



Pelagic trawl survey in Bulgarian marine area, December 2015

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Institute of Oceanology – BAS, Varna, Bulgaria

The survey was carried out during the period 03.12.2015 – 20.12.2015 in Bulgarian Black Sea area on board of F/V EGEO 3” in execution of National Programs of Bulgaria for data collection in 2015.

List of authors:



BULGARIAN ACADEMY OF SCIENCES
INSTITUTE OF OCEANOLOGY
VARNA

Associate Professor Violin Raykov, Ph.D.
Chief assistant Maria Yankova, Ph.D.
Associate Professor Petya Ivanova, Ph.D.
Associate Professor Kremena Stefanova, Ph.D.
Chief assistant Elitssa Stefanova, Ph.D.
Chief assistant Antoaneta Trayanova, Ph.D.

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1 Biological results from International Pelagic Trawl Survey in December 2015

1.1. Summary

Pelagic Trawl Survey was accomplished in December 2015 in the Bulgarian Black Sea area. Scientific team has produced a biological analysis of the results obtained in the marine area.

The analysis is based on the reference species biomass (*Sprattus sprattus*) at the scale of the whole survey. Furthermore, an analysis of the distribution and abundance of the rest species caught as by-catch, is presented. The Black Sea Sprat (*Sprattus sprattus*) is a key species for the Black Sea ecosystem. Together with the anchovy, sprat is one of the most abundant, planktivorous, pelagic species. The level of its stocks depends on the conditions of the environment mainly and on the fishing effort.

The changes in the environment due to anthropogenic influence, affect the dry land as well as the world ocean. The level of the sea pollution and its “self-purifying” ability are completely different. There is a clear indication of changes in the nature equilibrium in the corresponding ecological niches.

The greatest impact in the world ocean has the commercial fishery, which directly devastates a significant part of the given species populations. As a result of this some of the species stocks are declined or depleted.

As a result of the excessive exploitation, altered habitats and climatic variations numerous of the commercial species are critically endangered or vulnerable.

The abundance of the given fish species generations is dependent on different abiotic and biotic factors. With great importance are: the level of fishing mortality, changes in trophic levels due to mass occurrence of the ctenophore *Mnemiopsis leidyi*, algal blooms which lead to hypoxia in the shallower waters with mass mortality of the bottom dwelling organisms and etc.

Recent state of the sprat stock biomass (aggregations) off Bulgarian Black sea coast show relative stability i.e. taking into consideration almost constant level of exploitation (in western and north-western part of the Black Sea) in the last years the stock possibly is underexploited yet.

All analyses are based on the biomass and density estimates and by geographical strata. All the teams calculated their standard statistical estimates using the same software.

This report presents successively the results obtained at these two levels. The regional reports are presented in an order following the coast, from the northern to southern part of the Black Sea. The document is completed by a series of tables and figures related to the biomass/abundance indices and length frequency distributions of the species included in the reference list.

2 Fishing vessel and fishing gear

The Pelagic Trawl survey (PT) was accomplished on board of fishing vessel “EGEO 3”,. The main characteristics of the ship are given bellow:



Picture 2.1. F/V Egeo

- | | |
|----------------------------|------------------|
| - Name - | “EGEO 3”; |
| - Length overall - | 17.5 m; |
| - Maximum molded draught - | 2 m |
| - Year of construction - | 2013 |
| - Effective power - | 265 kW |
| - Gross tonnage - | 23.5 t; |
| - Speed - | 11 knots; |
| - Number crews - | 5 |
| - Number researchers - | 2 |

The dimensions of the pelagic trawl (Pict. 2.2 and 2.3) employed are as follows:

- type of pelagic trawl 50/35 – 74 m
- Length of the head rope – 40 m.
- Horizontal spread of trawl – 16 m
- Vertical spread of trawl – 7 m
- Mesh size of the net – 7x7 mm.
- Effective part of wing spread – 27 m.
- Pelagic doors – 3.5 m².

The hauls were carried out during the day with single haul duration between 30 - 40 min., depending on hydro-meteorological conditions at average trawl speed 2.7 knots (2.7-2.9).



Picture2.2. Pelagic trawl of F/V “EGEO 3” employed in the study.



Picture 2.3. Catches in trawl codend of F/V “EGEO 3”

3, Material and Methods

Pelagic Trawl survey was accomplished with accordance with National Programs for Data Collection in Fisheries sector of Bulgaria for 2015. The study held during the period of December 2015, in the area enclosed between Durankulak and Ahtopol (Bulgaria) with total length of coastline of 370 km. Study area encloses waters between 42°05' and 43°45' N and 27°55 and 29°55 E.

During the survey, total 36 mid-water hauls were carried out in Bulgarian area (December 2015). 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

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 in four strata according to depth – Stratum 1 (15 - 35 m) Stratum 2 (35 – 50 m), Stratum 3 (50 – 75 m) and Stratum 4 (75 – 100 m).

The study area in Bulgarian waters was partitioned into 128 equal in size not overlying fields, situated at depth between 10 - 100 m. At 36 of the fields chosen at random, sampling by means of mid-water trawling was carried out (Fig.3.1.1.).

Each field is a rectangle with sides 5' Lat × 5' Long and area around 62.58 km² (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.

The arrangement of stations is represented on the map of Fig.3.1.1. As a result of the trawling survey a biomass index was calculated.

