PREFACE

Amphibian skin has, over the last century, proven to contain a treasure-trove of biologically active compounds. Since that time further investigation has added hundreds of such compounds to the list of active substances from amphibian skin. Peptides, proteins, bufadienolides steroids and alkaloids (tetrodotoxins, biogenic amines and lipophilic alkaloids) represent the main compounds found in the amphibian skin. This book discusses the anatomy of amphibians. It also provides topics on the ecological significance and conservation strategies of these animals.

Chapter 1 – Hydropower, generated primarily from hydroelectric dams, is a renewable energy source that provides electricity and helps reduce carbon emissions. In the Mediterranean region the construction of dams is also considered fundamental for maintaining water supplies during the summer, primarily for irrigation and domestic water consumption. The construction of dams results in the conversion of small temporary streams and ponds into permanent reservoirs. The massive alteration in hydrologic cycles and associated physical and chemical processes creates new conditions that promote the population growth of invasive species which are detrimental to the reproduction of most indigenous amphibian species. Additionally, the flooding of certain areas affects multiple critical habitats used by adult amphibians, and flooding may additionally impose barrier effects to amphibian movement and dispersion. Utilizing a case study in Portugal, the Baixo Sabor Dam, the authors propose steps to compensate for the impact on amphibian communities, namely the creation of small temporary ponds. Preliminary monitoring results are also analysed and discussed.

Chapter 2 – Metallothioneins (MT) are well conserved ubiquitous metal-binding proteins that constitute a large superfamily of small, cysteine-rich peptides present in some prokaryotes and in all eukaryotes in so far examined. Recently, comparative analyses integrating phylogenetics, functional, and structural approaches unraveled the complex evolutionary pattern of vertebrate metallothioneins, characterized by gene duplication and gene loss events. Data also disclosed for the vertebrate metallothioneins a complexity in the primary structures that might suppose some functional differences in proteins. Up today, there is no evidence of metallothionein multiplicity in reptiles and amphibians and the apparent simplicity of these metallothionein systems contrasts with the MT complexity in mammals, where four members (MT1 to MT4) where found, with a 13-fold amplification of MT1 in humans. To provide a more up-to-date view of the metallothioneins in non-mammalian tetrapods, the still scarce knowledge concerning the primary structure and the evolution of metallothioneins in amphibians has been improved. Data demonstrate an unexpected diversity of metallothionein sequences among amphibians, accompanied by remarkable features in their phylogeny. Phylogenetic analysis also reveals the complexity of vertebrate metallothionein evolution, made by both ancient and more recent events of gene duplication and loss.

Chapter 3 – The ability of amphibians to survive in such a broad diversity of habitat types - potentially pathogen-rich aquatic and terrestrial environments such as slow-flowing rivers, ponds, ditches, the deep leaf-litter and upper soil zones of the forest floor, underground burrows and spaces beneath and holes in rotting logs - may be attributed to the evolution of many different morphological, physiological, biochemical and behavioral adaptations. Thus, an efficient anti-microbial and anti-fungal defense system operating on the skin surface is an evident requirement for survival.

Noxious or toxic secretions in the skin, and the presence of biogenic amines, peptides, bufadienolides, tetrodotoxins and lipophilic alkaloids were provided years ago. Since that time, further investigation has added hundreds of such compounds to the list of active substances from amphibian skin, which has proven to contain a treasure-trove of biologically active compounds. Many of the compounds afford a chemical defense against predators and play several roles, either in the regulation of the physiological functions of the skin and providing protection from skin infections.

The type of biologically active substance found in amphibians appears to have phylogenetic significance. Bufonid toads have cutaneous secretion in high levels of bufadienolids steroids and indole alkylamines. Phenolic amines are typical from *Leptodactylid* frogs, while peptides are the abundant in *Hylid* frog skin secretions. The review discusses the importance of the chemical defense for amphibians, for both defense against predators and survival in microorganism-rich environments.

Chapter 4 – Paleontological record contains facts about the way fish went out of water to live on land. Devonian seas (416-359 Ma) were abundant in fish. This was indeed the "age of fish". Diversified ichthyofauna of that time included Rhipidistia and Dipnoi (class Sarcopterygii). The Late Devonian strata (385-359 Ma) yield remains of animals which advanced in their evolution from fish of the subclass Crossopterygii to amphibians abundant in Carboniferous bogs (359-299 Ma). The skull as well as dental, auditory and locomotory systems of present-day amphibians are close to those of Rhipidistia and Dipnoi. The latter possessed paired fins with a fleshy base consisting of a series of bony supporting elements. In course of further evolution, the fins gradually evolved to limbs. In the Devonian, they developed into the limbs of the early amphibians capable of bearing their weight on land.

In Siberia, in the river basin of Vilyui (left tributary of the Lena r.), in the Emyaksin Formation (Devonian-Carboniferous boundary strata, 370-350 Ma), endemic ichthyofauna of the class Sarcopterygii has been reported since 1967 (Emyaksin-Khayata locality), which shows a wide spectrum of evolutionary diversifications.

Warm, shallow pools of fresh water abounded in cyanobacteria. Their remains were first found by the author on the scales of fossil fish Sarcopterygii at the Emyaksin-Khayata locality.

