## METHODOLOGICAL GUIDE FOR MONITORING THE STRUCTURE OF ICHTHIOCENOSES

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## METHODOLOGICAL GUIDE FOR MONITORING THE STRUCTURE OF ICHTHIOCENOSES

This paper presents a set of documented procedures, a list of steps to follow, so anyone knows what must be done in order to monitor the structure of ichthiocenoses.

The methodology includes:

- activities and materials,
- instructions for using tools and materials,
- forms.

Using a unified methodology for monitoring ongoing ichthio-fauna reduces activity time, effort required, and the results are reproducible and comparable with other teams.

## Chaper 1. THEORETICAL CONSIDERATIONS ON FISH COLLECTION FOR THE STUDY OF ICHTHIOCENOSES

The study of fish fauna has evolved with the improvement of catching means. Traditional tools only allowed a qualitative assessment, which resulted in obtaining lists of species present in a given region. Through the diversification of sampling and catching there appeared the possibility of describing and using working methods (procedures) that are standardized and identifiable as methodologies. The introduction of fishing through electro-narcosis, which is a much less selective method, allowed for quantitative studies, estimates of composition on species and age, and the relationship between various species to be addressed. Concepts like ichthiocenoses or fishing communities emerged as a consequence of ecological research.

Currently, in European countries, fish fauna is subject to ongoing monitoring activities, under the application of the Water Directive 2000/60 EC. In Romania, the legal context has been aligned to the European one by Law 310/2004, which, among other provisions, introduced fish as part of water quality assessment.

The term "monitoring", increasingly used today, derives from the Latin "moneo" which means warning, guidance, recommendation. It became widespread through its English form, i.e. "monitoring", with meanings related to permanent observation, supervision, guidance.

For the purposes of this guide, monitoring is the process of collecting data over a longer period of time, by repeating sampling periodically in order to allow observation of the dynamics of fish community descriptors.

The elements of a monitoring program (Boyle, Kay \& Pond, 2001).

- A set of goals chosen by people. The momentum of a monitoring program is the assessment of the progress made in achieving predetermined goals. Therefore, at the basis of any program lie clearly established objectives which are correlated with the requirements of information users.
- A conceptual model of the world. The model shows how we see the world in the context of achieving our goals. It serves to delimit the system proposed for monitoring and provides the framework that correlates the indicators among them, in the general context of the monitored system.
- A set of indicators. Indicators characterize the analyzed system in a manner accessible to users.
- Methodology for data collection. The choice of procedures that address practical and technical issues related to data collection must be established in a manner that ensures their accuracy, consistency and statistical robustness.
- Methodology for calculating indicators. The data collected must be handled in order to obtain values for reaching indicators. Again, in this case, a method that ensures their accuracy, consistency and statistical robustness is required.
- A process for synthesis. The synthesization of information from the indicators to get an overview of the system status is essential for achieving the purpose of the program.
- Methodology for reporting. Indicator values and the results of the system's assessment should be reported to different categories of information users. The methodology, and especially its presentation in a clear, useful and timely manner for decision making is crucial to the usefulness and success of the monitoring program.

Attributes of a monitoring program (Boyle, Kay \& Pond, 2001).

- Be relevant and provide support for decisions,
- Consider the space-time scale,
- Rely on a conceptual model of the system that takes into account the relationship between society and the environment,
- Allow an overall integrated assessment of the system,
- Be adaptive and flexible,
- Be practical.


### 1.1. GENERAL PRINCIPLES ON THE COLLECTION METHODOLOGY IN FISH STUDIES

## General information on collecting fish

Collecting, for the purposes of this guide, refers to the capture of a number of fish using various tools, as part of the monitoring of fish populations that make up communities.

In general, early studies on a stream aim at inventorying existing species in a water biotope, and some of the most important characters of each species: abundance, size, food, etc. At this level, the initial data are few and any added information adds to the knowledge; the tools used and the collection protocol do not matter very much. This way one will gain useful but not sufficient information about fish.

A proper investigation must take into account several aspects:

- estimating the harvest stock by commercial fishing, subsistence or sport;
- assessment of ecological indexes: constancy, dominance, cenotic affinity index, etc.
- monitoring the effects of pollution, the study of behavior and / or ecology of a particular species of interest, etc.

Each of these studies need to obtain specific information, which may be obtained only by using certain collection techniques (protocols, processes), in particular in the case of protected species.

At this level of approach, the choice of instruments and the manner of using them are important. Today, in Europe, the following methods are mainly used for collection:

- reversible electro-narcosis particularly useful in small / medium rivers, and some lakes,
- classic mesh gear, used mainly in lakes,
- baited hooks (tethers) used in addition to other means of study.

The following chapters outline how to make this choice based on two aspects:

- biological, by defining the objectives of the collection, the type of information that is intended to be achieved,
- statistics, including criteria for assessing the effectiveness of the procedure regarding the achievement of these objectives.


## 1. 2. BIOLOGICAL ASPECTS IN CHOOSING AND USING COLLECTING TOOLS

### 1.2.1. When the objective of the study is to obtain qualitative and semi-quantitative information

The simplest goal is to obtain qualitative and semiquantitative information about a species in a particular place at a particular time. It is the goal of many biological studies covering feeding habits, growth, maturity. An essential step in achieving such a study is to obtain a sufficient number of fish for the information to be useful. Information is collected on the field or in the laboratory by examining in detail the fish caught. Information obtained from each specimen is useful, but is even more important as it can be compared to a larger number of cases (e.g. it is important to find that the stomach of a 55 cm pike contains two goldfish, but the outcome of the study would be more relevant if we could examine the stomach contents of a number of individuals). For such cases, the only requirement of the fishing gear is to allow the collection of a large number of specimens.

Much more common is when the goal of the study is the distribution of fish, wanting to know the size composition of the fish stock or the share of the population that is considered in some stage of sexual maturity. In this case it is very important to ensure that there is no sampling error (certain age and sexual maturity groups may be over or under-represented in the sample, due to the application of selective collection methods).

In a more complex investigation comparisons between different sampling sections within the same campaign or comparisons between the results of the collection in the same point but at different times are of interest.

It is not very interesting in itself to know that $55 \%$ of the 50 cm pikes in a particular lake were mature in 1995, without reference to a previous situation; for example, in 1970 the proportion of the of 50 cm pike was $30 \%$. In this way, the information becomes understood as an indicator of changes in
the population's characteristics (as a result of pollution or overexploitation).

In the same way, knowing that the average size of a barbel in a river is 13.2 cm with a maximum of 25 cm , increases in importance (scientific and practical) when measured against the fact that in another similar river (but polluted by municipal waste), the average size of the barbel is 18.5 cm , maximum 30 cm.

For comparisons of this type the collection methods used are very important. We must be sure that differences of 5.2 cm in the example above are not due to the use of different gears or when and where they were used (if fishing was done in the time and place of breeding it favored the capture of larger specimens). This confidence can be achieved using simple but standardized tools and by repeating collections in the same place and season for a longer period of time.

## 1. 2. 2. When the objective of the study is assessing abundance

## The abundance of parts of the population

The study of abundance raises specific issues. The ultimate goal is knowing absolute abundance (number or $\mathrm{kg} / \mathrm{ha}$ of fish populations).

In practice, due to the fact that the population of a species is represented by eggs, larvae, juveniles and adults, with significant differences in size, shape and behavior, it is difficult to determine abundance in a single operation; data should be collected separately for each stage of development.

In some cases the distinction between stages of development is very clear, but for other species it is difficult to assess. As in many cases small individuals, sub-adults have the largest contribution to the total biomass, it is important to make a clear indication of the category of population that will be evaluated and to choose the most appropriate tools to collect it. The easiest way is to proceed based on size (i.e. we want to collect only fish larger than 5 cm ). This simplifies things operationally, but may be associated with serious errors if the tools are de-calibrated or if the capture includes prey species.


