Methodology for pelagic research in the Bulgarian Black Sea waters

1. Preface

In Pelagic survey (PTSBS) conducted in Bulgarian marine zone, both manuals of MEDITS (Methodology_for_demersal_research_in_the_Bulgarian_Black_Sea_waters_L.pdf) and MEDIAS (http://www.medias-project.eu/medias/website/handbooks-menu/handbooks/MEDIAS-Handbook-(April-2021)/) have been followed.

All the data collected from pelagic and bathy pelagic research expeditions were entered in the program developed by COISPA, **Biondex Script** (version 3.1) running in program RStudio Version 1.1.463, through fisheries data processing software, including statistical data processing, in accordance with the requirements of the European Commission Regulations. Before entering the data in the Biondex script, are organized in the format of MEDITS tables, being verified with the RoME script (version 0.2.01) to perform multiple data checks. The RoME package includes functions related to single checks for a total of 55 functions ("facility functions"), associated with a certain control in the tables TA, TB, TC, TD, TT, TE and TL. The results obtained by running this script are saved in the OUTPUT folder, like JPEG, TIFF and CSV files and are presented like maps and tables that include data related to: * the surface of the researched square (Km^2, m^2); * the average mass per unit area (g/m^2, t/Km^2); * the mass limits variation per unit area; * the total biomass values (t); * the abundance index ($individuals/km^2$). Pelagic expeditions for the assessment of turbot and dog fish agglomerations provide additional information for the calculation of the catch effort per unit CPUE (kg*h-1) and of catch per unit area CPUA (kg/km^2) in the researched areas. The collected data are stored in the IO-BAS database, as well as in a special module created within EAFA Bulgaria.

2. *Research vessel and gears*

The Pelagic Trawl survey (PT) was accomplished on the board of research vessel *HaitHabu* (Pic. 1.1; 1.2.). The main characteristics of the ship are listed below.

Picture 1.1. R/V *HaitHabu*

R/V *HaitHabu*

IMO: 8862686
MMSI: 207139000
Call sign: LZHC
Flag: Bulgaria [BG]
AIS Vessel Type: Other
Gross Tonnage: 142
Length Overall x Breadth Extreme:24.53m × 8m Crew: 6

Picture 1.2.. Catch of the OTM

3. *Material and Methods*

Pelagic Trawl surveys were accomplished following National Programs for Data Collection in the Fisheries sector of Bulgaria for 2020-2021. The study was conducted during the period July 2020 , in the area enclosed between Durankulak and Ahtopol (Bulgaria) with a total length of the coastline of 370 km. The study area encloses waters between 42°05' and 43°45' N and 27°55 and 29°55 E.

During the survey, a total of 37mid-water hauls were carried out in the Bulgarian area (July 2020). The survey took place during the day and the following types of data were collected:

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

3.1. *Sampling design*

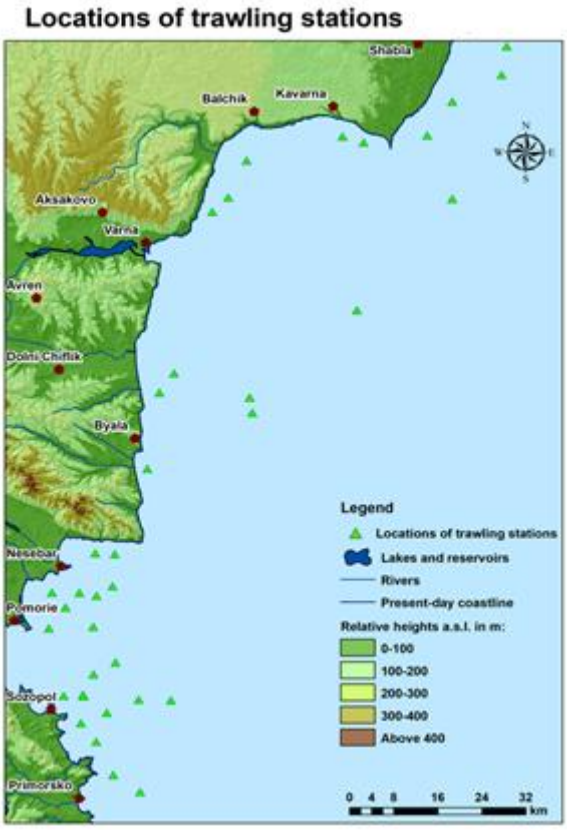
To establish the abundance of the reference species (*Sprattus sprattus*) in front of the Bulgarian coast a standard methodology for stratified sampling was employed (Gulland, 1966). To address the research objectives the region was divided into 3 strata according to depth: Stratum 1 (15 - 30 m), Stratum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

