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***TRANSYLVANIAN REVIEW OF  
SYSTEMATICAL AND ECOLOGICAL  
RESEARCH***

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**10**

***The Wetlands Diversity***

**Editors**

**Angela Curtean-Bănăduc, Doru Bănăduc & Ioan Sîrbu**

**Sibiu - Romania**

**2010**







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### **Editors**

**Angela Curtean-Bănăduc, Doru Bănăduc & Ioan Sîrbu**

“Lucian Blaga” University of Sibiu,  
Faculty of Sciences,  
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**Sibiu - Romania  
2010**

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## IN MEMORIAM

### *Theodor Schwann* (1810-1882)

The name of *Theodor Schwann* is known worldwide in connection with the development of cell theory. In addition, many contributions to biology/physiology are related to his name, such as the discovery of Schwann cells in the peripheral nervous system, the discovery and study of pepsin, the discovery of the organic nature of yeast and the first use of the term metabolism.

Born in 1810 in Neuss, a town situated on the Lower Rhine, he attended primary school and his first secondary school classes in his home town. *Schwann* attended the last years of secondary school in Köln (Cologne). After obtaining the school-leaving certificate in 1829, he began his studies in medicine at the University of Bonn, changing later to Würzburg and then in 1836 to the University of Berlin, where he learned under the famous physiologist Professor Johannes Peter Müller. Influenced by Müller's work, *Schwann* was the first among Müller's students to break with vitalism and work towards a physico-chemical explanation of life. His Professor also called *Schwann's* attention to the process of digestion, which *Schwann* considered in 1837 to be dependent on the presence of a specific ferment, which he named pepsin. This was the first enzyme isolated from animal tissue.

In 1838, *Schwann* became Professor at the Catholic University of Louvain and later in Liège. During his research work he found out that both sugar- and starch-fermentation are the result of life processes. *Schwann* worked also on the contraction of muscles and the structure of nerves and discovered the striped muscles in the upper part of the oesophagus and the myelin integument of additional axons, named Schwann cells. *Schwann* coined the term "metabolism" to describe the chemical changes taking place in living tissues and formulated as well the basic principles of embryology.

In the discussion between *Schwann* and Matthias Schleiden (1804-1881) during the time they spent together in Berlin (1837-1838) - Schleiden being a botanist who studied the microscopic plant structure - their discussions turned to the nuclei of plants and animal cells. *Schwann* remembered seeing similar structures in the cells of notochord and instantly realized the importance of connecting the phenomena. Following their observations, discussions and confirmations of research, *Schwann* and Schleiden conceived the hypothesis that all living things were composed of cells, the cell being the elementary structural unit of every organism, whereupon they formulated the cell theory. They coined the term "unicellular" for living organisms made up of one cell and "multicellular" for organisms made up of more than one cell.

In 1839, *Theodor Schwann* published his famous work "Microscopic Investigations on the Accordance in the Structure and Growth of Plants and Animals", which contained the first statement of their joint cell theory. In the course of the verification of cell theory which included the whole field of histology, *Schwann* proved the cellular origin and development of the most highly differentiated tissues and with his generalization created the base for modern histology.

For his research work and discoveries, *Theodor Schwann* was honoured in 1845 with the Copley-Medal and in 1875 with the medal "Pour le Mérite" for science and arts. He passed away in Cologne/Köln in 1882 in the age of 71 years.

In 2010, the year of the 200<sup>th</sup> anniversary of his birth, *Theodor Schwann's* work is still as important as it was during his lifetime, an important milestone in the history of science.

*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 already the presence of the medium and long-term significant change in the "average weather" all over the world, the most common key words which reflect the general concern are: heating, desertification, rationalisation and surviving.

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 approaches and efforts.

With the fact in mind that these approaches and efforts should be based on genuine scientific understanding, the editors of the *Transylvanian Review of Systematical and Ecological Research* series launch a second annual volumes dedicated to the wetlands, volumes resulted mainly as a result of the *Aquatic Biodiversity International Conference*, Sibiu/Romania, 2007-2009.

The term 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.

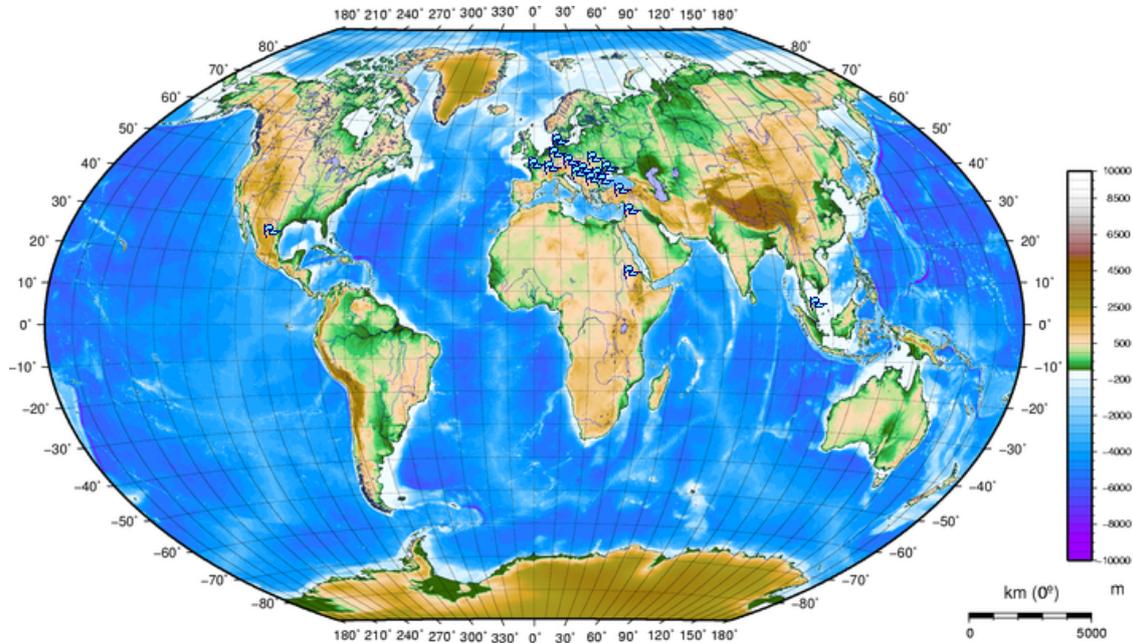
**Marine/Coastal Wetlands** - Permanent shallow marine waters in most cases less than six metres deep at low tide, includes sea bays and straits; Marine subtidal aquatic beds, includes kelp beds, sea-grass beds, tropical marine meadows; Coral reefs; Rocky marine shores, includes rocky offshore islands, 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; peat swamp 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 sub-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 world ...

This third volume included various researches from diverse wetlands around the world.



The subject areas (R) 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*

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**WATER TEMPERATURE AS AN APPLICABLE PARAMETER  
WITH A HIGH INDICATIVE VALUE  
FOR THE GENERAL CONDITION OF A RIVER-ECOSYSTEM,  
DRAWING ON THE EXAMPLE  
OF THE RIVER TRATTNACH IN UPPER AUSTRIA**

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**KEYWORDS:** Austria, Trattnach River, water temperature, anthropogenic impact, Te.M.P., retention basin, waste water treatment plant, thermal pollution.

**ABSTRACT**

Water temperature is considered one of the most essential regulating parameters in aquatic ecosystems. Water quality and biocoenoses are highly affected by this factor. Because of the intensive interrelations with other physical and chemical parameters, water temperature has a high indicative value when considering the general condition of a river-ecosystem and the intensity of anthropogenic impacts. The measurement-system Te.M.P. allows broadly based surveys on water temperature with relatively little effort.

In the river Trattnach in Upper Austria the Te.M.P. system was used to collect comprehensive data over the whole river-length in a timeframe of two years. The results indicate evidence of anthropogenic warming of the water. The main causes for the temperature change are a retention basin in the upper reach, the intensive morphological reshape of the stream course combined with a lack of riparian vegetation, as well as residual flow reaches and point source discharges.

The warming of the river water has diverse consequences on the river-ecosystem and its fauna and flora. In fish, the salmon family especially is negatively affected, whereas species that belong to the cyprinids can even benefit from higher temperatures. Based on these results, strategies and considerations for the improvement of the temperature regime and therefore of the whole ecological situation in the river Trattnach are going to be developed.

**ZUSAMMENFASSUNG:** Die Wassertemperatur als ein anwendbarer Parameter mit hohem Indikationswert für den allgemeinen Zustand eines Flussökosystems, dargelegt am Beispiel der Trattnach in Oberösterreich.

Die Wassertemperatur ist in Gewässerökosystemen der wesentliche steuernde Parameter für vielfältige biologische Regelkreise. Sie hat großen Einfluss auf die Wasserqualität und die Biozönose von Flüssen und Bächen. Durch die intensiven Zusammenhänge mit vielen anderen Parametern, die chemische und physikalische Beschaffenheit des Wassers bestimmen, eignet sich die Wassertemperatur als Indikator für den Gesamtzustand eines Gewässers sowie für die Intensität der anthropogenen Eingriffe. Das praxistaugliche Messsystem Te.M.P. bietet eine einfach handhabbare Möglichkeit, die Wassertemperatur kontinuierlich zu erheben.

An der Trattnach in Oberösterreich konnten damit bereits umfangreiche Daten über den gesamten Längsverlauf über einen zeitlichen Horizont von zwei Jahren gesammelt werden. Die Ergebnisse geben Hinweise auf eine anthropogen verursachte Erwärmung des Gewässers und die bedeutsamsten Ursachen für diese. Als Hauptursachen für die Erwärmung konnten ein Rückhaltebecken im Oberlauf, starke morphologische Veränderungen in Verbindung mit fehlender Beschattung, Restwasserstrecken sowie punktuelle Einträge identifiziert werden.

Die Erwärmung hat vielfältige Auswirkungen auf ein Gewässer und dessen Biozönose. Bei den Fischen sind vor allem die Salmoniden negativ betroffen, während Cypriniden sogar davon profitieren können. Deshalb sollen, abgeleitet aus den Ergebnissen der Untersuchungen, verschiedene Maßnahmen zur Reduktion der Erwärmung in der Trattnach angedacht werden.

**REZUMAT:** Temperatura apei ca parametru aplicabil cu mare valoare indicatoare pentru starea unui ecosistem de râu, exemplificat prin râul Trattnach din Austria superioară.

Temperatura apei este, în ecosistemele acvatice, parametrul esențial ce condiționează diversele circuite biologice de reglare. Are de multe ori o mare influență asupra calității apei și a biocenozelor de râuri și pârauri. Prin interrelațiile intensive cu mulți alți parametri determinanți pentru componența chimică și fizică a apei, temperatura apei se pretează ca indicator pentru starea generală a unui corp de apă, precum și pentru intensitatea intervențiilor antropogene. Sistemul practic de măsurare Te.M.P. oferă o posibilitate simplă de mânăuire, ce permite măsurarea continuă a temperaturii apei.

Cu acest sistem, a fost posibilă colectarea de date ample asupra întregului curs al râului Trattnach în Austria superioară, pe o perioadă de doi ani. Rezultatele indică încălzirea apei, datorită unor influențe antropogene și cauzele principale ale acestora. Ele sunt legate de un bazin de stocare a apei, în cursul superior, puternice schimbări morfologice combinate, cu lipsa de umbrire, segmente de râu cu volum mic de apă, precum și surse punctuale de deversări.

Încălzirea are consecințe multiple asupra cursului de apă și a faunei și florei sale. În cazul peștilor sunt influențate negativ, mai ales, salmonidele, pe când ciprinidele pot chiar profita de situație. Drept consecință a rezultatelor cercetărilor întreprinse sunt luate în considerare diferite măsuri pentru reducerea încălzirii apei în râul Trattnach.

## INTRODUCTION

Water temperature is a natural function of solar radiation, climate conditions, exchange processes with the environment and the hydrology of the river. Especially the inflow of spring- and ground-water, precipitation and the discharge are highly relevant for the temperature conditions in a river system (Jungwirth et al., 2003; Kromp-Kolb and Gerersdorfer, 2003; Frey et al., 2003).

Under natural conditions the water temperature increases from cool spring-influenced head waters to warmer lower reaches in summer time. In winter time, however, the ground water is relatively warm compared to the surface water. Therefore upper reaches are referred to as winter-warm and summer-cold. Studies have also shown that there is a distinct connection between water temperature and fish regions (Kromp-Kolb and Gerersdorfer, 2003; Gumpinger and Scheder, 2008). The maximum morning temperatures correlate to a great extent with the borders of the fish regions. In the upper salmon reaches, the maximum morning water temperatures are fluctuating between 4 and 10 C. In the metarhithral zone the range lies between 10 and 16°C, in the hyporhithral zone between 16 and 20°C, and in the epipotamal zone the maximum morning temperatures can reach more than 21°C.

Even during a day, considerable fluctuations in water temperature can be observed. With increasing distance from the source an increase of the daily amplitudes can be detected. In the middle reaches the daily amplitudes can reach up to 10 C. Further downstream, the bigger water volumes lead to a decrease of daily amplitudes again (Jungwirth et al., 2003).

For the whole aquatic ecosystem the water temperature is one of the most constitutive regulating parameters. Temperature is decisive for the food supply, for the chemical and physical circumstances like the solubility of different gases or the water density, and for the tolerance of organisms towards diseases and parasites. Therefore, the species assemblage and the abundance of species are tightly linked with the water temperature (Jungwirth et al., 2003; Kromp-Kolb and Gerersdorfer, 2003; Frey et al., 2003).

The high dependence of aquatic biocoenoses on the water temperature is due to the fact that nearly all the aquatic species are poikilothermic. The body temperature of fish ranges at about 0.1-1 C above the temperature of the surrounding aquatic environment (Beitinger et al., 2000). Survival, growth and reproduction of an individual are basically strongly regulated by the water temperature and, collaterally, by the interconnected water-parameters. Examples for processes regulated directly by the water temperature are the induction of migrations to spawning habitats or the time required for the development of eggs and fry. This indicates that different species and also different developmental stages of a species show diverse preferences and tolerances towards water temperature.

Therefore, aberrant temperature conditions have various, mainly negative effects on the aquatic fauna. Increasing temperatures result in an increased activity particularly in fish. They are not able to find enough nourishment to obtain their metabolism, and if temperatures exceed a certain threshold value, successful spawning or normal egg development is rendered impossible. The salmonid species are more severely affected than cyprinids. But it is particularly brown trout (*Salmo trutta* Linnaeus, 1758) and grayling (*Thymallus thymallus* Linnaeus, 1758) that sport fishers are most interested in. Hence, thermal pollution in lotic systems is not only of ecological, but also of economic interest.

The water temperature is influenced by a variety of natural factors, but human impacts outbalance natural impacts by far. The most common anthropogenic influences are linked to the intensification of the effects of natural energy flows, like changing the hydrologic regime by lowering the discharge, removing the alluvial vegetation or causing punctual impacts. In general, human impacts lead to an increased temperature, only in very few cases to a cooling.

A fundamental value concerning water temperature is the discharge and the volume of the waterbody, respectively. This variable affects the intensity of the diverse impacts on the water temperature decisively. At decreasing discharges the volume also decreases while the water surface-depending on the river-morphology - in most cases remains more or less the same. Therefore, during low flow situations the energy flow from the sun impinges on a similar surface, but on a much smaller volume. This leads to a more rapid warming in the daytime and a faster cooling at night. Particularly in residual flow reaches this phenomenon plays an important role.

Especially the relation between the solubility of oxygen and water temperature has far-reaching consequences. Increasing temperatures go along with a decrease of solute oxygen, but also with an increase of metabolic and other biological processes, which, again, causes a higher oxygen demand. The discrepancy between supply and demand that is caused by thermal loads can lead to threatening situations for aquatic ecosystems.

In the last centuries, people made considerable efforts to control brooks and rivers, for which intensive structural alterations were necessary. Watercourses were canalised and riparian areas were drained. The hydromorphological changes resulted in a wide spectrum of ecological problems. The collateral hydrological alterations are particularly relevant for the temperature regime. The canalisation of a streambed entails the homogenisation of water temperature along the whole cross section (Meier et al., 2004). Transverse control structures lead to a warming in the backwater; in residual flow reaches the small water volume is warmed up faster than at normal discharge in the daytime and cooled down more easily at night (Moosmann et al., 2005; Küttel et al., 2002; Frey, 2003; Meier et al., 2004). In backwater situations the length of the impoundment and the design of the outlet determine the intensity of changes in water temperature. In epilimnic outlets, where warm surface water is discharged to the river, enormous warming effects can occur downstream of the impoundment. Vice versa, the river water is cooled down if water is fed back from deeper, hypolimnic layers. In such cases the fish fauna can convert from a bioconenosis dominated by cyprinids to a community with high proportions of salmonids in the tailwater.

The intensive use of rivers and their surrounding areas often comes along with a lack of riparian vegetation. Under natural conditions, the shade cast by the alluvial vegetation reduces the influence of the solar radiation und therefore avoids unnatural warming effects. The reduction or removal of the vegetation along rivers is considered one of the biggest anthropogenic interferences on the temperature regime (Moosmann et al., 2005). Particularly at small discharges the enlarged radiation due to clear-cuttings leads to a considerably negative impact.

Furthermore, point source discharges are relevant for the thermal situation of a waterbody. Examples for such impacts are wastewater treatment plants, industrial effluents, as well as ponds and drainages. Precipitation on large sealed areas combined with a rapid run-off to the receiving rivers can also cause thermal peaks, especially in summer (Rossi, 2004).

All the mentioned human impacts are additionally superposed by the global climate change. Studies have already proven that the climate change goes along with the rising of water temperatures, a shift of the fish regions and changes in the biocoenoses, for example by the increased invasion of alien species. It is anticipated that thermophilic species and species with a broad tolerance range can benefit from the expected changes, while species which prefer cool temperatures will have to cope with a loss of adequate habitats. Those species, like most the salmonids are, will be forced to migrate to upstream regions in the future (Kromp-Kolb and Gerersdorfer, 2003; Hari and Güttinger, 2004).

Additionally to problems concerning temperature conditions, interruptions of the longitudinal continuum, the lack of suitable habitats or problems with the sediment transport often occur in anthropogenicall reshaped water bodies in Central Europe. Hence, the water temperature only reflects part of the whole situation. But the authors' experiences have shown that measuring the water temperature is an appropriate way to get an idea of the general condition of a river and of the intensity of human impacts on such a system.

### MATERIALS AND METHODS

The following study is based on the sampling of temperature data by means of a measurement system called Te.M.P. This measurement package was developed by the "Consultants in Aquatic Ecology and Engineering" in Wels ([www.blattfisch.at](http://www.blattfisch.at)) in cooperation with the Federal Secondary College of Engineering in Steyr. Te.M.P. works with data loggers provided by Maxim/Dallas, which can be programmed and read out by means of a software especially developed for that purpose. The sensors are combined with a battery and have the exact size of a coin cell. They are protected by means of water-tight aluminium or plastic casings (8 x 3.5 cm) (Fig. 1). The advantage of aluminium casings compared to those made of plastic is a better thermal conductivity and hence a more accurate measurement - on the other hand, the costs are higher for metal casings.



Figure 1: The main components of the Te.M.P.-System, a sensor combined with a battery, a water-tight metal casing and a steel rope to fix the measurement unit.

The measuring units - consisting of sensors and casings - are fixed in the watercourse using a steel rope that is tied to trees, roots or other immovable structures. Alternatively, they can be put into tubes that have been driven into the bedsediments. This fixing-method is advisable if vandalism or problems with floods are probable to occur. The data loggers can be programmed to measure the water temperature in intervals of minutes, hours or days. A precision of 0.5 C or 0.0625 C can be selected. To sum it up, the measurement system Te.M.P. is easy to apply, low-priced, and allows the sampling of a large amount of data.

The aim of the present study in the river Trattnach in Upper Austria was the collection of data showing the thermal development along the whole river length. The study focused on the different anthropogenic impacts along the river course. In the course of the project conception, morphological, hydrological and point source impacts were identified as sources of thermal pollution in the Trattnach River.

The following project design was chosen: 29 measurement units were installed, 26 of which were put directly into the Trattnach River, one into a small tributary and two into the Innbach River, which merges with the river Trattnach (Fig. 2). The sensors were programmed to measure the water temperature once an hour with a precision of  $0.0625^{\circ}\text{C}$ . The readout was performed by members of the local fishing club at regular intervals of several months. A total of 254.040 single datasets were recorded over the course of one year. After the first assessment cycle some data had to be excluded because of obviously incorrect measurement. It turned out that some units were washed ashore in the course of floods and were then measuring the air temperature instead of the water temperature. Only the comprehensible, correct data was edited in a database. In more than two years around 450.000 single temperature values were sampled using the method described.

### DESCRIPTION OF THE PROJECT AREA

The catchment area of the river Trattnach (Fig. 2) covers  $196.4\text{ km}^2$ . The source is situated at an altitude of  $620\text{ m a.s.l.}$  The brook flows through a hilly landscape until it merges with the river Innbach at an altitude of  $290\text{ m a.s.l.}$ , the latter being a direct tributary of the Danube River. The difference in altitude from the source to the mouth of the river Trattnach equals  $330\text{ m}$ , its total length is  $47\text{ km}$ .

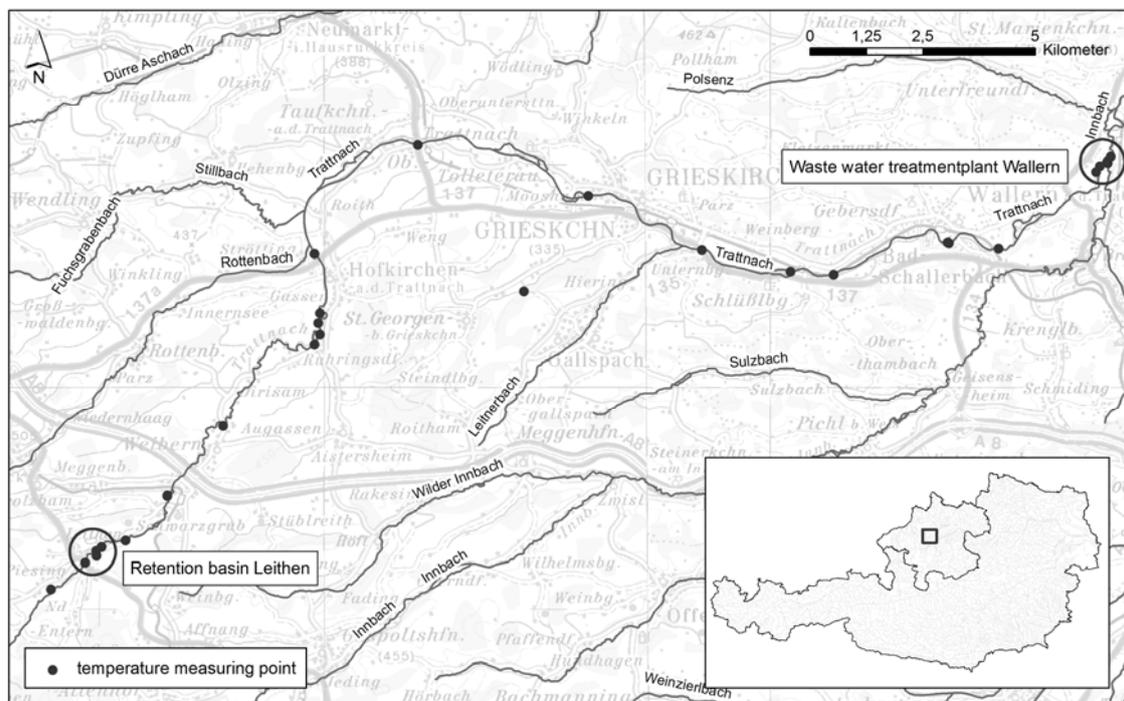


Figure 2: Overview of the project region, localisation of the temperature measuring points and selected aspects of special interest.

The mean discharge is indicated with 2.3 m<sup>3</sup>/s at the water gauge at Bad Schallerbach, upstream of which 184 km<sup>2</sup> of the catchment are situated (Hydrografisches Zentralbüro, 2008).

Some specific problems can be identified for the type of river the Trattnach represents. It is one in a set of rivers that are situated in the foothills of the Alps and that are - under natural conditions - characterised by a moderate to gentle slope and a winding or meandering morphology. These facts result in slow velocities, diverse bank structures, a high diversity in width and depth and consequentially in large habitat diversity (Wimmer et al., 2007).

Due to their size and location, such rivers are impaired by a variety of human activities. Their potential floodplain provides optimal areas for agriculture and settlement. Hence, the Trattnach River has, like many others of its kind, been forced into a canalised riverbed and is dominated by massive control structures, particularly from the town Hofkirchen to the river mouth. Moreover, the riparian vegetation has been reduced to a minimum or even removed totally.

The fish fauna was especially focused on in this study. Haunschmid et al. (2006) have defined special Leitbilder for different characteristic river stretches in different Austrian bioregions. In the Trattnach River, the brown trout is considered a core species in the whole river reach. From the mouth of a tributary called Rottenbach Brook downstream, the Trattnach River is defined as hyporhithral with the grayling, the burbot (*Lota lota* Linnaeus, 1758), the stone loach (*Barbatula barbatula* Linnaeus, 1758), the common minnow (*Phoxinus phoxinus* Linnaeus, 1758), and the European bullhead (*Cottus gobio* Linnaeus, 1758) occurring as additional core species. Further important species in this river stretch are, for example, the barbell (*Barbus barbus* Linnaeus, 1758) and the nase (*Chondrostoma nasus* Linnaeus, 1758) (Haunschmid et al., 2006).

## RESULTS AND DISCUSSIONS

The study at hand focussed on a variety of different impact factors. One of them was the retention basin "Leithen", as it was suspected to represent one of the main impacts on the temperature regime of the Trattnach River. This assumption was substantiated in the course of the project. The basin is not only used as a retention area during floods, but also as a swimming lake, and is therefore permanently connected to the river. The most significant temperature impacts normally occur between May and September, because at that time the water is warmed up intensively by solar radiation. In the basin, water temperatures of more than 29 C were measured in the summer of 2008. The problem is that just the particularly warm surface water is discharged into the river, thus increasing its water temperature significantly. This influence can be observed over a distance of at least two kilometres. Further downstream, the thermal impact of the retention basin is mingled with effects of other human impacts.

In the summer of 2008, the warming averaged +1.8 K when comparing the mean daily temperatures up- and downstream of the basin (Fig. 3). The maximum difference reached more than +3 K. Compared to the hot and dry summer of 2008, the year 2009 was relatively cool and rainy in Austria. But still, enormous warming effects caused by the basin could be detected in the summer of 2009 (Fig. 4). From May to September, the temperatures downstream of the basin were higher than upstream by +1.38 K on average, the maximum difference of mean daily temperatures reached +2.5 K (Figs. 3 and 4).

This dramatic warming has a huge influence on the whole river. It can be assumed that the whole biocoenosis is affected and its assemblage disturbed.

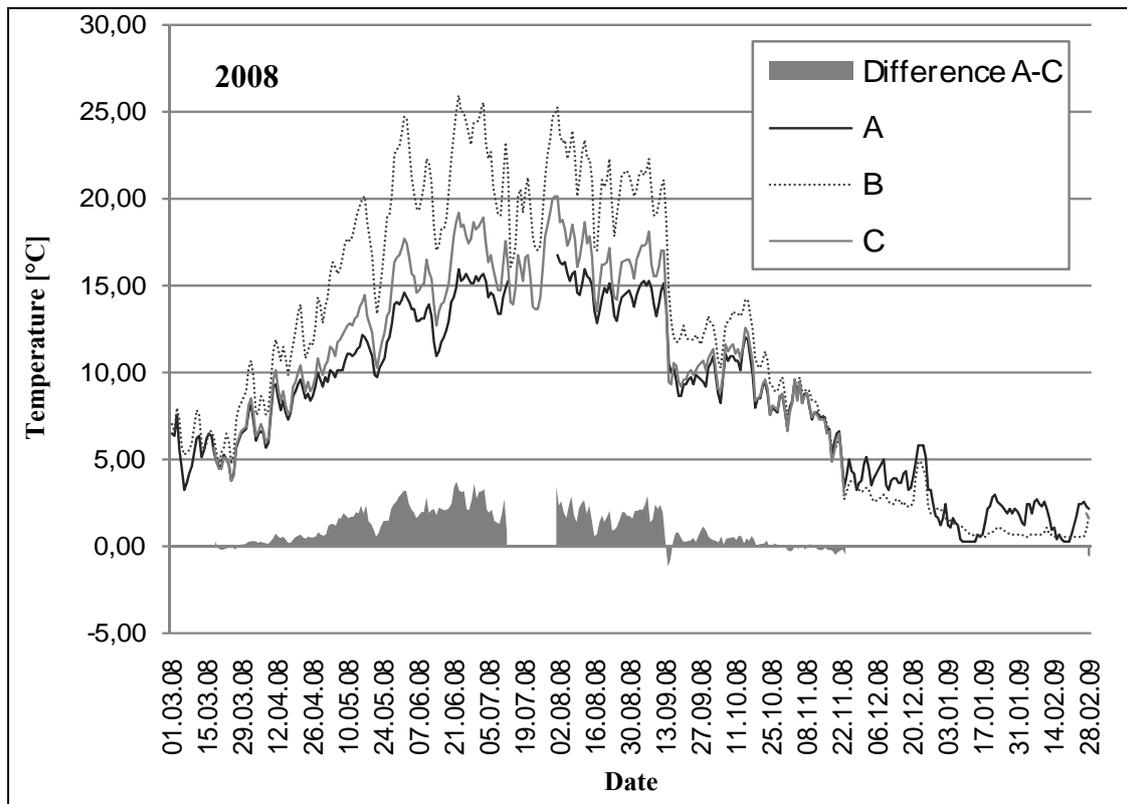


Figure 3: Water temperature situation at the retention basin “Leithen”, mean daily temperatures 2008; (A) natural reach upstream of the retention basin, (B) directly in the retention basin, (C) downstream of the retention basin.

The wastewater treatment plant “Wallern” is an example for a point source impact. Four wastewater treatment plants are installed within the catchment area of the Trattnach River, next to other point source discharges like a thermal spa and some fish ponds. The wastewater treatment plant “Wallern” is dimensioned for 65.000 population equivalents. Currently 6.2-6.5 Mio m<sup>3</sup> of sewage water per year are discharged into the Trattnach River (Anderwald and Nenning, 2003).

To what extent sewage water impairs the river depends on its ratio to the discharge in the riverbed (Frey et al., 2003). The ratio in wastewater treatment plant “Wallern” averages 1:10. The temperature of sewage water is supposed to lie between 10 C and 20 C throughout the year (Huber and Glasner, 2005). This fact results in different seasonal effects on the water temperature of the Trattnach River downstream of the plant. While the clarified water is warmer than the river water from September to April, the measurements have shown that it is cooler in the summer months. In the summer of 2008 (May-August) the discharge of the plant was colder than the river water by an average of -1.6 K. The maximum difference equalled -5.975 K. Over the course of the rest of the year the mean difference between the warmer water of the wastewater treatment plant and the cooler water of the Trattnach River accounted for +2.9 K (max. +6.863 K). The given values refer to the temperature of the undiluted sewage water. If the temperatures of the river water recorded at the measuring points upstream and downstream of the sewage plant are compared, the difference in the summer months of 2008 averaged -0.12 K, during the rest of the year 2008 +0.33 K (Fig. 5).

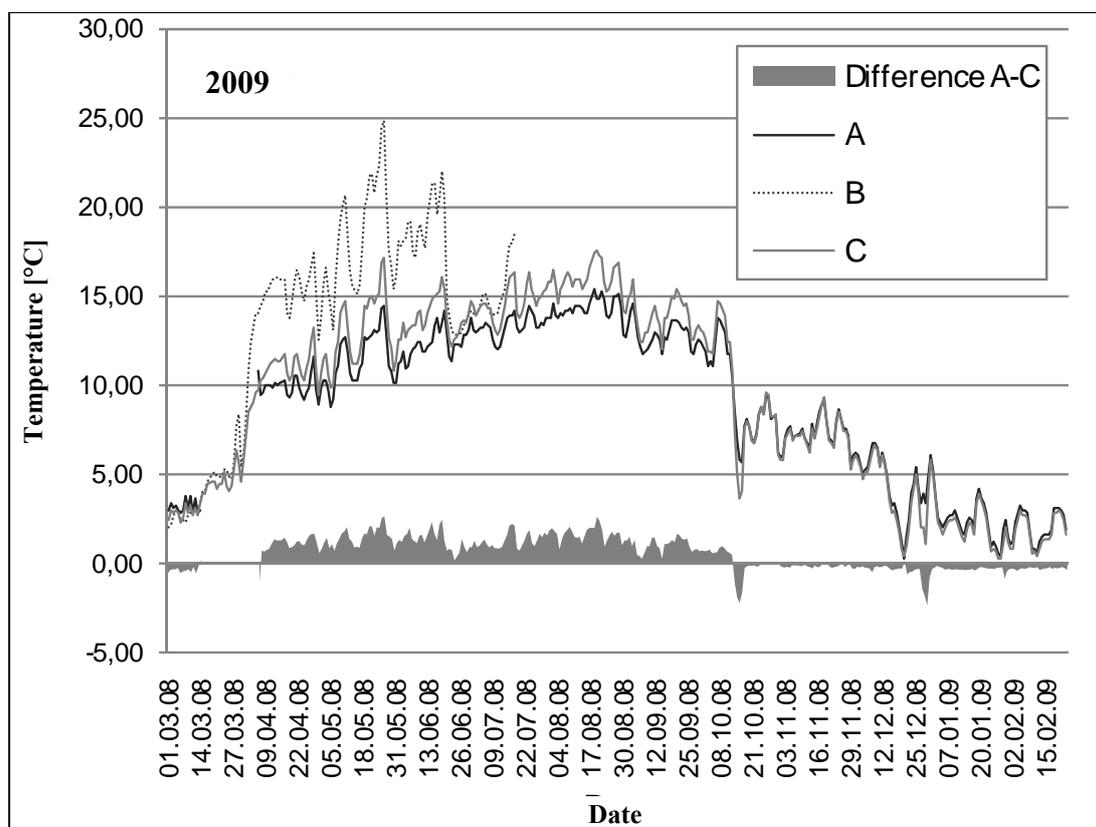


Figure 4: Water temperature situation at the retention basin "Leithen", mean daily temperatures 2009; (A) natural reach upstream of the retention basin, (B) directly in the retention basin, (C) downstream of the retention basin.

These warming and cooling tendencies are overlaid by the influences of precipitation and show distinctive seasonal effects. In summer, warm surface run-off is fed into the waste water treatment plant by means of a combined sewer system. Data has shown that the cooling effect of the plant discharge in summer is correlating negatively with the precipitation. The clarified sewage water is warmed up and has nearly the same temperature as the river water in the case of a rainfall. In spring, the water in the wastewater treatment plant is also warmed up by precipitation. In autumn and winter, however, rainfall leads to a cooling of the water from the sewage plant in comparison to the river water. It can be assumed that the intensity of the influence of the precipitation correlates with the sealing of the soil surface. In urban areas warmer run-off is drained to sewage plants and transported to the receiving rivers.

To sum it up, the wastewater treatment plant "Wallern" causes a cooling of the river water in summer when there is no rain. If there is precipitation, however, the water is warmed up a bit. In all other seasons the influence of the plant tends to result in a warming. Especially the warming during the winter is supposed to have negative physiological effects on fish, particularly on salmonids, as their activities are naturally reduced at that time.

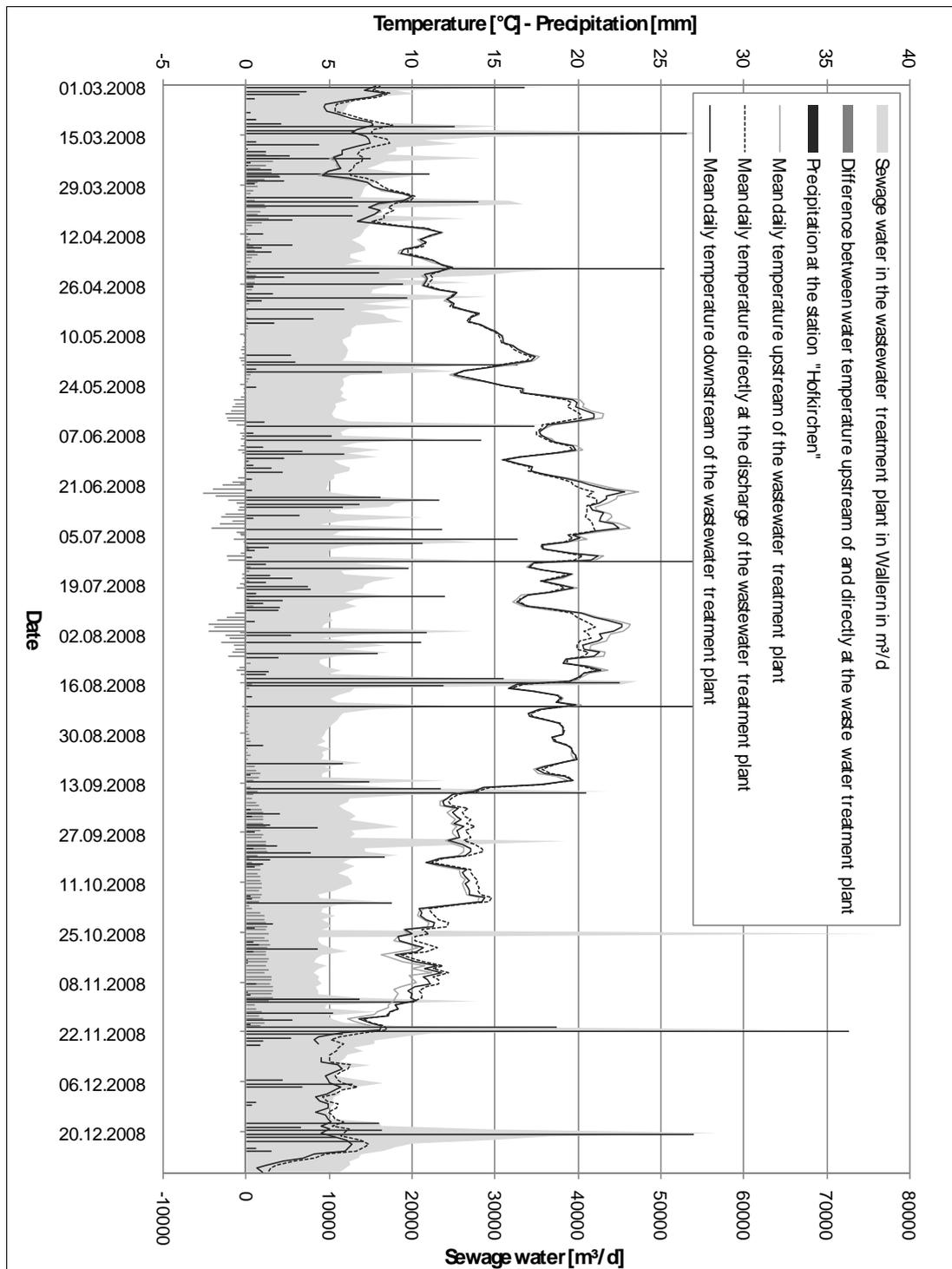


Figure 5: Water temperature situation at the wastewater treatment plant "Wallern", mean daily temperatures 2008.

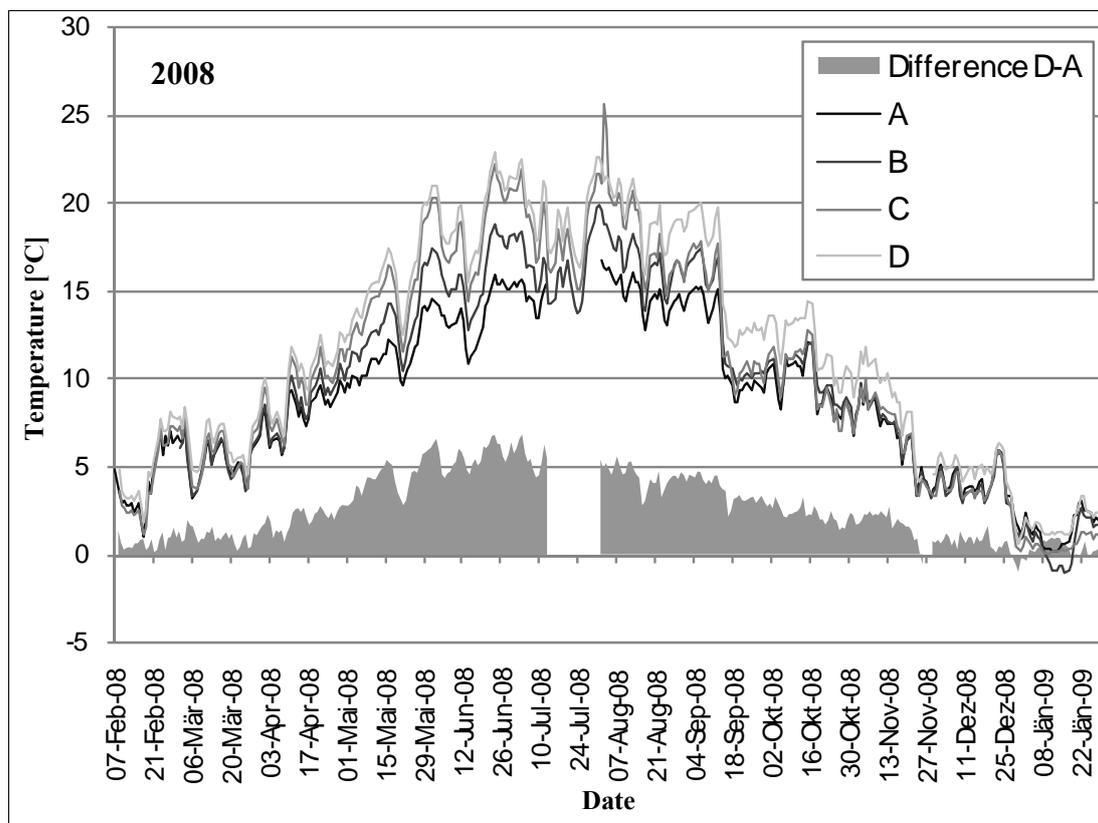


Figure 6: Water temperature development over the river length, mean daily temperatures 2008; (A) measuring point in a relatively natural reach upstream of the retention basin (42 km upstream of the confluence), (B) measuring point downstream of the retention basin, about 1.1 km downstream of point A, (C) measuring point 19.4 km upstream of the confluence, (D) measuring point at the confluence.

Apart from the mentioned local impacts, there are further general problems along the whole river system. The massive river draining, the intensive use of the potential floodplain and the lack of riverine vegetation are likewise responsible for changes in the temperature regime of the Trattnach River. Over the whole course a successive warming can be detected especially in the warm seasons. The water temperature increased, for example, in August 2008 by an average of +4.52 K within a river stretch of 42 km (Fig. 6). At the same time in 2009 the warming equaled +4.39 K on average (Fig. 7). In the after noon hours, even much higher singular short-time warming could be observed.

As temperature changes always have an outcome on the biocoenosis, the connection to the fish fauna was of high relevance for the present project. The anthropogenic warming shows the most intensive effects in spring and summer. Therefore, especially those species that spawn in spring, like grayling, nase or barbell, are highly negatively affected. Apart from the adverse temperature conditions they have to cope with further problems like the lack of appropriate spawning habitats, discontinuities along their migration routes or problems with fine sediments.

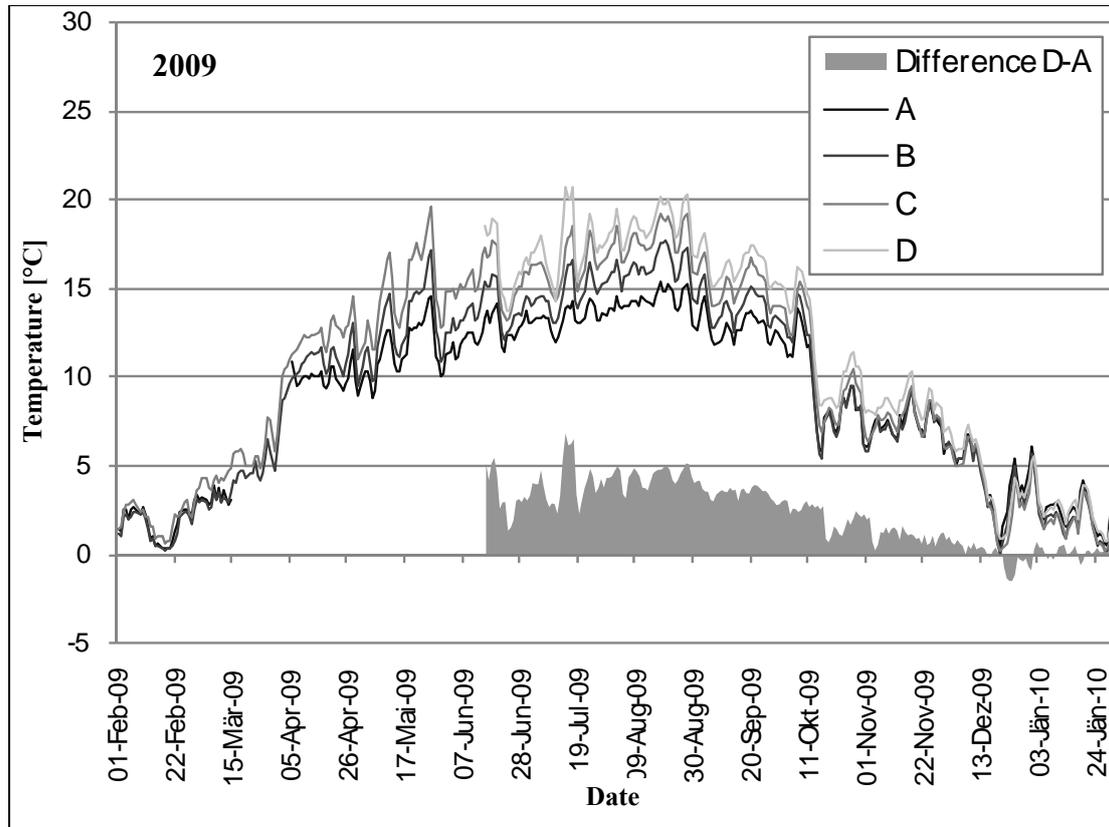


Figure 7: Water temperature development over the whole river length, mean daily temperatures 2009; (A) measuring point in a relatively natural reach upstream of the retention basin (42 km upstream of the confluence), (B) measuring point downstream of the retention basin, about 1.1 km downstream of point A, (C) measuring point 19.4 km upstream of the confluence, (D) measuring point at the confluence.

As mentioned above, particularly salmonids were brought into focus in the course of the study at hand, because they are massively negatively influenced by an increase of the water temperature. The grayling has to cope with problems almost over the whole river length. Especially in the middle and lower reaches, the rapid temperature increase in spring leads to an only small time fence in which spawning is possible; after March, the water temperature exceeds the grayling's optimum temperature for spawning. Therefore, its reproduction is assumed to be massively constrained in the Trattnach River. Surveys on the fish fauna have verified this assumption (Siligato and Gumpinger, 2004).

The brown trout is considered a core species over the whole course of the Trattnach River. However, in the lower reaches it takes a back seat compared to other species. The massive warming tendencies in the middle and lower reaches, caused by the morphological reshape of the river bed, result in serious problems for this species. In these reaches, thus, the stocking with salmonids turns out to be only possible with an adverse cost-benefit ratio and high loss rates. As salmonids, the naturally predominant fish family in the Trattnach River, cannot cope with the increased water temperature, a shift in the species assemblage was detected in the course of a survey (Siligato and Gumpinger, 2004) and affirmed by informal information from local fishermen.

In summary, the anthropogenic warming of the river Trattnach causes a permanent stress situation for the fish fauna which in turn results in a higher sensibility towards other negative environmental impacts. Eventually, sensitive species are replaced by tolerant ones.

### **CONCLUSIONS**

Our rivers are exposed to a wide range of different human impacts. These impacts have different consequences which interact and mingle in the aquatic ecosystem. Therefore it is difficult to identify and separate different causes (Küttel et al., 2002).

But it is scientifically approved that the water temperature is one of the most important abiotic parameters in an aquatic ecosystem. Nearly all chemical and biological processes are directly or indirectly controlled by it. Hence, the measurement of the water temperature allows an overview of the general status of a river-system.

This conclusion could be substantiated in the course of several different projects, for example in the river Trattnach in Upper Austria. In that river, the most important thermal impacts could be identified by means of constant temperature measurement. The data is used as a basis for projects that will aim to improve the situation.

### **ACKNOWLEDGEMENTS**

The authors wish to thank to the fishing club "Innbach-Trattnach", who commissioned and financed this project. Furthermore special gratitude has to be expressed to Mr. Höfner and his team, who read out the data for the project. Moreover they wish to thank to Mr. Berg and the Federal Secondary College of Engineering in Steyr for the constant refinement of the Te.M.P. measuring package.

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## NATURAL, TEMPORARY DRY RIVER BANKS AND THEIR IMPORTANCE FOR A SPECIAL PART OF BIODIVERSITY

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**KEYWORDS:** natural banks, ephemeral species and communities, proto-soil pioneers.

### ABSTRACT

Along natural river banks the interacting hydrological and morphological dynamics give rise to a mosaic of habitats that emerge and alter constantly as a result of erosion, sedimentation and re-deposition processes. Different habitat structures occur along the upper and lower river reaches, depending on slope, flow velocity and the grain sizes of the substrate. They are settled by varying ephemeral, annual, biennial and perennial pioneer species, all of which require proto-soil sites to develop.

Lowland rivers with water level fluctuations that temporarily lay bare broad parts of the river banks at low water levels provide fine-grained proto-soil sites for very specific plant communities, respectively biocoenoses which have adapted to these subsidiary bed sites. They are settled by ephemeral species that complete their life cycle within the 2-3 months of low water levels. Given that low water periods do not occur annually these species or their communities may not be found each year either.

The morphological dynamics loss as a result of river canalization and bank construction measures have led to a dramatic decrease in species that develop along the dynamic bank sites and are well adapted to or dependant on these sites. It concerns all species that develop their succession or occur exclusively on dynamic sites, as well along the mountain headwaters as along the middle and lower reaches of the waters. On numerous European rivers, especially in the foothills of the Alps, embankment constructions led to a dramatic decrease in and a large-scale extinction of proto-soil pioneers such as e.g. the tamarisk *Myricaria germanica*. Furthermore, as a result of the loss of the morphological dynamics, Black Poplar (*Populus nigra*) and White Willow (*Salix alba*) successions have become impossible along many rivers.

The Water Framework Directive considers natural or near-natural morphological river bank structures. Appendix I of the EU Habitats Directive also accounts for relevant river bank habitats with regard to the Natura 2000 network.

Using the Danube example comparing with other European rivers, a general survey on the very specific habitat range of the subsidiary bed as well as the proto-soil sites will be given, pointing out their particular relevance within the river-floodplain ecosystem complex.

A final point regarding the existence of mainly the ephemeral species as well as all species that are bound to proto-soil sites is that the canalization of the Lower Danube as a navigable waterway (bank protection measures, rockfill and dams) will bring the destruction of such habitats, along the river banks and around the islands. As a result this very specific biodiversity element will disappear or few relicts will survive and occur in uncharacteristic communities.

**ZUSAMMENFASSUNG:** Natürliche, zeitweilig trockenfallende Flussufer und ihre Bedeutung für eine besondere, ihnen eigene Biodiversität.

Im Zusammenwirken von hydrologischer und morphologischer Dynamik entsteht an natürlichen Flussufern ein Mosaik von Lebensräumen, die infolge von Abtrag, Anlandung und Umlagerung immer wieder neu entstehen und sich verändern. Diese Lebensräume sind von Oberlauf zu Unterlauf strukturell unterschiedlich, was mit dem Gefälle, der Fließgeschwindigkeit und den Korngrößen des Substrats zusammenhängt. Sie werden von unterschiedlichen Arten besiedelt, die alle Rohbodenstandorte brauchen, um sich entwickeln zu können, wobei es sich um kurzlebige, einjährige, zweijährige und mehrjährige Pionierarten handelt.

An Tieflandflüssen mit Wasserstandsschwankungen, die bei Niedrigwasser mitunter breite Uferstreifen temporär freilegen, bieten sich feinkörnige Rohbodenstandorte für ganz spezifische Pflanzengesellschaften bzw. Biozönosen an, die an diese Standorte im Niedrigwasserbett angepasst sind. Die Besiedlung erfolgt durch kurzlebige Arten, die ihren gesamten Lebenszyklus innerhalb von 2-3 Monaten der Niedrigwasserperiode abschließen. Da es nicht alljährlich ausgeprägte Niedrigwasserperioden gibt, so sind solche Arten bzw. ihre Gemeinschaften auch nicht jedes Jahr zu finden. Dank des im Boden verbleibenden Diasporenreservoirs können sie jedoch auch nach mehrjährigem Ausbleiben an trocken fallenden, schlammigen oder auf überschlickten Sandflächen wieder auftreten.

Der Verlust an morphologischer Dynamik durch Begradigung der Flüsse und Uferausbau haben zum drastischen Rückgang von Arten geführt, die sich an dynamischen Uferstandorten entwickeln und an derartige Standorte gut angepasst sind bzw. auf deren Existenz angewiesen sind. Dieses betrifft gleichermaßen alle Uferarten, die sich nur auf dynamischen Standorten entwickeln bzw. verjüngen können, sowohl am Gebirgslauf, als auch am Mittel- und Unterlauf der Gewässer. Der Uferverbau führte an vielen Europäischen Flüssen vor allem am Fuß der Alpen zu einem drastischen Rückgang und zu großräumigem Verschwinden von Rohbodenpionieren wie z. B. der Deutschen Tamariske *Myricaria germanica*. Weiterhin ist an vielen Flüssen durch den Verlust an morphologischer Dynamik die Verjüngung von Schwarzpappel (*Populus nigra*) und Silberweide (*Salix alba*) nicht mehr möglich.

Die Wasserrahmenrichtlinie trägt den natürlichen bzw. naturnahen morphologischen Uferstrukturen Rechnung. Auch die Flora Fauna Habitat Richtlinie erfasst in Anhang I Habitats, die an natürlichen Ufern vorkommen und für das Netzwerk Natura 2000 von Bedeutung sind.

Am Beispiel der Donau (im Vergleich zu anderen europäischen Flüssen) wird diese sehr spezifische Spanne von Lebensräumen im Niedrigwasserbett sowie der Rohbodenstandorte insgesamt aufgezeigt und auf ihre Bedeutung im Ökosystemkomplex Fluss-Aue hingewiesen.

Schwerwiegend ist für die Existenz besonders der kurzlebigen sowie aller auf Rohbodenstandorte angewiesenen Arten die Tatsache, dass durch den Ausbau der Unteren Donau zur Schifffahrtsstraße (Uferbefestigungen, Steinschüttungen, Dämme) derartige Lebensräume mit ihrem gesamten Artenspektrum sowohl an den Uferbänken, als auch um die Inseln verloren gehen werden und damit dieser ganz spezifische Teil der Biodiversität fehlen oder nur noch sehr spärlich und in untypischer Zusammensetzung auftreten wird.

**REZUMAT:** Maluri naturale de râuri temporar eliberate de sub apă și importanța lor pentru o parte specială a biodiversității.

Prin interacțiunea dintre dinamica hidrologică și morfologică pe malul râurilor se formează un mozaic de habitate, care datorită proceselor de eroziune și depunere sunt în continuă transformare, își schimbă locul și se redezvoltă în locuri învecinate. Aceste habitate se diferențiază, din punct de vedere structural, de la cursul superior spre cursul inferior, ceea ce este dependent de pantă, viteza de curgere a apei și componența granulometrică a substratului. Aceste locuri sunt, colonizate de diferite specii, care necesită pentru dezvoltarea lor substraturi nude, ele fiind de obicei specii pioniere efemere, anuale, bianuale sau perene.

Pe cursul inferior al râurilor, cu oscilații de nivel, care în timpul etiajului, eliberează temporar de sub apă benzi mai mult sau mai puțin late de-a lungul malului, există protosoluri nude cu fracțiuni granulometrice fine, care oferă stațiuni pentru colonizarea cu plante, comunități de plante, respectiv, biocenoze caracteristice, bine adaptate acestor condiții ale albiei minore. Colonizarea are loc prin specii anuale, terofite, care își desfășoară și își incheie tot ciclul de viață în timp de 2-3 luni ale perioadei de etiaj. Deoarece nu în fiecare an există perioade mai lungi de ape joase, sau, uneori, lipsind total, nu putem găsi astfel de specii sau comunități în fiecare an. Grație rezervorului de diaspori din sol, aceste specii adaptate condițiilor albiei minore, pot apărea în stațiuni măloase sau nisipoase, sau pe nisipuri acoperite de un strat subțire de mâl de pe maluri descoperite de apă, chiar dacă mai mulți ani de-a rândul n-au putut să se dezvolte.

Pierderea morfodinamicii prin rectificarea râurilor și consolidarea malurilor a dus la reducerea drastică a numărului de specii, care se dezvoltă în condiții de hidromorfodinamică, fiind bine adaptate la aceste condiții sau având nevoie de astfel de condiții pentru existența lor. Această situație are repercusiuni asupra tuturor speciilor de mal, care se pot dezvolta, respectiv se pot regenera doar în stațiuni dinamice, atât pe cursul superior, cât și cel mijlociu și inferior al râurilor. Consolidarea malurilor a dus în cazul multor râuri europene, mai ales la poalele Alpilor, la o reducere drastică și chiar dispariție la scară mare a speciilor pioniere de protosoluri. Un exemplu, în acest sens, este cățina mică (*Myricaria germanica*). De asemenea, datorită pierderii morfodinamicii și a depunerii de sedimente noi pe maluri, în zona ripariană a multor râuri, regenerarea naturală a plopului negru (*Populus nigra*) și a salciei albe (*Salix alba*) nu mai este posibilă.

Directiva Cadru Apă ia în considerare structurile morfologice naturale sau seminaturale. De asemenea și Directiva pentru Floră, Faună și Habitate cuprinde în Anexa I habitatele care se dezvoltă pe maluri naturale, fiind relevante pentru rețeaua Natura 2000.

Luând, drept exemplu, Dunărea în comparație și cu alte râuri europene, lucrarea prezintă această parte specifică a ariei ripariene, în care se dezvoltă habitatele din albia minoră și a protosolurilor, în general, arătând importanța lor în complexul ecosistemic de interacțiuni râu-luncă.

Cu consecințe negative pentru existența și supraviețuirea acelor specii, care au nevoie de aceste stațiuni de locuri nude, pioniere, este faptul, că, prin transformarea Dunării inferioare într-o cale navigabilă (consolidări de maluri, construcții de diguri) se vor pierde astfel de habitate, împreună cu întregul lor cortegiu de specii, atât pe malurile Dunării, cât și pe ostroave, ele fiind în prezent într-o stare naturală sau seminaturală. Astfel va lipsi acea parte specifică a biodiversității, sau va exista doar într-o formă foarte redusă în habitate fragmentate și atipice.

## INTRODUCTION

Along the natural river banks the interacting hydrological and morphological dynamics of the river give rise to a complex mosaic of habitats that emerge and alter constantly as a result of changing water levels, and of erosion, sedimentation and re-deposition processes. Due to these processes different habitat structures occur along the upper, middle and lower river bank reaches, depending on slope, flow velocity and the grain sizes of the present sediments. They are colonised by various ephemeral, annual, biennial and perennial pioneer species, all of which requiring proto-soil sites to develop.

Lowland river stretches with water level fluctuations that temporarily lay bare broad parts of the river banks at low water levels provide fine-grained proto-soil sites to very specific plant communities respectively biocoenoses which have adapted to these minor bed sites. They are settled by ephemeral species that complete their life cycle within the 2-3 months of low water levels. Given that low water periods do not occur annually these species respectively their communities may not occur each year either and can be missing also over a period of several years.

The loss of the morphological dynamics as a result of river training and bank construction measures on European rivers led to a dramatic decrease in species that develop along the dynamic bank sites and are well adapted to, or depend on these sites. It concerns all species that develop their succession or occur exclusively on dynamic sites, as well along the mountain head waters as along the middle and lower reaches of the waters. On numerous European rivers, especially in the foothills of the Alps, embankment constructions led to a dramatic decrease in and a large-scale extinction of proto-soil pioneers such as e.g. the Tamarisk *Myricaria germanica*, which disappeared on the Upper Rhine, and on the Upper Danube and the most of its tributaries (Haeupler and Schönfelder, 1988). Furthermore, as a result of the loss of the morphological dynamics Black Poplar (*Populus nigra* L.) and White Willow (*Salix alba* L.) succession have become impossible along many rivers or is existing only on a very small scale.

When analyzing the river banks of some European rivers the differences in biodiversity are clearly visible: the Loire and Allier rivers in France show strong morphological and hydrological dynamics and related to this fact presents a large variety of habitats and species (Dister et al., 1989; Schneider, 1996; Geerling et al., 2006); the Rhine and Elbe rivers have a reduced morphodynamics providing habitats for pioneer species in a few sections only whereas in the Lower Danube are numerous islands bordered by dynamic river banks with a high biodiversity.

## MATERIALS AND METHODS

Within the frame of its various projects the WWF Institute for Floodplain Ecology analyzed the bank areas and their pioneer settlements along a number of European rivers such as Loire and Allier in France, Upper Rhine, Elbe as well as Central and Lower Danube including the Danube Delta. Focus was put on two major aspects: 1. a general recording of colonization settlements on dynamic pioneer sites occurring as a result of erosion and aggradation as well as re-deposition of substrates; they may occur both above and below mean water level; 2. the study of pioneer sites in the minor bed that are laid bare with sinking water levels and where fauna and flora dispose of a short time span to develop.

Pioneer plant settlements have been studied in the respective river basins on chosen spots along transects and samples were taken according to the 7-point Braun-Blanquet scale for the evaluation of abundance-dominance (1964). Given that site conditions change on a very small scale, surveys were made on surfaces of the following sizes: 1.0 m x 1.0 m, 0.5 m x 1 m, or 0.5 m x 0.5 m. Subsequent to a summarized tabular illustration of the various associations, their species composition and share in life-forms, especially therophytes, have been studied for every association of the respective river basin. Comparative studies have been conducted all the same for chosen associations of the Loire, Allier, Rhine, Elbe and Danube rivers. Special attention has been paid here to the biodiversity of the biocoenosis in the rivers' minor beds. A comprehensive study on pioneer colonization occurring on Loire and Allier was published earlier (Schneider, 1996). In this study its results have thus merely been considered in summary and for comparison. The most representative pioneer associations have been chosen for every river basin studied and have been compared to one another as for their biocoenosis and biodiversity (Rhine at more dates, the most recent in 2009, Elbe 1994-1996, Danube Kopacki Rit 1990, Lower Danube from 1990 ongoing to 2009).

## RESULTS AND DISCUSSION

It became clear in all river basins studied that the development of habitat structures closely depends on the hydrological and morphological dynamics. A further crucial point consists in the structures determined by vegetation; they are of vital importance for settlement or habitat use by various animal species.

### Loire and Allier rivers

Thanks to a nearly unrestricted dynamics on the Loire and Allier rivers, pioneer settling is not only bound to floodplain sites of the minor bed, but occur on sites that lie considerably above mean water level as well. In the event of higher floods river bed sands are transported even up to the highest reaches of islands and river adjacent - floodplains, situated up to 2 or 3 m above mean water level. However, provided that higher floods do not occur frequently, these elevated spots are rapidly covered by the development of successive communities, from an open vegetation to closed and stable covers. The limiting factor for sites situated at lower level is their longer exposition to flood, only especially adapted species may survive to these conditions.

