H. invalida* has considerably expanded its range, and now it may be found at the most upstream site - at river-km 1425 and in the Sava River - at Usce village 60 km upstream from its mouth into the Danube. Penetration of Danube water by an invasive species *Corophium curvispinum* has caused a shrinkage in the distribution of the species *C. robustum*. Similarly, the snail *Theodoxus fluviatilis*, as one of currently not so numerous species that came from the Western Europe region such as Rhine or Atlantic coast, among which are *Corbicula fluminea* and *C. fluminalis*, was recorded in this study, and in the Danube tributary Sava (Martinovic-Vitanovic et al., 2008), well as in the Sava lake (reservoir at 9 - 4 Sava r-km) (authors unpublished data). Martinovic-Vitanovic and Kalafatic (2002a) registered *Corbicula fluminea* and *C. fluminalis* in the Serbian Danube stretch and ever since 2001 these mussel species are the most common ones in the middle and lower Danube stretch with tributaries Sava and Tisa (Martinovic-Vitanovic and Kalafatic, 2002a and 2002b; Martinovic-Vitanovic et al., 2004, 2006, 2008).

Ketelaars (2004) reported that range extensions of Ponto-Caspian species have probably been facilitated by the alteration of waters through pollution, eutrophication and other forms of human impacts that changed habitat characteristics.

The freshwater ecosystems aquatic community respond to both the effects of pollution and to hydro-morphology changes. The appearance and distribution of organisms in rivers is significantly influenced by: flow, habitat/substrate and water physical-chemical properties variations (pH, temperature, transparency, oxygen concentration, nutrients, toxic substances, pollutants, etc.). Species composition, their frequency of occurrence in benthic communities as well as quantitative aspect of assemblages are used as basis for the characterisation and assessment of the freshwater bodies ecological status (Chapman ed., 1997).

Cardoso and Free (2008) discuss invasive alien species incorporating them into ecological assessment in the context of the Water Framework Directive as currently the principal document covering management of inland, transitional and coastal waters in the EU. Its implementation regarding ecological assessment, using biota to develop approaches in order to detect a response to a specific pressure of invasive alien species owing to their ability to significantly alter the structure and functioning of aquatic ecosystems, is very important.

Generally, apart from influence of the substrate/habitat types and water flow, the diversity of benthic invertebrate community in the Danube is mostly affected by the permanent presence of biodegradable organic pollution received from tributaries and/or with poorly treated industrial or communal waste waters. Thus, besides the changes in overall characteristics of the Danube in the riverine, transitional and lacustrine zones resulting from river damming (Chapman, 1997), the saprobic status of the Danube in Kladovo - Iron Gate II reservoir (Djerdap II reservoir), as judged by the bottom fauna, corresponds to mesosaprobic conditions (Martinovic-Vitanovic and Kalafatic, 2002a; Martinovic-Vitanovic et al., 1999b, 2004, 2006, 2007). These results are in keeping with Damjanovic and Vulic (2004) data who monitored organic pollution of the Danube course in Serbia through biological oxygen demand (BOD) value. The authors reported that BOD tends to increase from Bezdan to Smederevo, whereas this tendency is less pronounced downstream from Smederevo to Radujevac.

In the period from 2005 - 2009, the saprobic status of the Danube at the Kladovo - 934 r-km, Iron Gate II reservoir (only samples containing *M. caspica* were analysed), estimateded on the basis of the bottom fauna bioindicators (Pantle and Buck, 1955; Moog et al., 2000), corresponds to mesosaprobic conditions, being in the range from beta-meso- to beta-meso- to alpha-mesosaprobity. The majority of recorded bioindicator species, which were most frequent and abundant, indicated beta-mesosaprobic conditions. These species had the greatest impact in the calculation of Saprobic index values of the benthocenoses at the investigated locality. *Manayunkia caspica* as well as *Polycelis tenuis*, *Enchytronia parva*, *Corbicula fluminalis*, and *C. fluminea* haven't individual indicator values (Sladecek, 1973). The values of Pantle-Buck's Saprobic Index S varied from 1.95 - 2.75 and estimated water quality, during the whole period of investigations, ranged from class II (50% of samples) to II - III (another 50% of samples with *M. caspica*). *Manayunkia caspica* has the greatest participation in total meio- and macrozoobenthic abundance at Kladovo site in samples which persist in water quality class II - III (S = 2.25 - 2.75). From 30 samples in all, 50% belong to class II, whereby this polychaeta was dominant and subdominant component of the bottom fauna total density. In the transition from water quality class II to III with Saprobic index ranging from S = 2.25 - 2.50 were 33% samples, and *M. caspica* dominated quantitativelly in ten samples. In another 17% of samples water quality was in the transition from class II to III with Saprobic index in the range from 2.50 to 2.75, where participation of *M. caspica* in benthocenoses total density was lower.

CONCLUSIONS

Findings of *M. caspica* at Kladovo over five years along 261 river km long studied Serbian Danube (with high occurrence frequency and high abundance in biocenoses) are the evidence that this Ponto-Caspian relict as invasive species shift the limit of its distribution up to 934 r-km in the Danube which is one of the main corridors for its spreading from the East (Romania, Bulgaria) to Central Europe. After dam construction (dam I) on the Danube in 1972, environmental conditions (substrate granulation i. e. type of habitat, water flow, and oxygen concentration in water) were changed and this polychaet finds suitable conditions nowadays only at Kladovo site as the most upstream location in Serbian (and Romanian) Danube stretch.

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FISH SPECIES DIVERSITY IN THE RIVERS OF THE NORTH-WEST BULGARIA

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KEYWORDS: North-West Bulgaria, biodiversity, indigenous species, conservation.

ABSTRACT
The rivers in the North-West Bulgaria belong to the Danube Basin. At present, they are slightly affected by industrial pollution and, except for their lower reaches, they are not regulated. In 2005, the fish species diversity in five rivers (Voinishka, Vidbol, Archar, Tsibritsa and Ogosta) was monitored. A total of 27 fish species belonging to seven families were recorded. Of them, 24 species are indigenous to the ichthyofauna of the Danube Basin. Most frequently occurred the species *L. cephalus*, *G. gobio*, *R. amarus* and *B. petenyi.* The greatest species diversity was registered in the rivers Ogosta (24 species), Lom (17 species) and Vidbol (16 species), and the lowest - in the Voinishka River (five species). 11 of the fish species recorded are of high conservation concern in Bulgaria. The main threats to fish populations are regulation by weirs and dikes in the lower reaches, water pollution, excessive fishing and poaching, and construction of micro-hydropower stations along the river courses.

ZUSAMMENFASSUNG: Die Fischfauna in den Flüssen Nord-West Bulgariens.
Die Flüsse im Nordwesten Bulgariens gehören zum Einzugsgebiet der Donau. Gegenwärtig leiden sie unter einer leichten Verschmutzung und lediglich in den Unterläufen bestehen wasserbauliche Einrichtungen. Im Jahr 2005 wurde die Fischartenzusammensetzung in fünf Flüssen (Voinishka, Vidbol, Archar, Tsibritsa und Ogosta) untersucht. Insgesamt wurden 27 Fischarten aus sieben Familien festgestellt, von denen 24 im Einzugsgebiet der Donau heimisch sind. Am häufigsten waren die Arten *L. cephalus*, *G. gobio*, *R. amarus* and *B. petenyi.* Die größte Artenvielfalt wurde in den Flüssen Ogosta (24 Arten), Lom (17 Arten) und Vidbol (16 Arten) nachgewiesen, während die niedrigste im Fluss Voinishka (fünf Arten) festgestellt wurde. 11 der nachgewiesenen Arten haben einen hohen Schutzstatus und sind für Bulgarien von großem naturschutzfachlichem Interesse. Die Hauptgefahren für die Fischpopulationen stellen die Dämme und Staumauern in den Unterläufen der Flüsse dar, außerdem die Wasserverschmutzung und die Überfischung. Dazu kommen noch Schwarzfischerei und die Errichtung zahlreicher kleiner Wasserkraftwerke in den Flussläufen.

REZUMAT: Diversitatea speciilor de peşti în râurile din nord-vestul Bulgariei.
Râurile din nord-vestul Bulgariei aparţin bazinului hidrografic al Dunării. În prezent, acestea sunt afectate în mică măsură de poluare şi, cu excepţia sectoarelor inferioare, nu sunt regulate. În 2005, s-a monitorizat diversitatea speciilor de peşti din cinci râuri (Voinishka, Vidbol, Archar, Tsibritsa şi Ogosta). În total, s-au identificat 27 de specii de peşti, aparţinând

la şapte familii. Dintre acestea, 24 de specii aparțin ihtiofaunei indigene a bazinului dunărean. Cele mai frecvente specii au fost *L. cephalus*, *G. gobio*, *R. amarus* şi *B. petenyi.* Cea mai mare diversitate a speciilor de peşti a fost înregistrată în râurile Ogosta (24 specii), Lom (17 specii) şi Vidbol (16 specii), iar cea mai scăzută în râul Voinishka (cinci specii). 11 dintre speciile de peşti înregistrate sunt strict protejate, în Bulgaria. Principalele ameninţări cu care se confruntă populațiile de peşti sunt regularizările râurilor cu stăvilare şi baraje, în zona inferioară, poluarea apei, pescuitul excesiv şi braconajul, precum şi construcția de microcentrale hidroelectrice de-a lungul cursurilor râurilor.

INTRODUCTION

The North-West Bulgaria and especially the West Balkan Mountains included in this region are known due to their high conservation priority. Two reserves - the Chuprene Biosphere Reserve and the Gornata Koriya Strict Nature Reserve, as well as 17 protected areas of national and international importance have been declared (Executive Environment Agency, Ministry of Environment and Water of Bulgaria). A proposal for designating the West Stara Planina Mountains as a nature park was made and the procedure has been under way (Vassilev et al., 1999). The status of the region and the development of the Bulgarian Natura 2000 network of protected areas necessitated having a complete knowledge about the fish species diversity in the rivers of the region.

There are few studies on the ichthyofauna of the North-West Bulgarian rivers. Drensky (1951) reported on the occurrence of some fish species. Michailova (1970) studied the ichthyofauna in the rivers of the West Stara Planina Mountains in 1963 - 1964. Summary information about the ichthyofauna of the Danube tributaries was published by Karapetkova (1994), Karapetkova and Zivkov (1995) and Karapetkova et al. (1998). Some unpublished and newly obtained data about the species diversity of the ichthyofauna in the West Balkan Mountains for the period 1985 - 1999 were summarized by Trichkova et al. (2004).

The goal of the present study was to make an ichthyological inventory of the rivers in the North-West Bulgaria in terms of species composition and conservation status.

MATERIAL AND METHODS

The study area comprises a territory of 9,105.38 km^2, which is approximately 8% of the territory of Bulgaria. It is characterized with a diverse landscapes and geographic conditions. The area is constructed of three structural entities - the Danube Plain, transitional “Predbalkan” and the Stara Planina Mountains, the latter two known as the Balkan Mountains. The variation in elevation is over 1,800 m. The area includes 13 main river catchments affiliated to the Danube River drainage basin. At present, the rivers are slightly affected by industrial pollution, and except for their lower reaches, they are not regulated. Their water is used mainly for irrigation and less for production of electricity. The water chemical characteristics of the rivers for the period 2000 - 2006 (mean values) were the following: water temperature in the range of 9.1 - 15.6^{o}C (10.7 - 22.2^{o}C for April-September period); pH range of 7.8 - 8.4; electro conductivity of 100 - 744 μS/cm; dissolved oxygen of 7.3 - 9.8 mg/l; dissolved solids of 66.7-496.3 mg/l; suspended solids of 17.8 - 68.1 mg/l; and Ca concentration range of 16 - 94 mg/l (Executive Environment Agency, Ministry of Environment and Water of Bulgaria).

In April-September 2005, the fish species diversity in five river basins: Voinishka, Vidbol, Archar, Tsibritsa and Ogosta (Tab. 1) was monitored. Totally, 40 sites along the entire reaches of the rivers except the uppermost reaches and estuaries, as well as some tributaries were sampled (Tab. 1). Sampling was made by electrofishing and beach seine.

Table 1: River lengths and catchment areas, location of sampling sites, North-West Bulgaria.

River	Length (km)	Catchment Area (ha)	Sites - Location, Coordinates (Latitude N, Longitude E, degrees), Altitude (m, a.s.l.)
Voinishka	60	27,700	(1) Bukovets, 43°55'42"N, 22°45'34"E, 58 m a.s.l. (2) 5 km upstream the river mouth, 43°55'13"N, 22°49'32"E, 46 m a.s.l.
Vidbol	62	33,000	(3) Valchek, 43°46'53"N, 22°4313E, 148 m a.s.l. (4) Stratsimirovo 43°49'36"N, 22°44'12"E, 106 m a.s.l. (5) Bozhuritsa 43°53'34"N, 02°45'00"E, 81 m a.s.l. (6) Dunavtsi, 2 km upstream (7) Dunavtsi, the bridge 43°54'11'N, 22°48'21"E, 44 m a.s.l. (8) Dunavtsi, downstream
Archar	59	36,500	(9) Dimovo, upstream 43°44'06"N, 22°43'05"E, 134 m a.s.l. (10) Dimovo, the bridge 43°44'18"N, 22°43'30"E, 134 m a.s.l. (11) Darzhanitsa 43°48'37"N, 22°5055E, 66 m a.s.l. (12) Archar, upstream (13) Archar, the old bridge 43°48'45"N, 22°55'08"E, 48 m a.s.l.
Lom	93	114,000	(14) Borovitsa (15) Ruzhintsi, upstream (16) Roglets 43°40'33"N, 22°55'61"E, 144 m a.s.l. (17) Dabova Machala (18) Staliiska Machala 43°43'19"N, 23°09'36"E, 44 m a.s.l. (19) Lom Town 43°48'30"N, 23°14'39"E, 38 m a.s.l.
Tsibritsa	88	93,400	(20) Klisuritsa (21) Slavotin 43°33'46"N, 23°04'00"E, 198 m a.s.l. (22) Between Slavotin and Doctor Yosifovo 43°32'08"N, 23°06'26"E, 191 m a.s.l. (23) Bezdenitsa, the bridge (24) Dolno Tserovene (25) Dalgodeltsi (26) Yakimovo (27) Valchedram 43°41'33"N, 23°27'06"E, 64 m a.s.l. (28) Zlatiya 43°44'58"N, 23°29'16"E, 39 m a.s.l. (29) Dolen Tsibar, upstream 43°47'51"N, 23°30'06"E, 41 m a.s.l.
Ogosta	144	315400	(30) Gavril Genovo (31) Montana Town 43°24'07"N, 23°13'52"E, 149 m a.s.l. (32) Michailovo (33) Hairedin (34) Miziya, upstream confluence with Skat River 43°41'30"N, 23°49'35"E
Shugavitsa, Ogosta basin			(35) Sumer, 43°20'46"N, 23°19'36"E, 189 m a.s.l.
Skat, Ogosta basin			(36) Tarnava 43°30'24"N, 23°53'08"E, 106 m a.s.l. (37) Altimir 43°31'39"N, 23°48'51"E, 79 m a.s.l. (38) Galiche 43°33'38"N, 23°51'44"E, 64 m a.s.l. (39) Miziya, upstream 43°40'13"N, 23°51'26"E, 49 m a.s.l. (40) Miziya, upstream confluence with Ogosta 43°41'09"N, 23°51'12"E, 33 m a.s.l.

RESULTS AND DISCUSSION

A total of 27 fish species belonging to seven families were recorded (Tab. 2). This amounts to about 35% of the ichthyofauna currently occurring in the Bulgarian part of the Danube River and its tributaries (unpublished data). In previous studies, the number of fish species reported in the same rivers ranged from 15 (Michailova, 1970) to 43 (Karapetkova, 1994). However, Michailova (1970) studied only the upper and middle reaches included in the mountain region, while Karapetkova (1994) included in her review the river estuaries as well. The uppermost parts (the high mountain parts) of the rivers and the river estuaries were excluded in our survey because of difficult accessibility.

Totally, 24 species recorded are indigenous to the ichthyofauna of the Danube Basin and three species are exotic in Bulgaria - *C. gibelio*, *P. parva* and *L. gibbosus*. Most frequently occurred the species *L. cephalus* (at 100% of the sites), followed by *G. gobio* and *R. amarus* (both at 75% of the sites) (Tab. 2). Frequent were also *B. petenyi*, *A. alburnus*, *Cobitis* sp. and *S. balcanica* occurring at over 50% of the sites. These results correspond to great extent to the findings of Michailova (1970), who reported as most frequent and abundant in the 1960s *B. petenyi*, *L. cephalus*, *G. gobio* and *A. bipunctatus*. The species *L. cephalus* and *B. petenyi* inhabited the entire reaches of the rivers being most abundant in the rivers Lom and Vidbol. *G. gobio* and *A. alburnus* occurred mainly in the middle and lower reaches and dominated in number in the Ogosta River. In our study, *A. bipunctatus* was found at 30% of the sites, only in four of the rivers (Tab. 2). However, it occurred along the entire reaches, as reported by Michailova (1970) as well, and it was most abundant in the Lom River. *R. amarus* was recorded in middle and lower reaches, abundant at sites with slow water and vegetation. It dominated in number in the Skat River, where most of the sites sampled were characterized by muddy substrate. *S. balcanica* was newly recorded in the rivers Vidbol and Voinishka and it was abundant at the sites 10, 16 and 25. The representatives of *Cobitis* sp. were not identified to species level. They occurred mainly in lower reaches and were newly found in the Archar River. *Cobitis* sp. was most abundant in the Tsibritsa River. *B. barbatula* occurred at 40% of the sites and it was first recorded in the Vidbol River.

The exotic species (*C. gibelio*, *P. parva* and *L. gibbosus*) which were absent until the 1960s (Michailova, 1970), and which occurred rarely in the subsequent periods (Karapetkova, 1994; Trichkova et al., 2004), during our inventory, were found at over 30% of the sites. Most frequent of them was *P. parva* (47.5%). It was first reported in the rivers Lom and Archar, while *L. gibbosus* in the rivers Lom and Vidbol. The two species were more common in the river lower reaches.

Recently, a considerable increase in abundance of *Neogobius* species in the Bulgarian section of the Danube and their range expansion upstream the tributaries has been observed (Polacik et al., 2008; our data). The results of our inventory confirm these observations and show that gobies were found more frequently than in the previous studies. Karapetkova (1994) reported *N. fluviatilis* and *P. marmoratus* only in the Ogosta River. Except in this river, *N. fluviatilis* was newly recorded in the rivers Lom, Archar and Vidbol, and *P. marmoratus* - in the rivers Archar and Vidbol. The species *N. melanostomus* was found for the first time in Ogosta and Archar rivers. The goby species occurred mainly in the river lower reaches.