The study area in Bulgarian waters was partitioned into 128 equal in size, not overlapping fields, situated at a depth between 16 - 92 m. At 37of the fields chosen at random, sampling employing mid-water trawling was carried out (Pic. 3.1.1).

Picture 3.1.1. Trawling operation

Each field was 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 were grouped in larger sectors, so-called strata, in which geographic and depth boundaries were 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.



Scheme.3.1.2. sampling scheme stations

3.2. *Onboard sample processing*

The data recorded and samples collected at each haul include (Gulland,1966):

- Depth, measured by the vessel's echo sounder
- GPS coordinates of start/end haul points
- Haul duration
- An 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 the conservation of sprat for stomach content examination.

3.3. *Laboratory analyses*

The samples collected onboard were processed in a laboratory for determination of age and food composition.

The age was established in otoliths under the binocular microscope.

The food spectrum was determined by separation of the stomach contents into taxonomic groups identified to the lowest possible level.

3.4. *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 a direct method for demersal species stock assessment (Foote,1996).

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) $a = D * hr * X2$
$D = V * t$

(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 a fraction of catch per unit effort from the 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 (km^2) 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 4):

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

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

$A = A1+A2+A3$

Average weighted 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 – an area of stratum 1, etc., A- size of total area.

Accordingly, total stock biomass for the whole marine area:

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

Where: $\overline{Ca}(A)$ - average weighted 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 7):

(9) MSY = 0.5*M*Bv

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

A 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

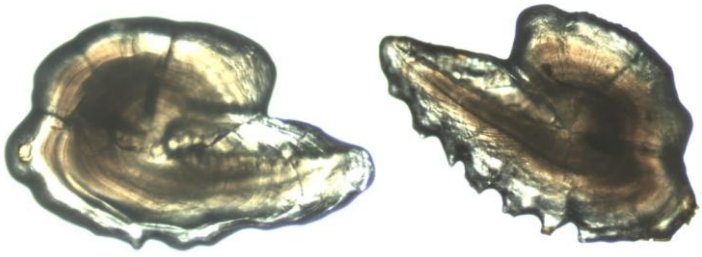
Several methods are available with the help of which total mortality (Z) can be estimated from length-frequency data. Thus, it is possible to obtain reasonable estimates of Z from the mean length in a representative sample or the slope of Jones' cumulative plot. In this article, a variety of approaches for analyzing length-frequency 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.

3.5. Age estimation

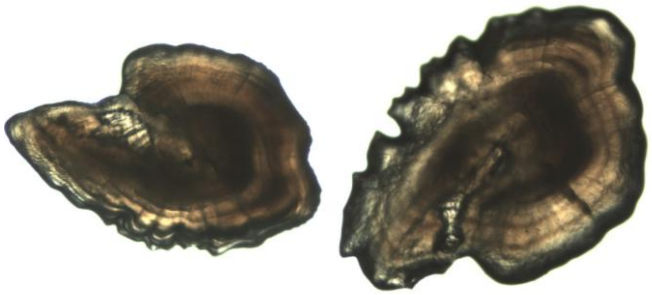
As it is well known, the Calcified Structures (CS) are usually used to assign age useful to obtain their growth model and so, to reconstruct age composition of exploited fish populations. Fish aging

implies the presence 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 aging are different: otoliths (sagittal, 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, aging criteria) regarding the age analysis are addressed by species. Otoliths are important for fish and fisheries scientists. Otoliths are playing role of balance, motion, and sound. These structures are effective from growth to death in the entire life cycle. They are most commonly used to determine growth age and for mortality studies. Research on otoliths began in the 1970s and continued to 21st century. Periodic growth increments in scales, vertebrae, fin rays, in cleithra, opercula, and otolith are used to determine annual age in many fish species.

