EFFUSE

Scientific education for advanced

PARTNERSHIP

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Project title:
Environment for the Future by Scientific Education
Partner name:
Pavol Jozef Šafárik University in Košice
Uzhhorod National University
Institute of Development of Carpathian Region
EU Contribution:
364 099, 41€

This educational material was produced with the financial support of the European Union within the project HUSKROUA/1901/6.1/0075 “Environment for the Future by Scientific Education” (EFFUSE) of ENI CBC Programme Hungary-Slovakia-Romania-Ukraine 2014-2020. Its contents are the sole responsibility of Pavol Jozef Šafárik University in Košice and do not necessarily reflect the views of the European Union. More information on the project is available on the links below:

https://effuse.science.upjs.sk/index.php/en/

EFFUSE team of authors

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INTRODUCTION

Living objects in the riverscape offer many opportunities for science education and research. The river ecosystem is dynamic and supports diverse life forms, each of which plays a key role in the ecological balance.

Different species of aquatic plants, algae and phytoplankton can be studied in terms of their adaptation to aquatic life, understanding the process of photosynthesis and water filtration processes and the basis of aquatic food webs and their role in maintaining the ecosystem.
Aquatic invertebrates, such as insects (horseshoe crabs, dragonflies), crustaceans (crayfish), molluscs (snails, bivalves) and invertebrates (leeches, aquatic worms), are essential for understanding food webs, biodiversity and the health of the river ecosystem. They are also indicators of water quality.

The study of different fish species can provide insights into their life cycle, breeding habits, survival patterns and adaptive mechanisms. Fish species vary depending on the river environment and are key to the study of ecology, evolutionary biology and conservation science. They can also serve as bioindicators of the ecological health of a river. Amphibians and reptiles that inhabit river environments can be studied in terms of their life cycles, their adaptation to aquatic and terrestrial environments and also as indicators of the environmental health of the environment and its pollution.

Of course, many other groups of vertebrates also live near rivers. In this publication, we focus in more detail on the fish and amphibians. Their requirements on the environment are characteristic and many species of these vertebrates are protected. This is why systematic scientific education is essential for understanding their bioindicator role.

The publication includes a separate chapter, devoted to present a perspective on science education towards sustainable development, as this global topic is largely absent in the curricula in Slovakia and Ukraine.

EFFUSE team

FISH AS INDICATORS - subject of scientific education

Fish (ACTINOPTERYGII) are a diverse group of vertebrates that sould be studied extensively in the field of scientific education. They are an important model organism for studying various biological processes. Importance of education about fish stems from the need:

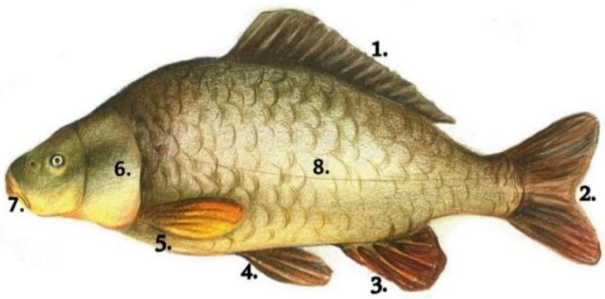
- understanding the role of fish in aquatic ecosystems
- importance of fish conservation and sustainable fishing practices
- learning about fish biology, behavior, and habitat requirements

Fish Morfology

The body of fish can be divided into the head (caput), trunk (truncus), and tail (cauda). Depending on the environment in which they live, the shape of the fish's body can vary. Species inhabiting swiftly flowing waters have a **torpedo-shaped** body to minimize resistance to the current (e.g., brown trout - *Salmo trutta morpha fario*, grayling - *Thymallus thymallus*). Fish living in stagnant or slowly flowing waters with vegetation have a **tall** and **laterally** flattened body, allowing them to maneuver skillfully among various obstacles (e.g., common bream - *Abramis brama*, rudd - *Scardinius erythropthalmus*). Fish that dwell near the bottom often have a flattened upper body, such as the wels catfish (*Silurus glanis*), or even completely flat, as seen in flatfish from the *Pleuronectidae* family. Predatory fish, like the northern pike (*Esox lucius*), have a cylindrical, torpedo-shaped, or fusiform body, with the dorsal and anal fins shifted toward the tail, enabling them to make a strong strike and quickly dart after prey.

Fins serve as the locomotor organs of fish. On their body (Fig. 1), we can observe **unpaired fins** (dorsal fin - *pinna dorsalis* (1), caudal fin - *p. caudalis* (2), and anal fin – *p. analis* (3)) and **paired fins** (ventral fins – *p. ventrales* (4), pectoral fins – *p. pectorales* (5)). In salmonid fish or invasive catfish of *the Ameiurus* genus, there is also a so-called **adipose fin** (*pinna adiposa*), but unlike the previous fins, it is not supported by rays; it consists of connective tissue. Various positions of ventral fins are recognized. They can be located anterior to the pectoral fins, known as **jugular** position, found, for example, in the freshwater burbot (*Lota lota*). In the **thoracic** position, they are situated directly beneath or just behind the pectoral fins, typical for perch-like fish (e.g., *Perca fluviatilis*), and in the **abdominal** position, they are placed behind the pectoral fins, approximately halfway along the body, a characteristic position for carp-like fish (e.g., *Cyprinus carpio*).

In the course of evolution, some species have experienced fusion of certain fins, such as the ventral fins, into a single unpaired ventral adhesive disc (e.g., in the European bullhead - *Ponticola kessleri*), or fins may be entirely absent (as in the European eel - *Anguilla anguilla*). Fins are reinforced at the front by **hard, unbranched rays**, also known as spines or thorns. Such strong, prominent spines can be found, for example, in the invasive brown bullhead catfish (*Ameirus nebulosus*) at the beginning of the dorsal and pectoral fins, where they are connected to a venom



gland. Therefore, handling this fish should be done with caution to avoid injury. **Soft branched rays** are located behind the hard rays and are soft to the touch (Fig. 2). The length of fins and the number of rays are important characteristics used in species identification.

Fig. 1. Fish body structure with indicated basic structures. 1. Dorsal fin – *pinna dorsalis*, 2. Caudal fin – *p. caudalis*, 3. Anal fin – *p. analis*, 4. Ventral fin – *p. ventralis*, 5. Pectoral fin – *p. pectoralis*, 6. Operculum – *skrela*, 7. Barbels, 8. Lateral line – *linea lateralis*.

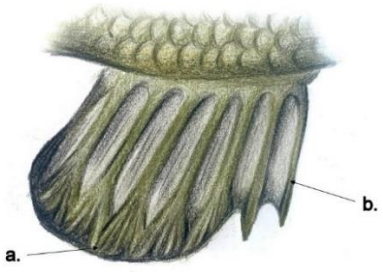


Fig. 2. Fin composed of rays. A. Soft rays branched at the apical end, B. Hard rays without branching.

On the sides of fish heads, there are **opercular bones** (screles) (6) that cover the gill cavity, protecting the gills. In the corners of the mouth, on the chin, or on the snout, **barbels** (7) may be present. These are often found in species that live near the bottom or search for food there, such as common carp (*Cyprinus carpio*), wels catfish (*Silurus glanis*), or stone loach (*Barbatula barbatula*). Sensory receptors located on the barbels significantly aid them in this foraging. The presence/absence of barbels and their number are also important identification features.

There are various types of caudal fins. **The heterocercal** fin, found, for example, in the sterlet *(Acipenser ruthenus*), has asymmetrical lobes, with the upper lobe being noticeably longer than the lower one. An important characteristic is that the vertebral column extends into the upper lobe of the fin. **The homocercal** fin is externally symmetrical, composed of two equally sized lobes, but internally it is asymmetrical. The terminal part of the vertebral column is bent upward, and during development, the dorsal lobe of the caudal fin is lost, and the ventral lobe is divided into two equal parts, making the fin a purely ventral structure. This type is the most common caudal fin type in bony fish, such as the gudgeon (*Gobio gobio*). In the **diphycercal** fin, the vertebral column extends straight back to the end of the body, and the fin develops symmetrically above and below it. A typical fish with this type of fin is the Senegal bichir (*Polypterus senegalus*) (Fig. 3).

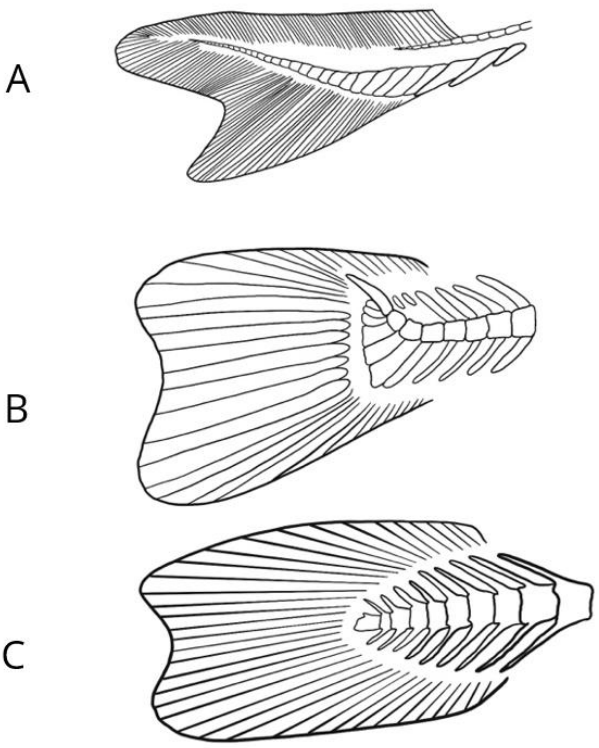


Fig. 3. Basic Types of Caudal Fins.

A. Heterocercal, fin B. Homocercal fin, C. Diphycercal fin

On the side of the body, there is the lateral line (linea lateralis) (8), which is a crucial sensory organ for fish. Through it, they can detect water movements caused by the presence of other fish, objects, or obstacles in the vicinity, allowing them to navigate well even in murky water. It usually extends from behind the gill arches to the area of the caudal peduncle (Fig. 4, A). In some fish, such as the European bitterling (Rhodeus amarus), it is very short and terminates in the front part of the body behind the head (Fig. 4, B). The length of the lateral line, its shape (straight – in the majority of species, wavy – e.g., sabre curvature), and the number of scales in the lateral line are often used as important identification features in fish species determination.

Fig. 4. Various Lengths of Lateral Lines in Two Different Fish: A. Lateral line running from the gill arch to the end of the tail peduncle in the stone moroko (Pseudorasbora parva). B. Short lateral line in the front part of the body in the European bitterling (Rhodeus amarus).

Another important identifying feature is the presence or absence of a **keel** - a longitudinal sharp edge on the ventral side of the body before the anal opening. The keel may or may not be covered with scales (Fig. 5).

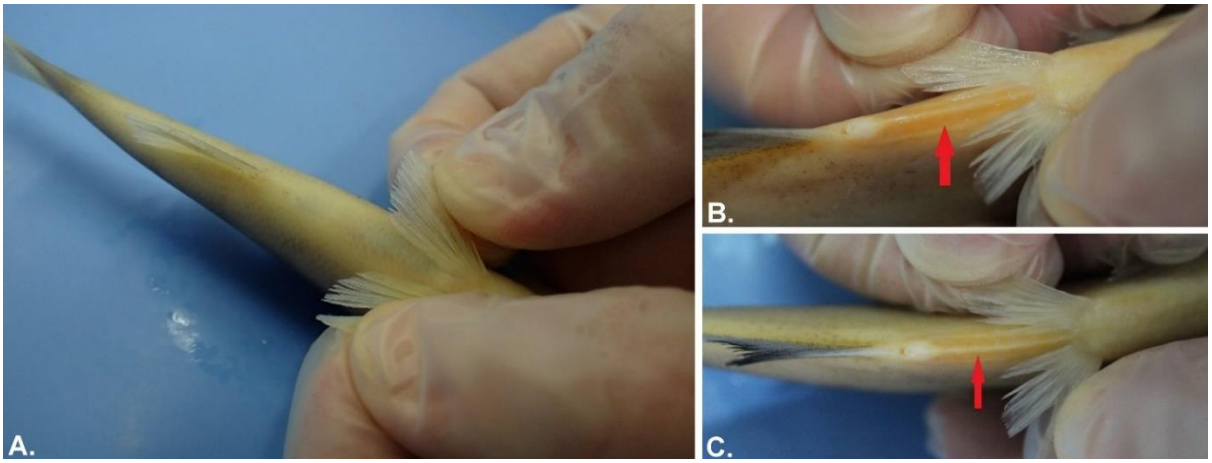


Fig. 5. Determining the Presence of a Keel in Two Different Fish Species: **A.** European perch without a keel between the pelvic and anal fins. **B.** Common bream with a present keel.

There are also different types of scales In fish,. Ganoid scales are known in sturgeons, cycloid scales in cypriniform fish, and ctenoid scales in perciform fish. **Cycloid** scales are round with a smooth outer surface, **ctenoid** scales have serrations on the outer edge, and **ganoid** scales have a rhomboid shape and are covered with ganoin (Fig. 6).

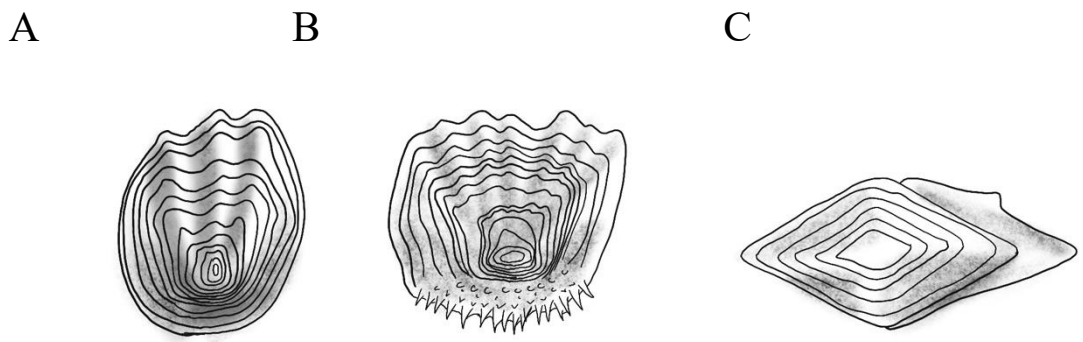


Fig. 6. Various types of scales in fish. A. Cycloid, B. Ctenoid, C. Ganoid.

The oral of fish can be horizontally split (**terminal mouths**) as seen, for example, in the perch (*Perca fluviatilis*) or rainbow trout (*Oncorhynchus mykiss*). They can be oriented upwards (**upper mouths, dorsal**) as seen in the silver carp (*Hypophthalmichthys molitrix*) or European bleak (*Alburnus alburnus*), or downwards (**lower mouths, ventral**) as in the nase (*Chondrostoma nasus*) (Fig. 7). However, sturgeons have their mouths located on the lower side of the head. The orientation of mouths can reveal a lot about the feeding ecology of a particular species. Terminal mouths are typical for predatory fish species, upper mouths are found in species that catch aquatic insects and feed on surface food. Lower mouths are characteristic of benthic fish species that inhabit the bottom and search for food there.

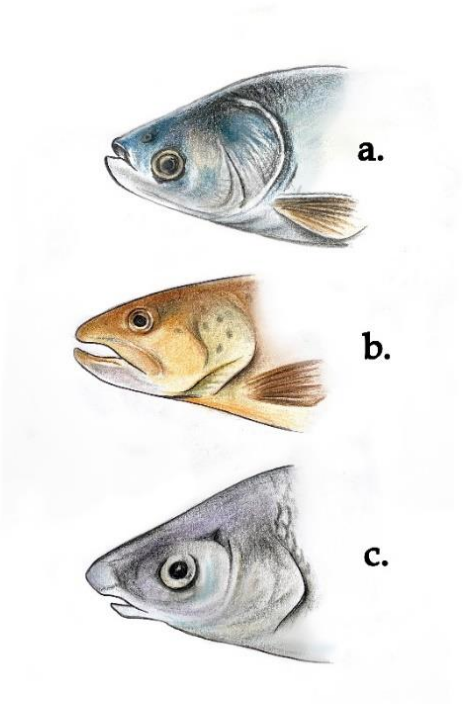


Fig. 7. Oral with different orientations. A. Upper, dorsal, B. Terminal, C. Lower, ventral.

During the spawning season, male carpids develop cornified formations on the head, as well as on other parts of the body, called spawning rashes, which irritate the females during spawning (Fig. 8).

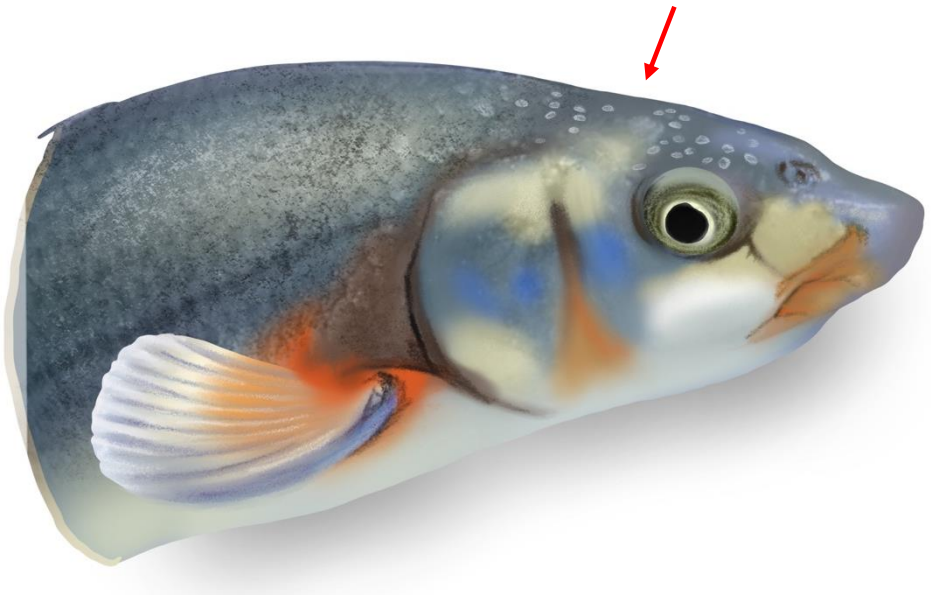


Fig. 8. Neresové vyrážky u samca podustvy severnej v období trenia.

Fish as Bioindicators in Aquatic Ecosystems

The transformation of aquatic ecosystems due to anthropogenic changes at the basin level has led to a significant deterioration in the state of fish populations in most water bodies in Ukraine. The goal of any reservoir reconstruction is to increase productivity or expand its use, but the structural complexity of ecosystems does not allow predicting all negative changes and processes that will develop as a result of human activity. In this context, there is a pressing need to determine ecological risks and indicative indicators at the biocenotic and population levels that would allow predicting negative changes in ecosystems. At present, the level of ichthyological research does not always clearly identify the processes in water bodies related to changes in species composition or population structure of fish. Therefore, there is a need to develop theoretical approaches to the use of fish as indicators of the state of hydroecosystems.

The use of structural features of fish populations and assemblages as bioindicator parameters has both advantages and disadvantages compared to aquatic invertebrates, algae, and higher aquatic plants. Among the advantages of this methodology are the relatively large size of the objects, the relative simplicity of determining the species affiliation of fish, the possibility of conducting research with minimal laboratory equipment, and the relatively simple determination of structural characteristics of fish populations.

The most significant disadvantages include difficulties in determining reliable indicators of population size for different fish species, the mobility of ichthyofauna representatives, allowing them to avoid unfavorable conditions, the factor of fish extraction for fisheries purposes, disrupting the structure of populations and assemblages.

Most Western European countries use biotic indices for standard water quality control. In recent decades, in Europe and the USA, there has been a trend in the development of biological methods for assessment within the framework of an ecosystem integrated approach. In Ukraine, there is currently interest among researchers in studying various approaches to using fish as indicators of hydroecosystem status. However, the use of fish as indicators has certain complexities, primarily associated with the following drawbacks:

1) Empirical data have some ambiguity;

2) Lack of reliable criteria for selecting absolutely adequate biological indicators for assessing the impact on ecosystems;

3) The problem of choosing a "standard" for comparing assessment results;

4) About 2/3 of biotic indices are based on benthic macroinvertebrates;

5) Fish are rarely considered as bioindicators;

6) The possibilities of bioindication based on the structural features of fish populations in water bodies in Ukraine are insufficiently studied;

7) The majority of studies on bioindication in Ukraine are carried out on large rivers and reservoirs, while small rivers are poorly researched in this aspect;

8) Problems of assessing environmental quality from anthropocentric and ecosystem positions and determining the optimal level of anthropogenic transformation of hydroecosystems;

9) Constant emergence of new threats to ecosystem stability – this requires expanding the possibilities of bioindication.

The identification of ichthyological indicators at biocenotic and population levels, characterizing the state of hydroecosystems, can be the basis for research aimed at predicting changes and preventing ecological risks in Ukrainian water bodies. Detailing the dependencies between quantitative indicators that characterize the structure and dynamics of ichthyocenoses and fish populations and the influence of key factors on ichthyofauna, on the other hand, allows revealing the features of ichthyocenoses' structure and quantitative characteristics of size-mass and gender structures of fish populations that correspond to a certain level of negative changes in hydroecosystems. Analyzing existing approaches and methods, we believe that the following five indicators at the population and cenotic levels will allow assessing transformations in water bodies:

1. **Size diversity of population individuals.** As known, each age group is represented by individuals of different sizes, depending on the quality of the environment. Therefore, their distribution by size classes will differ. To assess the current state of the environment, the most informative analysis would be conducted on young fish and species with a short life cycle, as the size diversity of older age groups with a long life cycle may result from actions that occurred in past periods. The coefficient of variation can be an indicative parameter in this case. If environmental conditions are favorable for fish development, individuals of the same species with a wide range of biological characteristics, such as different lengths and body masses, will survive and coexist. However, if environmental conditions are unfavorable and negative factors affect them, the action of stabilizing selection occurs, which eliminates extreme variants and maintains a certain phenotype with a narrow range of variation in indicators. Accordingly, the coefficient of variation for each parameter will be low in its value.

2. **Size-weight structure of the population**. Indicators of the species population structure can be an indirect reflection of the impact of negative factors. Significant dynamics in the size-weight structure of individuals allows us to speak about the presence of excessive catch and undermining of the population. This indicator can be used for species with sexual dimorphism in size.

3. **Gender ratio.** This is an especially important indicator for fish with sexual dimorphism. Under certain conditions, there may be significant deviations from the "normal" gender ratio due to the effects of various natural and/or anthropogenic factors. The sexual structure of individual fish species can change significantly, but the ratio is generally close to 1:1. Considering this dependence, it can be stated that the prevalence of females over males can be an indicator of the level of extraction and the state of fish populations in the studied water bodies.