Most rare during our survey were *A. aspius*, *B. bjoerkna* *C. nasus*, all *Romanogobio* species and *V. vimba*. In the past, the rheophilic Danube species *A. aspius*, *B. barbus* and *C. nasus* run far upstream the river mouths reaching the upper parts of middle reaches, where they coexisted with *B. petenyi* (Michailova, 1970). We found these species only in the biggest rivers Ogosta and Lom at single sites restricted to the lowest reaches (Tab. 2). *V. vimba* was found in the lower reaches, only in the Ogosta River, as in previous study (Karapetkova, 1994).

R. albipinnatus was recorded in the Vidbol River near Dunavtsi, where it was previously reported (Karapetkova, 1994). Other Danube species predominantly eurytopic, such as *B. bjoerkna*, *E. lucius*, *L. idus*, *R. rutilus* and *P. fluviatilis* were also found comparatively rare predominantly in the river lowest reaches. *R. kessleri* and *R. uranoscopus* which were reported as common and frequent by Michailova (1970) and Karapetkova (1994), during our survey, were recorded only at single sites in the rivers Lom and Skat, respectively.

16 species reported in previous studies were not found during our inventory. Among them are the species from the uppermost reaches *P. phoxinus*, *S. trutta fario* and the introduced *O. mykiss* in the rivers Ogosta and Lom (Michailova, 1970; Karapetkova, 1994), where sampling was not performed by us. The other species absent from the present list, but reported by Karapetkova (1994), are mainly limnophilic and eurytopic Danube species, found usually in the estuaries of the tributaries, such as: *A. brama*, *C. carpio*, *S. erythrophthalmus*, *T. tinca*, *M. fossilis*, *S. glanis*, *G. baloni*, *G. cernuus*, *G. schraetser*, *S. lucioperca* and *B. stellatus*. Our results do not confirm the finding of *P. platygaster* in the Vidbol River near Dunavtsi (Karapetkova, 1994). However, recently, this species is extremely rare and listed as critically endangered in the newly updated Red Book of Bulgaria (Stefanov et al., 2008).

The greatest species diversity was detected in the Ogosta River (24 species), followed by the rivers Lom (17 species), Vidbol (16 species), Archar (15 species), Tsibritsa (12 species), and the lowest - in the Voinishka River (five species). The fish community in the biggest river Ogosta was dominated by *A. alburnus* and *G. gobio* along the entire reaches, together with *B. barbus* - in the lower reaches. In the biggest Ogosta tributary Skat River, where 11 species were recorded, dominant were *R. amarus* at all sites and *C. gibelio* near Altimir. In the Lom River, most abundant were *A. bipunctatus*, *B. petenyi* and *L. cephalus*, while in the Vidbol River - *B. petenyi* and *L. cephalus*.

Some of the fishes recorded are of high conservation concern in Bulgaria. Seven species are under the protection of the Biological Diversity Act of Republic of Bulgaria (2002) (Tab. 2). *A. aspius*, *R. amarus*, *R. albipinnatus*, *R. uranoscopus* and *S. balcanica* are considered of high conservation priority and listed in Appendix 2; while *B. barbus* and *B. petenyi* are considered under a special regime of conservation and regulated use in nature and listed in Appendix 4. Ten species are listed in the newly updated, according to the IUCN criteria, Red Book of R. Bulgaria (Stefanov et al., 2008). Of them, two species are considered as endangered, five species as vulnerable, and three species as data deficient (Tab. 2). The present results confirm that most of these species, such as *A. aspius*, *B. barbus*, *R. kesslerii* and *R. uranoscopus*, are comparatively rarely found in the Danube tributaries.

One of the main threats to fish populations in the region is the river regulation by weirs and dikes in the lower reaches. This resulted in degradation or lost of suitable feeding and spawning habitats and caused the absence of Danube fish species upstream in some of the rivers. For instance, at site 13, in a pool of a big weir, abundant populations of *N. fluviatilis*, *N. melanostomus* and *E. lucius* were found. However, the high weir wall stopped these and other species from migrating upstream the Archar River. Another threat to fish populations in the rivers is water pollution. Since there are no big industries in the region, this is mainly due to deposition of household wastes and cattle grazing around the towns and villages. Serious threat is also excessive fishing and poaching. Potential threat to fish populations is the construction of micro-hydropower stations along the river courses. The extensive development of this river-based activity in the near future is expected to become a major concern in the region.

Table 2: List of species recorded in the rivers of North-West Bulgaria, occurrence and conservation status in Bulgaria. Numbers of sites are according to the table 1. BDA - Biological Diversity Act (2002); RB - Red Book of Republic of Bulgaria (in press); EN - Endangered, VU - Vulnerable, DD - Data Deficient.

Species	Sites	Frequency of occurrence (%)	Conservation status
Cyprinidae			
Alburnoides bipunctatus (Bloch, 1782)	6, 9-12, 14-18, 30, 35	30	RB-DD
Alburnus alburnus (Linnaeus, 1758)	9, 17-29, 31-40	60	
Aspius aspius (Linnaeus, 1758)	34	2.5	BDA-2; RB-VU
Barbus barbus (Linnaeus, 1758)	19, 32-34	10	BDA-4; RB-VU
Barbus petenyi (Heckel, 1847)	1-19, 20, 24, 30-35	67.5	BDA-4; RB-DD
Blicca bjoerkna (Linnaeus, 1758)	24	2.5	
Carassius gibelio (Bloch, 1782)	7, 8, 16, 20-29, 33, 35, 37	40	
Chondrostoma nasus (Linnaeus, 1758)	33	2.5	
Gobio gobio (Linnaeus, 1758)	1, 2, 5-7, 20-29, 30-40	75	
Leuciscus cephalus (Linnaeus, 1758)	1-40	100	
Leuciscus idus (Linnaeus, 1758)	6, 19, 34	7.5	
Pseudorasbora parva (Temminck and Schlegel, 1842)	9, 19, 20-29, 32, 33, 36-40	47.5	
Rhodeus amarus (Bloch, 1782)	1-19, 27, 31-40	75	BDA-2
Romanogobio albipinnatus (Lukasch, 1933)	7	2.5	BDA-2; RB-VU
Romanogobio kesslerii (Dybowsky, 1862)	18	2.5	RB-EN
Romanogobio uranoscopus (Agassiz, 1828)	37	2.5	BDA-2; RB-EN
Rutilus rutilus (Linnaeus, 1758)	7, 8, 34, 37	10	
Vimba vimba (Linnaeus, 1758)	32, 33	5	RB-DD
Cobitidae			
Cobitis sp.	11, 12, 18, 19, 20-40	62.5	
Sabanejewia balcanica (Karaman, 1922)	1, 2, 5, 10, 14-30, 35	55	BDA-2; RB-VU

Species	Sites	Frequency of occurrence (%)	Conservation status
Balitoridae			
Barbatula barbatula (Linnaeus, 1758)	5, 9-20, 23, 30, 35	40	RB-VU
Esocidae			
Esox lucius Linnaeus, 1758	8, 13	5	
Centrarchidae			
Lepomis gibbosus (Linnaeus, 1758)	6, 19, 26-29, 31-35, 37	30	
Percidae			
Perca fluviatilis Linnaeus, 1758	7, 8, 19, 27, 34	12.5	
Gobiidae			
Neogobius fluviatilis (Pallas, 1811)	8, 13, 19, 34	10	
Neogobius melanostomus (Pallas, 1814)	13, 34	5	
Proterorhinus marmoratus (Pallas, 1814)	7, 11, 12, 32, 33, 38	15	

CONCLUSIONS

As a result of the inventory of six main rivers in the North-West Bulgaria in 2005 period, a total of 27 fish species were finally recorded. Of this 27 species, 24 species are indigenous to the ichthyofauna of the Danube Basin, including 11 species of conservation concern according to national environmental legislation and red list. This shows that the rivers maintain comparatively high fish species diversity and have preserved their indigenous ichthyofauna. However, they suffer some negative impact, such as construction of weirs and dikes in the lower reaches, water pollution, excessive fishing and poaching, etc. Compared to previous studies, the non-indigenous species have become common, the goby species expanded their range upstream, while other species decreased their range and number considerably (*A. aspius*, *B. barbus*, *C. nasus* etc.), or have become very rare (*R. kesslerii*, *R. uranoscopus*). In order to ensure the optimum protection and preservation of the indigenous ichthyofauna, especially in terms of the high conservation priority of the studied region, the courses of the rivers Ogosta, Lom, Archar and Vidbol were proposed as potential Natura 2000 sites and new protected areas in Bulgaria.

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ASSESSMENT OF WATER MICROBIOLOGIC POLLUTION IN DURRES'S MARINE HARBOUR BASIN (ALBANIA)

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KEYWORDS: Albania, Durres, Apollonia Beach, pollution in coastal areas, water microbiologic pollution, microbiologic assessment, heterotrophic bacteria, total coliforms.

ABSTRACT

The objects of this study are four sampling areas of Durres's harbour basin, where microbiological research was made in sea water.

The period of sample-taking was between July and October 2008. In order to compare the level of water microbiologic pollution in areas of Durres's harbour basin is also studied a beach area near the harbour.

The sampling areas were: Ferry Terminal (FT), Fishery Harbour (FH), East Zone (EZ), Fuel Quay (FQ) and Apollonia Beach (AB). Durres's marine harbour is located in the southern part of the Adriatic Sea, in the south of Durres, in the northern part of Durres Bay.

It is known that marine harbours bring pollution in coastal areas because of the services that are performed there. The aim is to assess the water microbiologic pollution and to compare it with the European standards.

The strategy used for this purpose consisted in water insemination with coverage method by means of Petri dishes according to respective dilutions in PCA (Plate Count Agar) culture media for heterotrophs and MacConkey culture media for total coliforms.

The number of colonies that are formed determines the number of cells at the moment of water insemination, respectively the number of heterotrophs in PCA culture media. The number of pink and red colonies that were formed determines the number of cells at the moment of water insemination, respectively the number of total coliforms in MacConkey culture media.

The measure of heterotrophic bacteria and total coliforms used is CFU/100 ml water.

The comparison of heterotrophs and total coliforms level in sampling areas has been the essence of this research.

The conclusions drawn from five sampling areas are as follows.

Apollonia Beach is within European standards. The richest area with heterotrophs is Fishery Harbor, which confirms the fact that it is the most polluted microbiologic area in the harbour basin of Durres. The richest area with total coliforms is Ferry Terminal, which reflects spills of organic substance, such as focal pollution.

The poorest area with heterotrophs and total coliforms is Fuel Quay. This shows the inhibition of heterotrophs and total coliform bacteria growth in this area. The inhibition itself may come as a result of petroleum or chemical wastes such as heavy metals. However, this remains to be confirmed.

ZUSAMMENFASSUNG: Bewertung der mikrobiologischen Verunreinigung des Wassers im Hafenbecken von Durres (Albanien).

Gegenstand dieser Studie sind vier Bereiche innerhalb des Hafenbeckens von Durres, in denen Proben zur mikrobiologischen Untersuchung entnommen wurden. Die Beprobung fand im Zeitraum von Juli bis Oktober 2008 statt. Um den Grad der mikrobiologischen Belastung des Wassers im Hafenbecken von Durres einschätzen zu können, wurde auch eine neben dem Hafengelände gelegene Bucht untersucht.

Die Probestellen lagen im Bereich des Fährenterminals (FT), des Fischereihafens (FT), des östlichen Hafengebiets (EZ), am Brennstoffkai (FQ) und in der Apollonia Bucht (AB). Der Seehafen von Durres liegt im südlichen Teil des Adriatischen Meeres, im Süden von Durres, am nördlichen Rand der gleichnamigen Bucht.

Es ist bekannt, dass Seehäfen durch die in ihnen entfalteten Tätigkeiten eine Verschmutzung der Küstengebiete verursachen. Ziel der Untersuchung ist es, die mikrobiologische Belastung zu bestimmen und sie mit den europäischen Standards zu vergleichen.

Die zu diesem Zweck verwendete Strategie bestand in der Insemination des Wassers unter Verwendung der Abdeckmethode mit Petri Platten entsprechend einer bestimmten Verdünnung in PCA Kulturmedien (Plate Count Agar) für heterotrophe Organismen und Mac Conkey für die Gesamt-Colibakterien.

Die Zahl der entstandenen Kolonien, bestimmen die Zahl der Zellen zum Zeitpunkt der Wasserinsemination, bzw. die Zahl der heterotrophen Organismen im PCA Kulturmedium. Die Zahl der pinkfarbenen und roten Kolonien, die sich gebildet haben, bestimmen die Zahl der Zellen zum Zeitpunkt der Wasserinsemination, bzw. die Zahl der Gesamt-Colibakterien im MacConkey Kulturmedium. Das für die Messung der heterotrophen Bakterien und Gesamt-Colibakterien verwendete Maß ist CFU/100 ml Wasser.

Der Vergleich von heterotrophen Bakterien und der Höhe der Gesamt-Colibakterien der verschiedenen Probestellen, bildet den Kern der durchgeführten Untersuchungen. Aufgrund des Vergleichs der Ergebnisse der fünf Probestellen kann gefolgert werden, dass die Apollonia-Bucht mit ihren Ergebnissen innerhalb der europäischen Standards liegt. Das an heterotrophen Bakterien reichste Gebiet ist der Fischereihafen, der sich als der am stärksten mikrobiologisch verschmutzte Bereich innerhalb des Hafenbeckens von Durres darstellt. Das an Gesamt-Colibakterien reichste Gebiet ist der Fähren Terminal, welcher den Eintrag organischer Stoffe, wie fäkale Verschmutzung widerspiegelt. Das an heterotrophen Bakterien und Gesamt-Colibakterien ärmste Gebiet ist der Erdölkai, was durch eine wachstumshemmende Wirkung bedingt ist. Die Wachstumsinhibition könnte ein Ergebnis der Verschmutzung durch Erdöl und chemische Stoffe wie Schwermetalle sein, was allerdings noch einer Bestätigung bedarf.

REZUMAT: Evaluarea poluării microbiologice a apei bazinul portului marin Durres (Albania).

Obiectul prezentului studiu îl constituie patru arii de cercetare în bazinul portului marin Durres, unde au fost efectuate cercetări microbiologice în apa marină.

Probele au fost prelevate pentru analize între iulie şi octombrie 2008. În vederea stabilirii gradului de poluare microbiologică a portului de la Durres, a fost studiată în mod comparativ şi o arie de golf, situată în apropierea portului. Punctele de probă alese sunt terminalul de bac (Ferry Terminal - FT), portul piscicol (Fishery Harbour - FH), zona estică a portului (East Zone - EZ), cheiul de alimentare cu carburanţi (Fuel Quay - FQ) şi plaja Apollonia (Apollonia Beach - AB). Portul marin Durres este situat în sudul Mării Adriatice, în sudul oraşului Durres, la marginea nordică a golfului cu acelaşi nume.

Este cunoscut faptul, că porturile marine determină poluarea zonelor de coastă, datorită activităţilor desfăşurate pe raza lor. Obiectivul este de a determina poluarea microbiologică a apei şi a o compara cu standardele europene.

Strategia folosită, în acest scop, constă în însămânţarea apei prin metoda acoperirii cu plăci Petri, în mod corespunzător cu o diluţie în mediu de cultură PCA (Plate Count Agar) pentru organisme heterotrofe şi mediu de cultură MacConkey, pentru bacterii coliforme totale.

Numărul coloniilor formate determină numărul celulelor în momentul însămânţării apei, respectiv numărul organismelor heterotrofe, în mediul de cultură PCA. Numărul coloniilor dezvoltate de culoare roz şi roşu determină numărul celulelor în momentul însămânţării apei, respectiv numărul total al bacteriilor coliforme în mediul de cultură MacConkey.

Pentru măsurarea cantităţii bacteriilor heterotrofe şi a totalului de coliforme s-a folosit CFU/100 ml apă. Comparaţia cantităţii bacteriilor heterotrofe şi a totalului de colibacterii în diferitele puncte de probă din zona portuară a constituit esenţa acestor cercetări.

Golful Apollonia corespunde standardelor europene. Aria cea mai bogată în heterotrofe este portul piscicol, ceea ce confirmă că este cea mai poluată zonă din punct de vedere microbiologic, în bazinul portului de la Durres. Zona cea mai bogată în coliforme este terminalul de bac, ceea ce reflectă intrarea de substanţe organice prin poluare fecală. Zona cea mai săracă în heterotrofe şi coliforme este cheiul de alimentare cu carburanţi. Acest fapt demonstrează inhibiţia creşterii heterotrofelor şi a bacteriilor coliforme în această arie. Inhibiţia ar putea fi rezultatul poluării cu produse petroliere sau al poluării chimice cum ar fi cea cu metale grele. Oricum, această presupunere rămâne să fie confirmată.

INTRODUCTION

Durres's Marine Harbour is located in the south of Adriatic Sea, in south of Durres, in the northern part of Durres Bay. Durres Bay is approximately 18 km long from north to south, with a coastline of seven km in the east. In the west of the coastline the depth is more than ten m. Also the Harbour is well-protected in the east and west from the breakwater (******).

The bay provides a safe anchorage area for the sea-crafts that wait for operation in Harbour. Apart from the services that provides, this harbour has its problems related to water pollution. Water pollution is a serious problem that is aggravated by the growth of human population and rapid industrialization. (*, **)

There are three groups of microorganisms used as indicators of faecal pollution: the group of coliforms represented by *Escherichia coli*, the group of faecal streptococcus, represented by *Streptococcus faecalis* and the group of clostridiums that is responsible for sulphite reduction, represented by *Clostridium perfringenes*. The first group is the most important. In order to determine water pollution are also used heterotrophic bacteria (Davis et al., 1990; Hysko, 2007; Madigan et al., 1996).

Despite many standards proposed about the water microbiologic quality, keeping these standards remains a difficult duty in the majority of the cases. For this reason, the purpose of this research is to determine the number of heterotrophic bacteria and total coliforms in the harbour sampling areas and comparing their level with the beach area (***,****,*****).

From the five studied areas, results that the richest one in heterotrophic bacteria is Fishery Quay. This shows that this quay causes the biggest pollution in the basin of Durres. The richest area with total coliforms is Ferry Terminal Quay. This demonstrates that this quay causes the biggest pollution which confirms spills of organic substance, such as faecal pollution.

MATERIALS AND METHODS

Water sampling stations

Areas where samples of water are taken in Durres's Marine Harbour (Figs. 1 and 2): Ferry Terminal (FT), Fishery Harbor (FH), East Zone (EZ), Fuel Quay (FQ), as well as the beach area at Apollonia Beach (AB). In the areas are taken samples in each ten days for the period July - October 2008 (****).

The depth of taking samples

The samples are taken in a depth of 20 - 30 centimetres, in the vicinity of quays, three meters from the pedestal of the quay (Hysko, 2007).