#### Abstract

Absolute abundance Even if the targeted population segment and the tools have been established, there is still a long way until the calculation of absolute abundance. Many tools catch fish in a volume of water which is loosely defined and variable, the capture depending on the distance at which fish may be caught and made to enter a variable hook, depending on the season. Even when the area or volume is defined (e.g. net or trawl), it rarely can be said that all of the fish in that volume were captured by the tool. The result can be expressed as the number of captures / tool area / hour or tone, but not in number of individuals per hectare, even if we know the active width and the speed of the trawl. There are places where the operation of the fishing tool and certain fish species can provide an accurate estimate, but these are rare cases (e.g. for certain species of fish that are close enough to the substrate to be within range of the trawl, but not so close as to escape beneath).

More often we encounter a situation where the collection does not allow a numerical or gravimetric estimate of absolute abundance. In such cases we need more sophisticated methodologies, with tagging, mortality analysis, etc. in the field of population dynamics.

Among these, a particularly important information is the increase in relative abundance. Material collecting is coordinated so as to allow the calculation of these indices under various conditions, places and seasons.

These indices can characterize abundance in a capture through specific values, i.e. unit of effort on the tool used, which remains constant even if a variable vulnerability of the species to the gear used is observed.

Thus, when using the same tool, changes must be small from one fishing to other, when performed under the same conditions and time of year. Due to changes in the behavior of fish depending on the season, it is expected that they will be reflected in the catch, e.g. catching fish in nets or traps. Such seasonal changes are more difficult to deal with than those when capturing is performed each year in the same season.


## 1. 2. 3. Different species

As a rule, any study on fish needs information on a number of species, and the relationships between species and their common features of particular interest. For example, the
number of species observed or the distribution of the number of individuals per species are commonly used to assess environmental quality. The value of such an index decreases if the tools cannot capture certain species in the area. Nets, for example, are very selective and therefore not suitable for the "multi-species" study of fish communities. Since most of them are selective tools, in practice it is necessary to combine two or more tools.

## 1.3 . Statistical aspects of choice and use the instruments for collecting

Catches can be characterized by statistics, based on standard error of the mean and standard deviation (also called variance when at the power of two). These statistical parameters can evaluate the success of the collection protocol.

Standard deviation (the spread of results for each collection) defines a validity interval of the mean and shows us how close to the correct value is the average of each collection. On the other hand, quantitative evaluations of standard deviation and standard error of the mean provide more accurate information about how good the collection system is or if a system is better than another. Trying to make quantitative estimates requires a clear definition of what one is trying to measure by using the collection protocol. This may increase the accuracy of the whole collection program.

## Systematic error

During collection and evaluation of indices, systematic errors can occur for various reasons (collection techniques, measurement methods, tools / equipment used), which can lead to altered results.

On the other hand there are random errors because we examine only part of the individuals (characters), and we consider the results valid for the entire population. The error is inversely proportional to the number of individuals (characters) analyzed. The greater the number of individuals is, the smaller the errors are.

The size of the error becomes more important than variability. A high variability is associated with different results between samples, while samples with high error give the appearance of good results, but gives wrong information systematically. Samples with high variability provide information
with low precision, but those with large error give false information.

Error control concerns both the collection and the analysis of samples. The error should be obvious when changing the method of collection. Except collections by rotenone, all gears are selective, especially for fish size, but also to other features. For example, nets are selective for more active individuals. Bait traps will attract hungrier individuals and the stomach contents thus obtained may be unrepresentative. Nets select based on the circumference of the individual, not age, and can give errors on growth.

Some systematic errors are acceptable, when we know the deviation and if it not too high. For example, the small size of the species escaping from the net may be accepted as normal.

Error can often come from the design of the collection protocol for the spatial-temporal component. For practical considerations collecting is based on site availability, water level and depth, weather, etc. Fish caught this way may not characterize the entire population. Before accepting these errors at least some checks in different situations should be made, in terms of season, weather and other features, in order to appreciate the size of the error thus introduced.

The avoidance of systematic errors can be done by checking the sources (for potential errors). This refers on the one hand to the equipment and methods of collection, and on the other hand to analysis and interpretation.

## Standard deviation, variance

The standard deviation is a measure of the distribution of individual values around the average. A lower value of variance indicates that the individual values are distributed more closely around the average. A greater dispersion indicates greater variability. Squared standard deviation is called variance.

Except for exceptional cases, when small lakes may be emptied and all the fish examined, it is difficult to carry out quantitative assessments for all individuals of a population. The value obtained by sampling (collection of evidence) differs from the real one and the essential problem is not that there are differences, but the average size of this difference compared to the precision required in the study. This requires a clear establishment of the collection. Sometimes this is easy to do and it can be noted at the outset that the variability is sufficiently small.

For example, it is necessary to establish, comparatively, the growth rate of the fish in two lakes, based on the length of three year old individuals at the end of September. We must consider whether this difference is biologically significant. If we consider that only differences over 5 cm are biologically significant, the data obtained can show that such differences are smaller than this value, and the variance is quite small in this case; however, if differences of 1 cm are considered biologically significant, then variance will be too high.

The size of the variance is determined by the natural variability of the measured parameter, by the organization of the collection and by the sample size (number of individuals). Also, the standard deviation is inversely proportional to the square root of the number of samples.

This means that in order to achieve a higher accuracy a much larger volume of observations is needed than in the accuracy increase report. Beyond a certain level, it becomes impractical or uneconomic to attempt to obtain better accuracy by increasing the sample size (number of individuals) collected in the perfected collection program or in other ways.

Of the statistical techniques that can be applied to increase the efficiency of collection schemes and reduce the size of the variance for the same number of samples (and costs), the largest utility lies with the stratification (of the collection) of samples from fish populations. Stratification can reduce systematic error sources and can result in unbiased samples with low variance for a heterogeneous population, with a large number of species, by grouping the population into "layers", within which conditions are relatively homogeneous.

Samples are collected from each layer and are then analyzed separately. For example, the "statistical population" could be known through all the points in the lake where bottom nets were placed, but it can be layered on areas depending on water depth and substrate.

Similarly, stratification can be applied to fish that are captured in order to examine age, maturity, and so on, but each group (by length) is considered separately to get age-length "keys". Through an appropriate choice of the stratification of subareas in the lake based on the same conditions we can obtain a small number of samples to be collected (sufficient to characterize each substrate), and the total number of samples can be greatly reduced.

### 1.4. ESTABLISHING THE COLLECTION PROGRAM

It is part of the investigating process concerning fish fauna and the environment in which fish live. The steps listed below are in a logical order:

- Defining the collection objectives, e.g. the study of the effects of increasing catches on certain classes of fish (size, age) in a lake,
- Identifying parameters to be measured (metric or gravimetric), e.g. the length of adult or sub-adult specimens for three species of fish,
- Selecting tools that will be used: sets of nets (mesh size $5,8,10 \mathrm{~cm}$ ), electro-fisher, tethers (baited hooks in a line with pater-noster type bait), etc.
- Choosing the collection scheme, e.g. using nets one night every month, in eight different stations, two in each distinct habitat in the lake.

One must pay particular attention to these principles whenever a new program for fish fauna investigation appears. In general, the program and collection procedures may undergo some changes over time, depending on objectives, approach and resources available. Such adjustments must be followed by a corresponding change in the collection protocol, which does not always happen.

The result of the permanent non-adjustment is that a large amount of samples prove inadequate in the study. Since this might be one of the most expensive parts of the study program, the procedures for achieving this must be carefully considered, not just at the beginning of the program, and then regularly reassessed.

## Chaper. 2. THE ELECTRO-NARCOSIS METHOD FOR CATCHING FISH

Presentation of methodology is in line with European standard SR EN 14011 Sampling of fish using electricity and SR EN 60335-2-86 Electrical appliances that household and similar purposes, security part 2-86 Particular requirements for electrical fishing equipment.

### 2.1. THE EFFECT OF ELECTRIC CURRENT ON FISH

Fish have a nervous system similar to other vertebrates. In the dorsal area the nerves, exiting the spinal cord, follow the myomeres and penetrate the muscles. In the frontal part of the head there is a negative charge which would explain why fish are attracted to the anode. The length and orientation of nerves has a determined role - the effect of current being more powerful as these fibers are longer and transversely crossed by electric field lines. Fish orientation in the electric field determines how it is affected, the strongest effect being when the fish is placed perpendicular to the field lines and head facing the anode.