The cells of cyanobacteria are preserved as microscopic fossil remains replaced by calcium carbonate (microfossils). During the life time, the cells of these microorganisms possess a jelly coat, so the whole of cyanobacterial community represents a jelly mass. Fishes and cyanobacteria inhabiting the water bodies lived in symbiosis. The relations between the symbionts were mutualistic (mutually beneficial). Cyanobacteria liberated oxygen on photosynthesis, which was dissolved in water and thus could be used by fishes. The products of vital activity of fishes such as carbon dioxide and decomposed excrements favored the flourishing of cyanobacteria. In shallow-water pools, Sarcopterygii used their fins to move on the basin floor. Later on, these exercises helped them to survive in the changing environmental conditions.

When water basins got too shallow, cyanobacteria protected fish scales from sunrays with their mucus which held moisture for a long time. As a result, the surface of scales had long remained moist and the skin elastic. Fishes could have lived till the rains or they could move from the dried basins to water-filled ones. They could adapt to biotopes with close-to-terrestrial environmental conditions.

It is likely that in the Late Devonian-Early Carboniferous, cyanobacterial, lake-type shallow-water basins located on the territory of present-day eastern Siberia were, for the fishes of that time, the sites of adaptation, the biotopes of transition from water to land.

Chapter 5 – The Coatzacoalcos River Basin is one of the most important hydrological and ecological regions of Mexico. The Coatzacoalcos estuary has experienced a fast industrial and urban growth which combined with other productive activities such as agriculture and cattle farming have triggered a severe impact in the region's ecosystems. Persistent organic pollutants (POPs) and lead have been identified in several environmental matrices in the region. So far, few efforts have been made to evaluate exposure and effects on wildlife in the area. Amphibian populations have been recognized as biomonitors of changes in environmental conditions. The purpose of this chapter is to present the synthesized main research found, through assessment of exposure biomarkers and sublethal effects in giant toads specimens collected from the Coatzacoalcos River downstream. Lead levels in the blood of toads range from 10.8 to 70.6 μg/dL and are significantly higher in industrial sites. The authors have found a significant decrease of the δ-ALAD activity in blood measuring 35.3 to 78% for the urban-industrial and industrial sites, respectively. In addition, they have identified a strong inverse relationship between the δ-ALAD activity and the levels of lead found in blood ($r=-0.84$, $p<0.001$). Total POPs levels in soil and toad tissues range from 660.5-2,712.9 ng/g d.w. to 55.6-1,2471.9 ng/g l.w., respectively. Differences in POP's levels by site type have not been found. DNA damage found in blood ranges from 0.7 to 4.8 (Olive tail moment) and 7.4 to 16.7 μm (Tail length). DNA damage has been found to be higher at industrial zones compared to urban zones. Our results suggest that the *Rhinella marina* can be considered as a good biomonitor of DNA damage and δ-ALAD activity inhibition. This study provides a data baseline on the PBTs pollution status in soil and giant toads of Coatzacoalcos River downstream, in Veracruz.

Chapter 6 – Despite wetland importance as habitat for early live stages of amphibian species and the legislation designed to protect wetlands, they are currently at risk from a number of sources that include coal mining. It has been estimated that half of the world's total wetlands have been lost already. Within Southern Africa, the trend is similar, with an estimation of more than half of the wetland area in South Africa being lost. A major concern to the conservation of wetlands as habitat for amphibian species in South Africa is the increase of coal mining activities. Coal mining is seen to have serious impacts on wetlands, with impacts ranging from total destruction, to impacts on water quality and change in water flow. South Africa possesses approximately 5 % of the global recoverable coal reserves in the world and is the fifth largest global coal producer.

Currently, about 77 % of South Africa's primary energy needs are provided by coal. Due to the relative lack of suitable alternatives, this situation is unlikely to change over the next 10 years. The Witbank Coalfields represent the largest conterminous area of active coal mining in South Africa and mine de-watering activities result in the discharge of approximately 50 Ml/d of mine water into the upper Olifants River catchment situated in the province of Mpumalanga. Acidification caused by the release of acid mine drainage (AMD) from coal mines in this grassland biome adversely affect wetland biodiversity, resulting in biomass accumulation and the extirpation of sensitive species.

Nevertheless, any detection of a biological recovery in wetlands impacted by AMD in this region requires knowledge of acidification threshold values of sensitive species. This can be used in appropriate biomonitoring programs for aquatic ecosystem resilience and post wetland restoration. The present study was undertaken to determine variation in growth and development of the different life stages of *Xenopus laevis* under pre-restoration conditions of a wetland impacted by AMD in the Mpumalanga region. The objective of the study was to determine the toxicity and adverse effects of AMD on the early live stages of *X. laevis*. Sites were selected along a longitudinal gradient to present various changes in pH values and metal bioaccumulation within the test wetland downstream of a coal mine. By employing static bioassays at each selected site, it was evident that the growth and development of different life stages of *X. laevis* was in close relationship with changes in pH values measured in the surface waters of the studied wetland, while bioaccumulation of the metals Al, Fe, Zn, Cu and Mn correlated positively with an increase in suspended solids. The prerestoration conditions at the different sampling sites indicated a reduction in hatching, reduced survival and growth, as well as the occurrences of malformation in tadpoles.

These indicators will be used in a future monitoring program to determine post wetland restoration conditions.