Taking and transporting sea water samples

The analyzed samples were taken in sterile glass vessels, which are sterilized in aerosteril for a two hours period at a 180°C temperature. On these vessels have been noted the date, the time and the place of samplings. The vessels with samples are put in termobox and are transported to the laboratory. One vessel is used for measuring the temperature (that should not be above 10°C) of transported vessels. If the samples are not assayed at the moment of arriving in laboratory the water is kept in fridge. It would be better if the water is assayed within six hours from the moment it was taken from the sampling area, but not more than 24 hours from the time of sampling (Hysko, 2007).

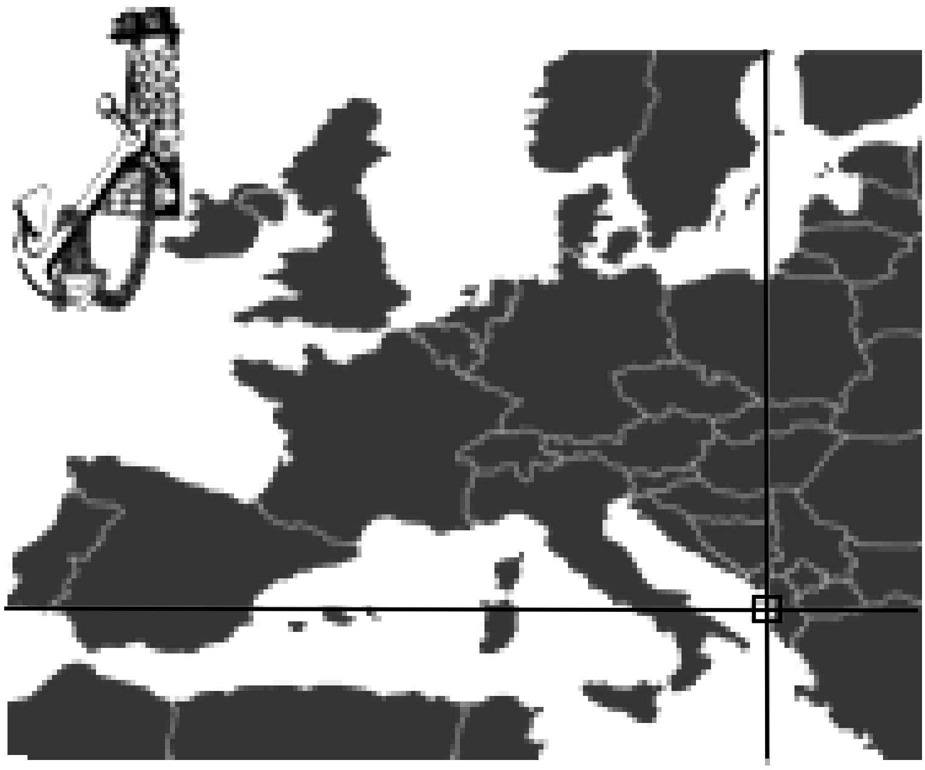

Figure 1: The Durres Harbour location.

Figure 2: The Durres Harbor.

Culture medium used

In this study there are used this culture medium: Plate Count Agar (PCA), MacConkey. The culture media are prepared from dry media in vessels with a covering thread and are sterilized in 121°C for 15 minutes (Hysko, 2007).

Determination of the number of heterotrophs

It is determined the number of heterotrophs grown in aerobic condition at 37°C. The water is inseminated in Petri plates in PCA with coverage method, without dilution or with dilution till 10^{-5}. One ml from each dilution is transferred in sterile Petri plates and than it is added 15 - 20 ml in PCA medium. It is mixed carefully and then the medium is allowed to become solid. The plates are incubated in thermostat in 37°C. The readings of plates are made after 24 and 48 hours. Then, the colonies formed in the surface and inside nutrient agar are counted. The number of colonies gives us the number of heterotrophs located in inseminated water. Calculations are made for one ml of water. The result is given per 100 ml of water (Hysko, 2007).

Determination of the number of total coliforms

The specific procedure followed in this respect is the same as for heterotrophs, but in this case is used MacConkey medium and are counted only red and pink colonies, due to the fact that the total coliforms dissolve lactose that is in this medium. Acidification of medium makes colonies of lactose positive bacteria to take red and pink colour (Hysko, 2007).

Statistical Data - processing

There are used these statistical indicators: arithmetic average, standard deviation and variation coefficient (Koni, 2005; ****).

The investigated area

The Harbour of Durres is very ancient and has a very important geographical position. It is not only the biggest harbour in Albania but also one of the most important harbours in the South Eastern Europe. It is situated 39 km away from the capital city, Tirana. It is located in the southern part of the Mediterranean Sea. The entrance canal is 8.5 m deep. It has a length of 3.65 miles and a width of 60 - 195 m. The general surface of the harbour is 1,467,000 m^2, where the water surface is approximately 674,000 m^2. There are 11 quays and the depth near the quays is 6.5 -10.2 m. The length of the operation quays is 2.2 km (******).

RESULTS AND DISCUSSION

Heterotrops in all water sampling stations along the coastal zone of Durres Area

The study is made in Durres's harbour in these places: Ferry Terminal (FQ), Fishery Harbor (FQ), East Zone (EZ), Fuel Quay (FQ) and in Apollonia Beach (AB) in July - October 2008.

Analysis of the number of heterotrophs for all the months when samples are taken is demonstrated in the tables 1, 2 and 3, and in the figures 3, 4, 5 and 6.

In the table 1 is calculated the absolute frequency, relative frequency and cumulative frequency of heterotrophs during the period July - October in all sampling stations for 100 ml water.

Table 1: Absolute frequency, relative frequency and cumulative frequency of heterotrophs according months, July, August, September and October in (FQ), (FH), (EZ), (FQ), (AB).

			Ferry terminal		Fishery harbour		East zone		Fuel quay		Apollonia Beach	
Month	Sample	Quantity	ab. freq. Heterotrophs	rel. freq. Heterotrophs	ab. freq. Heterotrophs	rel. freq. Heterotrophs	ab. freq. Heterotrophs	rel. freq. Heterotrophs	ab. freq. Het Heterotrophs	rel. freq. Heterotrophs	ab. freq. Heterotrophs	rel. freq. Heterotrophs
July	water	100 ml	728.750	76	955.000	81	18.100	28	55.100	70	74.500	56
Aug	water	100 ml	45.275	5	71.025	6	11.675	18	19.263	24	46.825	35
Sept	water	100 ml	160.783	17	143.483	12	34.567	53	4.650	6	9.350	7
Oct	water	100 ml	26.650	3	12.300	1	550	1	200	0	3.350	2

The figure 3 shows that the richest month with heterotrophs is July for all stations studied, where in head is the Fishery Harbour with 955.000 CFU/100 ml.

Then comes the Ferry Terminal, where the number of the heterotrophs is relatively big.

With a big difference from the first stations come respectively Apollonia Beach, Fuel Quay and in the end East Zone. This shows that July is the richest month with organic substance that brings a large growth of heterotrophs.

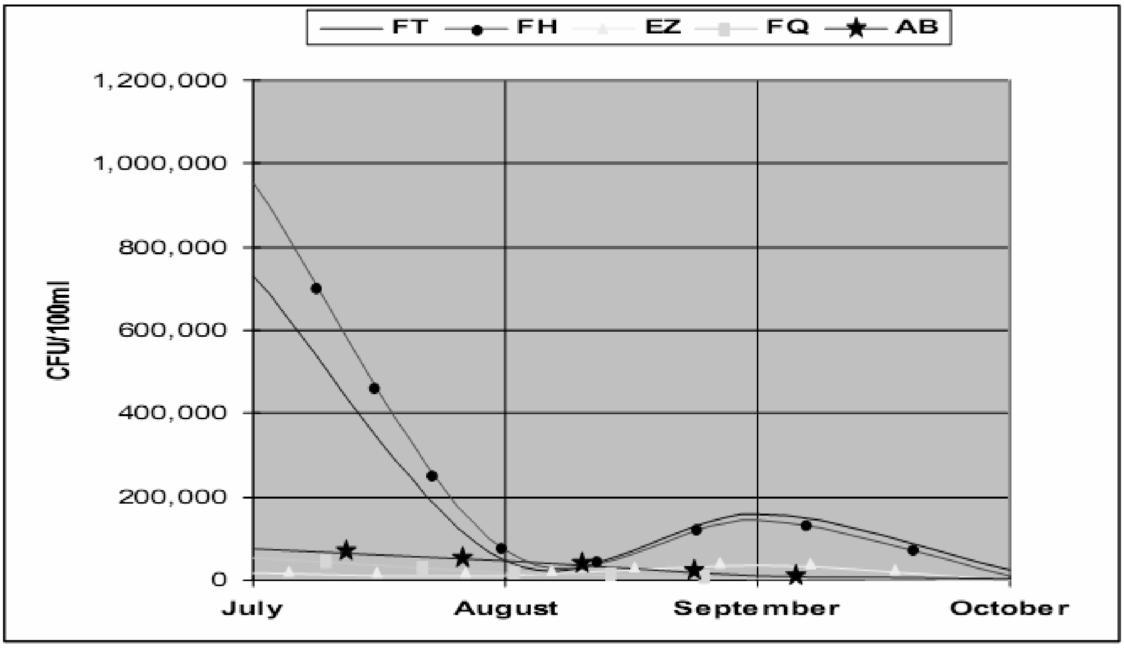

Figure 3: Heterotrophs number according to months
in (FT), (FH), (EZ), (FQ) and (AB).

The month of August has an apparent downfall of the number of heterotrophs in all sampling stations, notably in Fishery Quay and Ferry Quay. This shows that nourishing substance is reduced, which means that the heterotrophs organisms may have consumed an important amount of nourishing substance in the previous months, as a result, in August growth is not considerable. The food is a restrictive factor in this respect, since temperature doesn't change more than 1 or 2^{o}C. Another reason may be that termohalin circulation of the water is minimized much, because of seasonal termoclin. The organic substance which is important for the development of heterotrophs in the surface, goes down at the bottom of sea, as a result the number of heterotrophs is reduced.

In the month of September, in station Ferry Terminal, was noticed the fact that a considerable growth of heterotrophs with 160.783 CFU/100 ml. Then come Fishery Harbour and East Zone. Whereas, in Apollonia Beach and Fuel Quay the falling is more drastic than in August.

This shows that Ferry Terminal, Fishery Harbour and East Zone have a considerable pollution due to the human influence. That causes this growth of heterotrophs, because of maximal activity in these quays.

In the October period was noticed a downfall number of the heterotrophs organisms in all the studied sampling stations. A determinative factor of this downfall is the decrease of the water temperature at 20°C, with a difference of seven grades compared to the month of July.

Table 2: Absolute frequency, relative frequency and cumulative frequency of heterotrophs according to seasons in (FQ), (FH), (EZ), (FQ) and (AB).

			Ferry terminal		Fishery harbour		East zone		Fuel quay		Apollonia beach	
Seasons	Sample	Quantity	ab. freq. Heterotrophs	rel. freq. Heterotrophs	ab. freq. Heterotrophs	rel. freq. Heterotrophs	ab. freq. Heterotrophs	rel. freq. Heterotrophs	ab. freq. Heterotrophs	rel. freq. Heterotrophs	ab. freq. Heterotrophs	rel. freq. Heterotrophs
Summer	water	100 ml	181.970	59	247.820	69	12.960	33	26.430	88	52.360	87
Autumn	water	100 ml	127.250	41	110.688	31	26.063	67	3.538	12	7.850	13

In the figure 4 is shown that summer is richer than autumn in heterotrops in all stations, with the exception of the East Zone where these dominates in autumn.

The richest station with heterotrophs in summer is Fishery Harbour with 247.820 CFU/100 ml, which shows that in this area there is a considerable amount of organic substance at the disposal of the heterotrophs. This shows fairly well that the pollution of this area is caused by the wastes and the fishing boats some of which are out of function submerged or floating in this quay for years. We shouldn't forget that some of the fishing boats use petroleum, often of a low quality and the surface of water is oily. Near the quay is situated the Dockyard which also causes pollution. Then comes Ferry Terminal with 181.970 CFU/100 ml. This area has a considerable pollution with organic substance which brings the development of heterotrophs. May be this pollution comes from Fishery Harbour, being nearby, or by spills of organic substance in this area. This remains to be confirmed.

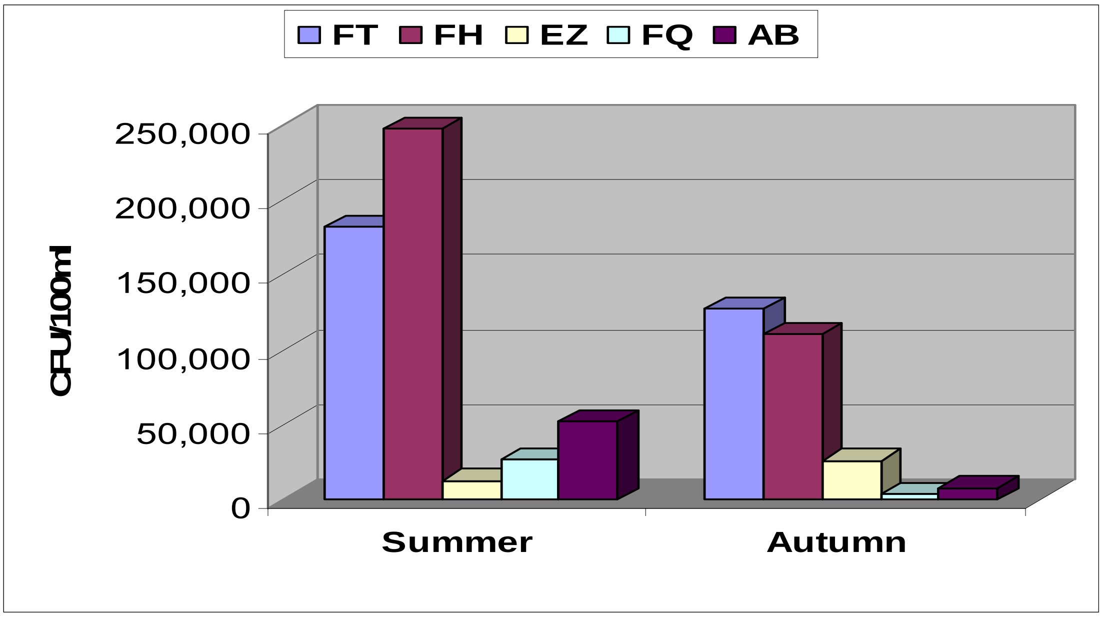

Figure 4: Heterotrophs number according to seasons
in (FT), (FH), (EZ), (FQ) and (AB) sampling stations.

In the third place comes Apollonia Beach with 52.360 CFU/100 ml, whereas Fuel Quay and East Zone are ranked at the end of the list. Even though Fuel Quay and East Zone are part of the Harbour basin, the number of heterotrophs is small. This shows the inhibition of heterotrophs bacteria growth in Fuel Quay. The inhibition itself may come as a result of petroleum or chemical wastes such as heavy metals, even though the food is abundant. This remains to be confirmed in future studies.

In autumn dominates Ferry Terminal with 127.250 CFU/100 ml. Then follows the Fishery Harbour with a little difference. In the third place ranges the East Zone, then comes Apollonia Beach and at the end Fuel Quay.

Table 3: Average, Standard Deviation and Variation Coefficient of heterotrophs in sampling areas in months July-October.

Place	Average (CFU/100 ml)	Standard Deviation	Variation Coefficient
FT	157,650	222,011.08	1.41
FH	186,872	282,008.24	1.51
EZ	18,783	27,275.96	1.45
FQ	16,256	20,508.47	1.26
AB	32,578	40,178.87	1.23

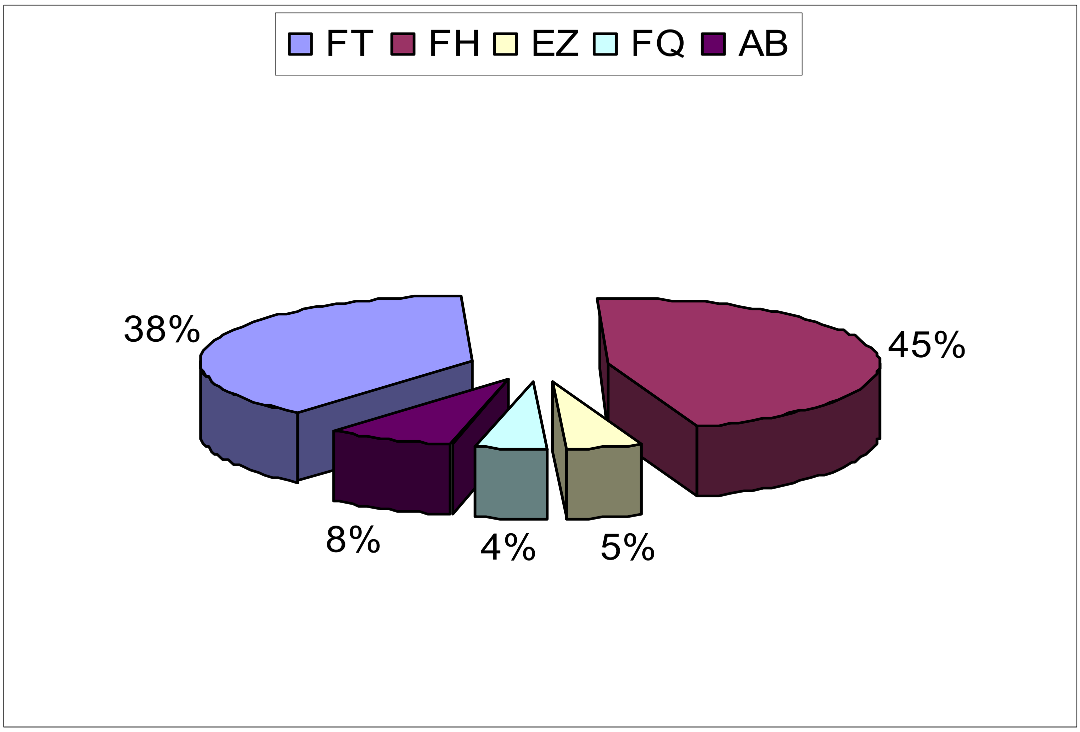

Figure 5: Heterotrophs average
in (FT), (FH), (EZ), (FQ) and (AB) sampling stations.

In the figure 5 is shown that Fishery Harbour contains the highest percentage of Heterotrophs with 45%, and then comes Ferry Terminal with 38%. Apollonia Beach has only 8% of all heterotrophs in five stations. In the end are ranked East Zone and Fuel Quay respectively with 5% and 4% of heterotrophs.

This confirms that Fishing Harbour and Ferry Terminal are shown as areas polluted with organic substances. Apollonia Beach is shown as a clean area, which does not have a big development of heterotrophs.

