TRANSYLVANIAN REVIEW OF SYSTEMATICAL AND ECOLOGICAL RESEARCH

12

The Wetlands Diversity

Editors

Angela Curtean-Bănăduc, Doru Bănăduc & Erika Schneider-Binder

Sibiu - Romania

2011

TRANSYLVANIAN REVIEW OF SYSTEMATICAL AND ECOLOGICAL RESEARCH

12

The Wetlands Diversity

Editors

Angela Curtean-Bănăduc, Doru Bănăduc & Erika Schneider-Binder

„Lucian Blaga" University of Sibiu,
Faculty of Sciences,
Department of Ecology and Environment Protection

Published based mainly on some of the scientific materials presented at the second and third "Aquatic Biodiversity International Conference" - Sibiu/Romania 2009-2011

Ecotur Sibiu
N.G.O.

"Lucian Blaga" University of Sibiu

International Association for Danube Research

Sibiu - Romania
2011

Scientifical Reviewers

John Robert AKEROYD
Sherkin Island Marine Station,
Sherkin Island - Ireland.

Ioan ARDELEAN
Romanian Academy, Institute of Biology Bucharest,
Bucharest - Romania.

Doru BĂNĂDUC
"Lucian Blaga" Univesity of Sibiu,
Sibiu - Romania.

Jürg BLOESCH
International Association for Danube Research,
Dübendorf - Switzerland.

Swaranjit Singh CAMEOTRA
Institute of Microbial Technology,
Chandigarht - India.

Kevin CIANFAGLIONE
University of Camerino,
Camerino - Italy.

Angela CURTEAN-BĂNĂDUC
"Lucian Blaga" Univesity of Sibiu,
Sibiu - Romania.

Constantin DRĂGULESCU
"Lucian Blaga" Univesity of Sibiu,
Sibiu - Romania.

Marian-Traian GOMOIU
Romanian Academy; "Ovidius" University.
Constanța - Romania.

Oriana IRIMIA-HURDUGAN
"Alexandru Ioan Cuza" University of Iaşi,
Iaşi - Romania.

Georg Albert JANAUER
University of Vienna,
Vienna - Austria.

Mike JOY
Te Kura Matauranga o nga Taonga a Papatuanuku Massey University,
Palmerston North - New Zealand

Maria LEICHTFRIED
Austrian Academy of Sciences, Institute for Limnology,
Mondsee - Austria

Mirjana LENHARDT
Institute for Biological Research,
Belgrade - Serbia

Eugenia LÓPEZ- LÓPEZ
National School of Biological Sciences, National Polytechnic Institute,
México D. F. - México.

Sanda MAICAN
Romanian Academy, Biology Institute of Bucharest,
Bucharest - Romania.

Peter MANKO
Prešov University,
Prešov - Slovakia.

IN MEMORIAM

Peter Simion Pallas
(1741-1811)

Peter Simion Pallas was born in Berlin on 22 September 1741, the son of professor of Surgery Simon Pallas. His father offered him the possibiliy to study with private tutors and help him constantly to find an interest in science and later to build a solid profesional basis. In these very early years of his life he took a special interest in natural history, an interest not to leave him until the end of his life.

Following his father he attending the Medical Institute of Berlin where graduates in 1758. He continue his studies in mathematics and sciences of medicine at Göttingen and Halle universities. In 1760 he moved to Leiden University and took his PhD in medicine.

He traveled throughout England where he researched the zoology of the marine coasts, and Netherlands being attracted by the Dutch museum collections. He settled for a while at Hague, and his new system of animal classification was praised by Georges Cuvier.

Returned to Berlin he starts to work on his Spicilegia Zoologica (1767-1780).

In 1767, was invited by the German origin Catherine II of Russia (Catherine the Great) to teach at the St. Petersburg Academy of Sciences.

He led an expedition between 1768-1774, to central Russia, Urals, West Siberia, Altay and Transbaikal collecting many natural history specimens. He explored also the Caspian Sea, the Ural, Altai Mountains and the upper Amur, reaching as far eastward as Lake Baikal.

In 1776, Pallas was elected as member of the Royal Swedish Academy of Sciences.

He was provided with the plants collected by other naturalists to compile the Flora Rossica (1784-1815), and started work on his Zoographica Rosso-Asiatica (1811-1831).

He also published an account of Johann Anton Güldenstädt's travels in the Caucasus.

Between 1793 and 1794, Pallas led a second expedition to southern Russia, visiting the Crimea, Black Sea and Caspian Sea coasts, Nipru Valley, etc. Pallas gave his account of the journey in his P. S. Pallas Bemerkungen auf einer Reise in die Südlichen Statthalterschaften des Russischen Reichs (1799-1801).

In his expeditions he covered with his interest a wide range of topics, including geology and mineralogy, reports on the native peoples and their religions, descriptions of new plants and animals, etc. The Empress bought Pallas's large natural history collection, and allowed him to keep them for life.

He issued the first time the idea of geographical variability of species and geographical differentiation of populations within the same species

Among his works we should add: Dissertatio inauguralis de infestis viventibus infra viventia (1760); Elenchus zoophytorum, sistens generum adumbrationes generaliores et specierum cognitarum succinctas descriptiones, cum selectis auctorum synonymis (1766); Naturgeschichte merkwürdiger Thiere (1769-1778); Reise durch verschiedene Provinzen des Russischen Reichs (1771-1801); Sammlungen historischer Nachrichten über die mongolischen Völkerschaften (1776-1801); Observations sur la formation des montagnes et sur les changements arrivés au Globe (1777); Novae species Quadrupedum e Glirium ordine (1778); Mémoires sur la variation des animaux (1780); Icones Insectorum praesertim Rossiae Sibiriaeque peculiarium (1781-1806); Species Astragalorum descriptae et iconibus coloralis illustratae (1800); Travels through the southern provinces of the Russian Empire (1802); etc.

He leaved Russia in 1810 and returned to Berlin. He died in 1811 in his country, letting behind a life of work and really impressive results in the field of natural sciences.

The Editors

CONTENTS

Preface

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

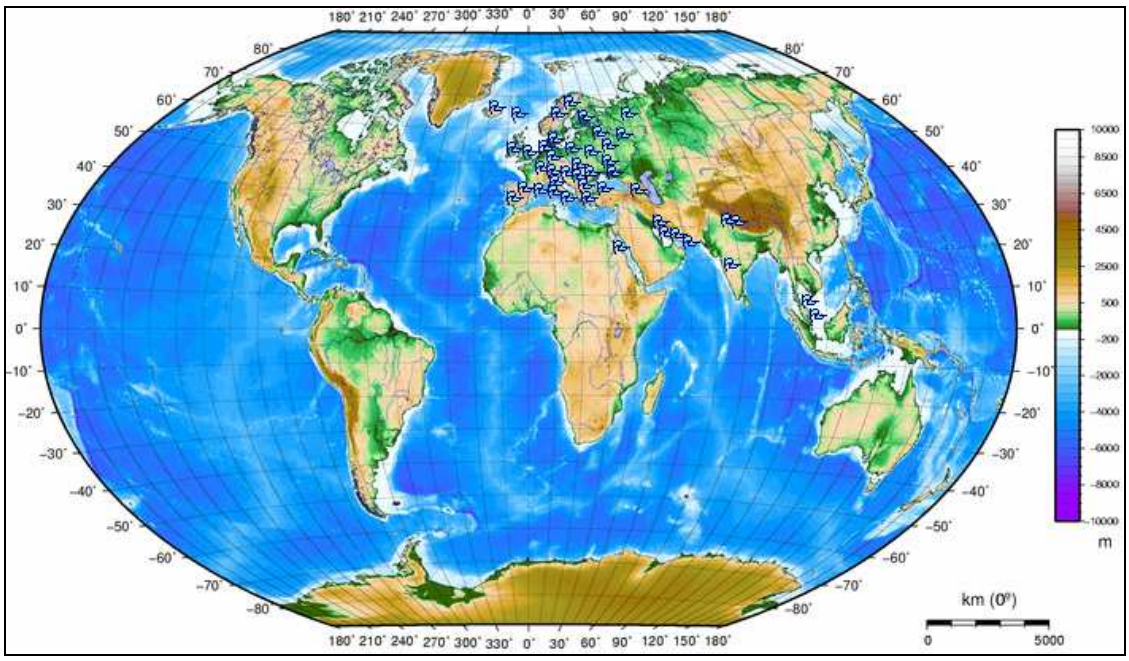
The causes, effects, trends and possibilities of human society to positively intervene to slow down this process or to adapt to it involve a huge variety of aproacess and efforts.

With the fact in mind that these aproaces and efforts shuld be based on genuine scientific understanding, the editors of the *Transylvanian Review of Systematical and Ecological Research* series launch a second annual volumes dedicated to the wetlands, volumes resulted mainly as a results of the *Aquatic Biodiversity International Conference*, Sibiu/Romania, 2007-2011.

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

The editors of the *Transylvanian Review of Systematical and Ecological Research* started and continue this new annual 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 fourth volume included variated researches from diverse wetlands around the world.



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

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

Acknowledgements

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

The Editors

Editorial Office:

„Lucian Blaga" University of Sibiu, Faculty of Sciences, Department of Ecology and Environment Protection, 5-7 Raţiu Street, Sibiu, Sibiu County, Romania, RO-550012, *Angela Curtean-Bănăduc* (banaduc@yahoo.com, angela.banaduc@ulbsibiu.ro)

ISSN 1841 - 7051

ARE THERE ENDEMIC VASCULAR PLANTS IN WET HABITATS OF EUROPE?

Carsten HOBOHM * and *Ines BRUCHMANN* **

* University of Flensburg, Department of Biology, Auf dem Campus 1, Flensburg, Germany, D-24943, hobohm@uni-flensburg.de
** University of Flensburg, Department of Biology, Auf dem Campus 1, Flensburg, Germany, D-24943, ines.bruchmann@uni-flensburg.de

KEYWORDS: endemism, distribution patterns, freshwater habitats, mires.

ABSTRACT

We extracted a list of vascular plant taxa associated with damp, wet and inundated environments from the database EvaplantE. At least 339 taxa which are endemic to Europe occur regularly in wet habitats. 103 of these are relatively closely associated and 236 are less closely associated with such habitats. 80 taxa are mainly associated with other habitat types but a minority of their populations also occurs under wet conditions. Only a few of the 339 taxa are hydrophytes which live in lakes or rivers. Far more taxa occur in habitats such as river banks or slopes in the surroundings of springs or waterfalls.

The distribution patterns of endemics associated with wet habitats show a relatively high diversity in Central and Western Mediterranean regions, the Alps and Atlantic regions and small numbers of endemics to the East of Europe. We did not find a clear north-south gradient with high numbers of endemics in the Canaries, Madeira Archipelago and Mediterranean and low numbers in Scandinavia and northern Russia.

Compared to rocks and screes, grassland, scrubs and heaths, or forests the number of endemics associated with wet habitats in Europe is low. We assume that this is due to the separation of water bodies in combination with the young age of wetlands and the low ecological continuity across wetland localities.

Absolute numbers of endemics in a region can be used as a measure for the responsibility in the framework of international nature conservation policies.

ZUSAMMENFASSUNG: Gibt es endemische Gefäßpflanzen in europäischen Feuchtgebieten?

Die hier vorgelegte Analyse bezieht sich auf für Europa endemische Gefäßpflanzen, die eine Affinität zu Süßwasserhabitaten oder anderen nassen bzw. feuchten Standorten aufweisen, oder auf diese beschränkt sind. Eine aktuelle Auswertung von EvaplantE ergab, dass mindestens 339 Taxa zu den für Europa endemischen Pflanzen nasser oder (wechsel) feuchter Standorte gehören. Etwa 103 von ihnen weisen eine enge Bindung, 236 eine weniger enge Bindung an derartige Standorte auf. 80 Taxa erscheinen zwar regelmäßig auch an feuchten Standorten, diese Sippen haben jedoch ihren Verbreitungsschwerpunkt in anderen terrestrischen Lebensräumen. Nur bei wenigen Taxa handelt es sich um Hydrophyten im engeren Sinne, d.h. um Besiedler der Wasserkörper von Flüssen oder Seen.

Das Bild der geographischen Verbreitung von Endemiten mit Affinität zu Feuchtstandorten im weitesten Sinne zeigt eine relativ große Diversität in den Regionen des zentralen und westlichen Mittelmeerraumes, den Alpen und in den atlantischen Gebieten.

Beide Gruppen weisen einen weniger ausgeprägten Nord-Süd-Gradienten auf als die Vertreter anderer Habitatgruppen, bei denen erwartungsgemäß das Zentrum der Endemiten im Mediterranraum, bzw. auf den Kanaren und den Inseln des Madeira-Archipels liegt.

Im Vergleich zu den Bewohnern von Fels- und Schuttfluren, Grasland, Gebüschen und Heiden, oder auch Wäldern in Europa ist die Zahl der Endemiten mit einer Affinität zu Feuchtgebieten vergleichsweise gering. Dies hängt möglicherweise mit der starken Isolation der einzelnen Feuchtgebiete in Kombination mit dem niedrigen Alter der meisten Still- und Fließgewässer in Europa und der geringen ökologischen Kontinuität innerhalb der Feuchtgebiete zusammen.

Die absolute Zahl der Endemiten kann als Maßstab für die relative Bedeutung einer Region und die Verantwortung der Zuständigen im Zusammenhang mit internationalen Naturschutzbestrebungen verwendet werden.

REZUMAT: Există oare plante vasculare endemice în zonele umede ale Europei?

Am extras o listă de plante vasculare care prezintă o afinitate spre habitate de apă dulce sau alte staţiuni umede, prin baza de date EvaplantE. Cel puţin 339 taxoni ai plantelor endemice din Europa aparţin celor legate de habitate de staţiuni umede. Dintre acestea aproximativ 103 taxoni sunt relativ strâns legaţi de zone umede, iar 236 au o afinitate mai puţin strânsă spre astfel de staţiuni. 80 de taxoni apar în mod frecvent şi în astfel de locuri, având însă punctul de greutate în răspândirea lor în alte habitate terestre. Doar la un număr redus de taxoni este vorba de hidrofite în sens strict, adică de plante legate de corpul apelor curgătoare sau de lacuri. De departe mai mulţi taxoni apar în habitate cum ar fi malurile râurilor sau pantele din jurul izvoarelor sau cascadelor.

Imaginea răspândirii geografice a endemitelor cu afinitate spre staţiuni umede în sens larg prezintă o diversitate relativ mare în regiuni ale spaţiului mediteraneean central şi vestic, în Alpi şi în zonele atlantice şi o diversitate redusă a endemitelor în partea de est a Europei. Nu am găsit un gradient nord-sud clar, cu un număr mare de endemite în Canare, Madeira şi zona mediteraneeană şi un număr redus în Scandinavia şi nordul Rusiei.

În comparaţie cu habitate de stâncării şi grohotişuri, de pajişti, tufărişuri sau de păduri din Europa, numărul endemitelor cu afinitate spre zone umede este relativ redus. Acest fapt ar putea fi în legătură cu izolarea diferitelor habitate umede în combinaţie cu vârsta scăzută a majorităţii habitatelor umede şi a continuităţii ecologice reduse în zonele umede.

Numărul absolut al endemitelor într-o regiune poate să servească drept reper pentru responsabilitatea factorilor de decizie în relaţie cu strădaniile politicilor internaţionale de conservare a naturii.

INTRODUCTION

Since De Candolle (1820; cf. also Rabitsch and Essl 2007) defined the term endemism in a biogeographical context, growing attention has been paid to the phenomenon and the meaning of endemism, in the international nature conservation policies. However, the relationship between endemism and habitat is a relatively new topic in vegetation science.

Perhaps Rikli (1943, 1946) was the first biogeographer who wrote a chapter about the habitats of endemic vascular plants. For the Mediterranean Basin and the surrounding regions, he described plant endemism in relation to rocky habitats, coastal habitats, garigue, semi-desert and desert, but not freshwater habitats, swamps or other wet habitats.

Few publications were found which dealt exclusively with endemics of wet habitats. Davis et al. (1997), for example, examined the Vernal Pools of California which harbor around 140 endemics in ephemeral freshwater communities and related vegetation types.

In Europe, at least 101 endemic vascular plant taxa inhabit bogs, mires, and fens, 255 occur on the lake shores, on river banks and in other freshwater-connected habitats (Hobohm and Bruchmann, 2009). 42 of the endemic vascular plant species or subspecies are relatively closely associated with mires. They comprise calciphilous and acidophilous taxa and representatives of lowlands and mountain areas (Hobohm, 2008b). In contrast to the distribution of endemic vascular plants of Europe as a whole (with high concentrations in the Mediterranean and only a few taxa in the north), inhabitants of moors are concentrated in the west of Europe.

For New Zealand, a figure of 264 endemic species is given for wetlands (McGlone et al., 2001). This relatively high number for an area which is much smaller than Europe might be explained by less marked effects of Pleistocene glaciations, higher precipitation rates, and the higher proportion of wetland areas in New Zealand in general (Davis et al., 1994, 1995).
For the swamp habitats and other freshwater wetlands of the Nile Delta in Egypt, McGinley (2008) gives eight endemic species (of a total of 553 plant species).

Information about single endemic plants and their habitats can be found on some websites (cf. Red List data of the IUCN, www.iucnredlist.orghttp://) and many regional floras.

However, only few comprehensive analyses on endemic vascular plant taxa deal with lakes, rivers, bogs, mires, swamps, or other aquatic habitats. We hypothesize that the proportion of endemics in wetlands is indeed relatively low because of the natural vulnerability of the habitats which can still be considered almost young.

Here, we focus on the endemic vascular plants of standing and running water, including e.g. ponds, springs, wet stream sides, river-banks (also those with summer-dry pioneer-vegetation on sandy or gravelly banks), bogs, mires and fens, including moorland, swamps, etc.

The aim of our analysis is to give an overview of endemic vascular plant taxa in wetlands and other wet habitats in Europe, to estimate the number of wetland endemics in Europe, and to characterize the ecology and distribution patterns of these plants in comparison with endemics of other habitat types. Furthermore, we want to underline the need to relate nature conservation efforts in Europe.

MATERIALS AND METHODS

For the purposes of assessing endemism, Fontain et al. (2007) and Tutin et al. (1996) divided Europe into regions which represent nations, groups of small nations or, in the case of Russia, parts of a nation, islands or groups of islands (Hobohm and Bruchmann, 2009; Figs. 1 and 2). Using a GIS analysis Bruchmann (2011) found the following area sizes for the 42 respective regions: Albania (28,657 km^2), Austria with Liechtenstein (84,128 km^2), the Azores (2,569 km^2), Belgium with Luxembourg (33,235 km^2), the Balearic Islands (5,100 km^2), Great Britain with the Orkneys, Zetland and the Isle of Man (230,790 km^2) and excluding Northern Ireland and the Channel Islands, Bulgaria (111,024 km^2), Canary Islands (7,556 km^2), Corsica (8,780 km^2), Crete with Karpathos, Kasos and Gavdhos (8,508 km^2), Cypres (9,138 km^2), former Czechoslovakia (127,692 km^2), Denmark (42,714 km^2), the Faroe Islands (1,484 km^2), Finland including Aaland Islands (335,313 km^2), France with the Channel Islands (539,527 km^2) and excluding Corsica, Germany (357,251 km^2), Greece without Crete (121,564 km^2), Ireland (the whole island; 83,924 km^2), Switzerland (41,493 km^2), The Netherlands (35,549 km^2), Spain with Gibraltar and Andorra (excluding the Balearic Islands; 494,053 km^2),

Hungary (93,002 km^2), Iceland (102,962 km^2), Italy without Sardinia and Sicily (25,0631 km^2), former Yugoslavia (255,252 km^2), Portugal (88,573 km^2), Madeira (774 km^2), Switzerland (41,493 km^2), Norway (excluding Svalbard and Jan Mayen; 320,915 km^2), Poland (311,695 km^2), Romania (237,396 km^2), the northern division of European Russia (1,463,824 km^2), the Baltic division (189,125 km^2), the central division of Russia (1,625,765 km^2), the south-western division (605,414 km^2), Ukraine the Crimean Peninsula (25,831 km^2) and the south-east of European Russia (953, 366 km^2), Sardinia (24,099 km^2), Svalbard comprising Bear Island and Jan Mayen (62,912 km^2), Sicily plus Malta (25,726 km^2), Sweden with Öland and Gotland (446,070 km^2) and the European part of Turkey at Imroz (23,877 km^2).

The database (EvaplantE; cf. Bruchmann and Hobohm, 2010; Hobohm and Bruchmann, 2009; Hobohm, 2008a) contains information about most endemic vascular plant taxa in Europe. Like a phone-book, such a list will never be complete and changes are still in progress. In general, we use a relatively broad term of what a species or subspecies is.

The database distinguishes between eight habitat groups, two of which contain most plants which live under more or less wet conditions: 1. freshwater habitats, comprising standing and running waters, including e.g. ponds, minerogene springs, wet stream sides, river banks and also wet to dry pioneer-vegetation on river banks etc. (in the following we call this group freshwater habitats s. l.), 2. bogs, mires, fens, swamps, including moorland, swamp-springs, wet or regularly inundated grassland etc. (in the following: mires s. l.). There is a wide overlap between both groups and therefore, many taxa are listed in both groups. Coastal habitats, like salt marshes or rocky habitats near the ocean, were excluded from this analysis.

We reduced and analyzed the recent version of the database (EvaplantE; version 12/2010) with a focus on plants living under wet conditions or in the succession stages following inundation. The analysis is founded on descriptive statistics. As a first step, we summed up numbers of families, species and subspecies for the whole of Europe, and numbers of species and subspecies per region. The number of endemics can be used as an important indicator or measure for international nature conservation policies and efforts (cf. Convention on Biological Diversity, Mittermeier et al., 2005).

Because the regions vary in size certain statistical methods cannot be applied. On the other hand, it is currently impossible to obtain serious numbers of European endemic vascular plant taxa per artificially defined grid cell.

Several regions are of similar size with a difference of less than 10% between the smaller and the larger region. This is the case for the central and northern part of European Russia, mainland France and mainland Spain, Finland, Norway and Poland, the states of former Yugoslavia, Italy, Romania and Britain, Czechia plus Slovak Republik and Greece (without Crete), Greece and Bulgaria, Bulgaria and Iceland, Iceland and Hungary, Hungary and Portugal, Austria and Ireland, Denmark and Switzerland, the Netherlands and Belgium plus Luxemburg, Ukraine in the Crimean Peninsula, Sicily plus Malta, Sardinia and the European part of Turkey, with Cyprus, Crete and Corsica. If we compare the number of regions with a similar area size or the same number of taxa, we can use these values as direct measurements for the density of endemics. The density of endemics in a particular region is also higher than in another one if the region is smaller and the number of endemics higher; we can thus make direct comparisons for very many pairs of regions. To estimate distribution patterns and altitudinal ranges we calculated absolute numbers and median values.

We then compared, qualitatively and semi-quantitatively, ecological conditions and distribution patterns for freshwater and wetland endemics in Europe with numbers of taxa associated with other habitats.

RESULTS AND DISCUSSION

The database EvaplantE comprises at least 339 plant taxa – species and subspecies – which occur more or less regularly in wetland communities (appendix). All of these are restricted to the boundaries of Europe, as defined in Fontain et al. (2007). This number is a minimum value because more than 20% of the listed taxa in EvaplantE have not yet been characterized in relation to ecological conditions and habitat.

However, some 103 taxa on the list are more or less closely associated with wetlands. This means that they do not normally occur in other habitat types such as rocky habitats, grasslands, scrub or heath communities, forests, saline habitats, ruderal places, urban habitats or arable land. Only few of them are hydrophytes living in standing or running waters. The other 236 taxa occur in both wetlands and other habitats. Some (c. 80) live secondarily in wetlands and primarily in other terrestrial habitats (appendix and Tab. 1).

Table number 1 illustrates the range of wet habitat types with examples of endemic vascular plants in different parts of Europe. The lists in the appendix and in the table number 1 show that hydrophytes associated with shallow standing or running waters are a minority. Most plant taxa are hemicryptophytes with a more or less strong affinity to wet conditions but not absolutely connected to open water bodies.

The largest systematic group is the family Asteraceae with 49 endemic taxa. This family is one of the two largest families in the world. The other is Orchidaceae, which has its main distribution in the tropics and subtropics; Asteraceae species occur mainly in the subtropical and temperate zones. Both families are characterized by a large proportion of wind-dispersed and insect-pollinated taxa. Further large families are Rosaceae (22), Brassicaceae (21), Scrophulariaceae (20), Apiaceae (19), Ranunculaceae (18), Poaceae (17), Cyperaceae (16), Caryophyllaceae (14), Orchidaceae (13), Liliaceae (12). All other families comprise only 10 or fewer endemic taxa.

Figure number 1 shows distribution patterns of endemic vascular plants associated with freshwater habitats s. l., figure number 2 shows patterns of endemics associated with mires s. l; the numbers in the south-west of Europe are higher than in the north-east. For taxa associated with freshwater habitats, s. l. the numbers in a triangle-shaped region between Spain, former Yugoslavia and Germany are much higher than in the rest of Europe. The highest absolute numbers of taxa associated with freshwater habitats s. l. were found in France and Spain. Austria, Italy and Germany also have high numbers (Fig. 1). The highest numbers of taxa associated with mires s. l. were found for France, Germany, Spain and Italy. Austria, former Yugoslavia and Great Britain all have the same number of taxa (Fig. 2). The numbers for freshwater-associated taxa are normally higher than for mires (Fig. 2). Some Atlantic, Baltic and northern regions such as the Faroe Islands, Azores, Ireland, the Baltic countries, Finland and northern Russia present similar values for both groups.

The comparison of regions with a similar area or number of taxa or with different area sizes but obviously higher densities shows that the concentration of endemism increases in most cases from the north-east to the south-west, but also from the Atlantic islands in the west to a region comprising France and neighboring countries, with the highest values for freshwater-related taxa s. l. more to the SE of Europe and for mires-associated taxa s. l. more to the temperate and Atlantic zone of the mainland in West Europe.

Table 1: Examples of European vascular plants which occur in wetlands.

Habitat types	Examples of endemic vascular plants (area within Europe)
Shallow standing or slow running waters, ponds, ditches, lake margins	*Apium inundatum* (Central and W Europe), *Baldellia alpestris* (Iberian Peninsula), *Callitriche brutia* (S, W and NW Europe), *Callitriche hamulata* (W, Central and N Europe), *Isoetes boryana* (France), *Isoetes setacea* (France, Spain, Portugal), *Ranunculus hederaceus* (W Europe), *Ranunculus revelieri* (Corsica, Sardinia, mainland France)
More or less rapid flowing waters, e.g. small rivers or irrigation channels	*Isoetes longissima* (Spain), *Isoetes malinverniana* (Italy), *Ranunculus fluitans* (many regions in Europe)
Banks of tidal rivers	*Angelica heterocarpa* (France), *Oenanthe conioides* (Germany), *Deschampsia wibeliana* (Germany)
Seasonally flooded or wet places, including seasonal pools, wet river banks, lake shores, sands or rocky ground	*Agropyron tanaiticum* (Central and E Europe), *Allium schmitzii* (Portugal), *Angelica razulii* (Iberian Peninsula), *Arenaria gothica* (Sweden, Switzerland?), *Centaurea margaritacea protogerberi* (E Europe), *Cyperus cyprius* (Cyprus), *Goniolimon graminifolium* (SW of former USSR), *Scrophularia trifoliata* (Corsica, mainland Italy, Sardegna), *Thorella verticillato-inundata* (France, Spain, Portugal), *Tragopogon floccosus* (Central Europe), *Verbascum banaticum* (E and SE Europe)
Seasonally inundated, wet or moist meadows and pastures	*Achillea asplenifolia* (Central Europe), *Alchemilla coriacea* (Alps and Iberian Peninsula), *Anthericum baeticum* (Spain), *Armeria arcuata* (Portugal), *Asparagus pseudoscaber* (Central Europe)
Moors, bogs, fens, swamps, marshes	*Allium suaveolens* (Central and S Europe), *Armoracia macrocarpa* (Danube Basin), *Aristolochia rotunda insularis* (Mediterranean), *Calamagrostis scotica* (Great Britain), *Dactylorhiza incarnata pulchella* (Ireland, Great Britain), *Dactylorhiza pseudocortigera* (Norway, Sweden), *Dactylorhiza sphagnicola* (W Europe), *Tofieldia calyculata* (many regions of Europe)
Shaded rocks and slopes near streams, humid and wet places close to springs, waterfalls or crater lakes, damp shady places and watercourses in woods, along orchards or on banks, woodland along rivers	*Arabis kennedyae* (Cyprus), *Carex lowei* (Madeira), *Centaurium microcalyx* (Iberian Peninsula), *Chrysosplenium oppositifolium* (S and W Europe), *Dryopteris aitoniana* and *D. maderensis* (Madeira), *Malus praecox* (Central and E Europe), *Syringa josikaea* (Carpathian mountains), *Soldanella pindicola* (Greece), *Trichomanes speciosum* (S and W Europe), *Veronica dabneyi* (Azores), *Sibthorpia peregrina* (Madeira, Portugal mainland), *Vaccinium padifolium* (Madeira)

For 203 of the 339 taxa in EvaplantE we obtained values for the altitudinal range. These taxa occur at all altitudes between sea level and the alpine zones. The average of the minima (median) is 300 m a.s.l., the average of the maxima 1,800 m a.s.l. This means that most endemics occur in the montane and not in the alpine zones (cf. Körner, 2002). *Gentiana bavarica* is found in damp places, e.g. moors with spring waters, wet alpine meadows, and snow-patches. This species represents the absolute maximum of 3,600 m a.s.l. in our list.

47 endemics are basiphytes, 39 acidophytes. Many species are indifferent to pH or not yet characterized.

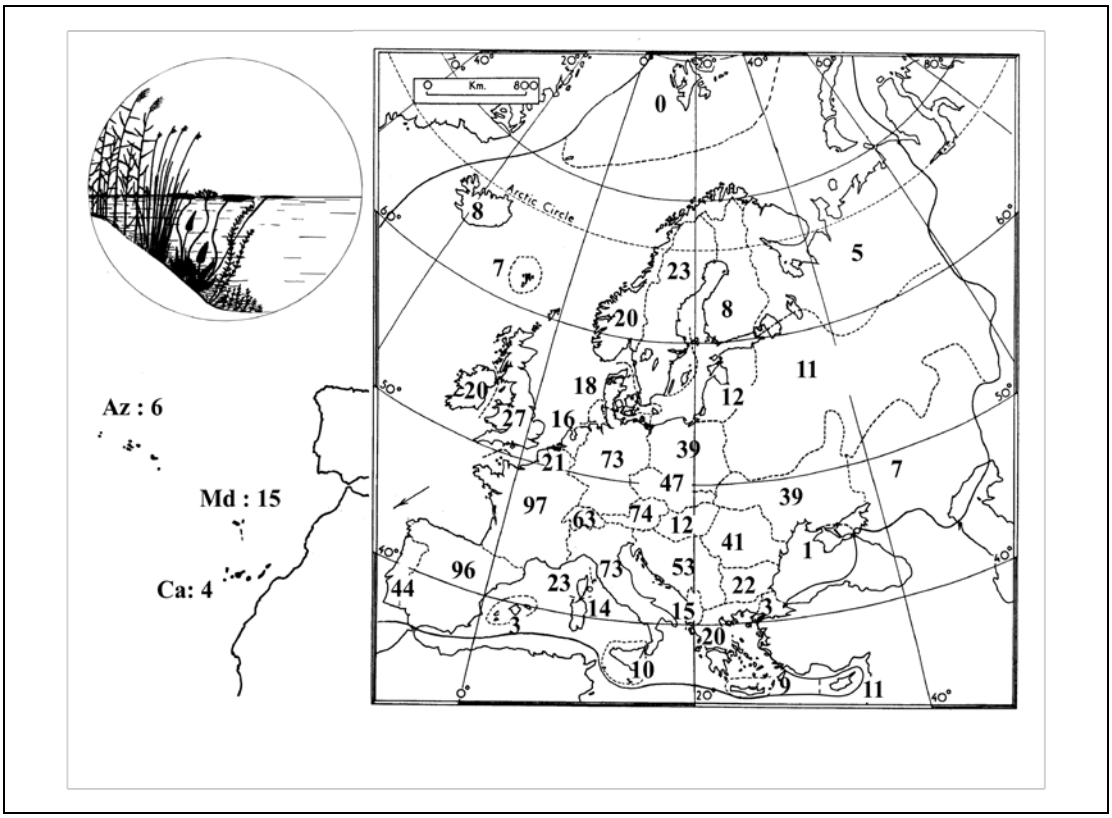


Figure 1: Numbers of European endemic vascular plants
which are associated with freshwater habitats s. l. in different regions of Europe (see text).

Our database is still incomplete. Approximately 20% of the taxa in EvaplantE are not characterized with respect to ecological conditions or habitat type. This means that the number of wetland endemics which we present here is a minimum number. We do not yet have all minimum and maximum for the altitudinal ranges, and we also assume that further information will alter our knowledge of the geographical distribution in some cases. Furthermore, molecular-genetic analyses have caused many systematic-taxonomical corrections in recent years. On the other hand, we do not have in present any indications or information from the scientific literature that could contradict the described tendencies.

The number of endemics in wetlands (339) is relatively low compared to endemics of rocky habitats and screes (2,756), grasslands (1,293), scrubs and heaths (1,107), or forests (761) (Bruchmann and Hobohm, 2010). The high endemism is generally related to the size of the respective area, age of the geological surface, ecological continuity, habitat diversity and richness of the species pool. Other parameters such as light, warmth or low productivity has also been discussed as factors promoting the richness of endemics in tropical rainforests, Mediterranean regions and high mountain zones (Jansson, 2003; Körner, 2002; Gaston, 1996; Huston, 1994; Latham and Recklefs, 1993). The separation-isolation factor both promotes and limits endemism. Genetic isolation is the precondition for speciation, and geographical separation promotes genetic isolation. On the other hand,

the richness of the species pool is also a precondition for speciation processes. The more the species in a region the more opportunities for speciation process. Distance and boundaries reduce dispersal opportunities, with the effect that islands at a far distant from the continents have relatively small numbers of species (cf. Kreft and Jetz, 2007; Kreft et al., 2007; Hobohm, 2000).

We assume that the relatively small number of endemics of wet habitats compared to other habitat types in Europe is due to the small total area size of the relating habitats, to the separation in combination with the young age of most habitats and low ecological continuity during different climate periods within the late Pleistocene and Holocene (Pott, 2010; Hobohm and Bruchmann, 2009).

The highest numbers, in absolute terms, of vascular plant endemics with an affinity to wet habitats (both groups) are found in France, with high concentrations of endemics in France and/or neighboring countries. We assume that this fact reflects a combination of conditions which favor endemism in general but especially in wet habitats. The area size of mainland France is much larger than e.g. Great Britain, Belgium plus Luxembourg, the Netherlands or Denmark. France is located within and between two high mountain ranges, the Pyrenees and the Alps. The country is also located between two marine environments which influence and stabilize climate. Therefore, three major climate regimes occur in France: Mediterranean, Atlantic and high-mountain climate. The mainland of France is connected with other species-rich regions – e.g. Iberian Peninsula, the Alps, Italy and some of the relict areas of glaciations periods are either part of the country or not far away.

All these factors together might explain the high numbers of European endemic vascular plant taxa associated with wet habitats in France and neighboring countries (Rull, 2004; Rosenzweig, 1995). However, this cannot easily be verified at the moment.

CONCLUSIONS

The distribution patterns of endemic vascular plant taxa in Europe differ depending on the habitat group to which they belong. Endemics associated with wetlands occur all over Europe with higher numbers to the west and south-west and at altitudes between sea level and 3,600 m a.s.l. As compared to the rocky habitats, grassland and forest, the wet habitats harbor a relatively small number of endemics.

The absolute number of endemics can be used as a measure for the responsibility in the framework of the international nature conservation policies.

ACKNOWEDGEMENTS

We are thankful to Linda Froome-Döhring for improving the English.

APPENDIX

Preliminary list of plants which are endemic to Europe and associated with wet habitats (according to the recent version of EvaplantE, December 2010).

Achillea asplenifolia
Achillea oxyloba
Aconitum napellus
Aconitum variegatum variegatum
Agropyron tanaiticum
Alchemilla anisiaca
Alchemilla coriacea
Alchemilla decumbens
Alchemilla demissa
Alchemilla filicaulis
Alchemilla fissa
Alchemilla incisa
Alchemilla inconcinna
Alchemilla pallens
Alchemilla pseudincisa
Alchemilla pyrenaica
Alchemilla reniformis
Alchemilla straminea
Alchemilla tenuis
Alchemilla undulata
Alchemilla versipila
Alchemilla xantho-chlora
Allium pendulinum
Allium schmitzii
Allium scorzoneri-folium
Allium suaveolens
Alyssum wulfenianum
Angelica heterocarpa
Angelica razulii
Anthericum baeticum
Apium inundatum
Arabis kennedyae
Arabis soyeri
Arenaria gothica
Aristolochia rotunda insularis
Armeria arcuata
Armeria maritima purpurea
Armoracia macrocarpa
Asparagus pseudoscaber
Atractylis cancellata gaditana
Baldellia alpestris
Bellis bernardii
Bellium bellidioides
Betula pubescens celtiberica
Brachypodium firmifolium
Brassica glabrescens
Buphthalmum salicifolium
Calamagrostis purpurea pseudopurpurea
Calamagrostis scotica
Callitriche brutia
Callitriche hamulata
Callitriche lusitanica
Callitriche platycarpa
Callitriche truncata
Calycocorsus stipitatus
Campanula herminii
Campanula pulla
Cardamine asarifolia
Cardamine pratensis crassifolia
Cardamine pratensis granulosa
Cardamine pratensis rivularis
Cardamine raphanifolia raphanifolia
Cardaria navasii
Carduus crispus multiflorus
Carduus personata
Carduus platypus
Carex arenaria
Carex bergrothii
Carex camposii
Carex cretica
Carex durieui
Carex frigida
Carex fuliginosa fuliginosa
Carex jemtlandica
Carex lainzii
Carex lowei
Carex nevadensis
Carex pulicaris
Carex randalpina
Carex serotina pulchella
Carex trinervis
Carum verticillatum

Centaurea arenaria sophiae
Centaurea macroptilon
Centaurea margaritacea appendiculata
Centaurea margaritacea donetzica
Centaurea margaritacea konkae
Centaurea margaritacea paczoskii
Centaurea margaritacea protogerberi
Centaurea rhenana savranica
Centaurium microcalyx
Cephalaria litvinovii
Cephalorhynchus cyprius
Cerastium azoricum
Cerastium brachypetalum doerfleri
Cerastium fontanum lucorum
Ceterach lolegnamense
Chaerophyllum ele-gans
Chondrilla chondrilloides
Chrysosplenium alpinum
Chrysosplenium oppositifolium
Cirsium bourgaeanum
Cirsium brachycephalum
Cirsium creticum triumfetti
Cirsium dissectum
Cirsium glabrum
Cirsium rivulare
Cirsium spinosissimum
Cirsium tymphaeum
Cirsium waldsteinii
Cochlearia glastifolia
Cochlearia officinalis pyrenaica
Coronilla globosa
Cymbalaria hepaticifolia
Cyperus cyprius
Cytisus multiflorus
Daboecia azorica
Dactylorhiza cordigera siculorum
Dactylorhiza incarnata coccinea
Dactylorhiza incarnata pulchella
Dactylorhiza maculata islandica
Dactylorhiza maculata schurii
Dactylorhiza majalis alpestris
Dactylorhiza majalis occidentalis
Dactylorhiza majalis praetermissa
Dactylorhiza majalis purpurella
Dactylorhiza pseudocordigera
Dactylorhiza sphagnicola
Dactylorhiza traunsteineri lapponica
Deschampsia littoralis
Deschampsia wibeliana
Diphasiastrum madeirense
Doronicum cataractarum
Dryopteris aitoniana
Dryopteris maderensis
Ebingeria elegans
Elymus alaskanus scandicus
Elymus alaskanus subalpinus
Epilobium alsinifolium
Epilobium fleischeri
Epilobium nutans
Erica tetralix
Erucastrum palustre
Eryngium viviparum
Erysimum creticum
Euphorbia uliginosa
Euphrasia calida
Euphrasia scottica
Festuca arundinacea uechtriziana
Festuca nitida
Festuca rivularis
Festuca rubra thessalica
Fraxinus pallisiae
Gagea julia
Galanthus nivalis
Galeopsis pyrenaica
Galium viridiflorum
Genista berberidea
Gentiana bavarica
Gentiana clusii
Gentianella ramosa
Geranium palmatum
Geranium rubescens
Geum rhodopeum
Goniolimon graminifolium
Gratiola linifolia
Gypsophila repens
Hemerocallis lilioasphodelus
Herniaria ciliolata
Hierochloe hirta hirta
Hierochloe odorata baltica
Huperzia dentata
Hypericum elodes
Inula helvetica
Iris spuria spuria
Isoetes azorica
Isoetes boryana
Isoetes heldreichii

Isoetes longissima
Isoetes malinverniana
Isoetes setacea
Isoetes velata asturicense
Isoetes velata tenuissima
Jasonia tuberosa
Juncus jacquinii
Juncus requienii
Juncus thomasii
Knautia godetii
Lathyrus neurolobus
Lathyrus palustris nudicaulis
Leontodon berinii
Leontodon pyrenaicus
Leucojum vernum
Limosella tenella
Lonicera nigra
Luzula sylvatica henriquesii
Lysimachia ephemerum
Malcolmia graeca
Malus praecox
Marsilea azorica
Melanoselinum decipiens
Mentha longifolia cypria
Meum athamanticum
Myosotis gallica
Myosotis lamottiana
Myosotis rehsteineri
Najas mircrocarpa
Narcissus cyclamineus
Narcissus jonquilla
Narcissus longispathus
Narthecium ossifragum
Narthecium reverchonii
Odontites kaliformis
Oenanthe conioides
Oenanthe divaricata
Oenanthe fluviatilis
Oenanthe lisae
Oenanthe tenuifolia
Oenothera ammophila
Origanum cordifolium
Oxytropis triflora
Papaver laestadianum
Papaver sendtneri agg.
Paradisea lusitanica
Pastinaca latifolia
Pedicularis foliosa
Pedicularis limnogena
Pedicularis pyrenaica
Pedicularis recutita
Pedicularis sylvatica hibernica
Pedicularis sylvatica sylvatica
Petagnia saniculifolia
Petasites kablikianus
Petasites paradoxus
Peucedanum aegopodioides
Peucedanum gallicum
Peucedanum lancifolium
Phoenix theophrasti
Pilularia globulifera
Pilularia minuta
Pinguicula grandiflora
Pinguicula leptoceras
Pinguicula nevadensis
Plagius flosculosus
Poa cenisia cenisia
Poa cenisia sardoa
Polygala amara
Polygala amarella
Primula clusiana
Primula deorum
Primula farinosa exigua
Pseudorchis albida albida
Ranunculus aconitifolius
Ranunculus barceloi
Ranunculus cordiger
Ranunculus flammula minimus
Ranunculus flammula scoticus
Ranunculus fluitans
Ranunculus hederaceus
Ranunculus kykkoensis
Ranunculus longipes
Ranunculus montanus
Ranunculus ololeucos
Ranunculus platanifolius
Ranunculus revelieri
Ranunculus wilanderi
Rheum rhaponticum
Rhododendron ponticum baeticum

Romulea revelierei
Rosa chionistrae
Rumex balcanicus
Sagina pilifera
Sagina saginoides nevadensis
Salix apennina
Salix bicolor
Salix cantabrica
Salix daphnoides
Salix glabra
Salix mielichhoferi
Salix repens
Salix salviifolia
Salix silesiaca
Sanguisorba dodecandra
Sanguisorba laterifolia
Saponaria cypria
Saponaria ocymoides
Saussurea alpina esthonica
Saussurea alpina macrophylla
Saussurea porcii
Saxifraga aquatica
Saxifraga clusii
Saxifraga hostii
Saxifraga hypnoides
Saxifraga mutata
Saxifraga oppositifolia amphibia
Saxifraga spathularis
Saxifraga umbrosa
Scorzonera fistulosa
Scrophularia alpestris
Scrophularia hirta
Scrophularia racemosa
Scrophularia trifoliata
Scutellaria minor
Securinega tinctoria
Sedum aetnense
Senecio doria legionensis
Senecio subalpinus
Sibthorpia peregrina
Silene asterias
Silene laconica
Silene pusilla
Silene saxifraga
Sisymbrella aspera aspera
Sisymbrella aspera praeterita
Sisymbrium supinum
Solanum patens
Soldanella pindicola
Symphytum officinale uliginosum
Syringa josikaea
Taraxacum fontanum group
Taraxacum schroeteranum
Thalictrum morisonii
Thalictrum speciosisimum
Thlaspi cepaeifolium cepaeifolium
Thlaspi cyprium
Thorella verticillato-inundata
Tofieldia calyculata
Tolpis azorica
Tragopogon brevirostris bjelorussicus
Tragopogon brevirostris longifolius
Tragopogon floccosus
Trichomanes speciosum
Trifolium saxatile
Trisetum fuscum
Vaccinium padifolium
Verbascum banaticum
Veronica dabneyi
Veronica repens
Viola cretica

REFERENCES

1. Bruchmann I., 2011 – Plant endemism in Europe: Spatial distribution and habitat affinities of endemic vascular plants, PhD-Thesis, University of Flensburg, Germany.
2. Bruchmann I. and Hobohm C., 2010 – Halting the loss of biodiversity: Endemic vascular plants in grassland of Europe, Grassland Science in Europe, 15, 776-778.
3. Candolle A. and De P., 1820 – Essai élémentaire de géographie botanique, in Dictionnaire des sciences naturelles 18, Flevrault, Strasbourg, 1-64.
4. Davis S. D., Heywood V. H. and Hamilton A. C. (eds) 1995 – Centres of Plant Diversity Vol. 2: Asia, Australasia and the Pacific. – IUCN Publications, Unit, Cambridge.
5. Davis S. D., Heywood V. H., Herrera-MacBryde O., Villa-Lobos J. and Hamilton, A. C. (eds) 1997 – Centres of Plant Diversity: Vol. 3, The Americas. IUCN Publications, Unit, Cambridge.
6. Davis G. W. and Richardson D. M. (eds) 1995 – Mediterranean-Type Ecosystems. Ecol. Studies 109, Berlin, Heidelberg, New York.
7. European Commission DG Environment 2007 – Interpretation manual of European Union habitats, EUR 27, 142 S.
8. Fontaine B., Bouchet P., Van Achterberg K., Alonso-Zarazaga M. A., Araujo R., Asche M., Aspock U., Audisio P., Aukema B., Bailly N., Balsamo M., Bank R. A., Barnard P., Belfiore C., Bogdanowicz W., Bongers T., Boxshall G., Burckhardt D., Camicas J. L., Chylarecki P., Crucitti P., Davarveng L., Dubois A., Enghoff H., Faubel A., Fochetti R., Gargominy O., Gibson D., Gibson R., Gomez Lopez M. S., Goujet D., Harvey M. S., Heller K.-G., Van Helsdingen P., Hoch H., De Jong H., De Jong Y., Karsholt O., Los W., Lundqvist L., Magowski W., Manconi R., Martens J., Massard J. A., Massard-Geimer G., Mcinnes S. J., Mendes L. F., Mey E., Michelsen V., Minelli A., Nielsen C., Nieto Nafria J. M., Van Nieukerken E. J., Noyes J., Papa T., Ohl H., De Prins W., Ramos M., Ricci C., Roselaar C., Rota E., Schmidt-Rhaesa A., Segers H., Zur Strassen R., Szeptycki A., Thibaud J.-M., Thomas A., Timm T., Van Tol J., Vervoort W. and Willmann R. 2007 – The European union`s 2010 target: Putting rare species in focus. Biol. Cons. 139, 167-185.
9. Gaston K. J. (ed.) 1996 – Biodiversity: A biology of numbers and difference. Blackwell Science, Oxford.
10. Hobohm C. 2008a – Ökologie und Verbreitung endemischer Gefäßpflanzen in Europa. Tuexenia 28, 7-22.
11. Hobohm C. 2008b – Gibt es endemische Gefäßpflanzen in Mooren Europas? Mitt. AG Geobot. in Schl.-Holst. und Hambg. 65, 143-150.
12. Hobohm, 2000 – Plant species diversity and endemism on islands and archipelagos, with special reference to the Macaronesian Islands. Flora 195, 9-24.
13. Huston M. A. 1994 – Biological diversity. Cambridge University Press, Cambridge.
14. Jansson R. 2003 – Global patterns in endemism explained by past climatic change. Proc. R. Soc. Lond. B. 270, 583-590.
15. Körner C. 2002 – Mountain biodiversity, its causes and function: an overview. In: Körner, C. and Spehn, E. M. (eds) – Mountain biodiversity: A global assessment. Parthenion Publishing, Boca Raton et al., 3-20.
16. Körner C. 2000 – Why are there global gradients in species richness? Mountains might hold the answer. Trends Ecol. Evol. 15/12, 513 f.
17. Kreft H. and Jetz W., 2007 – Global patterns and determinants of vascular plant diversity. PNAS 104: 5952-5930.
18. Kreft H., Jetz W., Mutke J., Kier G. and Barthlott W. 2008 – Global diversity of island floras from a macroecological perspective. Ecology letters 2008/11, 116-127.
19. Latham R. E. and Ricklefs R. E. 1993 – Global patterns of tree species richness in moist forests: energy-diversity theory does not account for variation in species richness. Oikos 67, 325-333.

20. McGinley M. 2008 – Nile Delta flooded savanna. In: Encyclopedia of Earth Cleveland C. J. (ed.) Url: http://www.eoearth.org/article/Nile_Delta_flooded_savanna (downloaded 5/7/2010).
21. McGlone M. S., Duncan R. P. and Heenan P. B. 2001 – Endemism, species selection and the origin and distribution of the vascular plant flora of New Zealand. Journal of Biogeography 28, 199-216.
22. Mittermeier R. A., Gil P. R., Hoffman M., Pilgrim J., Brooks T., Mittermeier C. G., Lamoreux J., da Fonseconda G. A. B., 2005 – Hotspots revisited: Earth`s biologically richest and most endangered terrestrial ecoregions. Cemex, Mexico City.
23. Noroozi J., Akhani H. and Breckle S.-W. 2008 – Biodiversity and phytogeography of the alpine flora of Iran. Biodiversity and Conservation 17, 493-521.
24. Pott R. 2010 – Klimawandel im System Erde. Berichte der RTG 22, 7-33.
25. Rabitsch W. and Essl, F. (eds) 2009 – Endemiten – Kostbarkeiten in Österreichs Pflanzen- und Tierwelt. Umweltbundesamt, Klagenfurth, Wien.
26. Rikli M. 1946 – Das Pflanzenkleid der Mittelmeerländer 2. Hans Huber, Bern.
27. Rikli M. 1943 – Das Pflanzenkleid der Mittelmeerländer 1. Hans Huber, Bern.
28. Rosenzweig M. L. 1995 – Species diversity in space and time. Cambridge University Press, Cambridge.
29. Rull V. 2004 – Biogeography of the `Lost World`: a palaeoecological perspective. Earth-Science Reviews 67, 125-137.
30. Tutin T. G. et al. (eds) 1996 – Flora Europaea Volumes 1-5. 2. editions or reprints, 581 + 469 + 385 + 505 + 452 pp., Cambridge University Press, Cambridge.

THE COENOLOGY AND CHOROLOGY OF VEGETATION FROM THE ORDER MAGNOCARICETALIA PIGNATTI 1953 IN SIBIU COUNTY (TRANSYLVANIA, ROMANIA)

Constantin DRĂGULESCU *

* "Lucian Blaga" University of Sibiu, Faculty of Sciences, Department of Ecology and Environmental Protection, Dr. Ion Raţiu Street 5-7, Sibiu, Sibiu County, Romania, RO-550012, ctindrg@yahoo.com

KEYWORDS: Magnocaricetalia associations; chorology; flower structure.

ABSTRACT

This paper is a part of a series dealing with the aquatic and paludal vegetation of Sibiu County (C. Drăgulescu, 2007, 2010). The author identifies thirteen plant associations and two sub-associations of the Order Magnocaricetalia in Sibiu County. The flower structure of the respective plant communities is found in the 73 phytocoenological samples given in tables 1 and 2. Half of these (37) were executed by the author in the years 1973-2010. After each place name where phytocoenoses of the Order are indicated, the bibliography shows that the author observed the respective coenoses (and the sign "!" is inserted), and a note is made whether there are samples in the respective place (mentioned as "smp"). The plant associations of the Order Magnocaricetalia comprise a small proportion (ca 0.1%) of the natural and semi-natural vegetation of Sibiu County, since the lakes, ponds and bogs are scattered far and wide and occupy small areas. The largest phytocoenoses of the Order lie in the Olt River meadows, between the villages of Racoviţa and Arpaşu de Jos; on the upper Sebeş River (Valea Frumoasei); on the upper Hârtibaci River (Rora Coveşului, Rora Bârghişului); and in the Ruscior meadow near Sibiu.

ZUSAMMENFASSUNG: Zönologie und Chorologie der Vegetation der Ordnung Magnocaricetalia Pignatti 1953 im Kreis Sibiu/Hermannstadt (Transylvanien, Rumänien).

Die Arbeit setzt die Reihe der Mitteilungen betreffend die Wasser- und Sumpfvegetation des Kreises Sibiu/Hermannstadt fort (C. Drăgulescu, 2007, 2010). Dabei geht es um 13 Assoziationen und zwei Subassotziationen der Ordnung Magnocaricetalia, die im Kreis Sibiu/Hermannstadt festgestellt wurden. Die floristische Zusammensetzung dieser Gruppierungen ist in den 73 phytozönologischen Bestandaufnahmen der tabellen 1 und 2 wiedergegeben. Die Hälfte dieser Aufnahmen (genauer 37) wurden vom Verfasser in den Jahren 1973-2010 erhoben. Nach jedem Ort, an dem Phytozönosen dieser Ordnung festgestellt wurden, sind Literaturhinweise angegeben, außerdem wird mit Ausrufezeichen vermerkt (!), wenn der Verfasser die Vegetationseinheiten selbst gesehen hat und ebenfalls ist festgehalten, ob es Bestandaufnahmen vom jeweiligen Fundort gibt (vermerkt mit "smp"/rel.). Die Gesellschaften der Ordnung Magnocaricetalia haben einen geringeren Anteil an der Gesamtheit der natürlichen und naturnahen Vegetation des Kreises Sibiu (cca. 0,1%), was sich aus den wenigen vorhandenen Seen, Tümpeln Sümpfen sowei ihrer geringen Ausdehnung ergibt. Die flächig größten Phytozönosen dieser Ordnung finden sich in der Aue des Olt-Flusses zwischen Racoviţa und Arpaşu de Jos, am Oberlauf des Sebeş-Flusses (Frumoasa-Tal) und des Harbachs/Hârtibaciu in den "Rora" genannten Gebieten von Coveş und Bârghiş sowie in der Aue des Ruşcior/Reussbachs neben Sibiu/Hermannstadt.

REZUMAT: Cenologia şi corologia vegetaţiei ordinului Magnocaricetalia Pignatti 1953 în judeţul Sibiu (Transilvania, România).

Lucrarea continuă seria comunicărilor referitoare la vegetaţia acvatică şi palustră a judeţului Sibiu (C. Drăgulescu, 2007, 2010). Autorul notează 13 asociaţii vegetale şi două subasociaţii din ordinul Magnocaricetalia identificate în judeţul Sibiu. Structura floristică a acestor grupări vegetale este redadă în cele 73 de relevee fitocenologice din tabelele 1 şi 2. Jumătate dintre acestea (mai exact 37) au fost efecuate de autor în peroada 1973-2010. După fiecare localitate unde s-au semnalat fitocenoze ale acestui ordin este indicată bibliografia, faptul că autorul a văzut cenozele respective (indicat cu semnul !) şi dacă există relevee efectuate în localitate respectivă (precizându-se cu "smp"/rel.). Grupările vegetale din ordinul Magnocaricetalia au o pondere mică în ansamblul vegetaţiei naturale şi seminaturale a judeţului Sibiu (cca. 0,1%), consecinţă a faptului că sunt puţine lacuri, bălţi şi mlaştini şi reduse ca suprafaţă. Cele mai întinse fitocenoze din acest ordin se află în lunca Oltului, între Racoviţa şi Arpaşu de Jos, pe cursul superior al Sebeşului (Valea Frumoasei), pe cursul superior al Hârtibaciului (Rora Coveşului, Rora Bârghişului) şi în Lunca Ruşciorului lângă Sibiu.

INTRODUCTION

The studied plant associations of the Order Magnocaricetalia make up the sequence following those of the Order Phragmitetalia in the process of colonising ponds and lakes. The typical species here are as follows: *Carex rostrata, Carex acutiformis, Carex appropinquata, Carex acuta (C. gracilis), Carex buxbaumi, Carex riparia, Carex vesicaria, Carex vulpina, Carex melanostachya, Peucedanum palustre, Poa palustris* and other species.

RESULTS AND DISCUSSION

The floristic structure of the respective plant communities is found in the 73 phytocoenologic samplings given in tables 1 and 2. Half of these (37) were executed by the author in the years 1973-2010. After each place's name, where phytocoenoses of the Order are indicated, bibliography shows that the author saw the respective coenoses (and the sign "!" is inserted), and a note is made whether there are samplings in the respective place (mentioned as "smp"). The plant associations of the Order Magnocaricetalia give a small quota (ca 0.1%) among the natural and semi-natural vegetation of Sibiu County, since the lakes, ponds and bogs come far and wide and have small areas. The largest phytocoenoses of the Order lie in the Olt River meadow, in between villages Racoviţa and Arpaşu de Jos; on the upper Sebeş River (Valea Frumoasei); on the upper Hârtibaci River (Rora Coveşului, Rora Bârghişului); and in the Ruscior meadow near Sibiu. Thirteen plant associations have been identified, along with two sub-associations, namely:

Magnocaricetalia elatae Pignati 1953

Magnocaricion elatae Koch 1926

Caricenion rostratae (Bálátová-Tuláčková 1963) Oberd. et al., 1967 (syn. Caricenion appropinquatae Bálátová-Tuláčková 1960)

Caricetum aproppinquatae Aszód 1936 (syn. Caricetum paradoxae Soó 1938): Rora Bârghişului along the Valea Lungă, within the limits of the village Pelişor (! with smp.).

Caricetum elatae Koch 1926 (syn. Scutellario-Caricetum elatae Passarge 1964): Crinţ (G. Anghel et al., 1965 under Carex elata as.).

Caricetum rostratae Rübel 1912: Arpaşu de Jos (I. Şerbănescu, 1963, 1964 with smp., C. Drăgulescu, 1999, !), Crinţ (G. Anghel and colab., 1965), Porumbacu de Jos (C. Drăgulescu, 1999); Lacul Doamnei (I. Resmeriţă and colab., 1977 reported C. rostrata-C. vesicaria as.).

Caricetum vesicariae Chouard 1924 (syn. Caricetum vesicariae Rübel 1933, Caricetum inflato-vesicariae Koch 1926): Boarta (! with smp.), Mohu-Veştem (C. Drăgulescu, 2004, !), Oaşa Mare la 1,270 m (A. Borza, 1959 with smp. under Caricetum inflatae-vesicariae, in which Carex vesicaria is probably present erroneously solely with +), Sibiu (!), between Sibiu and Şura Mică, on the right-hand side of the road to Ocna Sibiului (! with smp. and E. Schneider-Binder, 1974 with smp.), between Tălmaciu and Veştem (!), Turnişor (E. Schneider-Binder, 1974, 1976 with smp., E. Schneider-Binder, 1974, !).

Caricenion gracilis (Neuhäusl 1959) Oberd. et al. 1967

Caricetum acutiformis Eggler 1933: Dumbrăveni (C. Drăgulescu, 2005, !), Ţapu (C. Drăgulescu, 2005, !); (syn. Caricetum acutiformis-ripariae Soó (1938) 1947): Broşteni (C. Drăgulescu, 1974), Păuca (C. Drăgulescu, 1974, 1977 with smp., !); ***caricetosum melanostachyae***: Cristian (E. Schneider-Binder, 1974, 1976), Pâr. Strâmb-Lunca Ruşciorului (E. Schneider-Binder, 1970 with smp., E. Schneider-Binder, 1974, 1976), Veştem (E. Schneider-Binder, 1976); ***caricetosum ripariae***: Cornăţel (! with smp.), Cristian (E. Schneider-Binder, 1974, 1976), Mohu (E. Schneider-Binder, 1974, !), Pâr. Strâmb-Lunca Ruşciorului (E. Schneider-Binder, 1970 with smp., E. Schneider-Binder, 1974, 1976), Tălmaciu (C. Drăgulescu, 1995, ! with smp.), Turnişor (E. Schneider-Binder, 1974), Veştem (E. Schneider-Binder, 1974, 1976). More recently, the sub-association has been classified as ***Galio palustris-Caricetum ripariae*** Bálátová-Tuláčková et al. in Grabherr et Mucina 1993 (syn. Caricetum ripariae Soó 1928, Caricetum acutiformis-ripariae Soó 1938).

Caricetum gracilis Almquist 1929: Arpaşu de Jos (I. Şerbănescu, 1964 with smp., C. Drăgulescu, 1999), Avrig (C. Drăgulescu, 1999, !), Bârghiş (!), Cristian in Lunca Mare (E. Schneider-Binder, 1974 with smp.), Pelişor (!), Rora Bârghişului (!), Rora Coveşului (! with smp.), Scorei (I. Şerbănescu, 1964 with smp.), Sibiu in Şesul Măcelarilor and V. Rogojinii (E. Schneider-Binder, 1974, 1976 with smp., C. Drăgulescu, 2004, ! with smp.), between Sibiu and Şura Mică, Lunca Ruşciorului and Câmpul Rezului also Pâr. Strâmb (E. Schneider-Binder, 1970 with smp., E. Schneider-Binder, 1974, 1976 with smp., !), Şeica Mare (!), Turnişor (E. Schneider-Binder, 1974, 1976, !), Turnu Roşu (C. Drăgulescu, 1999, !), Veştem (C. Drăgulescu, 2004, !); Carex gracilis + Juncus inflexus: Movile (Şt. Csuros, A. Kovacs, 1962 with smp.).

Carici-Menyanthetum Arpaşu de Jos: (I. Şerbănescu, 1963 with smp., I. Şerbănescu, 1964 with smp.), Arpaşu de Sus at Lacul Tătarilor (!), Avrig (!), Vf. Ghihan, 1,350 m alt. (C. Drăgulescu, 1995 with smp., !).

Caricetum vulpinae Soó 1927: Cristian (E. Schneider-Binder, 1974, 1976, C. Drăgulescu, 2004, !), Lunca Ruşciorului (E. Schneider-Binder, 1976), Miercurea Sibiului (!), Turnişor (E. Schneider-Binder, 1974, 1976, C. Drăgulescu, 2004, !), Sibiu (E. Schneider-Binder, 1974, 1976, !) in Şesul Măcelarilor (! with smp.), Veştem (E. Schneider-Binder, 1974, 1976 with smp., C. Drăgulescu, 2004, !).

Carex buxbaumii as.: Arpaşu de Jos: (I. Şerbănescu, 1963, 1964 with synthetic smp. with Pojorta and Horezu, C. Drăgulescu, 1999).

Eleocharitetum palustris Ubrizsy 1948 (syn. Eleocharitetum palustris Schennikow 1919, Alismato-Eleocharitetum Mathe et Kovács 1967): Axente Sever (C. Drăgulescu, 2005, !), Avrig (C. Drăgulescu, 1999, !), Cantonul Rozdeşti (C. Drăgulescu, 1995 with smp., !), Copşa Mică (C. Drăgulescu, 2005, !), Cristian (!), Gura Râului (C. Drăgulescu, 2004, !), Mediaş (C. Drăgulescu, 2005, !), Miercurea Sibiului (!), Orlat (C. Drăgulescu, 2004, !), between Pârâul Ruşciori and Pârâul Strâmb (! with smp.), Porumbacu de Jos (C. Drăgulescu, 1999, !), Racoviţa (C. Drăgulescu, 1999, !), Râu Mare (C. Drăgulescu, 2004, !), Sadu (C. Drăgulescu, 1995 with smp., !), Tălmaciu (C. Drăgulescu, 1995 with smp., C. Drăgulescu, 2004, !), Tălmăcel (C. Drăgulescu, 1995, !), Târnăvioara (C. Drăgulescu, 2005, !), Turnişor (C. Drăgulescu, 2004, ! with smp.), Veştem (C. Drăgulescu, 2004, !).

The association is included by some phytocoenologists in the alliance Oenanthion aquaticae of the Order Oenanthetalia aquaticae.

Phalaridetum arundinaceae Libbert 1931 (syn. Poo palustris-Phalaridetum arundinaceae Passarge 1955): Brădeni-Netuş (Şt. Csuros, A. Kovacs, 1962, ! with smp.), Ocna Sibiului (C. Drăgulescu, 1994), Sibiu in proximity of ditch running parallel with the railway to Ocna Sibiului (!), upstream from Veştem near the confluence with the Hârtibaci River (! with smp.).

Other authors say the association falls into al. Phalarido-Glycerion Passarge 1964 or Phalaridion arundinaceae Kopeck 1961 of the Order Nasturti-Glyceretalia.

Poaetum palustris Resmeriţă et Raţiu 1974: Turnişor (! with smp.), Lunca Pâr. Ruşciori between Sibiu and Şura Mică (! with smp.).

Ranunculo flammulae-Gratioletum officinalis Borhidi et Juhász 1985: Racoviţa (C. Drăgulescu, 1999, !), Scorei (I. Şerbănescu, 1964 with smp., C. Drăgulescu, 1999), Tălmaciu-Şuvară (! with smp.), Tălmăcel "La Lac" (!), Turnu Roşu (C. Drăgulescu, 1999, !).

A. Borhidi and M. Juhász, who made a description of the association in Hungary, say that it falls into the Class Littorelletea, since the samplings executed show quite a lot of species in this class, as well as the class Isoëto-Nanojuncetea.

Part of the phytocoenoses is in Frumoasa Site of Community Interest. In order to prevent the loss, an interdiction to drain should be imposed, even more so as they preserve a number of rare species, as for instance *Carex appropinquata, Carex melanostachya, Peucedanum palustre, Iris sibirica, Orchis incarnata, Orchis laxiflora elegans, Menyanthes trifoliata, Potentilla palustris* and others.

Table 1a: Coenotaxons: Caricetum aproppinquatae (1-2), Caricetum rostratae (3-4), Caricetum vesicariae (5-8), Caricetum acutiformis (9), Caricetum acutiformis caricetosum ripariae (10-14), Caricetum acutiformis caricetosum melanostachyae (15), Caricetum gracilis (16-22).

Species/Sampling	1	2	3	4	5	6	7	8	9	10	11
Magnocaricetalia elatae											
Carex acutiformis	.	.	.	.	.	.	.	.	**3-4**	**+**	**+**
Carex ovalis	+	.	.	.	.	.	.	+	.	.	.
Carex riparia	.	.	.	.	.	.	+	.	+–2	**4**	**4**
Carex rostrata	2	1	**3**	**3**	.	.	.	.	.	.	.

Carex vesicaria	.	.	.	.	**4**	**3**	**2-4**	**3**	.	+	.
Carex vulpina	+	+	.	.	1	+	+–1	2	+–1	+	+
Carex appropinquata	**3**	**3**	.	.	.	.	.	.	.	.	.
Carex acuta	.	.	.	.	.	+	2	+	.	+	.
Carex melanostachya	.	.	.	.	.	.	1	.	.	.	.
Peucedanum palustre	+	+	.	+	.	.	.	.	.	.	.
Lysimachia vulgaris	.	.	.	.	.	.	+	+	.	.	.
Lythrum salicaria	+	1	.	.	.	.	.	+	.	.	+
Poa palustris	.	.	.	.	.	.	.	.	.	+	.
Valeriana officinalis	1	+	.	.	.	.	.	+	.	.	.
Myosotis caespitosa	.	.	.	.	.	.	.	.	.	.	.
Gratiola officinalis	.	.	.	.	.	+	+	.	.	.	+
Ranunculus flammula	.	.	.	.	.	.	+	.	.	.	.
Veronica scutellata	.	.	.	.	.	.	+	.	.	.	.
Equisetum fluviatile	.	.	.	.	.	.	.	.	.	.	.
Euphorbia palustris	.	.	.	.	.	.	+	.	.	.	.
Phragmitetalia											
Phragmites australis	.	.	1	.	.	.	+	.	.	.	+
Typha latifolia	.	.	.	.	.	.	.	.	.	.	.
Typha angustifolia	.	.	.	.	.	.	.	.	.	.	.
Glyceria maxima	.	.	.	.	.	.	+	.	.	.	.
Iris pseudacorus	.	.	.	.	.	+	1-2	.	.	+	.
Oenanthe aquatica	.	.	.	.	.	.	.	.	.	+	.
Eleocharis palustris	.	.	.	.	+	1	1	.	+	.	+

Berula erecta	+	.	.	.	.	.	.	.	.	.	.
Lycopus europaeus	.	.	.	.	.	+	.	+	+	.	+
Butomus umbellatus	.	.	.	.	.	.	.	.	.	.	.
Alisma plantago-aquatica	.	.	.	.	.	.	.	.	+	.	.
Equisetum palustre	.	.	.	+	.	.	.	2	+	.	+
Mentha aquatica	.	.	.	.	.	+	.	+	.	.	.
Galium palustre	+	+	.	+	+	1	+	+	.	.	+
Scutellaria galericulata	.	.	.	.	.	+	.	.	.	.	.
Nasturtio-Glycerietalia											
Glyceria fluitans	.	.	.	.	+		.	.	.	.	.
Glyceria notata	.	.	.	.	.	.	.	+	.	.	.
Mentha longifolia	+	.	.	.	.	.	.	+	.	.	.
Myosotis scorpioides	+	1	.	.	.	.	.	+	.	.	+
Scrophularia umbrosa	.	.	.	.	.	.	.	.	.	.	.
Veronica anagallis-aquatica	.	.	.	.	.	.	.	.	+	.	.
Veronica beccabunga	.	.	.	.	.	.	.	.	.	.	.
Myosoton aquaticum	+	.	.	.	.	.	.	.	.	.	.
Scheuchzerio-Caricetea fuscae											
Eriphorum latifolium	.	.	+	+	.	.	.	+	.	.	.
Carex lepidocarpa	.	+	+	+	.	.	.	.	.	.	.
Carex nigra	.	.	.	1	.	.	.	.	.	.	.
Carex echinata	+	.	.	1	.	.	.	.	.	.	.
Carex flava	.	+	.	.	.	.	.	.	.	.	.
Agrostis canina	.	.	.	+	.	.	.	.	.	.	.
Oxycocco-Sphagnetea											

Sphagnum spp.	.	.	.	2-3	.	.	.	.	.	.	.
Eriophorum vaginatum	.	.	.	1	.	.	.	.	.	.	.
Bidentetea											
Bidens tripartita	.	.	.	.	.	.	.	.	.	.	.
Polygonum hydropiper	.	+	.	.	.	.	.	.	.	.	+
Polygonum lapathifolium	.	.	.	.	.	.	.	.	.	.	.
Molinio-Arrhenatheretea											
Juncus conglomeratus	.	.	.	+	.	.	.	.	.	.	.
Juncus effusus	.	.	.	.	.	.	+	+	.	.	.
Juncus inflexus	.	.	.	.	.	.	.	.	.	.	.
Juncus subnodulosus	.	.	.	.	.	.	.	.	.	.	.
Ranunculus repens	.	.	.	.	.	.	+–2	+	.	.	+
Ranunculus acris	.	.	.	.	.	.	.	.	.	1	.
Lysimachia nummularia	+	1	.	.	+	.	+	.	.	+	+
Lychnis flos-cuculi	.	.	.	.	.	.	.	+	.	.	.
Symphytum officinale	.	.	.	.	.	+	+	.	.	+	+–1
Agrostis stolonifera	+	1	.	.	+	1	.	+	.	.	+
Rumex crispus	.	.	.	.	+	.	.	.	.	.	.
Potentilla reptans	.	.	.	.	.	.	.	.	.	.	+
Deschampsia caespitosa	.	.	.	.	+	1	.	+	.	.	.
Filipendula ulmaria	+	.	.	.	.	.	.	.	.	1	.
Potentilla anserina	.	.	.	.	.	.	.	.	.	.	.
Serratula tinctoria	.	.	.	.	.	.	.	.	.	+	.
Polygonum bistorta	.	.	.	.	.	+	.	.	.	.	.