Electric fishing seeks to interfere with the neural transmission path between the central nervous system and the muscles. By blocking the internal signal and overcoming it with the artificial signal, electric fishing redirects the neural signal and the muscular reaction. Fish can still roam undisturbed past the electric barrier if they are oriented in the proper position.

Once in the electric field, the behavior of the fish will depend on its spatial position in the beginning. The expected reaction is involuntary swimming in a predictable direction (toward the anode). If the force of the muscle contractions is too great, spinal injuries may occur.

Electric fishing is selective according to size. Larger fish tend to be more vulnerable due to the electric gradient, head to tail voltage. A big fish intersects more field lines than a small one (Figure 1).

There is an important difference between dimensional selectivity, catch efficiency and mortality. While the efficiency of the catch increases with the length of the fish, mortality depends mainly on the response to the length and frequency of impulses.

Reversible fishing through electro-narcosis differs fundamentally from the so-called "electric fishing" practiced by poachers. Because they use alternating current or pulsed frequencies above 50 Hz , fish and other aquatic organisms are killed in an indiscriminate manner, typically by spinal fracture as a result of muscle spasms.


Figure 1. The effect of electric current is particularly strengthened if the fish is large and is closer to the anode

### 2.2. ELECTRIC FISHING

A series of devices and related components, known as a unit, are used for the production and use of electrical current in fishing (Photo 1, 2, 3).


Photo 1. Portable fishing device


Photo 2. Fixed fishing device with cable reel


Photo 3. Power fishing device, for lakes, with compound anodes.

## THE ELECTRIC FISHING UNIT

The electric fishing unit has the following components:

1. Power source - continuous or alternating current generator, driven by a small size internal combustion engine with power from 3.5 to 15 kW . It can be used as a source and a dry NiCd battery or 12 V acid batteries.
2. Transformer - rectifies the current forming continuous current impulses up to 700 volts. It may also contain an electronic device for producing impulse trains of adjustable frequency from 10 to 120 impulses per second.

## 3. Electrodes:

The cathode is inert and consists of copper stripes that are placed in water or dragged behind the operator with portable devices. For more powerful devices, it can take the form of a cylinder of wire mesh, metal sheet or the metal hull of the boat.

The anode is the active electrode to which fish are attracted. It usually has a circular shape and is placed on an insulated handle with which it is carried through the water. Its shape and dimensions are of great importance for fishing efficiency.

In order to achieve a current density corresponding to the local conditions and fishing purposes the relationship between the surface of the anode and that of the cathode is very important. The cathode's surface must typically be $4-5$ times larger than that of the anode. When the anode is too big the current flow is too high, which leads to overloading the generator and even the engine.

Current recommendations prohibit the use of the anode to support the fish net, in order to reduce exposure to the effects of current and to eliminate the danger of direct contact between operators and the anode when recovering the fish.

Some companies provide anodes of different sizes and shapes to be used according to local conditions, along with fishing equipment.
4. Connection cables must allow easy handling of the active electrode under safe operating conditions. For fixes devices cables must have a length of 1-2 hundred meters, which requires finding an optimal ratio between weight and the safety of the insulation.
5. The following are required as accessories:

- fish nets with insulated handles to recovering fish,
- 20-50 I pots for temporary storage of fish and isolating the effects of the electric current,
- protective gear.


## Proper protection of the operators is crucial because of the hazardous working conditions. It is mandatory to use insulated long boots, insulated gloves and automatic devices for switching off the power if necessary.

### 2.3. FACTORS INFLUENCING ELECTRIC FISHING

Fish located inside a continuous electric field move toward the anode and when they get near it they enter into a state of electro-narcosis, lying on their side, making them very easy to catch. This condition is reversible and stops in one or two minutes after the fish is removed from the electric field.

The physiological effect of the electric current on the fish is influenced by several factors:

## The characteristics of the electric current

Alternating current produces much more powerful shocks and fish are paralyzed in place (tetany). When the frequency is high (approximately 50 Hz ) it can cause fractures of the spine, bleeding gills and other serious injuries (Matthew, 1968).

Best suited for research fishing in freshwater is continuous current transmitted as impulses. They increase the efficiency of current and thus extend its range (Figure 2). One can use less powerful currents, which means reducing the risk for operators and fish and using smaller and lighter devices (Cowx, 1990).

From a practical point of view we can make the following observations:

The power supply will have to increase in direct proportion to:

- water conductivity,
- the size, or depth, of the body of water,
- the surface of the electrodes (multiple or compound electrodes).


## Water conductivity

It varies depending on the amount of soluble salts which, by dissociation, form ions. They facilitate the movement of current
through water. The more conductive the water the bigger the discharge; it requires the use of more powerful sources.

In waters with very low conductivity electric fishing is very difficult, the extreme solution being to increase conductivity by dissolving salts in water by (Lenon and Parker, 1958; Zalewsky et al., 1989).

Conductivity changes over time, being related to factors such as: floods, leaves falling in the water, high temperature, etc.

## Species and size

Many authors consider that mobile species are more sensitive to current than sedentary ones (Timmermans, 1967).

Benthic fish usually take refuge in the mud during the formation of an electric field. Eels and sucker fish larvae (Eudontomyzon) leave the mud under the influence of the current and move toward the anode. Their own electric potential - in most species of fish - is $1-4 \mathrm{~V}$, for trout is $1,2-2 \mathrm{~V}$.

If the fish is large the effect of the current is stronger, because, at the same density of the electric field, a big fish intersects more field lines than a small one. Potential lines are arranged concentrically around the electrodes, being denser near them. Thus, a current that has effect on a 50 cm fish at a distance of 2 m will have an impact on a 10 cm fish only up to a distance of 20 cm (Figure 1).

Thanks to its outstanding efficiency, the electric fishing method is recommend in all studies on fish fauna. It is also the most protective fishing method currently used.


Figure 2. Schematic representation of the effect of various types of electric current on fish. Continuous electric current provides the most extensive galvano-narcosis and galvano-stunning area; alternating current produces no galvanostunning.

### 2.4. THE USE OF THE ELECTRIC FISHING UNIT

Depending on the size and type of the fishing device it can be carried on the back by one of the processors (Photo 1) or may be left on the shore, while the operator moves the anode, which must have a connecting cable of about 100 m (Photo 2). Also, if necessary, the device, or only the operator of the anode, can move with the aid of a boat.

In the case of high power devices you can use up to 5 anodes, which allows you to cover the entire width of the river.

It is advisable, particularly for quantitative studies, to use two small-meshed nets, which bar the full width of the bed, both upstream and downstream of the fishing area, thus catching all fish in that sector (Figure 3).


Figure 3. The use of barrier nets to stop fish from running from the sample surface

Each operator is accompanied by 1-3 people who collect the fish from the water by means of electrically insulated fish nets (Photo 3).

Those who collect the fish must be in close proximity to a plastic storage vessel (floating on the water) that can isolate the fish caught from the effect of the electric current, thus enabling their release (after measurements) in the best possible state.

Usually we record the length and width of the river (area fished) and the duration of the fishing, in order to compare results from different stations (catch / equal effort unit, CPUE).

### 2.5. ADVANTAGES AND DISADVANTAGES OF ELECTRIC FISHING

## Advantages of electric fishing:

- Allows a more complete fishing than any other method; all species are subject to the effect of the electric current in comparable proportions;
- Allows fishing in places that are hard to reach for other methods (pits with roots and other obstructions on the course of major rivers or small ponds invaded by vegetation, etc.);
- It is the least destructive method for the aquatic fauna.

By using continuous current the fish are left practically unaffected and can be released on the spot, in a good state. This allows repeated tests (even when dealing with small populations) and the study of rare, protected species.

## Disadvantages of electric fishing:

- prohibitive cost of equipment,
- distrust of authorities, who issue fishing authorizations with great difficulty,
- risk of injury (when using improvised equipment),
- incompletely known effects on other aquatic organisms.


