Material and methods for Pelagic trawl survey in the Bulgarian Black Sea area

Pelagic Trawl survey is accomplished with accordance with Work plan for Data Collection in Fisheries sector of Bulgaria. The study is conducted during the spring and autumn season, in the area enclosed between Durankulak and Ahtopol (Bulgaria) with total length of coastline of 370 km. Study area encloses waters between 42o05' and 43o45' N and 27o55 and 29o55 E. During the survey, total 36 mid-water hauls (per season) is carried out in Bulgarian area. The survey undergoes during the day and the following types of data were collected:

- Coordinates and duration of each trawl
- Sprat total catch weight
- Separation of the by-catch by species
- Composition of by-catch
- Conservation of the samples.

Sampling design

To establish the abundance of the reference species (Sprattus sprattus) in front of the Bulgarian coast a standard methodology for stratified sampling was employed (Gulland, 1966;). To address the research objectives the region was divided in 3r strata according to depth – Stratum 1 (15 - 30 m) Stratum 2 (35 – 50 m), Stratum 3 (50 – 100m)

The study area in Bulgarian waters was partitioned into 128 equal in size not overlying fields, situated at depth between 16 - 92 m. At 37of the fields chosen at random, sampling by means of mid-water trawling was carried out.

Each field is a rectangle with sides 5' Lat × 5' Long and area around 62.58 km-2 (measured by application of GIS), large enough for a standard lug extent in meridian direction to fit within the field boundaries. The fields are grouped in larger sectors – so called strata, which geographic and depth boundaries are selected according to the density distribution of the species under study. At each of the fields only one haul with duration between 30 - 40 min. at speed 2.7-2.9 knots was carried out.

As a result of the trawling survey a biomass index was calculated.

Onboard sample/processing

The data recorded and samples collected at each haul include:

- Depth, measured by the vessel's echo sounder;
- GPS coordinates of start/end haul points;
- Haul duration;
- Abundance of sprat caught;
- Weight of total sprat catch;
- Abundance and weight of other large species;
- Species composition of by-catch;
- 4% Formaldehyde solution with marine water was used for conservation of sprat for stomach content examination.

Laboratory analyses

The samples collected onboard were processed in the laboratory for determination of age and food composition. The age was established in otoliths under binocular microscope. The food spectrum was determined by separation of the stomach contents into taxonomic groups identified to the lowest possible level.

Statistical analyses

Swept area method

This method is based on bottom trawling across the seafloor (area swept), weighted with chains, rock-hopper and roller gear, or steel beams. Widely used direct method for demercal species stock assessment.

The main point of the method: the trawl doors are designed to drag along the seafloor for defined distance. Trawling area was calculated as follows:

$$(1)\quad a = D * hr * X2$$
$$D = V * t$$

where: *a* – trawling area, *V* – trawling velocity, *hr* X2* – trawl door distance, *t* –trawling duration (h), *D* – dragged distance on the seafloor;

(2) $D = 60 * \sqrt{(Lat_1 - Lat_2)^2 + (Lon_2 - Lon_1) * \cos(0.5 * (Lat_1 + Lat_2))}$

(3) $D = \sqrt{VS^2 + CS^2 + 2 * VS * CS * \cos(dirV - dirC)}$,

where, VS is vessel velocity, CS - present velocity (knots), dir*V* vessel course (degrees) and *dirC*- present course (degrees).

Stock biomass is calculated using catch per unit area, as fraction of catch per unit effort from dragged area:

(4) $(\frac{C_{w/t}}{a/t}) = C_{w/a} kg/sq.km$

where: *Cw/t* – catch per unit effort, a/t – trawling area (km2) per unit time;

Stock biomass of the given species per each stratum could be calculated as follows:

(5) $B = (\overline{C_{w/a}}) * A$

where: $\overline{C_{w/a}}$ - mean CPUA for total trawling number in each stratum, A- area of the stratum.