4**. Individual morphological variability** and the presence of phenodeviations. To determine the level of variability in studying a natural population as a holistic genetic-evolutionary system, it is promising to account for the stability of individual development based on such features as the level of fluctuating asymmetry and the number of phenodeviations. The latter, as a specific group of changes occupying an intermediate position between qualitative and quantitative characteristics, indicating hereditary deviations from the norm, are highly variable and occur with different frequencies. Usually, various levels of deviations are

encountered in natural populations, with their frequency being low, but in individual cases, their frequency significantly increases. In addition, there is another approach to assessing the stability of individual fish development in conditions of anthropogenic pressure on ecosystems – the analysis of morphological bilateral characteristics, which determines the variability of these characteristics on the left and right sides of the body.

Among clearly defined signs, such as those that do not require very careful examination of fish, various abnormalities in the structure and topography of the lateral line organs are encountered.

The main types of anomalies in fish in the studied region were: spine deformation, underdevelopment of one gill cover, and distorted fins. Their proportion was 1%, and the primary cause of such changes is close-relative crossbreeding. In summary, it should be noted that the frequency of appearance of any phenodeviant significantly depends on the fish's living conditions. The most important environmental factors influencing the frequency and degree of manifestation of these anomalies include temperature, excessive or deficient fish feed supply, gas regime in the water body, water pH, and pollution levels. The presence of phenodeviants in the population can be considered an indirect indicator of a decrease in genetic diversity and homeostasis of development. Genes or combinations of genes that do not manifest themselves in a well-balanced genotype and optimal living conditions are determined when there is a disturbance in genetic balance and an unfavorable environment.

A large number of asymmetrical manifestations in fish indicate a decrease in the viability of their natural populations under the influence of powerful anthropogenic pressure, including pollution, and can be used as indicative indicators of environmental conditions.

Skeletal Anomalies. The Connection Between the Frequency of Skeletal Anomalies in Aquatic Vertebrates and Pollution Has Been Experimentally Confirmed. For example, the chloroorganic pesticide Kepon induced scoliosis in minnows, while exposure to heavy metals in fish resulted in spine deformities and fractures. Therefore, monitoring involves a careful examination of fish to detect obvious anomalies, with possible further X-ray examination to identify hidden deformations, such as vertebral fusions. It is challenging to survey gill rakers and dorsal fins. Planktonic and zooplankton samples can be beneficial for detecting pathologies in larvae and anomalies in young fish.

The number of cases of skeletal anomalies in fish increases every year. Examples of anomalies include dorsal fusions and distortions, vertebral compression (flattening), head abnormalities, and fin anomalies. Such disturbances are found in most natural populations, but they are most commonly observed in polluted waters.

Skin Ulcers, observed in many fish species, are often referred to as "ulcerative syndrome." The main cause of ulcer development in fish is a high level of water pollution with hydrocarbons and an increase in populations of microorganisms potentially pathogenic to fish. The percentage of diseased fish is higher in the spring, so the season should be taken into account when fishing for monitoring purposes. Additionally, microbiological tests should be conducted on samples taken from bottom sediments and the water column.

Fin Erosion is one of the most common fish diseases, clearly linked to pollution.

The causes of erosion are complex and may include chemical agents affecting the epithelium, a deficit of dissolved oxygen in the water, and secondary bacterial infection. Systematic bacterial infection is not necessarily linked to fin erosion, although many bacterial species can be isolated from a sample taken from the ulcer. Monitoring this indicator is recommended, taking into account the season, fish size, species sensitivity, living conditions, and migration.

Tumors are found in almost all fish species, and they often have an infectious nature. Tumors can be caused by extreme water pollution and viral infections. There is also evidence that contamination with aflatoxin can induce liver tumors in minnows. Fish tumors are potentially useful indicators for monitoring the aquatic environment.

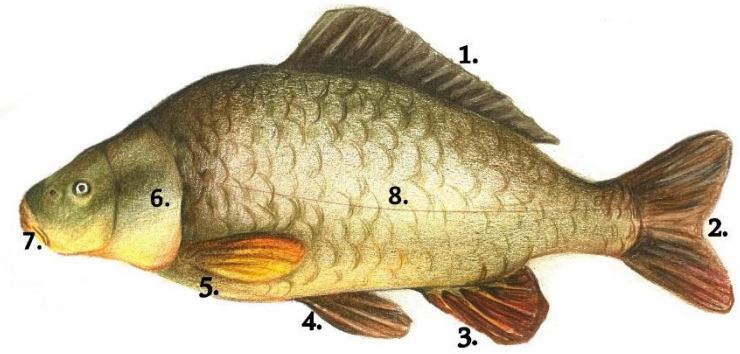
5. **Species and Taxonomic Diversity**. Information on the taxonomic diversity of functional groups of hydrobionts is an indicator of environmental conditions. The species and taxonomic diversity will have maximum values for certain average water quality indicators and will decrease towards very clean, oligotrophic, oligosaprobic, very dirty hypertrophic, and polysaprobic water bodies. It should also be noted that the diversity of fish species depends on many hydrological, hydrobiological, hydrochemical, and other factors. Among the most important are factors such as current strength, depth, transparency, salinity, gas regime, food base, etc. All the factors mentioned above have both direct and indirect effects on specific species and overall on the structure of ichthyocenosis.

It is known that the relationship between diversity and stability of ecosystems is sometimes contradictory. The stability of biosystems increases with an increase in diversity, but at the same time, it is noted that diversity itself is formed at the expense of ecosystem stability.

In this way, as a result of analyzing various approaches in the field of water quality bioindication and the state of hydroecosystems, it should be noted that the perspective of using ichthyological indicators is evident. They are advisable to use as bioindicators at the population and cenotic levels. At the population level, the following indicators are promising for bioindication: - size diversity of population individuals using the variation index; size-weight structure of the population with indicators of average long-term data on length or body mass; gender structure with indicators of increasing or decreasing the proportion of individuals of one sex; individual morphological variability of individuals and the number of phenodeviations. At the cenotic level, it is advisable to use indicators such as the number of fish species, diversity indices, and the diversity of fish with different degrees of steno- and eurybionticity.

Fish - Tasks to Practice

Task 1. Name the structures marked in the picture in both Slovak and scientific terms..



1. .....

2. .....

3. .....

4. .....

5. .....

6. .....

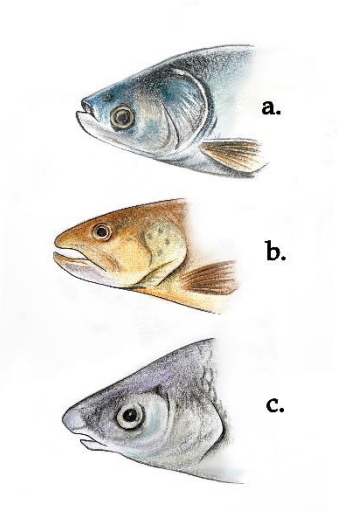
7. .....

8. .....

Task 2. What is a homocercic and heterocercic caudal fin. Try to explain and draw. Also give an example of a fish species in which the type of fin is found.

homocercic	heterocercic

...ral ...each
...he ...gory (phytophagous, zoophagous, omniphagous).



a.

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b.

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c.

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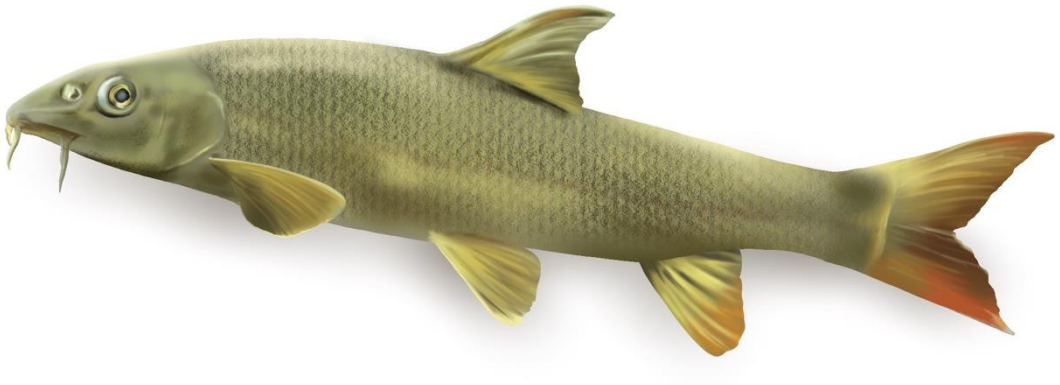
Task 4. Match each fish with its technical scientific name and the position of its pelvic fins.

A.



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Correct answer: *Barbus barbus*

B.

.....

Correct answer: *Perca fluviatilis*

C.

.....

Correct answer: *Lota lota*

D.

Which orders fish have the adipose fin and try to draw it in the picture.

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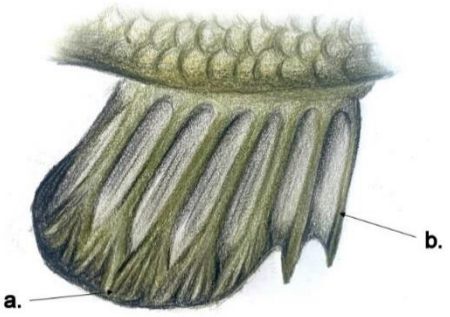
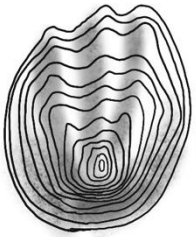
Task 5: Name the parts marked on the picture that make up the fin.

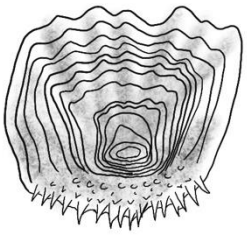


a. .....
b. .....

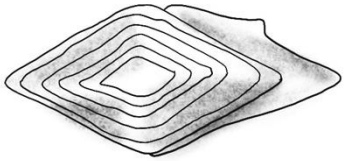
Task 6: What is the professional name for the scales fish depicted in the pictures? Assign each to a fish species in which they occur



A



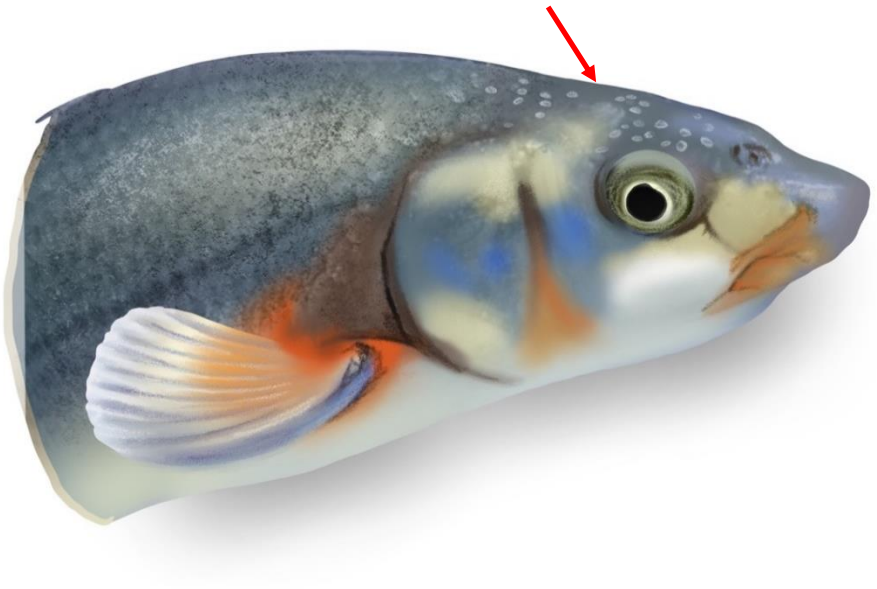
B



C

a. .....
b. .....
c. .....

Task 7. What is the structure marked in the picture called, and what is its purpose?



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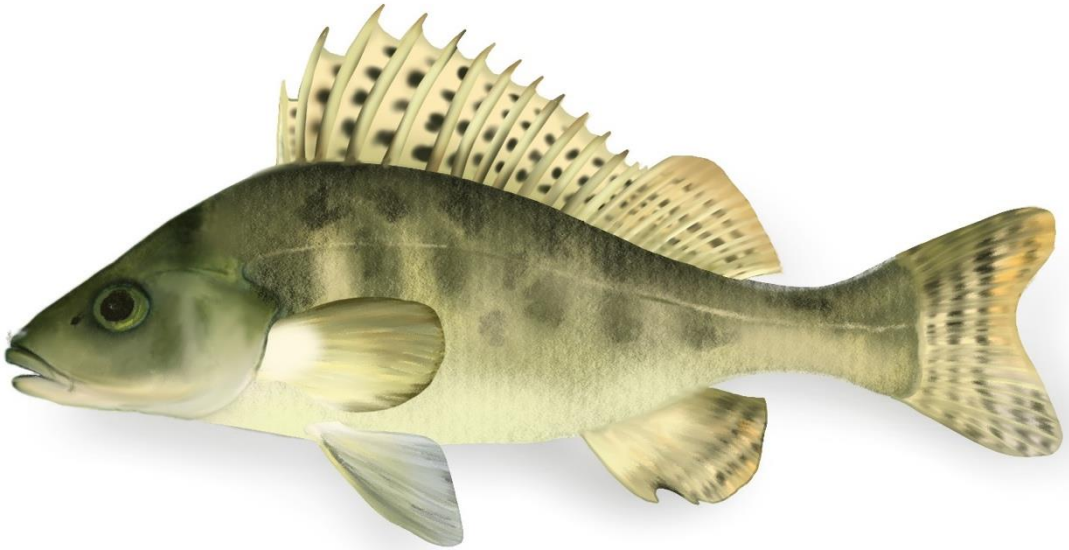
Task 8. Using identification keys and fish atlases, assign the correct scientific and Slovak names of the order and specific species to each fish image. In the table (Table: Fish Orders), write down the identified fish orders under the images and try to list the basic characteristics that are characteristic for them using literature.

(Note: The specific names and characteristics would depend on the actual content provided in the images and the literature you are referring to.)



A.

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B.

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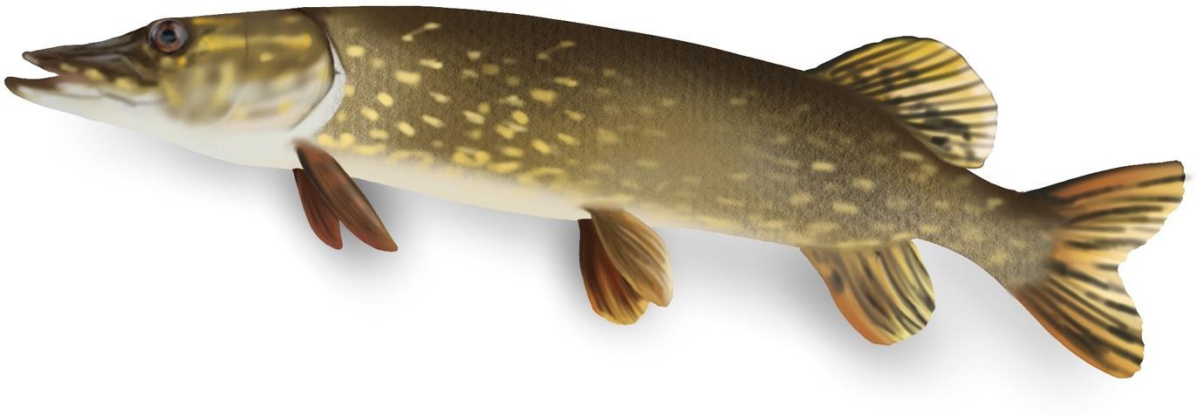

C.

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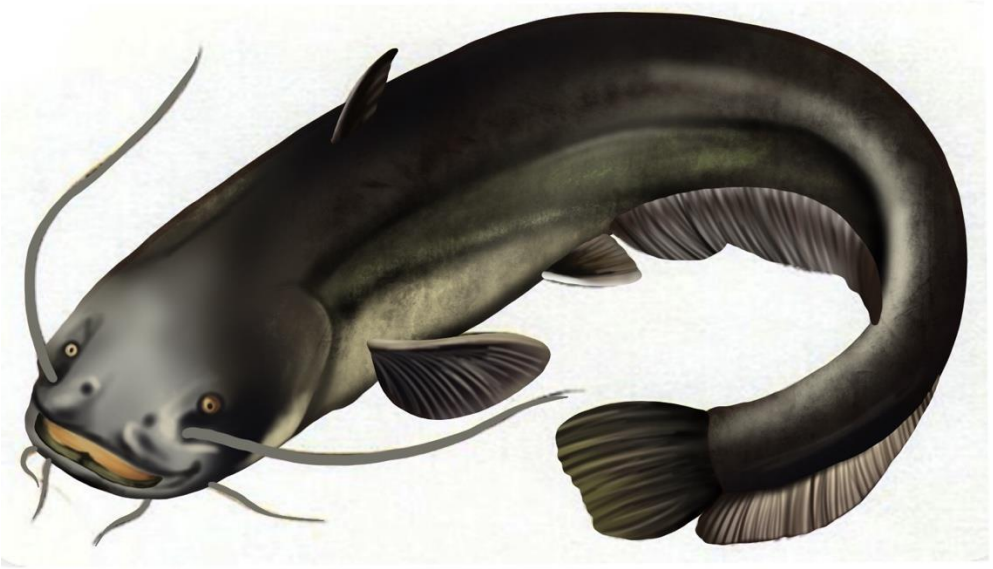

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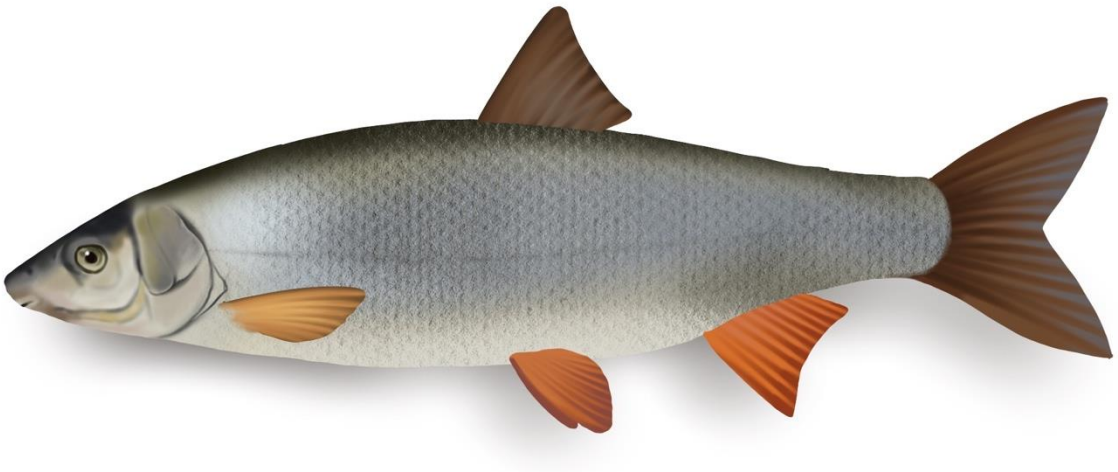

H.

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EUROPEAN UNION

Identified Order	Characteristic Features

Field Task

Using an identification key and fish atlases, try to fill in the table with the species spectrum of fish and their quantity that you managed to catch in the field. Assign to each species their order, orientation of the mouth, number of pairs of barbels, type of caudal fin, scientific name of the position of the ventral fins, feeding category (phytophage, zoophage, omnivore), and whether it is a native or invasive species.

location and date	species	No.	row	mouth orientation	number of pairs of barbels	type of caudal fin	position of ventral fins	feeding category	native or invasive

AMPHIBIANS AS INDICATORS- subject of scientific education

Amphibians (LISSAMPHIBIA) are a fascinating group of animals that play a crucial role in the environment and have a unique life cycle that is closely tied to water. As a result, the study of amphibians can be an excellent way to learn about both biology and ecology. Amphibians are also important indicators of environmental health, as they are sensitive to changes in water quality, temperature, and other factors. Because they have permeable skin that allows them to absorb oxygen and other substances from their surroundings, they are particularly susceptible to pollution and other forms of environmental degradation. As a result, declines in amphibian populations can be an early warning sign of broader ecological problems.

Overall, the study of amphibians provides a valuable opportunity to learn about both the natural world and the scientific process. By exploring the biology and ecology of these fascinating animals, we can gain insights into the complex interactions between organisms and their environments, and develop a deeper appreciation for the importance of conserving biodiversity.

Amphibians are the sole descendants of the first terrestrial vertebrates that ventured onto land. Morphologically and physiologically, they differ radically from other terrestrial vertebrates, following a distinct evolutionary trajectory from reptiles, birds, and mammals belonging to the monophyletic taxon Amniota. Currently, there are over 8600 species of amphibians, with more than 100 new species described each year. This class of vertebrates is divided into **three orders**: Anura (frogs and toads), Caudata (salamanders), and Gymnophiona (caecilians). The order Anura, comprising frogs and toads, is the most diverse, while caecilians remain the least studied.

Amphibians Morfology and Physiology

Many amphibians, as implied by their class name, are **amphibious**, inhabiting both aquatic and terrestrial environments. Similarly, our amphibians are tied to **aquatic environments** during the breeding season in spring. However, after this period, some species leave the water and dwell in the surrounding **terrestrial environment**, where they find suitable **food, shelters, and hibernation sites**. Other species, such as aquatic frogs, are more closely associated with aquatic environments and are present in or near water throughout their activity period, even hibernating at the bottom of water bodies during the winter. The need for both types of habitats increases **the requirements for their conservation**. Their environmental sensitivity is associated with a combination of typical features such as permeable skin, shell-less eggs increasing sensitivity to contaminants, UV radiation, and ectothermia leading to sensitivity to temperature and precipitation regime changes. Due to their sensitivity to environmental changes, they can serve as critical **bioindicators** of ecosystem health. Recent analyses by the International Union for Conservation of Nature (IUCN) revealed that up to a quarter of organism species are at risk of extinction due to human activities, with amphibians holding the unfortunate lead with 41% of endangered species. The main threats negatively affecting amphibian populations include **(1) habitat loss or alteration, (2) pollution, (3) climate change, (4) infectious diseases and parasites, (5) introduction of non-native competing or predatory species, and (6) commercial utilization,** often involving the simultaneous interaction of multiple factors.

With some exceptions, fertilization is **external** in most frogs and toads, internal in most salamanders (except families Hynobiidae, Cryptobranchidae), and all caecilians employ indirect internal fertilization using an everted cloacal region forming a copulatory organ (*phallodeum*) (Hilman et al. 2009). In our salamanders, we specifically refer to **indirect internal fertilization**, where copulation does not occur. Instead, the male deposits a **spermatophore** (a bundle of sperm) in an aquatic (e.g., newts) or terrestrial (e.g., salamander) environment. The female either takes it directly from the male's cloaca to hers, inserts it into her cloaca with her hind limbs, or retrieves it herself, often with or without direct guidance from the male. Fertilization of eggs then occurs in the female's reproductive tract.

