



World News of Natural Sciences

An International Scientific Journal

WNOFNS 18(1) (2018) 1-51

EISSN 2543-5426

Laboratory Manual on General and Special Ichthyology

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ABSTRACT

The manual presents the main methods for studying fish. The general features of the external structure of fish are described: the shape of the body, external organs, the structure and functions of the fins, the types of scales, and the structure of the lateral line. The indicators of morphometric studies of fish with the use of meristic and plastic features according to the classical schemes of measuring fish are also presented. A short description of the statistical method of comparative study of two data samples using the Student's test is given. A method for determining the age of fish by scales is described, as well as a methodology for calculating the annual growth of fish. General information on anatomical structure of fishes is also given. Furthermore, methods of studying the fertility of fish, as well as assessing the nutrition of fish, are described. Particular attention is paid to creating fish preparations: the production of the skeleton of fish, the design of fixed moist preparations and the preparation of a stuffed fish. The presented material has visual illustrations, accompanied by short biological information and forms of tabular material design. The proffered material will be useful for students of the biological and ecological profile, students of natural and agrarian faculties, post-graduate students and young scientists.

Keywords: fish, ichthyology, morphology, fins, scales, anatomy

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INTRODUCTION

Ichthyology (from the Greek. Ιχθυολογία, from ιχθύς – fish, λόγος – teaching) is a biological science research objects of which are Cyclostomes and Fish. At the present stage ichthyology studies taxonomy, evolution, morphology, anatomy and embryology of fish, species and age structure of populations and their quantities, migration and geographical distribution of fish in waters of different climatic zones, etiology and ecology of fish.

The purpose of ichthyology is theoretical and practical justification of conservation, restoration and protection of rare and endangered species, developing ways to increase fish productivity of reservoirs, predicting the state of fish fauna in environmental conditions.

Ichthyology is divided into:

- General Ichthyology (morphology, anatomy, ecology, phylogeny, evolution and distribution of fish);
- Special ichthyology (distinctive signs and biology of certain species of fish);

Because of fact that the fish is the most numerous group of vertebrates, and has more than 33,4 thousand species ichthyology evolved as a separate solid section of zoology [1]. As an independent science it is closely related to hydrobiology, hydrology and ecology.

The development of ichthyology has contributed to emergence of such areas as fish histology, fish embryology, fish physiology, biochemistry of fish, fish parasitology, genetics and breeding of fish, fishing, industrial fishing.

Laboratory work № 1

The main parts, body shape and outward features of fish

Objective: Learn about the diversity of body shapes of fish, structure of their outer organs.

Materials and equipment: fresh fish, set fixed preparations of fish (10–20 species). Tables: "The external structure of fish", "Fish body shape", "Location and types of fish mouth". Tools: tweezers, dissecting needles, cuvettes.

Basic theoretical information

The main parts of fish body. The body of fish consists of three parts: head, trunk and tail.

The head part is the distance from the mouth to the back edge of the gill cover.

The trunk part is the distance from the end of head to the anus or to the beginning of the anal fin.

The tail part is the distance from the anus (from the beginning of the anal fin) to the end of the tail fin.

There are mouth, eyes, nose and gill openings, spiracles on the head of the fish. Some fish have whiskers which are the bodies of touch and taste (catfish, burbot, eel). Shape of fish head is very diverse. Hammerhead shark's head is shaped like a hammer, on the edge of which there are the eyes [2]. American paddlefish has a head with a large spatulate extension. The upper jaw of some species is stretched into a long xiphoid appendage, which is used in the attack (swordfish); sometimes it has sawtooth appendage (fish-saw). Mouth of pipe-fish is stretched into a long tube [3]. Hagfishes and lampreys have it turned into a kind of suction cup.

The head part has the following: *the snout* which is the distance from the head to the front vertical of eye; *the space behind eyes* is from the back vertical of the eye to the distal end of the gill cover; *cheek* is part of the rear vertical of the eye to the rear edge preopercular bone; *forehead* is the space between the eyes.

At the bottom part of the head there are placed: *chin* which is the part of the head from the beginning of the mandible to the place of connection or attachment of gill membranes; *throat* is the distance from the gill membranes to the base of the pectoral fin; *symfizys* is the place of joining of the bones of the lower jaw.

Cartilage and sturgeon fish have the holes named spiracles behind eyes, which are remains of not functioning gill slits. Sometimes the fish head has arms as outgrowths of thorns and spines.

The location and structure of the mouth of fish depends on the nature of power. There are the following types of mouth (Fig. 1):

- 1) *Upper (half-upper) mouth* – lower jaw is protruding upwards (white carp, bleak, sabrefish);
- 2) *Terminal mouth* – the upper and lower jaw are equal in length (perch, pike);
- 3) *Lower (half-lower) mouth* – the upper jaw (or rostrum) is strongly protruding (cartilage, sturgeon);

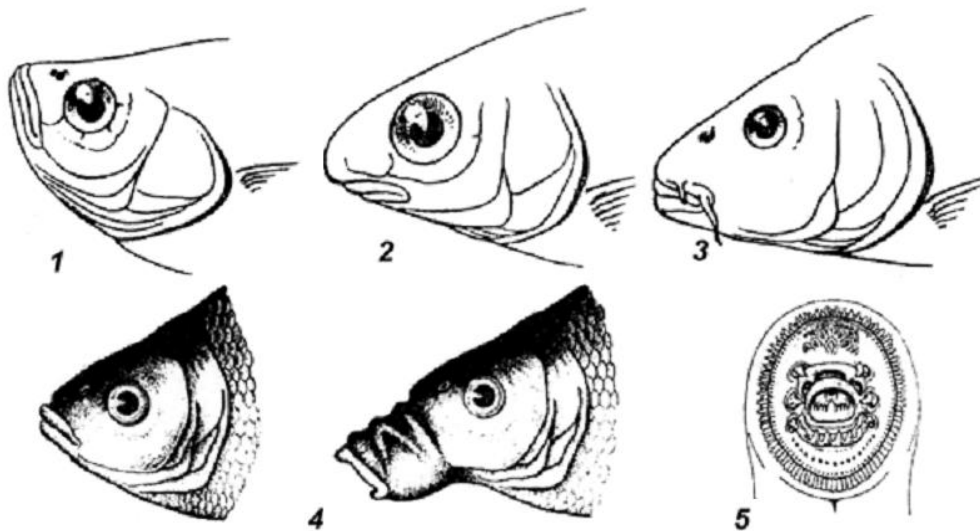


Figure 1. Forms of fish mouth: 1 – upper; 2 – lower; 3 – terminal; 4 – retractable; 5 – funnel-shaped.

Fish, which feed near the bottom (benthophages) typically have the lower (or half-lower) mouth and planktophages have the upper mouth. Except sharks (predators) which have the lower mouth because of the head hydrodynamic body – the rostrum [5]. Some fish have mouth which is able to push, forming a tube, through the nomination of premaxillary bones (bream, carp). Mouth of hagfishes and lampreys due to parasitic or hemiparasitic way of power is armed with horny "teeth" and has a funnel shape (sucking mouth).

Location of the eyes of fish depends on the environment. Eyes of bottom fish are at the top of the head or above the midline of the body. Eyes of pelagic species are on the sides of the head, at about the axis along the body [5, 18-22].

Head of fish ends with gill slits or holes, the amount of which may vary: in lampreys – 7 pairs; in hagfishes 1 to 15 pairs on each side; sharks – from 5 to 7 pairs; in chimeras – 4 pairs of gill openings covered with folds of skin; all bony fishes – 1 pair closed by the gill cover.

Shapes of fish body. Fish are adapted to living conditions mainly by form of the body. Most of them are streamlined: sharp head from which body gradually thickens, reaches its greatest thickness in the middle and tapers to the tail. Thereby fish body crashes into the water column with less stress (Fig. 2).

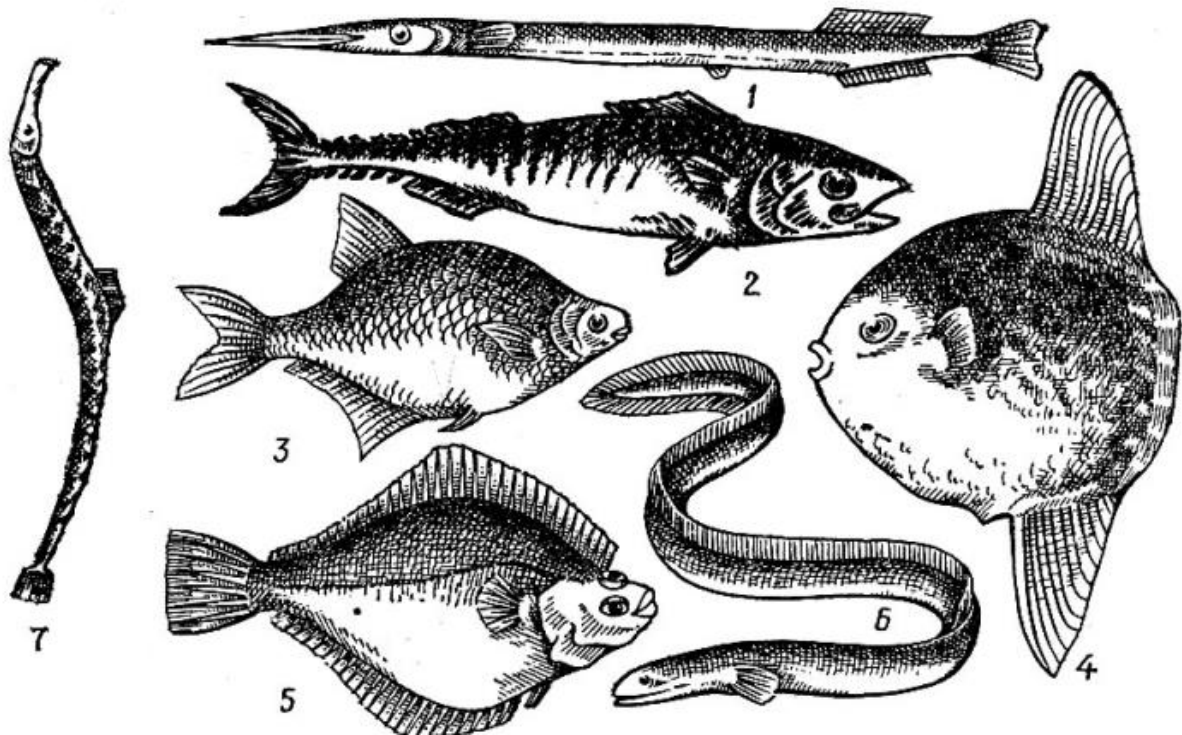


Figure 2A. Shapes of fish body: 1 – sagittal (garfish, pike); 2 – torpedo shaped (mackerel); 3 – bream shaped (bream); 4 – symmetrically flattened laterally (ocean sunfish); 5 – asymmetrically flattened laterally (flounder); 6 – eel shaped (eels, lampreys).