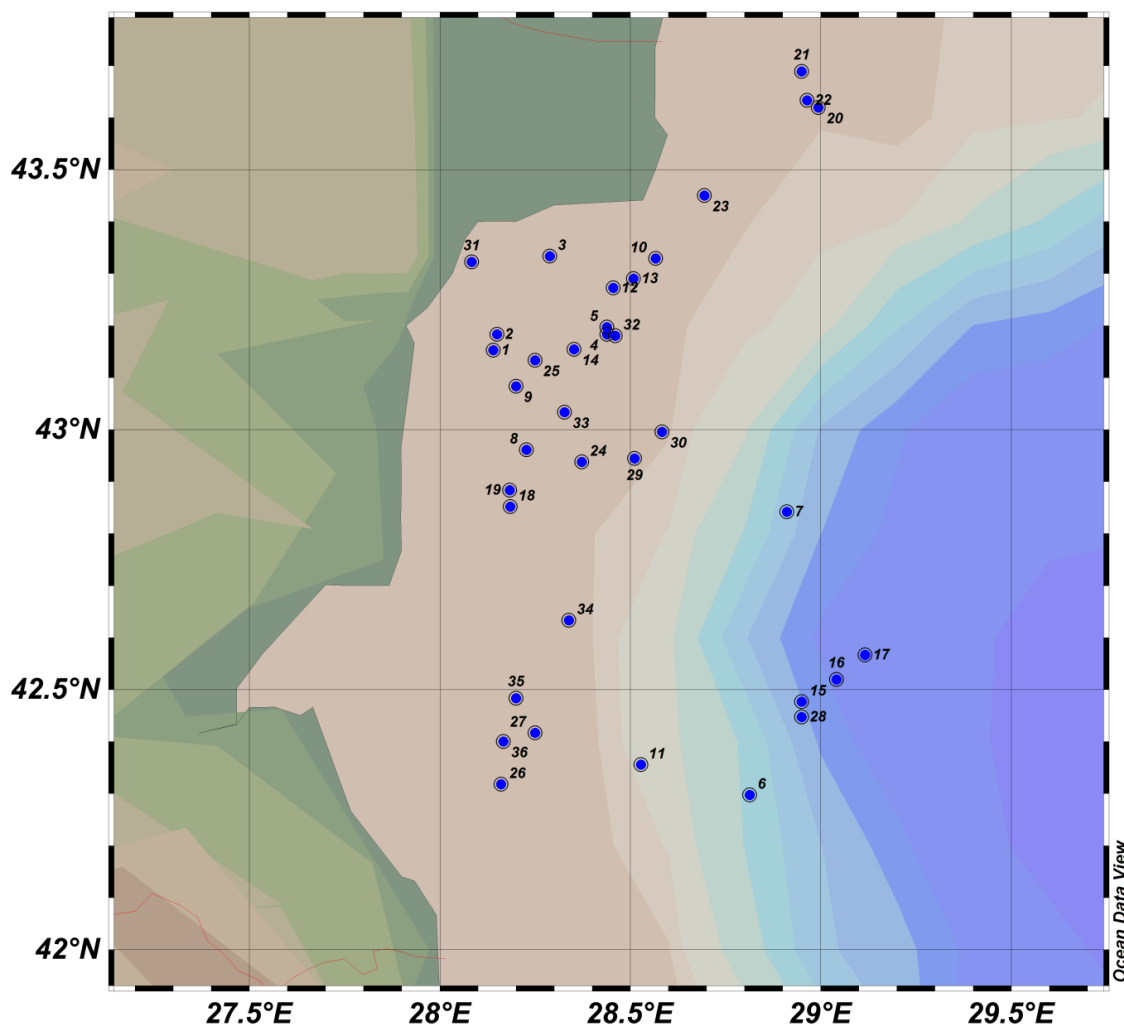


Figure 3.1.1. Research area and plan of the sampling fields.

3.2 Onboard sample processing

The data recorded and samples collected at each haul include:

- Depth, measured by the vessel's echosounder;
- 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.

3.3 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.

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 direct method for demersal 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:

$$(1) \begin{aligned} a &= D * hr * X^2 \\ D &= V * t \end{aligned}$$

(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), $dirV$ 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:

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

Where: $C_{w/t}$ – 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 4):

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

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

$$A = A1 + A2 + A3$$

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

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

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:

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

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:

$$(9) \quad MSY = 0.5 * M * B_v$$

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

Relative yield-per-recruit model with uncertainties

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

where: $U = 1 - (L_c / L_\infty)$

$m = (1 - E) / (M/k) = k/Z$

$E = F/Z$ – exploitation coefficient.

Age and growth Length-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 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 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 in 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, cleithra, 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 species¹⁶. 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 “asteriscus” 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 (Aydin, 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.

3.5.1 Otolith Preperation for sprat and turbot

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 ($\pm 0,1$ cm), total weight ($\pm 0,01$ g), sex, maturation stage (I-V), gonad weight ($\pm 0,01$ g) 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.

3.5.2 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 the size of the otolith; X4 is good for sprat and X1 for turbot.



Fig 3.5.2.. Binocular stereo microscobe with top and side illumination (Yoraz, 2015)

3.5.3. Age readings and commenting on annuluses

In order to prevent bias, during age reading reader should not refer length and weight of that fish. But information on the date of the catch and gonadal state is very important.

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 phalericus*)

In sprat left and right otoliths shows isometric growth. These are small and transparent (Fig 2). Age readings can be done over otolith surface by clear ring views. Due to summer and winter growths there are two different nucleus formation in the center; spring recruits has opaque, late fall recruits has hyaline rings which is taken into consideration during age readings (Pisil, 2006).



TL: $a - 6.2$ cm; $b - 6.7$ cm



b

Figure 3.5.4. Sprat otoliths

3.5.5. Sprat age reading protocol

1. Dissected otoliths rinsed and treated 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 (hyalines) better by unchanging the color contrasts. That's why magnification 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 hemisphere 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 yolk 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 continuous concentric rings prolonged close to real hyalin ringed are counted together with the real one as one age. This ring may be either a 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.
9. 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 unseparable, 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 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).

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 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 (Fig3.6.2.Table 3.6.2.);

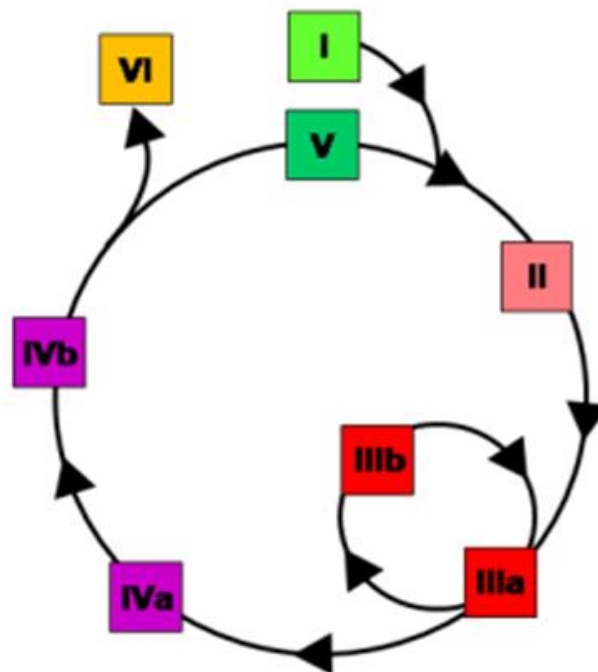


Figure3.6.2. Scale with six maturity stages in sprat (Name of the stages are given in Table 3.6.3)

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
C

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) b. Spawning (active)	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 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, +/- POF PG1, PG2, CA, VT1,VT2, VT3, HYD, POF
IV.a Cessation	Baggy appearance; bloodshot; grey-red translucent in color; atretic oocytes appear as opaque irregular grains; few residual eggs may	PG1, PG2, POF, atretic