Even though Fuel Quay and East Zone are part of Harbour basin, the number of heterotrophs is small. This shows the inhibition of heterotrophs bacteria growth in Fuel Quay. The inhibition itself may come as a result of petroleum or chemical wastes such as heavy metals, despite plenty of food. This remains to be confirmed in future studies.

In the figure 6 is shown that the area with the biggest variation of the number of heterotrophs is Fishery Harbour, whereas the area with the smallest variation is Apollonia Beach. This confirms that Fishing Harbour has the biggest pollution from human activities, which brings this variability of number of heterotrophs.

Apollonia Beach is an area relatively clean, where the number of heterotrophs per 100 ml doesn’t change considerably during this period of study compared with the station inside the Harbour. The Fuel Quay has a little variability compared with other areas of the Harbour. This shows the inhibition of heterotrophs bacteria growth in this area. The inhibition itself may come as a result of petroleum or chemical wastes such as heavy metals. This remains to be confirmed (Hysko, 2007; Koni 2005; Strum and Kirk, 1988; ****, *****).

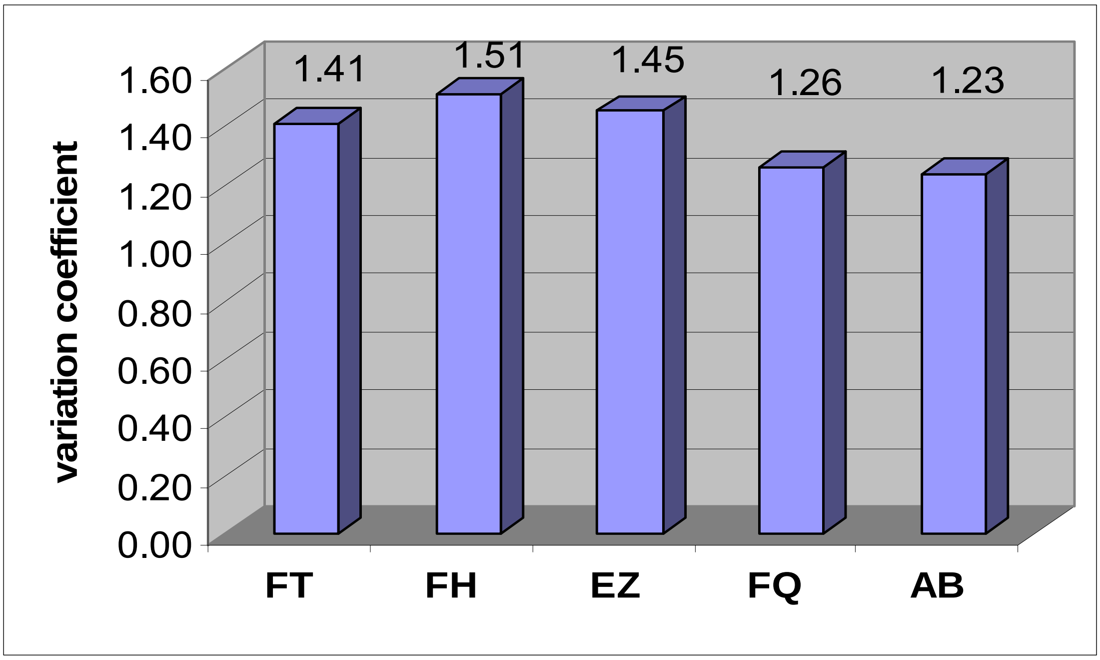

Figure 6: Heterotrophs Variation Coefficient
in (FT), (FH), (EZ), (FQ) and (AB) sampling stations.

Total coliforms in all water sampling stations along the Durres coastal zone

Analysis of the number of total coliforms for all the months when samples are taken is demonstrated in the tables 4, 5 and 6, and in the figures 7, 8, 9 and 10.

In the table 4 is calculated the absolute frequency, relative frequency and cumulative frequency of total coliforms during the period July - October in all sampling stations for 100 ml water.

Table 4: Absolute frequency, relative frequency and cumulative frequency of total coliforms according the months July, August, September and October in (FQ), (FH), (EZ), (FQ), (AB) sampling stations.

			Ferry terminal		Fishery harbour		East zone		Fuel quay		Apollonia beach	
Months	Sample	Quantity	ab. freq. Heterotrophs	rel. freq. Heterotrophs	ab. freq. Heterotrophs	rel. freq. Heterotrophs	ab. freq. Heterotrophs	rel. freq. Heterotrophs	ab. freq. Heterotrophs	rel. freq. Heterotrophs	ab. freq. Heterotrophs	rel. freq. Heterotrophs
July	water	100 ml	26,100	45	50,450	69	250	5	0	0	3,550	40
Aug.	water	100 ml	5,875	10	2,450	3	900	19	350	49	1,550	17
Sept.	water	100 ml	21,533	37	17,133	24	3,167	69	367	51	3,100	35
Oct.	water	100 ml	4,800	8	2,700	4	300	6	0	0	700	8

From the figure 7 we notice that in July there is a large amount of total coliforms in Fishery Harbour with 50.450 CFU/100 ml.

The Ferry Terminal area is positioned on the second place. The two areas are above imperative level in bathing beach water (10.000 CFU/100 ml). It is a well-known fact that the faecal coliforms originate from human and livestock intestines. They contribute with a high percentage of about 97% of total coliforms. Since the fish do not have a permanent coliform flora, the presence of coliforms in the fish bodies is an obvious evidence of water pollution in their habitat. So we can say the fact that there is faecal pollution of water in two areas, most notably in Fishery Harbour. Apollonia Beach is a clean area, because the number of total coliforms is below imperative level in bathing beach water with 3.550 CFU/100 ml. East Zone and Fuel Quay are below guide level (500 CFU/100 ml). This shows that in July, in these two stations may be poured chemical substance that inhibits total coliforms growth.

In August, in the five studied areas the number of total coliforms per 100 ml water is below imperative level in bathing beach water. This confirms that in August faecal pollution in the surface of water is small. May be the termohalin water circulation is reduced furthermore because of seasonal termoclin. In this way, the materials that cause pollution are deposited at the bottom of sea.

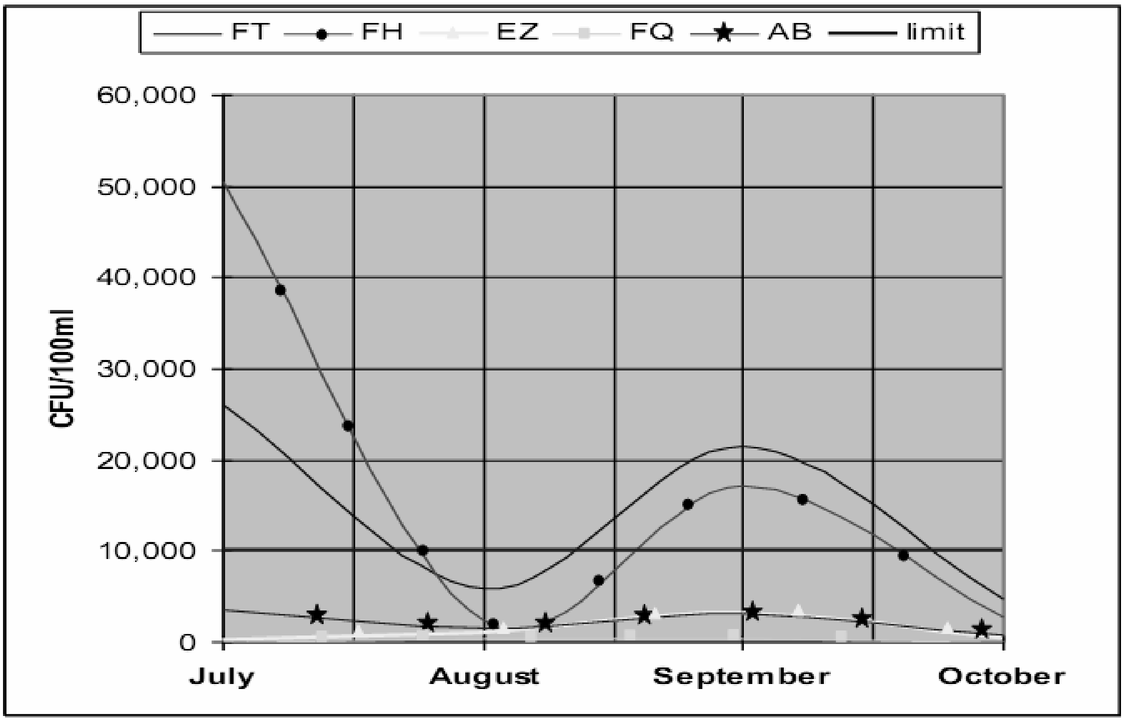

Figure 7: Total coliforms number according to months in (FT), (FH), (EZ), (FQ) and (AB), compared with imperative level in bathing beach water (10.000 CFU/100 ml).

Table 5: Absolute frequency, relative frequency and cumulative frequency of total coliforms according seasons in (FQ), (FH), (EZ), (FQ) and (AB).

			Ferry terminal		Fishery harbour		East zone		Fuel quay		Apollonia beach	
Seasons	Sample	Quantity	ab. freq. Heterotrophs	rel. freq. Heterotrophs	ab. freq. Heterotrophs	rel. freq. Heterotrophs	ab. freq. Heterotrophs	rel. freq. Heterotrophs	ab. freq. Heterotrophs	rel. freq. Heterotrophs	ab. freq. Heterotrophs	rel. freq. Heterotrophs
Summer	water	100 ml	9.920	36	12.050	47	770	24	280	50	1.950	44
Autumn	water	100 ml	17.350	64	13.525	53	2.450	76	275	50	2.500	56

In September was a high growth of total coliforms above the imperative level of bathing beach water in Ferry Terminal and Fishing Harbour. This confirms that in September we have a good termohalin circulation of water and the nourishing substance comes in the surface or may be we have an increase of faecal pollution. East Zone and Apollonia Beach are considered as stations within European standards. Fuel Quay continues to be below guide level. This may indicate an area polluted with toxic substance. However, this remains to be confirmed.

In October there is a minimal growth of total coliforms in all stations. This happens because of the decrease of temperature in the surface of water, which inhibits their growth. As in the case of heterotrophs, there are two peaks of development of total coliforms, in July and in September.

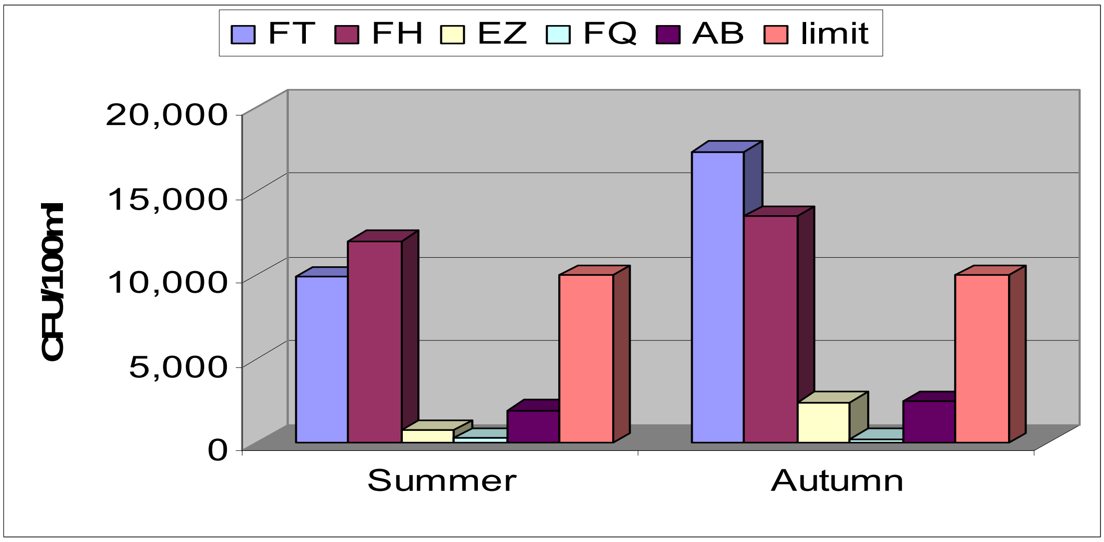

Figure 8: Total coliforms number according to seasons
in (FT), (FH), (EZ), (FQ) and (AB) compared with imperative level
in bathing beach water (10.000 CFU/100 ml).

From the figure 8 in all water sampling stations, the number of total coliforms is bigger in autumn than in summer, except Fuel Quay, where the number is the same in these two seasons. This shows that at the end of summer the faecal pollution is expected to increase.

In summer only Fishery Harbor has a number of total coliforms below imperative level in bathing beach water.

In autumn, Ferry Terminal is classified as the most polluted area with 17.350 CFU/100 ml. After Ferry Terminal is classified Fishing Harbor, both of which are above imperative level in bathing beach water. Then comes Apollonia Beach with 2.500 CFU/100 ml, East Zone and Fuel Quay. These three stations have a number of heterotrophs below imperative level in bathing beach water.

It is important to point out that in Fuel Quay the number of total coliforms is below guide level (500 CFU/100 ml). This confirms that in this area there are poisonous substances which inhibit their development.

Table 6: Average, Standard Deviation and Variation Coefficient of total coliforms in sampling areas in months July - October.

Place	Average (CFU/100 ml)	Standard Deviation	Variation Coefficient
FT	13,222	15,067.39	1.14
FH	12,706	16,821.06	1.32
EZ	1,517	2,960.23	1.95
FQ	278	473.23	1.70
AB	2,194	1,837.94	0.84

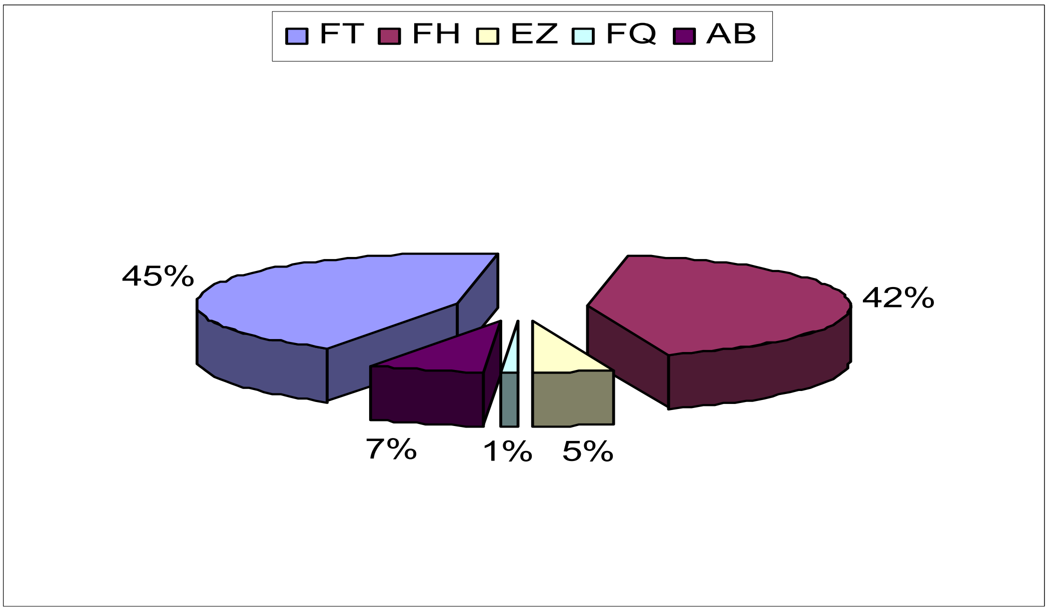

Figure 9: Total coliforms Average in (FT), (FH), (EZ), (FQ) and (AB) sampling stations.

Ferry Terminal is the station with the biggest growth of total coliforms (45%). This shows there is a continuous faecal pollution. There is a tube that pours polluted water directly in that area. May be this is one reason of the pollution. Fishing Harbor is considered a polluted zone (42%). Apollonia Beach is in third place with 7%, whereas East Zone and Fuel Quay have respectively 5% and 1%.

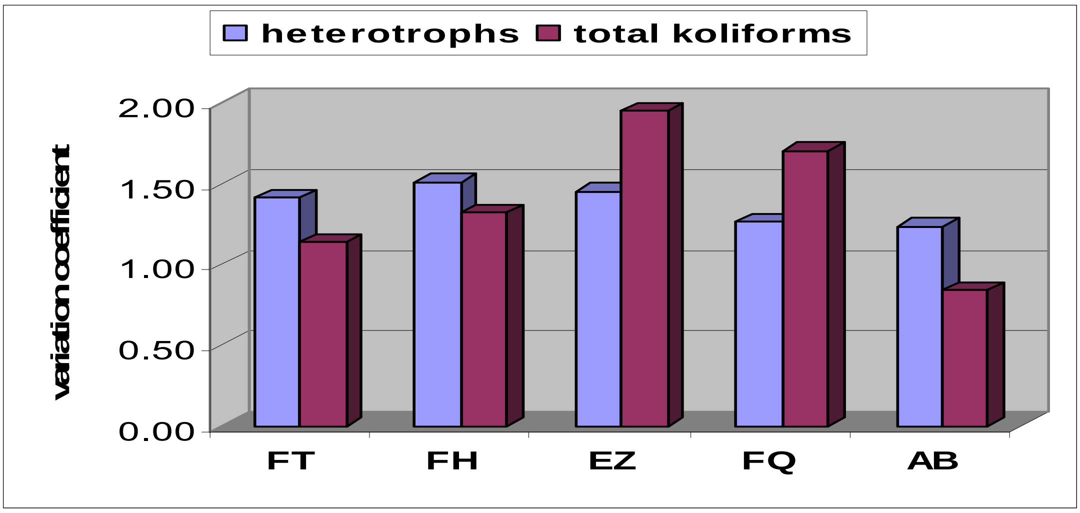

Figure 10: Variation Coefficient of number of heterotrophs compared with number of total coliforms in (FT), (FH), (EZ), (FQ) and (AB) sampling stations.

In the figure 10 is shown that variability of heterotrophs number is bigger than the number of total coliforms in Ferry Terminal, Fishing Harbor and Apollonia Beach, whereas in East Zone and Fuel Quay the variability of heterotrophs is smaller than that of total coliforms.

This confirms once more that the number of total coliforms is more stable than the number of heterotrophs in Ferry Terminal, Fishing Harbor and Apollonia Beach. This means that in these stations the environmental factors for the development of the total coliforms are more convenient than for heterotrophs (***; Hysko, 2007; Koni, 2005; ****; *****).

Temperature Effect

In the table 7 and in the figure 11 is shown the relation between a biotic factor (bacteria growth) and an abiotic factor (temperature) in costal area of Durres.