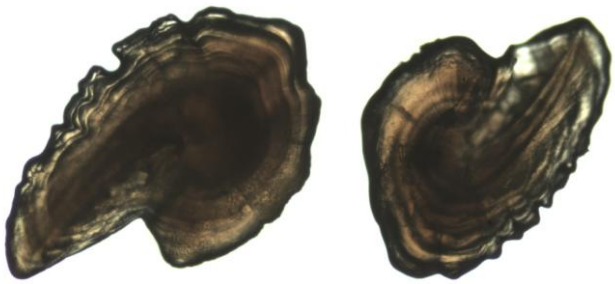
Researchers used otolith reference collections and photographs in publications to aid in identification (Pic. 3.5.1). Otoliths have a distinctive shape that is highly specific but varies widely among species.



6 cm (0+) 7.5 cm (1+)



8.2 cm (1+) 9 cm (2+)



9.5 (3+) 10.7 (4+)

Picture 3.5.1. Otoliths of sprat

Biologists, taxonomists, and archaeologists, based on the shape and size of otoliths determine fish predators feeding habits (Kasapoglu and Duzgunes, 2014). In teleost fishes, otoliths are the main CS

for age determination and it is widely used in fisheries biology. On the other hand, analyzing O_2 isotopes in their structure is useful to determine fish migrations between freshwater and sea as well as species and stock identification. Otoliths are the balance and hearing organs for the fish. They are three types located on the left and right side of the head in 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. 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 restoration or regeneration.
- Having the 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 $CaCO_3$ accumulations on the otoliths.

3.5.1. Otolith preparation for sprat

For the sampling of fish for otolith extraction from the overall samples is very important to have representative samples for the catch. The number of otoliths needed is lower for the species having a smaller size range than the species having a larger size range. According to the availability, 5 fish for each length group may be better for age readings to be representative of the population. Each of the individuals should be recorded individually with the 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 the 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.

3.5.2. Preparation of the otoliths for the age determination

Otoliths are put into small black convex glasses containing 96% ethyl alcohol for age readings under the binocular stereo microscope which is illuminated from top and sides (Fig. 3.5.2.1) (Polat and

Beamish, 1992). The magnifying level depends on the size of the otolith; X4 is good for sprat and X1 for turbot.

