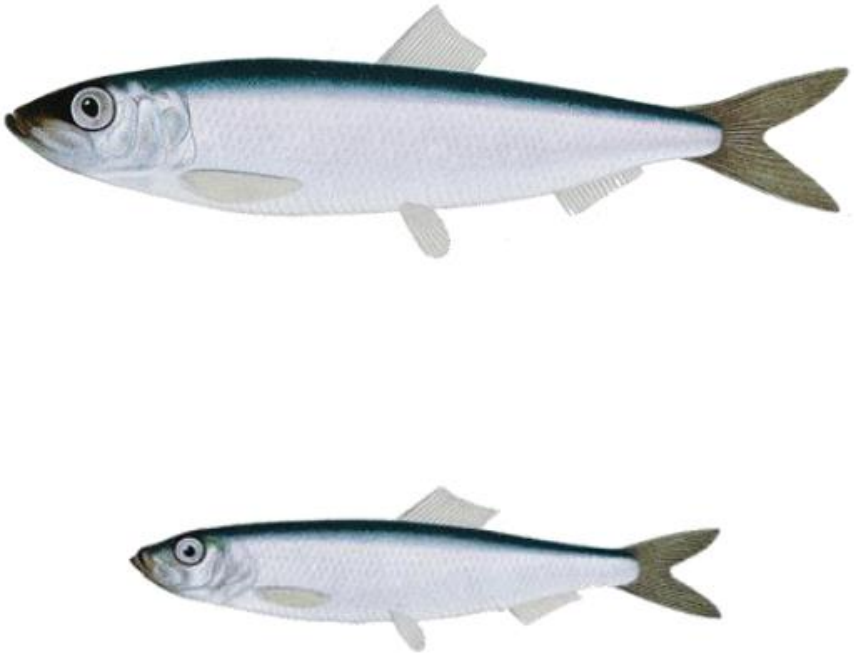
Collection and processing of pelagic fish biological material.



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1. Collection of Herring Biological Material

1.1 Collection of Herring Biological Material in the Central Part of the Baltic Sea (sub-regions 25-29 and 32, excluding the Gulf of Riga)

Herring biological samples are primarily collected by participating in commercial fishing trips (approximately nine trips per year). Samples are collected on randomly selected vessels based on fishing conditions and species composition of the catch. Within one trip, both herring and sprat biological samples are collected. In pelagic trawl fishing, the species composition of small pelagic fish (herring, sprat, sandeel, pipefish, etc.) is assessed by sorting one crate (30 kg) from the catch. In cases where only one species (herring, sprat) dominates the catch, a smaller quantity of 10 kg from the crate can be sorted. From the sorted portion, 200 sprat and 200 herring individuals are selected for mass measurements and biological analysis. If the number of individuals of a particular species does not reach 200 in the sorted portion, the missing individuals can be selected from the remaining portion. Herring biological samples are randomly selected and consist of at least 200 fish. The biological sample is analysed by conducting mass measurements and biological analysis. All information is documented in a biological analysis form, initially indicating:

- Date
- Vessel
- Fishing gear
- Fishing duration (minutes)
- Fishing sub-region
- Crate weight (kg)

An example of a completed biological analysis form is shown in Figure 3.

1.2 Mass Measurements

Fish in the biological sample are measured (length to the end of the caudal fin) and sorted into length groups with a 0.5 cm interval. Then, the fish in each length group are counted and weighed, and the following information is recorded in the biological analysis form:

- Length group (cm)
- Quantity
- Weight (g)

1.3 Biological Analysis

- Biological analysis is conducted on up to 10 randomly selected fish from each length group. In a standard biological analysis, the following information is recorded for each fish:
- Length group (cm)
- Mean weight of the length group (g)
- Gender (1 - male; 2 - female)
- Maturity stage (based on a 7-point scale) (Figure 4)

During the biological analysis, otoliths are collected from each fish using a scalpel for age determination. Otoliths are placed in special paper envelopes (Figure 1) or plastic molds (Figure 2). Each envelope is labelled with a fish's sequence number, which corresponds to the sequence number in the biological analysis form. Information about the vessel, date, and range of otolith sequence numbers in the envelope is indicated on each envelope. Age determination of the fish is later performed in the laboratory, and the obtained information is recorded in the respective biological analysis form or database. When determining the fish's age, information about its population affiliation (Gulf of Riga, Central Baltic, or Southern region herring) and run (spring or autumn spawning fish) is also recorded.