## Chaper 3. THE METHODOLOGY OF COLLECTING FISH FAUNA THROUGH REVERSIBLE FISHING (ELECTRONARCOSIS)

### 3.1. EQUIPMENT

All parts of the body likely to come into contact with the electrodes must be protected with insulated and waterproof clothing. Work equipment must be used according to the weather and, in need, headphones for protection against noise. For increasing fishing efficiency it is recommended that the team wears polarized glasses and caps with visors that help prevent light reflected from the water surface and increase your visibility of the fish.

1. Life jacket is mandatory in deeper water (above de knee) and fishing from a boat.
2. Fish nets must have handles made of insulating materials, the net must be knotless, not to hurt the fish.
3. The pots/containers for fish must be made of insulating material, be of suitable size, depending on the number of fish collected. When necessary, they have to be equipped with aerators to ensure better recovery and survival of fish.
4. Communication equipment for emergencies.
5. First aid kit must be present and equipped for Cardio Pulmonary Resuscitation, including instructions.
6. Fishing from a boat (with engine) requires the presence of a suitable fire extinguisher.

### 3.2. FISHING DEVICE

It consists of: source, control box, cables, switches and electrodes.

Current used may be continuous or continuous in impulses; alternating current is harmful to fish and will not be used.

Equipment must comply with IEC 60335-2-86.
Portable equipment must:

- have safety switches,
-be sufficiently light to be transported in difficult conditions,
- be in such a way as allow it to be abandoned quickly (special harness),
- have batteries that do not leak.

Generator equipment must be protected against oil spills, acid or oil.

Equipment must be calibrated to allow comparison of results.

### 3.3. SAFETY

Regarding safety and security at work, various aspects have to be covered, related to: working in the field, aquatic activities, using various equipment and hazardous substances and especially electric current. Personnel should be trained for accidents involving electrocution, drowning, falls, poisoning with exhaust gas.

The theoretical and practical training includes knowledge regarding:

- first aid, cardio-pulmonary resuscitation,
- preventing car accidents,
- the use and handling of boats, water safety,
- fieldwork: weather, orientation, knowledge of the area, personal safety
- knowledge and use of equipment and materials, maintenance,
- safety during electric fishing,
- the use and handling of hazardous chemicals.

Regarding communications, the following aspects are taken into account:

- checking the route and confirming arrival at the workplace,
- recording and observing the route, the work and travel schedule,
- contact: telephone, addresses for police, ambulance, fire department, mountain rescue, hospitals and first aid points in the work area.

As for personal protection, the following are required:

- work and protective equipment,
- medical records for all participants,
- personal contacts, phones,
- clinical examinations and vaccinations.


## Personal protective equipment

The employer shall provide appropriate protective clothing; besides thermal protection it should not have buckles, buttons or parts that could lead to cables or vegetation getting tangled in/around them. Zippers, buckles and/or other conductive metal parts are also prohibited.

Staff will wear boots with insulating soles during fishing; in waters deeper than 75 cm fishing will be carried out from the boat.

Wearing lifejackets is compulsory in waters deeper than 50 cm .

## Electrical equipment

Must meet standard SREN 60335-2-86.
Current sources are connected to the electrodes by electrical control panels.

The batteries should be dry and stored in a separate box from the rest of the equipment.

Power generators must be specially constructed, without grounding, with protection systems against burns caused by the exhaust box.

Control switchboards must have visual markings and clear indicators for connectors and controls.

Hand electrodes must have grips (rods), made of insulating material and the conductor has to be on the inside.

The backpack equipment must be powered by batteries, have special tilting and floating switches and the harness must allow a quick release (with one hand).

## Boats

Must be large enough to hold the equipment and fishing team.

The boats must be as stable as possible and have a work platform with non-slip surface, fitted with handrails at the bow.

Electrical equipment has to allow anchoring against slipping or changes in position.

Except for boats specifically designed by the manufacturer of the fishing equipment (where the aluminum hull acts as a cathode), they must be of insulating materials.

All mooring or reinforcement items shall be of insulating material - not from cable or chains.

Auxiliary equipment (fish nets, buckets, containers for fish) must be insulated.

Outboard engines must have plastic hoods or insulating covers.

Maintenance of equipment is only performed by specialists; unauthorized intervention is prohibited.

It is advisable to have spare units to replace malfunctioning equipment in field situations.

Risk Categories
Electrocution - Is a direct threat, which could result in death or damage to health; indirectly, it can cause falls or other trauma of the affected or companions. Direct effects are: electrical burns, cardiac or respiratory arrest.

Main sources of risk are:

- physical contact with energized electrodes,
- contact with the water in the electric field,
-shocks due to defective, non-insulated or improvised equipment.

Drowning - Working in water has a permanent risk of drowning; life jackets should be worn at all times.

Fires - Fishing generators with heat engines get really hot, electric batteries can ignite materials if accidental contact appears between terminals. Appropriate fire extinguishers must be present.

Falls, slips - Cables and ropes should be kept away from motors or moving parts and / or well secured to avoid tripping or slipping.

Trauma, blows - Given the limited space on the boat and the use of long tools, operators must always be careful not to hit colleagues with fish nets or anodes; care should be taken not to cause sudden movements that could tilt the boat and / or make others slip or fall.

## Safety measures

Each team will have someone in charge with work safety who will participate in activities.

Electro-narcosis devices pose an electrocution hazard if not used as directed. During work staff must wear properly insulated equipment. They will use insulating gloves and pantboots. If the boots get wet inside discontinue fishing until their replacement.

## Do not touch electrode active elements during operation.

Support staff will enter the water to collect fish or other objects only after the current has stopped and the anode was removed. Fishing can be continued only after the removal of all persons (except the work team) from the water.

It is prohibited to modify fishing gear, connections and switches; any intervention by unauthorized personnel is also prohibited. It is prohibited to fish near people in the water, domestic animals, etc. Fishing is interrupted during storms and rains.

During fishing, the safety officer must know the location of the nearest first-aid kit and have alarm means (mobile phone, radio). Although the authority is assigned to the safety officer, each team member has the responsibility to request the amendment of an action or to withdraw if safety rules are not observed.

At the beginning of the day the team leader will make a short training and will assign clear and precise tasks to team members.

Connections and switches will be checked in the field before starting work, in standstill.

The generator will be started only after immersing the cathode and after all team members have confirmed that they are ready to work.

At the beginning of each fishing session check if all team members are properly equipped and check the integrity of the equipment.

A system of visual signals should be adopted for communication between working team and the fixed device operator on shore (recommended hands-free radiotelephone).

It is prohibited to fish during floods and rain.
It is prohibited to fish alone.

During storage equipment must be locked and kept in appropriate conditions (protected from moisture, dirt, shock).

When working with your device on shore it will be in a stable and fixed position. The generator will not be moved during operation unless it is designed to be portable.

When fishing from the boat, the staff will be properly trained. All metal parts of the equipment must be "grounded" (connected) and secured against movement. The boat must be stable (does not sway or tilt).

Wearing lifejackets is compulsory.

### 3.4. OBJECTIVES IN FISHING

Scientific fishing is conducted in order to gather information about the structure of fish communities.

The information is recorded using worksheets to be completed for each sample taken. It is advisable to use standard forms to allow inter-comparison with other study program and easy implementation in electronic databases. It is better if the same person is responsible for filling out all records.

Usually, the monitored parameters are:

- abundance of fish,
- composition (species),
- population structure (age, size, sex).


## The size and number of fishing stations:

To ensure that the data on abundance and the age structure are valid, sufficient samples must be collected. The number of samples depends on the differences between stations (stations should include all habitat types); if necessary, one may consider a follow-up of changes in the population (for migratory species population size varies greatly in time).