Fertilization in our frog species occurs externally, always in an external aquatic environment; hence, we refer to this as **external fertilization**. During the breeding season, males attract females for mating through species-specific acoustic calls. Thanks to these vocalizations, we can determine the presence of a particular species and even estimate the number of calling males at a given location directly without capturing them. Typically, prominent dark-pigmented epidermal structures called **nuptial pads** occur on the fingers of their front limbs, serving to secure the male to the female (**amplexus**) (Fig. 1a,b). We recognize two types of amplexus: *amplexus axillaris*, where the male holds the female with its front limbs (toads, frogs, tree frogs) (Fig. 1a); *amplexus inguinalis* – the male holds the female in front of its hind limbs (common frog, agile frog) (Fig. 1b). During this attachment, both sexes synchronously release eggs and sperm into the aquatic environment. The fusion of sex cells thus occurs in the external environment.

Fig. 1a. Common toads (Bufo bufo) in amplexus (amplexus axillaris) at the Vinné site.

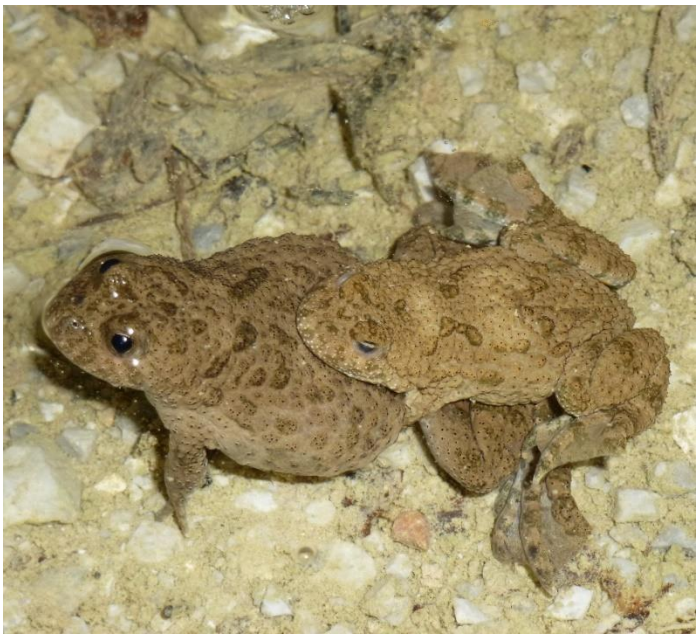

Fig. 1b. Common frogs (*Rana temporaria*) in amplexus (amplexus inguinalis) in the Muráň region.

The eggs of amphibians are anamniotic, lacking **internal embryonic membranes** (amnion, chorion, allantois), and **without a shell**, which is present in other tetrapod eggs. Amphibian eggs are surrounded by various gelatinous layers and are highly **susceptible to desiccation**. Therefore, eggs are always laid either in a moist terrestrial environment or directly into the aquatic environment. Our amphibians exclusively lay eggs in aquatic environments, either **individually** (e.g., newts), in the form of strings (toads from the Bufonidae family), or clusters (genus Pelophylax, Bombina, Hyla) (Fig. 2).

The shape and size of the egg clutch, the way they are **positioned** in the environment (attached to vegetation or not), the **type of aquatic environment** where they are laid (larger/smaller water

body, permanent/temporary, etc.) can provide valuable information about the species responsible for laying them. Therefore, these developmental stages, along with **tadpoles/larvae**, which are morphologically different among species, can be very helpful **in monitoring species composition**.

Fig. 2. String-like egg clutch of the common toad (Bufo bufo) and cluster of the common frog (genus Rana).

For all our amphibians, **indirect development** with a larval stage is typical from an ontogenetic perspective. The larval development usually takes place in water and concludes with **metamorphosis** when the immature young individual (**juvenile**) loses larval characteristics and transitions to land, where it mainly breathes air through relatively undeveloped lungs. The larval stages of our amphibians breathe through **gills**. In addition to these organs, **the skin** of amphibians plays a significant role in breathing in this group of vertebrates (60 – 80%, Zwach 1990), as it is permeable to respiratory gases and water. During the hibernation period, it ensures up to 100% gas exchange. The highly permeable skin allows them to absorb water effectively from the surroundings, but at the same time, it poses a risk of rapid water loss. The skin's surface is moist due to secretions from mucous glands and is densely covered with a network of blood capillaries. In addition to these glands, amphibians also have toxic glands with external secretion. They are scattered either irregularly or clustered into structures, which can be seen as raised formations behind the eyes (**parotoids** – poison glands, e.g., toads, salamanders) (Fig. 3). These toxins serve amphibians not only for protection against danger but also against parasites and various skin diseases, such as fungal infections.

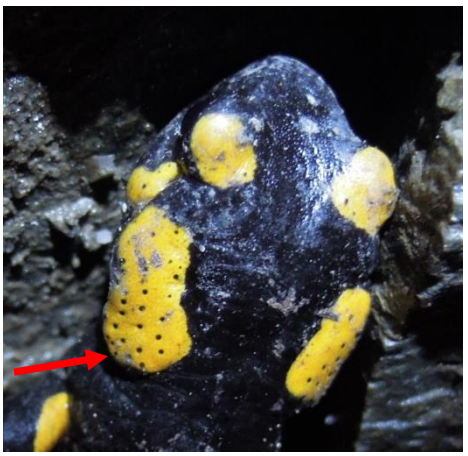

Fig. 3. Parotoid glands in the fire salamander (*Salamandra salamandra*).

Frogs have a developed chest bone (*sternum*), and they usually lack ribs, whereas in salamanders, it is the opposite. A crucial feature in the frog skeleton is the fused bones radius and ulna (*radioulna - os antebrachii*) in the forelimb and *tibia* and *fibula* (*tibiofibula, os cruris*) in the hindlimb. Another distinctive feature is the termination of the spine with a long rod-like bone (*urostyle*), which formed by the fusion of several terminal vertebrae (Fig. 6). These bones, preserved in pellets, reveal the presence of amphibians in the diet of predators.

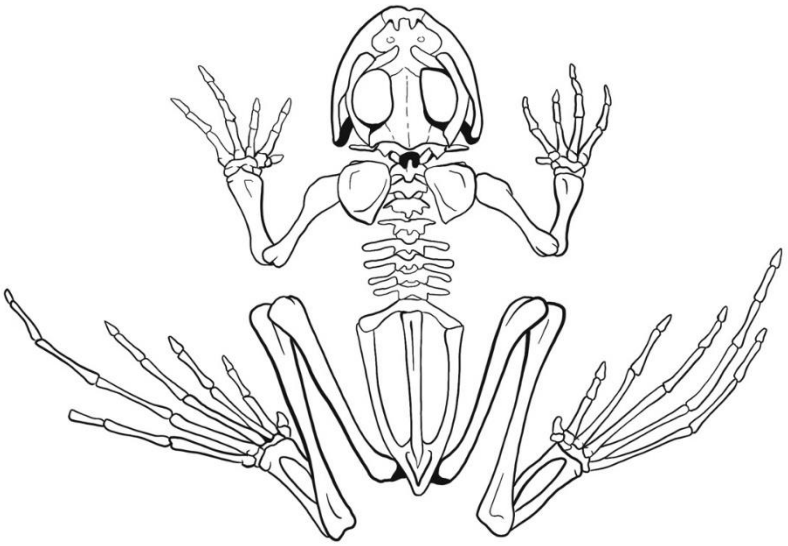

Fig. 6. Frog skeleton. A. *os antebrachii*, B. *os cruris*, C. *urostyl*.

Amphibians Species

Fire Salamander (*Salamandra salamandra*) is only representative of the genus *Salamandra* in Slovakia territory. Together with all newts, we classify it into the family Salamandridae. It inhabits moist deciduous or mixed forests, especially beech forests. Behind the eyes, there are parotid poison glands through which it releases a toxic secretion in danger (Fig. 5). Pores through which it releases the toxic secretion are also present in two lines on both sides of the spine from the end of the head to the end of the tail. Males do not have as pronounced secondary sexual characteristics (e.g., crests, colorful markings) as newts do. Therefore, determining the gender of this species can sometimes be complicated. Males differ from females by longer limbs and a slender body. Females, on the other hand, have a wider head. The most significant difference is observed in the shape of the cloaca. In males, it is swollen compared to the flat shape in females (Fig. 7b, c). However, sometimes it is not possible to determine the gender of a salamander unequivocally based on this determination feature. The female does **not lay eggs**; instead, after indirect internal fertilization (see above), the eggs develop in her reproductive tracts, and she lays well-developed larvae (still in egg cases). These larvae are laid primarily in streams or small pools.

Fig. 7a. Fire Salamander found during hibernation in a mine near Ružín.

Fig. 7b. Flat shape of the cloaca in a female.

Fig. 7c. Swollen shape of the cloaca in a male.

All our newt species are **oviparous**. They lay eggs individually, either attaching them freely to aquatic vegetation or "wrapping" them in the leaves of aquatic plants. The cloaca of males is much more swollen and pigmented compared to females (Fig. 8). In the spring, during the breeding season, newts are in the so-called aquatic phase, later in the summer and autumn, they live terrestrially (terrestrial phase). Two species, the Danube crested newt (*Triturus dobrogicus*) and the great crested newt (*Triturus cristatus*), are classified as "large newts," characterized by a much more robust body compared to the genera *Lissotriton and Ichthyosaura*. In the Czech Republic, the smooth newt (*Triturus vulgaris*) is also included in this category.

Fig. 8. Comparison of the cloaca of a female (left) and a male (right) of the Carpathian newt (*Lissotriton montandoni*).

The great crested newt (*Triturus cristatus*) has a gray-brown to brown-black colored body from above with large oval dark spots and small white dots on the sides. The belly is yellow to

orange, also with large dark spots. In the aquatic phase, males have a large toothed crest, which is interrupted in the pelvic area and starts from the eye level. The crest continues onto the tail, with a noticeable pearly white to silver stripe running along the center of the tail. This species has longer limbs, and when the front and hind limbs are laid longitudinally, they touch each other.

The Danube crested newt (*Triturus dobrogicus*) is very similar to the previous species (Fig. 9). However, the body is slimmer, and the limbs are more delicate. When the front and hind limbs are laid longitudinally, they cannot touch each other (Fig. 10). The back is light brown, light greenish-brown, gray-brown to brown-black. The sides are often dotted with white dots and dark round spots. The ventral side ranges in color from bright yellow through orange to red, with dark spots. During the breeding season, the male has a prominent crest on the dorsal side, reaching up to eye level, interrupted in the pelvic area. A distinct edge continues onto the tail, and in the central part, a bluish stripe with a pearly sheen can be observed (Fig. 11). In places of common occurrence, the Danube crested newt and the great crested newt can interbreed.

Fig. 9. Danube crested newts rescued from a vertical shaft in a field near the village of Streda nad Bodrogom.

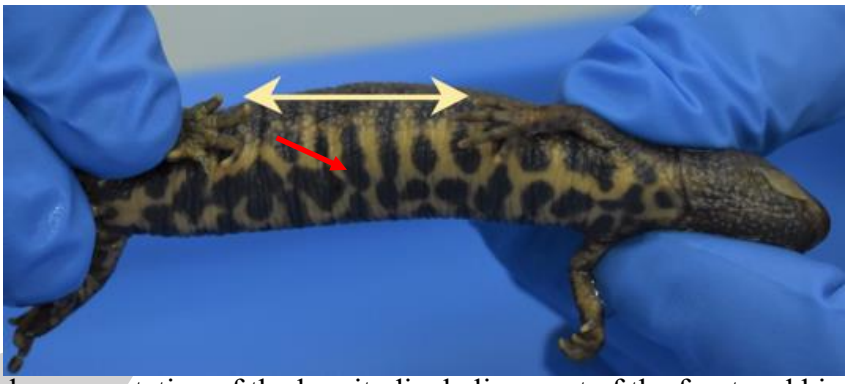

Fig. 10. Visual representation of the longitudinal alignment of the front and hind limbs in the Danube crested newt.

Fig. 11. Bluish stripe with iridescent shimmer in the middle of the tail in the Danube crested newt at the Streda nad Bodrogom site.

Alpine newt (*Ichthyosaura alpestris*) – The male has a dark gray to dark brown color on the back, with a low black-yellow stripe running through the center and a blueish tint on the sides, along with irregularly shaped black spots (Fig. 12). The belly is red without dark spots. Females are less brightly colored, with a marbled pattern on the back, and the belly is without spots (Fig. 13).

Fig. 12. Male Alpine newt discovered in the Ćićarija mountain range (Croatia).

Fig. 13. Male (left) and female (right) Alpine newt in the Czech Republic.

The smooth newt (*Lissotriton vulgaris*) is our most widespread newt. The coloration is highly variable, with the back and sides being light yellow-brown to brownish-gray. During the breeding season, the male has a prominent crest that is not interrupted in the pelvic region and smoothly transitions to the tail (Fig. 14B). There is a bluish stripe on the lower side of the dorsal crest. Dark oval spots are present on the body, and similar spots are also found on the belly. Two dark stripes extend from the tip of the nose along the side of the head, with one passing through the eye (Fig 14A,B). Females are less colorful, having small dots instead of larger dark spots, which may even be absent. Dark stripes on the head are also present in females.

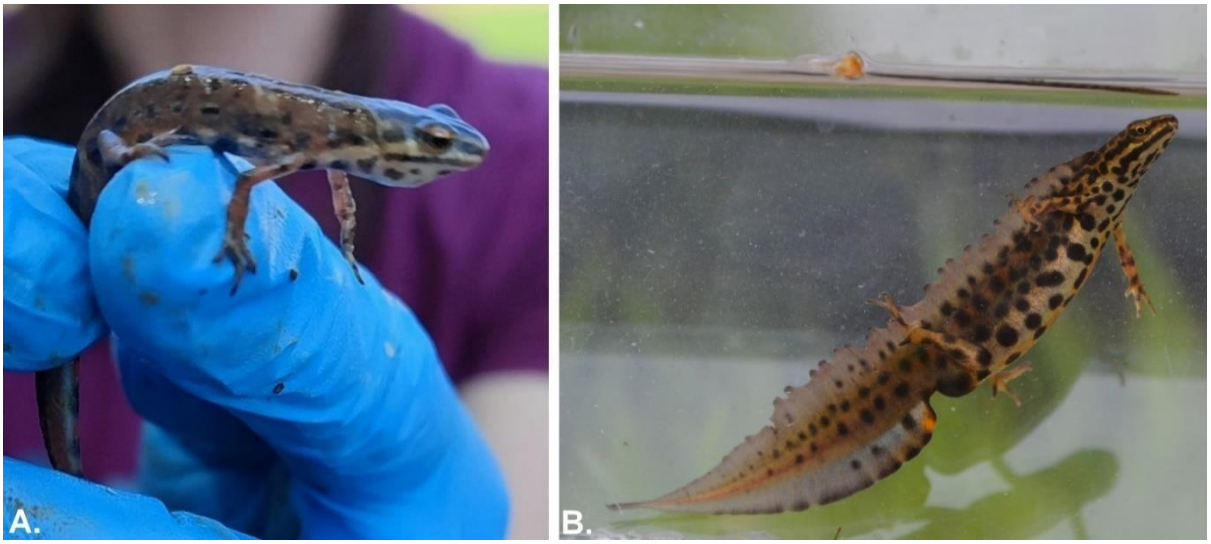

Fig. 14. Smooth newt with visible dark stripes on the head (A, B); a typical-looking male during the breeding season (B).

The Carpathian newt (*Lissotriton montandoni*) is an endemic species to the Carpathians. It gives a square impression due to the presence of skin ridges running along the sides of the back. The back is sandy yellow, yellowish-brown to olive-green, covered with small inconspicuous spots or dots. There are no dark spots on the yellow to orange belly. During the breeding season, males have a prominent thread-like appendage at the end of the tail (Fig. 15).

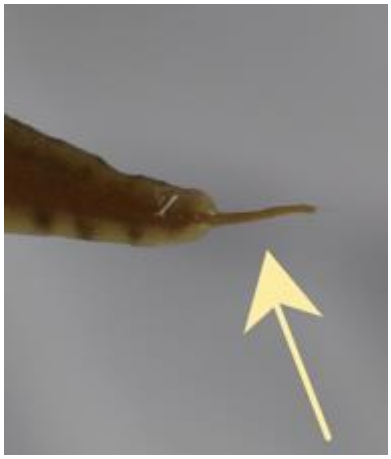

Fig. 15. Prominent thread-like appendage at the end of the tail in the male Carpathian newt during the breeding season.

In Slovakia, two species of fire-bellied toads are found: the fire-bellied toad (Bombina bombina) and the yellow-bellied toad (Bombina variegata). Where their ranges overlap, they can interbreed. The fire-bellied toad inhabits lowlands and hills up to 400 meters above sea level. The warts are bluntly rounded, giving a smoother feel to the touch. The ventral side displays a spotted pattern of red, orange, or yellow, covering a smaller area. The spots on the belly are separated from the spots on the limbs (Fig. 16D). Additionally, the spot on the palm of the front limbs does not extend into the first finger (Fig. 16C, upper image). The yellow-bellied toad is more robust and is associated with forested areas at medium altitudes. The warts are more pronounced and rougher to the touch. The yellow pattern on the belly covers a larger area (over 50%), and the spots on the belly are fused, interconnected with the spots on the limbs (Fig. 16E). The spots on the palms extend into the first finger (Fig. 16C, lower image).

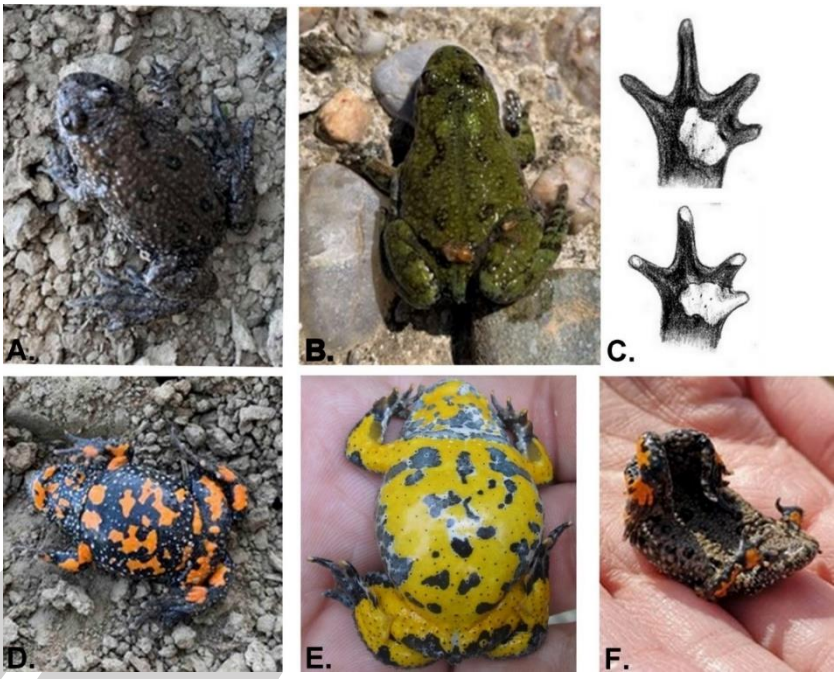

Fig. 16. Variability in the color of the dorsal side of fire-bellied toads (A, B), palm spot not extending into the first finger in the red-bellied toad (C, top), palm spot extending into the first finger in the yellow-bellied toad (C, bottom), small belly spots separated from spots on the limbs in the red-bellied toad (D), large belly area covered with spots and their connection to spots on the limbs in the yellow-bellied toad (E), defensive posture in danger (F).

The fire-bellied toad (*Bombina bombina*) primarily inhabits permanent water bodies such as ponds, oxbow lakes, blind river arms, or water reservoirs in sand pits. In contrast, **the yellow-bellied toad** (*Bombina variegata*) prefers smaller temporary water bodies, such as puddles on forest paths, marshes, waterlogged meadows, and small ponds in abandoned quarries or sand pits.

The European tree frog (*Hyla arborea*) is an arboreal frog species adapted to a tree-dwelling lifestyle. **Equipped with circular discs** on the tips of its fingers, it can climb vertically even on very smooth surfaces. The skin on the dorsal side is usually green, but it can change color depending on the environment or stress (Fig. 17 A,B). Therefore, its coloration can be highly variable. A dark stripe runs along the sides of the body, from the nostrils through the eye, eardrum, base of the front limbs, to the level of the hind limbs. The belly is white. Males have a **prominent vocal sac**, which they use to produce characteristic sounds to attract females for mating (Fig. 17 C). The European tree frog prefers open, well-lit habitats near smaller to moderately sized water bodies with rich herbaceous vegetation along the shoreline, the presence of trees, and meadow habitats. Various ponds, blind river arms, and smaller ponds are common breeding sites for this frog species.

Fig. 17. European Tree Frog. A. View of the dorsal and lateral sides of the European Tree Frog, B. European Tree Frog with significantly changed color on the dorsal side of the body, C. Discs on the tips of the fingers and the vocal sac in a relaxed state.

The spadefoot toad (*Pelobates fuscus*) is restricted to lowlands and foothills in our region, with its occurrence tied to sandy or clayey soils, or areas with loose substrates. This frog has a slit-like pupil, a triangular head with a distinctly elevated crown. There is a pronounced **stiff metatarsal bump** on the hind legs. The coloration varies from gray to olive-brown with irregular darker spots (Fig. 18). Red dots are sometimes present on the sides. It burrows into the substrate using its metatarsal bumps and can bury itself up to 1 meter deep during the day, enduring winter in this way. It is nocturnally active.

Fig. 18. A. Spadefoot Toad rescued from a vertical shaft in a field near the village of Streda nad Bodrogom, B. individual found in Hatfe near the village of Viničky.

The common toad (*Bufo bufo*) belongs to the so-called explosive breeders, meaning they mate once a year in large numbers over a short period during the spring. It is widespread across the entire territory of Slovakia. It is a terrestrial frog that seeks water only during the breeding season. After this period, it disperses into the surrounding terrestrial environment. It is nocturnally active. Females are significantly larger than males. Both genders have parotoid glands (parotids) behind their eyes, through which they release a toxic secretion. The coloration of the dorsal side is highly variable, ranging from gray through brown to olive-green, but it is always monochromatic, with irregular dark brown spots often present on this background. The ventral side is whitish with darker irregular spots. It is important to note that the body is prominently warty (Fig. 19). For breeding, it utilizes various medium to large permanent water bodies but generally does not have very specific requirements for breeding sites. During terrestrial life, it inhabits various types of forests, open wetlands, dry or waterlogged meadows, field edges, orchards, or even human-altered landscapes.