Figure 3.5.2.1. Binocular stereo microscope with top and side illumination

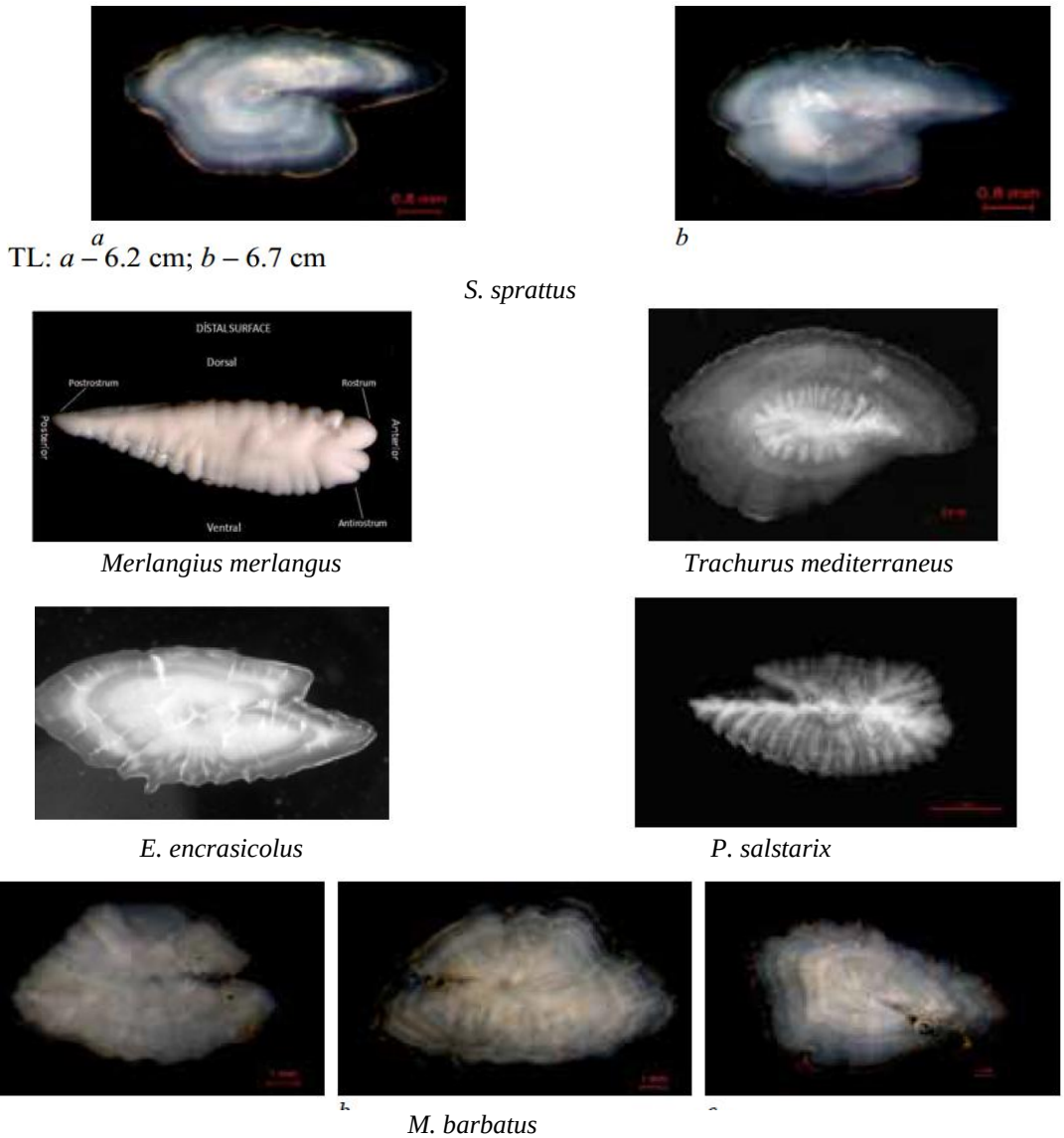
3.5.3. Age readings and commenting on annuluses

To prevent bias, during age reading reader should not refer the length and weight of that fish. But information on the date of the catch and gonadal state is very important. The first step is to clarify the place of the center and the first age ring. After that, observation of the successive rings, whether they are continuous or not is important.

Finally, determination of the fish in growth or just at the end of the growth period by checking characteristics of the ring at the edge of the otolith to decide it is opaque or hyaline. After these procedures otoliths can be read under these protocols which are very important to provide data on age to determine realistic population parameters and reduce uncommon procedures and biases by standardized age reading criteria.

3.5.4. Sprat (Sprattus sprattus)

In sprat left and right otoliths show isometric growth. They are small and transparent (Fig. 3.5.4.1). Age readings can be done over the otolith surface by clear ring views. Due to summer and winter growths, there are two different nucleus formations in the center; spring recruits have opaque, late fall recruits have hyaline rings which are taken into consideration during age readings (Pısıl, 2006).



a *b*

TL: *a* – 6.2 cm; *b* – 6.7 cm

S. sprattus

Merlangius merlangus

Trachurus mediterraneus

E. encrasicolus

P. salstarix

M. barbatus

Figure 3.5.4.1. Sprat, anchovy, horse mackerel, red mullet, bluefish otoliths

3.5.5. Age reading protocol

1. Dissected otoliths rinsed and treated with 96% ethyl alcohol and stored dry.
2. Readings were 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 was set considering the biggest otolith size which fitted the visual capacity of the lens. It was aimed not to change magnification rate which might enable false rings visible in bigger otoliths and permitted to see true rings (hyalines) better by unchanging the color contrasts. That's why magnification rate X4 was selected for the sprat otoliths.

4. Otolith samples were observed from the distal surface as a whole, broken ones were not used.
5. Birthday of the sprat is accepted as 1st of January as the common principle for the fish living in the Northern hemisphere in line with the sub-tropic fish growth models.
6. Central point surrounded by the hyaline rings which is one in some cases or two for the others is formed after the end of consumption of yolk sac and starting of free feeding, known as "stock rings". Next opaque accumulation is known as "first-year growth ring". This ring keeps its circular form in the postrostrum region. Both, this ring and the next hyaline ring forming "V" shape in the rostrum, are accepted as first age rings.
7. Tiny and continuous concentric rings prolonged close to the real hyaline ringe are counted together with the real one as one age. This ring may be either a very tiny and opaque one inside the hyaline band or tiny hyaline ring near the outer edge of the opaque ring.
8. Sprat and some other short-lived species have a very fast growth rate, especially in the first two years. Width of the growth bands after 2nd year ring becomes relatively narrower. This issue should be kept in mind in the older age ring readings.