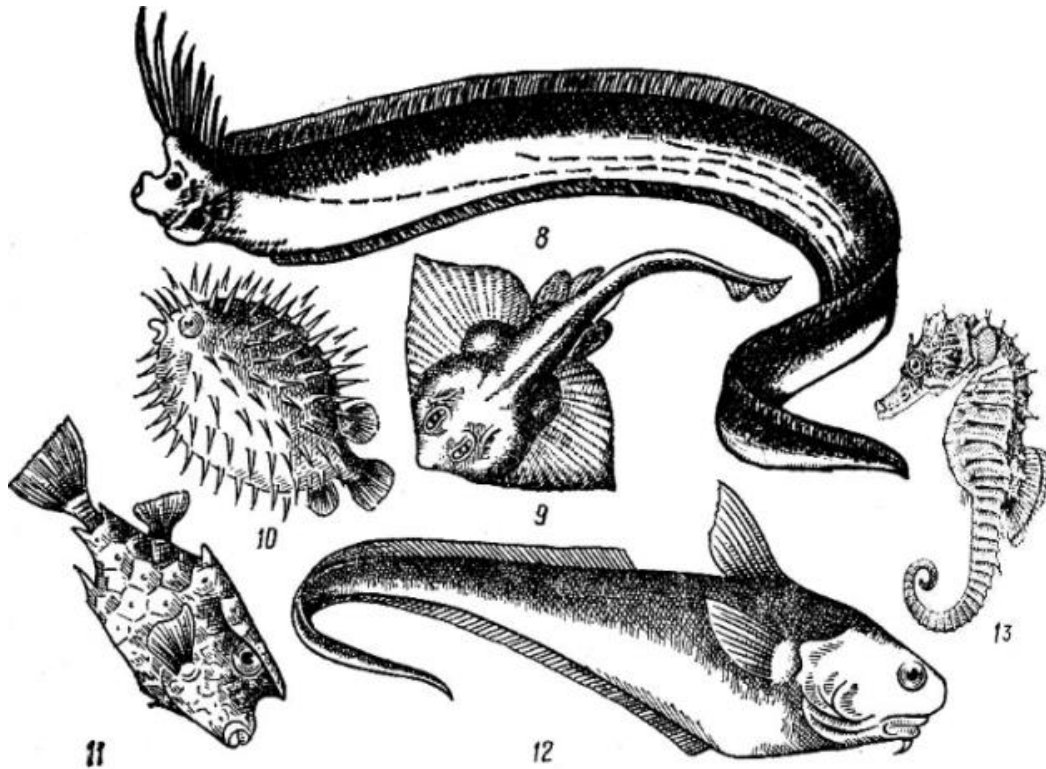


Figure 2B. Shapes of fish body: 7 – needle shaped (pipe- fish); 8 – tape-like (king herring); 9 – flattened in the spinal ventral direction (skate, monkfish); 10 – spherical (fish ball, fish-urchin); 11-cube-shaped (yellow boxfish); 12 –makrurus shaped (makrurus); 13 – unusual (sea horse).

Progress of work

1. To examine carefully the collection of fish and ungroup them by the shape of the body. Pay attention to the location and number of gill openings, the size and placement of the eyes.
2. Make a schematic drawing of fish and note all of the body parts.
3. To determine the boundaries of all body departments on 2–3 species of fish.
4. Select the forms of the body, most commonly found in fish of our waters.
5. Show the types of fish heads with various location of mouth.

Questions for individual work

1. List the body shapes of fish that live in the pelagic zone.
2. Name the body shapes of bottom fish.
3. What kind of body shape has pike, garfish?
4. What type of swimming is inherent for lamprey, hagfishes and eel?
5. Which fish have an unusual body shape?
6. What are the cheek, neck, and chin?
7. What is a tail stem?
8. How many pairs of gill openings have lampreys, hagfishes, sharks, chimeras, rays, bony fishes?

Laboratory work № 2

Fins of fish, their designation, structure and function

Objective: To examine the structure and function of fish fins, explore the caudal fin shape, learn to write formulas of fins.

Materials and equipment: A set of fixed fish – 10–20 species. Table: "The types of tail fins" "Location of pelvic fins", "Modifications of fins". Tools: tweezers, dissecting needles, cuvettes.

Basic theoretical information

Fins help fish to balance the body and are involved in the movements. The size, shape, number, position and function of them are various. Fins are divided into paired, which correspond to higher vertebrates' limbs and unpaired.

The paired are: pectoral P (*pinna pectoralis*), abdominal V (*pinna ventralis*). The unpaired include: dorsal D (*pinna dorsalis*) and anal A (*pinna analis*), tail C (*pinna caudalis*). Catfish, salmon, characids, bagridae have fat fin behind the dorsal fin (*pinna adiposa*), which does not have fin rays [6].

The pectoral fins are typical for most fish and are absent in Moray eels and Cyclostomes [7]. Rays have significantly increased pectoral fins and they are the main body of the movement. Flying fish have highly developed pectoral fins, allowing them to hover in the air.

Pelvic fins occupy various positions due to the displacement of the center of gravity caused by the decline in the abdomen and concentration of internal organs in front of the body (Fig. 3).

Abdominal position – pelvic fins are in the middle of the belly (sharks, Clupeiformes, Cypriniformes).

Thoracic position – pelvic fins are shifted to the front of the body (Perciformes).

Jugular position – pelvic fins are ahead of the thoracic and on the throat (cod).

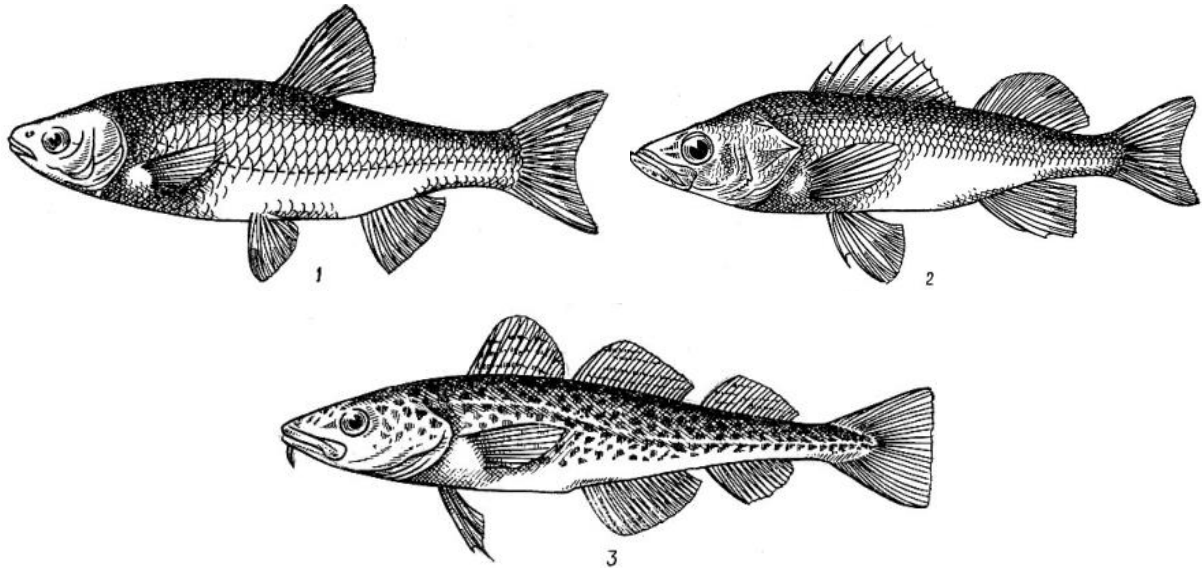


Figure 3. Positions of pelvic fins: 1 – abdominal; 2 – thoracic; 3 – jugular.

Pelvic fins of some species have turned into thorns (three-spined stickleback), some other`s – into a suction cup (pinohor). Rear beams of pelvic fins of males of sharks and rays have turned into copulative organs claspers. Pelvic fins are absent in eels and cyclostomes.

Dorsal fins may be three (Gadiformes), two (Perciformes) or one (Cypriniformes, Clupeiformes). The position of the dorsal fin can be varied: pike`s is shifted back Clupeiformes and Cypriniformes have it in the middle of the body, the fish with a massive front of the body one of the fins is located near the head (perch, cod).

Anal fin acts as keel usually there is one anal fin but codfish has two. Flounder, eel and catfish have well developed in length anal fin and it is used as movement body by fish.

Caudal fin has variety of different buildings.

Depending on the size of the upper and lower blades it is distinguished:

Isocercal type – the upper and lower blades of fin are identical (mackerel, tuna, carp);

Hipocercal type – elongated lower blade of fin (flying fish);

Epicercal type – elongated upper lobe of the caudal fin (shark Acipenseriformes).

Several types are distinguished by the shape and location relatively to the end of the spine:

Protocercal – as a fin fringe (lamprey larvae of some fish) (Fig. 4, 1);

Heterocercal – asymmetric tail fin with considerably elongated upper blade, which comes to the end of the spine (cartilage, sturgeon) (Fig. 4, 2);

Homocercal – externally fin has vane structure, but modified last body of vertebra (urostyl) comes to the top of the blade (bony fish) (Fig. 4, 3);

Diphycercal – tail fin merges with the dorsal and anal (Lungfish) (Fig. 4, 4).

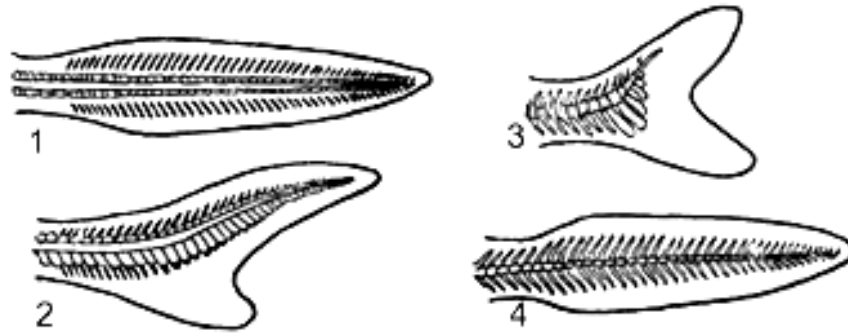


Figure 4. Types of caudal fins off ish: 1 – protocercal; 2 – heterocercal; 3 – homocercal; 4 – diphyccercal.

The bony fish have the following types of tail fins: forcipate (herring), sinuate (salmon), truncated (codfish), round (gobies, burbot), semilunar (tuna, mackerel), sharp (eel-pout). The fastest swimmers are fish with forcipate, sinuate and semilunar tail fin.

The complete absence of caudal fin is rather rare phenomenon, for example, seahorse (Fig. 5).



Figure 5. Seahorses body.

The support of fins is fin rays. There are branched and unbranched rays. The latest can be segmented and able to bend or unsegmented and tough (prickly), which in turn can be smooth or jagged. Solid (tough) and unbranched rays are always at the beginning of fin and branched soft rays are always at the end of the fin.

The shape and the number of rays in the fins, especially dorsal and anal are specific signs and expressed through the formula of fin. Every fin in this formula is signed by the first letter of the Latin name of the fin (see above). The number of unbranched (prickly) rays is indicated by Roman letters, and soft (branched) by Arab. For example, the formula of dorsal fin (pinna dorsalis) of zander looks like D XIII–XV, I–III 19–23 – this means that the fish has two dorsal fins, the first of which has 13–15 prickly rays, and the second has 1–3 prickly rays and 19–23 branched.

Progress of work

1. All species need to be examined and sketched: paired and unpaired fins; branched and unbranched and also segmented and unsegmented fin rays; position of thoracic and abdominal fins.
2. Find fish that: do not have paired fins; have modified fins; with one, two and three dorsal fins.
3. Determine all types and forms of the tail fin.
4. To make the formula of spinal and anal fins for species listed by teacher.