Caltha palustris	+	.	.	.	.	.	.	.	.	.	.
Stellaria graminea	.	.	.	.	.	.	.	.	+	.	.
Trifolium hybridum	.	.	.	.	.	+	+	.	.	.	+
Trifolium repens	.	.	.	.	.	.	.	.	.	.	.
Thalictrum lucidum	+	+	.	.	.	+	.	.	.	.	.
Thalictrum simplex	.	.	.	.	.	.	.	.	.	.	.
Vicia cracca	.	.	.	.	.	.	.	.	.	.	.
Stachys officinalis	.	.	.	.	.	.	.	.	+	.	.
Holcus lanatus	.	.	.	.	.	.	.	.	.	.	.
Sanguisorba officinalis	.	.	.	.	.	+	.	.	.	+	.
Cirsium canum	.	.	.	.	.	1	.	+	.	+	.
Viola stagnina	.	.	.	.	.	+	.	.	.	.	.
Taraxacum officinale	.	.	.	.	.	.	.	.	.	.	.
Rhinanthus minor	.	.	.	.	.	.	.	.	.	.	.
Cardamine pratensis	.	.	.	.	.	.	.	.	.	+	.
Arrhenatherum elatius	.	.	.	.	.	.	.	.	.	.	.
Plantago lanceolata	.	.	.	.	.	.	.	.	.	.	.
Lathyrus pratensis	.	.	.	.	.	.	.	.	.	.	.
Alopecurus pratensis	.	.	.	.	+	.	.	.	.	+	.
Briza media	.	.	.	.	.	.	.	.	.	.	.
Festuca pratensis	.	.	.	.	.	.	.	.	.	.	.
Cynosurus cristatus	.	.	.	.	.	.	.	.	.	.	.
Succisa pratensis	.	.	.	.	.	.	.	.	.	.	.
Trifolium pratense	.	.	.	.	.	.	.	.	.	.	.
Galium boreale	.	.	.	.	.	+	.	.	.	.	.

Carex cespitosa	.	.	.	.	.	.	.	.	.	.	.
Cirsium rivulare	.	.	.	.	.	.	.	.	.	.	.
Iris sibirica	.	.	.	.	.	.	+	.	.	+	.
Sanguisorba officinalis	.	.	.	.	.	.	+	.	.	.	.
Allium angulosum	.	.	.	.	.	.	.	.	.	.	.
Salicetea purpureae											
Salix cinerea	+–1	.	.	.	.	.	.	+	.	.	.
Salix triandra	.	.	.	.	.	.	.	.	.	.	.
Salix fragilis	.	.	.	.	.	.	.	.	.	.	.
Salix alba	.	.	.	.	.	.	.	.	.	.	.
Salix purpurea	.	.	.	.	.	.	.	+	.	.	.
Galio-Urticetea											
Angelica sylvestris	+	.	.	.	.	.	.	.	.	.	.
Cirsium oleraceum	.	+	.	.	.	.	.	.	.	.	.
Miscellaneous											
Eupatorium cannabinum	.	.	.	.	.	.	.	+	.	.	.
Mentha arvensis	.	.	.	.	.	.	.	+	.	.	.
Galium uliginosum	.	.	.	.	.	.	.	.	.	.	.
Aster novi-belgii	.	.	.	.	.	.	.	.	.	.	.
Carex hirta	.	.	.	.	.	.	.	.	.	.	.
Rorippa sylvestris	.	.	.	.	.	.	.	.	.	.	.
Sonchus asper	.	.	.	.	.	.	.	.	.	.	.
Plantago major	.	.	.	.	.	.	.	.	.	.	.
Cirsium arvense	.	.	.	.	.	.	.	.	.	.	.
Vicia tetrasperma	.	.	.	.	.	.	.	.	.	.	.

Vicia hirsuta	.	.	.	.	.	.	.	.	.	.	.
Juncus compressus	.	.	.	.	.	.	.	.	.	.	.
Galium rubioides	.	.	.	.	.	.	.	.	.	.	.
Convolvulus arvensis	.	.	.	.	.	.	.	.	.	.	.
Polygonum minus	.	.	.	.	.	.	.	.	.	.	.
Helianthus decapetalus	.	.	.	.	.	.	.	.	.	.	.
Carex distans	.	.	.	.	.	.	.	+	.	.	.
Carex pallescens	.	.	.	+–1	.	.	.	.	.	.	.
Betula pubescens	.	.	.	+	.	.	.	.	.	.	.
Orchis incarnata	.	.	.	.	.	.	.	+	.	.	.
Orchis laxiflora elegans	.	.	.	.	.	.	.	+	.	.	.
Lotus corniculatus	.	.	.	.	.	.	.	.	.	.	.
Poa pratensis	.	.	.	.	.	.	.	.	.	.	.
Pedicularis palustris	.	.	.	.	.	.	.	+	.	.	.
Achillea millefolium	.	.	.	.	.	.	.	.	.	.	.
Rumex acetosa	.	.	.	.	.	.	.	.	.	.	.
Selinum carvifolia	.	.	.	.	.	.	.	.	.	.	.
Scirpus sylvaticus	.	.	.	.	.	.	.	1	.	.	.
Leucanthemum vulgare	.	.	.	.	.	.	.	.	.	.	.
Juncus articulatus	.	.	.	.	.	.	.	.	.	.	.
Drepanocladus aduncus	.	.	.	.	2	.	.	.	.	.	.
Ranunculus polyanthemos	.	.	.	.	.	.	2	.	.	.	.
Polygonum amphibium	.	.	.	.	.	.	+	.	.	.	.
Artemisia vulgaris	.	.	.	.	.	.	.	.	.	.	.
Heracleum sphondylium	.	.	.	.	.	.	.	.		.	.

Table 1b: Coenotaxons: Caricetum aproppinquatae (1-2), Caricetum rostratae (3-4), Caricetum vesicariae (5-8), Caricetum acutiformis (9), Caricetum acutiformis caricetosum ripariae (10-14), Caricetum acutiformis caricetosum melanostachyae (15), Caricetum gracilis (16-22).

Species/Sampling	12	13	14	15	16	17	18	19	20	21	22
Magnocaricetalia elatae											
Carex acutiformis	+	.	.	+	.	.	.	.	.	.	.
Carex ovalis	.	.	.	.	.	.	.	.	+	.	.
Carex riparia	**3-4**	**4**	**3-4**	+–1	.	.	.	.	.	.	.
Carex rostrata	.	.	.	.	.	.	.	.	.	.	.
Carex vesicaria	.	.	.	.	.	.	.	.	.	.	.
Carex vulpina	+	1	+	.	+	+–2	.	.	+	+	.
Carex appropinquata	.	.	.	.	.	.	.	.	.	.	.
Carex acuta	.	.	.	+	**4-5**	**2-4**	**3**	**3**	**4**	**4**	**5**
Carex melanostachya	+	.	.	**3**	.	+–1	.	.	.	.	.
Peucedanum palustre	.	.	.	.	.	.	.	.	.	.	.
Lysimachia vulgaris	.	.	+	.	.	.	.	+	+	.	+
Lythrum salicaria	+–2	+	+	+	.	+	+	.	.	.	+
Poa palustris	.	.	.	.	.	.	.	.	.	.	.
Valeriana officinalis	.	.	1-2	.	.	.	.	.	+–1	.	.
Myosotis caespitosa	.	.	.	.	.	+	.	.	.	+	.
Gratiola officinalis	+–2	.	.	+	.	+	.	.	.	+	.
Ranunculus flammula	.	.	.	.	.	.	.	.	.	+	.
Veronica scutellata	.	.	.	.	.	+	.	.	.	.	.
Equisetum fluviatile	.	.	.	.	+	.	.	.	.	.	.
Euphorbia palustris	.	.	.	.	.	.	.	.	.	.	.
Phragmitetalia											
Phragmites australis	.	.	+–1	.	.	+	.	+	.	+	+

Typha latifolia	+	.	.	.	.	+	.	.	.	+	.
Typha angustifolia	.	2	.	.	.	.	.	.	.	.	.
Glyceria maxima	.	+	.	.	+	1	.	.	.	.	.
Iris pseudacorus	+	.	+	+	.	+	.	.	.	+	.
Oenanthe aquatica	.	.	.	.	.	.	.	.	.	.	.
Eleocharis palustris	+–2	1	.	+–1	.	+	.	.	.	2	.
Berula erecta	.	.	.	.	.	.	.	.	.	.	.
Lycopus europaeus	.	+	+	.	.	+	.	.	.	.	.
Butomus umbellatus	+	.	.	1	.	.	.	.	.	.	.
Alisma plantago-aquatica	+	+	.	+	.	+–2	.	.	.	.	.
Equisetum palustre	.	.	.	.	.	.	.	1	+	+	.
Mentha aquatica	.	.	.	.	.	+–1	+	.	+	.	.
Galium palustre	.	+	+	.	.	+–1	.	.	.	.	.
Scutellaria galericulata	.	.	.	.	.	+	.	.	.	.	.
Nasturtio-Glycerietalia											
Glyceria fluitans	.	.	.	.	+–1	+	.	.	.	+	.
Glyceria notata	.	.	.	.	.	.	.	.	.	.	.
Mentha longifolia	.	.	+	.	.	.	+	.	.	.	.
Myosotis scorpioides	.	.	.	.	.	.	.	+–1	+	.	.
Scrophularia umbrosa	.	.	.	.	.	.	.	.	.	.	.
Veronica anagallis-aquatica	.	.	.	.	.	.	.	.	.	.	.
Veronica beccabunga	.	.	.	.	.	.	.	.	.	.	.
Myosoton aquaticum	.	.	.	.	.	+	.	.	.	.	.
Scheuchzerio-Caricetea fuscae											
Eriophorum latifolium	.	.	.	.	.	.	.	+	.	.	.

Carex lepidocarpa	.	.	.	.	+	.	.	.	+	.	.
Carex nigra	.	.	.	.	.	.	.	.	.	.	.
Carex echinata	.	.	.	.	.	.	.	.	.	.	.
Carex flava	.	.	.	.	.	.	.	.	.	.	.
Agrostis canina	.	.	.	.	.	.	.	.	.	.	.
Oxycocco-Sphagnetea											
Sphagnum spp.	.	.	.	.	.	.	.	.	.	.	.
Eriophorum vaginatum	.	.	.	.	.	.	.	.	.	.	.
Bidentetea											
Bidens tripartita	+	.	.	.	.	.	.	.	.	.	.
Polygonum hydropiper	.	.	+	.	.	.	.	.	.	.	.
Polygonum lapathifolium	.	+	.	.	.	+	.	.	.	.	.
Molinio-Arrhenatheretea											
Juncus conglomeratus	.	.	.	.	.	.	.	.	+	.	.
Juncus effusus	.	+	+–1	.	.	.	.	.	+	.	.
Juncus inflexus	.	.	+	.	.	+	.	+–2	+	.	.
Juncus subnodulosus	.	.	.	.	+	.	+	.	.	.	.
Ranunculus repens	+	.	+	+	.	+	.	1	+	1	.
Ranunculus acris	.	.	.	.	.	+–3	.	.	1-2	.	.
Lysimachia nummularia	+	+	+	+	.	.	.	+–1	1-2	+	.
Lychnis flos-cuculi	.	.	.	.	.	+–1	.	.	+	.	.
Symphytum officinale	.	.	+	.	+	+	.	+	+	.	+
Agrostis stolonifera	.	+	+	.	.	+	.	.	+–1	+	.
Rumex crispus	.	.	.	.	.	+–2	.	.	.	.	.
Potentilla reptans	+	+	+	+	+	+	.	.	+–1	.	.

Deschampsia caespitosa	.	.	+	.	.	+–1	.	+	.	.	.
Filipendula ulmaria	+	.	.	.	.	+	.	.	.	.	.
Potentilla anserina	.	.	.	.	.	.	.	.	+	.	.
Serratula tinctoria	.	.	.	.	.	+	.	.	.	.	.
Polygonum bistorta	.	.	.	.	.	+	.	.	+	.	.
Caltha palustris	.	.	.	.	.	.	1	.	+	.	.
Stellaria graminea	.	.	.	.	.	+	.	.	+	.	.
Trifolium hybridum	.	.	.	.	.	+	.	.	+	.	.
Trifolium repens	.	.	+	.	.	+	.	.	.	.	.
Thalictrum lucidum	.	.	+	.	.	.	.	.	+	.	.
Thalictrum simplex	.	.	.	.	.	+	.	.	.	.	.
Vicia cracca	.	.	+	.	.	+	.	.	.	.	.
Stachys officinalis	.	.	.	.	.	+	.	.	.	.	.
Holcus lanatus	.	.	+–1	.	.	.	.	+–1	+	.	.
Sanguisorba officinalis	.	.	.	.	.	.	.	.	.	.	.
Cirsium canum	.	.	+	.	.	.	.	+	1	.	.
Viola stagnina	.	.	.	.	.	+	.	.	.	.	.
Taraxacum officinale	.	.	.	.	.	+	.	.	.	.	.
Rhinanthus minor	.	.	.	.	.	+	.	.	+	.	.
Cardamine pratensis	.	.	.	.	.	+	.	.	+	.	.
Arrhenatherum elatius	.	.	.	.	.	+	.	.	.	.	.
Plantago lanceolata	.	.	.	.	.	+	.	.	.	.	.
Lathyrus pratensis	.	.	+	.	+	+	.	.	+	.	.
Alopecurus pratensis	+–1	.	.	+	.	+	.	.	.	.	.
Briza media	.	.	.	.	.	+	.	+	+	.	.

Festuca pratensis	.	.	.	.	.		.	.	+	.	.
Cynosurus cristatus	.	.	.	.	.	.	.	+	.	.	.
Succisa pratensis	.	.	.	.	.	.	.	+	+	.	.
Trifolium pratense	.	.	.	.	.	.	.	.	+	.	.
Galium boreale	.	.	.	.	.	.	.	.	.	.	.
Carex cespitosa	.	.	.	.	.	.	.	.	+–1	.	.
Cirsium rivulare	.	.	.	.	.	.	.	.	+–1	.	.
Iris sibirica	.	.	.	.	.	.	.	.	.	.	.
Sanguisorba officinalis	.	.	.	.	.	.	.	.	.	.	.
Allium angulosum	.	.	.	.	.	+	.	.	.	.	.
Salicetea purpureae											
Salix cinerea	.	.	+	.	.	.	.	.	.	.	.
Salix triandra	.	.	.	.	.	.	.	.	.	.	2
Salix fragilis	.	.	.	.	.	+	.	.	.	.	.
Salix alba	.	.	+	.	.	.	.	.	.	.	.
Salix purpurea	.	.	.	.	.	+	.	.	.	.	.
Galio-Urticetea											
Angelica sylvestris	.	.	.	.	.	.	.	.	+	.	+
Cirsium oleraceum	.	.	.	.	.	.	.	.	.	.	.
Miscellaneous											
Eupatorium cannabinum	.	.	.	.	.	.	.	.	.	.	.
Mentha arvensis	.	.	+	.	.	.	.	.	.	.	.
Galium uliginosum	.	.	.	.	.	.	.	.	+	.	.
Aster novi-belgii	.	.	.	+	.	.	.	.	.	.	+
Carex hirta	+–2	.	.	.	.	+	.	.	.	.	+

Rorippa sylvestris	+	.	.	.	.	+–2	.	.	+	.	+
Sonchus asper	.	.	.	.	.	+	.	.	.	.	.
Plantago major	.	.	.	.	.	+	.	.	.	.	.
Cirsium arvense	.	.	.	.	.	+	.	.	.	.	.
Vicia tetrasperma	.	.	.	.	.	+	.	.	.	.	.
Vicia hirsuta	.	.	.	.	.	+	.	.	.	.	.
Juncus compressus	+	.	.	.	.	.	.	.	.	.	.
Galium rubioides	+	.	.	.	.	.	.	.	.	.	.
Convolvulus arvensis	+	.	.	.	.	.	.	.	.	.	.
Polygonum minus	+	.	.	.	.	.	.	.	.	.	.
Helianthus decapetalus	+	.	.	.	.	.	.	1-2	.	.	.
Carex distans	.	.	.	.	.	.	.	.	.	+	.
Carex pallescens	.	.	.	.	.	.	.	.	.	.	.
Betula pubescens	.	.	.	.	.	.	.	.	.	.	.
Orchis incarnata	.	.	.	.	.	.	.	.	+	.	.
Orchis laxiflora elegans	.	.	.	.	.	.	.	.	+	.	.
Lotus corniculatus	.	.	.	.	.	.	.	.	+	.	.
Poa pratensis	.	.	.	.	.	+–1	.	.	+	.	+
Pedicularis palustris	.	.	.	.	.	.	.	.	+	.	.
Achillea millefolium	.	.	.	.	.	.	.	.	+	.	+
Rumex acetosa	.	.	.	.	.	.	.	.	+	.	.
Selinum carvifolia	.	.	.	.	.	.	.	.	+	.	.
Scirpus sylvaticus	.	.	+	.	.	.	.	.	+	.	.
Leucanthemum vulgare	.	.	.	.	.	.	.	.	+	.	.
Juncus articulatus	.	.	.	.	.	+	.	.	+	.	.

Drepanocladus aduncus	.	.	.	.	.	+	.	.	.	.	.
Ranunculus polyanthemos	.	.	.	.	.	.	.	.	.	.	.
Polygonum amphibium	.	.	.	.	.	.	.	.	.	.	.
Artemisia vulgaris	.	.	.	.	.	.	.	.	.	.	+
Heracleum sphondylium	.	.	.	.	.	.	.	.	.	.	+

The places and the dates of the samplings:

1. the place known as Rora Bârghişului, on Valea Lungă, in the village Pelişor; about 460 m in altitude; 29th of June 2006;

2. the bogs at Arpaşul de Jos (I. Şerbănescu, 1964);

3. Arpaşul de Sus, the place known as Mlaca Tătarilor; 520 m in altitude; 29th of June 2006; two samplings synthetis;

4. in between Sibiu and Şura Mică, on the right-hand side of the road to Ocna Sibiului; cca. 420 m. (E. Schneider-Binder, 1974);

5. in between Sibiu and Şura Mică, on the right-hand side of the road to Ocna Sibiului, cca. 420 m, 3rd of June 2005;

6. the Sibiu district "Turnişor", ponds in "Lunca Mare", two sampling synthesis (E. Schneider-Binder, 1974);

7. the village Boarţa, bound for the village Buia, on the left-hand side of the road, cca. 350 m, 25th of May 2008;

8. the village Păuca, at V. Păucii and in meadow of the Secaşul Mic, cca. 330-340 m in altitude, 30th of May 1973, three sampling synthesis (C. Drăgulescu, 1974);

10. the Sibiu district "Turnişor", ponds in "Lunca Mare" along the Cibin River (E. Schneider-Binder, 1974);

11. in between the villages Tălmaciu and Sadu, next to the former railway bridge, 420 m in altitude, two sampling synthesis (C. Drăgulescu, 1995);

12. the "Pârâul Strâmb" Stream, cca. 420 m in altitude, three samplings synthesis (E. Schneider-Binder, 1970, 1974);

13. the village Mohu, pond by the railway station (E. Schneider-Binder, 1974);

14. in between the villages Caşolţ and Cornăţel, on the right bank of the river Hârtibaci, 403 m in altitude, 29th of June 2008, two samplings synthesis;

15. the marshy area "Pârâul Rusciorul – Pârâul Strâmb", cca. 420 m in altitude, three sampling synthesis (E. Schneider-Binder, 1974);

16. west of Arpaşu de Jos Village, two sampling synthesis (I. Şerbănescu, 1964);

17. Ruscior Stream meadow, Pârâul Strâmb Stream, between Sibiu and Şura Mică villages, cca. 420 m in altitude, eight sampling synthesis (E. Schneider-Binder, 1970, 1974);

18. north of the village Scorei (I. Şerbănescu, 1964);

19. the village Movile, two sampling synthesis (Şt. Csűrös, A. Kovács, 1962);

20. the vicinity of Rora Coveşului, cca seven km north of the village, 490 m in altitude, 23rd of June 2006, two samplings synthesis;

21. Sibiu, the place known as "Şesul Măcelarilor" (E. Schneider-Binder, 1974);

22. Cristian Village, on "Lunca Mare" of Cibin River (E. Schneider-Binder, 1974).

Table 2a Coenotaxons: Caricetum vulpinae (1-3), Eleocharitetum palustris (4-10), Carici-Menyanthetum (11-13), Phalaridetum arundinaceae (14-16), Poaetum palustris (17-18), Ranunculo flammulae-Gratioletum officinalis (19-20).

Species/Sampling	1	2	3	4	5	6	7	8	9	10
Magnocaricetalia elatae										
Carex ovalis	+	+	.	.	.	.	.	.	.	+
Carex vulpina	**3**	**3**	**3**	.	.	.	+	+	1	.
Carex melanostachya	.	+	.	.	.	.	.	.	.	.
Carex acuta	+–1	.	.	.	.	.	.	.	.	.
Carex elata	.	.	.	.	.	.	.	.	.	.
Peucedanum palustre	+	.	.	.	.	.	.	.	.	.
Lysimachia vulgaris	+	.	+	.	.	.	.	.	.	.
Lythrum salicaria	+	+	+	+	.	.	.	1	+	.
Poa palustris	.	.	.	.	.	.	+	.	.	.
Valeriana officinalis	.	.	.	.	.	.	.	.	.	.
Myosotis caespitosa	.	.	+	.	.	.	.	.	.	.
Gratiola officinalis	+	+	.	+	.	.	+	.	.	.
Ranunculus flammula	.	.	.	+	.	.	.	.	.	+
Menyanthes trifoliata	.	.	.	.	.	.	.	.	.	.
Phragmitetalia										
Phragmites australis	+	+	.	.	.	.	.	.	.	.
Typha latifolia	.	.	.	.	.	.	.	+	.	.
Iris pseudacorus	+–1	+	+	.	.	.	.	.	.	.
Oenanthe aquatica	.	.	.	.	.	.	.	+	1	.
Eleochraris palustris	.	.	.	**4**	**3**	**4**	**3-5**	**4**	**3**	**4**
Berula erecta	.	.	.	.	.	.	.	+	1	.
Lycopus europaeus	.	.	.	.	.	.	+–1	+	1	.
Butomus umbellatus	.	.	.	.	.	.	.	.	.	.

Alisma plantago-aquatica	.	.	.	.	.	.	.	+	+	.
Equisetum palustre	+	.	+	.	.	.	.	.	.	.
Mentha aquatica		.	.	.	.	.	.	.	+	.
Galium palustre	+	+–1	+	.	.	.	.	.	+	1
Phalaris arundinacea	.	.	.	.	.	.	.	.	.	.
Rorippa amphibia	.	.	.	.	.	.	.	.	+	.
Scutellaria galericulata	+	.	.	.	.	.	.	.	.	.
Nasturtio-Glycerietalia										
Epilobium hirsutum	.	.	.	.	.	.	.	.	.	.
Glyceria notata	.	.	.	+	.	.	+–2	+–1	.	.
Leersia oryzoides	.	.	.	.	.	.	.	.	.	.
Mentha longifolia	.	.	+	.	.	.	.	+	.	+
Myosotis scorpioides	.	+	.	.	+	.	.	.	.	+
Scrophularia umbrosa	.	.	.	.	.	.	.	.	.	.
Veronica beccabunga	.	.	.	.	+	.	.	.	.	.
Myosoton aquaticum	.	.	.	.	.	.	.	.	.	+
Scheuchzerio -Caricetea fuscae										
Eriophorum latifolium	.	.	.	.	.	.	.	.	.	.
Carex nigra	.	.	.	.	.	.	.	.	.	.
Carex echinata	.	.	.	.	.	.	.	.	.	.
Carex flava	.	.	.	.	.	.	.	.	.	.
Potentilla palustris	.	.	.	.	.	.	.	.	.	.
Isoeto-Nanojuncetea										
Ranunculus sceleratus	.	.	.	.	.	.	.	.	.	.

Juncus articulatus	.	.	.	+	.	.	+	+	.	+
Alopecurus aequalis	.	.	.	.	.	+	.	.	.	.
Callitriche cophocarpa	.	.	.	.	.	+	.	.	.	.
Molinio-Arrhenatheretea										
Scirpus sylvaticus	+–1	.	.	.	.	.	.	.	.	.
Juncus effusus	+–1	+–2	2	.	.	.	.	.	.	.
Juncus conglomeratus	1	.	.	.	.	.	.	1	+	.
Juncus inflexus	.	.	+	.	.	.	.	.	.	.
Juncus atratus	.	2-3	.	.	.	.	.	.	.	.
Ranunculus repens	1-2	1-2	.	+	+	+	.	+	1	1
Ranunculus acris	+	.	1	.	.	.	.	.	.	.
Ranunculus auricomus	+–1	.	.	.	.	.	.	.	.	.
Lysimachia nummularia	+	+	+	.	.	.	+–2	+	.	1
Lychnis flos-cuculi	+	+	.	.	.	.	.	.	.	.
Symphytum officinale	+	+	+	.	.	.	.	.	.	.
Agrostis stolonifera	+–1	+–1	2	+	+	.	+–1	+	1	1
Agrostis canina	.	.	.	.	.	.	.	.	.	.
Rumex crispus	+	.	.	.	.	.	.	.	.	.
Potentilla reptans	+	.	+	.	.	.	.	.	.	.
Deschampsia caespitosa	.	1	1	.	.	+	.	.	.	.
Filipendula ulmaria	.	.	+	.	.	.	.	.	.	.
Potentilla anserina	+–1	.	.	.	.	.	.	.		.
Geranium palustre	.	.	.	.	.	.	.	.	.	.
Trifolium hybridum	+	+–1	+	.	.	.	1	.	.	.
Trifolium repens	.	.	+	.	.	.	.	.	.	.

Thalictrum lucidum	+	.	+	.	.	.	.	.	.	.
Vicia cracca	+	.	+	.	.	.	.	.	.	.
Stachys officinalis	.	.	+	.	.	.	.	.	.	.
Holcus lanatus	.	+	+	.	.	.	.	.	.	.
Orchis laxiflora elegans	.	+	.	.	.	.	.		.	.
Alopecurus pratensis	1-2	.	+	.	.	.	.	.	.	.
Cirsium canum	1-2	+	+	.	.	.	.	.	.	.
Taraxacum officinale	+	.	.	.	.	.	.	.	.	.
Sanguisorba officinalis	+–1	.	.	.	.	.	.	.	.	.
Poa pratensis	+–1	.	.	.	.	.	.	.	.	.
Plantago lanceolata	+	.	.	.	.	.	.	.	.	.
Rumex acetosa	+	.	.	.	.	.	.	.	.	.
Polygonum bistorta	+	.	.	.	.	.	.	.	.	.
Achillea asplenifolia	.	+	.	.	.	.	.	.	.	.
Miscelaneous										
Lythrum virgatum	.	.	.	.	.	.	.	.	.	.
Carex distans	.	.	+	.	.	.	+	.	.	.
Cardamine pratensis	1	+	.	.	.	.	.	.	.	.
Glechoma hederacea	+	.	.	.	.	.	.	.	.	.
Salix purpurea	.	.	.	.	.	.	.	+	.	.
Carex hirta	.	.	.	.	.	.	.	+	.	.
Equisetum arvense	.	.	.	.	.	.	.	.	.	.
Drepanocladus fluitans	.	.	.	.	.	.	.	.	.	.
Nardus stricta	.	.	.	.	.	.	.	.	.	.
Luzula sudetica	.	.	.	.	.	.	.	.	.	.

Philonotis sp.	.	.	.	.	.	.	.	.	.	.
Sphagnum spp.	.	.	.	.	.	.	.	.	.	.
Agrostis capillaris	.	.	.	.	.	.	.	.	.	.
Dactylorrhiza maculata	.	.	.	.	.	.	.	.	.	.
Briza media	.	.	+	.	.	.	.	.	.	.
Festuca rubra	.	.	.		.	.	.	.	.	.
Carex pallescens	.	+	.	.	.	.	.	.	.	.
Sagina procumbens	.	.	.	.	.	.	.	.	.	.
Cynosurus cristatus	.	.	.	.	.	.	.	.	.	.
Prunella vulgaris	.	.	.	.	.	.	.	.	.	.
Alnus glutinosa	.	.	.	.	.	.	.	.	.	.
Salix cinerea	.	+	.	.	.	.	.	.	.	.
Festuca arundinacea	.	+	.	.	.	.	.	.	.	.
Galium rubioides	.	+	.	.	.	.	.	.	.	.

Table 2b Coenotaxons: Caricetum vulpinae (1-3), Eleocharitetum palustris (4-10), Carici-Menyanthetum (11-13), Phalaridetum arundinaceae (14-16), Poaetum palustris (17-18), Ranunculo flammulae-Gratioletum officinalis (19-20).

Species/Sampling	11	12	13	14	15	16	17	18	19	20
Magnocaricetalia elatae										
Carex ovalis	.	.	.	1	+	.	.	.	+	+
Carex vulpina	.	.	.	.	.	+	.	+	.	.
Carex melanostachya	.	.	.	.	.	.	.	.	.	.
Carex Acuta	.	.	.	+	.	.	.	.	.	.
Carex elata	+	.	.	.	.	.	.	.	.	.
Peucedanum palustre	.	.	.	.	.	.	.	.	.	.
Lysimachia vulgaris	.	.	.	+	+	.	.	.	.	.
Lythrum salicaria	.	.	.	1	+	+	+	+	.	+

Poa palustris	.	.	.	.	1	.	**3**	**3-4**	.	.
Valeriana officinalis	.	.	.	.	.	+	.	.	.	.
Myosotis caespitosa	.	.	.	.	.	.	.	.	+	.
Gratiola officinalis	.	.	.	.	.	.	.	+	.	**2**
Ranunculus flammula	.	.	.	.	.	.	.	.	**2**	**3**
Menyanthes trifoliata	**3**	**3**	**4**	.	.	.	.	.	.	.
Phragmitetalia										
Phragmites australis	.	.	.	1	1	+	.	.	.	.
Typha latifolia	.	.	.	.	.	.	.	.	.	.
Iris pseudacorus	.	.	.	.	.	.	.	.	.	.
Oenanthe aquatica	.	.	.	.	.	.	.	.	.	.
Eleochraris palustris	.	.	.	.	.	.	.	.	+	.
Berula erecta	.	.	.	.	.	.	1-2	.	.	.
Lycopus europaeus	.	.	.	+	.	+	.	.	.	.
Butomus umbellatus	.	.	.	.	.	.	.	+	.	.
Alisma plantago-aquatica	.	.	.	.	.	.	.	.	.	+
Equisetum palustre	.	.	.	.	.	.	.	.	.	.
Mentha aquatica	.	.	.	.	.	.	.	.	.	.
Galium palustre	+	+	+	.	+	+	+	+	+	+
Phalaris arundinacea	.	.	.	3	3	4	.	.	.	.
Rorippa amphibia	.	.	.	.	.	.	+	1	.	.
Scutellaria galericulata	.	.	.	.	.	.	.	.	.	.
Nasturtio-Glycerietalia										
Epilobium hirsutum	.	.	.	.	.	+	.	.	.	.
Glyceria notata	.	.	.	.	.	.	1-2	+–1	.	+

Leersia oryzoides	.	.	.	.	.	.	.	.	.	.
Mentha longifolia	.	.	.	+	1	+	1	+	.	+
Myosotis scorpioides	.	.	+	.	+	+	+	.	.	+
Scrophularia umbrosa	.	.	.	.	+	.	.	.	.	.
Veronica beccabunga	.	.	.	.	.	.	+	.	.	.
Myosoton aquaticum	.	.	.	.	.	.	.	.	.	.
Scheuchzerio-Caricetea fuscae										
Eriophorum latifolium	+	.	+	.	.	.	.	.	.	.
Carex nigra	+	+	.	.	.	.	.	.	.	.
Carex echinata	1	1	2	.	.	.	.	.	+	+
Carex flava	2	2	+	.	.	.	.	.	.	.
Potentilla palustris	+	.	.	.	.	.	.	.	.	.
Isoeto-Nanojuncetea										
Ranunculus sceleratus	.	.	.	.	.	.	+	.	.	.
Juncus articulatus	.	.	.	.	.	.	.	.	.	+
Alopecurus aequalis	.	.	.	.	.	.	.	.	.	.
Callitriche cophocarpa	.	.	.	.	.	.	.	.	.	.
Molinio-Arrhenatheretea										
Scirpus sylvaticus	.	.	.	.	.	.	+	.	.	.
Juncus effusus	.	.	+	.	.	.	.	.	1	.
Juncus conglomeratus	.	.	.	.	.	.	1	.	.	.
Juncus inflexus	.	.	.	.	.	.	.	+	.	.
Juncus atratus	.	.	.	.	.	.	.	.	.	.
Ranunculus repens	.	.	.	+	1	.	.	.	.	.
Ranunculus acris	.	.	+	.	.	.	.	.	.	.

Ranunculus auricomus	.	.	.	.	.	.	.	.	.	.
Lysimachia nummularia	.	.	.	+	+	.	.	+	.	+
Lychnis flos-cuculi	.	.	+	.	.	.	.	.	.	.
Symphytum officinale	.	.	.	.	.	+	.	+	.	.
Agrostis stolonifera	.	.	.	1	1	+	1	.	.	+
Agrostis canina	.	.	.	1	.	.	.	.	1	.
Rumex crispus	.	.	.	.	.	.	.	+	.	.
Potentilla reptans	.	.	.	+	.	.	.	.	.	.
Deschampsia caespitosa	.	.	.	.	.	.	.	.	1	.
Filipendula ulmaria	.	.	.	.	.	.	.	.	.	.
Potentilla anserina	.	.	.	.	+	.	.	+	.	.
Geranium palustre	.	.	.	.	.	+	.	.	.	.
Trifolium hybridum	.	.	.	.	.	.	.	.	.	.
Trifolium repens	.	.	+	.	.	.	.	.	.	.
Thalictrum lucidum	.	.	.	.	.	.	.	.	.	.
Vicia cracca	.	.	.	.	.	.	.	.	.	.
Stachys officinalis	.	.	.	.	.	.	.	.	.	.
Holcus lanatus	.	.	+	.	.	.	.	.	.	.
Orchis laxiflora elegans	.	.	.	.	.	.	.	.	.	.
Alopecurus pratensis	.	.	.	.	.	.	.	.	.	.
Cirsium canum	.	.	.	.	.	.	.	.	.	.
Taraxacum officinale	.	.	.	.	.	.	.	.	.	.
Sanguisorba officinalis	.	.	.	.	.	.	.	.	.	.
Poa pratensis	.	.	.	.	.	.	.	.	.	.

Plantago lanceolata	.	.	.	.	.	.	.	.	.	.
Rumex acetosa	.	.	.	.	.	.	.	.	.	.
Polygonum bistorta	.	.	.	.	.	.	.	.	.	.
Achillea asplenifolia	.	.	.	.	.	.	.	.	.	.
Miscelaneous										
Lythrum virgatum	.	.	.	.	.	.	.	+	.	.
Carex distans	.	.	.	.	.	.	.	+	.	.
Cardamine pratensis	.	.	.	.	.	.	.	.	.	.
Glechoma hederacea	.	.	.	.	.	.	.	.	.	.
Salix purpurea	.	.	.	.	.	.	.	.	.	.
Carex hirta	.	.	.	.	.	.	.	.	.	.
Equisetum arvense	.	.	.	.	.	.	+	.	.	.
Drepanocladus fluitans	.	.	+	.	.	.	.	.	.	.
Nardus stricta	.	.	+	.	.	.	.	.	.	.
Luzula sudetica	.	.	+	.	.	.	.	.	.	.
Philonotis sp.	.	.	1	.	.	.	.	.	.	.
Sphagnum spp.	.	.	+	.	.	.	.	.	.	.
Agrostis capillaris	.	.	+	.	.	.	.	.	.	.
Dactylorrhiza maculata	.	.	+	.	.	.	.	.	.	.
Briza media	.	.	+	.	.	.	.	.	.	.
Festuca rubra	.	.	1	.	.	.	.	.	.	.
Carex pallescens	.	.	+	.	.	.	.	.	.	.
Sagina procumbens	.	.	+	.	.	.	.	.	.	.
Cynosurus cristatus	.	.	+	.	.	.	.	.	.	.
Prunella vulgaris	.	.	+	.	.	.	.	.	.	.
Alnus glutinosa	+	.	.	.	.	.	.	.	.	.

Salix cinerea	+	.	.	.	.	.	.	.	.	.
Festuca arundinacea	.	.	.	.	.	.	.	.	.	.
Galium rubioides	.	.	.	.	.	.	.	.	.	.

The places and the dates of the samplings:

1. Sibiu, the place known as "Şesul Măcelarilor", in the proximity of the railway, cca. 430 m in altitude, two sampling synthesis, 20th of May 1987 and 27th of April 2004;

2. the village Veştem, in the meadow of Cibin River, two sampling synthesis (E. Schneider-Binder, 1974);

3. in between Sibiu and the village Şura Mică, in the meadow of the Ruşcior Stream, cca. 410 m in altitude, 10th June 2005;

4. the village Tălmaciu, Valea Sadului, 400 m in altitude, 30th of June 1980;

5. the village Sadu, Valea Sadului, 440 m in altitude, 4th of August 1978;

6. in between the forest districts of Rozdeşti and Şerbănei, 1,250 m in altitude, 8th of July 1980;

7. in between the streams Pârâul Ruşciori and Pârâul Strâmb, cca. 420 m in altitude, four sampling synthesis, 3th of June 2004, 10th of June 2005;

8. the Sibiu district of "Turnişor", on the meadow of the Cibin River, 425 m in altitude, 5th of June 2004;

9. the Sibiu district of "Turnişor", on the meadow of the Cibin River, 425 m. in altitude, fifth June 2004;

10. the village Racoviţa, NE location outside the village, cca. 440 m in altitude, 11th of September 2010;

11. south of the village Arpaşul de Jos, three sampling synthesis (I. Şerbănescu, 1963);

12. the bogs near the village Arpaşul de Jos, three sampling synthesis (I. Şerbănescu, 1964);

13. below the peak Ghihan, 1,350 m in altitude (C. Drăgulescu, 1995);

14. near the confluence of the rivers Cibin and Hârtibaci, 385 m in altitude, 9th of June 2008;

15. upstream from the village Veştem, near the confluence of the rivers Cibin and Hârtibaci, 385 m in altitude, 9th of June 2008;

16. the village Netuş, on the river Hârtibaci, 475 m in altitude, 29th of June 2008;

17. the Sibiu district of "Turnişor", on the meadow of the Cibin River, 425 m in altitude, two sampling synthesis, 5th of June 2004;

18. the meadow of the stream Ruşciori, in between Sibiu and the village Şura Mică, cca. 420 m in altitude, two sampling synthesis, 3th of June 2004;

19. the village common of Scorei (I. Şerbănescu, 1964);

20. the locality Tălmaciu, on the bank of Sadu River, in the Şuvară natural reserve, 420-425 m in altitude, two sampling synthesis, 13th of May 2004, 12th of June 2006.

REFERENCES

1. Anghel G., Niedermaier K., Burcea P., Stângă N. and Şerbănescu I., 1965 – Studiu şi cartarea păşunilor din corpul Crinţ- Munţii Cibinului, Com. bot. Bucureşti, III, 47-80. (in Romanian)
2. Borza A., 1959, Flora şi vegetaţia Văii Sebeşului, Edit. Acad. Bucureşti.
3. Cristea V., 1981 – Flora şi vegetaţia din Podişul Secaşelor, Teză doctorat Univ. „Babeş-Bolyai Cluj-Napoca, Fac. Biologie, geografie şi geologie. (in Romanian)
4. Csűrös Ş. and Kovács, A., 1962 – Cercetări asupra vegetaţiei din raioanele Sighişoara şi Agnita, Contr. bot. Cluj, 205-232. (in Romanian)
5. Drăgulescu C., 1974 – Flora şi aspecte de vegetaţie din împrejurimile localităţii Păuca (jud. Sibiu), Lucrare diplomă Univ. Babeş-Bolyai Cluj-Napoca, Fac. Biologie-Geografie. (in Romanian)
6. Drăgulescu C., 1994 – Vegetaţia lacurilor dintre Mândra şi Ocna Sibiului, Acta oecologica Univ. Sibiu, I, 1-2, 57-61. (in Romanian)
7. Drăgulescu C., 1995 – Flora şi vegetaţia din bazinul Văii Sadului, Edit. Constant Sibiu. (in Romanian)
8. Drăgulescu C., 1999 – The hydrophylous and hygrophylous flora and vegetation from the upper and middle Olt River valley, Transylv. Rev. Syst. Ecol. Res., 1, Sibiu, 13-30.
9. Drăgulescu C., 2004 – The vegetation of the Cibin Valley (Southern Transylvania), Kanitzia Sombathely, 12, 25-42.
10. Drăgulescu C., 2005 – The hydrophilous and hygrophilous flora and vegetation of Târnave rivers, Transylv. Review of System. and Ecol. Research, 2, The Târnava River basin, Sibiu, 13-30.
11. Drăgulescu C., 2007 – Contributions to the knowledge of Sibiu County's aquatic vegetation, Acta oecologica XIV, 1-2, Sibiu, 25-40.
12. Drăgulescu C., 2007 – The riverside thickets of the Saxon Villages area of south-east Transylvania in Curtean-Bănăduc A., Bănăduc D., Sîrbu I., 2007, The Saxon Villages Region of southeast Transylvania, Transylvanian review of systematical and ecological research, 4. Sibiu, 43-54.
13. Drăgulescu C., 2010 – Study of vegetation of Phragmitetalia order in the range of Sibiu county, Romanian Journal of Aquatic Ecology, I, 13-30.
14. Oroian S. 2009 – Flora şi vegetaţia satelor săseşti din sud-estul Transilvaniei, Edit. University Press Târgu Mureş. (in Romanian)
15. Resmeriţă I., Puşcaru-Soroceanu E., Morariu I., 1977 – Dare de seamă peivind organizarea şi desfăşurarea celei de a 10-a consfătuiri naţionale de geobotanică, Com. bot. Bucureşti, 5-16. (in Romanian)
16. Schneider-Binder E., 1970 – Vegetaţia acvatică şi palustră dintre pârâul Strâmb (Râsloavele) şi Ruşciorul, St. şi com. Muz. Brukenthal Sibiu, şt. nat., 15, 187-214. (in Romanian)
17. Schneider-Binder E. 1974 – Flora şi vegetaţia Depresiunii Sibiului şi a dealurilor marginale, Teză doctorat, Univ. "Babeş-Bolyai" Cluj, Fac. Biologie-Geografie. (in Romanian)
18. Schneider-Binder E., 1976 – Caracterizare generală a vegetaţiei Depresiunii Sibiului şi a dealurilor marginale, St. şi com. Muz. Brukenthal Sibiu, Şt. nat., 20, 15-46. (in Romanian)
19. Şerbănescu I., 1963 – Fâneţele de la sud de Arpaşul de Jos, Com. bot. Bucureşti, II, p. II-a, 93-106. (in Romanian)
20 Şerbănescu I., 1964 – Cercetări geobotanice în depresiunea Făgăraşului, Anuar. Comit. geol. Bucureşti, 36, II, 311-370. (in Romanian)

PIONEER PLANT COMMUNITIES ON STREAM BANKS OF THE TROPICAL RAINFOREST IN THE KHAO SOK NATIONAL PARK (SOUTHERN THAILAND)

Erika SCHNEIDER-BINDER *

* Karlsruhe Institute for Technology – University of Land Baden-Württemberg and National Research Centre of the Helmholtz-Society, Institute of Geography and Geoecology, Dept. WWF-Institute for Floodplain Ecology, Josef Strasse 1, Rastatt, Germany, D-76437, & "Lucian Blaga" University of Sibiu, Faculty of Science, Department of Ecology and Environmental Protection, Dr. Ion Rațiu Street 5-7, Sibiu, Sibiu County, Romania, RO-550012, erika.schneider@kit.edu, erika.schb@t-online.de

KEYWORDS: Thailand, Thai Khao Sok National Park, riparian pioneer plants, stream bank communities, tropical rain forest, cliffy limestone area, adaptation to changing water levels.

ABSTRACT

Riparian pioneer plants and their river bank communities were studied at a stream tributary of the Sok River in the tropical rainforest of the Khao Sok National Park/Southern Thailand. One stand-forming species is the Willow-Leaved Water Croton *Homonoia riparia* Lour. (Euphorbiaceae family), it shows a willow-like habitus and colonizes habitats comparable to those of some European willow species. A second species belongs to the Araceae family, *Cryptocorine albida* Parker, forming well-developed stands on gravel sites between boulders and coarse rubble of the river bank and in the river bed. This species is well adapted to the specific conditions of changing water levels dependent on the rainy and dry periods of the year and are characteristic riparian elements of the studied area.

ZUSAMMENFASSUNG: Pionierpflanzengesellschaften an Bachufern des tropischen Regenwalds im Khao Sok Nationalpark Südthailands.

An einem Gewässerlauf im tropischen Regenwald des Nationalparks Khao Sok/Südthailand wurden die Pionierbesiedler auf den Kiesbänken untersucht. Die eine bestandbildende Art ist die buschartig wachsende *Homonoia riparia* Lour. (Euphorbiaceae), die einen Weiden ähnlichen Habitus hat und auch ähnliche Habitate besiedelt wie einige unserer europäischen Weiden. Bei der anderen handelt es sich um eine Araceae und zwar die Wasserkelchart *Cryptocorine albida* R. N. Parker, die dichte Bestände auf dem Kies zwischen den Geröllblöcken und dem Grobschotter der Ufer bildet. Die Arten sind gut an die spezifischen Bedingungen der wechselnden Wasserstände in Abhängigkeit von Regen– und Trockenzeit angepasst und gehören zu den charakteristischen Fluss begleitenden Arten im Gebiet.

REZUMAT: Comunităţi de plante pioniere de pe malurile pârâurilor din pădurea pluvială tropicală a Parcului Naţional Khao Sok din Tailanda de Sud.

Pe malurile unui pârâu, afluent al râului Sok din pădurea tropicală a Parcului Naţional Khao Sok din Tailanda de sud au fost studiate plantele pioniere şi comunităţile lor. Una dintre ele, crescând sub formă de tufă, asemănătoare după habitus cu unele specii europene de sălcii, este *Homonoia riparia* Lour. (Euphorbiaceae), colonizând şi habitate similare, iar cealaltă este o specie din familia Araceae *Cryptocorine albida* R. N. Parker, care formează grupări bine închegate între bolovani şi pietriş din albia şi de pe malurile pârâului. Speciile studiate sunt bine adaptate la condiţiile specifice de nivele schimbătoare ale apei în funcţie de perioada uscată şi cea ploioasă din decursul anului, fiind elemente tipice zonei ripariene din aria studiată.

INTRODUCTION

The vegetation of stream and riverbanks is closely related to the hydrological and morphological dynamics of the water course, changing with the slope from the mountains to the plains. The different stretches are characterized by a changing flow velocity and the grain size of sediments, starting with the size of boulders and ending up with gravel and sands as well as fine silty deposits. Vegetation repartition depends on the period of flooding as well as on the height, duration and periodicity of floods, the latter differing in various geographical and comparable climatic zones around the world. In temperate zones the flood dynamics follows four seasons, in tropical areas the periodicity of floods and the adaptation to this periodicity is determined by the change of two seasons, i.e. the wet, rainy season and the dry period of the year. The species occurring on the river banks and in the river bed of the tropical area's small streams are well adapted to these seasonal changes of the two periods.

Large tropical rivers, their floodplains and floodplain forests are frequently in the focus of biodiversity studies and are known for their outstandingly high biodiversity. However, the plant communities along the river banks of small water courses, in particular in mountainous areas, generally receive less attention by research as compared to the larger ones. To be pointed out in this respect are the rivers and streams in the cliffy limestone area of tropical rainforests in Southeast Asia including those of the Thai-Malay Peninsula. The highly structured relief in this area offers many macro and microhabitats for different species.

Along smaller, more or less shaded and boulder-rich streams of the rainforest areas in Southern Thailand (Khao Sok National Park), willow-like bushes and small plants forming dense stands amongst the rocks and having perfectly adapted to the water dynamics stand out. These species occur as well in the river bed itself in places where gravel bumps arise between the ramifications and in braided areas. They settle on the finer and coarser sands that have been ground between the boulders and river gravels, this is where they find perfect conditions to spread their network of roots.

The species described is first the willow-like shrub *Homonoia riparia* (Euphorbiaceae family) that colonizes habitats comparable to European pioneer stands of willows, especially of the Purple Willow (*Salix purpurea*) and the willow *Salix elaeagnos*, and further on smaller Araceae of the Cryptocorine genus that occur on the South-Eastern Asian mainland with three species: *Cryptocorine albida* Parker, *Cryptocorine crispatula* Engler and *Cryptocorine leptospiralis* (Roxburgh) Kunth (Jacobsen, 1991). These species have perfectly adapted to the seasonal changes of rainy and dry periods and cope well with the dramatic and rapidly growing floods.

MATERIALS AND METHODS

Khao Sok National Park has been established in 1980 and is situated in the South of Thailand in the Surat Thani province, about 72 km north-east of Khao Lak, on the southern western coast of the country on the Malay peninsula on the Andaman Sea/Indian Ocean, at an absolute altitude of 300-600 m. Together with Malaysia's Taman Negara National Park (4,362 km^2) the Khao Sok National Park constitutes the second protected area of significant size of the Thai/Malay Peninsula (Henley, 1997).

The initially established area covered a surface of 645.52 km^2, today it extends to more than 739 km^2. Lying contiguous with the two Wildlife Sanctuaries Klong Saen and Klong Nakha as well as the National Park Sri Phangnga, we can consider the whole protected forest area as a complex of 4,000 km^2 (Henley, 1997). Due to its size the area of Khao Sok National Park and the surrounding protected sites are of great significance as habitats for the survival of large mammals (tiger, elephant, leopard and others). Wildlife inventories are far from being complete, the number of species confirmed in 1997 by Henley included 48 mammal and 184 bird species. The inventory of further fauna groups such as fish, amphibians, reptiles and insects is only a beginning and is subject to a number of studies. The same is true for plants, so that new species are constantly being discovered. Among the most important discoveries ranges a new species of *Rafflesia*, the world largest flower with 80 cm in diameter (Henley, 1997).

Owing to its very specific landscape consisting of rugged, individual limestone mountain ridges that emerged 60-140 million years ago, the Khao Sok National Park disposes of a very well conserved tropical, semi-deciduous rainforest showing an extremely varying mosaic of macro- and micro-habitats. A major part of this diversity is due to the National Park's water network of running waters that usually show a high gradient and are characterized by uncountable waterfalls, Sip Et Chan, Bang Lab Nam, Tang Nam, Ton Kloi and Thara Sawan being among the best-known. The rocky river beds with their broad, rounded stones, gravelly beds and clear, rapidly running waters offer habitats to characteristic rheophile species. The main river of the National Park is the Sok River on the southern border of the National Park, collecting the waters from the small tributary rivers and streams. The Sok River and its tributaries are exposed to the dynamics of discharge and water levels, changes that depend on the rainy and the dry periods of the year, the latter lasting from October to February.

Even though the area is classified within the evergreen tropical rainforests of the paleotropical-Indo-Malaysian biome group (Walter and Breckle, 1984), in this case we speak about a semi-deciduous rainforest, given that the areas also shelters species that cast their leaves during the dry season which is not very pronounced though. The Khao Sok area actually displays features of both the tropical evergreen forest and the rainforest given that rainfall is known to occur here in every month of the year (Henley, 1997), even though less frequently during the "dry" period. With an annually recorded precipitation of 3,500 mm the Khao Sok National Park is the wettest region of Thailand.

The studied stream (10-15 m width), tributary of the Sok River, shows the characteristics of a mountainous stream with a stony river bed, dominated by boulders, gravel of different sizes and sand, a greater slope and coming along with this a high flow velocity of the water. The water temperature of the studied water body has been of around 24 degrees.

On this stony, boulder- and gravel-rich stream in the Kao Sok National Park, the morphological structure of the river banks and their pioneer vegetation have been analysed twelve years ago, in February 1999, during the dry period.

Among the pioneer species that require open gravel and sand areas to settle and that occur in the stony river bed and along the banks, some could immediately be determined on-site on the basis of the Manual on the Flora of Thailand (Mc Makin, 1993). From the tiny specimen of a species growing densely amongst the rocks most of the plants had already withered. However, a number of flowering specimen could be found as well and could be taken home for a closer determination. Given that they were not listed in the Manual on the Flora of Thailand (Mc Makin, 1993), the plants could merely be assigned to the Araceae group on-site. A further examination in the laboratory based on relevant literature allowed a determination up to the *Cryptocorine albida* group (Jacobsen, 1991; Cook, 1996). Since the species is a morphologically variable one, its determination required a number of comparative studies within the *Cryptocorine albida* group (Tab. 1; Figs. 1 and 2). During the following two years the latter could also be observed as for their behavior as an aquarium plant.

Furthermore, phytocoenological studies have been carried out on-site (Tab. 2; Fig. 3). In this respect areas of 1 m^2 have been recorded according to abundance-dominance values obtained by means of the Braun-Blanquet (1964) method. These areas were laid out differently (1 x 1 m, 0,5 x 2 m) depending on the morphological structure of the ground (narrow or broad banks; slender, elongated gravel strips situated between boulders). Cross-sections have additionally been drawn to represent position and repartition of the riparian pioneer plants and their river bank communities along ecological gradients.

RESULTS AND DISCUSSIONS

The Willow-Leaved Water Croton *Homonoia riparia* Lour. (Euphorbiaceae family), has been identified (Mc Makin, 1993) as pioneer plant occurring on the gravel banks between the anastomoses in the river bed and on the river banks.

Homonoia riparia Lour. is a willow-like shrub – fact expressed also by the plant's English name which is Willow-leaved Crotton – with alternate, linear-lanceolate leaves and pendulous spikes of small, brown flowers (Mc Makin, 1993). It is a characteristic rheophilous species, the plants growing, as described by Richards (1996), "by fast-flowing streams, or in their beds, often have linear-lanceolate leaves much narrower than those of related species living in the interior of the forest. This is particularly marked in Malaysian species of Homonoia". This description also corresponds to the situation of the specimen occurring in the streams of Khao Sok National Park in Thailand.

For the species of the Cryptocorine genus, the studied plant material has in a first step been identified as belonging to the *Cryptocorine albida*-group. The genus Cryptocorine includes about 50 species which are distributed in tropical regions of Asia (Cook, 1996). They are living in and on rivers, as submerged, emerged or temporarily terrestrial plants and adapted to seasonal changes between high and low water levels. Many of the species are cultivated in Europe as decorative aquarium plants (Eichner, 2001; Greger, 1997; Horst, 1992; Jacobsen, 1991; Schmidt, 2006; Titz, 2002).

The specimen studied in Khao Sok National Park present morphological variability as for their leaves and structure of inflorescences (Tab. 1). Due to this fact comparative studies of different material of the *Cryptocorine albida* group were needed. The group includes the three species *Cryptocorine albida*, *Cryptocorine crispatula* and *Cryptocorine leptospiralis* (Jacobsen, 1991; Eichner, 2001; Titz, 2002), which are aquatic or amphibian plants with subterranean rhizomes, and a leaf rosette.

Table 1: Comparison of morphological characteristics of the species of *Cryptocorine albida* group (following data from Jacobsen, 1991; Cook, 1996; Eichner, 2001; Schmidt, 2006; Schöpfel, 1995; Schüssel, 2000; Titz, 2002; and own observations for *Cryptocorine albida*).

	I	II	III
Species	*Cryptocorine albida*	*Cryptocorine crispatula*	*Cryptocorine leptospiralis*
Rhizom, thickness	0, 8 cm.	–1 cm, occasionally irregularly broadened.	0.5-1.5 cm, occasionally irregularly broadened.
Leaves	10-30 cm in lenght, one-two cm broad lanceolate, green to brown even, leaf margin not undulate or bullate.	10-70 cm lenght, 0.2-4 cm broad Very variable, narrowly linear to lanceolate, green to brown, smooth to undulate to bullate or with a fine denticulate margin.	10-40 cm, 0.3-1.0 (–1.5) Linear to lanceolate green to brownish, flat to undulate.
Inflorescence Spathe limb	2,5 cm (– 4 cm), yellowish grayish shortly reflexed to spirally twisted; opening usually visibly extended, irregular markings varying from lines to spots (reddish to brownish).	Three-eight cm, Yellowish grayish or greenish more or less open spirally twisted, irregular markings, lines to circular spot, purple.	10-30 cm More or less spirally twisted. Markings larger on the spathe limb, more or less circular spots, reddish.
Inflorescence tube	Five-15 cm	Six-30 cm	Five-20 cm
Inflorescence opended kettle base of the spathe	0.8-1.5 cm	one-2.5 cm	10 cm
Inflorescence spadix female flowers; female part below and separated by a slender sterile axis of the spadix.	Four-seven with more or less vertical, ovate, pistils; olfactory bodies more or less club-shaped, frequently concave in the center.	Four-six with horizontal to vertical, round to ovate pistils; Olfactory bodies more or less regularly lobate.	Four-seven, with more or less horizontal, ovate to rounded pistils Upper part of olfactory body more or less regularly lobate.

The species occur in adjacent but not, or if so only slightly overlapping distribution areas (Jacobsen, 1991). The distribution area of *Cryptocorine albida* concentrates on the Malay Peninsula and extends only marginally to the northern part of Southeast Asia, i.e. the main distribution area of *Cryprtocorine crispatula*. For *Cryptocorine leptospiralis* the core area of its distribution is India (Jacobsen, 1991).

The examination of the studied plant material (Figs. 1 and 2) clearly showed the fact that the biological samples taken in Khao Sok National Park were to be attributed to *Cryptocorine albida* Parker, in spite of the fact that few plants showed slightly dentated leaf margins that resemble to the leaf forms of *Cryptocorine crispatula*.

Figure 1: Flowers of *Cryptocorine albida* Khao Sok National Park.

Ecology

Cryptocorine albida is an amphibious species which is well adapted to the specific requirements of changing water levels. With its well developed subterranean rhizomes that form a dense network of roots it is comfortably anchored in the soil and in a good position to endure even very strong currents at the moment of floods, which arise with the monsoon rains. According to studies conducted by Horst (1992) on the Kam Phuam and Nang Yon rivers, the plant consists of about 95% of roots and of merely 5% of foliage. The water levels obtained during the rain period on the river studied exceeded normal levels by 1.5-2 (–3) m but did not represent any problem for these well adapted plants.

Figure 2: *Cryptocorine albida* drawn following living plants (original).

During flowering time, i.e. from December, in some cases even from September to February (Jacobsen, 1991) the species grows emersed or occurs on completely dry spots on sandy, stony ground between larger rocks and boulders in the high floods river bed (Fig. 3). At falling water levels the long tubes of the spathe ensure that the opening is up above the water surface and that the flowers may well develop. Later on, with further falling water levels many plants bordering the river bank or adjacent to it are completely emerse or terrestrial. This is how we found them in the area of Khao Sok National Park. The flowers diffuse a penetrating and unpleasant aroma for man but it attracts small flies that ensure their pollination. Seeds are dispersed by the water, they settle on sandy spots and develop as pioneer vegetation on sites that are laid bare by the water. Dispersal by animals may occur as well (Cook, 1996).

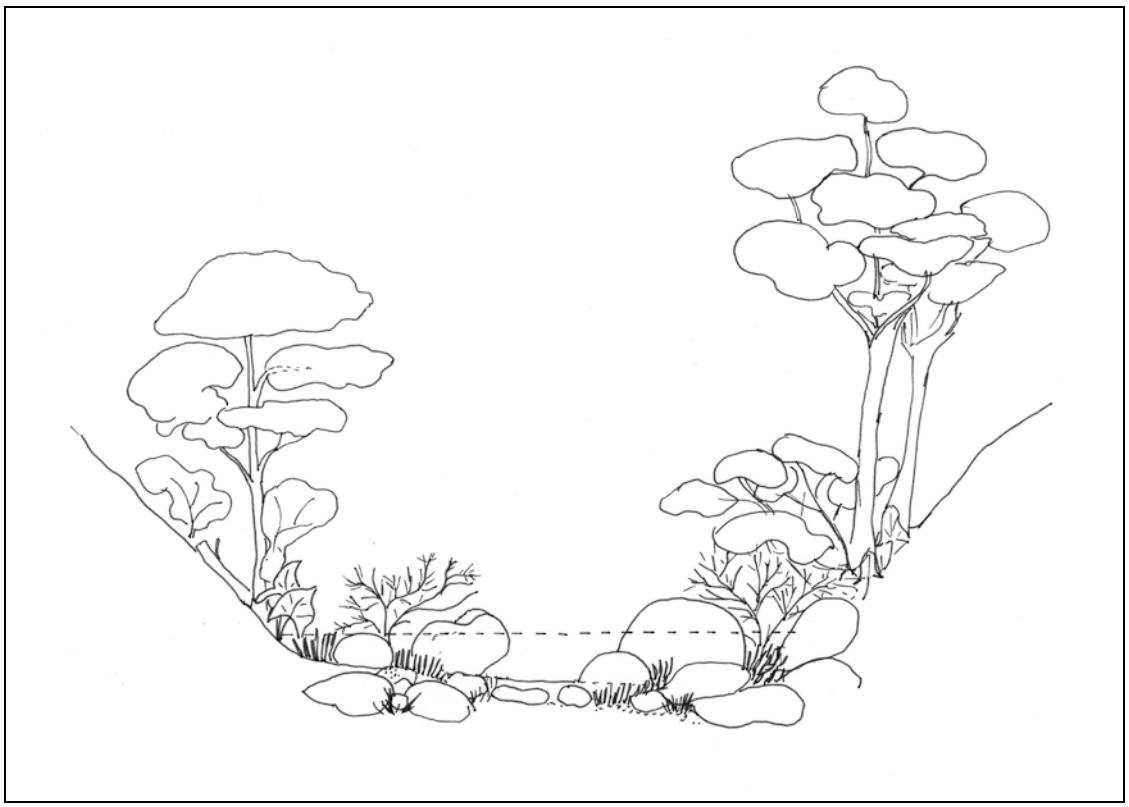


Figure 3: Cross section through the stream valley with repartition of vegetation:
between the stones and boulders the specimens of *Cryptocorine albida*
and the shrubs of *Homonoia riparia* (original).

The vegetation in the river bed and on the river banks is distributed following the ecological gradients (Fig. 3) demonstrating the tolerance of plants to a dynamic stream with changing water levels. Dense stands of *Cryptocorine albida* with a high coverage rate could be observed along the river banks between rocks (Fig. 4).

Samples taken from the chosen spots prove their abundance-dominance and great constancy. At some spots, there is a close interaction with *Homonoia riparia* bushes (Tab. 2). The dense *Cryptocorine albida* river bank vegetation offers diversified microhabitats to invertebrates that have also adapted to these conditions.

Table 2: *Cryptocorine albida* community.

Number of sampling	1	2	3	4	5	6	7	8	9	10	
Covering degree %	100	100	80	85	95	50	85	80	100	100	
Species											F
Cryptocorine albida	5	5	4	4	5	3	4	4	5	5	V
Cryptocorine albida var.	+	.	.	+	.	.	.	.	.	.	I
Homonoia riparia	1	2	+	1	.	1	2	.	.	+	III
Tall herbaceous Araceae	+	.	.	.	.	.	.	.	.	.	I

All samples were taken on from the stream banks in the Khao Sok National Park on 27th February 1999; F = Frequency classes.

Figure 4: Stands of *Cryptocorine albida* in the studied river bed in the Khao Sok National Park (foto Erika Schneider).

CONCLUSIONS

On the studied river banks of the Khao Sok National Park of Thailand two species have been stated as characteristic colonizers of dynamic, free river banks with gravels of different grain sizes between the boulders of the river bed. These are the scrub species *Homonoia riparia* which locally forms small galleries along the river banks, occurring as well in the stony river bed as well, and also the herbal species *Cryptocorine albida*, which is known in Europe, as a decorative aquarium plant along with other species of the Genus *Cryptocorine*.

The species are well adapted to the conditions of faster flowing river and to water level changes in the rainy and dry periods of the year.

Due to the large morphological variability of the species *Cryptocorine albida*, a comparative study with other species of the *Cryptocorine albida* group revealed necessary (Jacobsen, 1991; Eichner, 2001; Titz, 2002).

Both ecology and phytocoenology of small rivers and streams are not studied to a satisfactory extend and require further, also comparative studies in similar areas.

In the complex ecosystems of tropical rain forests the riverine vegetation is very important also as habitat for different animal species too.

No danger has yet been observed in such rivers, given that the conservation conditions of the natural habitats in the cliffy limestone area of Southern Thailand are remarkably good. It reveals the importance of protecting this kind of habitat from man's interference, even beyond the national park zones, so e.g. from river training and bank protections works.

REFERENCES

1. Braun-Blanquet J., 1964 – Pflanzensoziologie. 3. Aufl., Wien, 865. (in German)
2. Cook C. D. K., 1996 – Aquatic Plant Book. SPB Academic Publishing, Amsterdam/New York, 228.
3. Eichner R., 2001 – Cryptocorine albida de Wit, meine Erfahrungen mit einer sehr variablen Art. Aqua planta 2, 50-54.
4. Greger B., 1997 – Cryptocorine albida – Weißlicher Wasserkelch. Das Aquarium 30 (2), 46-47. (in German)
5. Henley T., 1997 – Reefs to Rainforests. Mangroves to mountains. A guide to South Thailand's Natural Wonders. Dawn and Happiness Resort Co., Ltd, Krabi/Thailand, 227.
6. Horst K., 1992 – Pflanzen im Aquarium. Ihre Funktion und Pflege, Kap. Aquarienpflanzen am natürlichen Standort 22-60, 2. Aufl., Ulmer Verlag Stuttgart. (in German)
7. Jacobsen N., 1991 – Die schmalblättrigen Cryptocorynen des asiatischen Festlandes. – Aqua-Planta, 1-9116, 2-33. (in German)
8. Mc Makin P. D., 1993 – Flowering plants of Thailand. A field guide. White Lotus Bangkok Cheney, second edition, 141.
9. Richards P. W., 1996 – The tropical rain forest an ecological study. Cambridge University Press, second edition, 575.
10. Schmidt J., 2006 – Der Grasblättrige Wasserkelch Cryptocorine crispatula Engler – Aqua Planta 3-06, 103-109, (in German)
11. Schöpfel H., 1995 – Der Gekräuselte Wasserkelch Cryptocorine crispatula. Das Aquarium 29, 7: 22-26. (in German)
12. Schüssel J., 2000 – Cryptocorine crispatula der Grasblättrige Wasserkelch. Aquarium live 4, 64-66. (in German)
13. Titz T., 2002 – Cryptocorine crispatula. Ein Wasserkelch in unterschiedlichen Gewändern Das Aquarium 36 (3), 35-37. (in German)
14. Walter H. and Breckle S.-W., 1984 – Ökologie der Erde Bd. 2. Spezielle Ökologie der tropischen und subtropischen Zonen. UTB Grosse Reihe, Gustav Fischer Verlag Stuttgart, 461. (in German)

MORPHOMETRIC MEASUREMENT AND FIELD ESTIMATION OF THE SIZE OF *CROCODYLUS NILOTICUS* IN LAKE NASSER (EGYPT)

Ashraf Hussein Ibrahim SALEM *

* Environmental Affairs Agency, South Area protectorates, Cairo, Egypt, ashraf2404@yahoo.com

KEYWORDS: Nile crocodile, morphometric measurements, snout vent length, total length, the health of lake Nasser, growth-rate.

ABSTRACT

Sixty-one crocodiles were caught between October 2009 and August 2010, within forty-two locations surveyed, based on capture-recapture and release surveys. Head length (HL), snout-vent length (SVL), total length (TL), right hind foot length (HFL), neck girth (NG), tail girth (TG), chest girth (CG), head depth (HD), head width (HW), Vent (V) and mass (W) were measured. HL was measured dorsally, while SVL and TL were measured ventrally. Simple linear regression equation was used to express the relationship between TL and crocodile measured parameters, and SVL and crocodile measured parameters were determined by the use of the regression equation (**y = a + bx**). The close significant correlation between parameters, exhibits the health of Nile crocodiles, as well as the healthily of lake Nasser ecosystem during the period of the survey. The snout-vent length increases relative to total length; this may be an indication of their behaviour, as small crocodiles have relatively longer tails than large crocodiles. The Nile crocodile's growth rate is extremely changeable and thus, predicting the age of individuals by using the size-age curves may result into huge errors, nevertheless, given the variability in growth rates of juvenile animals, it is not possible to accurately estimate the age using regression methods.

RÉSUMÉ: Mesures morphométriques et estimation sur le terrain des dimensions de *Crocodylus niloticus* de Lac Nasser (Egypte).