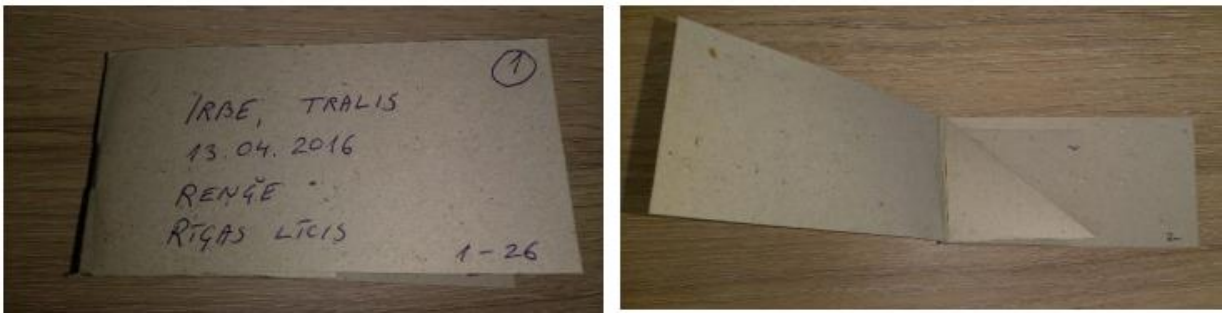


Figure 1. Envelopes for storing otoliths.

Figure 2. Plastic templates for storing otoliths. The number on the edge of the template allows for identifying the fish's serial number in the biological analysis.

1.4 Data Entry and Quality Control

Information from each fishing trip's biological analysis forms is entered into an Excel database format. During data entry, the information is cross-checked with the vessel's electronic log, and if necessary, the fishing location and catch weight are clarified. After data entry, a quality control process is carried out, evaluating the intervals of fish biological parameters (length group, average weight within each length group, sex, maturity stage, age, population, race) and generating scatter plots to visually assess the relationship between fish length and weight. Similarly, the relationship between fish age and length, as well as age and weight, is visually inspected. To evaluate individual body condition (plumpness), the Fulton's condition factor is calculated using the formula $F = W/L\text{^}3 \times 100$, and its value ranges are checked. Possible erroneous entries are reviewed, and if necessary, corrected in both the biological analysis forms and the Excel database file.

2. Collection of herring biological material in the Gulf of Riga (sub-region 28.1)

Biological samples of sprat are collected by organizing the researcher's participation in commercial fishing trips or through collaboration with fishing companies. In the first scenario, the biological samples are collected on the deck of the fishing vessel during industrial fishing trips using pelagic trawls. In the second scenario, sample collection takes place on the deck of the vessel or at the port. Samples are collected at least once a month on a randomly selected vessel, based on fishing conditions. On-site sample collection, with the researcher participating in the fishing trip, is organized at least once a quarter, following a random selection principle.

Biological samples of herring from industrial fishing in the Gulf of Riga are separately collected from the open part of the pelagic trawl fishing and from static gear and net fishing along the coast. The volume and locations of sample collection are as follows:

- Trawl fishing: 3 samples per month (during active trawl fishing) from randomly selected vessels.
- Static gear fishing: 1 sample every 10 days (from the end of April to the beginning of July) from each geographic area of the Gulf of Riga (east, south, west).
- Net fishing: up to 3 samples during the period from the end of April to the beginning of July (during the spawning season).

In the case of collecting biological samples in collaboration with fishing companies, the companies collect samples after random selection based on the catch. Each sample consists of at least 200 fish. Information about the fishing gear, catch date, and vessel or company is recorded for each sample. The collected samples, whether fresh or frozen, are transported to the "BIOR" Institute for analysis in the laboratory. The type of analysis depends on the fishing gear. All information is documented in written form on the biological analysis card.