Among the annual or biennial species occurring mainly on sand and gravel aggradations of the flood bed, many show their adaptation to prolonged draughts by a xeromorphic appearance or further specific adaptations (Schneider, 1996). Some species occurring on sand and gravel sites have adapted to these conditions with a short life cycle. They use the moisture remaining immediately after the area falls dry for their development. Ephemeral species with very short roots die off when the soils fall dry, e.g. Early Sand-Grass (*Mibora minima* (L.) Desv.), Common Whitlowgrass (*Erophila verna* (L.) Bess., Hairy Bittercress (*Cardamine hirsuta* (L.), Muse Ear (*Cerastium dubium* (Bast.) Guép.) and Mouse-ear Cress (*Arabidopsis thaliana* (L.) Heynh.). Even here only succulent species or species showing comparable adaptation characteristics to extreme draughts may persevere at higher temperatures and with lacking moisture.

Completely different demands are made on annuals occurring in areas that fall dry below the mean water level. In this case the limiting factor is the very short period before the next water climb. Ephemeral annual vegetation develops on gravel and sand areas that fell dry and on alluvial mud-covered sand and slime areas. They are settled by phytocoenoses of Cyperetalia fusci such as *Cyperus fuscus* L., Marsh Cudweed (*Gnaphalium uliginosum* L.), Small Fleabane (*Pulicaria vulgaris* Gaertn.), Necklace Weed (*Veronica peregrina* L.) and Water Mudwort (*Limosella aquatica* L.). The neophyte species *Illysanthes attenuate* (Muhl.) Small. and *Illysanthes gratioides* (L.) Benth. occur here as well. Large areas are covered by the low-growing Yellow Cress (*Rorippa sylvestris* (L.) Bess.) (Schneider 1996, and other own, unpublished research data).

The ephemeral species of the gravel and sand substrata sites in the minor bed are, just as the pioneer associations occurring in the muddy areas below mean water level, in a position to complete their life cycle within a very short time and to secure their survival by means of an abundant seed production (Dister, 1980; Schneider, 1996 and other own field data). Characteristic of such stands on Loire and Allier rivers are phytocoenoses of the *Corrigiola littoralis*- *Chenopodietum* association that show a huge therophyte share for all forms and that are generally very abundant species (Schneider, 1996). This becomes especially apparent in comparison with correspondent associations from the Elbe River that occurs under comparable site conditions, they do, however, reveal considerably poorer in species (Fig. 1, Tab. 1).

The habitat structures that are conditioned by the substrates' grain sizes (gravel, sand, silt) and by small pioneer vegetation allow settlements of animal species that are well adapted to these conditions: specialized ground beetles (Carabidae) and Cicindellidae (*Cicindella hybrida* L.) and spiders such as e.g. *Arctosa cinerea* Fabricius. Gravel areas in the river bed offer not only places for pioneer settling of plants, but also nesting and breeding places to species such as the Little Ringed Plover (*Charadrius dubius* Scopoli) and feeding habitats to various bird species (Dister et al., 1989; and other own field data).

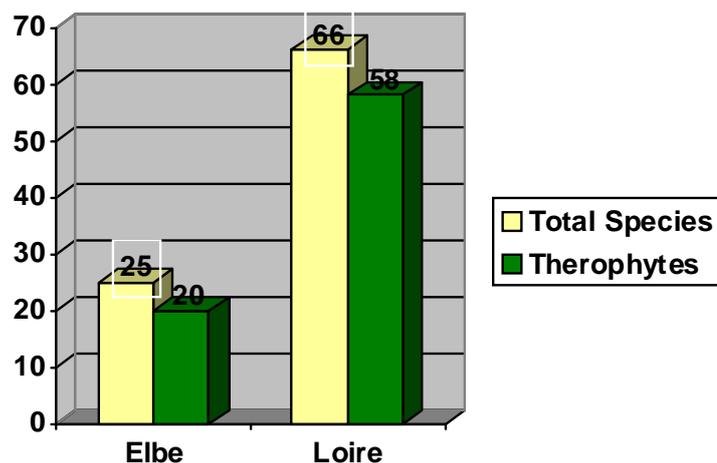


Figure 1: Comparison between total species number and therophytes in plant communities with *Corrigiola littoralis* on the Loire River/ France (Schneider, 1996) and Elbe River (Schneider, 1994, unpublished field data).

Table 1: Comparison of pioneer plant communities above the mean water level (on the base of the frequency) on the rivers Allier/France, Elbe/Germany and Olt-Danube/Romania.

	Serial number	1	2	3	4	5
	River	A	E	E	E	Olt
	Number of samples	9	5	7	10	6
Biof.		F%	F%	F%	F%	F%
T	<i>Corrigiola littoralis</i>	<b>100</b>	<b>60</b>	-	-	-
T-H	<i>Poa annua</i>	-	20	<b>100</b>	<b>100</b>	<b>67</b>
T	<i>Cerastium glutinosum</i>	-	-	<b>86</b>	<b>50</b>	<b>83</b>
T	<i>Myosurus minimus</i>	-	20	14	<b>70</b>	-
T	<i>Gnaphalium uliginosum</i>	33	<b>100</b>	-	-	-
T	<i>Limosella aquatica</i>	-	20	-	-	-
T	<i>Cyperus fuscus</i>	11	-	-	-	-
T	<i>Polygonum aviculare</i>	67	<b>80</b>	43	<b>80</b>	-
T, H	<i>Plantago intermedia</i>	22	100	14	10	-
T	<i>Xanthium albinum</i>	-	60	14	20	-
T	<i>Xanthium italicum</i>	22	-	-	-	17
T	<i>Polygonum lapathifolium</i>	100	100	-	-	-
T	<i>Matricaria inodora</i>	11	-	57	10	-
T	<i>Bromus mollis</i>	-	-	14	-	17
T, H	<i>Spergularia rubra</i>	33	60	14	80	-
T, H	<i>Lepidium virginicum</i>	11	-	14	-	-
T	<i>Bidens tripartita</i>	-	20	-	30	-
T	<i>Herniaria glabra</i>	11	-	-	80	-
T	<i>Capsella bursa pastoris</i>	-	-	-	20	50
T	<i>Veronica peregrina</i>	33	-	-	-	33
T	<i>Portulaca oleracea</i>	44	40	-	-	-
T	<i>Chenopodium album</i>	22	60	-	-	-
T	<i>Ranunculus sceleratus</i>	-	40	-	-	-
T	<i>Chenopodium rubrum</i>	-	100	-	-	-
T	<i>Juncus bufonius</i>	-	20	-	-	71
T	<i>Polygonumhydropiper</i>	-	20	-	-	17
T	<i>Chenopodium botrys</i>	22	-	-	-	-
T	<i>Atriplex hastata</i>	22	-	-	-	-
T	<i>Chenopodium polyspermum</i>	22	-	-	-	-
T	<i>Amarantus chlorostachys</i>	67	-	-	-	-
T	<i>Eragrostis minor</i>	22	-	-	-	-
T	<i>Plantago arenaria</i>	44	-	-	-	-
T	<i>Medicago arabica</i>	33	-	-	-	-
T	<i>Lindernia procumbens</i>	22	-	-	-	-
T	<i>Arenaria serpyllifolia</i>	22	-	-	-	-

Table 1 (continued): Comparison of pioneer plant communities above the mean water level (on the base of the frequency) on the rivers Allier/France, Elbe/Germany and Olt-Danube/Romania.

T	<i>Echinocloa crus galli</i>	-	40	-	-	-
	Serial number	1	2	3	4	5
	River	A	E	E	E	Olt
	Number of samples	9	5	7	10	6
Biof.		F%	F%	F%	F%	F%
T	<i>Rumex palustris</i>	-	40	-	-	-
T	<i>Ambrosia artemisifolia</i>	-	40	-	-	-
G, H	<i>Rorippa sylvestris</i>	33	<b>100</b>	<b>71</b>	<b>80</b>	17
G, H	<i>Rumex acetosella</i>	22	-	-	-	-
G	<i>Allium schoenoprasum</i>	-	-	-	40	-
H	<i>Agrostis stolonifera</i>	22	-	29	20	33
H	<i>Alopecurus geniculatus</i>	-	20	71	30	-
H	<i>Saponaria officinalis</i>	22	-	-	-	-
H	<i>Taraxacum officinale</i>	-	-	57	20	17
H	<i>Plantago major</i>	-	-	71	20	-
H	<i>Lolium perenne</i>	-	-	57	-	-
H	<i>Plantago lanceolata</i>	-	-	14	-	33
H	<i>Aster lanceolatus</i>	-	-	14	-	-
H	<i>Potentilla reptans</i>	-	-	-	20	-
H	<i>Potentilla anserina</i>	-	-	-	-	50
G, H	<i>Phalaris arundinacea</i>	-	-	14	-	10
H, C	<i>Trifolium repens</i>	-	-	29	-	-
H, C	<i>Poa trivialis</i>	-	-	-	50	-
A	<i>Ranunculus aquatilis f. terr.</i>	-	20	-	-	67
A	<i>Alisma plantago aquatica</i>	-	-	-	-	50
P	<i>Populus nigra</i> (juv.)	44	20	-	-	-
P	<i>Salix alba</i> (juv.)	-	20	-	-	-

Column 1: Allier river/France /A;

Columns 2, 3, 4: Elbe River/Germany/E;

Column 5: Olt River on the mouth into the Danube/Romania

Column 1 and 2: as. **Corrigiolo-Chenopodietum**

Column 3, 4 and 5: as. **Poo-Cerastietum dubii**

Species with low frequency values: column 1: H, C *Artemisia vulgaris* (11%), A, H *Rorippa amphibia* (11%); column 3: G, H *Carex praecox* (14%), G *Elymus repens* (14%), T, H *Leonurus marrubiastrum* (14%); T *Peplis portula* (17%), *Polygonum amphibium f. terrestris* (17%).

T = Therophyte,

H = Hemicryptophyte,

G = Geophyte,

C = Chamaephyte,

P = Phanerophyte,

A = Aquatic plant (bioforms following Ellenberg et al., 1991).

### Rhine River (Middle and Northern Upper Rhine)

Compared to Loire and Allier (Tab. 1), pioneer settlements on the Rhine River are considerably less frequent given that the conditions for pioneer colonization are very limited. This is due to river development measures taken on the Rhine River and to an almost lacking of morphological dynamics. Pioneer settlements on sites where erosion and aggradation do still occur exist only in very few places (Dister, 1980). The removal of river bank reinforcement carried out recently on short stretches along the Upper Rhine River near Rastatt and in the Kühkopf-Knoblochsaue nature reserve area represent a first step in giving a chance to settlers of protosoil sites (Dister et al., 2003). Pioneer settlements in the minor bed may occur on a larger scale along dynamic old branches of the Rhine that are connected to the water level dynamics of the main river. Characteristic vegetation communities of ephemeral species occur downstream the Iffezheim hydropower station, for instance along the old river branches in the nature reserve Rastatter Rheinaue (Wintersdorf and Plittersdorf; Tab. 2, column 2), along the former Rhine River branch near Illingen (Illinger Altrhein) (Tab. 2, column 3) as well as along the Northern Upper Rhine in the Nature Reserve Kühkopf-Knoblochsaue (Dister, 1980).

During longer and distinct low water periods, as was the case for instance in late summer and autumn of 2009, Mudwort covers (*Cypero-Limoselletum*) were observed on fine-grained substrates of silt and mud (Fig. 2). Individual species do generally not reach high coverage values; occur however steadily and in very characteristic species compositions. Besides the Water Mudwort (*Limosella aquatica*), Marsh Cudweed (*Gnaphalium uliginosum*) and Smale Galingale (*Cyperus fuscus*) occur with great steadiness (Tab. 2). In some spots a close interaction of Needle Spike Rush (*Eleocharis acicularis* (L.) R. et Schult.) phytocoenoses may be observed. In the area of Illinger Altrhein the Sinking Riccia (*Riccia rhenana*) occurred on muddy sites that had fallen dry but were still moist.

Table 2: Pioneer plant communities in the river bed, below the mean water level on the rivers Rhine and Danube on the base of frequency.

	Serial number	1	2	3	4	5	6	7	8
	River	E	R	R	KR	CD	C	DD	DD
	Number of samples	6	10	20	6	5	5	5	5
Biof.			F%	F%	F%	F%	F%	F%	F%
T	<i>Myosurus minimus</i>	33	-	-	-	-	-	-	-
T	<i>Gnaphalium uliginosum</i>	17	50	25	<b>100</b>	-	20	<b>80</b>	<b>80</b>
T	<i>Limosella aquatica</i>	<b>100</b>	<b>90</b>	<b>100</b>	<b>100</b>	-	-	-	20
T	<i>Cyperus fuscus</i>	-	-	50	<b>100</b>	20	-	<b>100</b>	<b>80</b>
T	<i>Heleochoa alopecuroides</i>	-	-	-	50	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>
T	<i>Polygonum aviculare</i>	<b>100</b>	-	-	-	-	-	-	-
T	<i>Xanthium italicum</i>	-	-	-	-	-	<b>100</b>	20	20
T	<i>Bidens tripartita</i>	-	20	-	-	-	80	-	-
T	<i>Ranunculus sceleratus</i>	-	30	-	-	40	-	80	60
T	<i>Chenopodium rubrum</i>	17	-	25	<b>100</b>	40	-	<b>80</b>	40
T	<i>Peplis portula</i>	-	-	45	-	-	-	-	-
T	<i>Lindernia procumbens</i>	-	-	-	67	-	-	-	40

Table 2 (continued): Pioneer plant communities in the river bed, below the mean water level on the rivers Rhine and Danube on the base of frequency.

	Serial number	1	2	3	4	5	6	7	8
	River	E	R	R	KR	CD	C	DD	DD
	Number of samples	6	10	20	6	5	5	5	5
Biof.			F%	F%	F%	F%	F%	F%	F%
T	<i>Lythrum tribracteatum</i>	-	-	-	<b>100</b>	-	-	-	-
T	<i>Echinochloa crus galli</i>	-	-	-	17	20	-	40	80
T	<i>Cyperus michelianus</i>	-	-	-	67	20	-	80	30
T	<i>Polygonum lapathifolium</i>	-	-	-	-	20	-	20	40
T	<i>Chenopodium polyspermum</i>	-	-	-	-	20	-	20	20
T	<i>Portulaca oleracea</i>	-	-	-	-	<b>60</b>	-	-	-
T	<i>Amarantus chlorostachys</i>	-	-	-	-	40	-	-	-
T	<i>Chenopodium album</i>	-	-	-	-	20	-	20	40
T	<i>Solanum nigrum</i>	-	-	-	-	20	-	20	-
T, H	<i>Potentilla supina</i>	-	-	-	-	60	-	60	40
T	<i>Cyperus serotinus</i>	-	-	-	-	80	-	80	80
T	<i>Mentha pulegium</i>	-	-	-	-	-	-	20	40
T	<i>Abutilon theophrasti</i>	-	-	-	-	-	-	-	60
T, H	<i>Plantago intermedia</i>	17	10	-	60	-	-	60	20
G, H	<i>Rorippa sylvestris</i>	-	-	-	17	20	-	<b>80</b>	40
C, H	<i>Trifolium repens</i>	17	-	-	-	-	-	40	-
H	<i>Alopecurus geniculatus</i>	57	-	-	-	-	-	-	-
H, C	<i>Poa trivialis</i>	85	-	-	-	-	-	-	-
H	<i>Agrostis stolonifera</i>	17	-	10	60	-	-	20	-
H	<i>Aster lanceolatus</i>	-	-	-	-	-	-	40	20
H	<i>Veronica anagallis aquatica</i>	-	40	15	-	-	-	-	-
H	<i>Glycyrrhiza echinata</i>	-	-	-	-	-	-	-	40
H	<i>Cirsium arvense</i>	-	-	-	-	-	-	60	60
H	<i>Lactuca tatarica</i>	-	-	-	-	-	-	40	-
H	<i>Rumex palustris</i>	-	-	-	-	-	-	20	40
A, H	<i>Veronica beccabunga</i>	-	20	10	-	-	-	-	-
A, H	<i>Rorippa amphibia</i>	-	70	90	100	-	40	40	20
A, H	<i>Oenanthe aquatica</i>	17	20	-	-	-	-	40	-
A, H	<i>Eleocharis acicularis</i>	67	10	25	-	-	-	-	-
A	<i>Callitriche obtusangula terr.</i>	-	20	30	-	-	-	-	-
A	<i>Myriophyllum spicatum terr.</i>	-	20	5	-	-	-	-	-
A	<i>Riccia rhenana</i>	-	-	25	-	-	-	-	-
P	<i>Salix alba</i> (juv.)	-	-	-	83	20	-	80	40
P	<i>Populus nigra</i> (juv.)	-	-	-	-	20	-	-	-
P	<i>Tamarix ramossissima</i>	-	-	-	-	60	-	-	-

As. **Cypero-Limoselletum**: Column 1: Elbe, Bucher Brack near to the town Tangermünde (unpublished samples from 1996); Column 2: Upper Rhine, old river branch near Rastatt-Wintersdorf, Nature Reserve Rastatter Rheinaue/Germany; Column 3: Upper Rhine, old branch "Illinger Altrhein" near Au a. Rhein, district Rastatt (field data 2009); 4: Central Danube/Kopački Rit, Croatia (unpublished field data 1990);

As. **Heleochoetum alopecuroidis** 5: Lower Danube, Cama-Dinu, Giurgiu County (2004); 6: facies with *Cyperus michelianus*: Lower Danube Ostrovul Călărași-Răul, Călărași County (2002);

As. **Dichostyli micheliani-Gnaphalietum** facies with *Heleochoa alopecuroides* Columns 7, 8: Danube Delta.

Other species with low frequency values: column 2: H *Myosotis palustris* (10%), Th-H *Poa annua* (10%); Column 3: A *Ranunculus aquatilis* (10%); column 7: T *Ambrosia artemisifolia* (20%), T *Capsella bursa pastoris* (20%), H *Lythrum salicaria* (20%), A *Mentha aquatica* (20%), T *Polygonum hydropiper* (20%), T *Rumex palustris* (20%); column 8: H *Scutellaria hastifolia* (20%).

T = Therophyte, H = Hemicyptophyte, G = Geophyte, C = Chamaephyte, P = Phanerophyte, A = Aquatic plant (bioforms following Ellenberg et al., 1991).



Figure 2: Muddy river banks on the river Rhine (old branch Illinger Altrhein) district Rastatt (foto E. Schneider-Binder, 2009).

Pioneer settlements occur rarely on sites with coarse sand and fine-grained gravel along the banks of the Rhine River. Yet they could be recorded on few sites where a however slight substrate aggradation and re-deposition occurred. During the already mentioned distinct low water period in autumn 2009 pioneer communities with prevailing Red Goosefoot (*Chenopodium rubrum* L.), in some spots even Many-seed Goosefoot (*Chenopodium polyspermum* L.), White Goosefoot (*Chenopodium album* L.) and Amanranth (*Amaranthus chlorostachys* Willd.) could thus be recorded on gravelly and slightly mud-covered sites along the Rhine River banks near Au am Rhein (rural district of Rastatt). Like other pioneer communities they do not regularly occur in the minor bed. Even on gravelly sites of the Rastatter Rheinaue, along the borders of the former gravel plant Peter, characteristic pioneer species of the gravel site such as *Epilobium dodonaei* Vill. and also *Chaenorhinum minus* (L.) Lange occurred as well as the species recorded on sandy and gravelly soils such as *Vulpia myuros* (L.) C. Gmel. and *Arenaria serpyllifolia* L.

#### **Central stretch of the Elbe River**

On the Central Elbe (Bucher Brack, Ringfurth, Rühstädt) one may observe some well developed pioneer communities along undeveloped river banks, in the foreland of the dams and in the groin fields along the banks. They are composed on the one hand of communities occurring on sandy pioneer sites with substrate dynamics and of communities living on fine-grained, silty substrates as occur mainly in the areas between the groins that lie bare at low water levels. *Corrigiola littoralis* L., mainly occurring in subatlantic-submediterranean areas may be observed here as well but rather plays an underpart. It usually develops together with species such as Yellowcress (*Rorippa sylvestris*), Marsh Cudweed (*Gnaphalium uliginosum*), Red Goosefoot (*Chenopodium rubrum*), i.e. a combination comparable to the phytocoenoses found on the Loire River (synthetic table of communities; Tab. 1) but much poorer in species. In Germany this community occurs rarely (Tüxen, 1979).

The muddy river bank communities occurring along the Rhine River on fine-grained substrates are characterized by Water Mudwort (*Limosella aquatica*) and may also be found along the Elbe River with a comparable species composition (Tab. 2, column 1). Moreover, on some spots of the open, flooded floodplain one may observe pioneer communities with Annual Blue Grass (*Poa annua* L.) and Mouse Ear (*Cerastium dubium*) that develop on a small scale in depressions of the alluvial meadows. This is the case on fine-grained substrates, i.e. on silty and clayey soils near Ringfurth. Further downstream the Elbe River (Rühstädt/Land Brandenburg) the phytocoenoses frequently comprises Sand Spurrey (*Spergularia rubra* (L.) Presl.) and Mousetail (*Myosurus minimus* L.). The occurrence of Rapturewort (*Herniaria glabra* L.) in some places is an indicator of very sandy alluvial soils. During summer time when these small patches in the outdrying depressions are rapidly overgrown by species forming offshoots such are *Elymus repens* (L.) Gould., *Agrostis stolonifera* L., *Potentilla reptans* L. and others.

#### **Central Danube (Kopacki Rit)**

On muddy banks that fell dry along the former branches (autumn 1990), broad muddy river bank communities have been observed along the southern central Danube in the Kopacki Rit (Croatia). They frequently comprised Water Mudwort (*Limosella aquatica*) together with Marsh Cudweed (*Gnaphalium uliginosum*), Small Galingale (*Cyperus michelianus* (L.) Link) and Brown Galingale (*Cyperus fuscus*) (Tab. 2, column 4). The development of *Heleochoa alopecuroides* (Pill. and Mitt.) Host indicates a transition towards pioneer communities occurring on the Lower Danube. Large-scale White willow (*Salix alba*) successions developed alongside with broad muddy river bank communities on the mean water level (Schneider, 2003).

### Lower Danube and Danube Delta

The banks of the Lower Danube River being largely undeveloped, ephemeral species and their communities find perfect conditions to develop during longer periods of low water levels. The banks of the numerous islands as well as of the new and constantly developing sand banks of the Lower Danube area (e.g. near river km 524, between km 515-505 in the Cama-Dinu area and in other places) provide ideal conditions to the development of pioneer species. The Lower Danube is among the very few river sections on European rivers where the whole development range occurs in all its stages, starting with pioneer settlements of ephemeral species to all phases of the softwood floodplain development and up to the most elevated spots of the floodplain respectively of the islands.

Among the pioneer communities developing in the minor bed Small Galingale (*Cyperus michelianus*) and Marsh Cudweed (*Gnaphalium uliginosum*) occurred in the Iron Gate area (island Moldova Veche) before the construction of the Iron Gates Power Plant (Morariu and Danciu, 1970; Morariu et al., 1973), though they were recorded on an island that has been flooded as a consequence of the hydropower station construction. They do, however, occur downstream as well in a comparable composition. A further community of the minor bed is *Heleochoa alopecuroides* (Tab. 2), it could be observed in various sections of the Lower Danube (Batin Island on the Bulgarian side, river km 530, near Călărași, river-km 507 Cama-Dinu area upstream Giurgiu) and in the Danube Delta (Tab. 2) Furthermore a less common pioneer community composed of *Cyperus flavescens* Jacq. and *Lindernia procumbens* (Krocker) Philcox has been recorded in the area near Cama-Dinu (2004) and Călărași islands (Schneider-Binder, 2008).

In periodically flooded depressions the phytocoenoses of the association Poo-Cerastietum dubii edified by Mouse Ear (*Cerastium dubium*) and Annual Blue Grass (*Poa annua*) has been observed in the major river bed/floodplain on muddy protosoils next to the Olt mouth into the Danube, however, it revealed less rich in species as compared to that of the Elbe River occurring in flooded depressions surrounded by pioneer grassland (Fig. 3).

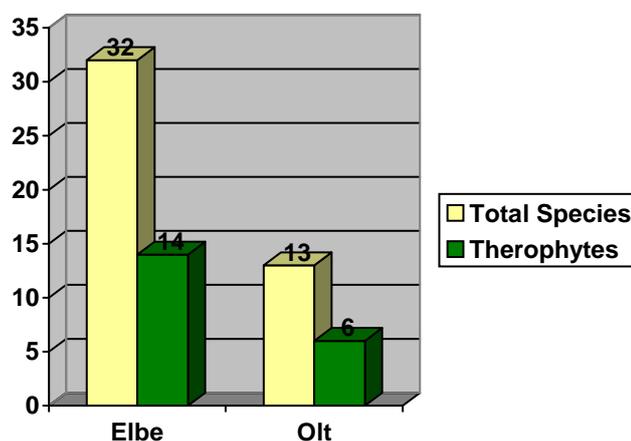


Figure 3: Pioneer plant communities with annual *Poa annua* and *Cerastium dubium* on the central stretch of the Elbe and on the mouth of Olt (Romania) into the Danube; the therophytes in the small short time flooded and vegetation free depressions are overgrowths after short time by biannual and perennial species.

The new, sandy islands that still develop constantly in the river bed thanks to the Danube River dynamics (e.g. at river km 524 upstream Giurgiu) or near Călărași/“Păcuiu lui Soare” Island, offer ideal conditions to pioneers such as Cocklebur (Xanthium) species, in particular *Xanthium strumarium* L., *Xanthium italicum* Moretti and also White Willow (*Salix alba*), Black Poplar (*Populus nigra*), Tamarisk (*Tamarix ramosissima* Ledeb.) and annual pioneer species such as between others *Chenopodium rubrum*, *Chenopodium polyspermum*, *Portulaca oleracea* L., *Heleochoa alopecuroides* and *Lindernia procumbens* (Tab. 2). They constitute a perfect habitat and breeding place for the Common Tern (*Sterna hirundo*) which is bound to such spots as it oviposits directly in the sand and breeds here. The sand islands with pioneer vegetation give also shelter to the European Otter (*Lutra lutra*) occurring on the Lower Danube. The temporarily dry falling river banks of the Danube and of its islands provide major feeding places for many bird species.

Just as on the Loire River’s open sand banks, one may find numerous species here that have perfectly adapted to these conditions such as e.g. the beetle *Cicindella hybrida* L., the larvae of which dig holes or smaller hollows in the bare banks and use them as hiding places in the event of high water levels. The ground beetles, characteristic of these sites, may be found as well on dry falling banks. Among them two species of genus *Bembidion* such as *Bembidion laticolle* Duftschmid 1812 and *Bembidion striatum* Fabricius 1792 (Tab. 3) are the most characteristic occurring along the Green Corridor of the Danube in considerable number on proto soils (Schneider Eckb., field data, 2004). These species were registered together with others during researches and monitoring of the epigeic beetles fauna on the Danube River banks between river km 500-521 and the area of Cama-Dinu islands (Schneider Eckb., in: Schneider-Binder et al., 2005).

Table 3: Comparison of the status of two characteristic river bank ground beetles in Central Europe and on the Lower Danube (monitored by Eckbert Schneider, 2004).

Species	Status of the species in Central Europe, basin of the river Rhine/Germany	Status of the species in South-East Europe/Lower Danube
<i>Bembidion laticolle</i> Duftschmid 1812	Extinct as a consequence of river embankment (Red list Germany, BfN 1998)	60% of river bank ground beetles in the Cama-Dinu area/Giurgiu County (2900 specimens)
<i>Bembidion striatum</i> Fabricius 1792	Red List Germany, BfN 1998): endangered species	4% of river bank ground beetles in the Cama-Dinu area/Giurgiu County (206 specimens)

When comparing the Ground beetle species occurring on the Danube to those observed on central European rivers (Müller-Motzfeld, 2004), *Bembidion laticolle* turns out to appear still frequently on the Lower Danube. It is, however, completely lacking on many developed European rivers (Tab. 3). The same is true for other proto soil settlers.

Comparing the studied riverbank sites on different European rivers it became very clear that the hydrological and morphological dynamics determine the structure and spatial distribution of the microhabitats and thus as well the colonization of certain plant and animal species. On rivers that are characterized by strong dynamics of the sites as is the case for Loire and Allier, the dynamics of flora and fauna is very pronounced all the same. It is reflected by the high diversity of pioneer protosoil species and their life communities.

Pioneer settlements with ephemeral species grow both on protosoils above mean water level and also in the minor bed (Tabs. 1, 2, 4 and 5). Ephemeral pioneer communities develop best in the minor bed. A large-scale succession of White Willow and Black Poplar still occurs on these rivers as well (Schneider, 2003).

Table 4: Settling/colonisation possibilities on natural river banks above and below the mean water level.

Above mean water level	Below mean water level
Settling of annual and perennial plants/communities;	Settling of annual, short living pioneer plants/communities in a larger scale
Annual species and communities are rapidly overgrown by biennial and perennial species	-
Natural regeneration of softwood: White Willow and Black Poplar stands	Possibilities for germination of White Willow and Black Poplar seeds and growing, during low water levels, but without long term surviving chances
Colonisation of pioneer ground beetles and spiders	Colonisation of pioneer ground beetles and spiders during the low water levels period
Breeding place for birds	-
Feeding place for birds	Feeding place for birds

The Corrigiolo-Chenopodietum association is the most frequent ephemeral pioneer community on Loire and Allier rivers and occurs in various forms (subunits, facies), both in the floodplain (major river bed) and in the minor bed. Numerous further associations of ephemeral species are frequent on Loire and Allier rivers.

On rivers like the Rhine, i.e. rivers that were subject to dramatic regulation and development measures and where morphodynamic processes can barely take place, pioneer settling may be observed to a minor degree. Communities are limited to few, undeveloped river bank sections or to banks with recently removed enforcements as well as along dynamic former branches that are connected to the dynamics of the Rhine River. Muddy river bank communities with the Water Mudwort (*Limosella aquatica*) as dominating species occurring mainly together with Brown Galingale (*Cyperus fuscus*) and Marsh Cudweed (*Gnaphalium uliginosum*) are characteristic of the Rhine River basin. Stands of Needle Spike Rush (*Eleocharis acicularis*) are observed as well in these places (Tab. 2, columns 2 and 3).

On the Elbe River, pioneer settlements consisting in ephemeral studied species may be found in the dyke foreland and occur frequently around groin fields. Poor *Corrigiola litoralis* plant communities can be observed on the Elbe River. Comparable forms of muddy river bank communities with *Limosella aquatica* are found there as well. The pioneer settlements are not only limited to the minor bed but occur also on dynamic sites in the flood bed. This is the case especially on spots that are connected to the dynamics of the river and in places where open soils emerge when ponds remain in depressions after the flood periods. The characteristic plant community of such categories of depressions is as mentioned above, a pioneer community composed of annual Blue Grass (*Poa annua*) and *Cerastium dubium* which occurs as well on the Lower Danube.

Muddy river bank communities develop on the southern central Danube, e.g. in the Kopacki Rit. As for their species composition they are an intermediary with regard to the pioneer communities of the Lower Danube. On largely undeveloped banks and in the area of the islands the Lower Danube still provides large-scale areas for pioneer settlements. This is where communities of Small Galingale (*Cyperus michelianus*) the Gramineae species *Helochloa alopecuroides* are dominant. Besides these, there are various further pioneer communities settling in the Danube's minor bed in various mosaics of microhabitats.

Table 5: Existing pioneer plant communities of the studied river basins.

	Loire/ Allier	Rhine	Elbe	Central Danube	Lower Danube/ Danube Delta
Corrigiolo- Chenopodietum	++	-	+	-	-
Cypero- Limoselletum	-	++	+	++	-
Myosurus minimus	-	-	+	-	-
Poo- Cerastietum dubii	-	(+)	++	-	+
Dichostyli- Gnaphalietum uliginosi	-	-	-	++	++ / ++
Heleochloetum alupecuroidis	-	-	-	-	++ / ++

In general we can observe a change in occurrences of characteristic habitats and species from west to east, but the most important factors remain the site conditions with their hydro- and morphodynamics.

### CONCLUSIONS

Given that these habitats are ephemeral and that they may be more or less pronounced from one year to another, they have not been studied intensely. The existence of species that are bound to and have adapted to protosoil sites has been dramatically endangered by river-training measures and drastically reduced respectively completely extinct on many rivers. This becomes clearly apparent when comparing the various rivers. Undeveloped rivers such as Loire and Allier e.g. are very rich in species. This is much less the case for the Rhine River and somewhat more for the Elbe as this river still shows undeveloped, dynamic sections. Dynamic pioneer sites do still exist on the Lower Danube as well, as its banks are largely left undeveloped and because the Danube comprises numerous natural or near-natural islands. The survival of species that have adapted to the pioneer sites, both plants and animals, depends on these. Their existence is thus threatened all over Europe in the event of river development measures.

The training of the Lower Danube as navigable waterway (bank protection measures, rockfills, dams) will bring along the destruction of such habitats, both along the river banks and around the islands. As a result this very specific biodiversity element which is bound to protosoils on river banks will disappear or few relicts will survive and occur in uncharacteristic compositions. When planning a sustainable development of the Danube River basin it is vital therefore to consider all aspects, including the conservation of well-operating floodplain ecosystems, natural and near-natural habitats, their structure and species diversity and the development of suitable models that account for the use on the one hand but consider nature conservation criteria as well.

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## ALIEN LIGNEOUS SPECIES EVALUATED BY POPULATIONAL INDICES IN THE DANUBE DELTA (ROMANIA)

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**KEYWORDS:** Danube Delta, alien species, ligneous species, population indices.

### ABSTRACT

In this article nine alien woody species within the Danube Delta were evaluated, from the perspective of certain population indices. The frequency and the abundance and the calculation of ecological significance index of these species were taken into account, both in fluvial and fluvial-maritime delta. Five zones of investigation were chosen so that they should comprise all the natural features of the Danube Delta. Within these areas, several vegetation units are included, as follows: Şontea-Fortuna, natural lake complex with reed beds, forests liable to flooding and meadows; Matița-Merhei lake complex, dominated by reed beds; Dunăvăț-Dranov artificial-canal complex, dredged with reed beds and meadows; Grindul Caraorman area with denuded sand dunes or with forest, reed beds and meadows; the seaside area (sector Sulina-Sfântu Gheorghe), area with vegetation specific to marine dunes, reed beds and planted forest. Subsequent to phytocoenological investigations, the species with the highest ecological significance index proved to be *Amorpha fruticosa*, as complementary species. The species *Eleagnus angustifolia*, *Robinia pseudoacacia*, *Lycium barbarum* and *Acer negundo* are associated species. *Fraxinus pennsylvanica*, *Morus alba*, *Gleditsia triacanthos* and *Ailanthus altissima* are accessory species. The most widespread species within the Danube Delta are *Amorpha fruticosa* and *Robinia pseudoacacia* within the fluvial delta, and *Elaeagnus angustifolia* within the fluvial-maritime delta.

**ZUSAMMENFASSUNG:** Fremdländische Gehölzarten im Donau-Delta (Rumänien), ausgewertet anhand von Populationsindices.

In vorliegender Arbeit werden neun allochthone Arten des Donau-Deltas anhand von einigen Populationsindices evaluiert. Dabei werden Frequenz und Abundanz sowie die Berechnung des ökologischen Signifikanz Indexes dieser Arten in Betracht gezogen. In beiden Bereichen des Deltas, dem fluvialen und dem fluvial-marinen, wurden fünf Untersuchungsgebiete ausgewählt, so dass alle natürlichen Kennzeichen berücksichtigt wurden. In diesen Gebieten wurden unterschiedliche Vegetationseinheiten berücksichtigt: der Komplex der natürlichen Seen von Şontea-Fortuna mit Schilfgebieten, überflutbarem Auwald und Wiesen; der Matița-Merhei von Schilfbetten beherrschte Seenkomplex; der Dunăvăț-Dranov von künstlichen Kanälen durchzogene Komplex mit Schilfbetten und Wiesen; das Dünengebiet von Caraorman, mit offenen spärlich bewachsenen oder bewaldeten Dünenbereichen, Schilfflächen und Wiesen; der Meer nahe Bereich (zwischen Sulina und Sfântu Gheorghe), ein von Meeresdünen, Schilf- und anderen

Riedflächen sowie gepflanztem Dünenwald geprägtes Gebiet. Entsprechend den phytocoenologischen Erhebungen erweist sich *Amorpha fruticosa* als die Art mit dem höchsten ökologischen Signifikanzindex, als eine komplementäre Art. *Robinia pseudoacacia*, *Lycium barbarum* und *Acer negundo* sind assoziierte Arten. *Fraxinus pennsylvanica*, *Morus alba*, *Gleditsia triacanthos* und *Ailanthus altissima* sind Begleitarten. Die am weitesten verbreiteten Arten sind *Amorpha fruticosa* und *Robinia pseudoacacia* im fluvialen sowie *Elaeagnus angustifolia* im fluvial-marinen Teil des Donau-Deltas.

**REZUMAT:** Specii lemnoase alohtone în Delta Dunării (România) evaluate pe baza unor indici populaționali.

În acest articol, sunt evaluate, din punct de vedere al unor indici populaționali, nouă specii lemnoase alohtone, din Delta Dunării. S-a avut în vedere studierea frecvenței și abundenței speciilor, atât în delta fluvială, cât și în cea fluvio-maritimă, precum și calcularea indicelui de semnificație ecologică. Au fost alese cinci zone de investigații, astfel, încât acestea să cuprindă toate caracteristicile naturale ale Deltei Dunării. În cadrul acestor zone, sunt cuprinse mai multe unități de vegetație, după cum urmează: Șontea-Fortuna - complex lacustru natural cu stufărișuri, păduri inundabile și pajiști; Matița-Merhei - complex lacustru, dominat de stufărișuri; Dunăvăț-Dranov - complex artificial de canale dragate, cu stufărișuri și pajiști; Grindul Caraorman - zonă de dune de nisip denudate sau cu pădure, stufărișuri și pajiști; cordonul litoral (sectorul Sulina-Sfântu Gheorghe) - zonă cu vegetație specifică dunelor marine, stufărișuri și pădure plantată. În urma investigațiilor fitocenologice, a rezultat că specia care prezintă indicele de semnificație ecologică cel mai ridicat este *Amorpha fruticosa*, ca specie complementară. Speciile *Elaeagnus angustifolia*, *Robinia pseudoacacia*, *Lycium barbarum* și *Acer negundo* sunt specii asociate. *Fraxinus pennsylvanica*, *Morus alba*, *Gleditsia triacanthos* și *Ailanthus altissima* sunt specii accesorii. Speciile cele mai răspândite în Delta Dunării sunt *Amorpha fruticosa* și *Robinia pseudoacacia* în delta fluvială, iar *Elaeagnus angustifolia* în delta fluvio-maritimă.

## INTRODUCTION

It is proven that the changes in the distribution of a species are natural phenomena within which the spreading area may extend or reduce. Thus, the species can colonize new areas beyond the natural existence space. Nevertheless, such events are rare and many times restricted by natural barriers (Wittenberg, 2005).

One of the main spreading vectors was represented by the Europeans settled within the new areas on the globe during colonization (Cogălniceanu, 2007). They had an important role in introducing economically valuable species in Europe and those from the new discovered areas in Europe (Botnariuc, 1961). The social values and options regarding the alien plants have changed throughout times. If in the past introducing certain alien plant species only supposed immediate economic benefit, at present, the awareness of the impact created by these species on biodiversity, economic system and health care is unanimously accepted (Lonsdale, 1999; Cox, 2004; Flemming and Svening, 2004; Lloret et al., 2005; Lambdon, 2008).

Despite all the anthropic activities carried out in the past, after 1989, it has been acknowledged that the deltaic territory still preserves an impressive diversity of habitats and species characteristic to wetlands in a relatively restrained space. Apart from its conservation status already gained after 1990, as a confirmation of its value, the Danube Delta's territory was integrated (2005) in Natura 2000 Network as an area of community interest and of avifauna importance.

## MATERIALS AND METHODS

Species mapping within Danube Delta has been fulfilled on basis of vegetation transects alongside channels within the lake complexes and Danube branches, by boat and within the rest of the delta, by foot. These transects, were registered with two GPS (Garmin 72) devices in order to note the presence in the territory of species and vegetation associations. Subsequently, the data has been interpreted by means of ArcView 3 programme and presented on a digital map. Another source that has contributed to completing the evaluation of species maps was the general forest arrangement and the maps of the 14 arrangements on the Danube Delta Biosphere Reserve.

In order to carry out the study, field trips have been made, as follows.

The vernal period - the monitoring of different development stages of the studied species, visual mapping on itinerary;

The aestival period - sample taking, visual mapping on itinerary, the monitoring of the key areas where repeated observation were done;

For the autumnal period, the monitoring specific to each species will be carried out, the completion of the phytocoenosis species list, observations on alien plant species frequency and abundance;

For field investigations, five different study areas have been selected (Fig. 1, Tab. 1) from the two sectors of Danube Delta.

In the fluvial delta, three areas have been selected:

1. Depression of Şontea - Fortuna;
2. Depression of Matița - Merhei;
3. Depression of Dunăvăț - Dranov.

In fluvial-maritime delta, two areas have been selected:

4. Grindul Caraorman (stationeries: a - Litcov channel; b - forest; c - south of village);

Sea side area (Sulina - Sfântu Gheorghe sector) (stationaries: a - Sulina; b - Sondei channel; c - Cășla Vădanei).

Within the fluvial delta, along channels and within the maritime delta, the littoral cordon area, the observations will be carried out by the squares method within well established routes. These routes were repeated during each vegetation period.

The phytocoenosis composition was determined by the elaboration of lists of species present at the time.

For a comparative research, regarding the phenology data, a minimum representative area was choosing for each species. This minimum area, constantly monitored, is established at 10 m<sup>2</sup> for the pasture and reed phytocoenoses and at 100 m<sup>2</sup> for the forest and brushwood phytocoenoses.

The phenological state is evaluated by means of the phenological system differentiated according to the vegetation type, system conceived by Ivan and Spiridon (1983), in which four stages are distinguished: vegetative stage, buttoning stage, inflorescence stage and fruit stage.

The practical method in the field will be enforced by writing down the stage in which the most (60-70%) specimens of each identified species are within the phytocoenosis structure. The approached quantities phytopopulational indices are coverage, abundance and frequency (Cristea et al., 2004).

The ecologic signification index represents the relation between frequency and abundance, thus showing the species position within the phytocoenosis.

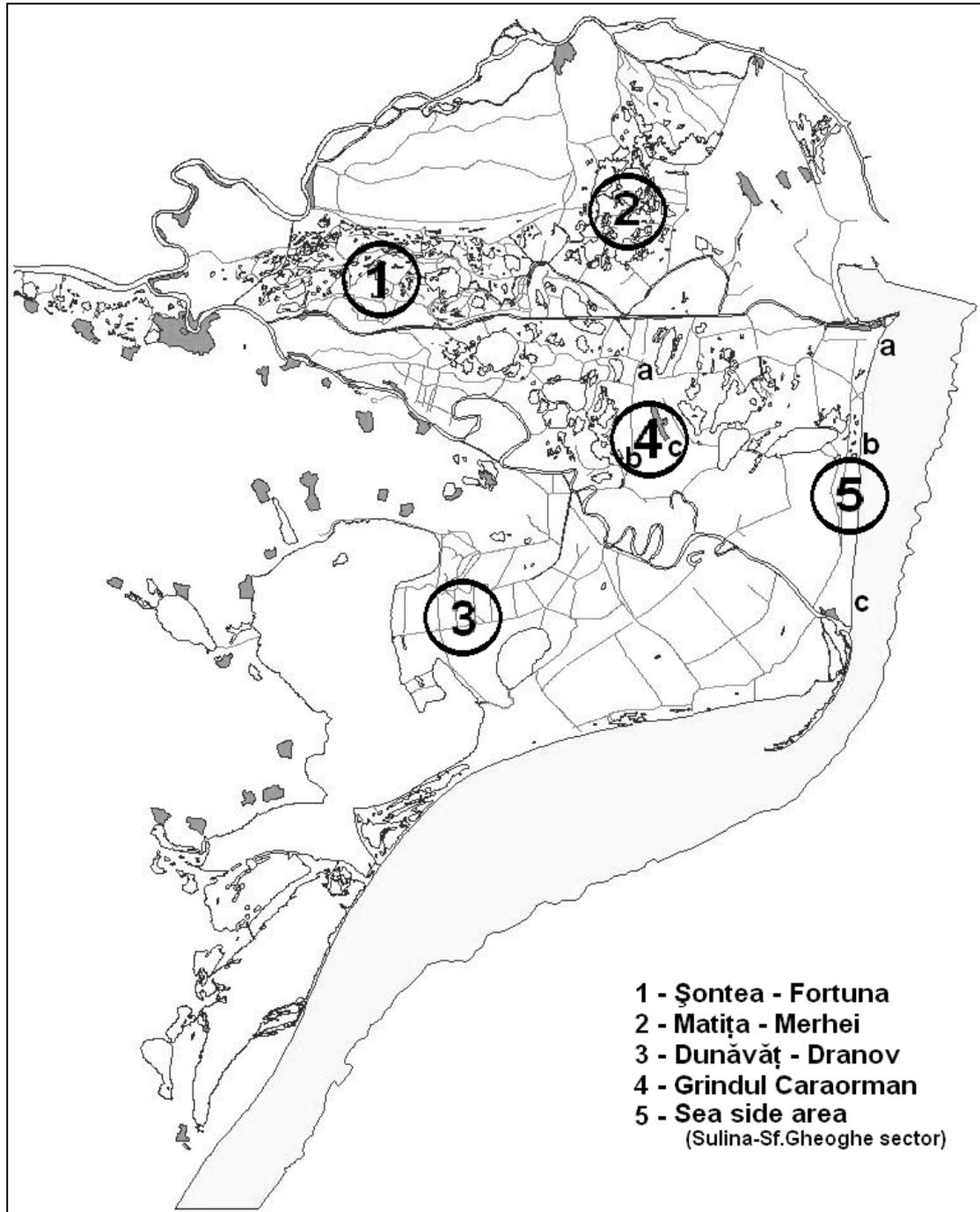


Figure 1: The location of study areas.

Table 1: Land use/cover percentage in study areas.

Vegetation units	Şontea-Fortuna	Matiţa-Merhei	Dunăvăţ-Dranov	Grindul Caraorman	Sea side area
	(%)				
Settlements	1.97	-	-	2.97	-
Fish ponds	1.20	0.01	0.22	0.24	-
Agriculture areas	20.35	-	-	-	-
Planted flood plain forest	9.47	0.07	-	0.96	4.51
Natural flood plain forest	15.78	1.02	0.31	0.78	-
Natural dune forest	-	-	-	5.62	-
Marshy vegetation	33.88	76.42	74.05	51.06	8.5
Lakes	13.20	21	21.73	0.07	1.39
Sea dunes	-	-	0.92	6.57	85.6
Steppe/dry areas	-	-	0.14	31.57	-
Grasslands	4.15	1.48	2.63	0.16	-

## RESULTS AND DISCUSSIONS

**1. Şontea - Fortuna Depression.** In comparison with the other studied areas, the complex represents diversity in vegetation, soils and higher altitude. The vegetation within the depression is prominently formed of shrubbery and meadows, and alongside channels is generally formed of natural forest easily flooded. The exception from this description is represented by the area included in Gârla Păpădia, Dunărea Veche and Sulina Branch. This one includes, mainly, planted forest vegetation. Draining is slow on the entire complex area, factor that is favourable to alien species development. From altitude perspective, Şontea - Fortuna Depression is included alongside banks: between 0 and 1 m on Olguţa and Războiniţa channels; between 1 and 2 m on Gârla Şontea, Păpădia Nouă, Păpădia Veche, Mitchina, Crânjeală and Mila 35 channels (northern part); between 2 and 3 m on Dunărea Veche, Păpădia and Mila 35 channels (Sulina Branch area).

Vegetation units which have been investigated in Şontea - Fortuna Depression are:

### ***Dunărea Veche***

Natural forests easily flooded: *Salicetum albae* 1924 s.l., *Salicetum cinereae* Zolyomi 1931, *Populetum marylandicae* Mititelu 1970 (ass. cult.)

Meadows on high dams: *Cynodonto - Poetum angustifoliae* (Rapaics 1926) Soo 1957, *Lolio-Plantaginetum majoris* (Linkola 1921) Beger 1930, *Bassietum sedoidis* (Ubrizsy 1949) Soo 1964, *Hordeetum murini* Libbert 1923 emend. Pass. 1964.

### ***Şontea Channel***

Mixed reed and club rush vegetation on organic soils: *Typhetum angustifoliae* (All. 1922) Pign. 1934, *Scirpo-Phragmitetum* W. Koch 1926;

Reed vegetation on mineral soils: *Scirpo-Phragmitetum* W. Koch 1926; mixed reed and club rush vegetation on mineral soils: *Scirpo-Phragmitetum* W. Koch 1926, *Typhetum angustifoliae* (All. 1922) Pign. 1934;

Natural forests easily flooded: *Salicetum albae* 1924 s.l., *Salicetum cinereae* Zolyomi 1931.

***Olguța Channel***

Mixed reed and club rush vegetation on mineral soils: Scirpo-Phragmitetum W. Koch 1926, Typhetum angustifoliae (All. 1922) Pign. 1934; Mixed reed and club rush vegetation on organic soils: Typhetum angustifoliae (All. 1922) Pign. 1934, Scirpo-Phragmitetum W. Koch 1926;

Natural forests easily flooded: Salicetum albae 1924 s.l., Salicetum cinereae Zolyomi 1931.

***Păpădia Channel***

Plantations of *Salix* sp., *Populus* sp., *Fraxinus pennsylvanica*, *Robinia pseudoacacia*;

Natural forests easily flooded: Salicetum albae 1924 s.l., Salicetum cinereae Zolyomi 1931.

Reed vegetation on mineral soils: Scirpo-Phragmitetum W. Koch 1926.

***Crânjală Channel***

Natural forests easily flooded: Salicetum albae 1924 s.l., Salicetum cinereae Zolyomi 1931, Salicetum triandrae Malcuit 1929; Mixed reed and club rush vegetation on mineral soils: Scirpo-Phragmitetum W. Koch 1926, Typhetum angustifoliae (All. 1922) Pign. 1934.

**2. Matîța - Merhei Depression.** The dominant vegetation within the complex is formed of reed associations (70%). Eracle, Iacob, Gârla Lopatna channels, as well as the linking channel between Old Danube and Bogdaproste Lake, are the access ways that make possible the entrance into the complex through the southern part. From altitude point of view, the depression has the lowest values, comprised between 0 and 1 metres, in the central part, while in the southern and western parts, there are values comprised between 1 and 2 metres. Drainage is slow on the entire depression area. This specific aspect, corroborated with the low heights, favours reed vegetation development. The salinity is very low, the same as in the rest of the lake complexes that belong to the studied fluvial Danube Delta.

The vegetation units that have been investigated within Matîța - Merhei Depression are:

***Lopatna Channel***

Reed and shrubbery vegetation on compact reed bed: Thelyptero - Phragmitetum Ștefan et al., 1995; Natural forests easily flooded: Salicetum albae 1924 s.l., Calamagrostio-Salicetum cinereae Soo et Zolyomi (1934) 1955.

***Bogdaproste Channel***

Reed and shrubbery vegetation on compact reed bed: Thelyptero - Phragmitetum Ștefan et al., 1995; Scirpo-Phragmitetum W. Koch 1926.

***Dunărea Veche***

Natural forests easily flooded: Salicetum albae 1924 s.l., Calamagrostio-Salicetum cinereae Soo and Zolyomi (1934) 1955, Salicetum triandrae Malcuit 1929.

***Eracle Channel***

Reed and shrubbery vegetation on compact reed bed: Thelyptero - Phragmitetum Ștefan et al., 1995; Scirpo-Phragmitetum W. Koch 1926.

**3. Dunăvăț - Dranov area.** Within this area, reed vegetation prevails. Alongside channels, the vegetation is formed of forests easily flooded. Draining, although it is changed on almost the entire area, is slow. From the altitude perspective, the arrangement is included between 0 and 1 m, rarely 2 m.

The vegetation units that have been investigated within Dranov Depression, on the main channels, Mustaca, Dunăvăț, Dranov and Lipovenilor Channel are: Reed and shrubby vegetation on compact reed bed: *Thelyptero - Phragmitetum* Ștefan et al., 1995; *Typhetum angustifoliae* (All. 1922) Pign. 1943; *Scirpo-Phragmitetum* W. Koch 1926;

Meadows on high dams: *Hordeetum murini* Libbert 1923 emend. Pass. 1964; *Cardarietum drabae* Timar 1950; Natural forests easily flooded: *Salicetum albae* 1924 s.l., *Calamagrostio-Salicetum cinereae* Soo and Zolyomi (1934) 1955, *Salicetum triandrae* Malcuit 1929.

**4. Caraorman Sand Dune.** The landscape is structured with dunes of diverse heights and interdunes spaces with various heights compared to the sea level. They also have various width and forms. This type of landscape influences the display of vegetation on the Caraorman Sand Dune area. Its display is dependent on the ecological gradients (the humidity, the salinity, and also the insolation) as well. Vegetation distribution is made up according to the land height, the soil granulometry, the depth of the ground water layer, and in some places, it is dependent on the dune slope inclination and exposition towards sunrays.

The most important role for the various plant communities is the role of the hydric regime. The ecological conditions reflect themselves very well in ligneous vegetation distribution, within the so called "hașmac" forests; the floristic composition from the herbaceous layer is represented according to the type of soil, the depth of ground water layer, and in the eastern part of the sand dune it depends on the ground water salinity as well.

The vegetation units that have been investigated on Caraorman Sand Dune are: shrubby - *Calamagrostio epigei-Hippophaetum rhamnoides* Popescu, Sanda, Nedelcu 1968; vegetation on high dunes - *Caricetum divisae* Slavnic 1948, *Saliceto (rosmarinifoliae) - Holoschoenetum vulgaris* Mititelu et al., 1973; *Cynodonto - Poetum angustifoliae* (Rapaics, 1926) Soo 1957; meadows on sand dunes - *Holoschoeno - Calamagrostetum epigeios* Popescu and Sanda 1978; *Plantaginetum arenarie* (Buia et al., 1960) Popescu and Sanda, 1987; *Ephedro-Caricetum colchicae* (Prodan 1939 n.n.; Morariu 1959) Sanda and Popescu 1973; mixed oak and ash tree forests - *Fraxino pallisae-angustifoliae-Quercetum roboris* Popescu et al., 1979.

A large part of the Crișan Channel length is within Caraorman Sand Dune. The vegetation existent alongside the channel alterns both in height and composition. In the northern part of the channel there is vegetation specific to fluvial delta, while on the rest of the channel, up to Caraorman locality, the vegetation becomes specific to fluvial - maritime delta.

The vegetation units that have been investigated on the Crișan Channel are the following.

Natural forests in general easily flooded - *Salicetum albae* 1924 s.l.; *Calamagrostio-Salicetum cinereae* Soo et Zolyomi (1934) 1955; shrubbery - *Calamagrostio-Tamaricetum ramosissimae* Simon et Dihoru (1962) 1963; mixed reed and club rush vegetation localised on mineral soils - *Scirpo-Phragmitetum* W. Koch 1926, *Typhetum angustifoliae* (All. 1922) Pign. 1934; meadows on high dams - *Cynodonto - Poetum angustifoliae* (Rapais 1926) Soo 1957; *Bassietum sedoidis* (Ubrizsy 1949) Soo 1964; *Hordeetum murini* Libbert 1923 emend. Pass. 1964; vegetation on high dunes - *Secaletum sylvestre*, *Ephedro - Caricetum colchicae*, *Artemisietum arenariae* Popescu and Sanda 1977.

**5. Seashore - Sfântu Gheorghe-Sulina sector.** The seashore may have width from a few dozen metres to a maximum of few hundreds metres and it has specific landscape, with the following land strips parallel with the line that separates land from sea: beach exposed to the waves, with not solified sand substrata, permanently wet, without vegetation; beach not exposed to the waves, with not solified sand substrata, wet at the surface, with pioneer vegetation, poor in species; high dunes with weakly fixed and not solified sand, slightly exposed to wind; middunes with sand partly fixed, where solification process has begun; low dunes with fixed sand and solification process more advanced, in complex with depressions where the sand is salinized, wet, frequently gleized. In this part, vegetation associations with dominant ligneous species are usually seen, depending on salinization degree and layer's humidity; marshes are gradually turned to surfaces with permanent water.

According to the specific micro landscape, there are biotopes with various life conditions under humidity aspect or water stagnation and salinity.

Within the depressions which have permanently wet sand substrata, very strongly salinised due to this reason, one can see the association *Salicornietum europeae*. On the depressions shores, the association *Suaedetum maritimae* appears. On the drier sand on flat places within depressions, the association *Aeluropetum littoralis* (Prodan, 1939) Șerbănescu 1965 is located.

The vegetation units that have been investigated in this study within this sector are the followings: shrubbery - *Calamagrostio epigei - Hippophaetum rhamnoides* Popescu, Sanda, Nedelcu 1968; *Calamagrostio - Tamaricetum ramosissimae* Simon et Dihoru (1962) 1963; sea shore vegetation on not fixed sands - *Atripliceto hastatae - Cakiletum euxinae* Sanda et Popescu 1999; *Argusietum (Tournefortietum) sibiricae* Popescu et Sanda 1975; *Plantaginetum arenariae* (Buia et al., 1960) Popescu and Sanda; *Juncetum acuti-maritimi* Popescu and Sanda 1972; *Elymetum sabulosi* Morariu, 1957.

#### **Species frequency and abundance**

As we already mentioned, for each G. P. S. point in the field (378 points) it correspond a plant association. In each plant association there were established the frequency and abundance of the studied species. According to this, we set up an average frequency and abundance for all nine species within Danube Delta territory (Figs. 2-10; Tabs. 2a and 2b).

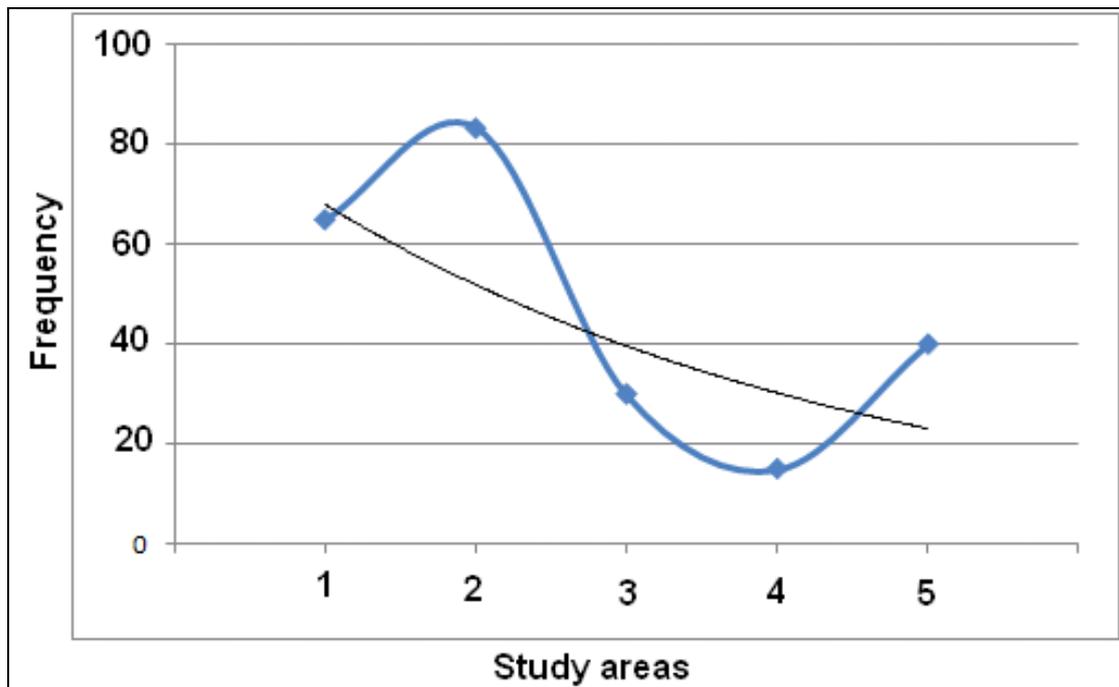


Figure 2a: Frequency of *Amorpha fruticosa* in Danube Delta.

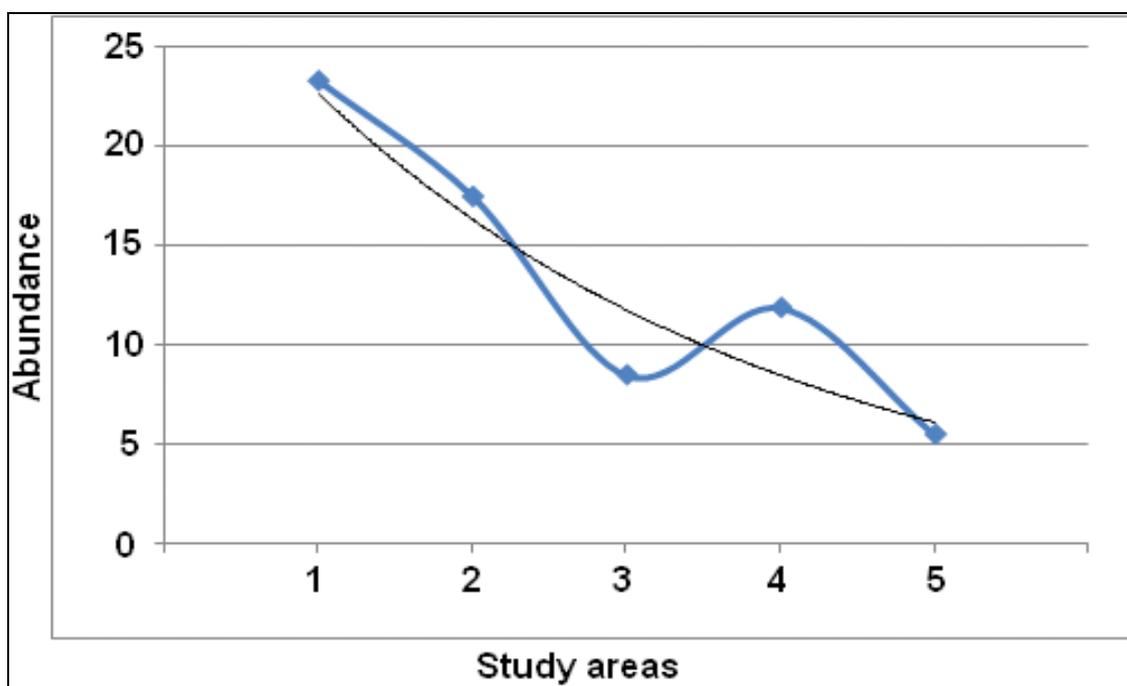


Figure 2b: Abundance (%) of *Amorpha fruticosa* in Danube Delta.