Soixante et un crocodiles ont été capturés entre Octobre 2009 et Aout 2010 dans 42 sites surveillées selon le système capture-recapture et relâchement. La longueur de la tête (HL), la longueur museau-cloaque (SVL), la longueur totale (TL), la longueur du membre postérieur droit (HFL), la circonférence du cou (NG), la circonférence de la queue (TG), la circonférence de la poitrine (CG), la hauteur de la tête (HD), la largeur de la tête (HW), le cloaque (V) et le poids (W) ont été mesurées. HL a été mesurée dorsalement, pendant que SVL et TL ont été mesurés sur le ventre. Des modèles simples de régression linéaire exprimant la relation entre TL et les paramètres mesurés du crocodile et entre SVL et les paramètres mesurés du crocodile ont été utilisés. La corrélation significativement proche entre les paramètres dénote l'état de santé du crocodile nilotique ainsi que l'état de santé de l'écosystème du Lac Nasser pendant la période de l'étude. La longueur museau-cloaque s'agrandit par comparaison avec la longueur totale; ceci peut être un indice vis à-vis de leur comportement, les crocodiles plus petits ayant des queues plus longues que les crocodiles plus grands. Le taux de croissance du crocodile de Nile est extrêmement variable donc, la prédiction de l'âge des individus en utilisant les courbes taille/âge se fait avec un taux d'erreur plus grand, néanmoins, étant donnée la variabilité du taux de croissance des juvéniles, il n'est pas possible d'estimer de manière précise l'âge en utilisant les méthodes de régression.

REZUMAT: Măsurători morfometrice şi estimarea pe teren a dimensiunilor *Crocodylus niloticus* din Lacul Nasser (Egipt).

61 de crocodili au fost capturaţi între octombrie 2009 şi august 2010 în 42 de locaţii, în sistemul captură-recaptură şi eliberare. Lungimea capului (HL), lungimea bot-orificiu cloacal (SVL), lungimea totală (TL), lungimea membrului posterior drept (HFL), circumferinţa gâtului (NG), circumferinţa cozii (TG), circumferinţa toracelui (CG), înălţimea capului (HD), lăţimea capului (HW), cloaca (V) şi greutatea (W) indivizilor au fost măsurate. HL a fost măsurată dorsal, în timp ce SVL şi TL au fost măsurate ventral. Au fost folosite ecuaţii de regresie simplă lineare exprimând relaţia între TL şi parametrii măsuraţi ai crocodilului şi între SVL şi parametrii măsuraţi ai crocodilului. Corelaţia semnificativă strânsă între parametri denotă starea de sănătate a crocodilului de Nil, şi starea de sănătate a ecosistemului Lacului Nasser pe perioada de studiu. Lungimea bot-orificiu cloacal creşte comparativ cu lungimea totală. Acest lucru poate fi o indicaţie a comportamentului lor, astfel încât crocodilii mai mici au cozi mai lungi decât crocodilii mai mari. Rata de creştere a crocodilului de Nil este foarte variabilă şi, deci, folosirea curbelor de corelaţie dimensiune – vârstă pentru a prezice vârsta indivizilor ar duce la erori destul de importante, cu toate acestea, datorită variabilităţii ratei de creştere a juvenililor, nu se poate estima cu acurateţe vârsta nici utilizând metoda regresiei.

INTRODUCTION

The prediction of the size and condition of crocodilians in the wild can be estimated according to **morphometric** relationships which allow for a basic of practical need arising from research on the ecology of crocodilians (Hutton 1987). On the other hand, animals' physiological state is related to its ability to survive as an individual and has direct consequences in terms of its reproductive success (Jakob et al., 1996).

The three methods tested by Jakob et al. (1996) are known as the ratio index or **relative condition factor (RCF),** where the mass of the individual is divided by the body size indicator (BSI – a linear measure of size). Although the RCF method (Le Cren, 1951) was intended for and has been used broadly in the scientific field of fisheries, it has previously been used for *Crocodylus niloticus* (Games, 1990; Leslie, 1997), *Crocodylus porosus* (Taylor, 1979) and for *Alligator mississippiensis* (Brandt, 1989). The second body condition was estimated by **the slope adjusted index**, which is similar to the ratio index, but for the calculation of the slope, (large) independent data set is used (Jakob et al., 1996). The third index, **the residual index**, uses the residuals of the body mass/BSI regression, after transformation of the data, to fit into a linear regression (normally in transformation). Jakob et al. (1996) suggests the use of the mass/BSI residual index, as this does not vary with body size, as is the case with the other mentioned indices. On the other hand, Green (2001) outlines a number of conditions for using this method which, when violated, leads to type I error, where the hypothesis has been rejected when it was true, or type II error, when the hypothesis failed to be rejected when a given alternative hypothesis was true (Zar, 1974).

In general, **r** values are high in reptiles (Paniagua et al., 1997), suggesting that residual indices generated by the least-squares ordinal method are likely to be more reliable for reptiles than for birds and mammals (Green 2001). The correlation between crocodilian age and size may be one of the most fundamental life history characters as reported by (Webb and Smith, 1987) and they suggested that the correlation between crocodilian age and size may be one of the most essential life history characters because it allows age, maturity and senescence to be estimated. On the other hand, in large ectotherms, demographic parameters are weakly related to age and growth. It is of primary interest as life-history phenomena are related to body size and that demographic parameters are more related to size than age (Hutton, 1987; Salem 2006).

Crocodiles are long-lived animals, the Nile crocodiles living for about a century, under optimal wild conditions (Branch, 1998). Long-term studies are therefore necessary to collect accurate growth rate data on crocodiles. Due to the high cost of equipment and the challenging logistics involved while working in an aquatic environment, there is little available long-term data on crocodilian growth rates in the wild. Captive crocodilians, under optimal pen densities, feeding and temperature conditions, will grow faster than those in the wild (Hutton, 1987), sometimes repetition "natural" growth rates (Chabreck and Joanen, 1979). It is difficult to assign ages to wild associates after just three-four years of growth (Cott, 1961; Hutton, 1984). Different size of the classes also exhibit differing growth rates, with the growth rate to size relationship changing at four-five years of age (Hutton et al., 1987). Webb et al. (1978) found that a linear growth pattern was exhibited for *C. porosus* up to 80 cm snout vent length (SVL), but this fitted line does not describe the growth rates for larger animals.

Smaller animals tended to have a much higher relative length-related growth rate than larger animals, while larger animals increased at a relatively higher rate in terms of mass.

There are significant influences on crocodilian growth due to the seasonal changes. Therefore crocodiles are ectothermic and they regulate their body temperature, by warming themselves in the sun and cooling in shaded places. The basking times hit the highest point before and after the midday (Cott, 1961; Modha, 1968). Crocodiles are nocturnally aquatic (Pooley, 1982), although they may be found on ground moving between habitats or lying on banks, on warm humid evenings (Pooley, 1982). Evenings are spent in the water, where body temperature is buffered by the cooler air temperatures, after which they hunt. Crocodilian growth slows during colder, winter months, and growth rates increase again with increased ambient temperatures (Sah and Stuebing, 1996). Ambient temperature plays an important role in growth rate (Hutton, 1987a) where sub-optimal temperatures can cause lower feeding and digestion rates. Which means that juveniles actually "get smaller" during the cooler period of the year and they decrease by 1.0-4.0 mm in total length per month (Chabreck and Joanen, 1979). In addition to ambient temperatures, fluctuating water levels, the way in lake Nasser, have a profound effect on the distribution and behaviour of wild crocodile populations (Pooley, 1982). Seasonal flooding allows crocodiles to exploit new habitats and food sources, this combined with warm weather normally results with increase in the growth rates (Hutton, 1987). This case resembles or is in harmony with other cases in South Africa, such as the lake Nasser's characteristics in general.

The aim of the study was to assess various morphometric and growth-rate parameters of the lake Nasser crocodile population and compare these to each other. Animals ranged in between 0.3/0.33 to 2.0 m total length were regularly capture, marked and release. Over one year of regularly studies and monthly trips to the lake Nasser, heads and body measurements were taken from crocodiles at the time of the capture. Generally these morphometric data were collected to allow estimation of animal size via heads and bodies measurement, and to allow quantitative analysis of functional allometric growth in relation to species ecology (Hutton, 1987a).

On the other hand, we can say that the morphometric relationships let the prediction of size and situation of crocodilians in the wild and perform a basic and practical need arising from research on the ecology of crocodilians (Hutton, 1987b). Furthermore the animals' physiological state is associated with its ability to survive as an individual and also has direct significance in terms of its reproductive achievement (Jakob et al., 1996).

MATERIALS AND METHODS

Study area (lake Nasser)

The fieldwork in the lake Nasser was conducted on 16 October, 2009 for the first trip and towards the late August, 2010 for the last trip. We censured the crocodile population using the spotlight surveys (Bayliss, 1987). The spotlight surveys were conducted, based on Boat-counts used to establish the number of Nile crocodiles in this lake. A five meter fiberglass-boat fitted with an outboard motor 25 hp Yamaha was used for each counting. The boat was always operated at an average speed of about five-15 kph 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, to approach the crocodile slowly to estimate its size.

The survey began after 15 to 30 minutes after sunset or as soon as the light became dark enough to use the spotlights. 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 fist observers. This estimation was based on experience and hard work, a lot of field works and crocodile survey trips in the lake Nasser (Platt et al., 2004; Salem 2006, 2009). During the survey, the beginning and endpoints of each survey route, and the distance traversed each crocodile sighting were logged with a Garmin etrix®GPS 12.

Capture-recapture surveys

Nile crocodiles were captured using a modified version of the methods described by Chabreck (1965a), Kofron (1989a) and the Florida Fish and Wildlife Conservation Commission (2003). These animals were all captured for a specific purpose, such as obtaining some morphometric measurements. Animals identified for capture were approached with a boat and captured with a steel snare attached to a 12 mm climbing rope. The standards self-locking were #1S-40 Snares, #2XX-72 and Snares #3XX-120 Snares. Thompson steel snares, supplied by Thompson Snares (Lynnwood, Washington, USA), were attached to the climbing rope by a steel coupling. The snare was kept open by stretching it over a Y-shaped frame attached to a four meter pole. The snare was positioned just behind the head of the crocodile and pulled tight. The self-locking mechanism on the snare prevented the crocodile from opening the snare and escaping. Crocodiles less than 2.0 m in TL were pulled into the boat, while bigger animals were pulled onto the shore. All crocodiles caught were physically restrained without the use of narcotics and were released within 15 minutes of being captured.

Capture-recapture surveys are the most important technique for estimating detection probability for individual animals in the study area (Williams et al., 2001). Additionally, recapture data can be used to calculate population parameters (i.e. survival), morphometrics of growth, body condition, reproduction, and basic biometrics. Animals were captured on all surveys using standard crocodilian capture techniques. Captured animals were assessed for several predetermined characters.

Animals were marked via two methods. The first utilizes caudal scale notching to mark each individual with a unique, three-digit code. The second utilizes natural tail markings to assign a unique, 20-40 digit code to each animal (Swanepoel, 1996). The advantage of the second method is that for sighted/resighted animals only a photograph of the tail must be taken to determine its individual identification. Crocodiles were captured nocturnally using a number of size-dependant capture techniques (Salem, 2010). Once captured and restrained, a number of morphometric measurements were taken and the animals were released within 10-15 minutes at the site of capture.

Morphometric comparisons

Morphometric measurements and abbreviations recorded are listed below (Webb et al., 1978; Leslie, 1997): 1. Total length (TL): distance from the tip of snout to tip of tail ventrally; 2. Snout-vent length (SVL): distance from the tip of snout to the posterior of first scale row after cloaca; 3. Neck Girth (NG)/Neck circumference (NC): circumference of neck at level of nuchal rosette (0.5 cm); 4. Tail girth (TG)/Base of tail circumference (BTC): circumference of the tail, immediately posterior to cloaca (0.5 cm); 5. Vent (V): measure of the cloacal length; 6. Hind Foot (HF): measure of the foot length from the heel to the tip of claw of the longest digit (one mm); 7. Chest Girth (CG): circumference of chest just posterior to front legs in cm; 8. Head width (HW): the maximum width of the head at the posterior margin of the quadratojuagals or at the level of jaw articulation; 9. Head depth (HD): maximum depth of head (0.1 cm); 10. Head length (HL): the distance from tip of snout to the median hind edge of supra-occipital bone (0.1 cm); 11. Total body mass (g); 12. Water depth (WT): water depth at capture site in cm; 13. Sex: male or female, M/F; 14. Sediment thickness (MD): measured from water surface to the rock bottom subtracting water depth in cm; 15. Cap Method (CM): capture method (snare, hand, tang, harpoon); 16. Habitat Description: at each capture site short notes of habitat had been recorded, as well as the structure of vegetation of the area.

Although SVL is the most variable parameters for details of animal's history, TL is the most important measure for estimation of animal's total length in the field and making some comparison between species and individuals in the natural habitats (Cott, 1961). Furthermore, the body mass is also important for other life history parameters, therefore the animals are scaled by mass (Western, 1979). Measurements one-seven were recorded using a standard plastic/fixable tape measure. Total length (TL), snout-vent length (SVL) and vent (V) were measured ventrally. Measurements 8, 9 and 10 were made using vernier calipers, sized according to the size of the animals. Animals were weighed using size-dependent Pesola spring scales and portable electronic fish scale (ANGYU) down to one decagram levels. Larger animals were not weighed due to the logistical difficulties. The crocodiles were assigned to classes based on their size, in this case based on the TL (Salem, 2010).

Morphometric measurements were compared between males and females (all size classes). These analyses took the form of linear and multiple regressions, using sex and location as "replica" variables. Although HL was used as a standard measure of body length in the past, as reported by Hutton (1984), the close correlation in this study between SVL and TL (R = 88.1% – 99.6%, where the very rare animals with damaged tails and obvious errors in recording had been removed) lends confidence to SVL. Therefore SVL was used in the analyses.

The following were measured by each group for every crocodile: head length (HL), snout-vent length (SVL), total length (TL), right hind foot length (RHF), neck girth (NG), tail girth (TG), chest girth (CG), and mass. HL was measured dorsally, while SVL and TL were measured ventrally. A measurement kit was provided with every crocodile that contained: a clipboard, pencil, string for measuring tail girth between scutes, a Pesola scale/portable electronic fish scale, and a flexible centimeter sewing tape. These kits were kept with the crocodile so that the same equipment would be used. Measurements were made with the flexible sewing tape to the nearest 0.1 cm. Mass was measured with 10-40 kg portable electronic fish scale (ANGYU) to the nearest 0.01 kg. The scales were calibrated before use, with a weight of known mass. Crocodiles were also marked individually with numbers of three size digit tags.

Temperature

The average temperature of the lake Nasser was calculated during the field work between 16 October, 2009 (the first trip) to the late August, 2010 (the last trip), using temperature data collected at the time of the capture of each crocodile during the mark-recapture experiment using a Digital Hydro-Thermometer. The temperature of the water was measured approximately 40 cm. below the surface, at the side of the boat. Average monthly temperatures, measured between the hours of 22:00 and 03:00, were used to standardize the water temperature for the duration of the project.

Water depth at time of the capture or sighting

Water depth during the field work was obtained during the capture and sighting of crocodiles in the lake Nasser manually, using water level tapes (max length 100 m). On the other hand, water level was obtained from the lake Nasser authorities, irrigation authorities and other sources. As shown in the flowing diagram in between 1964 to 2010 (Fig. 1).

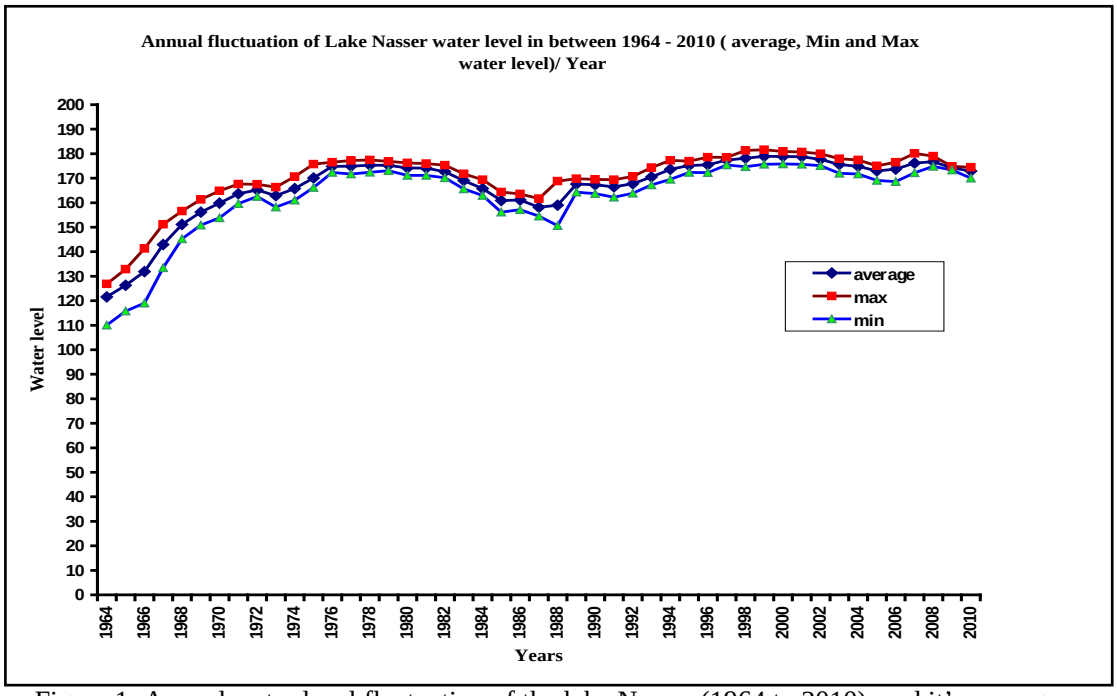


Figure 1: Annual water level fluctuation of the lake Nasser (1964 to 2010), and it's average, minimum and maximum fluctuation values.

RESULTS

Morphometric data

A total of 528 + 81 (81 hatchling crocodiles caught, violating the environmental law and returned to the lake, including one in Luxor Airport and 80 in Red Sea airport Egypt 2010) crocodiles were sighting. 61 crocodiles were captured between October, 2009 and August, 2010. Yearlings made up the majority of the crocodile individuals, reflecting their natural dominance in the population (Tabs. 1a, b) Male and female hatchlings were pooled due to the difficulties. In determining the sex of hatchlings in the field, non-lethal techniques were used (Tab. 1). The number of crocodile individuals captured per size class from 2009/2010 was determined, from the lake Nasser region, by using nocturnal, and boat based techniques. Hatchling's sex could not be determined reliably in the field using non-lethal methods.

Regression analysis

Simple linear regression equations were computed, expressing the relations between TL and VL, HFL, NG, TG, CG, HD, V and W, and SVL and TL, HFL, HFL, NG, TG, CG, HD, V and W.

Snout-vent length – Total length relation

Crocodile hatchlings were removed from the analysis due to the low increase in snout-vent length with increase in the total length than the other class sizes. For the specific study between the total length and snot vent length relationship, the slope of other size show highly significant relationship ($r = 0.996$, $P < 0.001$), which indicates a significantly higher increase in SVL relative to the increase in TL as animals grew longer. Male and female adult individuals showed significantly different rates of increase and intercepts, with males showing a relatively higher increase of SVL: TL than females. In this specific scientific study, the intercept for females was however non-significant.

Snout-vent length relationships: Head length

A significant relationship was found between head length and snout-vent length for all size, with the HL increasing at a higher rate relative to SVL in smaller animals (Fig. 2). The HL: SVL regression slope decreased step-wise as crocodiles grew, with adults having the lowest head-length increase relative to SVL ($r = 0.986$, $p < 0.001$). Hatchling head length measurements showed more variation than other size and so the intercept for this regression was not significant.

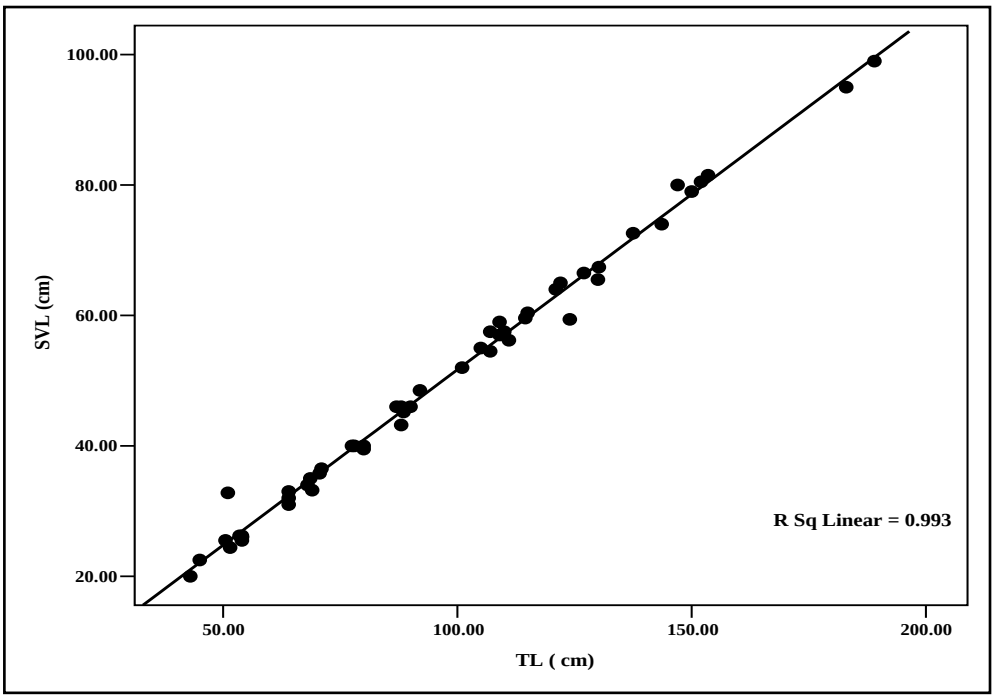


Figure 2: Linear regression between snout-vent length and total length.

Snout-vent length – head length relation

A significant relationship was found between head length and snout-vent length for all sizes ($r^2 = 0.986$, $p < 0.001$) (Fig. 3). Hatchling head length measurements showed a greater variation than other sizes and so the regression was not significant.

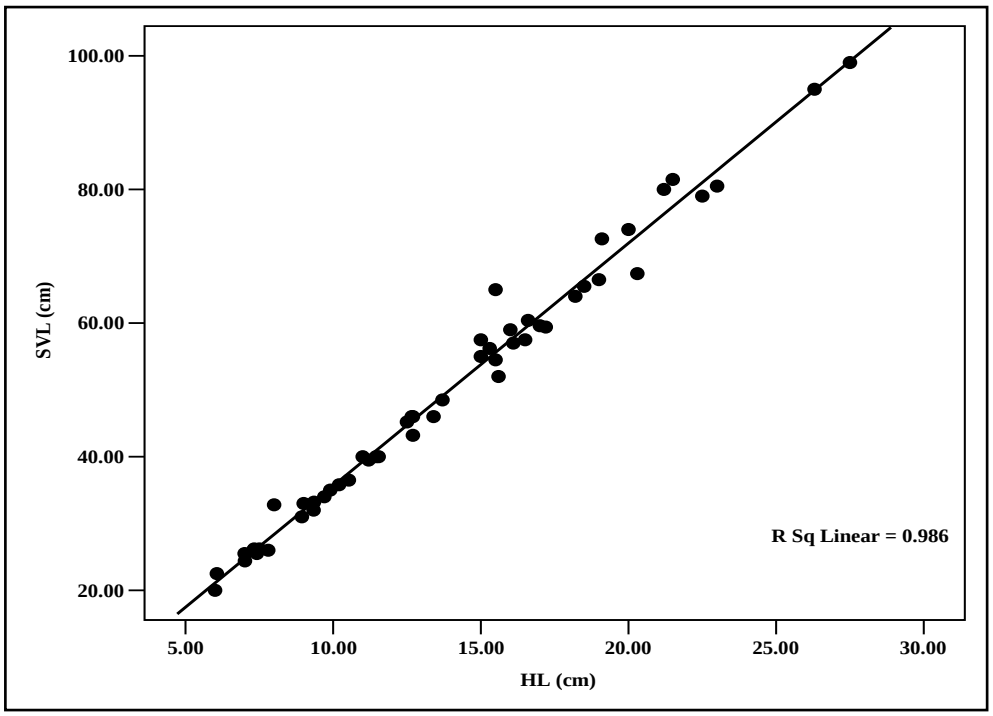


Figure 3: Linear regression between snout-vent length and head length.

Snout-vent length – head depth relation

The regression between HD and SVL shows that larger animals have significantly deeper heads than smaller animals ($r^2 = 0.975$, $p < 0.001$, Fig. 5).

The regression trends for juvenile and subadults sizes show significant variation in the rate of increase of HD with increasing SVL. The rate of increase of HD with increasing SVL did not differ between sexes ($p > 0.05$).

Table 1: Crocodile morphometric measurements.

SVL (cm)		TL	HL	HW	HD	NG	TG	CG
Trip 1	1	110	15	7.22	5.36	22	24.5	28.5
	2	50.5	7	3.4	2.4	10	10.3	11.5
	3	43	6	3	2.01	8.5	8.5	11
	4	147	21.2	9.5	7.6	32	35	35
	5	109	16	7	5	22	24	22.5
Trip 2	6	51	8	3.15	2.51	10	10.5	9.8
	7	54	7.3	3.35	2.41	11.3	11.5	11
	8	77.5	11	5.29	3.78	15	18	16
	9	80	11.5	5.29	3.72	16.5	18	17
	10	71	10.5	4.83	3.39	15	16	14
	11	78	11.4	5.21	3.42	15.5	17	16
	12	54	7.41	3.48	2.16	10.5	11	10
	13	45	6.06	3.10	2.08	9.1	9	7.8
Trip 3	14	54	7.5	3.63	2.36	10.3	11	10.8
	15	101	15.6	6.98	4.82	20.5	23.6	22.6
	16	87	12.6	6.85	4.33	19.5	21.4	21.5
	17	64	8.94	4.29	3.2	12.7	14.3	13.2
	18	107	15.5	6.63	4.92	21	21.5	22.7
	19	70.6	10.2	4.73	2.9	14	14.8	14.6
	20	152	23	11.1	8.4	36	37.8	39
	21	150	22.5	10.2	6.41	30.2	34	34.2
	22	130	20.3	9.19	6.45	28	30.7	29.4
Trip 3	23	88.5	12.5	5.58	3.72	16	18.2	17.4
	24	68.6	9.9	4.73	2.89	13.4	14	13.5
	25	88	13.4	6	4.17	17.8	19.2	18.7
	26	80	11.2	4.81	3.50	15.3	16.5	15.5
	27	54	7.8	3.66	2.33	10.4	11.3	10.5
	28	88	12.7	5.76	3.65	17.3	18.2	18.4
	29	127	19	8.73	6.23	27	27.8	28
	30	109	16.1	7.63	5.31	23.7	25.5	23.9
	31	68	9.7	4.46	2.89	13	13.3	13.3
	32	189	27.5	11.8	9.53	41	47	45
	33	114	17	7.57	5.25	22.7	25.9	24
Trip 4	34	90	12.7	6.02	4.22	17.2	17.7	17.5
	35	115	16.6	8.04	5.22	21	23.2	22.4
	36	107	16.5	7.69	4.77	22.3	23.3	22.2
	37	64	9.33	4.20	2.9	11.8	13.2	12.3
	38	183	26.3	11.6	9.57	40.1	43	40.2
	39	53.5	73.2	3.49	2.34	9.6	9.7	9.7
	40	144	20	9.49	6.62	26.7	30.7	28.7
Trip 5	34	90	12.7	6.02	4.22	17.2	17.7	17.5
	35	115	16.6	8.04	5.22	21	23.2	22.4
	36	107	16.5	7.69	4.77	22.3	23.3	22.2
	37	64	9.33	4.20	2.9	11.8	13.2	12.3
	38	183	26.3	11.6	9.57	40.1	43	40.2
	39	53.5	73.2	3.49	2.34	9.6	9.7	9.7
	40	143	20	9.49	6.62	26.7	30.7	28.7
Trip 6	41	69	9.34	4.44	3.03	12.3	12.8	12.1
	42	64	9	4.26	2.91	12.6	12	12.6
	43	121	18.2	8.53	5.93	25	25.7	25.4
	44	153	21.5	10	7.29	32	34	34.6
	45	137	19.1	9.43	6.39	27.3	29.6	28.5
	46	92	13.7	6.35	4.21	16.7	19.2	18
	47	111	15.3	7.57	5.03	22	25	24
	48	51.5	7.01	3.29	2.14	8.5	9.5	8.6
	49	105	15	7.02	4.87	20.2	23	22.4
	50	130	18.5	8.80	6.48	27	30	28
	51	124	17.2	8.064	5.778	24.4	26.5	27.3
51-60 hatchling			53					
	61	122	15.5	8.97	6.205	26.8	28.4	29.3
*81		≈ 33/35	*81 hatchling crocodiles were caught in violation of environmental laws; had been returned to lake.					

Table 2: Crocodile morphometric measurements.

	No.	W. kg	Vent	Sex	SVL	HF	A.T.	W.T.	W.D.
Trip 1	1	3.71	2.2	F	57.5	21	26.3	28.6	150
	2	0.35	1.75	M	25.5	10	31	27.5	0
	3	0.19	1.65	M	20	7.5	25.6	27.9	20
	4	10.5	3.35	F	80	25	29.3	27.5	20
	5	3.81	2.2	F	59	18	28.5	27.6	25
Trip 2	6	0.66	1.8	M	32.8	4.3	22.5	22	60
	7	0.36	1.5	M	26	4	22	22.5	40
	8	1.33	2.1	F	40	6.2	22	22	50
	9	1.35	0.8	M	40	8	24.4	22.32	0.6
	10	0.99	2	F	36.5	6.5	20.1	22.5	0.25
	11	1.17	2.1	F	40	6.6	20.4	22.5	0.3
	12	0.34	1.3	M	25.5	4.5	21.5	22.5	0.25
	13	0.24	1.7	M	22.5	3.7	20	22.8	0.4
Trip 3	14	0.39	1.9	F	26.2	4.6	26.5	24.2	0.4
	15	3.18	2.2	F	52	8.7	27.1	25	0.25
	16	2.14	2.2	F	46	6.7	27	24.1	0.5
	17	0.71	1.5	M	31	5.3	26	24	0.3
	18	3.12	2.2	F	54.5	8.6	26	24.2	0.5
	19	0.92	1.8	M	35.8	5.4	27	25	0.45
	20	12.5	3.6	F	80.5	12.5	25.6	26.2	0.9
	21	10.3	3.1	F	79	13	25.6	26.2	1.3
	22	7.36	2.9	F	67.4	11.6	24	26.7	1
Trip 3	23	1.68	2.6	F	45.2	7.1	27.6	26.5	0.7
	24	0.77	1.3	M	35	5.4	33.5	29.9	1
	25	1.92	1.7	F	46	7	31.5	27.5	0.25
	26	1.2	1.8	M	39.5	6	29.7	28	0.5
	27	0.4	0.9	M	26	4.3	27.6	28.2	0.3
	28	1.7	2	M	43.2	6.2	27.4	27.6	0.25
	29	6.42	3.2	F	66.5	10	27.4	27.6	1
	30	4.6	2.8	M	57	8.3	27.4	27.6	0.6
	31	0.87	1.7	M	34	5.7	27.4	26.6	0.5
	32	–	3.6	F	99	14.6	27.4	26.6	1.5
	33	4.76	2.2	M	59.6	8.6	27.4	26.6	0.9
Trip 4	34	1.82	2.1	M	46	6.4	28.3	27.8	60
	35	3.88	2.3	M	60.4	10	29	28	29.4
	36	3.83	3.5	M	57.5	9	29	30	0.5
	37	0.65	1.8	M	32	5.2	29.1	29.4	0.78
	38	20.6	3.7	F	95	14.4	28.3	27.8	1
	39	0.35	1.6	M	26.2	4.2	32.4	29.9	0.5
	40	7.93	3	F	74	11.6	30.7	28.8	0.6
Trip 5	34	1.82	2.1	M	46	6.4	28.3	27.8	60
	35	3.88	2.3	M	60.4	10	29	28	29.4
	36	3.83	3.5	M	57.5	9	29	30	0.5
	37	0.65	1.8	M	32	5.2	29.1	29.4	0.78
	38	20.6	3.7	F	95	14.4	28.3	27.8	1
	39	0.35	1.6	M	26.2	4.2	32.4	29.9	0.5
	40	7.93	3	F	74	11.6	30.7	28.8	0.6
Trip 6	41	0.73	1.3	M	33.2	5.6	30.2	28.4	40
	42	0.58	1.6	M	33	4.9	30.5	28	40
	43	5.18	2.5	F	64	10	33.8	29.6	0.5
	44	11.48	3	F	81.5	12.2	28	28.2	0.6
	45	7.86	3.1	F	72.6	10.3	32	29	0.4
	46	2.8	2	M	48.5	7.5	32	28.8	0.5
	47	4.52	2.2	F	56.2	8.6	34.1	29.3	0.8
	48	0.35	1.2	M	24.4	3.6	34.3	29.7	0.65
	49	3.26	2.3	M	55	9	31.5	30	0.45
	50	6.94	3.1	F	65.5	10.2	31.5	30	1
	51	5.62	2.4	F	59.4	10.1	31.6	30.2	0.75
51-60 hatchling					17.5				
	61	122	15.5	8.97	6.205	26.8	28.4	29.3	6.4	2.3	F	65	10.5	35.2	30.5	0.38
*81		≈ 33/35			*81 hatchling crocodiles were caught in violation of environmental laws; had been returned to lake.											

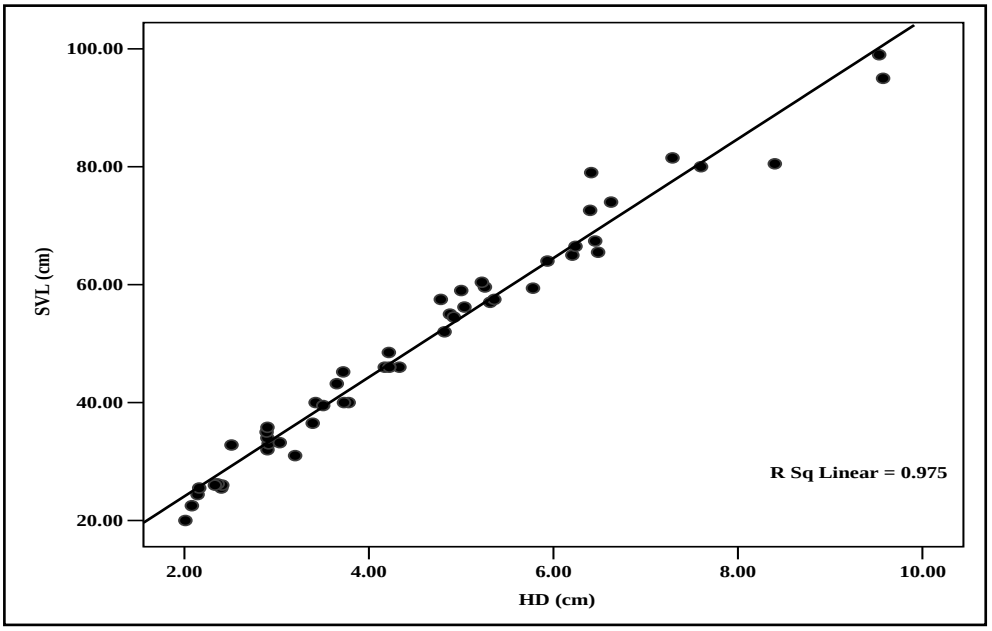


Figure 5: Linear regression between snout-vent length and head depth.

Snout-vent length – head width relation. A significant relation was found between SVL and HW, (Fig. 3) (r^2 = 0.978, $p < 0.001$). The slope of the regression shows a rapid increase in snout vent length width with the increase of head length; however that may depend on some factor, such as the food source, which is not a problem in our case.

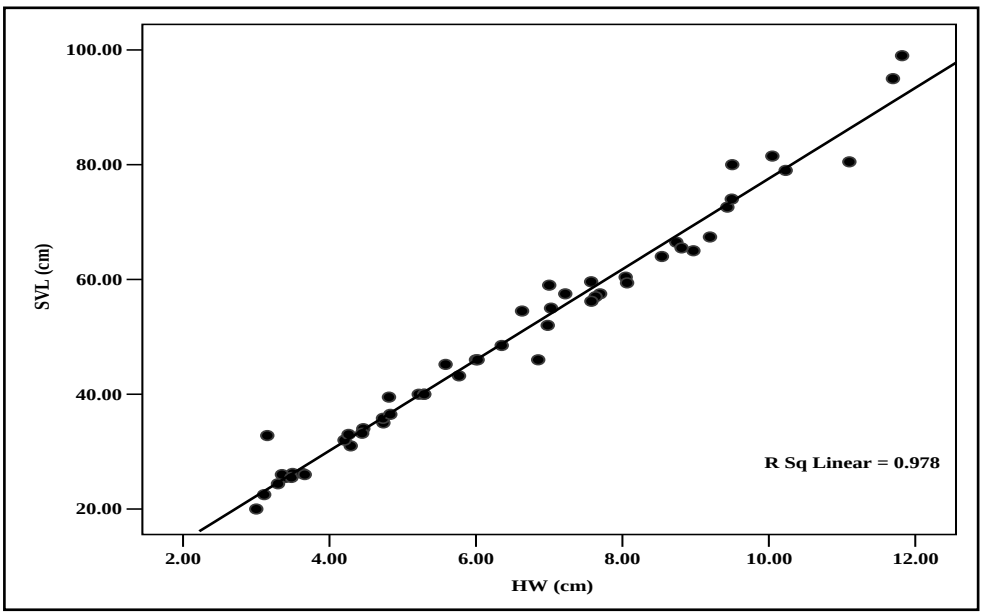


Figure 4: Linear regression between snout-vent length and head width.

Snout-vent length – tail girth relation

The regression analysis of the relation between the tail girth and snout-vent length shows a highly significant relation ($r^2 = 0.978$, $p < 0.001$, Fig. 6).

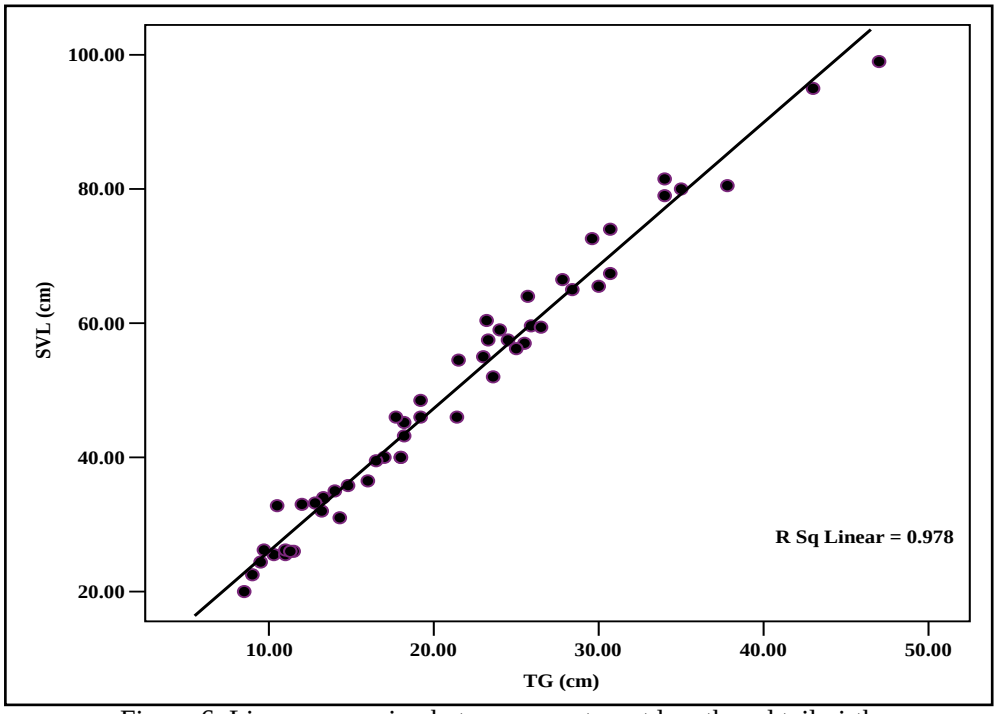


Figure 6: Linear regression between snout-vent length and tail girth.

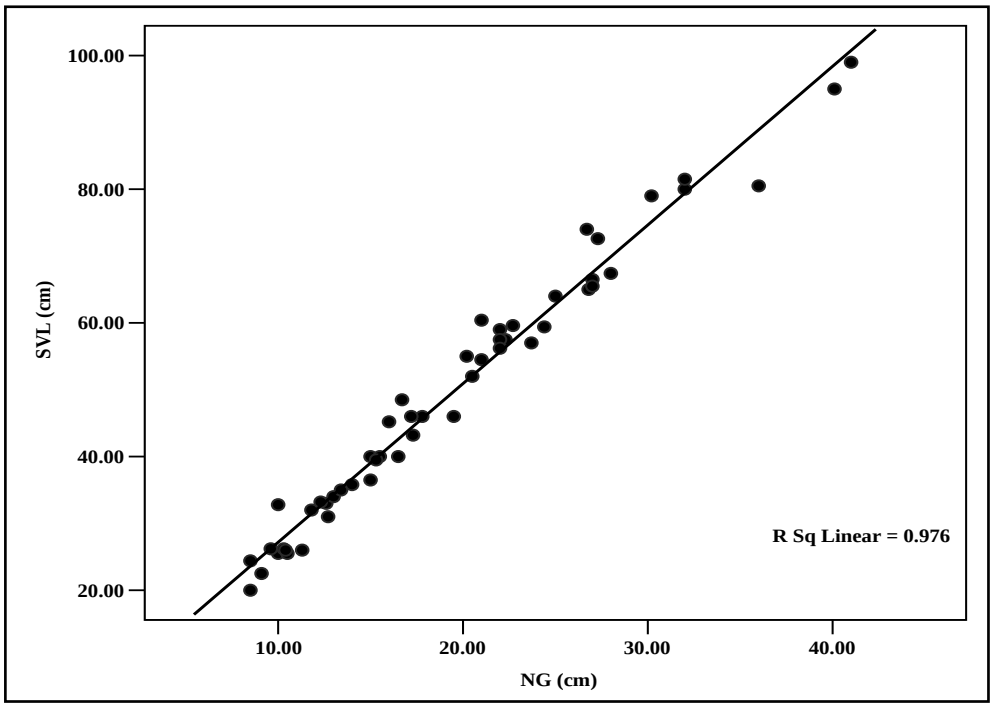


Figure 7: Linear regression between snout-vent length and neck girth.

Snout-vent length – chest girth relation. The regression analysis of the relation between chest girth and snout-vent length, shows a high significant relation ($r^2 = 0.966$, $p < 0.001$, Fig. 8).

Snout-vent length – body mass relation. The regression analysis of the relation between the body mass – snout-vent length is presenting a highly significant relation between the two parameters ($r^2 = 0.848$, $p < 0.001$). This relation, as we discussed before, may be dependent on the nutrients and the amount of food, and on the time of feeding; this relation is also dependant on location, or the location and time play a significant role in that case.

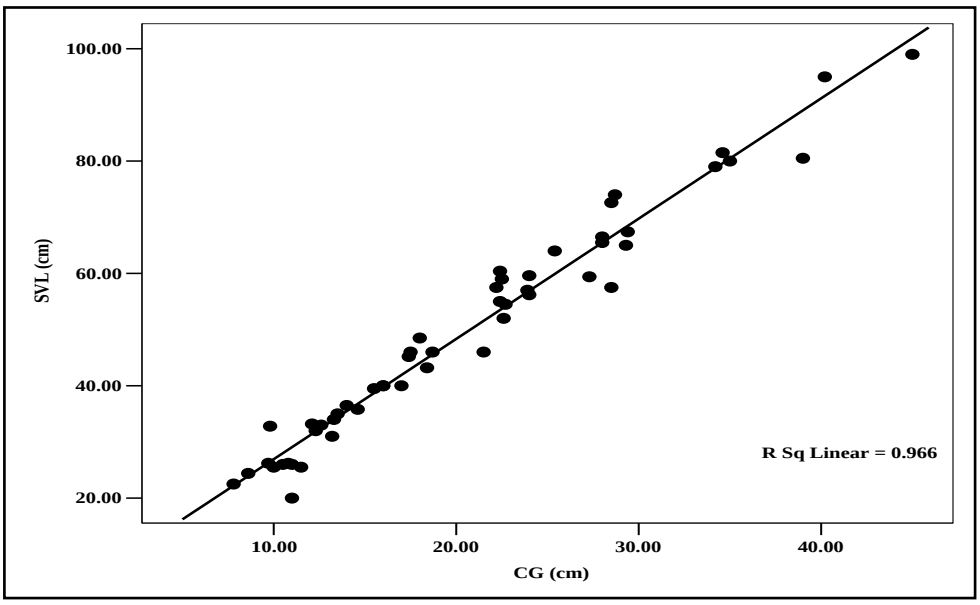


Figure 8: Linear regression between snout-vent length and chest girth.

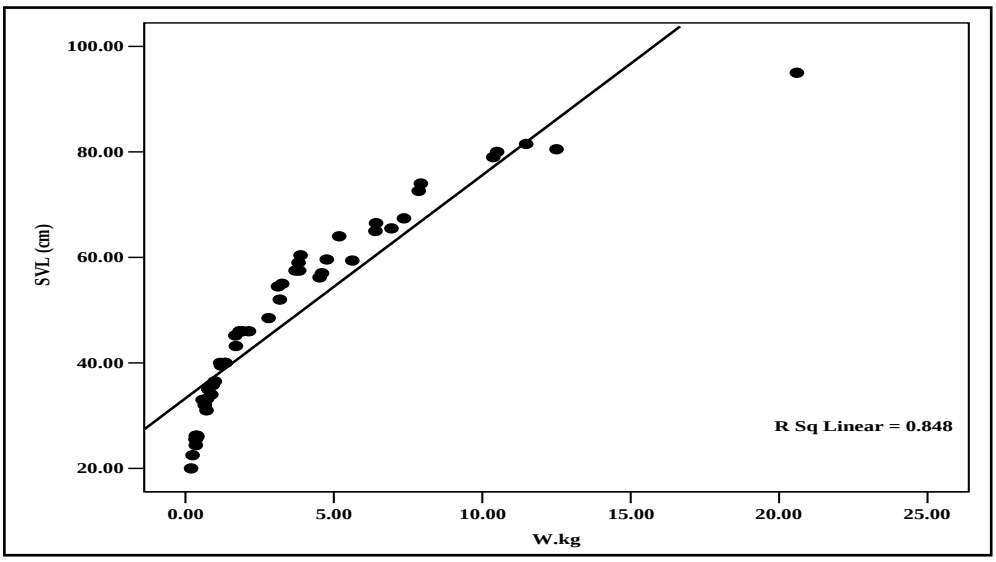


Figure 9: Linear regression between snout-vent length and body mass.

Snout-vent length – vent relation. The regression analysis of the relation between the Vent and the snout-vent length presents a significant relation ($r^2 = 0.783$, $p < 0.001$, Fig. 10)

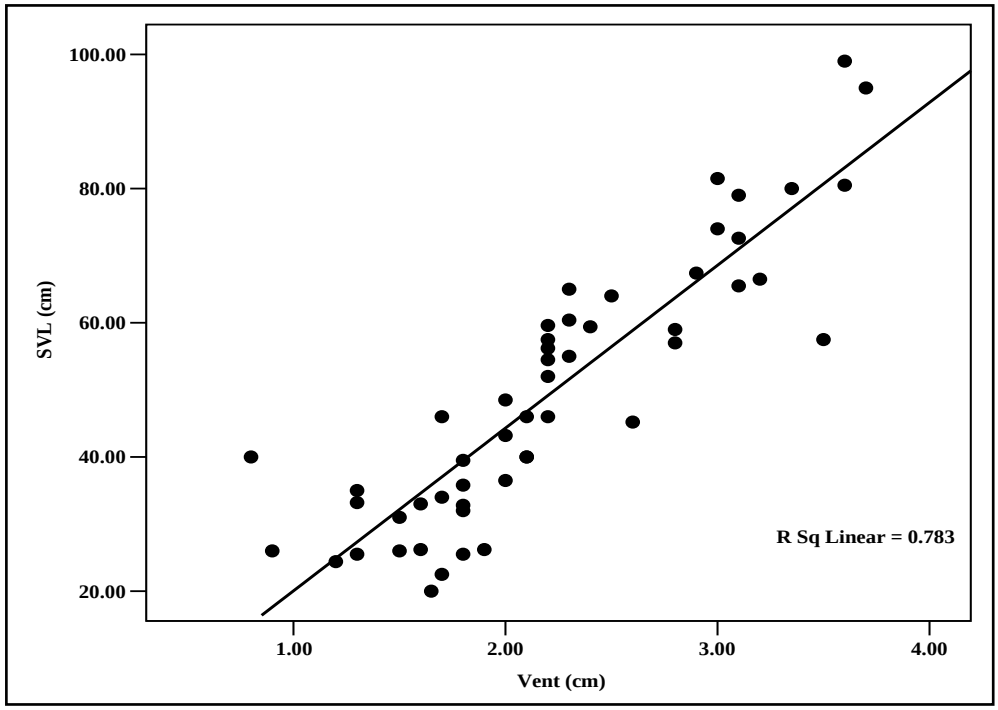


Figure 10: Linear regression between snout-vent length and vent size.

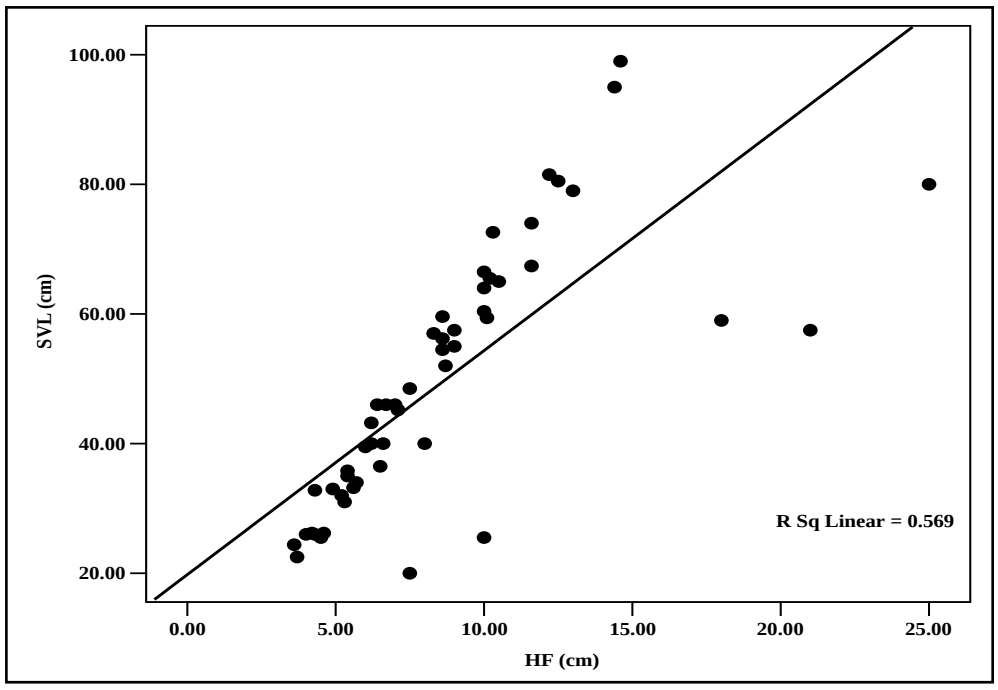


Figure 11: Linear regression between snout-vent length and hind foot length.

Hind foot length – snout-vent relation. The regressions analysis shows a moderate relationship ($r^2 = 0.569$, $p < 0.001$, Fig. 11).

Relations between total length and the other parameters

Lake Nasser population showed a significant relation between head width and total length for all sizes, with TL increasing relative to HW ($r^2 = 0.98$, $p < 0.001$, Fig. 12). Hatchling head length measurements showed more variation than other sizes and so the regression was not significant.

There was also a significant relation between TL and HD of Nile crocodiles from lake Nasser, presented in figure 13 ($r^2 = 0.977$, $p < 0.001$). On the other hand, the regression between TL and NG ($r^2 = 0.978$, $p < 0.001$, Fig. 14) shows a highly significant relation, which means that as the animals increase in total length, the neck girth increased rapidly; however, that may depend on some factors such nutrients, but in our case the nutrients in lake Nasser are not a problem for animal growth.

The regression analysis of the relation between the tail girth and total length shows a highly significant relation ($r^2 = 0.982$, $p < 0.001$, Fig. 15). On the other hand, the regression between total length and chest girth, also shows a high significance ($r^2 = 0.968$, $p < 0.001$, Fig. 16), while the relations between the total length and the vent size ($r^2 = 0.763$, $p < 0.001$, Fig. 18) and, respectively, the hind foot length ($r^2 = 0.548$, $p < 0.001$, Fig. 19) are moderately significant; the significance is high in the case of the body mass and the total length relation ($r^2 = 0.852$, $p < 0.001$, Fig. 17), which means there were no problem in the nutrients/food sources for crocodiles in lake Nasser.

Table 3: Regression results for Total length relationship with measurement parameters (SVL, HL, HW, HD, NG, TG, CG, W.Kg, Vent and HF).

Animal parameters		The relationship	r	P Value
TL	SVL (cm)	Snout-vent length: total length	0.996	$p < 0.001$
	HL (cm)	Head length: total length	0.994	$p < 0.001$
	HW (cm)	Head width: total length	0.990	$p < 0.001$
	HD (cm)	Head depth: total length	0.988	$p < 0.001$
	NG (cm)	Neck girth: total length	0.989	$p < 0.001$
	TG (cm)	Tail girth: total length	0.991	$p < 0.001$
	CG (cm)	Chest girth: total length	0.984	$p < 0.001$
	Weight (kg)	Body mass: total length	0.923	$p < 0.001$
	Vent (cm)	Vent: total length	0.873	$p < 0.001$
	HF (cm)	Hind foot length: total length	0.740	$p < 0.001$

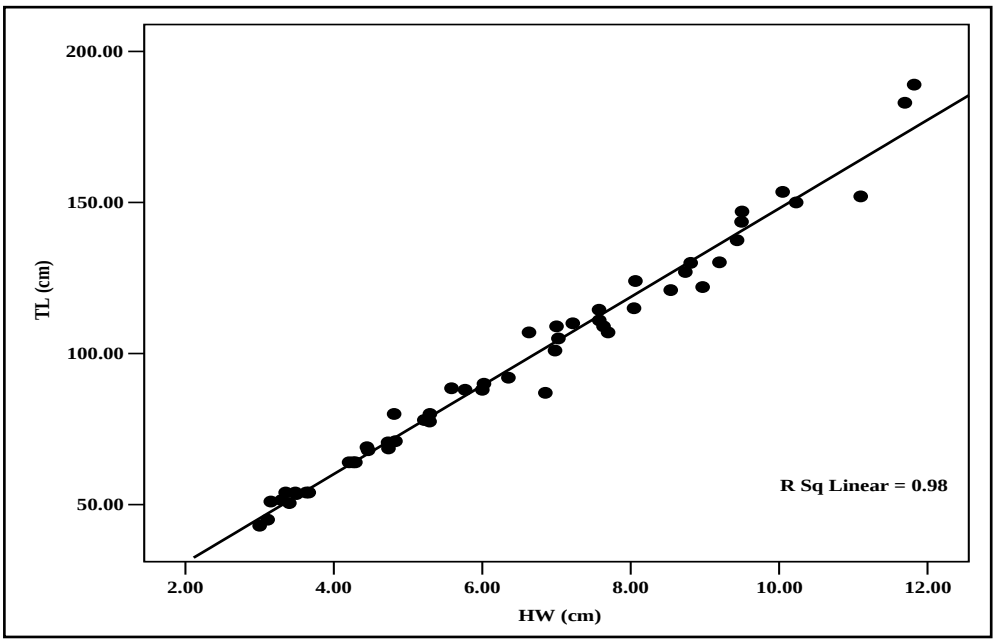


Figure 12: Linear regression between total length and head width.

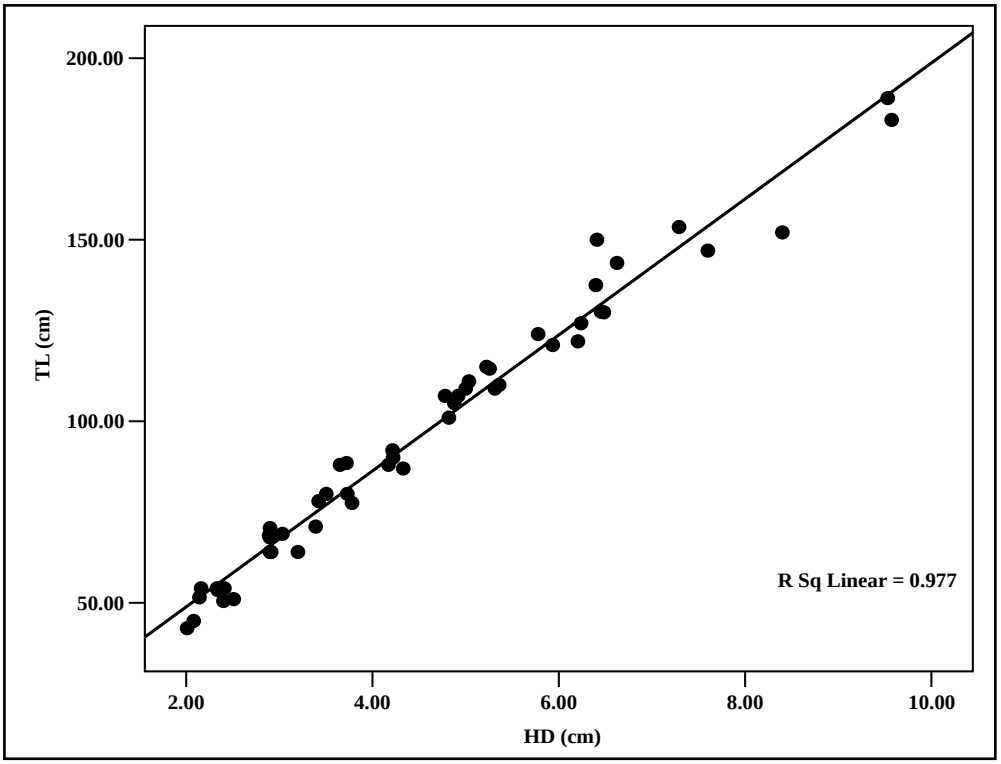


Figure 13: Linear regression between total length and head depth.

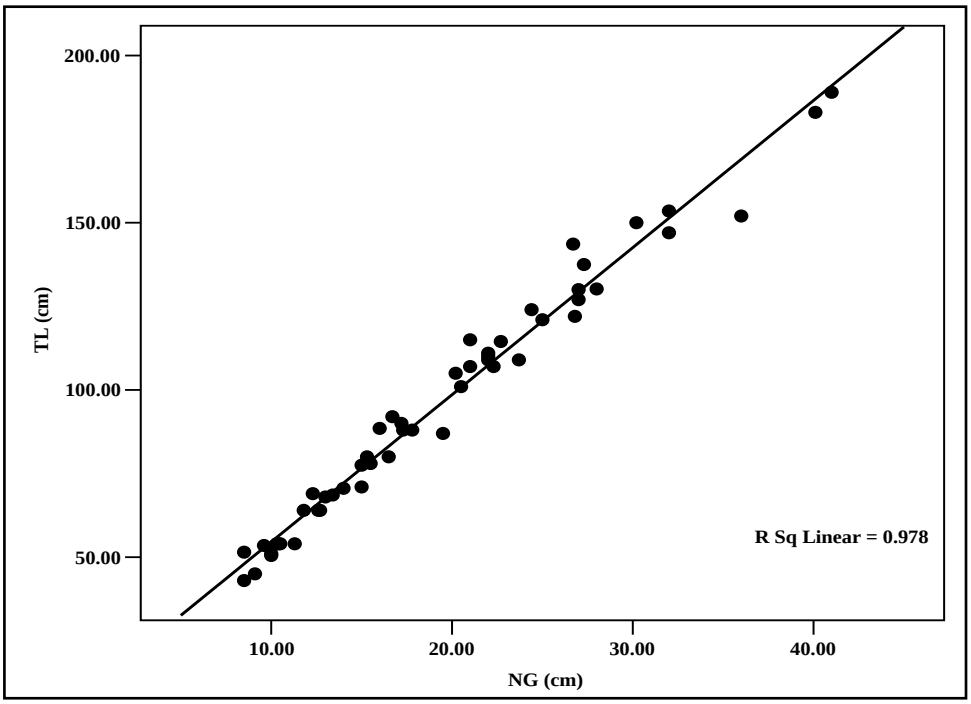


Figure 14: Linear regression between total length and neck girth.

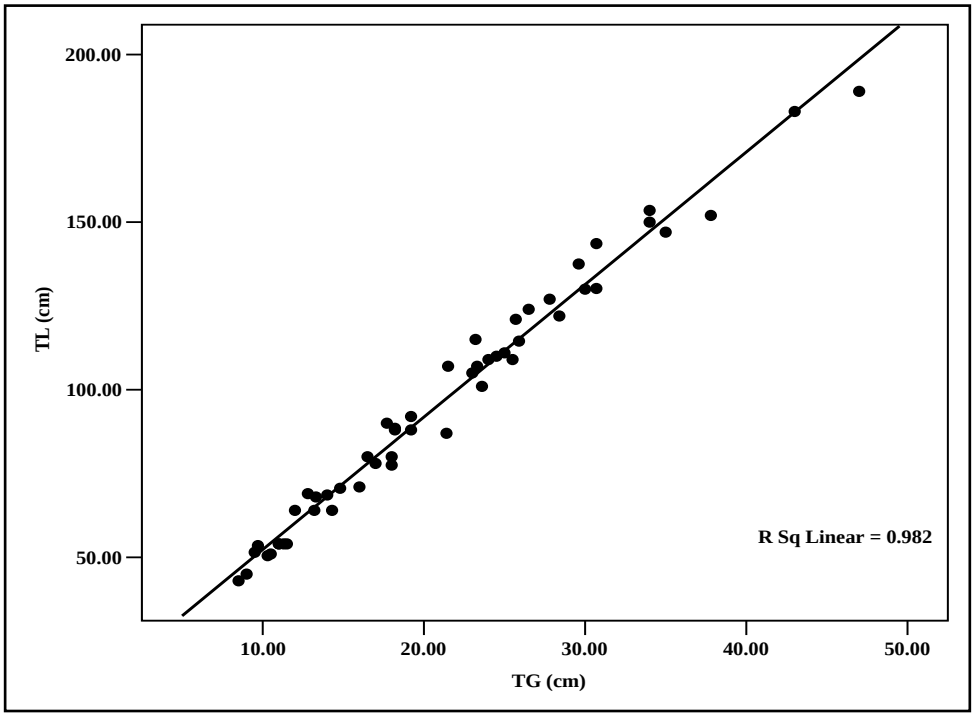


Figure 15: Linear regression between total length and tail girth.

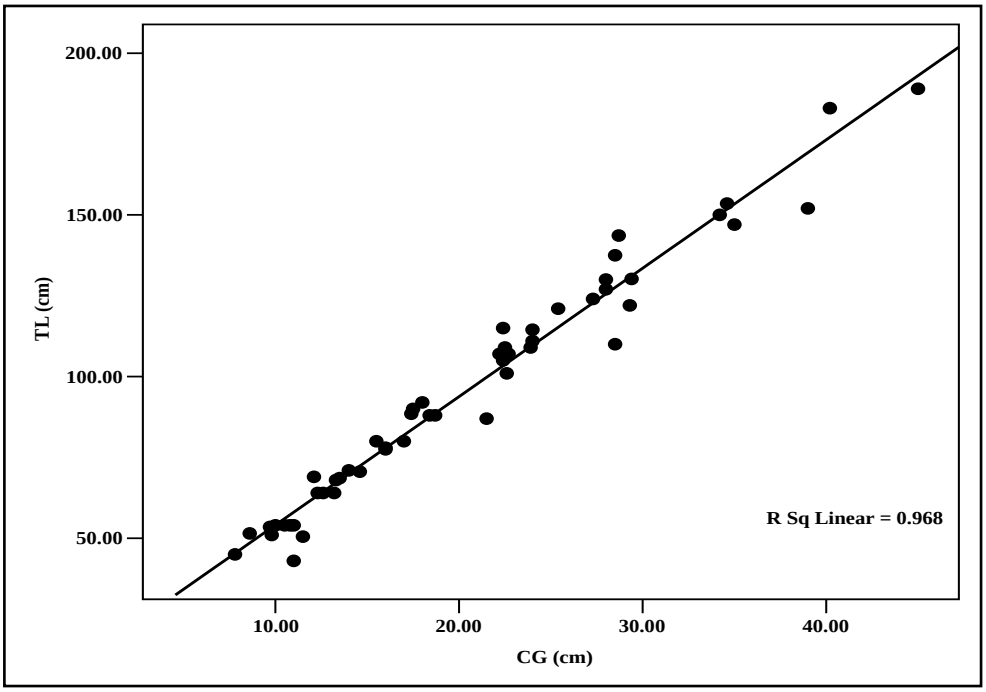


Figure 16: Linear regression between total length and chest girth.

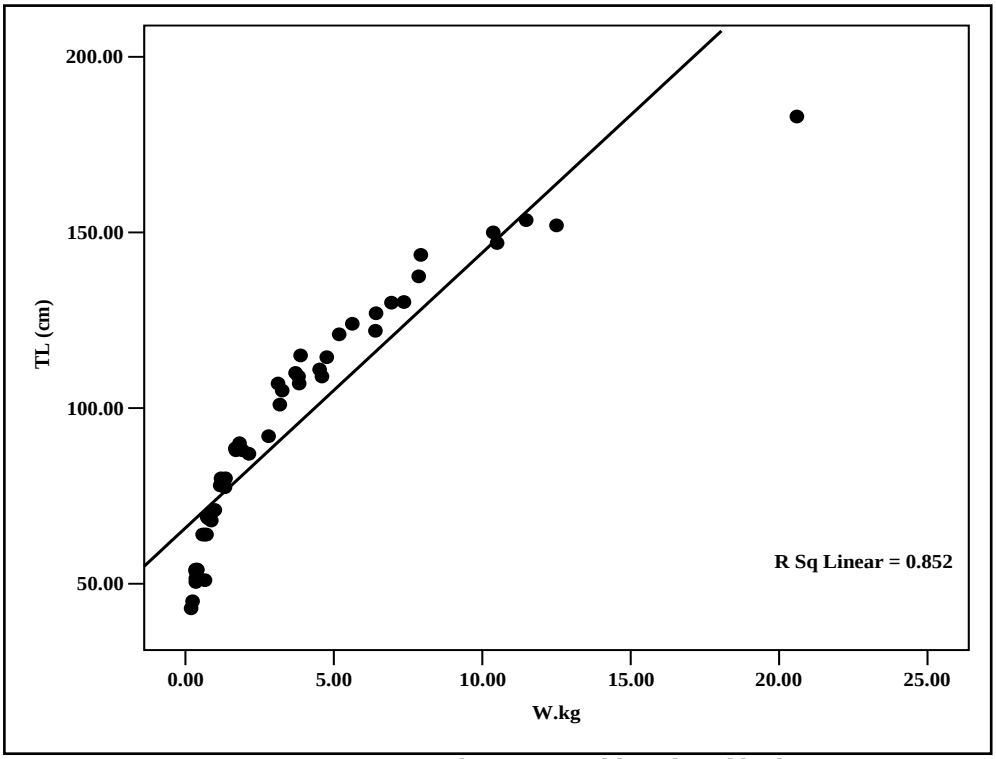


Figure 17: Linear regression between total length and body mass.

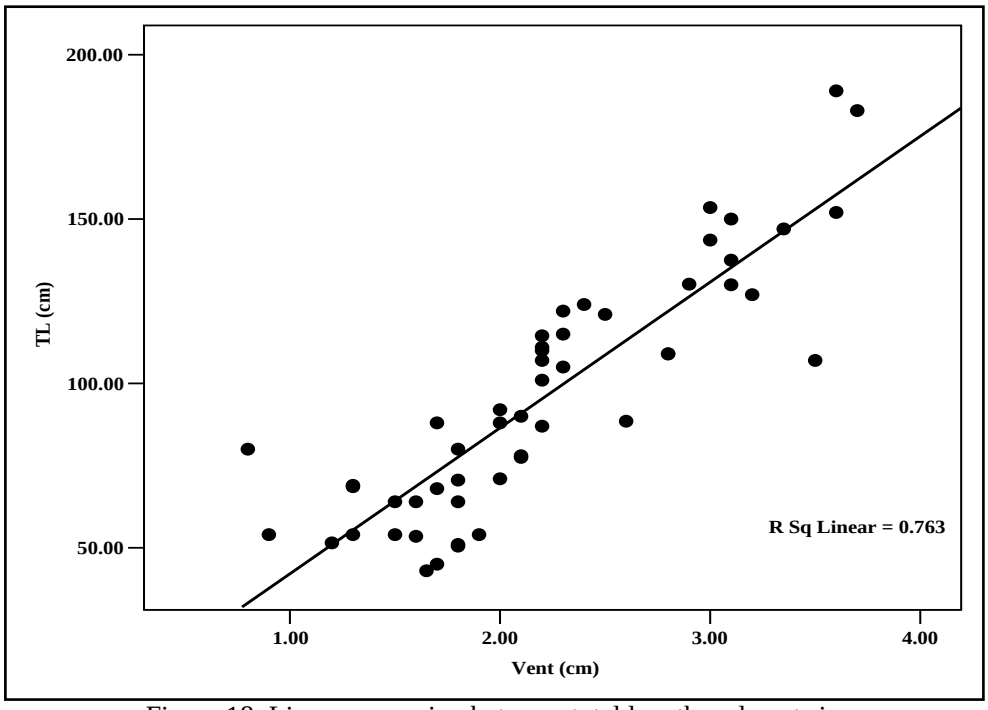


Figure 18: Linear regression between total length and vent size.