IV.b. Recovery	<p>remain</p> <p>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</p>	<p>oocytes, residual HYD</p> <p>PG1, PG2, atretic VT oocytes</p>
V. Resting	<p>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.</p>	<p>PG1, PG2 +/- atretic oocytes</p>
VI. Abnormal	<p>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</p>	<p>Abnormal tissue</p>
<p>MALES (SG: <i>Spermatogonia</i>; PS: <i>Primary spermatocytes</i>; SS: <i>Secondary spermatocytes</i>; ST: <i>Spermatids</i>; SZ: <i>Spermatozoa</i>; SSB: <i>Spawning stock biomass</i>)</p>		
I. Immature	<p>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).</p>	<p>SG, PS</p>
II-Preparation	<p>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.</p>	<p>SG, PS, SS, potentially few ST</p>
III. Spawning	<p>a. Spawning(inactive)</p> <p>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</p> <p>c. Spawning (active)</p> <p>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..</p>	<p>SG, PS, SS, ST, SZ</p> <p>SG, PS, SS, ST, SZ</p>
IV.a Cessation	<p>Baggy appearance (like an empty bag when cut open); bloodshot; grey to reddish brown translucent in color; residual sperm may be visible</p>	<p>SG, PS, atretic SS, ST and SZ</p>

IV.b. Recovery	in sperm duct. 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 was determined as '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 1985). The ovary free female weight and the ovary weight were determined: Three tissue samples of ~ 50 mg were removed from different parts of the ovary and their exact weight 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 and 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:

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

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):

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

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

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:

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

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:

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

$$(15) \quad \log M = -0.2107 - 0.0824 \log W_{\infty} + 0.6757 \log K + 0.4627 \log T^{\circ}C$$

where: L_{∞} , W_{∞} and k – parameters in von Bertalanffy growth function; $T^{\circ}C$ - average annual temperature of water, ambient of the investigated species.

Food composition

Zooplankton collection, preservation and laboratory analysis

Samples were collected by vertical plankton Juday net, 0.1 m² mouth opening area, 200 µm mesh size, from 2 meters above the bottom to the surface at integrated sampling layer. Total of 26 zooplankton samples were collected from 26 stations during the cruise. Before sample preservation, the gelatinous species (*Aurelia aurita*, *Pleurobrachia pileus*, *Mnemiopsis leidyi*) were removed, rinsed, measured and counted on board. The samples were preserved in 4% buffered to pH 8-8.2 with disodiumtetraborate (borax) (Na₂B₄O₃·10H₂O) formalin solution (Korshenko&Aleksandrov, 2006).

Species were identified according to Morduhay-Boltovskoy (1968, 1969, and 1972). Species quantity was determined by the method of Dimov (1959). Biomass was estimated using species individual weight by Petipa, 1959.

To estimate the importance of each food item among the forage, an Index of Relative Importance IRI (Pinkas et al., 1971) was calculated as follows– equation 14:

$$(16) \quad IRI = (CN + CW) * F$$

where: CN – is the percentage of food item i in total number; CW - is percentage of food item i in total weight; F – is percentage of occurrence frequency in the food item i.

To estimate the importance of each food item among the stomach contents, IRI expressed on a percent basis (Cortes, 1997) was also calculated:

$$(17) \quad \%IRI_i = \frac{100 * IRI_i}{\sum_i^n IRI_i} \quad \text{where: } n - \text{ is the total number of food categories considered at a given taxonomic level.}$$

Zooplankton collection, preservation and laboratory analysis

Total of 11 zooplankton samples were collected from 11 monitoring stations. Samples were collected by vertical plankton Juday net, 0.1 m² mouth opening area, 150 µm mesh size, from 2 meters above the bottom to the surface at integral sampling layers. Before sample preservation, the gelatinous species (*Aurelia aurita*, *Pleurobrachia pileus*, *Mnemiopsis leidyi*, *Beroe ovata*) were removed, rinsed,

measured and counted on board. The samples were preserved in 4% buffered to pH 8-8.2 with disodiumtetraborate (borax) ($\text{Na}_2\text{B}_4\text{O}_3 \cdot 10 \text{ H}_2\text{O}$) formalin solution (Korshenko&Aleksandrov, 2012).

Species were identified according to Morduhay-Boltovskoy (1968, 1969, and 1972) manuals. Species quantity was determined by the method of Dimov (1959). Biomass was estimated using species individual weights by Petipa, 1959.

Sprat feeding

The study of sprat diet was based on stomach content analysis of 132 fish individuals collected in front of Bulgarian Black Sea coast in the period 03.12 – 19.12.2015. The coordinates of the zooplankton sampling sites and sites of sprat diet were presented at Table 1. Two of stations were located at coastal area (st. 6 and 7) and shelf (30-200m).

After the each trawl catch about 20-25 sprat individuals were separated and immediately preserved in 10 % formaldehyde seawater solution. The total length (TL, to the nearest 0.1 cm) and weight (to the nearest 0.01 g) of 12 randomly selected sprat specimens from each sample were measured in laboratory conditions. Each stomach of selected animals was weighted with analytical balance (with 0.0001 g precision) after its removal. The food mass of each individual has been calculated as a difference between the weights of full and empty sprat stomach.

Zooplankton species composition of the sprat food and prey number of the stomach content was investigated under binocular microscope "Olympus". The biomass of prey was estimated by multiplication of the registered number of consumed mesozooplankton species by their individual weights (Petipa, 1959).

The following indices were calculated: stomach fullness index (SFI) as a percent of body mass: $(\text{stomach content mass}/\text{fish mass}) \times 100$; and index of relative importance - IRI, Pinkas et al. (1971): $\text{IRI} = (\text{N} + \text{M}) \times \text{FO}$; where N - the proportion of prey taxa (species) in the diet by numbers (abundance); M - the proportion of prey taxa (species) in the diet by mass; FO- frequency of occurrence among fish.

Table 3.6.4. Location of stations corresponding to the sampling programme during the monitoring exercise (03.12-19.12.2015) in front of Bulgarian coast.