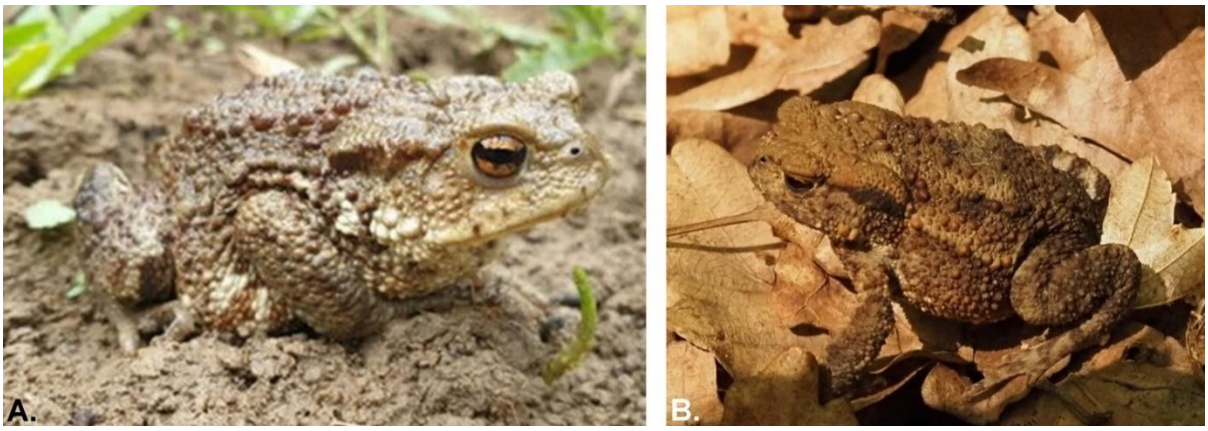

Fig. 19. Common Toad (Bufo bufo). A. Adult individual, B. Subadult individua.

The green toad (*Bufotes viridis*) is known for its adaptation to steppe and forest-steppe habitats, tolerating higher temperatures, dry conditions, and salinity. It is considered a pioneer species capable of colonizing newly available water bodies and persisting successfully in human-altered landscapes such as field edges, gardens, construction sites, quarries, and even highly urbanized areas like large cities. For breeding, it often seeks shallow, well-heated, smaller water

bodies such as puddles, ponds in quarries, sand pits, or on the edges of fields and construction sites. In urban environments, it may lay its eggs in fountains. Compared to the previous species, it is smaller in size. It also has parotoid glands behind its eyes, secreting toxic substances. The dorsal coloration is whitish to gray with less or more defined green to brownish-green spots. The ventral side is often light with dark irregular spots (Fig. 20).

Fig.20. Green toad (Bufotes viridis) with beautiful green bordered spots on grey-brown ground.

Frogs can be ecologically divided into two separate groups: **terrestrial and aquatic**.

As the name suggests, terrestrial frogs like the common frog (*Rana temporaria*), moor frog (*Rana arvalis*), and agile frog (*Rana dalmatina*) inhabit land, except during the breeding season. They have brown body coloring, and usually, a wide dark spot extends from the back of the eye over the eardrum to the front limb.

On the other hand, the bodies of aquatic frogs (also known as green frogs), including the marsh frog (Pelophylax ridibundus), pool frog (*Pelophylax lessonae*), and their hybrid, the edible frog (*Pelophylax esculentus*), are colored in various shades of green, but they can also be brown. As the ecological name "aquatic" implies, these frogs are bound to aquatic environments year-round. They stay either directly in the water or in its closer or more distant surroundings (short-legged frog). The marsh frog prefers larger bodies of **water such** as oxbow lakes, lakes, or rivers, as well as artificial ones like gravel pits or ponds. It is closely tied to aquatic environments and typically hibernates in them. In contrast, the short-legged frog is less bound to aquatic environments and primarily hibernates on land. It prefers natural, overgrown smaller bodies of **water characteristic of wetlands**. The edible frog can survive in **both types of habitats** and mostly forms mixed populations with the previous parental species of aquatic frogs. The common frog, on the other hand, prefers moist and at least partially shaded environments with the presence of water bodies. It often inhabits forests with various streams and ponds. It can also penetrate meadows with higher herbaceous vegetation or quarries with water patches.

The different lifestyles of these two ecologically distinct groups are reflected in various adaptations visible on their bodies. For example, terrestrial frogs have a wider gap between their eyelids, which, when viewed from above, extends to the outlines of the head. In aquatic

frogs, **these eyelids are more shifted towards** the top of the head, and the gap between them is small, not reaching **the outlines of the head**. Terrestrial frogs typically have **less prominent webbing** on their hind limbs. Conversely, aquatic frogs have **a better-developed webbing**. Terrestrial frogs usually **have a dark "V"-shaped pattern behind the head**, with the tip pointing towards the head. In aquatic frogs, a **light stripe** often runs along the center of the back (Fig. 21).

As mentioned earlier, there are three representatives of aquatic frogs in Slovakia. **Two are referred** to as **parental species** (marsh frog and pool frog), and the third **representative** is a **hybrid** (edible frog), resulting from the crossbreeding of the parental species. The hybrids, edible frogs, are maintained in our nature thanks to their peculiar way of reproduction called **hybridogenesis**. This is a relatively complex issue that you can read about in various scientific publications because scientists pay great attention to aquatic frogs due to their reproductive peculiarities. Morphologically, these three representatives are **very similar**, so determining the species based solely on body characteristics is insufficient and needs confirmation through **molecular methods**. For curiosity, here are some physical characteristics that we observe in aquatic frogs in the field to approximately classify them into species: total body length, length of the thigh, length of the shin, length of the metatarsal tubercle, and length of the first toe on the hind limb, presence/absence of yellow pigment on the thighs and sides of the body, color of the resonators in males, presence/absence, and extent of spotting on the belly.

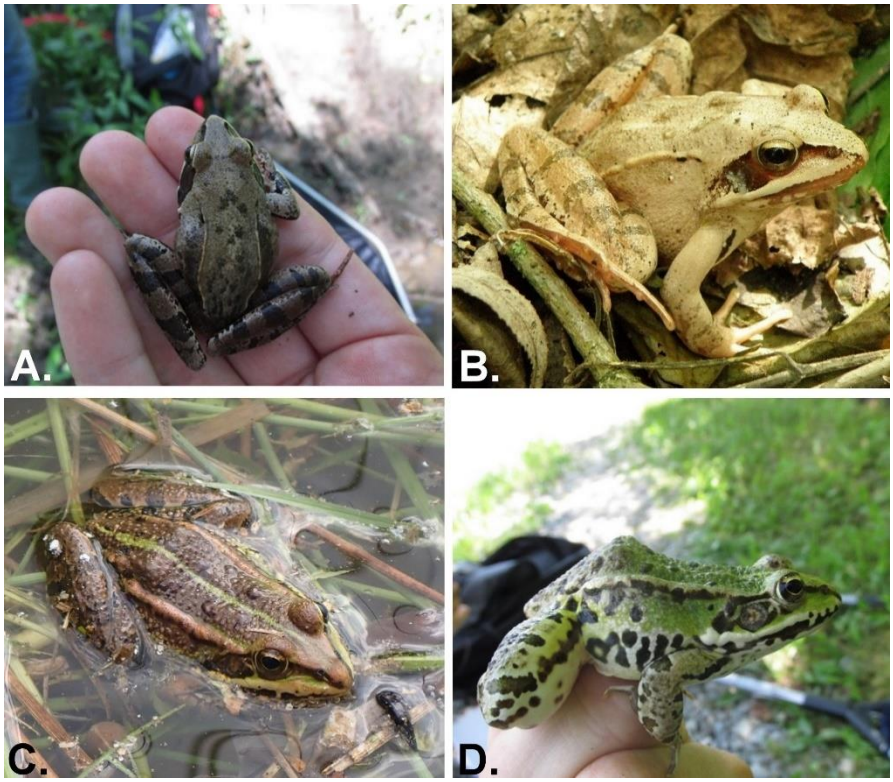

Fig. 21. Comparison of terrestrial and aquatic frogs. A. Common Frog (Rana temporaria), a terrestrial frog with a dark spotty pattern on the head. B. Marsh Frog (Pelophylax ridibundus), an aquatic frog with a light stripe running along the back.

Since all our amphibians are **protected by law**, capturing and handling them is prohibited. In this regard, a special permit from the Ministry of the Environment, Department of State Administration of Nature Protection, is required.

Amphibians as Bioindicators of Water Quality

One of the modern and most promising methods for the ecological assessment of environmental quality is bioindication, which allows detecting the degree and intensity of pollutant impact, as well as tracing the dynamics of ecosystem degradation over time and space and presenting data in an integrated form. Natural reactions of animal organisms to the quality of the environment can often be extrapolated to humans.

Amphibians meet all the requirements imposed on animals used for bioindication. Amphibian species found in the study areas have clear and convenient features for research, and their eggs and/or larvae are sensitive to pollution. Morphophysiological parameters of amphibian organisms reflect the state of the local habitat. Amphibians do not exhibit a pronounced tendency for migration, and they are characterized by a high level of polymorphism. All these factors allow the successful use of amphibians as bioindicators.

To detect adaptations of amphibians arising from the influence of water body pollution, we propose conducting research at three levels: at the population level (assessing population size, gender and phenotypic composition), at the individual level (evaluating morphology, physiology, and developmental stability), and at the cellular level (assessing the stability of nuclear structures).

The determination of the species and quantitative composition of amphibians should be carried out continuously using standard methodology for every 100 meters of shoreline. The sexual and phenotypic structure of populations is preferably analyzed in the spring without removing animals from their environment.

Research on the population structure of amphibians is crucial as it allows assessing the degree of ecological plasticity of the population and the species as a whole. Population size is one of the most important evaluative criteria for the state and dynamics of a population, reflecting its status and biological progress or regress.

The sexual composition of the majority of tailless amphibian populations can be easily determined by the presence of vocal sacs and nuptial pads in males. The ideal ratio in a population should theoretically be close to 1:1, with an equal proportion of males and females in the reproductive part of the population, resulting in a higher reproductive potential.

Fig. 22: Male Lake Frog with Resonators

Reducing the number of females in a population is detrimental, as it leads to a decrease in reproductive potential and impoverishment of the genetic structure. Interestingly, there is an

intriguing perspective in the literature that suggests the loss of males due to adverse factors is somewhat beneficial. In this scenario, the reproductive capabilities of the population do not suffer or suffer to a much lesser extent than the loss of females. Additionally, there is a selection of genotypes that are resistant to adverse factors, thus facilitating a microevolutionary process.

The sexual structure of amphibian populations can serve as an indicator of pollution, allowing for rapid, reliable bioindication of water without removing animals from populations.

Phenotypic Structure of Amphibian Populations

Genetic heterogeneity in natural populations is expressed in intrapopulation polymorphism. In several frog species, the so-called "striata" morph, characterized by a light dorsomedial stripe, and the "maculata" morph, a spotted characteristic, are observed. It is known that in many amphibian species, populations most susceptible to anthropogenic influence show an increased frequency of the striata morph. Genetic analysis indicates that this is a monogenic mutant.

The dominant allele of a diallelic autosomal gene, "striata," determines the presence of the stripe (complete dominance). Thus, "striata" serves as an excellent phenotypic marker to study phenotypic manifestations of changes in the genetic structure of populations.

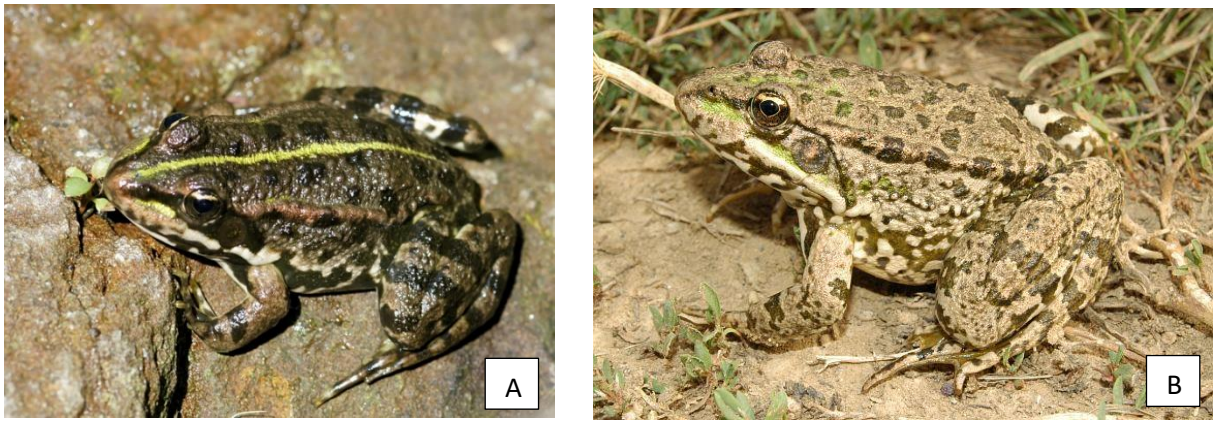

Fig. 22: Morph "striata" of the Lake Frog (A),
Morph "maculata" of the Lake Frog (B)

Taking into account the influence of non-selective elimination on the genetic structure of the population, as well as several features of the striata morph, it can be concluded that the high prevalence of this phenotype in polluted water is due to the advantages it receives under these conditions. The striata morph is characterized by a higher level of oxidative-reductive processes, hemoglobin content, reduced sodium permeability, and the content of certain metals at a greater body mass. The change in the phenotypic structure of amphibian populations in polluted water is associated with the different adaptive value of phenotypes, manifested in their selective mortality.

Therefore, the ratio of striata and maculata phenotypes in amphibian populations can be a convenient indicator for bioindication of pollution.

Morphological indicators of amphibians against anthropopressure

Measurements of standard morphological parameters were conducted using a caliper with an accuracy of 0.1 mm.

The sizes of morphological features are largely influenced by the surrounding environment, and the average values of many characteristics can be reliable markers of negative changes occurring in the habitat of amphibians.

Living in polluted waters is usually associated with changes in the external indicators of amphibians (body length and mass). It is known that amphibians in polluted water are smaller in size. This may be due to the accumulation of toxic substances in their bodies and disturbances in metabolism.

For bioindication, it is advisable to use both the entire complex of characteristics (males and females) and individual, most informative ones, as an increase in the concentration of heavy metals in the water leads to a significant change in all morphological features.

Teratogenesis in Amphibian Populations

Under conditions of environmental stress, the diversity of anomaly types and the overall frequency of aberrations change, so the variety and frequency of anomalies can be an indicator of the degree of transformation of the natural environment. In our opinion, a high frequency and variety of anomaly types can be an indicator of chronic stress, caused by an increased content of heavy metals in the water. According to literature data, anomalies result from a critical disturbance of developmental stability. The frequency and variety of congenital morphological anomalies can be highly effective in environmental monitoring systems.

Developmental Stability

The assessment of developmental stability is conducted based on the fluctuating asymmetry indicator. The degree of water pollution compared to the norm is determined by the disruption of developmental stability based on fluctuating asymmetry. The fluctuating asymmetry indicator reflects morphogenetic homeostasis.

Summary:

For rapid primary bioindication of the ecological state of water bodies and rivers, it is advisable to use population characteristics of tailless amphibians, such as the ratio of males to females, the proportion of the striata morph, and the assessment of fluctuating asymmetry. For an extended, in-depth assessment of the ecological state of water bodies and rivers, the entire complex of tested population, organismic, and cytological characteristics sensitive to environmental pollutants may be recommended. This complex includes (in addition to characteristics for primary bioindication) variations of morphometric parameters, indices of internal organs, and feeding, detection of the spectrum of phenodeviants, assessment of ontogenetic homeostasis based on the level of stability of nuclear structures. High birth rates and a wide variety of phenodeviants can be recommended as indicators of water body and river pollution with heavy metals. The bioindication test system can be used in operational environmental monitoring systems and for ecological forecasting of the situation's development.

Amphibians - Tasks to Practice

Task 1. Try to explain the concepts "**amphibian and indirect development**" associated with amphibians.

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Task 2. Try to write about the three orders (scientific and Slovak names) into which the class of amphibians is divided, and specify which ones occur in our territory.

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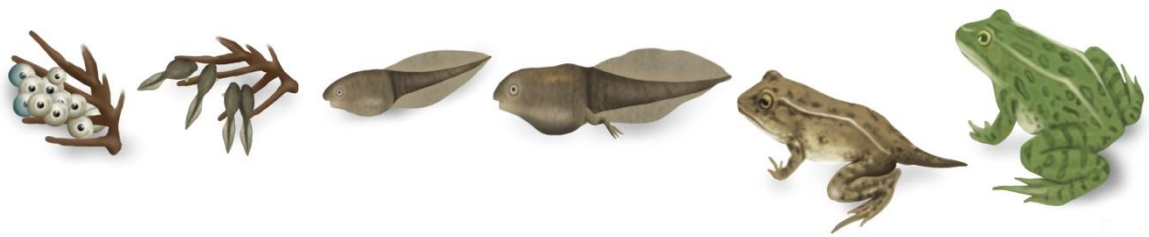
Task 3. How would you name, in one word, the process depicted in the picture? (correct answer: metamorphosis)



Task 4. Try to classify the amphibian eggs based on their visual assessment into the genera where they occur. Do the eggs have a calcareous shell? What are they susceptible to?

.....

.....

Task 5. With which three body organs do amphibians breathe, and in what developmental stage?

Task 6. Professionally name the types of fertilization and explain how they occur in our anuran and caudate amphibians.

Anuran amphibians -

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.....

Caudate amphibians -

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.....

.....

Task 7. All our amphibians are legally protected. What does this mean for us?

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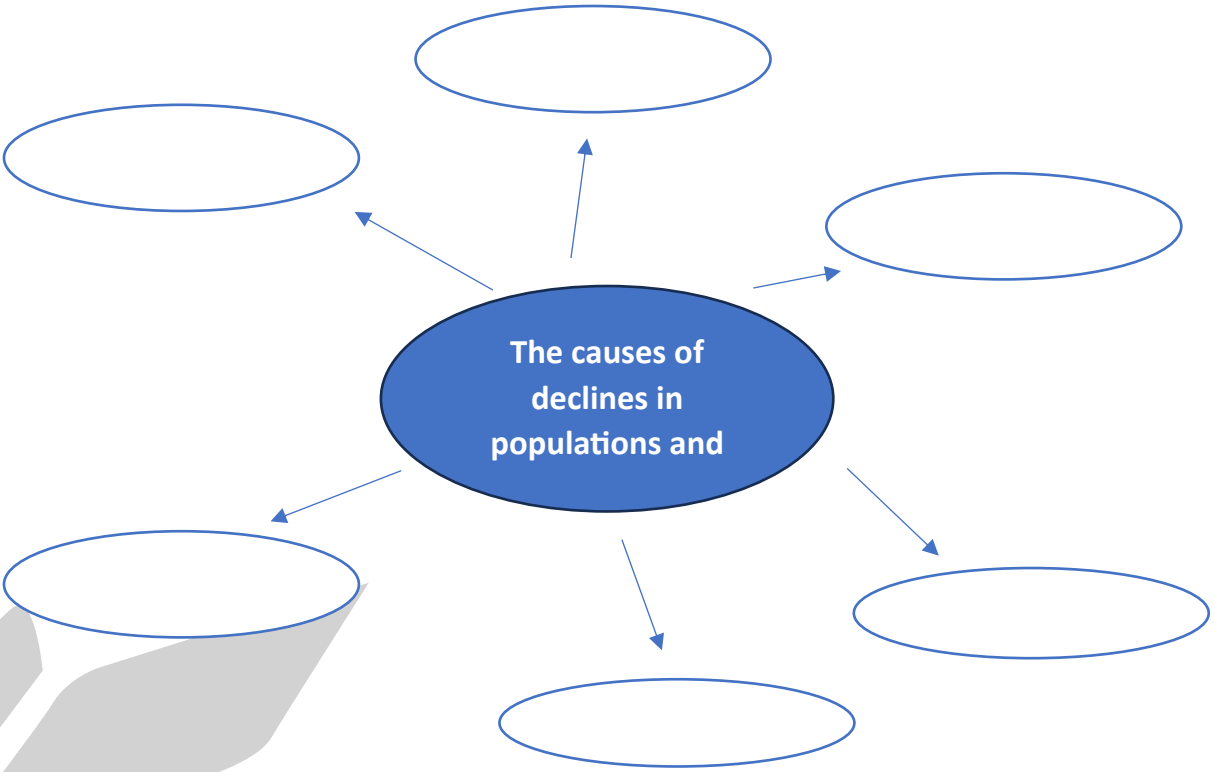
Task 8. Amphibians are currently among the most endangered vertebrates globally. Try to reflect on the factors threatening amphibian populations today and causing the extinction of some species.



Task 9.

You already know the meanings of the terms oviparous and ovoviviparous. Try to classify our amphibian species into the respective categories based on their reproductive methods.

Oviparous	Ovoviviparous

Task 10:

Imagine you are in the field and come across a spotted salamander. Based on what criteria could you determine whether it's a male or female? Does this amphibian species exhibit secondary sexual characteristics that allow for a straightforward distinction between genders? If yes, you can also draw these characteristics.

.....

Task 11:

Among the so-called "large newts" in our fauna, we classify species of newts that have larger sizes compared to other newt species. These large newts can be distinguished by their greater dimensions.

.....

Task 12.

Try to match the species with their morphological descriptions.