The variance of biomass estimate for each stratum is (equation 6):

(6) $VAR(B) = A^2 * \frac{1}{n} * \frac{1}{n-1} * \sum_{i=1}^{n} [Ca(i) - \overline{Ca}]^2$

Total area of the investigated region is equal to the sum of areas of each stratum:

$$A = A1 + A2 + A3$$

Average weightened catch per whole aquatic territory is calculated as follows:

(7) $$\overline{Ca}(A) = Ca1 * A1 + Ca2 * A2 + Ca3 * A3 / A$$

where: *Ca1*- catch per unit area in stratum 1, *A1* – area of stratum 1, etc., *A*- size of total area.

Accordingly, total stock biomass for the whole marine area to:

(8) $$B = \overline{Ca}(A) * A$$

where: $\overline{Ca}(A)$ - average weightened catch per whole investigated marine area, A – total investigated marine area.

Estimation of Maximum Sustainable Yield (MSY)

The Gulland's formula for virgin stocks is used – equation 9:

(9) MSY = 0.5*M*Bv

where: M – coefficient of natural mortality; Bv – virgin stock biomass.

Relative yield-per-recruit model with uncertainties

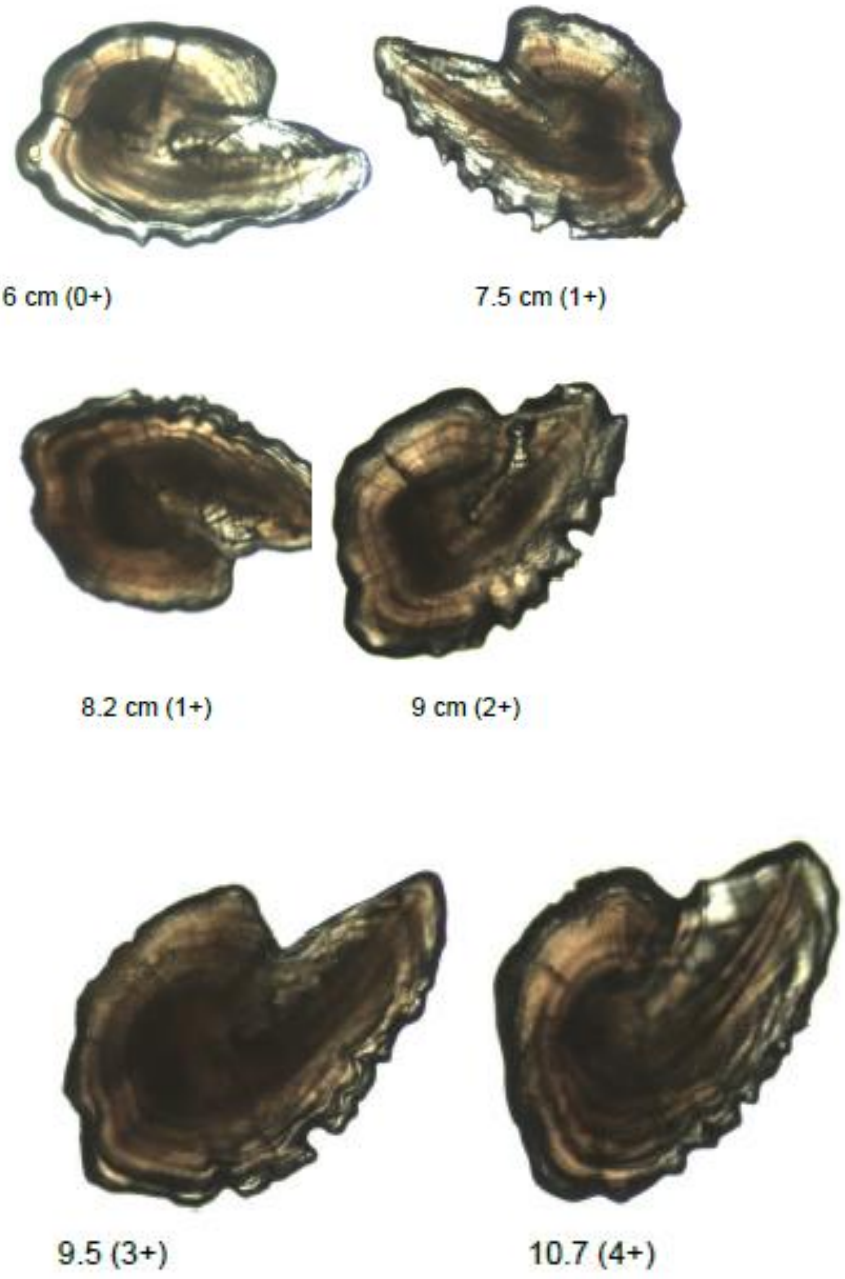
(10) $$Y'/R = E * U^{M/k}\left\{1 - \frac{3U}{(1+m)} + \frac{3U^2}{(1+2m)} - \frac{U^3}{(1+3m)}\right\}$$