Questions for individual work

1. What fins are attributed to paired and unpaired? What Latin letters are they designated by?
2. Which fish fatty fin is characteristic for?
3. What types of fin rays can be identified? How do they differ?
4. Where are the pelvic fins? What determines their position?
5. Give examples of modifications of fins.
6. What functions do fish fins perform?
7. What types of tail fins are characteristic for fish?

Laboratory work № 3

Morphometric analysis of fish

Objective: To learn the circuit of fish measuring and learn how to conduct morphometric analysis.

Materials and equipment: Set of fresh or fixed fish – 10–15 species. Tools: ruler, dimensional board, calipers, measuring tape, dissecting needles.

Basic theoretical information

Complete morphometric analysis involves the study of a large number of plastic and meristic features, which are recorded in protocols of experiments.

Plastic features are the features that may vary with age of fish or can be influenced by environmental conditions. For example, the length of the head, body, tail stalk, weight, the largest and the smallest height of the body and so on.

Meristic features are species-specific steel signs which are characteristic for some populations and species. They are determined by calculation. For example, the number of scales in the lateral line, the number of gill rakers, rays in the fins, vertebrae, pyloric appendages, etc.).

As an example, the measurement scheme of carp fish (Cyprinidae) and pumpkinseed sunfish (Centrarchidae) fish is considered (Fig. 6, 7).

Plastic features:

ab (L) – *the absolute length of the fish.* The distance from the top of the snout to the vertical lowered from the end of the longer blade of caudal fin straightened in the normal condition;

ac – *length by Smith.* The distance from the front edge of the snout to the end of the middle caudal fin rays;

ad(l) – *industrial or ichthyological length.* The distance from the top of the snout to the end of scaly cover, or to the base of middle caudal fin rays if fish has naked body;

od (lcor) – *trunk length*. The distance from the most remote point of the gill cover to the base of middle caudal fin rays.

an (lr) – *snout length*. It is measured from the top of the snout to the front edge of the eye;

np(do) – *eye diameter*. The horizontal diameter of the eye is typically determined;

po (po) – *behindeye distance*. The distance from the rear edge of the eye to the most remote point of the gill cover;

ao (lc) – *the length of the head*. The distance from the top of the snout to the most remote point of the gill cover;

lm – *the height of the head near the nape*. It is measured as a vertical length held by the rear edge of the eye;

gh (H) – *the maximum height of the body*. The vertical distance from the highest point of the back to the belly (the height of the fins is not accounted);

ik (h) – *the lowest height of the body or height of the tail stem*. It is measured as well the distance between the nearest points of the dorsal and ventral edges of the tail stalk;

aq (aD) – *anterdorsal distance*. Distance in a straight line from the top of the snout to the front edge of the dorsal fin bases;

sd (pD) – *postdorsal distance*. It is measured from a vertical, down from the end of the dorsal fin to conventional axle of body, by the end of scaly cover (or base of the caudal fin);

y₁d (pl) – *length of the tail stem*. The distance from the vertical edge of the rear bases of the anal fin to the conditional axis of the body by the end of scaly cover

qs (ID) – *length of dorsal fin D*. It is measured from the base of the first ray to the base of the last;

tu (hD) – *maximum height of dorsal fin D*. The height of the biggest ray of this fin is measured;

yy₁ (IA) – *length of basis of anal fin A*. The distance from the base of the first to the base and the last ray;

ej(hD) – *maximum height of anal fin A*. The length of the biggest ray of this fin is measured;

vx (IP) – *length of pectoral fin P*. Distance from the anterior point of basis of fin to the farthest point of its blade;

zz_1 (**IV**) – *length of pelvic fin V*. The distance from the anterior point of basis of ventral fin to its farthest point;

vz (**PV**) – *pectoventral distance*. It is measured the distance from the anterior point of bases pectoral fins to the anterior point of bases of ventral fin;

zy (**VA**) – *ventroanal distance*. It is measured the distance from the anterior point of basis of ventral fin to the front point of the anal fin basis

Meristic features:

ll – *the number of scales in the lateral line*. The number of scales permeated by lateral line canals is counted;

D – *the number of rays in the dorsal fin*. Branched and unbranched rays are counted separately. Content of dorsal fin formula, for example, might be: D III 9 (where, III – number of unbranched rays 9 – of branched);

A – *the number of rays in the anal fin*. It is determined the same way as in the dorsal fin;

P – *the number of rays in the pectoral fins*. Accounting of rays is conducted with a magnifying glass, as the lower unbranched rays of pectoral fins are very small;

V – *the number of rays in the ventral fin*. Unbranched rays must be separated from each other by dissecting needle.

C – *the number of rays in the caudal fin*. The longest top unbranched ray, the number of branched rays and the longest lower unbranched ray is pointed. For example, C I 12 I.

The most important component of the morphometric analysis of fish is a statistical analysis of the results. The arithmetic mean (M) is determined by dividing the sum of the values of option (x_i) on the number (n): $M = \sum x_i / n$.

Besides the arithmetic mean value (M) the standard deviation (σ), the average error of measurement ($\pm m$) and the differences coefficient of Mayr (CD) is calculated. To compare the variability of different traits relative measure of variation or the coefficient of variation is calculated (CV): $CV = \sigma / M \times 100\%$. If the value of the received CV is less than 20%, the sample can be considered as representative in case of large values of CV more researches are conducted. The reality of differences of morphometric parameters of various fish samples are determined by Student t-test: $t = (M_1 - M_2) / \sqrt{(m_1 + m_2)}$, where M_1 , M_2 are the arithmetic mean and the m_1 , m_2 are the average errors of two samples.

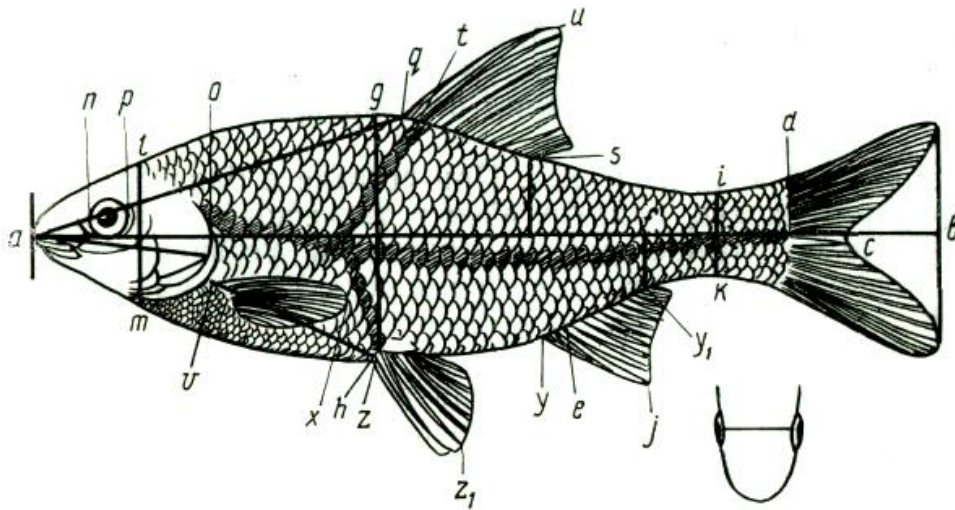


Figure 6. Scheme of measurement of main features of carp fish (Cyprinidae) on the example of common roach (*Rutilus rutilus*). Shaded rows explain calculation of the scales in the lateral line, above and below it. Under the main picture it is shown the measuring of width of forehead.

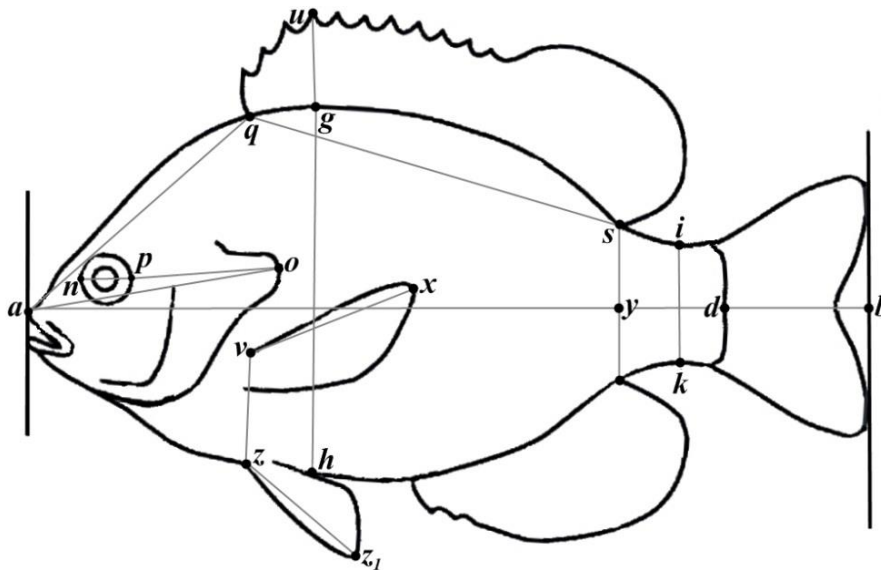


Figure 7. Scheme of measurements of the pumpkinseed sunfish: ab (L) – total body length; ad (l) – body length to the end of the scale cover; gh (H) – maximum body depth; ik (h) – minimum body depth; l caud – length of caudal peduncle; lv – length of ventral fin; aq (aD) – antedorsal distance; sd (pD) – postdorsal distance; qs (ID) – length of dorsal fin base; gu (hD) – height of dorsal fin at the level of the fourth unbranched ray; vz (PV) – distance between pectoral fin base and ventral fin base; vx (IP) – length of pectoral fin base; ao (lc) – length of head; np (do) – horizontal diameter of eye; po (po) – postorbital distance [8].

Progress of work

1. To examine carefully the external structure of the fish.
2. Place the fish on the right side of the measuring board. Head of fish should lie to the side of the mechanical measuring boards and snout should touch zero.
3. The work requires two performers: one holds dimensions other registers data. All linear dimensions are indicated in millimeters. Morphometric analysis of small fish, as well as its individual parts (diameter of the eye, body height, the length of the snout) should be conducted using calipers. During the measurements it is necessary to observe the correct position of the fish, do not squeeze it with hand or instrument.
4. Conduct a statistical analysis of the results.
5. To make a special table for each type of fish (Table 1):

Table 1. Morphometric analysis of fish

Species of fish							
№	Feature	№ sample			Statistical figures		
		1	2	3	$M \pm m$	σ	CV
Plastic features::							
1.	<i>ab (L)</i>						
2.	<i>ac</i>						
3.	<i>ad(l)</i>						
4.	<i>od (lcor)</i>						
...
22.	<i>zy(VA)</i>						
Meristic features							
1.	<i>l.l.</i>						
2.	<i>D</i>						
...
6.	<i>C</i>						

Questions for individual work

1. What parameters of the fish are necessary to be measured?
2. Which method is carried out in morphometric study?
3. How many fish should be investigated? Which part?
4. What is plastic and meristic features of fish?
5. How the statistical study of material should be conducted?

Laboratory work № 4

External covers of fish, the lateral line, determining the age of fish by scales

Objective: To explore the variety of external covers of fish, learn how to determine the formula of lateral line and learn the technique for determining the age of fish scales.

Materials and equipment: A set of fixed fish – 10–20 species. Preparations: scales of different species of fish. Table: "The structure of different types of fish scales", "The structure of the lateral line of fish". Photos of scales of different species of fish. Tools: binocular microscope, glass, cuvettes, tweezers, dissecting needles.