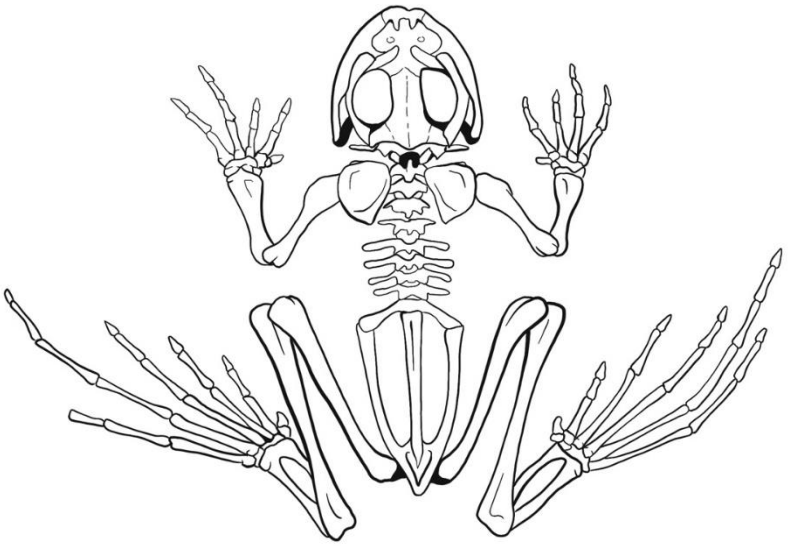
1	
2	
3	
4	
5	

Species
1. *Lissotriton vulgaris*
2. *Ichthyosaura alpestris*
3. *Triturus dobrogicus*
4. *Lissotriton montandoni*
5. *Triturus cristatus*

A. This is one of our most colorful newts. Females have marbling on the upper side of the body, and the belly is orange without spots. During the breeding season, males have a low but distinct black and yellow stripe extending from the head to the tail along the back. On the lower edge of the tail, there is a bluish stripe. The body is covered with prominent dark spots, and there are two dark stripes on the side of the head, one passing through the eye.
B It is the most widespread newt in our territory. The belly is whitish to orange with dark spots. During the breeding season, males have a prominent crest on the back from the head to the tail, uninterrupted in the pelvic area. On the lower edge of the tail, there is a bluish stripe. The body shows distinct dark spots, and two dark stripes run from the tip of the nose on the side of the head. Females are less conspicuous in color, with small dots instead of larger dark spots. Dark stripes are present on the head.
C It is a Carpathian endemic species. There are no dark spots on the belly, and skin ridges extend on both sides of the back, making the cross-section of the body angular from above. During the breeding season, males have a prominent thread-like extension at the end of the tail.
D The body is brown to dark reddish-brown from above. Dark spots are present on the back and sides. The belly is brightly orange-red with dark spots. The dorsal crest in males reaches up to the eye level, interrupted in the pelvic area, and continues on the tail. Limbs are short, and when pressed longitudinally against the body, the front and hind limbs do not touch.
E The body is dark gray from above with dark black spots and small white spots on the sides. The belly is yellow to orange with dark spots. The strongly serrated crest in males starts from the head at the eye level, and on the sides of the tail, there is a pearly shiny stripe. Limbs are longer, and when pressed longitudinally, the front and hind limbs touch each other.

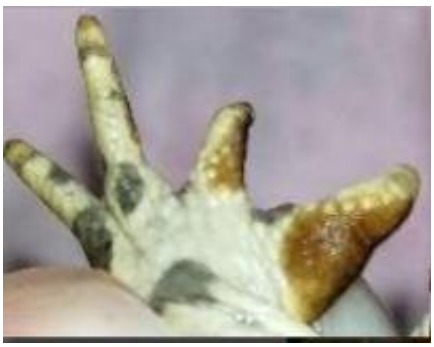
Task 13.

Try to name the professionally marked bones that occur on the skeleton of frogs.



Task 14.

What is the name of the pigmented structure on the fingers of the front limb in frogs? In which gender does it occur and what is it for?



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Task 14.

How would you distinguish a yellow-bellied rabbit from a red-bellied rabbit? What else do we have to take into account when determining the individuals that overlap the areas of occurrence of these two species?

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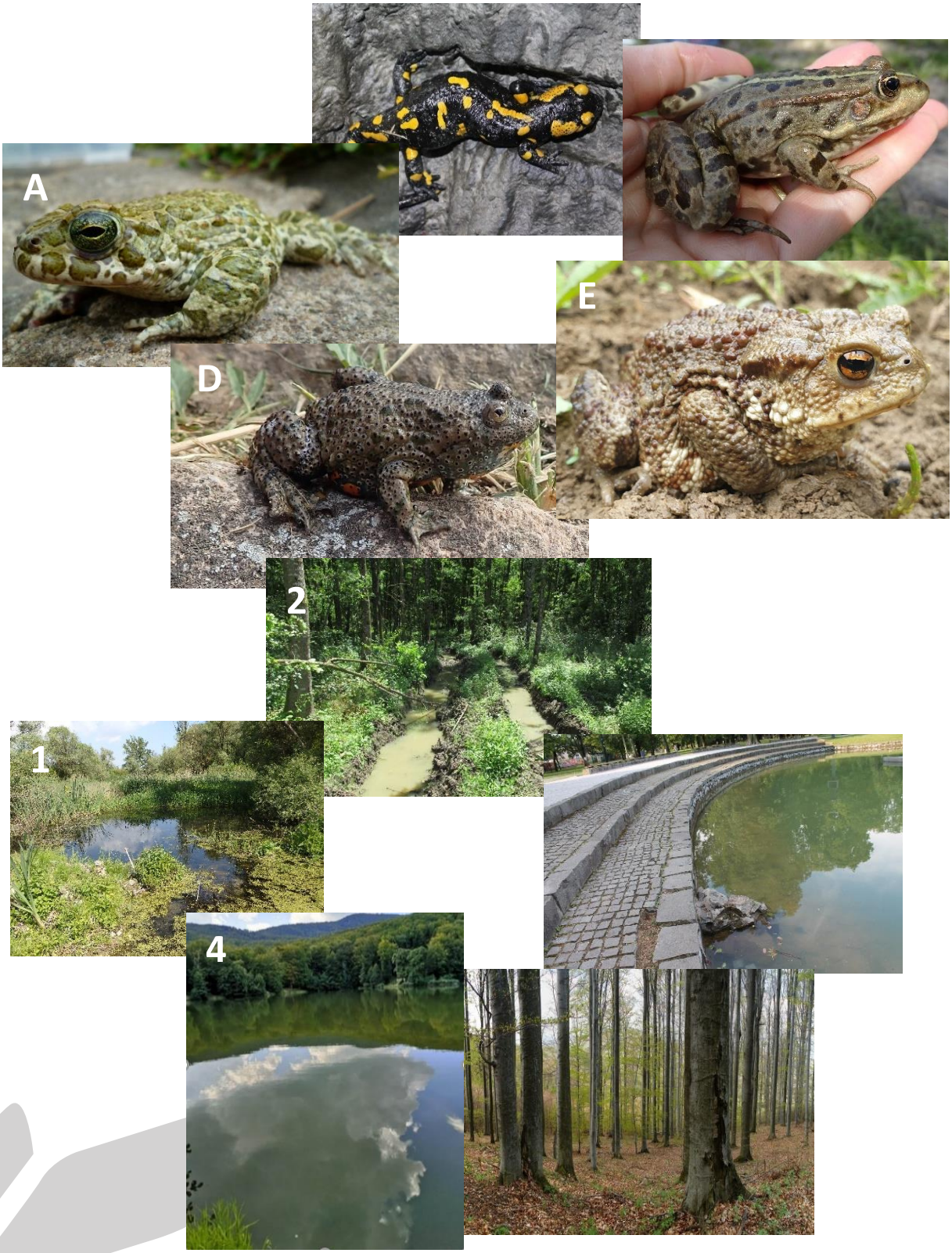
Task 15.

Name and assign suitable terrestrial or aquatic environments to each species where we could find them.



Task 16.

What is the name of the frog species in the picture and what is it known for? In which regions of Slovakia could we encounter this species?

.....

Task 17.

How would you distinguish between two separate species from the Bufonidae family that occur in our region? Try to list their distinct identification features.

Common Toad	Common Toad ....

Task 18.

Try to think and identify the species of amphibian in the picture. What are the structures labeled in the picture called? What gender is it likely to be?

.....

Task 19.

How many species of frogs are found in Slovakia, and into what basic ecological groups are they divided? Can you categorize individual species into these groups and explain the basic differences between these groups?

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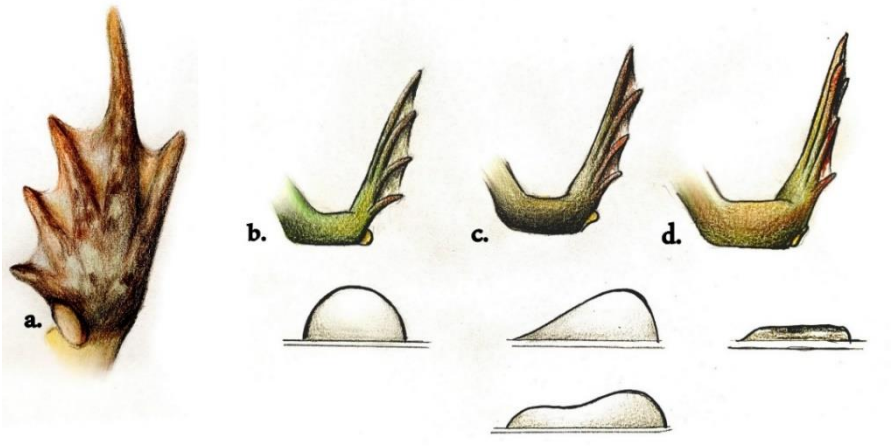
Task 20. **"I have a bump on my heel, so what?"**

Some amphibians have a more or less pronounced bump on their hind limbs. What is its purpose? Based on the following characteristics of frogs, assign the heel bumps to individual species.



Choose one of the options and assign it to the following characteristics.

A	B	C	D

Pelobates fuscus - It is a small frog with a hidden lifestyle and little dependence on aquatic environments. It has a prominent metatarsal tubercle on its hind limbs, which serves the toad for burrowing into the soil. It hibernates on land, likely very deep in burrows that it usually digs on its own.

Pelophylax ridibundus - It is our largest amphibian closely tied to aquatic environments, hibernating mainly at the bottom of water bodies, occasionally on land. It inhabits larger water surfaces such as lakes, gravel pits, or rivers.

Pelophylax esculentus - It is a hybrid not considered a true species. Morphologically (including the shape of the metatarsal tubercle) and in its characteristics, it falls between the common frog and the pool frog. It hibernates on land and in water, living year-round in or near water.

Pelophylax lessonae - It is the smallest among the green frogs, usually hibernating on land in wooded marshes or deciduous forests. It has a high crescent-shaped metatarsal tubercle on its hind limbs. Among all water frogs, it is the least dependent on water, preferring smaller water bodies with denser vegetation.

Based on the knowledge you already have about amphibians, try to illustrate the most important elements in the habitat that need protection for their successful survival.

For the amphibian capture, you will need a dip net, rubber boots, and surgical gloves. The capture and handling of individuals will be performed by an expert. Your task is to observe the expert's work attentively and, using a key or literature, identify the species. Try to fill in the following tables. Take note of relevant qualitative parameters (e.g., color, spotting, etc.) in captured individuals. Record selected body measurements that the expert reads with a caliper. After recording all important information, thoroughly photograph each individual.

Using the identification key and atlases, try to identify the amphibians you observed in the field and create your own field notebook.

Slovak name of the species	Scientific name of the species	Date	Location	GPS coordinates	Habitat type	Number of individuals

Location	Date	Species	Body Color	Belly Spots	Yellow Color on Thighs/Sides	Body Length	Thigh Length	Shank Length	Shape of the Heel Bump	Pupil shape	Iris color	Photo number

Do you hear croaking?

Using the vocalization key on the website [https://soundcloud.com/], try to identify the species of frog. Use the table below. If a particular species is present, record it in the last column.

Species	Sound Characteristics	Presence
Common toad	https://soundcloud.com/mark-wilkinson1-1/common-toad-mating	
European green toad	https://soundcloud.com/marco-pesente/bufotes-balearicus-call	
Common frog	https://soundcloud.com/nordicnature/common-frog-rana-temporaria	
Agile frog	https://soundcloud.com/alexandre-roux-994988095/rana-dalmatina	
Common spadefoot toad	https://soundcloud.com/user-650307165/aba-moczarowa-rana-arvalis	
Moor frog	https://soundcloud.com/wildlife-sound-recording/marsh-frog	
European tree frog / European common frog	https://soundcloud.com/user-123934429/pelophylax-lessonae-pelophylax-kl-esculentus-rnn76	
Common toad	https://soundcloud.com/krzysztof-konieczny-blog/hyla-arborea	

Do males or females exhibit vocalizations?

Capture and Handling of Tadpoles

Tadpoles are very fragile, so handling them should be done very carefully and only by an expert. At each location, don't forget to measure the water temperature where you find tadpoles. Observe captured tadpoles using a magnifying glass in a Petri dish or a small transparent container. Don't forget to photograph the tadpole from the ventral, lateral, and dorsal sides. Try to find the respiratory opening. On which side is it located? Have you observed any deformities in the tadpoles? What are they? Based on morphological features (presence of respiratory opening, limbs, tail, etc.), try to classify the tadpole into the corresponding developmental stage (more information in the Study Text). Record all these observations in a table.

Location	Date	Habitat	Position of respiratory opening	Deformities	Gosner stage

At the end, take a collective photograph of all the captured tadpoles in the Petri dish along with a scale (e.g., ruler, grid paper) from the same distance and angle. You can use a camera mounted on a tripod, with the Petri dish positioned vertically. Release all the captured tadpoles back to their capture site without causing harm. With these photographs, you will be able to work with them at school.

Evaluation of Field Observations in School

Compare the occurrence of amphibians at the visited locations.

Which species and in what numbers did you observe at each location? Consider what factors could have influenced their activity, and record your findings in a table.

Location	Species	Number	Selected Water Quality Parameters

Tasks focused on the analysis of photographs:

1. By analyzing the photos you have taken, compare the developmental stages of the barnacles and possibly also the amount of deformities within the site and between sites. Study the information in the study text.
2. Measure the length of the fins from the photos taken using the Image J program. Instructions for working with Image J can be found at effuse.science.upjs.sk in the section for teachers
3. From the recorded total composition of animals at the site, determine which species could be prey or predators of tadpoles and metamorphosed (transformed) frogs..

"Write a verbal conclusion on the species composition of amphibians at the sites:

HABITATS AS INDICATORS – subject of scientific education

Aquatic, Riparian, and Wetland Plants and Habitats as Indicators of Water Body Status

Plant communities serve as crucial visual indicators of ecosystem health. Through phytosociological studies or analysis of the behavior of species forming communities, it is possible not only to assess climatic and edaphic resources but also to monitor changes in the ecosystem associated with both natural and anthropogenic factors. However, considering the difficulty of identifying communities without specialized training, it is more practical to use biotopes - the places where vegetation exists, treated as ecosystem types with spatial and temporal aspects.

A biotope is a more convenient object for classification and mapping of ecosystems. The degree of representativeness and preservation of aquatic and riparian biotopes can be applied to assess the state of water bodies and conduct subsequent monitoring. The names, scope, and understanding of biotopes are based on the "National Catalog of Biotopes of Ukraine" (2018), considering regional interpretations of biotope types in other catalogs. Identification and determination of biotopes are carried out based on the presence of characteristic species, compliance with typical structure, and the specific ecological characteristics of a particular biotope, as described in the mentioned catalogs, or by using a specially developed key for determining biotope types.

Criteria for Biotop Assessment

In analogy to the system developed by us, considering the experience of European countries (Germany, Norway, Austria, etc.), for assessing threats and determining the conservation status of rare habitat types in the Ukrainian Carpathians and Zakarpattia lowlands, the evaluation of aquatic and riparian biotopes and surrounding vegetation as indicators of water body status should be conducted based on two main criteria:

1. *Loss of area* (range) where a specific biotope type is prevalent.

2. *Qualitative changes in the biotope* (its degradation).

For the convenience and universality of assessment, it is proposed to conduct the evaluation for each criterion across three categories.

Criterion "*Area Loss*" (LA):

1 – Complete loss of area, total destruction, or very significant reduction in area – a threat of complete disappearance (today, only a small part of the original biotope area exists, or without the application of special conservation and management measures, complete disappearance may occur in the near future) – area reduction by 75-100%.

2 – Strong or substantial reduction in area (significant threat of biotope disappearance in this area; on adjacent areas, the biotope has disappeared, or there are negative trends in area reduction within the entire watercourse or locally) – area reduction by 25-75%.

3 – Absence or minor reduction in area, or remains unchanged; potentially stable – area reduction by 0-20%.

Criterion "*Quality Loss*" (QU):

1 – Completely destroyed – the biotope has undergone such qualitative changes that typical or natural variants of the habitat are entirely destroyed, or the biotope is threatened with complete qualitative destruction (ruin) (threatened by complete destruction) or has undergone negative qualitative changes almost throughout its distribution within the watercourse, so that the typical natural structure and species composition remain only in a few or only one locality, and there is a threat of complete destruction of the biotope in a short time – qualitative changes have occurred by 75-100%.

2 – Significant qualitative changes – the biotope has undergone qualitative changes to the extent that the loss of a qualitative state covers most of the distribution within the watercourse, or the typical natural structure and species composition of the biotope have disappeared/transformed in several localities; qualitative changes have occurred by 25-75%. This point also includes biotope disturbance due to the penetration and spread of invasive species.

3 – No or minor qualitative changes, invasive species have penetrated, but they constitute a small percentage (1-5%) of the plant cover.

Rules of assessment

Criterion	*Criteria for evaluating the indicator*		
Area Loss **(Area Loss – LA)**	**1** **Well** Biotope not at risk (proper functioning)	**2** **Satisfactory** Biotope under threat	**3** **Unsatisfactory** Biotope under threat of disappearance (Disrupted functioning)
	Absent or slight reduction in area; or remains unchanged; potentially present – reduction in area by 0-20%	Strong or significant reduction in area (significant threat of biotope disappearance in this section, adjacent areas have lost the biotope, or there are negative trends in area reduction within the entire watercourse (watercourses) or locally) – reduction in area by 25-75%	Complete loss of area, complete destruction, or very strong reduction in area – threat of complete disappearance (today, only a small part of the previous biotope areas exists, or without the application of special conservation and management measures, its complete disappearance may occur in the near future) – reduction in area by 75-100%
Qualitative change of biotope (Quality Loss – QU)	**1** **Well** Biotope not at risk (proper functioning)	**2** **Satisfactory** Biotope under threat	**3** **Unsatisfactory** Biotope under threat of disappearance (Disrupted functioning)

	Qualitative changes are absent or minor; invasive species have penetrated, but constitute a small percentage (1-5%) of the plant cover.	Significant qualitative changes (properties) – the biotope has undergone qualitative changes to the extent that the loss of qualitative status has occurred over a large part of the distribution section within the watercourse, or the typical natural structure, species composition of the biotope have disappeared/transformed in several locations, qualitative changes have occurred by 25-75%; this point also includes biotope disturbance due to the penetration and spread of invasive species.	Completely destroyed – the biotope has undergone such qualitative changes that typical or natural variants of the habitat are completely destroyed, or the biotope is under the threat of complete qualitative destruction (ruin) or has undergone negative qualitative changes almost throughout the distribution section within the watercourse, so that the typical natural structure, species composition remain only in a few or only one locality, and there is a threat of complete destruction of the biotope in a short time – qualitative changes have occurred by 75-100%.

The assessment of the water body/stream status is carried out simultaneously using two criteria. The overall rating was calculated as the arithmetic mean of the sum of categories of the two factors. Based on the average ratings in the survey points along the watercourse, an assessment is established for the biotope within the entire distance of the watercourse. In case the average indicator falls within the range between whole numbers of categories, the indicator is always rounded up to the higher category (2-3 → 2).

Activities focusing on the theme of sustainable development offer the teacher the opportunity to develop students' critical thinking skills and encourage alternative ideas and provocative questions in the classroom. The learning gains momentum and the atmosphere encourages an active approach to the issues discussed. At the same time, the underlying theme is 'playing with the unknown' for which there is no clear solution yet, which is in contrast to learning about already known facts that have already been discovered. A prerequisite for a 'fruitful' discussion or any active activity in an area that is unfamiliar to pupils is sufficient knowledge, which pupils should possess.

In the framework of the EFFUSE cross-border project, this handbook has been developed for biology students, prospective teachers or practising teachers who are interested in planning environmental education in a targeted way. In this handbook, we offer a concept for education focused on the topic of water in the context of sustainable development. The concept is designed in three modules **Theoretical background**, **Global issues** and **Sustainable development**.

The core of education for sustainable development should be educational activities aimed at adapting to climate change, mitigating its impact and reducing its negative consequences. There is a need to emphasise greater effectiveness of education. Education reduced to rote memorisation of known facts cannot bring value to society. On the contrary, creating a conducive classroom climate that encourages creativity, innovation, exploration, hands on science or alternative learning exclusively outdoors is a way to overcome the obstacles that hinder a pupil's intellectual growth.

The basic knowledge needed to progress to the level of understanding global problems and designing possible solutions is covered in the first module, Theoretical Foundations. The module is divided into three thematically distinct units, **Water and its "Faces"**, **The Water Cycle** and **Water as the Essence of Life**.

After the theoretical background, the second module introduces students to more detailed **Global Water Issues** that are directly or indirectly related to human use of water resources. They already have some knowledge of many of them, so it is appropriate to initiate a discussion at the beginning of the module and then to adapt the content of the module. Suitable formulations of global issues might be **Water as an Endangered Natural Resource** and **Water as a natural disaster.**

Following the module focused on learning about the nature of global issues related to the topic of water, students move on to the most challenging part of the learning process. In the third - final module, **Sustainable Development**, they design solutions to problem-solving tasks, using the knowledge acquired in the previous two modules**. Water Protectors, Water as a Factor in the Biodiversity of Organisms** and **Sustainable Project Proposals.**

MODUL Theoretical and Practical Backround

Water - Same and Always Different

Activity 1: The many faces of water

Objective	to highlight the importance and significance of water for humans and other living organisms
Time:	45 minutes
Aids:	papiere, písacie potreby, stopky

Short desription: The activity is an introduction to water, presenting a comprehensive view of water and its importance not only for humans, but also for life on Earth. Pupils work in groups during the activity, ideally with at least 4-5 members in each group.

Procedure: The activity takes place in five rounds, alternating between a short guiding word by the teacher and student activities in groups. The teacher explains the rules of the game to the pupils. The first group sends one member to the teacher. The teacher shows him one word. The pupil's task is to use pantomime to try to show or act out the word to the other members of their group. It is important that the pupil who is showing does not say anything or make any sounds until his group has guessed it.

Terms and context need to understand:

water as an environment, as a necessary condition of life, as a part of our household, as a place of relaxation, as a dangerous element

ROUND 1:. One pupil uses pantomime to show or play the word to other members of his group, who try to guess the word. If successful, the group gets a point. In this way the groups take turns and the pupils take turns in presenting in the same way

Words for the first round:

river, ocean, ice, cloud

Tip: In addition to pantomime, the teacher can also introduce a description of the word or a drawing of the word without expressing it verbally. In each round there are several words from which the teacher can choose at least as many as there are play groups.

After the first round, the teacher asks the students what they think the theme of the activity is. They know the clues, they are the words they have guessed so far during the group work. The pupils should come to the conclusion that the topic studied is water. The teacher will point out that in the first round water was represented in different states. Through the first round, pupils should review the states of water as well as the places on Earth where water is found in different forms.

ROUND 2: The second round will be conducted in the same way. Pupils take it in turns to pantomime acting, or describing or drawing, to make the words available to their group as quickly as possible.