where: U = 1-(Lc/L∞), m = (1-E)/(M/k) = k/Z, E = F/Z – exploitation coefficient.

Lenght-converted catch curve

A number of methods are available with the help of which total mortality (Z) can be estimated from length-frequency data. Thus it is possible to obtain reasonable estimates of Z from the mean length in a representative sample, or from the slope of Jones' cumulative plot. In this article, a variety of approaches for analysing lengthfrequency data are presented which represent the functional equivalent of [age structured] catch curves; these "length-converted catch curves" are built around assumptions similar to those involved in age-structured catch curves.

Age estimation



As it is well known, the Calcified Structures (CS) are usually used to assign age useful to obtain their growth model and so, to reconstruct age composition of exploited fish populations. Fish ageing implies the presences in the CS of a structural pattern, in terms of succession of opaque and translucent zones and the knowledge of the periodicity of this deposition pattern.

Calcified structures available for fish ageing are different: otoliths (sagittae, lapilli, asterischi), scales, vertebrae, spines and opercular bones (Panfili et al., 2002). For the selected stocks the CS utilized is the sagittae. The most important aspects (difficulties, extraction, storage, preparation method, ageing criteria) regarding the age analysis are addressed by species. Otoliths are important for fish and fisheries scientists. Otoliths are playing role balance, motion and sound.

These structures are effective from growth to death in entire life cycle.

They are most commonly used for age in order to determine growth and mortality research. Research on otoliths began in 1970s and continues to 21st century. Periodic growth increments which in scales, vertebrae, fin rays, incleithra, opercula and otolith are used to determine annual age in many fish species.

Researchers have used otolith reference collections and photographs in publications to aid in identifications. Otoliths have a distinctive shape which is highly specific, but varies widely among species.

Biologists, taxonomists and archaeologists, based on the shape and size of otoliths determined fish predators feeding habits (Kasapoglu and Duzgunes, 2014). In teleost fishes, otoliths are the main CS for the age determination and it is widely used in fisheries biology. On the other hand analysing O2 isotopes in their structure is useful to determine fish migrations between fresh water and sea as well as species and stock identification. Otoliths are the balance and hearing organs for the fish. They are in three types located on the left and right side of the head in the semi rings; “sagitta” in the saccular, “lapillus” in the lagenar and “asteriskus” in the utricular channels. Place, size and shape of these three types are different by species, the biggest one is sagitta and the smallest one is asteriscus. So, sagitta is the one mostly used in age determination in bony fishes (Aydın, 2006). Other reasons for the preference to otoliths are:

- Their formation in the embryonic phase which shows all the changes in the life cycle of the fish;

- Existence in the fish which have no scales;

- Giving better results than the scales and more successful age readings in older fish

than their scales;

- No resorption or regeneration;

- Having same structure in all the individuals in the same species (Jearld, 1983).

On the other hand, their disadvantages are the obligation of dissecting the fish and some failures in age determination due to crystal like formations by irregular CaCO3 accumulations on the otoliths.