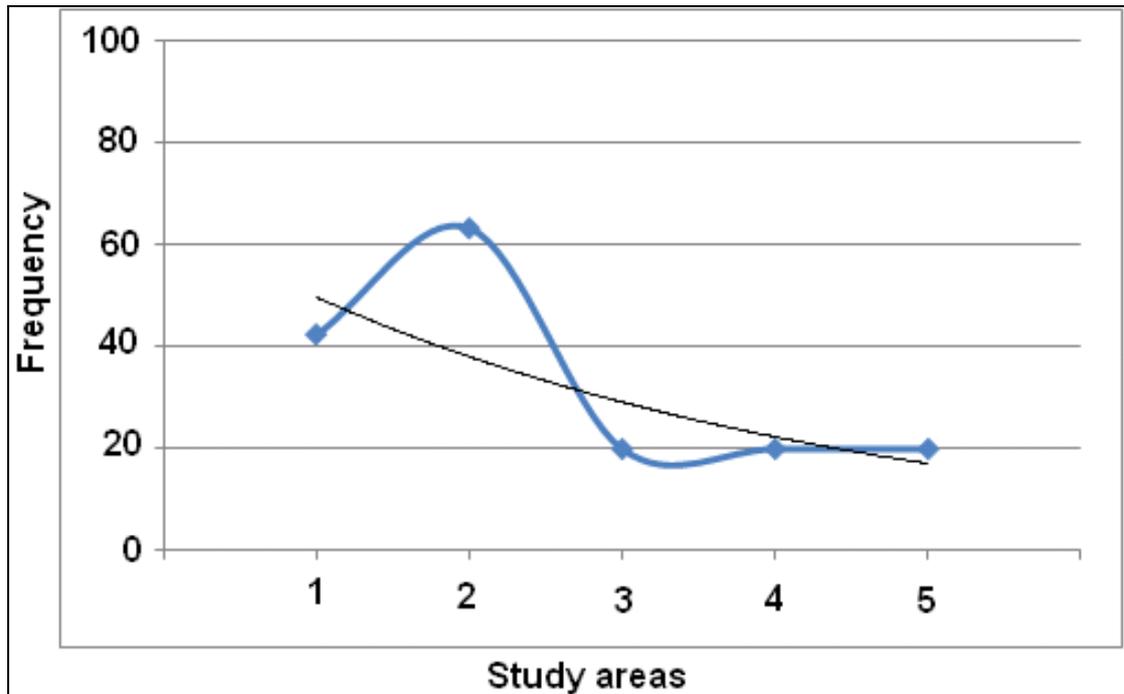


Figure 3a: Frequency of *Robinia pseudacacia* in Danube Delta.

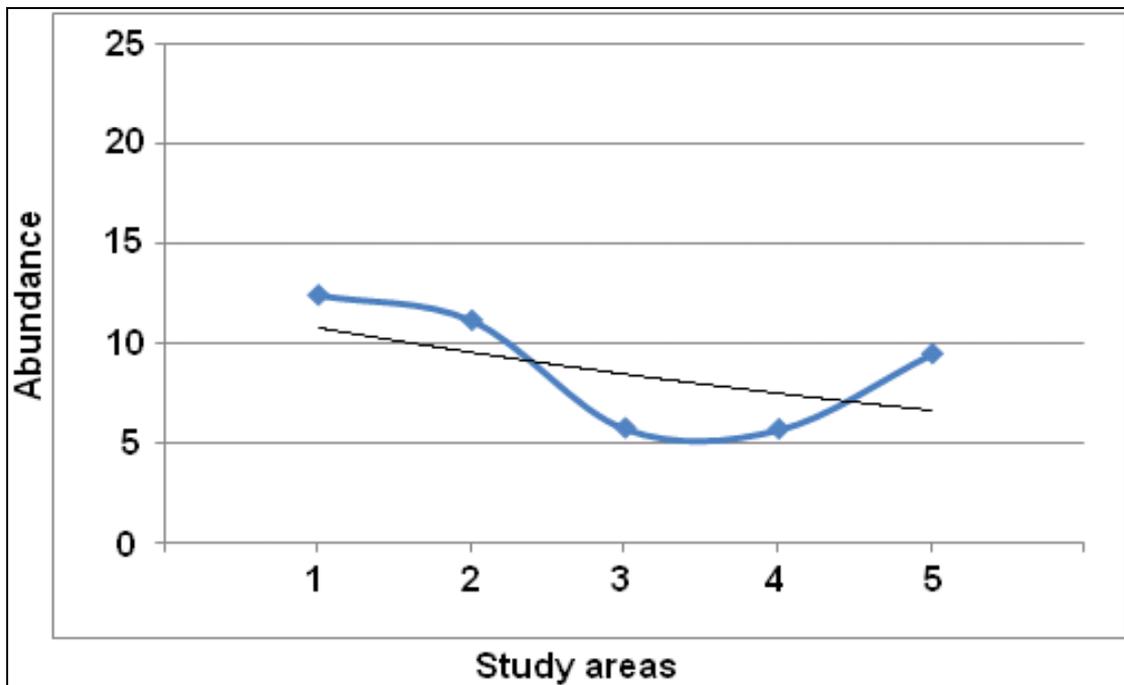


Figure 3b: Abundance (%) of *Robinia pseudacacia* in Danube Delta.

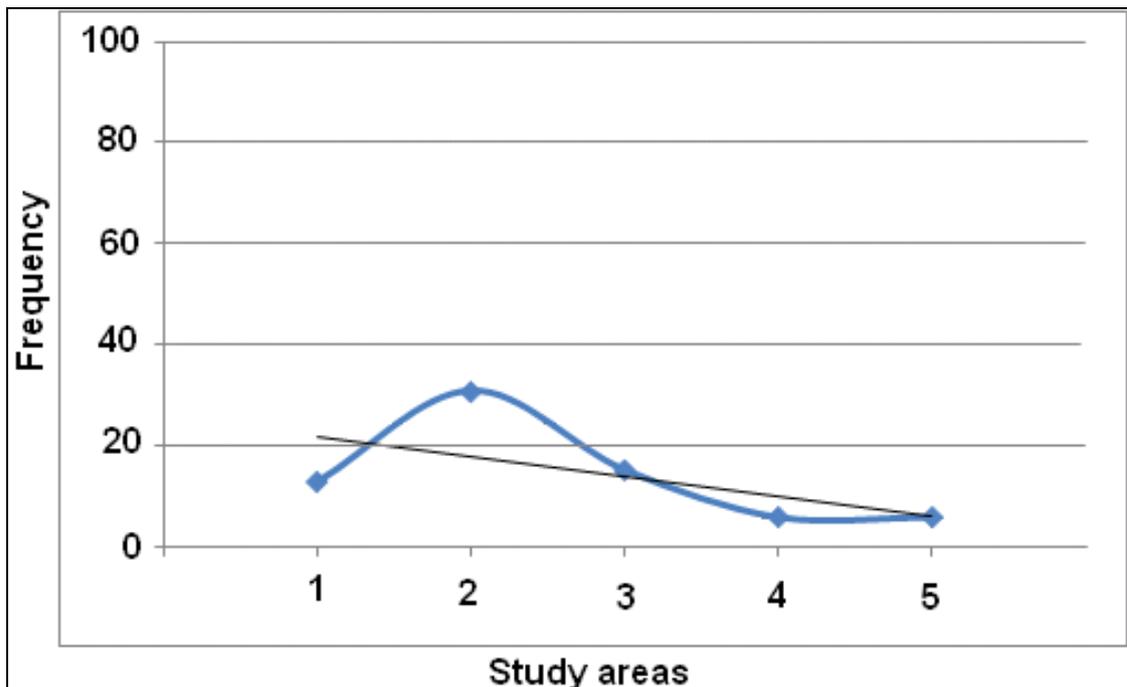


Figure 4a: Frequency of *Gleditsia triacanthos* in Danube Delta.

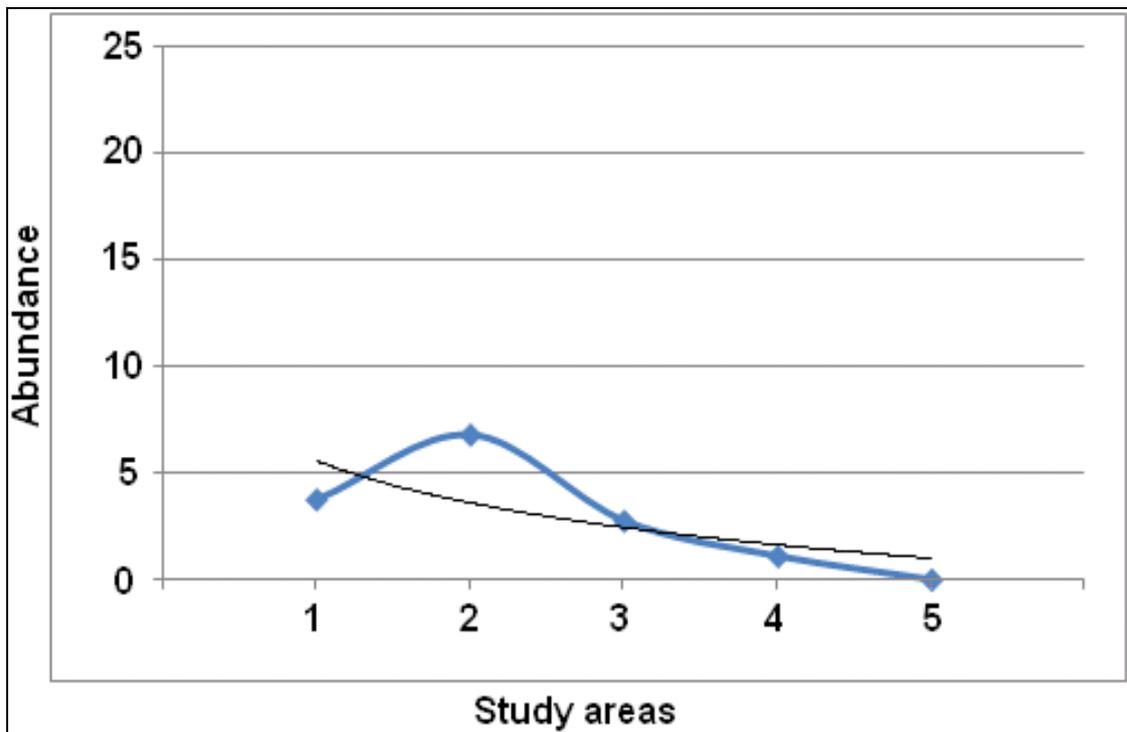


Figure 4b: Abundance (%) of *Gleditsia triacanthos* in Danube Delta.

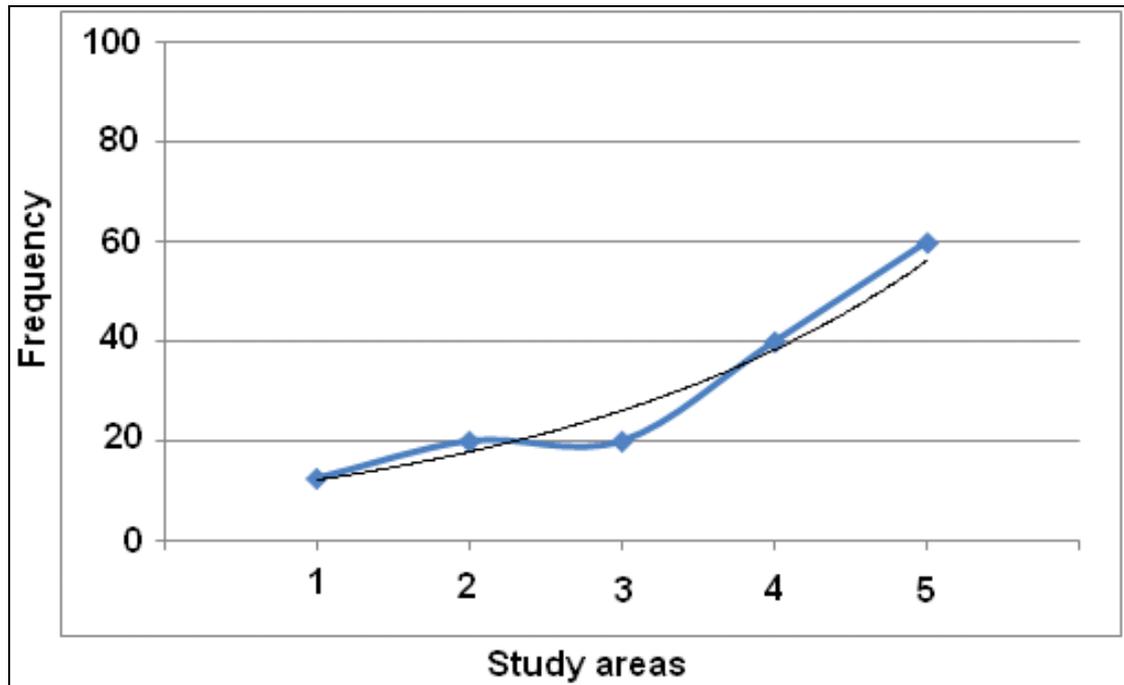


Figure 5a: Frequency of *Elaeagnus angustifolia* in Danube Delta.

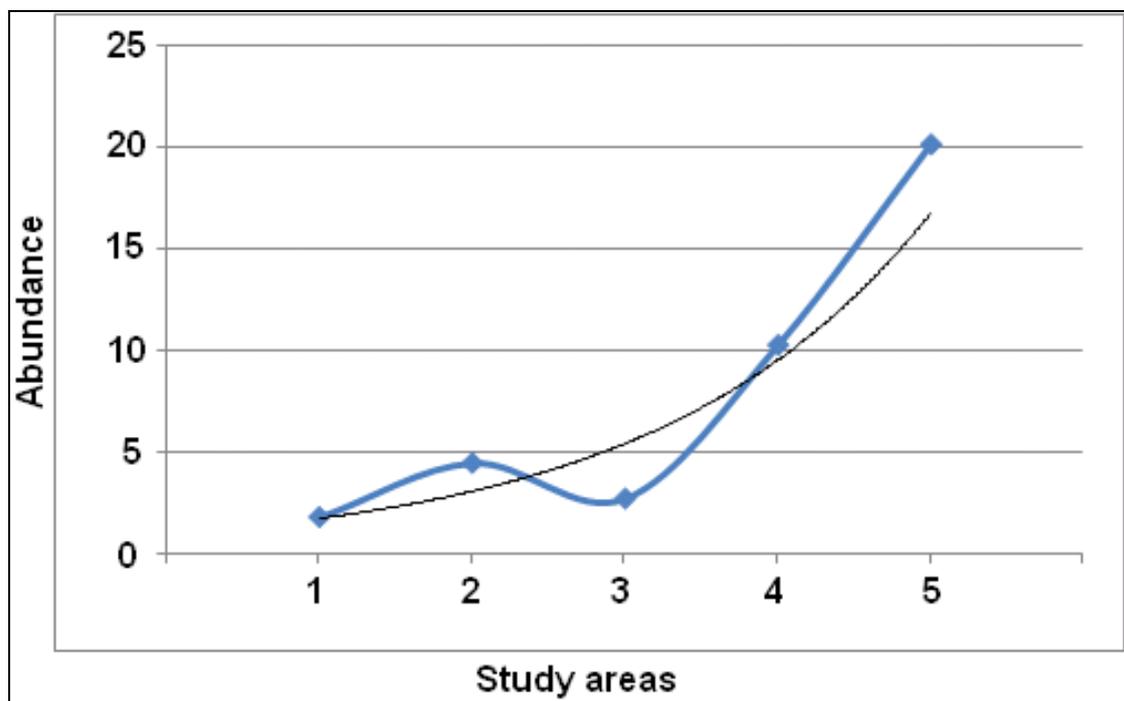


Figure 5b: Abundance (%) of *Elaeagnus angustifolia* in Danube Delta.

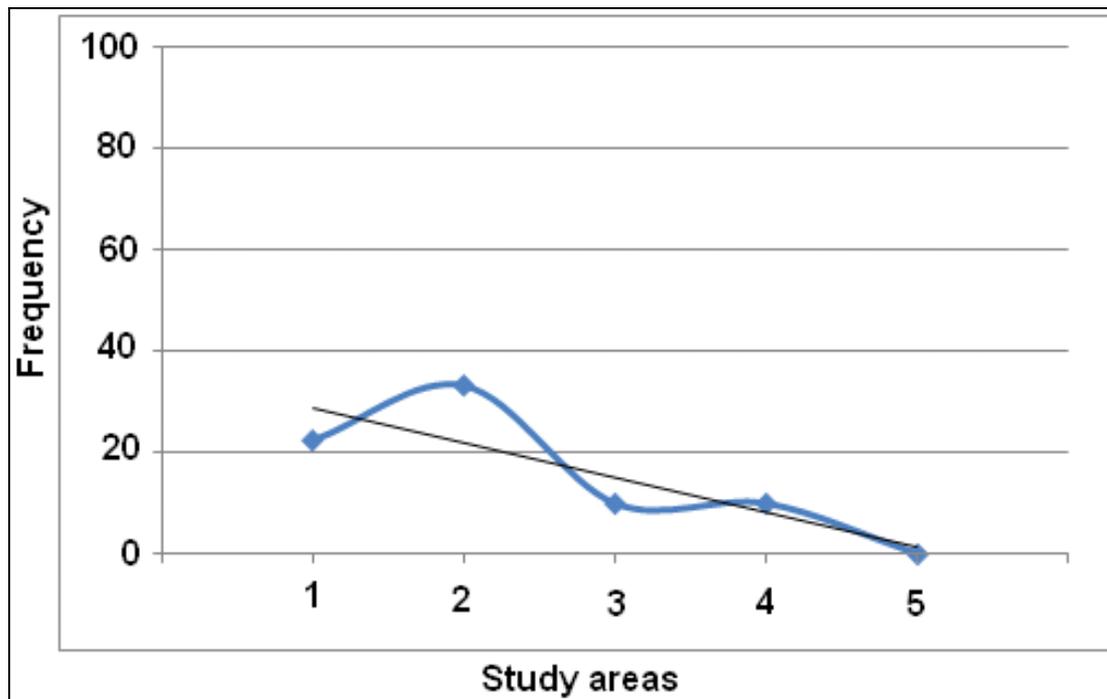


Figure 6a: Frequency and abundance (%) of *Ailanthus altissima* in Danube Delta.

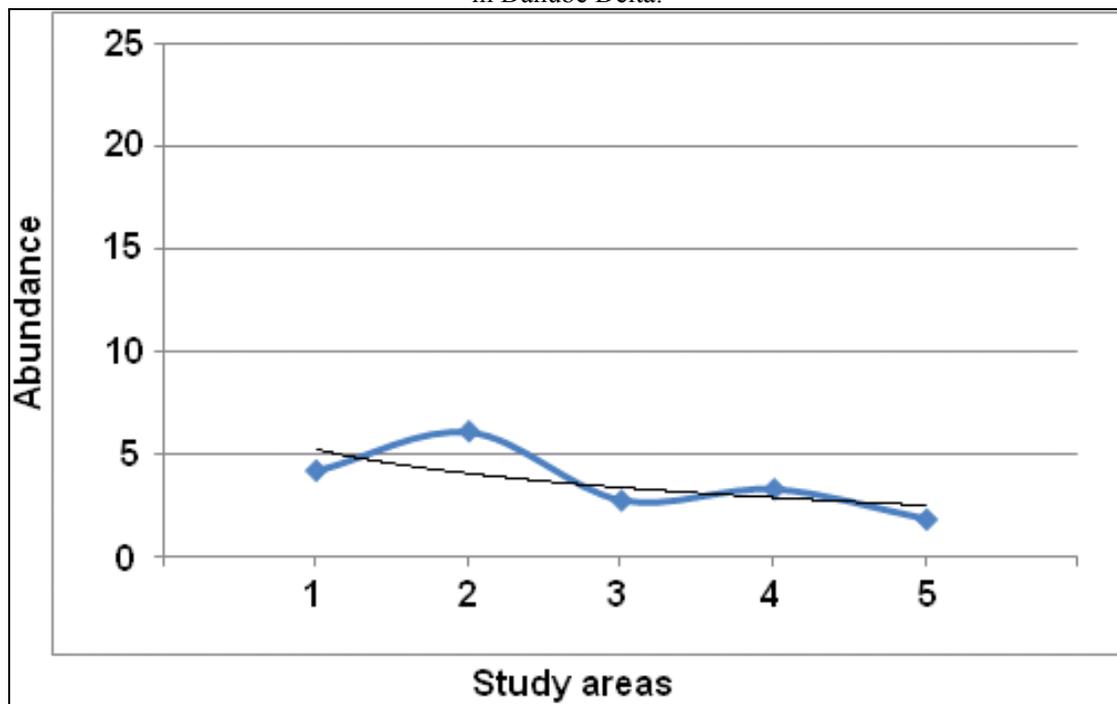


Figure 6b: Abundance and abundance (%) of *Ailanthus altissima* in Danube Delta.

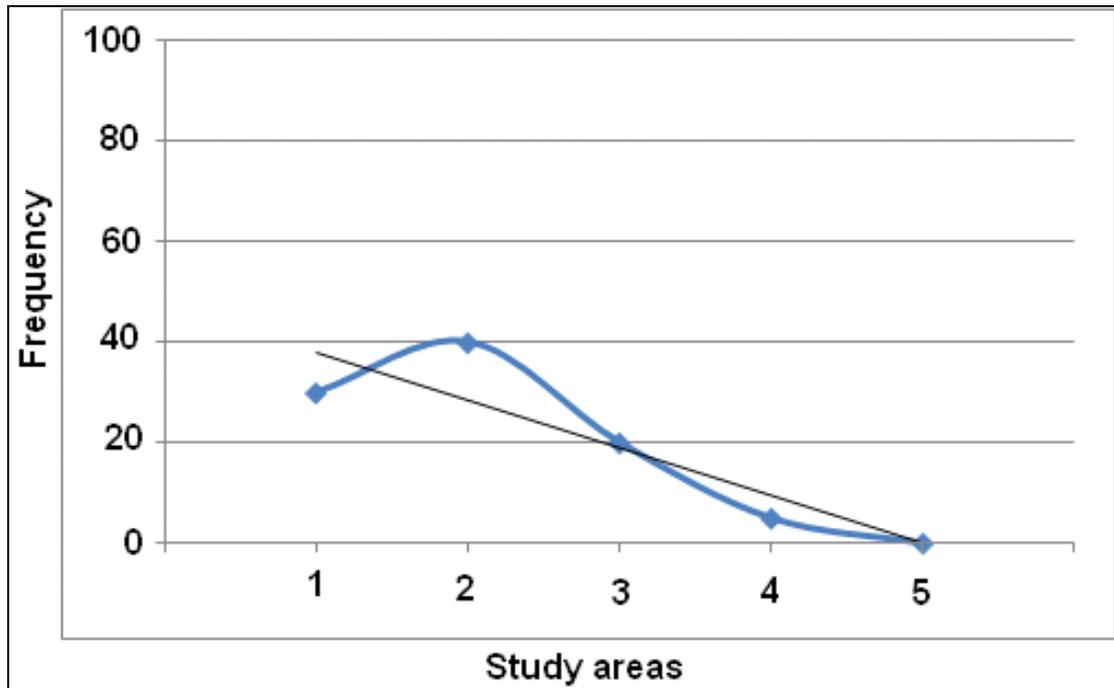


Figure 7a: Frequency of *Acer negundo* in Danube Delta.

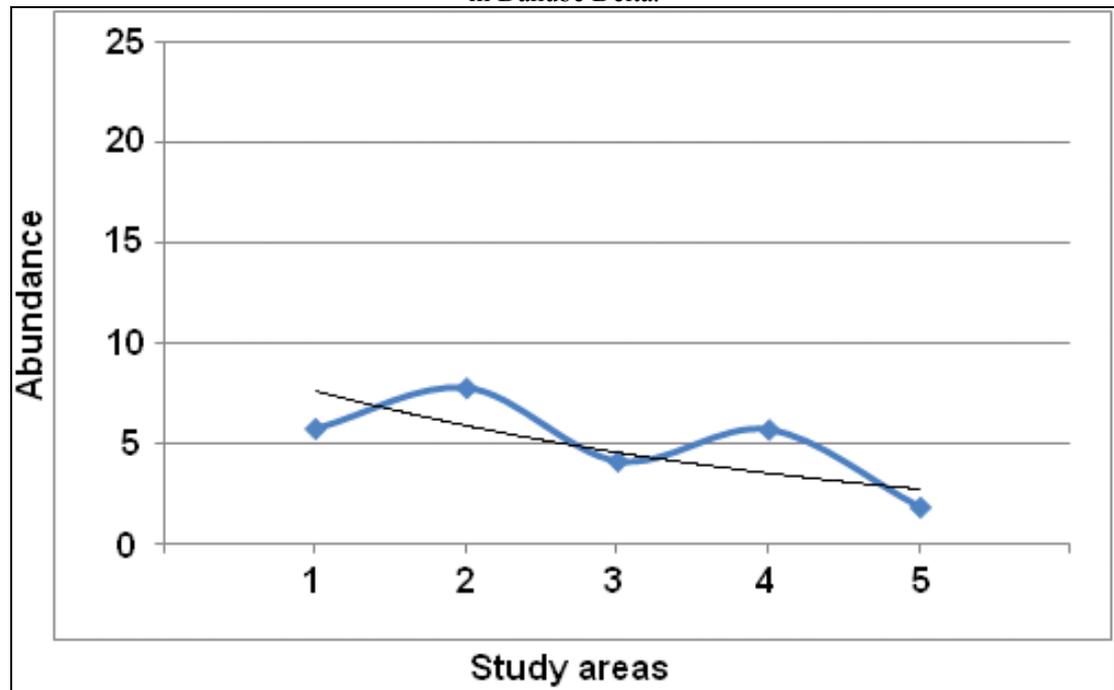


Figure 7b: Abundance (%) of *Acer negundo* in Danube Delta.

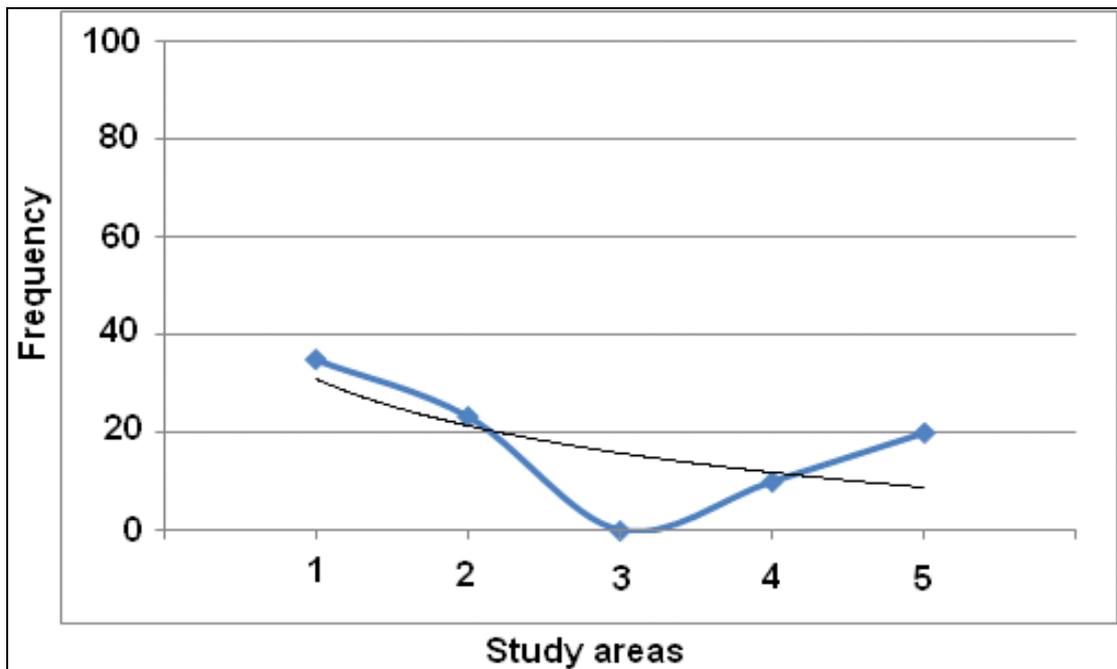


Figure 8a: Frequency of *Morus alba* in Danube Delta.

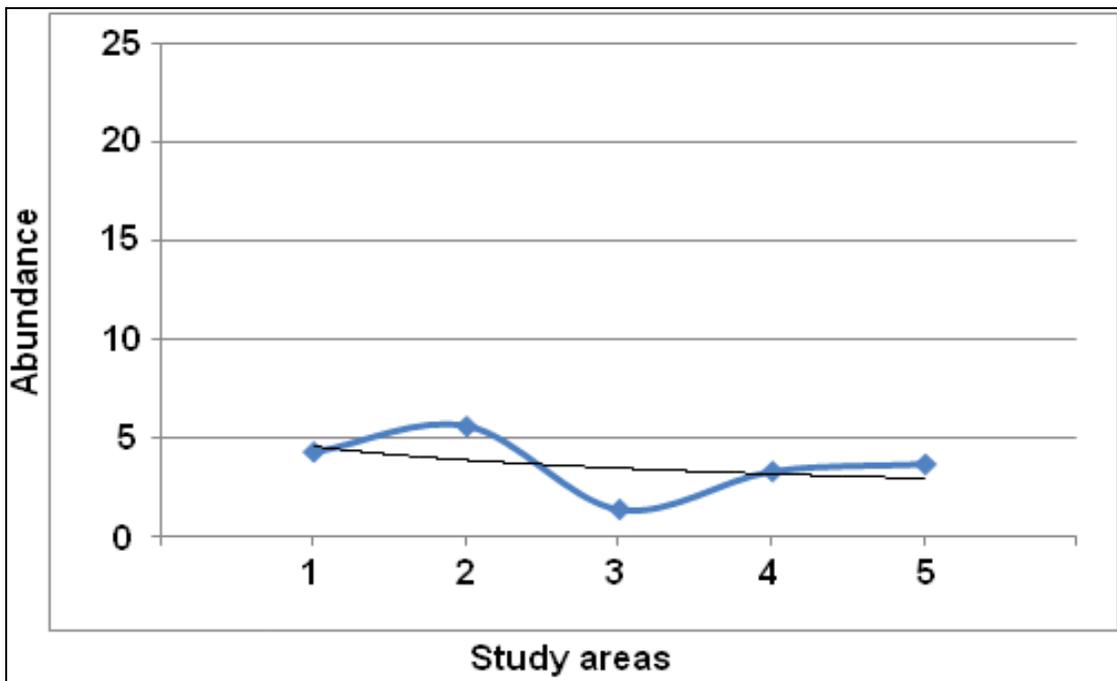


Figure 8b: Abundance (%) of *Morus alba* in Danube Delta.

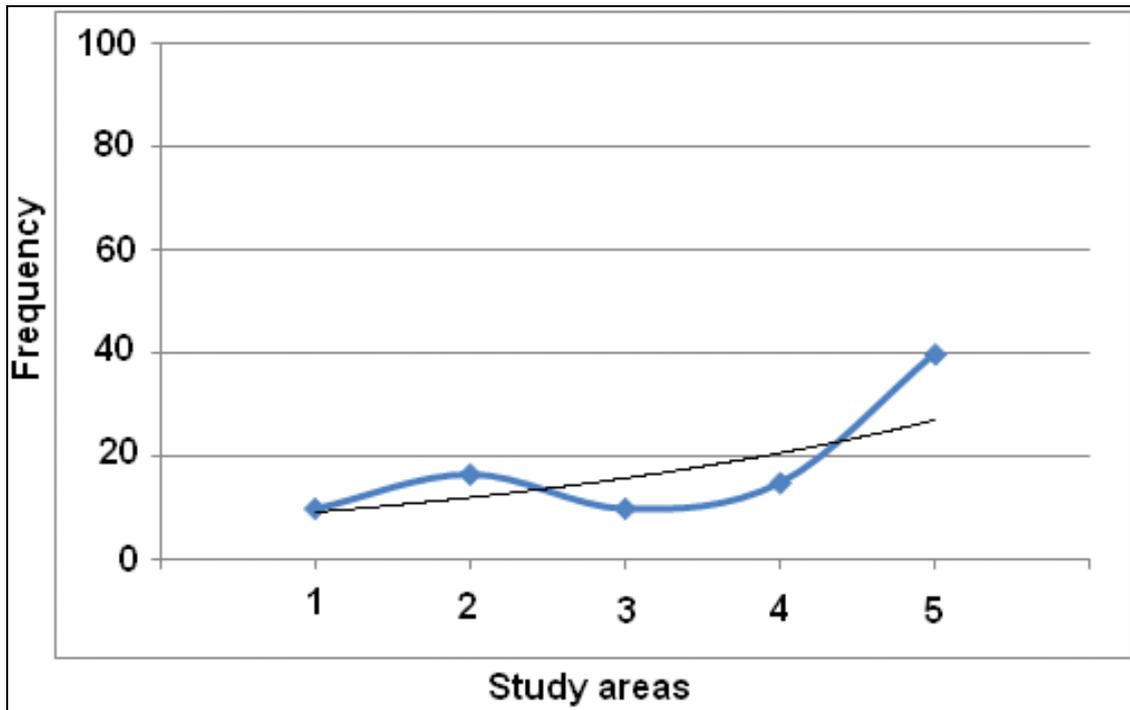


Figure 9a: Frequency of *Lycium barbarum* in Danube Delta.

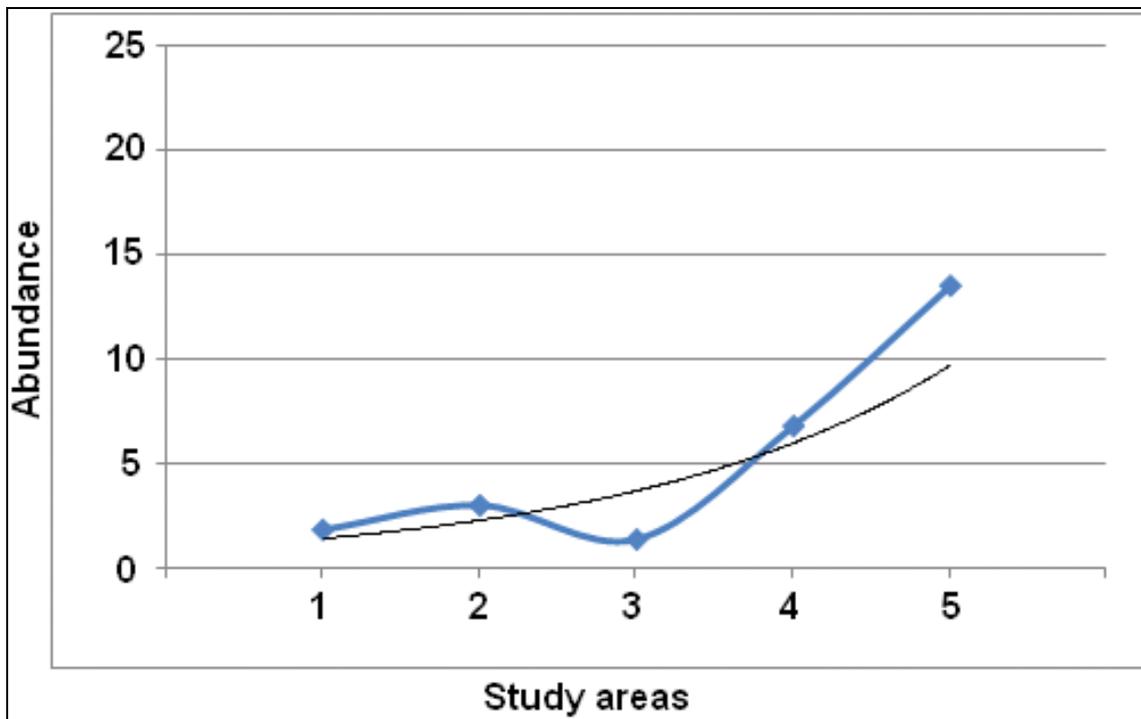


Figure 9b: Abundance (%) of *Lycium barbarum* in Danube Delta.

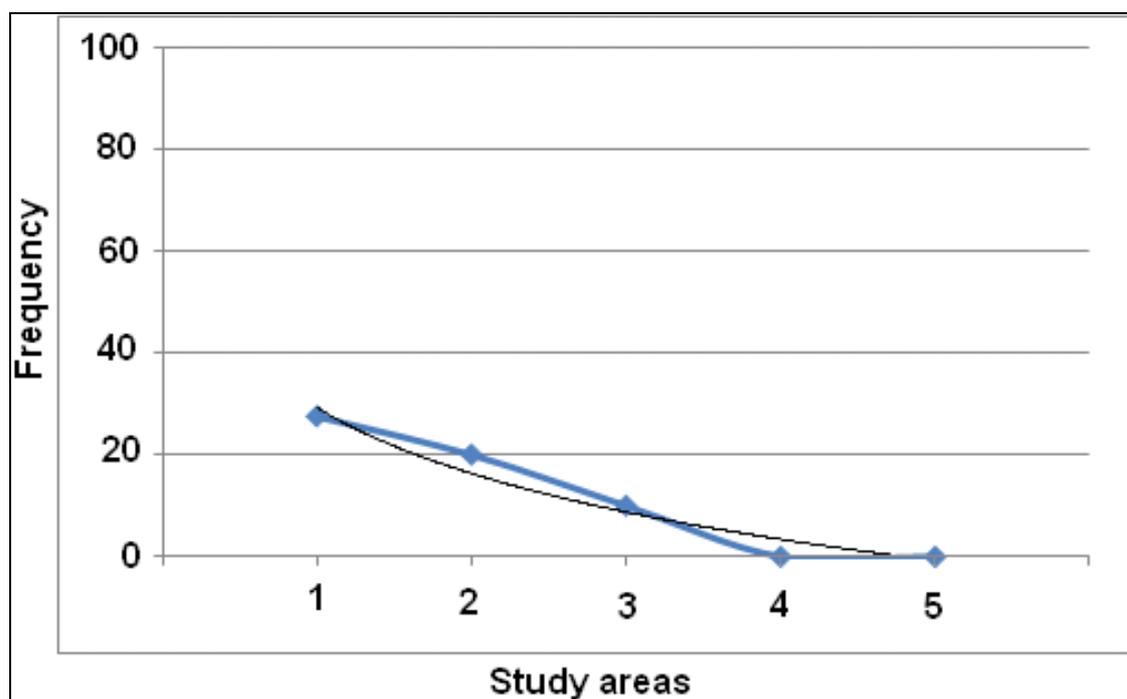


Figure 10a: Frequency of *Fraxinus pennsylvanica* in Danube Delta.

Table 2a: Species abundance within delta channels in proportion to coverage index (%).

Study area	<i>Amorpha fruticosa</i>	<i>Robinia pseudacacia</i>	<i>Gleditsia triacanthos</i>	<i>Elaeagnus angustifolia</i>	<i>Ailanthus altissima</i>
Șontea - Fortuna					
Șontea	65	40	1	3	10
Mila 35	60	30	1	5	5
Stipoc	20	2	10	1	1
Iulia	25	6	5	-	1
Draghilea	15	-	-	-	-
Trofilca	5	-	-	-	-
Olguța	5	4	-	-	1
Crânjeală	20	2	1	-	-
Sireasa	30	5	-	1	5
Candura	10	1	-	-	-
Războinița	5	-	-	-	-
Păpădia	5	10	2	-	1
Average abundance	22.1	11.1	3.3	2.5	4.2
Dunăvăț-Dranov					
Mustaca	40	30	15	5	13
Dunăvăț	50	20	20	10	10
Dranov	50	5	5	10	5
Matița-Merhei					
Dunărea Veche	20	10	5	1	1

Table 2a continued.

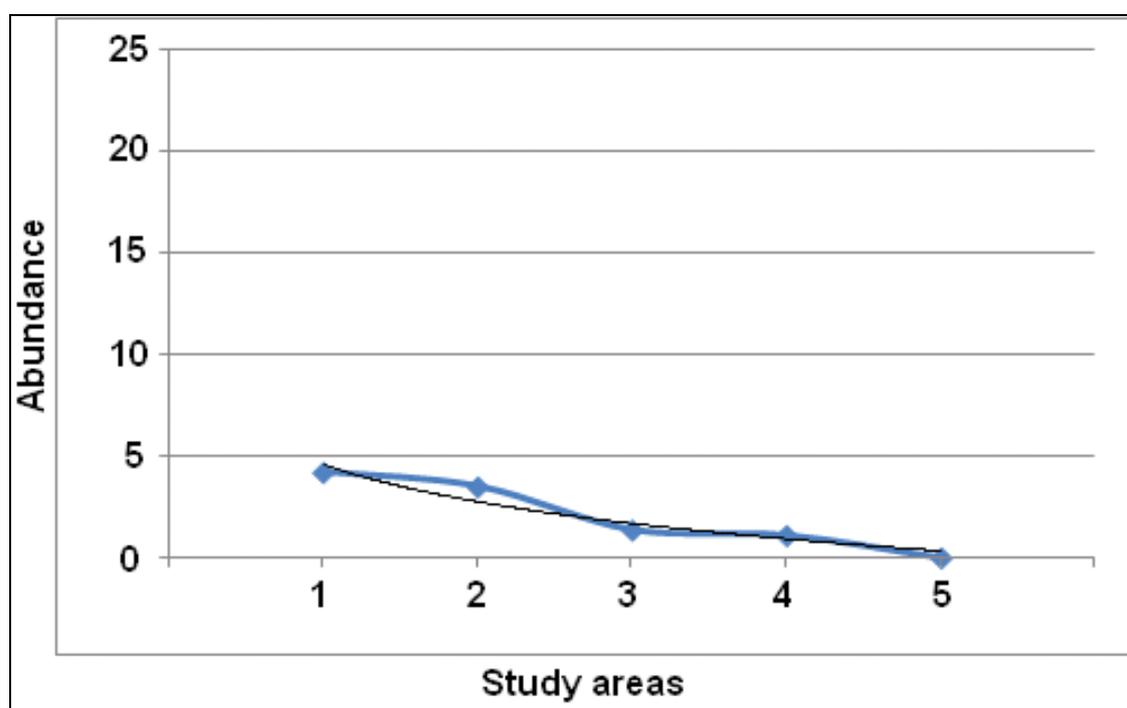
Bogdaproste	10	5	5	-	1
Răducu	1	-	-	-	-
Lopatna	5	-	-	3	-
Average abundance	9.0	7.5	5.0	2.0	1.0
Caraorman					
Crișan north	35	15	1	1	1
Crișan south	10	5	-	10	1
Stationary a	5	-	-	5	-
Stationary b	5	1	-	15	6
Stationary c	5	-	-	10	-
Average abundance	12	7	1	8.2	2.6
Cordon litoral					
Stationary a	10	10	-	10	1
Stationary b	10	5	-	1	-
Stationary c	10	5	-	30	-
Average abundance	10.0	6.6	0.0	13.6	1.0
Cocoș	25	15	10	1	3
Lejai	20	10	5	1	1
Tărăța	10	5	-	-	7
Crasnicol	10	5	5	3	-
Lipovenilor	15	5	-	5	-
Belciug	5	-	-	-	-
Perișor	10	10	-	6	10
Canalul de Centură	10	10	1	8	2
Average abundance	22.3	11.5	8.7	5.4	6.4

Table 2b: Species abundance within delta channels in proportion to coverage index (%).

Study area	<i>Acer negundo</i>	<i>Morus alba</i>	<i>Lycium barbarum</i>	<i>Fraxinus pemsylvanica</i>
Șontea - Fortuna				
Șontea	15	10	1	15
Mila 35	10	10	1	1
Stipoc	15	1	1	5
Iulia	5	1	-	1
Draghilea	-	-	-	5
Trofilca	-	-	-	-
Olguța	-	-	-	5
Crânjeală	1	-	-	-
Sireasa	1	1	1	5
Candura	-	-	-	-
Războinița	-	-	-	-
Păpădia	-	-	-	-
Average abundance	7.8	4.6	1.0	5.3
Dunăvăț-Dranov				
Mustaca	10	5	5	4
Dunăvăț	10	10	5	5
Dranov	15	4	5	10
Cocoș	5	5	1	1
Lejai	1	10	-	-
Tărăța	-	1	-	-
Crasnicol	1	1	-	1

Table 2b continued.

Lipovenilor	1	-	-	-
Belciug	6	-	-	-
Perișor	-	5	1	1
Canalul de Centură	4	10	1	1
Average abundance	5.9	5.6	3.0	3.3
Matița-Merhei				
Dunărea Veche	5	-	1	1
Bogdaproste	3	-	-	-
Răducu	-	-	-	-
Lopatna	2	1	-	-
Average abundance	3.3	1.0	1.0	1.0
Caraorman				
Crișan north	10	1	5	1
Crișan south	5	1	10	-
Stationary a	-	-	1	-
Stationary b	1	1	1	-
Stationary c	-	-	-	-
Average abundance	5.3	1	4.25	1
Cordon litoral				
Stationary a	1	10	10	-
Stationary b	-	-	5	-
Stationary c	-	1	10	-
Average abundance	1.8	5.5	8.3	-

Figure 10b: Abundance (%) of *Fraxinus pennsylvanica* in Danube Delta.

### Ecological significance

According to the ecological classes for abundance (A), frequency (F) and ecological significance (W), the ecological study on ligneous alien species has been carried out, too (Tab. 3). The phytopopulational indices mentioned above have the highest relevance in identifying their way of adaptation. The species that presents the highest index of ecological significance and which is included within the class of complementary species (W4) (Fig. 11), is *Amorpha fruticosa*. Its abundance and frequency have indicated the highest values in Danube Delta. Being given these conditions, this species, although planted in the past (decisive factor in the actual frequency), is best adapted. The fact that it is a shrub confers it an advantage in its installation in the shrub layer and not only, from where it competes with the indigenous species. This phenomenon was firstly mentioned by Borza, by identifying the vegetal association *Salicetum triandrae* Malcuit 1929; *subas. amorphosum fruticosae* Borza 1954.

Table 3: Ecological significance index according to abundance and frequency classes.

Abundance (A)		Frequency (F)		Ecological significance index (W)	
(%)	Class	(%)	Class	(%)	Class
A1 < 5	sporadic	F1 < 10	very rare	W1 (0.1-1)	accidental
A2 (5-17.4)	subrecedent	F2 (10.1-25)	rare	W2 (1-5)	accessory
A3 (17.5-37.4)	recedent	F3 (25.1-45)	accessory	W3 (5-10)	associate
A4 (37.5-62.5)	codominant	F4 (45.1-70)	frequent	W4 (10-20)	complementary
A5 > 62.5	dominant	F5 (70.1-100)	very frequent	W5 > 20	characteristic

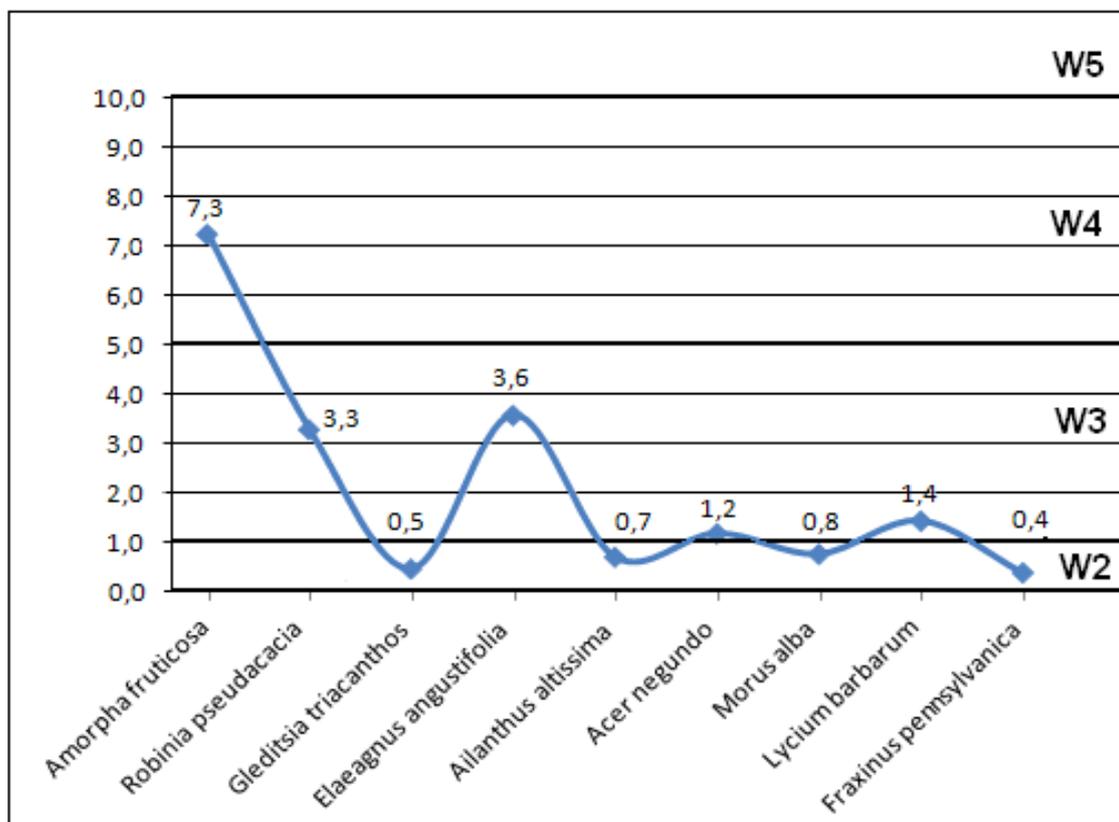


Figure 11: Ecological significance of alien ligneous species in Danube Delta.

The species *Eleagnus angustifolia* and *Robinia pseudoacacia* are fit the index of ecological significance W3 (Fig. 11), respectively associated species, with the highest values. Although they display adaptation differences in different sectors of Danube Delta, namely in fluvial-maritime delta and respectively fluvial delta, the two species were deliberately introduced within both sectors. This adaptation phenomenon was observed in time, naturally, through the differences of frequency and abundance.

Other species included at the same index of ecological significance (W3), but with lower values, are *Lycium barbarum* and *Acer negundo*. The abundance-frequency of these species, as compared to those two formerly mentioned, are more closely related to anthropic activities. The species with the lowest ecological significance (W2) are accessory species. They are maintained within forest arrangements in an increased number of individuals. Their presence within natural habitats depends on this factor. However, both in natural (frost, floods, drought) and artificial conditions (cutting, burning) of stress, these species reveal a high capacity of regenerating and a potential of spreading higher than in normal conditions as compared with the indigenous species.

### CONCLUSIONS

Alien plant species have a higher frequency, which are widespread in both natural habitats and in those controlled by humans, for example: *Amorpha fruticosa*, *Robinia pseudoacacia*, *Acer negundo*, *Morus alba*, *Fraxinus pennsylvanica*, *Ailanthus altissima*, *Lycium barbarum*, *Gleditsia triacanthos* and *Elaeagnus angustifolia*.

From the alien ligneous plant species, the most widespread in Danube Delta are *Amorpha fruticosa*, *Robinia pseudoacacia* in fluvial delta and *Elaeagnus angustifolia* in maritime-delta.

Another action that can be efficient at Danube Delta level is the general monitoring that involve prevention of new species introduction; specific monitoring of the key areas by succession of vegetation and species monitoring on the base of existent spread, phenology, development and habitats data and by human interventions (dredging, clearings or burning).

The most affected habitats are natural forests easily flooded; meadows on high dams; low dunes with fixed sand and solification process more advanced and seashore vegetation on not fixed sands.

### ACKNOWLEDGEMENTS

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**AQUATIC AND SEMI-AQUATIC HETEROPTERA (NEPOMORPHA)  
FROM THE SULINA - SFÂNTU GHEORGHE CANAL  
(DANUBE DELTA, ROMANIA)**

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**KEYWORDS:** Danube Delta, Sulina Channel, Nepomorpha, habitats, species diversity.

**ABSTRACT**

Aquatic and semi-aquatic Heteroptera communities from the northern part of the Sulina - Sfântu Gheorghe Canal were investigated. Nine sampling stations were chosen to reflect the large variety of aquatic habitats present in the area. 15 species representing the group were sampled, a total of 471 adults and 157 larvae, mostly the common species, but also the rare *Sigara (Halicorixa) mayri* (Fieber 1860), *Sigara (Halicorixa) stagnalis* (Leach 1817) and *Mesovelina vittigera* Horvath 1895. Two species, *Micronecta vittigera* and *Micronecta (Dichaetonecta) scholtzi* (Fieber 1860) are mentioned for the first time from the Danube Delta, as well as in southern Romania. Aquatic habitats are strongly influenced by climatic conditions, heavy rains creating large flooded areas lasting for several days, which retreat into smaller puddles or marshes where the aquatic populations, including our target group, take cover, feed and reproduce.

**ZUSAMMENFASSUNG:** Die aquatischen und semiaquatischen Heteropteren (Nepomorpha) des Sulina - Sfântu Gheorghe - Kanals (Donaudelta, Rumänien).

In dieser Studie wurden die Gemeinschaften der aquatischen und semiaquatischen Heteropteren aus dem nördlichen Abschnitt des Sulina - Sfântu Gheorghe - Kanals untersucht. Um die große Vielfalt der aquatischen Habitate dieses Gebietes zu widerspiegeln, wurden neun Probestellen gewählt, an denen 15 Arten dieser Gruppe, mit einer Gesamtanzahl von 471 Adulten Exemplaren und 157 Larven gesammelt wurden. Dabei geht es größtenteils um weit verbreitete, aber auch seltene Gattungen, wie *Sigara (Halicorixa) mayri* (Fieber 1860), *Sigara (Halicorixa) stagnalis* (Leach 1817) und *Mesovelina vittigera* Horvath 1895. Zwei, Arten, *Micronecta vittigera* und *Micronecta (Dichaetonecta) scholtzi* (Fieber 1860), werden erstmals für das Donaudelta und den gesamten südlichen Teil Rumäniens angegeben. Die aquatischen Habitate sind stark durch das hydrologische Regime und die klimatischen Bedingungen beeinflusst, wobei bei den eher seltener auftretenden Regengüssen große überschwemmte Gebiete entstehen, die mehrere Tage dauern, bevor sich das Wasser in Teiche und in weniger ausgedehnten Sümpfe zurückzieht, wo die aquatischen Populationen (die untersuchte Gruppe eingeschlossen) ihre Verstecke finden, sich, ernähren und vermehren.

**REZUMAT:** Heteropterele acvatice și semiacvatice (Nepomorpha) de pe Canalul Sulina-Sfântu Gheorghe (Delta Dunării, România).

Comunitățile de Heteroptere acvatice și semiacvatice, din partea nordică a Canalului Sulina - Sfântu Gheorghe, au fost investigate pentru studiul de față. Nouă stații de colectare au fost alese pentru a reflecta marea varietate de habitate acvatice, prezente în zonă. 15 specii aparținând grupului au fost capturate, cu un total de 471 adulți și 157 larve, majoritatea specii larg răspândite, dar și specii rare ca *Sigara (Halicorixa) mayri* (Fieber 1860), *Sigara (Halicorixa) stagnalis* (Leach 1817) și *Mesovelina vittigera* Horvath 1895. Două specii, *Micronecta vittigera* și *Micronecta (Dichaetonecta) scholtzi* (Fieber 1860) sunt, la prima menționare pentru Delta Dunării și pentru întreaga regiune sudică a României. Habitatele acvatice sunt puternic influențate de regimul hidrologic și de condițiile climatice, ploile puternice care în general nu apar prea frecvent în deltă creând areale inundate extinse, care persistă pentru mai multe zile, retrăgându-se sub forma unor bălți sau mlaștini, mai puțin întinse, unde populațiile acvatice, inclusiv grupul studiat, se adăpostesc, se hrănesc și se reproduc.

### INTRODUCTION

The Danube Delta is a landmark of life diversity at European level, and one of the most important protected areas of Romania. According to the Danube Delta Biosphere Reserve Administration, 1839 floral and 3590 fauna species are present on the reserve, as well as 30 different ecosystem types ([www.ddbra.ro](http://www.ddbra.ro)), making the Danube Delta a unique habitat for the European continent. Although the wild life was intensely studied in the last centuries, some groups remain uncharted in the Danube Delta, mostly because researchers that study those groups chose not to extend their research in the area. In contrast, other groups, such as birds, for example, were studied by hundreds of biologists so far, who presented results regarding all the aspects of almost each species' life.

Aquatic and semi-aquatic Heteroptera, presently known as Infraorder Nepomorpha (Aukema, 2004), are one of the groups with very few mentions in the Danube Delta (Paina, 1975; Kis and Davideanu, 1994). Insects associated, more or less, to water surfaces, they form, together with other groups, the nekton and the epineuston, inhabiting a large variety of micro biotopes, from those lacking in vegetation, to those completely covered with vegetation (Andersen, 1982; Davideanu, 1999; Ilie, 2009). Favorable habitats for the group are ponds, lakeshores, slow flowing creeks or little bays formed at the shore of rivers, all frequent on the Danube Delta. Most species are resistant to moderate or even heavy human impact in the habitat, as well as to the presence of vegetation (Andersen, 1982; Davideanu, 1999).

The present study aimed to investigate a first sector of the eastern part of the Danube Delta, opening the group research in the area. Species composition and habitat characteristics were given equal importance in the field study, in order to obtain a clearer picture of the group's life in the area. We chose a sector in the easternmost part of the reserve, close to the Black Sea, on the littoral canal that connects Sulina and Sfântu Gheorghe.

### MATERIALS AND METHODS

The investigated area (around 400 m long and 100 m wide) is located between the city of Sulina, on the west, and the Black Sea, on the east, on a plane, sandy territory. From north to south, a series of canals connects Sulina Branch with Sfântu Gheorghe Branch, forming a complex often referred to as the Sulina - Sfântu Gheorghe Canal. The canals are locally named Bursuca, Împutița and Tătaru (from north to south), our samplings being made on the first two. Two roads cross the area, one connecting Sulina with a small tourist beach (known as Sulina Beach), and another one, perpendicular on the first one, which follows the canals.

Nine sampling stations with 17 sampling points were chosen for the present study (Fig. 1), covering a large variety of habitats suitable for the studied group. The principle was to investigate sampling stations as different as possible in the terms of habitat conditions, in order to detect a larger number of species. The number of individuals was of secondary importance, being discussed only to point out a specific characteristic of the habitat.



Figure 1: Sulina sampling area (habitats depicted as white shapes, sampling points as black dots).

The description of sampling station is as follows.

**SU1:** A swamp sector formed by rain water collected on a lower area resulted from the construction of two local roads. The station is L-shaped, the two sides are perpendicular, following the two roads. Its location is about 1 km south of Sulina Beach, close to the right shore of the Sulina - Sfântu Gheorghe Canal.

The two sides of the station are 20 m long, the one following the road, going east to the Black Sea, and 15 m long, the one parallel with the canal; the water depth is around 10-15 cm, the shores are sandy and the bottom is muddy, with a low amount of organic detritus. Hygrophilous vegetation is present throughout the station.

Two sampling points were studied, one on each side, totalizing 15 m of samplings: first sampling point was chosen on the canal side of station, area on an advanced clogging state, with abundant low height hygrophilous and hydrophilous vegetation (Fig. 2); the second sampling point was chosen on the east-west oriented part of the station, with slightly deeper, clear water, with fermentation marks present and no vegetation at shores (Fig. 3).



Figure 2: Sampling station SU1.



Figure 3: Sampling station SU1.

**SU2:** A portion of the right shore of the Sulina - Sfântu Gheorghe Canal. The station is located about 1 km south of Sulina Beach, on a widening of the course, where the water is almost stagnant. Both hydrophilous (*Elodea* sp., *Lemna* sp.) and hygrophilous vegetation (*Typha* sp., *Phragmites* sp.) are abundant (Figs. 4 and 5). Cattle marks are present at the shore (Fig. 6), the sector being probably used for watering. Plastic bottles and other garbages are also found in the water, due to the lack of speed of the water. The canal is frequently circulated by motor-boats, creating powerful waves towards the shores.



Figure 4: Sampling station SU2.



Figure 5: Sampling station SU2.



Figure 6: Sampling station SU2.

The sampling station is around 10 m long, 1 to 1.5 m wide, the water depth not exceeding 30 cm. Two sampling points were chosen, on the extremities of the station, totalizing 9-10 m of samplings. On the downstream one, no individual was collected.

**SU3:** A swamp sector formed by rain water collected on a lower area resulted from the construction of two local roads. Unlike SU1, the sector is at a constant level, therefore the swamp is much larger, and the water level is very low.

The station is located in the vicinity of Sulina Beach (around 100 m away); the size of the swamp is around 400 m<sup>2</sup> (a 20 per 20 m square), the shores are sandy, the water depth is under 20 cm, and hydro- and hygrophilous vegetation is abundant.



Figure 7: Sampling station SU3.



Figure 8: Sampling station SU3.

Around 10 m were sampled, from two sampling points: one on the typical part of the swamp (Fig. 7), with water invaded by hydrophilous vegetation (*Nymphaea* sp., *Lemna* sp., *Potamogeton* sp.) and a second one on an advanced clogging area (Fig. 8), with very low water level and thick *Typha* sp. and *Phragmites* sp. formations.

Access to the inner part of the station was very difficult, due to quicksand and unstable bottom therefore the samplings were made at shores.

**SU4:** A swamp sector, 500 m south of SU3, down the road parallel to the Sulina - Sfântu Gheorghe Canal. The swamp represents the remains of a larger wet area, formed by rain water collected below the lifted sector on which the road is constructed.

The station is quasi-circular, with around 10 m in diameter. Water is shallow (10-15 cm), turbid and fermented. Reed (*Phragmites* sp.) and Bulrush (*Typha* sp.) densely covers most of the station (Figs. 9, 10 and 11), excepting some marginal parts.

Cattle marks are present (Fig. 9), indicating its use for watering, and the station is located in the close proximity of a tourist facility, presenting the risk of anthropic intervention (tourists often collect reed flowers as a souvenir).

Two sampling points were chosen: one on a densely vegetated area (Fig. 10) and one on an opening in the vegetation, with larger open water sector (Fig. 11); about 10 m were sampled.



Figure 9: Sampling station SU4.



Figure 10: Sampling station SU4.



Figure 11: Sampling station SU4.

**SU5:** Left shore of the Sulina - Sfântu Gheorghe Canal, about 100 m west of Sulina Beach. The station is represented by a canal widening before the bridge leading to the beach (Fig. 12). Station parameters are: 15 m long, around 2 m wide, depth growing up to 40-50 cm away from the shore. The water flow is almost stagnant, and the current is directed towards the shore, and not on the normal flowing direction of the canal. No signs of human impact are present, except periodical disturbance from boat circulation.

Hydrophilous (*Lemna* sp., *Elodea* sp.) and hygrophilous vegetation (*Typha* sp.) is present only on the extremities of the station (Fig. 13), the central part being clear (Fig. 14).



Figure 12: Sampling station SU5.



Figure 13: Sampling station SU5.



Figure 14: Sampling station SU5.

Three sampling points were investigated, totalizing 12-15 m of samplings, one on each extremity and one between the downstream extremity and the central part, different in vegetation composition: a station only with aquatic vegetation, one only with hygrophilous vegetation, and one with both types of vegetation present.

**SU6:** Right shore of the Sulina - Sfântu Gheorghe Canal, 50 m downstream SU5, after the bridge leading to Sulina Beach. Station is 15 m long, 1-1.5 m wide and with water level growing away from the shore up to 30-35 cm. The canal sector upstream the station is used for boat parking and fishing, therefore the flow speed is slow, and the current often comes towards the shore and not in the normal flowing direction (the situation is similar to SU5, but its frequency is lower in SU6).

Both aquatic and hygrophilous vegetation is scarcely represented (Figs. 15 and 16). Two sampling points were chosen, one on open water and one in a sector with denser vegetation (Fig. 17). About 10 m were sampled. Although the sector is intensely used for fishing, the water is clear and free of garbage. Like all other canal sectors, it is influenced by waves produced by boat circulation.