Words for the second round:

human, frog, mosquito, plankton,

Based on the guessed words, the teacher can point out that not only humans but also other living organisms depend on water, but this dependence can take different forms. For some animals, such as fish, water is their lifelong habitat. Frogs such as toads, for example, seek water at breeding time to lay their eggs. These hatch into tadpoles, which develop in the water. Once it has transformed into a frog, the toad no longer needs an aquatic environment. It only needs to take in water, just like us humans and all land animals. The mosquito, whose larvae develop in water, is the same way. In addition to thirst, the stork is drawn to the water by its food, which is found near and in the water.

ROUND 3: As a follow-up to the discussion in the second round, the teacher can draw attention to humans and their use of water in life. He can let the pupils try to list all the things we need water for, e.g. for drinking, washing, washing clothes, watering flowers, generating electricity, relaxation, sports, etc. The vocabulary from this round can be used as a guide or clue.

Words for the third round:

electricity, swimming, boat, toilet

ROUND 4: The next round focuses on water as an element that can cause considerable problems for I. Such a water element can be an avalanche, a flood, a falling iceberg (hail). Water shortages or lack of drinking water can also be a problem that the teacher can point to.

Words for the fourth round:

avalanche, flood, desert, tsunami

ROUND 5: The last round is dedicated to the water cycle in the earth.

In the last round on the water cycle in the earth, pupils have to guess words related to the water cycle in nature, e.g. sea, sun, soil, cave, rain, clouds, evaporation, precipitation. It is useful to choose a smaller number of words, e.g. five as in the example given in each round.

Words for the fifth round:

cave, soil, evaportion, rain

After the pupils have guessed the words, create a water cycle in the landscape using the paper with the words that the pupils were asked to guess in each round. You can expand the vocabulary as needed.

CONCLUSION

At the end, the teacher and the pupils review all the possible 'faces' of water that they have introduced in this activity. For the review, the teacher can use a concept map (mind map) with water as the central target word.

Activity 2: Adventure travel of raindrops

Objective	to create water cycle in microworld using easily available aids
Time:	45 minutes / work in pairs for one week
Aids:	.....

Short desription: Pupils try to draw listened story about the journey of a raindrop.

Procedure: The teacher reads the story about the journey of a raindrop the to the pupils about how the drop traveled around the world and what it saw and experienced on its way. Pupils listen and at the same time record the path of the drop in writing or drawing on a prepared drop cut out of paper. After finishing, the students present their creation and repeat what they heard in the story. There is a discussion about what the individual students picked up, what concepts they encountered, what they learned. /Forms of water sources/

Water – Constant Cycle

Activity 1: Creation of water cycle in microworld

Objective	to create water cycle in microworld using easily available aids
Time:	45 minutes / work in pairs for one week
Aids:	.....

Short desription: The activity is an introduction to water, presenting a comprehensive view of water and its importance not only for humans, but also for life on Earth. Pupils work in groups during the activity, ideally with at least 4-5 members in each group.

Procedure: The activity takes place in five rounds, alternating between a short guiding word by the teacher and student activities in groups.

Activity 2: Creation of educational material focusing on the water cycle

Objective	creation of digital educational card aimed to the water cycle.
Time:	45 minutes / work in pairs for one week
Aids:	Grafic tablet or notebook

Short desription: The activity is focused on the development of digital skills, and consolidates existing knowledge about the water cycle. The prerequisite is that the students have already become familiar with the topic and the creation of a digital card supports the linking of knowledge about the water cycle in the nature.

Procedure: The activity is individual work or work inpairs if necessary. Is implemented during lesson when teacher can guide pupils to correct understanding. Before activity is is necessary to check pupils comptencies to work in grafic desinprogram.

Water as the Essence of life

Activity 1: Microworld of water Mikroriasy , rozsievky – odkaz na kľúč

Objective	Observation of water sample from lake under microscope.
Time:	45 minutes / individual work
Aids:	Water sample, microscope

Short desription: Overall, the microworld of water is a rich and diverse area of study, spanning the fields of physics, chemistry, and biology. In terms of biology, the microworld of water is teeming with life. Tiny organisms like plankton, algae, and bacteria are crucial parts of aquatic ecosystems, playing key roles in nutrient cycling and food chains. These organisms are often microscopic, requiring specialized equipment to observe and study.

Procedure: Observe water samples from different sources under a microscope. Determine if small algae or plankton are present in the samples. For more detailed analyses we present the rules for the analysis of the microbial composition of water.

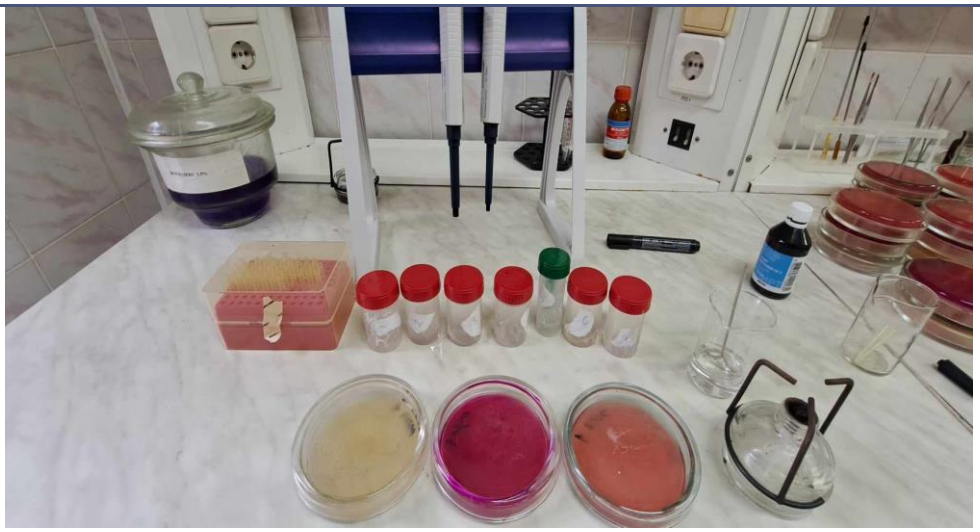
General Rules for Water Sampling

Samples from open water bodies are taken using special instruments called bathometers, which are lowered to a certain depth. The instrument opens, allowing water to enter, and is then brought to the surface for analysis. When taking samples, certain rules must be followed:

1. If there is a source of contamination, three water samples are taken from such a water body: above the source of contamination, opposite it, and downstream.
2. Water samples from wells are taken twice: in the morning before water use and in the evening after water use.
3. From ponds, lakes, and rivers, water samples are taken from a depth of 0.5-1 m and at a distance of 1-2 m from the shore.

Water samples are collected in well-cleaned, dry glass bottles with tightly sealed stoppers. For microbiological analysis, 2 liters of water are required for each sample. A accompanying form is attached to each sample, indicating the name and location of the source, the date of sampling, the exact sampling location, air temperature, precipitation data on the day of sampling and for the last 10 days, water temperature, a brief description of the water body, and the purpose of the analysis. Samples are delivered to the laboratory immediately.

The content of mesophilic aerobes and facultative anaerobes per 1 ml of water is determined. Two volumes are plated from each sample, ensuring that the number of colonies ranges from 30 to 300. For the analysis of tap water, 1 ml of contaminated water is added to each of the two dishes – 0.01 and 0.001 ml. When determining the microbial count in heavily polluted and sewage water, the samples are diluted with sterile water to create serial dilutions.



During planned routine examination, a partial list of indicators is determined, including the degree of overall microbial contamination regardless of bacterial type TBC, the content of total coliforms bacteria group (BGCP), E.coli, and enterococci.

From the collected sample, 2 ml of water is taken using a sterile pipette: 1 ml is added to a sterile bacteriological dish, and 1 ml to a test tube with 9 ml of sterile water. The test tube represents the first dilution (1:10). Using another sterile pipette, the contents of the first test tube are mixed, and 1 ml of the dilution is transferred to a second test tube with 9 ml of sterile water (second dilution 1:100), and 1 ml is added to a second bacteriological dish. The same procedure is repeated for subsequent dilutions. Afterward, 10-12 ml of sterile melted and cooled to 45°C nutrient agar (MNA) is poured into each dish. The dishes are quickly sealed and agitated with rotational movements to mix the MNA with water. A corresponding mark is made on the lid of each dish. Two repetitions are performed for each dilution. After the MNA solidifies, the dishes are inverted and placed in an incubator at a temperature of 35-37°C for three days. The number of colonies in each dish is counted, the arithmetic mean is calculated, and taking into account the dilution, the total number of microorganisms per 1 ml is computed. It is preferable to use the dilution that yields 100-200 colonies for calculation. Water quality is assessed based on the following criteria for the number of microbes per 1 ml: microbiologically clean water - up to 100; questionable - 100-500; poor - over 500 (Table 1).

According to GOST 2874-54, for drinking water, the total number of microbes per 1 ml when seeding undiluted water and incubating for 24 hours at a temperature of 37°C should not exceed 100. TBC for open water bodies and well water should not exceed 1000. During the sanitary assessment of water conducted by sanitary-epidemiological stations, the accepted indicators include coliform titer and coliform index.

Table 1 Microbiological Safety Indicators for Drinking Water

№	Indicator Name	Units	Norms
1	Number of bacteria per 1 cm3 of water (TBC)	CFU /1 cm^3	Not more than 100
2	Number of coliform bacteria per 100 cm3 of water	CFU /1 dm^3	Not more than 3

3	Number of thermotolerant coliform bacteria per 100 cm3 (FC index)	CFU /1 cm^3	Absence
4	Number of pathogenic microorganisms per 1 dm3 of water	CFU /1 dm^3	Absence
5	Number of coliphages per 1 dm3 of water	CFU /1 dm^3	Absence

Determining Coliform Bacteria in Water

Coliform bacteria include gram-negative, non-spore-forming, oxidase-negative rods that ferment lactose with the production of acid and gas at 37°C within 1-2 days. The category of coliform bacteria includes bacteria from the *Enterobacteriaceae* family, such as *Citrobacter*, *Enterobacter*, and *Klebsiella*. These bacteria are released into the environment through human and warm-blooded excreta and serve as quantitative indicators of the degree of fecal contamination in water. The most common method used today is the fermentation (titration) method, which determines the proliferation of coliform bacteria by seeding different volumes of the tested liquid. Lactose-peptone medium with an indicator and fermentation tubes are used as the accumulation environment.

Depending on the water source, the following are investigated:

- During purification and disinfection stages, 100, 10, 1, and 0.1 cm^3 of water are seeded.

- In tap water, three volumes of 100 cm^3, three volumes of 10 cm^3, and three volumes of 1 cm^3 are examined.

The specified volumes are introduced into bottles and vials with lactose-peptone medium with floats. Seeds of 100 and 10 cm^3 of water are carried out in bottles and vials, respectively, with 10 and 1.0 cm^3 of concentrated medium (for 1000 cm^3 of distilled water: 100 g of peptone, 50 g of glucose, 50 g of sodium chloride, 100 cm^3 of Andrade indicator). Samples of 1.0 cm^3 of water are sown in vials containing 10 cm^3 of normal concentration medium (for 1000 cm^3 of distilled water: 10 g of peptone, 5 g of glucose, 5 g of sodium chloride, 10 cm^3 of Andrade indicator). Seeds are incubated for 24 hours at 37°C. The absence of changes in the cultures allows concluding the study at this stage and giving a negative result. In the presence of cloudiness, acid, and gas in such a vial, seeding is performed on Petri dishes with Endo medium. The presence of red colonies with a metallic sheen on the Endo medium and rod-shaped gram-negative bacteria in smears, with a negative oxidase test, indicates the presence of coliform bacteria in the samples.

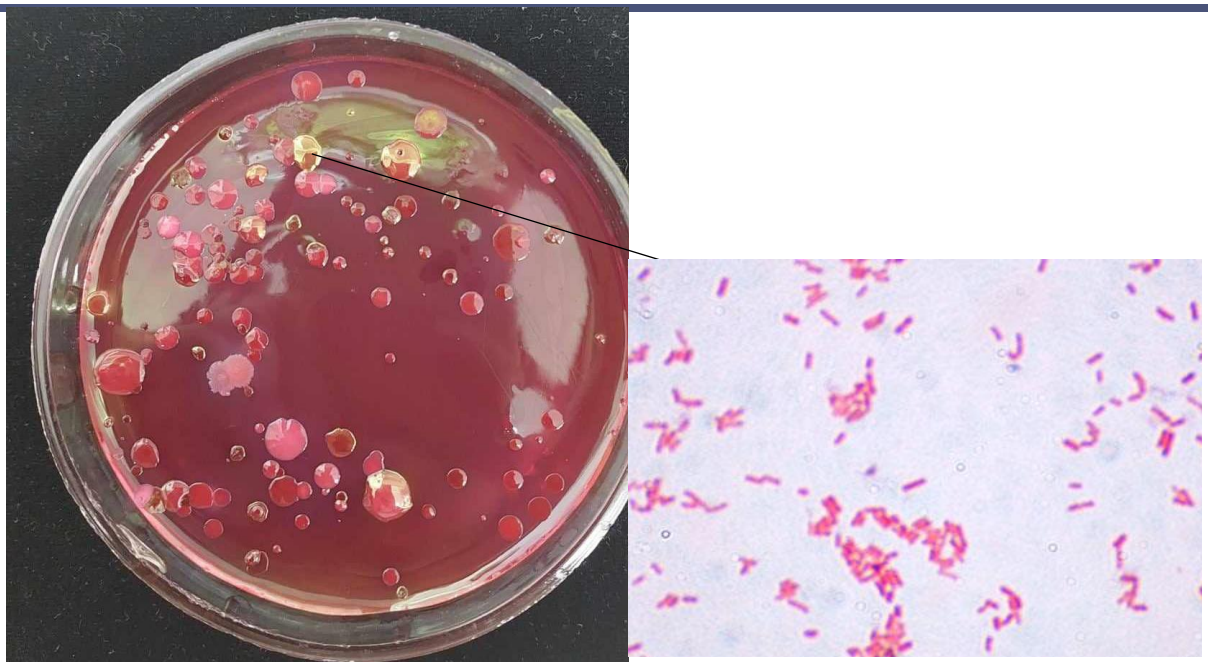
Drinking water is considered suitable for consumption if it contains up to 100 microbial cells per 1 ml. The degree of biological contamination of water is assessed by the coliform titer and coliform index. The coliform titer is the smallest volume of water from which one *coliform cell* can be seeded. The coliform index is the quantity of *coliform cells* found in 1 liter of water. According to current standards for tap water, the coliform titer should not be less than 300, and the coliform index should be more than 3. In large cities, more stringent requirements are imposed on water quality based on bacteriological indicators: coliform index not exceeding 2, and coliform titer not less than 500. In case of bacterial contamination of water above permissible standards, additional research is conducted to detect the presence of bacteria, indicators of fresh fecal contamination, such as coliforms. Their presence is determined by their

ability to ferment lactose to acid and gas at 43°C in the presence of inhibitors of foreign microorganisms (Table 2).

Table 2 Determination of the Coliform Bacteria Index (Coliform Index) when inoculating 333 cm³ of water:

Number of Positive Results in Water Analysis			Coliform Index (Coli-Index)	Coliform
three bottles of 100 cm³ each	three vials of 10 cm³ each	three vials of 1 cm³ each		
0	0	0	<3	>333
0	0	1	3	333
0	1	0	3	333
1	0	0	4	250
1	0	1	7	143
1	1	0	7	143
1	1	1	11	91
1	2	0	11	91
2	0	0	9	111
2	0	1	14	72
2	1	0	15	67
2	1	1	20	50
2	2	0	21	48
2	2	1	28	36
3	0	0	23	43
3	0	1	39	26
3	0	2	64	16
3	1	0	43	23
3	1	1	75	13
3	1	2	120	8
3	2	0	93	11
3	2	1	150	7
3	2	2	210	5
3	3	0	240	4
3	3	1	460	2
3	3	2	1100	09
3	3	3	>1100	>0,9

Bacteria of the coliform group (BGC) are a key indicator when assessing bacterial contamination of drinking water, the degree of purification and disinfection of sewage, water from water supply sources, seas, and swimming pools. Some countries (such as the USA and the UK) have adopted enterococci as standards. Enterococci are part of the normal microbiota of the human intestine and are present in significant quantities in sewage. The ratio of BGC to enterococci can be used to assess the sanitary condition of water bodies. In water bodies contaminated with untreated sewage, BGC predominates over enterococci. In the case of sewage disinfection, BGC dies off more rapidly, and the ratio of the number of BGC to the number of enterococci decreases almost to one.



Sowing a water sample on Endo medium.

Escherichia coli at 1000x magnification (Gram staining). https://sk.wikipedia.org/wiki/Escherichia_coli

Fig. Determination of bacteria of the coliform group on Endo medium."

Activity 2: Basic messurement of of water parameters

Objective	Observation of water parameters in the nearest water source
Time:	45 minutes / work in pairs for one week
Aids:	Work with database

Short desription: Participants engage in a week-long, 45-minute activity, working in pairs to observe and record water parameters in the nearest water source. Utilizing a database, they collect valuable data to understand the quality and characteristics of the water. This activity enhances observational skills and environmental awareness by actively involving participants in monitoring and understanding local water quality, facilitated by a user-friendly database.

Procedure:

1. Introduction (5 mins):- Emphasize the importance of water parameter monitoring.

Introduce the week-long task: observing and recording data from the nearest water source.

2. Database Tutorial (10 mins): Briefly explain how to use the database. Specify the parameters to observe (e.g., temperature, pH).

3. Field Observation (30 mins, spread over one week): Pairs regularly visit the water source, recording parameters in the database. Encourage noting any changes or anomalies.

4. Data Review (5 mins): At week-end, participants analyze collected data. Discuss findings within pairs, identifying patterns.

For messuremnet of basic parameters by messurement system Vernier see Anexes (page 82)

Activity 3: Water as living area

Objective	Creation of video scenario - story for animated video focused on animals living in the water, or surraundings
Time:	45 minutes / work in pairs
Aids:	Pen, paper

Short desription: During this 45-minute activity, participants will work in pairs to create a captivating video scenario centered around the theme "Water as a Living Area." The focus will be on exploring the diverse ecosystems of aquatic environments and the fascinating animals that inhabit them.

Procedure: 1. Introduction (5 mins): Discuss the importance of water ecosystems and introduce the goal: creating an animated video on aquatic life.

2. Brainstorming and Storyboarding (25 mins): Pairs brainstorm ideas and create a rough storyboard, emphasizing creativity in depicting water habitats and diverse animal life.

3. Scriptwriting (10 mins): Participants write a concise script focusing on engaging storytelling and educational content about the chosen aquatic environment.

4. Presentation (5 mins): Pairs present their video scenarios to the group, encouraging feedback and discussion.

MODUL Global Water Issues

Water as an endangered natural resource

Activity 1: Total water supply in the world

Objective	Finding out which countries have the largest reserves of drinking water
Time:	10 minutes /individual work / work in pairs / work in groups
Aids:	internet

Short desription: The activity is focused on developmnet of critical thinking and and the ability to distinguish a reliable source of information.

Procedure: The activity is individual work or work inpairs or groups depending on depending on the age of the pupils. The task is to find answers to the following questions:

Q.1. In which country is the largest supply of drinking water?
Q.2. Why?
Q.3. Is there enough drinking water in this country?
Q.4. Which country has the greatest shortage of drinking water?
Q.5. How do you think it should be resolved?

Activity 2: The role of the rainforest in global weather regulation

Objective	Observation of water sample from lake under microscope.
Time:	45 minutes / individual work
Aids:	Water sample, microscope

Short desription: Participants engage in a 45-minute individual activity exploring the crucial role of the rainforest in global weather regulation. Through observation of a water sample from a lake under a microscope, they gain insights into the microscopic life forms that contribute to the delicate balance of this ecosystem. The activity aims to foster appreciation for the intricate connections between microscopic organisms and the larger ecosystems, highlighting the essential role of rainforests in global weather regulation.

Procedure: **1. Introduction (5 minutes):** Briefly discuss the vital role of rainforests in global weather regulation. Introduce the objective: observing a water sample from a lake under a microscope to understand the microscopic life within.

2. Sample Collection (10 minutes): Provide participants with water samples from a local lake. Emphasize the importance of sampling from a natural environment to capture diverse microscopic organisms.

3. Microscope Setup (5 minutes): Instruct participants on the proper use of microscopes. Assist in preparing the microscope slides with the collected water samples.

4. Observation (20 minutes): Participants individually observe the water samples under the microscope. Identify and note the various microscopic life forms present, such as algae, protozoa, and microorganisms.

5. Discussion (5 minutes): Conclude the activity with a brief discussion on the observed microscopic life forms. Relate the findings to the interconnectedness of microscopic organisms and their role in the rainforest's ecosystem.

Water Pollution

Activity 1: Water pollution

Objective	Be aware of the different forms of water pollution
Time:	45 minutes / individual work
Aids:	4 jam jars, pieces of waste, edible oil, litmus paper and vinegar

Aim: to reflect on pollution in our environment, to highlight the major sources of pollution, the impacts of pollution and how to eliminate it

Water is most often described as a colourless, clear, odourless and tasteless liquid. Yes, this is true if we think of tap water. But look at our streams, rivers and lakes. We can hardly think of them as colorless, transparent, odorless liquids.

Supporting information for implementation:

Concepts: water pollution, agriculture, industry, human settlements, transport, waste PROCEDURE: Teacher prepares 4 samples of water pollution and labels each sample with a number: 1. cloudy water - for example, water from a river or water with soil mixed in, 2. Macro pollution - water with small pieces of waste (e.g. a piece of foil, a rusty screw, a PET bottle cap, etc.) 3. water with oil on the surface, 4. clear water with a little vinegar or citric acid. At the beginning, the teacher asks the students which of the following samples represents clear water. With further questions, the teacher asks how the pupils came to the conclusion that one sample is clean, and also initiates a discussion on the topic of what makes each sample dirty.

For each sample, the teacher can ask the same questions: 1. What is contaminating the water sample? 2. What could have caused the contamination? 3. How might such pollution affect life in or near the water? 4. How can we help the water with such pollution?

Step 1 : The first sample of cloudy water may be natural in nature. Slightly brown coloured water may contain dissolved clay particles. Green water, on the other hand, may contain algae or cyanobacteria that colour it green. As an example of how water can be purified, the teacher can use filter paper to point out the purification process that treats water before it enters our homes. But who purifies water in nature? In nature, our streams, rivers and streams have a natural ability to purify, but the banks of these waters must be natural and the life in the water must be in balance with each other. And the most natural stream or river can only address its cleansing capacity up to a certain degree of pollution. Too much pollution can upset the natural balance in the water, and even completely destroy aquatic life, lakes

Step 2 For the second sample, we encounter pollution that the water can't cope with the waste. In this sample, we need to highlight a problem that has become a global problem and is troubling people all over the world. Waste pollutes all our rivers, seas and oceans. Some waste heaps have grown to the size of large islands. Just type 'great pacific garbage patch' into a search engine on the Internet and look at the pictures or videos. At the same time, it is important to point out that the source of waste is us humans, our dwellings and our homes, and that waste dumped freely into the countryside is a burden that nature cannot easily cope with. - The teacher can specifically draw attention to plastics, which break down in the water and break off into smaller and smaller pieces up to microscopic size, and thus can enter the bodies of other animals and even humans.