2.1 Analysis of herring biological samples from trawl fishing

For herring biological sample analysis from trawl fishing, the fish in the sample are measured (from the tip of the snout to the end of the tail) and sorted into length groups with a 0.5 cm interval. Then, the fish in each length group are counted and weighed, and the following information is recorded on the biological analysis card:

- Length group (in centimetres)
- Number of fishes
- Weight (in grams)

Biological analysis is performed on up to 10 randomly selected fish from each length group. The standard biological analysis includes the following information for each fish:

- Length group (in centimetres)
- Weight (in grams)
- Gender (1 - male; 2 - female)
- Gonad maturation stage (according to a 7-point scale)

During the biological analysis, otoliths are collected from each fish using a scalpel for age determination. The otoliths are placed in special otolith envelopes or plastic molds (Figure 1 or Figure 2). Each otolith envelope is labelled with a fish's serial number, which corresponds to the serial number on the biological analysis card. Information about the vessel, date, and range of otolith serial numbers in the envelope is indicated on each of the envelopes. The determination of fish age is later performed in the laboratory, and the obtained information is recorded in the respective biological analysis card or database. When determining fish age, information about population affiliation (Gulf of Riga, Central Baltic, or Southern Region herring) and run (spring or autumn spawning fish) is also recorded.

2.2. Analysis of herring biological samples from gillnet and net fishing

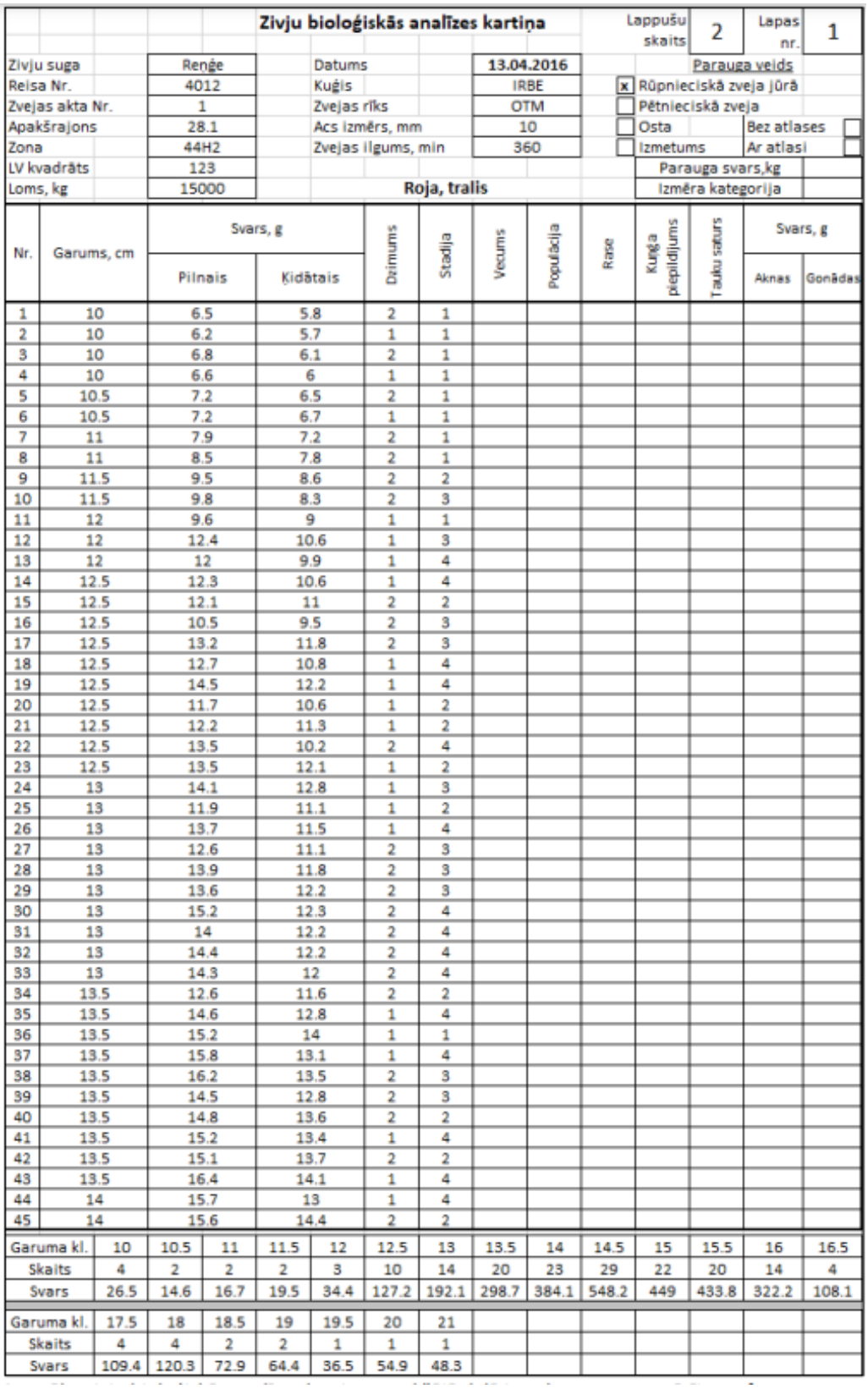
For herring biological sample analysis from static gear and net fishing, a complete biological analysis is performed on sequentially selected 100 fish, and the following information:

- Length, cm
- Weight, g
- Gutted weight, gender (1 - male; 2 - female)
- Maturation stage (according to a 7-point scale) (Figure 4)

During the biological analysis, otoliths are collected from each fish using a scalpel to determine their age. The otoliths are placed in special fish logbooks (Figure 1) or plastic templates (Figure 2). The fish logbook page is marked with the fish's sequence number, which corresponds to the sequence number on the biological analysis card. Each logbook contains information about the vessel, date, and range of otolith sequence numbers. Fish age determination is later performed in the laboratory, and the obtained information is recorded in the respective biological analysis card or database. When determining fish age, information about population affiliation (Riga Bay, Central Baltic, or Southern Region herring) and race (spring or autumn spawning fish) is also recorded.

2.2 Data Input and Quality Control

Information from the biological analysis cards is entered in Excel database format. During data entry, information about the fishing square, fishing duration, and catch weight is added based on electronic vessel logs and coastal fishing statistics. After data entry, quality control is performed by evaluating the intervals of fish biological parameters (length, weight, gender, maturation stage, age, population, race) and creating a scatter plot to visually check the relationship between fish length and weight. Similarly, the relationship between fish age and length, as well as age and weight, is visually examined. To assess the individual body weight and length ratio (condition), the Fulton's condition factor is calculated using the formula F = (W/L^3) × 100, and its value limits are checked. Possible erroneous entries are verified and, if necessary, corrected in both the biological analysis card and the Excel database file.



Zivju bioloģiskās analīzes kartiņa

Lappušu skaits: 2 | Lapas nr.: 1

Zivju suga	Reņģe	Datums	13.04.2016	Parauga veids	
Reisa Nr.	4012	Kuģis	IRBE	[x] Rūpnieciskā zveja jūrā	
Zvejas akta Nr.	1	Zvejas rīks	OTM	[ ] Pētnieciskā zveja	
Apakšrajons	28.1	Acs izmērs, mm	10	[ ] Osta	Bez atlases [ ]
Zona	44H2	Zvejas ilgums, min	360	[ ] Izmetums	Ar atlasi [ ]
LV kvadrāts	123			Parauga svars,kg	
Loms, kg	15000	Roja, tralis		Izmēra kategorija	