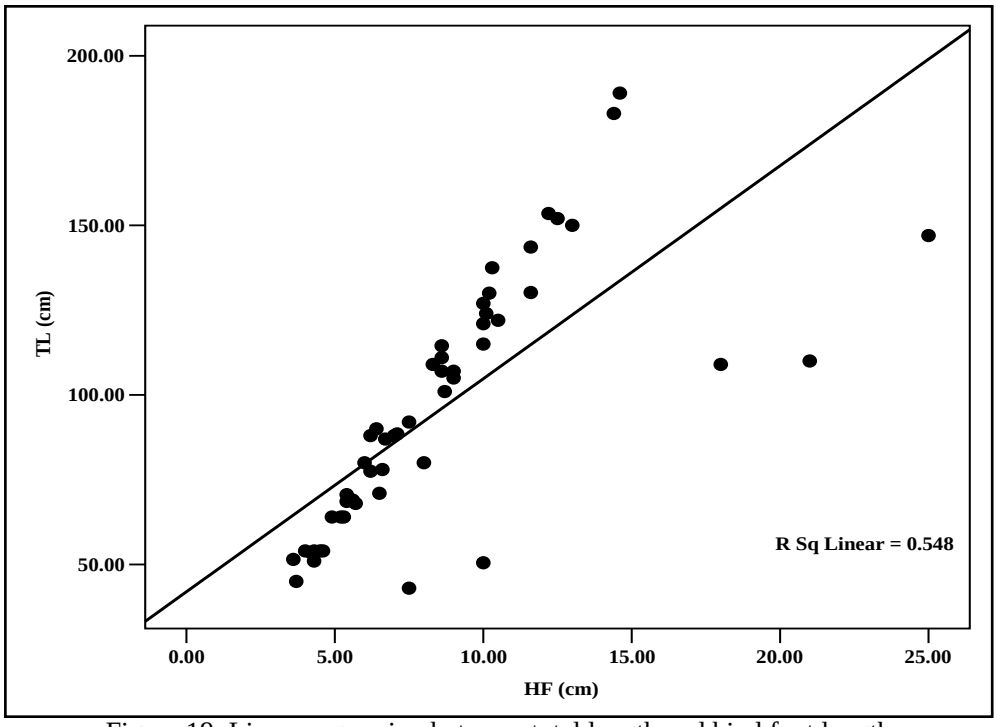


Figure 19: Linear regression between total length and hind foot length.

CONCLUSIONS

A number of biotic and abiotic factors interact to determine growth rates, morphometrics and body condition indices of crocodilians. In the lake Nasser, the most significant influences were water level and temperature, diet and possibly the fast-flowing nature of the lake Nasser itself, or, in other words, water fluctuation. While these influences were not unique to this crocodilian population, their combined effects may distinguish the physical characteristics of the individuals, differentiating the lake Nasser population from other populations. The body design of crocodilians, while showing extreme conservation throughout their evolution, is phenotypically flexible, allowing minor adjustments in response to environmental stimuli, as confirmed by extant genera (Richardson et al., 2002).

The close significant correlations and relations between parameters, exhibits the health of Nile crocodiles or, in other words, the close to ideal status of the lake Nasser ecosystem, during the time of the study with minor adjustments in response to environmental stimuli. This is observable although lake Nasser's ecosystem is affected by several types of sub-surface pollutants, which means that the Nile crocodile needs further research to estimate its ability to survive, and what effect will the pollutants have on its life history and at what degree. The close relation between parameters illustrated that there is no problem with the nutrients and food sources in lake Nasser

ACKNOWLEDGMENTS

I would like to acknowledge the support of the Mohamed Bin Zayed Species Conservation Fund. I would like to point out that without such support, this project would not have come to light and we wouldn't have been able to complete this unique work on the lake Nasser. On the other hand, the success of this project would not have been possible without the enthusiastic support of the MBZ Conservation Fund. I would like to acknowledge NCS, South Area Protectorate researcher, EEAA, Mr. McGrath, the Canadian journalist who wrote about the Nile crocodile in Egypt based on our project and Mr. Shaltout and the Ecology Department, Tanta University. Special thanks to Transylvanian Review of Systematical and Ecological Research editorial board members for their continuous support, help and kindness.

REFERENCES

1. Branch B., 1998 – Field Guide to Snakes and Other Reptiles of Southern Africa, third edition, Struck Publishers, Cape Town, South Africa.
2. Brandt L. A., 1991 – Long-Term Changes in a Population of Alligator mississippiensis in South-Carolina. Journal of Herpetology, 25, 419-424.
3. Chabreck R. H. and Joanen T. 1979 – Growth Rates of American Alligators in Louisiana. Herpetologica, 35, 51-57.
5. Cott H. B. 1961 – Scientific Results of an Inquiry into the Ecology and Economic Status of the Nile Crocodile (Crocodylus niloticus) in Uganda and Northern Rhodesia. Transactions of the Zoological Society of London, 29, 211-279.
6. Games I. 1990 – The Feeding Ecology of Two Nile Crocodile Populations in the Zambezi Valley. PhD Thesis, University of Zimbabwe, Harare.
7. Green A. J. 2001 – Mass/Length Residuals: Measures of Body Condition or Generators of Spurious Results? Ecology, 82, 1143-1483.
8. Hutton J. M. 1987 – Growth and Feeding Ecology of the Nile Crocodile Crocodylus niloticus at Ngezi, Zimbabwe. Journal of Animal Ecology, 56, 25-38.
9. Huton J. M., Webb G. J. W., Manolis S. C. and Whitehead P. J. 1987 – Techniques for Ageing Wild Crocodilians. In: Wildlife Management: Crocodiles and AlligatorsSurrey Beatty and Sons Pty Ltd., Chipping Norton-Australia. 211.
10. Huton J. M. 1987b – Morphometrics and Field Estimation of the Size of the Nile Crocodile. African Journal of Ecology, 25, 225-230.
11. Huton J. M. 1984 – Population Ecology of the Nile Crocodile, Crocodylus niloticus, Laurenti, 1768, at Ngezi, Zimbabwe. PhD Thesis, University of Harare, Zimbabwe.
12. Hutton J. M. 1987a – Growth and Feeding Ecology of the Nile Crocodile Crocodylus niloticus at Ngezi, Zimbabwe. Journal of Animal Ecology, 56, 25-38
13. Hutton J. M. 1987b – Morphometrics and Field Estimation of the Size of the Nile Crocodile. African Journal of Ecology, 25, 225-230.
14. Hutton J. M. 1987c – Techniques for Ageing Wild Crocodilians. In: Wildlife Management: Crocodiles and Alligators Webb G. J. W., Manolis S. C. and Whitehead P. J. (eds), Surrey Beatty and Sons, Pty Ltd., Sydney.
15. Hutton J. M., Webb G. J. W., Manolis S. C. and Whitehead P. J. 1987 – Techniques for Ageing Wild Crocodilians. In: Wildlife Management: Crocodiles and Alligators. pp. 211. Surrey Beatty and Sons Pty Ltd., Chipping Norton-Australia.
16. Jakob E. M., Marshal S. D. and Uetz G. W. 1996 – Estimating Fitness: A Comparison of Body Condition Indices. Oikos, 77, 61-67.
17. Kofron C. P. 1993 – Behavior of Nile Crocodiles in a Seasonal River in Zimbabwe. Copeia, 463-469.
18. Le Cren E. 1951 – The Length-Weight Relationship and Seasonal Cycle in Gonad Weight and Condition in the Perch (Perca fluviatilis). Journal of Animal Ecology, 66, 1504-1512.
19. Leslie A. J. 1997 – The Ecology and Physiology of the Nile crocodile, Crocodylus niloticus, in lake St Lucia, South Africa. PhD Thesis, Drexel University, PA, USA.
20. Modha M. L. 1968 – Basking Behaviour of the Nile Crocodile on Central Island, lake Rudolf. East African Wildlife Journal, 6, 81-88.
21. Paniagua Di´Az C., Keller C. and Andreu A. C. 1997 – Hatchling Success, Delay of Emergence and Hatchling Biometry of the Spur-thighed Tortoise, Testuda graeca, in South-western Spain. Journal of Zoology, 140, 109-125.
22. Pooley A. C. 1982a – The Status of African Crocodiles in 1980. In: Crocodiles, Proceedings of the 5th Working Meeting of the Crocodile Specialist Group 174. IUCN, The World Conservation Union, Gland-Switzerland.

23. Richardson K. C., Webb G. J. W. and Manolis S. C., 2002 – Crocodiles: Inside Out. Surrey Beatty and Sons Pty Ltd, Australia.
24. Sah S. A. M. and Stuebing R.B. 1996 – Diet, Growth and Movements of Juvenile Crocodiles Crocodylus porosus Schneider in the Klias River, Sabah, Malaysia. Journal of Tropical Ecology, 12, 651-662.
25. Swanepoel D. G. J. 1999 – Movements, Nesting and the Effects of Pollution on the Nile Crocodile Cocodylus niloticus in the Olifants River, Kruger National Park. MSc Thesis, University of Natal.
26. Taylor G. W. 1973 – Nile crocodile in the Okavango Delta: A Report on a Wildlife Population for Botswana Game Industries. Rep. No. 1. Botswana Game Industries, Francistown, Botswana.
27. Taylor J. A. 1979 – The Foods and Feeding Habits of Subadult Crocodylus porosus Schneider in Northern Australia. Australian Journal of Wildlife Research, 6, 347-359.
28. Wallace K. M. and Leslie A. J. – The Diet of the Nile crocodile (Crocodylus niloticus) in the Okavango Delta, Botswana. Journal of Herpetology, in press.
29. Webb G. J. W. and Smith A. M. A., 1987 – Life History Parameters, Population Dynamics and the Management of Crocodilians. In: Wildlife Management: Crocodiles and Alligators G. J. W. Webb, S. C. Manolis and P. J. Whitehead (eds), Surrey Beatty and Sons, Pty Ltd, Australia.
30. Webb G. J. W., Messel H., Crawford J. and Yerbury M. J. 1978 – Growth Rates of Crocodylus porosus (Reptilia: Crocodilia) from Arnhem Land, Northern Australia. Aust. Wild. Res., 5, 385-399.
31. Webb G. J. W., Messel H., Crawford J., and Yerbury M. J. 1978 – Growth Rates of Crocodylus porosus (Reptilia: Crocodilia) from Arnhem Land, Northern Australia. Australian Journal of Wildlife Research, 5, 385-399.
32. Williams B. K., Nichols J. D. and Conroy M. J. 2001 – Analysis and Management of AnimalPopulations. Academic Press, New York.

RIVER FRAGMENTATION AND CONNECTIVITY PROBLEMS IN THE GANGE RIVER OF THE UPPER HIMALAYA (INDIA): THE EFFECT ON THE FISH COMMUNITIES

Velaichamy JEEVA *, *Sudhir KUMAR* **,
Dharmendra VERMA ** and *Harcharan Singh RUMANA* ***

* Indian Council of Forestry Research and Education, Directorate of Extension, Environmental Impact Assessment Division, P.O. New Forest, Dehradun, India, IN-248006, vjeeva@icfre.org
** Indian Council of Forestry Research and Education, Directorate of Extension, Environmental Impact Assessment Division, P.O. New Forest, Dehradun, IN-248006, India, sudhir@icfre.org
*** Indian Council of Forestry Research and Education, Directorate of Extension, Environmental Impact Assessment Division, P.O. New Forest, Dehradun, India, IN-248006, vermad@icfre.org
**** Mantech Consultant, New Delhi, India, hsrumana@gmail.com, harcharan_r@hotmail.com

KEYWORDS: Himalaya, Ganges River, Nayyar River, migratory fish, human impact.

ABSTRACT

Sound ecological practices in the development of hydropower projects are essential to protect the fragile ecosystem of the Himalayan Mountains in northern India. Evidence is growing that the increasing number of river valley projects causes habitat fragmentation and degradation of both terrestrial and aquatic habitats. The impact was studied on the migratory fish known as the Mahseer, a flagship species, and the need to conserve the aquatic ecosystem in the upper Himalaya of Uttrakhand. The proposed construction site of the Kotlibhel hydro-electric project stage-II for the generation of 530 MW on the river Ganga/Ganges near the village of Kaudiyala, and the upstream rivers Alaknada, Bhagirathi and Nayar, were studied for the biodiversity of the riparian flora and aquatic fauna to assess the importance of river connectivity for conservation. Recommendations are emphasized for habitat restoration and connectivity to improve the riparian habitats for the Mahseer and to conserve the river Nayyar, which has the potential for conserving the native fish diversity.

ZUSAMMENFASSUNG: Die Unterbrechung des Flusskontinuums und Konnektivitätsprobleme am Ganges im oberen Himalayagebiet: Auswirkungen auf die Fischgemeinschaften.

Umweltverträgliche ökologische Praktiken in der Entwicklung von Wasserkraftprojekten sind wesentlich für den Schutz des fragilen Ökosystems in den Himalaya-Gebirgen Nordindiens. Die Erkenntnisse verstärken sich dahingehend, dass die steigende Zahl von Flussprojekten Habitatzerstückelungen verursachen und zu einer Degradation sowohl von terrestrischen als auch aquatischen Lebensräumen führen. Untersucht wurden die Auswirkungen auf die wandernden, als Mahseer bekannten Fischarten – eine Leitart – und die Notwendigkeit des Schutzes der aquatischen Ökosysteme im oberen Himalayagebiet von Uttrakhand. Im Hinblick auf den vorgeschlagenen Bau der Etappe II des Kotlibhel Wasserkraftprojektes zur Erzeugung von 530 MW am Ganges nahe des Dorfes Kaudiyala und der oberstrom liegenden Flüsse Alaknada, Bhagirathi und Nayar wurde die Artenvielfalt der Uferflora und der Gewässerfauna untersucht, um die Bedeutung der Konnektivität für den Schutz der Arten zu bewerten. Dabei wurden Empfehlungen für die Habitatrenaturierung und Konnektivität zur Verbesserung der Lebensräume für den Mahseer gegeben sowie für den Schutz des Nayyar Flusses, der das Potential für den Schutz der ursprünglichen Biodiversität besitzt.

REZUMAT: Fragmentarea râului şi probleme de conectivitate pe râul Gange în zona superioară a munţilor Himalaia: efecte asupra comunităţilor de peşti.

Practicile ecologice compatibile cu dezvoltarea unor proiecte hidroelectrice sunt esenţiale în vederea protecţiei ecosistemului fragil al munţilor Himalaia din India de nord. Se dovedeşte din ce în ce mai mult, că numărul crescând al proiectelor pe râuri are drept consecinţă fragmentarea şi degradarea atât a habitatelor terestre, cât şi a celor acvatice. A fost studiat impactul acestor proiecte asupra speciei de peşti migratori cunoscută sub numele de Mahseer, specie indicatoare, şi necesitatea conservării ecosistemului acvatic în partea superioară a munţior Himalaya din Uttrakhand. Construcţia propusă a fazei II a barajului hidroelectric de la Kotlibhel pentru generarea de 530 MW pe Râul Gange în apropierea satului Kaudiyala şi pe râurile Alaknada, Bhagirathi şi Nayar situate în amonte au fost studiate din punct de vedere a biodiversităţii florei ripariene şi a faunei acvatice pentru a se putea aprecia importanţa conectivităţii pentru conservarea speciilor. Sunt date măsuri pentru refacerea habitatelor şi a conectivităţii în vederea îmbunătăţirii habitatele ripariene pentru Mahseer, precum şi pentru conservarea râului Nayyar, acesta deţinând potenţialul pentru conservarea diversităţii native a speiilor de peşti.

INTRODUCTION

The riparian zones are among the most diverse, dynamic, and complex habitats on the world's continents (Naiman and Décamps, 1997) as they are considered to be the most important natural pathways for plants and animals, especially migratory fish (Forman and Godron, 1986; Malanson, 1993; Schneider and Sharitz, 1988; Nilsson et al., 1991; Johansson et al., 1996; Planty-Tabacchi et al., 1996). The increased fragmentation due to dams is reported to disrupt the natural dispersal pathways of the riparian communities (Hanson et al., 1990; Fahrig and Merriam, 1994) and alter the structure. Such specific alterations have however been documented only for migratory fish (Petts, 1984) and considerable works have been reported on the impact of dams on aquatic ecosystems and fish life cycles in America and Europe. No systematic efforts have been made so far on Himalayan riparian ecosystems, especially in the flagship fish species due to the habitat fragmentation. Therefore, it was thought desirable to assess the impact of the proposed dam on the flora and fauna, which are important components of the ecosystem in the study area in Terhi Garhwal Himalaya, Uttrakhand, India.

The Mahseer is an important flagship species (Simberloff, 1998) distributed along the Himalaya in India, Pakistan, Bhutan and Bangladesh. Golden mahseer (*Tor putitora*) is one of the main game and food fish of Uttrakhand, is largely confined to lotic habitats (streams, rivers). The fish migrates considerable distances upstream in search of suitable spawning grounds. The migratory Mahseer is a column feeder, omnivorous fish and attains a maximum length of 275 cm (9 ft) and a maximum weight of 54 kg (118 lb) (Talwar and Jhingran, 1991; Wikipedia, 2006). Becouse of its size, golden colour, beautiful appearance and flavour, the fish is exploited along the Himalayan foothills. Stocks of the Himalayan mahseer are reported of being depleted and it is considered an endangered species (Singh and Sharma, 1998; Khan and Sinha, 2000; Sharma, 2003). Further, habitat degradation and fragmentation has also been reported, to contribute to their decline in numbers, in the Himalayan stretch of the Ganges (Nautiyal, 1984, 1989, 1990, 1994). Therefore, it is very important to understand the vital links within the study area to restore the habitat connectivity.

MATERIALS AND METHODS

Study area

The proposed Kotlibhel H. E. Project Stage-II is located on the river Ganges, the confluence point where the rivers Alaknanda and Bhagirathi meet and flow between the Tehri and Pauri Districts of Uttarakhand (Figs. 1 and 2). The basin up to the proposed dam site near the village Kaudiyala lies between Longitude from 78°09'10" E to 80°10'31" E and Latitude from 29°45'03" N to 31°29'57"N.

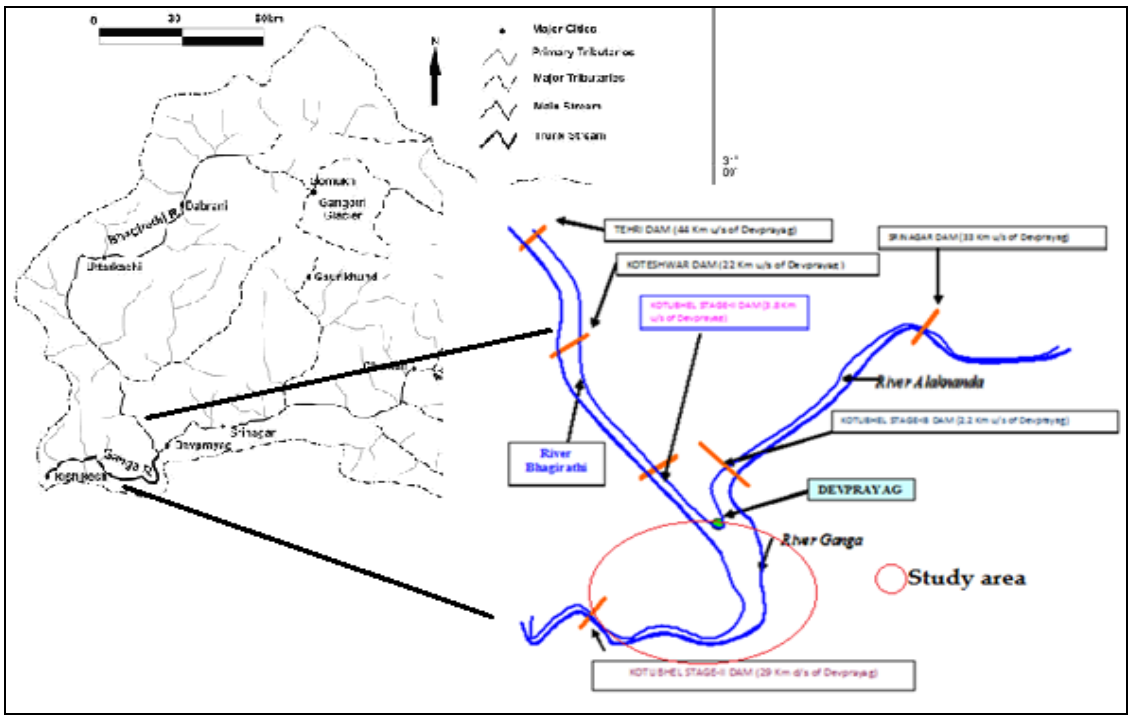


Figure 1: The studied area.

The proposed dam will be a 58.6 m high concrete gravity dam across the river Ganges which will completely deprive the upstream migration of the economically important migratory fish Mahseer both to rivers Alaknanda and Bhagirathi. The proposed project is part of the cascade development of the Kotlibhel project in three stages, proposed by the National Hydropower Power Corporation, India and the study was conducted during 2006-2007.

Methodology

Two reference sites were identified in the project area for the collection of water samples and obtaining data related to the aquatic primary production and production of Mahseer (*Tor tor*, *Tor putitora*). For limnological analyses of the aquatic environment, standard methods outlined in Mackereth et al. (1978) were followed. The vegetation samples for phytosociological study were collected according to Misra (1968) and Kershaw (1973) and were quantitatively analyzed for density, frequency and abundance according to Curtis and

McIntosh (1950). The tree species diversity was determined using Shannon Wiener (Shannon and Wiener, 1963). Concentration of dominance was measured by Simpson Index (Simpson, 1949). Fish, including their spawns, fry and fingerlings were captured and identified with the help of keys of Jayaram (1981) and Menon (1987). Benthic macro-invertebrates were studied (Welch, 1948) and the collected specimens were preserved in 8% formalin solution and identified up to the generic level with the aid of keys given by Usinger (1950) and Tonapi (1980). The benthic macro-invertebrates were also identified with the help of keys given by Edmondson (1959) and Pennak (1953). Secondary reports were also consulted (Sharma and Ashutosh Mishra, 2002; Nautiyal and Lal, 1982).

RESULTS AND DISCUSSIONS

The water quality analysis was carried out at various places covering sections of river Bhagirathi, river Alaknanda, river Nayar and the river Ganges, to have a holistic view of it during monsoon and pre- monsoon periods. The details of various sites on the river are given in table number 1 (a, b). The parameters analyzed, showed a significant variation for the river Nayar, as compared to rivers Bhagirathi, Alaknanda and Ganges. This could be naturally due to the fact that, the Nayar is a spring fed and the rest are glacial fed. However the slight variation in the case of the river Ganges, from Bhagirathi and Alaknanda could be due to other anthropogenic factors, such as pilgrim pressure and ecotourism near the village Kaudiyala.

The quantitative distribution of the benthic macro-invertebrates collected from the 39 km long stretch of the study area is presented in table number 2 for all the seasons in the rivers Nayar and Ganges. A total of 18 species of the macro-zoo benthos have been identified from this stretch of the river. It is evident from the study that the highest population density was observed in the river Nayar as compared to the river Ganges. The high diversity in the Nayar River is due to the spring fed, river substratum consisting of bed rock, boulders, cobbles and gravels, which supports the survival, growth and reproduction of macro-benthic organisms. Observations further show a close relationship between abundance of the fish presence and population density of macro-zoobenthos with phytobenthos.

Phytobenthos consume the carbon dioxide present in the water and therefore, is considered as a water purifier. The data indicate (Tab. 3) that the bottom substratum of the rivers Ganges and Nayar is enriched in green matters or phytobenthos. Though the density of phytobenthos is good throughout the year, the highest abundance is observed from October to March. The concentration of phytobenthos decreases during the period April – September, just at the onset of the ablation period, as the river water becomes turbid and torrential. The richness of phytobenthos in the river Ganges could be due to its joining with Nayar River.

It is evident that Mahseer dominates and is found healthier in the river Nayar than that is found in river Ganges (Tab. 4). This is due to the spring-fed streams of the region that have relatively high temperature (10.0 to 27.5°C), low current velocity (0.4 to 1.2 m/s), and a relatively confined channel basin (4.4 to 18.5 m). The dissolved oxygen content is lower than in the glacier-fed streams (6.6 to 8.8 mg/L). However, pH was slightly higher (7.0-7.9) and total alkalinity was much higher (97-148 mg/L). The current velocity and water temperature have great influence on the periphyton density in the streams. It was higher in spring-fed streams of Nayar than in the Ganges glacier-fed. The high temperature, moderate current, good periphyton coupled with high substrate heterogeneity, enhance the density of macrobenthic fauna in spring-fed streams that have provided a good base for the development of mahseer and contribute greatly to the commercial fishery at Satpuli in the foothills of Himalaya.

Table 1a: The water quality parameters in the rivers of upper Himalaya in Uttrakhand.

Sl. no.	Parameters	Unit	Bhagirathi		Alaknanda	
			Pr. M	Po. M	Pr. M	Po. M
A. Physical Parameters						
1	Temperature	^{o}C	14	15	16	16
2	pH	–	8.2	8.1	8.0	7.9
3	Conductivity	uS/cm	170	185	182	190
4	Total Dissolved Solids	mg/1	105	118	113	120
5	Dissolved Oxygen	mg/1	9.08	8.2	9.5	8.0
6	Salinity	Ratio	0.104	0.100	0.106	0.102
7	Turbidity	NTU	12	10	15	12
B. Chemical Parameters						
8	Total Alkalinity	mg/1	72	74	62	62
9	Calcium Hardness	mg/1	54	52	44	45
10	Magnesium Hardness	mg/1	20	24	22	26
11	Total Hardness	mg/1	74	76	66	71
12	Chloride	mg/1	20	19	22	22
13	Iron	mg/1	0.08	0.05	0.06	0.04
14	Nitrate as N	mg/1	0.3	0.02	0.22	0.16
15	Phosphate	mg/1	0.04	0.02	0.02	0.01
16	Manganese	mg/1	Not Detected		Not Detected	
17	Biological Oxygen Demand	mg/1	< 2	< 2	< 2	< 2
18	Chemical Oxygen Demand	mg/1	2.8	2	2.0	2
C. Bacteriological characteristics						
19	E-Coli	N/100 ml	10	6	5	4
	Total Coliform	N/100 ml	220	200	240	180
Pr. M. = Premonsoon; Po. M = post monsoon.						

Table 1b: The water quality parameters of the rivers in the upper Himalaya in Uttrakhand.

Sl. no.	Parameters	Unit	Ganges		Nayar River	
			Pr. M	Po. M	Pre. M	Post. M
A. Physical Parameters						
1	Temperature	^{o}C	15	16	22	20
2	pH	–	8.0	8.0	7.0	7.9
3	Conductivity	uS/cm	174	180	200	205
4	Total Dissolved Solids	mg/1	110	115	126	132
5	Dissolved Oxygen	mg/1	9.5	8.7	6.6	8.8
6	Salinity	Ratio	0.104	0.100	0.103	0.102
7	Turbidity	NTU	20	12	5	12
B. Chemical Parameters						
8	Total Alkalinity	mg/1	60	64	97	148
9	Calcium Hardness	mg/1	47	48	72	70
10	Mangesium Hardness	mg/1	21	21	56	48
11	Total Hardness	mg/1	68	69	128	118
12	Chloride	mg/1	22	20	12	12
13	Iron	mg/1	0.06	0.04	Not Detected	
14	Nitrate as N	mg/1	0.22	0.16	0.22	0.18
15	Phosphate	mg/1	0.02	0.01	Not Detected	
16	Manganese	mg/1	Not Detected		Not Detected	
17	Biological Oxygen Demand	mg/1	< 2	< 2	< 2	< 2
18	Chemical Oxygen Demand	mg/1	2.22	2	3.0	4
C. Bacteriological characteristics						
19	E-Coli	N/100 ml	5	4	5	4
	Total Coliform	N/100 ml	200	200	200	180
Pr.M = Premonsoon; Po.M = post monsoon.						

Table 2: Quantitative distribution of Zoo benthos in rivers Ganges and Nayar.

Season of the year	**Spring**				**Summer**				**Monsoon**				**Winter**			
	Average		Range		Av.		Ra.		Av.		Ra.		Av.		Ra.	
Order	G	N	G	N	G	N	G	N	G	N	G	N	G	N	G	N
Periphytons- Density No/Cm^2																
Green Algae	190	230	11-232	28-542	187	212	8-83	17-483	68	236	3-88	12-108	59	89	5-28	16-89
Diatoms	270	490	20-125	29-289	236	212	12-896	19-483	86	389	7-73	249	39	168	7-20	11-178
Zooplanktons No/L																
Protozoan	27	32	2-12	12-37	22	38	1-18	8-22	15	22	2-8	4-19	11	18	1-5	6-19
Rotifers	20	25	1-11	4-16	18	20	2-11	4-11	10	18	1-9	1-19	15	16	2-6	4-13
Copepods	18	28	2-10	4-20	16	19	3-8	4-21	12	21	2-4	3-16	10	13	1-5	2-10
Cladocerans	14	24	2-11	3-19	14	16	2-9	2-23	11	17	3-7	4-18	9	11	2-4	2-15
Macro invertebrates No/M^2																
Ephemeropt.	89	112	3-22	26-48	58	98	2-16	2-16	45	81	2-11	3-19	39	78	1-15	1-17
Odonata	93	118	4-12	13-35	46	67	2-22	4-29	36	53	1-9	2-15	27	43	2-7	3-19
Diptera	112	125	5-9	11-56	73	89	3-18	6-26	52	84	2-8	7-15	35	62	1-5	2-15
Coleoptra	76	128	6-11	12-52	67	94	3-21	5-32	48	68	4-7	4-18	33	76	1-4	3-11
Hemiptera	82	136	5-8	15-58	71	67	2-20	2-28	53	79	2-8	3-11	42	63	2-4	1-14
Trichoptera	67	78	4-11	3-22	58	63	3-18	2-22	43	72	1-5	3-10	23	45	1-6	1-12
Nematoda	8	12	2-4	2-7	6	8	2-5	4-7	4	8	2-2	4-8	2	6	1-1	1-5
Annelida	9	8	1-4	8	4	5	2-5	2-8	3	4	1-3	4-7	2	3	1-2	1-3
Earthworms	10	12	2-5	12	7	7	2-4	2-5	5	4	1-1	1-2	2	2	1-1	1-1
G = Ganges River; N = Nayar River.																

The Mahseer population undertakes migration from the foothill sector of the Ganges, often ascending into the rivers Bhagirathi and Bhilangana (Sehgal, 1972; Nautiyal et al., 2001) the only possible route. The present survey reveals that the fish have easy access to the spring-fed placid streams of Nayar, that provide a congenial environment (Nautiyal and Lal, 1984) because of the lower current velocity, more potential breeding grounds are available in the spring fed streams (0.35-1.7 m/s) than in the glacier-fed streams (0.68-2.5 m/s). Also the availability of the coarse bottom of boulders, rocks or pebbles substratum provides base for biotypes for enrichment of macro benthos. It is obvious that the proposed Kotlibhel Stage II may obstruct the upstream migration of mahseer. Also the suitable riparian habitat would get submerged because of the proposed reservoir.

A detailed floral diversity was assessed in the submergence area of the proposed Kotlibhel Stage II. The study area showed rich herbaceous diversity in terms of distribution and density of species with dominant and co-dominant species specific to riparian zone (Tabs. 5 and 6). The entire stretch of the floral diversity in the project area will be lost due to the proposed intervention resulting in the land use change.

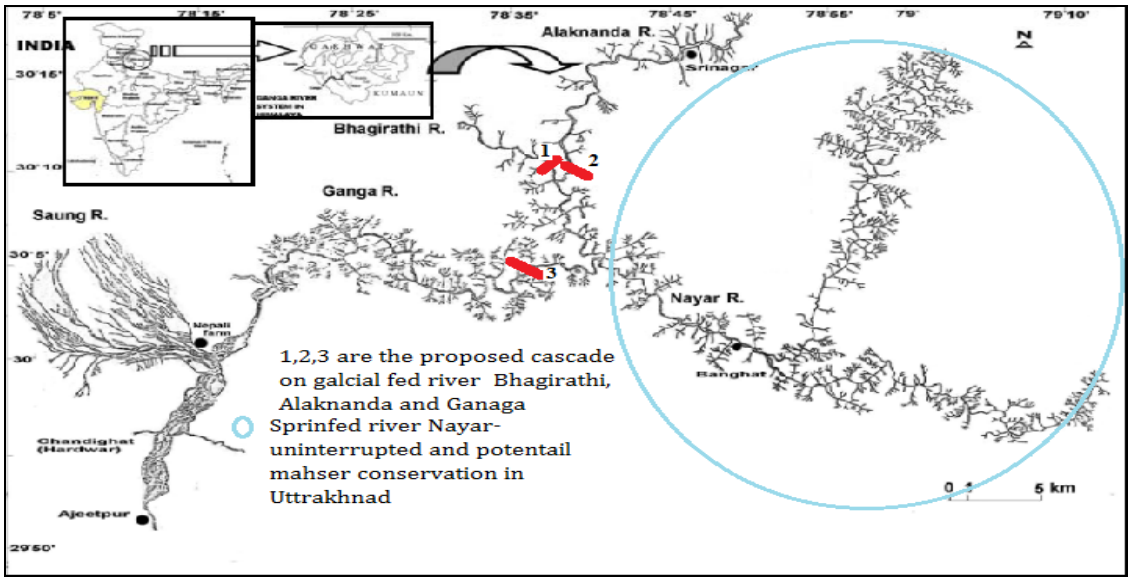


Figure 2: The river Nayar in the upper Himalayas
of Uttrakhand uninterrupted by the development activities.

Table 3: Percentile contributions of important fish species in terms of number and weight in the rivers Ganges and Nayar.

Species	River Ganges (% contribution)		River Nayar (% contribution)	
	By number	By weight	By number	By weight
Schizothorax sp.	18.6	20.4	20.2	18.9
Tor sp.	23.2	25.8	26.6	28.5
Tor putitor	24.6	26.4	27.8	30.4
Labeo sp.	8.2	7.6	8.1	9.6
Garra sp.	7.8	5.8	5.3	6.2
Barilius sp.	4.2	3.9	6.2	2.4
Puntius sp.	5.4	3.9	1.8	1.3
Glyptothorax sp.	5.5	2.7	2.3	2.1
Nemacheilus sp.	1.9	2.7	2.1	1.4

Table 4: Phytosociological analysis of herbaceous vegetation at the left bank of the river Ganges in the study area.

Name of the species	F	D	RDe	R F	R Do	IVI
Herbs						
Achyranthes aspera	18	0.64	3.54	3.7	0.85	8.09
Barleria cristata	27	0.73	4.04	5.56	11.65	21.24
Dicleptera roxburghiana	18	1.64	9.09	3.7	3.84	16.63
Eupatorium adenophorum	18	1.91	10.61	3.7	10.2	24.51
Hamiltonia suaveolens	18	0.36	2.02	3.7	12.09	17.81
Lannea nudicaulis	9	0.09	0.51	1.85	2.1	4.46
Lepidagathis cuspidata	36	2.55	14.14	7.41	7.74	29.28
Triumfetta rhomboidea	18	1.00	5.56	3.7	3.99	13.25
Seedlings of Trees						
Adina cordifolia	9	0.09	0.51	1.85	0.66	3.02
Ehretia laevis	18	0.27	1.52	3.7	13.61	18.83
Syzygium cumini	18	1.82	10.1	3.7	0.56	14.37
Toona ciliata	18	0.82	4.55	3.7	0.21	8.45
Seedlings of Climbers						
Atylosia volubilis	9	0.09	0.51	1.85	0.22	2.58
Atylosia scarabaeoides	9	0.09	0.51	1.85	0.11	2.46
Cissampelos pareira	36	0.55	3.03	7.41	0.48	10.91
Dioscorea bulbifera	18	0.27	1.52	3.7	0.15	5.37
Ichnocarpus frutescens	27	0.45	2.53	5.56	0.6	8.68
Pueraria tuberosa	9	0.09	0.51	1.85	2.51	4.87
Ventilago denticulata	9	0.18	1.01	1.85	0.58	3.44
Seedlings of Shrubs						
Carissa opeca	18	0.27	1.52	3.7	4.45	9.67

Murraya koenigii	36	1	5.56	7.41	3.48	16.44
Name of the species	**F**	**D**	**RDe**	**R F**	**R Do**	**IVI**
Murraya paniculata	9	0.18	1.01	1.85	0.2	3.07
Woodfordia floribunda	9	0.09	0.51	1.85	0.05	2.4
Grasses						
Arundinella pumila	9	0.18	1.01	1.85	0.02	2.88
Capillipedium parviflorum	9	0.18	1.01	1.85	0.27	3.13
Chloris dolichostachya	9	0.27	1.52	1.85	0.03	3.4
Nyraudia arundinacea	9	0.36	2.02	1.85	1.31	5.18
Oplismenus burmanii	9	0.36	2.02	1.85	0.02	3.89
Oplismenus compositus	18	1.18	6.57	3.7	1.32	11.59
Saccharum spontaneum	9	0.27	1.52	1.85	13.73	17.1

*__F__ = Frequency, **D** = Density/m^2, **RDe** = Relative Density, **RF** = Relative Frequency, **RDo** = Relative Dominance, **IVI** = Importance Value Index, **H'** = Species Diversity 1.28, **CD** = Concentration of Dominance 0.6

Table 5: Phytosociological analysis of plants in herbaceous vegetation at the right bank of the river Ganges.

Name of the species	**F**	**D**	**RDe**	**R F**	**R Do**	IVI
Herbs						
Achyranthes aspera	80	2.6	10.74	10.26	2.12	23.12
Aerva sanguinoenta	50	1.1	4.55	6.41	0.42	11.37
Ageratum conyzoides	70	4.8	19.83	8.97	16.77	45.58
Anisochilus carnosus	10	0.1	0.41	1.28	0.11	1.8
Artemisia nilagarica	10	0.9	3.72	1.28	0.08	5.08
Barleria cristata	20	0.3	1.24	2.56	1.08	4.89
Bidens pilosa	50	2.7	11.16	6.41	0.86	18.43
Blumea mollis	10	0.1	0.41	1.28	0.1	1.8
Boehmeria platyphylla	10	0.1	0.41	1.28	0.14	1.84

Boehravia diffusa	10	0.3	1.24	1.28	0.23	2.75
Name of the species	**F**	**D**	**RDe**	**R F**	**R Do**	**IVI**
Cassia tora	10	0.2	0.83	1.28	0.64	2.74
Celosia argentea	10	0.2	0.83	1.28	0.61	2.72
Cleome viscose	10	0.1	0.41	1.28	0.32	2.02
Commelina benghalensis	10	0.2	0.83	1.28	0.08	2.19
Eupatorium adenophorum	10	0.7	2.89	1.28	8.62	12.79
Euphorbia hirta	20	0.6	2.48	2.56	0.54	5.58
Justicia prostrate	10	0.2	0.83	1.28	0.1	2.21
Lepidagathis cuspidatus	30	0.6	2.48	3.85	0.49	6.82
Malvastrum coromandelianum	20	0.4	1.65	2.56	0.44	4.66
Nicandra physaloides	20	0.3	1.24	2.56	9.88	13.68
Oxalis corniculata	20	0.5	2.07	2.56	19.5	24.14
Parthenium hysterophorus	10	0.2	0.83	1.28	0.26	2.37
Passiflora foetida	10	0.1	0.41	1.28	0.01	1.71
Rumex hatatus	10	0.3	1.24	1.28	3.03	5.55
Urena lobata	10	0.2	0.83	1.28	0.7	2.81
Solanum nigrum	10	0.1	0.41	1.28	0.14	1.84
Seedlings of Climbers						
Atylosia scarabaeoides	10	0.1	0.41	1.28	0.01	1.71
Cissampelos pareira	10	0.1	0.41	1.28	0.05	1.74
Cissus repanda	10	0.1	0.41	1.28	0.03	1.73
Ipomoea hederifolia	10	0.1	0.41	1.28	0.11	1.81
Grasses						
Apluda mutica	10	0.2	0.83	1.28	0.12	2.22
Arthraxon lancifolius	10	1.4	5.79	1.28	28.01	35.08
Cynodon dactylon	10	0.1	0.41	1.28	0.12	1.81

Eragrostis tenella	10	0.1	0.41	1.28	1.6	3.29
Nyrandia arundinacea	10	0.2	0.83	1.28	0.06	2.17
Oplismenus burmannii	40	1.5	6.2	5.13	0.57	11.89
Oplismenus compositus	10	0.8	3.31	1.28	1.1	5.68
Seedlings of Shrubs						
Anisomeles indica	10	0.1	0.41	1.28	0.15	1.84
Lantana camara	10	0.3	1.24	1.28	0.06	2.58
Murraya koenigii	10	0.1	0.41	1.28	0.09	1.79
Nepeta graciliflora	40	0.6	2.48	5.13	0.38	7.98
Woodfordia floribunda	10	0.1	0.41	1.28	0.01	1.71
Sedge						
Eriophorum comosum	10	0.2	0.83	1.28	0.24	2.35
Kyllinga nemoralis	10	0.2	0.83	1.28	0.02	2.13

F = Frequency, **D** = Density/m^2, **RDe** = Relative Density, **RF** = Relative Frequency, **RDo** = Relative Dominance, **IVI** = Importance Value Index, **H'** = Species Diversity 1.91, **CD** = Concentration of Dominance 0.87.

In general, the riparian flora species are dependent on free spaces generating disturbing events for their germination and recruitment on the one hand and on flowing water for their dispersal on the other hand (Forman and Godron, 1986; Malanson 1993; Planty-Tabacchi et al., 1996; Jansson et al., 2000; Ward et al., 2002). Both processes (i.e. recurrent disturbance due to fragmentation and habitat connectivity through flowing water) will be impacted. Implications on the management of fragmentation, that affect the migratory fish species is of great concern. Hence, restoration program is prioritized to avoid genetic erosion of the species (e.g. hatchery and stocking programs). In case of the river fragmentation by dams or weirs, development of connectivity as a practical management are undertaken to restore the genetic integrity of populations. The approach proposed here is to conserve and preserve the existing habitat i.e. the river Nayar a spring fed river which is totally devoid of any developmental activities in the study area. The use of a larger spatial scale sampling design could be used for reaching at this specific goal.

The riparian floral species such as *Arundinella pumila, Capillipedium parviflorum, Chloris dolichostachya, Nyraudia arundinacea, Oplismenus burmanii, Oplismenus composites* and *Saccharum spontaneum* shall be taken into account for the enrichment of the habitats of the river Nayar

CONCLUSIONS

To conclude, our study demonstrates the importance of considering several species of riparian flora and aquatic fauna when investigating the effect of fragmentation mediated by humans. We have shown that the suitable habitat ecology is important to understand the influence of fragmentation on the integrity of fish populations. This means that to conserve connectivity through restoration programmes needs to be prioritized and targeted according to the specificity of each species. The riparian habitat of river Nayar will be the undisturbed area available for conservation as the other rivers Alaknada and Bhagirathi are fragmented due to various hydropower projects and the connectivity becomes negligible. Future experimental studies are needed to ascertain that the restoration programmes have contributed to the connectivity and conservation of the important fish fauna.

REFERENCES

1. Curtis J. T. and McIntosh R. P., 1950 – The interrelations of certain analytic and synthetic phytosociological characters, Ecology, 31, 434-455.
2. Edmondson W. T. (ed.), 1959 – "Ward and Whipple's Fresh-Water Biology." John Wiley and Sons, New York.
3. Fahrig L. and Merriam G., 1994 – Conservation of fragmented populations. Conservation Biology 8: 50-59.
4. Forman R. T. T. and Godron M. 1986 – Landscape Ecology. Wiley, New York, USA.
5. Hanson J. S., Malanson G. P. and Armstrong M. P., 1990 – Landscape fragmentation and dispersal in a model of riparian forest dynamics. Ecological Modelling 49, 277-296.
6. Jansson R., Nilsson C. and Reno B., 2000 – Fragmentation of riparian floras in rivers with multiple dams. Ecology 81, 899-903.
7. Jayaram K. C., 1981 – The Freshwater Fishes of India, Pakistan, Bangladesh, Burma and Sri Lanka- A Handbook. Zoological Survey of India, Calcutta. The Director Zoological Survey of India, 475.
8. Johansson M. E., Nilsson C. and Nilsson E., 1996 – Do rivers function as corridors for plant dispersal?, J. Veg. Sci., 7, 593-598.
9. Khan M. A. and Sinha M., 2000 – Status of Mahseer Fisheries in north and north eastern India with a note on their conservation. Journal Inland Fisheries Society, India, 32 (1): 28-36.
10. Kershaw K.R., 1973 – Quantitative and Dynamic Plant Ecology (London: Edward Arnold Ltd.) 308pp.
11. Malanson G. P., 1993 – Riparian Landscapes. Cambridge University Press, Cambridge, UK.
12. Mackereth F. J. H., Heron J. and Talling J. F., 1978 – Water analysis: some revised methods for limnologists. Freshwater Biology v. 36, p. 1-121, 1978.
13. Menon A. G. K., 1987 – The fauna of India and the adjacent countries. Pisces Vol. IV. Teleostei- Copitoidea. Part 1. Homolopteridae. Zool. Surv. India.
14. Misra R., 1968 – Ecology Work Book. Oxford and IBH Publishing Company, New Delhi.
15. Naiman R. J. and Decamps H., 1997 – The ecology of interfaces – riparian zones. Annual Review of Ecology and Systematics 28: 621-658.
16. Nautiyal P. 1984 – Natural history of Garhwal Himalayan Mahseer Tor putitora (Ham.) and breeding biology. Proceedings of Indian Academy of Science (Animal Science), 93: 97-106.
17. Nautiyal P., 1989 – Mahseer Conservation, Problem and Prospects. J. Bom. Nat. His. Soc., 86: 32-36.
18. Nautiyal P., 1990 – Natural history of Garhwal Himalayan mahseer: Growth rate and age composition in relation to fishery, In: R. Hirano and I. Hanyu (eds), feeding and breeding ecology Proc. Sec. Asian Fish. Forum, Tokyo, Japan. Asian Fish. Soc. Manila, 769-772.
19. Nautiyal P., 1994 – The Himalayan Mahseer Tor putitora (Ham). In: P. Nautiyal, (ed.), Mahseer, The Game Fish. Jagdamba Prakashan. Dehradun, 4-43.
20. Nautiyal P. and Lal. M. S., 1984 – Preliminary observations on the migratory behaviour of the Garhwal Himalayan mahseer. Journal of the Bombay Natural History Society 81, 204-208.
14. Nautiyal P. and Lal M. S., 1982 – Recent records of Garhwal mahseer (Tor putitora) with a note on its present status. Journal of the Bombay Natural History Society 79, 639-695.
15. Nautiyal P., Bahuguna S. N. and Thapliyal R. P., 2001 – The role of eclogical factors in governing the direction, time and purpose of migration in Himalayan Mahseer Tor putitora (Ham.). Applied Fisheries and Aquaculture 1, 133-138.

16. Nilsson C., Ekblad A., Gardfjell M. and Carlberg B., 1991 – 'Long-term effects of river regulation on river margin vegetation', J. Appl. Ecol., 28, 963-987.
17. Petts G. E., 1984 – Impounded rivers: perspectives for ecological management. John Wiley and Sons, Chichester, UK.
18. Planty-Tabacchi A.-M., Tabacchi E., Naiman R. J., Deferrari C. and Decamps H., 1996 – Invasibility of species-rich communities in riparian zones. Conservation Biology 10, 598-607.
19. Pennak R. W. 1978 – Freshwater Invertebrates of United states. John Wiley and Sons, New York, 803.
20. Pennak R. W., 1953 – Freshwater Invertebrates of United States. Ronald Press Co., New York, 769.
21. Schneider R. L. and Sharitz R. R., 1988 – Hydrochory and regeneration in a bald cypress-water tupelo swamp forest. Ecology 69, 1055-1063.
22. Sehgal K. L., 1972 – Coldwater fisheries and their development in India for sport and profit. Silver Jubilee ICFRI Souvenir: 125-31.
23. Sharma R. C., 2003 – Fish diversity and their ecological status in protected areas of Uttaranchal. Verma, S.R. (ed.) Protected Habitats and Biodiversity, Nature Conservators Publ. 8, 617-638.
24. Simberloff D., 1998 – Flagships, umbrellas and keystones: is single species management pass´e in the landscape era? Biol. Conserv. 83, 247-257.
25. Singh D. and Sharma R. C. 1998 – Biodiversity, ecological status and conservation priority of the fish of the river Alaknanda, a parent stream of the river Ganges (India). Aquatic Conservation: Marine and Freshwater Ecosystems 8, 761-722.
26. Shannon C. E. and Wiener W., 1963 – The Mathematical Theory of Communication, University of Illinois Press, Champaign, IL, USA, 144.
27. Sharma A. P. and Ashutosh M. 2002 – Present status and prospects of Mahseer fishery in Garhwal Region of Central Himalaya (English). In: Cold water fisheries in the trans Himalayan countries; FAO Fisheries Technical Paper (FAO), no. 431 Petr T. (ed.) Swar S. B. (ed.)/FAO, Rome (Italy). Fisheries Dept., 257-272.
28. Simpson E. H., 1949 – Measurement of diversity. Nature 163, 688.
29. Talwar P. K. and Jhingran A. G., 1991 – Inland fishes of India and adjacent countries, vol. 1 and 2. Oxford and IBH Publishing Company Pvt. Ltd., New Delhi.
30. Tonapi G. T., 1980 – Fresh Water Animals of India (An Ecological Approach) 341. New Delhi: Oxford and IBH. Publ. Co. 1980.
31. Usinger R. L. 1950 – Aquatic Insects of California: With Keys to North American Genera and California Species. University of California Press, Berkeley Los Angles, London.
32. Ward J. V., Tockner K., Arscott D. B. and Claret C. 2002 – Riverine landscape diversity. Freshwater Biology 47, 517-539.
33. Welch P. S. 1948 – Limnology. McGraw Hill Book Co. Inc., New York.

THE DANUBE IN SERBIA – ECOLOGICAL STATUS AND MANAGEMENT ISSUES

Ana BORISAVLJEVIC *

* University of Belgrade, Faculty of Forestry, Kneza Viseslava 1, Belgrade, Serbia, CS-11030, ana.borisavljevic@sfb.bg.ac.rs, borisavljevic.ana@gmail.com

KEYWORDS: flora, fauna, ecological status, bioindicators, hydromorphological alterations, management, Danube.

ABSTRACT

Many stretches of the river Danube have lost their ecological value in recent decades as a result of anthropogenic pressure from intensive agriculture, new infrastructure projects, more intensive navigation and the construction of hydro-power plants. The greatest alterations have happened in recent decades also in the investigation area, which is a part of the Middle Danube, from 1,433 to 845 km, and extends into Serbia. The content of this review paper is based on the analysis and use of topographical maps, data on waterline and discharges for five hydrological stations on the Danube in Serbia, analysis of the floral and faunal bioindicators, and a review of relevant literature. We describe indications of ecosystem alterations, following which we offer discussion on management issues, and make recommendations and comments. This paper synthesizes existing knowledge on the Danube in Serbia and, at the same time, warns of proposals that can become real projects that may have extreme adverse consequences for the Danube ecosystem.

ZUSAMMENFASSUNG: Die Donau in Serbien – ökologischer Zustand und Managementfragen.

In den letzten Jahrzehnten haben viele Abschnitte der Donau unter dem Druck menschlicher Tätigkeiten durch intensive Landwirtschaft, neue Infrastrukturprojekte, intensivere Schifffahrt und den Bau von Wasserkraftwerken ihren ökologischen Wert verloren. Derartige, große Veränderungen fanden in den letzten Jahrzehnten auch in dem untersuchten Donauabschnitt statt, der sich als Teil der mittleren Donau in Serbien von Strom-Kilometer 1,433 zu 845 erstreckt. Der Inhalt dieser Abhandlung beruht auf einer Analyse topografischer Karten, von Daten betreffend Wasserstands- und Abflussverhältnisse fünf hydrologischer Pegelstationen an der serbischen Donau sowie von Bioindikatoren aus Flora und Fauna und einer unter Berücksichtigung relevanter Fachliteratur. Neben der Hervorhebung der ökologischen Veränderungen und einer folgenden Diskussion über Managementfragen, werden auch umsetzbare Empfehlungen und dazu entsprechende Anmerkungen gegeben. Die Arbeit liefert eine Synthese der Kenntnisse über die Donau in Serbien und warnt gleichzeitig vor Ideen, die reelle Projekte werden können und extreme gegensätzliche Konsequenzen für das Donau-Ökosystem haben können.

REZUMAT: Dunărea în Serbia – starea ecologică şi probleme de management.

În ultimele decenii multe sectoare ale Dunării şi-au pierdut valoarea lor ecologică datorită presiunilor antropogene din partea agriculturii intensive, noi proiecte de infrastructură, navigaţie mai intensivă şi construcţii de hidrocentrale. Astfel de schimbări remarcabile au avut loc în deceniile trecute şi în aria de investigaţii, care e parte a Dunării mijlocii, de la kilometrul fluvial 1,433 la 845 în Serbia. Conţinutul prezentei lucrări se bazează pe analiză şi folosirea hărţilor topografice, analiza datelor privind cotele apelor şi a debitelor pentru cinci staţii hidrometrice ale Dunării din Serbia, a unor bioindicatori ai florei şi faunei, precum şi a literaturii relevante de specialitate. Pe lângă prezentarea alterărilor ecosistemului şi a discuţiilor asupra problemelor de management, sunt date de asemenea recomandări aplicabile şi unele remarci adiţionale. Lucrarea sintetizează cunoştinţele existente asupra Dunării în Serbia şi în acelaşi timp atrage atenţia asupra ideilor care pot deveni proiecte reale şi care au consecinţe extrem de adverse pentru ecosistemul Dunării.

INTRODUCTION

By enacting the Water Framework Directive in 2000, the EU improved its water policy and stated that rivers, lakes, groundwater and coastal areas should achieve "good ecological status" by 2015 as a base for the sustainable use of water resources across Europe (EU 2000, article 4; WWF 2001). This new water policy was triggered by rapid deterioration of water quality and degrading river ecosystems. However, reaching this goal is a great challenge in environmental protection for the EU member states.

Floodplain ecosystems have important functions, indicating ecological status of the entire river and providing water resources protection (EU 2000). Barrett (1999) underscores that wetlands and floodplains are used for water quality improvement as a valuable ecoengineering application. River floodplains are recognized as flood retention areas and natural sinks of nutrient load (Haycock et al., 1993; Barrett, 1999; Mölder and Schneider, 2010). Moreover, floodplains can be core areas for the restoration of whole, degraded riverine landscapes (Zsuffa and Bogradi 1995). However, surface connectivity between stream and riparian zones has been reduced and floodplains have been fragmented by channelizing and dredging river beds (Tockner et al., 1998). About 80% of the historical floodplain in large rivers has been lost during the last 150 years mainly through significant man-made hydromorphological alterations (WWF 1999; ICPDR 2005)

The Danube River has the most international basin in the world; it is a transit navigable line of international importance and has experienced a long history of human occupation (JDS 2008; Sommerwerk et al., 2009). Many reaches of the Danube have lost their ecological value during the past decades due to anthropogenic pressure by intensive agriculture, new infrastructure projects, intensified navigation, construction of hydropower plants, and flood protection schemes (dykes). Many of these projects and activities are in conflict with the principles and objectives of river ecosystem protection. For instance, the construction of dams, such as Gabcikovo and Iron Gate, has caused severe and adverse changes upstream and downstream in river ecosystems (Perisic, 2001; Klaver et al., 2007). However, there are still river stretches quite close to the natural state. Gornje Podunavlje in Serbia, Kopacki Rit in Croatia, Gemenc and Béda-Karapancsa in Hungary, crosscut by state-political borders, are parts of a huge floodplain ecosystem area. As very high biodiversity has been recorded, they are considered as an integral area, protected and listed as the Reserve of Biosphere, "Amazon of Europe".

The Serbian stretch in the Middle Danube is 588 km long. Most part of the territory of the Republic of Serbia (81,000 km² or 92%) belongs to the Danube River basin or to the Black Sea catchment (Ministry and Institute Jaroslav Cerni 2001). After ratifying the Framework Agreement on the Sava River basin and the Danube River Protection Convention in 2002 and 2003, respectively, Serbia became a member state of the ICPDR and the ISRBC, contributing to the international aims regarding sustainable and integrated management of the Danube River and the Danube region. The Republic of Serbia ratified the Ramsar Convention and has nine Ramsar sites so far (only one of them, Gornje Podunavlje is on the Danube). Determining wetland areas in Serbia as possible Natura 2000 Network of the EU is in progress. It is necessary to identify other valuable stretches of the Danube in Serbia as well and to inform the public to promote the conservation and protection of these areas.

This paper ensued from the research focusing on review, analysis and synthesis of hitherto data, published studies and reports which observed the Danube at the whole course or some parts of the Serbian stretch. Beside the aim to contribute to the national status assessment for the Danube, this work has an intention to underline the significance of floodplains and support future research on floodplains and their protection in Serbia. When considering the Danube stretch in Serbia, we discuss about three main issues:

– pointing all the main changes that have happened on the Danube in Serbia emphasizing the ecological alterations;
– management issues and applicable recommendation.

MATERIALS AND METHODS

Study area

The river basin of the Danube, which has a total length of 2,850 kilometres, can be divided into three main sections and the delta. The Upper Danube extends from the source in the Black Forest in Germany to the confluence with the Morava River near the Slovakian capital of Bratislava, the Middle Danube extends from Bratislava to the breach valley of the Iron Gate (border between Romania and Serbia), and the Lower Danube is formed by the Romanian-Bulgarian lowlands and the large Romanian floodplains downstream of Călăraşi-Silistra (border between Romania and Bulgaria). Finally, the Danube discharges into the Black Sea; the delta is the second largest in Europe (Schneider et al., 2009; Sommerwerk et al., 2009). The investigation area is a part of the Middle Danube, from 1,433 to 845 rkm (i.e. from Bezdan to the mouth of the Timok River) which extends in Serbia. This stretch, in terms of the alterations of waterline and discharge, hydromorphology, species diversity changes on the one hand and pollution/water quality, dredging and impact of other activities, on the other hand, are studied.

The Tisa River and the Sava River, as the most important tributaries, have the confluences in the Danube in the vicinity of Slankamen and Belgrade, respectively, and the impact in terms of the discharge and water quality. The Tisa River (966 km, 766 m³/sec) is the longest tributary of the Danube, with the largest sub-basin in the Danube River basin and the second largest discharge, after the Sava River (940 km, 1,564 m³/sec) (Liepolt, 1967). Beside the Sava and the Tisa, there is a third most important tributary of the Danube in Serbia, the Great Morava (494 km, 256 m^3/s). It begins from the confluence of the Juzna Morava (Southern Morava) and the Zapadna Morava (Western Morava), near the small town of Stalac. Even 85% of the catchment of mentioned Morava River system belongs to the territory of

Serbia and it has a tremendous value in economical sense. The last can be applied also to the Timok River, which at first begins as Svrljiski Timok, receiving other "Timok rivers" with different attributes in front of this name. After 184 km Timok flows into Danube and it is a point where the Danube River leaves the territory of Serbia. The other tributaries on the Serbian stretch of the Danube are Tamis/Timiş, Karas/Caraş, Nera, Pek and Cerna (Ministry for agriculture, forestry and water management and Institute for water management Jaroslav Cerni, 2001).

After entering Serbia near Bezdan, the Danube flows in the direction north-south meandering through lowlands, then under the geomorphological influence of Fruska Gora mountain it continues in the direction of east. Before the confluence of the Tisa, it turns to the south; then receiving water from Sava in Belgrade, turns to east entering the Djerdapska Gorge. On its course in Serbia, the Danube has 42 meanders (ref. dr. I. Todorovic, IAD) and a lot of river icelands (such as Sarengradska ada, Krcedinska ada, Veliko ratno ostrvo). The average discharge of the Danube in Bezdan is 2.263 m^3/s and in Veliko Gradiste 5.466 m^3/s. The fall is 37,6 m and this river has the largest hydro potential on the territory of Serbia. (Ministry and Institute Jaroslav Cerni, 2001).

The Danube River changes its discharge regime along its course under the influence of different factors. It belongs to the glacial type till Bratislava, but after the influence of confluence of larger rivers such as the Tisza and the Sava, it is pluvial. The Danube discharge in Serbia peaks in June and July, and it is low in September, November and December (Ministry and Institute Jaroslav Cerni, 2001)

Figure 1: The Danube stretch in Serbia. Cartography: Jürgen Christmann (WWF Institute for Floodplain Ecology, Rastatt);
Gauging stations are in Bezdan, Apatin, Novi Sad, Zemun and Banatska Palanka.

Material and data analysis

The content of this review paper is based on analyzing and using topographical maps, data on waterline and discharges from 1967 to 2006 (however, there are unavailable data on discharge for most of years) for five hydrological stations (Bezdan, Apatin, Novi Sad, Zemun and Banatska Palanka). The analysis of waterline in last few decades gives a possibility to conclude about river bed incision. On the base of identification of floral and faunal bioindicators, several valuable conclusions on the ecological quality of the habitats and, further, on the ecological status of the floodplains can be made. Unfortunately, due to lack of species data for entire Serbian stretch of Danube, for some parts of the reach profound registration of flora and fauna species is not possible. Interviews with international and local experts were helpful, too. Published studies, project reports and journal papers, which observed Danube at whole course or some stretches in Serbia, were used. Using two internet available bibliographic databases, "ISI Web of Knowledge" and "Science Direct – Scopus", we selected scientific papers which deal with the whole Danube course. We find significant some sources that do not deal with this stretch, but another stretches of the Danube or even another river.

RESULTS AND DISCUSSIONS

Serbian Danube stretch is divided in two especially in terms of ecological state of the floodplains: the first, reach from entering the Danube in Serbia till the confluence of the Tisa and the second, from the confluence of the Tisa till the border between Serbia, Romania and Bulgaria. Determining wetland areas in Serbia as possible Natura 2000 sites is ongoing. Gornje Podunavlje and Koviljsko-Petrovaradinski Rit are considered to be listed as Natura 2000 sites and already a RAMSAR sites as well (Institute for Nature Conservation 2007, 2010). According to the Joint Danube Survey 2, the Special natural reserve "Gornje Podunavlje" and floodplain forests upstream of the Tisa confluence appertain to the intacted floodplain areas with high or very high ecological value (1 and 2 class of the given scale – nearly natural and slightly modified). Despite the legal-based protection of "Gornje Podunavlje" there is still a wide range of problems that threaten to the water and marsh ecosystems, which are thus, recommended to be protected by stricter measures (Panjkovic, 2005).

However, 1972 and the construction of the Iron Gate I (which is the largest single hydropower dam along the Danube; the second largest is upstream the Gabcikovo Dam) was the key year for a wide range of alterations in river ecosystem of the Danube at Serbian stretch as well as downstream (Fig. 1) (Vukov et al., 2008; Klaver et al., 2007; Schneider et al., 2009). These are reduced flow velocity (Figs. 2, 3 and 4), migration barriers, increased sedimentation upstream and decreased sedimentation downstream of the dam, hydromorphological alterations and loss of floodplains owing to increased water level and backwaters nearly up to Belgrade (Vukov, Igic, Boza, Anačkov and Janauer 2008; McCartney, Sullivan and Acreman, 2001). A pristine ecosystem of the stretch from Belgrade to the Iron Gate, before its construction was much more diverse (Perisic, 2001). Even though it had been settling for millennia, due to anthropogenic pressure in the last decades, the second sector of the Danube floodplains in Serbia, from Tisa confluence until the Timok confluence, is considerably altered and damaged. For instance, the streber habitats in the gorge section of the Iron Gate have been destroyed by building of two Iron Gate dams (WWF, 1999)**.** Before the construction of the Iron Gate reservoir, *Acipenser ruthenus, Huso huso, Acipenser stelatus, Acipenser guldenstaedti, Alosa pontica* used to go upstream for reproduction (Bănărescu, 1964).

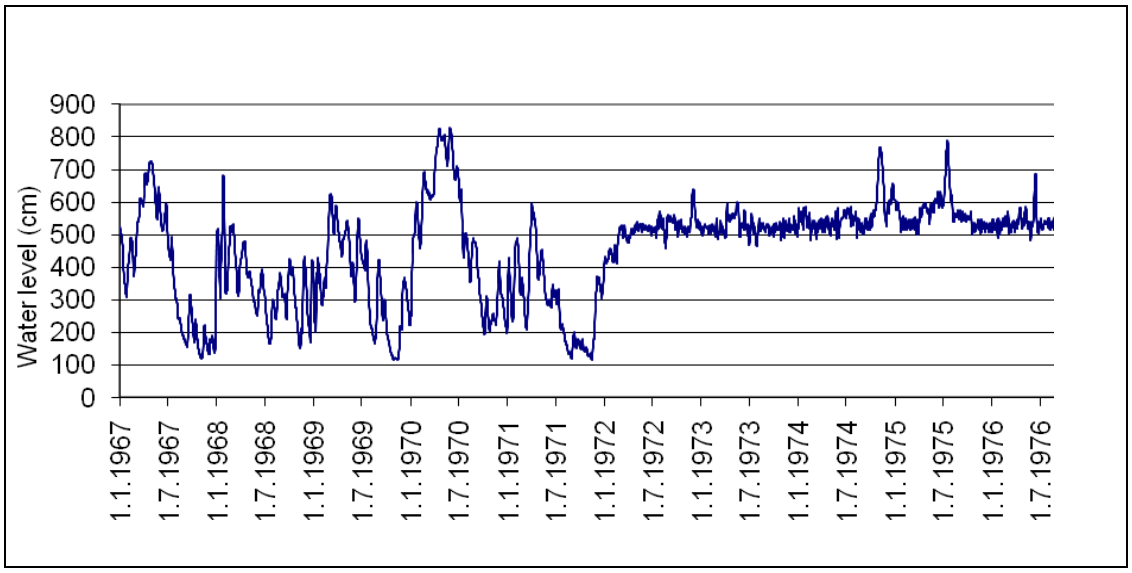


Figure 2: Banatska Palanka gauge (1,076.6 rkm; 62.85 m above the sea level).

Upstream, on the Veliko Ratno River island near Belgrade, *Amorpha fruticosa* has spread and occupied 37% of total island area in last two decades (Šinžar-Sekulić et al., 2008). After the construction of canal network of the hydrosystem "DTD" (Danube – Tisa – Danube) half a century ago, area of marshes in Gornje Podunavlje and the region of Backa was lowered for 11 times (Panjkovic, 2005). It resulted in dying wetland poplar and willow forest (Herpka, 1979). The competitiveness of *Amorpha fruticosa*, *Fraxinus pennsylvanica*, *Acer negundo* and *Solidago serotina* changed the structure of associations in Gornje Podunavlje (Panjkovic, 2005).

The ICPDR (2009) defined four significant water management issues in the Danube River Basin District Management Plan – hazardous substances, hydromophplogical alternations, organic pollution and nutrient pollution which apply also for the Danube stretch in Serbia. Besides worsening water quality, the most significant hydromorphological alterations on the Danube are longitudinal continuity interruptions (dams, weirs), lateral connectivity interruptions (loss of floodplains, bank reinforcements), and hydrological alterations (water abstraction and hydro-peaking). Moreover, four main impacts of hydromorphological alterations on the riverine habitats are recognized: decline of species biodiversity, decline of species abundance, altered population composition, hindrance of species migration and a corresponding decline in naturally reproducing fish populations (ICPDR 2005, 2009).

According to Schwarz (2008a), there is no clear evidence of channel incision at the stretch between the Tisa confluence and the Iron Gate.

Analization of gauge data in the period of 1967-2006, we concluded and confirmed Schwarz's claim that there is almost no channel incision. Dredging is not ample – in 2004 one-year permits for dredging of 24 million m^3 from the Danube were issued, but most of dredging companies are not operable and there is no enough capacity (Joint Danube Survey 2 – Final Scientific report 2008, Schwarz 2008b). However, there is a claim about illegal dredging.

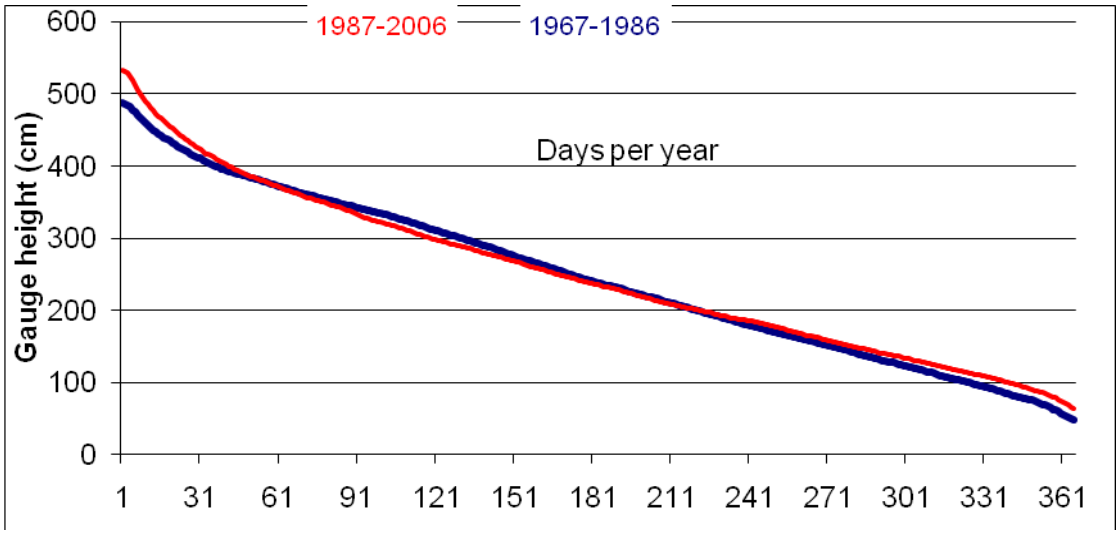


Figure 3: Mean exceeded gauge heights in Novi Sad (1967-2006).

Detailed maps show the floodplain areas recommended for restoration which can retain floods, improve water quality and increase biodiversity. The area between Gemenc and Kopacki Rit (Hungary, Croatia, and Serbia) was listed as a proposed restoration area with large-scale rehabilitation possibilities. The present settlement structure requires a detailed investigation to determine a concise delineation (WWF, 1999). In the largest area of the Gornje Podunavlje, dykes are too close to the river course and should be moved from the river bed to give more life to the valuable floodplains (oral communication Dister, 2010). Such restoration becomes rather urgent since climate change increases the risk and frequency of extreme floods (European Parliament and Council 2007; Fig. 4). For instance, Barredo (2007) showed that from the 1950s to the 1990s, the number of floods in European river basins was rising from 11 to 64 per decade, while the first five years of 21st century already showed 104 floods.