The solution is to clean the waters of waste and not to create unnecessary new waste (waste minimisation, sorting, recycling, etc.).

The teacher can gradually pick up the litter from the water and, with the help of the pupils, sort it correctly into the appropriate containers.

Step 3: The third sample is water with oil on the surface. It is ideal to use dark edible oil so that the effect is clearly visible.

The teacher guides the pupils to try to find an example of such pollution. Oil on water in this case simulates the behaviour of oil on water in oil tanker accidents. Poisonous, dangerous substances enter the water and the oil slick itself on the surface kills many animals that come into contact with it. Getting rid of the oil slick will only solve the surface problem. Getting rid of the hazardous substances that have entered the water and that have dissolved in it remains a problem.

Step 4: The last sample looks clean but contains acid, which we can prove with litmus paper. Not all contamination is visible to the naked eye. The contamination may not have any color. The amount of colourless chemicals that enter the water from industry will change the chemical composition of the water, which can have a worse impact on aquatic life than previous types of pollution. In nature, we often see a combination of these pollutions. For example, after heavy rainfall, fine soil material washes off our fields and into rivers, along with the chemicals used in farming and often the rubbish that stood in the way between the field and the river.

Step 5: In the last step, the teacher will review all types of pollution, pointing out their most important sources as well as ways to eliminate them. Special attention should be paid to the waste produced by the pupils themselves, which is a serious global problem.

Activity 2: Lack of drinking water

Objective	Tvorba clanku do miestnych novin o priemyslenej cinnosti, ktora ovplyvnuje vodne toky
Time:	3 months / individual work
Aids:	Camera, video editor

Short desription: Participants engage in a three-month individual activity focused on creating an article for local newspapers addressing the industrial activities impacting water bodies. Utilizing a camera and video editor, they document and present their findings to raise awareness about the lack of drinking water.

Procedure:

1. Introduction (1 week) Present the issue of industrial impact on local water sources. Provide resources for understanding the problem.
2. Research and Documentation (4 weeks): Research local industrial activities affecting water. Use a camera to document relevant visuals and gather historical and current impact information.
3. Article Drafting (4 weeks): Draft newspaper articles, focusing on clarity, accuracy, and a compelling narrative.
4. Video Compilation (2 weeks): Compile footage using a video editor to create a visually impactful presentation.
5. Review and Editing (1 week): Review and edit articles and videos for coherence and engagement.
6. Submission (1 week): Submit final articles and videos to local newspapers and share on social media.

This three-month individual project empowers participants to investigate and communicate the impact of industrial activities on local water bodies. The combination of a written article and a visually compelling video aims to raise awareness and prompt action on the issue of water scarcity.

Water as Natural Disasters

Activity 1: Water-related disasters of the world

Objective	Creation of word report about water disaster
Time:	3 months / individual work
Aids:	Camera, video editor

Short desription: Participants embark on a three-month individual project to create a comprehensive word report addressing water-related disasters worldwide. Utilizing a camera and video editor, they aim to visually enhance their findings, shedding light on the critical issue of water-related disasters.

Procedure:

1. Introduction (1 week): Introduce global water-related disasters and provide essential resources.
2. Research and Compilation (6 weeks): Conduct research on various water-related disasters. Compile data, statistics, and case studies for a comprehensive overview.
3. Visual Documentation (4 weeks): Use a camera to capture impactful visuals, emphasizing affected areas and rescue operations.
4. Report Drafting (6 weeks): Draft a detailed word report based on research and visual documentation. Include clear organization, informative content, and potential solutions.
5. Video Integration (2 weeks): Utilize a video editor to seamlessly integrate captured footage into the report.
6. Review and Editing (2 weeks): Review and edit the report and video compilation for coherence and accuracy.
7. Presentation (1 week): Present the report, fostering discussion on mitigation strategies and global implications.

Activity 2: Regional report of water disaster

Objective	Creation of reginal report about water disaster
Time:	3 months / individual work
Aids:	Camera, video editor, paper

Short desription: education is a critical component of disaster risk reduction and management. By educating people about water disasters, we can build more resilient communities that are better prepared to face the challenges of a changing climate.

Procedure: Creating a regional report on water disasters involves several steps. Here are some general guidelines to help you get started:

1. Define the scope: Determine the region you want to focus on and the types of water disasters you want to cover. This could include floods, hurricanes, tsunamis, droughts, or other water-related hazards. You can make short videos about water desasters near your area.
2. Gather data: Collect data on past water disasters in the region, including their frequency, severity, and impact. You can find this information from government agencies, non-governmental organizations, academic institutions, and other reliable sources.

3. Analyze the data: Look for patterns and trends in the data. Identify the most common types of water disasters in the region, the areas that are most vulnerable, and the factors that contribute to their occurrence.
4. Assess the risks: Based on the data analysis, evaluate the risks associated with water disasters in the region. Consider the potential impact on people, infrastructure, and the environment.
5. Develop recommendations: Based on the risk assessment, develop recommendations for reducing the risks associated with water disasters. This could include improving infrastructure, enhancing early warning systems, and promoting community preparedness.
6. Write the report: Use the data, analysis, and recommendations to write a comprehensive report on water disasters in the region. Be sure to include an executive summary, introduction, methodology, findings, conclusions, and recommendations.
7. Review and edit: Review the report carefully to ensure that it is accurate, clear, and concise. Edit the report for grammar, spelling, and punctuation errors.
8. Disseminate the report: Share the report with relevant stakeholders, including government agencies, non-governmental organizations, and the public. This will help raise awareness about the risks associated with water disasters in the region and promote action to reduce their impact.

Water protectors

Activity 1: Sources of drinkikng water

Objective	Inform where are ithe nearest sources of drinking water, what is quality
Time:	45 minutes / work in pairs for one week
Aids:	Work with database

Short desription: Participants engage in a week-long, 45-minute activity working in pairs to identify and assess the nearest sources of drinking water. Using a database, they collect and analyze information on the location and water quality of these sources. This activity empowers participants to proactively identify and assess nearby drinking water sources, promoting awareness of water quality. The use of a database facilitates organized data collection for informed decision-making

Procedure:

1. Introduction (5 minutes): Emphasize the importance of knowing the location and quality of drinking water sources. **I**ntroduce the task: identifying and assessing the nearest sources of drinking water.

2. Database Familiarization (10 minutes): Provide a brief tutorial on how to use the database for recording information. Specify parameters for assessing water quality (e.g., purity, accessibility).

3. Field Observation (30 minutes, spread over one week): Pairs visit the designated water sources, recording relevant information in the database. Assess the cleanliness, accessibility, and overall quality of each source.

4. Data Analysis (5 minutes): At the end of the week, participants review and analyze the collected data. Discuss findings within pairs, identifying reliable and accessible drinking water sources.

Activity 2: Protection the surroundings of springs and watercourses

Objective	Garbage collection
Time:	45 minutes / work in pairs for one week
Aids:	Work with database

Short desription: Embark on a week-long, 45-minute endeavor in pairs to protect the surroundings of springs and watercourses. Focusing on garbage collection, participants utilize a database for identifying pollution hotspots, showcasing the impact through on-site photos, and presenting measurable results. Utilizing a database for strategic site selection and documenting real-time improvements through photos enhances the impact of the garbage collection effort.

Procedure: **1. Kick-off (5 minutes):** Convey the importance of safeguarding springs and watercourses from pollution. Introduce the task: garbage collection in identified areas prone to excessive pollution.

2. Database Utilization (10 minutes): Provide a quick guide on using the database to pinpoint pollution hotspots. Emphasize the importance of accurate documentation.

3. Site Identification (10 minutes): Pairs select and mark locations with potential pollution. Document initial conditions through photos for visual impact.

4. Garbage Collection (20 minutes, spread over one week): Pairs engage in garbage collection at the chosen sites. Capture 'before' and 'after' photos to illustrate the impact of the cleanup.

5. Data Compilation (5 minutes): Input collected data into the database. Summarize results, showcasing the volume of collected garbage and the improved state of the surroundings.

Water as Factor of Organisms Biodiversity

Activity 1: Biological diversity of the rainforest – water means life

Objective	Creation of reginal report about water disaster
Time:	3 months / individual work
Aids:	Camera, video editor

Short desription: Participants embark on a three-month individual project focused on creating a regional report highlighting the critical link between water and the biological diversity of the rainforest. Utilizing a camera and video editor, they aim to visually capture and convey the importance of water in sustaining life within this ecosystem.

Procedure:

Step 1: Introduction (1 week): Introduce the significance of water for the biological diversity of the rainforest. Provide resources for understanding the interdependence of water and the ecosystem.

Step 2: Research and Documentation (6 weeks): Conduct in-depth research on the role of water in sustaining rainforest biodiversity. Utilize a camera to document visuals showcasing the diverse flora and fauna dependent on water.

Step 3: Report Drafting (6 weeks): Draft a detailed regional report, emphasizing the intricate connection between water availability and the vitality of the rainforest. Incorporate findings from research and visual documentation.

Step 4: Video Compilation (2 weeks): Use a video editor to compile recorded footage, creating a visually compelling presentation. Ensure the video complements and enhances the written content of the regional report.

Step 5: Review and Editing (2 weeks): Review the report and video compilation for coherence, accuracy, and engagement. Make necessary edits to refine the final deliverables.

Step 6: Presentation (1 week): Participants present their regional reports and videos, highlighting the significance of water in sustaining the rainforest's biological diversity. Encourage discussion on conservation efforts and the impact of water-related issues on this ecosystem.

Supporting information for implementation

Provide access to reputable sources on rainforest biodiversity and water ecosystems. Offer guidance on effective research methodologies and data collection techniques. Facilitate a workshop on using cameras and video editors for participants unfamiliar with these tools. Encourage collaboration and information sharing among participants to enhance the comprehensiveness of their reports.

Activity 2: Biodiversity of invertebrates

Objective	Determination of invertebrate species (genera, families) using the identification key at regional level
Time:	1 season (from spring to autumn) / individual work
Aids:	Camera, notepad, planctonic net, metal sieve, tweezer, magnifying glass, small vials or boxes for transport to lab, alcohol, rubber boots, raincoat

Short desription: The activity is focused on identifying of different water invertebrates using detemination key. Various species of water invertebrates show various level of tolerance to changes of chemical and physical phactors of waters. Many of them (e.g. molluscs, crustaceans, ...) are very good bioindicators of water quality. Presence or absence of such animals at selected place could help us to consider condition of water habitat.

Procedure: Research requires repeated visits at the locality of interest within vegetation season from spring to autumn, at least once a month. It is suitable to use a combination of various observation methods (visual observing on rocks and vegetation, water filtering with planctonic net, searching under stones and in organic material, sifting fine sediments, ...) durind every visit. It is good to change type of weather (sunny, cloudy, rainy) within study period because different species prefer activity in different weather condition. In the case of water streams observtion is better to observe such places in the time of normal water level. High water level with strong stream power causes hiding of majority of water animals. Findings of animals could be determined visually (or using a magnifying glass) documented by camera and species which are hardly identifiable in the field (e.g. plankton, larvae, ...) should be stored in small boxes and transported into the lab. Every determined species should be writen into list of species. It is suitable to record also number of specimens of particular species. It is often useful to compare numbers of species specimens along period of water monitoring.

Supporting information for implementation – Key for determination of water invertebrates Supplement 2.

Activity 3: Biodiversity of amphibians

Objective	Determination of amphibian species using the identification keys at regional level
Time:	3 months / individual work
Aids:	Identification keys, camera, audiorecorder, notepad, raincoat, headlamp, rubber boots

Short desription: Morphological and physiological characteristics make amphibians one of the most sensitive organisms on earth. Therefore, they serve as excellent bioindicators. The species composition of amphibians is important for knowing their occurrence and habitat preferences in a given area, and at the same time for knowing the ecological condition of this area.

Procedure: Research requires regular visits to the locality of interest, at least once a week. Visits should be made at different times of the day (even in the evening, some amphibians are active during the day, some at night) and in different weather (some amphibians are active in/after the rain, others in sunny weather). Findings should be documented with audio and photo recordings and identified using the identification keys. For identification of species composition we can use also egg clutches or larval stages, that are species specific, but more experiences and knowledge of observer are needed in this type of determination.

Supporting information for implementation – If manipulation of the animal is necessary for species determination, it is important to have an approved permission for species protected by law.

Activity 4: Biodiversity of fishes

Objective	Determination of fish species using the identification key at regional level
Time:	3 months / individual work
Aids:	Camera, notepad, nets, transparent box, rubber boots

Short desription: Fish are excellent bioindicators in aquatic environment. The species composition of ichthyofauna is important for knowing its occurrence and habitat preferences in a given area, and at the same time for knowing the ecological condition of this area.

Procedure: It is important to visit various aquatic habitats such as ponds, streams, rivers, dead arm rivers, gravel pits, lakes etc. According to the type of observed water area it is necessary to adapt the capture method to be as effective as possible. Findings should be documented with photo recordings and identified using the identification keys. It is good to record total number of individuals per species and anomalies if they occur.

Supporting information for implementation – Prior to capturing fish it is important to have appropriate permissions.

Sustainable Project Proposals

Activity 1: Transfer of frogs

Objective	Creation of reginal report about water disaster
Time:	3 months / individual work
Aids:	Camera, video editor

Short desription: The most effective measure to prevent the death of migratory amphibians is often to transfer them - especially frogs and toads. Contact the local protectional organisations in your area to find out about the possibility of getting involved in this conservation activity.

Procedure: Practical involvement of pupils is possible in all three supporting stages of the event.

1. barrier construction (as the most physically demanding stage that lasts 1-2 days) the main activities are making wooden pegs, digging a trench, hammering the pegs into the ground, fixing the foil, burying the foil, carrying and feeding the pegs, etc.

2. Once the barriers are erected and the frogs begin to migrate, the second stage begins - the transfer of the frogs. These are usually organised every day (in the evening at the peak of the migration or throughout the day).

3. Once the migration is over, the barriers need to be removed so that they do not prevent the toads from getting back into the forest environment.

Activity 2: Project proposal for the creation of a rain garden

Objective	Creation of reginal report about water disaster
Time:	3 months / individual work
Aids:	Camera, video editor

Short desription: Participants undertake a three-month individual project focused on proposing and creating a rain garden to address water-related issues in their region. Utilizing a camera and video editor, they document the process and present a regional report outlining the importance of rain gardens in mitigating water disasters.

Procedure: Research local rain garden success stories and case studies. Identify and visit existing rain gardens for inspiration and to understand their impact. Collaborate with local environmental organizations for expertise and potential partnerships. Find information on suitable native plants for rain gardens in the region.

1: Introduction (1 week): Introduce the concept of rain gardens and their role in water management. Provide resources on the benefits of rain gardens and their potential impact on local water-related issues.

2. Research and Site Visits (6 weeks): Research rain garden designs and best practices for the local climate and geography. Visit potential sites, documenting current water-related challenges and opportunities for rain garden implementation.

3. Proposal Development (6 weeks): Create a comprehensive proposal for the establishment of a rain garden, addressing local water disaster concerns. Include details on design, plant selection, and maintenance.

4. Implementation (8 weeks): Execute the proposed rain garden project, documenting the process with a camera. Record the stages of construction, planting, and any community involvement.

5. Video Compilation (2 weeks): Utilize a video editor to compile footage, showcasing the creation and impact of the rain garden. Ensure the video complements the detailed regional report.

6. Report Writing (3 weeks): Draft a regional report summarizing the rain garden project, its goals, and observed outcomes. Include data on water quality improvement and any community engagement.

7. Review and Editing (2 weeks): Review the report and video compilation for coherence, accuracy, and engagement. Make necessary edits to refine the final deliverables.

8. Presentation (1 week): Present the regional report and video to the community, emphasizing the positive impact of the rain garden on local water-related challenges. Encourage community involvement and future sustainability of the rain garden.

ANNEX

How to proceed with measurements using vernier sensors

If the school has a computer-supported laboratory with Vernier sensors, we can use them to measure selected chemical parameters. The Vernier measurement system offers a wide range of sensors and software programs that can be used for simple measurements in the laboratory as well as in the field.

The basic components needed for measurements are:

Measurement Interface Unit

A small, portable device with a touch screen and intuitive control. It is used for data collection and evaluation either independently or with the option to transfer the acquired data to a computer. Equipped with a Wi-Fi system and Bluetooth interface, which allows it to connect to a variety of software programs on tablets, mobile devices, or computers. Each LabQuest package includes a charger and a USB cable for connecting it to a computer.

Picture 1: Measurement Interface Unit LabQuest2

1) Vernier Measurement Sensors

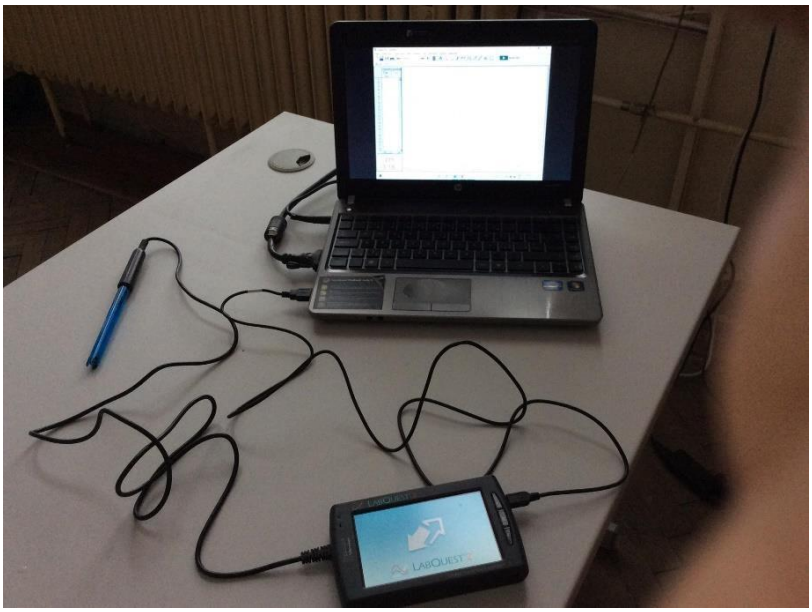
Vernier measurement sensors encompass a wide range of nearly 90 wired and wireless measurement sensors for chemistry, biology, physics, geography, technical, and environmental sciences. Each sensor package includes a storage solution with instructions for its use and potential calibration.

2) Software Equipment

For more in-depth data processing (e.g., with students during lessons or in the school laboratory), a wide range of applications and programs can be used to process data from Vernier devices. The most commonly used ones are the computer program Logger Pro 3.16 (available for both Windows and Mac) and the LabQuest Viewer or Graphical Analysis applications, which are also functional on Android and iOS platforms.

Picture 2: Vernier pH Sensor in Storage Solution

Picture 3: Example of Sensor Connection to the Measurement Interface Unit



Picture 4: Example of Connecting a Sensor to LabQuest on a Computer with LoggerPro Software. When the computer is connected to a projector, measurements or experimental analysis can be projected and discussed with students directly in the classroom.

Measurement procedure with measurement sensors

1. Determination of Acidity/Alkalinity - pH Sensor (PH-BTA)

The Vernier pH sensor can be used wherever traditional pH determination methods are applicable (indicators, pH strips, titration, etc.). Unlike chemical indicators that only provide a visual color change, this sensor allows for the automatic reading of the pH value of a solution and its analysis. This sensor does not require constant calibration because it comes with pre-stored calibration to respond to standard pH values in the range of 0-14 with a measurement deviation of +/- 0.02 pH (as specified by the manufacturer). Before measuring pH using the sensor, it's a good practice to measure pH traditionally as well (e.g., using indicator strips) and compare it to the value measured by the sensor. This way, you can easily determine whether the sensor needs calibration.

!!! All Vernier sensors are highly sensitive electronic devices, so it's important to follow the manufacturer's precise instructions when working with them.

Tools: pH sensor, LabQuest 2 measurement unit, syringe with distilled water, paper towels

Procedure:

Before using the pH sensor, follow these steps:

1. Unscrew the cap from the storage bottle and carefully remove the sensor.

Picture 1: pH Sensor in the Storage

2. Rinse the sensor from the bottom, especially around the measuring bulb, thoroughly with distilled or deionized water from a chemical syringe.
3. Connect the sensor to the interface, and you can proceed with measuring the pH value.

Picture 2: pH Measurement

4. Submerge the sensor in the solution, wait for the value to stabilize, and read it. Only immerse the sensor in the measured solution to about the lower third of its length; there's no need to fully submerge it.
5. After reading the pH value, rinse the sensor with distilled water, pass it through the bottle's cap, and screw it into the storage solution.

NOTE: If there is little solution left in the storage bottle, you can top it up with water, but not repeatedly. Over time and for longer-term storage of the sensor, it's necessary to prepare a storage buffer solution with pH 4 as instructed, which is included in the sensor package.

For more information, click on this link https://pmsdelta.sk/product/ph-senzor/#toggle-id-1 or contact the Vernier systems supplier for Slovakia, which is PMS Delta, s. r. o.,. https://pmsdelta.sk/.

2. Zisťovanie tvrdosti vody - senzor vodivosti (CON-BTA)

The conductivity sensor can be used in the laboratory or in the field to measure the conductivity of solutions or to study the total concentration of ions.

Measuring conductivity is one of the most common environmental tests for water samples. It allows us to easily detect any changes in the ionic composition of a watercourse or lake. It does not provide information about the specific types of ions in the solution but gives information about the overall level of dissolved solids (TDS). Specific ion specifications can be determined using traditional methods (e.g., titration, analytical evidence, etc.). There is an approximately linear relationship between conductivity and concentration, so, for specific ions, conductometry can be used to determine the concentration of these ions in the solution.

When sampling in watercourses, it's best to take samples away from the shore and below the surface to better represent water quality. If you don't have the opportunity to measure directly in the field, you can take samples in fully filled containers (to prevent evaporation and reaction with CO2, which could lead to the creation of products that might distort the measurement).

Tools: Conductivity sensor, LabQuest 2 measurement unit, syringe with distilled water, paper towels.

Procedure:

Before using the conductivity sensor, follow these steps:

1. Rinse the end of the sensor with distilled water. Water droplets in the measurement opening could contribute to the distortion of our measurement, so dry them by blowing or shaking the sensor in the air. Connect the sensor to LabQuest.