Nr.	Garums, cm	Svars, g		Dzimums	Stadija	Vecums	Populācija	Rase	Kuņģa piepildījums	Tauku saturs	Svars, g	
		Pilnais	Kidātais								Aknas	Gonādas
1	10	6.5	5.8	2	1							
2	10	6.2	5.7	1	1							
3	10	6.8	6.1	2	1							
4	10	6.6	6	1	1							
5	10.5	7.2	6.5	2	1							
6	10.5	7.2	6.7	1	1							
7	11	7.9	7.2	2	1							
8	11	8.5	7.8	2	1							
9	11.5	9.5	8.6	2	2							
10	11.5	9.8	8.3	2	3							
11	12	9.6	9	1	1							
12	12	12.4	10.6	1	3							
13	12	12	9.9	1	4							
14	12.5	12.3	10.6	1	4							
15	12.5	12.1	11	2	2							
16	12.5	10.5	9.5	2	3							
17	12.5	13.2	11.8	2	3							
18	12.5	12.7	10.8	1	4							
19	12.5	14.5	12.2	1	4							
20	12.5	11.7	10.6	1	2							
21	12.5	12.2	11.3	1	2							
22	12.5	13.5	10.2	2	4							
23	12.5	13.5	12.1	1	2							
24	13	14.1	12.8	1	3							
25	13	11.9	11.1	1	2							
26	13	13.7	11.5	1	4							
27	13	12.6	11.1	2	3							
28	13	13.9	11.8	2	3							
29	13	13.6	12.2	2	3							
30	13	15.2	12.3	2	4							
31	13	14	12.2	2	4							
32	13	14.4	12.2	2	4							
33	13	14.3	12	2	4							
34	13.5	12.6	11.6	2	2							
35	13.5	14.6	12.8	1	4							
36	13.5	15.2	14	1	1							
37	13.5	15.8	13.1	1	4							
38	13.5	16.2	13.5	2	3							
39	13.5	14.5	12.8	2	3							
40	13.5	14.8	13.6	2	2							
41	13.5	15.2	13.4	1	4							
42	13.5	15.1	13.7	2	2							
43	13.5	16.4	14.1	1	4							
44	14	15.7	13	1	4							
45	14	15.6	14.4	2	2							

Garuma kl.	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15	15.5	16	16.5
Skaits	4	2	2	2	3	10	14	20	23	29	22	20	14	4
Svars	26.5	14.6	16.7	19.5	34.4	127.2	192.1	298.7	384.1	548.2	449	433.8	322.2	108.1

Garuma kl.	17.5	18	18.5	19	19.5	20	21
Skaits	4	4	2	2	1	1	1
Svars	109.4	120.3	72.9	64.4	36.5	54.9	48.3

Figure 3. Fish biological analysis card. The bottom part displays the form for measuring weight.