Otolith Preperation for sprat

Sampling of the fish for otolith extraction from the overall samples is very important to have representative samples for the catch. Number of otoliths needed is lower for the species having smaller size range than the species having larger size range. According to the availability 5 fish for each length group may be better for age readings to be representative for the population. Each of the individuals should be recorded individually with place of catch, date and ID number. These steps are useful for the process:

- For each fish total length (±0,1 cm), total weight (±0,01g), sex, maturation stage (I-V), gonad weight (±0,01g) are recorded.
- Sagittal otoliths of each fish are removed by cutting the head over eyes after all individual measurements. Then, rinsed and immersed in 96% ethyl alcohol to get rid of organic

wastes/residuals and finally kept in small chambers in plastic roomed boxes with the sample number and other operational information.

Preperation of the otoliths for the age determination

Otoliths are put into small black convex glasses containing 96% ethyl alcohol for age readings under binocular stereo microscope which is illuminated from top and sides (Fig 3) (Polat ve Beamish, 1992). Magnifying level depends on the size of the otolith; X4 is good for sprat and X1 for turbot.

Age reading protocol

1. Dissected otoliths rinsed and threated with 96 % ethyl alcohol and stored dry.

2. Readings are carried out by inspecting the whole otolith in 96% ethyl alcohol in black colored convex glass bowl under reflected light against a dark background.

3. Magnification set considering the biggest otolith size which is totally fit the visual capacity of the lens. It is aimed not to change magnification rate which may enable false rings visible in bigger otoliths and permits to see true rings (hiyalins) better by unchanging the color contrasts. Thats why magnifation rate X4 is selected for the sprat otoliths.

4. Otolith samples observed from distal surface as a whole, broken ones are not used.

5. Birthday of the sprat accepted as 1st of January as the common principle for the fish living in the Northern semiphere in line with the sub-tropic fish growth models.

6. Central point surrounded by the hyalin rings which is one in some cases or two for the others, is formed after the end of consumption of yolc sac and starting of the free feeding, and known as “stock rings”. Next opaque accumulation is known as “first year growth ring”. This ring keeps its circular form in the postrostrum region. Together with this ring and the next hyalin ring forming “V” shape in the rostrum, is accepted as first age rings.

7. Tiny and continious consantric rings prolonge close to real hyalin ringed are counted togetherwith the real one as one age. This ring may be either very tiny and opaque inside the hyaline band or tiny hyaline ring near the outer edge of the opaque ring.

8. Sprat and some other short lived species has very fast growth rate especially in the first two years. Width of the growth bands after 2nd year ring has relatively getting narrower. This issue should be kept in mind in the older age ring readings.

Number of tiny and weak hyaline rings, known as false rings, in the opaque region, is not so high and, their seperation from age rings is rather easy. When they are so much and unseperable, these otoliths should not be used.

Sex and Maturity Estimation

The European sprat (Sprattus sprattus L.) is a small short-lived pelagic species from the family Clupeidae. Sprat has a wide distribution including shelf areas of the Northeast Atlantic, the Mediterranean Sea and the Baltic Sea. Sprat is most abundant in relatively shallow waters and tolerates a wide range of salinities. Spawning is pelagic in coastal or offshore waters and occurs over a prolonged

period of time that may range from early spring to the late autumn. Sprat is an important forage fish in the North Sea and Baltic Sea ecosystems. Commercial catches from pelagic fisheries are mainly used for fish meal and fish oil production. Three subspecies of sprat have been defined i.e. Sprattus sprattus sprattus L., distributed along the coasts of Norway, the North Sea, Irish Sea, Bay of Biscay, the western coast of the Iberian peninsula down to Morocco, Sprattus sprattus phalericus, R) in the northern parts of the Mediterranean and the Black Sea, and Sprattus sprattus balticus S. in the Baltic Sea. Knowledge about stock structure, migration of sprat and mixing of populations among areas is limited. Questions have been raised about the geographic distribution and separation of stocks and their interaction with neighboring stocks (ICES 2011). The apparent overlap e.g. between North Sea sprat and English Channel sprat seems very strong, whereas the overlap between North Sea sprat and Kattegat sprat is not as strong and varies between years. A distribution wide phylo-geographic study showed that sprat in the Western Mediterranean is a subgroup of the Atlantic group and that these two populations are closer to each other than to sprat in the Eastern Mediterranean and Black Sea (Debes et al., 2008).