Date	Zooplankton station	Coordinates		Depth (m)	Station of sprat feeding
		Longitude	Latitude		

5.12.2015	1	28.435	43.0892	75	Stomach content
13.12.2015	2	28.3563	43.1417	45.9	Stomach content
13.12.2015	3	28.2812	43.0801	41	Stomach content
12.12.2015	4	28.5197	43.3174	48.1	Stomach content
4.12.2015	6	28.1583	43.0983	27.8	Stomach content
4.12.2015	7	28.15	43.1833	18.9	Stomach content
3.12.2015	8	28.1312	42.9778	33	Stomach content
18.12.2015	9	28.2083	42.3983	84	Stomach content
18.12.2015	10	28.0777	42.1826	58.6	Stomach content
19.12.2015	11	27.9242	42.3445	37.3	Stomach content
5.12.2015	12	28.411	42.98	85	Stomach content

4. Results

4.1 Abundance index and biomass

Introduction

Total 36 hauls in the Bulgarian marine area were carried out on board of F/V "EGEO" Total number of identified species was 17, from which 13 fish, 1 crustacean, 1 mollusc, 1 macrozooplankton and 1 ascidians species. The most frequent species in the total of hauls (in terms of presence/absence) were (in decreasing order): *S.sprattus* (100%) and *A.aurita* (95.28%), as the rest of species(*E.encarsicolus*, *A.immaculata*, *N.melanostomus*, *G.niger*,*M.merlangius*,*Tr.mediterraneus*,*Sq.acanthias* and *P.maxima* have negligible preseamnce in the catches during December 2015. In terms of weight the biggest share holds sprat with 92.8 %, followed by *Aurelia aurita* (7.2%).

In the Bulgarian Black Sea area were realized 36 hauling in the period 03/12 – 20/12/2015. The towing time was of 30 and 40 minutes, on depths between 18.9 m and 85.6 m, being surveyed area between Ahtopol and Durankulak.

The surveyed area in Bulgarian waters was about 8135.40 km². The sprat was observed on depths after 18 m.

The densest sprat aggregations were detected in the shallowest stratum 50-75 m with average 7318.46 kg.km⁻² and with average from all strata of 4889.887442 kg.km⁻²

Global comments for Fish and other species

Fish

Sprattus sprattus L.

Small pelagic species, inhabiting the continental shelf area up to 100 – 120 m.

The survey in 2015 was following the previous conducted surveys and areas. During the survey the highest biomass indices were established in stratum localized far from the shore -50-75 m in Bulgarian marine area.

The biomass index in this stratum was 20 151.54 t. In the rest of the strata the biomass was 2-3 times lower than in the shallowest stratum. The size composition ranged from 6 to 12.5 cm, the age ranged from 0+ to 4-4+, as oldest age groups and young-of-the-year was presented with low percentage.

Whiting (*Merlangius merlangus*)

Whiting inhabits layer near the sea bottom and feed on predominantly with sprat. The species is predator on sprat and is important food component for top

predators as turbot and dolphins. Whiting in December was not usual in the catches. This could be related with relatively high temperatures of SST and bottom temperatures.

Other species

Anchovy (*E. encrasicolus* L)

The species is migratory and pelagic. In the period of the survey, species not suppose to migrate in front of the Bulgarian for spawning but all observed individuals were with high level of maturation.

Scad (Tr.mediterraneus)

Migratory, small pelagic species, occurring in North-western part of the Black Sea in the time of spawning. In December, only in 4 hauls were detected, as the specimen belonged to one cohort with lengths not exceeding 5 cm (young-of-the-year)

Red mullet (Mullus barbatus)

Benthic, commercially important species, inhabiting coastal zone. Only few speciemen were detected in 2 hauls.

Round goby (N.melanostomus)

The species is benthic dwelling, with relatively good share in mid-water trawls. Only few speciemen were detected in 6 hauls.

Comments about *Sparttus sprattus* biomass by stratum in the Bulgarian area.

Table 4.1.1. Swept area method from Bulgarian marine area

CPUA mean		B (kg)	Ax	No Fields
3700.1	15-30	7641206	2065.14	33
4031.9	30-50	7317251.6	1814.82	29
7318.5	50-75	20151537	2753.52	44
3664.1	75-100	9171966.4	2503.2	40
		44281961	9136.7	146

The total surveyed area in Bulgarian part was 9136.7 km² and total estimated biomass was 44 281.961 t (*Table 4.1.1.*).

Biomass index

Biomass index 15-30m

The highest biomass index in Northern and Southern part of investigated area was detected as follows: 3370.7 t (North) and 3204.3 t (South)

Biomass index 35-50m

Two high values was estimated in southern part at the same strata: 4952.1 and 4733.6 kg/m², respectively.

Biomass index 50-75m

Extremely high value of biomass index (t) was detected in 50-75m in front of Burgas Bay, namely 18 102 t, as the CPUE kg/km² was 2400, the highest from this research. Other high values were 5243.4 ,5825.9 and 6554.2 tons – in front of Durankulak, northern part.

Biomass index 75-100m

The highest cvalue of biomass index in this strata was 4369.5 tons, detected at station F19 in southern part of Bulgarian marine area.

Table. 4.1.2. Descriptive statistics of Biomass (t) of sprat

Mean	306009.1561
Standard Error	49866.84854
Median	234078.2565
Mode	301700.8639
Standard Deviation	299201.0913
Sample Variance	89521293006
Kurtosis	18.59996662
Skewness	3.821712536
Range	1772492.576
Minimum	37712.60799
Maximum	1810205.184
Sum	11016329.62
Count	36
Confidence Level(95.0%)	101235.0839