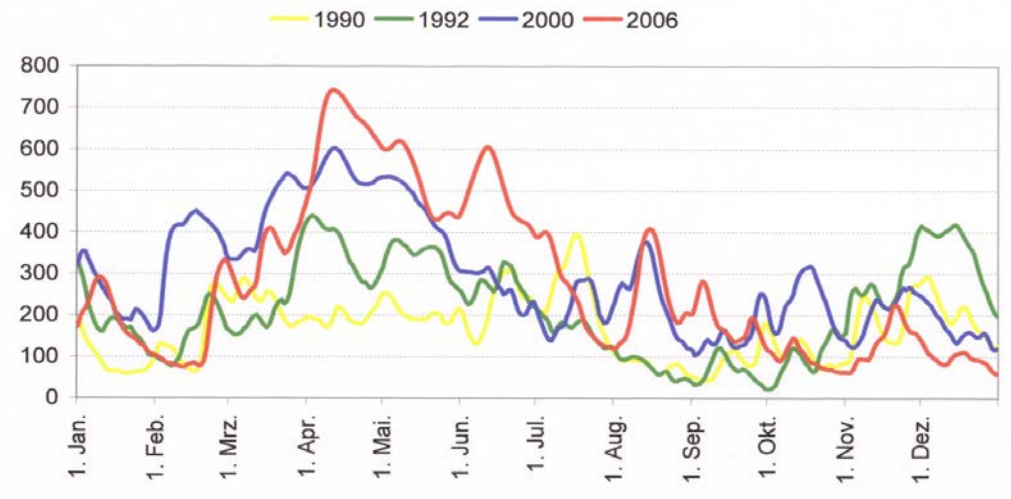


Figure 4: Water level of the Danube for specific years.

The contribution of Serbia to the EU Danube strategy is a responsibility on national level including five adopted projects: (1) the construction of a new Zezelj bridge in Novi Sad, (2) the removal of unexploded ordnance, (3) the removal of sunken German ships from World War II, (4) the implementation of RIS in Serbia, and (5) hydrotechnical works in critical sectors near Apatin (Ostojic Barjaktarevic, 2010). However, sound environmental impact assessments of projects and strict adherence to the environmental laws, such as in the case of the bridge constructions near Belgrade and Novi Sad or the modern harbor center in Apatin, are a must and an obligation. Nevertheless, in the master plan for water management, decision makers plan to construct a new large hydropower plant near Novi Sad, which would totally destroy Gornje Podunavlje and Kopacki Rit, adding new risks of extreme floods and considerably affecting the Danube-Drava National Park in Hungary. Therefore, this proposal raises understandable concern and we find it completely unacceptable.

Finally, since the Republic of Serbia strives to the European Union membership, it is recommended to work more on meeting the requirements given in the Water Framework Directive 2000/60/EC and the Flood Directive 2007/60/EC as well as to use assigned funds in a wise manner. Further, the river basin management of the Great Morava, must be implemented as the water quality of this tributary is worse than that of the Danube (JDS 2008). The Ministry for water management initiated work on the integrated water resources management plan for the Great Morava, but not in cooperation with other countries in the river basin – Bulgaria and Macedonia (15% of the catchment). However, transboundary management is necessary as rivers and pollution do not respect national borders. A recent testimony, after a lot of alarming examples in the past, is the shocking ecological catastrophe “Ajka” in 2010 coming from Hungary, contaminating the downstream stretch of the Danube in Serbia. Therefore, transboundary and integrated water resources management is required as a complex and comprehensive task setting up a framework for ensuring long-term use and improvement of the ecological status of all waters as well as a fulfillment of the principle “upstream and downstream users” (UN Water 2008; Kojiri 2008). Moreover, water management issues must be more incorporated into other development policy sectors of the country.

CONCLUSIONS

The Serbian stretch of the Danube can be divided into two parts in terms of ecological state of the floodplains: the first, reach from entering the Danube in Serbia till the confluence of the Tisza and the second, from the confluence of the Tisza till the border between Serbia, Romania and Bulgaria. Floodplain areas, recommended for restoration, are designed. In this sense, the urgency for action in benefit of floodplains is greater as climate change increases the risk and frequency of extreme floods. Realizing some ideas and projects would totally destroy valuable parts of the Danube.

Finally, although this paper deals with the Danube in Serbia, it can be noticed that upstream and downstream influences are considered and the need for more intensive cooperation between countries in the Danube River basin is recognized.

ACKNOWLEDGMENTS

I am grateful to the German Environment Foundation (DBU) that supported financially my research stay at the WWF Institute for Floodplain Ecology. I would like to acknowledge supervising by Moelder A. and Schneider E., and the Province Institute for Nature Conservation in Novi Sad assistance.

REFERENCES

1. Barredo J., 2007 – Major flood disasters in Europe: 1950-2005, Nat Hazards, 125-148.
2. Barrett K., 1999 – Ecological Engineering in Water Resources: The Benefits of Collaborating with Nature. Water International, 24, 182-188.
3. European Parliament and Council, 2000 – Directive 2000/60/EC of the European Parliament and of the Council.
4. European Parliament and Council, 2007 – Directive 2007/60/EC of the European Parliament and of the Council.
5. International Commission for the Protection of the Danube River, 2005 – Danube Basin Analysis. ICPDR – International Commission for the Protection of the Danube River, Vienna.
6. International Commission for the Protection of the Danube River, 2009 – Danube River Basin District Management Plan.
7. Institute for Nature Conservation, Republic of Serbia, Novi Sad E., 2007 – Nomination of Gornje Podunavlje for RAMSAR site in Serbia.
8. Institute for Nature Conservation, Republic of Serbia, Novi Sad, E., 2010 – Nomination of Koviljsko-Petrovaradinski Rit for RAMSAR site in Serbia.
9. Haycock N., Pinay G. and Walker C., 1993 – Nitrogen Retention in River Corridors: European Perspective. Ambio, 340-346.
10. Herpka I., 1979 – Ecological and biological characteristics of autochthonous poplars and willows in swamp forests of the Danube Basin. Poplar Research Institute, Novi Sad.
11. Joint Danube Survey 2 – Final Scientific report, 2008 – ICPDR – International Commission for the Protection of the Danube River, Vienna.
12. Klaver G., Os B., Negrel P. and Petelet-Giraud E., 2007 – Influence of hydropower dams on the composition of the suspended and riverbank sediments in the Danube. Environmental Pollution, 148, 718-728.
13. Kojiri T., 2008 – Importance and necessity of integrated river basin management. Physics and Chemistry of the Earth, 33, 278-283.
14. Liepolt R., 1967 – Limnologie der Donau. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart.
15. McCartney M., Sullivan C. and Acreman M., 2001 – Ecosystem Impacts of Large Dams. International Union for Conservation of Nature and Natural Resources and the United Nations Environmental Programme.
16. Ministry for agriculture, forestry and water management and Institute for water management Jaroslav Cerni, 2001 – Master plan for water management in Serbia.
17. Mölder A. and Schneider E., 2010 – On the beautiful diverse Danube? Danubian floodplain forest vegetation and flora under the influence of river eutrophication. River Research and Applications, 9999, n/a.
18. Ostojic Barjaktarevic, 2010 – Priority projects on the Danube in Serbia.
19. Panjkovic B., 2005 – Aquatic and semiaquatic vegetation of Apatinski and Monostorski Mars.
20. Perisic M., 2001 – Iron Gate Water Reservoir – truth and mistakes. Geoinstitute, Belgrade.
21. Schneider E., Dister, E. and Doepke, M., 2009 – Lower Danube Green Corridor. Kraft-Druck, Ettlingen.
22. Schwarz U., 2008a – Assessment of the balance and management of sediments of the Danube. Slovenian National Commitee for IHP Unesco, Ljubljana.
23. Schwarz U., 2008b – Genesis and typology of riparian and fluvial landforms of the Kopački Rit within the Danube floodplain corridor in Croatia and Serbia. pp. 318-321. Vienna
24. Sommerwerk N., Baumgartner C., Bloesch J., Hein T., Ostojić A., Paunović M., Schneider-Jakoby M., Siber R. and Tockner K., 2009 – The Danube River Basin. The Rivers of Europe Tockner K., Robinson C. and Uehlinger U. (eds), Elsevier, London.

25. Tockner K., Schiemer F. and Ward J., 1998 – Conservation by restoration: the management concept for a river-floodplain system on the Danube River in Austria. Aquatic Conservation: Marine and Freshwater Ecosystems, 8, 71-86.
26. UN Water, Task Force on Transboundary Waters, 2008 – Transboundary Waters: Sharing Benefits, Sharing Responsibilities. Zaragoza.
27. Vukov D., Igic R., Boza P., Anačkov G. and Janauer G., 2008 – Habitat and Plant Species Diversity along the river Danube in Serbia. pp. 127-131. Vienna.
28. WWF, 2001 – Elements of Good Practice in Integrated River Basin Management: A Practical Resource for Implementing the EU Water Framework Directive. Brussels.
29. WWF Danube-Carpathian-Programme and WWF-Auen-Institut, 1999 – Evaluation of Wetlands and Floodplain Areas in the Danube River Basin. WWF-Auen-Institut, Rastatt.
30. Zsuffa I. and Bogradi J. J., 1995 – Floodplain Restoration by Means of Water Regime Control. Phys. Chem. Earth, 20, 237-243.

THE ANALYSE OF THE TROPHIC RESOURCES UTILISATION BY THE CONGENERIC SPECIES *BARBUS BARBUS* (LINNAEUS, 1758) AND *BARBUS MERIDIONALIS* RISSO, 1827 IN TÂRNAVA RIVER BASIN

*Doru BĂNĂDUC *, Letiția OPREAN *,*
*Alexandru BOGDAN *** and *Angela CURTEAN-BĂNĂDUC **

* "*Lucian Blaga*" University of Sibiu, Faculty of Sciences, Dr. Ioan Rațiu Street 5-7, Sibiu, Romania, RO-550012, ad.banaduc@yahoo.com, letitia.oprean@ulbsibiu.ro, angela.banaduc@ulbsibiu.ro,
** Romanian Academy, "Acad. *David Davidescu*" Centre of Studies and research for Agrosilvicultural Biodiversity, Bucharest, Romania, alecutbogdan@yahoo.com

KEYWORDS: *Barbus barbus*, *Barbus meridionalis*, trophic supply, trophic demand, trophic niche dimension, trophic niche overlapping, Târnava Basin, Romania.

ABSTRACT

The study performed in the Târnava River basin shows that the congener species *Barbus meridionalis* and *Barbus barbus* have relatively narrow trophic niches with a high degree of overlapping. In the analysed fish associations, *Barbus meridionalis* has a relative advantage in competing for food with *Barbus barbus* due to the larger use of the trophic resources most available in the environment and due to a significant overlapping of its trophic niche with a smaller number of fish species, (*Barbus meridionalis* with four fish species, *Barbus barbus* with six fish species). The situation may explain at least partially the increasing presence of the first and the decrease of the second species' presence in the Târnava River basin.

RÉSUMÉ: L'Analyse de l'utilisation des niches trophiques par les espèces congénères *Barbus barbus* (Linnaeus, 1758) et *Barbus meridionalis* Risso, 1827 dans le bassin de la rivière de Târnava.

L'étude effectuée dans le bassin de la Târnava relève le fait que les espèces congénères *Barbus meridionalis* et *Barbus barbus* présentent des niches trophiques relativement étroites et le degré de superposition des niches de ces espèces est relativement important. Dans les communautés de poissons étudiées, *Barbus meridionalis* détient un avantage compétitif comparé à *Barbus barbus* grâce à l'utilisation plus importante des ressources trophiques les plus disponibles dans le milieu et due à la superposition signifiante des niches trophiques avec un plus petit nombre d'espèces de poissons (*Barbus meridionalis* avec quatre espèces, *Barbus barbus* avec six espèces). Cette situation peut expliquer au moins partiellement la distribution croissante de la première espèce et la diminution de la seconde dans le bassin de Târnava.

REZUMAT: Analiza utilizării resurselor trofice de către speciile congenere *Barbus barbus* (Linnaeus, 1758) și *Barbus meridinalis* Risso, 1827 în bazinul râului Târnava.

Studiul realizat în bazinul Târnava relevă faptul că speciile congenere *Barbus meridionalis* și *Barbus barbus* prezintă nișe trofice relativ înguste, iar gradul de suprapunere al nișelor trofice ale acestor specii este mare. În comunitățile de pești studiate, *Barbus*

meridionalis este în avantaj în competiţie pentru hrană cu *Barbus barbus* datorită utilizării în proporţii mai mari a celor mai disponibile resurse trofice din mediu și suprapunerii nişelor trofice într-un grad semnificativ cu un număr mai mic de specii de peşti (*Barbus meridionalis* cu patru specii de peşti, *Barbus barbus* cu şase specii de peşti). Acestă situaţie poate explica măcar parţial distribuţia în creştere a primei specii şi restrângerea distribuției celei de a doua, în bazinul Târnava.

INTRODUCTION

In the last few decades it was noticed that the *Barbus barbus* (Linnaeus, 1758) populations restrained their distribution and *Barbus meridionalis* (Risso, 1827) extended it in the Romanian lotic systems in comparison to the middle of the XXth century data (Bănărescu and Bănăduc, 2007). As long as the habitats where these congeneric fish species co-exist, they suffered in general by the same types of human impact. *Barbus meridionalis* is considered a more sensitive species to the human impact than *Barbus barbus* is (Bănăduc, 2007), another explanation that these species' habitat changes influence their different range modification. Could the trophic relations bring an explanation in this respect?

Barbus barbus and *Barbus meridionalis* were always distinguished by the fishermen of the Romanian national territory (Băcescu, 1947) and were discussed in terms of scientific knowledge for that time in the monographycal ichtyological fauna of Romania (Antipa, 1909). Here are given general data on the geographic spread of both species in Romania, noting however that since that time, confusions between these two species were not possible.

Concerning these species' food,worms and insect larvae are noted (Antipa, 1909). Bănărescu (1964) noted general data: for *Barbus barbus* there are, aquatic insects, worms, crustaceans, plants and roes, and for *Barbus meridionalis* there are benthic aquatic invertebrates (tendipedes, ephemeropterans, trichopterans, gamarids, oligochaetes) and less plants. Neither of these two species was studied in a comparative approach in the following half of the century.

In terms of the protective approach, *Barbus barbus* is considered of Least Concern (IUCN, 2011) and *Barbus meridionalis* is considered of being Near Threatened (IUCN, 2012) and belong to Habitats Directive Annex 2. From the economic point of view, these two fish species are considered as being of local interest especially for anglers, (Bănărescu, 1964).

This species is usually directly threatened by pollution, habitat destructions and water abstraction, (Bănărescu and Bănăduc, 2007), but all of these threats can also have an indirect effect on the fish when negatively influencing their natural macroinvertebrates trophic resources.

The natural trophic (qualitatively and quantitatively) offer is a very important element which induces these species' population structures and competition?

The (natural/anthropogenic modified) aquatic habitat elements variability are modified in an important manner, by the biotic interrelations and model the aquatic biodiversity structure (Momeu et al., 1999; Kutzenberger, 2008; Ciubuc, 2009; Bănăduc, 2010; Sisma-Ventura, 2010).

The limits of the variability, in the actual trends of climate change (IPCC, 2007) will be pushed further, as well as the adaptation potential of aquatic macroinvertebrates and fish. The habitat limitations, modifications and destruction, forced the aquatic biota to be, at least in some cases or periods of time, confined to some more or less refugia sectors, where due to the abnormal/high fish densities, the pressure on the trophic offer (with spatial and temporal effects in terms of quality and availability) can be very high with effects on the fish populations' structure. The questions are, would different feeding strategies and possibilities

allow some species to be more competitive in such circumstances or not, and if these strategies are working, are the congeneric species included? Are these strategies significantly different enough to allow an advantage for one or another species to win the competition for food?

Târnava Basin was studied for these targeted purposes due to its variation in time and space in climate, geology, relief, hydrology, plants, invertebrates and vertebrates communities, and human impacts' by categories and strength, (Curtean-Bănăduc et al., 2005; 2007). This variability of the physico-chemical factors and biotic interactions offer different situations in which the two congeneric species co-exist or not and can be realised; also a comparative study in relation with the invertebrates trophic offers variation.

MATERIAL AND METHODS

There were analyzed individuals of the species of interest and also of the other sampled fish species, of three sectors (S1-3) on the Târnava Mare River and of three sectors (S4-6) on the Târnava Mică River (Fig. 1). In these lotic sectors are stable/permanent populations of *Barbus barbus* and/or *Barbus meridionalis*. These fish associations sectors were selected based on some older studies/sampling sectors (Bănăduc, 2005), regarding the fish communities structure and their biotope conditions, as to be appropriate for this study targets.

The fish communities structure (Tab. 1) was achieved in terms of relative abundance, based on the quantitative samples obtained by elecrtrofishing in one hour and effort unit, on five-seven longitudinal sections of 100 m in length for each river sector. After fish identification, the fish were released into their habitat, except individuals required analyzing the intestinal content, they were immediately preserved in formaldehyde 4%, formaldehyde was also injected into the digestive tract. For each considered fish community, we analyzed the fish diet (Tabs. 2-7) of the species with a higher than 4% relative abundance.

For the fish diet analysis, a minimum of 25 adult individuals for each species and for each station were dissected and the intestinal content were analyzed – the identifiable fragments of the ingested organisms; at the individuals which ingested detritus this was reported as present.

Ten macroinvertebrate quantitative samples per station, in different points were done, totalling 70 benthic macroinvertebrates samples. The quantitative benthic macroinvertebrates samplings were realized with an 887 cm^2 surface Surber Sampler with a 250 μm mesh net. To name the ecologic niche trophic dimension we will use the "trophic niche" syntagm. The biological material was fixed immediately after its sampling in 4% formaldehyde solution, then preserved in 70% alcohol, and included in the "Lucian Blaga" University of Sibiu, Department of Environmental Sciences, Hydrobiology Laboratory collections.

The trophic supply was estimated based on the benthic macroinvertebrates groups average density values, which was transformed in terms of relative abundances. The trophic demand was estimated through the food items transformation in terms of relative abundance – each systematic group proportion as part of the conspecific individuals groups for each sampling station. For the sampled fish species trophic niche dimension assessment, the following indexes were determined: the standardized Levins index (Bs) and the standardized Hurlbert index (BA) (Krebs, 1989). Also, for the ecologic niches overlapping degree assessment Pianka index (O_{jk}) (Gomoiu and Skolka, 2001) has been determined.

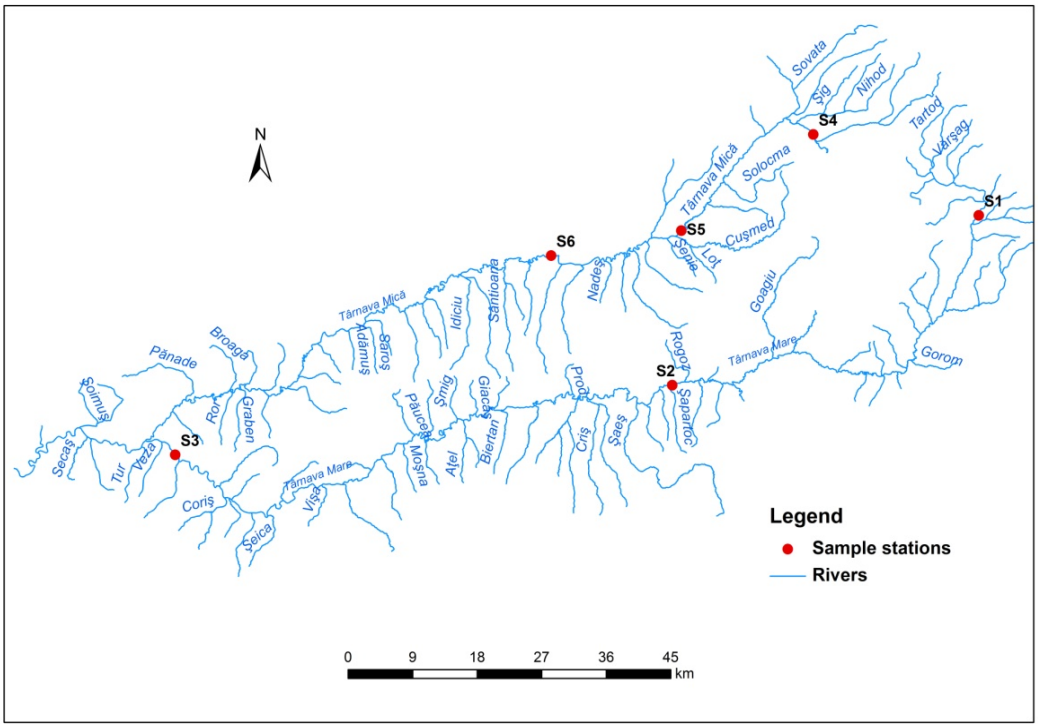


Figure 1: The Târnave rivers biological material sampling sectors (S1-S6).

RESULTS AND DISCUSSIONS

Based on the relative abundance of the fish species, the studied fish communities can be grouped in four classes (Fig. 2): I. communities in which the numerical dominant is the species *Gobio g.*, following with abundances higher than 10% *Squalius c.*, *Alburnoides b.*, *Barbus m.*, present in S2 and S6; II. communities in which numerically co-dominants are *Squalius c.*, *Sabanejewia b.* and *Gobio g.*, present in S3 and S5; III. communities in which numerically co-dominants are *Squalius c.* and *Barbus m.*, present in S1; IV. communities in which numerically co-dominants are *Alburnoides b.* and *Barbatula b.*, present in S4.

The relative abundance of *Barbus b.* vary between 1.6% and 28.57%, and that of the species *Barbus m.* is between 8.58% and 23.93% (Tab. 1). With the exception of the altitudinal lowest S3 river sector, *Barbus m.* present higher abundances than *Barbus b*. The values of the standardized Levins index (Tabs. 2, 3, 4, 5, 6, and 7), for the six fish analyzed communities indicate that the *Barbus b.* and *Barbus m.* species have relatively narrow trophic niches.

The non-parametric Kruskal-Wallis test, indicates that there are no statistically significant differences between the trophic spectrum of the two analyzed *Barbus* species; their trophic spectrum is predominantly formed from chironomidae larvae, alongside which appear in variable proportions of oligochetes, trichopterans, and ephemeropterans larvae (Tabs. 2, 3, 4, 5, 6, and 7). In the digestive tube of 35% of the *Barbus barbus* individuals and 27% of the *Barbus meridionalis* individuals detritus was identified, but no conclusions can be drawn regarding the use of detritus of a deliberate food or if it reaches the digestive tract with the ingestion of food organisms. In this context, it can be revealed that there is a possible higher selectivity of the food items of *Barbus meridionalis* in comparison with *Barbus barbus*, and in this context, a competitive advantage for the first species.

Comparing the *Barbus barbus* and *Barbus meridionalis* species' diet, with the trophic offer (Tabs. 2, 3, 4, 5, 6, and 7) it is found that they consume chironomids and oligochetes, which are more abundant as a resource in the environment, as well as ephemeropterans and trichopterans, whose abundance in the environment is lower. The Hurlbert index values indicate that *Barbus meridionalis* usually consumes the most available resources in the environment in a higher proportion than *Barbus barbus* – in the diet which appears with higher frequency groups with lower relative abundance in the environment. This should provide an advantage for the feeding success of *Barbus meridionalis* populations.

The analysis of the degree of overlapping of trophic niches based on the Pianka index (Figs. 2, 3, 4, 5, 6, and 7) indicates that the trophic niches of *Barbus barbus* and *Barbus meridionalis* species are significantly overlapping, also there is a high degree of overlapping between the trophic niches of *Barbus barbus*, *Barbus meridionalis* and *Alburnoides bipunctatus* (Fig. 3), *Barbus meridionalis*, *Sabanejewia balcanica* and *Gobio gobio* (Fig. 4), *Barbus barbus*, *Barbus meridionalis*, *Gobio gobio*, *Sabanejewia balcanica*, *Gobio kessleri* and *Alburnoides bipunctatus* (Fig. 5); *Barbus barbus*, *Orthrias barbatulus*, *Sabanejewia balcanica* and *Barbus meridionalis* (Figs. 5, 6).

Table 1: The fish communities' structure, analyzed in Târnava Basin (A – relative abundance, S1-S6 sampling stations).

Species	Sampling station A (%)					
	S1	S2	S3	S4	S5	S6
Eudontomyzon danfordi Regan, 1911	2.56	0	0	0	0	0
Squalius cephalus (Linnaeus, 1758)	36.75	19.5	15.71	5.15	20.7	13.33
Phoxinus phoxinus (Linnaeus, 1758)	8.55	0	0	0	0	0
Alburnoides bipunctatus (Bloch, 1782)	5.98	10.9	8.58	43.30	0	25.19
Chondrostoma nassus (Linnaeus, 1758)	1.71	14.8	0	2.06	0	0
Rhodeus sericeus (Pallas, 1776)	0	0	0	0	18.7	0
Gobio gobio (Linnaeus, 1758)	3.43	38.3	11.43	5.15	20.7	35.55
Romanogobio kesslerii (Dybowski, 1862)	0	0	10.0	0	0	0
Barbus barbus (Linnaeus, 1758)	5.98	1.6	28.57	5.15	4.4	5.19
Barbus meridionalis Risso, 1827	23.93	12.5	8.58	10.32	13.8	10.38
Barbatula barbatula (Linnaeus, 1758)	5.13	1.6	0	24.74	4.4	5.93
Sabanejewia balcanica (Karaman, 1922)	1.71	0.8	17.13	4.13	17.3	4.43
Cottus gobio (Linnaeus, 1758)	4.27	0	0	0	0	0

The values of the standardized Levins index (Tabs. 2, 3 4, 5, 6, and 7), for the six fish analyzed communities indicate that the *Barbus barbus* and *Barbus meridionalis* species have relatively narrow trophic niches.

The non-parametric K-W test, indicates that there are no statistically significant differences between the trophic spectrum of the two analyzed *Barbus*, their trophic spectrum is predominantly formed from chironomidae larvae, alongside which appear in variable proportions larvae of oligochetes, trichopterans, and ephemeropterans (Tabs. 2, 3, 4, 5, 6, and 7). In the digestive tube of 35% of the *Barbus barbus* individuals and 27% of the *Barbus meridionalis* individuals detritus was identified, but no conclusions can be drawn regarding the use of detritus of a deliberate food or if it reaches the digestive tract with the ingestion of food organisms. In this context can be revealed a possible higher selectivity of the food items of *Barbus meridionalis* in comparison with *Barbus barbus*, and in this context a competitive advantage for the first species.

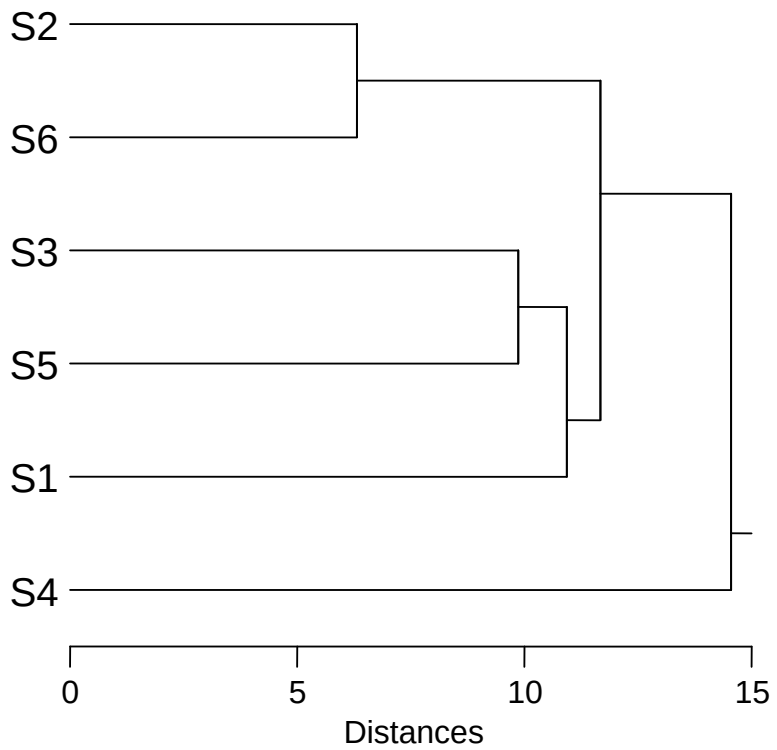


Figure 3: Cluster analysis of the fish communities from the six rivers sectors (S1-S6) considered along the rivers Târnava Mare and Târnava Mică, based on the relative abundance of the fish species present in each community.

Comparing the *Barbus barbus* and *Barbus meridionalis* species diet, with the trophic offer (Tabs. 2, 3, 4, 5, 6, and 7) it is found that they consume in addition to chironomids and oligochetes, which are more abundant as a resource in the environment, ephemeropterans and trichopterans whose abundance in the environment is lower. The Hurlbert index values indicate that *Barbus meridionalis* usually consumes the most available resources in the environment in a higher proportion than *Barbus barbus* – in the diet of which appear with higher frequency groups with lower relative abundance in the environment. This should provide an advantage for the feeding success of *Barbus meridionalis* populations.

The analysis of the degree of overlapping of trophic niches based on the Pianka index (Figs. 3, 4, 5, 6, 7, and 8) indicates that the trophic niches of *Barbus barbus* and *Barbus meridionalis* species are significantly overlaping, also there is a high degree of overlapping between the trophic niches of *Barbus barbus*, *Barbus meridionalis* and *Alburnoides bipunctatus* (Fig. 3), *Barbus meridionalis*, *Sabanejewia balcanica* and *Gobio gobio* (Fig. 4), *Barbus barbus*, *Barbus meridionalis*, *Gobio gobio*, *Sabanejewia balcanica*, *Gobio kessleri* and *Alburnoides bipunctatus* (Fig. 5), *Barbus barbus*, *Orthrias barbatulus*, *Sabanejewia balcanica* and *Barbus meridionalis* (Figs. 6 and 7).

Table 2: The structure of fish species' diet in S1 station and ecological niche size values (A – relative abundance of the groups in the diet, F – frequency of groups in the diet, Bs – standardized Levins index, FT – Smith index, BA – standardized Hurlbert index).

Fish species	**Diet**			**Benthos structure – trophic supply (%)**	**Ecologic niche dimension indexes**	
	Structure	**A%**	**F%**		Bs	BA
Squalius cephalus	Oligochaeta Ephemeroptera Plecoptera Trichoptera Chironomidae detritus plants	10.71 28.57 14.29 14.29 32.14 + +	21.74 86.95 52.17 52.17 86.95 21.74 13.04	Coelenterata – 63.02 Oligochaeta – 7.4 Gastropoda – 1.96 Acarina – 1.35 Amphipoda – 0.36 Ephemeroptera – 3.74 Odonata – 0.042 Plecoptera – 1.65 Trichoptera – 1.7 Heteroptera – 0.048 Blepharoceridae – 0.8 Chironomidae – 17.93	0.268	0.186
Alburnoides bipunctatus	Trichoptera detritus	100 +	100 44		0	0.017
Barbus barbus	Oligochaeta Trichoptera Chironomidae detritus	4.35 50 45.65 +	20.83 100 95.83 21.74		0.098	0.063
Barbus meridionalis	Trichoptere Chironomide detritus	33.33 66.67 +	95.33 81.43 38.10		0.067	0.111
Cottus gobio	Ephemeroptera Plecoptera Trichoptera Chironomidae	71.88 21.88 3.12 3.12	92 84 68 44		0.064	0.059
Phoxinus phoxinus	Oligochaeta Ephemeroptera Plecoptera Trichoptera Chironomidae	1.12 91.01 5.62 1.12 1.12	22.73 95.45 40.90 54.55 18.18		0.017	0.044
Barbatula barbatula	Ephemerptera Chironomidae	69.90 30.10	89.47 26.32		0.061	0.073

Table 3: The structure of fish species' diet in S2 station and ecological niche size values (A – relative abundance of the groups in the diet, F – frequency of groups in the diet, Bs – standardized Levins index, FT –Smith index, BA – standardized Hurlbert index).

Fish species	**Diet**			**Benthos structure – trophic supply (%)**	**Ecologic niche dimension indexes**	
	Structure	**A%**	**F%**		Bs	BA
Squalius cephalus	Oligochaeta Ephemeroptera Trichoptera Chironomidae detritus plants	4.12 41.24 36.08 18.56 + +	5 75 75 35 5 10	Oligochaeta – 49.64 Hirudinea – 0.51 Acarina – 0.19 Ephemeroptera – 1.49 Trichoptera – 7.96 Tipulidae – 0.45 Chironomidae – 39.76	0.329	0.074
Alburnoides bipunctatus	Hirudinea Oligochaeta Ephemeroptera Trichoptera Coleoptera Chironomidae detritus plants	1.79 1.79 14.28 53.57 1.79 26.78 + +	4.76 4.76 76.19 90.48 4.76 71.43 4.76 4.76		0.256	0.183
Chondrostoma nasus	Oligochaeta Detritus	100 +	100 33.33		0	0.495
Barbus barbus	Oligochaeta Ephemeroptera Trichoptera Chironomidae plants	21.87 15.63 40.63 21.87 +	30.43 13.04 82.61 39.13 4.35		0.418	0.254
Barbus meridionalis	Oligochaeta Trichoptera Chironomidae detritus	7.69 26.93 65.38 +	33.33 68.18 5.17 9.09		0.15	0.526
Gobio gobio	Oligochaeta Ephemeroptera Trichoptera Chironomidae detritus	6.12 0.68 7.48 85.72 +	15 15 60 90 15		0.057	0.518
Barbatula barbatula	Ephemeroptera Trichoptera Chironomidae detritus	5.56 50 44.44 +	63.16 89.47 26.32 10.53		0.203	0.259
Sabanejewia balcanica	Trichoptera Chironomidae detritus	9.09 90.91 +	19.05 95.45 23.81		0.033	0.457

Table 4: The structure of fish species' diet in S3 station and ecological niche size values (A – relative abundance of the groups in the diet, F – frequency of groups in the diet, Bs – standardized Levins index, FT –Smith index, BA – standardized Hurlbert index).

Fish Species	**Diet**			**Benthos structure – trophic supply (%)**	**Ecologic niche dimension indexes**	
	Structure	**A%**	**F%**		Bs	BA
Alburnoides bipunctatus	Oligochaeta Ephemeroptera Trichoptera Coleoptera Chironomidae detritus plants	13.27 7.14 18.37 4.08 57.14 + +	18.18 22.73 86.36 9.09 95.45 9.09 4.54	Oligochaeta – 15.82 Amfipoda – 1.05 Collembola – 1.05 Ephemeroptera – 2.1 Trichoptera – 3.1 Coleoptera – 2.28 Heteroptera – 1.01 Tipulidae – 1.79 Chironomidae 71.8	0.178	0.506
Squalius cephalus	Hirudinea Oligochaeta Ephemeroptera Trichoptera plants	1.88 66.04 1.89 30.19 +	4.76 52.38 23.81 61.90 23.81		0.1	0.164
Sabanejewia balcanica	Oligochaeta Ephemeroptera Trichoptera Chironomidae Tipulidae	20.17 4.20 1.68 73.11 0.84	21.05 26.32 47.37 84.21 5.26		0.081	0.909
Barbus barbus	Oligochaeta Chironomidae detritus	3.95 96.05 +	13.04 100 4,35		0.009	0.77
Barbus meridionalis	Chironomidae Detritus	100 +	100 4		0	0.717
Gobio gobio	Oligochaeta Ephemeroptera Trichoptera Chironomidae detritus	20.75 1.24 0.83 77.18 +	29.17 45.83 37.5 95.83 16.67		0.062	0.898
Romanogobio keslerii	Ephemeroptera Trichoptera Chironomidae	14.81 7.41 77.78	76 44 100		0.065	0.484

Table 5: The structure of fish species' diet in S4 station and ecological niche size values (A – relative abundance of the groups in the diet, F – frequency of groups in the diet, Bs – standardized Levins index, FT –Smith index, BA – standardized Hurlbert index).

Fish species	**Diet**			**Benthos structure – trophic supply (%)**	**Ecologic niche dimension indexes**	
	Structure	**A%**	**F%**		Bs	BA
Gobio gobio	Oligochaeta Ephemeroptera Trichoptera Chironomidae	1.71 6.84 20.51 70.94	12.5 62.5 62.5 70.83	Nematoda – 0.49 Oligochaeta – 11.96 Collembola – 0.3 Ephemeroptera – 11.37 Plecoptera – 18.06 Trichoptera – 19.98 Coleoptera – 0.44 Blepharoceridae – 0.44 Chironomidae – 36.96	0.091	0.617
Barbatula barbatula	Hirudinea Oligochaeta Ephemeroptera Trichoptera Chironomidae	0.14 4.33 0.41 7.17 87.96	4.35 52.17 8.70 34.78 86.96		0.031	0.466
Alburnoides bipunctatus	Oligochaeta Ephemeroptera Plecoptera Trichoptera Coleoptera Chironomidae	0.30 46.99 0.60 28.92 0.30 22.89	13.64 86.36 68.18 68.18 9.09 68.18		0.2	0.398
Barbus barbus	Oligochaeta Trichoptera Chironomidae	1.29 10.73 87.98	36 76 92		0.03	0.463
Barbus meridionalis	Oligochaeta Trichoptera Chironomidae detritus plants	2.99 3.99 93.02 + +	29.17 29.17 83.33 25 4.17		0.017	0.423
Squalius cephalus	Ephemeroptera Plecoptera Trichoptera Chironomidae	36 2.66 26.67 34.67	80 30 55 55		0.234	0.547
Sabanejewia balcanica	Oligochaeta Chironomidae	7.14 92.86	48 100		0.017	0.419

Table 6: The structure of fish species' diet in S5 station and ecological niche size values (A – relative abundance of the groups in the diet, F – frequency of groups in the diet, Bs – standardized Levins index, FT –Smith index, BA – standardized Hurlbert index).

Fish Species	**Diet**			**Benthos structure – trophic supply (%)**	**Ecologic niche dimension indexes**	
	Structure	**A%**	**F%**		Bs	BA
Gobio gobio	Ephemeroptera Trichoptera Chironomidae	8.82 8.82 82.36	17.39 26.09 95.65	Turbelariata – 0.71 Hirudinea – 0.28 Oligochaeta – 18.32 Gastropoda – 0.75 Acarina – 1.34 Ephemeroptera – 1.79 Trichoptera – 6.2 Heteroptera – 0.27 Chironomidae – 70.34	0.049	0.656
Sabanejewia balcanica	Trichoptera Chironomidae	9.09 90.91	13.64 90.91		0.022	0.763
Barbus barbus	Chironomidae	100	100		0	0.702
Barbatula barbatula	Trichoptera Chironomidae	22.22 77.78	65.22 78.26		0.059	0.603
Barbus meridionalis	Ephemeroptera Chironomidae detritus	18.52 81.48 +	68.18 100 13.64		0.048	0.349
Squalius cephalus	Ephemeroptera Odonata Trichoptera Chironomidae	9.37 1.56 67.19 21.88	36 4 80 80		0.108	0.123

Table 7: The structure of fish species' diet in S6 station and ecological niche size values (A – relative abundance of the groups in the diet, F – frequency of groups in the diet, Bs – standardized Levins index, FT – Smith index, BA – standardized Hurlbert index).

Fish Species	**Diet**			**Benthos structure – trophic supply (%)**	**Ecologic niche dimension indexes**	
	Structure	**A%**	**F%**		Bs	BA
Gobio gobio	Ephemeroptera Chironomidae	19.48 80.52	18.18 90.91	Turbelariata – 0.84 Oligochaeta – 21.95 Gastropoda – 0.84 Acarina – 1.27 Ephemeroptera – 1.76 Trichoptera – 3.38 Heteroptera – 0.42 Chironomidae – 69.54	0.057	0.323
Squalius cephalus	Ephemeroptera Chironomidae	62.5 37.5	90.48 66.66		0.11	0.044
Alburnoides bipunctatus	Oligochaeta Ephemeroptera Trichoptera Chironomidae detritus	3.11 35.11 14.22 47.56 +	29.17 50 29.17 70.84 8.34		0.212	0.125
Barbatula barbatula	Oligochaeta Ephemeroptera Trichoptera Chironomidae	2.02 5.48 2.31 90.18	40 28 28 80		0.028	0.734
Barbus meridionalis	Oligochaeta Ephemeroptera Trichoptera Chironomidae detritus plants	3.98 3.48 8.46 84.08 + +	34.78 39.13 47.83 82.61 8.70 4.35		0.05	0.535
Barbus barbus	Trichoptera Chironomidae	66.67 33.33	90.47 76.19		0.1	0.001
Sabanejewia balcanica	Oligochaeta Ephemeroptera Trichoptera Chironomidae	2.94 2.94 3.92 90.20	40 28 28 100		0.028	0.363

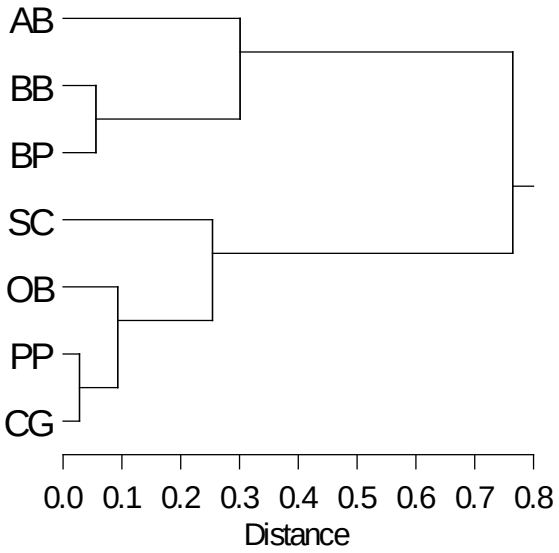


Figure 4: Cluster analysis based on the Pianka index values
for the trophic niches overlapping for the fish species of sector S1
(AB – *Alburnoides bipunctatus*, BB – *Barbus barbus*, Bp – *Barbus meridionalis*,
SC – *Squalius cephalus*, OB – *Orthrias barbatulus*, PP – *Phoxinus phoxinus*, CG – *Cottus gobio*).

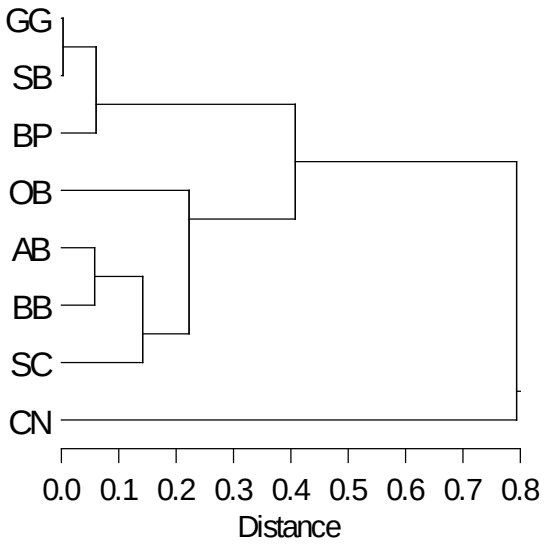


Figure 5: Cluster analysis based on the Pianka index values
for the trophic niches overlapping for the fish species of sector S2
(GG – *Gobio gobio*, SB – *Sabanejewia balcanica*, AB – *Alburnoides bipunctatus*,
BB – *Barbus barbus*, Bp – *Barbus meridionalis*, SC – *Squalius cephalus*,
OB – *Orthrias barbatulus*, CN – *Chondrostoma nassus*).

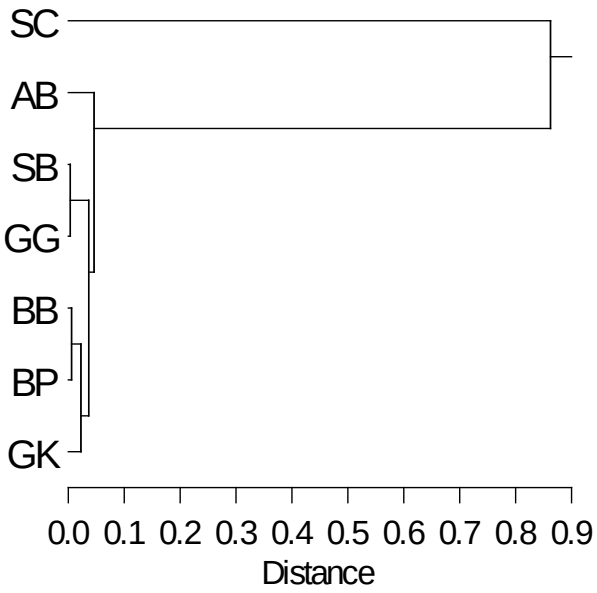


Figure 6: Cluster analysis based on the Pianka index values
for the trophic niches overlapping for the fish species of sector S3
(GG – *Gobio gobio*, SB – *Sabanejewia balcanica*, AB – *Alburnoides bipunctatus*,
BB – *Barbus barbus*, Bp – *Barbus meridionalis*, SC – *Squalius cephalus*, GK – *Gobio kessleri*).

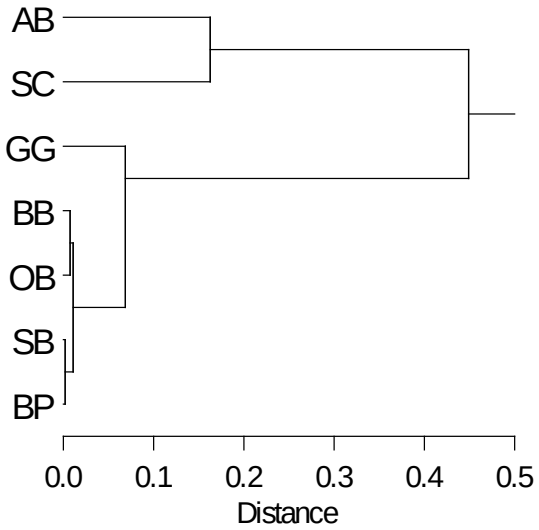


Figure 7: Cluster analysis based on the Pianka index values
for the trophic niches overlapping for the fish species of sector S4
(GG – *Gobio gobio*, SB – *Sabanejewia balcanica*, AB – *Alburnoides bipunctatus*,
BB – *Barbus barbus*, Bp – *Barbus meridionalis*, SC – *Squalius cephalus*,
OB – *Orthrias barbatulus*).

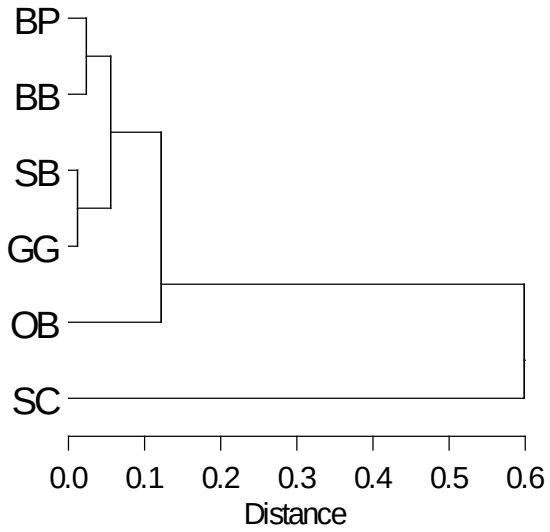


Figure 8: Cluster analysis based on the Pianka index values
for the trophic niches overlapping for the fish species of sector
S5 (GG – *Gobio gobio*, SB – *Sabanejewia balcanica*, AB – *Alburnoides bipunctatus*,
BB – *Barbus barbus*, Bp – *Barbus meridionalis*, SC – *Squalius cephalus*,
OB – *Orthrias barbatulus*).

CONCLUSIONS

Apparently, at least in the researched Târnava Basin, in the context of both congeneric studied fish species, there is relatively narrow and no statistically significant different trophic niches/trophic niches significantly overlapped; *Barbus meridionalis* is in an advantage in the competition for food with *Barbus barbus* due to: using the most available resources in the environment; overlapping trophic niches with a lower number of fish species, (*Barbus meridionalis* with four fish species, *Barbus barbus* with six fish species).

The study results reveal the higher competitiveness in this respect of *Barbus meridionalis* in comparison with *Barbus barbus*, a situation which can at least partially explain the expansion of the first species and the diminishing range of the second one in the last few decades in the Târnava Basin.

AKNOVLEDGEMENTS

This work was supported mainly by the European Social Fund through Sectoral Operational Programme-Human Resources Development 2007-2013, project POSDRU 89/1.5/S/63258 “Postdoctoral school for zootechnical biodiversity and food biotechnology based on the eco-economy and the bio-economy required by ecosanogenesis”.

REFERENCES

1. Antipa G., 1909 – Fauna Ichtiologică a României, Academia Română, Publicaţiunile Fondului Vasile Adamachi, XVI, Institutul de arte grafice „Carol Göbl", Bucureşti, 294. (in Romanian)
2. Badea L. et al., 1983 – Geografia României I, Geografia Fizică, Edit. Academiei Române, (in Romanian).
3. Băcescu M., 1947 – Peştii, aşa cum îi vede ţăranul pescar român/Fish as are seen by the Romanian peasants fisherman, Institutul de cercetări piscicole al României, Monographia, 3, 1946, 75-80, 123-129. in Romanian
4. Bănăduc D, 2005 – Fish associations – habitats quality relation in the Târnave rivers ecological assessment, Transylvanian Review of Systematical and Ecological Research, 2, Edit. Universităţii „Lucian Blaga" din Sibiu, ISSN 1841-7051, ISBN 973-739-141-1, 123-136.
5. Bănăduc D., 2007 – Middle Olt River (Transylvania, Romania) – special area for conservation (Natura 2000 site) proposal for Barbus meridionalis Risso, 1827 and associated fish species, Acta Ichtiologica Romanica, Vol. II, 37-42, 2007.
6. Bănăduc D, 2010 – Hydrotechnical works impact on Cyclostomata and Cottidae species in the Rodna Mountains and Maramureş Mountains Natura 2000 sites (Transylvania-Maramureş, Romania), Repede River – a study case, Transylvanian Review of Systematical and Ecological Research, 9, pp. 175-184, 2010.
7. Bănărescu P., 1964 – Pisces-Osteichthyes, (in Romanian), Fauna R. P. R., Edit. Acad. R. P. R., 13, 959, Bucureşti.
8. Bănărescu P. M. and Bănăduc D., Habitats Directive (92/43/EEC) fish species (Osteichthyes) on the Romanian territory, Acta Ichtiologica Romanica, Vol. II, pp. 43-78, 2007.
9. Beck, W. M., 1977 − Environmental requirements and pollution tolerance of common freshwater Chironomidae, Environmental Monitoring Series, E. P. A. 600/4, 77-124.
10. Botnariuc N. and Spătaru P., 1963 – Étude de la nourriture naturelle de la brême (Abramis brama) dans le complex d'étangs Crapina-Jijia (zone inondable du Danube), Véstn. Českoslov. Společn. Zool., vol. 27, fasc. 2, p. 130-146. in French.
11. Cărăuş I. S., 1952 – Tratat de Ichtiologie/Treaty of ichthyology, Edit. Academiei Republicii Populare Române, 112-113. in Romanian
12. Ciubuc C., 2009 – Order Trichoptera (Insecta) from the Apuseni Nature Park (Transylvania, Romania), Transylvanian Review of Systematical and Ecological Research, 2, The Arieş River Basin, 97-124.
13. Curtean-Bănăduc A. et al., 2001 – Impactul antropic asupra Târnavelor şi Ampoiului (in Romanian), Sibiu.
14. Curtean-Bănăduc A., Bănăduc D. and Sîrbu I. (eds), 2005 – Transylvanian Review of Systematical and Ecological Research – The Târnava River Basin, 2: 1-182.
15. Curtean-Bănăduc A., Bănăduc D. and Sîrbu I. (eds), 2007 – Transylvanian Review of Systematical and Ecological Research – The Saxon Villages Region of southeast Transylvania, 4: 1-216.
16. Dimitriu M., 1938 – Contribution à l'étude de la nourriture des sterlets (Acipenser ruthenus) du Danube, Ann. Inst. Nat. Zootehn., vol. 6, p. 1-12. (in French)
17. Gomoiu M. T. and Skolka M., 2001 – Ecologie, metodologii pentru studii ecologice, Ovidius University Press, Constanţa, 170. (in Romanian)
18. Gyurkó Ş. and Robert A., 1959 – Contribuţiuni la studiul tractusului digestiv şi al nutriţiei la scobar (Chondrostoma nasus), ibidem, an. 18, nr. 3, p. 61-70. in Romanian
19. Krebs J. C., 1989 – Ecological methodology, Addison Wesley Longman Inc., 745.
20. Kutzenberger H., 2008 – Threats for Danube Region Biodiversity – Global trends and local impacts and changes in biogeographical patterns, Transylvanian Review of Systematical and Ecological Research, 6, The Wetlands Diversity, 149-154.

21. Leonte V., 1959 – Contribuţii la cunoaşterea răspîndirii hranei şi ritmului de creştere la puietul de sturioni în Dunăre, ibidem, an. 18, nr. 4, p. 9-18. in Romanian
22. Momeu L., Rasiga A., Peterfi Ş., Kozma A., 1999 – Algal communities of the Cibin River and of some wetlands situated on the upper and middle course of the Olt River, Transylvanian Review of Systematical and Ecological Research, 1, The upper and middle Olt River Basin, 49-67.
23. Munteanu I., 1957 – Câteva observaţiuni asupra ritmului de creştere şi a hranei la morunaş (Vimba vimba n. carinata Pallas) din Complexul Razelm, ibidem an. 16, nr. 4, p. 55-59. in Romanian
24. Munteanu I., 1959 – Contribuţiuni la cunoaşterea hranei şi dezvoltării puietului de crap pe diverse zone de reproducere din Deltă şi Razelm, ibidem, an. 18, nr. 3, p. 75-79. in Romanian
25. Posea G. et al., 1983 – Enciclopedia Geografică a României (in Romanian), Edit. Ştiinţifică şi Enciclopedică, Bucureşti.
26. Rădulescu I. and Suceveanu N., 1959 – Contribuţii la cunoaşterea hranei şi parazito-faunei somnului pitic (Ameiurus nebulosus) din apele româneşti, ibidem, an. 18, nr. 3, p. 71-74. in Romanian
27. Roşu, A., 1980 − Geografia Fizică a României, Edit. Didactică şi Pedagogică, Bucureşti, 234.
28. Seager J., Milne I., Rutt G., Crane M., 1992 − Integrated biological methods for river water quality assessment, Riwer water quality, P. Newman et al. (eds), 399-415.
29. Sisma-Ventura G., Barinova S., Greenbaum N. and Tavassi M., 2010 – 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), Transylvanian Review of Systematical and Ecological Research, 8, The Wetlands Diversity, 10, 137-150.
30. Sîrbu I., 2005 – The freshwater molluscs from the Târnava rivers basin (Romania), Transylvanian Review of Systematical and Ecological Research, 2, The Târnava River Basin, 51-60.
31. Teodorescu R., 1942 – Contribution à l'étude du dévélopment des larves de sandre (Lucioperca sandra). La nutrition et l'ossification du système osseux, Anal. I.C.P., vol. 1, p. 29-42. in French
32. Vasiliu G. D., 1947 – Nouvelles données sur la nutrition naturelle de la Sandre (Sander lucioperca) dans les eaux de la Roumanie, Not. Biol., vol. 5, p. 181-198. (in French)
33. Vasiliu G. D. and Cristescu A., 1946 – Données nouvelles sur les variations dans l'alimentation naturelle chez la Truite commune en Roumanie, ibidem, vol. 4, p. 64-124. in French
34. Vasiliu G. D. and Nicolau A., 1947 – Recherches sur la biologie de la nutrition naturelle chez Aspius aspius dans les eaux de la Roumanie, ibidem, pp. 250-261. (in French)
35. Vasiliu G. D. and Costea E., 1947 – Contribution à connaissance de la biologie de la nutrition chez Vimba vimba n. carinata dans les eaux de Roumanie, Not. Biol., vol. 5, p. 229-236. in French.
36. Yacoub H., 2009 – Najas ssp. Growth in relation to environmental factors in Wadi Allaqi (Nasser Lake, Egypt), Transylvanian Review of Systematical and Ecological Research, 8, The Wetlands Diversity, 1-40.

HEAVY METAL CONCENTRATIONS IN AN IMPORTANT MANGROVE PALM (*NYPA FRUTICANS*), IN REMBAU-LINGGI MANGROVE FOREST (PENINSULAR MALAYSIA)

Tengku Nurul Aishah AZIZ * and *Nor Rasidah HASHIM* **

* University Putra Malaysia, Faculty of Environmental Studies, UPM Serdang MY-43400, Selangor, Malaysia, rasidah@env.upm.edu.my
** Forest Research Institute Malaysia, Tropical Forest Biodiversity Division, Selangor, Malaysia, Kepong, MY-52109, rasidah@env.upm.edu.my

KEYWORDS: Malaysia, mangroves, *Nypa fruticans*, heavy metals.

ABSTRACT

Plants and microorganisms are becoming commonly used worldwide to remediate heavy metal pollution. However, plant species vary in their ability to take up heavy metals or in their tolerance to high concentrations of heavy metals in soils or water. In this study we analyzed heavy metal (cadmium, chromium, copper, lead, and zinc) concentrations in an important mangrove palm in Malaysia: *Nypa fruticans*. The plant samples as well as samples of surrounding sediment were taken from the Rembau-Linggi mangrove forest in the south-western part of the Peninsular, Malaysia. The results showed that uptake of heavy metals by the roots of *Nypa fruticans* were good for all heavy metals except Cu. However, only Zn was easily translocated to the leaves. Therefore, further studies are required to determine whether heavy metals such as Cr, Cu and Cd will become more available for uptake by *N. fruticans* during episodes of increased heavy metal runoff or pollution of mangrove forests. A suplementary reason for the study of heavy metals accumulation in *Nypa fruticans* is the fact that some parts of this plant are used to obtain some alimentary products.

ZUSAMMENFASSUNG: Schwermetallkonzentrationen in einer wichtigen Mangrovenpalme (*Nypa fruticans*) im Rembau-Linggi Mangrovenwald (Malayische-Halbinsel).

Pflanzen und Mikroorganismen werden weltweit bereits allgemein zur Beseitigung der Schwermetallverschmutzung eingesetzt. Die Pflanzenarten unterscheiden sich jedoch in ihrer Fähigkeit Schwermetalle aufzunehmen oder in ihrer Toleranz gegenüber hohen Konzentrationen von Schwermetallen in Boden oder Wasser. In dieser Studie wurden die Konzentrationen der Schwermetalle Cadmium, Chrom, Kupfer, Blei und Zink in einer Mangrovenpalme, *Nypa fruticans* in Malaysia untersucht. Die Pflanzenproben und auch jene aus den Sedimenten der Umgebung wurden im Rembau-Linggi Mangrovenwald im Südwesten der Malayischen Halbinsel entnommen. Die Ergebnisse zeigen, dass die Aufnahme der Schwermetalle durch die Wurzeln von *Nypa fruticans* für alle Schwermetalle außer Kupfer gut war. Jedoch allein Zink wurde leicht in die Blätter transportiert. Daher müssen weitere Untersuchungen durchgeführt werden, um zu bestimmen, auf welche Art und Weise Schwermetalle wie Cr, Cu und Cadmium leichter verfügbar sind, um von *Nypa fruticans* während Ereignissen mit hohen Schwermetalleinträgen oder Verschmutzung im Mangrovenwald aufgenommen werden können. Ein zusätzlicher Grund für Untersuchungen der Schwermetallspeicherung in *Nypa fruticans* ist die Tatsache, dass verschiedene Teile dieser Pflanze zur Herstellung einiger Lebensmittel genutzt werden.

REZUMAT: Concentraţiile de metale grele dintr-un palmier de mangrove important, *Nypa fruticans*, din pădurea de mangrove Rembau-Linggi, Malaezia peninsulară.

În întreaga lume, plantele şi microorganismele sunt folosite frecvent în combaterea poluării cu metale grele. Oricum, speciile de plante variază din punctul de vedere al capacităţii lor de a acumula metale grele sau al toleranţei lor la cantităţi mari de metale grele din apă sau sol. În această lucrare noi analizăm concentraţiile de metale grele (cadmiu, crom, cupru, plumb şi zinc) dintr-un important palmier de mangrove din Malaezia: *Nypa fruticans*. Mostrele din plantă precum şi mostrele din sedimentele din jurul plantei au fost luate din pădurea de mangrove Rembau-Linggi, din partea de sud-vest a Malaeziei peninsulare. Rezultatele au arătat că absorbţia de metale grele cu ajutorul rădăcinilor de *Nypa fruticans* s-a realizat foarte bine cu excepţia cuprului. Oricum, doar zincul a fost transportat până la frunze. De aceea, în continuare trebuie făcute studii pentru a afla dacă metalele grele, precum Cr, Cu şi Cd, pot fi absorbite cu uşurinţă de *Nypa fruticans* atunci când există scurgeri sau poluări majore în mangrove. Un motiv suplimentar pentru studiul acumulării metalelor grele în *Nypa fruticans* este faptul că din diverse părţi ale acestei plante sunt obţinute unele produse alimentare.

INTRODUCTION

Heavy metal contamination in our natural resources (air, soil, freshwater, oceans and wetlands) resulting from anthropogenic activities is one of the major modern environmental problems (Kabata-Pendias and Pendias, 1992; Alloway, 1990; Peters et al., 1997; Ester et al., 2000). Heavy metals are non-biodegradable and can persist for long periods in aquatic and terrestrial environments or may be taken up by microorganisms and plants (Boularbah et al., 2006). For this reason, plants and microorganisms are commonly used to remediate heavy metal pollution worldwide. Nonetheless, leaf detritus, trunks and stems enriched in heavy metals need to be disposed of properly to avoid heavy metal reintroduction into the ecosystem as well as to prevent heavy metal bioaccumulation in humans and animals (Peters et al., 1997).

Heavy metals are one of the main pollutant classes accumulating in mangrove ecosystems (Harbison, 1986; Peters et al., 1997). The heavy metals may originate from various sources including urban and household runoffs, industrial effluents, boating activities and agricultural runoff (Stark, 1998). Although mangroves and estuaries are vulnerable to heavy metal pollution, the fine sediments seem to be effective at sequestering the heavy metals while the plants living in these wetland ecosystems seem to vary in their ability to take up heavy metals (Freedman and Hutchinson, 1980; Peters et al., 1997; Blaylock and Huang, 2000; MacFarlane and Burchett, 2002).

In the mangroves of Malaysia, *Nypa fruticans* is the main free-standing native palm species. Besides its ecological importance, this species has socioeconomic values for the people living in or near the mangroves because it has several uses such as cigarettes (dried leaves), food (fruits) and fermented drink (sap). In this study, we analyze selected heavy metal concentrations in *Nypa fruticans*.

MATERIAL AND METHODS

Sampling area and sample collection

The sampling area for this study is the Rembau-Linggi mangrove (2.43° North, 102.06° East) in the south-western part of Peninsular Malaysia. The Rembau-Linggi mangrove is one of the few remaining mangrove areas in the West coast of the peninsula. In many parts of the mangrove area, the originally waterlogged soil was drained so as to allow the non-native oil palm (*Elaeis guineensis*) to be planted. Nowadays it is not uncommon to find mangrove species such as *Nypa fruticans* and *Sonneratia caseolaris* growing on a narrow riverbank adjacent to oil palm plantations in the mangrove areas (Nazli and Hashim, 2010).

As mentioned previously, the chosen study species is *Nypa fruticans*. We sampled five trees (five leaves per tree and several root cuttings), and the top sediment near the base of the chosen trees in October 2009. The trees were located along the banks of the Rembau River, one of the tributaries of the Linggi River.

Laboratory analyses of heavy metals

The laboratory analyses for this study were conducted at the Faculty of Environmental Studies, University Putra Malaysia. The sediment samples were first air dried at room temperature. Then, the samples were further dried in the oven above 65°C for 24 hours. The samples were later sieved into one mm in size and finely ground. The sediment samples were digested using the Aqua Regia method. For strong digestion, the samples were refluxed for several hours in a mixture of three parts of hydrochloric acid and one part of nitric acid. Lastly, the samples were filtered and determined using the Atomic Absorption Spectroscopy (ASS).

The leaves and roots were air dried at room temperature and were later allowed to evaporate to dryness over a steam bath. Then the dried roots and leaves were finely ground. This was followed by dry ashing the samples, i.e. burning the samples in a muffled furnace at 550°C until they became white ash. The ash was then dissolved in a minimum quantity of nitric acid and warm water. Finally, the samples were filtered and determined using the Atomic Absorption Spectroscopy (ASS).

RESULTS AND DISCUSSIONS

The results from the determination of heavy metal concentrations in the plant parts and sediments are summarized in the table 1. It was found that the heavy metal concentrations in the plant parts and sediments did not show a consistent trend except for Cadmium and Chromium (Roots > Leaves > Sediments). Below we provide the details of the results.

Cadmium (Cd)

The roots had the highest concentration of Cd, followed by the leaves and the sediments. Although the range of the means is very small (0.010-0.013 mg/kg), the differences were statistically significant ($p < 0.05$).

Chromium (Cr)

The roots had the highest concentration of Cd, followed by the leaves and the sediments. The differences between the means were statistically significant ($p < 0.05$).

Copper (Cu)

The roots had the highest concentration of Cd, followed by the sediments and the leaves. The differences between the means were statistically significant ($p < 0.05$).

Lead (Pb)

The sediment had the highest concentration of Cd, followed by the roots and the leaves. The differences between the means were statistically significant ($p < 0.05$).

Zinc (Zn)

The leaves had the highest concentration of Cd, followed by sediments and the roots. The differences between the means were not statistically significant ($p < 0.05$).

In order to provide a complete discussion of the results we need to consider the environmental factors in which the plants lived because it is well known that the amounts of metal uptake from the soils are influenced by soil factors such as pH, redox potential, organic matter content, fertilizer application, and by plant factors including plant species, cultivars and age (Freedman and Hutchinson, 1980; Adriano, 1986; Alloway, 1990). In this sense our discussion will be limited because we do not have such important supplementary information. Nonetheless, some discussion can still be made by comparison with previous findings.

Nazli and Hashim (2010) conducted special heavy metal analysis of *Sonneratia caseolaris* samples taken from the same Rembau-Linggi mangrove area but a little further downstream, in the year 2007. Compared to their sediment samples, the sediments in this study contained lower amounts of all the heavy metals (Tab. 2). This is not surprising at all because the lower stream of the mangrove receives more heavy metal runoff from several more rivers. In fact, the concentrations of certain heavy metals in the sediments from Nazli and Hashim's study sites were comparable to those obtained from coastal areas in the northern part of the peninsula and from paddy fields in Sabah (Tab. 2).

Moreover, Nazli and Hashim (2010) found the fact that the roots of *Sonneratia caseolaris* had the highest concentrations of Cr, Pb and Zn, whereas Cd was concentrated in the leaves. In contrast, *Nypa fruticans* in our study displayed the highest concentrations of Cr, Cd and Cu in the roots, whereas Zn was found to be higher in the leaves than in the roots. The results from these two studies, especially for Cr, were consistent with those found by Shewry and Peterson (1976), Yim and Tam (1999) and MacFarlane and Burchett (2002), who suggested the fact that physiological barriers and lack of transport mechanisms in plants hinders the translocation of certain heavy metals from roots to shoots.

In this study, it was found that *Nypa fruticans* had higher concentrations of Cd in its roots than in either the sediments or leaves. This finding is in contrast with the suggestion made by Thornton (1981) that Cd is mostly unavailable for uptake by plants and that the uptake is inhibited by the presence of large amounts of other metal ions, especially Zn. However, it was found that the concentrations of Pb in *N. fruticans* were higher in the roots than in the leaves, which is in line with Adriano (1986), who suggested that most of Pb taken up by plants are accumulated in the roots and only a small amount is translocated to the shoots.

Table 1: Concentrations of heavy metals (µg/g) in plant parts and sediments from Rembau-Linggi mangrove; means within the same column denoted by the same letter are not statistically significant at $p < 0.05$. Values are Mean ± SEM.

	Cd	Cr	Cu	Pb	Zn
Leaf	0.012 ± 0.001^{a}	0.215 ± 0.006^{a}	0.084 ± 0.008^{a}	0.251 ± 0.013^{a}	1.484 ± 0.027^{b}
Root	0.013 ± 0.001^{b}	0.243 ± 0.007^{a}	0.202 ± 0.036^{b}	0.437 ± 0.049^{b}	0.877 ± 0.073^{a}
Sediment	0.010 ± 0.000^{c}	0.174 ± 0.008^{b}	0.706 ± 0.142^{c}	0.492 ± 0.017^{b}	1.028 ± 0.290^{ab}

Table 2: Concentrations of heavy metals (µg/g) in plant parts and sediments from several studies; Values are Mean ± SEM. N/A = not available. ND = not detected. References: [1]This study, [2]Nazli and Hashim (2010); [3]Peters et al. (1997), [4]Idris et al. (2009), [5]Yap et al. (2009).

		Cd	Cr	Cu	Pb	Zn
Rembau-Linggi mangrove[1]	*Nypa fruticans* (Leaf)	0.012 ± 0.001	0.215 ± 0.006	0.084 ± 0.008	0.251 ± 0.013	1.484 ± 0.027
	Nypa fruticans (Root)	0.013 ± 0.001	0.243 ± 0.007	0.202 ± 0.036	0.437 ± 0.049	0.877 ± 0.073
	Sediment	0.010 ± 0.000	0.174 ± 0.008	0.706 ± 0.142	0.492 ± 0.017	1.028 ± 0.290
Rembau-Linggi mangrove (lower stream)[2]	Sediment	0.8 ± 0.5	6.0 ± 0.6	31.9 ± 2.0	83.1 ± 3.1	4.3 ± 0.1
Various places[3]	*Rhizophora* spp. (Leaf)	0.04-0.24	N/A	0.12-11	0.43-27	0.03-32
Northern Peninsular Malaysia[4]	Coastal Sediment	N/A	6	4	35	31
Rice Paddy (Sabah, Borneo)[5]	Leaf	0.203 ± 0.023	1.020 ± 0.088	1.243 ± 1.566	ND	1.214 ± 0.556
	Root	0.190 ± 0.028	1.86 ± 0.458	9.252 ± 11.28	1.565 ± 4.686	2.306 ± 0.932
	Sediment	0.776 ± 0.139	2.075 ± 0.482	ND	ND	21.094 ± 11.248

CONCLUSIONS

We conclude that the uptake of heavy metals by the roots of *Nypa fruticans* in this study was good for all heavy metals except Cu. However, only Zn could be easily translocated to the leaves. Therefore, further studies need to be made to determine whether heavy metals such as Cr, Cu and Cd will become more available for the uptake by *N. fruticans* during episodes of increased heavy metal runoffs or pollution into the mangroves.

ACKNOWLEDGMENTS

We would like to express our thanks to Mohd Fazlin Nazli, Wan Faridah Akmal Wan Jusoh, Raja Nurul Nadia Raja Alang (UPM) and the villagers of Kampung Sungei Timun for their assistance throughout this project. We would also like to acknowledge the faculty and laboratory staff of the Faculty of Environmental Studies, University Putra Malaysia for their support.