Maturity Stages of Sprat

It is very important to use standardized maturity scales for sprat (and all species) to evaluate sampling strategies and timing for accurate classification of maturity in order to provide reliable maturity determination for both sexes. For sprat, small gonad size and the batch spawnings by several cohorts of eggs over a long period of time are the main challenges for standardizing a maturity scale.

According to the ICES (2011), present standardized maturity scales of sprat include 6-stages for both sexes (Fig 3.6.2.Table 3.6.2.)

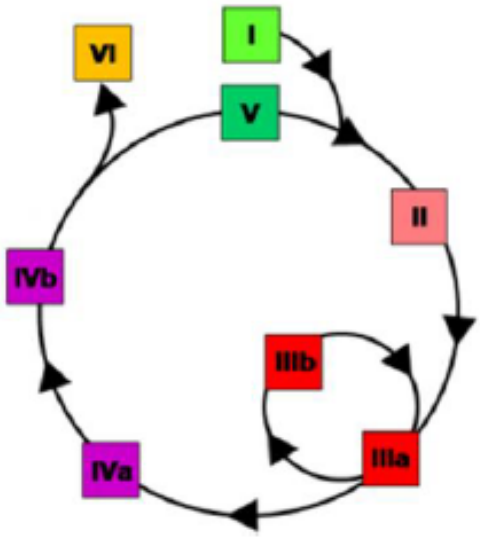


Figure3.6.2. Scale with six maturity stages in sprat (Name of the stages are given in Table 3.6.3)

In particular, specimens without visible development have been combined into Immature and Preparation, whereas the spawning stage has been sub-divided into a non-active spawning stage (maturing and re-maturing characterized by visible development of gametes) and an active spawning stage indicated by hydrated eggs/running milt. The integration of maturing and re-maturing into the

spawning stage allows an accurate determination of maturing and spawning specimens and reliable assessment of the spawning fraction of the population.

Table 3.6.3. Macroscopic and histological characteristics of gonadal development stages

Stages	Macroscopic Characteristics	Histological characteristics
FEMALES (OG: Oogonia, PG1: Early previtellogenic oocytes, PG2: Late previtellogenic oocytes, CA: Cortil alveoli oocytes, VT1: Early vitellogenic oocytes, VT2: Mid vitellogenic oocytes, VT3: Late vitellogenic oocytes, HYD: Hydrated oocytes, POF: Postovulatory follicles, SSB: Spawning stock biomass).		
I-Immature	Juvenile: ovaries threadlike and small; transparent to wine red and translucent in color; sex difficult to determine; distinguishable from testes by a more tubular shape; oocytes not visible to the naked eye	OG+/-PGI
II-Preparation	Transition from immature to early maturing; oocytes not visible to the naked eye; ovaries yellow-orange to bright red in color; ovaries occupy up to half of the abdominal cavity. This stage is not included in SSB.	PG1, PG2, CA
III. Spawning a. Spawning (inactive)	Maturing and re-maturing: yolked opaque oocytes visible to the naked eye; ovaries change from semi-transparent to opaque yellow-orange or reddish in color as more oocytes enter the yolk stage; ovaries occupy at least half	PG1, PG2, CA, VT1, VT2, VT3, +/- POF