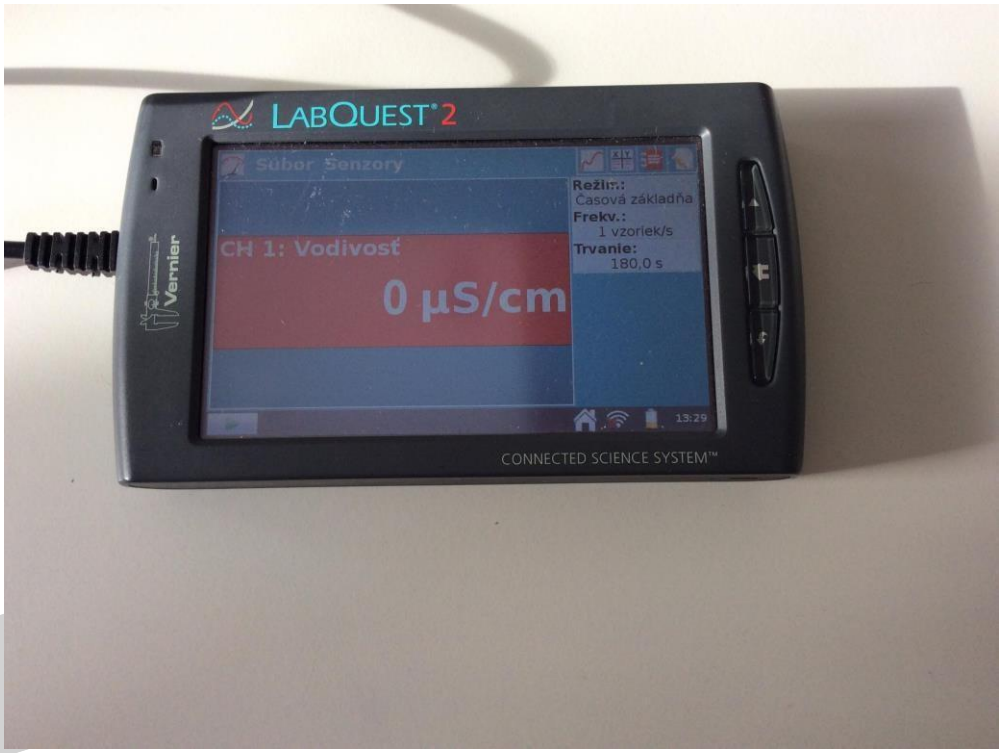
2. This sensor has a switch on the side for measurement ranges. This may lead to the assumption that if, for example, we expect a low concentration of ions in the measured solution, we should switch thi… …ch to the 0-200 µS range. However, Vernier's conductivity sensor is equip… …matic identification (auto-ID) circuits, which means when used with … …llection software, the sensor is recognized, and it configures the … …he most appropriate parameters. Therefore, it is not necessary to manually … …witch; what's important is...

   If calibration is needed, please refer to for more detailed information. https:// …cz/katalog/manualy/sk/CON-BTA.pdf

Picture 4: Measurement Range Switch - Use it only during calibration

4. After connecting the sensor to the interface, conductivity values will appear in the red field. By clicking in the red field, a window will open where you can select the option "Zeroing." (You can also change the measurement units here, or begin sensor calibration.)

Picture 5: LabQuest after connecting the conductivity sensor



Picture 6: After zeroing - you can start the measurement

5. Insert the sensor into the sample to be measured. The oval cutout must be completely submerged in the water, and there should be no air bubbles around it. Also, do not submerge the sensor completely, including the handle, as it is not waterproof.
6. Gently stir the sample and wait for the measured value in the data collection software to stabilize. At temperatures below 15°C or above 30°C, it may take a longer time for the values to stabilize.
7. After completing the measurement, rinse the sensor with distilled water, and you can continue with the next sample (refer to step 1). Also, after finishing the measurement, rinse and dry the sensor with a towel. Always store the sensor in a dry state.

For more information, click on https://www.vernier.cz/katalog/manualy/sk/CON-BTA.pdf or contact the Vernier systems supplier for Slovakia, which is PMS Delta, s.r.o. https://pmsdelta.sk/.

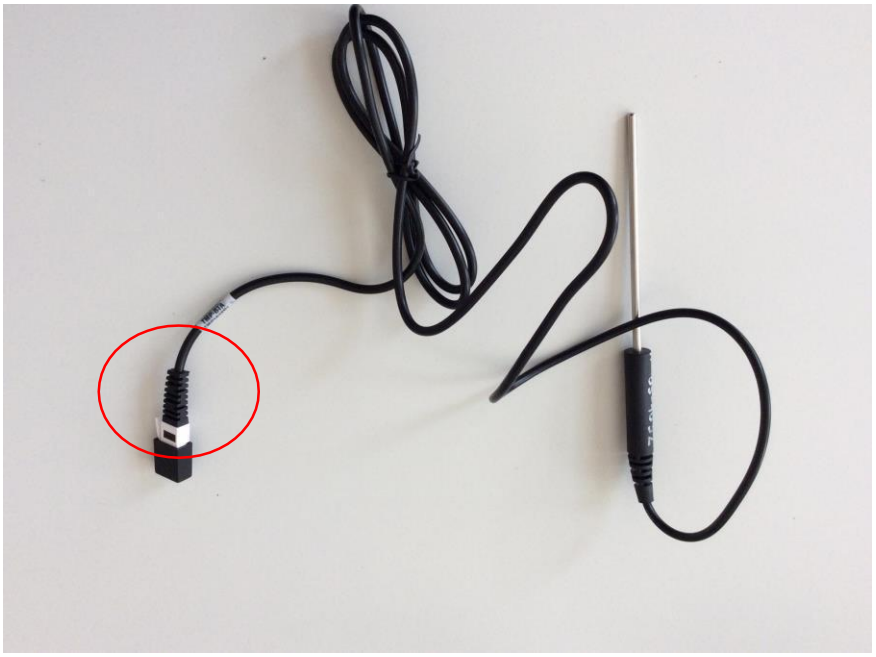
3. Temperature Measurement - Stainless Steel Thermometer (TMP-BTA)

The Vernier stainless steel thermometer is a durable laboratory thermometer suitable for various applications. It can be used in biology, physics (e.g., various temperature experiments), chemistry (e.g., monitoring temperature changes during chemical reactions), and geography. When measuring the temperature of aqueous solutions, the sensor can be continuously submerged in a temperature range of -40°C to 150°C.

Tools: Thermometer, LabQuest 2 measurement unit, syringe with distilled water, paper towels.

Procedure:

1. Connect the sensor to the interface. Newer sensor packages have a USB connector with a protective cover that should be removed before connecting.



Picture 7: Stainless Steel Thermometer with the protective cover of the USB connector (highlighted in the red circle)

Picture 8: Temperature Measurement

2. Start the data collection software or wait until the temperature value stabilizes. The software will automatically identify the thermometer and set up the standard data collection settings.
3. After using the sensor, always rinse it thoroughly and dry it with a paper towel before storing it.

This Vernier temperature sensor does not require calibration; it is supplied pre-calibrated. In exceptional cases, calibration can be performed according to the procedure provided https://pmsdelta.sk/wp-content/uploads/senzory/docs/TMP-BTA_Nerezovy_teplomer.pdf, where you can also find more extensive technical information.

4. Measurement of nitrate levels - nitrate ion-selective electrode (no3- bta)

The Vernier nitrate ion-selective electrode (ISE) is used to measure the concentration of nitrate ions (NO3-) in aqueous solutions.[3] Sources of nitrates in freshwater samples can include wastewater, runoff from fertilized fields, or livestock facilities.

Before use, it's recommended to check the contents of the nitrate ISE package, which should include:

1. Electrode with a storage bottle.
2. A 30 ml bottle with a high-concentration calibration solution (100mg/l NO3-).
3. A 30 ml bottle with a low-concentration calibration solution (1mg/l NO3-).
4. A bottle for short-term immersion of the ISE.

Picture 9: Contents of the nitrate ISE package

For the accuracy and efficiency of measurements, it is recommended to perform this two-step process before using the nitrate ISE:

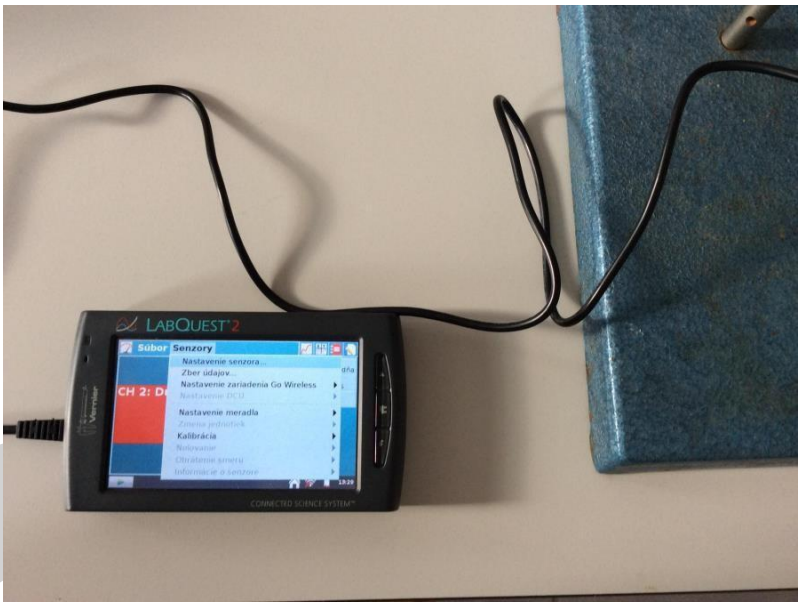
I: Preparing the Nitrate ISE for Use

- Immerse the electrode in the high-concentration calibration solution for 30 minutes. The ISE should not be entirely at the bottom of the container, but the white reference marks at the tip must be submerged in the solution, and there should be no air bubbles around them.

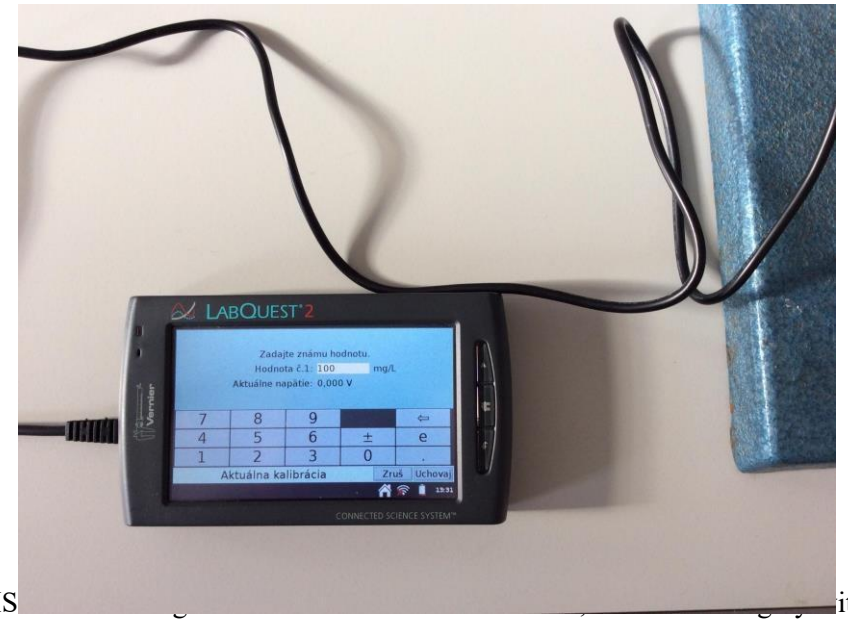
Picture 10: Before use, allow the electrode to stand for 30 minutes in a container with a high-concentration standardization solution. The lower end of the sensor should not touch the bottom of the container..

II: Calibration of ISE (Using LabQuest)

- After 30 minutes have passed, connect the ISE to LabQuest. In the top menu, select "Sensors" and click on "Calibrate" (NOTE: The system may display the name of the connected sensor - so by clicking on "Nitrate Electrode," you proceed to Calibrate).



- Next, a window will open asking for value number 1. Enter the value 100 (the concentration of the standardization solution is 100 mg/l, in which the electrode has been soaked).
- • After the voltage value stabilizes, click on "Save."



- Select the IS ith distilled water, and gently dry it with a towel. Place the ISE in a container with a low-standard solution. Also, ensure that it is not completely at the bottom of the container, but that the white contacts are submerged in the solution, and no air bubbles are created around the white marks.

Picture 13: Calibration Procedure

- Enter the value 1 for calibration point 2 (corresponding to the low-concentration calibration solution).
- Click Save again after the voltage value stabilizes.
- If you want to save this calibration (so that you don't have to repeat it multiple times when using it in different classes during teaching), follow these steps:
  a) Touch **the Storage tab.**
  b) Click **Save Calibration to the Sensor** and then **OK**, which will complete the calibration process..

Picture 14: Completing the Nitrate ISE Calibration

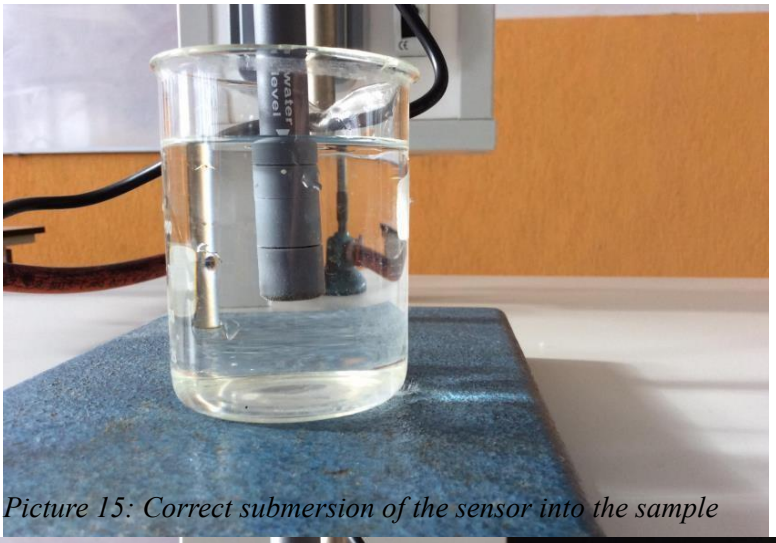
- After calibrating the ISE, rinse it with distilled water and dry it.

Once we have the ISE calibrated, we can proceed to measure the amount of nitrates in the samples.me prejsť k meraniu množstva nitrátov vo vzorkách.

Procedure for measuring NO3 – concentration

Tools: nitrate ISE, LabQuest 2 measurement unit, syringe with distilled water, paper towels

1) Insert the end of the ISE into the sample to be measured. It is sufficient for the electrode to be submerged in the sample just slightly above the white reference dots. You do not need to fully immerse it since the electrode handle is not waterproof either.



Picture 15: Correct submersion of the sensor into the sample

Pict

2) Allow the ISE to rest until the measured value stabilizes, then record it.

NOTE: In some measurements, especially if the sensor detects high NO3- concentrations in the sample, it may take several minutes, which can negatively affect the electrode for subsequent samples. Therefore, if you have an approximate idea of the concentrations in your samples, it's a good practice to conduct measurements from the lowest concentration sample to the highest.

3) After each reading from the ISE, rinse it with distilled water and dry it before storage.

For more information, click on https://pmsdelta.sk/wp-content/uploads/senzory/docs/NO3-BTA_Dusicnanova_ISE.pdfn or contact the Vernier systems supplier for Slovakia directly, which is the PMS Delta company, s. r. o. https://pmsdelta.sk/.

5. Measuring the amount of dissolved ammonium ions - ammonium ion-selective electrode (ISE) (NH4-BTA)

This electrode is used to measure the quantity of dissolved ammonium ions NH4+ in solution. The presence of ammonia in water is most commonly a result of field fertilization and can also occur through the decomposition of organic residues. The amount of ammonium ions in water significantly affects the pH of water samples, as there is an equilibrium relationship

$$NH_3(aq) + H^+(aq) \leftrightarrow NH_4^+(aq)$$

In more acidic solutions, higher concentrations of H+ ions shift the reaction to the right, resulting in higher concentrations of ammonium ions NH4+. At higher pH values, the equilibrium shifts toward the reactants, so there will be more NH3 in the solution. At pH values lower than 7.5, essentially only ammonium ions are present in the solution. When analyzing relatively hard water, this issue does not apply, as hard water is naturally buffered against pH changes. In drinking water, the ammonium content should not exceed 0.5 mg/l, but in natural water bodies, higher values can be measured.

The packaging of the ammonium ISE should contain:

1. Electrode with a storage bottle (with a sponge)
2. 30 ml bottle with a high-concentration calibration solution (100mg/l NH_4^+ ako N)
3. 30 ml bottle with a low-concentration calibration solution (1mg/l NH_4^+ ako N)
4. Short-term soaking bottle for the ISE

Picture 17: Ammonium ISE in the packaging

For accuracy and efficient measurements, it is recommended to follow a two-step preparation process for the ammonium ISE before use, similar to the nitrate electrode:

I: Soak the ISE in a high-concentration standardization solution for 30 minutes.

II: Calibrate the electrode using LabQuest.

For more detailed information, please refer to the following link:
https://pr...ent/uploads/senzory/docs/NH4-BTA_Amoniova_ISE.pdf

Co... ...g the Ammonium ISE

...sis: ammonium IS... ...bQuest 2 measuring unit, syringe with distilled water, paper towels

...orrectly calibrated. If the sensor, after being connected to the

LabQuest, displays a value such as 1 mg/L and is not in a solution with a concentration of 1 mg/L, you need to recalibrate it.

2) Insert the ISE's tip into the sample being tested. It is sufficient for the electrode to be slightly immersed in the sample, just above the white reference dots. There's no need to fully submerge it since the electrode handle is not waterproof.

Picture I *, it is*

3) Allow the ISE to sit undisturbed until the measured value stabilizes, then record it.
4) After each reading with the ISE, rinse it with distilled water and dry it before storing it

NOTE: If you plan to store the electrode for a short time (up to 24 hours), store it in a storage bottle filled to ¾ with the high-concentration calibration solution. If you don't plan to use the sensor for an extended period (more than 24 hours), moisten the sponge at the bottom of the storage bottle for long-term storage with distilled water and screw the cap with the sensor.

Picture 19: Procedure for long-term storage of the sensor

Thread the electrode through the bottle cap and seal it. Ensure that the white reference point is inside the bottle, and make sure the bottom part of the electrode does not touch the sponge, as this could damage the sensor.

Picture 20: Properly stored electrode in the storage bottle (the lower edge of the sensor does not touch the sponge, which is moistened with distilled water).

For more information, please visit https://pmsdelta.sk/wp-content/uploads/senzory/docs/NH4-BTA_Amoniova_ISE.pdf or contact the Vernier systems supplier for Slovakia, PMS Delta, s. r. o., at https://pmsdelta.sk/

6. Measuring dissolved oxygen levels - dissolved oxygen sensor (DO-BTA)

The dissolved oxygen sensor offers a wide range of tests and experiments to determine the level of dissolved oxygen as a fundamental indicator of life in aquatic environments. It can be used to monitor dissolved O2 in aquariums, detect changes in its concentration due to photosynthesis and respiration of aquatic organisms, measure oxygen consumption in waters containing organic residues where oxygen is consumed during decomposition, or establish a relationship between the amount of dissolved oxygen and water temperature.

The contents of the dissolved oxygen sensor kit should include:

1. Dissolved oxygen sensor with a membrane cap
2. Calibration standard of sodium sulfite (2.0 M Na2SO3) and its safety data sheet
3. Electrode filling solution for dissolved oxygen sensor, a pipette, and its safety data sheet
4. Spare and additional components (replacement blue membrane cap, polishing strips for the sensor, calibration bottle)

Picture 21: Contents of the dissolved oxygen sensor kit

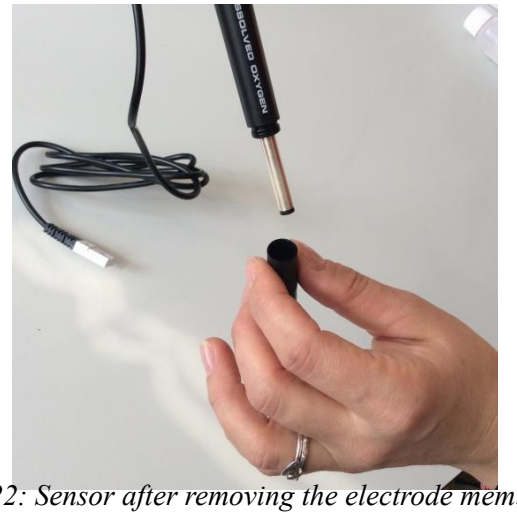
The dissolved oxygen sensor is delivered pre-calibrated. This calibration is sufficient for classroom use. However, for increased measurement accuracy, especially when working directly in a watercourse or lake, it's advisable to recalibrate after repeated use.

You can find the calibration procedure at https://pmsdelta.sk/wp-content/uploads/senzory/docs/DO-BTA%20Senzor%20rozpusteneho%20kyslika.pdf

Preparing the dissolved oxygen sensor for use

I: Sensor preparation

a) Remove the blue protective cover from the end of the sensor.
b) Unscrew the membrane cap at the end of the sensor.



Picture 22: Sensor after removing the electrode membrane cap

a) Fill the membrane cap with 1 ml of dissolved oxygen electrode fill solution using a pipette.

Picture 23: Filling the electrode membrane cap

a) Carefully screw the electrode membrane cap back onto the end of the sensor.
b) Place the sensor in a container with 100 ml of distilled water.

II. Sensor Activation

Connect the sensor in the container with 100 ml of distilled water to the interface. Turn on the data collection program and leave the sensor like this for 10 minutes.

Data Collection Using the Dissolved Oxygen Sensor

Tools: Calibrated and activated dissolved oxygen sensor, LabQuest 2 data collector, syringe with distilled water, paper towels

1) Click on the red field to zero the sensor.
2) Immerse the end of the sensor into the sample to a maximum depth of 10 cm. Immersing the sensor deeper could potentially damage it.
3) Gently stir the sample around the sensor. The sensor collects oxygen from the water as it passes through its membrane, so the water around the sensor should be in constant motion. In stagnant water, dissolved O2 readings might appear to drop.
4) Wait for the reading on the display to stabilize and record it.

NOTE: If you plan to reuse the sensor within 24 hours, store it in a container with distilled water (the sensor should be submerged to a depth of about 2.5 cm). However, if you won't be using the sensor for an extended period, disassemble the membrane cap and rinse the inside and outside with distilled water. Briefly dry it in the open air, gently pat the internal parts of the electrode with a paper towel, and screw the cap back onto the electrode body.

For more information, click on https://pmsdelta.sk/wpcontent/uploads/senzory/docs/DO-BTA%20Senzor%20rozpusteneho%20kyslika.pdf or contact the Vernier systems supplier for Slovakia directly, which is PMS Delta, s.r.o. at https://pmsdelta.sk/

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