REFERENCES

1. Adriano D. C., 1986 – Trace elements in the terrestrial environment. Springer-Verlag. New York. 533.
2. Alloway B. J., 1990 – Heavy metals in soils. John Wiley and Sons. New York. 306 p.
3. Blaylock M. J., Huang J. W., 2000 – Phytoextraction of metals. In: Raskin L., Ensley B. D. (eds), Phytoremediation of toxic metals: Using plants to clean up the environment. John Wiley and Sons. New York. 53-70.
4. Boularbah A., Schwartz C., Bitton G., Aboudrar W., Ouhammou A. and Morel J. L., 2006 – Heavy metal contamination from mining sites in South Morocco: 2. Assessment of metal accumulation and toxicity in plants. Chemosphere, 63, (5), 811-817.
5. Ester van der Voet, Jeroen B. G., Udo de Heaes H. A., 2000 – Heavy metals: A problem solved? Methods and models to evaluate policy strategies for heavy metals. Kluwer Academic. Netherlands. 4-47.
6. Freedman B. and Hutchinson T. C., 1980 – Pollutant inputs from the atmosphere in soils and vegetation near a nickel-copper smelter at Sudbury. Canadian Journal of Botany, 58, 108-132.
7. Harbison P., 1986 – Mangrove muds – A sink or source for trace metals. Marine Pollution Bulletin, 17, 246-250.
8. Idris W. M. R., Rahim S. A., Rahman Z. A., Lihan T., Hashim A., Yusuf N. M., 2009 – Komposisi geokimia sedimen pantai di Pulau Langkawi, Kedah, Malaysia (in Malay). Sains Malaysiana, 38, (3), 313-320.
9. Kabata-Pendias A. and Pendias H., 1992 – Trace elements in soils and plants, second edition CRC Press. Boca Raton. 365.
10. MacFarlane G. R. and Burchett M. D., 2002 – Toxicity, growth and accumulation relationships of copper, lead and zinc in the grey mangrove Avicennia marina (Forsk.) Vierh. Marine Environmental Research, 54, 65-84.
11. Nazli M. F. and Hashim N. R., 2010 –Heavy metals concentrations in an important mangrove species, Sonneratia caseolaris, in Peninsular Malaysia. Environment Asia, 3 (special issue), 50-55.
12. Peters E. C., Gassman N. J., Firman J. C., Richmond R. H., Power E. A., 1997 – Ecotoxicology of Tropical marine ecosystems. Environmental, Toxicology and Chemistry, 16, 12-40.
13. Shewry P. R. and Peterson P. J., 1976 – The uptake and transport of chromium by Barley seedings (Hordeum vulgare L.). Planta, 132, 209-214.
14. Stark J., 1998 – Heavy metal pollution and macrobenthic assemblages in soft sediments in two Sydney estuaries, Australia. Marine and Freshwater Research, 49, 533-540.
15. Thornton I., 1981 – Geochemical aspects of the distribution and forms of heavy metals in soils. In: Effect of Heavy Metal Pollution on Plants. Edit. N.W. Lepp, Vol. 2, Metals in the Environment. Applied Science Publishers. London. 1-33.
16. Yap D. W., Adezrian J., Khairiah-Ismail B. S. and Ahmad-Mahir R., 2009 – The uptake of heavy metals by paddy plants (Oryza sativa) in Kota Marudu, Sabah, Malaysia. American-Eurasian Journal of Agriculture and Environmental Sciences, 6, (1), 16-19.
17. Yim M. W. and N. F. Y. Tam, 1999 – Effects of wastewater-borne heavy metals on mangrove plants and soil microbial activities. Marine Pollution Bulletin, 39, 179-186.

DETECTION OF RANGE FINDING TEST OF MERCURY CHLORIDE IN YELLOW-FIN SEA BREAM (*ACANTHOPAGRUS LATUS*)

Aliakbar HEDAYATI *, *Alireza SAFAHIEH* *, *Ahmad SAVARI* * and *Jasem GHOFLE* **

* Khorramshahr University of Marine Science and Technology, Department of Marine Biology, Khorramshahr, Iran, Marinebiology1@gmail.com
** South Iranian Aquaculture Research Center, Ahwaz, Iran.

KEYWORDS: Range Finding Test, Mercur Chloride, *Acanthopagrus latus*.

ABSTRACT

Toxicity tests allow the determination of pollution effects, providing direct evidence of the biological responses of marine organisms to contaminants. Fifty-four yellow-fin sea bream, all immature males of the same size (120 g final body weight average) were obtained from Mahshahr creeks with hooks in an Upon capture. In the laboratory, the fish were maintained in a seawater re-circulatory system (300-litre tanks) equipped with physical/biological filters and with aeration. All samples were acclimatized for one week in a 15 litre aerated fibreglass tank containing 46‰ saltwater maintained at 25°C under a constant 12:12 L:D photoperiod. Acclimatized fish were fed daily with a live feed (fresh shrimp) and daily we checked water quality and water parameters. Hg^{2+} tested concentrations were 20, 50, 100, 200, 500, 1,000, 2,000, 5,000 and 10,000 µg/l. Groups of six male yellow-fin sea bream (120 g) were exposed for 96 hours to each of the Range Finding Tests for LC_{50}, in a fibreglass tank equipped with aeration, with 100 litre of test medium. The control group was exposed to filtered sea water in similar conditions. Test medium was not renewed during the assay and no food was provided to the fish. Values of pH, temperature and salinity were measured at times 0, 24, 48, 72 and 96 hours. At the end of the bioassay, range values were determined as 500-1,000 µg/l (50% of mortality between 500 and 1,000). Range Finding Test values indicated that mercury is toxic to *A. latus*. The range obtained in the present study, compared with corresponding values published for other fish species, shows differences in the ranges of mercury for different species and even for different time periods; lower range values for *A. latus*, compared with most species, confirm the sensitivity of *A. latus* to low mercury doses.

RÉSUMÉ: Détection de la dose limite de chlorure de mercure par le range finding test chez le pagre à nageoires jaunes *Acanthopagrus latus* (Houttuyn, 1782).

Les testes de toxicité permettent la détermination des effets de la pollution, en fournissant des preuves directes de la réponse biologique des animaux marins aux contaminants. Cinquante-quatre pagres à nageoires jaunes, tous des males juvéniles (120 g moyennes du poids corporel final) ont été obtenus des ruisseaux de Mahshahr avec des crochets dans une capture Upon. Au laboratoire, les poissons ont été maintenus dans un système recirculant l'eau de mer (des réservoirs de 300 litres) équipés avec des filtres physiques et biologiques et aérateur. Tous les exemplaires ont été acclimatés pendant une semaine dans 15 réservoirs en fibre de verre avec aérateur contenant 46‰ eau salée

maintenues à 25˚C avec une photopériode constante de 12:12 jour/nuit. Les poissons acclimatés ont été nourris chaque jour avec de la nourriture vivante (crevettes fraîches) et les paramètres de l'eau et la qualité de l'eau ont été vérifiés tous les jours. Les concentrations testées de Hg^{2+} ont été de 20, 50, 100, 200, 500, 1000, 2000, 5000 et 10000 µg/l. Des demi-douzaines de pagres à nageoires jaunes males (120 g) ont été exposées pendant 96 h à chaque test de détection de la dose limite (Range Finding Test) pour LC50, dans un réservoir de fibre de verre équipé avec aérateur dans 100 litres de milieu de test. Le groupe témoin a été maintenu dans de l'eau de mer filtrée dans des conditions similaires. Le milieu de test n'a pas été renouvelé pendant le test et les animaux n'ont pas reçu de nourriture. Les valeurs de pH, température et salinité ont été mesurées après 0, 24, 48, 72 et 96 h. A la fin du test, le domaine de valeurs a été déterminé comme 500-1000 µg/l (50% de la mortalité entre 500 et 1000). Les valeurs du Range Finding Test ont indiqué que le mercure est plus toxique pour le *A. latus*. Les valeurs obtenues dans la présente étude, par comparaison avec les valeurs correspondantes données dans la littérature de spécialité pour d'autres espèces de poissons, montrent des valeurs limite de toxicité du mercure différentes pour des espèces de poissons différentes et des intervalles de temps différentes, mais ce qui compte, la valeur inferieure du Range Finding Test pour *A. latus* est comparable avec celle de la plupart des espèces et confirme la sensibilité de *A. latus* aux concentrations faibles de mercure.

REZUMAT: Detectarea limitelor de toxicitate (range finding test) la clorura de mercur pentru *Acanthopagrus latus* (Houttuyn, 1782), Fam Sparid.

Testele de toxicitate permit determinarea efectelor poluării, furnizând dovezi directe ale răspunsului biologic al organismelor marine la contaminanţi. 54 de exemplare de *Acanthopagrus latus* (Houttuyn, 1782), Fam. Sparidae, masculi juvenili de aceleaşi dimensiuni (120 g greutate medie finală) au fost capturaţi în pâraiele Mahshahr cu cârlige într-o captură Upon. În laborator, peştii au fost menţinuţi într-un sistem de apă marină recirculată (bazine de 300 litri) echipat cu filtre fizice/biologice şi aerator. Toate exemplarele au fost aclimatizate timp de o saptamână în 15 rezervoare de fibră de sticlă cu aerator conţinând 46‰ apă sărată menţinută la 25˚C şi la o fotoperioadă constantă 12:12 L:D. Peştii aclimatizaţi au primit zilnic hrană vie (crevete proaspete) iar calitatea apei şi parametrii apei au fost controlaţi zilnic. Concentraţiile testate de Hg^{2+} au fost de 20, 50, 100, 200, 500, 1000, 2000, 5000 şi 10000 µg/l. Grupuri de şase masculi de *Acanthopagrus latus* (120 g) au fost expuse timp de 96 h la fiecare dintre Range Finding Test pentru LC50, în acvariu de fibră de sticlă echipat cu aerator şi conţinând 100 l de mediu de testare. Grupul martor a fost expus la apă de mare filtrată în condiţii similare. Mediul de test nu a fost înlocuit pe parcursul experimentului şi animalele nu au primit hrană. Valorile pH-ului, temperaturii şi salinităţii au fost măsurate după 0, 24, 48, 72 şi 96 h. La sfârşitul experimentului, domeniul de valori toxice a fost găsit a fi între 500-1000 µg/l (50% mortalitate între 500 şi 1000). Valorile Range Finding Test au indicat că mercurul este mai toxic pentru *A. latus*. Valorile obţinute în prezentul studiu, prin comparaţie cu valorile similare publicate în literatura de specialitate pentru alte specii de peşti, se încadrează într-un domeniu diferit pentru mercur faţă de alte specii şi chiar intervale de timp diferite, dar ceea ce este important, valoarea inferioară a Range Finding Test pentru *A. latus* este comparabilă cu cea de la majoritatea speciilor şi confirmă sensibilitatea *A. latus* la doze mici de mercur.

INTRODUCTION

Aquatic ecosystems are typically monitored for pollution of heavy metals using biological assays. Fish species are often the primary consumers in any aquatic ecosystems and thus metal concentration in fish can act as an environmental indicator of the state of any aquatic system. Aquatic organisms have been reported to accumulate heavy metals in their tissues, several times higher than the ambient levels. Fishes have been used for many years, for the determination of the pollution status of water, and are thus regarded as excellent biological markers of metals in aquatic ecosystems.

Mercury (Hg) is a liquid metal at ambient temperatures and pressures. It forms salts in two ionic states: mercury (I) and mercury (II). Mercury (II), or mercuric salts, are much more common in the environment than mercury (I) or mercurous salts. These salts, if soluble in water, are bioavailable and considered toxic. Mercury also forms organo-metallic compounds, many of which have industrial and agricultural uses (Boening, 2000).

Mercury in fish was already recognized as a public health and ecological problem in the 1960's. It was commonly assumed that local point sources (industrial effluents, utility emissions, fungicide applications) are the main sources, and many studies focused on water around point source contamination sites nearby.

Although mercury chloride is not the most toxic mercury compound from the marine environment (Boudou and Ribeyre, 1997), it is the key form between the gaseous metal, transported through atmosphere, and the methyl mercury, that bio accumulates in organisms. Once it enters the organism, mercury can draw various immunotoxic effects.

Toxicity tests allow the determination of these effects, providing direct evidence of the biological responses of marine organisms to contaminants. Due to the fact that organisms from different species vary in their sensitivity towards chemical substances, it is difficult to set species protection standards with regard to pollutants from the environment. Extrapolation from one species to another is, therefore, difficult if their relative sensitivities are not known (Van Straalen et al., 1994).

The present study was conducted to determine the acute toxicity of the heavy metal compound $HgCl_2$ in a statistic system to the marine fish *Acanthopagrus latus* (Houttuyn, 1782). This species was selected for bioassays because it can easily be raised under laboratory conditions. It fulfils most of the requirements of a model species and is available throughout the year.

MATERIAL AND METHODS

Fifty four yellow-fin sea bream, all immature male of the same size (120 g final body weight average) were obtained from Mahshahr creeks with hooks in a Upon capture (only healthy fish, as indicated by their activity and external appearance, were used in the experiments), the fish were maintained alive on board in a fibreglass tank and, on return to shore, transferred to a 300 l aerated vat, filled with sea water, in order to be transported to the nearby laboratory. In the laboratory (Mariculture Research Station of the South Iranian Aquaculture Research Center, Mahshahr, Iran), the fish were maintained in a seawater re-circulatory system (300 l tanks), equipped with physical/biological filters and with aeration, from October to November.

All samples were acclimated for one week in 15 aerated fibreglass tanks, containing 46 ppt. saltwater maintained at 25° C under a constant 12:12 L:D photoperiod. Acclimatized fish were fed daily with live fresh shrimp and water quality and parameters were checked also daily.

The tested Hg^{2+} concentrations were found to be 20, 50, 100, 200, 500, 1,000, 2,000, 5,000 and 10,000 µg/l, Groups of six male yellow-fin sea bream (120 g) were exposed for 96 h to each of the Range Finding Test for LC_{50}, in fibreglass tank equipped with aeration, with 100 l of test medium. The control group was exposed to filtered sea water in similar conditions.

The bioassay was performed in a controlled room, at a temperature of 25 ± 1°C and under a natural photoperiod (12hL: 12hD). The test medium was not renewed during the assay and no food was provided to the animals. The values of pH, Temperature, and salinity were measured at time of 0, 24, 48, 72 and 96 h. At the end of the bioassay, range values were determined as 500-1,000 µg/l (fifty percent of mortality between 500 and 1,000) (Boyd and Tucker, 1992).

RESULTS

There was 100% mortality at 10,000 µg/l concentration within the first 4 h after dosing, and 100% mortality at 5,000 µg/l within the 14 h, whereas 100% mortality for 2,000 µg/l was at 42 h and for 1,000 µg/l, at 54 h.

The mortality of yellow-fin sea bream for mercury chloride doses of 20, 50, 100, 200, 500, 1,000, 2,000, 5,000 and 10,000 µg/l were examined during the exposure times in 24, 48, 72 and 96 h for Range Finding Test (Tab. 1). There is a significant increase seen in the number of dead yellow-fin sea bream with increasing concentration, for the samples exposed during the period of 24-96 h.

Table 1: Cumulative mortality of yellow-fin sea bream (n = 6, each concentration) at Range Finding Test.

Concentration (µg/l)	No. of dead yellow-fin sea bream			
	24 h	48 h	72 h	96 h
Control	–	–	–	–
20	–	–	–	–
50	–	–	–	–
100	–	–	–	–
200	–	–	–	–
500	–	–	–	–
1,000	1	3	6	6
2,000	2	6	6	6
5,000	6	6	6	6
10,000	6	6	6	6

Because of no mortality at 500 µg/l and 100% mortality at 1,000 µg/l, we found that the main range is between 500-1,000 µg/l; the mortality of yellow-fin sea bream for different mercury chloride concentration, examined during exposure times of 24, 48, 72 and 96 h for Range Finding Test is in figures 1-4.

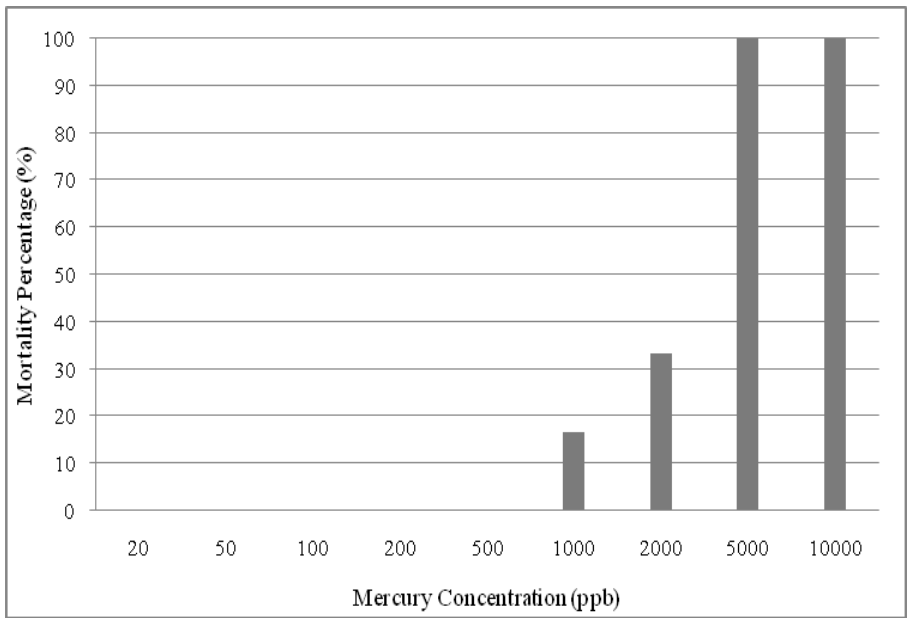


Figure 1: Mortality percentage of yellow-fin sea bream exposed to mercury chloride in 24 h.

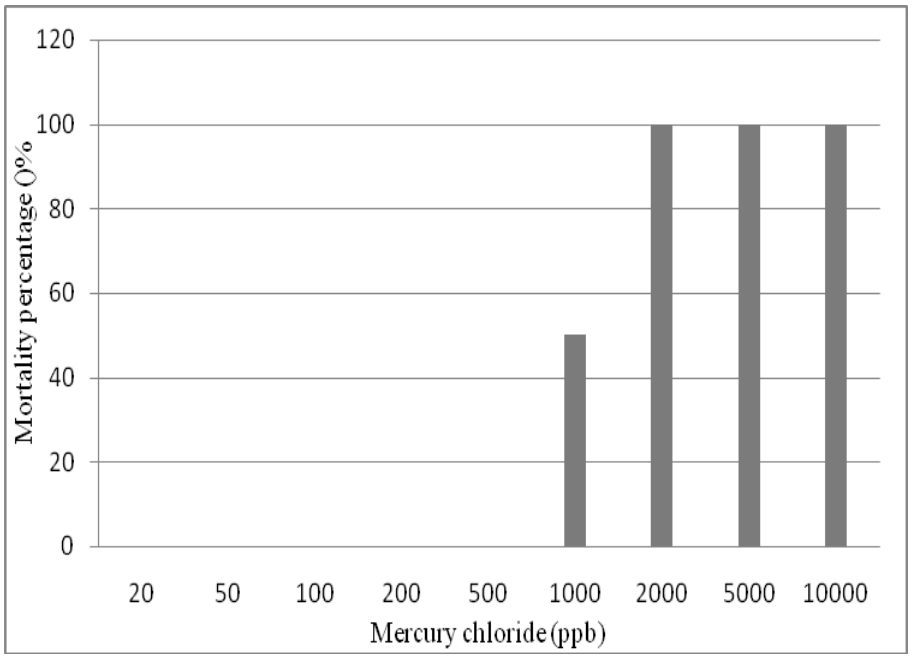


Figure 2: Mortality percentage of yellow-fin sea bream exposed to mercury chloride at 48 h.

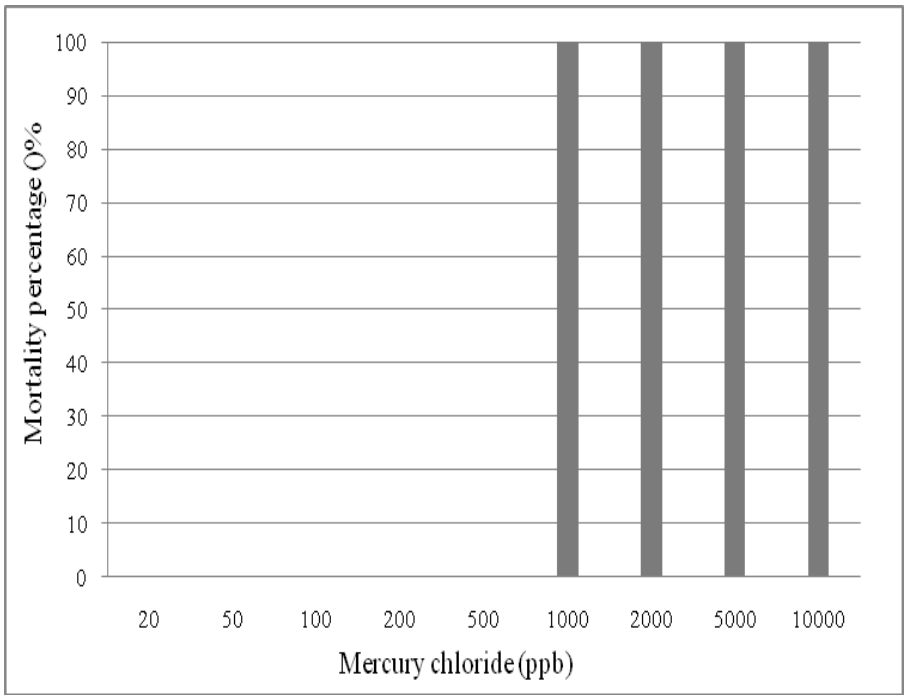


Figure 3: Mortality percentage of yellow-fin sea bream exposed to mercury chloride in 72 h.

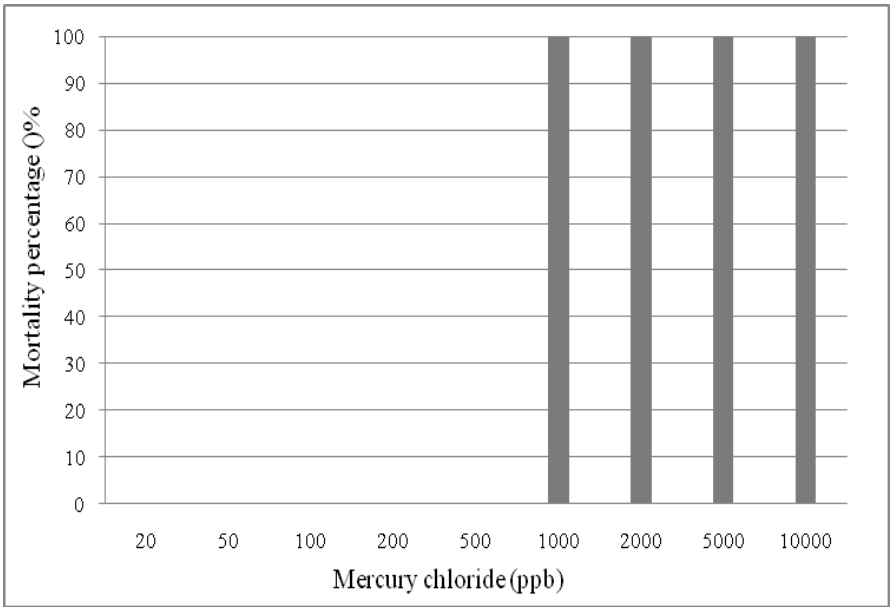


Figure 4: Mortality percentage of yellow-fin sea bream exposed to mercury chloride in 96 h.

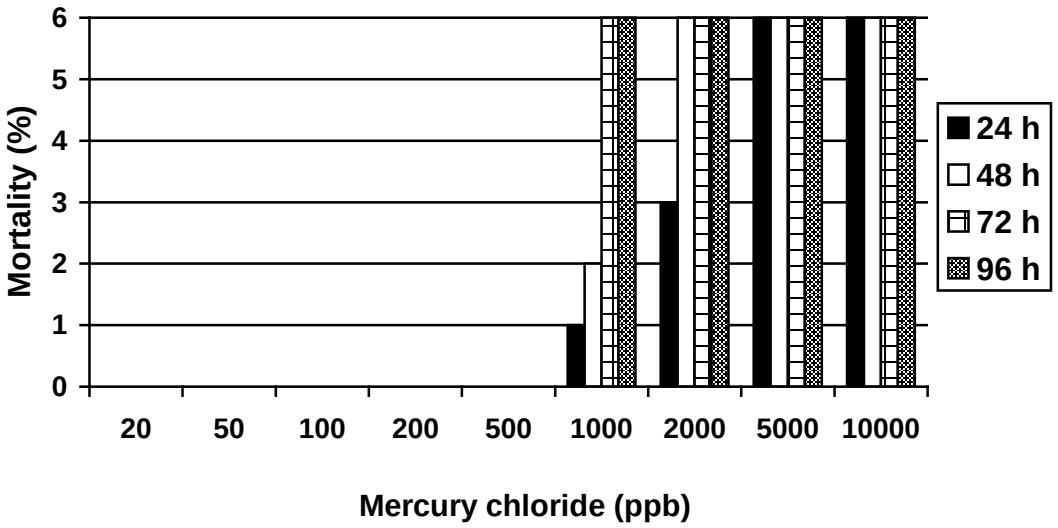


Figure 5: Mortality percentage of yellow-fin sea bream exposed to mercury chloride.

DISCUSSIONS

Toxic effects of mercury and its compounds depend on the chemical form of mercury. Organic forms of mercury are generally more toxic to aquatic organisms than are the inorganic forms. $HgCl_2$ can be converted into highly toxic methyl mercury by methylation through chemical or biological processes.

Factors influencing mercury levels can be divided into exogenous (characteristics of the water body) and endogenous (characteristic of the individuals or species). Exogenous factors include pH, sulphur and organic matter (e.g., dissolved organic carbon). Endogenous factors include species, habitat and food preferences, metabolic rate, age, growth rate, size, mass, and diet.

According to Gooley et al. (2006), mercury is one of the metals raising concerns in aquaculture and has a LC_{50} of 10-40 µg/l and only one µg/l accepted for the safe levels; LC_{50} values for other heavy metals are higher than mercury (cadmium 80-420, cooper 20-100, zinc 1,000-10,000, lead 1,000-40,000 µg/l). Chowdhury et al. (2006) show the 96 h LC_{50} for the juvenile trout as 11 µg/l (95% CI = 9.2 – 11.9 µg/l).

The 96 h LC_{50} value for catfish exposed to Hg^{2+} under static test was determined to be 570 µg/l (Elia et al., 2000). The 96 h LC_{50} value of mercury chloride for chub was found to be 205 µg/l and 96 h LC_{50} for trout, 814 µg/l (Verep et al., 2007). On the estuarine fish *Pomatoschistus microps*, LC_{50} of copper and mercury at 96 h were 568 µg/l and 62 µg/l, respectively (Vieira et al., 2009).

The concentrations of trace metals that resulted in mortality of *H. rubra* were investigated by exposing juveniles to acute concentrations of Cu, Zn, Hg and Cd for 96 h. Hg resulted in more sudden mortality rate after 24 h exposure, compared to Cu, yet produced a 96 h LC_{50} of 173 µg/l Hg (Gorski, 2007).

EPA studies (1997) on many aquatic species show vast ranges of LC_{50} for mercury chloride, which for saltwater fish was 36 µg/l (juvenile spot) to 1,678 µg/l (flounder), higher than the one for saltwater invertebrates: 3.5 µg/l (mysid shrimp) to 400 µg/l (soft clam).

This result emphasis that yellow-fin sea bream is sensitive to mercury chloride and have low range value.

According to FAO/UNEP (1991), the 96 h LC_{50} values of mercury chloride are, for cat fish 350 µg/l, rainbow trout, 220 µg/l, striped bass, 90 µg/l, and brook trout, 75 µg/l.

The 96 h LC_{50} values of mercury chloride is 37 µg/l for fathead minnow, 160 µg/l for bluegill sunfish, 903 µg/l for rainbow trout, 200 µg/l for rainbow trout, and lower in invertebrates: two µg/l for crayfish, five µg/l for cladocera, 10 µg/l for *Gammarus* sp., five µg/l for blue mussel, 15 µg/l for prawn, and three µg/l for limpet (Eisler, 1987).

Rathore and Khangarot (2002) reported that the acute toxicity of $HgCl_2$ increases with the increase of temperature. Cairns et al. (1981) reported similar trends for other metals. Khangarot and Ray (1987) also observed that the toxicity of copper abruptly decreased with an increase in pH of the Cu-containing medium. Acute toxicity studies are the very first step in determining the water quality requirements of fish. These studies obviously reveal the toxicant concentrations (LC_{50}) that cause fish mortality even at short exposure. Therefore, studies demonstrating the sensitivity of genotoxic effects of heavy metals in aquatic organisms, particularly in fish, are needed. Thus, it can be concluded from the present study that fish are highly sensitive to $HgCl_2$ and their mortality rate is dose dependent.

Comparison of the values reported earlier with those obtained in the present study may not be meaningful, because various factors may influence bioassay techniques, like differences in fish (e.g., species, weight, size) and other environmental factors (temperature, variations in pH of the water, total hardness of water, dissolved oxygen). Sprague (1969) observed variability in acute toxicity even in a single species and single toxicant depending on the size, age, and condition of the test species along with the experimental factors. Gupta et al. (1981) reported that the differences in acute toxicity may be due to changes in water quality and test species.

Chronic toxicity values are much lower than acute values and highlight the adverse effects of relatively low concentrations of mercury in water (i.e., < one µg/l).

In aquatic toxicology, if LC_{50} concentration is smaller than 1,000 µg/l, the chemical is highly toxic, and if it is between 1,000-10,000 µg/l, then it is considered to be moderately toxic (Louis et al., 1996), therefore we report mercury chloride to be highly toxic to yellow-fin sea bream and may cause many damages to these fish.

Range values indicated that mercury is more toxic to *A. latus*. The range obtained in the present study (500-1,000 µg/l) compared with the corresponding values that have been published in the literature for other species of fish, show different range of mercury in different species and even different time periods, but, what is important is lower range values for *A. latus*, compare with most species confirm the sensitivity of *A. latus* to low mercury doses.

ACKNOWLEDGMENTS

The authors of this scientific paper are thankful to the Director and Staff at the Mariculture Research Station, Mahshahr, Iran, for providing necessary facilities for the experiment, and the University of Marine Science and Technology, Khoramshahr, Iran, for support during the tenure of this project. We would like to express our special thanks to Dr. Movahedinia for assistance during the trials.

REFERENCES

1. Boudou A. and Ribeyre F., 1997 – Aquatic ecotoxicology: from the ecosystem to the cellular and molecular levels. Environ. Health Perspect. 105 (Suppl. 1), 21-35.
2. Boening D., 2000 – Ecological effects, transport, and fate of mercury: a general review. Chemosphere. 40, 1335-1351.
3. Cairns J. Jr., Buikema Jr., A. L., Heath A. G. and Parker B. C., 1981 – Effects of temperature on aquatic organism sensitive to selected chemicals. Va. Water Resources Res. Center Bull. 106, 1–88.
4. Eisler R., 1987 – Mercury hazards to fish, wildlife and invertebrates. U.S. fish and wildlife research center, 10.
5. Elia A. C., Dörr A. J. M., Mantilacci L., Taticchi M. I. and Galarini R., 2000 – Effects of mercury on glutathione and glutathione-dependent enzymes in catfish (Ictalurus melas R.), in: Markert, B. and Friese, K. (eds), Trace Elements—Their Distribution and Effects in the Environment: Trace Metals in the Environment, Vol. 4., Elsevier Science, Amsterdam.
6. FAO/UNEP, 1991 – Operation of the prior informed consent procedure for banned or severely restricted chemicals in international trade, Joint FAO/UNEP program, Rome, Geneva, (amended 1996).
7. Gooley G. J., Gavine F. M. and Olsen L., 2006 – Biological Systems to Improve Quality and Productivity of Recycled Urban Wastewater. A Joint Project of: Department of Primary Industries, Victoria.
8. Gupta P. K., Khangarot B. S. and Durve V. S., 1981 – The temperature dependence of the acute toxicity of copper to a freshwater pond snail, Viviparus bengalensis L. Hydrobiologia 83, 461–464.
9. Gorski J, 2007 – The effects of trace metals on the Australian abalone, Haliotis rubra. PhD thesis. RMIT University.
10. Vieira L. R., Gravato C., Soares A. M. V. M., Morgado F. and Guilhermino L., 2009 – Acute effects of copper and mercury on the estuarine fish Pomatoschistus microps: linking biomarkers to behavior. Chemosphere 76, 1416–1427.
11. Louis A. H, Diana L. W. Patricia H. and Elizabeth R. S., 1996 – Pesticides and aquatic Animals, Virginia Cooperative Extension, Virginia State University, Virginia.
12. Rathore R. S. and Khangarot B. S., 2002 – Effect of temperature on the sensitivity of sludge worm Tubifex tubifex (Muller) to selected heavy metals. Ecotoxicol. Environ. Saf. 53, 27-36.
13. Sprague J. B., 1969 – Measurement of pollutant toxicity to fish: I. Bioassay methods for acute toxicity. Water Res. 3, 793-821.
14. Straalen N. M. van, Leeuwangh P. and Stortelder P. B. M., 1994 – In Ecotoxicology of soil organisms. Donker M.H. (ed.), Eijsackers H and Heimbach F. CRC. Press.
15. Verep B., Sibel Besli E., Altionk I. and Mutlu C., 2007 – Assessment of Mercuric chloride toxicity on Rainbow trouts and cubs. Pakistan Journal of Biological Sciences. 10, (7), 1098-1102.

BACTERIAL COMMUNITY STRUCTURE IN THE WETLANDS OF HORTOBÁGYI NEMZETI PARK (HUNGARY)

*Viera STRAŠKRÁBOVÁ *, Gyula LAKATOS *** and *Péter KERESZTÚRI **

* Academy of Sciences of Czech Republic, Institute of Hydrobiology, Na sádkách 7, České Budějovice, Czech Republic, CZ-37005, straskraba@volny.cz
** Debrecen University, Institute of Biology and Ecology, Department of Applied Ecology, Debrecen, HU-4010, lakgyu@delfin.unideb.hu
*** Debrecen University, Institute of Biology and Ecology, Department of Applied Ecology, Debrecen, HU-4010, pkereszturi@gmail.com

KEYWORDS: Hungary, soda wetlands, shallow pools, macrophytes, aquatic bacteria communities, fluorescent in situ hybridization (FISH), 16S RNA.

ABSTRACT

Aquatic microbial community composition was investigated in the pools of wetlands in Hortobágy National Park. Fifteen sites were studied in areas with high and low impact of cattle, and with low and high water salinity. Abundances of heterotrophic flagellates, picocyanobacteria and bacteria were estimated. Seven groups of bacteria and *Archaea* were detected quantitatively. *Archaea, Actinobacteria, α-* and *β-Proteobacteria* were the most numerous (up to 50-80% of total counts). High total abundances (up to 75 x 10^6 ml^{-1}) were found at sites with a high concentration of organic matter, and/or with a high conductivity. In the absence of microbial grazers, high growth rates (μ d^{-1}) close to one were observed among *Archaea*, *α-, β-* and *γ-Proteobacteria* as well as in the subcluster R-Bt. Only one group – *Actinobacteria* – did not grow.

RÉSUMÉ: La structure des communautés bactériennes dans les flaques d'eau du Parc National Hortobágyi (Hongrie).

On a étudié la composition des communautés bactériennes aquatiques des plaines humides du Parc National Hortobágyi. 15 sites, situées dans des zones soumises à un impact élevé ou très bas des troupeaux et ayant une salinité de l'eau basse ou élevée. L'abondance des flagellées hétérotrophes, des picocyanobactéries et des bactéries a été estimée. Sept groupes de bactéries et Archaea ont été détectées de point de vue quantitatif. *Archaea, Actinobacteria, α-* et *β-Proteobacteria* ont été les plus nombreuses (jusqu'a 50-80% du nombre total). Dans des sites avec une concentration élevée de matière organique et/ou conductivité élevée on a retrouvé des abondances totales élevées (jusqu'a 75 x 10^6 ml^{-1}). Dans l'absence des consommateurs primaires microbiens, on a observé des taux de croissance élevées (μ d^{-1}) près de un chez *Archaea*, *α-, β-* et *γ-Proteobacteria*, ainsi que chez le sous cluster R-Bt. *Actinobacteria* n'a pas enregistré de croissance. Dans des sites avec une concentration élevée de matière organique et/ou conductivité élevée on a retrouvé des abondances totales élevées (jusqu'a 75 x 10^6 ml^{-1}). Dans l'absence des consommateurs primaires microbiens, on a observé des taux de croissance élevées (μ d^{-1}) près de un chez *Archaea*, *α-, β-* et *γ-Proteobacteria*, ainsi que chez le sous cluster R-Bt. *Actinobacteria* n'a pas enregistré de croissance.

REZUMAT: Structura comunităţilor bacteriene din bălţile Parcului Naţional Hortobágyi (Ungaria).

S-a studiat compoziţia comunităţilor microbiene acvatice, din bălţile zonelor umede ale Parcului Naţional Hortobágyi. Au fost prelevate probe din 15 staţii în zone cu impact mare şi impact scăzut al prezenţei turmelor de vite, precum şi cu salinitate mare şi scăzută a apei. S-a estimat abundenţa flagelatelor heterotrofe, a picocianobacteriilor şi a bacteriilor. Şapte grupe de bacterii şi de Arhaea au fost detectate cantitativ. *Archaea, Actinobacteria, α-* şi *β-Proteobacteria* au fost cele mai numeroase (până la 50-80% din numărul total). Abundenţe totale ridicate (până la 75 x 10^6 ml^{-1}) s-au găsit în staţii cu o concentraţie ridicată de materie organică, şi/sau conductivitate ridicată. În absenţa consumatorilor primari microbieni, rate de creştere mari (μ d^{-1}) aproape de unu au fost observate la *Archaea*, *α-, β-* şi *γ-Proteobacteria* precum şi la subclusterul R-Bt. *Actinobacteria* nu a crescut.

INTRODUCTION

The alkaline soda wetlands in Hortobágy National Park (Tisa Basin, Hungary) are a special type of shallow aquatic systems – temporary ponds and marshes of different sizes and depths, with seasonal fluctuation of water level. They are characterized by high concentrations of sodium, magnesium and calcium cations with prevailing carbonate and hydrocarbonate anions. They are rich in organic matter, dark suspended particles and dark sediment. In the past century, round 1960, an intensive agriculture threatened these wetlands (Gőri et al., 2000). Later, begining in 1972, the area was protected and the dominated wetland types survived and recovered after the unfavourable conditions. The EU Habitats Directive includes a protection of Pannonic salt steppes and salt marshes, predominantly found in Great Plain (Boros, 2003). In that area, important stagging and roosting sites of migrating water fowl and shorebirds are located and a specific flora (including aquatic macrophytes) is protected. Historically, that area had been used for livestock breeding, which is now maintained in an extensive way. Our study was performed in two groups of sites differing in conductivity – in Hortobágy puszta and in Egyek puszta of Hortobágy National Park.

Numerous botanical and zoological studies were carried out in alkaline wetlands and lakes of Hungary (refered by Boros, 2003). Vegetation was studied in the first phase of rehabilitation (Gőri et al., 2000). In shallow lakes and wetland pools also periphyton on reed was also studied (Lakatos et al., 1998). Bacterial communitiy composition was less frequently studied, in Hungarian alkaline wetlands (Borsodi et al., 2007; Vladar et al., 2008), as well as in other wetlands of similar type (D'Auria et al., 2010). Practically, all these studies focused on bacteria in periphyton and in the sediment layer, and specifically on bacterial sulphate oxidizers and their function. In contrast, we studied bacterial community composition in the water phase, identified *Archaea* and all main groups of *Eubacteria* quantitatively and tried to characterize complex microbial assemblages in free water. In order to differentiate between bacterial groups for their ability of growth or resistance against flagellate grazing, we performed preliminary tests after laboratory incubation.

MATERIAL AND METHODS

Samples for bacterial and heterotrophic nanoflagellate (cell range of two-20 μm) abundances, and for picocyanobacterial counts were fixed with formaldehyde (2% final concentration, v/v), concentrated on 0.2 μm pore size black polycarbonate filters (OSMONIC INC., Livermore, USA), stained by DAPI (final concentration 0.1 mg mL^{-1}, wt/vol) and enumerated by epifluorescence microscopy (Olympus BX 60), details in Šimek et al. (2001). Picocyanobacteria were distinguished by autofluorescence of chlorophyll on the same filters.

Analysis of bacterioplankton communities was performed using group-specific rRNA-targeted oligonucleotide probes (CARD-FISH – catalysed reporter deposition fluorescence in situ hybridization). We applied CARD-FISH protocol (Pernthaler et al., 2002; Sekar et al., 2003) employing the oligonucleotide probes (ThermoHybaid, Interactiva Division, Ulm, Germany) targeted at respective groups. The proportions of FISH-positive bacteria were detected directly by inspecting 500-1,000 cells in the replicate samples via epifluorescence microscopy (Olympus AX-70). The following groups were assessed: three subclasses of *Proteobacteria* – alpha, beta and gamma (probes ALPH968, BET42a, GAM42a), the *Cytophaga/Flavobacterium/Bacteroidetes* group (probe CF319a), the *Actinobacteria* group (probe HGC69a), domain *Archaea* (probe ARCH915, Loy et al., 2002). Moreover, within *Betaproteobacteria* subclass a subcluster of *Rhodoferax* sp. BAL47 (probe R-BT065) and the cluster *Polynucleobacter* (probe PnecABCD445) were assessed, which are frequently found in freshwater (Zwart et al., 2002; Hahn et al., 2005).

In two samples from the sites with lower conductivity, sampled in the morning, the ability of growth of particular bacterial groups and of heterotrophic flagellates after incubation was tested as follows. Sample was pre-filtered (i) through five μm pore size polycarbonate filter (zooplankton grazers removed) and (ii) through 0.8 μm pore size filter to remove all grazers including protozoans (heterotrophic flagellates and ciliates). Subsamples were taken and preserved immediately after prefiltration, and the rest incubated in the darkness at laboratory temperature for 72 hours and then preserved. Bacterial and heterotrophic protist abundances and bacterial community composition were determined similarly like in the original samples.

SITE DESCRIPTION AND SAMPLING

10 sites were sampled in Hortobágyi puszta near Nyirőlapos (47°34 – 47°35 N, 21°15 – 21°16 E, 93 – 97 m a.s.l.). Another five sites were sampled in Egyek puszta near Meggyes (47°33.3 – 47°33.4 N, 20°53.3 – 20°53.8 E, 92 – 100 m a.s.l.). Sampling was performed on May 20, 2008, starting after short heavy rain at 10 a.m., and proceeding with sunny weather until five p.m. Water depth, Secchi disc transparency, temperature, oxygen saturation, pH, electrode-potential and conductivity were measured in the field. Due to the sunny weather and increasing sun radiation, water temperature rose from 17°C in the morning up to 25°C in the afternoon, as well as oxygen saturation (surface) from 22 to 51%, and pH from 6.5 to 8.5 due to increasing photosynthesis. Surface layer was sampled in places where the depths varied from seven to 50 cm. Secchi disc depths varied from one to 22 cm.

The first three sites were characterized by low amounts of suspended matter compared to the rest. Sites one – 10 showed low alkalinity values (except of site no. 8), whereas all the sites from Egyek puszta (nos. 11-15) showed values above 1,450 μS cm^{-1} (Tab. 1).

Macrophytes were of similar composition in all sites, while the species richness was the highest in sites nos. one-three (average 10 taxa per site), it was lower in sites four-10 (average seven taxa per site) and the lowest in the sites 11-15 (only five taxa per site). From 37 taxa found in the 10 sites, Nos one-10, only 16 occurred in the sites (Nos. 11-15) from Egyek puszta. The taxa found at least in five sites of the whole area sampled (including sites with high conductivity), were as follows: *Bolboschoenus maritimus* (L.) Palla, *Eleocharis palustris* (L.) Roem et Schult., *Lemna minor* L., *Phragmites australis* (Cav.) Trin. ex Steudel, *Rorippa silvestris* (L.) Besser, *Rumex crispus* L., *Scirpus lacustris* (L.) Palla and *Typha angustifolia* L.

Table 1: Abiotic parameters (ranges) in the sites.

Sites	Temperature °C	pH	% O_2 saturation	Redox-potential mV	Conductivity µS cm^{-1}	Suspended matter mg L^{-1}
1, 2, 3	17.5 – 17.9	6.5 – 7.2	22.1 – 26.8	187 to 204	460 – 680	4 – 16
4, 5, 6, 7, 9, 10	21.5 – 23.6	6.7 – 7.9	32.6 – 42.0	164 to 189	219 – 780	246 – 440
8	22.7	8.2	36.0	147	1,532	6,762
12, 13	23.9 – 25.1	7.4 – 8.4	30.6 – 38.9	184 to 189	1,452 – 1,753	23 – 36
11, 14, 15	24.0 – 25.6	8.1 – 8.3	45.2 – 51.4	127 to 155	1,439 – 1,641	193 – 272

RESULTS AND DISCUSSION

The sites can be grouped according to abiotic characteristics (Tab. 1). The first group, sites one, two and three, were not influenced by cattle, they had rich littoral flora and the lowest concentration of suspended matter. These sites, especially site three, were visited by water fowl, especially site three with a large flock of spoon bills (*Platalea leucorodia* L.). The sites showed the lowest values of temperature, pH and oxygen saturation, but the highest redox-potential. Total bacterial (DAPI) abundances were by one order lower than in all other sites (Tab. 2). Among microbial groups after hybridization, the representatives of the domain *Archaea* were scarce there, compared to all the other sites. They shared the lowest percentage in DAPI and the lowest abundances per ml (0.118 – 0.165 x 10^6 ml^{-1}). In all other sites the range of *Archaea* abundances was 0.449 – 2.453 x 10^6 ml^{-1}.

The second group of sites, from four to 10, were in the area affected by cattle. Compared to the first group, they showed higher temperature, pH and oxygen saturation, and also by more than one order higher suspended matter concentration, but lower redox-potential. One site – no. eight was quite different, with a very high suspended matter (the highest from all sites) and high conductivity (comparable with the values found in Egyék puszta), and with the lowest ox/red potential (Tab. 1). The total bacterial (DAPI) abundance was the highest out of all the sites (Tab. 2) as well as the share (%) of γ-*Proteobacteria* in DAPI. Abundance of *Actinobacteria* was the highest there: 20.2 x 10^6 ml^{-1}. The site was small, a deep and muddy ditch in the cattle tracks.

The third group of sites, from 11 to 15, were sampled later in the day, in the area with a higher conductivity, affected by cattle. As expected, the afternoon temperature, pH and oxygen saturation were the highest than all other sites, and the redox-potential was the lowest (Tab. 1). Bacteria from *Polynucleobacter* cluster were less numerous. They only reached 0.53 – 0.70 x 10^6 ml^{-1} in sites 11, 12, 14 and 15, whereas values above 2 x 10^6 ml^{-1} were frequent in other site groups. Two sites of the group (12 and 13) were distinguished from sites 4-7, 9-11, 14 and 15 by a lower concentration of suspended matter and a slightly higher redox-potential. Site No. 13 showed the lowest pH value among all "afternoon" samples (7.44) and also slightly higher *Polynucleobacter* abundance – 2.63 x 10^6 ml^{-1}.

In the sites one-three autotrophic picoplankton cell counts were around 23,000 per ml (Tab. 2). In the other sites of low alkalinity, they varied from zero up to 180,000 per ml, while in the high alkalinity sites, they reached values of 5,900,000 per ml (comp. Tabs. 1 and 2). Heterotrophic nanoflagellates were very scarce (500 – 1,000 ind. per ml) in all samples, but they increased in number during incubation without zooplankton grazers (see below). Ciliates were found in one case – less than 100 per ml.

Table 2: Microbial data in sites. DAPI – total microscopic counts with DAPI stain, APP – autotrophic picoplankton cell counts. Bacterial groups in percentage of DAPI counts: subclasses α, β, γ – *Proteobacteria*, C/F – *Cytophaga/Flavobacterium/Bacteroidetes* group, Act – *Actinobacteria* group, Arch – *Archaea* domain, R-Bt – subcluster of *Rhodoferax* sp., Pnec – *Polynucleobacter* cluster (not determined in site no. eight due to high interference by detritus particles).

SITE	DAPI 10^6 ML^{-1}	APP 10^3 ML^{-1}	A%	B%	Γ%	C/F%	ACT%	ARCH %	R-BT %	PNEC %
1, 2, 3	2.8 – 6.3	20.2 – 26.0	2.2 – 2.5	5.4 – 26.3	0.5 – 1.1	1.4 – 6.8	2.1 – 13.5	0.2 – 6.1	0.8 – 2.1	0.5 – 21.9
4, 5, 6, 7, 9, 10	6.6 – 32.8	0 – 182.2	1.2 – 13.8	2.4 – 85.5	0.3 – 2.6	3.6 – 7.6	13.4 – 66.2	0.5 – 40.7	0.2 – 7.1	0.7 – 18.0
8	75.7	1,142.8	9.0	5.1	6.5	8.0	26.7	4.9	1.4	n.d.
12, 13	14.5 – 28.1	5.8 – 376.3	1.1 – 3.5	10.3 – 28.8	2.5 – 2.6	4.9 – 9.1	3.1 – 8.2	3.1 – 17.7	2.2 – 2.3	1.9 – 9.3
11, 14, 15	18.1 – 66.3	0 – 5894.4	0.9 – 4.7	6.9 – 19.4	0.1 – 1.1	5.0 – 5.5	3.3 – 20.0	10.5 – 22.0	1.2 – 3.1	1.0 – 2.9

in samples with a high concentration of suspended matter. Besides, even when the concentration of detritus particles was low, samples were turbid and the volume concentrated through the filter was thus limited. So the slight differences among samples were not statistically significant and might be caused either by difficulties at microscopic counting and/or by heterogeneous distribution of microbes in the samples.

The ability of growth and resistance against protozoan grazing of microbial groups was investigated in samples from site three and nine. Both had similar conductivity and comparable pH and temperature values. The main differences were: absence of cattle, lower concentration of suspended matter and higher redox-potential in site three, compared to site nine, which was heavily impacted by cattle. Due to turbidity (as explained above) the volume filtered was not sufficient to elaborate all analyses in replicates (Tabs. 3 and 4).

Changes in the microbial community during three days of incubation were investigated in two treatments: without protozoan grazers and with protozoans (two replicates in each treatment). In the tables 3 and 4 apparent growth rates per day (μ d^{-1}) are shown. They are calculated as follows:

$$\mu = \frac{\ln X_2 - \ln X_1}{3}$$

where X_2 and X_1 are abundances of a respective microbial group at the end and at the start of the three day incubation.

Table 3: Changes in bacterial community structure after three day incubation (filtered through five μm – without zooplankton grazers). a, b – two replicates. HNF – heterotrophic nanoflagellates, μ in d^{-1} – growth rate per day (or % loss). n.d. – not determined. For other explanations see the table 2.

Site	HNF μ in d^{-1}	α μ in d^{-1}	β μ in d^{-1}	R-Bt μ in d^{-1}	Pnec μ in d^{-1}	γ μ in d^{-1}	C/F μ in d^{-1}	Act μ in d^{-1}	Arch μ in d^{-1}
No 3a	0.85	0.25	0.67	0.20	0.29	0.23	0.37	0.15	– 44%
No 3b	n.d.	0.38	0.43	0	0.24	n.d.	0	n.d.	0.74
No 9a	0.56	0.51	– 18%	0.45	– 29%	0.64	– 42%	– 50%	0.55
No 9b	n.d.	0.37	0.36	0.56	0.42	n.d.	0.68	n.d.	0.55

Table 4: Changes in bacterial community structure after three days of incubation (filtered through 0.8 μm – without protozoan and zooplankton grazers). a, b – two replicates. μ in d^{-1} – growth rate per day. n.d. – not determined. For other explanations see the table 2.

Site	α μ in d^{-1}	β μ in d^{-1}	R-Bt μ in d^{-1}	Pnec μ in d^{-1}	γ μ in d^{-1}	C/F μ in d^{-1}	Act μ in d^{-1}	Arch μ in d^{-1}
No 3a	0.88	0.29	0.47	0.40	0.22	0.78	0.24	0.64
No 3b	0.58	0.71	0.75	0.63	n.d.	0.35	n.d.	0.90
No 9a	0.93	0.52	1.03	0.39	1.05	0.59	0	0.85
No 9b	0.76	1.10	1.43	0.70	n.d.	0.55	n.d.	0.95

In the treatment with protozoans (Tab. 3), flagellates in sample three showed a high growth rate increasing from 519 up to 6,747 ind. per ml (ciliates were not detected). Initial total abundance of bacteria was 2.8 x 10^6 ml^{-1} per ml and it slightly increased to 3.6 x 10^6 ml^{-1} ($\mu = 0.09 d^{-1}$). In this treatment, bacterial growth rates are "apparent", since their growth rates decreased by protozoan grazing. There were bacterial groups increasing even at that high grazing pressure, especially *β-* and *α-Proteobacteria* and *Polynucleobacter*. High differences among replicates, rather low growth rates or decreased abundances were found in the other groups.

In sample nine, flagellates grew slower, from 1,000 to 5,320 ind. per ml. The initial bacterial abundance was 6.6 x 10^6 ml^{-1} and it increased up to 9.4 x 10^6 ml^{-1} ($\mu = 0.12 d^{-1}$). In that case, almost all groups (except of *Actinobacteria*) showed higher growth rates (at least in one replicate) than in sample three. Subgroups R-Bt (both replicates) and *Polynucleobacter* (one replicate) showed higher growth rates than the total *β-Proteobacteria*.

In the treatment without grazers (Tab. 4), both total bacterial abundance and all groups detected increased, except in *Actinobacteria*. Total bacteria in sample three increased from 3.9 to 8 x 10^6 ml^{-1}, whereas in sample nine they grew from 6.6 to 14.4 x 10^6 ml^{-1} (total growth rates $\mu = 0.23 – 0.26$ d^{-1}). High growth rates (> 0.50) in both samples were observed for *α-* and *β-Proteobacteria, Cytophaga/Flavobacterium* and *Archaea*, as well as for both subgroups R-Bt and *Polynucleobacter*. In sample nine, growth rates far higher than in sample three were observed for R-Bt, *Polynucleobacter* and *γ-Proteobacteria*. On the contrary, *Actinobacteria* in both samples and *γ-Proteobacteria* in sample three did not show any increase in growth rate in the treatment without grazers compared to the other treatment.

The microbial community found in our samples had similar composition like in the pelagial of other aquatic systems, except for the very low occurrence of protistan grazers. In cultivation experiments it appeared that free swimming heterotrophic flagellates in situ were under strong control of zooplankton (larger than five μm). During our sampling, we found frequently both cladocerans and copepods. Ciliates were probably present in periphyton attached to host plants and they occurred in considerable numbers in soils (Szabó, 2000).

As expected, total bacterial abundances (DAPI) as well as bacterial growth rates, were "bottom up" controlled by organic matter and/or by phosphorus concentration (Šimek et al., 2006). However, among our sites it seems that another "bottom up" controlling parameter might be the conductivity (difference between sites one-10, and 11-15). Chemical parameters connected with conductivity, alkalinity and pH were shown to be important in controlling limnological processes, especially phytoplankton production (Talling and Talling, 1965; Talling, 2010). Similarly, water chemistry along with geographical location was found to explain most of the variation in the occurrence and composition of bacterial communities (Jezberová et al., 2010; Šimek et al., 2010).

In the sites 1-10, there were variations in suspended matter and in conductivity. Sites 11-15 had lower variation of conductivity, but they varied in suspended matter and pH (table number 1). Correlation coefficient between DAPI counts and suspended matter in the set of all 15 samples was 0.60 whereas in the set of 10 samples (1-10) it was 0.92. Correlation between DAPI counts and conductivity in all samples was only 0.53, but in 10 samples (1-10) it was 0.80 (Tab. 5).

The bacterial community composition found in our samples was compatible with the group composition in reservoirs and lakes: main groups were α- and β-*Proteobacteria*, *Actinobacteria* and *Archaea* (Šimek et al., 2001; Sekar et al., 2003; Zwart et al., 2002; Mašín et al., 2003). Abundances of bacterial groups in sites were expressed in percentage of DAPI (Tab. 2). For correlation with environmental parameters we expressed them in counts (10^6 ml^{-1}) and compared with suspended matter concentration, with pH and conductivity. In table number five there are correlation coefficients for $P < 0.05$ or < 0.01 or < 0.001, either for all samples (15 sites) or for 10 selected sites (one – 10) in Hortobágy puszta. Conductivity was correlated with suspended matter with the highest significance, but only in 10 selected samples (not shown in table). Two groups – *Archaea* and *β-Proteobacteria* (surprisingly) did not significantly correlate with any of the abiotic variables. The subgroups of *β-Proteobacteria* – RB-t and *Polynucleobacter* – correlated ($P = 0.05$ only) just with pH and conductivity, and with suspended matter, respectively. In other groups, the correlation with suspended matter was rather high for all sites. In the 10 selected sites, correlation with pH and conductivity was also remarkable.

The top down effect by grazing upon bacterial community composition was studied and documented in different aquatic ecosystems (Šimek et al., 2001a; Šimek et al., 2001b; Šimek et al., 2005; Corno et al., 2008). High growth rates of heterotrophic flagellates after removing zooplankton were repeatedly found (Šimek et al., 2006). *β-Proteobacteria* were found as a group rather selectively grazed by protists and growing fast under considerable grazing pressure. Our data from wetland pools did not show this phenomenon quite clearly – subgroups RB-t and Pnec as well as *α-Proteobacteria* and even *Archaea* often grew faster than the whole group of *β-Proteobacteria* (Tabs. 3 and 4). The reason for the lesser importance of *β-Proteobacteria* in the wetland pools might be the prevalence of vascular plants among primary producers, shallowness of pelagial and scarcity of phytoplankton (except of small picocyanobacteria). A tight relationship between phytoplankton primary production and growth of *β-Proteobacteria* was found in reservoirs (Šimek et al., 2008). The growth of *Archaea*, on the other hand, probably was controlled by dissolved organic matter released from sediment, which might explain why they did not correlate with suspended solids.

Table 5: Correlation between abundances of bacterial groups and abiotic parameters. susp. m. – suspended organic matter, conduct – conductivity, ALL – all sites, SEL – selected 10 sites (1-10), * P < 0.05, ** P < 0.01, *** P < 0.001, n.s. – not significant. Other explanations in table 2.

	susp. m. ALL	susp. m. SEL	pH ALL	pH SEL	conduct. ALL	conduct. SEL
DAPI	0.605*	0.925***	0.575*	0.690*	0.533*	0.804**
alpha	0.835***	0.924***	n.s.	0.801**	n.s.	0.690*
Act	0.723***	0.718*	n.s.	n.s.	n.s.	n.s.
gama	0.980***	0.995***	n.s.	0.659*	n.s.	0.843**
C/F	0.789***	0.960***	0.598*	0.713*	0.527*	0.804**
RB-t	n.s.	n.s.	n.s.	0.702*	0.539*	n.s.
Pnec	n.s.	0.719*	n.s.	n.s.	n.s.	n.s.

It was surprising why *Actinobacteria*, an abundant group in aquatic environments (Glöckner et al., 2000), did not grow in the absence of grazers and, at the same time, they were not much grazed. As shown by Horňák et al. (2005), they were not preferred as food by flagellates, whereas *β-Proteobacteria* and other "larger" bacteria were selectively grazed. *Actinobacteria* were tightly correlated with suspended organic matter – they might prefer to stay in sediment.

CONCLUSIONS

Microbial community in water phase of wetland pools consists of bacteria, picocyanobacteria and a low number of heterotrophic nanoflagellates (HNF). Eucaryotic phytoplankton and ciliates were almost absent. As shown in incubations, HNF were under heavy control of zooplankton grazers in the pools and grew fast after they were removed.

Bacterial community composition was similar to what was found in different aquatic systems, but the share and growth rates of particular groups differed from the situation found in lakes and reservoirs. The main difference was found in *β-Proteobacteria* group, which was less numerous and slowly growing, though their two subgroups *RB-t* and *Polynucleobacter* grew faster. *β-Proteobacteria* also showed no relation to suspended matter and/or conductivity.

High growth rates after removal of HNF were observed in *α-Proteobacteria, γ-Proteobacteria, Cytophaga/Flavobacterium* and *Archaea.* On the other hand, *Actinobacteria* did not grow and they were almost not grazed. A significant correlation between suspended matter and conductivity was found for the abundances of all groups except of *Archaea.*

Acknowledgements

The authors are grateful to the staff of Debrecen University for the efficient help in the field sampling and for chemical data from the sites. We appreciate an efficient help of the scientific and technical staff during microbial analyses at the Institute of Hydrobiology, especially by Šimek K. and Malá R.. The expedition and elaboration of data were supported by the mobility fund of ALTER-net network of Excellence (GOCE-CT-2003-505298).

REFERENCES

1. Gőri S., Lakatos G., Aradi C., Kiss M. K. and Bitskey K., 2000 – The vegetation of the Meggyes Marsh in the starting phase of rehabilitation, Acta Bot. Croat., 59, 403-409.
2. Boros E., 2003 – Alkaline lakes, National Ecological Network 4, R. Érdi (ed.), published by Authority for Nature Conservation, Ministry for Environment and Water, Budapest, Hungary, 28.
3. Lakatos G., Grigorszky I. and Biró P., 1998 – Reed-periphyton complex in the littoral of shallow lakes, Verh. Internat. Ver. Limnol., 26, 1852-1856.
4. Borsodi A. K., Rusznyák A., Molnár P., Vladár P., Reskoné M. N., Tóh E. M., Sipos R., Gedeon G. and Márialigeti K., 2007 – Metabolic activity and phylogenetic diversity of reed (Phragmites australis) periphyton bacterial communities in a Hungarian shallow soda lake, Microbial Ecology, 53, 612-620.
5. Vladár P., Rusznyák A., Márialigeti K., Borsodi A. K., 2008 – Diversity of sulfate-reducing bacteria inhabiting the rhizosphere of Phragmites australis in lake Velencei (Hungary) revealed by a combined cultivation-based and molecular approach, Microbial Ecology, 56, 54-75.
6. D'Auria G., Barón-Rodríguez M. M., Durbán-Vincente A., Moya A., Rojo C., Latorre A. and Rodrigo M. A., 2010 – Unravelling the bacterial diversity found in the semi-arid Tablas de Daimiel National Park wetland (central Spain), Aquatic Microbial Ecology, 59, 33-44.
7. Šimek K., Pernthaler J., Weinbauer M. G., Horňák K., Dolan J. R., Nedoma J., Mašín M. and Amann R., 2001a – Changes in bacterial community composition, dynamics, and viral mortality rates associated with enhanced flagellate grazing in a mesotrophic reservoir, Appl. Environ. Microbiol., 67, 2723-2733.
8. Pernthaler A., Pernthaler J. and Amann R., 2002 – Fluorescence in situ hybridization and catalyzed reporter deposition for the identification of marine bacteria, Appl. Environ. Microbiol., 68, 3094-3101.
9. Sekar R., Pernthaler A., Pernthaler J., Warnecke F., Posch T. and Amann R. 2003 – An improved protocol for quantification of freshwater Actinobacteria by fluorescence in situ hybridization, Appl. Environ. Microbiol., 69, 2928-2935.
10. Loy A., Lehner N., Lee J., Adamczyk H., Meier J., Ernst K. H., Schleifer M. and Wagner M., 2002 – Oligonucleotide microarray for 16S rRNA gene-based detection of all recognized lineages of sulfate-reducing prokaryotes in the environment, Appl. Environ. Microbiol., 68, 5064-5081.
11. Zwart G., Crump B. C., Agterveld M., Hagen F. and Han S. K., 2002 – Typical freshwater bacteria: an analysis of available 16S rRNA gene sequences from plankton of lakes and rivers, Aquat. Microb. Ecol., 28: 141-155.
12. Hahn M. W., Pöckl M. and Wu Q. L., 2005 – Low intraspecific diversity in a Polynucleobacter subcluster population numerically dominating bacterioplankton of a freshwater pond, Appl Environ. Microbiol., 71, 4539-4547.
13. Šimek K., Horňák K., Jezbera J., Nedoma J., Vrba J., Straškrábová V., Macek M., Dolan J. R. and Hahn M. W., 2006 – Maximum growth rates and the possible life strategies of different bacterioplankton groups in relation to phosphorus availability in a freshwater reservoir, Environmental Microbiology, 8, 1623-1624.
14. Szabó A., 2000 – Estimated biomass of Ciliata (Protozoa) communities in alkaline soils of the Hortobágyi National Park, Hungary, Opusc. Zool. Budapest, 32, 129-138.
15. Talling J. F. and Talling I. B., 1965 – The chemical composition of African lake waters. Intern. Rev. Hydrobiol., 50: 421-463.
16. Talling J. F., 2010 – pH, the CO_2 system and freshwater science, Freshwater Reviews, 3, 133-146.

17. Jezberová J., Jezbera J., Brandt U., Lindström E. S., Langenheder S. and Hahn M. W., 2010 – Ubiquity of Polynucleobacte necessarius ssp. asymbioticus in lentic freshwater habitats of a heterogeneous 2000 km^2 area, Environmental Microbiology, 12, 658-669.
18. Šimek K., Kasalický V., Jezbera J., Jezberová J., Hejzlar J. and Hahn M. W., 2010 – Broad habitat range of the phylogenetically narrow R-BT065 cluster representing a core group of the betaproteobacterial genus Limnohabitan, Applied and Environmental Microbiology, 76, 631-639.
19. M. Mašín, K. Šimek, J. Jezbera, J. Nedoma, V. Straškrábová, J. Hejzlar, 2003 – Changes in bacterial community composition and microbial activities along the longitudinal axis of two differently loaded canyon-shaped reservoirs, Hydrobiologia, 504, 99-113.
20. Šimek K., Armengol J., Comerma M., Garcia J. C., Kojecká P., Nedoma J. and Hejzlar J., 2001b – Changes in the epilimnetic bacterial community composition, production and protist-induced mortality along the longitudinal axis of a highly eutrophic reservoir, Microbial Ecology, 42, 359-371.
21. Šimek K., Horňák K., Jezbera J., Mašín M., Nedoma J., Gasol J. M., Shauer M., 2005 – Influence of top-down and bottom-up manipulations on the R-BT065 subcluster of β-Proteobacteria, an abundant group in bacterioplankton of a freshwater reservoir, Applied and Environmental Microbiology, 71, 2381-2390.
22. Corno G., Caravati E., Callieri C., Bertoni R., 2008 – Effects of predation pressure on bacterial abundance, diversity and size – structure distribution in an oligotrophic system, Journal of Limnology, 67, 107-113.
23. Šimek K., Horňák K., Jezbera J., Nedoma J., Znachor P., Hejzlar J. and Seďa J., 2008 – Spatio-temporal patterns of bacterioplankton and community composition related to phytoplankton composition and protistan bacterivory in a dam reservoir, Aquatic Microbial Ecology, 51, 249-262.
24. Glöckner F. O., Zaichikov E., Belkova N., Denissova L., Pernthaler J. and Amann R., 2000 – Comparative 16S rRNA analysis of lake bacterioplankton reveals globally distributed phyllogenetic clusters including an abundant group of Actinobacteria, Applied and Environmental Microbiology, 69, 5053-5065.
25. Horňák K., Mašín M., Jezbera J., Bettarel Y., Nedoma J., Sime-Ngando T. and Šimek K., 2005 – Effects of decreased resource availability, protozoan grazing and viral impact on a structure of bacterioplankton assemblage in a canyon-shaped reservoir, Microbial Ecology, 52, 315-327.

CONSERVATION STATUS OF THE HAWKSBILL TURTLE (*ERETMOCHELYS IMBRICATA*) IN SOME ISLANDS OF THE PERSIAN GULF

*Ruhollah ZARE *, Seyyed Mohammad Bagher NABAVI ***
and *Mahdieh EFTEKHAR VAGHEFI **

* Chabahar Maritime and Marine Science University, Marine Biology Department, Chabahar, Iran ru_zare@yahoo.com
** Department of Environment, Marine Environment Bureau, Tehran, smbnabavi@yahoo.com
*** University of Marine Science and Technology, Marine Biology Department, Khorramshahr, juniper_90@yahoo.com

KEYWORDS: Persian Gulf, *Eretmochelys imbricata*, nesting season, conservation.

ABSTRACT

Globally, marine turtles are experiencing serious threats to their survival and are considered as species of international conservation concern. Due to this status, they are listed in the International Union for the Conservation of Nature's "Red List of Threatened Animals"; and in the Appendix 1 of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Over-hunting for their shells and meat and the collection of eggs from turtle nests are among the factors contributing to the endangered status of turtles. They are also threatened by coastal development, pollution and pathogens, global warming and fisheries. Fisheries impact is thought to be a leading cause of marine turtle death and injury worldwide and can occur when turtles bite baited hooks, become entangled in fishing lines, are crushed by dredges, or otherwise held underwater by a variety of fishing methods.

In the Persian Gulf islands, turtle declines are worsened by the breakdown of traditional conservation practices, the use of powered boats in turtle hunting, commercial sale, large-scale harvesting of eggs in the rookeries and habitat destruction. In these islands, the most common species is the hawksbill turtle (*Eretmochelys imbricata*). The nesting season of this species occurs from March to May. In this study, we have investigated nesting activity of hawksbill turtles on the islands of Shidvar, Lavan, Qeshm, Hormuz, Farour and Hendurabi Islands. In addition, we have monitored all human activities in the nesting beaches and neighbouring areas (such as coastal development, artificial light, pollutants and beach destruction) and recorded the natural predators of eggs, hatchlings and nesting females.

Our results show some serious threats to hawksbills survival, especially in Shidvar Island (due to high egg harvesting and in some cases killing adult females), Hendurabi Island (hunting for their meat) and Qeshm and Kish Islands due to coastal development, habitat destruction and artificial lights. Our study shows that unfortunately, the available conservation programs aren't sufficient to prevent local people from egg harvesting. In concern to the conservation importance of hawksbill turtles and by using this information, we can prevent the increased depletion of these stocks in these areas and protect nesting habitats. In addition, continuous monitoring of nesting beaches, their conditions and nesting activity of sea turtles can help us in designing better conservation programs.