Figure 15: Sampling station SU6.



Figure 16: Sampling station SU6.



Figure 17: Sampling station SU6.

**SU7:** Right shore of the Sulina - Sfântu Gheorghe Canal, about 200 m downstream SU6. The station has stagnant water, being located after a pile of rocks remaining from the road construction, which block the normal water flow.

The station is about 6-7 m long, 2 m wide and about 1 m deep, the bottom being formed by rocks from the road construction, covered with algae (Fig. 18). The shore is steep, and dense pointy bushes emerge from it, shadowing the water (Fig. 19). Hygrophilous vegetation is scarce, aquatic vegetation absent and the water is filled with organic scraps (small branches, leaves, etc.), brought by the waves made by motor-boats. 10 m of samplings were taken from a single sampling point, covering the entire station.



Figure 18: Sampling station SU7.



Figure 19: Sampling station SU7.

**SU8:** A small puddle, in the proximity of the road leading to Sulina Beach, close to SU6. The station is teardrop-shaped, 2.5 m long and 1 m wide, at its maximum extension. The genesis of the station is similar to SU4 (the remnant part of a former larger humid area).

The bottom is sandy, water is shallow (around 25-30 cm), low height hygrophilous vegetation is abundant (Fig. 20), and the water is clogged by algae (*Spyrogira* sp.) (Fig. 21). Plastic cups and bottles are a consistent presence, showing that the station is used by tourists as a garbage container. About 5 m of samplings were taken from a single sampling point, covering the entire habitat.



Figure 20: Sampling station SU8.



Figure 21: Sampling station SU8.

**SU9:** Large swamp sector formed by rain water collected on a lower area resulted from the construction of the road leading to Sulina Beach, about 200 m west of the beach.

The station is square shaped, about 50 per 50 m, water depth, 15 to 20 cm in the largest part of the station, thick Bulrush (*Typha* sp.) formations are covering the habitat (Fig. 22), *Mentha* sp. plants are frequent on the sectors with very low water. Few marginal sectors have deeper (around 50 cm) and open water (Fig. 23), with intense fermentation marks (Fig. 24).

Two sampling points were chosen, one on the typical Bulrush section, and one on an open water sector, totalizing 15 m of samplings.

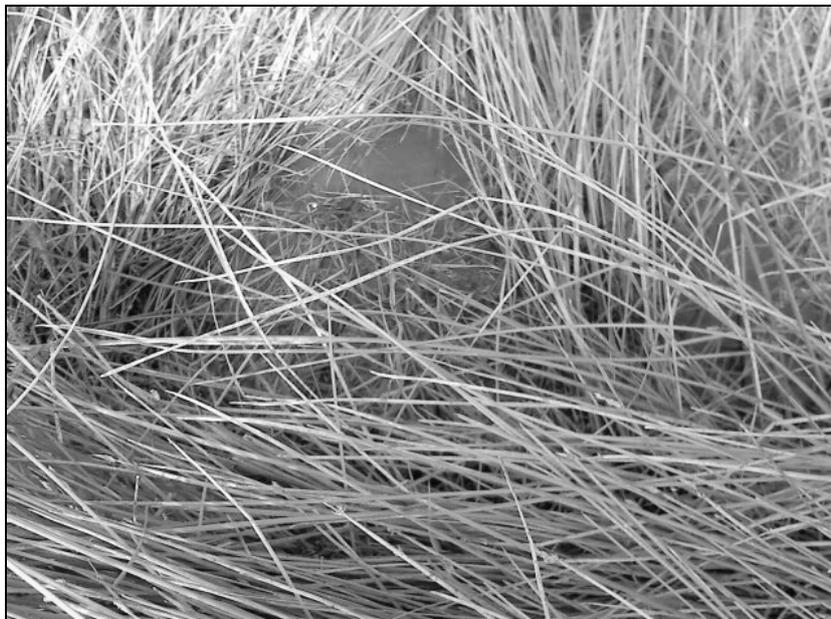


Figure 22: Sampling station SU9.



Figure 23: Sampling station SU9.



Figure 24: Sampling station SU9.

Samplings were similar in all sites, but the structure of each station was taken into concern. One sample of 5 to 15 meters in length was taken, in order to cover the entire habitat (water surface and body, hydrophilous and hygrophilous vegetation, where present, station bottom); the samples were collected during a single campaign in September 2010, with an entomological net with a 60 cm<sup>2</sup> mesh-size. Identification of the species was made at a stereo binocular by the morphological features or by the configuration of genitalia, where necessary, using data from known specialists (Jansson, 1986; Davideanu, 1999). Species and higher taxa nomenclatures are according to the system developed by Aukema (2004).

## RESULTS AND DISCUSSIONS

628 individuals (adults and larvae) belonging to 15 aquatic and semi-aquatic Heteroptera species from six families inhabit the chosen sampling stations: *Sigara (Halicorixa) mayri* (Fieber 1860), *Sigara (Halicorixa) stagnalis* (Leach 1817), *Sigara (Sigara) assimilis* (Fieber 1848), *Sigara (Sigara) striata* (Linnaeus 1758), *Sigara (Vermicorixa) lateralis* (Leach 1817) and *Micronecta (Dichaetonecta) scholtzi* (Fieber 1860), from the Corixidae Family, *Aquarius paludum* (Fabricius 1794), *Gerris (Gerris) argentatus* Schummel 1832 and *Gerris (Gerris) lacustris* (Linnaeus 1758), from the Gerridae Family, *Microvelia (Microvelia) reticulata* (Burmeister 1835), from the Microveliidae Family, *Mesovelia furcata* Mulsant and Rey 1852 and *Mesovelia vittigera* Horvath 1895, from the Mesoveliidae Family, *Notonecta (Notonecta) glauca glauca* Linnaeus 1758 and *Notonecta (Notonecta) viridis* Delcourt 1909, from the Notonectidae Family, and *Plea minutissima minutissima* Leach 1817, from the Pleidae Family. Species' distribution is presented in the table 1. Alongside the species' adults, an important number of larvae were collected, showing good reproductive conditions for the group. Their identification at species level is impossible (Andersen, 1982; Jansson, 1986). Furthermore, in three of the stations, SU1, SU3 and SU6, female individuals belonging to the Corixidae Family were sampled, again impossible to differentiate at species level (Jansson, 1986), therefore presented as *Sigara* sp. and *Micronecta (Dichaetonecta)* sp.

Table 1: Aquatic and semi-aquatic Heteroptera sampled in the area (number of individuals).

Species	Station	SU	SU	SU	SU	SU	SU	SU	SU	SU
		1	2	3	4	5	6	7	8	9
<b>Family Corixidae</b>										
<i>Micronecta (Dichaetonecta) scholtzi</i>		-	2	-	-	-	-	-	-	-
<i>Micronecta (Dichaetonecta) sp.</i>		-	-	-	-	-	1	-	-	-
<i>Sigara (Halicorixa) mayri</i>		8	-	-	-	-	-	-	-	-
<i>Sigara (Halicorixa) stagnalis</i>		28	-	-	-	-	-	-	-	-
<i>Sigara (Sigara) striata</i>		-	-	-	5	-	-	7	-	-
<i>Sigara (Sigara) assimilis</i>		1	-	-	-	-	-	-	-	-
<i>Sigara (Vermicorixa) lateralis</i>		208	-	4	10	-	-	-	-	-
<i>Sigara</i> sp.		87	-	1	-	-	-	-	-	-
Corixinae (larvae)		140	-	-	-	10	-	3	-	-
<b>Family Gerridae</b>										
<i>Aquarius paludum</i>		-	-	-	-	-	-	4	-	-
<i>Gerris (Gerris) argentatus</i>		1	-	1	3	-	-	-	-	-
<i>Gerris (Gerris) lacustris</i>		-	-	-	1	-	-	-	-	-
Gerridae (larvae)		-	-	-	-	2	-	-	-	2
<b>Family Veliidae</b>										
<i>Microvelia (Microvelia) reticulata</i>		-	-	1	-	-	-	-	7	4
<b>Family Mesoveliidae</b>										
<i>Mesovelia furcata</i>		1	-	-	-	9	-	1	-	-
<i>Mesovelia vittigera</i>		-	5	4	1	22	-	-	-	-
<b>Family Notonectidae</b>										
<i>Notonecta (Notonecta) glauca</i>		-	-	1	-	-	-	-	-	3
<i>Notonecta (Notonecta) viridis</i>		-	-	-	-	-	-	-	-	4
<b>Family Pleidae</b>										
<i>Plea minutissima</i>		-	10	3	-	10	-	-	1	12

At the first glance, the area provides excellent conditions for the target group, found in every habitat investigated, even in the ones with poorer conditions, such as SU6 or SU8. The number of species is not very high (15 out of 67 already found in Romania - Davideanu, 1999; Ilie, 2009), and the majority are the most frequent ones for our country (Davideanu, 1999; Ilie, 2009; Olosutean and Ilie, 2008a, 2010a, 2010b; Ilie and Olosutean, 2009), and are usual inhabitants of the Danube area (Kis and Davideanu, 1994; Nosek et al., 2007; Skern et al., 2010). However, species like *S. mayri* and *M. vittigera* are rare sightings in Romanian fauna, the first species being sampled only once in the past, again in the Danube Delta, at Periprava, north-west of Sulina (Kis and Davideanu, 1994), and the last being found so far only in southern Transylvania (Ilie and Davideanu, 2002; Olosutean and Ilie, 2008b). *D. scholtzi* has a slightly different situation: it is known as a common species in Transylvania (Ilie, 2009) and Moldavia (Davideanu, 1999), but was not mentioned nowhere in the southern part of Romania (the southernmost sample comes from Bacău - Davideanu, 1999).

Analyzing the communities from a faunistic point of view, we can easily observe that the Corixidae are dominant, as the number of species (6 out of 15, or 40%), as well as the number of individuals (362 adults out of 471, or 76.85%). Of great importance in that aspect is station SU1, which provides four Corixidae species (three of them - the rare *S. mayri*, *S. stagnalis* and *S. assimilis* - not sampled elsewhere in the area), and a large number of individuals (332 corixid adults, representing 70.49% from the total adults sampled, and 140 larvae, representing 89.17% from the collected larvae).

As for the habitats, they are under the influence of ever-changing conditions caused by the particular configuration of the area: sandy substratum and high level of the water table lead to the accumulation of large water quantities at every heavy rain (Fig. 25). After such an event, many of the stations investigated in the present study became part of a few very large puddles (Figs. 26 and 27), which were covering several km<sup>2</sup>, making possible for members of the group to migrate over large areas with little effort. Given the fact that they are poor fliers, this acts as a huge colonial advantage, therefore their strong presence in the studied area.



Figure 25: Local flooding after heavy rain.

Even more, road construction creates anthropic barriers, stopping the normal water drainage and creating conditions for swamp formation. In that order, suitable habitats for feeding and reproduction are temporarily larger than usual offering excellent conditions for aquatic and semi-aquatic Heteropterans. In other words, the sampling stations we investigated act as refuges or as nurseries for the group, the individuals spreading throughout the region at high waters.



Figure 26: SU8 before the rain.



Figure 27: SU8 after the rain.

Such a nursery, for *A. paludum*, was found in SU7 (Fig. 28). A large number of different instar larvae were spread throughout the station, guarded by adults. Almost all sampled and observed adults were coupled for reproduction. The entire group was clustered on a limited lenitic area (around 2.5 m away from the shore), found beneath a pile of rocks, and tended to stay inside that area even during the samplings (the adults performed their known evasive manoeuvres - jumping, fast rowing - but did not surpass the stagnant water limit).

Powerful waves made by motor-boats had different influence on the individuals: adults seem not to be bothered, while all the larvae were jumping off the water at strong waves, and the small ones even at little waves made by the net during the sampling, showing gradual habituation with the given conditions.

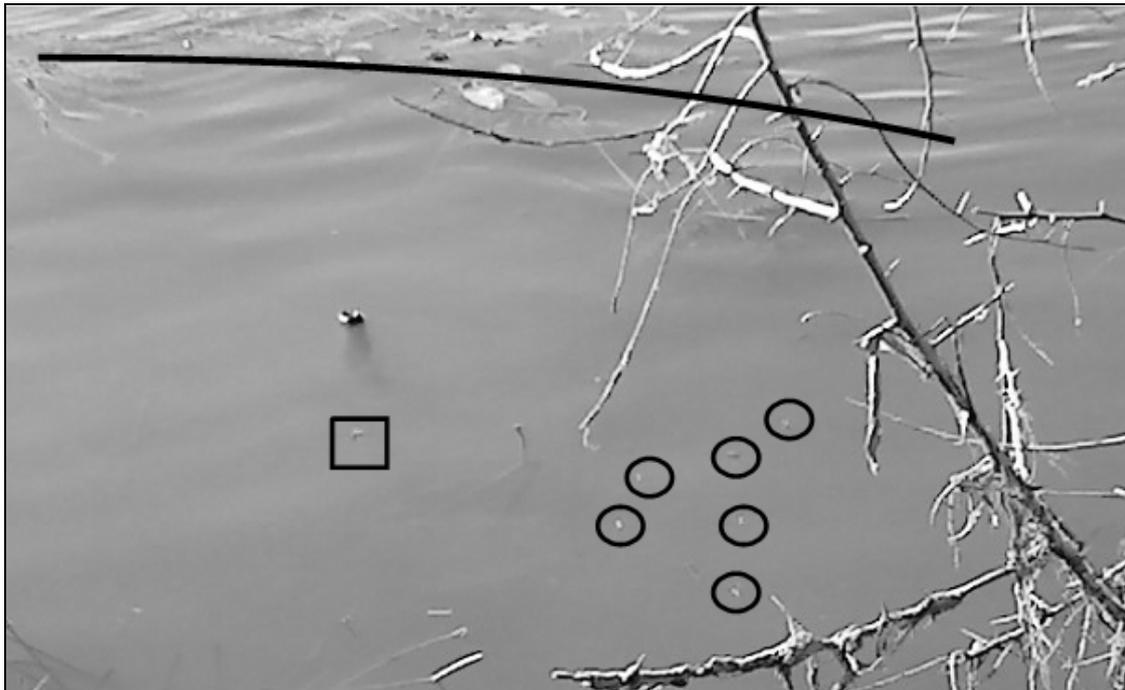


Figure 28: *A. paludum* nursery in SU7  
(squares - adults; circles - larvae; curved line - stagnant water limit)

Because the stations are, in fact, the lowland areas where the flooding water retreats last, their water quality suffers several transformations: eutrophication and fermentation are the most common ones (Figs. 29 and 30), favoured by high summer temperatures and condensed nutrient quantities from the remaining waters. An interesting situation is the change in water colour that occurred at 24-36 hours after the rain. The darker water colour (Fig. 31) might come from tannins dissolved from plants, or from dissolved soil substances, and was observed in other parts of the Danube Delta too (at Sfântu Gheorghe, E. Schneider-Binder, in verbum). The actual cause and its influence on the insects need further investigation.



Figure 29: Water quality transformations: *Spyrogira* sp. growth in SU8.



Figure 30: Water quality transformations: fermentation in SU9.

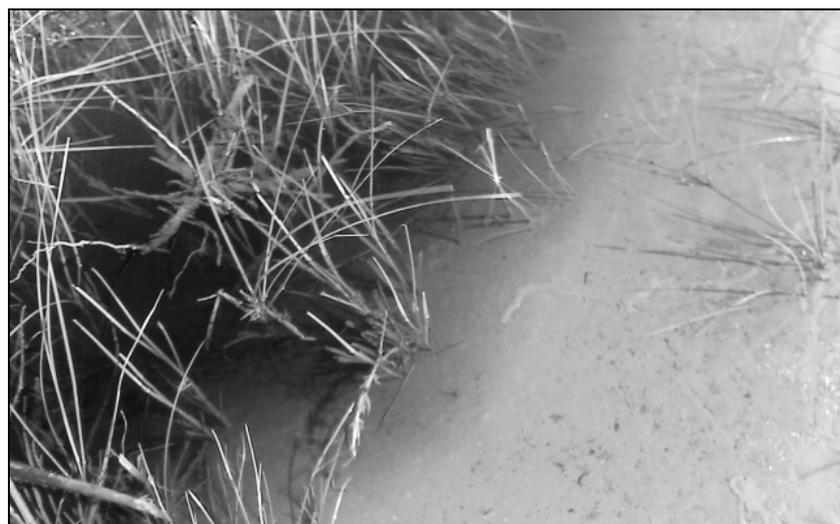


Figure 31: Water quality transformations: colour change in SU8.

## CONCLUSIONS

An important number of species and individuals were collected from a relatively small area (around 4 ha), showing a high potential from the group's point of view. Each station presents a different community structure, confirming previous studies that indicated the preference for a specific micro-niche of most species.

*G. lacustris* is the most notorious and wide spread species of the group probably because is not respecting the previous rule, and is adaptable to all kinds of habitat conditions. However, we found only one individual in the entire sampling area, and very few gerrids altogether, the most plausible causes being the lack of large stagnant open waters, preferred by those species (the abundance of aquatic and semi-aquatic vegetation that covers almost all stagnant water areas is not proven as a restrictive factor for the gerrids, but most species need open waters with a slow flow).

Some of the sampling stations we chose are a temporary condition, remains of much larger flooded areas. Every heavy rain is modifying the configuration of the wetlands, creating the possibility for the species to migrate through immense areas, in order to find trophic resources or to reproduce. Anthropogenic barriers, e.g. lifted areas used for road construction, play an important restrictive role in the migration of the individuals, dividing those flooded areas.

Being small parts of a once larger water surface leads to a particular water composition and quality: fermented water and eutrophication are frequent, as well as a high quantity of dissolved salts, including sodium chloride from the sea water. At high waters, the conditions change, forcing the species to adapt to unstable and ever-changing habitats, a situation that seems favorable for the group.

Anthropic impact on different degrees was present in most stations: powerful waves and fuel marks from motor-boats, cattle watering, plastic bags, bottles and other types of garbage. Species distribution and number of individuals seem not to be influenced by this factor, proving again the well known fact that most species are insensible to moderate human impact, some being even favored by it. This fact, correlated with their tolerance to the permanent change of conditions, presents the group as very versatile and easily adaptable to habitat interference.

From a strictly faunistic point of view, we have two species, *D. scholtzi* and *M. vittigera*, that are new mentions for the Danube Delta area, and also for the entire southern Romania. In fact, all the species are new to the Sulina area, but all except the two presented before were sampled till now elsewhere in the Danube Delta. It might be concluded that the area has an important potential for the group's researchers, and additional studies are welcomed.

As a final general conclusion, we can state the fact that the area is offering a large variety of habitats for aquatic and semi-aquatic Heteroptera, fact that can be easily extrapolated to the entire Danube Biosphere Reserve: small ponds, marshes, slow flowing river sectors, lakes are forming the largest part of the Danube Delta, and they are frequently inhabited by our group. In other words, the large variety of aquatic habitats should provide support for a large variety of species, a fact partially proved by this study and opened for further discussions.

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**ANT DIVERSITY ON *SONNERATIA CASEOLARIS* TREES  
IN REMBAU-LINGGI MANGROVE FOREST,  
PENINSULAR MALAYSIA**

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**KEYWORDS:** Peninsular Malaysia, mangrove forests, ant communities, distribution, abundances.

**ABSTRACT**

Ants are reported to inhabit mangrove trees in various localities throughout the world. However, little is known about their species diversity. In this study, we investigated the diversity and abundance of arboreal ants on mangrove apple trees, *Sonneratia caseolaris* Engl. in the Rembau-Linggi mangrove, Peninsular Malaysia. We collected nests and foraging individuals found on the trees (branch or foliage). In total we sampled 699 ants of nine species (from four subfamilies), of which 62% were obtained from the nests, 30% from tree branches and 8% from the foliage. *Dolichoderus* sp. was the most frequent and abundant species on the trees, followed by *Crematogaster* sp. Our study has shown that ants in the mangrove are diverse and sometimes living in abundance even on a single *S. caseolaris* tree. We suggest future studies that focus on other mangrove plants in order to increase our database on mangrove ant diversity.

**ZUSAMMENFASSUNG:** Die Ameisenvielfalt an *Sonneratia caseolaris* Bäumen in den Rembau-Linggi Mangroven Wäldern der Malaysia Halbinsel.

Ameisen werden an Mangrovenbäumen in verschiedenen Orten rund um die Welt gemeldet. Dennoch weiß man sehr wenig über ihre Artendiversität. In vorliegender Arbeit werden die Ergebnisse einer Untersuchung der Diversität und Abundanz von Baumameisen an Mangroven Apfelbäumen *Sonneratia caseolaris* Engl. der Rembau-Linggi Mangrove/Malaysia Halbinsel vorgestellt. Es wurden Nester und fressende Individuen von den Bäumen d. h. von Ästen und Laub gesammelt. Insgesamt wurden 699 Ameisenindividuen, zugehörig zu neun Arten aus vier Unterfamilien erfasst, von denen 62% aus den Nestern, 30% von Ästen und 8% vom Laub stammen. *Dolichoderus* sp. war an Bäumen die häufigste und abundant vertretene Art, gefolgt von *Crematogaster* sp. Unsere Studie belegt, dass die Ameisen in Mangroven vielfältig sind und manchmal in großer Menge an einem einzigen *Sonneratia caseolaris* Baum leben. Wir empfehlen zukünftige Studien auf andere Mangrovenpflanzen auszurichten, um auf diese Weise unsere Datenbasis zur Diversität der Ameisen in Mangroven zu vergrößern.

**REZUMAT:** Diversitatea furnicilor pe arbori de *Sonneratia caseolaris* în pădurile de mangrove Rembau-Linggi ale peninsulei Malaysia.

Furnici, având ca habitat arbori de mangrove, sunt menționate din diferite părți ale lumii. Totuși, există foarte puține date asupra diversității lor specifice. În studiul de față, este prezentată diversitatea și abundența furnicilor arboricole pe mărul de mangrove, *Sonneratia caseolaris* Engl., din mangrovele Rembau-Linggi ale peninsulei Malaysia. Au fost colectate cuiburi și indivizi de furnici hrănindu-se pe arbori (ramuri și frunziș). În total, au fost colectate 699 de specimene, aparținând la nouă specii (din patru subfamilii), din care 62% din cuiburi, 30% de pe ramuri și 8% de pe frunziș. *Dolichoderus* sp. a fost cea mai frecventă și abundentă specie pe arbori, urmată de *Crematogaster* sp. Studiul nostru a dovedit că furnicile din mangrove sunt diverse, trăind în abundență, uneori, chiar pe un singur arbore de *Sonneratia caseolaris*. Sugerăm ca studiile viitoare să se axeze pe alte specii din pădurile de mangrove, pentru a mări baza de date asupra diversității furnicilor de mangrove.

## INTRODUCTION

Ants are a very common and widespread group of insects belonging to the order Hymenoptera (Daly et al., 1978; Borror et al., 1989). There are 16 subfamilies of ants with 296 genera and more than 9,000 described species in the world (Alonso et al., 2000). The habitats of ants include peat swamps, mangrove forests and mountain forests at 1,500 m above sea level (Pfeiffer and Linsenmair, 2000).

Ants are reported to inhabit mangrove trees in various localities throughout the world (Nielsen, 1997, 2000; Dejean et al., 2003; Ward and Harris, 2005; Hashim et al., 2010). They are often abundant, particularly in the canopy, with most of them nesting in the hollow branches (Dejean et al., 2003; Hogarth, 2007) and one found to be a mud-nesting species, *Polyrhachis sokolova* (Nielsen, 1997). Their abundance suggests that ants are important for the plant community through their interactions with the herbivores (Cannicci et al., 2008). One obvious species is the weaver or tailor ant, *Oecophylla smaragdina* that feeds on sugary secretion of coccids bugs that suck plant sap (Hogarth, 2007), and by the presence of their pheromone, these ants can deter the leaf beetles, *Rhyparida wallacei* from feeding on the leaves of *Rhizophora mucronata* (Offenberg et al., 2004).

Although the distribution of ants in the mangrove has been well studied, little is published about their diversity (Clay and Andersen, 1996; Nielsen, 2000; Dejean et al., 2003; Hogarth, 2007). There are 24 ant species recorded in northern Australia (Clay and Andersen, 1996; Nielsen, 2000) which make up as the only available data on mangrove ant diversity so far. A recent study was conducted by Hashim (2010) who found four species of predominantly ground-dwelling ants living in fragmented mangrove forests in Peninsular Malaysia.

In this study, we investigated the arboreal ants living on mangrove apple trees, *Sonneratia caseolaris* Engl, attempting to find out how many species of ants inhabit *S. caseolaris* trees and how abundant is each ant species.

## MATERIALS AND METHODS

### Study area

This study was carried out in the Rembau-Linggi mangrove forest (2°26.7' north, 102°3.5' east), Peninsular Malaysia (Fig. 1). The fieldwork took place along Rembau River (one of the tributaries of Linggi River) in November 2009, during the rainy season. Here the *Sonneratia caseolaris*, living in association with other mangrove species such as *Rhizophora*, Sea Hibiscus and Nipa Palms, covers a total of about 9 km of the river.

### Sampling methods

To determine the number of ant species on *S. caseolaris* trees, collection activities were carried out on eight selected trees along 4.5 km of the Rembau River (Fig. 1). Collection of nests and foraging individuals in the canopy took place at high tide, because our boat could get closer to the trees (Nielsen, 1997). In each tree, the nests and foraging individuals were collected by hands or using forceps, for a total of two days (with about 45 minutes spent at each tree). Foraging ants were collected from the tree branches and foliage. The nests were easily collected because they were mostly made up of folded leaves. Immediately after being collected, all specimens were placed in vials containing 70% ethanol. Identification of all specimens was carried out in the laboratory at the Faculty of Agriculture, Putra University of Malaysia and Forest Research Institute Malaysia (FRIM).

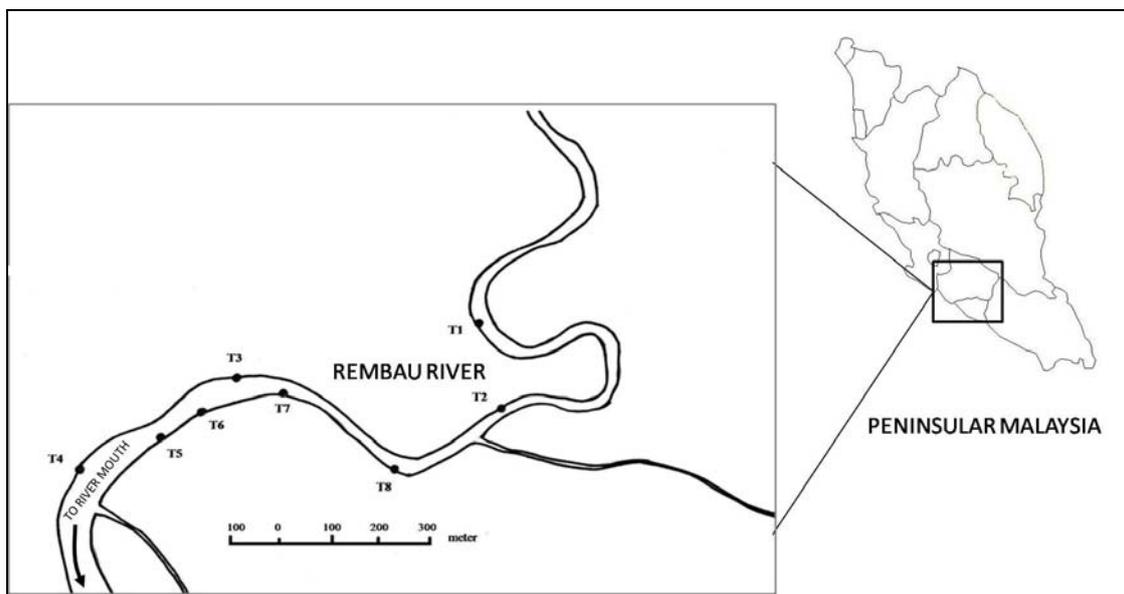


Figure 1: Map of study site (sampled trees are T1-T8).

### RESULTS

We have collected 699 individuals from the eight *S. caseolaris* trees (Tab. 1). A total of 63% of the sampled individuals were taken from the nests, 29% from tree branches and another 8% from the foliage. Nine species were found on the sampled trees consisting of four subfamilies: Dolichoderinae, Formicinae, Myrmicinae and Pseudomyrmicinae.

The most frequently found genus was *Dolichoderus* sp. which was present on all trees excepting T6. This was followed by *Crematogaster* sp., present on five trees (T1, T2, T3, T5 and T7). Other genus (*Tapinoma* sp., *Iridomyrmex* sp., *Technomyrmex* sp., *Polyrhachis* sp., *Tetraponera* sp., *Camponotus* sp. and unidentified sp.) were found on 1 to 3 of sampled trees.

A major collection of *Dolichoderus* sp. was obtained from four out of five nests (391 individuals), which is the highest abundance of ants recorded in this study (Fig. 2). The ants of this species were also found foraging on branches (34 individuals) and foliage (31 individuals). An abundant species on the branches was *Crematogaster* sp. with 122 individuals compared to 15 individuals collected on foliage (Fig. 2).

Fifty-four individuals of *Tapinoma* sp. were collected from T3 and T7 (Tab. 1). Of these, 35 were sampled from one nest, 13 were found on branches and others were found on foliage (Fig. 2). The only species that was found on T6 was *Iridomyrmex* sp., with most of them foraging on branches. Another species that can be found on both branches and foliage was *Polyrhachis* sp. (8 individuals). This species was found on three trees (T3, T4 and T8). Meanwhile, four species, namely *Technomyrmex* sp., *Tetraponera* sp., *Camponotus* sp., and an unidentified species (a single male specimen) were found on the tree branches or foliage only.

Table 1: The presence/absence of species on each sampled tree (1 - presence; 0 - absence. T1 = tree 1, T2 = tree 2, T3 = tree 3, T4 = tree 4, T5 = tree 5, T6 = tree 6, T7 = tree 7, T8 = tree 8).

Species	T1	T2	T3	T4	T5	T6	T7	T8	Total no. of individuals
<i>Dolichoderus</i> sp.	1	1	1	1	1	0	1	1	457
<i>Crematogaster</i> sp.	1	1	1	0	1	0	1	0	137
<i>Tapinoma</i> sp.	0	0	1	0	0	0	1	0	54
<i>Iridomyrmex</i> sp.	0	0	0	0	0	1	0	1	23
<i>Technomyrmex</i> sp.	0	0	1	0	0	0	1	0	15
<i>Polyrhachis</i> sp.	0	0	1	1	0	0	0	1	8
<i>Tetraponera</i> sp.	0	1	0	0	0	0	0	1	3
<i>Camponotus</i> sp.	0	0	1	0	0	0	0	0	1
Unidentified species	0	0	0	0	1	0	0	0	1
Total no. of species on each tree	2	3	6	2	3	1	4	4	699

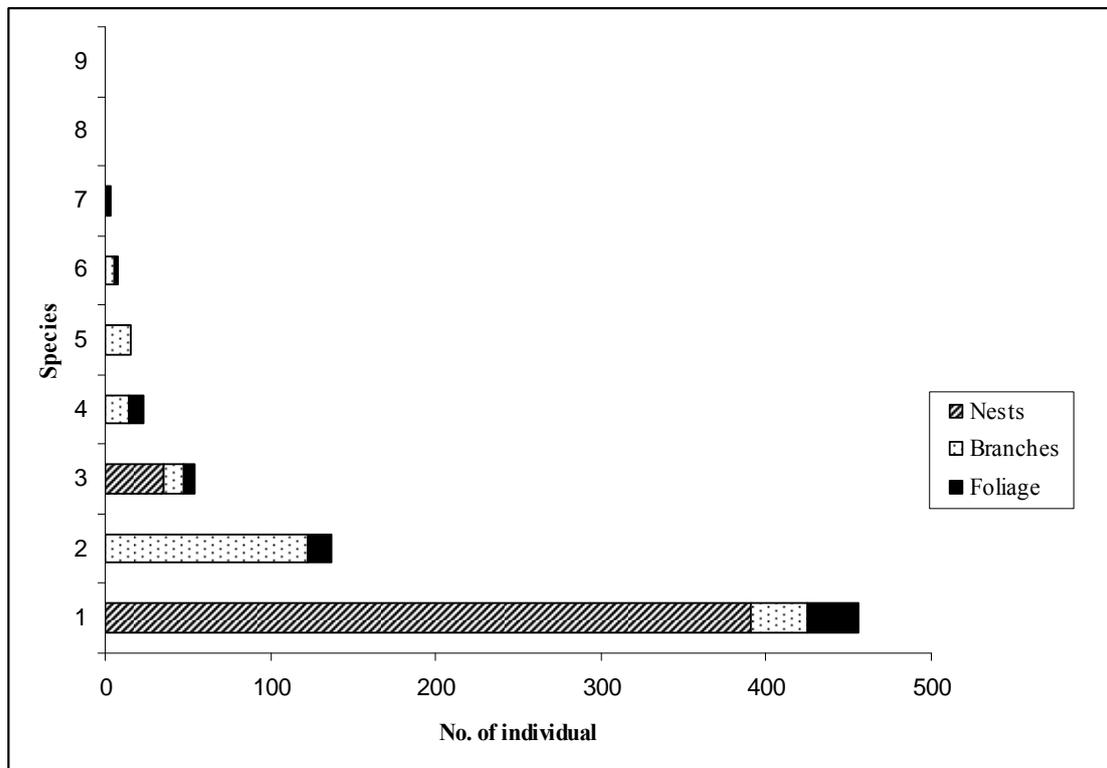


Figure 2: Number of ants according to source of collection; species number according to Tab. 1.

## DISCUSSIONS

The present study results suggest that *S. caseolaris* trees of Rembau-Linggi mangrove forests support a rich and abundant ant fauna. Two ant genus, *Dolichoderus* sp. and *Crematogaster* sp., are dominant on *S. caseolaris* trees. Both species are attracted to plants that produce nectars and *Sonneratia* is one of the best examples (Ng and Sivasothi, 2001). Similar to our study, *Crematogaster* sp. was the major forager on tree branch compared to other species in Australian mangroves (Clay and Andersen, 1978; Nielsen, 2000).

One of the most remarkable findings of this study was the discovery of a nest and foraging individuals of *Tapinoma* sp. Our present findings are in agreement with Nilesen (2000), who hypothesized that *Tapinoma* sp. forages outside the nest as no coccids is to be found in it. Another interesting species, *Iridomyrmex* sp., with more than 20 individuals were found on two trees in our study area. The findings are inconsistent with Clay and Andersen (1996), who inferred that this species do not forage extensively into the mangroves.

In our study, a low abundance of *Tetraoponera* sp. was recorded probably because we could not sample the individuals, as they tend to remain inside hollow twigs and feed on tiny insects found in these twigs (Nielsen, 2000; Ng and Sivasothi, 2001). In contrast, the collected ants of *Technomyrmex* sp. were all foragers on branches. This specific genus is reported to be the dominant ants in Nansei Island, Japan (Terayama, 1989) but these ants have been introduced into New Zealand and may pose a threat to the native ants (Ward and Harris, 2005). Also in this study, we found several individuals from the genus *Polyrhachis*, which is the most common in mangrove foliage (Ng and Sivasothi, 2001), also being the most captured throughout Australian mangroves (Clay and Andersen, 1996).

## CONCLUSIONS

In conclusion, our present study has shown that the arboreal ants are diverse and can be found in abundance even on a single *S. caseolaris* tree. We suggest future studies to focus on other mangrove plants in order to increase our database on mangrove ant diversity.

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## CADDISFLY (INSECTA, TRICHOPTERA) ASSEMBLAGES IN THE VIȘEU RIVER BASIN (ROMANIA)

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**KEYWORDS:** Romanian Carpathians, Maramureș Mountains, Vișeu River basin, Țâșla River, Vaser River, Ruscova River, Frumușeaua River, Trichoptera larvae communities.

### ABSTRACT

This study presents the description of the structure and diversity analysis of the Trichoptera larval communities of the Vișeu River basin. The paper is based on quantitative benthic macro-invertebrates and qualitative caddisfly samples, collected in 2007 (June - September), from 29 sampling stations. In the reference zone, 30 caddisfly species were identified, belonging to 19 genera and 11 families, representing 11.24% of the Romanian Trichopteran fauna. The caddisfly larval communities exhibit high diversity on the Frumușeaua River basin, on the upper Vișeu River course and also on the Ruscova River basin. Here, the habitats characteristic for Trichoptera show good condition, and human impact is insignificant. These areas should be managed towards aquatic biodiversity conservation. Caddisflies exhibit their lowest diversity in the Țâșla Stream, between the confluence with the Bălăsâna Stream and that with the Vinișoru Stream (area affected by pollution from mining exploitation) and in the Vișeu River 50 m upstream of the Moisei locality (the sector affected by the Borșa locality impacts). These sectors require the rehabilitation of the aquatic habitats.

**RESUMEN:** Asociación de fríganos (Insecta, Trichoptera) en la cuenca del Río Vișeu (Rumania).

Este estudio presenta la descripción de la estructura y el análisis de la diversidad de la comunidad de larvas de Trichoptera de la cuenca del Río Vișeu. El artículo se basa en muestras cuantitativas de macro-invertebrados bentónicos y muestras cualitativas de los fríganos, recolectadas en 2007 (Junio-Septiembre), en 29 estaciones de recolecta. En la zona de referencia, se identificaron 30 fríganos, pertenecientes a 19 géneros y 11 familias, que representan el 11.24% de la fauna de Trichoptera de Rumania. La comunidad de larvas de fríganos presenta alta diversidad en la cuenca del Río Frumușeaua, en la parte alta del curso del Río Vișeu y también en la cuenca del Río Ruscova. Aquí los hábitats característicos para los Trichoptera presentan un buen estado y el impacto humano es insignificante. Estos hábitats deben ser manejados con enfoque hacia la conservación de la diversidad acuática. Los fríganos presentan la más baja diversidad en el arroyo Țâșla, entre la confluencia con el arroyo Bălăsâna y la confluencia con el arroyo Vinișoru (área afectada por la contaminación por explotación minera) y en el Río Vișeu 50 m río arriba de la localidad Mosei (sector afectado por el impacto de la localidad de Borșa). Estos sectores requieren de rehabilitación.

**REZUMAT:** Comunități de trichoptere (Insecta, Trichoptera) în bazinul râului Vișeu (România).

Lucrarea prezintă descrierea structurii comunităților larvelor de trichoptere, din bazinul hidrografic Vișeu și analiza diversității acestor comunități. Datele prezentate în lucrare se bazează pe probe cantitative de bentos și calitative de trichoptere, colectate în anul 2007 (iunie - septembrie), din 29 stații de prelevare.

În zona de referință, au fost identificate 30 specii de trichoptere, aparținând la 19 genuri și 11 familii, acestea reprezintă 11,24% dintre speciile de trichoptere semnalate în România.

Comunitățile larvelor de trichoptere prezintă diversitate mare în bazinul Frumușeaua, în cursul superior al Vișeului și în bazinul Ruscova. Aici, habitatele caracteristice trichopterelor prezintă o stare bună, iar impactul antropic este nesemnificativ. Aceste zone trebuie manageriate, în sensul conservării biodiversității acvatice.

Cea mai mică diversitate a trichopterelor se înregistrează în Țâșla, între confluența cu Bălăsâna și confluența cu Vinișoru (sector afectat de poluarea de la exploatarea miniere) și în Vișeu, 50 m amonte de localitatea Moisei (sector afectat de impactul generat de localitatea Borșa). În aceste sectoare, se impun măsuri de reabilitare a habitatelor acvatice.

## INTRODUCTION

This study presents description of the structure and diversity analysis of Trichoptera larvae communities of the Vișeu River basin.

The Vișeu River is a second order tributary of Danube localized in the north part of the Romanian territory. The majority of the Vișeu River basin was included in the Maramureș Mountains Nature Park.

Vișeu River has its sources in the Rodna Mountains, 80 km length, 1606 km<sup>2</sup> catchment basin and a multiannual average flow at the confluence with Tisa River of 30.7 m<sup>3</sup>/s. Some of the most important tributaries of Vișeu River are (from upstream to downstream): Țâșla River (20 km length, 106 km<sup>2</sup> drain surface), Vaser River (42 km length, 422 km<sup>2</sup> drain surface, 9 m<sup>3</sup>/s multiannual average flow at the confluence with Vișeu River) and Ruscova River (39 km length, 435 km<sup>2</sup> drain surface, 11 m<sup>3</sup>/s multiannual average flow at the confluence with Vișeu River). (Roșu, 1980; Badea et al., 1983; Posea et al., 1982)

Actual research on the macroinvertebrate communities in this area are few, in this respect we have to mention the study concerning the benthic macroinvertebrates too along Vișeu River (Staicu et al., 1998), Vișeu River and some tributaries ecological assessment based on macroinvertebrate communities (Curtean-Bănăduc, 2008) and the studies regarding mayfly assemblages and stonefly assemblages in the Vișeu watershed (Curtean-Bănăduc, 2009; Curtean-Bănăduc, 2010).

## MATERIALS AND METHODS

This paper is based on quantitative benthic macroinvertebrates and caddisflies qualitative samples, sampled in 2007 (June - September), in 29 sampling stations (Fig. 1).

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 overexploitation of the river bed mineral resource and exploitation of riverine lands, in order to highlight the Trichoptera species diversity, and also the variation of the communities' structure.

In each station were sampled quantitative samples from five points, in order to highlight the micro-habitats specific diversity. In the study period 290 quantitative benthic macroinvertebrates samples were sampled and analyzed.

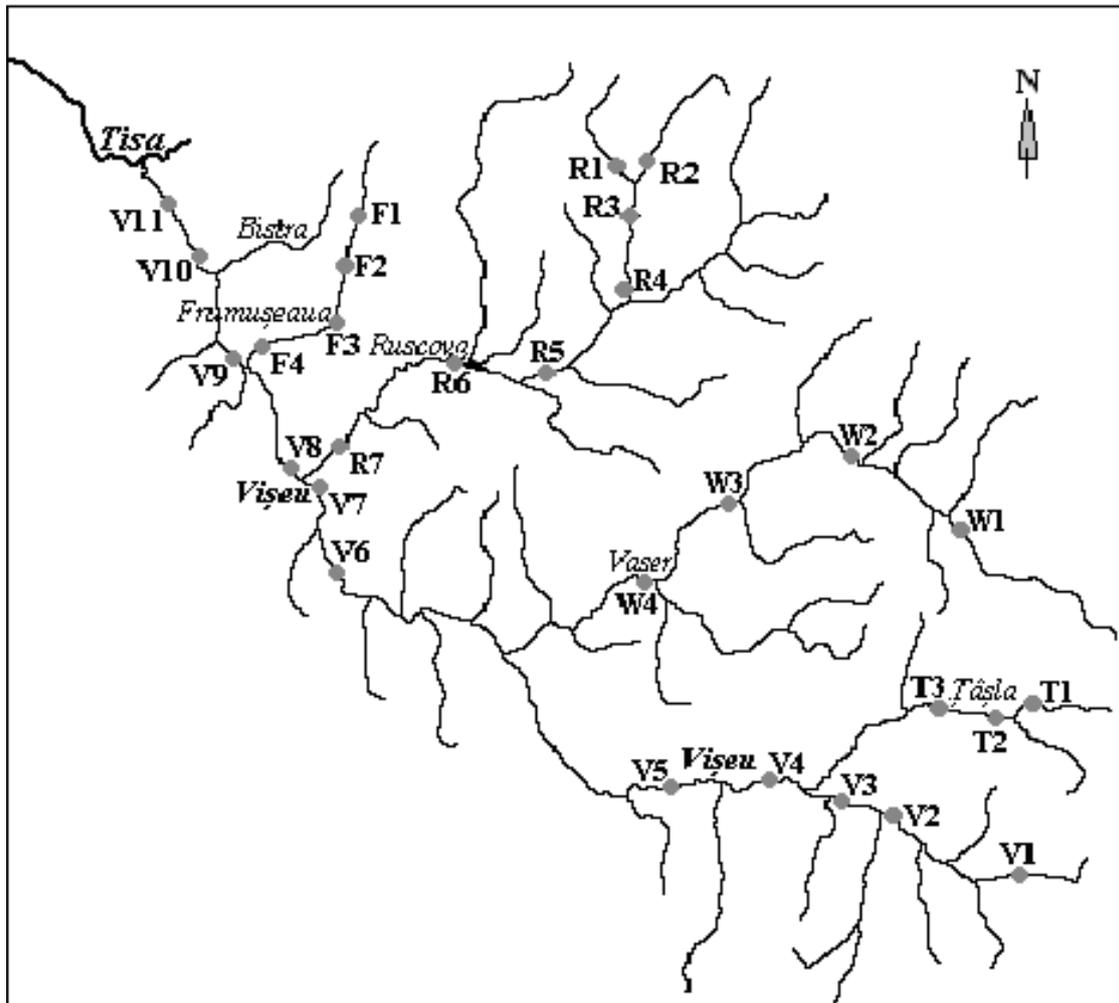


Figure 1: The Vișeu Basin sampling stations (V1-V11, T1-T3, W1-W4, R1-R7, F1-F4) layout.

The benthic macroinvertebrates quantitative samples were carried out with an 887 cm<sup>2</sup> surface Surber Sampler, with a 250  $\mu$ m mesh net. The sampled biological material was fixed in 4% formaldehyde solution at which NaHCO<sub>3</sub> was added.

The analyzed biological material included 3250 Trichoptera larvae in life cycle periods which allowed their identification to species level.

For the quantitative structure description of the caddisfly communities we have used the relative abundance (A%) and the statistical density (Ds). For the communities' diversity quantifying, the heterogeneity index Simpson (Gomoiu and Skolka, 2001; Krebs, 1989) were determined, based on the quantitative samples.

## RESULTS AND DISCUSSIONS

In the reference zone 30 caddisfly species were identified, belonging to 19 genera and 11 families. The identified caddisfly species list of the Vișeu River basin, with the specific sampling sites (V1 - V11, T1 - T3, W1 - W4, R1 - R7, F1 - F4 - sampling stations):

### Fam. Hydropsychidae

*Hydropsyche pelucidulla* (Curtis 1834) – V4, V5, V6, V7, V9, V10, V11, W2, W3, W4, R4, R5, R6, R7, F1, F2, F3, F4

*Hydropsyche fulvipes* Curtis 1834 – V9, V10, R7, F1

*Hydropsyche instabilis* (Curtis 1834) – V6, V8, V10, V11, R6, F2, F4

*Hydropsyche angustipennis* (Curtis 1834) – V6, V9, V11, R4, R7, F1, F2, F3, F4

### Fam. Polycentropodidae

*Plectrocnemia conspersa* (Curtis 1834) – V2

*Polycentropus flavomaculatus* (Pictet 1834) – V2

### Fam. Philopotamidae

*Philopotamus montanus* (Donovan 1813) – V1, V3, V4, T1, R3, F2

*Philopotamus variegatus* (Scopoli 1763) – W1, W2

### Fam. Brachycentridae

*Brachycentrus montanus* Klapalek 1892 – W4, R1, R2, R3, R4

### Fam. Goeridae

*Goera pilosa* (Fabricius 1775) – V4, R1, R2, R3, R4, R5

*Silo pallipes* (Fabricius 1781) – V3

### Fam. Limnephilidae

*Drusus bruneus* Klapalek 1898 – V1, V2, V3, V4

*Drusus discolor* (Rambur 1842) – V1, T1, T2, R4, R5, R6

*Halesus digitatus* (von Paula Schrank 1781) – V2, R1, R2, R3

*Limnephilus rhombicus* (Linnaeus 1758) – V3, V8, F2, F3

*Limnephilus lunatus* Curtis 1834 – V10, F1, F2, F3, F4

*Potamophylax latipennis* (Curtis 1834) – V2

*Mesophylax impunctatus* Mc Lachlan 1884 – R2

*Chaetopteryx polonica* Dziedzielewicz 1889 – R6

### Fam. Phryganeidae

*Oligotricha striata* (Linnaeus 1758) – F2, F3

### Fam. Beraeidae

*Ernodes articularis* (Pictet 1834) – R7

### Fam. Sericostomatidae

*Sericostoma personatum* (Kirby and Spence 1826) – V9, V10, V11, W1, W2, W3, W4, R2, R3, R4

### Fam. Glossosomatidae

*Glossosoma conformis* Neboiss 1963 – W3, W4

*Glossosoma boltoni* Curtis 1834 – V2, F4

### Fam. Rhyacophilidae

*Rhyacophila fasciata* Hagen 1859 – V1, V2, V3, V4, V6, V7, V8, V9, V10, V11, T3, R4, R5, R6, R7, F1, F2, F3, F4

*Rhyacophila nubila* Zetterstedt 1840 – R6, F2

*Rhyacophila tristis* Pictet 1834 – V2, V3, R1, R2, R3, R4, R7, F1

*Rhyacophila flava* Klapalek 1898 – F2, F3, F4

*Rhyacophila obliterated* Mc Lachlan 1863 – R4, R7

*Rhyacophila philopotamoides* Mc Lachlan 1879 – R7

The caddisfly species with the widest distribution in the Vişeu River basin is *Rhyacophila fasciata* (present in 19 of the 29 studied lotic sectors) and *Hydropsyche pelucidulla* (present in 18 of the 29 studied lotic sectors). The species with the most restricted distributions are *Plectrocnemia conspersa*, *Polycentropus flavomaculatus*, *Silo pallipes*, *Potamophylax latipennis*, *Mesophylax impunctatus*, *Chaetopteryx polonica*, *Ernodes articularis* and *Rhyacophila philopotamoides* sampled only in the one of the 29 studied lotic sectors.

The species *Mesophylax impunctatus* and *Chaetopteryx polonica* were identified exclusively in quantitative samples.

In the reference zone, the caddisflies had the highest species diversity (10 species) in the Tomnatec Stream 200 m upstream the confluence with Pop Ivan Stream (F2 - Frumuşeaua River basin) (Tab. 1).

The caddisflies present the lowest species diversity (one species) in the Țâşla Stream 100 m downstream the confluence with Bălăsâna Stream and at the confluence with Vinişoru Stream (T2 and T3) - sector affected by the mining exploitations pollution, and in the Vişeu River 50 m upstream the Moisei locality (V5) - sector affected by the impact generated by the Borşa locality (Tab. 1).

The caddisfly larvae communities with the highest registered heterogeneity (according the Simpson Index) are present in the Vişeu Stream (V2), Ruscova River 50 m downstream the Socolău and Rica confluence (R4) and Frumuşeaua River (F2 and F4) (Tab. 1).

The numerical weight of the caddisflies larvae in the benthic macroinvertebrate communities vary, in the reference area, between 74.88% in the Țâşla Stream 2 km upstream the confluence with Bălăsâna River (T1) and 3.23% in the Ruscova River 50 m upstream the confluence with Bardiu River (R5). In the upper Vaser course (W1 and W2) and in Vişeu 50 m upstream the Moisei locality (V5) the Trichoptera were not present in the quantitative samples, the reported species being identified in qualitative samples (Tab. 1).

Analyzing the similarity of the caddisfly larvae communities in the 29 sampled lotic sectors, on the basis of the species relative abundance (Fig. 2), allow us to classify these communities as following:

- communities in which numerically dominant are the species of the family Hydropsychidae, present in F1, F2, F3, F4, V6, V7, V10, V11 and R7;
- communities in which numerically co-dominant are the species *Brachycentrus montanus* and *Rhyacophila tristis*, present in R1, R2, R3 and R4;
- communities erected by *Hydropsyche pelucidulla*, *Drusus discolor* and *Rhyacophila fasciata*, present in R5 and R6;
- communities in which numerically co-dominant are the species *Sericostoma personatum* and *Glossosoma conformis*, present in W3 and W4;
- communities in which numerically dominant is the species *Rhyacophila fasciata*, present in T3 and V8.

Table 1: The structure of caddisfly communities presents in the 29 lotic sectors analyzed in the Maramureș Nature Park and the numerical weight of this systematic group in the benthic macroinvertebrate communities (P - Trichoptera numerical weight in the benthic macroinvertebrate community structure, A% - relative abundance of each species, qs - qualitative samples).

Sampling station	P (%)	Reversed Simpson Index (1-I)	The specific structure of the Trichoptera larvae community	A (%)
V1	29.80	0.634	<i>Philopotamus montanus</i> <i>Drusus bruneus</i> <i>Drusus discolor</i> <i>Rhyacophila fasciata</i>	7.69 23.07 53.84 15.38
V2	12.25	0.881	<i>Plectrocnemia conspersa</i> <i>Polycentropus flavomaculatus</i> <i>Drusus bruneus</i> <i>Halesus digitatus</i> <i>Potamophylax latipennis</i> <i>Glossosoma boltoni</i> <i>Rhyacophila fasciata</i> <i>Rhyacophila tristis</i>	11.12 16.66 22.22 5.55 11.12 5.55 16.66 11.12
V3	36.0	0.761	<i>Philopotamus montanus</i> <i>Silo pallipes</i> <i>Drusus bruneus</i> <i>Limnephilus rhombicus</i> <i>Rhyacophila fasciata</i> <i>Rhyacophila tristis</i>	14.29 7.14 42.86 21.43 7.14 7.14
V4	22.53	0.777	<i>Hydropsyche pelucidulla</i> <i>Hydropsyche instabilis</i> <i>Philopotamus montanus</i> <i>Goera pilosa</i> <i>Drusus bruneus</i> <i>Rhyacophila fasciata</i>	11.76 5.89 35.29 17.65 11.76 17.65
V5	qs	0	<i>Hydropsyche pelucidulla</i>	-
V6	13.33	0.685	<i>Hydropsyche pelucidulla</i> <i>Hydropsyche instabilis</i> <i>Hydropsyche angustipennis</i> <i>Rhyacophila fasciata</i>	42.86 14.29 14.29 28.56
V7	16.05	0.447	<i>Hydropsyche pelucidulla</i> <i>Rhyacophila fasciata</i>	66.66 33.34
V8	18.3	0.564	<i>Hydropsyche instabilis</i> <i>Limnephilus rhombicus</i> <i>Rhyacophila fasciata</i>	21.43 21.43 57.14
V9	4.0	0.624	<i>Hydropsyche pelucidulla</i> <i>Hydropsyche fulvipes</i> <i>Hydropsyche angustipennis</i> <i>Sericostoma personatum</i> <i>Rhyacophila fasciata</i>	3.23 6.45 45.16 12.9 32.26
V10	12.0	0.814	<i>Hydropsyche pelucidulla</i> <i>Hydropsyche fulvipes</i> <i>Hydropsyche instabilis</i> <i>Limnephilus lunatus</i> <i>Sericostoma personatum</i> <i>Rhyacophila fasciata</i>	31.25 12.5 6.25 6.25 12.5 31.25

Sampling station	P (%)	Reversed Simpson Index (1-I)	The specific structure of the Trichoptera larvae community	A (%)
V11	3.74	0.594	<i>Hydropsyche pelucidulla</i> <i>Hydropsyche instabilis</i> <i>Hydropsyche angustipennis</i> <i>Sericostoma personatum</i> <i>Rhyacophila fasciata</i>	61.54 7.69 15.39 7.69 7.69
T1	74.88	0.513	<i>Philopotamus montanus</i> <i>Drusus discolor</i>	53.24 46.76
T2	58.32	0	<i>Drusus discolor</i>	100
T3	51.72	0	<i>Rhyacophila fasciata</i>	100
W1	qs	-	<i>Philopotamus variegatus</i> <i>Sericostoma personatum</i>	- -
W2	qs	-	<i>Hydropsyche pelucidulla</i> <i>Philopotamus variegatus</i> <i>Sericostoma personatum</i>	- - -
W3	12.73	0.570	<i>Hydropsyche pelucidulla</i> <i>Sericostoma personatum</i> <i>Glossosoma conformis</i>	7.69 53.85 38.46
W4	7.89	0.674	<i>Hydropsyche pelucidulla</i> <i>Brachycentrus montanus</i> <i>Sericostoma personatum</i> <i>Glossosoma conformis</i>	7.69 15.38 46.15 30.78
R1	13.33	0.762	<i>Brachycentrus montanus</i> <i>Goera pilosa</i> <i>Halesus digitatus</i> <i>Rhyacophila tristis</i>	37.5 12.5 12.5 37.5
R2	23.32	0.760	<i>Brachycentrus montanus</i> <i>Goera pilosa</i> <i>Halesus digitatus</i> <i>Sericostoma personatum</i> <i>Mesophylax impunctatus</i> <i>Rhyacophila tristis</i>	30.43 17.40 13.04 8.70 qs 30.43
R3	19.58	0.767	<i>Philopotamus montanus</i> <i>Brachycentrus montanus</i> <i>Goera pilosa</i> <i>Halesus digitatus</i> <i>Sericostoma personatum</i> <i>Rhyacophila tristis</i>	29.16 25.0 8.33 4.17 4.17 29.16
R4	27.38	0.848	<i>Hydropsyche pelucidulla</i> <i>Hydropsyche angustipennis</i> <i>Brachycentrus montanus</i> <i>Goera pilosa</i> <i>Drusus discolor</i> <i>Sericostoma personatum</i> <i>Rhyacophila fasciata</i> <i>Rhyacophila tristis</i> <i>Rhyacophila oblitterata</i>	4.55 4.55 18.18 4.55 22.73 4.55 9.08 22.73 9.08

Sampling station	P (%)	Reversed Simpson Index (1-1)	The specific structure of the Trichoptera larvae community	A (%)
R5	3.23	0.760	<i>Hydropsyche pelucidulla</i> <i>Goera pilosa</i> <i>Drusus discolor</i> <i>Rhyacophila fasciata</i>	25.0 12.5 37.5 25.0
R6	12.35	0.758	<i>Hydropsyche pelucidulla</i> <i>Hydropsyche instabilis</i> <i>Drusus discolor</i> <i>Chaetopteryx polonica</i> <i>Rhyacophila fasciata</i> <i>Rhyacophila nubila</i>	35.71 19.04 21.45 qs 19.04 4.76
R7	14.77	0.805	<i>Hydropsyche pelucidulla</i> <i>Hydropsyche fulvipes</i> <i>Hydropsyche angustipennis</i> <i>Ernodes articularis</i> <i>Rhyacophila fasciata</i> <i>Rhyacophila tristis</i> <i>Rhyacophila oblitterata</i> <i>Rhyacophila philopotamoides</i>	32.25 19.35 16.13 3.23 9.68 3.23 3.23 12.90
F1	17.21	0.723	<i>Hydropsyche pelucidulla</i> <i>Hydropsyche fulvipes</i> <i>Hydropsyche angustipennis</i> <i>Limnephilus lunatus</i> <i>Rhyacophila fasciata</i> <i>Rhyacophila tristis</i>	38.28 3.57 33.16 7.14 3.57 14.28
F2	23.65	0.838	<i>Hydropsyche pelucidulla</i> <i>Hydropsyche instabilis</i> <i>Hydropsyche angustipennis</i> <i>Philopotamus montanus</i> <i>Limnephilus rhombicus</i> <i>Limnephilus lunatus</i> <i>Oligotricha striata</i> <i>Rhyacophila fasciata</i> <i>Rhyacophila nubila</i> <i>Rhyacophila flava</i>	31.75 12.19 9.75 2.43 9.75 4.87 2.43 14.65 9.75 2.43
F3	20.88	0.796	<i>Hydropsyche pelucidulla</i> <i>Hydropsyche angustipennis</i> <i>Limnephilus rhombicus</i> <i>Limnephilus lunatus</i> <i>Oligotricha striata</i> <i>Rhyacophila fasciata</i> <i>Rhyacophila flava</i>	29.15 12.27 20.43 5.59 4.20 25.16 3.20
F4	29.15	0.822	<i>Hydropsyche pelucidulla</i> <i>Hydropsyche instabilis</i> <i>Hydropsyche angustipennis</i> <i>Limnephilus lunatus</i> <i>Glossosoma boltoni</i> <i>Rhyacophila fasciata</i> <i>Rhyacophila flava</i>	15.38 30.79 7.69 7.69 15.38 15.38 7.69

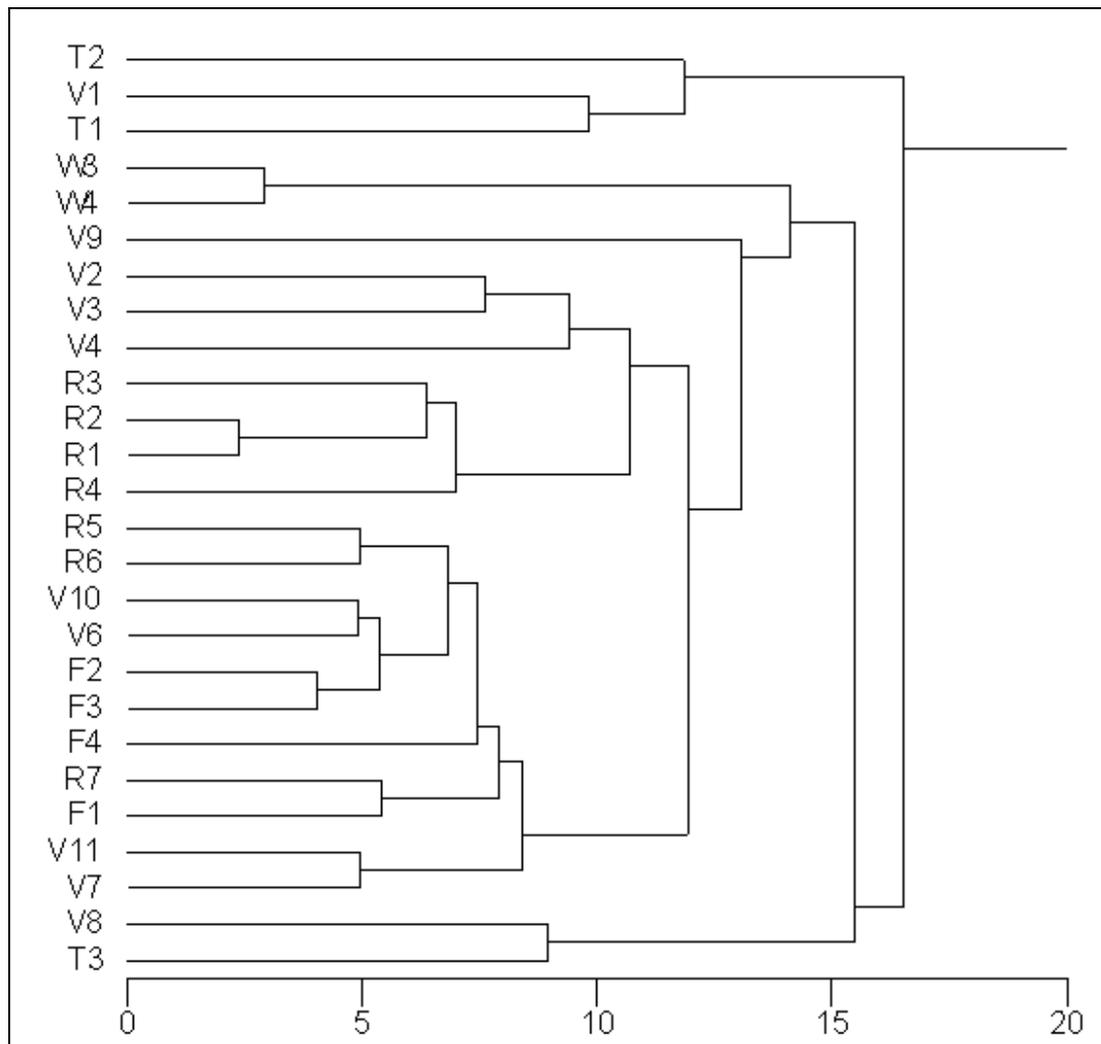


Figure 2: Tree diagram based of the caddisfly species relative abundance (A%), of the 29 lotic sectors (euclidean distances, V1 - V11, T1 - T3, W1 - W4, R1 - R7, F1 - F4 - sampling stations).