The minimum number of stations depends on the coefficient of variation between stations (CV). The coefficient of variation is the ratio between the standard deviation between stations / arithmetic mean of abundance. Abundance is the number of fish / station.

| Coefficient of variation <br> CV | Minimum number of <br> stations <br> $\mathbf{n}$ |
| :---: | :---: |
| 0,2 | 3 |
| 0,4 | 4 |


| 0,6 | 9 |
| :---: | :---: |
| 0,8 | 16 |

Table 1 The minimum number of stations in relation to the coefficient of variation

The surface off which the sample is collected depends on the size of the body of water and the habitats.

| Size of river | Minimum length for fishing |
| :--- | :--- |
| Small streams, width $<5 \mathrm{~m}$ | 20 m on the entire width |
| Small streams, width $5-15 \mathrm{~m}$ | 50 m on the entire width |
| Rivers and channels $>15 \mathrm{~m}$ | $>50 \mathrm{~m}$ on one or both sides |
| Large bodies of water (rivers, <br> lakes), shallow $<70 \mathrm{~cm}$ | $200 \mathrm{~m}^{2}$ |
| Deep lakes $>1 \mathrm{~m}$ | $>50 \mathrm{~m}$ near the sea |

Table 2 Minimum length traveled for a sample

### 3.5. REVERSE FISHING THROUGH ELECTRONARCOSIS

### 3.5.1. Describing activities

Preparation for travels:

- Obtaining fishing permits and licenses,
- Staff training,
- Logistics: materials, equipment, fuel, spare parts,
- Information on access roads, local conditions.

Preparation at station point:

- Informing authorities and local population (warning signs),
- Team organization, establishing responsibilities,
- Recognition and guidance toward fishing site,
- Recording physical and geographical data,

Fishing:

- Connect and check cables and insert electrodes into the water,
- Check switches,
- Connect the power,
- Collect fish in the electric field.
3.5.2. Fishing in small rivers (that can be crossed by foot)

Will be fished from shore or with trouser-boots. When the width of the river so requires, it is preferred to use two anodes, i.e. one anode every 5 m in width. Fishing is usually done upstream do that muddy water does not reduce effectiveness. Operators must move slowly, covering the habitat with sweeping movements of the electrode to remove the fish out of hiding (Figure 4).


Figure 4. Two ways of movement during fishing in order to completely cover the surface of the sample area

In order to facilitate the capture of fish in rapid waters the fish nets must be adjacent to and downstream of the anode. To estimate absolute density, cross nets barring the stream (Figure 3) and the Lurry method (which consists in repeating the fishing until stocks are exhausted) must be used. For relative estimates partial barriers can be used (such as fords, if species are mobile, non-territorial).

Use portable equipment or access areas via long cables. If conditions allow (uniform depth) the generator can be placed on a small raft.

### 3.5.3. Fishing in large rivers

May be fished electrically or in combination with other methods. Electric fishing is effective especially along the banks. When water exceeds 70 cm it is necessary to use a boat.

Operators should be placed so as to have access to the effective area of the electric field with the fish net (stunning and narcosis).

The boat must move downstream (in such a way as to ensure access to bushes and other hiding places) or upstream, when the flow is high.

In rapid waters it is necessary to move the boat at the same speed as the water by using the engine or oars, so the boat has the same speed as the immobilized fish drifting in the water.

In slow waters is not necessary to synchronize the movement of the boat with the flow of water; the boat can be controlled, as needed, from the shore, with ropes.

If the river is large it is more difficult and dangerous to place cross nets. In large rivers one must follow a combination of impulse forms and frequencies in order to optimize the capture of target species.

Absolute estimation of densities in large rivers is difficult. In these bodies of water a stable representative sample of the number and specific composition, abundance, is actually a series of sub-samples, numerically proportionate to the diversity of habitat types present. For this reason a stratified sampling methodology is required. In terms of quality, a larger amount of data can be obtained by fishing in shore areas, in different types of habitat.

When the power of the generator allows, efficiency can be increased by increasing the number of anodes (Figure 4). Compound anode systems may be mounted on board (Photo 3). Depending on conductivity, current consumption can rise substantially, requiring appropriate generators and control panels. Often, it is only possible to fish in areas near the shore, with some efficiency, while deep water fish will escape.

## Chaper 4. FISHING WITH MESH GEAR

This method takes into account standard SR-EN 14757 Sampling of fish using nets with different mesh sizes.

The team will collect fish using mesh gear, fixed and mobile: gillnets, pots, baskets, drag net at night.

For this purpose, in advance, the major types of habitat and appropriate places for the use of various tools will be identified. Fishing should cover all habitat types and be distributed uniformly, randomly.

Fish will be identified as species level and examined pathologically. After identifying the species, specimens collected will be measured and weighed (at least) by age. Specimens will be preserved to verify measurements and for museums. It is better if a single person handles the registration cards and tags and then another one checks whether the data is complete and accurate.

## Selecting the fishing grounds

Initially, the team assesses the presence and expansion of major habitat types. Selection should be made using temperature data, dissolved oxygen, bathymetry data and shoreline maps. Tools should be placed based on equal coincidental representation of habitat types and not on maximum catch.

## Tools and boats

Gillnets - For fishing in lakes we use the so-called Swedish nets. These are trammel nets with different mesh sizes. The benthic ones are assembled from 12 panels of netting with a height of 1500 mm , a width of 2500 mm , mesh size from 5 to 55 mm and wire thickness from 0.1 to $0,25 \mathrm{~mm}$. Pelagic nets are assembled from 11 panels with a height of 6 m , a total length of 27.5 m . It is recommended that the weights and buoys be included in their cord, to avoid snagging and reduce the disentanglement effort.

Fyke nets are made of mesh with 5 mm openings, diameter is 600 mm , with wings of $5-10 \mathrm{~m}$.

The drag net has a height of 2 m and a length of 10 m and is made of 5 mm mesh.

Boats used with nets must be large enough for 4 people, equipped with oars and motor, stable so as to allow those launching and collecting pelagic nets to stand, and must have smooth edges, devoid of hooks.

During work it is mandatory to wear rescue belts and equipment with no buttons.

## Choosing the habitat

This step is carried out on the first day, before starting to collect the biological material. It is good to consider (for subsequent collections) at least two major types of habitat: coastal and pelagic.

Depending on the size of the lake, 2 to 10 sample stations may be required. Apart from the places selected according to the
standard protocol, the team will designate one or two "most appropriate stations". In lakes with depths greater than 8 m one will take into account the existence of vertical stratification (hypo-, meta- and epilimnion). In shallow lakes we will have a single pelagic habitat.

## Recommendations:

- Choose the stations considered characteristic for the micro-habitat.
- If you encounter a homogeneous mass of plants in the pelagic area it is better to move toward its edge.
- Avoid heavy traffic or recreational areas (boats, anglers, swimmers etc.).
- Avoid areas with oxygen under $2 \mathrm{mg} / \mathrm{l}$.

It is advisable to inform the residents nearby about the purpose of the fishing session and that you have all necessary permits; it is advised to avoid disruption of the activities of the local population.

| Lake <br> surface ha | Baskets | Shore <br> gillnets | Open water <br> gillnets | Trammel |
| :---: | :---: | :---: | :---: | :---: |
| 5 | 1 |  | 1 | 1 |
| 20 | 2 |  | 3 | 1 |
| 50 | 4 | 1 | 3 | 3 |
| 150 | 6 | 1 | 5 | 4 |

Table 3 The effort required to assess fish communities

## Selecting sites for gillnets

The aim is to sample 3 types of habitat; if the hipolimnion is extended more effort will be allocated to it.

- The hipolimnion must be fished from the base, the deepest point with enough oxygen.
- The metalimnion will be fished with nets placed along its shore outline and not across. The weight line needs to be on the thermocline.
- The epilimnion will be fished with nets placed randomly toward the middle of the lake at depths greater than 3 m , with the float line at a depth of 1.5 m .


## Selecting locations for shore gillnets

One must evaluate the number of nets needed according to the surface of the lake, the length of the shoreline and the diversity of the habitats.

## .4.1. FISHING IN LAKES WITHOUT THERMAL STRATIFICATION

## A. shallow

All gillnets are placed at the bottom, the first in the deepest area. The other nets must be placed about midway between the center and one of the shoreline habitats. Every fourth gillnet will be placed perpendicular, with one end on shore.
B. deep

As above, but every third gillnet placed on the surface, in the pelagic area.