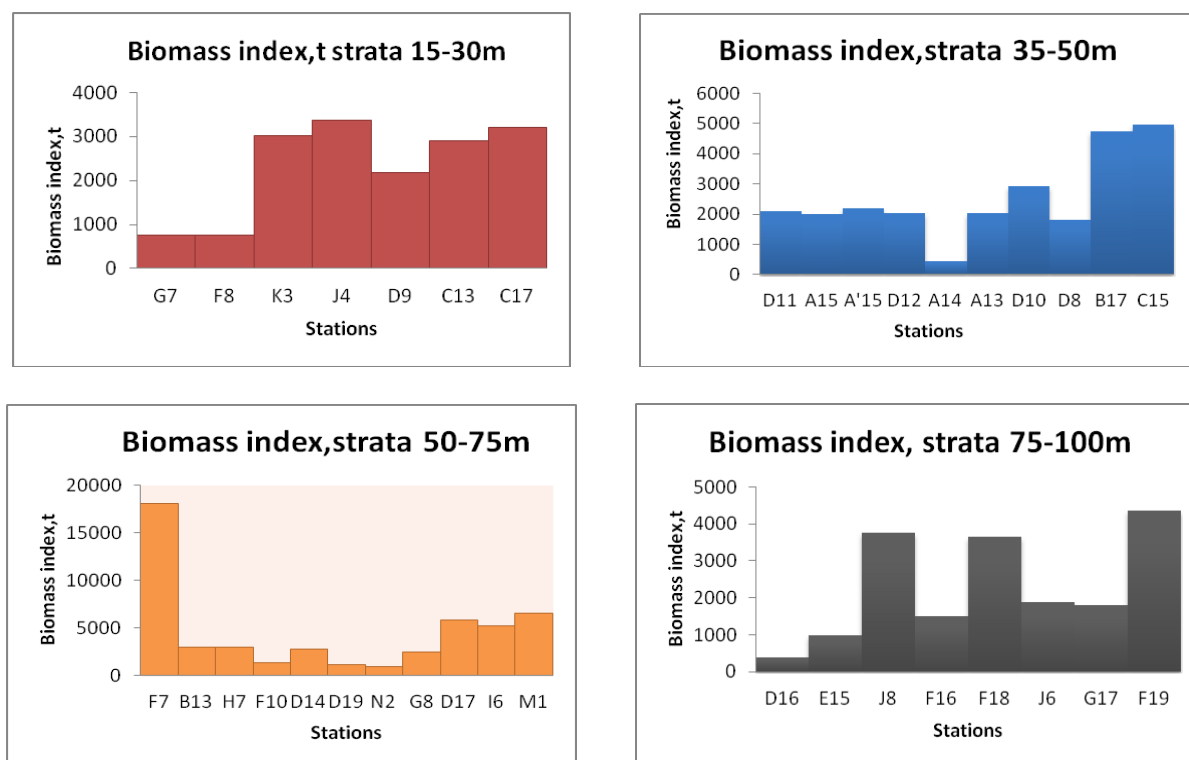


Figure 4.1.1. Biomass index (t.km-2) in Bulgarian marine area

Table 4.1.2. Statistical analysis and validation of the abundance by stratum

A. 10-35m

Mean	315.4082
Standard Error	58.24529
Median	400
Mode	400
Standard Deviation	154.1025
Sample Variance	23747.59
Kurtosis	-1.32117
Skewness	-0.82655
Range	362.8571
Minimum	100

Maximum	462.8571
Sum	2207.857
Count	7
Confidence Level(95.0%)	142.5211

B.35-50m

Mean	343.6666667
Standard Error	59.75350602
Median	280
Mode	280
Standard Deviation	188.9571772
Sample Variance	35704.81481
Kurtosis	0.504131134
Skewness	0.858804168
Range	620
Minimum	60
Maximum	680
Sum	3436.666667
Count	10
Confidence Level(95.0%)	135.1718214

C.50-75m

Mean	617.5325
Standard Error	195.0487
Median	400
Mode	400
Standard Deviation	646.9035
Sample Variance	418484.1
Kurtosis	6.575226
Skewness	2.405945
Range	2280
Minimum	120
Maximum	2400
Sum	6792.857
Count	11
Confidence Level(95.0%)	434.5957

D.75-100m

Mean	308.75
Standard Error	70.31657
Median	245
Mode	500
Standard Deviation	198.8853
Sample Variance	39555.36
Kurtosis	-1.49279
Skewness	0.335273
Range	550
Minimum	50
Maximum	600
Sum	2470
Count	8
Confidence Level(95.0%)	166.2723

4.2. Catch per unit area

The calculated catches per unit area (CPUA) for the Bulgarian Black Sea area by strata are represented on Fig. 4.2.1.

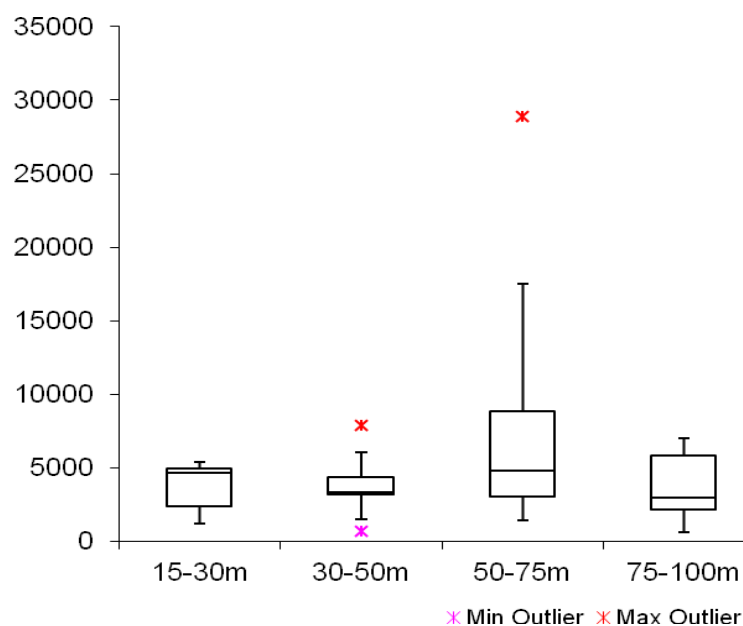


Figure 4.2.1.. Box plots of CPUA kg.km^{-2} means and hinges from different strata in Bulgarian area

CPUA kg/km^2

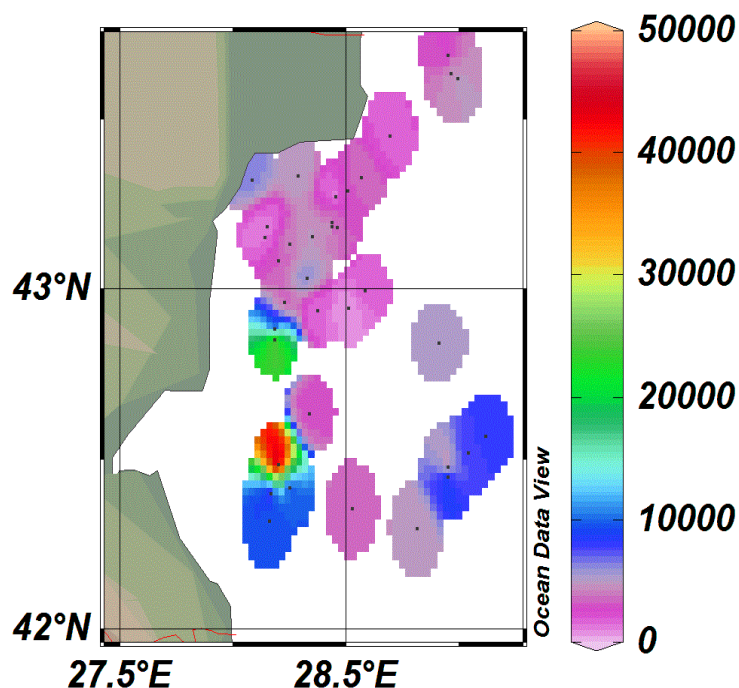


Figure 4.2.2.CPUA kg.km^{-2} from surveyed area.

Stratum 10-35m

In stratum 10-35m CPUA kg.km^{-2} : the lowest value was 1205 and the highest one was 5386 kg.km^{-2} detected in northern part of the coast.