Table 7: Linear Correlation between physic parameters (temperature) and heterotrophs (CFU/100 ml) in (FT), (FH), (EZ), (FQ) and (AB) in July - October 2008.

	df	SS	MS	F	Significance F
Regression	1	8904871.439	8904871	0.013616	0.907540184
Residual	54	35315783700	6.54E + 08		
Total	55	35324688571			

As we show from the table 7 and the figure 11, the Regression Coefficient is R^2 = 0.907. This confirms that temperature (20 - 27°C) is a determinant factor in growth of microorganisms (Strum and Kirk, 1988).

May be others environment factor influence to the growth of bacteria in this area. This should be proved in future studies.

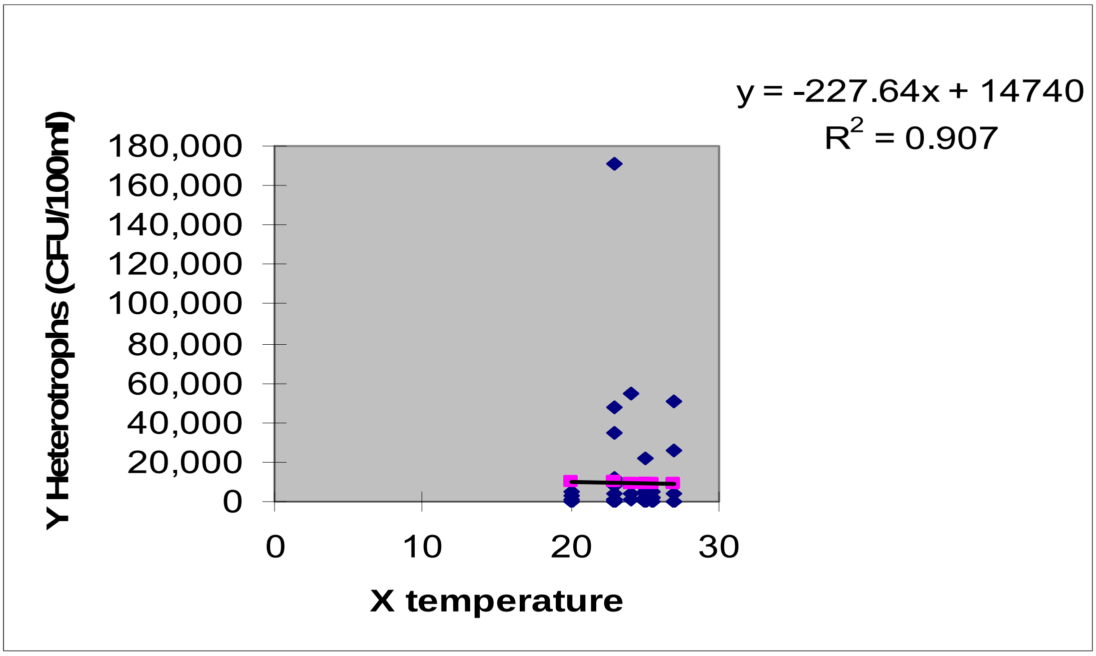

Figure 11: Linear Correlation between physic parameters (temperature) and heterotrophs (CFU/100 ml) in (FT), (FH), (EZ), (FQ) and (AB) in July - October 2008.

CONCLUSIONS

The richest area with heterotrophs is Fishery Harbour, which confirms the fact that it is the most microbiologic polluted area in Harbour of Durres.

The richest area with Total Coliforms is Ferry Terminal. This indicates the presence of an inflow of organic wastes in this area, such as faecal pollution.

Apollonia Beach is within European standards.

The poorest area with heterotrophs and total coliforms is Fuel Quay. This shows the inhibition of heterotrophs bacteria growth in this area. The inhibition itself may come as a result of petroleum or chemical wastes such as heavy metals. However, this remains to be confirmed.

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ENVIRONMENTAL EFFECT AND THREAT OF BALLAST WATER

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KEYWORDS: Environmental risk, aquatic species, ship, nuisance, management.

ABSTRACT

In recent years, many people have become increasingly aware that the globalization of trade, the increased speed of travel, the massive volume of cargo shipments, and rising tourism has combined to increase the chance of accidental introductions of foreign species through Ballast Water into indigenous habitats.

Aquatic species arrive through a variety of mechanisms - unintentionally, when attached to vessel hulls or carried in vessel ballast water, and intentionally, when imported for aquaria display, as live seafood for human consumption, or as a transplant to increase sport fishing opportunities.

Ballast water is an important way of transferring aquatic nuisance species (ANS) all over the world. ANS include algae, shellfish, developing larvae, eggs and microorganisms. Due to the lack of natural predators in the new environment, they are often able to thrive and outgrow the native species. This domination can cause massive ecological, economical, and public health problems such as degradation of habitat, alteration of water quality, blockage of flow in drainage and irrigation canals, or even transition of diseases to humans. The economic, social, recreational, and ecological losses/costs attributable to aquatic invasive species are difficult to quantify.

The International Maritime Organization (IMO) regards the introduction of harmful aquatic organisms and pathogens to new environments via ballast water, as one of the four greatest threats to the world's oceans. At least one foreign marine species is introduced into a new environment every nine weeks. In response to this, the International Marine Organization (IMO), a specialized agency of the United Nations with 166 member states, established the Ballast Water Management Convention (BWM) in 2004. Ballast water management (BWM) for vessels includes all measures that aim to prevent unwanted aquatic nuisance species from being transported between ports in the ballast. In this paper the ballast water management methods are explained under three categories; the exchange, the treatment and the isolation of the ballast water. The advantages and disadvantages of these methods are also discussed.

Ballast water has been identified as one of the four greatest threats to the world's oceans. The transfers of aquatic species and aquatic invasive species are perhaps the biggest environmental challenge facing the global shipping industry in this century.

ZUSAMMENFASSUNG: Auswirkungen von Ballastwasser auf die Umwelt und die davon ausgehenden Gefahren.

In den letzten Jahren sind viele Menschen sich zunehmend dessen bewusst geworden, dass die Globalisierung des Handels, die wachsende Geschwindigkeit des Verkehrs, das riesige Volumen von Schiffsfrachten und wachsender Tourismus dazu geführt haben, die Chance der durch Ballastwasser zufällig in die natürlichen Habitate eingeführten fremden Arten zu vergrößern.

Aquatische Arten gelangen durch unterschiedliche Mechanismen in andere Gebiete; unabsichtlich - wenn sie am Schiffskörper haften oder im Ballastwasser des Schiffsrumpfs enthalten sind - oder absichtlich, wenn sie für Aquarien eingeführt werden, als lebende Meeresfrüchte zum Verzehr durch den Menschen bestimmt sind oder durch Einbringen als Fischbesatz in andere Gebiete kommen, um die Möglichkeiten der Sportfischerei zu erhöhen. Ballastwasser ist ein wichtiger Weg lästige Wasserorganismen (ANS) rund um den Erdball zu verbreiten. Die so genannten „Aquatic Nuisance Species“, umfassen Algen, Schalentiere, sich entwickelnde Larven, Eier und andere Mikroorganismen. Bedingt durch den Mangel an natürlichen Räubern in der neuen Umgebung sind sie oft in der Lage sich prächtig zu entwickeln und die nativen Arten zu überwuchern. Diese Dominanz kann erhebliche ökologische, wirtschaftliche und solche der öffentlichen Gesundheit verursachen wie beispielsweise Verminderung der Habitatqualität, Veränderungen der Wasserqualität, Blockierung des Durchflusses in Be- und Entwässerungsgräben, oder sogar Übertragung von Krankheiten auf den Menschen. Die wirtschaftlichen, sozialen, den Erholungswert betreffenden und ökologischen Verluste bzw. Kosten, die den aquatischen invasiven Arten zuzuschreiben sind, können schwer quantifiziert werden.

Die Internationale Organisation der Meere (IMO) sieht die Einführung von schädlichen Wasserorganismen und Krankheitserregern über das Ballastwasser in eine neue Umgebung als eine der vier größten Gefahren für die Weltmeere an. Mindestens alle neun Wochen wird eine fremde marine Art in eine neue Umgebung eingeführt. Als Antwort darauf hat die Internationale Organisation der Meere (IMO), eine spezialisierte Agentur der Vereinten Nationen mit 166 Mitgliedstaaten, 2004 ein Übereinkommen zum Management von Ballastwasser (BWM) getroffen. Das Management von Ballastwasser (BWM) für Schiffe umfasst alle Maßnahmen, die darauf ausgerichtet sind, den Transport unerwünschter, schädlicher aquatischer Arten im Ballastwasser aus einem Hafen in einen anderen zu verhindern. In vorliegender Arbeit werden die in drei Kategorien gegliederten Methoden des Ballastwasser Managements erläutert und zwar der Austausch, die Behandlung sowie die Isolation des Ballastwassers. Die Vor- und Nachteile dieser Methoden werden ebenfalls diskutiert.

REZUMAT: Efectele şi ameninţarea asupra mediului a apei-balast.

În ultimii ani, mulţi oameni au devenit din ce în ce mai conştienţi de faptul că globalizarea comerţului, creşterea vitezei de transport, volumul masiv al încărcăturilor transportate şi creşterea turismului sau aceşti factori combinaţi, cresc şansele introducerilor accidentale de specii străine prin apa de balast, în noi habitate.

Speciile acvatice sosesc printr-o varietate de mecanisme - neintenţionat, atunci, când sunt ataşate de coca vaselor sau transportate în apa de balast a navelor şi intenţionat, când sunt importate ca exemplare vii pentru acvaristică, ca hrană vie pentru consumul uman, sau pentru creşterea oportunităţilor pentru pescuitul sportiv.

Apa utilizată ca balast reprezintă o cale importantă de transferare a speciilor acvatice dăunătoare (SAD) în întreaga lume. SAD includ alge, crustacee, moluşte, larve, icre şi microorganisme. Datorită lipsei prădătorilor naturali în noul mediu, acestea sunt adesea capabile să prospere şi să se dezvolte mai repede decât speciile native. Această dominare poate cauza probleme majore ecologice, economice şi de sănătate publică, cum ar fi degradarea habitatelor, degradarea calităţii apei, blocarea debitelor în canalele de scurgere şi de irigaţii sau chiar transmiterea unor boli la oameni. Pierderile/costurile economice, sociale, recreaţionale şi ecologice atribuite speciilor acvatice invazive sunt dificil de cuantificat.

Organizaţia Maritimă Internaţională (OMI) consideră că introducerea organismelor dăunătoare şi patogene în noi medii, odată cu apa utilizată ca balast, este una dintre cele patru ameninţări majore pentru oceanele lumii. Cel puţin o specie marină străină este introdusă într-un mediu nou, la fiecare nouă săptămâni. Ca răspuns la aceasta, Organizaţia Maritimă Internaţională (OMI), o agenţie specializată a Naţiunilor Unite cu 166 de state membre, au stabilit Convenţia pentru Managementul Apei de Balast (MAB) în 2004. Managementul apei de balast (MAB), pentru nave, include toate măsurile care au ca scop prevenirea transportului în balast, a speciilor acvatice nedorite între porturi. În această lucrare, metodele de management a apei utilizată ca balast sunt explicate în trei categorii: schimbarea, tratarea şi izolarea apei balast. Avantajele şi dezavantajele acestor metode sunt de asemenea discutate.

INTRODUCTION

Commercial vessels - ranging from large cruise ships to large boats - discharge a variety of pollutants including toxic chemicals, oily wastes, grey water laden with nutrients, and ballast water contaminated with invasive species. The ballast water has been marked as the major vector of unintentional bioinvasion. However, it is generally taken note of only when their impacts are felt. Since the invasions are vast and even their impacts are unknown, the environmental problem arises when this ballast water contains aquatic life. Many cases of marine bioinvasion have been reported and their harmful effects on the ecosystem and human health have been documented.

For example in San Francisco Bay a new invasive species establishes itself every 14 weeks from ballast water discharges. Aquatic invasive species are estimated to cost the United States of America $9 billion annually, much of which is borne by the Great Lakes region.

WHAT IS BALLAST WATER

Balance and stability of modern ship is provided with ballast water, when a ship empties its cargo, it takes in water as ballast to maintain its stability and structural integrity. Conversely, when it loads cargo, the ballast water is discharged usually in vicinity of ports just prior to loading the cargo. The ballast water is carrying thousands of aquatic species which are small enough to pass through a ships ballast water intake ports and pumps. These carried organisms include bacteria, cysts and larvae of various aquatic plant and animal species. Shipping transfers approximately three to five billion tones of ballast water internationally each day. A similar volume may be transferred domestically within countries and regions each year, bringing the total global ballast water movements to around ten billion tones per year. Translocation of organisms through ships is considered to be one of the most important issues that are threatening the naturally evolved biodiversity, and consequences of such invasions are being realized increasingly in the recent years. An analysis of global shipping traffic provides the broad reach of shipping as a vector for transporting aquatic species.

BALLAST WATER AS A VECTOR

The International Convention for the Prevention of Pollution from ships (MARPOL 73/78) adopted an equipment-based approach to oil pollution control. All ships were required to adopt Separated Ballast Tanks (SBT's) and Crude-oil Washing (COW) technologies. Most tanker owners had absolutely no reason to support Separated Ballast Tank (SBT's), Crude-Oil Washing (COW), or double-hull requirements. Yet, all three standards proved far more effective in changing behaviour and ensuring compliance because non-compliance of equipment requirements can more easily be detected than discharge violations.

It seems that species in which the adults attached to the seabed or are too large to be taken on in ballast water are unlikely to be transferred in ballast. The problem is compounded by the fact that all marine species have life cycles that include planktonic stage or stages, even large adult or attaching to the seabed are capable of transferring through ballast water during their planktonic phase. It is estimated that at least 7000 to possibly more than 10000 different species of marine microbes, plants and animals may be carried globally in ballast water each day. For example in San Francisco Bay (U.S.A) a new invasive species establishes itself every 14 weeks from ballast water discharges.

THREATS OF BALLAST WATER

Not all of the aquatic species which are carried with ballast water survive during voyage, since the ballasting and deballasting cycle and the inside conditions of ballast tanks could be undesirable to carried organism. However, for those do survive during a voyage and are discharged, depending on receiving environmental conditions and predation by and/or competition from native species, the chances of surviving might be further reduced. When all factors are favourable, an introduced species may survive to establish a reproductive population in the host environment. Therefore, ability of resistance to the ballast tank condition of voyage and to the both grazers and predators, high tolerance to abiotic factors, high reproduction rate and/or fast vegetative growth, hermaphroditic reproduction capability, and ability to hibernate or rejuvenate under unfavourable/favourable conditions are some of the success determinative factors of an organism invasion as a result of invasion whole ecosystems are being changed. The impacts directly experienced by the society can be economical (loss of fishery or water as a resource) and human health related (for example, cholera epidemic, toxicity due to micro algal contamination). The ecological impacts are complex and dependent on the interaction between the invader and the native community. Although the three above impacts are all inter-linked and influence each other.

ECOLOGICAL IMPACTS

An introduced plant or animal species, which appears a successful invader in its new environment, can cause a range of ecological impacts. Competing with native species for space and food; preying upon native species; altering habitat; altering environmental conditions (e. g. increased water clarity due to mass filter-feeding); altering the food web and overall ecosystem, and displacing native species, reducing native biodiversity and even causing local extinction.

The main attributes of the ecological impacts of the harmful aquatic bio-invasions is irreversibility and sever increasing over time. The impacts of aquatic bio-invasions are comparable with the ship sourced pollution, oil spills. Ecological impacts of oil spill are most likely to occur very quickly, be catastrophic and acute and considerably visible. Impacts are decreasing over time as soon as oil degrades and clean up. An aquatic bio-invasion initially impacts may be non-existent to minor and invisible. The more population, the more impacts in an insidious, chronic and irreversible manner over the time. Oil spills have been known for a long time and human developed a large range of cleaning-up strategies and methodologies. On the other hand, the invading species have been established huge viable populations in new environment which are almost impossible to remove in the present. No successful control and/or eradication of the aquatic invasion species have been recorded till the present. The invasive species cases are more difficult to treat in open water than in enclosed waters such as the small bays. What's more, they are almost undetectable in their early invasion stage.

ECONOMIC IMPACTS

Economic losses on human society are caused by aquatic species invasion in a number of ways including: species as a result of competition and/or predation, and/or through habitat changes by species invading; invading species can be a fouling one and have destructive effects on coastal infrastructure, facilities and industry; reduction in shipping efficiency and recreational and tourism beaches; introduced pathogens and toxic species impact human health and secondary economic impacts as a result of increasing in monitoring, testing, diagnostic and spread of illness among society and loss of social productivity and even death may be driven from bio-invasion; bio-diversity is lost and secondary impacts on economic results; the cost of the problem solving including research, monitoring, education, communication, regulation, compliance, management, and control the case.

THREAT TO HUMAN HEALTH

Although in modern time's quarantine procedures are conventionally addressed, shipping through ballast water remains a potential significant vector for pathogens and toxic organism. Ballast water can transfer a range of bacteria, viruses and species of micro-algae, including toxic species that may form harmful algae blooms or red tide. Red tide is well documented for paralytic shellfish poisoning (PSP), which can cause illness and even death in humans.

CONCLUSIONS

Ballast water has been identified as one of the four greatest threats to the world's oceans. Its transfers of aquatic species and aquatic invasive species are perhaps the biggest environmental challenge facing the global shipping industry in this century.

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MICROORGANISMS WITH BIOTECHNOLOGICAL POTENTIAL PRESENT IN OIL RESIDUES POLLUTED AQUIFERS AND GROUNDWATER

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ABSTRACT

Samples collected from two types of aquatic sites accidentally or chronically contaminated with oil residues were taken under study, i. e. aquifer from Ovidiu Pod site, Constanța County, area crossed by an oil pipe for fuel transport, and groundwater from the Boldeşti-Scăieni oil park, Prahova County. The water samples were analyzed in terms of physical, chemical and microbiological characteristics; more complex studies were performed on 15 isolates of hydrocarbon oxidizing bacteria, the most important microorganisms involved in the natural degradation of polluting hydrocarbons. The bacterial strains were characterized morphologically and physiologically, also by quantifying their capacity to synthesize several degradative enzymes, extra cellular tensioactive compounds and by quantifying their degradation potential on different types of hydrocarbons and crude oil. The bacterial strains exhibiting superior degradative performances were analyzed by means of the "BIOLOG" system for identification of bacteria, establishing their presumptive taxonomic affiliation as to: *Aeromonas*, *Brevundimonas*, *Micrococcus*, *Pseudomonas* and *Vibrio* genera. These microorganisms, could be of potential interest for rehabilitation of environments polluted with oil residues by means of ecological bioremediation techniques.

RĖSUMĖ: Microorganismes à potentiel biotechnologique présentes dans les eaux souterraines et les nappes phréatiques polluées avec des résidus pétroliers.