### 4.2. FISHING IN LAKES WITH THERMAL STRATI FICA- <br> TION

## A. with oxygenated deep waters

A layer of oxygenated water $>2 m$ thick below the metalimnion and expansion area of this layer exceeds $50 \%$ of the lake.

Gillnets are installed one by one as follows:

1. first one in the maximum depth oxygenated area toward the center of the lake,
2. metalimnion at the bottom following the outline of the thermocline, in randomly chosen types of habitat,
3. the epilimnion floats at a depth of 1.5 m halfway between the center of the lake and one of the different habitat types,
4. bottom, away from the first one in a random direction,
5. metalimnion, see A2,
6. seaside,

The additional ones follow A4, A6, A3, A4.

## B. with non-oxygenated deep waters

1. at the deepest level with oxygen, near the center of the lake,
2. metalimnion or bottom epilimnion (see A2). The net can be placed at the same depth with B1, but away from it in a random direction,
3. epilimnion between the waters (see A3)
4. coastal area,
5. same as B1,
6. same as B2,
7. same as B3,
8. same as B4,
9. same as B5,

## Selecting coastal stations

First of all, set:

1. Number of passive stations (coastal baskets and nets).
2. Proportion of shoreline in each macro-habitat. A macrohabitat must cover at least $10 \%$ of the shoreline to be considered major.
3. Physical habitat types that are in major habitats.

## Selection of specific collection points

1. If the number of major habitats is equal to that of stations, choose one at random.
2. If the number of coastal stations is greater than that of the major habitats, randomly choose a physical habitat and distribute the others so as to be uniformly distributed on the surface.

## Selecting sites for fishing with a trammel

Fishing with a trammel (after sunset) differs from other methods in that it is an active method. Although the method is effective, it gives results only under certain conditions: shore with beach with small slope, water up to 1 m deep, hard and smooth substrate.

The ideal place is about 50 m long, with clean shore, so that the net can be raised directly on the shore. In such circumstances mark the place during the day in order to fish after sunset. Measure and record the fished area. It is best if a third team member assists while fishing to carry equipment, record the surface covered, help carry buckets and other materials, etc.

Inform the administrator and show the authorization before going.

### 4.3. FISHING WITH MESH GEAR

## Preparation

While two team members are in charge of collecting physico-chemical data on the boat, the person ashore is preparing baskets and nets.

When the boat returns the nets and baskets are laid down. They are placed at the points identified according to the established collection scheme. Fishing with a trammel takes place after sunset, in the place marked during the day.

## Recovering the nets and processing the catch

The first task in the morning is recovering the nets and baskets.

These are addressed in turn. Only after completely processing one net does one move to the next. Fish caught must be released into the water at the end of the activity. In case of mortality it is necessary to destroy it (according to the law) and inform authorities.

### 4.4. IDENTIFICATION AND MEASUREMENT OF FISH, RELEASE

## General

In order to meet the minimum requirements the following indicators will be used, also useful for standardized methods of assessing water quality: composition (species and age).

Fish will be handled so as to avoid losses and injuries. Aeration of water stored in containers is essential to reduce fish mortality due to handling.

## Identifying the fish

It will be made for species, using morphological characters. For specimens with blurred characters (hybrid, alike, juvenile) specimens will be preserved, and will be identified in the laboratory.

## Measurements

Length and, where appropriate, the weight will be measured (for the calculation of gravimetric abundance). In the case of overlapping age classes scales can be harvested (from the left side of the fish, above the lateral line) in order to assess age. If the number for a sample species exceeds 30 individuals, it is considered sufficient to determine the age structure of the population.

## Anesthetics, disinfectants

Anesthetics are useful for handling large, active species. Disinfectants are recommended if the equipment is used in different water bodies, to prevent the spread of diseases and parasites.

## Releasing the fish

Except specimens for museum collections (voucher) and those preserved for laboratory identification (confusing characters) all fish will be released as soon as processing is finished. Processing and release will be made after every catch, length traveled.

Fish will be released in a quiet and deep portion, near the shore. Mortality should be evaluated and marked as a percentage of the total sample.

## Results

Electric fishing results will contain the following parameters:

- inventory of species present, the specific composition,
- abundance of each species will be reported to the total number of individuals captured and for $100 \mathrm{~m}^{2}$ fished,
- age structure will be reported for each species.

Age can be determined by the frequency of lengths or read on scales. The age structure can be expressed as average length for each age class together with standard deviation and number of fish in the sample. For abundant species the number of individuals in each age class has to be recorded in order to identify the absence of certain age classes. If the determination of age is too expensive or too complicated, size distribution may be sufficient to assess the structure of the population.

- additional measures, optional: weighing and calculating the biomass of each species.
- abnormalities and parasites.


## Data quality control

Data quality is influenced by the methodology used, the accuracy and precision of measurements.

Quality assurance is done by:

- adequate training of field team,
- application of standardized methodology,
- verification of completion of records,
- clear and accurate definitions,
- clear definition of the objectives of the study.


## Recording of data

Details about physical features, fishing team, effort, goals, equipment, catch, etc. will be recorded in a standard record. It will
be compiled and distributed by the organizer of the fishing session, with a unique look and content (Annex 3).

## Chapter 5. PROCESSING AND INTERPRETING DATA RESULTING FROM FISHING

Raw data obtained from fisheries are lists of species, sizes and weights associated with individuals; they are supplemented by information on age, sex, morphological abnormalities, external parasites or other detectable conditions.

Such data must be processed and interpreted using basic information on the biology and ecology of the species identified, and with indicators and statistics that can be computed using existing formulas. Through complex processing of the data from fishing we are able to draw conclusions about the status of those habitats and alterations of environmental factors. The value of fish as biological indicators of water quality is recognized and appreciated by current legislation in Europe, North America and other parts of the world.

### 5.1 THE USE OF FISH AS INDICATORS OF WATER QUALITY

Since 2007 Romania has adopted and implemented the European legal framework. In the field of water resources management, the Water Directive 2000/60/EC has brought important changes implemented in national law by Law no. $310 / 2004$. Among other changes, it introduces biological monitoring as the main method for the surveillance of water quality and indicates fish fauna as an element to be introduced and used in water quality assessment activities.

The advantages of using fish as ecological indicators
The aquatic ecology studies of various authors state that a number of characters specific to fish populations make them particularly useful in the assessment of environmental degradation:

1) the fish are present in all aquatic environments, often in highly polluted waters;
2) generally, they have stable populations and are not subject to strong seasonal fluctuations (as in many invertebrates);
3) The fish are integrators for the responses of other departments of the ecosystem because they depend on them for breeding, food or shelter:

- fish take the food from different levels of the food chain, thus integrating all components of the ecosystem; their biomass depends on primary and secondary production,
- fish have a relatively long life; the analysis of the population's age structure and the calculation of the growth rhythm provides data about the history of the population.

4) fish are easily identifiable on the ground and allow a rapid appreciation of the ecological quality of the station;
5) there is a lot of information about the biology of fish, and many institutions that collect data on them;
6) the interest of policy makers and the public for the status of fish stocks is higher than that for microorganisms and invertebrates;
7) unlike chemical methods that provide "a lot of data and little information", Biotic Integrity Indexes allow fair decisions to be made, based on very sketchy data.

Undoubtedly, the use of the highest trophic level makes it difficult to precisely determine the mechanisms responsible for changes; hence the need for a detailed study of the ecosystem.

Under the Water Directive, the key descriptors of fish fauna are:

- composition by species,
- abundance,
- typical species
- composition by age.