ZUSAMMENFASSUNG: Der Schutzstatus der Echten Karettschildkröte (*Eretmochelys imbricata*) auf einigen Inseln im Persischen Golf**.**

Meeresschildkröten sind weltweit großen, ernstzunehmenden Gefahren ausgesetzt, die ihr Überleben gefährden und stehen daher international im Blickpunkt des Naturschutzes. Bedingt durch diesen Status wurden sie in die "Rote Liste der gefährdeten Tierarten" der IUCN aufgenommen. Sie sind ebenfalls im Anhang I des Übereinkommens über den internationalen Handel mit gefährdeten Arten frei lebender Tiere und Pflanzen (CITES) aufgelistet. Überjagung wegen ihres Gehäuses und ihres Fleisches sowie das Einsammeln von Eiern aus ihrem Gelege sind einige der Faktoren, die zum Gefährdungsstatus der Schildkröten beigetragen haben. Sie sind ebenso gefährdet durch die Entwicklung an den Küsten, durch Verschmutzung und pathogene Stoffe, globale Erwärmung und Auswirkungen der Fischerei. Letztere gehören wohl weltweit zu den Hauptursachen für Tod und Verletzung der Schildkröten und kann sich ereignen, wenn sie gelegte Köder anbeißen, sich in den Fischernetzen verfangen, von Schwimmbaggern gequetscht oder auf andere Art durch verschiedene Fischfangmethoden unter Wasser gehalten werden.

Auf den Inseln im Persischen Golf hat sich die Abnahme der Schildkröten durch den Zusammenbruch der traditionellen Schutzpraktiken, die Nutzung von angetriebenen Booten zur Schildkrötenjagd, gewerblichen Verkauf, großmaßstäbliches Sammeln der Eier aus den Nestern und Lebensraumzerstörung verschlechtert. Auf diesen Inseln ist die Echte Karettschildkröte (*Eretmochelys imbricata*) die meistverbreitete Art. Die Brutzeit der Art dauert von März bis Mai. In vorliegender Studie wurden die Nistaktivitäten der Echten Karettschildkröte auf den Inseln Shidvar, Lavan, Qeshm, Hormuz, Farour und Hendurabi untersucht. Zusätzlich wurden alle menschlichen Tätigkeiten (Küstenentwicklung, künstliches Licht, Verschmutzung und Buchtenzerstörung) in den Nistbuchten und benachbarten Gebieten aufgenommen sowie die natürlichen Räuber von Schildkröteneiern, die geschlüpften Tiere und nistenden Weibchen erfasst.

Unsere Ergebnisse belegen einige ernste Bedrohungen für das Überleben der Echten Karettschildkröte, vor allen auf der Insel Shidvar (durch hohe Eierernte und in einigen Fällen Töten adulter Weibchen) und Insel Hendurabi (wegen Jagd auf Schildkrötenfleisch) sowie den Inseln Qeshm und Kish bedingt durch Küstenentwicklung, Lebensraumzerstörung und künstliches Licht. Unsere Studie zeigt, dass bedauerlicherweise vorhandene Schutzprogramme ungenügend sind um die lokale Bevölkerung von der Eierernte abzuhalten. In Anbetracht der Bedeutung der Echten Karettschildkröte für den Naturschutz und unter Verwendung dieser Informationen, können wir einer weiteren Abnahme und Verschlechterung der Bestände in den genannten Gebieten vorbeugen und die Bruthabitate schützen. Zusätzlich kann uns ein fortlaufendes Monitoring der Brutbuchten, ihrer Bedingungen und der Nisttätigkeit der Meeresschildkröten helfen, bessere Schutzprogramme zu entwickeln.

REZUMAT: Starea de conservare a broaştei ţestoase (*Eretmochelys imbricata*) în câteva insule din Golful Persic.

Broaştele ţestoase marine sunt ameninţate în mod global şi se consideră că trebuie protejate şi conservate la nivel internaţional. Datorită acestui statut ele sunt incluse în „Lista roşie a animnalelor ameninţate" a Uniunii de Conservare Mondială, în Apendixul 1 al Convenţiei asupra comerţului internaţional de specii periclitate din flora şi fauna mondială. Vânarea excesivă pentru carapace şi carne şi colectarea ouălelor din cuiburi sunt câteva elemente care au condus la declararea de specie periclitată a broaştelor ţestoase marine. De asemenea, ele sunt ameninţate de dezvoltarea activităţilor de coastă, poluare şi agenţii

patogeni, încălzirea globală şi impactul produs de pescării. Impactul produs de crescătoriile de peşte se consideră a fi una din cauzele principale ale morţii ţestoaselor marine şi a rănirii lor în întreaga lume şi care se poate produce în momentul în care ţestoasele muşcă din momeala din cârlige, când rămân blocate în plasele de pescuit, când sunt strivite de dragă sau sunt tinute sub apă prin diferite metode de pescuit în care nimeresc.

În insulele din Golful Persic, declinul populaţiilor de ţestoase marine este înrăutăţit de renunţarea la practicile de conservare, folosirea bărcilor cu motor la vânarea ţestoaselor marine, de comercializarea lor, de colectarea ouălelor din cuiburi pe scară largă şi de distrugerea habitatelor. În aceste insule, cea mai comună specie este ţestoasa cioc de şoim (*Eretmochelys imbricata*). Perioada de cuibărit al acestei specii este din martie până în mai. În acest studiu, noi am observant activitatea de cuibărit a ţestoasei cioc de şoim în insulele Shidvar, Lavan, Qeshm, Hormuz, Farour şi Hendurabi. În plus, noi am monitorizat întreaga activitate umană de pe plajele unde acestea cuibăresc şi de pe zonele învecinate (precum dezvoltarea activităţii de coastă, lumina artificială, poluanţii şi distrugerea plajei) şi am înregistrat prădătorii naturali ai ouălelor, cuibăritului şi femelelor cuibăritoare.

Rezultatele noastre demonstrează prezenţa unor ameninţări foarte serioase către supravieţuirea ţestoasei cioc de şoim, în special în insula Shidvar (datorită colectării intense de ouă şi câteodată a uciderii femelelor adulte) în insula Hendurabi (din cauza vânătorii pentru carne) şi în insulele Qeshm şi Kish, din cauza dezvoltării activităţii de coastă, a distrugerii habitatului şi a luminilor artificale. Studiile noastre demonstrează, din nefericire, că programele de conservare în vigoare nu sunt suficiente pentru protecţia împotriva colectarii ouălelor de către localnici. Având în vedere importanţa conservării ţestoaselor cioc de şoim şi folosirea acestor informaţii, noi putem interveni în stoparea epuizării acestor stocuri în aceste zone şi protejarea habitatelor de cuibărit. Mai mult decât atât, monitorizarea continuă a plajelor de cuibărit, condiţiile şi activitatea de cuibărit a ţestoaselor marine ne pot ajuta în redactarea unor programe mult mai bune de conservare.

INTRODUCTION

Globally, populations of sea turtles are declining (Gärdenfors, 2001). All species of sea turtles are listed in Appendix I of the CITES (1986). This means that all turtle species are considered endangered by international trade to such an extent that if commercial trade is not eliminated with respect to these species, they will become extinct. Nesting density of hawksbill turtles is low throughout its range (Meylan, 1998).

Over-hunting of marine turtles for their shells, meat and over-collecting of eggs from nests are some factors attributing to the endangered status of turtles (Carr and Meylan, 1980; Davis, 2005). It is believed that the decline of turtle populations in the Persian Gulf has been accelerated by the breakdown of traditional conservation practises, the use of powered boats in turtle hunting, commercial sale, habitat degradation, incidental by-catch in fishing gear, and the large scale harvesting of eggs in rookeries (Zare et al., 2008). The factors that are known to cause decline in sea turtle populations are generally similar but differences do exist in terms of importance for different populations i.e. in different parts of the world, and with changing laws and technologies through time (Carr et al., 1982; Mast et al., 2006). For example, before the widespread use of trawlers and high seas gill-nets, turtle mortality caused by fishing was minimal but laws were not in force then to protect turtles and their products. Hence, there was widespread hunting of turtles for meat, shell and leather (Mast et al., 2006; Mrosovsky, 1997). Eggs were also collected extensively for food. Seas were not as polluted then, hence mortality

caused by plastics, tar balls, pollutant induced diseases were not as extensive. Similarly, the degree of importance of factors threatening turtles in different parts of the world does differ. A constant cause for decline, independent of time, is when mortality is greater than recruitment. Mortality and recruitment vary, depending on predation, food availability and quality, habitat quality, and many other factors (Groombridge and Luxmoore, 1989; IUCN, 2003). Because the life cycle of a sea turtle is complex, and includes large periods of time and large expanses of the planet, mortality can occur at many places and many times during an individual turtle's life (Bowen and Karl, 1996). Natural threats are indiscriminate and may affect any species (Carr, 1972; Carr et al., 1982; Mrosovsky, 1997). Natural predation on eggs and hatchlings is thought to be kept in check by natural balances of predator prey relationships. Predation is so high that it is obvious that a number of terrestrial, marine and avian species depend on sea turtles as a source of protein. Anthropogenic threats to nesting habitats are again indiscriminate and driven more by coastal development, industrialization and the recreational opportunities provided by coastal environments (Broderick et al., 2006; IUCN, 2003; Mrosovsky, 1983; Zare, 2008).

In addition to fulfilling statutory requirements, the purposes of our study were: 1) to relocate eggs from nests deposited in sites threatened by natural processes or human activities and thus maximize hatchling recruitment; 2) to accurately survey sea turtle nesting patterns to document historical trends and assess natural and anthropogenic factors affecting nesting patterns and densities; 3) to assess the success of sea turtle recruitment and of hatchery operations in terms of nesting success, hatching success and total hatchlings released, and 4) to inform and educate the public about sea turtles and their conservation.

MATERIAL AND METHODS

Study area

Qeshm is the largest island in the Persian Gulf near the straits of Hormoz. Shibderaz Village is in the centrally-located southern coastline of Qeshm Island which is currently the only stretch used in the entire island by Hawksbill Turtles for nesting and laying eggs. The coastline, therefore, represents an important and strategic Hawksbill hotspot.

Shidvar Island has an area of eight km^2, a coastline of 5.5 km of which two km of the northern and eastern shores is suitable for the nesting of turtles.

Hendourabi Island has a coastline of 20 km of which some two km are suitable for marine turtle nesting. Most parts are rocky shores, but there are some small and large sandy beaches suitable for nesting in the east, north east and south.

Lavan Island is an Iranian island in the Persian Gulf. It has an area of 76 km^2. The island has one of the four major terminals for export of crude oil in Iran and some suitable nesting areas are being destroyed.

Hormuz Island is an Iranian island in the Persian Gulf. It is located in the Straits of Hormuz and is part of the Hormozgān Province. It has an area of 42 km^2 (Fig. 1).

Daily beach surveys commenced at sunrise or 6:00 AM. Each nest location was initially recorded relative to the nearest building, street, or other landmark. These locations were later cross-referenced to the nearest survey marker. Nest and false crawl locations were recorded using Global Positioning System (GPS) receivers. The field survey also attempted to assess the suitability of the beaches for turtle nesting. The assessment only looked at the surface level of the beach area at high water mark (through which the turtle would crawl) and the nesting area (above high water mark). When there were many nests requiring relocation, additional trips were occasionally necessary. After recording all pertinent information, the crawl marks were obliterated to avoid duplication.

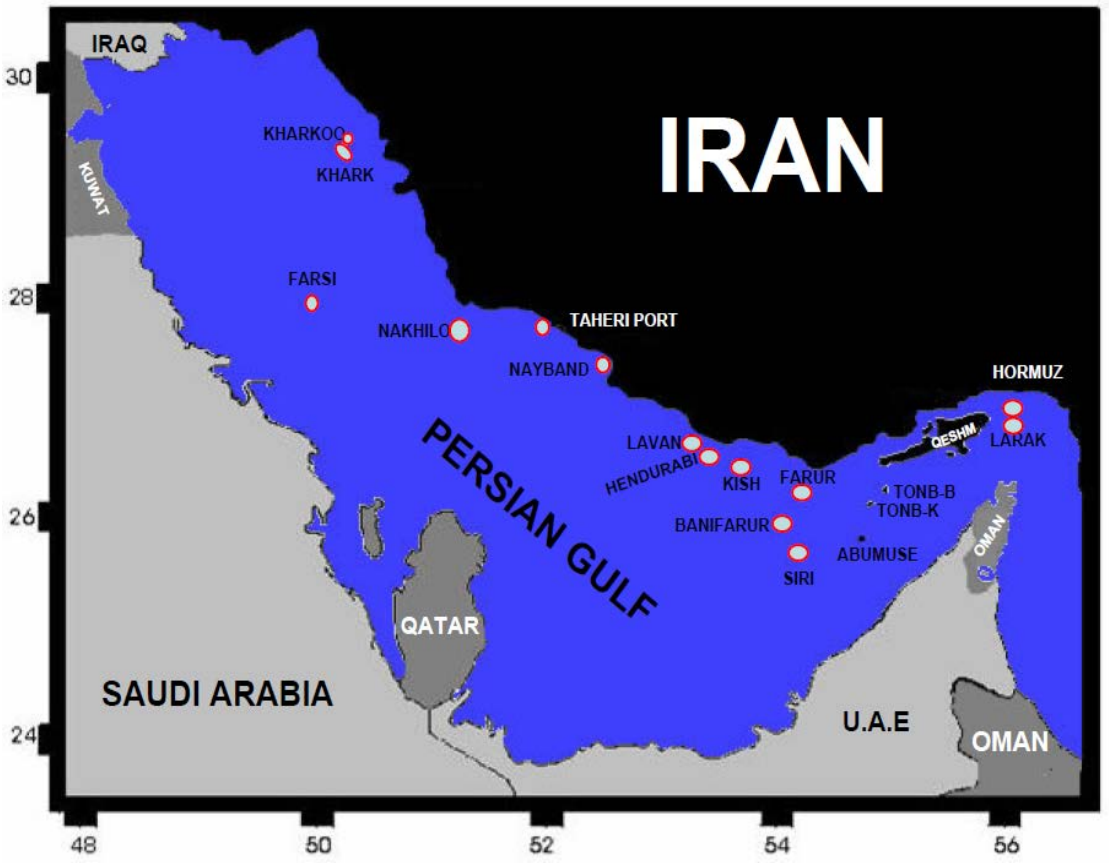


Figure 1: Persian Gulf and the studied islands.

RESULTS

Number of nests

The number of nests represents the actual tally of records recorded during the surveys, some of which may not represent a good estimate for the month due to limited surveys conducted on certain beaches during certain months (Tab. 1).

Table 1: Total number of hawksbill nests in the studied area.

Island	Total number of nests	Beach length (km)
Shidvar	36	2.1
Lavan	24	3.2
Qeshm	56	6.7
Hormuz	34	3.4
Farour	35	4.6
Hendurabi	46	5.7
Overall	231	25.7

Threatening factors

During the study at different islands, we observed various factors threatening sea turtles and their nesting habitats (Tab. 2; Figs. 2-7).

Figure 2: Residential use of nesting habitat in Shidvar Island.

Table 2: Current anthropogenic threats to sea turtle populations in some studied islands.

Threat	Shidvar	Lavan	Qeshm	Hormuz	Farour	Hendurabi
Habitat alteration and loss	+	+	+	–	–	–
Beach armouring (concrete walls)	–	+	+	–	–	–
Beach nourishment/sand mining	–	+	+	–	–	–
Beach cleaning and beach driving	–	–	+	–	–	–
Human presence on beach	+	+	+	+	–	+
Artificial light	–	–	+	–	–	–
Boat strikes	+	+	+	+	–	+
Animal predation at rookeries	+	–	–	+	+	–
Oil pollution	?	+	+	?	–	–
Other pollution sources	+	+	+	+	–	+
Fishing and incidental capture	–	+	+	+	–	–
Shrimp trawling	–	–	+	–	–	–
Pelagic fishing gear	+	+	+	+	–	–
Gill nets	–	–	+	–	–	–
Traditional and commercial fishing	+	+	+	–	–	–
Eggs harvests	+	+	–	–	–	–
Adult harvests	+	+	–	–	–	+

Figure 3: Striking a green turtle with a fishing boat in Hormuz Island area.

Figure 4: Killing of a hawksbill turtle in Hendurabi Island for meat usage.

Figure 5: Erosion of nesting beach due to wave action in Shidvar Island.

Figure 6: Physical obstacles in nesting habitat in Shidvar Island.

Figure 7: Fishermen and their boats in nesting habitats in Shidvar Island.

DISCUSSION

In the past there was a great deal of hawksbill turtle nesting at the Persian Gulf but egg collection and entanglement of adult females was rife. All measures that prevent sea turtles from being killed would be of priority. These are:

– conservation measures or techniques that reduce the incidental catch of adult and juvenile turtles in fishing gears e.g.: (i) use of TEDs in trawlers (shrimp and fishing); (ii) regulate or ban the use of high seas gill-nets; (iii) regulations to protect turtles or restrict the use of fishing methods harmful to turtles off their nesting grounds during the nesting season;

– conservation measures to curb the hunting and trade of live turtles, adults and juveniles, for meat and other turtle products;

– conservation measures to curb commercial exploitation of eggs, both legal and illegal;

– conservation measures to curb the destruction of nesting grounds by beachfront development, seawalls, land reclamation, etc.;

– conservation measures to curb the destruction of feeding grounds by trawlers, pollution, land reclamation, etc.;

– conservation measures to prevent the killing or drowning of turtles in man-made structures (e.g. oil rigs) or by powered watercrafts.

– Conservation measures to curb marine pollution to reduce the mortality of hatchlings, juveniles and adults caused by marine debris like plastic bags, tar balls, styrofoam, etc.

– Conservation measures to prevent the inducement and spread of diseases that may be anthropogenically related, e.g. fibriopapillomas.

Main Threatening Factors of sea Turtles in Persian Gulf (Zare et al., 2008):

- in most parts of the nesting sites, egg collection for traditional usage as well as aphrodisiac purposes;
- in some parts, turtles harvested for their meat for the same purposes;
- different kinds of pollution are common, like debris, oil and light;
- by-catch in fishery activities is another major threat, as the turtle habitats are important fishing grounds too;
- natural predators such as those found in other parts of the world are common, like mammals, birds, crabs and insects;
- density of the body pits in the small beaches prevent hatchlings from reaching the sea,
- mismanagement causes problems in some parts;
- being hit by boats is common and increasing in some parts;
- different kinds of coastal area development are another threat to the nesting sites and turtles;
- other natural factors like erosion is common in most places, especially in small beaches.

Some of the conservation programs:

- listing of sea turtles as “Endangered animals” in the country;
- there was a fine of about 3,200,000 Rials for each turtle killed which doubled in the past year – 6,400,000 Rials, or about $US 700;
- there is also a fine for egg collection, about $US 233 for each egg;
- designation of the nesting sites as “Under management and control Area” like Mond protected area and sheedvar wildlife refuge;
- monitoring of the sites by DOE guards.

In order to respond to a critical conservation situation, as is the case of these sea turtle populations, an agreement must meet some requirements: (1) it must include all, or most of the countries involved in the problem; (2) it must be an “agile” organization, capable of facing a dynamic situation without getting bogged down with time consuming formalities; (3) it must turn words into actions very rapidly; and perhaps also, (4) it must have the capability to implement and execute a comprehensive program, and (5) if possible, it is preferable that the agreement is a binding one. It is quite clear that the institution must have a level of credibility with the different stakeholders.

In summary, these data undoubtedly confirm that there is still significant hawksbill turtle nesting in Persian Gulf islands. It is hoped that further work at these sites will yield status information and allow prioritisation of conservation efforts.

REFERENCES

1. Baillie J., Groombridge B., 1996 – IUCN Red List of Threatened Animals. IUCN, Gland.
2. Bowen, BW., Karl, S.A., 1996 – Population genetics, phylogeography, and molecular evolution. In: Lutz PL, Musick, JA (eds) The biology of sea turtles. CRC Press, Boca Raton, FL, p 29-50.
3. Broderick A. C., Frauenstein R., Glen F., Hays G. C., 2006 – Are green turtles globally endangered? Glob EcolBiogeogr, 15, 21-26.
4. Carr A. F. 1972 – Great reptiles, great enigmas. Audubon 74, (2), 24-34.
5. Carr A. F., Hirth H. and Ogrean L., 1966 – The ecology and migrations of sea turtles, 6. The hawksbill turtle in the Caribbean Sea. American Museum Novitates, 2248, 1-29.
6. Carr A. F., Meylan A. 1980 – Extinction or rescue for the hawksbill? Oryx 15, (5), 449-450.
7. Carr A. F., Meylan A. B., Mortimer J. A., Bjorndal K. A., Carr T. 1982 – Preliminary survey of marine turtle populations and habitats in the western Atlantic. NOAA Tech. Memo. NMFSSEFC.
8. Davis F. R. 2005 – Saving sea turtles: the evolution of the IUCN Marine Turtle Group. Endeavour 29, 114-118.
9. Gärdenfors U., 2001 – Classifying threatened species at national versus global levels. Trends Ecol Evol 16, 511-516.
10. Groombridge B. 1994 – 1994 IUCN Red List of Threatened Animals. IUCN, Gland
11. Groombridge B, Luxmoore R. A., 1989 – The green turtle and hawksbill (Reptilia: Cheloniidae): world status, exploitation and trade. Secretariat of the Convention on International Trade in Endangered Species of Wild Fauna and Flora, Lausanne
12. Groombridge B., 1982 – The IUCN Amphibia-Reptilia red data book, part 1: Testudines, Crocodylia, Rhynocehapalia, IUCN, Gland.
13. Hutchinson B. J., Mast R. B., Pilcher N. J., 2007 – IUCN-SSC Marine Turtle Specialist Group quarterly update. Mar Turtle Newsl 117, 13-14.
14. IUCN, 2003 – Guidelines for application of IUCN Red List criteria at regional levels: Version 3.0. IUCN Species Survival Commission. IUCN, Gland and Cambridge.
15. IUCN-CMC. (IUCN Conservation Monitoring Centre), 1986 – 1986 IUCN Red List of Threatened Animals. IUCN, Gland and Cambridge.
16. Mast R. B., Seminoff J. A., Hutchinson B. J., Pilcher N. J., 2006 – The role of the IUCN Marine Turtle Specialist Group in setting priorities for sea turtle conservation. Mar Turtle Newsl 113, 16-18.
17. Meylan A., 1998 – Hawksbill turtles still endangered. Nature 391, 117.
18. Meylan A. B, Donnelly M., 1999 – Status justification for listing the hawksbill turtle (Eretmochelys imbricata) as Critically Endangered on the 1996 IUCN Red List of Threatened Animals. Chelonian Conserv Biol 3, 200-224.
19. Mrosovsky N. 1983 – Conserving sea turtles. British Herpetological Society, London.
20. Mrosovsky N. 1997 – IUCN's credibility critically endangered.Nature 389, 436.
21. Schuster C., Robinson A. C., Mulipola A., Butler D. J. and Time S., 1994 – Status of the Sea turtle s in Western Samoa.
22. Witzell W. N. and Banner A. C. 1980 – The Hawksbill turtle in Western Samoa. Bulletin of Marine Science. 571-574.
23. Zare R., Eftekhar M. 2009 – Nesting behaviour and some biological characteristics of hawksbill turtles in Shidvar Island, Persian Gulf (Iran). International Conference on the Marine Ecosystems 2009 (INCOMES 2009), 26th to 28th May 2009, Awana Porto Malai, Langkawi, Malaysia.
24. Zare R., Nabavi S. M. B., Eftekhar M. 2008 – Nest site location of hawksbill sea turtle (Eretmochelys imbricata) on Shidvar Island, Hormozgan Provinc, International Congress of Documenting, Analysing and Managing Biodiversity in the Middle East, Amman, Jordan.

25. Zare R., Nabavi S. M. B., Eftekhar M. 2008 – Breeding biology of hawksbill turtle, Skretting Australasian Aquaculture 2008 International Conference and Trade Show.
26. Zare R., Nabavi S. M. B., Eftekhar M. 2008 – Identification of spawning habitats of Hawksbill turtle (Eretmochelys imbricata) In some of Iranian Islands of Persian Gulf, International Conference on Biodiversity Conservation and Management, Cochin University of Science and Technology, Cochin, Kerala, India.
27. Zare R., Nabavi S. M. B., Eftekhar M. 2008 – Nesting activity of Hawksbill turtle (Eretmochelys imbricata) in Shidvar Island (Iran), International Conference on Biodiversity Conservation and Management, Cochin University of Science and Technology, Cochin, Kerala, India.
28. Zare R., Nabavi S. M. B., Eftekhar M. 2008 – Placement and predation of nests in hawksbill sea turtles (Eretmochelys imbricata) in Shidvar Island, IRAN, S kretting Australasian Aquaculture 2008 International Conference and Trade Show.
29. Zare R., Nabavi S. M. B., Eftekhar M. 2008 – Status of nesting beaches of Hawksbill turtle (imbricata in Shidvar Island (Iran) World Aquaculture, Busan, Korea.

PHOSPHATE-SOLUBILIZING BACTERIA *MICROBACTERIUM PARAOXYDANS* ISOLATED FROM THE RHIZOSPHERIC SYSTEM OF WHEAT (*TRITICUM AESTIVUM*)

*Gurdeep KAUR *, Jasminder KAUR **, K. S. DADHICH ** and *Swaranjit Singh CAMEOTRA ****

* Dolphin (PG) College of Life Sciences, Department of Microbiology, V. P. O. Chunni Kalan. 25, kms. Chandigarh-Fatehgarh Sahib Road, Punjab, India.
** Home Science, Chandigarh Administration, Chandigarh, India.
*** MTCC and Gene Bank, Ida Imtech, Sector 39A, Chandigarh, India, IN-160036, ssc@imtech.res.in

KEYWORDS: Phosphate-solubilizing bacteria, *Microbacterium paraoxydans*, phosphate solubilization, Tri-calcium phosphate, rhizosphere.

ABSTRACT

Most agricultural soils contain large reserves of phosphorus (P), a considerable portion of which accumulates as a consequence of regular applications of "P" fertilizers and, sooner or later, influences adjacent aquatic and terrestrial ecosystems. However, the greater part of the phosphorus in soil, *c*. 95-99%, is present as insoluble phosphates and hence cannot be utilized directly by plants. In the present study, bacterial strains isolated from rhizospheric soil were characterized that have the potential to solubilise insoluble inorganic phosphate. A total of 224 isolates was obtained by the screening of four soil samples from the rhizosphere of berseem clover *(Trifolium alexandrium)* (85), wheat *(Triticum aestivum)* (48), rape *(Brassica rapa* subsp. *rapa* (39) and oat *(Avena sativa)* (52). Of 224 isolates, 44 were found to be phosphate solubilizers and their percentage in the rhizosphere of berseem, wheat, sarson and oat was 21.2%, 20.8%, 17.9% and 17.3% respectively. Of these 44 phosphate-solubilizing bacteria (PSB), only four isolates showed maximum zone of solubilization on a Pikovskayas agar plate. From these four isolates, one (W1-PSB2) from the rhizosphere of wheat showed maximum zone of solubilization (27.0 mm), solubilization efficiency (207.14%) and phosphate solubilization activity (3.0). The isolate W1-PSB2 was found to have released 472.0 µg/ml soluble "P" from 0.5% Tri-calcium phosphate in 100 ml of PKV broth, with decrease in pH of 3.98 from initial pH 7.0, after seven days of incubation period. Phosphatase enzyme production was 33.0 µmol p.N.P.P/ml/hour. Siderophore production activity was found to be 1.5. The efficient PSB isolate W1-PSB2 on the basis of 16s-rRNA sequences was identified as *Microbacterium paraoxydans* (Gene Bank accession no. FJ871123). This is the first report of *Microbacterium paraoxydans* capable of phosphate solubilization.

RÉSUMÉ: La bactérie solubilisatrice de phosphate *Microbacterium paraoxydans* isolée du système rhisosphèrique du blé (*Triticum aestivum)***.***

La plus part des sols agricoles contiennent des grandes stocks de phosphore (P) dont une partie considérable s'accumule suite à l'application des engrais phosphoriques et influencent plus tôt ou plus tard les écosystèmes terrestres et aquatiques environnants. Malgré cela, une part plus importante du phosphore du sol, environ 95-99% est présent sous la forme insoluble des phosphates, qui sont inutilisables par les plantes. Dans l'étude ci-dessous nous

décrivons des souches bactériennes, isolées à partir des sols rhisosphèriques, ayant le potentiel de solubiliser le phosphate inorganique insoluble. Nous avons obtenu 224 isolats par le screening de quatre échantillons de sols des rhizosphères de trèfle d'Alexandrie *(Trifolium alexandrium)* (85), blé *(Triticum aestivum)* (48), rave *(Brassica rapa* subsp. *rapa)* (39) et avoine cultivée *(Avena sativa)* (52). A partir des 224 isolés, 44 ont été prouvés des solubilisateurs de phosphates et leur pourcentage dans les rhizosphères mentionnées a été de 21.2%, 20.8%, 17.9% and 17.3% respectivement. De ces 44 bactéries solubilisatrices des phosphates (PSB) seulement quatre ont montré une zone maximale de solubilisation sur la gélose pour dénombrement Pikovskaya. Un de ces quatre isolats, W1-PSB2, appartenant à la rhizosphère du blé, a montré le maximum de la zone de solubilisation (27,0 mm), de l'efficacité de solubilisation (207,14%) et de l'activité de solubilisation du phosphate (3,0). L'isolat W1-PSB2 a libéré 472.0 µg/ml "P" soluble à partir de 0,5% tri calcium phosphate dans 100 ml milieu PKV avec une baisse du pH de 7,0 à 3,98 après sept jours d'incubation. La production de l'enzyme phosphatase a été de 33.0 µmol p.N.P.P/ml/heure. L'activité de production du sidérophore a été de 1,5. L'isolat PSB efficace W1-PSB2 a été identifié suite au séquençage 16s-ARNr comme *Microbacterium paraoxydans* (no. accès Gen Bank FJ871123). Ceci est la première mention de *Microbacterium paraoxydans* capable de solubiliser le phosphate.

REZUMAT: *Microbacterium paraoxydans*, bacteria care solubilizează fosfatul, izolată din sistemul rizosferic al grâului *(Triticum aestivum)*.

Majoritatea solurilor agricole conţin rezerve mari de fosfor (P), cea mai mare parte provenind din amendarea regulată a solului cu îngrăşăminte fosfatice care, mai devreme sau mai târziu, influenţează ecosistemele acvatice sau terestre învecinate. Oricum, o mare parte a fosforului din sol, aproximativ 95-99%, este prezent sub formă de fosfaţi insolubili şi de aceea nu este accesibil plantelor. În acest studiu, sunt descrise tulpinile bacteriene izolate din solurile rizosferice care au capacitatea de a solubiliza fosfatul anorganic insolubil. Un total de 224 izolări au fost obţinute prin screening-ul a patru mostre de sol din rizosfere de trifoi egiptean *(Trifolium alexandrium)* (85), grâu *(Triticum aestivum)* (48), rapiţă *(Brassica rapa* subsp. *rapa)*, (39) şi ovăz *(Avena sativa)* (52). Din cele 224 izolate, 44 s-au dovedit a fi capabile să solubilizeze fosfatul iar procentul lor în rizosferele de trifoi, grâu, rapiţă şi ovăz a fost de 21,2%, 20,8%, 17,9% şi 17,3%. Din cele 224 izolate, 44 s-au dovedit a fi capabile să solubilizeze fosfatul iar procentul lor în rizosferele de trifoi, grâu, muştar şi ovăz a fost de 21,2%, 20,8%, 17,9% şi 17,3%. Din cele 44 bacterii capabile să solubilizeze fosfatul (PSB) doar patru izolate au indicat zona maximă de solubilizare pe agar Pikovskaya. Din aceste patru izolate, un izolat denumit W1-PSB2, din rizosfera de grâu a atins valorile maxime pentru zona de solubilizare (27,0 mm), eficienţa solubilizării (207,14%) şi activitatea de solubilizare a fosfatului (3,0). S-a descoperit că izolatul W1-PSB2 a eliberat 472.0 µg/ml de "P" solubil din soluţia de 0,5% trifosfat de calciu la 100 ml de amestec PKV cu descreşterea pH-lui la 3,98 de la pH-ul iniţial de 7,0 după o perioadă de şapte zile de incubare. Producţia de fosfatază a fost de 33,0 µmol p.N.P.P/ml/oră. Activitatea de producere a sideroforilor a fost de 1,5. Izolatul PSB eficient denumit W1-PSB2 a fost identificat, pe baza secvenţei 16s-ARNr a fi *Microbacterium paraoxydans* (Banca de gene – nr. acces FJ871123). Acesta este prima semnalare de *Microbacterium paraoxydans* capabil să solubilizeze fosfatul.

INTRODUCTION

The availability of phosphorous in the soil is somehow limited, notwithstanding its ample distribution in nature, showing the need for the application of soluble fertilizers for adequate plant growth (Vassilev, 2001). To increase the availability of phosphorus for plants, large amounts of fertilizer are being applied to the soil. A large proportion of phosphorus fertilizers, after application are quickly transformed into the insoluble form (Omar, 1998). Therefore, a very small percentage of the applied phosphorus is available to plants, making continuous application necessary (Abd Alla, 1994). Phosphorus is often the first nutrient in which the agricultural soils become deficient. Moreover, unlike carbon and nitrogen, which can be fixed from the atmosphere, phosphorus has no gaseous form. Phosphorus deficiencies are widespread in the soils throughout the world and phosphorus fertilizers represent a major cost for agricultural production. Numerous microorganisms, especially those associated with roots, have the ability to increase plant growth and productivity (Ulrich et al., 2009). Many soil fungi and bacteria are known to solubilize inorganic phosphates. Soil microorganisms have enormous potential in providing soil phosphates for plant growth. Several mechanisms like lowering of pH by acid production, ion chelation and exchange reactions in the growth environment have been reported to play a role in the phosphate solubilization by PSMs (Abd-Alla, 1994; Whitelaw, 2000). Phosphorus biofertilizers, in the form of microorganisms can help in increasing the availability of accumulated phosphates for plant growth by solubilization (Gyaneshwar et al., 2002). Application of phosphates, along with phosphate solubilizing bacteria (PSB) improved P uptake by plants and yields indicating that the PSBs are able to solubilize phosphates and mobilize phosphorus in crop plants (Rogers, 1993). In this respect, bio-fertilization technology has played a part in minimizing production costs and at the same time, avoided the environmental hazards (Galal et al., 2001). Application of PSMs in the field has been reported to increase crop yield. In the present study, bacterial strains having the potential to solubilize insoluble phosphate were isolated and the isolate showing maximum solubilization was gnomically characterized.

MATERIAL AND METHODS

Collection of soil samples

The soil samples were collected from a depth of six-15 cm from different agricultural lands of the Balachaur area of S. B. S. Nagar district in Punjab. Samples were collected from the rhizosphere of berseem, wheat, sarson and oat plants, aseptically in small plastic bags/bottles, with the help of sterile spatula and prior to their processing, kept at 4ºC.

Processing of samples

10 g of soil sample was suspended in 90 ml of sterilized distilled water, mixed thoroughly and 10 fold serial dilutions were made with sterilized distilled water. From these dilutions, 100 µl was spread plated on Pikovskaya's (PVK) agar plates and then incubated at 30ºC. The colonies were observed for two-seven days. Phosphate solubilizing bacteria showing a zone of clearance were streaked again on PVK agar plates to check their purity and phosphate solubilization ability. The pure strains forming zone of clearance were maintained by streaking on nutrient agar slants and stored at 4ºC.

Solubilization index

A pin point inoculation of bacterial isolates was made on PVK agar plates and incubated at 28ºC for seven days with continuous observation for colony diameter. Solubilization index was evaluated by using the formula:

$$SI = \frac{\text{Colony diameter + halo zone diameter}}{\text{Colony diameter}}$$

Quantitative estimation of phosphate solubilization in liquid medium

Quantitative estimation of phosphate solubilization activity was carried out in 50 ml PVK broth, amended with 0.5% tri calcium phosphate. Each flask received 200 µl of the two days old culture of approximate 1.0 O.D at 600 nm. Inoculated flasks and un-inoculated controls were incubated at 28ºC on a rotary shaker at 120 rpm. On each sampling day, a sub sample of the culture supernatant was aseptically withdrawn from each flask. Three ml was used for pH measurement and the remaining two ml was centrifuged and used for the colorimetric estimation of released phosphorous by paramolybdate blue method (Jackson, 1973). It is expressed in terms of $\mu g ml^{-1}$ phosphorous released in culture medium.

Assay for Phosphatase enzyme production (De Souza et al., 1999)

The Phosphatase activity was measured by the release of nitro phenol from p. nitro phenyl phosphate (p.NPP) by the culture isolates. The absorbance was read at 418 nm and the activity was expressed as $\mu mol.p.NPP.ml^{-1}\ h^{-1}$.

Siderophore producing activity

Siderophore production was detected by using Chrome-Azurol-S (CAS) Agar Medium (Schwyn and Neilands, 1986). Formation of a Yellow Orange halo around the colony was considered positive for siderophore production.

Morphological and biochemical characterization of the most efficient isolate (W1-PSB2)

For identification by biochemical means, Bergy's Manual of Systemic Bacteriology, Vol. 1, and Manual for the Identification of Medical Bacteria by Cowan and Steel (Second edition, Cambridge University press) were followed. Colony morphology, Cell morphology, Motility, Endospore formation, Indole production, MR, VP, Citrate utilization, Catalase test, Oxidase test, Urease test, Acid production from different sugars were also studied.

Identification of the most efficient isolate (W1-PSB2)

Isolate W1-PSB2 was genomically characterized by using 16s rRNA sequencing. Genomic DNA was isolated by using the CTAB method; PCR amplification was done using universal primers (27F and 1492R). The PCR product was analysed on 1% agarose gel. Sequences were determined following the dideoxy chain-terminator cycle method using ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit and analysed by ABI 310 genetic analyzer (Applied Biosystems, USA). Homology search was performed using blast at NCBI (http://blast.ncbi.nlm.nih.gov).

RESULTS

Population density of phosphate solubilizing bacteria in the rhizospheric soil of selected plants

Soil samples from the S.B.S. Nagar district were used for enumeration of total bacteria and phosphate solubilizing bacteria on a Pikovskayas (PVK) agar medium. The population of total bacteria on PVK agar medium, ranged from 39-85×10^3 cfu/g of the rhizospheric soils of different crop plants, whereas the population of bacteria showing zone of solubilization ranged from 7-18×10^3 cfu/g (Tab. 1). In the present study, a total of 224 isolates were obtained by screening of four soil samples from the rhizosphere of berseem (a leguminous crop) (85), wheat (cereal crop) (48), sarson (an oil seed crop) (39) and oat (a forage crop) (52). Out of these, 44 isolates were found to be phosphate solubilizers. In the rhizosphere of berseem, the population size of phosphate solubilizing bacteria was the highest (18×10^3 cfu/gm of soil). The percentage of phosphate solubilizers in the rhizosphere of these four selected agricultural crops was in the range from 17.3% to 21.2% (Tab. 1).

Qualitative screening

Out of 44 phosphate solubilizing bacterial isolates, four isolates B1-PSB1, B1-PSB2, W1-PSB1 and W1-PSB2 produced more than five mm zone of solubilization, isolate W1-PSB2 from rhizosphere of Wheat crop showed maximum zone of solubilization (27.0 mm) among selected isolates (Tab. 2; Fig. 1). Phosphate solubilizing bacterial isolate W1-PSB2 showed maximum solubilizing efficiency (207.14%) followed by other efficient isolates B1-PSB2 (157.14%), W1-PSB1 (87.55%) and B1-PSB1 (66.6%) respectively. Similarly, the highest phosphate solubilizing activity was shown by isolate W1-PSB2 (3.0) followed by B1-PSB2 (2.57), W1-PSB1 (1.8) and B1-PSB1 (1.66) respectively (Tab. 3).

Phosphate solubilization in the broth medium

The "P" content released into the medium from 0.5% tri calcium phosphate was 472.0 µg. P. ml^{-1} by W1-PSB2 isolate. It was followed by B1-PSB2 isolate, which liberated 260.0 µg. P. ml^{-1} into the medium (Tab. 4) and the remaining bacterial isolates liberated "P" content in the range of 224.0 to 132.0 µg. P. ml^{-1}.

Phosphatase production by the selected isolates

The maximum phosphatase production activity was found in isolate B1-PSB2 (34.79 µmol. p.NPP.ml^{-1}.h^{-1}). It was followed by W1-PSB2 isolate producing (33.0 µmol. p.NPP.ml^{-1}.hrs^{-1}) into the medium and the remaining bacterial isolates secreted phosphatase enzyme in the range of 30.0 –32.54 µmol. p.NPP.ml^{-1}.h^{-1} (Tab. 5).

Siderophore production ability

The values obtained by z/c ratio indicated that W1-PSB2 isolate had the maximum siderophore production ability (1.5), followed by B1-PSB1 (1.44), B1-PSB2 (1.41) and W1-PSB1 (1.31) (Tab. 6.).

Table 1: Percentage of phosphate solubilizing bacteria in cultivated soil from the rhizosphere of selected plants.

Crop (December – March)	Total bacterial population (cfu×10^3/g soil)	Population of "P" solubilizers (cfu×10^3/g soil)	Percentage of "P" solubilizers
Berseem *(Trifolium alexandrium)*	85	18	21.2%
Wheat *(Triticum aestivum)*	48	10	20.8%
Rape *(Brassica rapa rapa)*	39	7	17.9%
Oat *(Avena sativa)*	52	9	17.3%

Table 2: Selection of efficient phosphate solubilizing bacterial isolates, obtained from the rhizosphere of selected plants. PSB represents Phosphate Solubilizing Bacteria, isolated from the soil sample of Berseem (B) and Wheat (W); Z = Diameter of the zone + Diameter of the colony. C = Diameter of colony.

Crop (Dec.-March)	Total no. of "P" Solubilizing Bacteria	No. of less efficient bacteria (> 5 mm zone of solubilization)	No. of more efficient bacteria (< 5 mm zone of solubiliza-tion)	Symbolic designation of "P" Solubilizing bacteria	Zone of solubilisation in (mm) at 30°C		
					Z-dia (mm)	C-dia (mm)	Z-C dia (mm)
Berseem	18	16	2	B1-PSB1	25	15	10
				B1-PSB2	18	7	11
Wheat	10	8	2	W1-PSB1 pppPPSPSB1P	15	8	7
				W1-PSB2	40	13	27
Sarson	7	7	0	–	> 5 mm	–	–
Oat	9	9	0	–	> 5 mm	–	–

In designation, PSB represents Phosphate Solubilizing Bacteria, isolated from the soil sample of berseem (B) and wheat (W)
Z = (diameter of zone + diam. of colony).
C = diameter of colony.

Table 3: Phosphate Solubilizing Efficiency and Phosphate Solubilizing Activity in the efficient phosphate solubilizing bacterial isolates. Solubilizing efficiency (% S.E.) = Z – C × 100/C; where, Z = Diameter of zone + Diameter of colony; C = Diameter of the colony; Z/C = Solubilizing Activity.

Bacterial isolates	Dia. of zone (Z) mm	Dia. of colony (C) mm	Solubilization efficiency (% S.E)	Solubilization activity (Z/C)
B1-PSB1	25	15	66.6%	1.66
B1-PSB2	18	7	157.14%	2.57
W1-PSB1	15	8	87.5%	1.8
W1-PSB2	40	13	207.14%	3.0

Solubilizing efficiency (% S.E.) = Z – C × 100/C; where, Z = (diameter of zone + diameter of the colony); C = diameter of the colony; Z/C = Solubilizing Activity.

Table 4: Phosphate Solubilizing Ability of selected isolates in liquid medium.

Bacterial isolates	Initial pH	Final pH	$T^{O}C$	"P"-released in the medium (µg. P. ml^{-1})
B1-PSB1	7.2	4.55	30	224.00
B1-PSB2	7.2	4.39	30	260.00
W1-PSB1	7.2	5.95	30	132.00
W1-PSB2	7.2	3.98	30	472.00

Table 5: Phosphatase production by the selected isolates.

S. no.	**Bacterial isolates**	**Phosphatase production (µ mol. p. NPP.$ml^{-1}h^{-1}$)**
1	B1-PSB1	32.54
2	B1-PSB2	34.79
3	W1-PSB1	30.00
4	W1-PSB2	33.00

Table 6: Siderophore Producing Ability of selected efficient phosphate solubilizing bacterial isolates.

Bacterial isolates	Diam. of zone (Z) in mm	Diam. of colony (C) in mm	Siderophore Production Activity (Z/C)
B1-PSB1	13	9	1.4
B1-PSB2	17	12	1.41
W1-PSB1	11	8	1.37
W1-PSB2	21	14	1.5

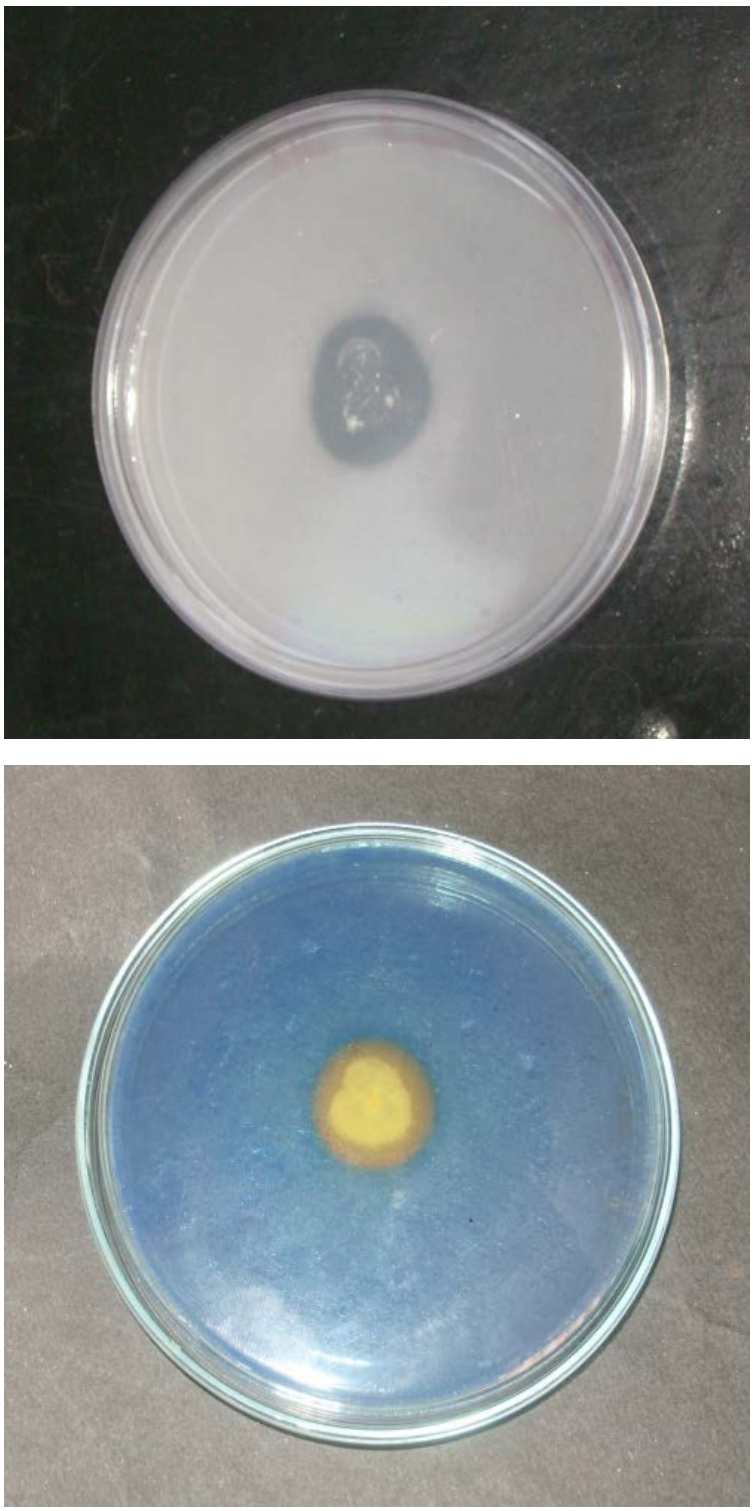


Figure 1a and 1b: Phosphate solubilization (clear zone) and siderophore production (formation of yellow coloured zone on CAS agar medium) by the isolate W1-PSB2.

GCCGTGCGGCGTTCTTACACATGCAAGTCGAACGGTGAACACGGAGCTTGCTCTG
TGGGATCAGTGGCGAACGGGTGAGTAACACGTGAGCAACCTGCCCCTGACTCTGG
GATAAGCGCTGGAAACGGCGTCTAATACTGGATATGTGACGTGACCGCATGGTCT
GCGTTTGGAAAGATTTTTCGGTTGGGGATGGGCTCGCGGCCTATCAGCTTGTTGGT
GAGGTAATGGCTCACCAAGGCGTCGACGGGTAGCCGGCCTGAGAGGGTGACCGG
CCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAA
TATTGCACAATGGGCGAAAGCCTGATGCAGCAACGCCGCGTGAGGGATGACGGCC
TTCGGGTTGTAAACCTCTTTTAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAG
AAAAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAG
CGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTCGCGTCTGCTG
TGAAATCCCGAGGCTCAACCTCGGGCCTGCAGTGGGTACGGGCAGACTAGAGTGC
GGTAGGGGAGATTGGAATTCCTGGTGTAGCGGTGGAATGCGCAGATATCAGGAGG
AACACCGATGGCGAAGGCAGATCTCTGGGCCGTAACTGACGCTGAGGAGCGAAA
GGGTGGGGAGCAAACAGGCTTAGATACCCTGGTAGTCCACCCCGTAAACGTTGGG
AACTAGTTGTGGGGTCCATTCCACGGATTCCGTGACGCAGCTAACGCATTAAGTTC
CCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACCTCAAAGGAATTGACGGGACCC
GCACAAGCCGGCGGAGCATGCGGATTAATTCCGATGCAACCGCGAAGAACCCTAC
CTAGCCTTGACATTAATACGAGGACCGGGCCAGAATGTCCACCTCTTTGGACACCC
TCGTAACCAGTGGTGCCATTGTTGTCGGTCCAGCCCTCGGTTCCGGGAATGTTGGT
TAGTCACGCACCGAGCGCCAACCCTCGCCATGTGCAGGCGTATGTGGACCCATGG
G

Figure: 2a 16s-rRNA sequence
of *Microbacterium paraoxydans* W1-PSB2.

Characterization of the most efficient isolate W1-PSB2

The strain W1-PSB2 is gram positive, short rods. It gave positive results for nitrate reduction, casein hydrolysis and MR tests and negative results for indole production, citrate utilization, Voges-Proskauer, urea hydrolysis, oxidase, starch, gelation, H_2S production and oxidation/fermentation. Carbohydrates utilized by this bacterial isolate were glucose, sucrose, lactose and mannitol. Physiological studies were shown that culture can grow in temperature ranged from 20°C to 37°C. The strain was tolerant up to 7% NaCl concentration. On the basis of 16S RNA gene sequence, this efficient phosphate solubilizer W1-PSB2 was identified as *Microbacterium paraoxydans* Laffineur et al. 2003 emend. Buczolits et al. (2008) with sequence similarity of 99% (Fig. 2a, b). The Gen Bank accession no. is FJ871123.

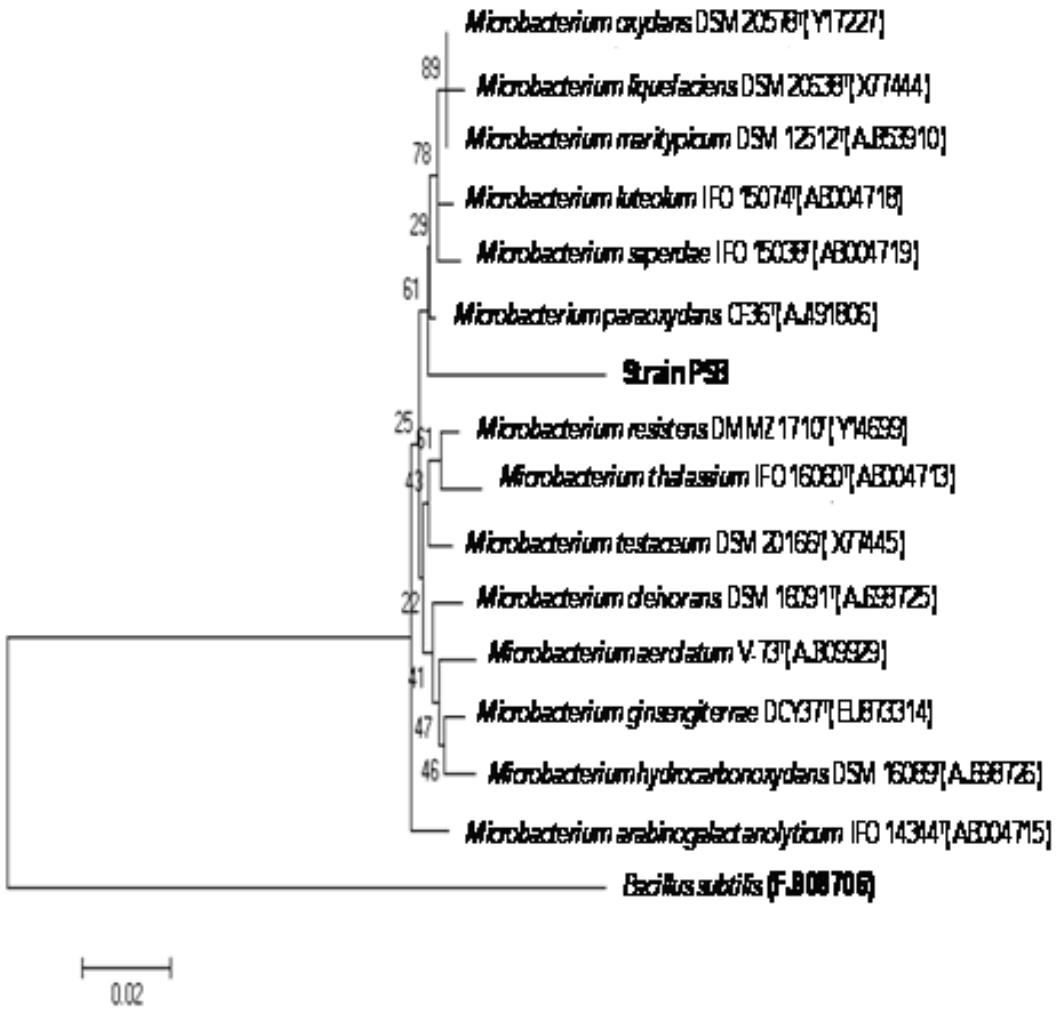


Figure 2b: The Neighbor-Joining (NJ) tree, inferred using MEGA software version 4 (Tamura et al., 2001) Bootstrap values expressed as percentage of 1,000 replications are given at the nodes. Bar equals 0.02% sequence variation.

DISCUSSION

Soil microorganisms are involved in a range of processes that affect phosphate solubilization and influence the subsequent availability of phosphate to plants (Richardson, 2001). The present investigation was carried out mainly to isolate and characterize efficient phosphate solubilizing bacteria present in the rhizospheric soil of important food crops grown in the Northern India. Mostly the genera of *Bacillus* sp., *Pseudomonas* sp. and *Rhizobium* sp. are known to exhibit higher efficiency in mobilizing phosphorus from the insoluble tri calcium phosphate. Here we are reporting for the first time a good phosphate solubilizing ability of *Microbacterium paraoxydans*; the bacteria also show good phosphatase and siderophore production ability. There is increasing evidence that phosphate solubilizing bacteria improve plant growth due to biosynthesis of plant growth substances and their action to release available phosphorous (Ponmurugan and Gopi, 2006). The use of phosphate

solubilizing bacteria in mixed cultures or co-inoculants with other microorganisms for improving the phosphate availability to the plants has been extensively studied. Phosphate solubilizing microorganisms are reported to dissolve insoluble phosphates by production of inorganic or organic acids, which results in the decrease of pH (Whitelaw, 2000). Most of the previous reports state that the calcium phosphates are dissolved by acidification. Therefore, any microorganism that acidifies its external medium will show some level of phosphate solubilization activity (Dave and Patel, 2003; Chung et al., 2005). Bacterial strains isolated in the present study solubilize the insoluble form of phosphate (tri-calcium phosphate) into soluble form and show decrease in pH by acidifying the external medium. Pot experiments (in which isolates are used as bio-inoculants to check the effect on plant growth and yield) are presently being carried out, which will give a better understanding of the use of these isolates as potential biofertilizers. The results will be the basis of a separate communication. The bio-potentiality of the isolates could be exploited in the fertilizer industries for sustainable agriculture systems.

This is the first report to the best of our knowledge, for the efficient phosphate solubilization by *Microbacterium paraoxydans* which could be further used as a potential bio-inoculant for field and environment applications.

AKNOWLEDGEMENT

This paper is dedicated to Mr. D. Singh, the father of SSC, who was a great support and a source of inspiration. We thank CSIR for financial assistance. This is IMTECH communication No. 011/2009.

REFERENCES

1. Abd-Alla M. H., 1994 – Phosphatases and the utilization of organic phosphorous by Rhizobium leguminosarum biovar viceae. Lett. Appl. Microbiol, 18, 294-296.
2. Chung H., Park M., Madhaiyan M., Seshadri S., Sony J., Cho H. and So T., 2005 – Isolation and characterization of phosphate solubilizing bacteria from rhizosphere of crop plants of Korea. Soil Biol. and Biochem. 37: 1970-1974.
3. Dave H. and Patel H., 2003 – Impact of different carbon and nitrogen sources on phosphate solubilization by Pseudomonas fluorescens, Indian Journal of Microbiology, I, 43, 33-36.
4. De Souza M.-J. B. D., Nair S and Chandramohan D., 1999 – Phosphate solubilizing bacteria around Indian peninsula. Indian J. Marine Sciences. 29, 48-51.
5. Galal Y.G., El-Gandaour J. A. and El-Akel F. A., 2001 – Stimulation of wheat growth and N fixation through Azospirillum and Rhizobium inoculation. A field trial with 15N techniques. In. W.J. Horst. (Ed.). Plant nutrition-Food security and sustainability of agro ecosystems. 666-667.
6. Gyaneshwar P. G, Kumar N, Parekh L. J and Poole P. S., 2002 – Role of soil microorganisms in improving phosphorus nutrition of plants. Plant Soil. 245, 83-93.
7. Halder A. K., Mishra A. K., Bhattacharyya P. and Chakrabarthy P. K., 1991 – Solubilization of rock phosphate by Rhizobium and Bradyrhizobium. J. Gen. Appl. Microbiol. 36, 81-92.
8. Jackson M. L., 1973 – Soil chemical analysis. Prentice Hall of India, New Delhi, India, 134.
9. Omar S. A., 1998 – The role of phosphate solubilizing fungi and vesicular arbuscular mycorrhiza (VAM) in growth of wheat plants fertilized with rock phosphate, W. J. Microbial. Biotechnology 14, 211-219.
10. Pikovskayas R. I., 1948 – Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. Mikrobiologiya 17, 362-370.
11. Ponmurugan P and Gopi C., 2006 – Distribution pattern and screening of phosphate solubilizing bacteria isolated from different food and forage crops. J Agronom 5, 600-604.
12. Richardson A. E., 2001 – Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. Austral J. Plant Physiol. 28, 897-906.
13. Rogers R. D. and Wolfran J. H., 1993 – Phosphorus, sulphur, and silicon related elements. 77, 137-140.
14. Schwyne B. and Neiland J. B., 1987 – Universal chemical assay for detection and determination of Siderophores. Anal Biochem 160, 40-47.
15. Ulrich A., Mally D. and Voora V., 2009 – Peak Phosphorus: Opportunity in the making why the phosphorus challenge presents a new paradigm for food security and water quality in the lake Winnipeg basin by International Institute for Sustainable Development (IISD), Winnipeg, Manitoba, Canada.
16. Vassileva N., Vassileva M., Azcon R., Fenice M., Fedrici F. and Barea J. M., 1998 – Fertilizing effect of microbially treated olive mill waste water on trifolium plants. Biores. Technol. 66, 133-137.
17. Vassilev N., Vassileva M., Fenice M., Federici F., 2001 – Immobilized cell technology applied in solubilization of insoluble inorganic (rock) phosphates and P plant acquisition. Biores. Technol., 79, 263-271.
18. Whitelaw M. A., 2000 – Growth promotion of plants inoculated with phosphate solubilizing fungi. Adv. Agronom. 69, 99-151.

ASYMMETRY STUDIES IN *RHYNCHORHAMPHUS GEORGI* (VALENCIENNES, 1846) (FAMILY: HEMIRAMPHIDAE) COLLECTED FROM THE SEA OF OMAN

*Juma AL-MAMARY * Laith JAWAD *, Hajer AL-MAMARY **, Manal AL-YARUBI *** and *Dawood AL-MAMARY **

* Marine Science and Fisheries Centre, Ministry of Fisheries Wealth, Sultanate of Oman, P.O. Box 427, Postal Code 100 Muscat, jumammry@gmail.com**,** laith_jawad@hotmail.com, almaamari007@hotmail.com
** High Technical College, Muscat, Sultanate of Oman, hajeralmamry@hotmail.com, manalalyarubi@hotmail.com

KEYWORDS: Oman Sea, fish, *Rhynchorhamphus georgi*, bilateral asymmetry.

ABSTRACT

The differential development of a bilateral character between the sides of an organism is known as asymmetry. The analysis of these phenomena has been carried out for five bilateral characters of *Rhynchorhamphus georgi* (Valenciennes, 1846) (Family: Hemiramphidae). Using a seine net, 126 specimens of *R. georgi* were collected from the Sea of Oman at the City of Muscat. Five bilateral characters were used to compare asymmetry in this species, and these are the five morphometric characters: (1) length of the pre-orbital distance (mm); (2) length of the post-orbital distance (mm); (3) eye diameter (mm); (4) head length (mm) measured from mouth to the posterior edge of operculum and meristic character; and (5) number of pectoral fin rays. The results showed that the level of asymmetry of the eye diameter character was higher than those of the rest of the characters studied. The possible cause of asymmetry in this species has been discussed in relation to different pollutants and their presence in the area. A trend of increase in the asymmetry values with fish length was noted in the post-orbital length and number of pectoral fin rays.

RÉSUMÉ: Etude de l'asymétrie chez *Rhynchorhamphus georgi* (Valenciennes, 1846) (Fam. Hemiramphidae), collecté dans la Mer d'Oman.

Le développement différencié des caractères bilatéraux dans un organisme est connu sous le nom d'asymétrie. L'analyse de ce phénomène a été conduite sur cinq caractères bilatéraux de *Rhynchorhamphus georgi* (Valenciennes, 1846) (Fam. Hemiramphidae). 126 spécimens de *R. georgi* ont été collectés à la seine dans la Mer d'Oman, devant la ville de Muscat. Cinq caractères bilatéraux ont été comparés pour déterminer l'asymétrie de cette espèce: quatre caractères morphométriques: (1) longueur de la distance préorbitale (mm); (2) longueur de la distance postorbitale (mm); (3) diamètre oculaire (mm); (4) longueur de la tête (mm): mesuré à partir de la bouche jusqu'au bord postérieur de l'opercule et un caractère méristique (5) nombre des rayons de la nageoire pectorale. Les résultats ont montré que l'asymétrie du diamètre oculaire a été plus grande que celle des autres caractères étudiées. La cause possible de l'asymétrie dans cette espèce a été discutée à propos de la relation avec les différents polluants et leur présence dans la région. Une tendance positive de l'asymétrie corrélée avec la longueur du poisson a été remarquée concernant la longueur postorbitale et le nombre des rayons dans les nageoires pectorales.

REZUMAT: Studii de asimetrie la *Rhynchorhamphus georgi* (Valenciennes, 1846) (Fam. Hemiramphidae), exemplare colectate în Marea Oman.

Dezvoltarea diferenţiată a unui caracter bilateral între două părţi ale aceluiaşi organism se numeşte asimetrie. Analiza acestui fenomen a fost efectuată pentru cinci caractere bilaterale la *Rhynchorhamphus georgi* (Valenciennes, 1846) (Fam. Hemiramphidae). 126 specimene de *R. georgi* au fost capturate cu ajutorul unei plase circulare din Marea Oman în dreptul oraşului Muscat. S-au utilizat cinci caractere bilaterale pentru compararea asimetriei la această specie, adică caractere morfometrice: (1) lungimea distanţei preorbitale (mm); (2) lungimea distanţei postorbitale (mm); (3) diametrul ochiului (mm); (4) lungimea capului (mm) măsurată de la gură la marginea posterioară a operculului şi un caracter meristic (5) numărul razelor din înotătoarea pectorală. Rezultatele au arătat că nivelul de asimetrie al diametrului ocular a fost mai mare decât al celorlalte caractere studiate. Cauza posibilă a asimetriei în această specie a fost discutată în concordanţă cu diferiţi poluanţi şi prezenţa lor în zona de studiu. Tendinţa de creştere a valorilor asimetriei este direct proporţională cu lungimea peştelui în ceea ce priveşte lungimea postorbitală şi numărul razelor înotătoarei pectorale.

INTRODUCTION

The differential development of a bilateral character between the sides of an organism is known as asymmetry (Van Valeen, 1962; Palmer and Strobeck, 1986; Leary and Allendrof, 1989). Fluctuating asymmetry results when a trait present on both sides of the body does not undergo identical development. It is also known that fluctuating asymmetry represents a measure of developmental sensitivity to environmental stress (Moller and Pomiankowski, 1993; Jawad, 2001, 2003, 2004; Jawad et al., 2010). Asymmetry usually increases under environmental stresses due to the failure of the homeostatic regulatory mechanism. These developmental effects might occur before the concentration of toxicants in the water or food reaches levels high enough to produce morbidity (Bengtson and Hindberg, 1985).

The only published study on the fluctuating asymmetry in Omani fish species is that of Jawad et al. (2010), therefore, the present study is considered a quantitative and qualitative addition to the previous study on Omani fish fauna. The present work studied fluctuating asymmetry in selected morphological characters of the teleost fish *Rhynchorhamphus georgi* collected from the Muscat coastal area in the middle region of the Sea of Oman.

Fish specimens of *Rhynchorhamphus georgi* were collected from Muscat coastal area, The Sea of Oman. The five bilateral characters used to compare asymmetry were as follows: (1) Length of the pre-orbital distance (mm): measured from mouth to the anterior edge of the orbit. (2) Length of the post-orbital distance (mm): measured from the posterior edge of the eye to the posterior edge of the operculum. (3) Eye diameter (mm): measured from the anterior to the posterior edges of the eye. (4) Head length (mm): measured from mouth to the posterior edge of operculum. Meristic characters: (5) Number of pectoral fin rays: a count of the total number of pectoral fin ray, including the most upper ray. Most characters were counted and measured under a binocular dissecting microscope. A magnifying glass was used for specimens too large to fit under a microscope.

The statistical analysis included calculating the squared coefficient of asymmetry variation (CV^2_a) for meristic and morphometric characters according to Valentine et al. (1973):

$$CV^2_a = (S_{r-l} \text{ X } 100/X_{r+l})^2$$

where S_{r-l} is the standard deviation of signed differences and X_{r+l} is the mean of the character, which is calculated by adding the absolute scores for both sides and dividing by the sample size. To obviate scaling problems associated with growth in morphometric characters, each measurement was divided by suitable general size measurements, e.g. head length was used as the standardizing measurement. Each of the morphometric characters was treated as such before obtaining the signed differences.

RESULTS

The results of asymmetry data analysis of the previously listed characters of *Rhynchorhamphus georgi* collected from the Muscat coastal area, in the middle region of The Sea of Oman are shown in table number 1. The highest value was recorded for the eye diameter and the lowest value for the head length.

Table 1: Squared coefficient asymmetry (CV2a) values and character means (X_{r+l}) of *Rhynchorhamphus georgi*.

Character	CV2a	N	Character mean	Individuals with asymmetry (%)
Preorbital length	2.44	126	14.10	31
Postorbital length	6.10	126	13.20	23
Eye diameter	121.30	126	2.37	56
Number of pectoral fin rays	24.20	126	10.80	43
Head length	0.51	126	36.6	12

The percentage of the individuals showing asymmetry in the eye diameter was the highest among the percentages recorded for the five characters (56% of the total fish studied). The lowest percentage was recorded in the individuals with asymmetry in head length (12% of the total fish studied). Individuals of *Rhynchorhamphus georgi* were grouped into length classes (Tab. 2).

An increasing trend in the asymmetry of postorbital length and number of pectoral fin rays in relation to fish length is obtained.

Table 2: Squared coefficient of asymmetry and character means (X_{r+1}) by size class of *Rhynchorhamphus georgi*.

Size Class	n	CV^2_a	Character mean X_{r+1}	Individuals with asymmetry (%)
Preorbital length				
17.1-18.0	1	0	12.7	0
18.1-19.0	23	1.22	13.5	13.04
19.1-20.0	63	3.56	13.9	20.6
20.1-21.0	32	1.8	14.6	21.8
21.1-22.0	5	0	15.6	0
25.1-26.0	7	0	18.7	0
Total	126			
Postorbital length				
17.1-18.0	1	0	11.8	0
18.1-19.0	23	14.1	12.3	26
19.1-20.0	63	23.3	13.1	17.4
20.1-21.0	32	27.3	13.6	25
21.1-22.0	5	30	14.2	35
25.1-26.0	7	49	17.8	100
Total	126			
Eye diameter				
17.1-18.0	1	0	2.36	0
18.1-19.0	23	121.4	2.35	45
19.1-20.0	63	120.6	2.37	67
20.1-21.0	32	117.0	2.33	89
21.1-22.0	5	114.5	2.30	100
25.1-26.0	7	121.4	2.35	43
Total	126			
Head length				
17.1-18.0	1	0	34.6	100
18.1-19.0	23	0.17	35.3	21.7
19.1-20.0	63	0.56	36.2	19.04
20.1-21.0	32	0.77	37.5	31.20
21.1-22.0	5	0	39	0
25.1-26.0	7	0	47.5	0
Total	126			
Number of pectoral fin ray				
17.1-18.0	1	0	11	0
18.1-19.0	23	19.1	11	21.7
19.1-20.0	63	21.8	10.8	20.6
20.1-21.0	32	34.9	10.7	31.2
21.1-22.0	5	41.2	11.9	20
25.1-26.0	7	159.7	11.2	50
Total	126			

DISCUSSION

There is some variation in the asymmetry values among the five morphological characters studied in *Rhynchorhamphus georgi*. In the present time it is impossible to evaluate the level of asymmetry of those characters and to determine if they are higher or lower than the average due to the lack of data regarding natural asymmetry in this part of the world. However, the eye diameter character showed higher asymmetry values than those of the remaining characters. High asymmetry in eye diameter was also recorded in several freshwater and marine fish species (Al-Hassan et al., 1990; Al-Hassan and Hassan, 1994; Jawad, 2001, 2003; Jawad et al., 2010). Such agreements in results of asymmetry might indicate the vulnerability of this character to the immediate changes in the environment. It is not possible at this stage to confirm such effect as the correlation between different environmental pollution and the morphology of the fish species in question is not available. However, based on previous studies in this field, it is possible to conclude that there is a direct correlation between environmental stress due to pollution and asymmetry in this species. Such environmental factors are present in the waters of the coastal area of The Sea of Oman.