Basic theoretical information

The scales of fish. Body of majority of fish is covered with scales. Small scales appear on the body of the young fish when it is on the transition from the stage of the late larvae to the stage early fry. Number of scales does not change, but their size increases with age. By building of scales it can be determined not only duration of the life of fish, but the rate of growth for each year or the transition to the spawning herd.

There are the following types of scales: placoid, ganoid, cosmoid and bone (cycloid and ctenoid) (Fig. 7).

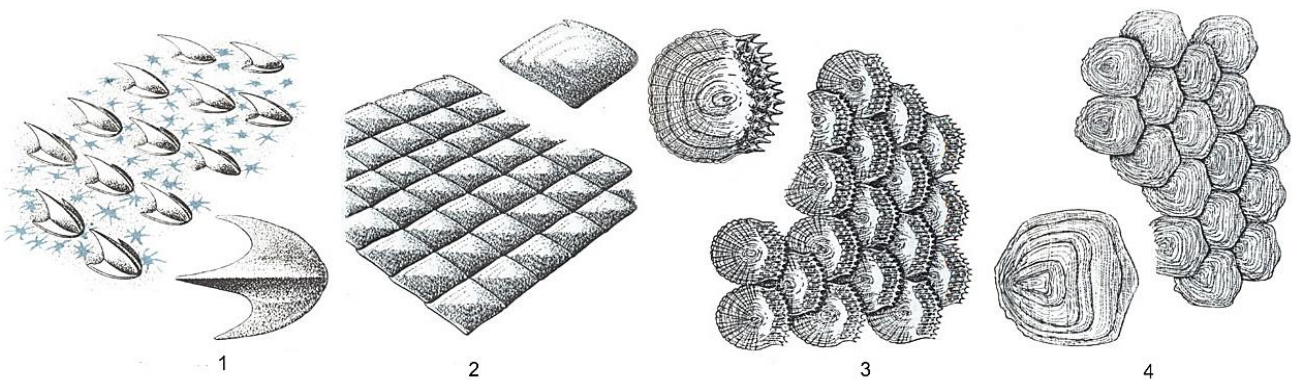


Figure 7. Different types of fish scales: 1 – placoid scales; 2 – ganoid scales; 3 – cycloid scales; 4 – ctenoid scales.

Placoid scales are characteristic for the sharks and stingrays. It consists of rhombic plates, which occur in the corium, and odontoid outgrowth, which reaches the surface of the body and directed to the rear end of the body of fish. It contains three layers: vitrodentin, dentin and pulp.

Ganoid scales have rhombic shape and a side ledge in a shape of tooth with which scales are interconnected, forming a kind of armor. It has three layers: ganoine, cosmine, isopedin. It occurs at the base of the upper lobe of caudal fin of sturgeon fish, gar pike and polypterus.

Cosmoid scales have rounded shape, it has no ganoine layer. It is typical for crossopterygian fish.

Bone scales were formed as a result of the simplification of ganoid, layers of ganoine and cosmine have disappeared only bone basis has remained. There are two types of scales, with a smooth rear edge – cycloid (carps, Clupeiformes et al.), and with serrated surface on the edge of scleritis – ctenoid (Perciformes, flatfish).

Scales on the body of fish are contained in a special leather pocket, from which only part of it peeps out. These pockets are clearly visible on the body of carp fish, when the scales are removed.

If the scales are observed with optical devices, the lines, each of which forms a ring can be seen. These rings are called scleritis. Their size increases with distance from the center of the scale, and outlines of each ring almost correspond to edge outlines of the scales. Rings are located less and thicker, creating wider and narrower areas. During the year, one broad zone of scleritis is usually formed (summer) and one narrow zone (autumn and winter), and the amount of dual zone corresponds to the age of the fish.

The fish grow unevenly during the year, which affects the growth scales. It increases due to the emergence of young scales with bigger size, which grow under the old. Thus, scales becomes thicker every year. It consists of the accrete flakes, the top of which is the smallest and oldest, and bottom one is the largest and youngest.

Lateral line (*linea lateralis – ll.*). The majority of fish has lateral line on each side of body, which is the kind of fish seism sensory sense organ that is able to accept low-frequency vibrations of water. It is a subcutaneous canal, which is covered with reniform sensitive epithelial cells which are connected with nerve endings. The canal is connected with the external environment by holes that penetrate the scales or skin of the fish body.

The lateral line has essential systemic importance. Its appearance is quite diverse. In some fish it runs along the sides of the body in the form of a straight line from the head to the base of the caudal fin (carp, perch, bream, etc.), in others it is intermittent (smelt) or have

bends over pectoral fins (sabrefish). Herring, gobies and some other fish have no lateral line, and the system of developed sensory canals on the head performs its functions.

Characteristics of lateral line are recorded using formula. To write the formula the number of lateral line scales along the lateral line, above and below it are counted. So the formula of lateral line ofide looks like $l.l. = 56 \frac{8-9}{4-5} 61$, that means: 56 is the lowest number of scales along the lateral linefor the species, 61 - the largest number of scales along the lateral line, 8-9 – the number of scales on the lateral line to the base of the dorsal fin, 4-5 – the number of scales in the lateral line to the base of pelvic fins. counting above and below the lateral line can not be always carried out accurately, so sometimes it is limited to only counting scales along the lateral line. Then the formula of lateral line ofideis following: $l.l. = 56-61$.

Progress of work

1. All fish should be divided into species and numbered with labels that are put in the gill slits.
2. Prepare sample books for scales of different species of fish.
3. Thoroughly clean the fish from dirt, slime and scales of other fish species.
4. Measure the fish – industrial and the absolute length should be determined. Small fish (up to 50 cm) should be measured to within 1 mm, and large (over 50 cm) – up to 0.5 cm.
5. To weigh each species of fish. Fish weighing more than 250 grams weighed to within 2-3 g, 40 to 250 g – 1 g, less weight – 0.5 g
6. To determine the right place for selection of scales for each species. Examine the scales of fish, to specify what type is it to determine the position of the lateral line.
7. Record formula of lateral line of fish, chosen by teacher.
8. To conduct a sampling of scales from the middle of body length of fish or above its lateral line using the scalpel or forceps.
9. After sampling wipe it of mucus (rinse with water and clean with a soft brush or wash in weak solution of ammonia), or put it to the scales book for further processing.
10. To dry scales, then put between two slides for its determination under the microscope. If scales are large dandruff can be used or the age rings can be determined with the naked eye.

11. To record the results into the Table 2:

Table 2. Biological parameters of fish

№	Fish species	Weight, g	Length, cm		Lateral line formula, <i>l.l.</i>	Age, years
			<i>L</i>	<i>l</i>		
1.						
2.						
3.						

Questions for individual work

1. What types of scales do fish have?
2. What fish are ganoid scales characteristic for?
3. What are the types of bone scales? How do they differ?
4. How does the growth of scales occur?
5. What is the lateral line, its function and structure?
6. How is lateral line formula determined?

Laboratory work № 5

Manufacturing of preparations from scales and determination of the growth rate of fish

Objective: Learn to make preparations from fish scales and master the method of determining of E. Lea about growth rate of fish of different age groups.

Materials and equipment: A set of fixed or fresh fish – 10–20 species. Preparations: scales of different species of fish. Table: "The growth and size of the fish." Tools: scalpel, forceps, dissecting needles, soft brush, microscope, magnifying glass of 10–20 increase, ruler, calipers, ocular micrometer.

Basic theoretical information

Manufacturing of preparations from scales. Selected samples of scales often need to be kept for long time, so permanent preparations should be made.

Selected fish scales are put for 1–10 minutes in a weak solution of ammonia. After this they are washed using detergent. To remove mucus and epidermal pellicle scales are wiped with a soft cloth.

The scales should be observed under a microscope or with a slight increase under binocular. It is selected 5–8 scales with the correct form of the intact edges, 3–4 of which, with most distinct annual rings are separated for survey.

Preparation № _____
Zander, № 400, 26.05.17.,
Zaporozhian Reservoir,
Viyskove village
48°22'30.75" N; 35°20'80.05" E
<i>L</i> – 34,0 cm, <i>l</i> – 28,0 cm, <i>m</i> = 350 g

Figure 8. The example of label for preparation of scales.

Then wet scales are placed between the two lenses of subject that are fixed quiescently. Labels which indicate the following information: the number of preparation, name and number of fish according to biological journal, date, place of collecting, station of selection, absolute length (L), industrial length (l) and weight of fish (m) are made on preparations (Fig. 8.).

The logbook is prepared for preparations, where numbers of preparations will be recorded. Thereafter, the finished product is stored in boxes.

Determining of the growth rate of fish. Growth of fish is defined as increasing of its weight and linear body sizes. Fish grow throughout life, but uneven: at a younger age, they grow faster. It is known that during puberty, migration, wintering or spawning their growth slows or even stops. The presence of age rings on scales allows determining the relationship between growth of fish scales and body (Fig. 9).

Analysis of growth allows making important economic conclusions about the profitability of growth and the maximum allowable age of fish in terms of the most rapid and fulling use of food resources of reservoirs.

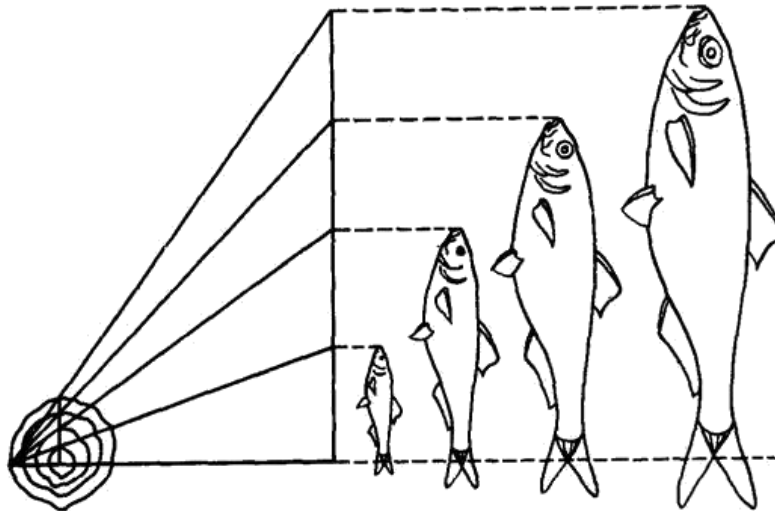


Figure 9. The ratio between the growth rate of the fish body and its scales (by Nikolskiy, 1963).

In his studies about age and growth E. Lev concluded that fish length and length of scales vary with age directly proportional in relation to each other:

$$\frac{L}{C} = \frac{l_n}{c_x}, \quad \text{whence} \quad l_n = \frac{L}{C} \cdot c_x$$

where L is fish length, mm; C is length of scales (from the center to the edge of the part in which age rings are determined); l_n is fish length at different ages, mm; c_x is length of scales in different years (from the center of the scales).

Knowing length of fish in the moment of its catch and in all the previous years, its annual growth can be determined:

$$t_1 = l_1; t_2 = l_2 - l_1; t_3 = l_3 - l_2 \dots$$

Progress of work

1. Determine the absolute length of each specimen.
2. To select fish scales and determine age.
3. Manufacture a preparation of scales.
4. Use calipers or eyepiece micrometer measure the length of scales from the center to the edge of each age ring.
5. to calculate a linear increase in years of life for each specimen using E. Lea formula, record the data into a Table 3:

Table 3. The growth rate of fish

№	Species	L , cm	Age	Linear increase ($t_1; t_2; t_3 \dots$), cm
1.				
2.				
3.				