Stratum 35-50 m

In the next stratum (35-50m) in two polygons were established high values of CPUA = 7564 kg.km^{-2} (B17), 7913 kg.km^{-2} (C17) – both in southern part, in front of Burgas Bay and in south-eastern direction from.

Stratum 50-75m

In stratum 50-75 m C_{PUA} in polygon one polygon, east from Burgas Bay extremely high value of C_{PUA} was detected (28 926 kg.km⁻²), as the second peak was in station M1 in the northern part, in front of Shabla (10 473 kg.km⁻²).

Startum 75-100m

At the most remote from the shore stratum the values of C_{PUA} were in the limits of 377 – 4369 kg.km⁻²

4.3. Catch per unit effort

Values CPUE kg.h⁻¹ from December pelagic trawl survey is presented on Fig.4.3.1.

CPUE kg/h

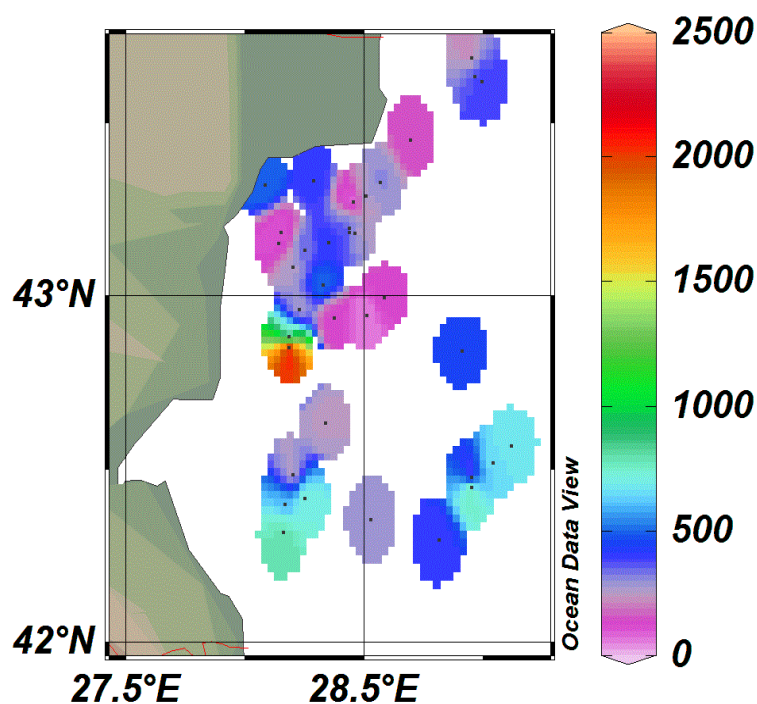


Figure 4.3.1. CPUE kg.h⁻¹ from surveyed area.

4.4. Size structure of *S.sprattus*

The size composition comprised of length classes (TL, cm) from 6.5 cm up to 12.5 cm in the samples from Bulgarian marine area, (Fig.4.4.1. to 4.4.14).

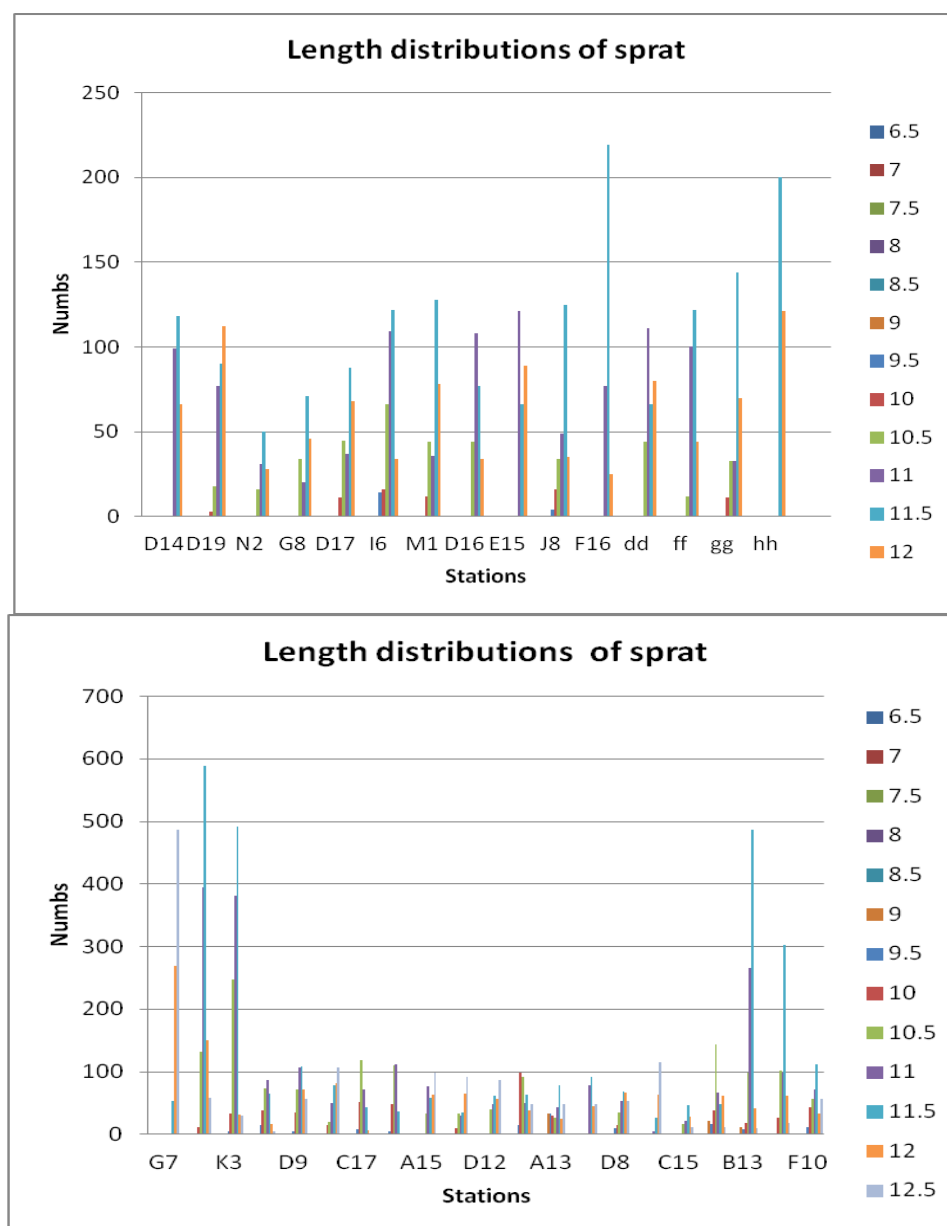


Figure 4.4.1. Share of size classes of sprat from Bulgarian marine area.