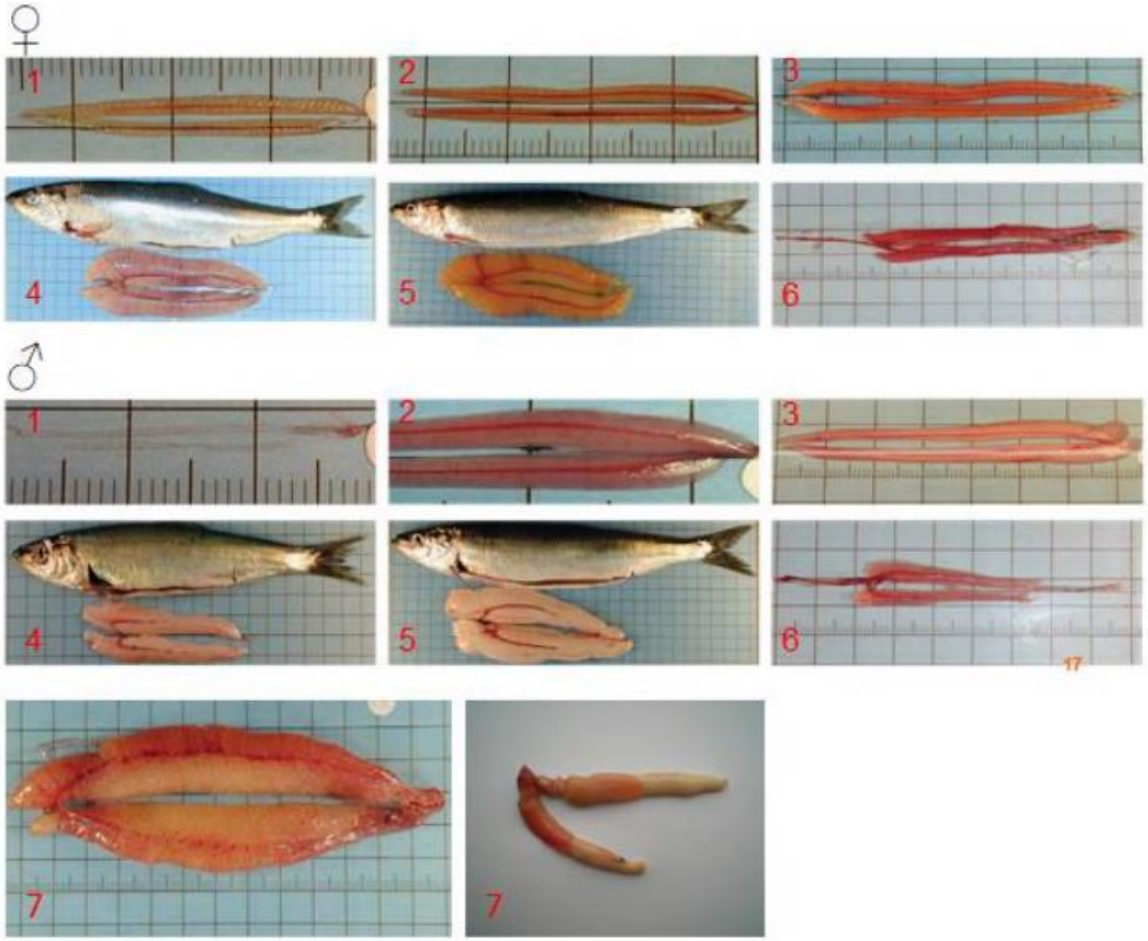


Figure 4. Gonadal maturation stages of herring (Kiselevich scale - 6+1 stages, ♀ - female, ♂ - male) (Image from DTU Aqua).

1. Juvenile stage (immature gonads, transparent and thread-like in males, forming a lance shape, yellowish and thicker gonads in females, with a wrinkled surface).
2. Undeveloped stage (stage before the first or subsequent maturation (after spawning)).
3. Maturation stage (gonads start to develop - individual oocytes are visible in females, testes turn whitish in males, but the gonads do not occupy the entire body cavity).
4. Maturation stage (well-developed gonads that occupy the majority of the body cavity).
5. Spawning stage (gonads occupy the entire body cavity or, in the case of incomplete filling, if spawning is in progress, gentle pressure on the gonads causes them to flow easily).
6. Post-spawning stage (distended gonads, often bloody, with a small amount of retained gametes inside. This stage gradually transitions to stage 2).
7. Abnormal stage (various developmental abnormalities - resorption, presence of both sexes in one individual, one undeveloped gonad, etc.).

3. Collection of sprat biological material

Biological samples of sprat are collected by organizing the researcher's participation in industrial fishing trips or sample collection in collaboration with fishing companies. In the first case, biological samples are collected on the deck of the fishing vessel during an industrial fishing trip using a pelagic trawl, while in the second case, sample collection takes place on the deck of the vessel or in the port. Samples are collected at least once a month on a randomly selected vessel, based on fishing conditions. Within one trip, the collection of both sprat and herring biological samples is organized. During the industrial trip, after catching sprat, the biological sample is randomly selected and consists of at least 200 fish. The collected samples, in fresh or frozen form, are transported to the Institute "BIOR" where they undergo analysis in the laboratory. Sample collection is also organized in collaboration with fishing companies. The collected samples in frozen form are transported to the Institute "BIOR" for laboratory analysis.