Questions for individual work

1. How are preparations from fish scales made?
2. What is the growth increase in fish?
3. How does the growth of fish scales and body occur?
4. What is the growth rate of fish?
5. List the age groups of fish, number of rings on the scales and designations of ages.

Laboratory work № 6

Working with the determinants of fish

Objective: Learn to use determinants of fish and identify fish species.

Materials and equipment: Set for determination: fixed preparations of various groups of cyclostomes and fish; cuvettes, dissecting needles, tweezers, magnifier (4–6-x); determinants of fish.

Basic theoretical information

Defining tables are compiled on the basis of the fundamentals (abstract) and the counter fundamentals (antithesis). There are numbers such as 1 (3) in front of each thesis and antithesis, the first of which is the thesis which is given without brackets, and the second is the antithesis is in parentheses. Definition comes to comparing characteristics that are resulted in the thesis and antithesis, and to the subsequent decision which of them corresponds to features of determined fish. If thesis or antithesis is suitable, but they do not have the title of taxonomic categories, the following serial number of theses should be read its contents should be compared with the contents of its antithesis. So, moving step by step, the title of determined taxonomic category should be reached. Using the table, it can be determined which class examined representative belongs to.

1 (2) Mouth has as a shape of sucking funnel or round, surrounded by antennae, jaws are absent. One nasal opening. Pair fins and their belts are absent. Body is eel-shaped and naked... Class cyclostomes *Cyclostomata*.

2 (1) Presence of jaws. Nasal opening is paired. There are paired fins and their belts.

3 (4) No skeletal gill covers. The body is covered with placoid scales or naked. Males have claspers in the pelvic fins... Class Chondrichthyes *Chondrichthyes*.

4 (3) Bony gill covers. The body is covered with bony or ganoid scales, sometimes it is naked... Class bony fishes *Osteichthyes*.

After defining the class of representative in this table, it is necessary to start work with determinants. After the determination Latin name of every taxonomic category, which the present representative belongs to and main features of the family is written to notebooks.

Progress of work

1. To examine carefully the collection of fish, to define gradually class family, genus and species of fish by determinants,.
2. Jot down main features of the family, whose members have been identified.
3. Record Latin names of studied fish.

Questions for individual work

1. What for are the determinants of fish used? How to use them properly?
2. What are the main features for determination the fish class, family, genus and species?
3. Which taxonomic groups include representatives of native fish fauna of reservoirs of the region?

Laboratory work № 7

Anatomical structure of bony fishes

Objective: Learn to make anatomical dissection and familiarize with the structure of the internal organs of fish.

Materials and equipment: Fresh fish – 5–8 species (preferably pike, perch, carp), wet preparations. Tables: "The internal structure of the fish", "Fish musculature", "The circulatory system of fish", "The respiratory system of fish", "The digestive system of fish", "The nervous system of fish", "The excretory system of fish", "The reproductive organs of fish." Tools: scissors, scalpel, dissecting needles, ruler, cuvettes, Petri dishes, tweezers.

Basic theoretical information

All of the internal organs of fish are in the body cavity coelom. Coelom is divided into two unequal parts by thin transverse membrane, which is located near the pectoral girdle; these parts are small anterior pericardial bag that includes heart and back abdominal one, where all internal organs are located (Fig. 10.).

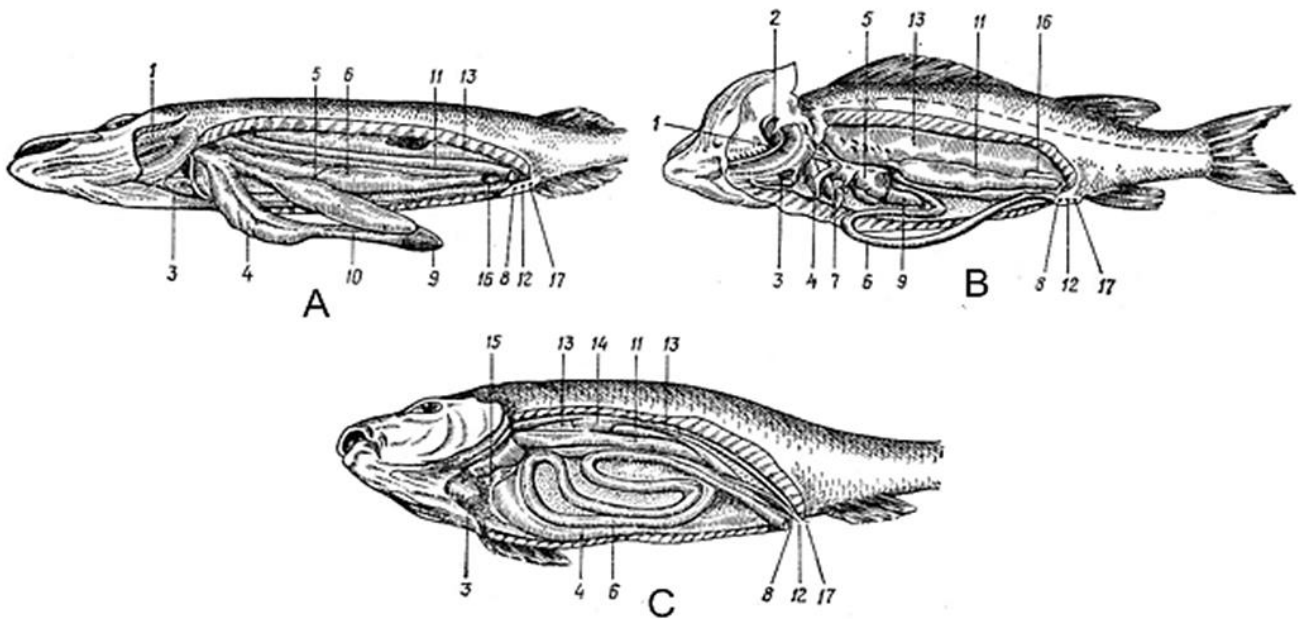


Figure 10. The internal structure of the fish (A – pike, B – perch, C – carp): 1 – gills; 2 – gill moiety; 3 – heart; 4 – liver; 5 – stomach; 6 – intestines; 7 – pyloric appendages; 8 – anus; 9 – spleen; 10 – pancreas; 11 v gonads; 12 – genital opening; 13 – swim bladder; 14 – kidney; 15 – the main kidney; 16 – bladder; 17 – urinary opening.

The muscular system of fish is divided into the somatic (body muscles) and visceral (muscles of internal organs). The somatic musculature consists of skeletal striated muscle comprising the muscle segments – myomeres separated by connective membranes – myosepts. This segmentation is typical for trunk and tail sections. Musculature of fins is more differentiated, so that fish can carry out complex movements. Significant development has muscular system of the respiratory system and gill covers. Visceral musculature of fish is represented by smooth muscles.

The respiratory system of fish is represented by gills, which include gill lobes located on the gill arches [9]. Gills develop from ectoderm and are derived from the skin. On each side the bony fish have four full gills, and one half gill (fifth gill arch is devoid of gill petals). At the outer end of the gills gill lobes are placed in two rows that are covered by transverse, thin pleats – petals that increase the contact area with water, i.e. respiratory surface. On the inside each branchial arch has gill stamens that form a kind of filter apparatus.

The digestive system is represented by the digestive tract and digestive glands. It begins with oral cavity. The teeth of the fish are not only in the jaws, but on other bones of the mouth and even on the tongue. Next is the pharynx, which goes into the esophagus, and into the stomach (most fish). The stomach can have a different number of pyloric appendages (salmon has multiple, perch has three). The intestine is behind stomach. A large liver with gall bladder is under the stomach. The pancreas is located along the gallbladder. In place of transition of the stomach into the duodenum there is a compact spleen. A specific hydrostatic body of fish (swim bladder) is located under kidneys and above intestines.

In the posterior part of fauces cavity there are pharyngeal teeth. Perch, burbot and pike have the upper and lower pharyngeal teeth. Carp fish have no upper teeth and lower are developed quite well, solid food is crushed by them. The function of the upper pharyngeal teeth has a solid horny formation – *millstone*. The number and structure of pharyngeal teeth is important systematic feature of the carp family.

Circulatory system of fish is closed and consists of the heart and blood vessels system. Two-chamber heart is located at the bottom of the body cavity. It includes the following sections: venous sinus, atrium, and ventricle. Unlike cartilaginous fish bony fish do not have a fourth department called arterial cone. Directly from ventricle large vessel departs – abdominal aorta, which has some expansion in the start – aortic bulb. Blood in the heart of fish is venous only.

Oxygen-rich blood is collected in remote gill arteries flowing into the roots of the dorsal aorta. Dorsal aorta divides into numerous arterial vessels through which blood flows to all internal organs. The main department of the aortic root forms the main arterial circle.

Venous blood from the tail section moves in odd tail vein, which is divided into two and passes through the kidneys. After it blood goes forward by the back cardinal veins, at heart level they merge with the front one, which carries blood from the head. As a result of the merger paired Cuvier`s ducts are formed that fall into the venous sinus. Blood from the intestinal flows through the portal vein to the liver and there it forms a portal system. Blood from the liver gets into the venous sinus by the hepatic vein. Bony fishes have one closed circle of circulation.

Hematopoiesis organs of bony fishes are the spleen, which is located in one of the bends of the intestine and is maroon, kidney, thymus, gill apparatus, blood vessel walls.

The nervous system of bony fishes is divided into central, peripheral and autonomic. Central nervous system is clearly differentiated into brain and spinal cord. The brain has five sections which are front, intermediate, middle, back and the medulla oblongata. Compared with the nervous system of cartilaginous fish, bony fish can be characterized by a smaller size of the forebrain, the lack of nervous substances in its cover and separation of the front brain cavity by the longitudinal partition. Most of the forebrain consists of the striatum. On top of the intermediate brain goes pineal gland at the bottom of an intermediate brain there is a funnel, which is connected to the pituitary gland. The average brain compared to other more developed departments. Cerebellum is large.

The spinal cord has segmented structure; the number of segments corresponds to the number of vertebrae. Two dorsal nerves that connect and branch out into three branches - dorsal, ventral and internal branch out from the both sides of each segment.

The autonomic system includes two beds of ganglia which are associated with spinal cord and brain, as well as with each other. It runs along the spinal cord and enters the brain, innervating internal organs of fish.

The peripheral nervous system is represented by nerves and nerve endings that are primarily associated with the senses of the skin.

Bony fish are able to distinguish the smell and taste, hear, see, perceive temperature and fluctuations in the environment.

Excretory organs are paired and ribbon-shaped *mesonephric* (truncal) kidneys that extend on either side of the spine above the bladder. Front slightly enlarged ends form the main kidney, which is well expressed in perch and carp. In the back part right and left kidneys merge. On the inside part of the kidneys there are ureters that are homologues of *Wolff ducts*. Ureters come out from kidneys and merge into unpaired duct, which opens by separate hole near the genital opening.

Reproductive organs of fish have a number of features. Males have the testes, females have ovaries and they are located on either side of the swim bladder. The degree of development depends on the season and the age of the fish. *Testes* are long paired organs on the upper edge of which there are spermaducts that open out by small genital opening. Ovaries are paired in the most fish (perch has unpaired). Elongated rear parts of the ovaries go into the output channel that opens by independent unpaired genital opening. Fertilization of most fish is external.