It is evident that the sizes classes from 7.5-9.00 cm are predominate, as the bigger classes were presented with low percentage. The situation with the absence (or low share) of the larger (oldest) individuals was the same in the period of 2007-2012 (Raykov et al., 2007, 2008, 2009, 2010).

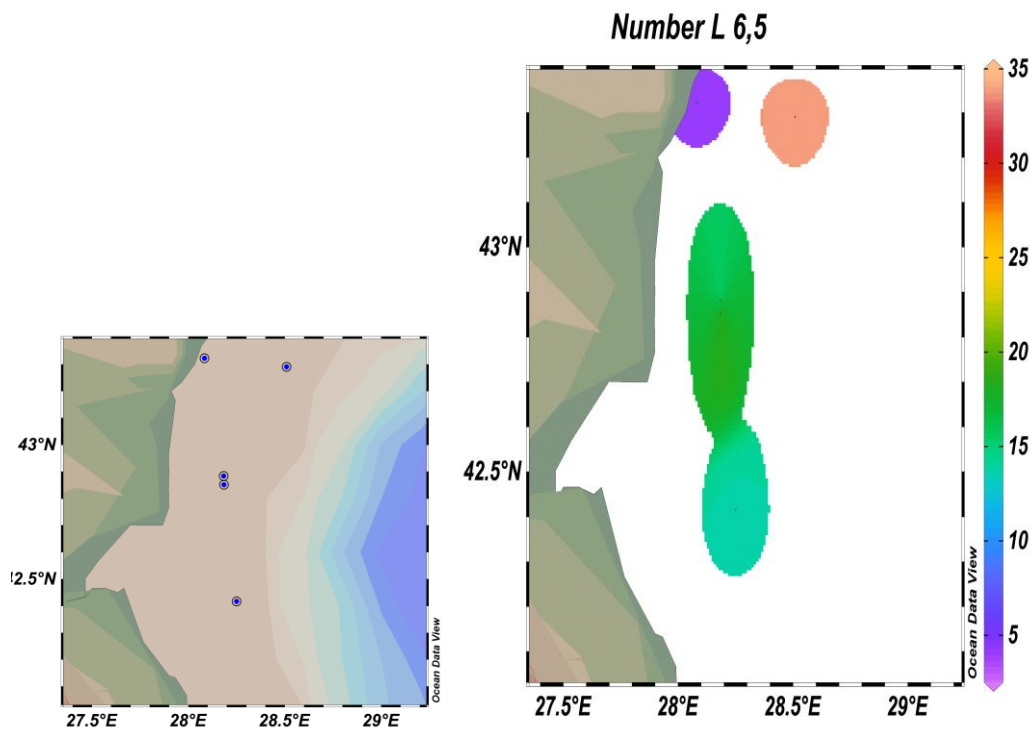


Figure 4.4.2. Sampling stations and spatial distribution of length class 6.5 cm of sprat in Bulgarian marine area, December 2015.

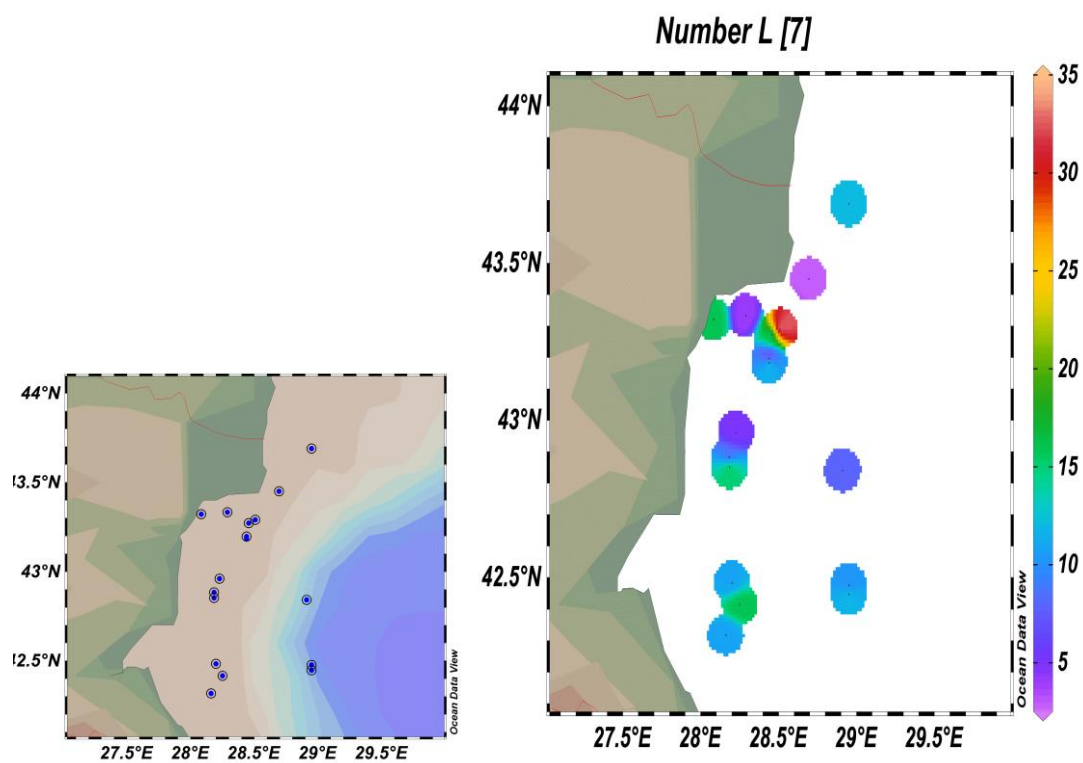


Figure 4.4.3. Sampling stations and spatial distribution of length class 7 cm of sprat in Bulgarian marine area, December 2015.