### CONCLUSIONS

The Trichoptera fauna of the Vișeu River basin presents a relative high species diversity. In the studied area 30 caddisfly species belonging to 19 genera and 11 families were identified, representing 11.24% of the Romanian Trichoptera fauna.

The caddisfly larvae communities present high diversity on the Frumușeua River basin (F1-F4), on the upper Vișeu River course (V2, V3, V4) and on the Ruscova River basin (R1-R7). Here the characteristic habitats of the Trichopterans present a good state, and the human impact is insignificant. These areas should be managed in the direction of the aquatic biodiversity conservation.

The caddisfly present the lowest diversity in the in the Țâșla Stream, between the confluence with Bălăsâna Stream and the confluence with Vinișoru Stream (T2 and T3) - sector affected by the mining exploitation pollution, and in the Vișeu River 50 m upstream the Moisei locality (V5) - sector affected by the impact generated by the Borșa locality. In these sectors rehabilitation measures are required for the aquatic habitats.

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**BIOLOGICAL AND GENETIC-BIOCHEMICAL PARAMETERS  
OF *CARASSIUS GIBELIO* POPULATION  
IN THE KAMCHIYA RIVER  
(BLACK SEA TRIBUTARY, BULGARIA)**

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**KEYWORDS:** *Carassius gibelio*, age and length composition, sexual ratio, growth rate, condition factor, electrophoresis, enzymes, general muscle proteins, genetic distance.

**ABSTRACT**

In Bulgaria, the species gibel carp, *Carassius gibelio* (Bloch, 1782), was first reported in the 1940s only in the Danube River and the Black Sea coastal lakes. At present, it is common in all water bodies from the Aegean and the Black Sea basins, and it is one of the dominant fish species in the Bulgarian lakes and reservoirs. In the Kamchiya River (Black Sea tributary) the species was first reported in 1974.

Totally 50 specimens of gibel carp were collected in the Kamchiya River in June 2009. Their standard length ranged from 95 to 220 mm, and weight from 25 to 290 g. They belonged to five age groups: 11 specimens of age 1+, 26 specimens of age 2+, 7 specimens of age 3+, 5 of age 4+ and one of age 5+. All individuals were sexually mature. The population consisted of females and males in the ratio 2:1. Growth rate and condition factor were studied and compared with other gibel carp populations.

General muscle proteins and five enzyme systems of 34 individuals were analyzed by electrophoresis. All samples had three allelic polymorphism in the *PROT-3\** locus, and the frequency of *PROT-3\*a* allele in the Kamchiya population was the highest (0.75) in comparison with other Bulgarian rivers and reservoirs. Two-allelic polymorphism with a null allele in the second esterase polymorphic zone (*EST-2\**) was found in the Kamchiya River. Expression of allele a (*EST-2\*a*) was observed. All other enzyme systems (MDH, LDH and SOD) had common electrophoretic patterns. The gene frequencies of polymorphic loci (*PROT-3\** and *EST-2\**) could be used as genetic markers for distinguishing of *C. gibelio* population in the Kamchiya River.

**ZUSAMMENFASSUNG:** Biologische und genetisch-biochemische Parameter der Populationen von *Carassius gibelio* im Kamtchiya Fluss (Zufluss des Schwarzen Meeres, Bulgarien).

Die Fischart Giebel *Carassius gibelio* (Bloch, 1782) wurde in Bulgarien erstmal in den 1940er Jahren in der Donau und in Küstenseen am Schwarzen Meer nachgewiesen. Aktuell ist

die Art in allen Gewässern von der Ägäis bis zum Schwarzen Meer verbreitet und eine der dominanten Spezies in den bulgarischen Seen und Stauseen. Im Kamchiya Fluss (ein Zufluss des Schwarzen Meeres) wurde die Art erstmalig 1974 vorgefunden.

Im Juni 2009 wurden insgesamt 50 Giebel im Kamchiya Fluss gesammelt. Ihre Standardlänge lag zwischen 95 und 220 mm, bei einem Gewicht von 25 bis 290 g. Die Tiere umfassten 5 Altersgruppen: 11 Individuen hatten ein Alter von 1+, 26 Individuen hatten ein Alter von 2+, 7 Tiere hatten ein Alter von 3+, 5 waren 4+ alt und eines 5+. Alle Tiere waren geschlechtsreif. Die Population bestand aus Weibchen und Männchen im Verhältnis 2:1. Die Wachstumsraten und der Konditionsfaktor wurden berechnet und die Daten mit denen anderer Giebel-Populationen verglichen.

Die allgemeinen Muskelproteine und fünf Enzymsysteme wurden bei 35 Individuen mittels Elektrophorese analysiert. Alle Proben hatten drei Allelpolymorphismen auf dem *PROT-3\** locus und die Häufigkeit des *PROT-3\*a* Allels war, verglichen mit anderen bulgarischen Flüssen und Seen, in der Kamchiya Population am häufigsten (0.75). Zwei Allel-Polymorphismen mit einem Null-Allel in der zweiten polymorphen Esterase-Zone (*EST-2\**) wurden im Kamchiya Fluss gefunden. Die Ausprägung des Allels a (*EST-2\*a*) wurde festgestellt. Alle anderen Enzym-Systeme (MDH, LDH und SOD) zeigten übliche elektrophoretische Muster. Die Genfrequenzen der polymorphen loci (*PROT-3\** und *EST-2\**) könnten als genetische Marker zur Unterscheidung der Giebel-Populationen im Kamchiya Fluss verwendet werden.

**REZUMAT:** Parametri biologici și genetic-biochimici ai populației de pești *Carassius gibelio* în râul Kamtchiza (afluent al Mării Negre, Bulgaria).

Specia de pești *Carassius gibelio* (Bloch, 1782) a fost semnalată, pentru prima dată, în Bulgaria, în anii 1940, în Dunăre și lacurile de coastă ale Mării Negre. În prezent, specia este răspândită în toate apele dintre Marea Egee până la Marea Neagră și una din speciile dominante în lacurile naturale și cele de baraj ale Bulgariei. În râul Kamchiya, afluent al Mării Negre, specia a fost găsită, pentru prima dată, în 1974.

În iunie 2009, au fost colectate în total 50 de specimene în râul Kamchiya. Lungimea lor standard era între 95 și 200 mm, la o greutate corporală de 290 g. Exemplarele reprezentau 5 categorii de vârstă: 11 indivizi, având vârsta de 1+, 26 de indivizi vârstă de 2+, 7 indivizi vârstă de 3+, 5 exemplare aveau vârsta de 4+ și un exemplar vârstă de 5+. Toate exemplarele erau mature sexual. Populația era compusă din femele și masculi, în proporția de 2:1. Au fost calculate rata de creștere și factorul de condiție, datele, fiind apoi comparate cu cele ale altor populații de caras.

Ansamblul de proteine musculare și cinci sisteme enzimice au fost analizate la 35 de indivizi, cu ajutorul electroforezei. Toate probele aveau trei alel-polimorfisme pe locusul *PROT-3\**, iar frecvența alelului *PROT-3\*a* avea, în comparație cu cea din alte râuri și lacuri din Bulgaria, cea mai ridicată valoare în râul Kamchiya (0.75). Două alel-polimorfisme cu un alel-zero în a doua zonă polimorfă a esterazei (*EST-2\**) au fost găsite în râul Kamtchiza. De asemenea a fost constatată expresia alelului a (*EST-2\*a*). Toate celelalte sisteme enzimice (MDH, LDH și SOD) arătau modele electroforetice obișnuite. Frecvențele genelor pe locurile polimorfice (*PROT-3\** and *EST-2\**) ar putea fi folosite ca markeri genetici, pentru diferențierea populațiilor de caras, în râul Kamtchiza.

## INTRODUCTION

In Bulgaria, the species gibel carp *Carassius gibelio* (Bloch, 1782) was first reported in the 1940s as occurring only in the Danube River (Drensky, 1948), and later, as established in some Black Sea coastal lakes (Stojanov, 1949; Drensky, 1951). At present, it is common in all water bodies from the Aegean and the Black Sea basins and is one of the dominant fish species in the Bulgarian lakes and reservoirs (Karapetkova and Zivkov, 1995; Trichkova and Zivkov, 2007; Trichkova et al., 2007).

There are few data published on gibel carp in Bulgarian water basins related to morphology (Sivkov, 2003), toxicology (Arnaudov et al., 2008a, b; Dobрева et al., 2008; Tomova et al., 2008a, b) and parasitology (Shukerova, 2005). Biological studies focused on age and sexual structure, age and length of maturity, growth rate and condition, as well as fecundity of gibel carp populations in some rivers and reservoirs (Zivkov and Stoyanova, 1976; Zivkov, 1980a; Trichkova and Zivkov, 2007; Trichkova et al., 2008; Zivkov et al., 2008; Trichkova and Zivkov, 2010). Genetic-biochemical data were published only by Apostolou et al. (2007).

The ichthyofauna of the Kamchiya River (a Black Sea tributary) was well studied in the past (Chichkoff, 1934, 1936; Karapetkova, 1970, 1974). The gibel carp was first recorded in the river system during sampling in the period 1967-1970 (Karapetkova, 1974). It occurred mostly in the lowest reaches and it was considered as introduced to the river together with stocked carp fingerlings (Karapetkova, 1974).

The goal of the present study was to make an analysis of some biological characteristics, such as: age and length composition, sexual ratio, growth rate and condition factor, as well as the genetic-biochemical parameters of *C. gibelio* population in the Kamchiya River.

## MATERIAL AND METHODS

The Kamchiya River is the biggest Black Sea tributary in Bulgaria. It is 245 km long and it has a catchment area of 5,358 km<sup>2</sup>. The river spring from the east Balkan Mountains at an altitude of 710 m a.s.l. The water course is characterized with a small mean altitude (327 m a.s.l.) and small mean slope value 2.9‰ (Marinov, 1957).

The sampling was made in the river lowest reaches in June 2009. The fish were caught using gill nets 50 m long with mesh sizes of 11-14, 24, 28, 30, 40-45, 60, 70 and 80 mm and hand net with mesh size 1.2 mm. Totally 50 specimens were collected. The standard body length (L) and weight (W) of all fish were measured to the nearest 1 mm and 0.01 g, respectively. The sex and reproductive stage of gonads were determined macroscopically. The age was determined by scales on a projector Lesergerät Carl Zeiss Jena at a magnification of 17.5x.

The correlation-regression analysis was made according to the standard biostatistical methods. The linear growth rate was estimated through back-calculations of length from diagonal radius of scales (S) according to the following equation:  $L = -15.7039 + 1.59445S$ ,  $R = 0.97$ . The comparison of growth rate of different populations was based on comparing mean back-calculated lengths of fish at the same age (Zivkov, 1972; Zivkov et al., 1999). The absolute mean weights, calculated at the same standard length values - 50, 100, 150 and 200 mm according to the equation  $W = 67 \times 10^{-6} L^{2.8475}$ ,  $R = 0.997$  were used as condition factor of *C. gibelio* population (Zivkov, 1980b, 1993, 1996; Raikova-Petrova and Zivkov, 1998).

34 individuals were analyzed by electrophoresis. For the analyses of the enzymes and non-enzyme protein systems, a homogenate of white dorsal muscle was used. The proteins were separated by horizontal starch gel electrophoresis according to Smithies (1955), modified by Dobrovolov (1973). Five enzymatic systems, such as: esterase (EC 3.1.1.1 - EST), lactate dehydrogenase (EC 1.1.1.27 - LDH), malate dehydrogenase (MDH), malic enzyme (MEP) and superoxide dismutase (EC 1.15.1.1 - SOD) were analyzed. Staining of different enzymes was performed according to Shaw and Prasad (1970). The buffer systems of Dobrovolov (1976) and Clayton and Gee (1969) were used for the electrophoresis. The nomenclature of loci and alleles used followed essentially the recommendation of Shaklee et al. (1990). Calculation of indices of genetic similarity and genetic distance was performed after Nei (1972).

## RESULTS AND DISCUSSIONS

### Age, length and sexual composition

The age and sexual composition of *C. gibelio* population analyzed by length classes is presented in the table 1. The sampled individuals belonged to 5 age groups: 11 specimens at age 1+, 26 at age 2+, 7 at age 3+, 5 at age 4+ and one at age 5+. All were sexually matured. The standard length of females ranged from 95 to 200 mm, and this of males from 110 to 220 mm; the weight of females ranged from 24.88 to 239.3 g, and of males from 42.17 to 290 g. The individuals at age class 2+ and within the length range of 140-159 mm dominated in the catches. Females dominated in the smaller age classes, while males in the bigger (4+, 5+).

Table 1: Age, length and sexual composition of *C. gibelio* population in the Kamchiya River; m - males, f - females, n - number of the analyzed specimens.

Length class, mm	1+		2+		3+		4+		5+		n
	m	f	m	f	m	f	m	f	m	f	
90-99		4									4
100-119	2	5	1								8
120-129			1	1							2
130-139			3	2							5
140-149			4	7							11
150-159				8							8
160-169					1	2	1	1			5
170-179					1	1	1				3
180-199							1				1
200-229						1	1		1		3
Total number	2	9	9	18	2	4	4	1	1		50

### Sexual ratio

The population consisted of females (64%) and males (36%) in a ratio of 2:1. In Bulgaria, in the 1950-1980s, populations in the reservoirs and fish-ponds consisted mainly of gynogenetic females, while males occurred occasionally in an average in the range from 0 to 1.5% (Paspalev and Pechev, 1955; Zivkov, 1980a). However, recently, bisexual populations are found with males ranging from 30 to 50% in the catches (Trichkova and Zivkov, 2007; Trichkova et al., 2008).

### Linear growth rate, length-weight relationship and condition factor

The mean linear annual increments of the population were the highest in the second and third year of life: 63 mm and 44 mm (Tab. 2a, b). In the following years they gradually decreased. The mean annual weight increments were very low in the first and second year of life and they had the highest values in the third and fourth year. At age 6 high values of mean length and weight were recorded but as this was represented by only one specimen the results are not statistically significant.

Table 2a: Back-calculated mean body lengths (L, mm) and weights (W, g) and corresponding absolute linear (t) and weight (T) annual increments of *C. gibelio* in the Kamchiya River; n - total number of specimens.

Age group	Generation	L1	W1	L2	W2	L3	W3	n
1+	2008	46.8	3.8	95	28.7			11
2+	2007	34.5	1.6	107	40.5	149	103	26
3+	2006	31.7	1.3	91.8	26	138.5	83.9	7
4+	2005	32	1.3	81	18	119	54	5
5+	2004	16	0.2	67	10.7	135.8	79	1
$L_{av.}$		36		99		143		
$t = L_n - L_{n-1}$		36		63		44		
$W_{av.}$			2		33		93	
$T = W_n - W_{n-1}$			2		31		60	

Table 2b: Back-calculated mean body lengths (L, mm) and weights (W, g) and corresponding absolute annual increments (t) of *C. gibelio* in the Kamchiya River; n - total number of specimens.

Age group	Generation	L4	W4	L5	W4	L6	W6	n
1+	2008							11
2+	2007							26
3+	2006	172.7	157.2					7
4+	2005	148.5	102	174	160.8			5
5+	2004	175.6	165	198	232	217	301.7	1
$L_{av.}$		164		178		217		
$t = L_n - L_{n-1}$		21		14		39		
$W_{av.}$			137		173		301.7	
$T = W_n - W_{n-1}$			44		36		128.7	

The linear growth rate of *Carassius gibelio* studied population is illustrated in the table 3.

The Kamchiya population is compared with other 16 populations in different rivers according to the mean back-calculated lengths at the same age (Zivkov, 1972; Zivkov et al., 1999). The results showed that it is characterized with a low growth rate.

Table 3: Linear growth of *C. gibelio* in different rivers (Trichkova et al., 2008).

Water Body/ References	Back-calculated mean body lengths (mm) at different age (years)						
	1	2	3	4	5	6	7
<b>Low Growth Rate</b>							
Nitra River (Sedlar et al., 1976)	48	93	123	167	199		
Danube River, Slovakia (Sedlar et al., 1980)	60	95	127	157	184	217	
Hron River (Sedlar, 1975)	56	84	131	161	197	239	
Dudvah River (Makara and Stranai, 1980)	72	107	135	154	172		
Danube River delta (Kururadze and Mariash, 1975)	107	124	136	172	205	215	
<b>Kamchiya River (our data)</b>	<b>36</b>	<b>99</b>	<b>143</b>	<b>164</b>	<b>178</b>	<b>217</b>	
Volga River delta (Gudkov, 1985)	73	115	154	198	219	244	262
Amur River (Nikolskii, 1950)	58	114	158	190	213	230	240
<b>Moderate Growth Rate</b>							
Danube River, Slovakia (Holcik, 1975)	76	146	166				
Danube River at Silistra (2004) (Trichkova et al., 2008)	77	162.5	186	214			
Ipel River (Barus and Lusk, 1978)	77	156	190	219			
Danube River at Vidin (1988) (Trichkova et al., 2008)	111	173	191	209	218		
Lower Dnieper (Movchan and Smirnov, 1983)		158	194	242	273	295	
Danube River (Marinov, 1966)	76	142	195				
<b>High Growth Rate</b>							
Dyie River (Barus and Lusk, 1978)	85	173	204	233	251		
Danube River at Baikal (1986) (Trichkova et al., 2008)	82	183	234	247	310		
Danube River delta (Kukuradze and Mariash, 1975)	104	162	238	260	291	324	348

The condition factor of the population, expressed by absolute mean weights, calculated at the same standard length values - 50, 100, 150, 200 mm according to the equation  $W = aL^b$  (Zivkov, 1980b, 1993, 1996) is shown in the table 4. The Kamchiya population is compared with different sites in the Danube (Trichkova et al., 2008). At 50 mm length it ranked third with weight of 4.6 g, while at 150 and 200 mm lengths it had the lowest mean weights.

Table 4: Condition of *C. gibelio* population in the Kamchiya River compared to the Danube River - Bulgaria (Trichkova et al., 2008). The condition is determined by the mean weights (W) calculated at the same length values ( $L = 50$  mm [ $L_{50}$ ];  $L = 100$  mm [ $L_{100}$ ]), according to the equation  $W = aL^n$ .

Water body	$W = aL^n$	Weight (g)					
		$L_{50}$	$L_{100}$	$L_{150}$	$L_{200}$	$L_{250}$	$L_{300}$
Danube River (Silistra)	$W = 349.782 \cdot 10^{-6} L^{2.5730}$	8.2	49	139	291.3	571.2	826.9
Kamchiya River	$W = 67 \cdot 10^{-6} L^{2.8475}$	4.6	33.2	105.3	238.8		
Danube River (Baikal)	$W = 47.837 \cdot 10^{-6} L^{2.9228}$	4.4	33.5	109.7	254.3	488.1	831.7
Danube River (Vidin)	$W = 23.127 \cdot 10^{-6} L^{3.0750}$	3.9	32.7	113.7	275.3	546.7	957.8

### Genetic-biochemical characteristics

The results from the genetic-biochemical analyses of Kamchiya gibel carp population were compared to two Bulgarian reservoirs - Drenovets and Poletkovtsi from the Danube Basin (Tab. 5, our data). All samples studied had two allelic polymorphism in the *PROT-3\** locus, and the frequency of *PROT-3\*a* allele in the Kamchiya population was the highest (0.75) in comparison with the two reservoirs (Fig. 1, Tab. 5).

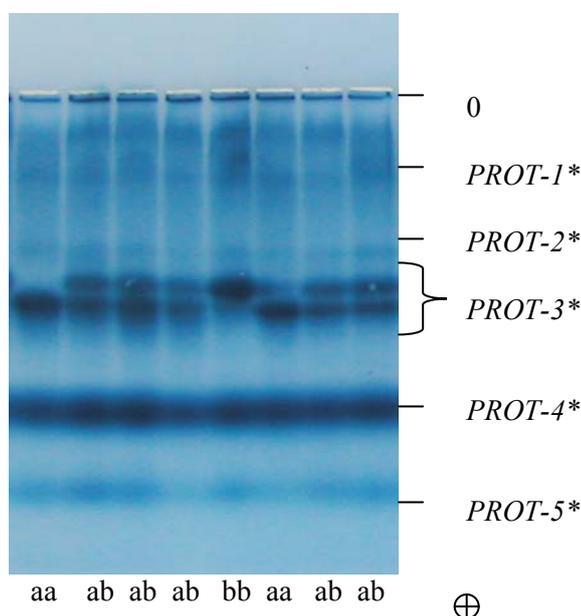


Figure 1: Starch gel electrophoresis on general muscle proteins (*PROT*) of *C. gibelio* (Kamchiya River), polymorphism on *PROT-3\** was marked, aa, ab and bb phenotypes, 0 - origin.

Table 5: Distribution of phenotypes on the polymorphic loci in *C. gibelio* populations, checking up the gene equilibrium: 1 - Drenovets Reservoir, 2 - Poletkovtzi Reservoir and 3 - Kamchiya River.

Characters	<i>PROT-3*</i>			<i>EST-2*</i>			<i>sMEP-1*</i>	<i>sMEP-2*</i>
Localities Phenotypes	1	2	3	1	2	3	2	2
aa	4/3.77	4/4.75		5/4.58			7/7.11	0/0.11
		18/19.13		25/25.02				
ab	8/8.47	10/8.47		6/6.85			2/1.78	2/1.78
		15/12.75						
bb	5 / 4.76	3/3.77		3/ 2.57	6/5.99		0/0.11	7/7.11
		1/2.13						
bc								
cc								
ac								
0				3/2.99	9/9.018			
Number of individuals	17	17	34	14	14	34	9	9
Criteria $\chi^2$	0.052	0.55	1.059	0.216	0.004	0.00006	0.139	0.139
Probability (P)	P > 0.75	P > 0.25		P > 0.50	P > 0.90		P > 0.50	P > 0.50
		P > 0.25			P > 0.99			
Gene frequencies								
a	0.471	0.389	0.750	0.572		0.486	0.889	0.111
b	0.529	0.611	0.250	0.428	0.423		0.111	0.889
c								
0				0.577	0.514			

The polymorphism is under Hardy-Weinberg equilibrium which confirms that the population in the Kamchiya River consisted of individuals from both sexes.

On the esterase electrophoregrams in the Kamchiya population, three zones with esterase activity were visualized (Fig. 2). They probably were coded from three loci (*EST-1\**, *EST-2\** and *EST-3\**). The *EST-2\** locus had two allele system of inheriting in the samples from Drenovets and Poletkovtzi reservoirs as well as from Kamchiya River. In the last two areas null alleles were found (Tab. 5). Polymorphism in the same locus with two alleles was observed in the Drenovets Reservoir. *EST-1\** and *EST-3\** loci were monomorphic in the Kamchiya River and in the reservoirs.

Malate dehydrogenase (*MDH*) - One *mMDH\** and two *sMDH\** loci were detected. Interloci hybrid fractions between *sMDH-1\** and *sMDH-2\** loci were established (Fig. 3). All loci in the river and reservoir populations were monomorphic (Fig. 3).

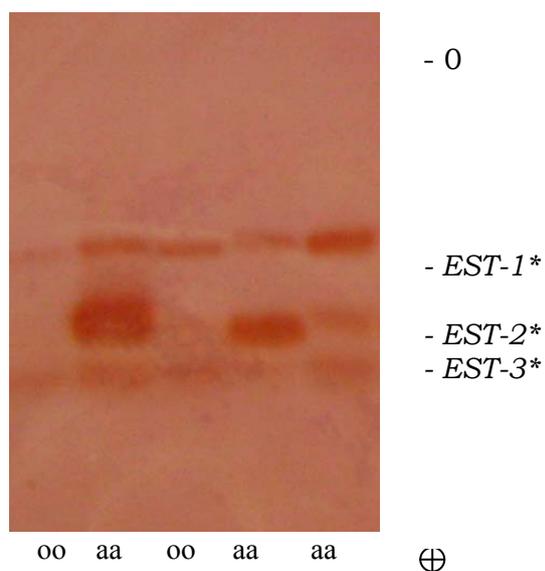


Figure 2: Esterase zymograms on starch gel by *C. gibelio* (Kamchiya River), aa and oo - phenotypes in *EST-2\** locus, 0 - origin.

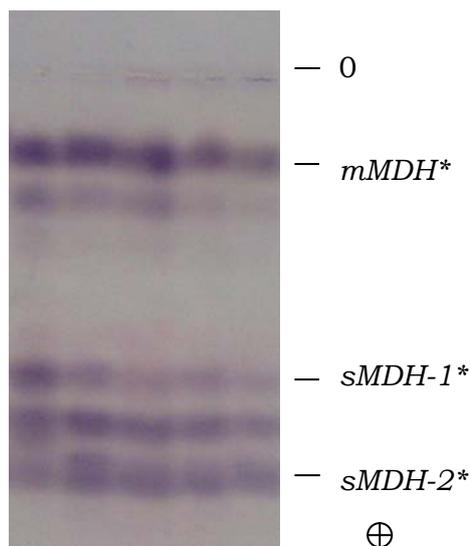


Figure 3: Malate dehydrogenase zymograms on starch gel by *C. gibelio* (Kamchiya River), 0 - origin.

Lactate dehydrogenase (*LDH*) - This enzyme was represented by 9 fractions, which were coded from four loci (*LDH-A\*1*, *LDH-A\*2*, *LDH-B\*1* and *LDH-B\*2*). In the reservoirs and in the Kamchiya River the four loci were monomorphic (Fig. 4).

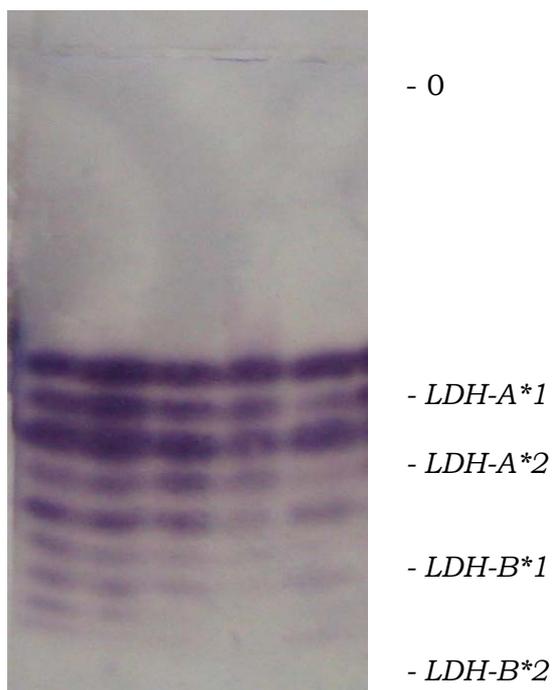


Figure 4: Lactate dehydrogenase zymograms on starch gel by *C. gibelio* (Kamchiya), 0 - origin.

Malic enzyme (*MEP*) - Three zones with enzyme activity, determined from three loci (*sMEP-1\**, *sMEP-2\** and *mMEP\**) were visualized on the electrophoregrams (Fig. 5). All of them were monomorphic in the samples from the Drenovets Reservoir and in the Kamchiya River (Fig. 5: no. 3 and 4). Only in the Poletkovtsi Reservoir, polymorphism in the *sMEP-1\** and *sMEP-2\** loci was found (Tab. 5).

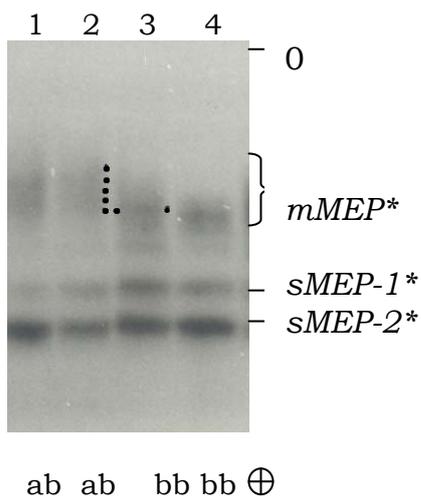


Figure 5: Malic enzyme zymograms on starch gel by *C. gibelio*, 0 - origin.

Superoxide dismutase (*SOD*) - *sSOD* was monomorphic in the reservoirs and in the Kamchiya River (Fig. 6).

The gene frequencies of polymorphic loci (*PROT-3\** and *EST-2\**) could be used as genetic markers for distinguishing of *C. gibelio* population in the Kamchiya River.

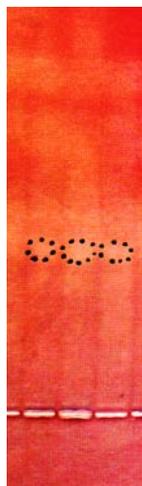


Figure 6: Superoxide dismutase zymograms on starch gel by *C. gibelio* (Kamchiya River), 0 - origin.

### CONCLUSIONS

The gibel carp appeared to have stable population in the estuary area of the Kamchiya River. The catches consisted of individuals belonging to 5 age groups, with standard length in the range from 95 to 220 mm and sexual ratio 2 females: 1 male.

The population was characterized with low growth rate and low condition factor.

The gene frequencies of polymorphic loci (*PROT-3\** and *EST-2\**) could be used as genetic markers for distinguishing of gibel carp population in the Kamchiya River.

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**NOCTURNAL SPOTLIGHT COUNT SURVEY METHODS  
FOR THE ESTIMATION OF ABUNDANCE, DISTRIBUTION AND  
POPULATION TREND ANALYSIS OF THE *CROCODYLUS NILOTICUS*  
IN LAKE NASSER  
(EGYPT)**

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**KEYWORDS:** Egypt, Lake Nasser, Nile crocodile, abundance, distribution.

**ABSTRACT**

Fortytwo locations were surveyed between October 2009 to August 2010 in order to facilitate the estimated count of the Nile crocodile (*Crocodylus niloticus*) in Lake Nasser. The study aims to quantitative survey and determine the crocodile population size in Lake Nasser (South Egypt) and to analyze the dynamics of the Nile crocodile population in this lake through spotlight survey methods. Double-observer counts technique was used to estimate detection probability of animals along the survey route. In total 1261.3 km were surveyed at 42 locations. 528 crocodiles were observed, the encounter rate or the total abundance being of 0.419 crocodiles/km, and the corrected abundance being 0.304 crocodiles/km. Of these, 81 (15.3%) were classified as EO (reflective eyes), and 441 (85.0%) were approached closely enough to estimate size; these included 133 (25.2%) juvenile, 249 (47.0%) subadults and 56 (10.6%) adults. On the other hand the hatchlings represented about 11 (2.0%) of the total count of crocodiles. In accordance with our survey this percentages exhibit positively skewed size class frequency distributions which may represent rapidly-growing population with high reproductive capacity. Such distribution may also indicate a high juvenile mortality. In these populations, the younger individuals are preponderant, which is an indication of strength and success of the species, being mostly due to victorious reproduction and survival of individuals. The crocodiles were not randomly distributed in between habitats along lake Nasser shoreline.

**RÉSUMÉ:** Méthodes d'étude par dénombrement nocturne a la lumière artificielle pour l'estimation de l'abondance, de la distribution et l'analyse des tendances populationnelles chez *Crocodylus niloticus* dans le Lac Nasser (Egypte).

Quarante deux locations ont été étudiées d'Octobre 2009 en Aout 2010 pour faciliter le dénombrement estimatif des crocodiles de Nile (*Crocodylus niloticus*) du Lac Nasser. L'étude a comme but l'estimation quantitative et de déterminer la taille de la population de crocodiles de Lac Nasser (Sud de l'Egypte) ainsi que d'analyser la dynamique de la population de crocodiles de Nile dans ce lac le long du trajet d'observation, par la méthode du comptage sous éclairage. La technique du comptage à deux observateurs a été employée afin d'estimer la probabilité de la détection des animaux sur le trajet d'observation. En total, 1261,3 km ont été étudiés sur 42 locations. 528 crocodiles ont été observés, l'abondance totale étant 0,419 crocodiles/km et l'abondance corrigée étant 0,304 crocodiles/km. De ceux, 81 (15,3%) ont été

classés EO (des yeux phosphorescents) et 441 (85,0%) étant suffisamment proches pour estimer leur taille. Parmi eux on a retrouvé 133 (25,2%) juvéniles, 249 (47,0%) subadultes et 56 (10,6%) adultes. D'une autre part, les juvéniles fraîchement éclos ont été en nombre de 11 (2,0%) du nombre total des crocodiles. Ces pourcentages peuvent exprimer de manière positive une distribution des fréquences des différentes classes de longueur qui pourrait être conforme avec une population à croissance rapide et capacité de reproduction importante. Mais ce type de distribution peut également représenter une mortalité juvénile accrue. Dans ces populations, les individus plus jeunes sont le mieux représentés, ce qui constitue un indicateur de la capacité reproductive et du succès de l'espèce, la plupart du temps étant due à une reproduction efficace et à la survie des individus. Les crocodiles ont été distribués de manière aléatoire dans des différents habitats des berges du lac Nasser.

**REZUMAT:** Metodele de studiu prin numărare nocturnă cu iluminat artificial pentru estimarea abundenței, distribuției și analiza tendințelor populaționale la *Crocodylus niloticus* în Lacul Nasser (Egipt).

Patruzeci și două de locații au fost studiate între octombrie 2009 și august 2010, pentru a facilita estimarea cantitativă a populațiilor de crocodil de Nil (*Crocodylus niloticus*), din Lacul Nasser. Articolul are ca scop studiul cantitativ și determinarea dimensiunilor populației de crocodil din Lacul Nasser (Egiptul de Sud) și analiza dinamicii populației de crocodil de Nil în acest lac, prin metoda studiului la lumină artificială. S-a folosit tehnica numărătorii cu doi observatori pentru estimarea probabilității detectării animalelor pe traseul de studiu. În total au fost studiați 1261,3 km din 42 de locații. Au fost observați 528 de crocodili, rata de întâlnire sau abundența totală, fiind de 0,419 crocodili/km, iar abundența corectată fiind de 0,304 crocodili/km. Din aceștia, 81 (15,3%) au fost clasificați ca EO (ochi reflectorizanti) iar 441 (85,0%) fiind suficient de aproape pentru a li se estima lungimea. Între aceștia, s-au regăsit 133 (25,2%) juvenili, 249 (47,0%) subadulti și 56 (10,6%) adulți. Pe de altă parte, juvenili, proaspăt eclozați, au fost în număr de 11 (2,0%) din numărul total de crocodili. Aceste procentaje pot exprima de manieră pozitivă o distribuție a frecvenței diferitelor clase de mărimi care ar fi concordantă cu o populație cu creștere rapidă și o capacitate reproductivă mare. Dar acest tip de distribuție poate, de asemenea, reprezenta o mortalitate juvenilă ridicată. În aceste populații, indivizii cei mai tineri sunt preponderenți, ceea ce se constituie într-un indicator al puterii reproductive și a succesului speciei, de cele mai multe ori datorat unei reproducere eficiente și supraviețuirii indivizilor. Crocodilii nu au fost distribuiți, aleatoriu, în diferitele habitate din zona limnicolă a lacului Nasser.

## INTRODUCTION

The Nile crocodile, *Crocodylus niloticus*, occurs throughout Lake Nasser from Aswan high dam to the Sudanese boundaries and the population extends up. A number of human-induced disturbances have led to the decline of the Lake Nasser crocodile population over the last ten years (Salem, in progress). These include extensive hunting, habitat destruction, and interference from local fishermen and overgrazing along the Lake Nasser shoreline, boat traffic and the extensive eggs collection from the wild by crocodile hunters and fishermen.

Generally, eggs and yearling are collected from nests and sold very expensively, since people are not aware that this will lead to a severe shortage in species abundance and in the end to the destruction of the Lake Nasser's ecosystem.

Messel et al., (1977) reported that the spotlight count method of surveying crocodilians was standardized in 1977 and was proved to be a very successful and dependable method over the last three decades (Letnic and Connors, 2006). On the other hand, this method is the most frequent technique to estimate crocodilian population trends as reported by Bayliss et al. (1986) and Thorbjarnarson et al. (2000). As a result of a reflective layer in the eye known as the tapetum lucidum (Grenard, 1991), crocodilian eyes reflect any bright light shone into them and exposed crocodiles can be located for distances up to 100 m.

Chabreck (1966), Hutton and Woolhouse (1989) and Combrink (2004) reported that the Nile crocodile location depends upon few factors such as vegetation density, vegetation types, but the strength of the spotlight and the relative position of the crocodile provides a detailed literature review and description of the spotlight survey method, mainly these factors may be limiting the occurrence of Nile crocodile during the water location, furthermore the experience of the observers in spotlight surveys. Spotlight counts have been used to estimate populations by providing the researcher with indices of population size and density (Bayliss et al., 1986; Letnic and Connors, 2006). Yet, spotlight counts are in effect inaccurate due to a number of factors causing visibility bias and for instances observers do not necessarily see all of the crocodiles at hand during a survey route (Bayliss et al., 1986). The probability of detection of crocodiles is influenced by several factors including: the type and structure of vegetation between the observer and the crocodile, Lake Nasser's khors width, frequency of bends (sinuosity), position of the crocodile (submerged, on land, etc.), the orientation or the angle of the crocodile in relation to the observer, and the wariness of the crocodile.

Additionally, at any given time, up to 38% of crocodiles may be underwater (Bayliss et al., 1986; Hutton and Woolhouse, 1989). Large crocodilians tend to be warier than the smaller size classes and often submerge when approached (Webb and Messel, 1979; Ron et al., 1998). Spotlight counts therefore represent an index of the total population and without an idea of how many animals there are in the system, there is no way to check on the relationship between spotlight counts and total population size (Woodward and Moore, 1993). However, in combination with population estimates, correction factors can be applied to spotlight count data to improve the accuracy of the method. Nevertheless, for trend analysis without the knowledge of population size, the assumption is made that the relationship between total population size and spotlight count density remains constant (Woodward and Moore, 1993).

#### **MATERIAL AND METHODS**

The fieldwork in the Lake Nasser was conducted from 16<sup>th</sup> of October 2009 to August 2010. We censused the crocodile population using a spotlight surveys (Bayliss, 1987). Spotlight surveys were conducted based on boat-counts used to establish the number of Nile crocodiles in the Lake Nasser. A five meter fiberglass-boat fitted with an outboard motor 25/15 hp Yamaha/ Mariner respectively, was used for every count. The boat was always operated at an average speed of about 5-15 km/h in order to scan the water and the lake Nasser shoreline of khors to pick up the crocodiles' reflective eyes. The average speed of the boat was reduced upon each sighting, in order to approach the crocodile more slowly and estimate its size. The team consisted of three/four persons, two were observers who spotted and counted the crocodiles (one of these persons also piloted the boat (showing the direction for motor boat driver), while the third member of the team recorded the sightings on a water proof notebook, and the fourth person drove the boat.

The survey began 15 to 30 minutes after sunset or as soon as the light became dim enough to use the spotlights. The crocodile eye shines (reflective eyes) were detected using a 100,000 to 200,000 Spot flood Q-beam spotlight "Brinkmann", and 12-volt headlights. When a crocodile was spotted, its total length (TL) was estimated by the first observers. This estimation was based on experience and hard work, a lot of field works and crocodile survey trip in Lake Nasser (Bayliss, 1987; Platt et al, 2004; Salem, 2009). During the survey the beginning and the endpoints of each survey route, and the distance traversed for each crocodile sighting were logged using a Garmin etrix® GPS 12.

All crocodiles sighted were classified by total length (TL) as hatchlings (H, < 40 cm), juveniles (Y, TL > 40 < 1 m), sub adults classified as (1.0 - < 1.5 m, 1.5 - < 2.0 m, 2.0 - < 2.5 m, (> 2.5 - < 3.0 m) and adult individuals were classified as (3.0 - < 3.5 m, 3.5 - < 4.0 m, 4.0 - < 4.5, 4.5 - < 5.0 m and > 5.0 m) on the other hand, crocodiles that submerged before TL could be determined were classified as 'eye shine only' (EO), to be useful for dynamic studies (modified from Platt et al., 2004; Salem, 2009).

The frequencies of individuals within each size classes were determined as absolute and relative value. Descriptive statistical analysis was used to estimate the skewed values in order to determine the population dynamics per location studied in Lake Nasser (modified from Salem, 2009).

Finally the observed distribution of crocodiles along Lake Nasser habitats was tested against a null model of random distribution using a chi-square ( $\chi^2$ ) analysis of fit test.

The total abundance or the encounter rate was calculated as the number of crocodiles observed per kilometre of surveyed route (as modified by Platt and Thorbjarnarson, 2000 and 2004). The general animal population studies resulted from one of two metrics: absolute counts or indices of population size. The difference between the two is that the former takes into account the issue of detectability, where just because an animal was not seen does not mean it wasn't there (Williams et al., 2002). The relationship between the count and true abundance can be shown as  $N = C/P$  where  $N$  = true abundance,  $C$  = count statistic, and  $P$  = detectability.

The purpose of a sampling technique is to estimate the number,  $N$ , of individuals present in an area from a sample count,  $C$ , of that area, the expected value of the count is given by  $E(C) = NP$ , where  $P$  is the detection probability (Nichols et al., 2000; Farnsworth et al., 2002) or index ratio (Bart and Earnst, 2002). Use of  $C$  (raw count data) to estimate the change in  $N$  over time (i.e. trend), requires the proportionality assumption (Thompson, 2002) that a trend in  $P$  does not exist (J. Bart, pers. com). Populations in different areas and populations counted with different methods cannot be compared quantitatively with indices (i.e.  $C$  values; Bart and Earnst, 2002). In order to make inferences from count data without anxiety with the knowledge that such inferences depend upon untested assumptions (Nichols et al., 2000), it is necessary to estimate  $N$ , preferably with methods that are grounded in statistical theory (Thompson, 2002). The standard form of such an estimate is given by this equation:  $E(C) = NP$ .

For this study it was determined essentially to find the true abundance of crocodiles in the lake Nasser, while concurrently discovering the basic relationship between the true abundance and indices of abundance to facilitate future monitoring efforts. To do this we will join standard, nocturnal spotlight surveys within double survey methods furthermore a collection of environmental variables were recorded for each survey period in attempt to calculate correction factors based on variation in the environment and the landscape.

Nichols et al. (2000) reported that double-observer counts can be used to estimate detectability of animals along the survey route, regardless of the difference in detection between different observers. During each of the surveys described below, two observers will

alternately act as Observer 1 and Observer 2, in order to record double-observer count statistics. The data will be analyzed with the program DOBSERV (Hines, 2000) and estimates of detectability, as well as inter-observer variation in detection, will be calculated.

The double observer technique implies that observers are present during all point counts in the route count. At each count (a visit to a single route), one observer is selected as "primary" and the other as "secondary". The primary observer identifies all crocodiles seen and reports to the secondary observer as well as to the other team member in the boat, and estimates their sizes and capture if there are a chance for capture, as well as detected, the position of the crocodile, if it's seen as EO (eyes only) and estimate their distance from the boat. The secondary observer records the animal detected by the primary observer but also surveys the area. Crocodiles detected only by the secondary observer are also recorded. At the end of each route (notes: the route is divided into 2 km sectors, then changes occurs in between observers), the number of crocodiles detected by the primary observer (1) and the number of crocodiles detected only by the secondary observer (2) are counted. A key element of the design is that each observer serves both primary and secondary roles on any group of counts. We recommend that observers alternate roles on consecutive counts, with one observer serving as primary at the first 2 km from the route count, secondary at the second count, primary at the third count, and so on. Under this design, each observer will serve as primary observer for half of the total route counts.

## RESULTS

### Spotlight surveys

#### Trip one

A total of 37 km were surveyed in Khor abuo Al hadid part 1 Dahmit area of the lake Nasser, 29 crocodile individuals were observed, the encounter rate or the total abundance = 0.784 crocodiles/km, and the corrected abundance = 0.297 crocodiles/km and note that, the corrected abundance calculated by removes H and Y individuals from the calculation. Of these individuals, 29 (100%) were approached closely enough to estimate size; these included 18 (62.1%) juvenile, 11 (37.9%) sub adults, no hatchlings, no adult and no EO crocodiles were observed during a spotlight survey.

On the other hand, in total of the 14 km surveyed at Khor El magharby of lake Nasser 9 crocodiles were observed, the encounter rate or the total abundance = 0.643 crocodiles/km, and the corrected abundance = 0.500 crocodiles/km. Of these, 1 (11.1%) were classified as EO, and 8 (89%) were approached closely enough to estimate size; these included 2 (22.1%) juvenile, 6 (66.7%) sub adults, furthermore no adults or hatchlings were observed during a spotlight survey.

26 km were surveyed at Khor Ambercab area, and 18 crocodiles were observed, (the encounter rate or the total abundance = 0.692 crocodiles/km, and the corrected abundance = 0.500 crocodiles/km). 5 (11.1%) were classified as EO, and 16 (88.9%) were approached close enough to estimate their size: 5 juveniles (27.8%), 10 sub adults (55.6%) and 1 adult (5.6%).

In total 32 km were surveyed at Khor Ahmed Badawy - W. Dahmit area at lake Nasser 14 crocodiles were observed, the encounter rate or the total abundance = 0.438 crocodiles/km, and the corrected abundance = 0.219 crocodiles/km. Of these, 3 (21.4%) were classified as EO, and 11 (78.6%) were approached close enough to estimate their size; these included 6 juvenile (42.9%), 3 sub adults (21.4%), 1 adult (7.1%) and one hatchling (7.1%).

From the 109 km surveyed at Khor abuo Al hadid part 1st Dahmit area, Khor El magharby, Khor Ambercab and Khor Ahmed Badawy at Dahmit east, 70 crocodiles were observed, (the encounter rate or the total abundance = 0.642 crocodiles/km, and the corrected abundance = 0.349 crocodiles/km). Of these, 6 (8.6%) were classified as EO, and 69 (98.6%) were approached close enough to estimate their size; these included 32 juveniles (45.7%), 29 sub adults (41.4%) and 2 adults (2.9%) and only one hatchling 1 (1.4%). (Tabs. 1 and 2)

Table 1: Crocodile sighting data - trip one.

Survey Date		Site Name		Crocodile sightings by size class (meters)											EO	Total	Survey (km)	Total Abundance	Corrected Abundance
				Hatchling		Yearling		Sub adults			Adults								
				H<40 cm	Y<1 m	1.0 - <1.5	1.5 - <2.0	2.0 - <2.5	>2.5 - <3.0	3.0 - <3.5	3.5 - <4.0	4.0 - <4.5	4.5 - <5.0	> 5.0					
Trip one October 2009	Khor Abuo El hadid	0	18	6	4	1	0	0	0	0	0	0	0	0	29	37	0.784	0.297	
	Khor El magharby	0	2	3	2	1	0	0	0	0	0	0	0	1	9	14	0.643	0.500	
	Khor Ambercab	0	5	9	1	0	0	1	0	0	0	0	0	2	18	26	0.692	0.500	
	Khor Ahmed Badawy - W. Dahmit	1	6	2	1	0	0	1	0	0	0	0	0	3	14	32	0.438	0.219	
Total		1	31	20	8	2	0	2	0	0	0	0	6	70	109	0.642	0.349		

Table 2: Crocodile sighting data - trip one: hatchlings, juveniles, sub adults, adults and eyes-only percentages.

Survey Date	Site Name	hatchling		yearling		sub adult		adult		EO	
		no.	%	no.	%	no.	%	no.	%	no.	%
Oct. 2009	Khor Abuo El hadid	0.0	0.0	18.0	62.1	11.0	37.9	0.0	0.0	0.0	0.0
Oct. 2009	Khor El magharby	0.0	0.0	2.0	22.2	6.0	66.7	0.0	0.0	1.0	11.1
Oct. 2009	Khor Ambercab	0.0	0.0	5.0	27.8	10.0	55.6	1.0	5.6	2.0	11.1
Oct. 2009	Khor Ahmed	1.0	7.1	6.0	42.9	3.0	21.4	1.0	7.1	3.0	21.4
Total		1.0	1.4	32.0	45.7	29.0	41.4	2.0	2.9	6.0	8.6

**Trip two**

In total, 18 km were surveyed at Khor near al hadid Dahmit West and 5 crocodiles were observed, (the encounter rate or the total abundance = 0.278 crocodiles/km, and the corrected abundance = 0.222 crocodiles/km). Of these, 2 (40%) were classified as EO, and 3 (60%) were approached close enough to estimate size; 1 juvenile (20%), 1 sub adult (20%), and 1 adult (20%).

49 km were surveyed at Khor Rahma part 1 and 2, 41 crocodiles were observed, (the encounter rate or the total abundance = 0.837 crocodiles/km, and the corrected abundance = 0.204 crocodiles/km). Of these, 6 (14.6%) were classified as EO, and 35 (85.4%) were approached close enough to estimate size: 31 juveniles (75.6%), 3 sub adults (7.3%), and 1 adult (2.4%).

10 km were surveyed at Mirwaw West (Khor hag Araby) and 3 crocodiles were observed, (the encounter rate or the total abundance = 0.300 crocodiles/km, and the corrected abundance = 0.200 crocodiles/km). All of them were approached close enough to estimate their size: 1 juvenile (33.3%) and 2 sub adults (66.6%).

Table 3: Crocodile sighting data - trip two.

Survey Date		Site Name		Crocodile Sightings by size class (meters)											EO	Total	Survey (km)	Total Abundance	Corrected Abundance	
				Hatchling		Yearling		Sub adults			Adults									
				H<40 cm	Y<1m	1.0 - <1.5	1.5 - <2.0	2.0 - <2.5	>2.5 - <3.0	3.0 - <3.5	3.5 - <4.0	4.0 - <4.5	4.5 - <5.0	> 5.0						
Trip one October 2009	Khor near al hadide Dahmit West	0	1	0	0	1	1	0	0	0	0	0	0	2	5	18	0.278	0.222		
	Khor Rahma	0	31	1	2	0	0	0	0	1	0	0	6	41	49	0.837	0.204			
	Mirwaw West	0	1	1	1	0	0	0	0	0	0	0	0	3	10	0.300	0.200			
	Khor al Manam	0	1	2	3	2	0	0	0	0	0	0	2	10	30	0.333	0.300			
Total		0	34	4	6	3	1	0	0	1	0	0	10	59	107	0.551	0.234			

(TL) as hatchlings (H < 40 cm), juveniles (Y, TL > 40 < 1 m), sub adults classified as (1.0 - < 1.5 m, 1.5 - < 2.0 m and 2.0 - < 2.5 m) and adult individuals were classified as (> 2.5 - < 3.0 m, 3.0 - < 3.5 m, 3.5 - < 4.0 m, 4.0 - < 4.5, 4.5 - < 5.0 m and > 5.0 m), crocodiles that submerged before TL could be determined were classified as 'eye shine only' (EO) the total abundance is the total number/survey distance, and correct abundance removes H and Y individuals from the calculation.

On the other hand, a total of 30 km were surveyed at Khor Al Manam Dabod part 1 and 10 crocodile individuals were observed, (the encounter rate or the total abundance = 0.333 crocodiles/km, and the corrected abundance = 0.300 crocodiles/km): 2 (20%) classified as EO, and 8 (80%) approached close enough to estimate their size: 1 juvenile (10%) and 7 sub adults (70%).

Finally, in total of 107 km were surveyed at Khor near al hadide Dahmit West, Khor Rahma part 1 and 2, Mirwaw West (khor hag Araby) and Khor Al Manam Dabod part 1, 59 crocodiles were observed, the encounter rate or the total abundance = 0.551 crocodiles/km, and the corrected abundance = 0.234 crocodiles/km (note that, the corrected abundance is calculated by removing H and Y individuals from the calculation). Of these, 10 (16.9%) were classified as EO, and 49 (83.1%) were approached close enough to estimate their size: 34 juvenile (57.6%), 13 sub adults (22.0%) and 2 adults (3.4%). (Tabs. 3 and 4)

Table 4: Crocodile Sighting Data - trip two: hatchlings, juveniles, sub adults, adults and eyes-only percentages.

Survey Date	Site Name	hatchling		yearling		sub adult		adult		EO	
		no.	%	no.	%	no.	%	no.	%	no.	%
Nov. 2009	Khor Rahma p1 and 2	0.0	0.0	31.0	75.6	3.0	7.3	1.0	2.4	6.0	14.6
Nov. 2009	Mirwaw West	0.0	0.0	1.0	33.3	2.0	66.7	0.0	0.0	0.0	0.0
Nov. 2009	Khor al Manam	0.0	0.0	1.0	10.0	7.0	70.0	0.0	0.0	2.0	20.0
Total		0.0	0.0	34.0	57.6	13.0	22.0	2.0	3.4	10.0	16.9

### Trip three

25 km were surveyed at Khor Allaqi part 1, 3 crocodiles being observed, (the encounter rate or the total abundance = 0.120 crocodiles/km, and the corrected abundance = 0.120 crocodiles/km): all approached close enough to estimate their size: 2 sub adults (66.6%) and 1 adult (33.3%).

25 km were surveyed at Khor Allaqi part 2 and 10 crocodiles were observed, (the encounter rate or the total abundance = 0.400 crocodiles/km, and the corrected abundance = 0.400 crocodiles/km). Of these, 3 crocodiles (30%) were classified as EO, and 7 (70%) were approached close enough to estimate their size: 6 sub adults (60%), and 1 adult (10%).

From the 50 km surveyed at Khor Allaqi part 1 and Khor Allaqi part 2, 13 crocodiles were observed, (the encounter rate or the total abundance = 0.260 crocodiles/km, and the corrected abundance = 0.260 crocodiles/km). 3 (23.1%) were classified as EO, and 10 (76.9%) were approached closely enough to estimate their size: 8 sub adults (61.5%) and 2 adults (15.4%). (Tabs. 5 and 6)

Table 5: Crocodile sighting data - trip three.

(TL) as hatchlings (H < 40 cm), juveniles (Y, TL > 40 < 1 m), sub adults classified as (1.0 - < 1.5 m, 1.5 - < 2.0 m and 2.0 - < 2.5 m) and adult individuals were classified as (> 2.5 - < 3.0 m, 3.0 - < 3.5 m, 3.5 - < 4.0 m, 4.0 - < 4.5, 4.5 - < 5.0 m and > 5.0 m), crocodiles that submerged before TL could be determined were classified as 'eye shine only' (EO) the total abundance is the total number/survey distance, and correct abundance removes H and Y individuals from the calculation.

Survey Date	Site Name	Crocodile sightings by size class (meters)										EO	Total	Survey (km)	Total Abundance	Corrected Abundance	
		Hatchling		Yearling		Sub adults			Adults								
		H, <40 cm	Y, <1m	1.0 - <1.5	1.5 - <2.0	2.0 - <2.5	>2.5 - <3.0	3.0 - <3.5	3.5 - <4.0	4.0 - <4.5	4.5 - <5.0						> 5.0
Trip 3 Dec. 2009	Khor Allaqi part 1	0	0	1	1	0	0	1	0	0	0	0	0	3	25	0.120	0.120
	Khor Allaqi part 2	0	0	3	3	0	0	0	1	0	0	0	3	10	25	0.400	0.400
Total		0	0	4	4	0	0	1	1	0	0	3	13	50	0.260	0.260	

Table 6: Crocodile sighting data - trip three: hatchlings, juveniles, sub adults, adults and eyes-only percentages.

Survey Date	Site Name	hatchling		yearling		sub adult		adult		EO	
		no.	%	no.	%	no.	%	no.	%	no.	%
Dec. 2009	Khor Allaqi part1	0	0.0	0	0.0	2	66.7	1	33.3	0	0.0
Dec. 2009	Khor Allaqi part2	0	0.0	0	0.0	6	60.0	1	10.0	3	30.0
Total		0	0.0	0	0.0	8	61.5	2	15.4	3	23.1

**Trip four**

25 km were surveyed at Khor Allaqi part 3 and 4 crocodiles were observed, (the encounter rate or the total abundance = 0.160 crocodiles/km, and the corrected abundance = 0.160 crocodiles/km). All 4 (100%) were approached close enough to estimate their size: 2 (50%) sub adults and 2 (50%) adults.

Table 7: Crocodile sighting data - trip four.

(TL) as hatchlings (H < 40 cm), juveniles (Y, TL > 40 < 1 m), sub adults classified as (1.0 - < 1.5 m, 1.5 - < 2.0 m and 2.0 - < 2.5 m) and adult individuals were classified as (> 2.5 - < 3.0 m, 3.0 - < 3.5 m, 3.5 - < 4.0 m, 4.0 - < 4.5, 4.5 - < 5.0 m and > 5.0 m), crocodiles that submerged before TL could be determined were classified as 'eye shine only' (EO) the total abundance is the total number/survey distance, and correct abundance removes H and Y individuals from the calculation.

Survey Date	Site Name	Hatchling		Yearling		Crocodile sightings by size class (meters)						EO	Total	Survey (km)	Total Abundance	Corrected Abundance	
		H, <sub>&lt;</sub> 40 cm	Y, <sub>&lt;</sub> 1m	Sub adults			Adults										
				1.0 - <1.5	1.5 - <2.0	2.0 - <2.5	>2.5 - <3.0	3.0 - <3.5	3.5 - <4.0	4.0 - <4.5	4.5 - <5.0						> 5.0
Trip 4 Mar. 2010	Khor Allaqi part3	0	0	1	1	0	0	1	0	1	0	0	0	4	25	0.160	0.160
	Khor Allaqi part4	0	0	1	1	0	0	0	1	0	0	0	3	3	25	0.120	0.120
Total		0	0	2	2	0	0	1	1	1	0	0	3	7	50	0.140	0.140

25 km were surveyed at Khor Allaqi part 4 and 3 crocodiles were observed, (the encounter rate or the total abundance = 0.120 crocodiles/km, and the corrected abundance = 0.120 crocodiles/km), all approached close enough to estimate their size; 2 sub adults (66.7%) and 1 adult (33.3%).

From the 50 km surveyed at Khor Allaqi part 3 and Khor Allaqi part 4, 7 crocodiles were observed, (the encounter rate or the total abundance = 0.140 crocodiles/km, and the corrected abundance = 0.140 crocodiles/km, all of them approached close enough to estimate their size: 4 sub adults (57.1%) and 3 adults (42.9%). (Tabs. 7 and 8)

Table 8: Crocodile sighting data - trip four; hatchlings, juveniles, sub adults, adults and eyes-only percentages.

Survey Date	Site Name	hatchling		yearling		sub adult		adult		EO	
		no.	%	no.	%	no.	%	no.	%	no.	%
Mar. 2010	Khor Allaqi	0	0	0	0	2	50	2	50	0	0
Mar. 2010	Khor Allaqi	0	0	0	0	2	66	1	33	0	0
Total	0	0	0	0	0	4	57.1	3	42.9	0	0

**Trip five**

25 km were surveyed at Khor El Tiri at El Madiq area, 23 crocodiles were observed, (the encounter rate or the total abundance = 0.920 crocodiles/km, and the corrected abundance = 0.840 crocodiles/km) - 2 (8.7%) classified as EO, and 21 (91.3%) approached close enough to estimate their size - 2 juveniles (8.7%), 18 sub adults (78.3%) and 1 adult (4.4%).

40 km were surveyed at Khor Chalbia at El Madiq area 15 crocodiles being observed, (the encounter rate or the total abundance = 0.375 crocodiles/km, and the corrected abundance = 0.325 crocodiles/km) - 5 (33.3%) classified as EO, and 10 (66.6%) approached close enough to estimate their size - 2 juveniles (13.3%) and 8 sub adults (53.3%).

58 km were surveyed at Khor Chalbia - Awad at El Madiq area 31 crocodiles being observed, (the encounter rate or the total abundance = 0.534 crocodiles/km, and the corrected abundance = 0.500 crocodiles/km) - 2 (6.5%) classified as EO and 29 (93.5%) approached close enough to estimate their size - 2 juveniles (6.5%), 23 sub adults (74.2%) and 4 adults (12.9%).

Table 9: Crocodile sighting data - trip five hatchlings, juveniles, sub adults, adults and eyes-only percentages.

Survey Date		Site Name		Crocodile sightings by size class (meters)										EO	Total	Survey (km)	Total Abundance	Corrected Abundance		
				Hatchling		Yearling		Sub adults			Adults									
				H<40 cm	Y<1m	1.0 - <1.5	1.5 - <2.0	2.0 - <2.5	>2.5 - <3.0	3.0 - <3.5	3.5 - <4.0	4.0 - <4.5	4.5 - <5.0						> 5.0	
Trip 5 April 2010	Khor El Tiri El Madiq area	0	2	15	2	1	0	1	0	0	0	0	2	23	25	0.920	0.840			
	Khor Chalbia	0	2	6	1	1	0	0	0	0	0	0	5	15	40	0.375	0.325			
	Khor Chalbia Awad	0	2	17	4	2	2	1	0	1	0	0	2	31	58	0.534	0.500			
	part of El Tiri and El Sayala	0	4	15	8	0	0	0	0	0	0	0	4	31	25	1.240	1.080			
Total		0	10	53	15	4	2	2	0	1	0	0	13	100	148	0.676	0.608			

Table 10: Crocodile sighting data - trip five, percentages.

Survey Date	Site Name	hatchling		yearling		sub adult		adult		EO	
		no	%	no	%	no	%	no	%	no	%
April 2010	Khor El Tiri El Madiq area	0	0	2	8.70	18	78.26	1	4.35	2	8.70
April 2010	Khor Chalbia	0	0	2	13.33	8	53.33	0	0	5	33.33
April 2010	Khor Chalbia Awad	0	0	2	6.45	23	74.19	4	12.9	2	6.45
April 2010	El Tiri and El Sayala	0	0	4	12.90	23	74.19	0	0	4	12.90
Total		0	0	10	10.00	72	72.00	5	5	13	13.00

25 km were surveyed at Last part of El Tiri and El Sayala area at El Madiq area and 31 crocodiles were observed (the encounter rate or the total abundance = 1.240 crocodiles/km, and the corrected abundance = 1.080 crocodiles/km) - 4 (12.9%) classified as EO, and 29 (87.1%) approached close enough to estimate their size - 4 juveniles (12.9%), 23 sub adults (74.2%) and 2 adults.

From the 148 km surveyed at Khor of El Madiq area 100 crocodiles were seen - (the encounter rate or the total abundance = 0.676 crocodiles/km, and the corrected abundance = 0.680 crocodiles/km) 13 (13.0%) classified as EO and 87 (87%) approached close to estimate their size - 10 juveniles (10%), 72 sub adults (72%) and 5 adults (5.0%). (Tabs. 9 and 10)

### Trip six

80 km were surveyed at Khor El sheikh Abellah el Madiq area and 33 crocodiles were observed, (the encounter rate or the total abundance = 0.413 crocodiles/km, and the corrected abundance = 0.188 crocodiles/km) - 7 (21.2%) classified as EO, and 26 (79.8%) approached close enough to estimate sizes - 18 juveniles (54.2%), 7 sub adults (21.2%) and 1 adult (3.0%).

30 km were surveyed at Wadi Abayad area part 1 area and 2 crocodiles were observed (the encounter rate or the total abundance = 0.067 crocodiles/km, and the corrected abundance = 0.033 crocodiles/km), both approached close enough to estimate their size - 1 juvenile (50%) and 1 sub adult (50%).

Finally in total of 165 km were surveyed at Khor El sheikh Abellah el Madiq area, at El Khor Al Ahmer El Madiq area, Khor Sa'ad (El gabana) Rahma A area and Wadi Abayad area part 1 area 61 crocodiles were observed, (the encounter rate or the total abundance = 0.370 crocodiles/km, and the corrected abundance = 0.218 crocodiles/km. Of these, 8 (13.1%) were classified as EO and 53 (87.9%) were approached close enough to estimate size: 25 (41.0%) juveniles, 27 (44.3%) sub adults and 1 (1.6%) adults (Tabs. 11 and 12).