Cette étude a été menée sur des échantillons prélevés au niveau de deux types d'environnement aquatique, contaminés, de manière accidentelle ou chronique par des résidus pétroliers: de la région Ovidiu Pod, département de Constantza, et un échantillon d'eau phréatique provenant de Boldeşti-Scăieni, département de Prahova. Quinze souches bactériennes hydrocarbon-oxydatives représentent les microorganismes les plus importants impliqués dans la biodégradation des hydrocarbures polluantes. Les souches bactériennes ont été caractérisées du point de vue morphologique et physiologique, y compris par la mise en évidence de leur activité de synthèse des enzymes de dégradation, des composés tensioactifs extracellulaires, ainsi que de point de vue de leur potentiel dégradatif envers des différents types d'hydrocarbures et de pétrole brut. Les souches bactériennes qui présentent la plus grande activité dégradative ont été analysées en utilisant le système "BIOLOG" pour identifier leur affiliation taxonomique. Ces microorganismes appartiennent aux genres: *Aeromonas*, *Brevundimonas*, *Micrococcus*, *Pseudomonas* et *Vibrio*. Les organismes identifiés présentent un intérêt potentiel dans la régénération des milieux aquatiques et terrestres contaminés par des résidus d'hydrocarbures, en utilisant des techniques écologiques de bioremédiation.

REZUMAT: Microorganisme cu potențial biotehnologic, prezente în acviferele şi pânza freatică poluate cu reziduuri petroliere.

S-au luat în studiu probe prelevate din două tipuri de situri acvatice, contaminate accidental sau cronic, cu reziduuri petroliere: acvifer din zona Ovidiu Pod, jud. Constanța, tranzitată de conducte de transport a combustibilului petrolier şi apă de strat de la Parcul petrolier Boldeşti-Scăieni jud. Prahova, unde se extrage, prelucrează şi stochează țiței semi-parafinos. Probele de apă au fost caracterizate fizico-chimic şi microbiologic, aprofundându-se studiul a 15 izolate de bacterii hidrocarbon-oxidante, cele mai importante microorganisme, implicate în degradarea naturală a hidrocarburilor poluante. Tulpinile bacteriene au fost caracterizate morfologic şi fiziologic, inclusiv prin evidențierea capacității de sinteză a unor enzime degradative, compuşi tensioactivi extracelulari şi a potențialului degradativ față de diferite tipuri de hidrocarburi şi țiței brut. Tulpinile bacteriene cu performanțe degradative superioare au fost analizate, folosind sistemul „BIOLOG”, pentru identificarea bacteriilor stabilindu-se apartenența taxonomică, prezumptivă la genurile: *Aeromonas*, *Brevundimonas*, *Micrococcus*, *Pseudomonas* şi *Vibrio*. Aceste microorganisme ar putea fi de potențial interes în reabilitarea mediilor poluate cu reziduuri petroliere, prin tehnici ecologice de bioremediere.

INTRODUCTION

The main sources of hydrocarbon pollution of the environment are the spills and leaks of petroleum products. The exploration and exploitation practices and the breaking of oil pipes lead to incessant pollution of soils and water, including aquifer and groundwater (Pritchard et al., 1992; Potter, 1993; Oudijk, 2005; Genske, 2007). The presence in the environment of various pollutants influences directly the diversity of microorganisms, causing alteration of the structure of microbial communities (Macnaughton et al., 1999; Abe et al., 2002; Röling et al., 2004). The microorganisms posses complex genetic mechanisms which allow them a rapid adaptation to various environmental conditions (Tschape, 1994; George-Okafor et al., 2005).

In recent times, an increasing amount of microbiological research has been devoted to bioremediation of oil-contaminated sites using various microbial species (Atlas, 1993, 1995; Al-Turki, 2009). Biodegradation of hydrocarbons can be executed by various microorganisms. More than 60 genera of bacteria, 80 genera of fungi and several microalgae genera contain species capable of hydrocarbons degrading (Dinamarca et al., 2003; Head et al., 2006; George-Okafor et al., 2009). The application of bioremediation capabilities of indigenous microorganisms to clean up pollutants is viable, economic and a gentle non-aggressive approach for natural ecosystems (Englert et al., 1993; Luptakova, 2006; Das and Mukherjee, 2007; Ştefănescu et al., 2009; Voicu et al., 2009). Some of these species are capable of degrading aliphatic hydrocarbons, other aromatic molecules, but few are capable to degrade both classes of molecules. However, the complete degradation of hydrocarbons with complex structure (PAHs) may require a community of microorganisms that sequentially exchange and than transform excreted metabolites as the molecules that is gradually broken down (Voicu et al., 2003; Kee et al., 2009). The rate of microbial degradation of hydrocarbons is affected by several physico-chemical and biological parameters including the number and species of microorganisms present, the conditions for microbial degradation activity (presence of nutrients, oxygen, pH, temperature, quantity, quality and bioavailability of the contaminants). Aerobic biodegradation is far more widespread and far more rapid than anaerobic degradation (Al-Turki, 2009). Our studies' objective was to characterize from microbiological point of view samples collected from two types of aquatic sites (aquifer and groundwater) contaminated with oil residues. A more complex morpho-physiological study of the 15 hydrocarbon-oxidizing bacterial strains isolated from the analyzed water samples was undertaken.

MATERIAL AND METHODS

The water samples under study were drawn from two types of sites contaminated accidentally or chronically with oil products: aquifer from Ovidiu Pod area, Constanța County, area crossed by an oil pipe for fuel transport, and groundwater collected from various sites (oil derricks, decantation station, pumping station) within the Boldeşti-Scăieni oil park, Prahova County, were semi paraffinic oil is extracted, processed and shipped.

Physico-chemical characterization of the water samples was performed by means of Aquamerck kits.

The microbiologic analysis of water samples, using selective media, aimed at assessing the presence of the main bacterial groups involved in the carbon, nitrogen, sulfur and iron cycles in the nature. Hydrocarbon-oxidizing bacterial populations were obtained on mineral media containing as sole carbon source, crude semi-paraffinic oil at a 5% concentration (v/v) and redox indicator. The density of the microorganisms developed on the used selective media (CFU/ml), was assessed by the MPN (most probable number) statistical method (Rodina, 1972). Among the microbiota present in the water samples, the hydrocarbon-oxidizing bacteria were studied more thoroughly, a number of 15 strains being isolated from the obtained populations.

Characterization of the hydrocarbon-oxidizing bacterial strains was done microbiologically and physiologically, including the use of the "BIOLOG" system for identification of bacteria.

Assessment of the synthesis capacity of some bacterial degradative enzymes belonging to the oxidoreductases (oxidase, catalase, reductase) and hydrolases (protease, cellulase, lipase-esterase) categories was performed by qualitative tests (Zarnea et al., 1992), using for confirmation a wide range of test methods either for:

- pointing out proteolytic properties: gelatine hydrolysis, Frazier method, photographic film test, indol and ammonia production, H_2S production by reducing the sulphur containing inorganic compounds, emphasizing H_2S production by means of filter paper impregnated with lead acetate; or for
- pointing out reducing properties (dehydrogenase); stain reduction (methylene blue, 2.6 dichlorophenol-indophenol), reduction of nitrates to nitrites or nitrogen.

Synthesis of extracellular tensioactive compounds in the mineral medium in the presence of both saturated aliphatic hydrocarbon (n-hexadecane) and semi-paraffinic crude oil, (5% v/v) was rendered evident by the kerosene emulsifying qualitative test (Kokub et al., 1991).

The tolerance to hydrocarbons and the biodegradation capacity were assessed under aerobic conditions against saturated aliphatic hydrocarbons (n-hexadecane), cyclic (cyclohexane) and monoaromatic (benzene) as well as against semi-paraffinic crude oil, in the mineral medium MM (Foght and Westlake, 1988) with hydrocarbons 5% (v/v). The tolerance to hydrocarbons and viability of bacterial cells following contact with the given hydrocarbons was evaluated by spectrophotometric determination of the OD at 660 nm, initially and finally (after ten days of aerobic incubation at 28°C temperature). The qualitative determination of the residual hydrocarbons was performed by extraction with organic solvents (benzene, methilene chloride), against a non-inoculated control.

RESULTS AND DISCUSSIONS

Bacteria represent the most important microorganisms involved in natural degradation of hydrocarbons. Bacterial strains capable of degrading numerous compounds present in the oil composition are ubiquitous in nature. Some of the most adequate sources for their isolation are the oil residues polluted soil and/or waters (George-Okofor et al., 2005; Head et al., 2006; Al-Turki, 2009).

In this general context, the aim of our researches was to characterize from the microbiological, biochemical and physiological point of view some samples collected from two types of aquatic sites, contaminated accidentally (aquifer) or chronically (groundwater) with oil products.

The microbiologic analysis (Tab. 1) revealed a bacterial density relatively similar in the water samples collected from both the aquatic sites, whose physico-chemical characteristics (Tab. 2) turned out to be favourable to the development of the five analyzed groups of microorganisms. The only exception is the group of sulphate reducing bacteria, present in a larger number in the groundwater samples due to a higher content of SO_4^{2-} in the water.

Table 1: Microbiological analysis of water samples.

Physiological groups of microorganisms	cfu/ml (limits) in water samples collected from the sites:	
	aquifer	groundwater
Heterotrophic aerobic, facultative anaerobic bacteria	3.0×10^7 - 1.5×10^9	9.5×10^2 - 1.2×10^7
Hydrocarbon oxidizing-bacteria	4.5×10 - 9.5×10^4	9.5×10 - 1.2×10^3
Denitrifying bacteria	4.0×10^2 - 2.5×10^4	4.5×10 - 9.5×10^3
Sulphate-reducing bacteria	2.5×10^2 - 9.5×10^2	4.5×10^2 - 1.5×10^7
Iron oxidizing bacteria	4.5×10^3 - 2.5×10^6	9.5×10 - 1.6×10^3

Table 2: Physico-chemical characteristics of water samples.

Tests	Concentrations (limits) in water samples collected from the sites:	
	Aquifer (Ovidiu Pod, Constanța County)	groundwater (Boldești-Scăieni oil park, Prahova County)
pH	7.2 - 8.0	6.0 - 7.0
NO_3^- (mg/ml)	0 - 25.0	10.0 - 25.0
NO_2^- (mg/ml)	0.025 - 0.40	0.075 - 0.10
PO_4^{3-} (mg/ml)	0	0 - 0.25
NH_4^+ (mg/ml)	0.2 - 0.4	0.2 - 0.4
O_2 (mg/ml)	9.0 - 18.5	51.6 - 65.0
Carbonate (mmol/l)	7.0 - 28.0	10.0 - 15.5
Total hardness (mmol/l)	5.5 - 11.5	0
SO_4^{2-} (mg/l)	300	300 - 900
Ca^{2+} (mg/l)	60.0 - 100.0	600 - 780
Cu^{2+} (mg/l)	0	0
Fe^{2+} (mg/l)	2.0 - 3.0	3.0 - 15.0
Al^{3+} (mg/l)	10.0 - 25.0	10.0 - 25.0
Pb^{2+} (mg/l)	0	0
Zn^{2+} (mg/l)	0.30 - 0.45	0 - 5.0

Out of the total of aerobic facultative anaerobic heterotrophic bacteria present, the group of hydrocarbon-oxidizing bacteria represented the most significant percent, which demonstrates their degree of adaptation to the environmental conditions, the presence of oil pollutants, respectively (Tab. 2).

Among present microbiota, a more depth morpho-physiological study of the 15 bacterial hydrocarbon-oxidizing strains from the analyzed water samples was undertaken (Tab. 3).

Table 3: The diversity of some hydrocarbon-oxidizing bacterial strains isolated from water samples under study; +, +++ = positive reaction, - = negative reaction, A = aerobic; A/FAN = aerobic/facultative anaerobic.

Isolation sources	Indicatives of bacterial strains	Cell morphology	Diffusible pigments	Gram reaction	Respiratory type	Identification after "BIOLOG" System
Aquifer	1	bacillus (rod-like)	-	-	A/FAN	*Aeromonas*
	2	coccobacillus	-	-	A/FAN	
	4	coccus (spherical)	-	+	A/FAN	*Micrococcus*
	5	coccobacillus	-	-	A/FAN	
	6	coccobacillus	+++ (piocyanine)	-	A/FAN	*Pseudomonas*
	7	cocobacillus	+++ (piorubine)	-	A/FAN	*Pseudomonas*
	9	cocobacillus	-	-	A/FAN	
Groundwater	10	bacillus (rod - like)	-	-	A/FAN	
	11	bacillus (rod - like)	-	-	A/FAN	
	12	bacillus (rod - like)	-	+	A/FAN	*Corynebacterium*
	17	bacillus (rod - like)	-	-	A	*Vibrio*
	19	coccus (spherical)	-	+	A	*Micrococcus*
	22	bacillus (rod - like)	-	-	A/FAN	*Brevundimonas*
	25	coccus (spherical)	-	-	A/FAN	
	26	bacillus (rod - like)	+++ (piocyanine)	-	A/FAN	*Pseudomonas*

As cellular morphology, the bacillary and coccobacillary shapes were dominant, while the spherical ones (cocci) were characteristic only for three isolates. The physiologic diversity of the microorganisms, analyzed by means of the "BIOLOG" System, was quite accentuated, indicating their presumptive taxonomic belonging to: *Aeromonas*, *Brevundimonas*, *Corynebacterium*, *Micrococcus*, *Pseudomonas* and *Vibrio* genera (Tab. 3). Subsequent researches based on molecular techniques will confirm this identification. Most of the isolates are Gram negative, possessing in their structure an outer, supplementary membrane which confers them an enhanced resistance towards hydrocarbons (Sikkema et al., 1995; Norman et al., 2002; Ramos et al., 2002). The predominant respiratory type of the bacterial strains is the aerobic/facultative anaerobic one (Tab. 3).

The bacterial enzymatic equipment contains predominately degradative enzymes belonging to reductases (dehydrogenases), oxidases, catalases, proteases categories (Tab. 4).

Hydrocarbons are water-insoluble, and bacteria optimize their transformation by extra cellular biosurfactant production or by specific mechanisms of adhesion/ desorption that allow them to achieve a physical contact with the hydrocarbons.

Biosurfactants are small detergent-like molecules with a hydrophilic head and lipophilic tail. Synthesis of bacterial tensioactive compounds favours degradation of hydrocarbons, leading to their dispersion and emulsification, improving thus their bioavailability, including stimulation of microbial adhesion onto the surface of the hydrophobic pollutants and facilitation of their trans-membrane transport (Jacobucci et al., 2001; Sen, 2006; Al-Turki, 2009). The majority of the analyzed bacterial strains have the capacity to synthesize extra cellular tensioactive compounds in the presence of both saturated aliphatic hydrocarbon (n-hexadecane) and semi-paraffinic crude oil. The figure 1 shows the results concerning the qualitative demonstration of the capacity to synthesize bacterial tensioactive compounds.

Table 4: Qualitative demonstration of the capacity to synthesize some bacterial enzyme Categories; +, ++, +++ = positive reaction, - = negative reaction.

Indicatives of bacterial strains	Ability of synthesis of the following enzyme categories:					
	Oxidase	Catalase	Reductases (dehydrogenases)	Protease	Cellulase	Lipases (esterases)
1	-	+	+++	+++	++	-
2	+	++	+++	++	-	-
4	+++	++	+++	-	-	-
5	++	-	+++	-	++	-
6	+	+++	+++	+++	-	-
7	++	++	+++	+++	-	-
9	++	+++	+++	-	-	-
10	+++	+	+++	-	-	-
11	-	+	+++	+++	-	-
12	+	+++	+++	+++	++	+
17	+	+	++	+++	+++	-
19	+	++	++	+++	+	-
22	-	-	++	+++	++	-
25	++	+++	+++	+++	-	-
26	+++	+++	+++	+++	-	++

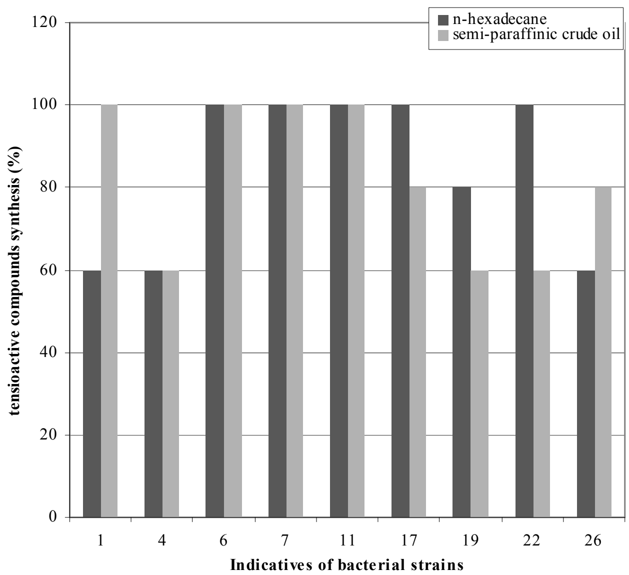

Figure 1: Qualitative demonstration of the capacity to synthesize bacterial tensioactive compounds.

Tolerance to various hydrocarbon categories as well as to complex hydrocarbon mixtures such as the semiparaffinic crude oil is different, each bacterial strain possessing its own intrinsic level of tolerance towards hydrocarbons. Best tolerated was the semi-paraffinic crude oil, and among the tested hydrocarbons were the n-alcans and the cycloalcans, hydrocarbons which are present in the composition of this type of oil (Tab. 5).

Table 5: Tolerance to hydrocarbons and degradation capacity of the bacterial strains under study; B. G. = bacterial growth (final OD) (Initial OD/660 nm = 0.038 - 0.095); C. V. = cellular viability (OD after 48 hour incubation, on LB medium).

Bacterial strain indicative	Monitored parameters after ten days cultivation on mineral medium with 5% (v/v) of the following hydrocarbons:								
	n - hexadecane			cyclohexane			semi-paraffinic crude oil		
	B. G.	%degradation	C. V.	B. G.	%degradation	C. V.	B. G.	%degradation	C. V.
1	1.026	88.7	0.602	0.560	72.2	0.432	0.674	91.2	0.282
2	0.382	8.6	0.933	0.255	19.3	0.905	0.286	13.3	0.981
4	0.503	49.2	1.268	0.340	73.0	1.259	0.559	61.4	1.079
5	0.536	8.9	0.706	0.184	46.2	0.537	0.350	8.6	0.672
6	1.923	89.3	1.150	0.185	54.4	1.016	2.935	71.2	1.581
7	1.933	90.4	1.118	0.111	22.7	0.955	3.059	89.6	1.192
9	0.439	51.3	0.561	0.120	38.2	0.244	0.144	9.3	0.703
10	0.800	9.4	1.045	0.276	48.3	0.976	1.087	70.7	1.207
11	1.852	53.2	1.220	0.727	51.5	0.909	2.054	74.2	1.439
12	0.704	42.4	0.673	0.645	58.3	0.548	0.622	69.9	0.507
17	2.821	92.1	1.839	0.287	49.7	0.966	2.259	75.6	1.381
19	2.293	76.2	1.595	0.374	69.9	1.020	2.075	88.7	1.284
22	2.402	89.7	1.711	0.199	45.4	0.240	2.678	89.8	1.260
25	0.404	10.4	0.731	0.221	36.8	0.225	0.938	17.8	0.991
26	0.378	49.7	0.589	0.468	62.1	0.671	2.164	76.1	1.315

The presence of a monoaromatic hydrocarbon (benzene), that is not included in the composition of this crude oil type, was poorly tolerated, the viability of several bacterial strains being affected following a longer contact with this solvent (Tab. 6).