b. Spawning (active)	of the body cavity; re-maturing ovaries may be red to grey-red or purple in color and less firm than an ovary maturing first batch, few hydrated oocytes may be left Spawning active. Hydrated eggs are visible among yolked opaque oocytes; hydrates oocytes may be running; ovaries fill the body cavity; overall color varies from yellowish to reddish.	PG1, PG2, CA, VT1,VT2, VT3, HYD, POF
IV.a Cessation IV.b. Recovery	Baggy appearance; bloodshot; grey-red translucent in color; atretic oocytes appear as opaque irregular grains; few residual eggs may remain Ovaries appear firmer and membranes thicker than in sub-stage IV.a; these characteristics together with the slightly larger size distinguish this stage from the virgin stage; ovaries appear empty and there are no residual eggs; transparent to wine red translucent in color	PG1, PG2, POF, atretic oocytes, residual HYD PG1, PG2, atretic VT oocytes
V. Resting	Ovaries appear more tubular and firmer; oocytes not visible to the naked eye; transparent or grey-white to wine red in color with well-developed blood supply; this stage leads to stage II.	PG1, PG2 +/- atretic oocytes
VI. Abnormal	a) infection; b) intersex - both female and male tissues can be recognized; c) one lobe degenerated; d) stone roe (filled with connective tissue); e) other	Abnormal tissue
MALES (SG: Spermatogonia; PS: Primary spermatocytes; SS: Secondary spermatocytes; ST: Spermatids; SZ: Spermatozoa; SSB: Spawning stock biomass)		

I. Immature	Juvenile: Testes threadlike and small; white-grey to grey brown in color; difficult to determine sex, but distinguishable from ovaries by a more lanceolate shape (knife shaped edge of distal part of the lobe).	SG, PS
II-Preparation	Transition from immature to maturing: Testes easily distinguishable from ovaries by lanceolate shape; sperm development not clearly visible; reddish grey to creamy translucent in color; testes occupy up to ½ of the abdominal cavity; this stage is not included in SSB.	SG, PS, SS, potentially few ST
III. Spawning a. Spawning (inactive)	Maturing and re-maturing: Testes occupy at least half of the body cavity and grow to almost the length of the body cavity; the empty sperm duct may be visible; color varies from reddish light grey, creamy to white; edges may still be translucent at the beginning of the stage, otherwise opaque; re-maturing testes may be irregularly colored with reddish or brownish blotches and grey at the lower edge with partly whitish remains of sperm	SG, PS, SS, ST, SZ
b. Spawning (active)	Spawning active: testes fill the body cavity; Sperm duct filled and distended throughout the entire length; sperm runs freely or will run from the sperm duct, if transected; color varies from light grey to white..	SG, PS, SS, ST, SZ
IV.a Cessation	Baggy appearance (like an empty bag when cut open); bloodshot; grey to reddish brown translucent in color;	SG, PS, atretic SS,

	residual sperm may be visible in sperm duct	ST and SZ
IV.b. Recovery	Testes appear firmer and the testes membrane appear thicker than in stage IVa due to contraction of the testes membrane; these characteristics together with the slightly larger size distinguish this stage from the virgin stage; testes appear empty and no residual sperm is visible in the sperm duct; reddish grey to greyish translucent in color.	SG, PS, potentially SS, atretic SZ
V. Resting	Testes appear firmer, development of a new line of germ cells; grey in color; this stage leads to stage II.	SG, PS, SS
VI. Abnormal	a) infection; b) intersex - both female and male tissues can be recognized; c) one lobe degenerated; d) other.	e.g. oocytes visible among spermatogenic tissues

Batch fecundity

All fish were measured to the nearest 1 mm in the Total Length (TL) and weighted to the nearest 1 g. Gonads of the fish were examined under a dissecting microscope for its external features such as turgidity and colour in order to determine a maturity stage. The sex ratio also calculated in this study (i.e., No. of males/No. of females (Simon et al., 2012). The female was determined by the macroscopic observation of matured ovary (Laevastu, 1965a).

Batch fecundity can vary considerably during the short spawning season, low at the beginning, peaking during high spawning season and declining again towards the end.

Annual egg production is the product of the number of batches spawned per year and the average number of eggs spawned per batch.

Batch fecundity of sprat was determined as 'Hydrated Oocyte Method'. (HUNTER et all 1985). Oilly hydrated females.were used. After sampling their body cavity was opened and they were 'preserved in a buffered formalin solution (HUNTER 1985). The ovary free female weight in the ovary weight were determined: Three tissue samples of - 50 mg were removed from different parts of the ovary and their exact were determined. Under binocular number of hydrated oocytes, in each of the three subsamples was determined.