25 km were surveyed at El Khor Al Ahmer El Madiq area 16 crocodiles being observed, (the encounter rate or the total abundance = 0.640 crocodiles/km, and the corrected abundance = 0.440 crocodiles/km), 1 (6.3%) classified as EO, and 15 (93.7%) approached close enough to estimate their size - 5 juveniles (31.3%) and 10 sub adults (62.2%). 30 km were also surveyed at Khor Sa'ad (El gabana) Rahma A area with 10 crocodiles observed, (the encounter rate or the total abundance = 0.333 crocodiles/km, and the corrected abundance = 0.300 crocodiles/km), all of them approached close enough to estimate their size - 1 juvenile (10%) and 9 sub adults (90%). (Tabs. 11 and 12)

Table 11: Crocodile sighting data - trip six.

(TL) as hatchlings (H < 40 cm), juveniles (Y, TL > 40 < 1 m), sub adults classified as (1.0 - < 1.5 m, 1.5 - < 2.0 m and 2.0 - < 2.5 m) and adult individuals were classified as (> 2.5 - < 3.0 m, 3.0 - < 3.5 m, 3.5 - < 4.0 m, 4.0 - < 4.5, 4.5 - < 5.0 m and > 5.0 m), crocodiles that submerged before TL could be determined were classified as 'eye shine only' (EO) the total abundance is the total number/survey distance, and correct abundance removes H and Y individuals from the calculation.

Survey Date	Site Name	Hatchling		Yearling		Crocodile sightings by size class (meters)						EO	Total	Survey (km)	Total Abundance	Corrected Abundance	
		Sub adults		Adults													
		H<40 cm	Y<1m	1.0 - <1.5	1.5 - <2.0	2.0 - <2.5	>2.5 - <3.0	3.0 - <3.5	3.5 - <4.0	4.0 - <4.5	4.5 - <5.0						> 5.0
Trip 6 May 2010	Khor El sheikh Abellah El Madiq	0	18	3	2	2	0	0	1	0	0	0	7	33	80	0.413	0.188
	El Khor Al Ahmer El Madiq	0	5	5	2	3	0	0	0	0	0	0	1	16	25	0.640	0.440
	Khor Sa'ad Rahma	0	1	5	2	2	0	0	0	0	0	0	0	10	30	0.333	0.300
	Wadi Abayad area part 1	0	1	1	0	0	0	0	0	0	0	0	0	2	30	0.067	0.033
Total	0	25	14	6	7	0	0	1	0	0	0	8	61	165	0.370	0.218	

Table 12: Crocodile sighting data - trip six, percentages.

Survey Date	Site Name	hatchling		yearling		sub adult		adult		EO	
		no.	%	no.	%	no.	%	no.	%	no.	%
May 2010	Khor El sheikh Abellah el Madiq	0	0.00	18	54.5	7	21.2	1	3.00	7	21.2
May 2010	El Khor Al Ahmer El Madiq	0	0.00	5	31.3	10	62.5	0	0.00	1	6.3
May 2010	Khor Sa'ad Rahma A	0	0.00	1	10.0	9	90.0	0	0.00	0	0.00
May 2010	Wadi Abayad area part 1	0	0.00	1	50.0	1	50.0	0	0.00	0	0.00
Total		0	0.00	25	41.0	27	44.3	1	1.6	8	13.1

**Trip seven**

40 km were surveyed at Wadi Abayad area part 2 - Khor Anter and 12 crocodiles were observed, (the encounter rate or the total abundance = 0.300 crocodiles/km, and the corrected abundance = 0.295 crocodiles/km) - 1 (8.3%) classified as EO and 11 (91.7%) approached close enough to estimate their size - 4 juveniles (33.3%) and 7 sub adults (58.3%).

Table 13: Crocodile sighting data - trip seven.

Survey Date	Site Name	Crocodile sightings by size class (meters)										EO	Total	Survey (km)	Total Abundance	Corrected Abundance	
		Hatchling		Sub adults			Adults										
		H<40 cm	Y<1m	1.0 - <1.5	1.5 - <2.0	2.0 - <2.5	>2.5 - <3.0	3.0 - <3.5	3.5 - <4.0	4.0 - <4.5	4.5 - <5.0						> 5.0
Trip 7 June 2010	Wadi Abayad area part 2 - Khor Anter	0	4	4	2	1	0	0	0	0	0	0	1	12	40	0.300	0.295
	Khor El Singari - east	0	3	2	3	1	0	0	0	0	0	0	2	11	39	0.282	0.274
	Khor Abuo Handal - east	0	3	2	0	0	0	0	0	0	0	0	1	6	35	0.171	0.171
	Khor Wadi El Arab east	0	1	0	4	6	0	4	5	0	0	0	0	20	40	0.500	0.500
	Khor El Fouly	0	7	8	2	0	0	0	0	0	0	0	0	17	45	0.378	0.378
	Khor Lantern (El Fanos) El Sibou	0	0	5	2	0	0	0	0	0	0	0	3	10	35	0.286	0.286
	Khor Galal - Hassan metwaly Islands	0	0	1	0	0	1	0	0	0	0	0	0	2	10	0.200	0.200
Total	0	18	22	13	8	1	4	5	0	0	0	7	78	244	0.320	0.320	

Table 14: Crocodile sighting data - trip seven: hatchlings, juveniles, sub adults, adults and eyes-only percentages.

Survey Date	Site Name	hatchling		yearling		sub adult		adult		EO	
		no.	%	no.	%	no.	%	no.	%	no.	%
June 2010	Wadi Abayad area part 2 - Khor Anter	0	0.00	4	33.33	7	58.33	0	0.00	1	8.33
June 2010	Khor El Singari - east	0	0.00	3	27.27	6	54.55	0	0.00	2	18.18
June 2010	Khor Abuo Handal (the main) east	0	0.00	3	50.00	2	33.33	0	0.00	1	16.67
June 2010	Khor Wadi El Arab east	0	0.00	1	5.00	10	50.00	9	45	0	0.00
June 2010	Khor El Fouly	0	0.00	7	41.18	10	58.82	0	0.00	0	0.00
June 2010	Khor Lantern (El Fanos) El Sibou	0	0.00	0	0.00	7	70.00	0	0.00	3	30.00
June 2010	Khor Galal - Hassan metwaly Islands	0	0.00	0	0.00	1	50.00	1	50	0	0.00
Total		0	0.00	18	23.08	44	56.41	9	11.5	7	8.97

39 km were surveyed at Khor El Singari - east area, 11 crocodiles being observed (the encounter rate or the total abundance = 0.282 crocodiles/km, and the corrected abundance = 0.274 crocodiles/km); 2 (18.2%) were classified as EO and 10 (81.2%) were approached close enough to estimate their size - 3 juveniles (27.3%) and 6 sub adults (54.6%).

A total of 35 km were surveyed at Khor Abuo Handal, 6 crocodiles were observed (the encounter rate or the total abundance = 0.171 crocodiles/km, and the corrected abundance = 0.171 crocodiles/km), 1 (16.7%) was classified as EO and 5 (83.3%) were approached close enough to estimate their size - 3 juvenile (50.0%) and 2 sub adults (33.3%).

40 km were also surveyed at Khor Wadi El Arab east, 20 crocodiles being observed (the encounter rate or the total abundance = 0.500 crocodiles/km, and the corrected abundance = 0.500 crocodiles/km); all crocodiles were approached close enough to estimate their size; these included 1 juvenile (5.0%), 10 sub adults (50.0%) and 9 adults (45.0%).

45 km were surveyed at Khor El Fouly and 17 crocodiles were observed (the encounter rate or the total abundance = 0.378 crocodiles/km, and the corrected abundance = 0.378 crocodiles/km). All 17 were approached close enough to estimate their size - 7 juveniles (41.2%) and 10 sub adults (58.8%).

35 km were also surveyed at Khor Lantern (El Fanos) El Sibou east, 10 crocodiles were observed (the encounter rate or the total abundance = 0.286 crocodiles/km, and the corrected

abundance = 0.286 crocodiles/km). 3 (30.0%) were classified as EO and 7 (70%) were approached close enough to estimate their size, all of them sub adults.

10 km were surveyed at Khor Galal - Hassan metwaly Islands and 2 crocodiles were observed (the encounter rate or the total abundance = 0.200 crocodiles/km, and the corrected abundance = 0.200 crocodiles/km), both approached close enough to estimate their size - 1 sub adult (50.0%) and 1 adult (50.0%).

A total of 244 km were surveyed at Wadi Abayad area part 2 - Khor Anter, Khor El Singari - east, Khor Abu Handal - east, Khor Wadi El Arab east, Khor El Fouly, Khor Lantern (El Fanos) El Sibou east and Khor Galal - Hassan metwaly Islands), 78 crocodiles being observed (the encounter rate or the total abundance = 0.320 crocodiles/km, and the corrected abundance = 0.320 crocodiles/km), 7 (8.97%) were classified as EO, and 71 (91.0%) were approached close enough to estimate their size - 18 juveniles (23.1%), 44 sub adults (56.4%) and 9 adults (11.5%). (Tabs. 13 and 14)

### **Trip eight**

40 km were surveyed at Wadi Abayad area part 3 - Khor Anter and 26 crocodiles were observed (the encounter rate or the total abundance = 0.650 crocodiles/km, and the corrected abundance = 0.475 crocodiles/km), 3 of these (11.54%) were classified as EO and 23 (88.5%) were approached close enough to estimate their size - 7 juveniles (26.92%), 4 sub adults (15.38%) and 12 adults (46.2%).

30 km were surveyed at Khor El Meqawel - Shatorma, 4 crocodiles were observed (the encounter rate or the total abundance = 0.133 crocodiles/km, and the corrected abundance = 0.274 crocodiles/km), all approached closely enough to estimate their size - 1 juvenile (25.0%), 2 sub adults (50.0%) and 1 adults (25.0%).

30 km were also surveyed at Khor Dahab - Tushka east and 16 crocodiles were observed (the encounter rate or the total abundance = 0.533 crocodiles/km, and the corrected abundance = 0.533 crocodiles/km), 2 (12.50%) classified as EO and 5 (87.5%) approached close enough to estimate their size - 11 sub adults (68.75%) and 3 adults (18.8%).

32 km were surveyed at El Genina and El Sheibbak Mid and third Khor and 15 crocodiles were observed (the encounter rate or the total abundance = 0.469 crocodiles/km, and the corrected abundance = 0.469 crocodiles/km). 4 of these crocodiles (26.67%) were classified as EO and 11 (73.3%) were approached close enough to estimate their size - 9 sub adults (60.0%) and 2 adults (13.3%).

20 km were surveyed at Khor Abel khany - Tunqula east and 3 crocodiles were observed (the encounter rate or the total abundance = 0.150 crocodiles/km, and the corrected abundance = 0.100 crocodiles/km). All approached close enough to estimate their size - 1 juvenile (33.3%), 1 sub adults (33.3%).

25 km were surveyed at Khor Abu Dirwa - Kushtamina West, 5 crocodiles were observed (the encounter rate or the total abundance = 0.200 crocodiles/km, and the corrected abundance = 0.200 crocodiles/km) and all were approached close enough to estimate their size - 3 sub adults (60.0%) and 2 adults (40.0%).

25 km were surveyed at Khor Rahma - Khor tip part 3 and 5 crocodiles were observed (the encounter rate or the total abundance = 0.200 crocodiles/km, and the corrected abundance = 0.120 crocodiles/km) All 5 were approached close enough to estimate their size - 2 juveniles (40.0%) and 3 adults (60.0%).

Table 15: Crocodile sighting data - trip eight.

(TL) as hatchlings (H < 40 cm), juveniles (Y, TL > 40 < 1 m), sub adults classified as (1.0 - < 1.5 m, 1.5 - < 2.0 m and 2.0 - < 2.5 m) and adult individuals were classified as (> 2.5 - < 3.0 m, 3.0 - < 3.5 m, 3.5 - < 4.0 m, 4.0 - < 4.5, 4.5 - < 5.0 m and > 5.0 m), crocodiles that submerged before TL could be determined were classified as 'eye shine only' (EO) the total abundance is the total number/survey distance, and correct abundance removes H and Y individuals from the calculation.

Survey Date	Site Name	Crocodile sightings by size class (meters)										EO	Total	Survey (km)	Total Abundance	Corrected Abundance		
		Hatchling		Yearling			Sub adults			Adults								
		H < 40 cm	Y < 1 m	1.0 - < 1.5	1.5 - < 2.0	2.0 - < 2.5	> 2.5 - < 3.0	3.0 - < 3.5	3.5 - < 4.0	4.0 - < 4.5	4.5 - < 5.0						> 5.0	
Trip 8 July 2010	Wadi Abayad area part 3 - Khor Anter	0	7	0	3	1	4	2	3	1	1	1	3	26	40	0.650	0.475	
	Khor El Meqawel	0	1	0	1	0	1	1	0	0	0	0	0	4	30	0.133	0.100	
	Khor Dahab - Tushka east	0	0	2	7	2	0	1	1	1	0		2	16	30	0.533	0.533	
	El Genina and El Sheibbak Mid and third Khor	0	0	2	4	3	1	0	0	1	0	0	4	15	32	0.469	0.469	
	Khor Abel khany - Tunqula east	0	1	1	0	0	0	0	0	0	0	0	1	3	20	0.150	0.100	
	Khor Abu Dirwa - Kushtamina West	0	0	1	1	1	1	0	1	0	0	0	0	5	25	0.200	0.200	
	Khor Rahma - Khor tip part 2	0	2	0	0	0	0	1	0	2	0	0	0	5	25	0.200	0.120	
	<b>Total</b>	<b>0</b>	<b>11</b>	<b>6</b>	<b>16</b>	<b>7</b>	<b>7</b>	<b>5</b>	<b>5</b>	<b>5</b>	<b>1</b>	<b>1</b>	<b>10</b>	<b>74</b>	<b>202</b>	<b>0.366</b>	<b>0.312</b>	

Table 16: Crocodile sighting data - trip eight - hatchlings, juveniles, sub adults, adults and eyes-only percentages.

Survey Date	Site Name	hatchling		yearling		sub adult		adult		EO	
		no.	%	no.	%	no.	%	no.	%	no.	%
July 2010	Wadi Abayad area part 3 - Khor Anter	0	0.00	7	26.92	4	15.38	12	46.2	3	11.54
July 2010	Khor El Meqawel	0	0.00	1	25.00	1	25.00	2	50	0	0.00
July 2010	Khor Dahab - Tushka east	0	0.00	0	0.00	11	68.75	3	18.8	2	12.50
July 2010	El Genina and El Sheibbak Mid and third Khor	0	0.00	0	0.00	9	60.00	2	13.3	4	26.67
July 2010	Khor Abel khany - Tunqula east	0	0.00	1	33.33	1	33.33	0	0.00	1	33.33
July 2010	Khor Abu Dirwa - Kushtamna West	0	0.00	0	0.00	3	60.00	2	40	0	0.00
July 2010	Khor Rahma - Khor tip part 2	0	0.00	2	40.00	0	0.00	3	60	0	0.00
Total		0	0.00	11	14.86	29	39.19	24	32.4	10	13.51

A total of 202 km were surveyed at Wadi Abayad area part 3 - Khor El Meqawel - Shatorma, Khor Dahab - Tushka east, El Genina and El Sheibbak Mid and third Khor, Khor Abel khany - Tunqula east and The tip part of Khor Rahma part 3) 74 crocodiles being observed (the encounter rate or the total abundance = 0.366 crocodiles/km, and the corrected abundance = 0.312 crocodiles/km. 10 of these (13.51%) were classified as EO and 64 (86.5%) were approached close enough to estimate their size - 11 juveniles (14.9%), 29 sub adults (39.2%) and 24 adults (32.4%). (Tabs. 15 and 16)

### **Trip nine**

24 km were surveyed at Khor Abu Al-Hadid part 2 Dahmit area and 18 crocodiles were observed (the encounter rate or the total abundance = 0.750 crocodiles/km, and the corrected abundance = 0.667 crocodiles/km). 7 of them (38.9%) were classified as EO, and 11 (61.1%) were approached close enough to estimate their size - 2 juveniles (11.1%), 7 sub adults (38.9%) and 2 adults (11.1%).

29.5 km were surveyed at Khor El Soker (Sugar) - Korth and 6 crocodiles were observed (the encounter rate or the total abundance = 0.203 crocodiles/km, and the corrected abundance = 0.203 crocodiles/km). 5 of these (83.3%) were approached close enough to estimate their size all of them sub adults.

Table 17: Crocodile sighting data - trip nine.

(TL) as hatchlings (H < 40 cm), juveniles (Y, TL > 40 < 1 m), sub adults classified as (1.0 - < 1.5 m, 1.5 - < 2.0 m and 2.0 - < 2.5 m) and adult individuals were classified as (> 2.5 - < 3.0 m, 3.0 - < 3.5 m, 3.5 - < 4.0 m, 4.0 - < 4.5, 4.5 - < 5.0 m and > 5.0 m), crocodiles that submerged before TL could be determined were classified as 'eye shine only' (EO) the total abundance is the total number/survey distance, and correct abundance removes H and Y individuals from the calculation.

Survey Date	Site Name	Crocodile sightings by size class (meters)										EO	Total	Survey (km)	Total Abundance	Corrected Abundance	
		Hatchling H<40 cm	Yearling Y<1m	Sub adults			Adults										
				1.0 - <1.5	1.5 - <2.0	2.0 - <2.5	>2.5 - <3.0	3.0 - <3.5	3.5 - <4.0	4.0 - <4.5	4.5 - <5.0						> 5.0
Trip 9 August 2010	Khor Abu Al-Hadid part 2	0	2	7	0	0	1	0	0	0	1	0	7	18	24	0.750	0.667
	Khor El Soker(sugar)	0	0	2	3	0	0	0	0	0	0	0	1	6	29.5	0.203	0.203
	Gaif Hussein (khor Ismail hagag)	0	2	0	1	0	0	0	0	0	0	0	0	3	30	0.100	0.033
	Khor Maria	8	0	0	0	1	0	0	1	1	1	0	6	18	30.8	0.584	0.325
	Dahmit east (abdellmu lla area)	2	0	3	1	0	0	1	1	0	0	0	3	11	30	0.367	0.300
	Dabod area (khor Bram)	0	0	0	1	2	0	0	0	0	0	0	3	6	22	0.273	0.273
	Dabod area (khor El Safih)	0	0	1	1	0	0	0	0	0	0	0	2	4	20	0.200	0.200
	Khor Emicool	0	0	1	1	0	0	0	0	0	0	0	2	4	20	0.200	0.200
Total	10	4	13	7	3	1	1	2	1	2	0	21	65	186.3	0.349	0.274	

30 km were surveyed at Khor Ismail hagag Garf Hussein area and 6 crocodiles were observed (the encounter rate or the total abundance = 0.100 crocodiles/km, and the corrected abundance = 0.033 crocodiles/km). 6 (100%) were approached close enough to estimate their size - 4 juveniles (66.7%) and 2 sub adults (33.3%).

Table 18: Crocodile sighting data - trip nine: hatchlings, juveniles, sub adults, adults and eyes-only percentages.

Survey Date	Site Name	hatchling		yearling		sub adult		adult		EO	
		no.	%	no.	%	no.	%	no.	%	no.	%
August 2010	Khor Abu Al - Hadid part 2	0	0.00	2	11.11	7	38.89	2	11.1	7	38.89
August 2010	Khor El Soker (Sugar)	0	0.00	0	0.00	5	83.33	0	0.00	1	16.67
August 2010	Garf Hussein (Khor Ismail hagag)	0	0.00	2	66.67	1	33.33	0	0.00	0	0.00
August 2010	Khor Maria	8	44.4	0	0.00	1	5.56	3	16.7	6	33.33
August 2010	Dahmit east (Abdellmulla area)	2	18.2	0	0.00	4	36.36	2	18.2	3	27.27
August 2010	Dabod area (Khor Bram)	0	0.00	0	0.00	3	50.00	0	0.00	3	50.00
August 2010	Dabod area (Khor El Safih) Khor Emicool	0	0.00	0	0.00	2	50.00	0	0.00	2	50.00
Total		10	15.4	4	6.15	23	35.38	7	10.8	21	32.31

30.8 km were surveyed at Khor Maria area, 18 crocodiles being observed (the encounter rate or the total abundance = 0.584 crocodiles/km, and the corrected abundance = 0.325 crocodiles/km). 6 of these (33.3%) were classified as EO and 12 (66.7%) were approached close enough to estimate their size - 8 hatchlings (44.4 %), 1 sub adult (5.6%) and 3 adults (16.7%).

30 km were surveyed at Abdellmulla area Dahmit east and 11 crocodiles were observed (the encounter rate or the total abundance = 0.367 crocodiles/km, and the corrected abundance = 0.300 crocodiles/km). 3 (27.3%) were classified as EO and 9 (81.8%) were approached close enough to estimate their size - 2 hatchlings (18.2%), 4 sub adults (36.4%) and 2 adults (18.2%).

22 km were surveyed at Khor al Manam part 2 Dabod area, 6 crocodiles being observed (the encounter rate or the total abundance = 0.273 crocodiles/km, and the corrected abundance = 0.273 crocodiles/km). 3 of these crocodiles (50%) were classified as EO and 3 (50%) were approached close enough to estimate their size, all sub adults.

20 km were surveyed at Khor El Safih Khor Emicool Dabod area 3 crocodiles being observed (the encounter rate or the total abundance = 0.150 crocodiles/km, and the corrected abundance = 0.150 crocodiles/km) - 1 (33.3%) classified as EO and 2 (66.7%) approached close enough to estimate their size, both sub adults.

From the total of 186.3 km surveyed at Khor Abu Al-Hadid part 2 Dahmit area, Khor El Soker (Sugar) - Khor Ismail hagag Garf Hussein area Korth, Khor Maria area, Abdellmulla area Dahmit east, Khor al Manam part 2 Dabod area and khor El Safih Khor Emicool Dabod area, 66 crocodiles were observed (the encounter rate or the total abundance = 0.354 crocodiles/km, and the corrected abundance = 0.279 crocodiles/km) 22 of these (33.3%) were classified as EO and 44 (66.6%) were approached close enough to estimate size - 11 hatchlings (15.4%), 4 juveniles (6.15%), 23 sub adults (35.4%) and 7 adults (10.8%) (Tabs. 17 and 18).

### An estimation of the total number of crocodiles from Nasser Lake

It was observed that nocturnal (night) counts provide a practical means of sampling crocodilian populations in many areas, this come in harmony with Chabreck (1966); Woodward and Marion (1978) and Botha (2005) but the main problem with estimating population size from nocturnal counts is that data involves the estimation of the proportion of total animals which are actually seen (Nichols, 1987). On the other hand, Woodward and Marion (1978) reported that data provided by nocturnal surveys probably vary from one area to another and with environmental conditions especially when we work in a huge area like the lake Nasser.

Table 19: The number of Nile crocodiles counted and the estimated probability and abundance of crocodiles detected using DOBSERV (Hines, 2000).

T	No.	Location name	X	P	SE (P)	N	SE (N)	Lower	Upper
T1	1	Khor el hadide part 1	10	1.000	0.0000	10	0.000	----	----
	2	Khor El hadide part 1 cont	19	0.9969	0.0064	19.06	0.270	19.00	20.86
	3	Khor El magharby	9	1.000	0.000	9.000	0.000	----	----
	4	Khor Ambercab	18	1.000	0.000	18.00	0.000	----	----
	5	Khor Ahmed Badawy (Dahmit east)	14	0.9941	0.0123	14.08	0.340	14.00	16.29
T2	6	Khor near al hadide	5	1.000	0.000	5.000	0.000	----	----
	7	Khor Rahma part 1	14	1.000	0.000	14.00	0.000	----	----
	8	Khor Rahma part 2	27	0.7500	0.2042	36.00	10.39	28.48	81.68
	9	Mirwaw West (Khor Hag Araby)	3	1.000	0.000	3.000	0.000	-----	-----
	10	Khor Al Manam	10	0.9375	0.0989	10.67	1.41	10.05	18.55
T3	11	Allaqi	7	1.000	0.000	7.00	0.000	-----	-----
T4	12	Allaqi	13	1.000	0.000	13.00	1.000	-----	-----
T5	13	Khor El Tiri El Madiq area	23	0.8754	0.1183	26.27	4.04	23.50	44.60
	14	Khor Chalbia	15	0.9949	0.0106	15.08	0.32	15.00	17.18
	15	Khor Chalbia Awad	31	0.8326	0.1325	37.23	6.52	32.15	64.64
	16	part of El Tiri and El Sayala	31	0.9149	0.0731	33.88	3.24	31.49	47.93
T6	17	Wadi Abayad area part 1	2	1.000	0.000	2.000	0.000	-----	-----
	18	Khor El sheikh Abellah El Madiq P1	7	1.000	0.000	2.000	0.000	-----	-----
	19	Khor El sheikh Abellah El Madiq p 2	26	0.9669	0.0360	26.89	1.39	26.10	33.83
	20	El Khor Al Ahme El Madiq	16	0.9467	0.0683	16.90	1.56	16.09	25.06
	21	Khor Sa'ad (El gabana) Rahma area	10	1.000	0.000	0.000	0.000	-----	-----

Table 19 (continued): The number of Nile crocodiles counted and the estimated probability and abundance of crocodiles detected using DOBSERV (Hines, 2000).

T	No.	Location name	X	P	SE (P)	N	SE(N)	Lower	Upper
T7	22	Wadi Abayad area part 2- Khor Anter	12	0.9917	0.0173	12.10	0.38	12.00	14.57
	23	Khor El Singari - east	11	0.8594	0.1905	12.80	3.18	11.17	29.58
	24	Khor Abu Handal (the main)- east	6	1.000	0.000	6.000	0.000	-----	-----
	25	Khor Wadi El Arab east	20	0.8889	0.1148	22.50	3.35	20.34	38.27
	26	Khor El Fouly	17	1.000	0.000	17.00	0.000	-----	-----
	27	Khor Lantern (El Fanos) El Sibou	10	0.9375	0.0989	10.67	1.41	10.05	18.55
	28	Hassan metwaly Islands	2	1.000	0.000	2.000	000	----	---
T8	29	Wadi Abayad area part 3- Khor Anter	26	0.8678	0.1201	30.08	4.71	26.67	50.78
	30	Khor El Meqawel	4	1.000	0.000	4.000	0.000	----	-----
	31	Khor Dahab- Tushka east	16	0.8889	0.1283	18.00	3.00	16.24	32.80
	32	El Genina and El Sheibbak Mid and third khor	15	0.8678	0.1545	17.29	3.48	15.27	34.54
	33	Khor Abel khany - Tunqula east	3	1.000	0.000	3.000	0.000	----	----
	34	Khor Abu Dirwa- Kushtamina West	5	1.000	0.000	5.000	0.000	----	----
	35	Khor Rahma - Khor tip part 2	5	1.000	0.000	5.000	0.000	----	----
T9	36	Khor abuo Al hadid part 2	18	0.9600	0.0506	18.75	1.33	18.07	24.74
	37	Khor El Soker (Sugar)	6	0.9600	0.0877	6.26	0.77	6.01	11.01
	38	Garf Hussein (khor Ismail hagag)	3	1.0000	0.000	3.00	0.00	----	----
	39	Khor Maria	18	0.9600	0.0506	18.75	1.33	18.07	25.74
	40	Dahmit east (abdellmulla area)	11	0.9505	0.0773	11.57	1.22	11.04	18.43
	41	Dabod area (khor Bram)	6	0.9600	0.0877	6.25	0.77	6.01	11.01
	42	Dabod area (khor El Safih) Khor Emicool	4	1.0000	0.000	4.00	0.00	-----	----
Total			528			553.1			

The numbers of crocodiles counted in Nasser Lake during nocturnal spotlight survey counts at 42 sites are 528. However, as explained above, this figure takes into account the influence of environmental factors/conditions and the variations in between different locations. Turner (1977) reported that counting of Nile crocodiles sometimes shows some difficulty due to their seasonal migrations. On the other hand, an aerial survey is very difficult to conduct in Nasser Lake for many reasons such the complicated process for license of helicopters, very expensive letting prices, and the difficulty of the Nasser Lake shorelines and landscape due to the presence of high mountains, hills and the difficult topography, so an estimation of Nile crocodile depends of the method described above. On using Double-Observer Counts and analyzed data on using DOBSERV program (Hines, 2000; see the table 19).

An average estimate of detectability was  $\approx 0.96$  in between the 42 locations and thus the total estimated count of about 553.1 crocodiles. According to the variation of Nasser Lake shoreline length by the water fluctuation, is given an estimation of the lake Nasser crocodiles according to its shore line length.

In accordance of a total of 528 Nile crocodile were observed along our survey of about 1261.3 km of survey route the total abundance or "the encounter rate" (0.419 crocodiles/km) and the corrected abundance (0.304 crocodile/km). It was noted that a considerable variation is manifested in the total abundance and in the in counter rate in between 42 locations (Tab. 20).

A chi-square test ( $\chi^2 = 254.2$ ,  $df. = 41$ ;  $P < 0.0001$ ) indicated the fact that the crocodile individuals were not randomly distributed in between habitats along the lake Nasser shoreline.

The obtained data, involve three important factors which are: the detectability factor (0.43), the total abundance or the encounter rare factor (0.419) and the corrected abundance factor (0.304). Based on the above three factors, according to the table 10, we can estimate the total number of Nile crocodiles in lake Nasser.

These factors can be multiplied separately by the lake shoreline length at a given water level, and we can find the estimated value of the crocodile's number. However, those values would be under some adjustment later when deducting the value of certain areas, showing that most of them are considered not suitable/less suitable habitats, which have proved to be qualified to zero number for crocodiles or through which data possibilities that were made at a number of them randomly during the field survey show that they do not contain any crocodile, and the probability of crocodiles by the non-existent, but even if it had seen one or more.

Our investigation and recognition post facto detected that the crocodiles walk during the migration when moving from one place to another, in other words, it was passing the road only and the nocturnal survey did not gave any evidence about the occurrence of any crocodile.

Moreover, the lake Nasser water level during the survey was in between 175 m a.s.l. and then dropped to 170 m a.s.l., meaning that the average level of lake Nasser during observations was about  $\approx 172/172.5$  m a.s.l. and this will give a high variation in lake Nasser shoreline total length by a significant amount.

According to the area estimated and counted or determined, using supervised classification and 3D modelling of habitat suitability, there are of about 900 to 1,000 km of shoreline occupied by habitats not suitable for Nile crocodile at low water level; this will be discussed in another paper, but mainly this will reduce the estimate of Nile crocodile by about 300-400 crocodiles, according to table 19. According to table 20, the mean number estimate of crocodiles at 172.5 m water level will be  $2262.5 \pm 237.8$  crocodiles, and at 175,  $3029.3 \pm 551.5$ . However, at 170 m water level, the estimated number will be only  $1529.1 \pm 278.4$ .

Most of our field study work was done around the mean water level, (172.5 m.a.s.l) so the average estimated number of lake Nasser crocodiles is  $2262.5 \pm 237.8$ . Based on the suitability factor, these estimations will be reduced by 300-400 crocodile individuals of the mean estimated number so, the mean estimated will be ranged in between  $1962.5 \pm 237.8$  to  $1862.5 \pm 237.8$  at the mean water level of lake Nasser 172.5 (m.a.s.l.)

Table 20: Mean Nile crocodiles estimation based on detectability, abundance (encounter rate), corrected abundance and lake Nasser water level and shoreline length;  $\pm$  SD - standard deviation,  $\pm$  SE Standard error of mean, M - mean.

Nasser Lake water level (m)	Shore -line length (km)	Lake Nasser crocodiles estimation upon				
		Detectability factor (0.43)	Total abundance or the encounter rate factor (0.419)	Corrected abundance factor (0.304)	Mean $\pm$ SD	Mean $\pm$ SE
150	3920	1646.4	1191.7	1685.6	1507.9 $\pm$ 158.5	1507.9 $\pm$ 274.5
155	4285	1799.7	1302.6	1842.6	1648.3 $\pm$ 173.3	1648.3 $\pm$ 300.1
160	4600	1932.0	1398.4	1978.0	1769.5 $\pm$ 186.0	1769.5 $\pm$ 322.2
165	4780	2007.6	1453.1	2055.4	1838.7 $\pm$ 193.3	1838.7 $\pm$ 334.8
170	3975	1669.5	1208.4	1709.3	1529.1 $\pm$ 160.7	1529.1 $\pm$ 278.4
M. 172.5	5882	2470.4	1788.1	2529.2	2262.5 $\pm$ 237.8	2262.5 $\pm$ 411.9
175	7000	2940.0	2128.0	3010.0	2692.7 $\pm$ 283.1	2692.7 $\pm$ 490.3
180	7875	3307.5	2394.0	3386.3	3029.3 $\pm$ 318.4	3029.3 $\pm$ 551.5

Table 21: Mean Nile crocodiles estimation based on detectability, abundance (encounter rate), corrected abundance and Nasser Lake water level and shoreline length;  $\pm$  SD: standard deviation,  $\pm$  SE Standard error of mean, M - mean.

Lake Nasser water level (m)	Shoreline length (km)	Lake Nasser crocodiles estimation upon				
		Detectability factor (0.43)	Total abundance or the encounter rate factor (0.419)	Corrected abundance factor (0.304)	Mean $\pm$ SD	Mean $\pm$ SE
150	3920	1646.4	1191.7	1685.6	1507.9 $\pm$ 158.5	1507.9 $\pm$ 274.5
155	4285	1799.7	1302.6	1842.6	1648.3 $\pm$ 173.3	1648.3 $\pm$ 300.1
160	4600	1932.0	1398.4	1978.0	1769.5 $\pm$ 186.0	1769.5 $\pm$ 322.2
165	4780	2007.6	1453.1	2055.4	1838.7 $\pm$ 193.3	1838.7 $\pm$ 334.8
170	3975	1669.5	1208.4	1709.3	1529.1 $\pm$ 160.7	1529.1 $\pm$ 278.4
M172.5	5882	2470.4	1788.1	2529.2	2262.5 $\pm$ 237.8	2262.5 $\pm$ 411.9
175	7000	2940.0	2128.0	3010.0	2692.7 $\pm$ 283.1	2692.7 $\pm$ 490.3
180	7875	3307.5	2394.0	3386.3	3029.3 $\pm$ 318.4	3029.3 $\pm$ 551.5

## DISCUSSION

### Spot light survey estimation

In accordance with Bayliss et al. (1986), Hutton and Woolhouse (1989), Brown et al. (2004), and Combrink (2004), the spotlight surveys have usually been used to assess and observe populations by providing indices of population size and density. On the other hand, the spotlight counts are innately inaccurate due to the visibility biases, and observers during the survey routes do not see all of the current crocodiles during a survey. Messel et al., (1977) reported that the spotlight count method of surveying crocodilians was standardized in 1977

and was proved to be a very successful and dependable method over the last three decades (Letnic and Connors, 2006). On the other hand, this method is the most frequent technique to estimate crocodilian population trends as reported by Bayliss et al. (1986) and Thorbjarnarson et al. (2000). As a result of a reflective layer in the eye known as the tapetum lucidum (Grenard, 1991), crocodilian eyes reflect any bright light shone into them and exposed crocodiles can be located for distances up to 100 m.

On the other hand, the probability of detection of crocodiles in lake Nasser influenced by several factors include the type and structure of vegetation along the shoreline of lake Nasser between the observer and the crocodile, khors width in lake Nasser, frequency of bends position of the crocodile - submerged, on land, the orientation of the crocodile, and the wariness of the crocodile; as well as the shoreline type sandy, rocky or whatever. All these factor influencing Nile crocodile estimation, and also we not forget the weather condition and waves level. Large crocodile tend to be more wary than smaller animals as reported by (Ron et al. 1998). Being performed over a significant period of time, from October 2009 to August 2010, the spotlight counts of all encounters with crocodiles in the Nasser Lake resulted in a reliable population structure and size class distribution pattern.

During the last 10 years, three surveys have been conducted for crocodiles in Nasser Lake. As reported by Ibrahim (1998) the first was conducted by the Nasser Lake Authority over 3 trips during February and March 1998 reaching no further than 20 km of the High Dam and they cover some areas of Khor El Ramla. The surveys carried out using crocodile counts and "Eyelids of Morning". In total, 13 crocodiles were detected ranging in size from < 2.5 - 5 m, and from this they estimated the total Nasser Lake population size to be no more than 1,000 crocodiles.

The second was conducted by the South Area Protectorates, Nature Conservation Sector of EEAA over a 6 day period, when the team travelled from the High Dam south to Khor Korosko via the west bank and returned north via the east bank, covering over 50% of the lake shoreline (Salem and Asran, 2006). 19 crocodiles were detected, ranging in size from 2 to 4 m, and from this they estimated the population to be less than 2,000 individuals for the entire Nasser Lake. The third was also conducted by the EEAA South Area Protectorates and estimated the total count of Nasser Lake Nile crocodiles by more 3,000-3,0000. The previous three surveys were conducted exclusively during the day and were created with technical errors. Additionally, the resulted estimates of population size were calculated using elementary relationships of detections/unit area, extrapolated to the area of the entire lake (i.e. not just suitable habitat). Although these surveys provide little precious quantitative data, they do provide insights regarding the distribution of Nile crocodiles and the perceptions of threats to the lake Nasser fishing community. The previous mentioned duo to a lot of reasons, such as an error methods of survey technique, and the use of detection/the unit area of lake Nasser which mainly not suitable habitats for the occurrence of the Nile Crocodile or sand nesting of Nile crocodile, deep water and so on.

In accordance to our recent surveys, it was observed that the density of Nile crocodile (/km) varies by location and time meaning that lake Nasser has a great variation in shorelines topography, habitats and degree of disturbances. For instance at Khors of El Madiq area, the encounter rate or the total abundance was 0.676 crocodiles/km, and the corrected abundance, 0.680 crocodiles/km, at Khor abuo Al hadid part 1 at Dahmit area, Khor El magharby, Khor Ambercab and Khor Ahmed Badawy at Dahmit the encounter rate or the total abundance was

0.642 crocodiles/km, and the corrected abundance, 0.349 crocodiles/km, while at Khor near al hadide Dahmit West, Khor Rahma part 1 and 2, Mirwaw West (khor hag Araby) and Khor al Manam Dabod part 1 the encounter rate or the total abundance was 0.551 crocodiles/km, and the corrected abundance, 0.234 crocodiles/km, meaning that shorelines of Khors of El Madiq area from the eastern part of lake Nasser, characterized by rocky cliffs and steeply sloping, rocky shorelines, though, contain patches of habitat similar to the ones from the western shore area presented above in the text, in other words suitable habitats for the Nile crocodile.

In contrast the trips 3 and 4 at Khor Al-Allaqi showed a low density of crocodile individuals (0.260 and 0.140, respectively), although these areas seem more suitable than the ones above. By working to discover the reasons, it was observed that this location is characterized by high density of fishermen, motor boats and by agricultural activities or reclamations, limiting the suitable habitats for crocodiles. Furthermore the last location was surveyed in winter, where the water temperature is low, also a significant reason for a low number of crocodiles; this site was also famous for some crocodile hunters. The low density locations have similar conditions.

In general we observed that larger crocodiles of over 4 meters are extremely wary, few of them being counted during surveys. Furthermore, they submerged at the first sign of approach explaining the low percentage of animals reported in this size class. However we can safely assume that about 10% of lake Nasser crocodiles are larger than 4 m. But the main reasons for the low percentages of large crocodiles are professional hunting for illegal skin trade, hunting sports, although at a very low scale, and lake Nasser fishermen hunting crocodiles to prevent negative impacts on their livelihoods (damage to fishing nets and depletion of the fishery and herds. So, there is a trend/habit to kill crocodiles for the above mentioned reasons in some areas along lake Nasser, meaning that the EEAA decisions makers and researchers should increase their public awareness for the importance of Nile crocodile and transmit that along lake Nasser fishermen.

The size frequency distributions of the Nile crocodile populations exhibit a more or less inverse J-shaped or positively skewed size frequency distribution, which may represent a rapidly growing population, with high reproductive capacity. Such specific distributions may indicate also a high hatchling/juvenile mortality as well (Harper, 1977), but nevertheless they seem to represent long-term stability, since in most stable populations, one would expect an excess of juvenile over mature individual (Goldberg and Turner, 1986). In all these Nile crocodile populations, the younger individuals are preponderant than the older individuals, which is an indication of strength and success of the species, mostly due to victorious growing and survival of these specific individuals (Salem, 2006).

According to Bayliss et al., (1986) and Hutton and Woolhouse (1989), 38% of crocodiles may be underwater, because large crocodilians tend to be more wary than the smaller size classes and often submerge when approached (Webb and Messel, 1979; Ron et al., 1998). Spotlight counts therefore represent an index of the total population and without an idea of how many animals there are in the system, there is no way to check on the relationship between spotlight counts and total population size (Woodward and Moore, 1993). However, in combination with population estimates, correction factors can be applied to spotlight count data to improve the accuracy thereof. Nevertheless, for trend analysis without the knowledge of population size, the assumption is made that the relationship between total population size and spotlight count density remains constant (Woodward and Moore, 1993).

Therefore, using the above assumption, the total count will be increased by about 38% than it was estimated before.

## CONCLUSIONS

During the period between October 2009 to August 2010, forty two locations were surveyed in order to and estimate the count of the Nile crocodile (*Crocodylus niloticus*) in lake Nasser. Mainly the study aimed to quantitatively survey and to determine the crocodile population size in lake Nasser, and to analyze the dynamics of the Nile crocodile population in lake Nasser (South Egypt), through spotlight survey methods. This study is important for the populations of Nile crocodiles that are continuously subjected to different kinds of threats, hunting and illegal skin trade. Furthermore, the local fishing communities around lake Nasser suggested that the crocodile population is currently large enough to be a threat to their livelihoods, through destruction of nets and a depletion of the fish stock. Nocturnal spotlight surveys were conducted based on Boat-counts used to establish the number of Nile crocodiles in the lake Nasser. Double-observer counts technique was used to estimate detection probability of animals along the survey route. In total 1261.3 km were surveyed at lake Nasser on 42 locations (Khor abuo Al hadid part 1 at Dahmit area, Khor El magharby, khor Ambercab and Khor Ahmed Badawy at Dahmit, Khor near al hadide 2 Dahmit West, Khor Rahma part 1 and 2, Mirwaw West (khor hag Araby) and Khor AL Manam Dabod part 1, Khor Allaqi part 1 and Khor Allaqi part 2, Khor Allaqi part 3 and Khor Allaqi part 4, Khors of El Madiq area, Khor El sheikh Abellah el Madiq area, at El Khor Al Ahmer El Madiq area, Khor Sa'ad (El gabana) Rahma A area and Wadi Abayad area part 1, Wadi Abayad area part 2- Khor Anter, Khor El Singari- east, Khor Abu Handal (the main)- east, Khor Wadi El Arab east, Khor El Fouly, Khor Lantern (El Fanos) El Sibu east and Khor Galal - Hassan metwaly Islands, Wadi Abayad area part 3- Khor El Meqawel - Shatorma, Khor Dahab - Tushka east, El Genina and El Sheibbak Mid and third khor, Khor Abel khany - Tunqula east, and the tip part of khor Rahma part 3 and Khor Abu Al-Hadid part 2 Dahmit area, Khor El Soker (Sugar), (khor Ismail hagag) Garf Hussein area, Khor Maria Maria area, (abdellmulla area) Dahmit east, (Khor AL Manam part 2) Dabod area and (khor El Safih) Khor Emicool Dabod area).

528 crocodiles were observed, the encounter rate or the total abundance = 0.419 crocodiles/km, and the corrected abundance = 0.304 crocodiles/km. Of these, 81 (15.3%) were classified as EO (reflective eyes), and 441 (85.0%) were approached closely enough to estimate size; these included 133 (25.2%) juvenile, 249 (47.0%) sub-adults and 56 (10.6%) adults; on the other hand the hatchlings represented 11 (2.0%) of the total count of crocodiles.

Our survey exhibit positively skewed size class frequency distributions which may represent a rapidly-growing population with high reproductive capacity. Such distribution may also indicate a high juvenile mortality. In these populations, the younger individuals are preponderant than older ones, which is an indication of strength and success of the species, mostly due to victorious reproduction and survival of individuals. On using DOBSERV program (Hines, 2000) the average detection probability in between different observers was  $\approx 0.96$ . On the other hand, a chi-square test ( $\chi^2 = 254.2$ ,  $df = 41$ ;  $P < 0.001$ ) indicated that crocodiles were not randomly distributed in between habitats along lake Nasser shoreline.

Our study was conducted around three important water levels of lake Nasser, minimum, maximum and the mean of them, the mean estimated number of crocodiles at the mean water level 172.5 (m.a.s.l.) was  $2262.5 \pm 237.8$  crocodile on the other hand, at the maximum, (175 m.a.s.l.) the mean estimated number was  $3029.3 \pm 551.5$  and at minimum (170 m.a.s.l.) was only  $1529.1 \pm 278.4$ .

Generally most of our study work was done around the mean water level, (172.5 m.a.s.l.) so the mean estimation number of lake Nasser crocodile is considered to be  $2262.5 \pm 237.8$ .

Based on the suitability factor, these estimative numbers will be reduced by 300-400 crocodile of the mean estimated number so the mean estimated will be ranged in between  $1962.5 \pm 237.8$  to  $1862.5 \pm 237.8$  at the mean water level of lake Nasser 172.5 (m.a.s.l.).

In accordance to the assumption discussed by Bayliss et al. (1986) and Hutton and Woolhouse (1989) for the estimation of the total count, 38% of crocodiles may be underwater, because large crocodilians tend to be more wary than the smaller size classes and often submerge when approached (Webb and Messel, 1979; Ron et al., 1998). Spotlight counts therefore represent an index of the total population and, without an idea of how many animals there are in the system, there is no way to check on the relationship between spotlight counts and total population size (Woodward and Moore, 1993). However, in combination with population estimates, correction factors can be applied to spotlight count data to improve the accuracy thereof. Nevertheless, for trend analysis without the knowledge of population size, the assumption is made that the relationship between total population size and spotlight count density remains constant (Woodward and Moore, 1993).

Therefore the total count using the above assumption will be greater by about 38% of crocodiles than that was estimated before.

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**THE INFLUENCE OF LOW STORM DISCHARGE ON NUTRIENTS  
AND ALGAL PERIPHYTON DYNAMICS OF A SMALL MEDITERRANEAN,  
MOUNTAINOUS, EPHEMERAL STREAM POND  
- OREN RIVER BASIN, CARMEL MOUNTAINS  
(ISRAEL)**

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**KEYWORDS:** Oren River basin, mountainous stream pond, storm flow, nutrient cycles, glass-slide experiments, periphyton-chlorophyll-*a*.

**ABSTRACT**

The influence of low storm discharge on a small Mediterranean mountain wetland pond in the upper reaches of the Oren River (Israel) was studied by algal colonization of artificial substrates and by measuring storm and inter-storm fluctuations in nutrients. During the rainy period, January - April 2007, environmental variables and chlorophyll-*a* were monitored to assess the relative importance of water discharges and nutrient concentrations for regulation of the periphyton biomass. Algal and cyanobacterial diversity and abundance were revealed and measured weekly for colonization of glass slides. The glass slides were placed at three different levels. The algal diversity increased at the middle level, including diatoms, greens and cyanobacteria. An increase of nutrient concentrations strongly correlated with rainy storm periods. Each low storm discharge was followed by recover and increases of algal accumulation on the slides, which was expressed by the elevated chlorophyll-*a* concentration. A succession from a *Spirogyra*-community to a macrophyte *Chara vulgaris* community took place over the study period. Biological requirements for nutrients constituted a major constraint for the tightly linked N and P cycles and their recognition. The impact of storm discharges on nutrients circulation and periphyton biomass correlated with an increase of anthropogenic activity after 2004 in the Oren River basin.

**ZUSAMMENFASSUNG:** Der Einfluss von Gewitterabfluss auf die Algendiversität und Nährstoffdynamik in einem Feuchtgebiet im Einzugsgebiet des Oren-Flusses, Carmel Gebirge (Israel).

In einem kleinen Speichersee eines montanen Feuchtgebietes im Mittelmeerraum am Oberlauf des Oren-Flusses (Israel) wurde der Einfluss von niedrigen Gewitterabflüssen auf die Algenbesiedlung künstlicher Substrate durch Messungen von Nährstoffschwankungen während Gewittern und zwischen Gewitterereignissen untersucht. Während der Regenperiode von Januar - April 2007 wurden die Umweltvariablen sowie Chlorophyll-*a* gemessen, um die relative Bedeutung des Wasserabflusses und der Nährstoffkonzentration für die Regulierung

der Periphyton Biomasse zu ermitteln. Die Diversität der Algen und Cyanobakterien sowie ihre Abundanz wurden erfasst und wöchentlich die Besiedlung auf ausgelegten Glasplättchen gemessen. Die Glasplättchen wurden in drei unterschiedlichen Niveaus angebracht. Die Algendiversität lag höher auf dem Plättchen in mittlerer Lage und umfasste Kieselalgen, Grünalgen und Cyanobakterien. Ein Anstieg der Nährstoffkonzentration war eng mit den Gewitterabflüssen verbunden. Jeder niedrige Gewitterabfluss war gefolgt von einer Wiederbesiedlung und dem Anstieg der Algenakkumulation auf den Glasplättchen, die anhand der hohen Konzentration von Chlorophyll-*a* ermittelt wurde. Eine Sukzession von *Spirogyra*-Gesellschaften zu Makrophyten-Gesellschaften der Armleuchteralge *Chara vulgaris* vollzog sich während der Untersuchungsperiode. Der biologische Bedarf an Nährstoffen war eine wesentliche Einschränkung für die eng verbundenen Stickstoff- und Phosphorkreisläufe und deren Erkennung. Der Einfluss der Gewitterabflüsse auf den Nährstoffkreislauf und die Periphyton Biomasse war mit einem Anwachsen menschlicher Tätigkeiten nach 2004 im Einzugsgebiet des Oren-Flusses verbunden.

**REZUMAT:** Influența debitelor mici de furtună asupra dinamicii diversității speciilor de alge din perifiton și a nutrienților într-un mic lac mediteranean, montan, temporar - bazinul râului Oren, munții Carmel (Israel).

Într-un mic lac, al unei zone umede mediteraneene montane, din cursul superior al râului Oren (Israel), a fost studiată colonizarea algală pe substraturi artificiale, prin măsurarea fluctuației nutrienților, în timpul perioadelor de furtună și perioadelor între evenimente de ploaie. În timpul perioadei ploioase, în ianuarie - aprilie 2007, au fost monitorizate și măsurate variabilele de mediu și clorofila-*a*, pentru a se putea aprecia importanța relativă a debitelor de apă și a concentrațiilor de nutrienți pentru reglarea biomasei de perifiton. A fost cercetată diversitatea și abundența algelor și a cianobacteriilor, măsurându-se, săptămânal, colonizarea lor pe lamele de sticlă, care au fost plasate la diferite nivele. Diversitatea algelor a crescut la nivelul mediu, fiind găsite diatomee, alge verzi și cianobacterii. O creștere a concentrației nutrienților a fost strâns corelată cu perioade de furtună. Fiecare debit mai mic, din timpul furtunilor și după furtuni, a fost urmărit, înregistrându-se recolonizarea și creșterea acumulării algelor pe lamele de sticlă, în baza concentrației de clorofilă-*a*. Succesiunea de la comunități de *Spirogyra* spre comunități de macrofite, edificate de *Chara vulgaris* a decurs în timpul investigațiilor făcute. Cerințele biologice de nutrienți au constituit un factor esențial de restricție pentru circuitele strâns legate de azot și fosfor și recunoașterea lor. Impactul debitului, în timpul furtunilor, asupra circulației nutrienților și biomasa de perifiton, a fost strâns corelat cu creșterea activităților antropice, după anul 2004, în bazinul râului Oren.

## INTRODUCTION

In small watersheds the quality of surface water is usually determined by nutrient loads penetrating into the aquatic ecosystem, as well as by the intensity with which self-purification processes occur (Biggs, 2000). In these ecosystems algae serve as primary producers in the nutrient utilization process, and in streams they develop on substrates (Barinova et al., 2005). The intensity of the self-purification process is directly linked to the amount of nutrients and the rate of their utilization (Bunn and Davies, 2000). Such processes can be detected by the species composition in the algal assemblage, the presence of indicative species, their abundance, and chlorophyll concentrations (Cardoso et al., 2005).

The examination of periphyton in streams using a glass-slide device is most suitable for the moderate depths and slow currents that are most common in springs, shallow lakes and along the margins of larger rivers (Round, 1981; Haslam, 1988; Allan, 1995; Biggs, 1996). In rivers and streams the periphyton abundance and composition correlates to the stream flow velocity (Paul et al., 1991; Bourassa and Cattaneo, 1998) and to the nutrient concentration (Biggs, 2000). In such ecosystem the major algae representatives are usually periphyton, and are therefore measurable. The use of the glass-slide method for trophic levels and structural diversity determination has revealed changes in micro-scale periphyton assemblage of many streams and rivers (Patrick, 1961; Peterson et al., 1983; Pringle, 1990). However, it must be employed carefully when filaments, and especially macrophytic forms, exist (Allan, 1995).

Streams in Mediterranean climate regions are physically, chemically, a biologically controlled by sequential, seasonal events of flooding and drying over an annual cycle. Correspondingly, aquatic communities undergo a yearly cycle, whereby abiotic (environmental) controls that dominate during floods are reduced when the discharge declines (Barinova and Nevo, 2010; Gasith and Resh, 1999). The influence of floods on water solution chemistry in Mediterranean ephemeral streams depends on the precipitation timing and intensity, properties of soil, antecedent moisture conditions, and the local physiographic characteristics, such as lithology, hydrology, topography (Marti and Sabater, 1996; Gasith and Resh, 1999; Butturini and Sabater, 2002). Mediterranean streams show high sensitivity to water solution enrichment processes, due to the constant lack of high quality water for the dilution of increasing anthropogenic inputs (Marti et al., 2001; Butturini and Sabater, 1998; Kronvang et al., 1999).

The purpose of this research is to study the influence of low storm discharge on a small Mediterranean mountainous stream pond, by studying artificial substrates for periphytic colonization. By measuring storms and inter-storm fluctuations in flow discharge, nutrient and chlorophyll-*a* concentrations (Chl-*a*), this study demonstrates the importance of flows and nutrients for regulating periphyton biomass.

### Study site

The Mount Carmel is a distinctive ridge in the north-west of Israel that is characterized by its proximity to the sea and its sharp borders with the adjacent lowlands. The vegetation of Mount Carmel is a typical Mediterranean forest, composed mainly of a complex of pine (*Pinus halepensis*), oak (*Quercus calliprinos*) and *Pistacia lentiscus*. The climate type is Mediterranean, with dry and hot summers and rainy winters. Precipitation commences in October and ends in May; most rain storms usually occur between November and March. The average annual rainfall in the upper parts of Mount Carmel ranges from 690-750 mm/year.

Nahal Oren is a typical mountainous ephemeral stream system with the drainage area of 35 km<sup>2</sup> and general gradient of 3%. The karstic nature of the Nahal Oren Basin is well exemplified by the relatively low drainage density of 3.2 km/km<sup>2</sup> (Wittenberg et al., 2004, 2007). Stream flows are generated after accumulated rainfall of 120-150 mm, while 'large floods' with a peak discharge > 5 m<sup>3</sup>/s (recurrence interval of five years), are generated in response to rainfall over 100 mm when the soil is saturated. Hence, 'large floods' occur not earlier than December, and their hydrographs are flash-type, with a sharp rising limb followed by slow recession. The slow recession is related to the spring flow water input, which during large floods may generate base flows that may last from several days up to a few weeks (Wittenberg et al., 2007).

Nahal Oren is the least polluted coastal river of Israel, and provides a habitat for 221 known species and subspecies ranks belonging to nine classes of algae and cyanoprokaryotes. Most of the species are cosmopolitan or widespread in both the Mediterranean and boreal realms. Ecologically, the majority of algal species are periphytic, which are typical of temperate alkaline low-discharge waters of low mineralization (Barinova et al., 2005).

The shallow carbonate aquifers of the Nahal Oren Basin drain by various springs: Ein Oren, and especially Ein Alon, springs (Fig. 1) being major sources of base flow in the upper basin. The hydrograph of Ein Alon spring rises quickly in response to rainfall events, and fluctuates according to rainfall intensity, whereas during the dry rain spells the discharge decreases greatly (Greenbaum et al., 2006).

The Nahal Oren Pond is a small 40 m long, 15 m wide and 1 m deep reservoir, functioning as partial barrier during floods. The upper Nahal Oren Basin (18 km<sup>2</sup>) drains into the pond in high-intensity rainstorms, resulting in floods from the beginning of December or later, when the ground becomes saturated. An average of two to three flow events occurs every year in the upper Oren Basin (Wittenberg et al., 2007).

#### **MATERIALS AND METHODS**

Temperature, electrical conductivity (EC), pH and total dissolved solids (TDS) were monitored using the HANNA HI 9813 apparatus (unfiltered sample of water) and a thermometer on a weekly basis. Coverage of benthonic algae on the pond bottom (%) was estimated on a weekly basis in four constant plots of 0.25 m<sup>2</sup>. Channel bed coverage was estimated a few days before and after flow events in three constant sites located 10, 100 and 500 m upstream of the pond inlet. Algae coverage percentage values represent the mean value of all the plots (pond) or observation sites (stream bed). Flow discharge was recorded at the hydrometric station, located about 150 m downstream of the pond outlet, and base flow was recorded manually at the pond inlet (Fig. 1). Daily rainfall was recorded at the Bet Oren gauging station (Fig. 1). Water samples from the pond were collected on a weekly basis, and at 3-4 hour intervals for selected storm events. Samples were filtered through a 0.2 µm polycarbonate membrane filter, and stored at 3°C until completion of analyses. The concentrations of N, as NO<sub>3</sub><sup>-</sup> and P, as PO<sub>4</sub><sup>3-</sup>, were measured using ion chromatography.

The glass slide experiments were conducted *in vitro* and *in situ* on artificial substrates. The *in situ* test was conducted on a permanent glass-slide device composed of a 1 m aluminium pole with 4 slide tips every 15 cm (Fig. 1), which were installed in a constant location with respect to water column stability for the duration of the test. The *in vitro* test was conducted in a 30 l plastic tank filled with pond water and sediments, which was placed in an exposed location at the University of Haifa. The *in vitro* test pH and conductivity were maintained close to the pond conditions, while temperatures were slightly higher. All glass slides were placed horizontally, and served as the artificial substrates.

Chl-*a* analysis was performed on the algal growth removed from a 10 cm<sup>2</sup> area on each slide, and was repeated four times using the spectrophotometric method for methanol extraction (Wetzel and Westlake, 1969). Descriptive statistics (mean, median, and range) were calculated for all sampling depths (Fig. 1). Inspection of algal species on the surface of the glass slides was performed using a dissecting Swift microscope under magnifications of 800 x.

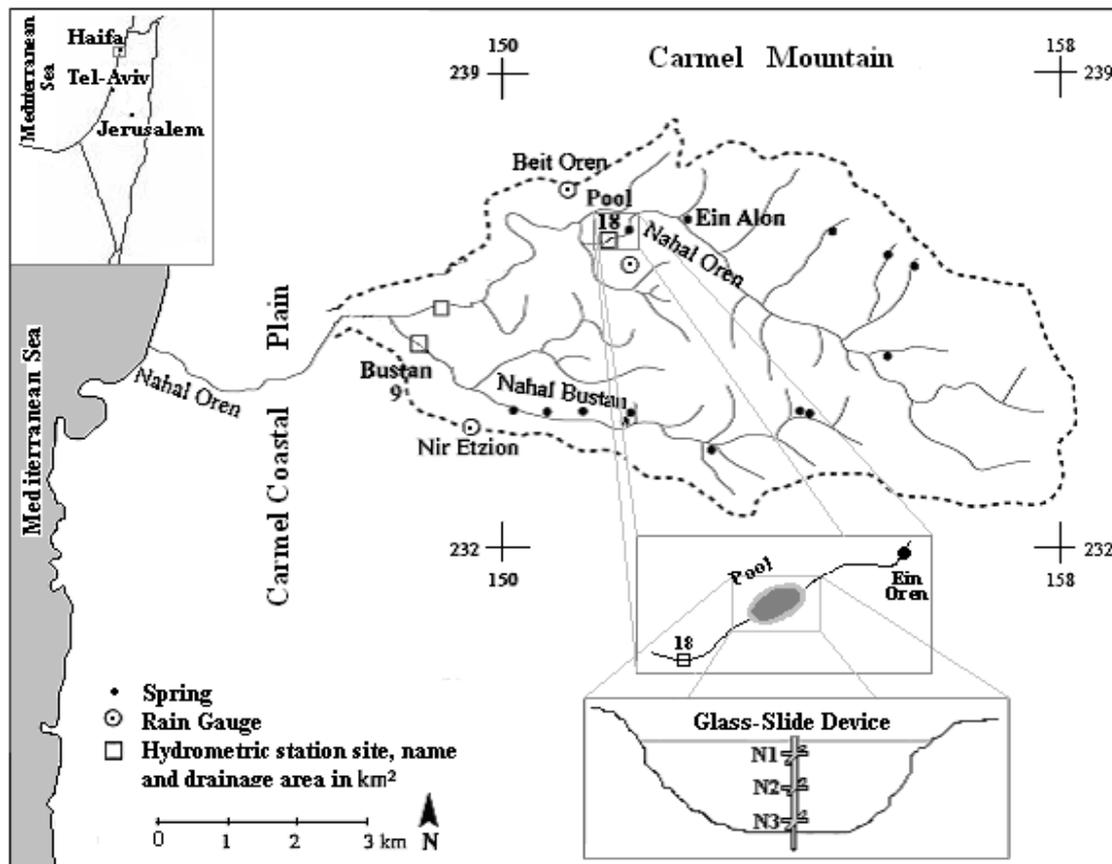


Figure 1: Nahal Oren Basin (after Wittenberg et al., 2004), the pond study site and location of monitoring equipment presented with the schematic diagram of permanent glass slides device. Notice that each measuring depth has 4 slides tips which contains a bundle of 4 slides therefore, a total of 16 samples of chlorophyll can be determined in each sampling depth.

## RESULTS

### Rainstorms characteristics

Annual precipitation during the winter of 2006-2007 was 571 mm/year, with intensities ranging from 15 to 40 mm/hour. During the study period 30 rainfall events were recorded with daily rainfall amounts of 3-38 mm/day, usually less than 35 mm/day. In fewer than 50% of the rainfall events daily rainfall was less than 15 mm/day. The mean inter-storm length ( $\Delta t$ ) was 14.5 days (3-51 days), indicating an extremely scattered rainy season. The first flow event on 5-7.01.2007 was generated after accumulated rainfall of about 100 mm. Later, when the ground became saturated, flows were generated after accumulated rainfall of about 30 mm (Fig. 2). Low magnitude flows that reached the pond inlet only were generated after accumulated rainfall of about 20 mm. Low storm discharge occupied about 5.5% of the time.