Tolerance to hydrocarbons was not always correlated with the capacity to their degradation. The degrading potential was maximal against the crude semi-paraffinic oil (61.4 - 91.2%) and n-hexadecane (42.4 - 92.1%). Cyclohexane, a cyclic aliphatic hydrocarbon, was used as carbon source to a lesser percent (19.3 - 73.0%) (Tab. 5).

Investigations of the degradative capacity against the tested hydrocarbon categories led to results concordant to the data in the literature, i. e. the sensitivity of microorganisms to hydrocarbons is inversely proportional to the Pow Log index of the hydrocarbons (Ramos et al., 2002). Thus, hexadecane, whose Pow Log value is > 4, is less toxic to bacteria and consequently easier to degrade as compared to cyclohexane, especially benzene, with a Pow Log index, ranging between values 1 - 4 and a much higher toxicity. The undertaken research ended with the selection of nine bacterial strains capable to tolerate and degrade both individual hydrocarbons and crude oil. These isolates are Gram negative bacteria belonging to *Aeromonas*, *Brevundimonas*, *Pseudomonas* and *Vibrio* genera, but were found also two Gram-positive strains belonging to *Micrococcus* genera. The hydrocarbon degradative capacity of some Gram-positive cocci is confirmed in literature (Nielsen et al., 2005). In the figure 2 the hydrocarbon degradation ability of the selected bacterial strains is presented.

Table 6: Tolerance to a monoaromatic hydrocarbon (benzene) of the bacterial strains under study; * = initial OD/660 nm = 0.038-0.095.

Bacterial strain indicative	Monitored parameters after ten days cultivation on mineral medium with 5% (v/v) benzene:	
	Bacterial growth (final OD)*	Cellular viability (OD after incubation on LB medium)
1	0.082	0.025
2	0.179	0.520
4	0.125	0.531
5	0.561	0.244
6	0.095	0.068
7	0.136	0.017
9	0.127	0.149
10	0.008	0.023
11	0.062	0.033
12	0.022	0.025
17	0.063	0.147
19	0.029	0.129
22	0.139	0.220
25	0.020	0.020
26	0.052	0.029

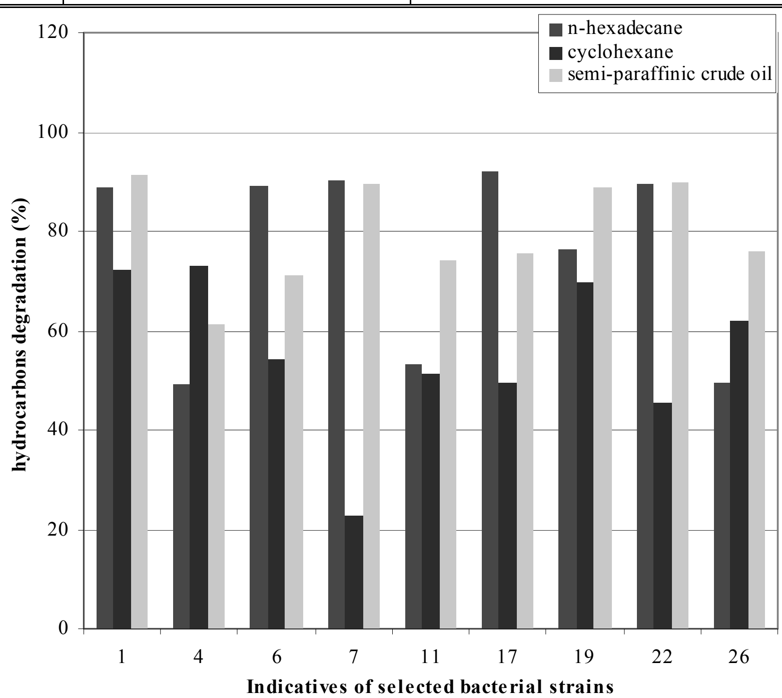

Figure 2: The hydrocarbon degradation ability of the selected bacterial strains.

These microorganisms could be of interest for the ecological technologies intended to recover sites contaminated with oil products or could be used as metabolic biomarkers for monitoring "in situ" hydrocarbon degradation (Young, 2005), or as bioindicators for detection of oil products present in the environment.

CONCLUSIONS

The microbial diversity founded in the studied water samples represented an ideal source for isolation of hydrocarbon-oxidizing bacteria, already adapted to the frequent presence of oil products in the environment.

The performed microbiological and physiological characterizations confirmed bacterial adaptation to the presence of such oil pollutants, e. g.: the group of hydrocarbon-oxidizing bacteria represented the most significant percent; most of the isolates are Gram negative, consequently more resistance to hydrocarbons; predominant respiratory type is the aerobe/facultative anaerobic one; in the enzymatic equipment predominant were degradative enzymes belonging to the oxido-reductases and hydrolases category; the capacity to increase of hydrocarbon bioavailability by synthesis of extracellular tensioactive compounds in the presence of hydrocarbons; good tolerance and degradative capacity against semi-paraffinic crude oil, present at various concentration in the water samples and against n-alcans which are present in the composition of this type of crude oil.

The undertaken researches allowed the screening of nine bacterial strains capable to tolerate and degrade both individual hydrocarbons and also crude oil. These microorganisms, belonging to *Aeromonas*, *Brevundimonas*, *Micrococcus*, *Pseudomonas* and *Vibrio* genera, used possibly as a consortium, could be of potential interest for rehabilitation of polluted environments by bioremediation technologies.

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AQUATIC HEALTH ASSESSMENT: A METHODOLOGICAL PROPOSAL FOR MEXICAN AQUATIC ECOSYSTEMS

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KEYWORDS: water quality index, trophic state index, multibiomarker response.

ABSTRACT

Aquatic ecosystem health and environmental stress concepts are analyzed to discuss upon the advances that in freshwater topics have been carried out in the National School of Biological Sciences (NSBS) Mexico. We comment and discuss the main approaches used in the search of the diagnostic symptoms that researchers of the NSBS have investigated historically. Different indicators (end points) of abiotic (chemical and physical environment) and biotic conditions (biodiversity, exotic species, sentinel organisms, biomarkers and bioindicators) have been assessed to analyze the freshwater ecosystem health. The use of biomarkers has proven to be useful in establishing evidence of exposure to pollutant chemicals and health damage to sentinel organisms. In addition, biomarkers have helped to establish causal relationships. An integrated protocol to freshwater ecosystem health assessment with water quality index, trophic state index, biomarkers battery and bioindicators, has been implemented, forming part of the new generation of quantitative assessment of freshwater ecosystem health. Different perspectives remain open to deepen in freshwater ecosystem health research. Diverse research projects have been carried out under this scheme by our staff in lakes of the Mexican Central Plateau: Health assessment of Yuriria Lake, and in rivers of the tropic rainforest: aquatic health assessment of Champoton River, among others. Multidisciplinary approaches are necessary.

SUMARIO: Evaluación de la salud de los ecosistemas acuáticos: Una propuesta metodológica para ecosistemas acuáticos mexicanos.

Los conceptos de estrés ambiental y salud de los ecosistemas acuáticos son analizados y discutidos sobre los avances que en materia de ecosistemas dulceacuícolas se han llevado a cabo en la Escuela Nacional de Ciencias Biológicas del Instituto Politécnico Nacional (ENCB), México. Comentamos y discutimos los principales acercamientos empleados en la búsqueda de los síntomas diagnósticos que investigadores de la ENCB han investigado históricamente. Diferentes indicadores (puntos finales) de condiciones abióticas (ambiente físico y químico) y bióticas (biodiversidad, especies exóticas, organismos centinela, biomarcadores y bioindicadores) han sido evaluados para analizar la salud de los ecosistemas dulceacuícolas. El uso de biomarcadores ha resultado una herramienta útil para establecer la evidencia de daños a la salud de organismos centinela expuestos a mezclas de contaminantes, así como para establecer relaciones causales. Nuestra experiencia nos ha llevado al diseño de un protocolo de trabajo para evaluar la salud de los ecosistemas dulceacuícolas integrado por un índice de calidad del agua, un índice de estado trófico, el uso de biomarcadores de alerta temprana y bioindicadores poblacionales. Dicho protocolo forma parte de la nueva generación de evaluaciones cuantitativas de la salud de los ecosistemas dulceacuícolas. Diferentes perspectivas siguen abiertas para profundizar aun más en la investigación de la salud de los ecosistemas acuáticos. Bajo este protocolo de trabajo hemos realizado diversos proyectos de investigación en embalses del altiplano mexicano (Evaluación de la salud de la Laguna de Yuriria), y en ríos del trópico húmedo (Evaluación de la salud del río Champotón), entre otros. El trabajo y la aproximación multidisciplinaria son necesarios para la evaluación de la salud de los ecosistemas dulceacuícolas.

REZUMAT: Evaluarea stării de sănătate a apelor: o propunere metodologică pentru ecosisteme acvatice mexicane.

Conceptele de sănătate a sistemelor acvatice şi de stress ecologic sunt analizate în vederea comentării progreselor făcute în ceea ce priveşte starea apelor dulci la National School of Biological Sciences (NSBS) Mexico. Autorii comentează şi discută pe marginea mai multor metode, folosite în investigarea simptomelor de diagnostic, pe care cercetătorii de la NSBS le folosesc în mod tradiţional. Diferiţi indicatori (puncte finale) ale condiţiilor abiotice (mediul fizic şi chimic) şi biotice (biodiversitate, specii invazive, organisme santinelă, biomarkeri şi bioindicatori) de mediu au fost evaluaţi în analiza stării de sănătate a ecosistemelor dulcicole. Utilizarea biomarkerilor s-a dovedit utilă în stabilirea gradului de expunere la substanţe chimice poluante şi în stabilirea daunelor produse sănătăţii organismelor santinelă. În plus, biomarkerii au ajutat la stabilirea relaţiilor cauzale. S-a implementat un protocol integrat de evaluare a stării de sănătate a ecosistemelor dulcicole, utilizand indici de calitate a apei, indici ai stării de troficitate, o gamă largă de biomarkeri şi bioindicatori, formând o parte a noii generaţii de evaluări cantitative a sănătăţii ecosistemelor dulcicole. Rămân deschise diferite perspective în aprofundarea cercetării, în acest subiect. În această schemă, personalul nostru a desfăşurat o serie de proiecte de cercetare în lacurile din Platoul Mexican Central: evaluarea stării de sănătate a lacului Yuriria, precum şi în râurile din pădurea tropicală: evaluarea stării de sănătate a râului Champoton, între altele. Este necesară o abordare multidisciplinară.

INTRODUCTION

The aquatic ecosystems are complex and dynamic, consisting of biotic and abiotic parts and processes (Sherry, 2003). The environment receives organic and inorganic strange compounds (xenobiotics) constantly, emitted by urban and industrial settlements. In the XXth century thousands of pollutants as polychlorinated biphenyls, organochlorine pesticides, polycyclic aromatic hydrocarbons, polychlorinated biphenyls and dibenzodioxins, were emitted to the environment (van der Oost et al., 2003). The final destiny of many of these pollutants is water, whether for direct inputs or different hydrologic and atmospheric processes (Stegeman and Hahn, 1994). The presence of a xenobiotic in an aquatic ecosystem is not, by itself, a toxic effects indicator (van der Oost et al., 2003), moreover these could have not an immediate effect; however, the total charge of pollutants has contributed to the changes observed in the structure and function of the aquatic ecosystems (Oertel and Salánki, 2003). The noxious effects upon the populations are frequently complex to detect in wild life organisms, due to the symptoms that can only be manifested after long periods of exposition. When the effect is finally clear, the destructive process could be very advanced to the point where the actions to solve or reduce the risk, could not be applied (van der Oost et al., 2003).

Since 1956, Seyle defined the stress as "the state showed by the specific syndrome, that consist in all the changes produced not specifically inside a biologic system"· Seyle applied his concept to organisms and sub organisms level (cellular, tissues, organ); however, since then the term of stress has been used in all biologic integration levels (Barret et al., 1976). Examples of environmental stress are the volcanic activity, the hurricanes, the floods, changes in the land use of soil, the introduction of exotic species. The sewage discharges of industrial and agricultural activities represent one of the main sources of stress in the aquatic ecosystems (Rapport and Whitford, 1999).

Cairns (2003) describe the environmental stress like an action, agent or condition that harms the structure or function of a biologic system. The responses to the environmental stress could be structural or functional. The structural changes are comprised of alterations in the species composition, trophic relations and indicator species, whereas the functional alterations involve the nutrient cycle and the energetic flow. The stress then, is the result of a degradation process, which is characterized by symptoms or responses like DNA damages, mortality, loss of biodiversity, of energy or nutriments, recruitment decrease and the reduction of primary and secondary production, and a resistance to the natural disturbance diminution.

Therefore, the responses of the biologic systems to the environmental stress are different in a hierarchical scale, due to the affectation, that can be at cellular level, organisms, populations, communities, ecosystems and regional.

The biomarkers, defined as a change in the biologic responses, are used as indicators that show that the organism has been exposed to a xenobiotic or that this had entered in the organism. The biologic responses, in this case, are observed as a deflection of the normal condition of the organism and its measure is at individual or suborganism level (van der Oost et al., 2003).

The bioindicators enable to identify responses as structural changes by age, genus proportion, fertility, somatic index (condition factor, hepatosomatic and gonadosomatic index) and so on. At higher levels of biologic organization, the diversity and equity alterations are also responses to stress.

Therefore, is evident that the ecosystems can be dysfunctional too, particularly when they are exposed to stress caused by the human activities (Rapport et al., 2000). Traditionally the health concept has only been applied to individuals and populations; however Rapport (1989) introduced the concept of aquatic ecosystem health as a metaphor in relation to the human health. Constanza (1992), considers ecosystem as sustainable when it keeps its structure and function over the time, facing the external stress. Rapport et al. (1998), defined a healthy ecosystem as the one that is free of stress and degradation, and keeps its organization and autonomy through the time, and is tough to stress. Calow and Forbes (1998) stated that the use of the term "ecosystems health" promotes the protection and preservation of these. A healthy ecosystem is made up of biotic communities and abiotic characteristics that form a self-adjustable and self-supportive unity (John, 2003). The biotic integrity of an ecological ecosystem is reflected by the health of the organisms that inhabit in that system. In the particular case of the aquatic ecosystems, the fish are excellent indicators of the system health (Adams et al., 1993).

The ecosystems health could be evaluated for vigour indicators (metabolism, productivity), organization (diversity), and resistance (Rapport et al., 1998).

HEALTH ANALISYS OF THE FRESHWATER ECOSYSTEMS IN THE NATIONAL SCHOOL OF BIOLOGICAL SCIENCES (NSBS)

The aquatic ecosystems health term, as it has been indicated, appeared in the 80's with Rapport's analogy of the human health. However this doesn't mean that the previous studies about the evaluation of the aquatic ecosystems hadn't considered the symptoms and the diagnostic of stressing agents in these ecosystems. Álvarez (1960), stated since then, that the use of natural resources should be performed, with the minimal deterioration to the useful species, without damaging them but obtaining the maximum production at the same time (making use of the sustainable concept before it was known as that). Afterwards, in 1981, Alvarez himself in his work named "Pláticas Hidrobiológicas", dedicate a chapter to the analysis of the effects caused by the introduction of new species of exotic fish to Mexico, pointing out some of the carps characteristics (*Cyprinus carpio* and *Carassius auratus*), of the trout (*Oncorhynchus mykiss*) and crappie in general, with specific cases about the introduction of these exotic species to the freshwaters in Mexico (Tequesquitengo, Pátzcuaro, Aljojuca and Tecuitlapa), and gives an exact account of disadvantages about these species introduction and some of their effects.

CASES OF STUDY IN THE LERMA RIVER BASIN

In the National School of Biological Sciences several health assessment studies, about the Lerma River's basin have been done, this is one of the rivers with most environmental damage and most anthropogenic influence in Mexico. In 1991, Soto-Galera and collaborators investigated the relation to the environmental deterioration with the ichthyofauna distribution in Lerma River. This is one of the first studies accomplished in the NSBS, where the fish community was used as environmental indicator. Likewise, López-López and Díaz-Pardo (1991), carried out a study about the distributional changes of the ichthyofauna in the La Laja River sub-basin, by this area ecological disturbance effects, taking notice of the damage generated by the anthropogenic influence in the lower portion of the sub-basin, that caused a restriction in the fish range distribution, provoking the extirpation of sensible species and favouring the presence of the more tolerant species. Despite the ecological disturbances detected, it was found out that the La Laja River operated as a refuge zone.

Again in 1998, Soto-Galera and collaborators carry out a fish community study in the Lerma River, this time classifying them in regard to the environmental degradation, as tolerant, medium tolerant and sensible. Remarkable changes were detected in the frequency of occurrence of the native species, with a 40% that disappeared or decreased its frequency of occurrence in nearly all the study sites in relation to historical records, previous to 1991. In this case, the sensible fish were found in waters with not marked human impact and with good water quality; moreover, it showed that these areas should be considered as conservation sites for these species. In this research a comparison in relation to 1991 was done and it was detected that the sub-basin with the best quality was the Turbio River in the middle portion of the basin while the most damaged zone was the upper portion of the Río Lerma. It was also detected the possible extinction of *Algansea barbata*, adding up three extinct species in the Lerma River basin. In the health condition assessment of the Lerma River, taking the fish assemblage as indicator an increase in the symptoms of environmental deterioration (loss of biodiversity and habitat, species extinction) was detected. The study showed that only a few restricted areas have good environmental quality.

Soto-Galera et al., (1999) put forward a particular study about the sub-basin of the Río Grande de Morelia. Showing that is a highly deteriorated zone and with a great habitat loss by anthropogenic activities. López-López and Dávalos-Lind (1998) investigated about the algal growth potential in a serial reservoir system in this sub-basin, finding out differences in the algal growth related to the land use of the adjacent areas; in this case a strain of *Selenastrum capricornutum* was used as environmental indicator (Recommended by the EPA protocol) and native microalgae.

Martínez-Tabche et al., (2001) studied the acetylcholinesterase activity and hemoglobin concentration (both of them early warning biomarkers), in *Limnodrilus hoffmeisteri* (Oligochaeta), exposed to sediments of the Ignacio Ramírez reservoir, located in the upper portion of the Lerma River. The sediments were characterized and lead was discovered in them. The results demonstrate that the biomarkers are useful indicators of the sediments toxicity in these sample organisms.