Progress of work

1. Make anatomical dissection of fish. Abdominal cavity should be cut the three sections. At first the wall of the abdomen just above and in front of the anus should be pierced by scalpel. Insert blunt end of scissors in the puncture and make the first cut, which should be along the abdomen forward along its midline and terminate on the basis of the pectoral fins. Continue to cut the abdominal wall and back to the front edge of the anal fin. It should not touch the hole on the left side of the body wall. Then completely remove the left wall of the body cavity. The third section should be made along the back edge of the belt of pectoral and abdominal fins.

2. After removing the wall of the abdominal cavity and the gill cover to examine the overall placement of internal organs.

3. For examination of digestive system cut all the ripples that connect parts of the digestive tract between them.

4. Make a scratch of gills, indicating their elements, and digestive tract, indicating its departments and depicts circuit of circulation.

Questions for individual work

1. What are pharyngeal teeth and millstone?
2. Tell the structure of the gill apparatus of fish.
3. What are the parts of the digestive system of fish?
4. Which species do have stomach and which do not? How does this relate to the nutrition of fish?
5. Which fish do have open bladder and which do have close bladder?
6. Tell the structure of the heart and circulatory system of fish.
7. What are the organs of fish that may perform hematopoietic function?
8. Reproduction organs of fish. Which fish have paired ovaries?
9. What are the parts in the brain of fish? What are their functions?

Laboratory work № 8

Definition of fertility and level of maturity of sexual products

Objective: Learn how to determine the maturity of gonads, calculate absolute and relative fecundity of fish.

Materials and equipment: fixed or fresh fish – 5–8 species. Tables: "Reproduction organs of fish." Tools: scissors, scalpel, dissecting needles, ruler, cuvettes, Petri dishes, tweezers, filter paper, foil, microscope, eyepiece micrometer, torsion, pharmaceutical and technical scales.

Basic theoretical information

Materials for determining the fertility of fish are taken simultaneously with sampling for establishing the age and growth. Numbers of samples for determination the age and fertility should be kept as all other data records and labels. However, the number of taken samples can be much less because of that numbers can gain spaces. After measurements and taking scales fish is dissected to determine the sex and sampling to establish fertility.

To determine the fertility of fish roe samples are taken in the middle section of roe in the fourth stage of maturity. Caviar is weighed on pharmaceutical scales and fixed in alcohol with 2% formalin solution (1:1). For fish that have large eggs (pike, catfish), sample weight should be 2–5 g, and for fish with fine caviar it should be 1 g. Sample is accompanied by a foil label, which indicates the date, place of catch, species, serial number fish in the journal, body length, total weight, weight of roe, the number of taken spawn.

Absolute fecundity of fish directly depends on the length of the body, so for its definition material must be collected at least 5 samples from individuals of each size class - bream, roach, silver bream, carp, tench, perch, sabrefish, rudd [10, 11]. For such fish as carp, pike, perch, herbivorous fish class interval of 3 cm is enough.

To determine the maturity of sexual products of fish, it is necessary to use a following universal scale:

Stage I – juvenile [12]. Immature individuals, poorly developed gonads, look like thin transparent strands, sex cannot be determined with the naked eye.

Stage II. Individuals that mature or individuals with sexual products that develop after spawning. Sex glands are small. The eggs are so small that they are invisible. However, the sex can be determined. There is quite thick blood vessel along the ovaries. Milt is transparent.

Stage III. The gonads are relatively well developed. Ovaries occupy from 1/3 to 1/2 of volume of abdomen and filled with small transparent eggs of different colors. The eggs are hardly separated from the internal partitions. Testes are tight and taut their surface is pink. After the cut their edges do not deliquesce and stay sharp.

Stage IV. Eggs and sperm have almost reached full development. The eggs are large enough and can be easily separated one from another. Color of ovarian of fish varies. Milt is milk-white, easily flows after pressing.

Stage V. Fluent individuals. Eggs and sperm are so mature that after the light pressing on the abdomen they freely flow from the genital opening. Volume of the gonads from the beginning of spawning to the end is rapidly declining. Stage is brief.

Stage VI. Slaughter. These are individuals that have spawned. Sex glands are small, puffy, swollen and full of blood. The eggs remaining in the glands dissolve. After a few days the inflammation goes down and gonads pass in the second stage of development.

If sex products are in the intermediate stage and it is difficult to identify, the designation consists of two numbers connected by a dash, but the stage to which the development of sexual products is closer, is put at the beginning.

Individual absolute fertility (IAF) is the number of mature eggs in the ovaries of a fish. It is calculated by weighting method:

$$IAF = g \times n$$

where: g – is ovarian mass, g & n – is a number of eggs in 1 g of sample.

Absolute fertility (AF) is an average number of eggs in females of certain group of fish (dimensional, age) for the spawning season.

The relative fertility of fish (RF) is the number of eggs per unit of weight or per unit of body length of fish. It is calculated by the formula:

$$RF = \frac{AF}{m}$$

where: AF – is absolute fertility; m – is fish mass, (g).

Working (physiological) fertility (WF) is the number of eggs that really laid by the female per spawning season (in fish culture it is measured by the number of eggs in 1 g or 1 cm³ of sample for a particular female). Other indicators of fertility are derived from absolute.

Progress of work

1. The fish is distributed by species and each individual is numbered.
2. Weigh and determine the absolute length.
3. Determine the age of the fish.
4. Make dissection of abdomen to determine the sex of individuals and maturity of sexual products by the six-point scale.
5. Remove the ovaries of females, weigh and select sample of 1 g to count eggs. If eggs are large enough samples may be 3–5 g. If it is necessary to fix eggs they are tied in cheesecloth with a label and placed in a bank with 2% formalin.
6. The eggs are divided by dissecting needles and counted in the Petri dish with a dark bottom. Number of eggs in sample is recorded and 20 eggs are measured in diameter. To determine the mass of raw eggs 100 eggs are dried and weighed using torsion balance accurate to 0.0005 g. For fish that spawn in portions a part of first sample is separated and then 1/2 – 1/5 part from it is taken for measurement.
7. When using the microscope eyepiece micrometer diameter of 200 eggs is measured, the other are counted, but no more than 500–1000 pieces. Caviar remaining after counting of 1000 pcs., is dried, weighed and counted by weight (the average weight of eggs).
8. Determine the absolute and relative fertility of each of the studied females. The data is pointed into a Table 4:

Table 4. Fish fertility

№	Species	Sex	Fish mass, g	Length, sm	Ovarian mass, g	Number of eggs in 1 g, pcs	Fertility	
							AF, thous. pcs.	RF, pcs./g
1.								
2.								
3.								

Questions for individual work

1. Provide a description of each stage of maturity of sexual products of fish.
2. What is the absolute fertility of fish?
3. What is the relative fertility of fish?
4. What is the population fertility of fish?
5. How does the fish fertility change with the age?
6. Which factors can be associated with increase in fertility of fish?

Laboratory work № 9

The study of fish nourishment

Objective: Learn basic methods of studying of fish nutrition, learn to select and analyze the sample material.

Materials and Equipment: Fresh fish – 5–8 species. Tools: scissors, scalpel, dissecting needles, ruler, cuvettes, Petri dishes, tweezers, filter paper, thick thread, microscope, torsion, pharmaceutical and technical scales, formalin.

Basic theoretical information

The study of fish nutrition is one of the problems of determination of patterns of stocks and catches of fish [13, 14]. Knowing the features of fish nutrition is used in the research of conditions of fish foraging, processes of acclimatization of new species, determination the causes of fluctuations in numbers, in studying of fish growth, in the process of determining the optimal fishing effort and the development of measures for the most efficient use of feed resources of reservoirs.

There are two methods of collecting and processing materials on studying of nutrition: *individual* and *group*.

In case of individual collecting each fish is analyzed separately in case of group collecting all tracts are collected from a group of fish, and their content is treated as one.

Material is collected by means of active gear (scraper, seines, trawls) which do not stay in the water for a long time. The sample for tests on nutrition should consist of 10-100 individuals, depending on the method of study and research purposes.

Collecting of samples for the study of young fish nutrition is carried out in bays and shallow parts of reservoirs using whitebait seine with length of 10 m, made of nylon netting №6. There must be at least 25 pcs. of each type of youth in the sample.

Simultaneously with sampling for nutrition study in the same places hydrobiological samples (benthos, plankton and nekton al.) are taken, it is necessary to conduct hydrochemical and hydrological studies. In the study of nutrition of young fish the data on the nature of the habitat (soil, vegetation, etc.), weather conditions is recorded additionally in the log.

To study the nutrition of fish in the places of their catch the collection must be carried out as follows:

1. The caught fish is measured and weighed;
2. The fish up to 20 cm is fixed completely;
3. From the fish longer than 20 cm only intestines should be taken, they are cut off from the esophagus to the anus;
4. Each intestine is wrapped in gauze with label fixed in 4% formaldehyde;
5. Samples must be fixed and stored in big glass banks;

To study the diurnal nutrition of fish samples are taken every 2 hours for 1.5 days.

The degree of filling for each part of gastrointestinal tract is assessed on a scale of Lebedev:

- 0 – empty;
- 1 – single;
- 2 – small filling;
- 3 – middle filling;
- 4 – plenty full stomach or intestine;
- 5 – mass, stretched intestine.

Filling of the gastrointestinal tract is recorded by three-digit number. For example, 321 – filling of esophagus – 3, stomach – 2, intestines – 1.

The extent of digestion is estimated as follows:

- 1 – organisms are well preserved;
- 2 – slightly digested organisms, species identification is possible;
- 3 – half-digested organisms partially damaged, but the definition for the separate parts is possible;
- 4 – organisms digested very heavily damaged, but the definition for the separate parts is possible;
- 5 – completely undefined mass.

The degree of digestion is also determined in each department of tract and recorded in three-digit number.

Within all the methods of digital processing of materials for determination of the nutrition of fish is the most accurate is the method of indexes. *Index of filling of intestines* quantifies the intensity of the nutrition of fish. It is determined by the ratio of the feed clot or its individual components to mass of fish. There are *general indexes of gut filling* (based on the weight of the whole feed clot) and *indexes of gut filling* (based on the weight of the individual feed components). Index of gut filling is expressed as a percentage, but often to prevent them from lodging in the form of fractions, it is accepted to multiply index of 10,000 or express in prodecimylus.

Progress of work

1. Carry out biological analysis of each individual, and define a standard industrial body length, weight and age of the fish.

2. Make the dissection of fish with scissors or a scalpel on the ventral side of the anus to the head. Gastrointestinal tract should be cut from the esophagus to the anus. To tie with a thread the front and rear end of the tract to prevent the loss of food.

3. Determine the weight of fish without internal organs, sex and stage of maturity of sexual products.

4. To assess the degree of filling of gastrointestinal tract with food by Lebedev's six-point scale;

5. Tract should be cut into three parts, the contents of each is move with a scalpel in a Petri dish. Bolus of food is dried by filter paper and weighed.

6. The content of each part is examined under a microscope. The extent of digestion is determined.

7. Determine the index of intestine filling. This requires the mass of gut to be multiplied by 1000, and then divided by the mass of fish. The results are recorded in prodecimylus – ‰ .

8. The results are added to the Table 5:

Table 5. Parameters of fish nourishment

No	Species of fish	Sex, stage of fertility	L, cm	L, cm	Mass, g	Age	Degree of intestine filling	The extent of digestion	The index of filling, $\frac{0}{000}$
1.									
2.									
3.									