Hydrated oocytes can easily be separated from all other types of oocytes because of their large size t their translucent appearance and their wrinkled surface which is due to formalin preservation. Batch fecundity was estimated based on· the average number of hydrated oocytes per unit weight of the three subsamples.

Gonadosomatic Index (GSI) was determined monthly. GSI was calculated as:

$$GSI = \frac{GW}{SW} X\ 100$$

where: *GW* is gonads weight and *SW* is somatic weight (represents the BW without GW).

For the estimation of sprat growth rate, the von Bertalanffy growth function (1938) is used, (according to Sparre, Venema, 1998):

(11) $L_t = L_\infty \{1 - \exp[-k(t - t_0)]\}$

(12) $W_t = W_\infty \{1 - \exp[-k(t - t_0)]\}^n$

where: L_t, W_t are the length or weight of the fish at age t years; L_∞, W_∞ - asymptotic length or weight, k – curvature parameter, t_0 - the initial condition parameter.

The length – weight relationship is obtained by the following equation:

(13) $W_t = qL_t^n$

where: q – condition factor, constant in length-weight relationship; n – constant in length-weight relationship.

Coefficient of natural mortality (M)

Pauly's empirical formula (1979, 1980) is applied:

(14) $\log M = -0.0066 - 0.279 * \log L_\infty + 0.6543 * \log k + 0.4634 * \log T°C$

(15) $\log M = -0{\cdot}2107 - 0{\cdot}0824 \log W_\infty + 0{\cdot}6757 \log K + 0{\cdot}4627 \log T°C$

where: $L\infty$, $W\infty$ and κ – parameters in von Bertalanffy growth function; $T°C$ - average annual temperature of water, ambient of the investigated species.

Feeding of sprat

The study includes analysis of stomach content composition of number of specimens, collected in front of the Bulgarian Black Sea coast during the year, and it encompasses additional analyses of the zooplankton species composition and biomass in the marine environment.

Per trawl catch, individuals will be separated and preserved in 10 % formaldehyde: seawater solution. The absolute length (TL, to the nearest 0.1 cm) and weight (to the nearest 0.01 g) of fish specimens were measured. Under laboratory conditions, the stomachs of the selected animals were weighted with analytical balance (to the nearest 0.0001 g). The food mass of each individual has been calculated as a difference between the weights of full and empty sprat stomach.

The stomach content was investigated under a microscope for the estimation of species composition and prey number. The prey biomass was estimated by multiplication of the number of consumed mesozooplankton species by their weights.

The following indices were calculated:

1. Stomach fullness index (ISF) as a per cent of body mass: (stomach content mass/fish mass) *100; and

2. Index of relative importance - IRI, Pinkas et al. (1971): IRI = (N+M) x FO; where N - the proportion of prey taxa (species) in the diet by numbers (abundance); M - the percentage of prey taxa (species) in the diet by mass; FO - frequency of occurrence among fish.

The zooplankton samples in the marine environment were gathered from the whole water layer (bottom- surface) with a plankton set (opening diameter d = 36 cm; mesh size 150 µm). The samples were fixed onboard ships with 4% formaldehyde: seawater solution (Korshenko & Aleksandrov, 2013).

The mesozooplankton species composition has been identified by “Guides for the Black and Azov Seas” (Morduhai-Boltovskii et al., 1968), and its quantity - by the method of Bogorov (Korshenko & Aleksandrov, 2013).

SELECTIVITY OF THE FHING GEAR

The change in mesh size of the codend is the basis of the analysis of the selectivity in the calculations. The mesh size (a, mm) of the trawl bag is shown in Fig. 3.8.1. The study of the variation in the trawl selectivity is based on calculations at the corresponding change in the size of the "eye" side.