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

3.6. *Sex and maturity estimation*

3.6.1. Sprat

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 that may range from early spring to 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 phylogeographic 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).

3.6.2. 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 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 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. 3.6.2.1, Table 3.6.2.1)

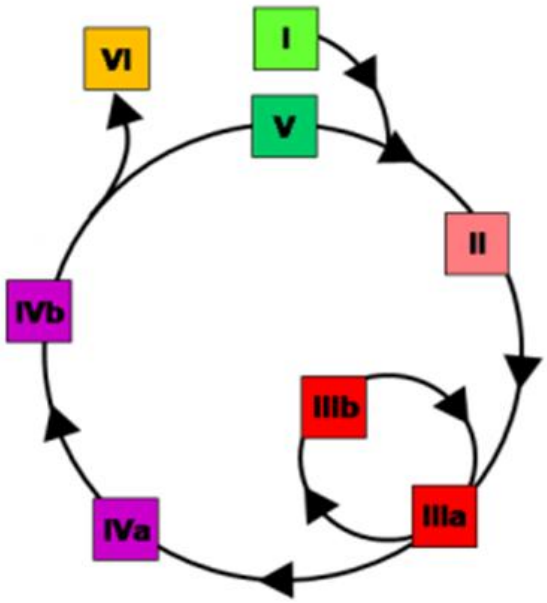


Figure 3.6.2.1. Scale with six maturity stages in sprat
(names of the stages are given in Table 3.6.2.1)

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 3.6.2.1. 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	*The transition from immature to early maturing; oocytes not visible to the naked eye; ovaries yellow-orange to bright red; 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 as more oocytes enter the yolk stage; ovaries occupy at least half of the body cavity; re-maturing ovaries may be red to grey-red or purple in color and less firm than an ovary maturing the first batch, few hydrated oocytes may be left*	*PG1, PG2, CA, VT1, VT2, VT3, +/- POF*
b. Spawning (active)	*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	*Baggy appearance; bloodshot; grey-red translucent in color; atretic oocytes appear as*	PG1, PG2, POF, atretic oocytes,

IV.b. Recovery	*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*	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 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; difficult to determine sex, but distinguishable from ovaries by a more lanceolate shape (knife-shaped edge of the distal part of the lobe).*	SG, PS
II-Preparation	*The transition from immature to mature: Testes easily distinguishable from ovaries by lanceolate shape; sperm development not 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*	*SG, PS, SS, ST, SZ*

c. Spawning (active)	*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* *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 IV.b. Recovery	*Baggy appearance (like an empty bag when cut open); bloodshot; grey to reddish-brown translucent in color; residual sperm may be visible in the sperm duct.* *Testes appear firmer and the testes membrane appears 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, atretic SS, ST and SZ* *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*

3.6.3. 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 turbidity and color to determine a maturity stage. The sex ratio was 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 mature ovary (Laevastu, 1965).

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 using the 'Hydrated Oocyte Method' (Hunter et al., 1985). Oily hydrated females were used. After sampling their body cavity was opened and they were preserved in a buffered formalin solution (Hunter et al., 1985). The ovary-free female weight and the ovary weight were determined. Three tissue samples of ca. 50 mg were removed from different parts of the ovary and their exact weight determined. Under a binocular, the 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, 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 the 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):

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

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

where

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

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

$$W_t = qL_t^n \quad (13)$$

where

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

Coefficient of natural mortality (M)

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

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

(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_{∞}, W_{∞} and κ – parameters in von Bertalanffy growth function, $T^{o}C$ - an average annual temperature of the water, ambient of the investigated species.

3.7. *Feeding of sprat (Sprattus sprattus, L)*

Per trawl catch, about 10/11 fish specimens were 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).

Cluster analysis (PRIMER 7.0.17) was used to group the data on the food spectrum of sprat from different depths and study stations.

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