Questions for individual work

1. What fish feed on phytoplankton?
2. What do bentophagous feed on?
3. Which method is used for determining of nutrition spectrum of predatory species?
4. How much food should fish consume?
5. What is the index of intestine filling?
6. What changes in the power range of fish occur throughout their lives?

Laboratory work № 10

Manufacturing of fish skeleton

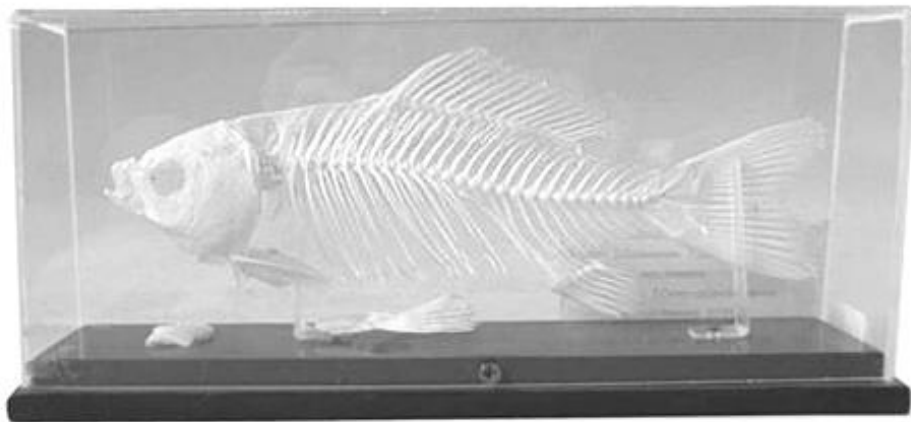
Objective: To learn the method of manufacturing a fish skeleton.

Materials and equipment: Fresh fish with length of 20–30 cm. Tools: scissors, scalpel, dissecting needles, ruler, cuvettes, tweezers, brush, thick thread, wire, plate, gasoline, hydrogen peroxide.

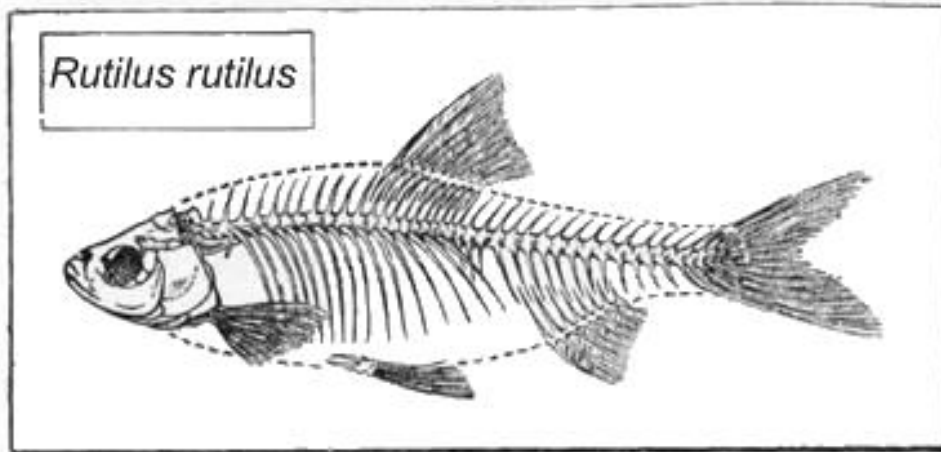
Basic theoretical information

A great exhibit and clearly material for studying the structure of fish is fish skeleton. Moreover its manufacturing is quite easily. There are several variants for placing fish skeleton, complete skeleton upright on a wired rack, half-skeleton on a black background to which it is attached by spine (Fig. 11). The biggest problem in the manufacturing of fish skeleton is that this work is very painstaking and requires a lot of attention, diligence and patience.

For choosing an object for the manufacturing of the skeleton, especially if the work is done for the first time attention must be paid to the species of fish that have a massive skeleton with thick bones. For example, carp fish (roach, carp, silver bream, and bream) have a thick edge and perch (pike, perch), herring (herring) and pike, on the contrary have thin and soft ribs, and work with them is difficult. Fish size also plays a significant role, do not use very large specimens, it is better to use medium-sized individuals it will greatly facilitate the process of making the preparation.



A



B

Figure 11. Variants of placement of fish skeleton. **A** – skeleton in an upright position at the wired rack; **B** – half-skeleton attached to the timber.

Progress of work

1. Wash the fish thoroughly to remove slime and scales. While cleaning fish from the scales it is important not to damage the fins.
2. Make a small incision on the ventral side of the body - in front of the anal fin, insert the edge of the scissors into this incision and cut through the skin of the fish to the pelvic fins. Make incision around the belly fins without cutting them. Bring cut up to the head. Remove any entrails of fish.
3. Remove fins of fish. Firstly remove abdominal fins with triangular bone in which they are held. The dorsal and anal fins need to be removed as follows: make two cuts by scissors on the sides of fins and remove them with their major bones. Cut off the tail fin with the last vertebra. Remove the pectoral fins. The remnants of the muscles should be removed from bases of fins by a brush, for this purpose only bases of fins can be immersed briefly to boiling water. When the fins are cleared of residual muscle, they should be whitened; they need to be put for some time in a solution of hydrogen peroxide. Then rinse them in water, spread and put on a plate, cover with sheet and leave to dry under the pressure.
4. Divide the fish head from the body. Remove the gills. Using a scalpel and needle it is necessary to clean maximally the skull and remove brain. Remove excess fat.
5. Remove fish muscles roughly through their cut along the spinous processes of the vertebrae. Clipped muscles should be pushed and cut at the beginning of ribs. Cleaning of the axial skeleton from muscles must be done with a scalpel, tweezers and brushes. If the muscles fade badly, hot water can be used - the fish need to be immersed to the boiling water for a very short time. The main thing is not overdo fish in hot water because the skeleton may disintegrate. It is necessary to scrape muscles very gently in a combination of ribs with the vertebrae so as not to tear them away from the spine. Clear ribs from the muscles.

6. To prevent darkening of the bones due to grease, it must be immersed in gasoline for several hours.

7. Further skeleton should be bleached in the solution of hydrogen peroxide. Bleaching of skeleton is held until the end of foam forming. Drying of skeleton should be carried out in the open sunlight, so it is additionally bleached.

1. 8. Skeleton should be dried. Connect all its components and set on a wooden stand by glue, thread and wire. If it is necessary (if the skeleton dark) can be painted with white dye (but often some details of the structure of the skeleton become invisible). It is possible to install skeleton on a plate to which background can be added with details that can characterize the living conditions of fish (bottom of the reservoir, snags, overgrown vegetation, etc.).

8. A label with the name of the type of fish (Latin), group number, name student, and the date of manufacture of the skeleton should be added to the completed skeleton.

Questions for individual work

1. Tell the structure of the axial skeleton of fish.
2. How is skeleton of limbs of fish built?
3. What structure does the head skeleton of bony fish have?
4. Tell the structure of cyclostomes skeleton.
5. Tell the structural features of the cartilaginous fish skeleton.
6. Provide a general description of the structure of the skeleton of bony fishes.

Laboratory work № 11

Manufacturing of wet preparation

Objective: To learn the method of manufacturing the wet preparation of fish.

Materials and Equipment: Fresh fish with length of 20–30 cm. Tools: glass cylinder with bung, glass plate, needle, nylon thread, scissors, scalpel, dissecting needles, electric stove, distilled water, 5% solution of formalin.

Basic theoretical information

For manufacturing of wet preparations internal organs of large fish can be used (gills, gastrointestinal tract, genitalia, swim bladder, etc.) as well as sections of the fish with the designation of internal organs, whole specimens of fish in order to create collections of members of fish fauna, fish in various stages of development in order to show clearly periods of ontogeny.

In the manufacturing of wet preparations there are certain rules:

1. The work should be done in anatomical rubber gloves.
2. The material for the manufacturing of preparations should be taken from the recently dead fish, but without obvious disease or injury.
3. At the beginning of work it is necessary to clean the fish from dirt and gently wash away mucus, without damaging the fins and scales.
4. For rapid penetration of fixing fluid it is necessary to inject it into the tissues or organs by syringe piercing the body (tissue) in different locations or through natural openings - blood vessels, gastrointestinal tract etc.
5. Volume of fixing fluid should be 10 times more than the amount of material.
6. Fixation of the material occurs within 3–7 days.
7. If the preparation is for exposition, the fish (or an organ) must be properly straightened, and placed in a glass cylinder so that it can be shown well.

It is easier to straighten the fresh specimens; they are very flexible and change shape or position well. After long-term preservation in the fixing liquids (alcohol, formalin) fish harden highly. For some softening of fixed specimens they should be put for some time in the water, which must be replaced periodically to remove the fixing liquid.

For straightening fish is put on the dissecting cuvette on a thin plate. To impart the required position, fish is fixed by needles and thin strips of parchment. Fins of fish are fixed by needles and wooden plates. Needles cannot be stuck directly into the body of the animal, as they rust and leave stains on the surface of the fish that are impossible to be washed off.

When the fish is straightened cuvette is poured with 5% formalin solution, and if it was placed on the board, it is lowered horizontally to capacity with formalin, where it is fixed for 3–7 days. Then it can be moved to the exposition cylinder.

Capacity, which is used for exposure do not need to be cylindrical, it can also be rectangular. Everything depends on the shape and size of the preparation. The size of the exposure containers must meet the size of the exhibit, which should not exceed $\frac{2}{3}$ of the volume of the vessel. This is important because reliable storage of the preparation requires a certain amount of preserving liquid. In addition, large specimens of fish that are placed to small vessels have very bad shape, their shape and size of the object is curved.

Usually the fish is not just put into cylinder, but it is attached to the glass, which is inserted into the tank by means of threads. Glass is chosen by the form and size of the cylinder its length should reach the edge of the stopper or lid and occupy the entire width of the cylinder. If the glass is narrower than the cylinder it is necessary to be fixed by thread or plugs (Fig. 12). The fish must be seen with all the details, the background, on which object is placed helps with it. Light fish should be placed to the dark glasses and dark fish to white. Mounted specimens are fixed to glass by sewing. The fish is pierced by needle near the side that will be on the glass, tightening and knotted by threads. Color of thread should be selected similar with color of glass or preparation or they would not fall into the eyes.

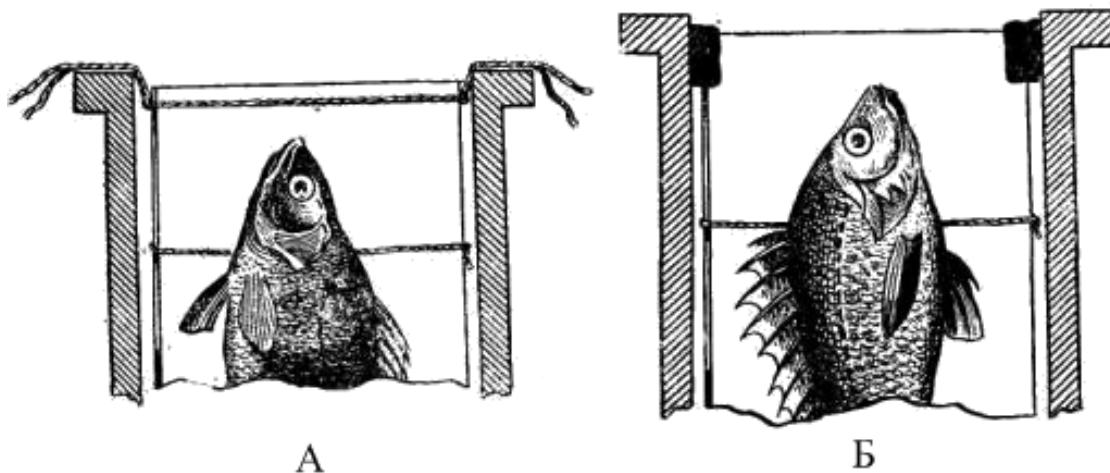


Figure 12. Fixed fish in a glass cylinder. A – glass which is strengthened using yarns;
B – glass, which is strengthened by using cork.