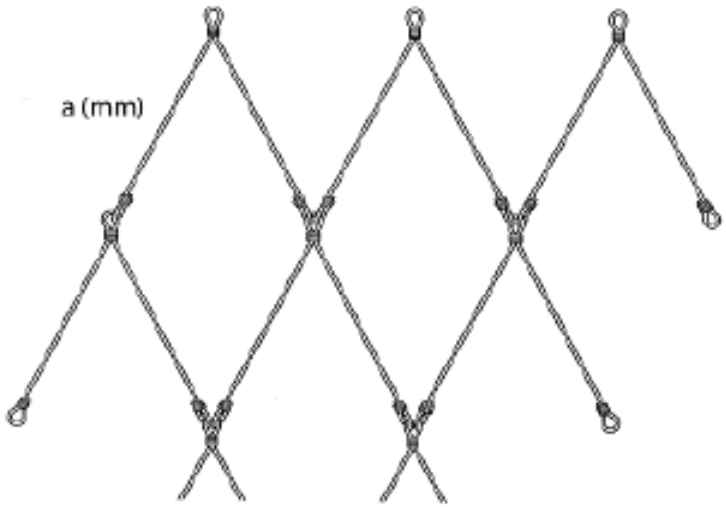


Figure 3.8.1. "Eye" of the codend and size a (mm)

Using the model of Tresthev (1974), it was worked out to construct an additional trawl bag to experimentally study the change in selectivity:

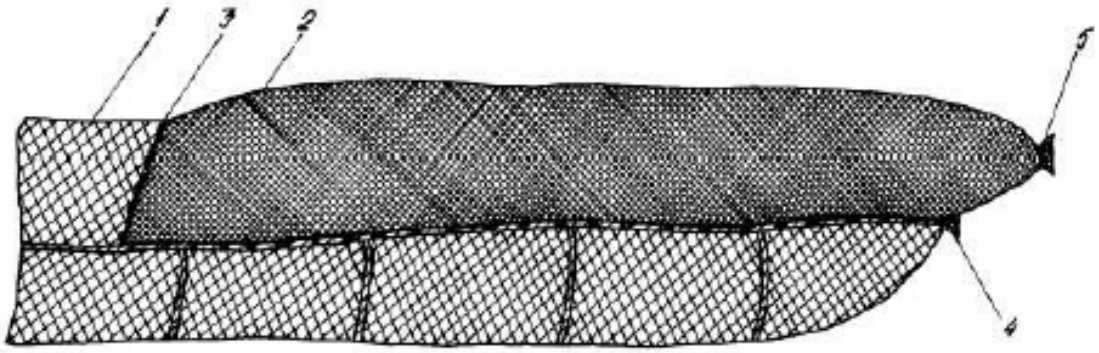


Figure 3.8.2 Codend bag scheme: 1 - main bag 2 - apron; 3 - connector, 4 - the main bag 5 - the trailer outer bag connection.

Linear size measurements were used to evaluate the following biological parameters:

L50, L25 and L75 the amount at which 50%, 25% and 75% of the individuals entered into the fishing gear are detained therein;

Selectivity factor (c) an extent of selectivity

The dimensional selectivity of the trawl bag is determined by the relationship between the probability p, the fish entering the bag and its size l (Holden, 1971). This link is described by the logistic function (Fryer, 1991):

$$p = \frac{e^{(v_1 + v_2 \mathbb{D})}}{(1 + e^{(v_1 + v_2 \mathbb{D})}}$$

where: *v1* represents the intersection of the abscissa, *v2* is the slope of the curve following log-transformation. The L50, L25 and L75 function values can be estimated from the following expressions:

$$L_{50\%} = \frac{v_1}{v_2} \qquad L_{25\%} = \frac{(-Ln(3) - v_1}{v_2} \qquad L_{75\%} = \frac{(Ln(3) - v_1}{v_2}$$

$$SR = L_{75} - L_{25} \qquad SF = \frac{L_{50}}{meshsize}$$

Suppose that fish of size: l1, l2,. . .1N enter the trawl bag. Small fish may loose through the mesh (ie, have a low probability of retention), but as they grow in length, the chance to get rid of the net decreases. At some point, because of their increased size, they can not get out of the net (their probability of retention equals 1).