Recently, Sedeño-Díaz and López-López (2007), assessed the historical records of water quality detecting environmental variations related to the anthropogenic impacts and measuring the water quality through a water quality index proposed by Dinus in 1987. The study showed that Lerma River is one of the most affected environments in Mexico. Afterward Sedeño-Díaz and López-López (2006a and 2006b) carried out a regional study of ten reservoirs located in priority hydrological areas (Arriaga et al., 2002) in the Lerma River basin. In this research the trophic state was diagnosed, the water quality index was calculated and the level of lipidperoxidation as early warning bioimarker was used in primary producers of these reservoirs. The results revealed environmental variations among the reservoirs and the xenobiotic presence that produce free radicals in the first levels of the web food, not detected by the water quality index.

OTHER RESERVOIRS CASES OF STUDY

Díaz-Pardo et al., (1998) analysed the Azteca Lake, making use of the phytoplankton assemblage as a health condition bioindicator. The investigation was divided in two: a) A short time analysis about the phytoplankton assemblage and their water quality conditions (1981-1982), and b) A long time variations analysis in the phytoplankton assemblage and their environmental changes (1984 - 1994). The study showed that during 1981 - 1982, the Azteca Lake was in a mesotrophic condition with a phytoplankton community dominated by Chlorophyta and Bacillariophyceae; however its evolution was decreasing on water quality, replacing the phytoplankton assemblages by cyanobacteria as the main component, reaching an eutrophic condition. In the lentic systems, the phytoplankton has a very important role in the food chain, being the first level (producers), for this reason this health assessment approach is interesting and unique in Mexico, for their long term tracking.

Lyons et al., (2000) introduced a biotic integrity index design in order to appraise the lake conditions in the Mexican Central Plateau, explaining that there is no existence of an integrated health indicator in Mexican lake ecosystems yet. Concluding that the qualification judgement and the species classification system should be improved and also take in consideration more information to standardize the sample proceedings.

The environmental deterioration has a high ecological price in Mexico. The historical track had detected several species in danger to become extinct, and many others were registered as extinct. Xochimilco, a place that still conserve many of its prehispanic attributes like the crop in *chinampas*, has experimented the extinction of two endemic fish species of the Mexico basin: *Evarra tlahuacensis* and *E. bustamantei*, and almost the eradication of another endemic species: *Girardinichthys viviparus*. López-López et al., (2006) conducted bioassays with *G. viviparus* in order to investigate the possible damage that this species will undergo in a restocking case in Xochimilco, one of their old natural environments. This approach used the response of oxidative stress and neurological damage biomarkers. The results showed that there is damage to the *G. viviparus* health, however the authors recommend perform more studies before restocking Xochimilco waters with this species, which is in danger (Sedeño-Díaz and López-López, 2009).

Tejeda-Vera et al., (2007) assessed the health condition of two fish endemic species of Goodeid in Mexico, *Goodea atripinnis* widely distributed over the Mexican Central Plateau, and *Ameca splendens*, more restricted and considered as a threatened species as López-López and Sedeño-Díaz (2009) stated. The study comprise a holistic evaluation that include two sentinel species, one reference site, the habitat quality assessment using a water quality index, the early warning assessment (to make evident the hepatic, neurological, oxidative stress and detoxification mechanism damages which results were integrated in an index), and the population bioindicators assessment (sex ratio, fertility, age, condition factor, hepatosomatic and gonadosomatic index and other morphometric measures). The results demonstrated that both fish species have responses at two organization levels: individual through the integrated biomarker response (IBR) and the population level (bioindicators responses). It also demonstrated that *Ameca splendens* is more sensitive to the environmental stress than *Goodea atripinnis* even in the reference site responses were detected in *A. splendens* and *G. atripinnis*.

Martínez-Jerónimo et al., (2008), developed acute and chronic toxicity studies using the cladoceran *Simocephalus mixtus* exposed to sediments of a lake polluted by hydrocarbon and heavy metals. This organism test response was compared with the *Daphnia magna* obtained one. In the acute toxicity test (acute toxicity), the individual mortality was mostly calculated, while in the chronic, other population parameters were determined. The severe exposition results revealed a 17% and 10% in mortality respectively. The chronic exposition showed reproduction damage, surviving reduction, adult size reduction and fertility damages. Martínez-Jerónimo (2008a, 2008b) introduced two evaluation protocols about the chronic toxicity in chlorophyte microalgae and acute toxicity for Martínez-Jerónimo and Espinoza-Chávez (2008) showed another toxicity protocol for fish larvae and juvenile organisms. These tests are designed to demonstrate mainly the toxic effects of pure compounds, natural and effluents waters with or without treatment.

DIAGNOSIS SYMPTOMS ASSESMENT DETECTED IN THE STUDIES PERFORMED IN THE NSBS

The studies performed in the NSBS about the freshwater ecosystems health, as it could be seen in the previous section, have been diverse and have been focused from different views, considering methodological approach and diverse influence confines from point studies in a single body of water to regional studies (including the basin and sub-basin level). The diagnosis symptoms development about the freshwater ecosystems health deterioration, previously studied in the NSBS, can be observed on table 1 and are analyzed below.

The diagnosis symptoms about the freshwater ecosystems assessment has been divided in four groups: a) Abiotic Indicators, b) Biologic Indicators, c) Influence Scope, d) Organization Level; a) The abiotic indicators comprise the physical and chemical measurable variations in the aquatic environment. The abiotic environment assessment has evolved from the physical changes visual observation to the physicochemical monitoring, and the environmental service assessment through water quality index application that qualify the use of the water. b) The biologists have considered the construction of systematic inventories which evaluation through the time allowed make evident the biodiversity loss, changes in the fish distribution, the species extinction, the presence or introduction of exotic species, changes in the population structure and changes in the trophic status of water bodies; all of them have high ecological significance aspects. The use of sentinel species has been an important advance as well. In these species the accumulative damages in the organism related to the xenobiotics and stressing factors by exposure in the aquatic environment have been directly evaluated; likewise, in this decade the use of early warning biomarkers and the population bioindicators have been incorporated to the researchers' working protocols in the NSBS in order to prove the biological responses of the sentinel species. c) Influence Scope. The freshwater ecosystems health assessments in the NSBS have had different coverage. From specific water bodies (Díaz-Pardo et al., 1998), comparative between two study sites (Tejeda-Vera et al., 2007), at basin level (Sedeño-Díaz and López-López, 2007), and even, at regional level in the Mexican Central Plateau (Lyons et al., 2000), using more comprehensive approaches and with more holistic vision. d) Organization Level. The first studies incorporated the population and community levels facilitating the ecosystem global damage identification (high ecological relevance); however, the use of the organism and suborganism approaches offer the tools to identify the damages caused by specific xenobiotic groups and their effect over target organisms that endanger the species stability in its habitat and permit the damage detection on lower organization levels, before they reach upper levels (populations, communities and ecosystem) where the effects could be permanent, this would permit the appearance of management, conservation or prevention strategies of the freshwater ecosystems.

Table 1a: Freshwaters ecosystems health symptoms classification studied in NSBS.

Chronologic Reference	Abiotic Indicators		
	Habitat Structural Changes	Physical and Chemical Environmental Quality	Environmental Services Assessment
Álvarez, 1960			
Álvarez, 1981			
Soto-Galera et al., 1991	**X**		
López-López et al., 1991	**X**		
Soto-Galera et al., 1998	**X**		
Soto-Galera et al., 1999	**X**		
López-López and Dávalos-Lind 1998	**X**	**X**	
Díaz-Pardo et al., 1998		**X**	**X**
Lyons et al., 2000			
Martínez-Tabche et al., 2001		**X**	
López-López et al., 2006	**X**	**X**	
Sedeño-Díaz and López-López 2006a	**X**	**X**	**X**
Sedeño-Díaz and López-López 2006b	**X**	**X**	
Sedeño-Díaz and López-López 2007	**X**	**X**	**X**
Tejeda-Vera et al., 2007	**X**	**X**	**X**
Martínez-Jerónimo et al., 2008	**X**	**X**	**X**
Martínez-Jerónimo, 2008a	**X**	**X**	**X**
Martínez-Jerónimo, 2008b	**X**	**X**	**X**
Martínez-Jerónimo and Espinoza-Chávez, 2008	**X**	**X**	**X**

Table 1b: Freshwaters ecosystems health symptoms classification studied in NSBS.

Chronologic Reference	Biologic Indicators								
	Biodiversity Loss	Species distribution changes	Species Extinction	Exotic species existence	Ppulation structural changes	Trophic status changes	Use of sentinel organisms	Early warning biomarkers	Population bioindicators
Álvarez, 1960									
Álvarez, 1981				X					
Soto-Galera et al., 1991	X	X	X	X	X				
López-López et al., 1991	X	X		X	X				
Soto-Galera et al., 1998	X	X	X	X	X				
Soto-Galera et al., 1999	X	X	X	X	X				
López-López and Dávalos-Lind, 1998						X	X		
Díaz-Pardo et al., 1998	X				X	X			
Lyons et al., 2000	X	X					X		
Martínez-Tabche et al., 2001							X	X	
López-López et al., 2006		X			X		X	X	
Sedeño-Díaz and López-López, 2006a							X	X	
Sedeño-Díaz and López-López, 2006b						X			
Sedeño-Díaz and López-López, 2007									
Tejeda-Vera et al., 2007					X		X	X	X
Martínez-Jerónimo et al., 2008							X		
Martínez-Jerónimo, 2008a							X		
Martínez-Jerónimo, 2008b							X		
Martínez-Jerónimo and Espinoza-Chávez, 2008							X		X

FINDING A METHODOLOGYCAL PROPOSAL

In the NSBS we are in the freshwaters ecosystems health diagnosis period. Methods and implements were modernized; currently, various levels of biological organization and signals of biological responses are integrated with the characterization of abiotic environment.

In the Ichthyology and Limnology laboratory of the NSBS a methodological protocol has been designed in order to assess the freshwaters ecosystems health (Fig. 1). This protocol joins the social-economic, environmental, and biological aspects. The social-economic and environmental areas are related to water quality and the bodies of water aptitude for anthropogenic uses through an index assessment that qualify the different uses of water and the trophic state diagnosis which besides its biologic involvement, affects the environmental services that bodies of water offer. The third aspect involves the sentinel species in different organization levels (suborganism, organism and population), identifying in each level the biological response signs to environmental stress (biomarkers battery and bioindicators). The integrated response, that is a result of the biomarkers battery analysis, permit to identify the effect of the sentinel species exposure to xenobiotic families or reactive oxygen species, stress responsible; and the use of biomarkers which are indicators of neurologic and hepatic damage.

Table 1c: Freshwaters ecosystems health symptoms classification studied in NSBS.

Chronologic Reference	Influence Scope		Organization Level		
	Local Level	Basin / Sub basin Levels	Regional level	Organism/ Suborganism	Population/ Community
Álvarez, 1960					
Álvarez, 1981			**X**		
Soto-Galera et al., 1991		**X**			**X**
López-López et al., 1991		**X**			**X**
Soto-Galera et al., 1998		**X**			**X**
Soto-Galera et al., 1999					**X**
López-López and Dávalos-Lind 1998			**X**		**X**
Díaz-Pardo et al., 1998	**X**				**X**
Lyons et al., 2000			**X**		**X**
Martínez-Tabche et al., 2001	**X**				
López-López et al., 2006			**X**	**X**	
Sedeño-Díaz and López-López 2006a		**X**	**X**		**X**
Sedeño-Díaz and López-López 2006b		**X**	**X**	**X**	
Sedeño-Díaz and López-López 2007		**X**			
Tejeda-Vera et al., 2007	**X**			**X**	**X**
Martínez-Jerónimo et al., 2008	**X**			**X**	**X**
Martínez-Jerónimo 2008a	**X**			**X**	
Martínez-Jerónimo 2008b	**X**			**X**	
Martínez-Jerónimo and Espinoza-Chávez, 2008	**X**			**X**	

The links between the environment quality, the biological responses and the social-economic aspects are integrated through water quality index (WQI), trophic state index (TSI) and the integrated biomarker response (IBR), that account for the studied ecosystem health. Joining the response or result of each index we have, as a result, the Freshwaters Ecosystems Health Integral Assessment (FEHIA).

The freshwaters ecosystems health integral assessments that are in the contemporary literature don't integrate into the same study water quality index, hepatosomatic index and early warning biomarkers battery response. This holistic approachment situates the NSBS investigations at the forefront of international bioevaluation and the aquatic ecosystems health diagnosis.

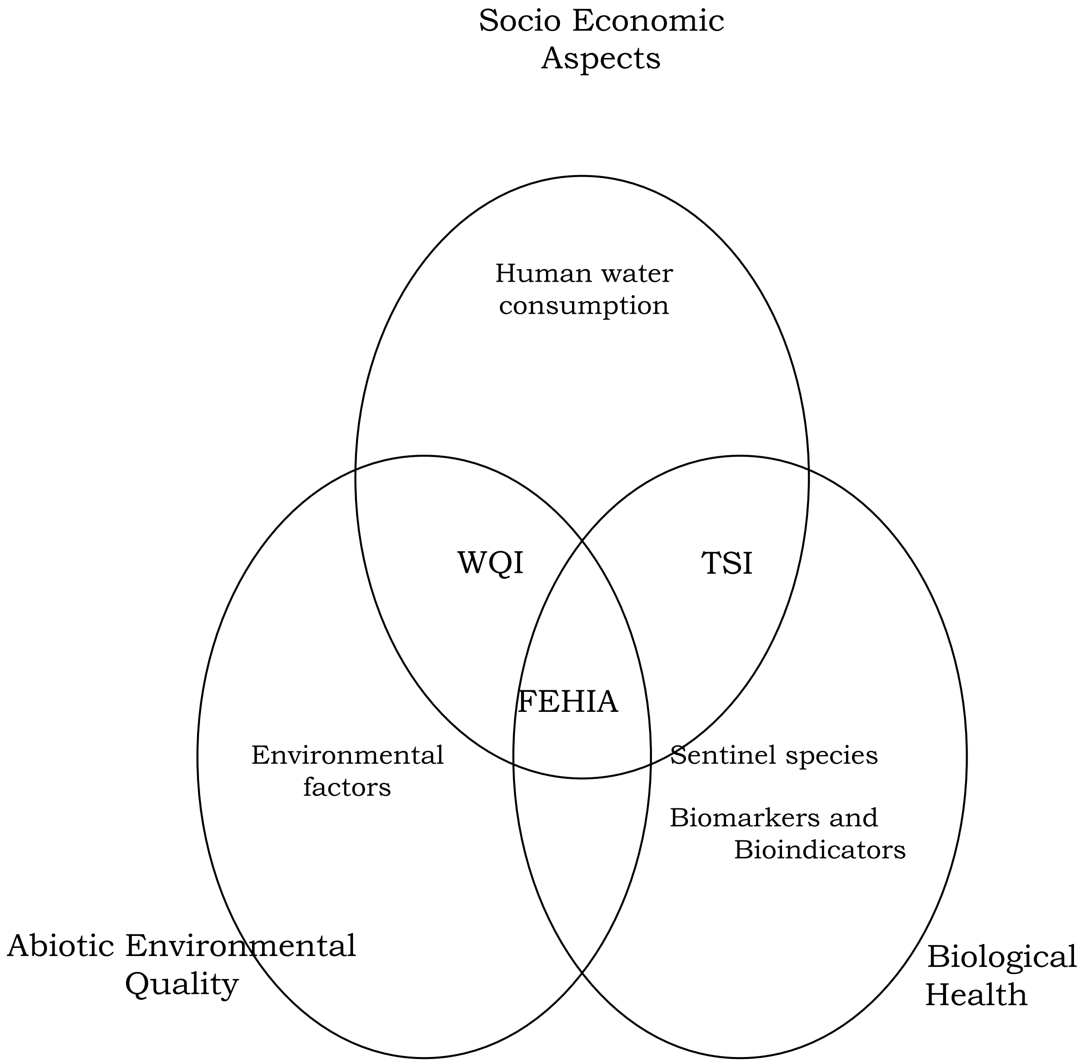

Figure 1: Aquatic ecosystems health assessment protocol: WQI = Water Quality Index, TSI = Trophic State Index and IBR = Biomarkers Integrated Response (BIR), FEHIA = Freshwaters Ecosystems Health Integral Assessment.

CONCLUSIONS

The aquatic ecosystems health concept alludes to the biotic and abiotic characteristics. In the NSBS had been carried out investigations with a freshwaters ecosystems health assessment approach. A methodological evolution has been experimented in the ways of dealing with the freshwater bodies' health scientific studies, comparing the direct and indirect stressing agent effects.

The incorporation of different levels organization have made possible to have a holistic vision about the affectation in the aquatic ecosystems health assessment.

PROSPECTS

From the analysis performed, many prospects are visualized:

- Mexico as a mega-diversity country has the duty of protect the biological species diversity in all their environments, but it is mandatory to elaborate systematic indexes to increase its own biodiversity knowledge. The environmental damage increase and many species have been lost without awareness of their existence.

- We should progress in the pristine sites research that permit, with its representative species, the formulation of ecological standards (benchmarks) in order to assess the fresh water ecosystems health.

- In Mexico the environmental legislation is still regulated through the environmental standard examination based on the determination of chemical species maximum permissible limit that are at the pollutant sources effluent. However, as it was mention, the numbers of new compounds, that are the results of chemical industry synthesis, increase day by day and are eventually deposited in the bodies of water. In the same way, the metabolites and resulting mixtures in the body of water cause effects of synergy, antagonism and toxic effects potentiation. For all these new compounds and body of water blends there are no environmental standards that point out the maximum permissible limit and there would never have the normative instruments. The bioevaluation should be added to normative instruments.

The traditional toxicology is far away from current necessities in the water body's bioassessment. It should opt for the research of end point assessments that do not correspond to the organism's death, since then the possibility of the early warning detection is scorned, and with this the opportunity of remedy and heal the damages before they reach upper organization levels where the damage is immutable.

- The sentinel organism used should be supported by native species that has a genetic register of the ancient environmental condition and react to stimulations that are not part of natural environmental variations.

- For NSBS the opportunity of create legislation based on the early warning well calibrated indicators is open and, for Mexico the necessity of change the environmental regulation instance from environmental to ecologic standards.

The aquatic ecosystems health has been acknowledged as a multidisciplinary concept that implies, beside the limnologists work, the integration of toxicologists, environmental engineers, sociologists and chemical scientists, only to cite some subjects, to the work team. It is important that NSBS open the possibility of a multidisciplinary work with the purpose of incorporate the different environmental visions to the health assessments. Nowadays the National Polytechnic Institute count on an Environmental Network, which is an opening area to increase the multidisciplinary work in the Ecosystems health assessments.

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