All wet preparations should be well sealed. For this, the glass cover of cylinder should be carefully solder by paraffin.

A label containing the following information: name of the species, the name of the preparation (internal structure, external structure, digestive system, etc.), date of manufacturing and name the one who made it should be attached to the preparation.

Progress of work

1. Thoroughly wash the glass cylinder, where the preparation will be, and rinse it with distilled water.
2. Thoroughly but gently wash the fish.
3. If you plan to manufacture wet preparation with a clear anatomical structure of the fish, it is necessary to make an anatomical a dissection of fish.
4. By using a long needle stretch nylon thread through the body of the fish (in the front and back of the body). The ends of the strands capture fish on a glass plate.
5. Open and if it is necessary fix all systems and organs of the fish so that they were visible.
6. To place the preparation in the cylinder and gently pour filtered 5% solution of formalin.
7. Within several days, change the solution until the residue disappears completely.
8. Heat the wax in a water bath and solder cover of cylinder.
9. Make and stick the label.

Questions for individual work

1. Provide a detailed description of the method of fixing of fish.
2. List the stages of production of wet preparations.
3. Provide a description of the organs (systems) of fish (according to manufactured drug).
4. How to fix fish in glass tanks?
5. What fixing solutions should be used in the manufacturing of preparations of fish?
6. How should wet preparations be kept?

Laboratory work № 12

Fish taxidermy

Objective: To learn the technique of fish taxidermy.

Materials and Equipment: Fresh fish. Tools: ruler, measuring tape, calipers, scalpel, crooked and simple scissors, needle, thread, cardboard, wood chips, straw, a solution of formaldehyde, paint, brushes, cotton, pencil, wire stand.

Basic theoretical information

Fish taxidermy is one of the most difficult processes of creating of preparations. Such work requires not only great accuracy, but plenty of experience [15-17].

Before the work it is necessary to make morphometric measurements of fish that will be useful during works to restore the shape and size of fish. The length of the abdominal and dorsal side of fish, and three trunk girths (circumference) must be also measured.

One of the key points in making of taxidermied fish is to preserve fish scaly cover during the removing of its skin because as a result of such manipulations scales often fall, especially in small fish. Firstly, all of fish fins should be stretched and necessary pasture of a building should be provided (depending on position of the exhibit). Fish needs to be put in 20–30% solution of formalin (or 80-90% alcohol) for fixing of the scales. In strong formalin (or alcohol) it stands for about 10-14 hours, which helps to perpetuate the scaly cover.

Incision is made on the ventral side along the median line for removing of skin. Bones and muscles are removed by parts as the separation of skin from the body. This greatly facilitates removing of skin, prevents skin tears and creases, on which scales usually fall. For this work crooked scissors must be used. Also massive scissors with big levers are needed to cut quickly and carefully the spine of fish.

Bones of fins are cut with scissors near the skin, so there are only fixed in formalin (alcohol) fins on the skin. It is necessary to monitor drying out of fins during work because then they can easily break. It can be provided as follows: straighten fins, glue them on one side with a thin paper like placing fin on the substrate. When preparation will be completed this paper can be easily washed and removed.

Muscles, fat and connective tissue that partially remained on the skin of the fish, is gently removed by scalpel. The skin cannot be bended or twisted because scales can fall down. If scales have felt, it is necessary to put it immediately to a solution of formalin

(concentration 1.2%). It will be necessary during finishing of taxidermied fish. The scales, which remain in the open air, rapidly change their shape, and warp so it is not suitable for inserts.

During the working with head eyes, tongue, brain and cheek muscles are removed. Cheeks are cut through the mouth opening; this work should be done very careful because cover should not be broken.

After cleaning, removed skin is immersed into a solution of arsenic or smeared by it on both sides. The skin can also be put for 10-20 minutes up to 3% solution of formalin.

Thick wire is fixed on a chosen stand. The long end of the wire is bent according to the contour and size of fish and attached by the free end to the stand, thus closing the contour of fish (Fig. 13.).

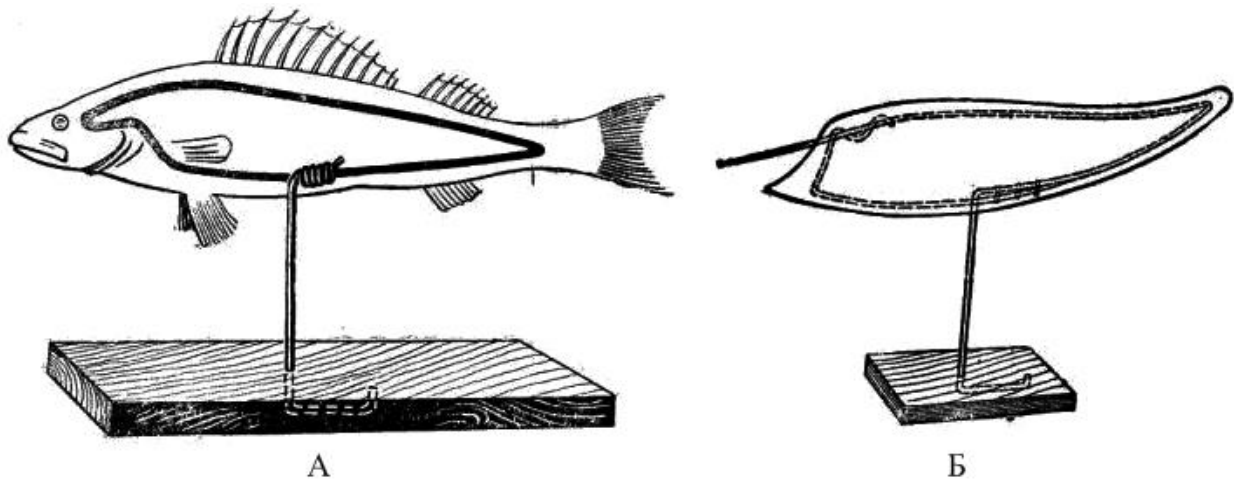


Figure 13. Manufacturing of contour for taxidermied fish: A – wired frame in taxidermied fish. B – securing the frame on a stand.

For making large taxidermied fish two wires are mounted into the stand, both ends of the wire contour are taken outside or second wire is put as a trellis.

Then contour is provided by the relevant position (pose of a future fish) and carcass begins to be formed using a soft wire (Fig. 14). Carcass is wrapped by thread, trying to avoid sharp corners and constrictions.

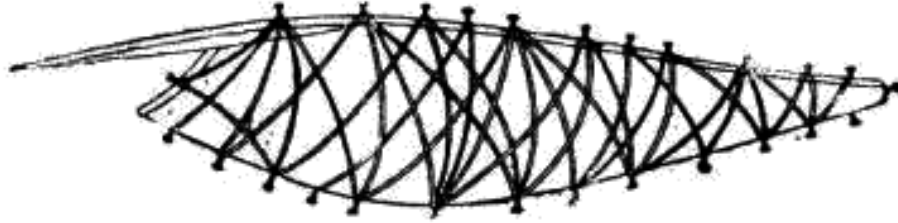


Figure 14. Formation of fish carcasses using a soft wire.

When the carcass is similar to the required size, forming continues with soft layered wool, which is imposed by thin layers. Each layer is smoothed by soft hair brush moistened in starch paste. Brush does not entail wool, but rather, glue and smooth it.

While putting skin on the carcass in some places sometimes it is necessary to enclose pieces of wool. This should be done by tweezers. Some parts of head are filled with plastic (crushed chalk mixed with glue) and cheeks are stuffed tightly with cotton wool. Skull of fish is skewered tightly to wire of the carcass; it becomes rigid when carcass is dry. The scales, which have previously fallen are carefully glued into places before the process of drying.

This method of forming of fish carcass makes taxidermied fish very light and allows giving it a particular position. In addition, the skin dries without wrinkles. Often in the manufacturing of stuffed fish clay is used and it changes the volume during the drying, and therefore skin settling, then stuffed begins to change shape. Taxidermied fish with clay or wooden cores is very heavy and brittle.

When taxidermied fish is dry, eyes are mounted firstly filling eye orbit with plastic (glue and crayons). The mass, which can tread around the eyes is carefully leveled and smoothen. Eyes can be made of colored synthetic material, picking up natural color, which is typical for a specific type of fish. Also for making eyes colored beads or buttons can be used.

Fish often needs to be painted, as natural color fades away or disappears. It is best to paint by oils dyes, delicately rubbing them on scales with oil or varnish. It should not be painted basing on the imagination of the color of the fish, a fresh fish or quality photo of fish should be as an example.

Dried taxodermed fish when the paint no longer stick to hands, is covered by colorless varnish or liquid joiner's glue.

It is very difficult to make a taxidermied fish from small fish, as loach, bitterling, etc. It is recommended to make wet preparations from them.

Progress of work

1. Choose the desired specimen of fresh and whole fish without pretreatment, do not wash and do not remove mucus.
2. Make the main morphometric measurements. Put the fish on a sheet of cardboard and cut around the contours of the body, they will be needed for restoring of external structure of fish at the time of stuffing.
3. Dunk the fish in a strong solution of formalin (alcohol). Remove the skin. Fix and treat the fins. Remove the content of the skull and treat the head of fish. Dunk the skin in a solution of 3% formalin.
4. Make the contour of stuffed fish, tighten the skin. Sew up carefully cuts of the skin using a needle and thread; it is necessary to make small stitches placed close to each other.
5. When the incisions are sewn up, start stuffing the fish with dry wood sawdust. Stuffing is conducted using a wooden stick through rictus of fish. It is necessary to preserve the natural shape and size of fish. Fish needs to be filled tightly, but not too much, to avoid breaking and changes of skin circuit is made previously.
6. Fix the fins. Spread them and squeeze between two cardboard plates, which should take the form of fins and slightly exaggerate their natural size.
7. The finished taxidermied fish is put to dry in sawdust and abdomen should be down. Fins must be straightened. Duration of drying depends on the size of fish (fish of medium size dried for 6-7 days).
8. After drying of exhibit label with following information is placed on a wooden stand: Ukrainian and Latin name of the species of fish, name and surname of the student, year.

Questions for individual work

1. How are morphometric measurements of fish made?
2. What determines the natural color of the fish?
3. What structure should have fins of fish?
4. What position of the mouth do fish have? Why is that?
5. What color of fish is provided by? Why do the dead fish quickly fade or get lighter?
6. At what temperature should stuffed fish be dried?
7. Under what conditions should bestuffed fish kept?

CONCLUSION

The basic methods that are used during ichthyological researches on natural and artificial water bodies of different types are considered. The brief theoretical material for each laboratory work and the issues for independent work are provided. Special attention is paid to the rules of formalization of the obtained material, logging of experiments protocol. Text is illustrated with drawings and diagrams. For students of biological specialties of higher education institutions.

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