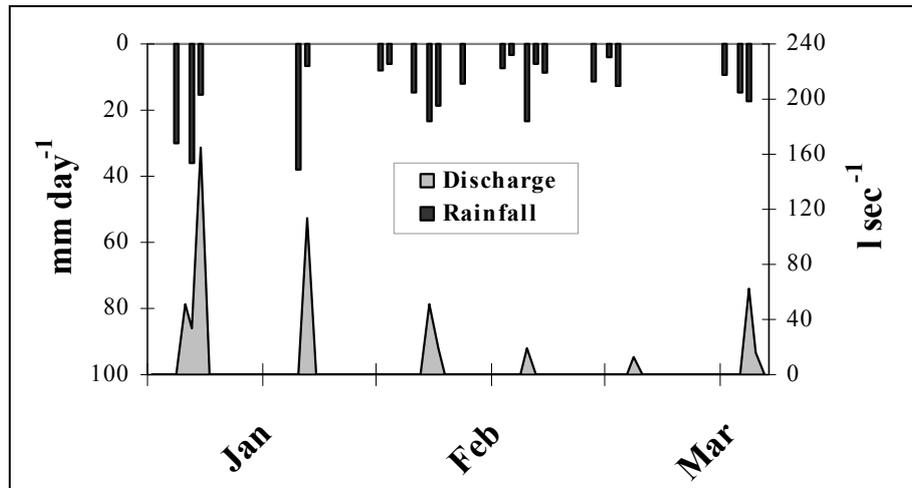


Figure 2: Temporal dynamics of discharge along the upper Nahal Oren Channel and daily rainfall at the Beit Oren gauging station during January-March 2007.

#### Stream flow characteristics

Only five rainfall events created flow in the upper Nahal Oren Basin, all of them being considered as small events (Wittenberg et al., 2007). The highest peak discharge of  $0.164 \text{ m}^3/\text{sec}$  was recorded during the 5-7.01.2007 storm. All storm hydrographs were flash-type, with a steep rising limb followed by a relatively short recession (Fig. 2). This trend is typical of semi-arid regions, in which moisture is minimal (Walling and Webb, 1986; Avila et al., 2002). In the current study flows were relatively short, and ranged from a few hours to 48 hours.

The pH and EC values observed were well within the limits of natural waters ( $7.8 \pm 0.5$  and  $400 \pm 150$  respectively in carbonates terrain) described by Bartram and Balance (1996). The mineralization variable controlled by the water salinity indicates slightly mineralized and alkaline runoff, which can be related to the relatively high base flows contribution to runoff. The N concentrations showed various patterns during the flow season. The first event had a relatively high N concentration, and later the concentration in flow discharge remained lower (Fig. 3). The P concentration in the current study was well above global standards,  $< 0.01 \text{ mg/l}$  (Fig. 3), for small drainage basins ( $10\text{-}100 \text{ km}^2$ ) (Bartram and Balance, 1996). A non-parametric mild correlation between N and P concentrations in flow events was found using the Spearman test ( $r = 0.66$ ,  $n = 25$ ,  $p < 0.01$ ). This correlation pointed out the importance of storm discharge in regulating the N and P concentrations.

The origin of high N and P concentrations can be related to flushing during the transition from dry conditions in summer to wet conditions in winter, as well as to storm flushing during flow regeneration after the end of the previous flow, common in the ephemeral flow regime of Mediterranean climate regions (Avila et al., 2002; Biron et al., 1999). However, the influence of traditional livestock pasturing, common throughout this study, and other anthropogenic inputs must also be considered.

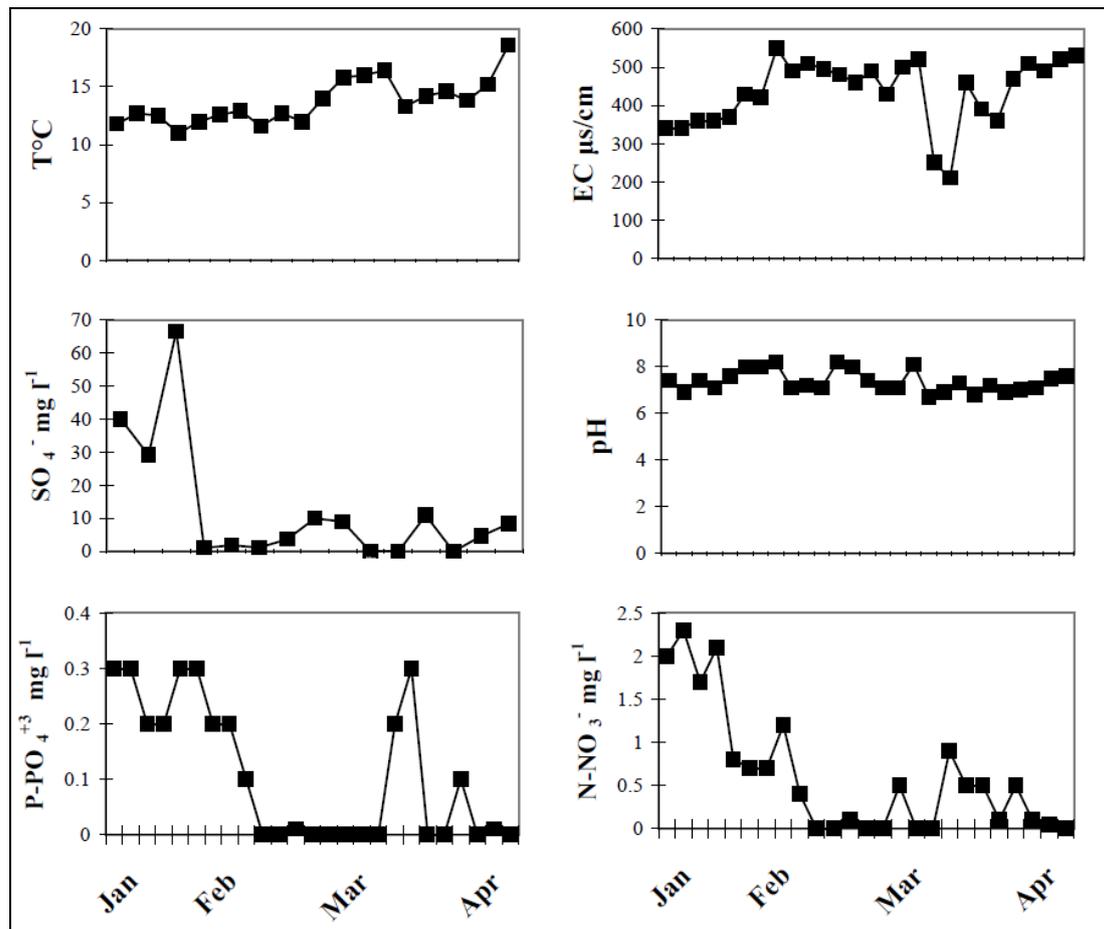


Figure 3: Temporal dynamics in flow water: chemical, physical variables during Jan.-April 2007.

Table 1: Summary of inter-storm variables in the Oren River channel and pond.

Variable	Oren channel	Oren pond
Discharge (L/sec)	0.04-5.0	
pH	7.1-8.3	6.9-8.1
EC (µs/cm)	480-620	460-543
TDS (ppm)	328-455	339-388
Temp (°C)	12.5-15.4	12-28
Depth (cm)	n.m.	40-85
Slide Chl <i>a</i> (mg cm <sup>-2</sup> )	n.m.	0.02-1.72
<i>Spirogyra</i> sp.%	10-85	< 20 to 90
<i>Chara vulgaris</i> %	< 10	< 10 to 90
N-NO <sub>3</sub> (mg/l)	0-0.3	0-1.2
P-PO <sub>4</sub> (mg/l)	0-0.01	0-0.2
Total N (mg/l)	n.m.	0-1.6
Total P (mg/l)	n.m.	0-0.6

Note: n.m. - not measured

### Base flow characteristics

Oren Pond inter-storm water input originates from a relatively small spring base flow that ranges from 0.4 to 5 l/sec. The pH and EC values (Tab. 1) show that the Oren base flow is fresh water, slightly mineralized and alkaline all year around. The typically circum-neutral to slightly alkaline nature of the stream water is related to rapid release of  $\text{Ca}^{2+}$  from calcite in soils and aquifers (Appelo and Postma, 1994).

The  $N_{\text{base-flow}}$  shows negative gradient of depletion downstream from the Ein Alon spring discharge source, with a mean N concentration of about 2 mg/l. The  $N_{\text{base-flow}}$  depletion along the stream bed from about 2 mg/l to 0-0.3 mg/l at the pond inlet was related to the large biomass of the periphyton community, and especially to the green filaments of *Spirogyra* sp. Filament patches covered between 5 and 85% of the total channel bed. The relatively low discharge during the winter of 2006-2007 did not drastically reduce the high occurrences of filament algae and the biofilm community (especially diatoms, red algae and cyanobacteria). Moreover, in some parts of the channel  $N_{\text{base-flow}}$  depletion can be mainly related to the dense bank vegetation (Avila et al., 2002). This phenomenon suggests that biological demand for N and P was a major process for the locally closed N and P cycles in Nahal Oren Basin.

### General pond characteristics

The pond water temperature fluctuated from 12°C to 21°C, decreasing after each flow event due to the inflow of cooler rain water, and rising during the intervals between storms. Water depth shows small fluctuations with no significant correlation with water temperature ( $r = -0.446$ ,  $n = 32$ ,  $P > 0.05$ ). The decrease in water level was mainly related to percolation into the bottom of the reservoir, as well as evaporation and water intake by vegetation. During storm flow the pond functions as a partial flow barrier, and therefore water outflow is related to the initial water level of the pond. Nutrient concentrations correspond to discharge, and usually remained high a few days after each storm, then declining rapidly.

Slides from the pond include planktonic-benthic forms, mainly diatoms, such as: *Navicula recens* (Lange-Bert.) Lange-Bert., *Amphora ovalis* (Kutz.) Kutz., *Nitzschia palea* (Kutz.) W. Sm., *Rhopalodia gibba* (Ehrb.) O. Mull., *Achnanthes* sp., etc, green filaments of *Spirogyra* sp., *Oedogonium* sp., and cyanobacteria (blue-green algae) such as *Phormidium autumnale* (Ag.) Gom., *Oscillatoria brevis* Kutz. ex. Gom., *Lyngbya* sp., *Tolypothrix* sp., *Aphanothece* sp., *Calothrix* sp., *Nostoc* sp., and *Chroococcus* sp. The glass slide Chl-*a* concentration was very low during the whole period of the experiment, ranging from 0.02 to 1.72 mg/cm<sup>2</sup> (Tab. 1). These concentrations only represent the uppermost water level N1 (Fig. 1). However, they can hardly represent Chl-*a* concentration, which was stored as bottom macrophytes. Bottom coverage by *Spirogyra* sp. and *Chara vulgaris* L. generally exceeded 60%.

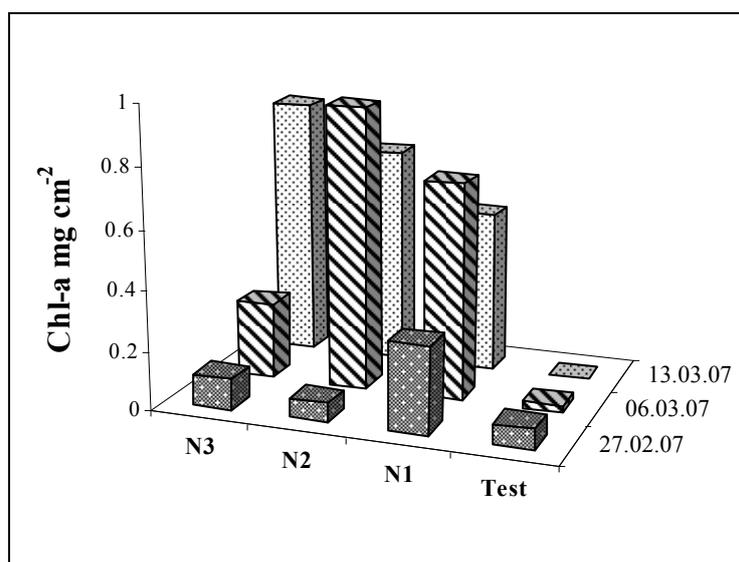


Figure 4: In vitro (test) and in situ mean Chl-*a* concentrations at three sampling dates (N1, N2 and N3 represent different depths).

## DISCUSSION

Similarly to other streams in Mediterranean regions, Nahal Oren Basin is controlled by seasonal events of flooding and drying over an annual cycle. In such ecosystems floods are the most important ecological factor, because it is a clear controlling mechanism of the biota: physically by the discharge, and chemically by nutrient cycling (Biggs, 2000). Many studies have shown that periphyton biomass usually decreases with increasing flood frequency and discharge (Scrimgeour et al., 1988; Biggs and Close, 1989; Clausen and Biggs, 1997), and increases substantially if total N and P reach thresholds of total P about 30  $\mu\text{g/l}$  and total N about 40  $\mu\text{g/l}$  (Dodds et al., 2002). In this study the effect of storm discharge on nutrient dynamics was determined by Chl-*a* concentrations that represent the periphytic colonization on artificial substrates. Trophic status classification (Padisak et al., 1991; Dodds et al., 1998) indicates that Chl-*a* concentrations (Tab. 1) were very low during the entire period of the experiment, and consistent with the ultra-oligotrophic to oligotrophic level. This phenomenon could be explained directly by the large occurrence of *Spirogyra* sp. on the pond bottom, and indirectly by the low discharge that did not reduce the high occurrences of filamentous algae. Therefore, we can conclude that Chl-*a* concentration was mostly stored as bottom macrophytes. On the other hand, intense flash floods are known to eliminate algae occurrence, and reduce invertebrate standing crop by 98% (Fisher et al., 1982). The biota recovery time from such events could range from weeks to months, when usually biomass of epilithic periphyton is not affected as severely as that of sedimentary algae (Dodds et al., 1996). The rapid recovery in the current study is physically related to the low storm discharge. At the same time, the relatively long mean inter-storm length (14.5 days) and the low flow discharge allowed a rapid biological uptake of nutrients. This phenomenon was observed in the in vitro experiment, when after few days the Chl-*a* concentration decreased to zero (Fig. 4). Simultaneously the pond concentration of Chl-*a* was slightly higher (Fig. 4), related to a relatively small flow event on 06.03.2007. Since base flow that reached the pond was depleted

throughout the study, only storm discharge could affect the accessibility of nutrients to the biota. Therefore, the typical relationship between discharge and Chl-*a* concentration in the upper Nahal Oren shows that after a short recovery time of a few days, Chl-*a* concentration increased (Fig. 5). The figure 6 shows that the increase in Chl-*a* concentration was significantly related to the time elapsed since the previous flow event. In broad terms, the increase of nutrient concentration during a storm event was followed by a short recovery and an increase of the algal accumulation on the slides, which was expressed by Chl-*a* concentration (Figs. 5 and 6). However, this method was only indicative for nutrient uptake by the epilithic periphyton, while most of it was stored in the green filaments and macrophytes.

The limiting factors of P and N in fresh water environments were well described by Guash and Sabater (1994), Mulholland et al. (1995) and Biggs (2000). Shortreed and Stockner (1983) showed that in the case of P enrichment the green filaments and some diatom forms flourished. The same trend was recognized in the current study, where for most of the study green filaments, cyanobacteria and some diatom forms prevailed. In this case it is suggested that storm flow serves as a sink for pond nutrients in all forms, and starts an in-pond cycle of nutrients. The lowest Chl-*a* concentration following the last flow event represents an exchange in the macrophytic community into a high quality water community of *Chara vulgaris* (Fig. 7). This exchange was observed on the pond bottom, and reflected the transformation into low discharge/standing water, poor nutrient ecosystem (Barinova et al., 2005). In this case, the nutrients were probably obtained via their roots as well as their shoots (Chambers et al., 1989), and thus the water column concentration could be misleading.

The prevailing fate of diatoms on the slide substrates fitted the classification of Barinova et al. (2005) for the Nahal Oren periphyton community. It was recognized from the *in vitro* test that the fate of the diatoms was highly proscribed by N and P concentrations, while the Chl-*a* was mostly stored in bottom macrophytes. This leads to the conclusion that the pond system serves as a reservoir for nutrient cycles, and suggests that the decomposition of green filaments of *Spirogyra* allowed the production of *Chara vulgaris* (Fig. 7). This principle of macrophytic primary production of entering the detritus food chain has already been discussed by Fisher and Carpenter (1976).

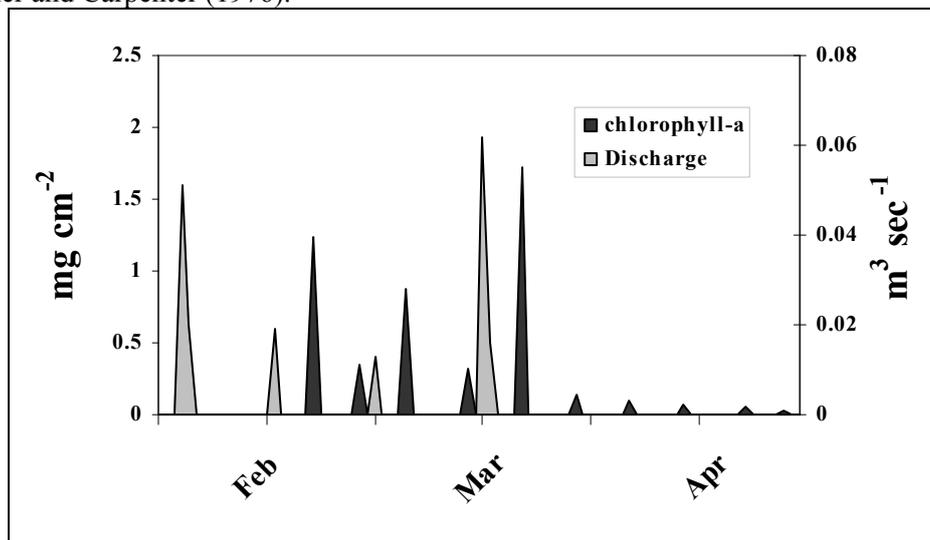


Figure 5: Temporal dynamics of storm discharge and mean Chl-*a* concentration in the pond. Note that the Chl-*a* concentration represents the uppermost depth level only and wasn't measured after the first event. The small black triangles represent sampling during base flow.

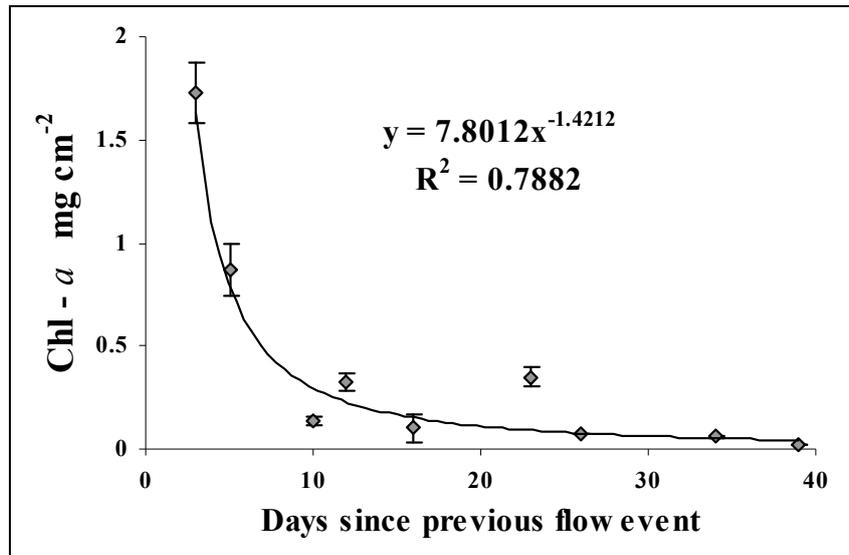


Figure 6: The relationship between mean and range of Chl-a concentration in the Oren Pond and time elapsed since previous flow event.

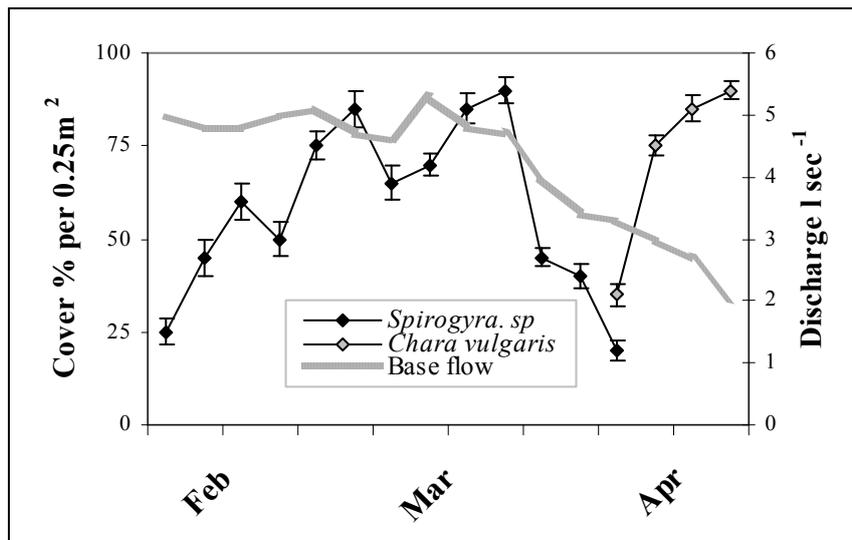


Figure 7: Exchange in the mean and range of the macrophytic community in the Oren Pond from *Spirogyra* sp. to *Chara vulgaris* under different base flow regimes.

## CONCLUSIONS

During the winter of 2006-2007, low storm discharge occupied about 5.5% of the time. The scattered rainy season with relatively low rainfall intensities produced small flows, and provided an insight into the inner cycle of nutrients in a small Mediterranean mountainous stream pond. The correlation between nutrient concentrations in the flow water indicates that storm discharge has a major role in nutrient dynamics. Moreover, the low discharge seems to act as a sink for nutrients. This trend was recognized in inter-storm intervals, when macrophyte channel coverage increased.

Slides Chl-*a* concentrations in the pond were very low during the whole experiment time, consistent with the ultra-oligo- till oligotrophic level and include benthic and plankto-benthic forms mainly diatoms, such as: *Navicula*, *Gomphonema*, *Nitzschia*, green filaments of *Spirogyra* sp. and cyanobacteria. The relationship between discharge and Chl-*a* concentration was determined by the time between two flow events, and was significantly related to the time elapsed since the previous event.

From the present specific study we conclude that: the pond system serves as a reservoir for the nutrient cycle, the biological demand for nutrients is a major process for the locally closed N and P cycles, and storm discharge regulates nutrient circulation and periphyton biomass.

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## VERTEBRATE SPECIES DISTRIBUTION IN SELECTED WETLANDS OF THE DANUBE RIVER BASIN

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**KEYWORDS:** Danube Basin, fish, amphibians, reptiles, birds, mammals.

### ABSTRACT

Riverine wetlands are key territories for the conservation of biodiversity on a river basin scale. The analysis of available data can offer valuable information underlining the importance of recent investigations for the researches in the field of wetlands biodiversity. Data collected for five vertebrate groups (fish, amphibians, reptiles, birds and mammals) in eight chosen riverine wetlands distributed in five countries of the Upper, Middle and Lower Danube were submitted to comparative analyses. The main components and cluster analyses of the data calculated for basic environmental parameters and the species composition of fish, reptile and birds fauna, presents visible differences between functioning floodplain wetlands well connected with the river and those with lacking connection to the river. The calculated mean values of similarity between the wetlands present for some vertebrate groups - with exception of fish and reptiles - significant differences. From all the environmental factors considered the connectivity with the main river is the key factor, correlated statistically significant with the variability of species number of two vertebrate groups, fish and reptiles (Pisces, Reptilia).

**ZUSAMMENFASSUNG:** Verteilung der Wirbeltierarten in ausgewählten Feuchtgebieten im Einzugsgebiet der Donau.

Die Feuchtgebiete der Flussauen sind Schlüsselbereiche für die Erhaltung der Biodiversität auf Einzugsgebietsebene. Analysen vorhandener Daten können wertvolle Informationen liefern und auf die Bedeutung rezenter Bestandaufnahmen für die Bearbeitung des Themas hinweisen. Aus acht ausgewählten, über fünf Länder verteilten Feuchtgebieten der Oberen, Mittleren und Unteren Donau gesammelte Daten von fünf Wirbeltiergruppen (Fische, Amphibien, Reptilien, Vögel und Säugetiere) wurden vergleichenden Analysen unterzogen. Die Hauptkomponenten und Clusteranalysen der erhobenen Daten berechnet anhand einiger grundlegender Umweltparameter und der Artenzusammensetzung der Fisch-, Reptilien- und der Vogelfauna lassen deutliche Unterschiede zwischen den gut funktionierenden, an den Fluss angebundenen Auengebieten und solchen mit schlechter Verbindung zum Hauptstrom erkennen. Die errechneten Mittelwerte der Ähnlichkeit zwischen den Feuchtgebieten zeigten für einzelne Wirbeltiergruppen - mit Ausnahme derer für Fische und Reptilien - signifikante Unterschiede. Von allen berücksichtigten Umweltfaktoren ist die Verbindung zum Hauptstrom der Schlüsselfaktor, der mit der Variabilität der Artenzahlen von zwei Wirbeltiergruppen, der Fische und der Kriechtiere (Pisces, Reptilia) statistisch signifikant korreliert war.

**REZUMAT:** Distribuția speciilor de vertebrate în zone umede, selectate din Bazinul Dunării.

Zonele umede din luncile râurilor constituie arii cheie pentru conservarea biodiversității, la scara bazinului hidrografic. Analiza datelor existente poate furniza valoroase informații, demonstrând totodată importanța de prelevări recente, pentru abordarea acestei teme de cercetare. Date colectate despre cinci grupe de vertebrate (pești, amfibieni, reptile, păsări și mamifere) din opt zone umede, distribuite în cinci țări din cursul superior, mijlociu și inferior al Dunării au fost supuse unor analize comparative. Analiza componentelor principale și analizele cluster ale datelor prelevate pe grupe de vertebrate și calculate pe baza unor parametri de mediu și a componenței faunei de pești, reptile și păsări prezintă diferențe evidente între zonele umede funcționale și bine conectate de fluviu, față de acelea cu conectivitate slabă. Valorile medii de similaritate, calculate pentru zonele umede studiate, prezintă pentru diferitele grupe de vertebrate - cu excepția celor pentru pești și reptile - diferențe semnificative. Dintre toți parametrii, luați în considerare, conectivitatea cu fluviul este factorul cheie, care a fost corelat statistic semnificativ cu variabilitatea numărului de specii a două grupe de vertebrate: pești și reptile (Pisces, Reptilia).

## INTRODUCTION

Wetland biodiversity studies as a part of recognition of wetland importance have been intensified worldwide, as also seen in the Danube River basin. In particular the vertebrate species are among the organisms most profoundly and for long time studied due to their attractiveness, vulnerability, conservation importance, indicator value and finally because of the economic and other social values of some of them (e.g. fishes). As a result the vertebrate biodiversity inventory of territories of European countries (also of riparian wetlands) might be considered almost accomplished. However, the relations between biodiversity and environmental factors in wetlands are not so well studied like in other aquatic ecosystems e.g. lakes and rivers (Mensing et al., 1998; Oertli et al., 2002; Meynecke et al., 2008). As Tockner et al. (2010) pointed out the wetland diversity and functions are result of interactions between natural and human induced stressors on one side and species tolerance abilities on the other. Diversity studies of single vertebrate groups in wetlands (Ficetola and De

Bernardi, 2004; Meynecke et al., 2008) or together with some other organism groups with indicator value in rivers (e.g. diatoms, macrophytes, macroinvertebrates, fish in the frame of EU Water Framework Directive (Johnson et al., 2006a, b; Hering et al., 2006) were intensified. Despite that, simultaneous assessments of different vertebrate groups in different wetlands along a river course are still seldom (Mensing et al., 1998; Brazner et al., 2007).

The recent compilation of data offers a possibility to compare several riparian wetlands by species number and composition of fish, reptiles, amphibians and birds reported recently and in the past. The compiled information will be applied for evaluation of wetland ordination and of relations between environmental factors and species numbers. Also, we will compare the four vertebrate groups by obtaining their group specific degree of similarity and pattern of differentiation between wetland sites within the Danube River basin.

## **MATERIAL AND METHODS**

### **Sites descriptions**

The Danube River is Europe's second largest river with 2,857 km length, up to 1.5 km width, 8 m depth in some places, and with a total catchment area of 801,463 km<sup>2</sup>.

As a result of reviewing several investigations of Danube wetlands in Austria, Croatia, Serbia, Bulgaria and Romania it was found that only 8 of them are studied relatively well and offered accessible data. This data includes information about species occurrence of vertebrate groups recently and in the past in these selected riverine wetlands. Moving from the west to the east, the case study sites in Austria are the following along the Danube River course: Greifenstein, Lobau and Regelsbrunn. The Greifenstein area is of prevailing lake like type and the uppermost situated wetland on sandstone rocks and chernozem-gleyic soil with the coordinates 48°06'50'' N and 16°47'40'' E. Lobau is also of lake like type situated on gravels, sands, clays, (alluvium) and fluvisols - calcareous soil with the coordinates 48°07'45'' N and 16°39'00'' E. Regelsbrunn is a wetland of prevailing channel-like type having the same geology and soil characteristics like Lobau at the coordinates 48°20'30'' N and 16°18'50'' E.

The Croatian wetlands are represented by the considerably larger Kopački rit - a complex floodplain area with shallow lakes and river channels as very significant sub-systems (for main hydrology characteristics see the table 1). The geology consists of thick sand deposits, (partly changing into gravel deposits), while the soil is diverse, presented by luvisol, humogley, eugley hypogley, eugley amphigley, gyttja deposits and sapropel. The geographic coordinates are 45°32' - 44' N and 18°44'-58' E (Mihaljević et al., 1999).

The chosen Serbian wetlands is the Koviljsko-Petrovaradinski Rit, which is second largest floodplain in Serbia (4,860 ha terrestrial and aquatic area) and also of lake-like type. Its geographic coordinates are comprised between 45°10'-15' N and 19°54'21'' - 20°07'18'' E. The geology consists of sedimentary alluvium and the soil is of sand and mud nature caused by specific water regime (Kovacevic et al., 1995; Budakov and Brankovic, 2002; Lazic et al., 2008a).

The Bulgarian island Belene has three separate aquatic water bodies (Peschin, Dyuleva bara and Murtvo blato swamps). They all were completely isolated from the river by dykes for many years and were fed by ground waters only, thus some of them converted to temporary waters. For the purpose of vertebrate diversity description, we find it appropriate to consider

the island as a whole unit due to its small size and the short distances between the single water bodies and their equal hydrological fate. The geology of the island consists of gravels, sands and clays, (alluvium), while the soil is fluvisols - calcareous. The latitude of the three water areas is about 43°40' N while the longitude amounts approximately to 25°12-15' E (Geological map of Bulgaria, 1989).

The Srebarna Lake was reconnected to Danube in 1994 after long period of isolation (since 1949); however, the connection effect is of limited significance because it functions as an inlet only mainly during the high water spring period. The geology of the lake is of limestones covered with clay, which do not allow the quick draining, thus favoring the water retention. The soil is gleyisols-eutric. Its geographic coordinates are 44°07' N and 27°03' E.

Despite the substantially larger size than of the previously described wetland sites the Danube Delta was considered also a single wetland for the purposes of vertebrate diversity analyses simply because literature sources treating diversity of vertebrate species related them to the whole delta area. It consists of four major subunits (Cotul Pisicii, Sulina, Capul Midia and Chilia Veche) whose geographic coordinates are comprised approximately between 44°20'- 45°27' N, and 28°10'- 29°42' E. The geological composition of this large territory is very diverse. The main components are light minerals, heavy minerals, clay minerals, quartz, feldspars, calcite, dolomite, silica etc. There is detailed information available about geology characteristics of each single delta subunit or large locality in Gâstescu and Știucă (2006). The soil types reported include alluvial limnosoils or underwater soils, gley soils, psammosols and sands, solonchaks, kastanozems, histosols and anthrosoils.

Some additional information about main topography and hydrology characteristics like size of aquatic area, altitude, distance from the river inflow into the Black Sea, channel and lake-like type connectivity to the main river etc. of the wetlands was compiled and partly presented and analyzed in the present paper (Tab. 1).

#### **Literature sources for vertebrate group composition**

The data of Austrian wetlands about vertebrates were extracted from the following references: Kummer et al. (1999), Schabuss and Reckendorfer (2002a, b) (fishes), Baumgartner (2004), Trauttmansdorff (1999) Janauer and Schiemer (2004), Hein and Schiemer (2004), Schiemer and Reckendorfer (2004), Funk et al. (2009) (amphibians, reptiles).

Birds are presented in Fruehauf and Wichmann (2006), while mammals are taken from Spitzenberger (2001, 2005).

The data about vertebrates of Kopački rit were collected from Mikuska (1981a, b); Popović and Mrakovčić (1990) (fish), Mikuska et al. (2004) (amphibians), Mikuška (2006a, b, c); Krčmar et al. (2007) (reptiles), Mikuška et al. (2002b); Mikuška et al. (2002a); Schneider - Jacoby et al. (2002); Mikuska et al. (2005); Mikuska et al. (2006a, b, c); Jurčević, Agić and Mikuska (2006), Radović and Mikuška (2009); (birds), Mikuska (1981a) (mammals).

The information about vertebrates of Serbian wetland Koviljsko-Petrovaradinski rit comes from the following sources: Stojsic, Kovacevic (1999); Puzovic (2000, 2008); Puzovic et al. (1999, 2003, 2009) for birds; Kovacevic et al. (1995); Vujasinovic (2007); Protection programme (1999, 2008); Panjkovic et al. (2007); Lazić et al. (2008b) for fishes; Protection programme, (1999, 2008); Official gazette of Republic of Serbia, no. 50/93; Vujasinovic (2007) for herpetofauna and Panjkovic et al. (2007); Official gazette of Republic of Serbia, no 50/93; Protection programme (1999, 2008); Kovacevic et al. (1995); Vujasinović (2007); Lazić et al. (2008b) for mammals.

The fish fauna data of the two Bulgarian Danube wetlands were collected from Pehlivanov (2000), Velkov et al. (2004), Pehlivanov and Pavlova (2009); Pehlivanov et al. (2005); Polacik et al. (2008). The information about occurrence of amphibia and reptilia in Bulgarian wetlands on Belene island and the Srebarna Lake was extracted from the following references: Kovatscheff (1905, 1912), Bouresh and Tsonkov (1933, 1934, 1941, 1942), Beškov and Beron (1964), Beshkov (1965, 1972, 1984, 1986), Tomov (1969, 1991), Oundzhiyan (2000).

Michev and Profirov (2003), Kambourova (2005a, b), Ivanov (2007) and Kostadinova and Gramatikov (2007) delivered data about bird fauna of above mentioned two Bulgarian wetlands. Gerasimov (1998) was used to provide information about mammalian species of the Srebarna Lake reserve, while for the Belene Island no data were available.

Papers about fish of Bănărescu (1964) and Oțel et al. (2006b), amphibians and reptiles Oțel and Torok (2006), birds Oțel et al. (2006a) and finally for mammals Oțel (2006), served to collect information about vertebrate species occurrence in the Danube Delta on the Romanian territory.

There are several environment variables selected (Tab. 1), which according to general research experiences are considered more or less determinant for biodiversity and are applicable to regarded wetlands. Unfortunately variables like shore line development were not among them because they were not applicable to the considered wetlands. The wetland water body of lake-like type is marked with 0, while 1 indicates channel type. Also a nominal variable was applied for quantifying degree of connectivity between the wetland and the surface water of the main river. The aquatic area similarly like ecosystem size is a basic feature strongly and positively related to species richness. The latitude, altitude and distance from the inflow (or from the source) are also biodiversity important characteristics already proven by numerous studies.

The compiled vertebrate data from selected well-studied wetlands of Austria, Croatia, Serbia, Bulgaria and Romania include detailed list of species names recently published (during the last 10 years) and published in the past (before the last 10 years). Due to their large volume, these species lists were not included in this paper but we composed a summary by giving the species number of mentioned groups in the table 2. Despite not published here the detailed species lists of different wetlands presented quantitatively on binary base (available, not available) were applied for ordination of wetlands by means of a cluster analysis based on distance measure calculated with Jaccard index. The obtained proximity averages of different vertebrate groups from matrixes between wetlands (similarity) were tested for significant differences by one-way ANOVA. The species numbers of the four groups were compared with environmental factors by rank correlation after Spearman. We also obtained another wetland ordination by calculating a principal component analysis (PCA) with their environmental characteristics. The multidimensional analyses were carried out by Canoco 4.5 (ter Braak and Smilauer, 2002) and the rest by PAST package (Hammer et al., 2001).

## **RESULTS AND DISCUSSION**

### **Environment characteristics and analyses**

All case studies (Tab. 1) but one situated in the Austrian upper Danube stretch are of lake-like type. The degree of connectivity clearly separated the Bulgarian wetlands from the others by their limited or lack of surface water connection to the Danube River. The aquatic area of compared wetlands differs considerably (about 3 order of magnitude).

Table 1: Topography and hydrology characteristics of Austrian (A), Croatian (HR), Serbian (SR), Bulgarian (BG) and Romanian (RO) wetlands, where latitude is presented with coordinates converted in decimal numbers, high, regular connectivity of wetland to the main river is indicated by 1 and lack of connectivity by 0; wetlands with prevailing channel-like hydrology are marked with 1 and those with lake-like hydrology with 0 and altitude is presented as meters a.s.l.

	Latitude decimal units	River -km	Aquatic area ha	Connectivity in rel. units	Channel-Lake rel. units	Altitude m a.s.l.
Greifenstein (A)	48.33	1943	48	1	0	175
Lobau (A)	48.13	1907	111	1	0	150
Regelsbrunn (A)	48.11	1896	69	1	1	145
Kopački rit (HR)	45.63	1397	4000	1	0	82
Koviljsko-Petrovaradinski rit (SR)	45.16	1250	512	1	0	74
Belene Island (BG)	43.66	566	314	0	0	18
Srebarna Lake (BG)	44.12	392	710	0.1	0	12
Danube Delta (RO)	45.00	25	22773	1	0	1

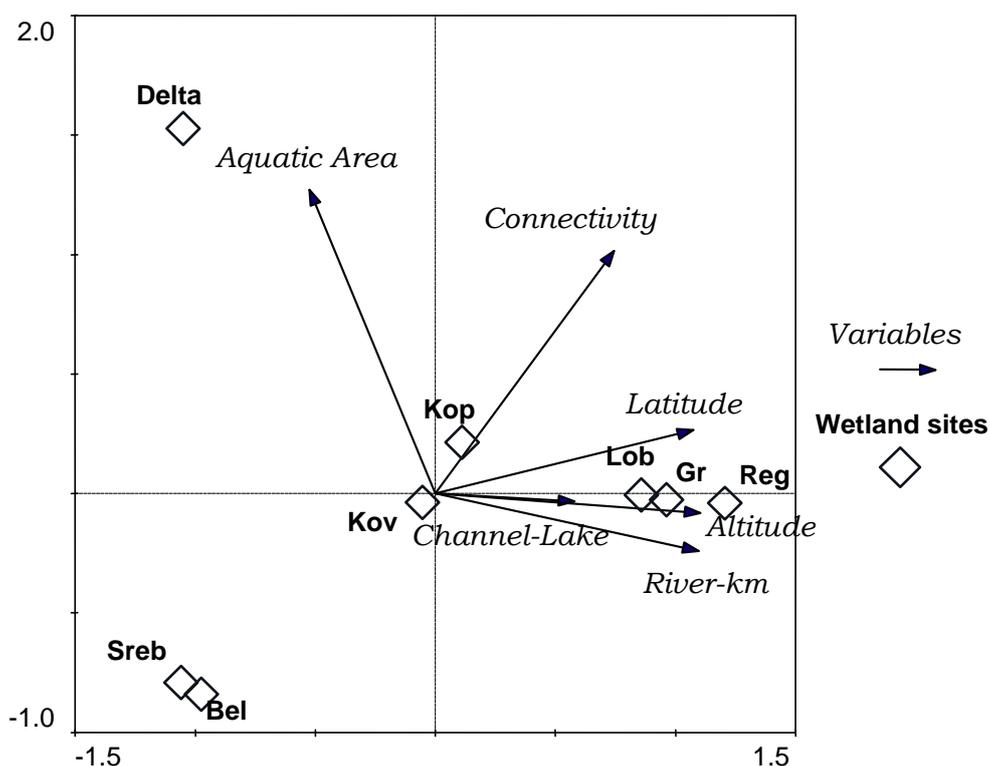


Figure 1: Ordination diagram (biplot) obtained by the principal component analysis (PCA) for 6 topographic and hydrologic environmental variables characterizing eight selected Danube River wetlands; abbreviated as follows: Delta - Danube River delta, Kop - Kopački rit, Lob - Lobau, Gr - Greifenstein, Reg - Regelsbrunn, Kov - Koviljsko-Petrovaradinski rit, Bel - Belene Island Sreb - Srebarna Lake. The first main axis accounted for 62.2%, while the second for 21.5% of variations.

By means of PCA calculated with the environmental data from the table 1 we obtained an ordination diagram of all wetlands (Fig. 1). The first main axis formed mainly by latitude, altitude, river distance and channel-lake character of wetlands ordered all but the Danube Delta in a pronounced gradient with Austrian (Regelsbrunn, Lobau, Greifenstein) and Bulgarian (Srebarna, Belene) groups as endpoints and Kopački rit and Koviljsko-Petrovaradinski rit as transition between them.

The Danube Delta was clearly separated due to its enormous aquatic area. The two Bulgarian wetlands owed their separated position on the diagram mainly to their substantial isolation from the river (low connectivity).

### **Distribution and analysis of vertebrate species number**

The table 2 summaries the number of species belonging to the main taxonomic vertebrate groups. As expected, the Aves are the group with the largest number of reported species, followed at an important distance by Pisces.

We tried to differentiate between recently (last 10 years) (prior to the last 10 years) published species data in order to find out some evidence for biodiversity loss. Unfortunately, only in very few cases the compiled data give such opportunity for comparison. Only the data presented from Kopački rit, have no gaps in the past and recent data in all considered groups, offering the opportunity to estimate the loss of biodiversity. Thus the strongest reduction of number of species is recorded for birds (70 species lost which is about a 25% of the initial number of species), while the other groups either showed no losses or only 1-6 lost species, which did not pass the 10% share of the total species number. The reported no changes in the number of bird species for Belene and Srebarna might easy lead to the erroneous conclusion that these wetlands remained not impacted. The reality is that most of their earliest data originated from the time when they were already disconnected from the river, which happened many years ago. Thus, the data gained 10 years before could not provide information about the pristine period before the disconnection. The number of amphibian species is ranked third, followed closely by the number of reported reptile species.

Logically, the compilation reveals no or less number of protected vertebrate species in the past than recently. The number of protected species has increased firstly due to the increasing pressure on global scale and secondly due to the local influences. Obviously the connectivity as a local factor might have affected stronger the fish species than other single vertebrate groups. However, the effects of global and local influences as well as historical and recent stages of biodiversity could hardly be separated reliably in this paper. The share (percentage) of protected species from the total species number seems to be the highest (about 100%) for Amphibia and Reptilia. For birds this percentage varies considerably between different countries but for fishes the value was similar in all five countries showing the lowest percentage from all presented vertebrate groups. Thus, the fishes seem to be among the less protected of all considered vertebrates. Unfortunately, detailed comments on protected species numbers are not appropriate and only strong tendencies revealed by compiled data might seem reliable.

Table 2: Distribution of species number of main vertebrate taxonomic groups in considered Danube wetlands; “recently” means data published within the last 10 years and “in the past” means data published before the last 10 years.

Taxons and categories of species number within them	Names of wetlands/country							
	Regelsbrunn Austria (A)	Lobau Austria (A)	Greifenstein Austria (A)	Kopački rit Croatia (HR)	Koviljsko-Petrovaradinski rit, Serbia (SR)	Belene Island Bulgaria (BG)	Stebarna Lake Bulgaria (BG)	Danube Delta Romania (RO)

#### Pisces

Total species number in the past	34			44		8	23	133
Total species number recently	36	34	49	44	46	2	26	
Number of protected species in the past								59
Number of recently protected species	6	10	12		14		8	

#### Amphibia

Total species number in the past			14	12		5		
Total species number recently	12	13	14	11	10	5	12	9
Number of protected species in the past				11		1	3	
Number of recently protected species	12	13	12	11				

#### Reptilia

Total species number in the past			1	12		2		
Total species number recently		8	7	12	6	2	1	11
Number of protected species in the past				10		1		
Number of recently protected species		8		10				

#### Aves

Total species number in the past			230	294		166	216	323
Total species number recently	250	250		229	192	166	216	
Number of protected species in the past				280	85	120	152	314
Number of recently protected species		27		280	114	120	152	

#### Mammalia

Total species number in the past			46	55				
Total species number recently		42		49	18		46	44
Number of protected species in the past				33				
Number of recently protected species		16	4	33	8		42	



Surprisingly, the cluster analysis carried out with species lists of most vertebrate groups presents ordination variations similar to the ordination calculated for wetland environmental characteristics by the PCA (Fig. 1). Fish species (Fig. 2) form an initial cluster including Kopački rit, Kovilj-Petrovaradin rit and Regelsbrunn to which Lobau Greifenstein and the delta joined later, while the Belene and Srebarna formed a second cluster despite the high geographic distance between both wetlands. Thus, the two Bulgarian wetlands according to their fish species composition differ markedly from the other sites probably due to their substantial isolation from the main river flow. The cluster analysis of wetlands with amphibian species does not show a distinct separation into two or more clusters, while the reptile species repeat to great extent the two-cluster separation observed for fish, however with some transposition of members within the first more numerous cluster.

Finally the birds classify the wetlands into two distinct clusters, the first of which is formed by Regelsbrunn Greifenstein and Kopački rit, the second one by Kovilj-Petrovaradin rit, Srebarna and Belene. The value of the delta joins the tree after the fusion of the two clusters, which is understandable due to its considerably richer bird fauna. The fish fauna of the delta was also distinctly richer than those of other wetlands and it joins as last site to the cluster of wetlands with preserved connectivity to the river, while the low connectivity of Srebarna and Belene separated them in a different cluster.

Thus, beside connectivity the differences between site classifications obtained for different vertebrate groups seem also to depend on the animal ecological preferences i.e. ability to inhabit at different scales of one or more environments. The average similarity of each vertebrate group between considered wetlands calculated from their Jaccard index matrix expresses more clearly the influence of number and scale of accessible habitats (Fig. 3). Thus, the birds group is able to occupy large scales of two or more environments.

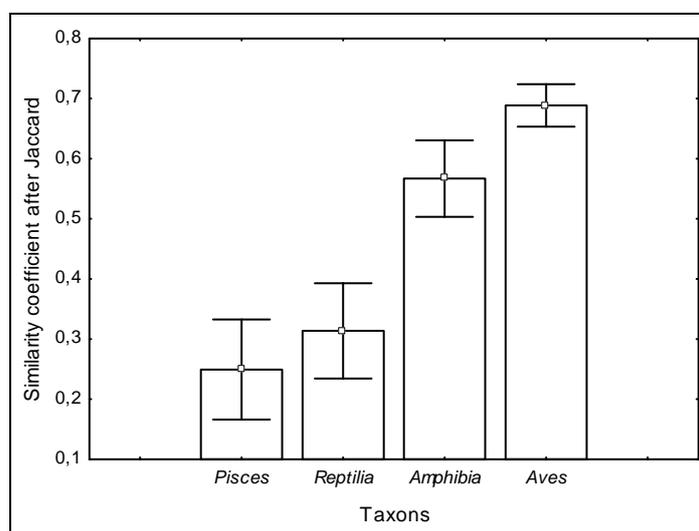


Figure 3: Similarity coefficients calculated between all wetland sites for four vertebrate groups after the Jaccard index presented by their averages and standard error for level of significance  $P < 0.95$ .

Amphibian organisms also inhabit aquatic and terrestrial environments and the ecotons between them. Therefore they both show higher similarity between sites than those restricted to one site (fish, reptiles to great extend). Similar differences in distribution of more mobile and less mobile amphibian species were reported by Ficetola and De Bernardi, (2004).

The absolute values of the similarity coefficients between different sites are group-specific indicating that wetlands are considerably closer after similarity calculated by amphibian and bird than by fish and reptile species. These differences are also statistically secured.

The one-way ANOVA shows that all but the difference between average similarity coefficients of Pisces and Reptilia are statistically significant for  $P < 0.05$ . Thus the more or less pronounced biotope preference and scale of distribution combined with variations in connectivity and other environmental characteristics of wetlands seem to determine the wetland classification obtained by cluster analysis.

Further, by means of Spearman's non-parametric coefficient, we tried to evaluate how far the environment factors presented in the table 1 are correlated with the species number variations of vertebrate groups. Two of the four groups (Pisces and Reptilia) showed statistically significant rank correlation with the connectivity variable. The relation between number of bird species and connectivity is also close to the significance boarder. However, such widely acknowledged relationship between number of species and area did not appear significant for all four groups probably due to striking area differences between compared sites.

Oertli et al. (2002), on hand of investigations of 80 Swiss wetlands also concluded that the influence of area on biodiversity is considerably lower than usually reported for non-wetland ecosystems (e.g. Broenmark et al., 1984). This might result from the large range of wetland altitude variation (210 to 2757 m a.s.l.), which the authors tried to eliminate by separating sites into two subsets.

In our study case the altitude correlates statistically significantly and positively only with the number of amphibian species. This illogical positive correlation is probably due to limited number of data and the close range (175 m) in which the altitude of the sites varied.

The other statistically significant correlations of amphibians with latitude and the river kilometers also seem senseless and are probably resulting from more detailed investigations of Austrian and Croatian than of Romanian and Bulgarian wetlands. Additionally the high inter-correlations between the altitude, latitude and the river km distance from the Black Sea (Fig. 1) might also contribute to the obtained correlations with amphibian species numbers.

However, the significant relations with connectivity seem to be indicative for species bound to a single environment, while the other two more mobile species groups inhabiting two or more environments, do not depend on connectivity. On the other hand one could assume that bird species number also correlates positively with connectivity, because the Gamma correlation coefficient distinctly form that of Spearman proved significant (Tab. 3). Thus, connectivity might increase fish diversity which in turn attracts birds especially the fish eating ones.

Table 3: Rank correlation coefficients after Spearman between species numbers of different vertebrate groups and environmental variables; coefficients in bold are significant at least for  $P < 0.05$ .

Environmental variables Vertebrate groups	Latitude	River km	Area	Connectivity	Channel-Lake	Altitude
Pisces	0.429	0.095	0.214	<b>0.764</b>	-0.082	0.095
Amphibia	<b>0.814</b>	<b>0.766</b>	-0.623	0.384	0.166	<b>0.766</b>
Reptilia	0.536	0.250	0.321	<b>0.757</b>	0.000	0.250
Aves	0.371	0.036	0.347	0.659	0.166	0.036

However, when commenting the obtained correlations it would be worth noting the fact that the applied species numbers is possible to include some uncertainty. The table 1 shows some gaps either for total number of species in the past or recently. In such cases in order to make calculations feasible, we were forced to assume the available species number from the past to be valid also recently or vice versa. For example we calculated the rank correlations for fishes by transferring the species number for Belene Island and Danube River delta reported in the past to the recent data and got a representative row of data in order to calculate the desired relationship. We assumed the fact that the introduced mistake is not crucial because the difference between the fish species number in the past and recently for Regelsbrunn, Kopački rit and Srebarna varies between 0 and 2 species.

### CONCLUSIONS

Unfortunately, with few exceptions, it was difficult on the basis of the compiled data to generalize reliably the degree of vertebrate biodiversity loss on a large scale in the selected Danube wetlands. It was out of scope of the project to afford the experts from different countries possibility to apply the same international conventions or to consider the peculiarities or to harmonize all national protection rules declaring definite species as protected or not. However, as known from many other reports, the loss of biodiversity, both in general and of protected species is considerable and beyond doubt.

The results of applied cluster analysis, separating wetland sites in such with high and low level of connectivity to the main river, clearly confirmed the reported potential of fish, amphibians and birds to respond to impacts of different nature (disturbed connectivity included) and at different scales. The degree of vertebrate species susceptibility to natural and anthropogenic influences is determined to great extend by their mobility inclusive the development of their ontogenesis stages in different environments. It is up to researchers and environmental managers to apply the different sensibility of vertebrate species for the purposes of ecological status monitoring and management by adapting them to the regional conditions. Thus, a monitoring based on most sensible vertebrates species (i.e. application of fish to detect connectivity disturbance) will help to optimize the management of wetland conservation and restoration under the complex conditions of global climate changes, which are especially crucial for wetland sustaining.

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## WATER POLLUTION AT SULTANSAZLIGI WETLAND AND DEVELI CLOSED BASIN (TURKEY)

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**KEYWORDS:** Turkey, Develi Closed Basin, Sultansazligi Wetland, water chemistry, water pollution.

### ABSTRACT

Sultansazligi Wetland Region is found at the middle of the Develi Plain in the boundary of Kayseri City in Central Anatolia, Turkey. Since 1994 this wetland area is a conservation area protected by the International Ramsar Agreement. Sultansazligi Wetland is one of the seven important wetlands of Turkey and the second most important bird habitat of Turkey. Sultansazligi Wetland Zone includes Camiz, Çöl, Yay Lakes, southern and northern Marshlands. The water level of Sultansazligi Wetland has dropped considerably in the recent years due to improper management policies and global warming. There is an irrigation water supply problem in the agriculture plots within the basin. Water pollution is also an important problem for Develi Closed Basin, this wetland being faced with the salinity, due to the irrigation return flow with high salt content. This study describes surface and groundwater pollution at Sultansazligi Wetland and Develi Closed Basin and gives some recommendations in order to prevent the water pollution.

**ZUSAMMENFASSUNG:** Gewässerverschmutzung im Sultansazligi Feuchtgebiet und dem geschlossenen Develi Becken (Türkei).

Das Gebiet des Sultansazligi Feuchtgebietes liegt in der Mitte der Develi Ebene an der Grenze der zentralanatolischen Stadt Kayseri in der Türkei. Es ist seit 1994 ein nach dem internationalen Ramsarabkommen geschütztes Gebiet. Das Sultansazligi Feuchtgebiet gehört zu den sieben bedeutendsten Feuchtgebieten der Türkei und steht an zweiter Stelle an Bedeutung als Lebensraum für Vögel. Es umfasst die Seen Camiz, Çöl, Yay sowie das südliche und das nördliche Sumpfland des Sultansazligi Feuchtgebietes. Der Wasserspiegel des Sultansazligi Feuchtgebietes ist in den letzten Jahren durch unangebrachte Bewirtschaftungspolitik und globale Erwärmung in beachtlichem Maße gesunken. Daher besteht ein Versorgungsproblem für die Bewässerung der landwirtschaftlichen Flächen. Die Gewässerverschmutzung ist ebenso ein wichtiges Problem für das geschlossene Becken von Develi, da das Feuchtgebiet mit einer Versalzung zu kämpfen hat, die sich aus dem Bewässerungsrückfluss mit hohem Salzgehalt ergibt. Die vorliegende Untersuchung befasst sich mit der Oberflächen- und Grundwasserverschmutzung im Sultansazligi Feuchtgebiet und dem Develi Becken und gibt einige Empfehlungen zur Verhütung der Wasserbelastung.

**REZUMAT:** Poluarea apei în zona umedă Sultansazligi și bazinul închis Develi (Turcia).

Aria zonei umede Sultansazligi este situată în mijlocul Câmpiei Develi, la marginea orașului central-anatolian Kayseri, în Turcia. Această zonă umedă constituie, din 1994, o zonă protejată, conform Convenției internaționale de la Ramsar. Aria Sultansazligi este una din cele șapte zone umede ale Turciei și a doua, în importanță, ca habitat pentru păsări. Zonei umede Sultansazligi îi aparțin lacurile Camiz, Çöl, Yay și zona sudică și nordică de mlaștini. Nivelul de apă al zonei umede Sultansazligi a scăzut considerabil, în anii recentți, datorită unei politici de gestiune nepotrivite și a încălzirii globale. Există o problemă de alimentare cu apă de irigație pentru culturile agricole din bazin. Poluarea apei, de asemenea, este o problemă importantă pentru bazinul închis Develi, fiind pusă față în față cu salinitatea, datorită refluxului de apă de la irigații, cu un ridicat conținut de sare. Studiul de față descrie poluarea apei de suprafață și a celei freatice, în zona umedă Sultansazligi și bazinul închis Develi, dând câteva recomandări pentru a preveni poluarea apei.

### INTRODUCTION

Develi Closed Basin is the sub-basin of Kızılırmak Basin in the border of Kayseri City in Turkey. Area of Develi Closed Basin (the research area) is 3,197 km<sup>2</sup>. Develi Plain is located at the center of Develi Closed Basin. Its average elevation varies between 1,070-1,150 m above the mean sea level. The location of the Develi Closed Basin can be seen in the figure 1. Sultansazligi Wetland is placed in Develi Plain and it is one of the seven important wetlands of Turkey and the second important bird habitat of the same country. Sultansazligi Wetland is also known as one of the most important wetlands of the Eastern Europe and the Middle East. There are Yay Lake, Çöl Lake, northern and southern Marshland areas in Sultansazligi. This wetland area is a conservation area protected by International Ramsar Agreement since 1994. Develi Closed Basin and Sultansazligi Wetland can be seen in the figure 2.



Figure 1: Location of the project area  
- Develi Closed Basin.

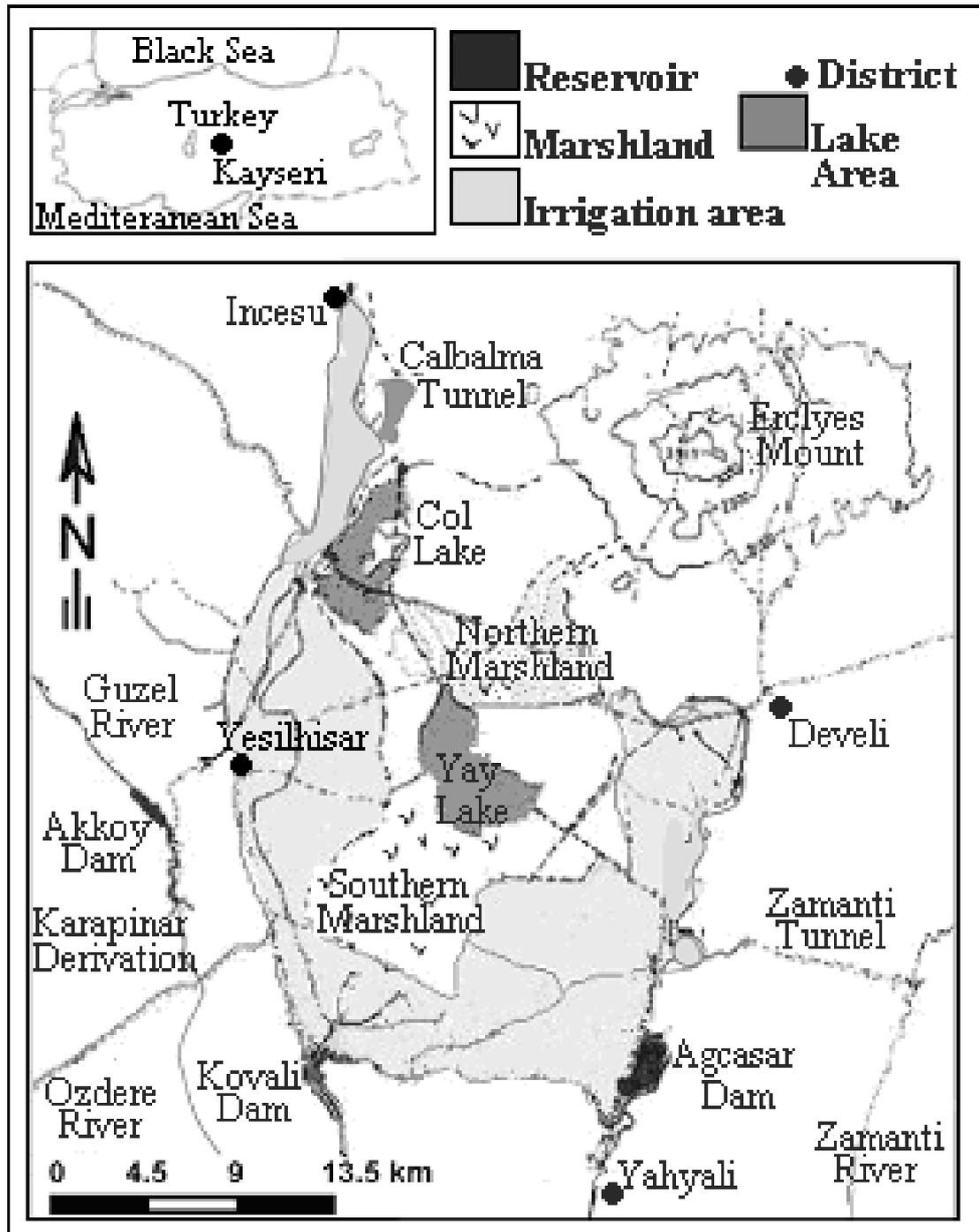


Figure 2: Develi Closed Basin and Sultansazligi Wetland.

Sultansazligi Wetland has water scarcity and pollution problems. There is only one waste water treatment plant at Develi Closed Basin which is not operating sufficiently. Wastewater treatment plant is under construction at Yahyali District and a wastewater treatment plant project will be completed for Yesilhisar District. Process of Yahyali and Yesilhisar Wastewater Treatment Plants will be “long aerated active sludge” (Gurer et al., 2008). Wastewater treatment process of Develi Wastewater Treatment Plant is “trickling filter” which is not suitable for Develi Region so this plant is not working efficiently. Also factories do not operate their industrial wastewater treatment plants regularly so both domestic and industrial wastewater pollute the Sultansazligi Wetland.

#### **Geomorphology, Geology and Hydrogeology of Develi Closed Basin**

There are basically three factors which affect the geomorphology of the Develi Closed Basin, Ecemis tectonic trench, Erciyes volcanic cone and rivers carrying sediment. Erciyes Mount is a volcanic cone, this mount developed in the large collapsing tectonic basin at the northern end of Ecemis Fault starting in the late Miocene (Erol, 1999). It is determined that Develi Closed Basin is located at the end of the elongated tectonic depression (Ecemis tectonic trench) along the northern Taurus Mountains. Rivers carried sediments to this tectonic depression basin and Quaternary alluvium formed at the middle of the basin, this alluvium plate is called as Develi Plain. Volcanic and magmatic rocks formed from the lava of the Erciyes Volcanic Cone.

Geological ages of the formations at Develi Closed Basin vary from Paleozoic to Quaternary. The figures 3a, b shows the geological map of Develi Closed Basin and I-I and II-II cross sections. The formations which are found at Develi Closed Basin can be listed as: Bozcaldag, Gümüsler, Dereköy, Kömürcütepe, Dünderli, Kuzuluk, Catalyesilkaya formations, Otlukaya Ofiyolite, Barakli, Cayraz, Cukurbag, Yesilhisar, Sarica formations, Susuzdag Volkanite, Salur limestone formation, Tahar Ignimbrite, Develi Tuff, Kizilkaya Ignibrite, Namlitarla, Kulpak Volkanites, Incesu Ignimbrite, Seksenverentepe Volkanite and Quaternary alluvium. At the south of Develi Closed Basin, there are metamorphic rocks such as limestone, schist and gneiss (Dünderli formation). At the northern side (nearby volcanic Erciyes Mountain), there are volcanic and magmatic rocks such as tuff, andesite and basalt (Develi tuff formation, Kulpak formation). At the east and west parts, there are volcano-sedimentary formations (Yesilhisar formation, Sarica formation). Sediment formation contains sand, gravel, clayey silt and silty clay at Develi Plain. Sediment particle size reaches from gravel and sand size to carbonate clay and silty clay size while going from the northern side to the middle part of Develi Plain. There is crystallized limestone, schist and gnays at the southern part of Develi Plain (MTA, 2005; Yildiz, 2007).