Looking at Table 4 we see how, in accordance with the recommendations of the Water Directive, a particular class of aquatic habitat quality is attributable using only fish fauna as indicator, based on fish community structure (ichthiocenosis).
\(\left.$$
\begin{array}{|l|l|l|l|}\hline \text { Element } & \text { Very good state } & \text { Good state } & \text { Moderate state } \\
\hline \text { Fish fauna } & \begin{array}{l}\text { Species composition and } \\
\text { abundance correspond } \\
\text { totally or nearly totally to } \\
\text { undisturbed conditions. } \\
\text { All specific species, } \\
\text { sensitive to disturbance } \\
\text { are present. }\end{array} & \begin{array}{l}\text { There are slight changes in species } \\
\text { composition and abundance from the } \\
\text { type specific communities, that can } \\
\text { be attributed to anthropogenic } \\
\text { impacts on physico-chemical or } \\
\text { hydro-morphological elements of } \\
\text { quality. }\end{array} & \begin{array}{l}\text { Composition and abundance of fish } \\
\text { species differs moderately from the } \\
\text { type-specific communities attributable to } \\
\text { the anthropogenic impact on physico- } \\
\text { chemical or hydro-morphological } \\
\text { elements of quality. }\end{array} \\
\text { communities can show a } \\
\text { little sign of } \\
\text { anthropogenic } \\
\text { disturbance but does not } \\
\text { indicate a deficiency in } \\
\text { reproduction or } \\
\text { development of any } \\
\text { particular species. }\end{array}
$$ \quad $$
\begin{array}{l}\text { Age structure of the fish communities } \\
\text { shows signs of disturbance } \\
\text { attributable to the anthropogenic } \\
\text { impact on physico-chemical or hydro- } \\
\text { morphological elements of quality. In } \\
\text { certain circumstances they may } \\
\text { indicate a deficiency in reproduction } \\
\text { or development of specific species, } \\
\text { to the extent that some age classes } \\
\text { may be missing. }\end{array}
$$ \quad \begin{array}{l}Age structure of fish communities shows <br>
major signs of anthropogenic <br>
disturbance to the extent that a <br>
moderate proportion of the type specific <br>

species are either absent or have very\end{array}\right]\)| lowndance. |
| :--- |

Table 4. Quality classes for fish fauna as listed in Law no. 310/2004

### 5.2. STATISTICAL INDICATORS USED TO ASSESS FISH COMMUNITIES

It is important to note that the data collected from field work can be structured in different ways.

Their stratification results in a very large pyramid with a base consisting of directly measurable raw data (temperature, concentration of various substances, lists of species).

The second layer of the pyramid, narrower, consists of statistics, averages of temperatures, concentrations, population size, the presence of certain ecological groups (guilds) etc.

The third layer is made up of the indicators, parameters, descriptors resulting from a calculation: constancy, dominance, reasonableness, etc.

The last layer consists of complex indices resulting from the synthesis of indicators like: ecological significance index, quality biotic indices etc.

In this way, through the aggregation and synthesis of data from the lower level, data are obtained from the upper levels of the pyramid.


#### Abstract

Abundence (A) represents the absolute number of individuals of a species present in an area. In our case absolute abundance is the number of specimens of a species in samples. The value of abundance determines whether species in an area are characterized as rare, less rare, abundant and very abundant.


Constancy (C) is a structural index that expresses continuity of occurrence of a species in a given habitat. The higher the value of this index the more adapted the species is to conditions in the biotope. Constancy is estimated by the relation:

$$
C_{A}=\frac{n_{p} A}{N_{p}} * 100
$$

$\mathrm{C}_{\mathrm{A}}=$ constancy of species A
$n_{p} A=$ number of samples in which species $A$ is present
$\mathrm{Np}=$ total number of samples examined
Depending on the value of this index, species are distributed in the following classes:

C1 - accidental, present in 1-25\% of samples;
C2 - accessories, present in $25,1-50 \%$ of samples;
C3 - constant, present in $50,1-75 \%$ of samples;

C4 - euconstant, present in 75,1-100\% of samples.
Dominance (D) shown the relationship between a number of a given species and the amount of individuals of other species with which they are associated, essentially expressing relative abundance (Ar).

Dominance is calculated with:

$$
\boldsymbol{D}_{\boldsymbol{A}}=\frac{n_{A}}{\boldsymbol{N}} * 100, \text { where }
$$

$\mathrm{D}_{\mathrm{A}}=$ dominance of species A
$\mathrm{n}_{\mathrm{A}}=$ total number of individuals of the species A found in the samples examined
$\mathrm{N}=$ total number of individuals of all species present in the samples examined

Depending on the values of the index, species are distributed in the following classes:

D1 - subrecedente - under 1,1\%;
D2 - recedent - between 1,1-2\%;
D3 - subdominant - between 2,1-5\%;
D4 - dominant - between 5,1-10\%;
D5 - eudominant - above 10\%.
Ecological significance index (W) represents the relationship between the structural (C) and the productive (D) index, showing the position of a species in the biocenosis.

It is calculated using the relation:

$$
W_{A}=\frac{C_{A} * D_{A} * 100}{10000}
$$

After the values for this index, species are divided into the following classes:

W1 - with values under $0,1 \%$;
W2 - with values between $0,1-1 \%$;
W3 - with values between 1,1-5\%;
W4 - with values between 5,1-10\%;
W5 - with values above $10 \%$.

W1 classes correspond to accidental species; W2 and W3 classes - accessories (accompanying) species; W4 and W5 classes - species characteristic for the given cenosis.

Jaccard coenotic affinity index (q) shows affinities between the species of a group in a biocenosis, based on common preferences for the conditions of the same living environment. The index enables characteristic (indicator) species to be highlighted as having the highest affinity. The cenotic affinity index can be represented graphically as a matrix or in the form of a dendrogram.

To estimate the affinity cenotic of representatives of a group identified on a specific area, we must calculate the affinity of each species with all others. Jaccard's formula is:

$$
\mathbf{q}=\frac{c}{a+b-c} * 100
$$

$\mathrm{a}=$ the number of samples in which species A is present, whether or not $B$ is present as well;
$b=$ the number of samples in which species $B$ is present, whether or not A is present as well;
$\mathrm{c}=$ the number of samples in which both species are present.

Species with values ranging from 70.1 to $100 \%$ are considered probative for that cenosis.

## Specific similarity index (Sorensen)

This index expresses the degree of similarity of communities in different habitats, paired two by two in terms of common species. They are estimated according to the Sorensen coefficient. The specific similarity index may be graphically represented as a matrix or in the form of a dendrogram, showing the degree of similarity between the two communities in percentages (common species,).

$$
\begin{aligned}
& r S \%=2 \frac{c}{a+b} * 100 \\
& \text { Where: } \quad \begin{aligned}
\mathrm{a} & =\text { number of species in habitat } \mathrm{A} \\
\mathrm{~b} & =\text { number of species in habitat } \mathrm{B} \\
\mathrm{c} & =\text { number of common species }
\end{aligned}
\end{aligned}
$$

Real diversity $\mathbf{H}(\mathbf{S})$ expresses the ratio between the number of species and the total number of individuals in a given biocenosis. The formula used to calculate the actual specific diversity is based on Shannon's relationship, as amended by Mac Arthur, Lloyd and Ghelardi:

$$
H(S)=k / N\left(\log _{10} N-\Sigma S_{r=1} \text { No. } \log _{10} \text { No. }\right)
$$

$\mathrm{H}(\mathrm{S})=$ real diversity (observed)
$\mathrm{k}=$ the conversion factor for changing the logarithm base from 10 to 2 , with a value: 3.321928 ;
$\mathrm{N}=$ total number of individuals in samples;
$\mathrm{S}=$ total number of species;
No. = number of individual found in species $r$ (abundence).
$\mathrm{Sr}=1 \mathrm{No} . \log 10$ No., where:
Real diversity will be higher if the index $\mathrm{H}(\mathrm{S})$ will have a higher value and vice versa. For the same number of individuals, diversity will increase with the number of species; for the same number of species, diversity will increase with the number of individuals.

Hypothetical maximum diversity $\mathbf{H}(\mathbf{S})$ max of a biocoenosis is reached when all the species present have the same number of individuals and is given by the relation:
$\mathrm{H}(\mathrm{S}) \max =\mathrm{k} \cdot \log _{10} \mathrm{~S}$
Equitability (relative diversity) is the ratio between the real diversity $\mathrm{H}(\mathrm{S})$ and the maximum diversity $\mathrm{H}(\mathrm{S})$ max.

$$
\mathrm{E}=\mathrm{H}(\mathrm{~S}) / \mathrm{H}(\mathrm{~S}) \max
$$

The value of equitability approaches zero when the species have a maximum difference between them and is equal to 1 when all species have equal frequencies. Given the real inequity in nature in terms of the distribution of individuals by species, the percentage obtained will show the percentage of the diversity of the community studied compared to an ideal community with the same number of species, but with equitably distributed individuals.