The biological sample is analysed by measuring the fish's mass and performing biological analyses. All information is recorded in a biological analysis card, which initially includes the following:

- Date
- Vessel
- Fishing gear
- Fishing duration in minutes
- Fishing subregion
- Total mass in kilograms

3.1 Mass Measurements:

The fish in the biological sample are measured (length up to the end of the caudal fin) and sorted into length groups with a 0.5 cm interval. Then, the fish in each length group are counted and weighed, and the following information is recorded in the biological analysis card:

- Length group in centimetres
- Quantity
- Mass in grams

3.2 Biological Analyses

Biological analysis is performed on up to 5 randomly selected fish from each length group up to 8.5 cm, up to 10 randomly selected fish from each 9.0 and 9.5 cm length group, and 15 randomly selected fish from each length group above 10.0 cm. In the standard biological analysis, the following information is recorded for each fish:

- Length group in centimetres
- Individual fish mass in grams with a precision of 0.1 g
- Gender (1 - male; 2 - female)
- Maturation stage (according to a 7-point scale)

Since sprat has batch-type spawning, the 6th stage is divided into three sub-stages (1st table).

During the biological analysis, otoliths are collected from each fish using a scalpel for age determination. The otoliths are placed in special envelopes. Each envelope's page indicates the fish's sequence number, which corresponds to the sequence number in the biological analysis card. Each envelope also contains information about the vessel, date, and range of otolith sequence numbers. Fish age determination is performed later in the laboratory, and the obtained information is recorded in the corresponding biological analysis card.

Recording of mass measurements and biological analyses, as well as the storage of otoliths, follow the same methods as for herring (see Figures 1-3).

3.3 Data Entry and Quality Control

Information from each trip's biological analysis cards is entered in Excel database format. During data entry, the fishing location and total mass are cross-checked and, if necessary, clarified using information from the vessel's electronic journal. After data entry, quality control is performed by evaluating the biological parameters of fish (length group, mean mass in each length group, gender, maturation stage, age) and creating a scatter plot to visually check the relationship between fish length and mass. The relationship between

Table 1.

Stage nr.	Title	Gonad description	
I	Juvenile stage	The gonads are small, elongated, thread-like, pale, and transparent. The gender cannot be determined without a microscope	
		Females	Males
III	The gonads are immature.	The ovaries are small, elongated, thin, pale yellow, transparent, or partially transparent. Oocytes are not visible to the naked eye; they are transparent, glass-like, and can be observed under a microscope, with a diameter of ≤0.2 mm.	The testes are very thin, semi-transparent, grayish, ribbon-shaped, and located along undeveloped blood vessels.
IV	Stage of maturation.	The ovaries enlarge and at the end of the stage occupy up to 2/3 of the body cavity. At the beginning of the stage, they are transparent, and at the end, they become yellowish. Oocytes are visible through the ovarian membrane, with a diameter of 0.2-0.3 mm at the beginning of the stage and 0.5 mm at the end. The oocytes are attached to each other and cannot be easily separated.	The testes enlarge and at the end of the stage occupy the majority of the body cavity. They are firm, initially grayish-white, and become white by the end of the stage. When cut, the cut surface retains its shape.
V	Stage of maturation.	The ovaries occupy the entire free space in the body cavity; they are not transparent and usually have a distinct yellow color. The oocytes have a diameter of 0.5-0.6 mm. The oocytes can be easily separated from each other.	The testes occupy the majority of the body cavity. They are firm and white. If cut, the cut surface changes its shape, squeezing out a small amount of sperm.

VI	Spawning stage	The ovaries have swollen, become watery, and the oocytes have separated from each other. When lightly pressed on the abdomen of the female, they are released outside through the genital opening.	Sperm easily escapes from the genital opening.
VI: VI-II	Post-spawning stage	The ovaries are small, bloody, usually without oocytes. The gonads later return to the II stage.	The testes are small and bloody, firm and opaque. They do not contain sperm and later return to the II stage.
VI-III	Stage of maturation of portions.	The next portion is developing. Ovaries and oocytes are similar to Stage III, but in a reddish color.	The next portion is developing. Testes are similar to Stage III, but they are smaller and reddish in color.
VI-IV	Stage of maturation of portions.	The next portion is developing. Ovaries and oocytes are similar to Stage VI, but they are reddish in color.	The next portion is developing. Testes are similar to Stage VI, but they are smaller and reddish in color.
VIII	Abnormal stage	Various developmental abnormalities can occur, such as resorption, hermaphroditism, underdeveloped gonads, and others.	