On the other hand, the low asymmetry value displayed by the head length character might be explained on the basis that this character is less vulnerable to environmental stresses. This may be the case when the developmental period of the head does not coincide with adverse environmental events (Jawad, 2003; Jawad et al., 2010). Other factors, including genetic ones, might be responsible for the asymmetry in these characters, but such factors cannot be discussed at this stage due to the lack of genetic data on the ichthyofauna of Oman.

The origin and cause of asymmetry in fishes can depend on several factors, one of which is environmental stress which leads to an increased level of asymmetry, but might occur at low levels before causing wide spread death (Bengtson and Hindberg, 1985).

Pollution of sea water and sediments by hydrocarbons, heavy metals, pesticides and organic matter is considered the main cause of environmental stress. This state of pollution is not unusual for the coastal environment of The Sea of Oman where different pollutants were reported to affect its water for at least the last twenty years (De Mora et al., 2004; De Mora et al., 2005; Al-Darwish et al., 2005; Tolosa et al., 2005; Abdel Gawad et al., 2008; Khan, 2008).

The environmental causes might be natural events, and several factors are known to produce nutritional deficiencies such as various pathogens and various population phenomena (Bengtson and Hindberg, 1985), and it is highly possible that these factors may be in action in Oman Sea waters as they seem to be common in the aquatic environment.

Several authors have shown a relationship between the coefficient of asymmetry and fish length (Al-Hassan et al., 1990; Al-Hassan and Hassan, 1994; Al-Hassan and Shwafi, 1997; Jawad, 2001; Jawad et al., 2010) where there was a trend of increase in the asymmetry with the increase in fish length. This trend is probably the result of incomplete development; character means are always lowest in smaller size classes (Valentine et al., 1973). The same results were obtained by Valentine et al. (1973) in selected fish species collected from California, USA and Jawad et al. (2010) in the carangid fish species, *Decapterus russelli* collected from the coastal north region of The Sea of Oman, Oman. They suggested two possible hypotheses that may account for such a trend; these are the ontogenetic changes which are an increase in asymmetry with size (age) and the possible historical process which is a secular increase in asymmetry.

CONCLUSIONS

An asymmetry in the bilateral characters of *Rhynchoramphus georgi* (Valenciennes, 1846) was observed. The eye lens diameter showed the highest asymmetry value among the characters studied. The possible cause of the asymmetry in this species has been discussed in relation to different pollutants and their presence in the area. A trend of increase in the asymmetry of the postorbital length and number of pectoral fin rays with the fish length was noticed.

ACKNOWLEDGEMENTS

We would like to thank the Ministry of Agriculture and Fisheries Wealth, Marine Science and Fisheries Centre and the Agriculture and Fisheries Development Fund for giving us the opportunity to work on the fish samples within the qualitative and quantitative distribution of marine organisms in Sultanate of Oman and to provide the appropriate financial support.

REFERENCES

1. Abdel Gawad E. A., Al-Azab M. and Lotfy, M. M., 2008 – Assessment of organic pollutants in coastal sediments, UAE., Environmental Geology, 54, 1091-1102.
2. Al-Darwish H. A., Abd El-Gawad E. A., Mohammed F. H. and Lotfy M. M., 2005 – Assessment of organic pollutants in the offshore sediments of Dubai, United Arab Emirates, Environmental Geology 48, 531-542.
3. Al-Hassan L. A. J. and Hassan S. S., 1994 – Asymmetry study in Mystus pelusius collected from Shatt al-Arab River, Basrah, Iraq, Pakistan Journal of Zoology, 26, 276-278.
4. Al-Hassan L. A. J. and Shwafi N. A. A., 1997 – Asymmetry analysis in two marine teleost fish species collected from the Red Sea coast of Yemen, Pakistan Journal of Zoology 29, 23-25.
5. Al-Hassan L. A. J., Al-Doubaikel A. Y., Wahab N. K. and Al-Daham N. K., 1990 – Asymmetry analysis in the catfish, Heteropneustes fossilis collected from Shatt al-Arab River, Basrah, Iraq, Rivesta di Idrobiologia 29, 775-780.
6. Bengtson B. E. and Hindberg M., 1985 – Fish deformities and pollution in some Swedish waters, Ambio 14, 32-35.
7. De Mora S., Fowler S. W., Wyse E. and Azemard S., 2004 – Distribution of heavy metals in marine bivalve, fish and coastal sediments in the Gulf and Gulf of Oman, Marine Pollution Bulletin 49, 410-424.
8. De Mora S., Fowler S. W., Tolosa I., Villeneuve J.-P. and Cattini C., 2005 – Chlorinated hydrocarbons in marine biota and coastal sediments from the Gulf and Gulf of Oman, Marine Pollution Bulletin 50, 835-849.
9. Jawad L. A., 2001 – Preliminary asymmetry analysis of some morphological characters of Tilapia zilli (Pisces: Cichlidae) collected from three localities in Libya, Bolletino del Museo regionale di Science naturale Torino 18, 251-257.
10. Jawad L. A., 2003 – Asymmetry in some morphological characters of four sparid fishes from Benghazi, Libya, Oceanological and Hydrobiological Studies 32, 83-88.
11. Jawad L. A., 2004 – Asymmetry analysis in the mullet, Liza abu collected from Shatt al-Arab River, Basrah, Iraq, Bolletino del Museo regionale di Science naturale Torino 21, 145-150.
12. Jawad L. A., Al-Mamry J. M., Al-Kharusi A. A. and Al-Habsi S. H., 2010 – Asymmetry in certain morphological characters of the carangid species Decapterus russelli, collected from Lemah coastal area, on the northern part of Oman Sea, Oceanological and hydrobiological studies 39, 55-62.
13. Khan N. Y., 2008 – Integrated management of pollution stress in the Gulf, in: Protecting the Gulf's Marine Ecosystems from Pollution, 57-92. Abuzinada A. H., Barth H.-J., Krupp F., Boer B., Al-Abdessalaam Z., (eds), Birkhauser, Basel.
14. Leary A. and Allendrof F. W., 1989 – Fluctuating asymmetry as an indicator of stress: implications for conservation biology, Trend in Evolution 4, 214-217.
15. Moller A. P. and Pomiankowski A., 1993 – Puntuated equilibria or gradual evolution: fluctuating asymmetry and variation in the rate of evolution, Journal of Theoretical Biology 161, 359-367.
16. Palmer A. R. and Strobeck C., 1986 – Fluctuating asymmetry: measurements, analysis and pattern, Annual Review of Ecology and Systematics 17, 391-421.
17. Tolosa I., De Mora S. J., Fowler S. W., Villeneuve J.-P., Bartocci J. and Cattini C., 2005 – Aliphatic and aromatic hydrocarbons in marine biota and coastal sediments from the Gulf and the Gulf of Oman, Marine Pollution Bulletin, 50, 1619-1633.
18. Valentine D. W., Soule M. E. and Samollow P., 1973 – Asymmetry in fishes: a possible statistical indicator of environmental stress, Fishery Bulletin, 71, 357-370.

19. Van Vallen L., 1962 – A study of fluctuating asymmetry, Evolution, 16, 125-142.

BIODEGRADATION OF DIESEL FUEL

Sonali BHAWSAR * and *Swaranjit Singh CAMEOTRA* **

* University of Pune, Department of Microbiology, Pune, Maharashtra, India, IN-411007.
** Institute of Microbial Technology (IMTECH), Sector 39ª, Chandigarth, India, IN-160036, ssc@imtech.res.in

KEYWORDS: biodegradation, recalcitrant, pollution, diesel, microflora, hydrocarbono-clastic potential.

ABSTRACT

Biodegradation is a cost efficient, eco-friendly clean-up technology for polluted environments. During this process, microbes play an essential role in the utilization and decontamination of the pollutant compounds. In this review, the pollutant under consideration is automobile diesel fuel, extensively used at the moment. It is a petroleum hydrocarbon made up of long chain alkanes (› C18), PAHs and BTEX are its major constituents. Their complete degradation has never been reported. Diesel is highly recalcitrant, has a poor solubility and contributes towards the pollution of ground water, marine and fresh water reservoirs and soil. But it is biodegradable and microflora from various environments is known to degrade it. This review gives a detailed account of the polluting effects of diesel, the process and mechanism of its biodegradation, the role of different microbes having degradation potential and their application strategies.

ZUSAMMENFASSUNG: Der biologische Abbau des Diesel Brennstoffes.

Der biologische Abbau ist wirksam auf Kostenniveau und dazu eine ökologisch umweltfreundliche Reinigungstechnologie der belasteten Umwelt. Während des Abbauprozess spielen die Mikroben eine wichtige Rolle in der Nutzung und Entgiftung der verunreinigten Komponenten. Aus dieser Perspektive gesehen, war der Schadstoff, den wir betrachtet haben, der bei Kraftfahrzeugen in großem Umfang verwendete Diesel-Kraftstoff. Dabei geht es um einen Kohlenwasserstoff des Erdöls und ein Alkan mit einer langer Kette (C18), deren Hauptaufbauelemente PAHs und BTEX sind. Über ihren vollständigen Abbau wurde bisher nicht berichtet. Das Dieselöl ist extrem widerstandsfähig, schwach löslich und trägt zur Verschmutzung des Grundwassernetzes, der Meeres- und Süßwasserreserven sowie des Bodens bei. Aber der Diesel-Kraftstoff ist biologisch abbaubar, wobei auch bekannt ist, dass die aus unterschiedlichen Medien stammende Mikroflora ihn abbauen kann. Die vorliegende Arbeit stellt im Einzelnen die Auswirkungen der Verschmutzung mit Dieselöl dar, geht auf den Prozess und die Mechanismen seines biologischen Abbaus, die Rolle der verschiedenen Mikroorganismen mit Abbaupotential sowie auf die Strategien ihrer Anwendung ein.

REZUMAT: Biodegradarea combustibilului Diesel.

Biodegradarea este eficientă la nivelul costurilor, o tehnologie clean-up eco-pritenoasă pentru mediul poluat. În acest proces, microbii joacă un rol esenţial în utilizarea şi decontaminarea componentelor poluate. Din această perspectivă, poluantul pe care l-am luat în considerare a fost combustibilul auto diesel, folosit pe scară largă. Este un hidrocarbon al

petrolului şi un alcalin cu lanţ lung (› C18), PAHs şi BTEX sunt elementele sale constituente principale. Nu a fost niciodată raportată degradarea lor completă. Diesel-ul este extrem de recalcitrant, este slab solubil şi contribuie la poluarea pânzelor freatice, a rezervoarelor de apă marină şi dulce şi a solului. Dar el este biodegradabil şi se ştie că microflora din diferite medii îl poate degrada. Acestă lucrare prezintă detaliat efectele poluării cu diesel, procesul şi mecanismul biodegradării sale, rolul diferiţilor microbi cu potenţial de degradare şi strategiile lor de aplicare.

INTRODUCTION

Diesel is an essential petroleum fuel used for all types of transport vehicles, from cars to trains. It is chiefly a hydrocarbon, containing paraffins, cycloparaffins, PAH's like naphthalenes, alkylbenzenes and aromatics like benzene, toluene, ethyl benzene and xylenes (BTEX) as its major constituents. Diesel and its components are hydrophobic oils with poor solubility and low volatility and thus are very recalcitrant to be biologically degraded (Abed et al., 2002; Rushton et al., 2007).

Awareness of environmental pollution among the scientific community and common people has put forth the negative effects of diesel, as well as its importance. It became evident from major accidental diesel spills, like that of 1969 (McCrary et al., 2003). The leakages during its underground storage at service stations contaminate ground water and soil on regular basis. All types of ecosystems like marine, freshwater and agricultural are found to be affected by diesel pollution. In addition, it has also imparted to air pollution. The exhaust soot produces in transport vehicles loads air with a considerable amount of hazardous particulate matter. These have contributed greatly to the ill health of all living things. Degradation of potable water supplies, destruction of fish populations and water birds from inland and marine waters, cardiovascular and pulmonary diseases, toxic effects on the kidneys and liver, occurrence of fatal diseases like cancers, immunological, foetal and genetic disorders are all the bad impacts which are solely caused by diesel pollution (Boonchan et al., 2000; Samanta et al., 2002). There is no alternate that could replace diesel, leading to its extensive use and resulting in pollution and recalcitrance in environment (Fig. 1).

Biodiesel obtained from vegetable oils could be an option for petroleum derived diesel. It can be blended with petroleum diesel to be used in diesel engines and it is easily biodegradable (Prince et al., 2008; Junior et al., 2009). But like petroleum diesel they are also known at polluters of aquatic systems, where they occur as greasy fractions (Zhang et al., 1998; Bhattacharyya et al., 2003; Novak et al., 1974). However, biodiesels alone also would not furnish the world's increasing demand for fuels.

In the literature, the potential of microorganisms, identified as degrading agents of several compounds indicates biological treatment as being the most promising alternative for reducing the environmental impact of oil spills (Facundo et al., 2001; Robert et al., 2003). The solution is provided by the biodegradation process, which not only transforms recalcitrant compounds into simpler nontoxic forms, but also reduces the negative effects of pollution created by diesel. The biodegradation of various chemicals, n-alkanes (Ueno et al., 2006; Mohanty and Mukherji, 2008), paraffin (Vieira et al., 2009), cycloparaffin (Beam and Perry, 1974), naphthalene (Seoud and Maachi, 1987; Heitkamp et al., 2003), alkyl benzene (Wilkes et al., 2000), ethyl benzene (Parameswarappa et al., 2008), benzene (Shim and Yang, 1999; Nicholson and Fathepure, 2004), toluene (Shim and Yang, 1999; Hanson et al., 1999) and xylenes (Chakraborty et al., 2005) which constitutes diesel is summarized in the table number 1. The review gives a detailed account of biodegradation strategies regarding diesel.

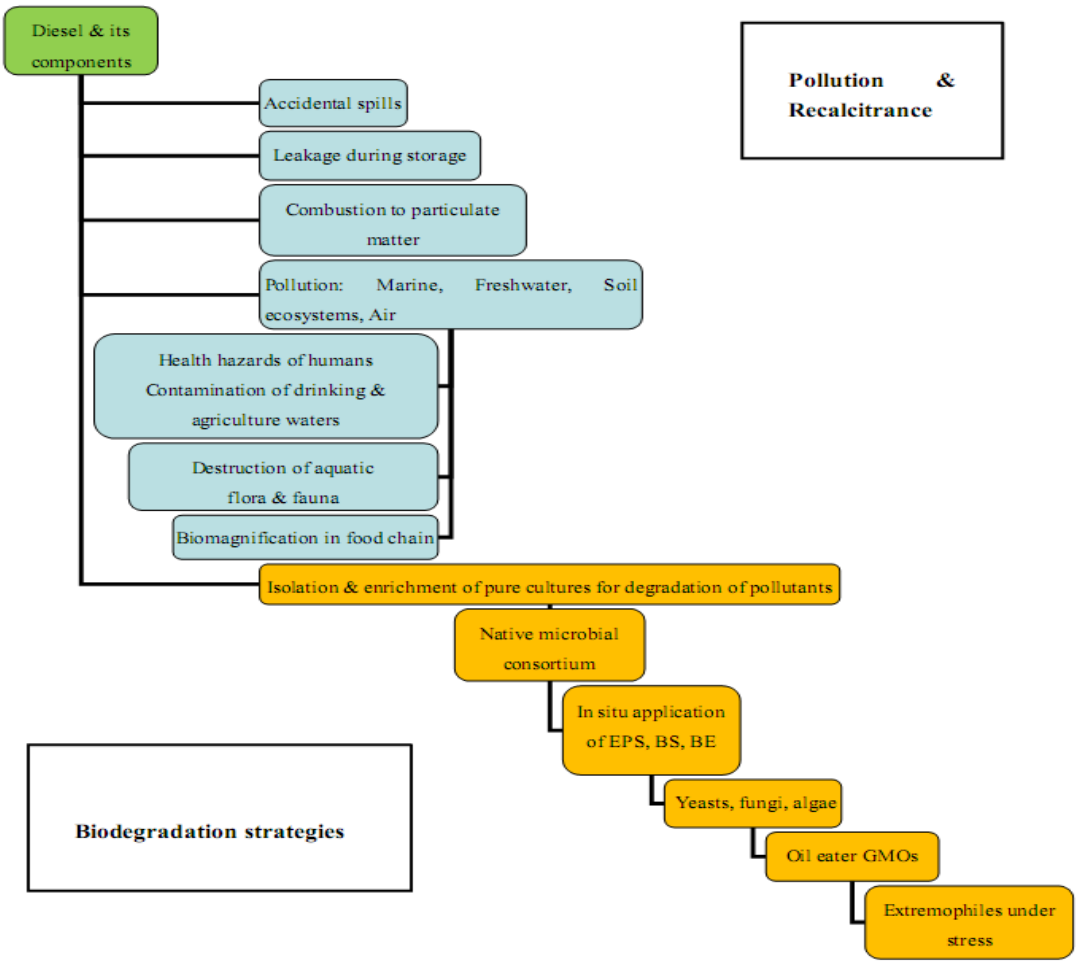


Figure 1: Diagrammatic presentation of diesel pollution and its biodegradation strategies.

Diesel degradation by pure cultures and bacterial consortia

Microorganisms and their metabolic activities play a crucial role in the removal, transformation or degradation of toxic contaminants in soil and water ecosystems. The widespread ability of microorganisms to assimilate hydrocarbons is of great significance in environmental decontamination (Davis et al., 1993) and the major consideration has been given to utilization of oil by pure and mixed cultures under laboratory and stimulated field conditions (Christofi and Iyshina, 2002; Noordman and Janssen, 2002; Bruheim et al., 1997). The strains of bacteria, fungi, yeasts and algae have been exploited for their application in diesel degradation. Pure culture of *Serratia marcescens* have been exploited for its diesel degradation potential, as well as corrosion markers for diesel transporting pipelines. A diesel and n-alkane degrading strain of *Rhodococcus* sp. has been developed as an inoculum to function in psychrotrophic conditions (Whyte et al., 1998). In vitro degradation studies of isolated *Pseudomonas fluorescens* showed that 30% of the phenanthrene in the diesel was degraded during a six month period, but none of the aromatic hydrocarbons (Sepi et al., 2001).

But mixed cultures or consortia are always preferred over single cultures of degrading strains. Complete degradation of more than one compound could be achieved from consortia of different degraders. Mixed culture might overcome the problems of degradation by single culture, such as nutritional stress and the abundant presence of toxic substances. In a consortium, a degradation sequence occurs where a second organism degrades partially degraded metabolite of the first and so on, until complete degradation. The best example of a microbial consortium is the intergeneric protoplast fusion between *Acinetobacter* sp. A3 and *Pseudomonas putida* DP99, for enhanced in vitro hydrocarbon degradation (Hanson and Desai, 1996). The parent *Acinetobacter* sp. A3 is alkane degrader, while *Pseudomonas* sp. is naphthalene degrader; the resulting fusants were capable of degrading both hydrocarbons. In their study, Dagher et al. (1997) have found that PAH-degrading genes of pseudomonads are located on plasmids and can be received by other bacterial genera in the soil by conjugation. Thus complete biodegradation of naphthalene and related compounds in the soil is the effect of the working bacterial consortia present in the soil.

Two diesel fuel degrading microbial consortia, consisting of bacteria belonging to the genera *Chryseobacterium, Acinetobacter, Pseudomonas, Stenotrophomonas, Alcaligenes* and *Gordonia* along with the fungus *Trametes gibbosa*, have remarkable biodegradation potential, stability and resistance to cryopreservation, and both of them appear very interesting candidates for bioaugmentation operations on diesel contaminated sites (Zanaroli et al., 2010). A bacterial consortium consisting of seven members was characterized by Richard and Vogel (1999). In this consortium, two isolates were able to degrade light aliphatic hydrocarbons and aromatic compounds. A third isolate degraded only aliphatic compounds. Remaining four isolates were unable to degrade any diesel components but increased the rate of diesel fuel mineralization to carbon dioxide. Thus the constituted consortium was efficient in degradation.

Yeasts that degrade diesel

A number of yeast strains have been exploited for their ability to degrade diesel and its components. Prabhakaran and Sivadas isolated important genera, like *Debaryomyces, Hamsenula, Kluvveromyces, Pichia, Saccharomyces, Candida, Geotrichum, Rhodotorula* and *Trichosporon* from Cochin backwaters, all of them with diesel degradation potential. A psychrotrophic *Yarrowia lipolytica* strain was also reported with diesel degradation activity at 10-20°C (Margesin and Schinner, 2006). Yeasts cultures of *Rhodotorula aurantiaca* UFPEDA 845 and *Candida ernobii* UFPEDA 862 were selected for bioremediation of areas contaminated by diesel, and during investigations they were found able to degrade almost completely in time various diesel components like decane, nonane, dodecane and tetradecane, 5 methyl-octane, octadecane, decane, nonane.

Diesel degrading Fungi

Filamentous fungi play an important role in degrading diesel and its constituents. Because of extensive and rapid hyphal growth and larger biomass production, they are preferred as potential biodegradation agents (Kenneth, 1995; Saadoun, 2002). The consortium of *Aspergillus fumigatus, Hormoconis resinae* and *Candida silvicola* isolated from diesel storage tanks showed diesel degradation in time (Fatima et al., 2005). Extremophile desert fungi *Fusarium lateritium, Drechslera* sp. and *Papulaspora* sp. possess the ability to degrade diesel at temperatures above 35°C and with salinity as high as 10%. Such fungi have great potential for bioremediation of diesel contaminated sites that prevail under temperature and salt stress conditions (Obuekwe et al., 2005).

Table 1: Biodegradation of individual components of diesel.

Sr. no.	Component of Diesel	Biodegrading culture	References
1.	n-Alkane	*Ps. aeruginosa*, *Exiguobacterium aurantiacum*, *Burkholderia cepacia*	Ueno et al., 2006 Mohanty and Mukherji, 2008
2.	Paraffin	Microbial consortium	Vieira et al., 2009
3.	Cycloparaffin	Unknown soil microorganisms	Beam and Perry, 1974
4.	Naphthalene	*Pseudomonas*, Microbial consortia	Seoud and Maachi, 2003 Heitkamp et al., 1987
5.	Alkyl Benzene/ Ethyl Benzene	Sulfate reducing bacteria *Ps. fluorescences*	Wilkes et al., 2000 Parameswarappa et al., 2008
6.	Benzene	*Ps. putida, Ps. fluorescences;* *Marinobacter*	Shim and Yang, 1999 Nicholson and Fathepure, 2004
7.	Toluene	*Ps. putida, Ps. fluorescences;* Microbial consortium	Shim and Yang, 1999 Hanson et al., 1999
8.	Xylene	*Dechloromonas*	Chakraborty et al., 2005

Algae used for diesel degradation

Algae are being exploited for their diesel biodegradation potential. Research studies showed that algae consortia were able to consume diesel present in the medium (Chavan and Mukherji, 2010).

Prospects of genetically modified microorganisms used in diesel biodegradation

Genetically engineered microbial consortia were designed by some research groups, in order to be used as tools for controlled and regulated bioremediation under varying environmental conditions. The group of Kapley (1999) has designed a four member consortium to degrade crude oil in soil and marine environment, with an additional phenotype of osmotolerance. Barac et al. (2004) demonstrated that endophytic bacteria *Burkholderia cepacia* engineered with the appropriate degradation pathway, improve the *in planta* degradation of toluene, one of the constituents of diesel.

The use of genetically modified microorganisms (GMOs) or the development of "pollutant eater microorganisms" would be an important remedy for degradation of presently unattackable organic pollutants. Genetic engineering also allows the enhancement of their degradative potential by increasing enzyme expression, the creation of mutants with altered enzyme activities, the combination of gene transfer and mutation in order to increase number of compounds that are degraded and the exploitation of new hosts that enhance degradation of specific chemicals (Barnum, 1995). Keeping aside the problems regarding GMOs, like their genetic stability and survival, legislative, ethical and perception issues concerned to the release of GMOs in the soil and marine environment, they could be very promising agents for biodegradation and clean-ups of contaminated environments.

Diesel degradation by indigenous microflora

Assessment of the degrading potential of indigenous soil bacteria present at contaminated sites has always been a method of choice for bioremediation of pollutant (Furukawa, 1994). The exploitation of novel microbial flora for bioremediation requires *in situ* investigations (McGillivray and Shiaris, 1994) and the use of indigenous soil microorganisms has been always in practice for the bioremediation of large oil spills (Christofi and Iyshina,

2002). Sharma and Rehman (2009) developed laboratory a scale method containing an indigenous bacterial consortium of *Moraxella saccharolytica, Alteromonas putrefaciens, Klebsiella pneumoniae, Aerogenes, Pseudomonas fragi* to eliminate diesel from the soil. In Talaie et al. (2009), it was investigated that the biodegradation of floating diesel fuel with the use of two gram negative strains, isolated from indigenous population of a reservoir tank of a gas station. An indigenous nitrogen fixing population was found to stimulate biodegradation of diesel in marine environment (Piehler et al., 1999). These indigenous microbial consortia decrease exogenous N requirements and reduce environmental impacts of bioremediation following petroleum pollution.

The degradation of diesel in rhizosphere of *Poa, Phleum, Agrostis*, *Pisum sativum* and *Trifolium pratense* was studied and in time diesel was eventually decomposed by unknown indigenous microbial flora (Pichtel and Liskanen, 2001). The synergism between *Mycobacterium hyalinum* and *Cladosporium* resulted in strong and complete degradation of diesel from contaminated sites in Karamay oilfield (Li et al., 2008). Indigenous bacteria *Acinetobacter calcoaceticus, Acinetobacter* sp., *Citrobacter freundii* and *Bacillus pumilus,* isolated from diesel contaminated soils, degraded diesel in two weeks, but it was also interesting to note that inoculation with the consortia did not show a higher degradation potential than the individual isolates (Singh and Lin, 2009). These results indicate that, the environmental conditions of contaminated sites play an important role in degradation, even though additional diesel-degraders has been introduced into the contaminated site.

Hong et al. (2005) isolated a *Pseudomonas aeruginosa* strain which showed very strong hydrocarbono-clastic potential. It could degrade diesel, crude oil, gasoline, benzene, toluene and xylenes, PAHs, such as naphthalene, phenanthrene and pyrene. Indigenous bacteria enriched from industrial contaminated soil were able to biodegrade high diesel oil concentrations at high biodegradation rates (Nikakhtari et al., 2009). Biodegradation of diesel oil was observed at an Arabian Sea sediment culture. This culture could use diesel as sole source of carbon and energy, and it was isolated in the vicinity of an oil field (Mukherji et al., 2004). Indigenous extremophiles are a very important source of hydrocarbons bioremediation in extreme environments. Many hydrocarbon-contaminated environments are characterized by low or elevated temperatures, acidic or alkaline pH, high salt concentrations, or high pressure. Margesin and Schinner (2001) suggested that hydrocarbon-degrading microorganisms, adapted to grow and thrive in these environments, like thermophiles, psychrophiles, acidophiles, alkalophiles, or barophiles play an important role in the biological treatment of polluted extreme habitats.

Microbial products used in diesel degradation

Microbial products, particularly bioemulsifiers and biosurfactants, have potential applications in pollution remediation. Their highest advantage over chemical treatments is that they are biodegradable (Awasthi et al., 1999; Georgiu et al., 1992), they are produced from renewable sources and can function under extreme environmental conditions (Fleck et al., 2000; Banat et al., 2000). They have a variety of potential applications in the remediation of organic- and metal-contaminated soils and promote biodegradation of hydrocarbons (Desai and Banat, 1997). Biosurfactants reduce surface tension, CMC and interfacial tension in aqueous solutions and hydrocarbon mixtures. They also favour the emulsification of hydrocarbons (Fig. 2), so that microorganisms could assimilate them as carbon and energy source (Rosenberg, 1981).

A. B. C.

Figure 2: Emulsification of diesel oil by bacteria A. and C.: Top layer of non-emulsified diesel B.: Emulsified diesel can be seen on aqueous layer of bacterial culture.

The usefulness of biosurfactants in emulsification of aqueous hydrocarbon mixtures has been clearly demonstrated (Aronstein et al., 1991). Studies of oil or hydrocarbon contaminated sand/soil have also indicated that microorganisms which produce biosurfactants can aid bioremediation when stimulated (Awasthi et al. 1999; Karanth et al., 1999). The screening studies of Balogun and Fagade (2008) detected the highest occurrence percentage of surfactant producing genera like *Pseudomonas*, *Arthrobacter*, *Proteus*, *Corynebacterium*, *Micrococcus* and *Klebsiella* amongst microbial population of diesel contaminated soils.

Bioremediation using biosurfactants provides potential for cost effective, contaminant specific treatments to reduce concentrations of individual or mixed environmental contaminants in soil environment (Bouchez et al., 1999). Among the bioemulsifiers and biosurfactants having biodegradation potential, rhamnolipids from *Pseudomonas aeruginosa* in solubilization of PAHs (Rouse et al., 1994; Herman et al., 1995), trehalose ester from *Rhodococcus erythropolis* to enhance PAHs solubilisation, or alasan from *Acinetobacter radioresistens* for PAHs extraction and solubilization (Navon-Venezia, 1995) have been used. Whang et al (2009) investigated the direct application of surfactin and rhamnolipid biosurfactants for enhanced biodegradation of diesel-contaminated water and soil. The study of Ganesh and Lin (2009) showed that biosurfactant producing bacteria were effective in diesel degradation. *Micrococcus* sp. isolated from oil contaminated sites showed considerable biosurfactant production and diesel degradation activity in the soils of different temperatures and micronutrient status (Santhini et al., 2009). Authors have indicated this species as a potential tool for biodegradation of diesel contaminated soil.

Though biosurfactants are the only microbial products having great potential for biodegradation, novel approaches have also been tried, like the stimulation of diesel degradation using protein hydrolysates and exopolysaccharides (EPS). The addition of protein hydrolysate solution to soil contaminated with diesel fuel led to an increase in diesel removal, as well as hydrocarbon degrading by microorganisms from the soil (Harrison et al., 1996). EPS produced by *Gordonia alkanivorans* were found to enhance bacterial diesel degradation (Chen et al., 2008). They increased cell floating, cell growth, and diesel biodegradation of indigenous or commercial-available, diesel-degrading bacteria.

CONCLUSIONS

Assuming that diesel is the principle hydrocarbon environmental pollutant, its removal by biodegradation presents a perfect solution for environmental clean-up. Biodegradation is a natural process and a metabolic ability of microorganisms to transform or mineralize contaminants into less harmful, non-hazardous substances, which are then integrated into natural biogeochemical cycles. Although it is influenced by several factors, such as nutrients, oxygen, pH value, composition, concentration and bioavailability of the contaminants and pollution history of the contaminated environment, it is environmentally sound, non-destructive, cost- and treatment-effective cleanup technology (Alexander, 1981). Amongst many strategies of biodegradation, the use of indigenous cultures seems to be a promising approach for in situ applications.

REFERENCES

1. Abed R. M. M., Safi N., Koster J., Beer D., El-Nahhal Y., Rullkotter J. and Pichel F. G., 2002 – Microbial diversity of a heavily polluted microbial mat and its community changes following degradation of petroleum compounds, Appl. Environ. Microbiol. 68, (4), 1674-1683.
2. Alexander M., 1981 – Biodegradation of chemicals of environmental concern, Sci. 211, 132-138.
3. Aronstein B. N., Calvillo Y. M. and Alexander M., 1991 – Effects of surfactants at low concentrations on desorption and biodegradation of sorbed aromatic compounds in soil, Environ. Sci. Technol. 25, 1728-1731.
4. Awasthi N., Kumar A., Makkar R. and Cameotra S. S., 1999 – Biodegradation of soil applied endosulfan in the presence of a biosurfactant, J. Environ. Sci. Health (B), 34, 793-803.
5. Balogun S. A., Fagade O. E., 2008 – Screening for surface-active agent producing bacteria from diesel oil polluted tropical soil, World Appl. Sci. J. 3, (6), 930-933.
6. Banat I. M., Makkar R. S. and Cameotra S. S., 2000 – Potential commercial applications of microbial surfactants, Appl. Microbiol. Biotechnol. 53, 495-508.
7. Barac T., Taghavi S., Borremans B., Provoost A., Oeyen L., Colpaert J. V., Vangronsveld J. and Lelie D., 2004 – Engineered endophytic bacteria improve phytoremediation of water-soluble, volatile, organic pollutants, Nature Biotechnol, 22, 583-588.
8. Barnum S. R., 1995 – Microbial biotechnology, in: Biotechnology, Chap. 5, 49-89.
9. Beam H. W. and Perry J. J., 1974 – Microbial degradation of cycloparaffinic hydrocarbons via co-metabolism and commensalism, J. Gen. Microbiol. 82, 163-169.
10. Bhattacharyya S., Klerks P. L. and Nyman J. A., 2003 – Toxicity to freshwater organisms from oils and oil spill chemical treatments in laboratory microcosms, Environ. Poll. 122, 205-215.
11. Boonchan S., Britz M. L. and Stanley G. A., 2000 – Degradation and mineralization of high-molecular weight polycyclic aromatic hydrocarbons by defined fungal-bacterial co cultures, Appl. Environ. Microbiol. 66, (3), 10.
12. Bouchez N. M., Rakatozafy H, Marchal R., Leveau J. Y. and Vandecasteele J. P., 1999 – Diversity of bacterial strains degrading hexadecane in relation to the mode of substrate uptake, J. Appl. Microbiol. 86, 421-428.
13. Bruheim P., Bredholt H. and Eimhjellen K., 1997 – Bacterial degradation of emulsified crude oil and the effect of various surfactants, Canadian J. Microbiol. 43, 17-22.
14. Chakraborty R., O'Connor S. M., Chan E. and Coates J. D., 2005 – Anaerobic degradation of benzene, toluene, ethyl benzene, and xylene Compounds by Dechloromonas strain RCB, Appl. Environ. Microbiol, 71, (12), 8649-8655.
15. Chavan A. and Mukherji S., 2010 – Response of an algal consortium to diesel under varying culture conditions, Appl. Biochem. Biotechnol. 160, (3), 719-729.
16. Chen L. T., Chang J. S. and Young C. C., 2008 – Exopolysaccharides produced by Gordonia alkanivorans enhances bacterial degradation activity for diesel, Biotech. Lett. 30, (7), 1201-1206.
17. Christofi N. and Ivshina I. B., 2002 – Microbial surfactants and their use in field studies of soil remediation, J. Appl. Microbiol. 93, 915-929.
18. Dagher F., Deziel E., Lirette P., Paquette G., Bisaillon J. G. and Villemur R., 1997 – Comparative study of five polycyclic aromatic hydrocarbon degrading bacterial strains isolated from contaminated soils, Canadian J. Microbiol., 43, 368-377.
19. Davis B., Eagle D. and Finney B., 1993 – Pollution and the soil, in: Soil management, 5th Edit, Chap. 18, 252-271.

20. Desai J. D. and Banat I. M., 1997 – Microbial production of surfactants and their commercial potential, Microbiol. Mol. Biol. Rev. 61, 47-64.
21. Facundo J. M., Vanessa H. and Teresa M. L., 2001 – Biodegradation of diesel oil in soil by a microbial consortium, Water Air Soil Poll. 128, 313-320.
22. Fatima M. B., Iwona B. B., Claire G.C., Edith E. G. and Lourdes M. I., 2005 – Degradation and corrosive activities of fungi in a diesel-mild steel-aqueous system, World J. Microbiol. Biotechnol. 21, (2), 135-142.
23. Fleck L. C., Bicca F. C. and Ayub M. A. Z., 2000 – Physiological aspects of hydrocarbon emulsification, metal resistance and DNA profile of biodegrading bacteria isolated from oil polluted sites, Biotech., Lett. 22, 285-289.
24. Furukawa K., 1994 – Genetic systems in soil bacteria for the degradation of polychlorinated biphenyls, in: G. R.Chaudhry (ed.), Biological degradation and bioremediation of toxic chemicals, Chapman and Hall Publishing Co. Chap. 2, 33-46.
25. Ganesh A. and Lin J., 2009 – Diesel degradation and biosurfactant production by Gram-positive isolates, African J. Biotechnol. 8, (21), 5847-5854.
26. Georgiou G., Lin S. C. and Sharma M. M., 1992 – Surface active compounds from microorganisms, Bio/Tech. 10, 60-65.
27. Hanson J. R., Macalady J. L., Harris D. and Scow K. M., 1999 – Linking toluene degradation with specific microbial populations in soil, Appl. Environ. Microbiol. 65, (12), 5403-5408.
28. Hanson K. G. and Desai A. J., 1996 – Intergeneric protoplast fusion between Acinetobacter sp. A3 and Pseudomonas putida DP99 for enhanced hydrocarbon degradation, Biotechnol. Lett. 18, (12), 1369-1374.
29. Harrison A. B., Ripley M. B., Dart R. K., Betts W. B. and Wilson A. J., 1996 – Enzy. Microb. Technol. 26, (5 and 6), 388-393.
30. Heitkamp M. A., Freeman J. P. and Cerniglia C. E., 1987 – Naphthalene biodegradation in environmental microcosms: estimates of degradation rates and characterization of metabolites, Appl. Environ. Microbiol. 53, (1), 129-136.
31. Herman D. C., Artiola J. F. and Miller R. M., 1995 – Removal of cadmium, lead, zinc from soil by rhamnolipid biosurfactant, Environ. Sci. Technol. 29, 2280-2285.
32. Hong J. H., Kim J., Choi O. K., Cho K. S. and Ryu H. W., 2005 – Characterization of a diesel-degrading bacterium, Pseudomonas aeruginosa IU5, isolated from oil-contaminated soil in Korea, World J. Microbiol. Biotechnol., 21, (3), 381-384.
33. Junior J. S., Mariano A. P. and Angelis D. F., 2009 – Biodegradation of biodiesel/diesel blends by Candida viswanathii, African J. Biotechnol. 8, (12), 2774-2778.
34. Kapley A., Purohit H. J., Chhatre S., Shanker R., Chakrabarti T. and Khanna P., 1999 – Osmotolerance and hydrocarbon degradation by a genetically engineered microbial consortium. Biores. Technol. 67, 241-245.
35. Karanth N. G. K., Deo P. G. and Veenanadig K., 1999 – Microbial production of biosurfactants and their importance, Curr. Sci. 77, 116-126.
36. Kenneth E. H., 1995 – Mechanisms for polycyclic aromatic hydrocarbon degradation by ligninolytic fungi, Environ. Health Perspec. 103, 41-43.
37. Li Y. Q., Liu H. F., Tian Z. L., Zhu L. H., Wu Y. H. and Tang H. Q., 2008 – Synergetic effect of Mycobacterium and filamentous fungi, Biomed. Environ. Sci. 21, (3), 181-187.
38. Margesin R. and Schinner F., 2006 – Effect of temperature on oil degradation by psychrotrophic yeast in liquid culture and in soil, FEMS Microbiol. Ecol. 24, (3), 243-249.
39. McCrary M. D., Panzer D. E. and Pierson M. O., 2003 – Oil and gas operations offshore California: Status, risks, and safety, Mar. Ornithol. 31, 43-49.
40. Saadoun I., 2002 – Isolation and characterization of bacteria from crude petroleum oil contaminated soil and their potential to degrade diesel fuel, J. Basic Microbiol., 42, 422-430.

41. M. B. Fatima, Iwona B. B., Claire G. C., Edith E. G., Lourdes M. I., 2005 – Degradation and corrosive activities of fungi in a diesel-mild steel-aqueous system, World J. Microbiol.Biotechnol, 21, (2), 135-142.
42. Obuekwe C. O., Badrudeen A. M., Al-Saleh E. J. Mulder L., 2005 – Growth and hydrocarbon degradation by three desert fungi under conditions of simultaneous temperature and salt stress, Inter. Biodeter. Biodegrad, 56, (4), (2005), 197-205.
43. Chavan A. and Mukherji S. 2010 – Response of an algal consortium to diesel under varying culture conditions, Appl. Biochem. Biotechnol. 160, (3), 719-729.
44. Kapley A., Purohit H. J., Chhatre S., Shanker R., Chakrabarti T. and Khanna P., 1999 – Osmotolerance and hydrocarbon degradation by a genetically engineered microbial consortium. Biores. Technol. 67, 241-245.
45. Barac T., Taghavi S., Borremans B., Provoost A., Oeyen L., Colpaert J. V., Vangronsveld J. and Lelie D., 2004 – Engineered endophytic bacteria improve phytoremediation of water-soluble, volatile, organic pollutants, Nature Biotechnol. 22, 583-588.
46. Barnum S. R. – Microbial biotechnology, in: Biotechnology, Chap. 5, 1995, pp. 49-89.
47. Furukawa K. – Genetic systems in soil bacteria for the degradation of polychlorinated biphenyls, in: G.R.Chaudhry (ed.), Biological degradation and bioremediation of toxic chemicals, Chapman and Hall Publishing Co. Chap. 2, 1994, 33-46.
48. McGillivray R. and Shiaris M. P., 1994 – Microbial ecology of polycyclic aromatic hydrocarbons degradation coastal sediments, in: G.R.Chaudhry (ed.), Biological degradation and bioremediation of toxic chemicals, Chapman and Hall Publishing Co. Chap. 6, 125-147.
49. Sharma A. and Rehman M. B., 2009 – Laboratory scale bioremediation of diesel hydrocarbon in soil by indigenous bacterial consortium, Indian J. Expt. Biol. 47, 266-269.
50. Talaie A. R., Talaie M. R. and Jafarzadeh N., 2009 – Optimizing biodegradation of floating diesel fuel Cont.Wastewater.
51. Piehler M. F., Swistak J. G., Pinckney J. L., Paerl H. W., 1999 – Stimulation of diesel fuel biodegradation by indigenous nitrogen fixing bacterial consortia, Microb. Ecol.
52. Pichtel J. and Liskanen P., 2001 – Degradation of Diesel Fuel in Rhizosphere Soil, Environ. Eng. Sci.18, (3), 145-157.
53. Li Y. Q., Liu H. F., Tian Z. L., Zhu L.H., Wu Y.H., Tang H. Q., 2008 – Synergetic effect of Mycobacterium and filamentous fungi, Biomed. Environ. Sci. 21, (3), 181-187.
54. C. Singh and J. Lin, 2009 – Isolation and characterization of diesel oil degrading indigenous microorganisms in Kwazulu-Natal, South Africa. African J. Biotechnol. 8, (12), 2774-2778.
55. Hong J. H., Kim J., Choi O. K., Cho K. S. and Ryu H. W., 2005 – Characterization of a diesel-degrading bacterium, Pseudomonas aeruginosa IU5, isolated from oil-contaminated soil in Korea, World J. Microbiol. Biotechnol. 21, (3), 381-384.
56. Nikakhtari H., Kumar P., Nemati M., Gordon H. A., 2004 – Biodegradation of diesel oil in a baffled roller bioreactor, J. Chem. Technol. Biotechnol., 84, (4), 525-532.
57. Mukherji S., Jagadevan S., Mohapatra G. and Vijay A., 2004 – Biodegradation of diesel oil by an Arabian Sea sediment culture isolated from the vicinity of an oil field, Biores. Technol. 95, (3), 281-286.
58. Margesin R. and Schinner F., 1999 – Biodegradation and bioremediation of hydrocarbons in extreme environments, Appl. Microbiol. Biotechnol. 56, 650-663.
59. Awasthi N., Kumar A., Makkar R. and Cameotra S. S., 1999 – Biodegradation of soil applied endosulfan in the presence of a biosurfactant, J. Environ. Sci. Health (B), 34, 793-803.
60. Georgiou G., Lin S. C. and Sharma M. M., 1992 – Surface active compounds from microorganisms, Bio/Tech. 10, 60-65.

61. Fleck L. C., Bicca F. C., Ayub M. A. Z., 2000 – Physiological aspects of hydrocarbon emulsification, metal resistance and DNA profile of biodegrading bacteria isolated from oil polluted sites, Biotech. Lett. 22, 285-289.
62. Banat I. M., Makkar R. S. and Cameotra S. S., 2000 – Potential commercial applications of microbial surfactants, Appl. Microbiol. Biotechnol. 53, 495-508.
63. Desai J. D. and Banat I. M. 1997 – Microbial production of surfactants and their commercial potential, Microbiol. Mol. Biol. Rev. 61, 47-64.
64. Rosenberg M. and Rosenberg E., 1981 – Role of adherence in growth of Acinetobacter calcoaceticus RAG-1 on hexadecane, J. Bacteriol. 148, 51-57.
65. Aronstein B. N., Calvillo Y. M., Alexander M., 1991 – Effects of surfactants at low concentrations on desorption and biodegradation of sorbed aromatic compounds in soil, Environ. Sci. Technol. 25, 1728-1731.
66. Karanth N. G. K., Deo P. G. and Veenanadig K., 1999 – Microbial production of biosurfactants and their importance, Curr. Sci., 77, 116-126.
67. Balogun S. A. and Fagade O. E., 2008 Screening for surface-active agent producing bacteria from diesel oil polluted tropical soil, World Appl.Sci. J. 3, (6), 930-933.
68. Bouchez N. M., Rakatozafy H., Marchal R., JLeveau Y. and Vandecasteele J. P., 1999 – Diversity of bacterial strains degrading hexadecane in relation to the mode of substrate uptake, J. Appl. Microbiol. 86, 421-428.
69. Rouse J. D., Sabatini D. A., Suflita J. M. and Harwell J. H., 1994 – Influence of surfactants on microbial degradation of organic compounds, Crit. Rev. Environ. Sci. Technol. 24, 325-370.
70. Herman D. C., Artiola J. F. and Miller R. M., 1995 – Removal of cadmium, lead, zinc from soil by rhamnolipid biosurfactant, Environ. Sci. Technol. 29, 2280-2285.
71. Navon-Venezia S., Zosim Z., Gottlieb A., Legmann R., Carmeli S., Ron E. Z. and Rosenberg E., 1995 – A new bioemulsifier from Acinetobacter radioresistans, Appl. Environ. Microbiol. 61, 3240-3244.
72. Whang L. M., Liu P.W., Ma C. C., Cheng S. S., 2009 – Application of biosurfactants, rhamnolipid, and surfactin, for enhanced biodegradation of diesel-contaminated water and soil, J. Haz. Mat. 164, (2-3), 1045-1050.
73. Ganesh A. and Lin J., 2009 – Diesel degradation and biosurfactant production by Gram-positive isolates, African J. Biotechnol. 8, (21), 5847-5854.
74. Santhini K., Myla J., Sajani S. and Usharani G., 2009 – Screening of Micrococcus sp. from oil contaminated soil with reference to bioremediation, Bot. Res. Int. 2, (4), 248-252.
75. Harrison A. B., Ripley M. B., Dart R. K., Betts W. B. and Wilson A. J. – Enzy. Microb. Technol. 26, (5 and 6), 388-393.
76. Chen L. T., Chang J. S. and Young C. C., 2008 – Exopolysaccharides produced by Gordonia alkanivorans enhances bacterial degradation activity for diesel, Biotech. Lett. 30, (7), 1201-1206.
77. Alexander M., 1999 – Biodegradation of chemicals of environmental concern, Sci. 211, (1981), 132-138.

A NEW PERSPECTIVE ON McKINNEY'S WASTEWATER MODEL

Letiţia OPREAN *
and *Horea OLOSUTEAN* **

* Lucian Blaga University of Sibiu, Faculty of Agricultural Sciences, Food Industry and Environmental Protection, Department of Biotechnologies, Raţiu Street 7-9, RO-550012, Sibiu, Romania, letitia.oprean@ulbsibiu.ro
** Lucian Blaga University of Sibiu, Faculty of Sciences, Department of Ecology an Environmental Protection, Raţiu Street 5-7, RO-550012, Sibiu, Romania, mesaje.facultate@yahoo.com

KEYWORDS: McKinney's Model, wastewater, activated sludge, correction, optimization.

ABSTRACT

Domestic wastewater represents one of the main problems for aquatic ecosystems located near urban areas, due to the supplementary contribution of organic and toxic substances. An effective treatment of such wastewater can assure the quality of those ecosystems, and this can only be achieved by the correct application of the proper mathematical model. To facilitate this outcome, Ross McKinney's Wastewater Model, the oldest complete model in the field, was discussed and optimized. Relationships between oxygen requirements and equivalents are deduced from the basic equations of the model, and presented in the form of constant based equations, using only three of the system's constants. A small correction is applied to the sludge recirculation – excess sludge removal part of the model, in order to give a more uniform structure to the model. Even, better a unitary approach of the model is presented, using the same equations for all the three types of wastewater treatment plant considered by McKinney.

RÉSUMÉ: Une nouvelle perspective sur le Modèle McKinney.

Les eaux usées ménagères représentent un des grands problèmes des écosystèmes aquatiques situées en proximité des agglomérations urbaines à cause de l'apport supplémentaire de substances organiques et toxiques. Une épuration efficace a le rôle de restaurer la qualité de ces écosystèmes, ce qui peut se faire uniquement en appliquant correctement le modèle mathématique de Ross McKinney, le plus ancien modèle complet d'une installation d'épuration, revu ici dans sa forme discutée et optimisée. Les relations entre les nécessaires et les équivalents d'oxygène sont déduites à partir des équations de base du modèle et sont présentées sous la forme des équations basées uniquement sur trois constantes du système. Une correction mineure a été appliquée à la partie du modèle décrivant le système de recirculation et l'élimination de l'excès de boue active, afin d'offrir une structure plus uniforme au modèle. De plus, on présente une approche unitaire du modèle, en utilisant les mêmes équations pour les trois types d'installations de traitement de l'eau usée prises en considération par McKinney.

REZUMAT: O nouă perspectivă asupra Modelului lui McKinney.

Apele uzate menajere reprezintă una din marile probleme ale ecosistemelor acvatice aflate în proximitatea aglomerărilor urbane, datorită aportului suplimentar de substanţe organice şi toxice. O epurare corespunzătoare are rolul de a asigura calitatea acestor ecosisteme acvatice, iar aceasta nu poate fi realizată decât prin aplicarea corectă a unui model matematic potrivit. În acest sens, modelul matematic al lui Ross McKinney, cel mai vechi model complet al unei instalaţii de epurare, a fost discutat şi de asemenea optimizat. Relaţii între necesarele şi echivalenţii de oxigen sunt deduse din ecuaţiile de bază ale modelului şi prezentate sub forma unor ecuaţii bazate pe constante, cu ajutorul a doar trei astfel de constante ale sistemului. O mică corecţie este aplicată acelei părţi a modelului care descrie sistemul cu recirculare şi eliminarea excesului de nămol activ, cu scopul de a oferi o structură mai uniformă modelului. În plus, este prezentată o abordare unitară asupra modelului, folosind aceleaşi ecuaţii pentru toate cele trei tipuri de instalaţii de tratare a apei uzate luate în considerare de McKinney.

INTRODUCTION

The waste water treatment is considered as one of the key element in sustaining the quality of a wide variety of the aquatic environments in the actual economic and human population context. High organic concentration of the effluent consist the main problem in the matter, leading to the modification of oxygen balances, eutrophication and, finally, to drastic changes in community composition in the affected areas, posing a clear danger to several protected aquatic species or to important aquatic habitats. A proper treatment of such wastewaters cannot be obtained in the absence of a specific mathematical model of the natural processes involved, and the choosing of the right model for a certain/specific station is considered as a problem in itself. Also, the application of the model must be as correct as possible in a certain circumstance, errors appearing throughout the application having as result an incomplete treatment and a high organic concentration of the effluent.

On this area of interest, Ross McKinney's model (1962) is the oldest mathematical model of the wastewater treatment process, based on Monod bacterial growth principles (1949). Although many other models were developed during the last 50 years, this one is considered a breakthrough in the field and the starting point for all the others, including the widely used models of Lawrence and McCarty (1970) or Eckenfelder (1971) (Orhon and Artan, 1994).

The model was used recently as a basis for model developers, and it still is a fertile ground for improvement (Vesilind, 2003; Graham and Smith, 2004; Ekama et al., 2006, 2007). Some parts of the model are not thoroughly exploited, even if they hide information interesting only for modelers, and not for wastewater engineers or plant operators. Even more, equations from the model are inconsistent, mostly because of a wrong starting expression.

The final equations for the three system types analyzed (without sludge recirculation, with sludge recirculation and with excess sludge removal) need a mathematical revision, since they can be expressed in a much simpler way.

MATERIAL AND METHODS

Basic equations

McKinney stated that protoplasm, although a heterogeneous mix of hundreds of substances, has a rather uniform chemical structure, hence the energy needed to produce one unit is constant, not depending on the chemical structure of metabolized material. From this specific point of view, it is possible to emit a clear relation between energy and synthesis.

Splitting the energy from the system into the one used for synthesis and the one used for respiration, McKinney described the system using two simple relations: *metabolized organic matter = synthesized protoplasm + energy required for synthesis*, reflected in equation [1], and *protoplasm accumulation = synthesized protoplasm – protoplasm consumed through endogenous respiration*, reflected in equation [2].

$$\frac{\Delta EO_m}{\Delta t} = \frac{\Delta EO_s}{\Delta t} - \frac{\Delta O_s}{\Delta t} \qquad [1]$$

$$\frac{\Delta EO_p}{\Delta t} = \frac{\Delta EO_s}{\Delta t} - \frac{\Delta O_e}{\Delta t} \qquad [2]$$

McKinney described the system with the use of oxygen (*O*) or oxygen equivalents (*EO*) required by the reactions.

He added indices to point out which component of the reaction was in discussion: *m* for organic substance metabolization, *s* for protoplasm synthesis, *e* for endogenous respiration and *p* for protoplasm accumulation.

With the use of reaction constants, he then described the relations between synthesis and energy, and between the bacterial mass and protoplasm synthesis or endogenous respiration:

$$\frac{\Delta O_s}{\Delta t} = k_1 * \frac{\Delta EO_s}{\Delta t} \qquad [3]$$

$$\frac{\Delta O_e}{\Delta t} = k_2 * M \qquad [4]$$

$$\frac{\Delta EO_s}{\Delta t} = k_3 * M \qquad [5]$$

Organic matter balance

The model contains a single equation for organic matter balance, regardless of the type of system used, representing the relation *substrate accumulation = material entering the system – material removed from the system – material consumed in reaction*:

$$V * \frac{\Delta C}{\Delta t} = Q * C_0 - Q * C - k_4 * V * C \qquad [6]$$

The symbols used to describe the equations of the model are presented in the table number 1.

Because it is a steady-state system, we can consider the left part of the equation equal to zero, and we can obtain the relation between C and C_0, by dividing to V and considering $T = Q/V$:

$$C = \frac{C_0}{k_4 * T + 1} \qquad [7]$$

Table 1: Notations used in describing the model.

Notations	
C	concentration of substances from the aeration basin
C_0	concentration of substances from the influent (initial concentration)
Q	influent capacity
M	mass of active bacteria
E	endogenous metabolism products
N	total mass of suspensions from the aeration basin
T	hydraulic retention time
V	volume of the aeration basin
t	time
X	recirculation fraction
w	influent fraction removed with excess sludge
s	sedimentation coefficient expressing the concentration of suspensions removed with excess sludge
k	process constant

Bacterial mass balance

At this point, McKinney offers three different equations, one for each type of wastewater system (Fig. 1), starting from the relation *bacterial mass accumulation = synthesized bacterial mass – endogenous consumption of bacterial mass – bacterial mass removed from the system*:

$$V * \frac{\Delta M}{\Delta t} = k_5 * V * C - k_6 * V * M - Q * M \qquad [8]$$

$$V * \frac{\Delta M}{\Delta t} = k_5 * V * C - k_6 * V * M - Q * x * M \qquad [9]$$

$$V * \frac{\Delta M}{\Delta t} = k_5 * V * C - k_6 * V * M - (1 - w) * x * Q * M - w * s * Q * M \qquad [10]$$

The equations refer to wastewater systems without sludge recirculation [8], with sludge recirculation [9] and with sludge recirculation and excess sludge removal [10], and they differ only by the last term, expressing the bacterial mass removed from the system.

If we apply the steady-state condition, divide by *V* and rearrange the terms, we can extract formulas for *M*, one for each of the presented systems:

$$M = \frac{k_5 * C}{\frac{1}{T} + k_6} \qquad [11]$$

$$M = \frac{k_5 * C}{\frac{x}{T} + k_6} \qquad [12]$$

$$M = \frac{k_5 * C}{\frac{1}{T} * (x - x * w + s * w) + k_6} \qquad [13]$$

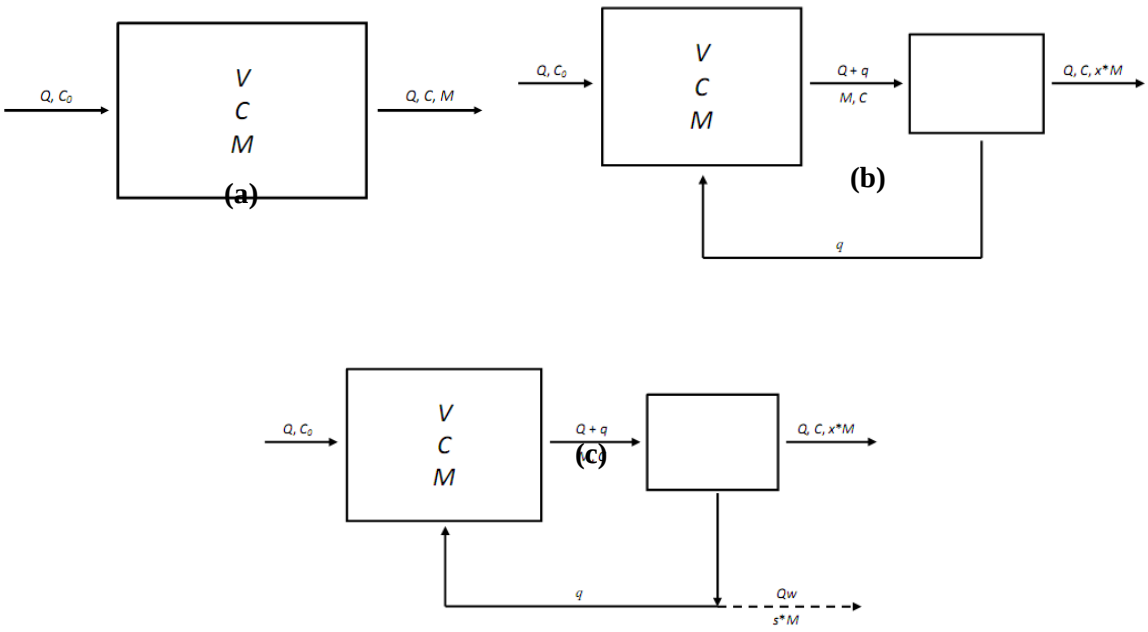


Figure 1: Wastewater treatment systems according to McKinney's Model:
a – without sludge recirculation, b – with sludge recirculation;
c – with sludge recirculation and excess sludge removal.

Endogenous metabolism

McKinney described this part of the process by the relation: *accumulation of mass in the endogenous process = substance produced by M trough endogenous metabolism – mass eliminated from the endogenous process*. For the three situations found in wastewater practice and described above, he gave three equations, differing only on the last term:

$$V * \frac{\Delta E}{\Delta t} = k_7 * M * V - Q * E \qquad [14]$$

$$V * \frac{\Delta E}{\Delta t} = k_7 * M * V - Q * x * E \qquad [15]$$

$$V * \frac{\Delta E}{\Delta t} = k_7 * M * V - Q * w * x * E - Q * w * s * E \qquad [16]$$

Again, if we consider a steady-state model, divide by *V* and rearrange the terms, we have three formulas for *E*, one for each type of system:

$$E = k_7 * M * T \quad [17]$$

$$E = \frac{k_7 * M * T}{x} \quad [18]$$

$$E = \frac{k_7 * M * T}{x + s * w} \quad [19]$$

Total mass balance

The total mass of suspensions, *N*, is the sum of *M*, *E* and two other components: N_t, the metabolizable biomass fraction that leaves the bioreactor unmetabolized, and N_m, unmetabolizable suspensions. The formulas for the three types of systems are, according to McKinney:

$$N = M * (1 + k_7 * T) + Nt + Nm \quad [20]$$

$$N = M * \frac{(1 + k_7 * T)}{x} + Nt + Nm \quad [21]$$

$$N = M * \frac{(1 + k_7 * T)}{x + s * w} + Nt + Nm \quad [22]$$

RESULTS AND DISCUSSION

McKinney's Model was the first complete model of a wastewater treatment plant, and it was successfully used and still is used by many such plants.

However, some equations are not fully investigated by the author, probably because they are not very important for plant operators, but they can prove useful to biochemists, microbiologists or other scientists in this area of interest.

Oxygen relations

Such equations are the ones showing the relations between oxygen necessaries and equivalents of the different system components, all of them constants and describable with the use of only three system constants: k_1, k_2 and k_3. These constants define, according to Ognean and Vaicum (1987), the relation between synthesis and energy (k_1), endogenous respiration (k_2) and bacterial growth (k_3).

For example, if we take equation [3], we can simplify Δt, since time is the same, and rearrange the terms:

$$\frac{\Delta EO_s}{\Delta O_s} = \frac{1}{k_1} \qquad [23]$$

If we combine equations [1] and [3], we have:

$$\frac{\Delta EO_m}{\Delta t} = (1 + k_1) * \frac{\Delta EO_s}{\Delta t} \qquad [24]$$

which gives us another relation:

$$\frac{\Delta EO_m}{\Delta EO_s} = k_1 + 1 \qquad [25]$$

Equation [3] can also be resolved for ΔEO_s:

$$\frac{\Delta EO_s}{\Delta t} = \frac{1}{k_1} * \frac{\Delta O_e}{\Delta t} \qquad [26]$$

which, inserted into equation [1], leads to:

$$\frac{\Delta EO_m}{\Delta t} = (1 + \frac{1}{k_1}) * \frac{\Delta O_s}{\Delta t} \qquad [27]$$

transformable into:

$$\frac{\Delta EO_m}{\Delta O_s} = 1 + \frac{1}{k_1} \qquad [28]$$

Furthermore, we can extract *M* from equations [4] and [5], and we can oppose the results:

$$\frac{\Delta EO_s}{\Delta t} * \frac{1}{k_3} = \frac{\Delta O_e}{\Delta t} * \frac{1}{k_2} \qquad [29]$$

Simplifying Δt, we have:

$$\frac{\Delta EO_s}{k_3} = \frac{\Delta O_e}{k_2} \qquad [30]$$

or

$$\frac{\Delta O_e}{\Delta EO_s} = \frac{k_2}{k_3} \qquad [31]$$

At this point, we can use these results to get relations between all parameters. For example, if we replace ΔEO_s from equation [28] with its equivalent from equation [31], we have the relation between ΔO_e and ΔEO_m:

$$\frac{\Delta EO_m}{\Delta O_e} = \frac{k_3 * (k_1 + 1)}{k_2} \qquad [32]$$

Relations between EO_p, on one side, and EO_s or O_e, on the other side, can be obtained by sequently replacing the equivalents of the two parameters from equation [31] into equation [2]:

for EO_s:

$$\frac{\Delta EO_p}{\Delta t} = \frac{\Delta EO_s}{\Delta t} - \frac{\Delta EO_s}{\Delta t} * \frac{k_2}{k_3} \quad [33]$$

$$\frac{\Delta EO_p}{\Delta t} = \frac{\Delta EO_s}{\Delta t} * (1 - \frac{k_2}{k_3}) \quad [34]$$

and, simplifying Δt:

$$\frac{\Delta EO_p}{\Delta EO_s} = 1 - \frac{k_2}{k_3} \quad [35]$$

for O_e:

$$\frac{\Delta EO_p}{\Delta t} = \frac{\Delta O_e}{\Delta t} * \frac{k_3}{k_2} - \frac{\Delta O_e}{\Delta t} \quad [36]$$

$$\frac{\Delta EO_p}{\Delta t} = \frac{\Delta O_e}{\Delta t} * (\frac{k_3}{k_2} - 1) \quad [37]$$

again, simplifying Δt:

$$\frac{\Delta EO_p}{\Delta O_e} = \frac{k_3}{k_2} - 1 \quad [38]$$

The relation between O_s and O_e can be easily deduced combining equations [3] and [31]:

$$\frac{\Delta O_s}{\Delta t} = \frac{k_1 * k_3}{k_2} * \frac{\Delta O_e}{\Delta t} \quad [39]$$

Simplifying Δt and rearranging the terms, we have:

$$\frac{\Delta O_s}{\Delta O_e} = \frac{k_1 * k_3}{k_2} \qquad [40]$$

EO_p's relations with O_s and EO_m are much difficult to extract. For the first case, we start with equation [2], in which we replace EO_s with its equivalent from equation [23], and O_e with its equivalent from equation [40]:

$$\frac{\Delta EO_p}{\Delta t} = \frac{\Delta O_s}{\Delta t} * \frac{1}{k_1} - \frac{\Delta O_s}{\Delta t} * \frac{k_2}{k_1 * k_3} \qquad [41]$$

transformable into:

$$\Delta EO_p = \Delta O_s * \frac{(k_3 - k_2)}{k_1 * k_3} \qquad [42]$$

or

$$\frac{\Delta EO_p}{\Delta O_s} = \frac{k_3 - k_2}{k_1 * k_3} \qquad [43]$$

For the second case, we start again from equation [3], replacing the elements from its right side with their equivalents from equations [25] and [32], respectively, obtaining:

$$\frac{\Delta EO_p}{\Delta t} = \frac{\frac{1+k_1}{\Delta EO_m}}{\Delta t} - \frac{\frac{1+k_1}{\Delta EO_m} * \frac{k_3}{k_2}}{\Delta t} \qquad [44]$$

If we simplify Δt, find the lowest common denominator and arrange the terms in a favorable way, we have:

$$\Delta EO_m * \Delta EO_p = \frac{(1+k_1)*(k_2-k_3)}{k_2} \qquad [45]$$

We obtained in this way all the possible relations between the oxygen necessaries and equivalents from McKinney's Model, using mathematical relations between only three system constants. The equations that reflect these relations are [23], [25], [28], [31], [32], [35], [38], [40], [43] and [45].

Model correction

Differential wastewater models from the '60s and the '70s are based on the same general material balances, but the terms of the equations are at the choice of the model developer. Because of this, it is possible for a certain parameter to be either inappropriate, or incomplete.

McKinney's Model is not free of this problem. In equation [10], the term defining *endogenous consumption of bacterial mass* is transcribed as (1-*w*)**x***Q***M*; as a difference, in equation [16], the term defining *substance produced by M trough endogenous metabolism* is *w***X***Q***E*, an incorrect approach, since w is the fraction removed from the system; the correct term, for the model's cursivity and consistency, would be (1-*w*)**X***Q***E* and the final equations for the wastewater plant with excess sludge removal will become:

$$E = \frac{k_8 * M * T}{x - w * x + w * s} \qquad [46]$$

$$N = M * \frac{(1+k_8 * T)}{x - w * x + s * w} + Nt + Nm \qquad [47]$$

The term *x*-*x***w*+*s***w* from these two equations is identical with the one from equation [13], making the model much more stable.

Even more, if we take the equations reflecting the value of *M* – [11], [12] and [13], E – [12], [18] and [21], and *N* – [13], [46] and [47], this term is valid for every type of wastewater system, and the equations for the sludge recirculation and excess sludge removal system are universal.

If we eliminate sludge removal from the system, *w*, influent fraction removed with excess sludge, becomes zero, and the term *x*-*x***w*+*s***w* reduces to *x*, transforming equation [13] into [12], [46] into [18], and [47] to [21]; if we eliminate recirculation, *x*, the recirculation fraction, becomes 1, because the entire sludge quantity is removed after a cycle, transforming equation [12] into [11], [18] into [17], and [21] into [20].

This approach is independent of the current model correction, because it can be also applied to equations [19] and [22].

CONCLUSIONS

McKinney's Model is the first complete model of a wastewater plant, and its structure and expression are the basis for many of the latter models.

Although criticized and considered obsolete (Benefield and Randall, 1977), it still raises interest in the modeling field, and can be discussed and improved.

Basic equations of the model can be interpreted in order to obtain constant relations between oxygen necessaries and equivalents from the system, which can be later used for any given system.

McKinney's choice of equations members can be characterized as inconsistent at some situations, and a much more coherent approach is presented.

The three different situations given by the author for wastewater plant types are, in fact, described by the system with sludge recirculation and excess sludge removal, the other two being particular situations of this one.

REFERENCES

1. Benefield L. D. and Randall C. W., 1977 – Evaluation of a comprehensive kinetic model for the activated sludge process. Journal of the Water Pollution Control Federation, 49, (7): 1636-1641.
2. Graham D. W. and Smith V. H., 2004 – Designed ecosystem services: application of ecological principles in wastewater treatment engineering. Frontiers in Ecology and the Environment, 2: 199-206.
3. Ekama G. A., Sötemann S. W. and Wentzel M. C., 2006 – Mass balance-based plant-wide wastewater treatment plant models – Part 3: Biodegradability of activated sludge organics under anaerobic conditions. Water SA 32, (3), 287-296.
4. Ekama G. A., Sötemann S. W. and Wentzel M. C., 2007 – Biodegradability of activated sludge organics under anaerobic conditions. Water Research 41, (1), 244-252.
5. Eckenfelder W. W. Jr., 1971 – Activated sludge and extended aeration, process design in water quality engineering – new concepts and developements. Vanderbilt Univ., Nashville, Tenn.
6. Lawrence A. W. and McCarty P. L., 1970 – Unified theory for biological treatment design and operation. Journal of the Sanitary Engineering Division: Proceedings of the American Society of Civil Engineers, 96, 757-778.
7. McKinney R. E., 1962 – Mathematics of completely-mixing activated sludge. J. Sanit. Eng. Div. (ASCE Proceedings) 88, 87-113.
8. Monod J., 1949 – The growth of bacterial cultures. Annu. Rev. Microbiol. 3, 371-393.
9. Ognean T. and Vaicum L. M., 1987 – Modelarea proceselor de epurare biologică. Edit. Acad. R.S.R., Bucureşti. (in Romanian).
10. Orhon D. and Artan N., 1994 – Modelling of activated sludge systems. Techomic Publishing Company, Lancaster, PA.
11. Vesilind P. A., 2003 – Water treatment plant design. Water Environment Federation.