## Multi-meter biotic indexes

The method of biotic indices based on the structure of fish communities appeared relatively late in relation to improvements in the capture and analysis of ichthio-fauna.

In 1990, Fausch defines biotic integrity as "the ability of an environment to support and maintain integrated and adaptive communities of organisms having a specific composition, a diversity and a functional organization comparable to that of the least disturbed habitats".

## physical variables:

temperature, turbidity
chemical variables: pH ; hardness, $\mathrm{O}_{2}$, alkalinity; minerals, organic matter, metals
hydrological regime: surface, distance from the source, rain, flow, current velocity
sources of energy: season; lighting;
availability in minerals, primary and
secondary production, exogenous organic matter
RESULTING BIOLOGICAL COMMUNITY
habitat structure: slope, substrate, depth, sinuosity, course width, shelters, ground and aquatic vegetation
biological interactions: reproduction, competition, parasitism, predatorism;

The main physical, chemical and biological environmental factors influencing the structure of the biological community in a lotic aquatic habitat.

Modified after Karr \& Dudley, 1981

Multi-meter fish indexes were materialized through the Index of Biotic Integrity (IBI) which, once created by Karr and Dudley in the 80's (U.S.A.), was then extended and adapted to the various aquatic habitats on all continents. However, IBI is primarily a method for medium-sized rivers in temperate climates.

The method is standardized and widely used in Environmental Agencies in the U.S. and Canada. It is the national official method in Spain, France, Belgium, and it is being tested in Poland, Lithuania, Switzerland, Romania, etc. Any fish biotic index uses parameters (descriptors, metrics) of the following categories: species richness and composition, abundance, trophic structure.

## Biological evaluation at multiple levels

It as drafted by the team at the BOKU University in Vienna led by Professor Stephan Schmutz after 2000.

The method analyzes the biological characteristics of ichthiocenoses, and the assessment is done on several hierarchical levels.

- individual,
- population,
- guild, species group using ecologically similar strategies,
- biocenosis,
- fauna.

The parameters analyzed are:

- the number of species characteristic for the type of habitat,
- the structure and assembly level of associations, fishery regions , the number and type of guilds,
- population size, density, biomass,
- reproductive success and age structure.

Systematics and biology of the species is fundamental to establishing the integrity of ichthiocenoses. In Europe, due to the relatively small number of species, these issues are clarified; we know the essential elements of their biology and ecology.

Ecological classifications allow us to group the fish using the following criteria:

- native / exotic species,
-replacement (invasive) species, which penetrated due to changes in environmental factors,
- rare species.

These criteria can be expanded to environmental groups ("guilds") using criteria such as: feeding particularities ("filters"), water velocity preferences, reproductive features, "nest" building, etc.

## Analysis of the assembly of species from an ecological perspective

Community structure is given by the ratio of species, and how they are grouped according to their preferences for certain factors of the environment and / or due to interaction with other species.

In order to understand how species are assembled, biologists have introduced criteria related to characteristics and ecological requirements of different species. Characterization of communities is done using metrics (descriptors) that can be grouped into different categories:
a. Fish zonation (of rivers)

Defined by the dominance of a species. The concept of "area" is associated with the related species; this zoning should be consistent with the division of the river on hydro-morphological criteria.

## b. Number of guilds and structure thereof

To do this, we take into consideration all species that are specific for the type of habitat. Ecological guilds are groups of species that exploit environmental resources in a similar way:

- thermal guilds, grouped by preference to temperature,
- guilds based on rheophilic degree,
- trophic guilds (predators, herbivores, plankton eaters),
- reproductive guilds (substrate used, building nests, caring for offspring),
- migration guilds.
c. Stock (abundance) of population provides information about the functionality of ichthiocenoses.
d. The demographic structure of the populations provides information on the functionality.

Based on dimensional dispersion diagrams we can identify standard natural populations to be compared with ecosystems affected by human impact.

## e. Further information

- population (input) controlled for certain species,
- catches made for industrial purposes or sports,
- general state indices (coefficient of fattening, prolificacy).

The analyzes must take into account that, at different ages, the same species changes its preferences for current
velocity, depth, types of food. Thus, it can be assigned to different guilds during development.

The multi-level evaluation method was the subject of a research program developed by a multinational consortium comprising 25 institutes funded by the EC - the Fish Program based Assessment Method for the Ecological Status of European Rivers FAME. The result of this is the European Fish Index (EFI).

## The purpose of knowing the structure of ichtiocenoses

The practical, applied value of the study of ichtiocenoses' structure is geared towards the following objectives:

- Knowing their current status, identifying and understanding the key indicators of the status and functionality of a ichthiocenosis; interactions with other environmental factors;
- Identifying the natural state of the structure, determining (theoretical reconstruction) the historical situation (baseline) without anthropogenic influence, and identifying the degree of alteration of the natural state,
- Ability to forecast the answer of an ichtiocenosis to changes in various environmental factors,
- Understanding and forecasting evolutionary trends of ichtiocenoses under the influence of global changes (climate) and / or chemical and morphological alterations induced by human activity,
- Assessing the quality of the aquatic habitat based on the structure of the fish community that inhabits it.


## ANNEX 1

## PREPARATORY OPERATIONS CHECKLIST

Before starting a field action these should be considered:<br>- verify the existence and operation of the equipment and materials necessary,<br>- obtain fishing permits and licenses,<br>- staff training (safety sheets),<br>- medical visit,<br>- identify access and evacuation routes, first aid points.

## ANNEX 2 <br> LIST OF NECESSARY MATERIALS

## General use

- File for the workstation,
- Map, description, sketch of the habitat
- Scrapbook,
- Sheets for sampling,
- Work manual,
- Fishing authorization, notice of access to the border area, boat and vehicle documents, business card, delegation,
- Sonar
- GPS unit with manual and spare batteries,
- Camera,
- Anchor and ropes,
- Brush for cleaning the equipment,
- Plastic film, sheet (for working in adverse conditions and protection of materials)
- Toolbox, duct tape, plastic bags, tightly closed bags,


## For collecting ichthiofauna

- Boat with necessary documents,
- Gillnets, pots, trammel, nets (barrier) for the closure of the fishery, fish nets,
- Electro-narcosis device with wires, coils, port-electrodes and electrodes,
- Long boots, gloves, rescue belts, polarizing glasses, hat with brim,
- Front lamps, flashlights,
- Buoys, ropes, anchors, warning signs,
- Basins, buckets, sealed jars for preservation,
- Electronic scales, ichthiometer, ruler, magnifying glass
- Formalin $37 \%$, beaker, large syringes, gloves and goggles,
- Iceboxes,
- Worksheets, universal markers, crayons, stickers.


## ANNEX 3

## DATA RECORDING SHEET

## LOCATION, TEAM, OBJECTIVES

## Station name:

$\qquad$
Type of water (stream, river, canal, lake):
River name: $\qquad$
GPS coordinates: $\qquad$
Team composition:
Fishing method:
Date:
Period of day:
Objectives (monitoring, verification, research, etc.): ......
Notes:
EQUIPMENT, CONDITIONS
Device model, type of net gear:
Manufacturer:
Settings:
Mesh size:
Water level:
Weather:
Conductivity:
Temperature:
Visibility, turbulence:
Anode type:
The use of barrier nets:
The number of crossings:
Notes: $\qquad$
STATION
Length:
Water width (average)
Average depth:
Maximum depth:
Fished area or length of shore traveled:
Water speed (class):
Dominant substrate type:
Habitat types:
Shore vegetation:
Aquatic vegetation:
Shade:
Plant debris (wood):

Altitude: ...
Slopes:
Notes:
CATCH
For each station:
List of species
Number of individuals
Maximum size for all species or specimens
Weight
Abnormalities, diseases, parasites
Sex
Maturity
Stomach content
Comments

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