There is unconfined aquifer under Develi Plain, its thickness is about 100-150 m, there is only one confined aquifer between Develi and Ilyasli village which is covering a small area (DSI, 1995). Sediment particle size of the aquifer decreases around Sultansazligi Wetland. There are flysch formations at the western part of the basin near Yesilhisar. Additionally there are many illegally opened wells at Develi Plain so excess groundwater abstraction is an important problem (ENCON, 1995; Yildiz, 2007; Güreer et al., 2008). Average aquifer transmissibility is 552 m<sup>2</sup>/day at the eastern, 100 m<sup>2</sup>/day at the south-western, 2,574 m<sup>2</sup>/day at the southern and 1,115 m<sup>2</sup>/day at the western part of Develi Closed Basin. It is determined that total aquifer inflow from the neighbour aquifers is 90.582\*10<sup>6</sup> m<sup>3</sup>/year (Gurer and Yildiz, 2007). Sustainable aquifer abstraction is computed as 65\*10\*10<sup>6</sup> m<sup>3</sup>/year by Turkish State of Hydraulic Works (DSI, 1995).

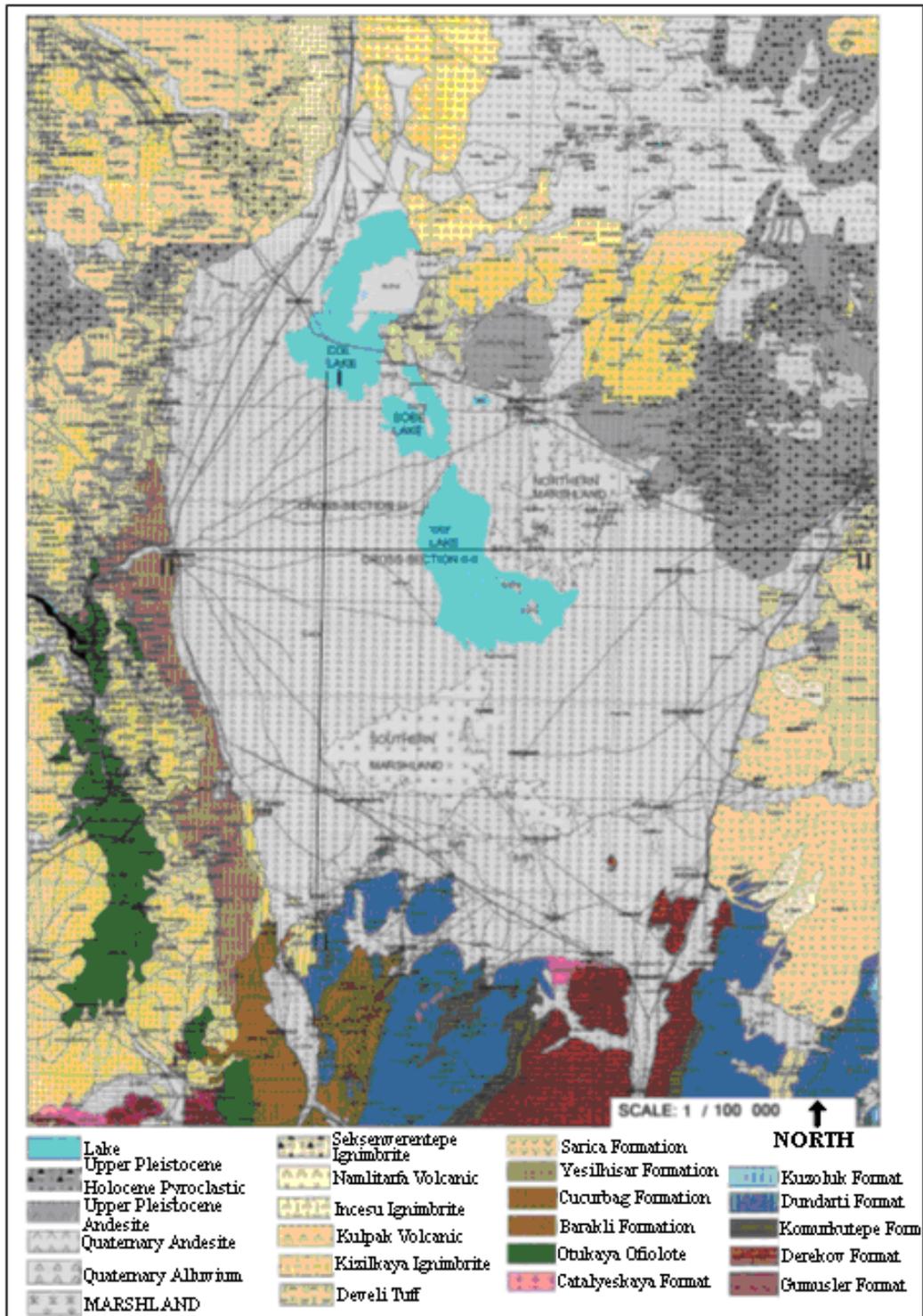


Figure 3a: Geology map of Develi Closed Basin (modified from MTA, 2005 and DSI, 1970).

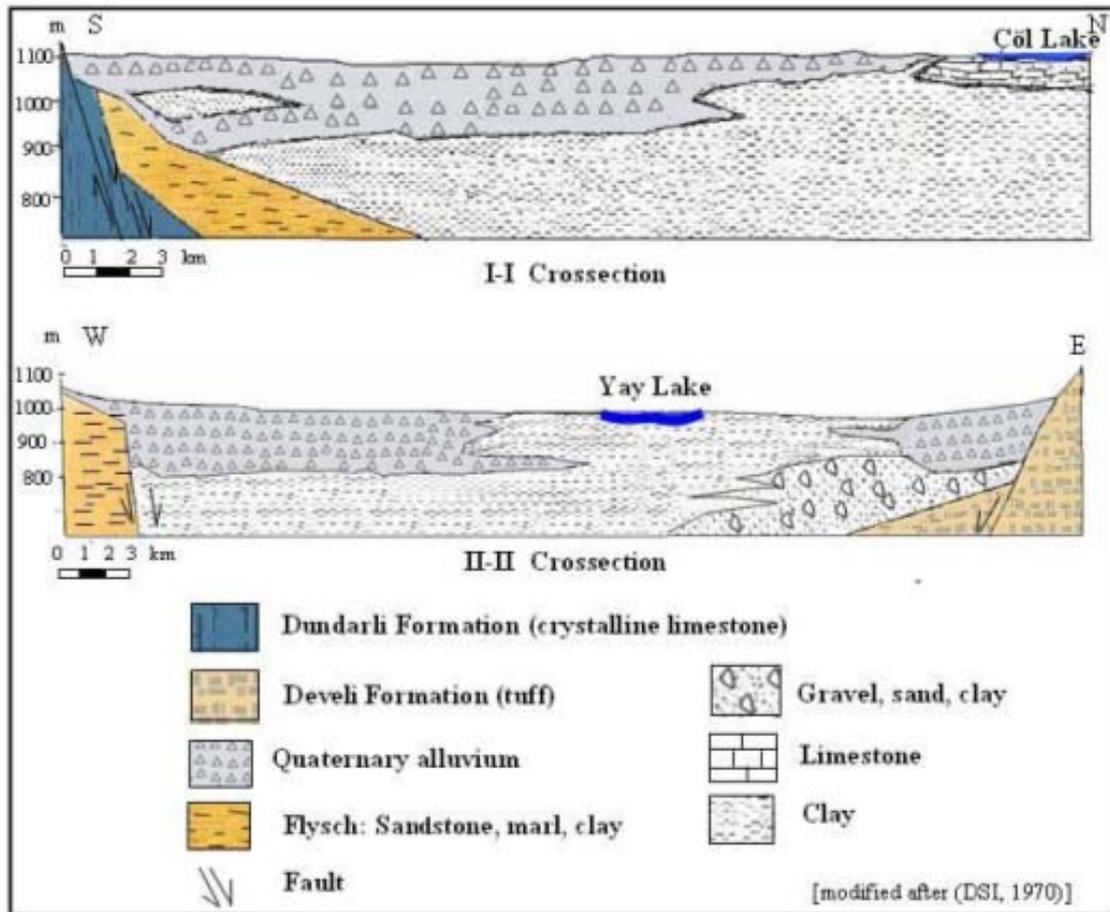


Figure 3b. Geology map of Develi Closed Basin (modified from MTA, 2005 and DSI, 1970).

## MATERIAL AND METHODS

This study describes surface and groundwater pollution at Develi Closed Basin and gives some recommendations in order to prevent the water pollution. Water samples from surface water, 22 deep wells and 16 springs, had been collected in 3 years (between 2003-2005) and chemical analysis of these water samples had been made by the 12<sup>th</sup> Regional Directorate of State of Hydraulic Works.

Surface water and groundwater contamination has been investigated at Develi Closed Basin after the water chemistry analysis. The figure 4 shows the groundwater sampling locations at Develi Closed Basin. Additionally former water chemistry analyses were supplied from Turkish State of Hydraulic Works.

## RESULTS

### Groundwater Pollution at Develi Closed Basin

The tables 1a, b shows the water chemistry analysis of the groundwater samples taken from the springs and the tables 2a, b shows the water chemistry analysis of the groundwater samples taken from the wells at Develi Closed Basin.

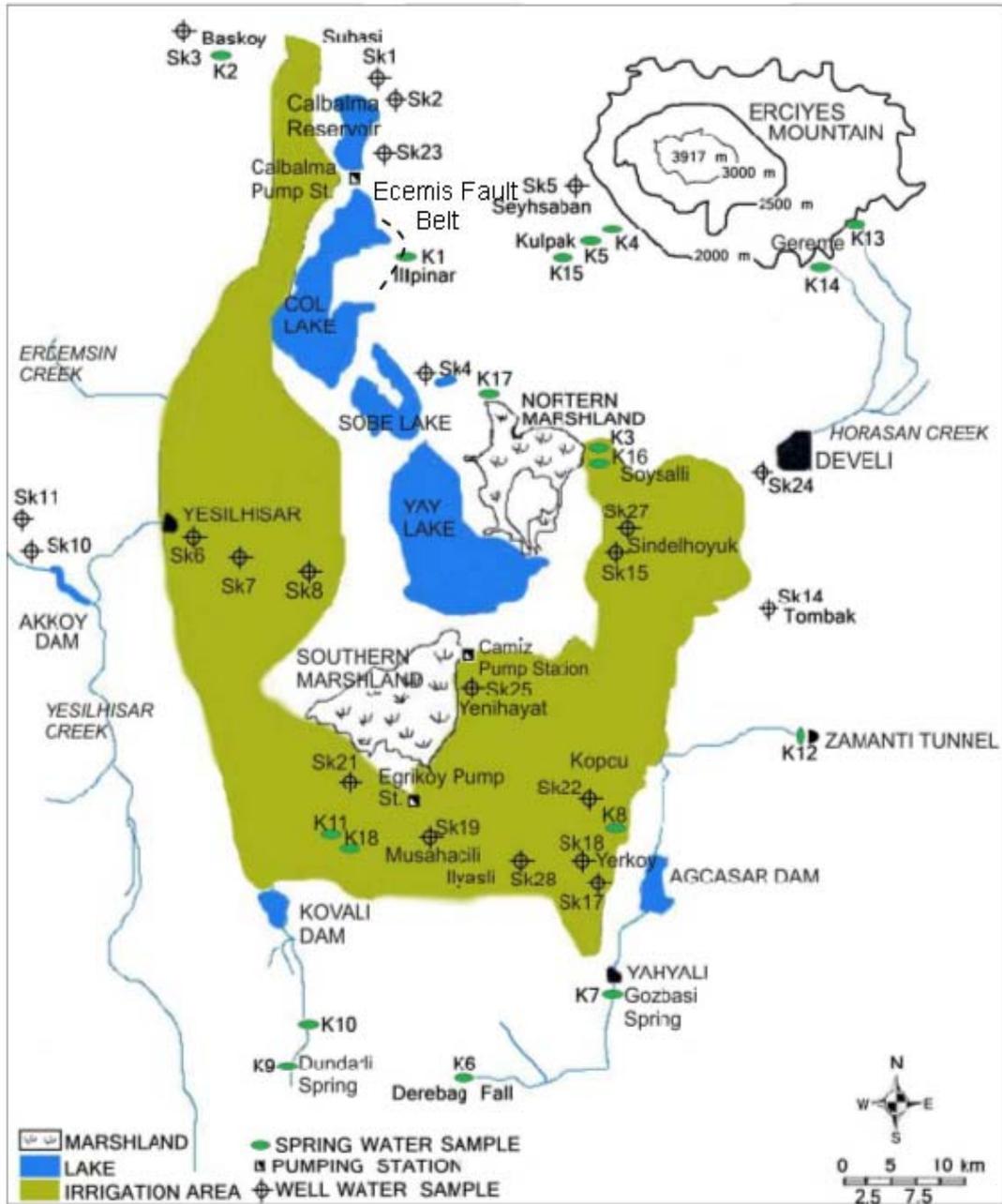


Figure 4: Water sample locations (K1 - K18, Sk1 - Sk31) at Develi Closed Basin for the water chemistry analysis.

Table 1a: Chemical analysis of the water samples taken from the wells in the study area;  
 \*TDS: Total dissolved solids; \*\*EC: Electrical conductivity.

Water Sample Location	pH	*TDS mg/l	**EC $\mu$ S/cm	Na+ mg/l	K+ mg/l	Ca++ mg/l	Mg++ mg/l	Cl- mg/l
SK1	7.98	116	189	14.71	1.2	5.411	8.628	19.9
SK2	8.26	3800	6605	1046.04	117.3	116.6	69.88	1549
SK3	7.9	412	496	26.44	3.9	53.91	16.53	14.2
SK3	7.49	415	472	30.35	4.7	49.3	17.5	13.8
SK4	7.39	140	206	19.77	2.0	8.818	6.684	17.4
SK 5	7.5	652	1274	17.24	9.8	54.91	22.48	220
SK 6	7.11	1645	1834	68.97	15.6	231.3	85.43	95.4
SK 6	6.89	1723	1756	95.41	9.8	211.4	102.7	133
SK7	7	1124	1358	62.99	7.8	152.7	53.59	78
SK7	6.73	1259	1382	97.71	9.0	150.9	61.86	122
SK8	8.3	650	1025	70.81	7.0	64.73	34.76	103
SK8	7.52	738	1000	94.26	7.0	70.94	35.97	147
SK10	8.17	265	386	10.12	1.6	53.51	4.375	14.2
SK11	8.46	164	223	5.98	1.2	32.26	3.16	4.25
SK11	8	254	223	8.74	1.2	47.29	5.833	3.19
SK14	7.54	480	719	22.07	1.2	91.98	14.58	30.5
SK14	7.59	599	780	63.68	3.5	85.17	20.54	77.3
SK15	7.15	319	465	28.74	4.7	31.86	18.23	45.4
SK15	6.8	432	617	55.87	9.4	32.06	23.09	81.5
SK17	7.9	267	275	2.53	0.4	55.11	6.684	3.19
SK18	7.8	422	488	3.68	0.4	88.98	9.844	5.67
SK18	7.6	333	476	3.68	0.4	28.26	6.076	7.45
SK19	8.23	616	730	63.68	3.1	58.12	30.5	28.4
SK19	7.8	551	643	60.23	3.9	57.11	7.656	34
SK20	7.5	627	836	52.88	2.3	84.77	25.64	70.2
SK20	7.63	485	698	59.31	3.9	67.13	6.562	56.7
SK21	8.53	340	441	29.89	1.2	30.06	23.7	25.5
SK21	8.01	363	425	37.93	2.0	32.46	21.75	55
SK23		1744	3010	354.90	29.1	149.5	62.35	621
SK24		260	323	22.12	4.6	30.13	12.47	16.2
SK25		5401	8240	1641.82	63.3	99.33	124.8	1925
SK29	7.2	852	1240	112.65	23.5	58.12	48.61	163
SK28	7	426	570	17.24	2.0	60.12	23.09	14.2
SK30	6.26	155	214	17.01	3.1	0	0	7.09
SK31	6.84	157	218	18.85	5.5	0	0	8.51

Table 1b: Chemical analysis of the water samples taken from the wells in the study area.

Water Sample Location	SO <sub>4</sub> - mg/l	HCO <sub>3</sub> mg/lt	CO <sub>3</sub> mg/lt	Hardness (F)	NO <sub>2</sub> (mg/l)	NO <sub>3</sub> (mg/l)	NH <sub>4</sub> (mg/l)	Boron (mg/l)
SK1	0.4803	65.902	0	4.9	0.003	4.05	0.089	0.2
SK2	326.12	565.05	9.601	57.85	0.023	8.11	0.722	13.7
SK3	6.2439	291.07	0	20.25	0.01	10.76	0.105	0.6
SK3	9.606	289.85	0	19.5	0	12.09	0.02	0.2
SK4	0.4803	85.428	0	4.95	0.003	2.45	0.05	0.3
SK 5	2.4015	325.85	0	22.95	0.043	2.24	0.19	4.1
SK 6	55.715	1093.5	0	92.85	0.01	28.88	2.48	1.8
SK 6	108.07	1063	0	95	0	31.51	2.09	1.3
SK7	43.707	723.7	0	60.15	0.01	12	0.86	1.3
SK7	60.038	756.65	0	63.1	0.007	7.8	0.83	1.4
SK8	49.951	308.15	10.8	30.5	0	19	0.23	1.3
SK8	81.171	302.05	0	32.5	0.003	23.74	0.21	1.4
SK10	11.047	164.75	5.401	15.15	0.007	17.68	0.064	0.5
SK11	0.4803	112.89	5.401	9.35	0.003	10.59	0.076	0.4
SK11	17.291	170.86	0	14.2	0	14.2	0.06	0.3
SK14	149.37	171.47	0	28.95	0.007	9.3	0.211	0.6
SK14	208.93	138.52	0	29.7	0.046	2.76	0.344	0.1
SK15	12.488	178.18	0	15.45	0.013	6.91	0.24	0.5
SK15	22.574	207.47	0	17.5	0		0	0
SK17	15.37	183.67	0	16.5	0.01	5.76	0.1	0.1
SK18	29.298	283.74	0	26.25	0.036	9.7	0.8	0.6
SK18	23.054	265.44	0	9.55	0	11.5	0.23	0
SK19	62.439	366.12	4.201	27.05	0.233	0.13	0.38	0.3
SK19	57.636	329.51	0	17.4	0	0	0.31	0.1
SK20	68.203	323.41	0	31.7	0	6.96	0.26	1.2
SK20	46.589	244.08	0	19.45	0	4.52	0.39	0.7
SK21	22.094	207.47	0	17.25	0.138	3.61	0.77	0.2
SK21	10.086	204.42	0	17.05	0.086	0.12	0.68	0
SK23	122.93	404.35	0		0	10.62	2.3	0
SK24	9.95	165.38	0		0	7.87	0.42	0
SK25	940.52	606.36	0		0	16.09	0	0
SK29	110.47	335.61	0	34.5	0		0	0
SK28	48.03	262.39	0	24.5	0		0	0
SK30	5.7636	122.04	0	8	0		0.06	
SK31	7.6848	115.94	0	6.6	0		0.2	0.1

Table 2a: Chemical analysis of the water samples taken from the springs in the study area;  
*\*TDS: Total dissolved solids; \*\*EC: Electrical conductivity.*

Water Sample Location	pH	*TDS mg/l	**EC $\mu$ S/cm	Na+ mg/l	K+ mg/l	Ca++ mg/l	Mg++ mg/l	Cl - mg/l
K1	7.53	653	1179	155.87	7.8	27.86	19.08	236
K1	7.37	776	1134	163.69	10.9	46.49	21.27	275
K2	7.81	286	350	20.69	1.6	34.47	12.15	7.09
K2	7.72	311	329	25.75	2.7	33.07	13.61	6.03
K3	7.49	85	126	5.75	1.2	9.82	3.889	3.55
K3	7.37	91	123	7.36	1.6	8.216	5.347	4.61
K4	7.49	36	69	2.30	1.6	1.804	2.917	1.77
K4	7.77	61	80	3.45	2.0	10.02	1.215	2.13
K5	7.3	56	82	2.99	1.2	9.82	0.608	2.13
K5	7.82	63	76	4.14	1.6	8.216	2.309	1.77
K6	8.7	246	292	1.15	0.4	49.3	8.75	1.77
K6	8.3	204	221	0.46	0.0	41.08	7.292	0.35
K7	7.9	325	381	1.61	0.8	64.13	11.42	2.48
K7	7.96	229	252	0.92	0.0	46.89	6.805	1.77
K8	7.38	506	653	25.98	1.6	87.98	15.31	44.7
K8	7.6	513	624	30.81	2.0	85.17	16.28	48.9
K9	7.95	135	227	0.69	0.0	41.28	7.899	5.67
K10	8.05	218	284	1.15	0.4	44.49	6.198	1.42
K10	8	229	251	0.69	0.0	47.29	6.805	1.06
K11	7.85	443	594	23.68	2.0	67.94	19.44	37.2
K11	7.66	360	601	32.88	2.3	65.13	21.87	33.7
K12	8.23	84	123	3.91	1.6	11.62	4.496	1.42
K12	8.37	71	129	6.67	1.6	10.22	6.562	4.61
K13	7.32	27	45	2.30	1.2	1.002	3.038	3.55
K14	7.66	43	59	4.14	1.6	2.004	3.038	1.06
K15		57	79.2	4.38	1.8	7.14	2.38	1.63
K16	6.6	172	224	21.15	4.7	12.02	7.292	10.6
K17	6.7	472	710	108.05	11.7	20.04	13.37	156
K18	7.1	622	876	34.25	2.7	76.15	41.32	46.1

Table 2b: Chemical analysis of the water samples taken from the springs in the study area; \*TDS: Total dissolved solids; \*\*EC: Electrical conductivity.

Water Sample Location	SO <sub>4</sub> - mg/l	HCO <sub>3</sub> mg/lt	CO <sub>3</sub> mg/lt	Hardness (F)	NO <sub>2</sub> (mg/l)	NO <sub>3</sub> (mg/l)	NH <sub>4</sub> (mg/l)	Boron (mg/l)
K1	2.88	203.2	0	14.8	0.003	2.97	0.11	3.2
K1	11.5	246.52	0	30.35	0	5.89	0.22	3.6
K2	2.88	207.47	0	13.6	0.007	10.9	0.06	0.2
K2	9.13	219.67	0	13.85	0	13.51	0.13	0.1
K3	0.48	59.8	0	4.05	0	4.13	0.07	0.2
K3	6.24	57.969	0	4.25	0	5.71	0.16	0
K4	1.92	23.188	0	1.65	0.023	2.91	0.33	0.4
K4	6.24	37.222	0	3	0	7.58	0.15	0
K5	0.96	37.832	0	2.7	0	2.97	0.16	0.4
K5	7.2	37.832	0	3	0	0	0.08	0
K6	13	164.75	6.601	15.9	0.01	2.15	0.25	0.6
K6	6.72	145.23	4.201	13.25	0	4.12	0.15	0.1
K7	30.3	215.4	0	20.7	0.01	6.56	0.2	0.6
K7	12.5	160.48	0	14.5	0	4.92	0.17	0.2
K8	36	293.51	0	28.25	0.033	9.04	0.165	0.6
K8	43.7	286.18	0	27.95	0	12.1	0.2	0.2
K9	1.44	1.8306	75.61	13.55	0	5.63	0.26	0.1
K10	5.28	161.09	0	13.85	0.003	5.32	0.12	0.4
K10	11.5	161.09	0	14.6	0	8.2	0.12	0.1
K11	49	244.08	0	24.95	0.049	14.97	0.308	0.6
K11	2.88	68.953	132	25.25	0	10.85	0.34	0.4
K12	1.44	53.087	6.601	4.75	0	2.76	0.2	0.4
K12	3.84	2.4408	33.9	5.25	0	4.16	0.17	0
K13	14.4	1.8306	0	1.5	0	3.83	0.14	0
K14	8.65	21.967	0	1.75	0	1.13	0.13	0
K15	2.52	36.852	0		0	3.35		0
K16	11.5	103.73	0	6	0		0	0
K17	9.61	152.55	0	10.5	0		0	0
K18	123	299	0	36	0		0	0

When the tables 1 and 2 are examined it is found that the nitrite concentration is higher than the maximum value ( $0.233 > 0.1$  mg/l) at the water samples (SK 19) taken from deep well so there is nitrite pollution at the water of this well and all water samples taken from the springs have good quality according to the Turkish Drinking Water Standard, except Ilipinar Spring (K1). Ilipinar Spring is located near Çöl Lake (Fig. 4). Ilipinar Spring flows at fault crack and fault zone affects the water quality of Ilipinar Spring. So Ilipinar Spring water is not suitable for the irrigation and drinking purposes.

According to the former water chemistry analysis of Soysalli and Dundarli Springs (spring samples: K3, K9 and K10), water of these springs had been polluted in 1998 and 1999, their ammonium concentrations are higher than the maximum ammonium value (0.5 mg/l) at this time period, but according to 2004 and 2005 analysis results, Soysalli and Dundarli Springs can be used as drinking water; their ammonium concentrations are lower than the maximum ammonium concentrations of Drinking Water Standard. Soysalli and Dundarli Springs are used as irrigation water in summer but these springs feed Sultansazligi Wetland during winter (Yildiz, 2007).

The figure 5 shows electrical conductivity (EC) and total dissolved solid (TDS) values of groundwater samples taken from wells at Develi Closed Basin. When (Fig. 5) is examined it is determined water samples of the deep wells SK2 and SK23 which are located near Calbalma Drainage Water Pumping Station have high EC and TDS values. Because drainage water accumulated at the pumping station pollutes the groundwater by infiltration. Additionally water sample of the shallow well SK25 which is located near the Southern Marshland of Sultansazligi Wetland has high EC and TDS. Because this shallow well is fed by the surface water of the Southern Marshland of Sultansazligi. Surface water of the Southern Marshland has high EC and TDS values due to the drainage water pollution.

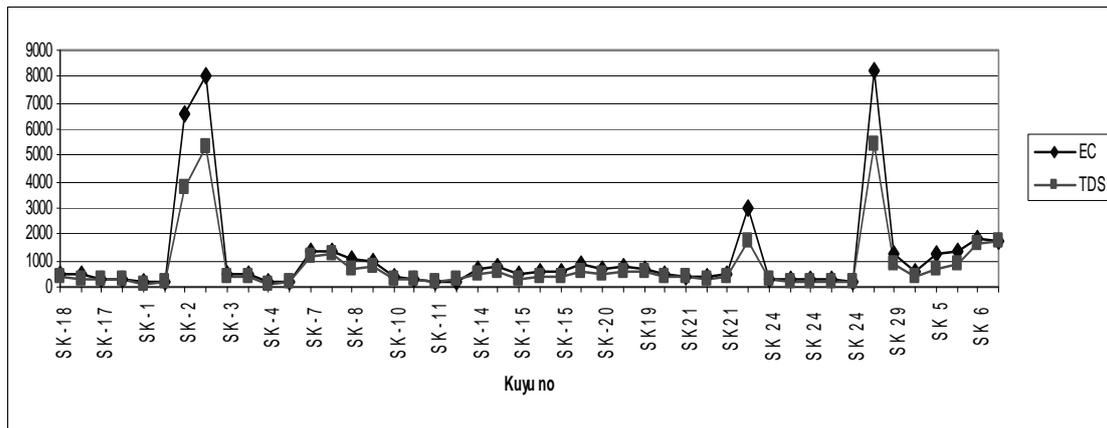
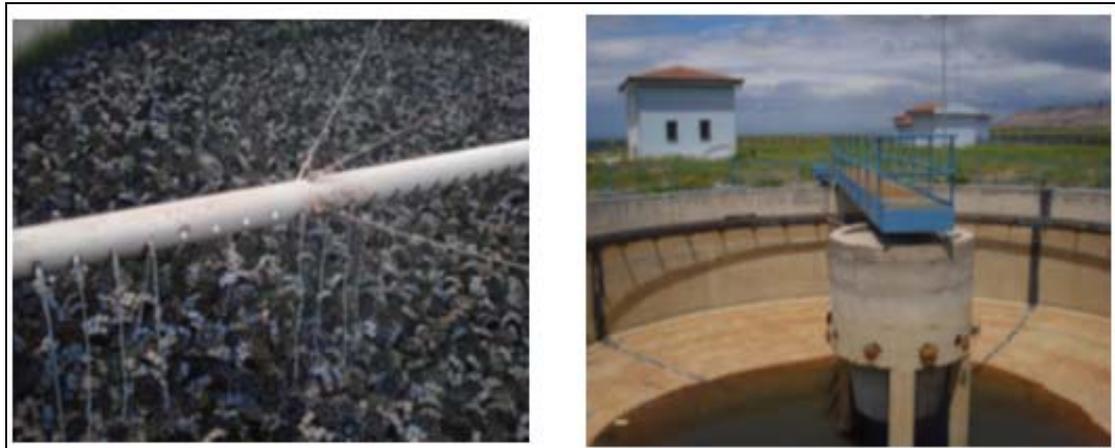


Figure 5: TDS and EC values of groundwater samples.

### Surface Water Pollution at Sultansazligi Wetland

Although there is wastewater and solid waste collecting network, there is not operated wastewater treatment plant at the end of collecting system at Yesilhisar and Yahyali Districts so domestic wastewater left to flow into open fields, near Sultansazligi Wetland. There is one waste water treatment plant at Develi District but it is not working efficiently because of unsuitable treatment process. Its treatment process is “trickling filter” (Fig. 6) but trickling filter is not suitable for the climate conditions of Develi District. Additionally industrial factories (textile, leather factories) do not use their industrial wastewater treatment plants efficiently, untreated industrial wastewater flows through Develi Wastewater Treatment Plant with domestic wastewater, so untreated industrial wastewater affects badly the quality of the treated wastewater outflow at Develi Wastewater Treatment plant (Gurer et al., 2008).

Yahyali Wastewater Treatment Plant has been under construction and a company is designing Yesilhisar Wastewater Treatment Plant project since 2010. Process of Yahyali and Yesilhisar Wastewater Treatment Plants will be “long aerated active sludge”.



Trickling filter tank

Sedimentation tank

Figure 6: Develi Wastewater Treatment Plant (photo Ferit Yuksel, 2008).

Farmers use pesticides and chemical fertilizers at Develi Closed Basin. For irrigating the land, "wild flooding" irrigation method is used about 95% of the region and therefore a big portion of the chemicals spread on soil is washed down to the water bodies by irrigation return flow to existing drainage network and consequently to Sultansazligi Wetland. According to the field trip at Develi Closed Basin and Sultansazligi Wetland, it is observed that drainage water having high salt content flows through Southern Marshland of Sultansazligi Wetland from Camiz Pumping Station (Figs. 7 and 8). Drainage water increases salt content of the surface water of Sultansazligi Wetland so surface water samples which had been collected from Sultansazligi Wetland have high EC values. Former surface water chemistry analysis of Sultansazligi Wetland in 1983 can be seen in the table 3 and surface water chemistry analysis in 1998, 2000 and 2003 can be seen in the table 4. When the surface water chemistry parameters of the same stations in tables 3 and 4 are compared: it can be said that EC, nitrate, ortho-phosphate and ammonium concentrations are increased and dissolved oxygen concentrations are decreased from 1982 to 1998, 2000 and 2003 because of water pollution at the surface water of Sultansazligi Wetland (Akcaakaya et al., 1983; Yildiz, 2007).

Table 3: Water chemistry analysis of Sultansazligi Wetland on 20<sup>th</sup> September 1982 (Yildiz, 2007).

Station no.	15-12-01-26	15-12-01-27	15-12-01-28
(Observation year)	1982	1982	1982
T (°C)	20	20	19
PH	7.6	7.5	8.5
EC (mmho/cm)	897	946	881
Ca (mg/l)	40.1	72.1	58.1
Mg (mg/l)	48.6	35.2	37.7
DO (mg/l)	3.4	3.3	11
NO <sub>2</sub> -N (mg/l)	0.002	0	0.008
NH <sub>3</sub> -N (mg/l)	0.9	0.71	0.91
O-PO <sub>4</sub> (mg/l)	0.06	0.03	0.07

Table 4: Water chemistry analysis of Sultansazlığı Wetland (Yildiz, 2007).

Station no	15-12-01-26	15-12-01-27	15-12-01-28	15-12-01-28
(Observation year)	1998	2003	1998	2000
T (°C)	16	24	17	24
PH	7.05	8.9	7	8
EC (mmho/cm)	1237	2898	1311	1752
Ca (mg/l)	-	214.6	-	-
Mg (mg/l)	-	155.6	-	-
DO (mg/l)	2.5	0.7	3.05	5.6
NO <sub>2</sub> -N (mg/l)	-	0.014	-	0.018
NH <sub>3</sub> -N (mg/l)	-	2	-	0.85
O-PO <sub>4</sub> (mg/l)	0.7	1.33	0.9	0.84



Figure 7: Camiz Pumping Station (photo by Ebru Yildiz)

Figure 8: Drainage water flowing in Sultansazlığı Wetland (photo by Ebru Yildiz)

The tables 5 and 6 show total dissolved solids (TDS) and electrical conductivity (EC) values of the surface water of Southern Marshland and Yay Lake before and after irrigation application at Develi Closed Basin. According to the tables 5 and 6 it is seen that irrigation return flow (drainage water) increases TDS and EC at Sultansazlığı Wetland and decreases the water quality of this wetland.

Table 5: Variation of TDS (mg/l) before and after irrigation (DSI, 1997).

Location of station	Yay Lake (15-12-01-52) av. max.		Southern Marshland			
			Egri Lake (15-12-01-27) av. max.		Sap Lake (15-12-01-28) av. max.	
Before irrigation	3422	4416	285	416	323	560
After irrigation	3634	6397	919	1345	917	1149

Table 6: Variation of EC (mmohms/cm) before and after irrigation (DSI, 1997)

Location of station	Yay Lake (15-12-01-52) av. max.		Southern Marshland			
			Egri Lake (15-12-01-27) av. max.		Sap Lake (15-12-01-28) av. max.	
Before irrigation	5346	6900	445	650	505	875
After irrigation	6081	10354	1557	2097	1550	1876

## **DISCUSSION**

It is determined that there is ammonium and nitrate pollution at groundwater (water samples SK2 and SK23) around Çöl Lake of Sultansazligi Wetland. Also EC of these groundwater samples are very high. There is only one waste water treatment plant at Develi Closed Basin so industrial and domestic waste water pollutes the surface water of Sultansazligi Wetland and groundwater of Develi Closed Basin. Drainage water flows through Sultansazligi Wetland and drainage water cause salt accumulation and decreases dissolved oxygen at the surface water of this wetland. This situation is very harmful for the aquatic fauna and flora living in this wetland.

Also untreated wastewater disposal pollute Sultansazligi Wetland. EC, nitrate, ortho-phosphate and ammonium concentrations are increased and dissolved oxygen concentrations are decreased from 1982 to 1998, 2000 and 2003 because of water pollution at Sultansazligi Wetland. Ministry of Environment and Forestry must enforce Develi Municipality to operate Develi Wastewater Treatment Plant efficiently and also Yahyali and Yesilhisar Wastewater Treatment Plants must be operated as soon as possible.

Irrigation water requirement is very high and surface water supplied from the reservoirs is not sufficient at Develi Closed Basin. So groundwater and the springs around Sultansazligi Wetland are used as irrigation water during the irrigation season.

There is water scarcity at Sultansazligi Wetland, only precipitation and salty drainage water feed Sultansazligi Wetland so fresh spring water can be used to feed Sultansazligi Wetland to decrease the salt concentration at the surface water of this wetland.

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**OXIDATIVE STRESS AND DNA DAMAGE  
ELICITED BY MICROCYSTIN-LR LEVELS  
AT IGNACIO RAMÍREZ RESERVOIR  
(MEXICO)**

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**KEYWORDS:** Microcystin-LR, DNA damage, oxidative stress, lipid peroxidation, catalase, Balb-C mice bioassays.

**ABSTRACT**

Increasing eutrophication of aquatic environments favors massive growth of phytoplankton species. Benefiting the most from these conditions are cyanobacteria such as *Microcystis aeruginosa*. Some strains of this microalga produce toxins such as microcystins, including microcystin-LR, which are associated with the death of various wild and domestic animal species. This is perceived as a public health problem in many countries worldwide, but there are a few data from Mexico. High performance liquid chromatography on phytoplankton samples at Ignacio Ramírez reservoir, in the Central Plateau of Mexico, indicates a maximum concentration of this toxin of 13.25 µg/g dry weight. This study aims to evaluate the acute effects of a fraction of the maximum concentration detected in Ignacio Ramírez of the microcystin-LR in male Balb-C mice using oxidative stress and DNA damage biomarkers. Commercial grade, pure microcystin-LR was injected intraperitoneally at a fraction of the maximum microcystin-LR found in Ignacio Ramírez reservoir in male Balb-C mice. After 0, 1, 3 and 24 hours, protein content, lipid peroxidation, catalase activity were assessed and DNA damage was evaluated by measuring DNA strand breaks in individual cells of the liver of treated mice. All biomarkers assessed showed a significant time-dependent increase. Microcystin treatment induced increase hepatic protein content at 1 h (40%), 3 h (37%) and 24 h (360%) compared to the control. The level of lipid peroxidation increased to 137 and 315% at 1 and 3 h after exposure, respectively, reaching a maximum of 1.228% after 24 h. Also, the microcystin-LR affected the catalase activity increasing to 80, 101 and 129% after 1, 3 and 24 h, respectively, compared to catalase activity of the control. Tail nucleus ratio of DNA showed a peak at 3 h of exposure and then a reduction. The largest increase in tail/nucleo ratio occurred during the first hour of exposition. The results indicate the fact that the potent toxicant microcystin-LR represents a possible public health risk at the study site where microcystin-LR was detected.

**RESUMEN:** Estrés oxidativo y daño al DNA provocado por Microcystina-LR en el embalse Ignacio Ramírez (México).

El incremento en la eutroficación de los cuerpos de agua ha favorecido el crecimiento masivo del fitoplancton, principalmente de las cianobacterias, tales como *Microcystis aeruginosa*. Algunas cepas de esta cianobacteria producen toxinas entre las que podemos mencionar las microcistinas, incluyendo Microcistina-LR, la cual se ha asociado con la muerte de varias especies animales, tanto silvestres como domésticas. Esta situación se ha convertido en problemas de salud pública en muchos lugares del mundo; en México poco se conoce acerca de este problema, existiendo pocos datos al respecto. En muestras de fitoplancton del embalse Ignacio Ramírez localizado en el altiplano mexicano, se detectó una concentración máxima de Microcistina-LR de 13.25 µg/g de peso seco. El objetivo del presente estudio fue evaluar los efectos agudos de una fracción de la máxima concentración de Microcistina-LR detectada en el embalse Ignacio Ramírez, en machos de ratones de la cepa Balb-C mediante el empleo de marcadores de estrés oxidativo y daño al DNA. Ratones de la cepa Balb-C fueron inyectados intraperitonealmente con Microcistina-LR pura, grado comercial, con una concentración menor del máximo detectado en el embalse Ignacio Ramírez. Después de 1, 3 y 24 horas de exposición, las proteínas totales, el nivel de lipoperoxidación y la actividad de la catalasa fueron cuantificadas; así mismo, el daño al DNA se determinó por medición del rompimiento de las cadenas de DNA en células individuales del hígado de los organismos expuestos. Todos los biomarcadores mostraron un incremento dependiente del tiempo de exposición. La exposición a la Microcistina-LR indujo un incremento en el contenido de proteínas hepáticas a 1 h (40%), 3 h (37%) y 24 h (360%) comparadas con el control. El nivel de lipoperoxidación se incrementó a 137 y 315% después de 1 y 3 h de exposición, respectivamente, alcanzado un máximo de 1,228% a las 24 h. La actividad de la catalasa se vio afectada también por la Microcistina-LR, incrementándose 80, 101 y 129% después de 1, 3 y 24 h de exposición, respectivamente, comparadas con la actividad de la catalasa del grupo control. En el ensayo Cometa, la relación de las colas y núcleos de DNA mostraron un pico a las 3 h de exposición, y posteriormente hubo una reducción. El mayor incremento en la relación cola/núcleo ocurrió durante la primera hora de exposición. Los resultados indican el potente efecto tóxico de la Microcistina-LR, representando un riesgo potencial a la salud pública en el sitio de estudio donde se detectó la Microcistina-LR.

**REZUMAT:** Stresul oxidativ și degradarea ADN-ului, induse de concentrațiile de microcistina-LR, în lacul de acumulare Ignacio Ramirez (Mexic).

Eutrofizarea tot mai intensă a mediilor acvatice favorizează creșterea masivă a speciilor de fitoplancton. Cel mai mult profită de acest fapt cianobacteriile ca *Microcystis aeruginosa*. Unele tulpini ale acestei microalge produc toxine ca microcistinele, inclusiv microcistina-LR, care este asociată cu moartea mai multor specii animale, sălbatice și domestice. Aceasta este percepută ca o problemă de sănătate publică, în multe țări din lume, dar în ceea ce privește Mexicul, datele sunt sărace. Analiza HPLC, efectuată asupra unor eșantioane de fitoplancton, din lacul de acumulare Ignacio Ramirez, din Podișul Central Mexican, indică o concentrație maximă a acestei toxine de 13.25 µg/g masă uscată. Prezentul studiu are ca scop evaluarea efectelor acute ale unei fracțiuni din concentrația maximă a microcistinei-LR, detectată în Ignacio Ramirez la șoarecele Balb-C mascul, utilizând biomarkerii pentru stress oxidativ și deteriorarea AND-ului. Microcistină-LR pură, în formula comercială, a fost

injectată intraperitoneal unor șoareci Balb-C, la o fracțiune din nivelul maxim de microcistină-LR, găsită în lacul de acumulare Ignacio Ramírez. După 0, 1, 3 și 24 ore, au fost măsurate conținutul de proteină, peroxidarea lipidelor și activitatea catalitică și s-a evaluat deteriorarea ADN prin măsurarea ruperii secvențelor ADN, în celule hepatice individuale, de la șoarecii tratați. Toți biomarkerii evaluați au arătat o creștere semnificativă, în directă dependență de timp. Administrarea de microcistină induce creșterea nivelului de proteină din ficat la 1 h (40%), 3 h (37%) și 24 h (360%), comparativ cu proba martor. Nivelul de peroxidare a lipidelor a crescut între 137 și 315% la 1 h respectiv 3 h după expunere, atingând un maxim de 1,228% după 24 h. Microcistina-LR afectează și activitatea catalitică, aceasta crescând la 80, 101 și 129% după 1, 3 și respectiv 24 h, comparativ cu activitatea catalitică din proba martor. Raportul cozii/nucleului ADN-ului a arătat un vârf la 3 h de la expunere, urmat de o diminuare. Cea mai mare creștere, în raportul cozii/nucleului, a avut loc în prima oră de după expunere. Rezultatele indică potențialul toxic al microcistinei-LR, reprezentând un risc posibil pentru sănătatea publică în locurile unde a fost detectată microcistina-LR.

### INTRODUCTION

In the past 30 years a remarkable rise in the eutrophication of natural and artificial water bodies has occurred world-wide, particularly near urban areas. Anthropogenic discharges have been identified as the primary cause behind these conditions. In response to this rising process of eutrophication, phytoplankton communities experience loss of biodiversity followed by dominance of cyanobacteria species and the emergence of massive cyanobacterial blooms, many of them toxic to other aquatic organisms (Smith, 2003).

The earliest known cases of human intoxication by ingestion of water contaminated with toxic cyanobacterial strains were described in Australia, England, China and South Africa (Falconer, 1993). Several episodes are known also from Brazil, the most serious thus far being the Caruaru incident (1996), where 50 haemodialysis human patients died following treatment with water contaminated with microcystins (Pouria et al., 1998).

The cyanobacterium *Microcystis aeruginosa* has frequently been linked to blooms. Some strains are able to produce microcystins such as microcystin-LR (MC-LR), the most powerful heptapeptide in this family (Kaebernick and Neilan, 2001). Massive deaths have occurred in aquatic and terrestrial species organisms associated to such type of blooms. These obvious threats have led the World Health Organisation (WHO) to establish a certain provisional guideline value for MC-LR in 1 µg/l of drinking water (WHO, 1998).

Microcystins are endotoxins whose high concentration in water is the result of cell lysis (Romanowska-Duda and Tarczynka, 2002) due to senescence or to treatment of water for human use by means of procedures such as prechlorination and algacide applications (Jones and Orr, 1994). They are able to spread across biological membranes and once released in water can persist for extended periods of time (Watanabe et al., 1992; Jones and Orr, 1994) before they are removed or by the biodegradation or by the photolysis.

Microcystins are considered potent toxicants nearly 1/8 as toxic as tetrodotoxin (Beasley et al., 2000). Microcystins specifically inhibit protein phosphatases PP1 and PP2A as a result of the covalent bond formed by the toxin and cysteine residues at positions 273 and 266. PP1 and PP2A inhibition interferes with intracellular signals that trigger and modulate cell growth and differentiation, and also cause the typical effects of protein hyperphosphorylation and hepatotoxicity. The latter manifests itself as hepatocyte deformation and a parallel increase in the phosphorylation of cytosol and cytoskeleton proteins, and triggers increased activity of hepatic enzymes such as aspartate aminotransferase and lactate dehydrogenase in plasma (Monserrat et al., 2003). If the amount of toxin is sufficiently high serious damage occurs to the liver, which experiences a massive irruption of blood leading ultimately to anaphylactic shock and the death of exposed animals (Carmichael, 1994).

Although the typical adverse effects elicited by microcystins are considered to be the result of PP1 and PP2A inhibition, recent evidence points to an alternative mechanism of toxicity involving oxidative damage. Several studies found lipid peroxidation (LPOX) levels and reactive oxygen species (ROS) content increase with exposure to microcystins in different species (Ding et al., 2003; Li et al., 2003; Yin et al., 2005; Pinho et al., 2005). Also, this toxin is a potent liver cancer promoter in laboratory mice (Bischoff, 2001). Hepatotoxicity and other toxic manifestations are well documented, but there is limited information on its role in induction of oxidative stress and DNA damage.

In vitro exposure of the mice and the common carp hepatocytes indicates that MC-LR is able to increase ROS content tenfold and reduce glutathione (Li et al., 2003). Javaral et al. (2006) have shown the fact that it induces oxidative stress in mice and reduces the activity of several antioxidant enzymes including superoxide dismutase (SOD) and catalase (CAT). Maidana et al. (2006) found the fact that this effect is also elicited in the hypothalamus of rats exposed to these compounds, resulting in memory alterations.

In vivo studies of mice hepatocytes indicate the fact that MC-LR induces DNA breakdown by endonuclease activation (Rao and Bhattacharya, 1996). However, it was found that the DNA damage may also be mediated by the action of ROS (Meneghini et al., 1997), which are known to react with certain transition metals such as Fe and Cu and to damage DNA via Fenton reactions and hydroxyl radical (OH) production, creating simple- and double-strand breaks (Meneghini, 1997; Lloyd and Phillips, 1999).

In Mexico, studies regarding MC-LR are very limited (Figueroa-Aldariz et al., 2006; López-López et al., 2007), and none of them address its effects at the level of oxidative stress and DNA damage. Thus, the aim of this study was to determine the effect caused in mice of the Balb-C strain, in oxidative stress and DNA damage, by exposure to MC-LR at a fraction of the highest concentration of MC-LR found at Ignacio Ramírez reservoir.

## MATERIALS AND METHODS

**Study area.** This study was conducted at Ignacio Ramírez Reservoir (19°27'35" N and 99°46'25" W), in the Lerma-Chapala Basin, the most important water system in the Mexican Central Plateau. The aquatic macrofauna includes at least three endemic species: the crayfish *Cambarellus moctezumae*, the fish *Menidia riojai* and *Girardinichthys multiradiatus*. The reservoir has a storage capacity of  $20.5 \times 10^6 \text{ m}^3$  and is used for agricultural purposes. The climate is temperate with rains in summer. The warmest months are May and June.

**Plankton collection and preparation of samples.** Cyanobacteria samples were collected with a Wisconsin-type 45- $\mu\text{m}$ -mesh plankton net and transported to the laboratory in plastic bottles at 4°C in total darkness. They were frozen until analysis. Collection took place in August 2004, usually the warmest month with the highest rainfall, when a bloom of *Microcystis aeruginosa* was detected.

**Identification and quantification of MC-LR.** The frozen samples were lyophilized using a Usifroid Mod. Rieutord equipment, and 50 mg lyophilized cells were extracted twice with 30 ml methanol and centrifuged at 1,500 g for 10 min. The supernatant was vacuum dried and dissolved in 1:3 ethyl acetate-isopropanol (v/v). The aqueous extracts were applied to the preconditioned C18 SPE silica cartridges to clean the samples. They were then dried, dissolved in 1,000  $\mu\text{l}$  methanol and analyzed with high performance liquid chromatography equipment (Beckman System Gold 126) provided with a diode array detector. A 4.6 x 250-mm 5C18-AR Comsomil column and a 5% solution of trifluoroacetate-methanol (8:2, v/v) in water were used as mobile phase. Flow velocity was 1 ml/min. Toxin concentration was estimated by comparing the peak of the area at 238 nm with standard MC-LR (Sigma). Standard solutions were specifically prepared at the following concentrations: 5, 8, 10, 20 and 50  $\mu\text{g} \text{ ml}^{-1}$  methanol.

**Toxicity tests.** Tests were conducted on 16 week old male mice of the Balb-C strain. The animals were acclimated for two weeks prior to testing, with water and food (Formula Diet 5008, Purina Feeds) supplied ad libitum. A cycle of alternating 12 h light/dark periods was used and the temperature was kept at  $22 \pm 0.5^\circ\text{C}$ . Once acclimation ended, mice were divided into four lots of five individuals each; three lots for toxin treatment and one control lot.

The mice were administered with a dose of 13.25  $\mu\text{g}/\text{kg}$  of MC-LR (Sigma, USA) in 0.9% saline, via intraperitoneal injection. This is equal to 1/1,000 of the concentration found in the solid fraction of plankton samples from the reservoir during August 2004 (Figuroa-Aldariz et al., 2006). The control mice group was administered only with saline solution. After 0, 1, 3 and 24 h, the mice were sacrificed by cervical dislocation. The liver was removed and DNA damage in hepatocytes was assessed by cell electrophoresis (Singh et al., 1988), as well as total protein content (Lowry et al., 1951), LPOX (Buege and Aüst, 1978) and CAT activity (Radi et al., 1991) in homogenized tissue were determined. All tests were triplicate performed.

**Statistical analysis.** Results were subjected to a one-way analysis of variance (ANOVA) and a Duncan test for mean differences. Differences were considered significant at  $p < 0.05$ .

## RESULTS AND DISCUSSION

The maximum concentration of MC-LR in lyophilized extracts from phytoplankton samples at Ignacio Ramírez Reservoir was 13.25  $\mu\text{g/g}$ , and 1/1,000 (13.25  $\mu\text{g/kg}$ ) of this amount was used in the toxicity tests. Increase in protein content is dependent on treatment duration with MC-LR (Fig. 1).

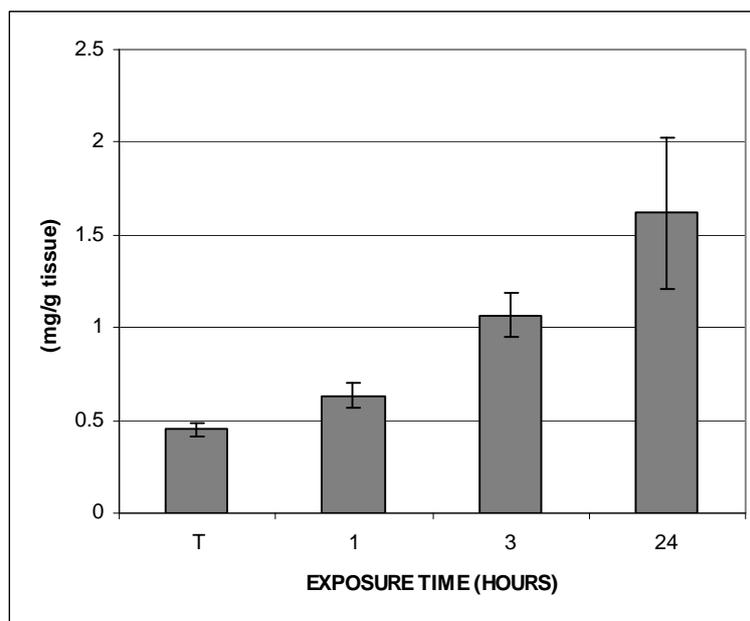


Figure 1: Variability of protein content over time in Balb-C mice treated with microcystin-LR (13.25  $\mu\text{g/kg}$ ); results show the mean of 5 replicates.

Protein content increased 40% with respect to controls in mice treated for 1 h, while those for 3 and 24 h exhibited increases of 137 and 360%, respectively.

LPOX levels also increased with time of exposure, to 137, 315 and 1,228% of initial values after 1, 3 and 24 h, respectively (Fig. 2).

CAT activity behaved much like LPOX, increasing with time of exposure, in this case to 80, 101 and 129% after 1, 3 and 24 h of treatment, compared to control values (Fig. 3).

Figure 4 illustrates DNA damage in hepatocytes by MC-LR exposure, expressed as tail/nucleus (T/N) ratio. The largest increase in this ratio occurred during the first hour. At 3 h, damage was still significant compared to basal values, but showed a tendency to decline. At the end of the study, DNA damage was only slightly higher than in control cells.

Results pertaining to the identification and quantification of MC-LR show this toxin is present at a concentration of 13.25  $\mu\text{g/g}$  in the lyophilized extract. It is worth pointing out that the values found at Ignacio Ramírez Reservoir are low compared to the concentrations found by other authors in different reservoirs worldwide. Baldia et al. (2003) report values ranging from 859 to 1,295  $\mu\text{g/g}$  in lyophilized extracts from Laguna de Bay, Philippines, while Ame et al. (2003) found the concentration of this toxin ranged from 5.8 to 2,400  $\mu\text{g/g}$  in lyophilized extract from San Roque Reservoir, Argentina, during the period 1998-2002. Such variability may be due to differences in sampling season, cyanobacterium population density, cell strain or diverse environmental factors.

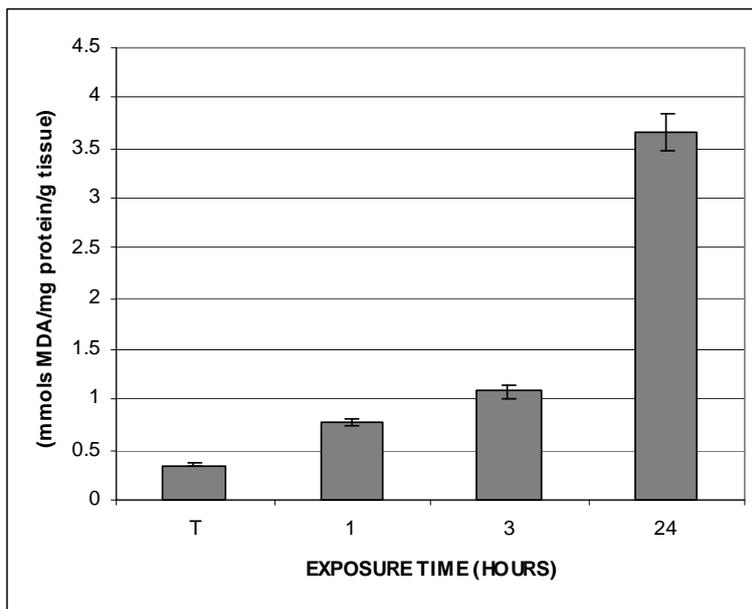


Figure 2: Variability of lipid peroxidation over time in Balb-C mice treated with microcystin-LR (13.25  $\mu\text{g}/\text{kg}$ ); results show the mean of 5 replicates.

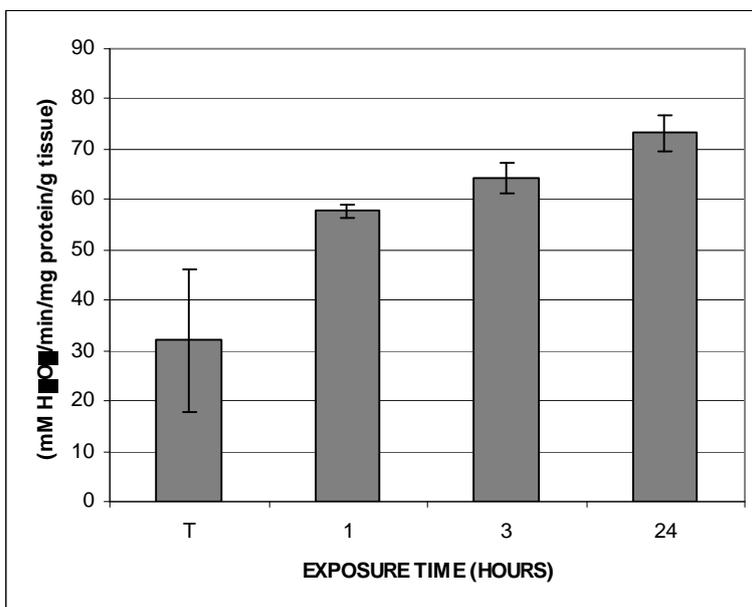


Figure 3: Variability of catalase activity over time in Balb-C mice treated with microcystin-LR (13.25  $\mu\text{g}/\text{kg}$ ); results show the mean of 5 replicates.

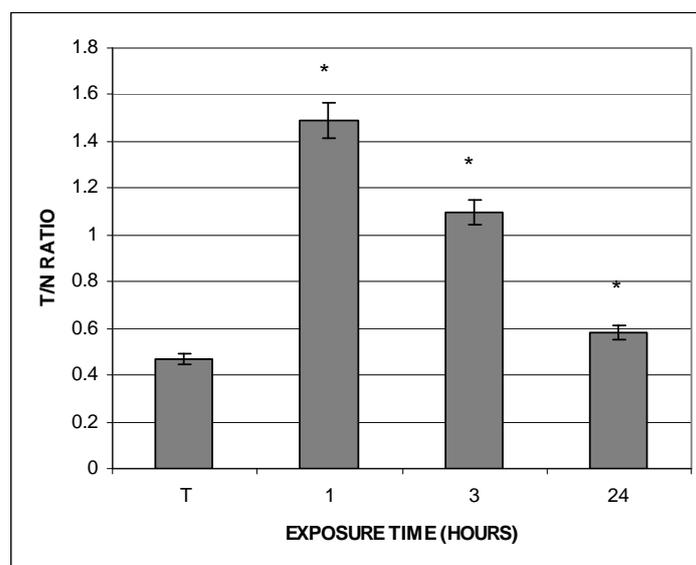


Figure 4: Variability of DNA damage over time in Balb-C mice treated with microcystin-LR (13.25 µg/kg); results are expressed as T/N ratio and show the mean of 5 replicates.

Even at concentrations considered as low as those found in the present study, bioaccumulation of cyanobacterial toxins may produce undesirable effects in natural ecosystems and also on public health. Several studies have shown that microcystins are accumulated by aquatic organisms at different trophic levels, from zooplankton (Ferra-Filho et al., 2002) to fish (Xie et al., 2005). The latter study, in Chaohu Lake, China, indicates these toxins are present in proportionately larger amounts in carnivorous (*Culter ilishaeformis*) and omnivorous (*Carassius auratus*) fish than in plankton-feeding (*Hypophthalmichthys molitrix*) or herbivorous (*Parabramis pekinensis*) fish. Thus, fish at the top of the food chain are at higher risk of exposure, and human populations consuming them must be alerted.

Several species inhabiting the studied reservoir, such as the silverside fish *Menidia riojai* and the crayfish *Camberellus moctezumae*, are marketed and consumed in nearby towns. Reservoir water is also used by surrounding communities for human and domestic animal consumption, irrigation and recreation with no prior treatment to remove this toxin.

The toxicity tests indicate the fact that MC-LR induces damage even at concentrations far below those found in the studied reservoir. Thus, protein content shows increase with duration of exposure to the toxin. Since MC-LR is highly toxic to cells, it is likely that once it enters the bloodstream, compensation mechanisms such as synthesis of stress proteins (Bischoff, 2001) are unleashed along with significant amounts of enzymes associated with oxidative stress, such as CAT, SOD and glutathione peroxidase, etc.

Moreover, the oxidative stress results show a substantial increase in LPOX levels and CAT activity with respect to the control group during the period of exposure. Microcystins can generate ROS in the cell and may consequently result in unbalanced cellular redox status, causing the cell to undergo an oxidizing process (Lathi et al., 1997; Monserrat et al., 2003). This fact has been confirmed also by other authors, including Li et al. (2003), who showed that in vitro exposure to 10 µg/l MC-LR for 6 h increased ROS content up to tenfold in mice and common carp (*Cyprinus carpio*) hepatocytes.

On the other hand, the cells of the aerobic organisms are equipped with a unique set of antioxidant enzymes, such as SOD and CAT, which help remove oxygen species in order to restore redox status in the cell (Carmichael and Falconer, 1993) and minimize toxin effect. It is thus possible that as LPOX and ROS formation increased in this study a parallel increase took place in CAT activity, which can be increased to compensate the effect oxidative of the ROS. There are several authors which have obtained similar results on other species in other studies. Li et al. (2005), for example, observed increases in the activity of several antioxidant enzymes, including CAT, in the fish *Misgurnus mizolepis* following 28 days of exposure to MC-LR. Furthermore, other authors found that CAT was inhibited (Pinho et al., 2005) at higher concentrations and periods of exposure in a crab species.

ROS formation is not only associated with effects at LPOX level, it may also induce DNA damage. Studies in cultures of human hepatocellular carcinoma, link DNA strand breaks to oxidation of purines and an increase in dose-dependent ROS content (Zegura et al., 2004). Furthermore, MC-LR may react with some transition metals, such as Fe<sup>+2</sup> or Cu<sup>+2</sup>, to yield modified bases like 8-hydroxydeoxyguanosine (8-OhdG). The latter is capable of inducing transversions and takes part in DNA intrastrand cross-links and formation of simple- and double-strand breaks (Meneghini, 1997; Lloyd and Phillips, 1999). This idea is supported by our in vivo results which show a significant increase in DNA damage along with rises in protein content, antioxidant enzymes and LPOX.

Our results indicate MC-LR induces DNA damage even at sublethal doses, from the first exposure hour. As time goes on, this damage is reverted, reaching close to normal values at 24 h, while CAT is augmented to 129% at the exposure end. This effect may be associated with the formation of ROS occurring once the toxin enters the cytoplasm and interacts with cell metabolism. It is significant that damage reversion begins at 3 h, probably due to the cell activates repair mechanisms, as well as is induced an antioxidant response by the values of CAT. More studies are necessary to confirm these observations. It is worth nothing that studies by Lankoff et al. (2004) on human lymphocytes exposed to MC-LR at sublethal concentrations from 1 to 25 µg/ml indicate the peak rate of apoptosis occurs after 24 h of treatment.

## CONCLUSIONS

MC-LR at sublethal concentrations (1/1,000 of 13.25 µg/kg) was shown to induce oxidative stress in hepatocytes of Balb-C mice and this effect was observed to be related to the DNA damage of the toxin. The low concentration of exposure provoked the increase in antioxidant response as well as DNA damage and their possible repair of DNA damage.

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