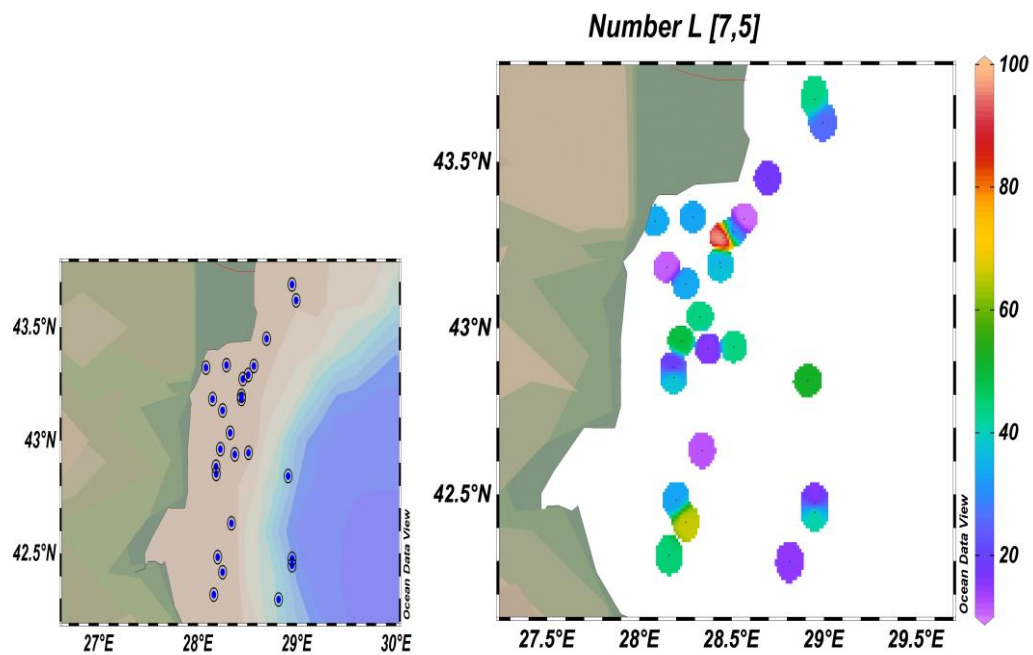


Figure 4.4.4. Sampling stations and spatial distribution of length class 7.5 cm of sprat in Bulgarian marine area, December 2015.

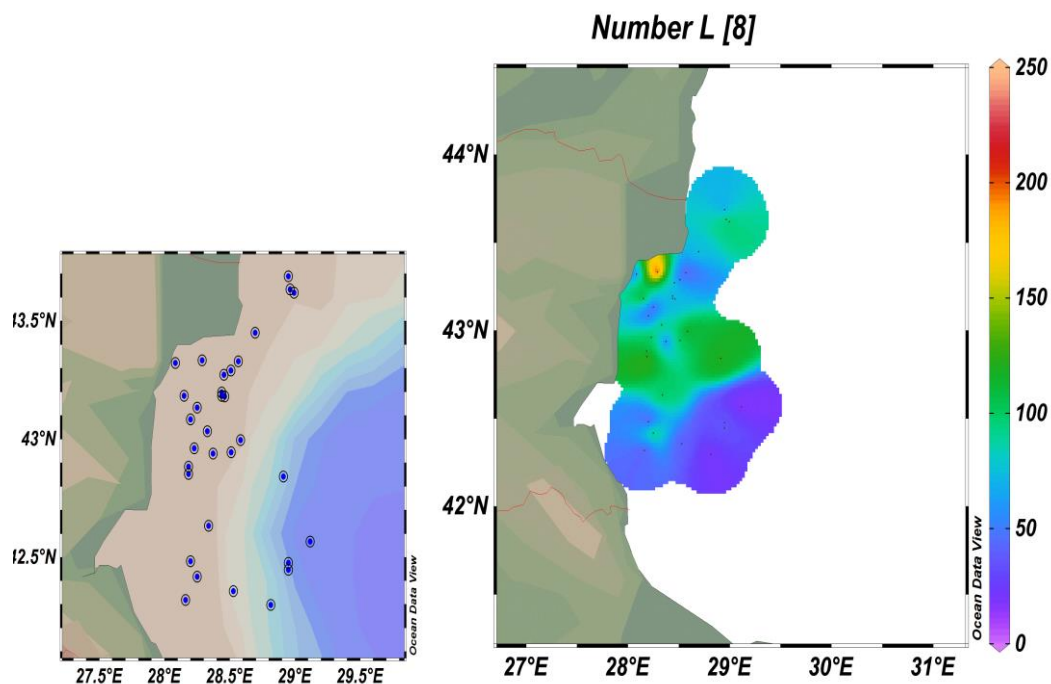


Figure 4.4.5. Sampling stations and spatial distribution of length class 8 cm of sprat in Bulgarian marine area, December 2015.

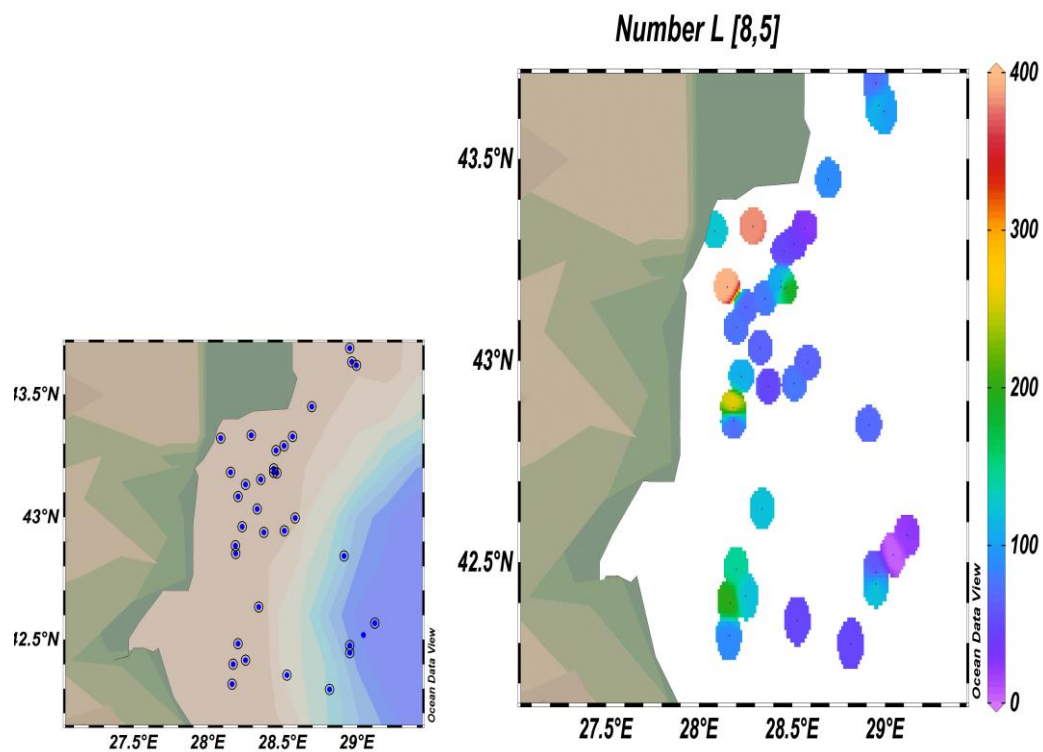


Figure 4.4.6. Sampling stations and spatial distribution of length class 8.5 cm of sprat in Bulgarian marine area, December 2015.

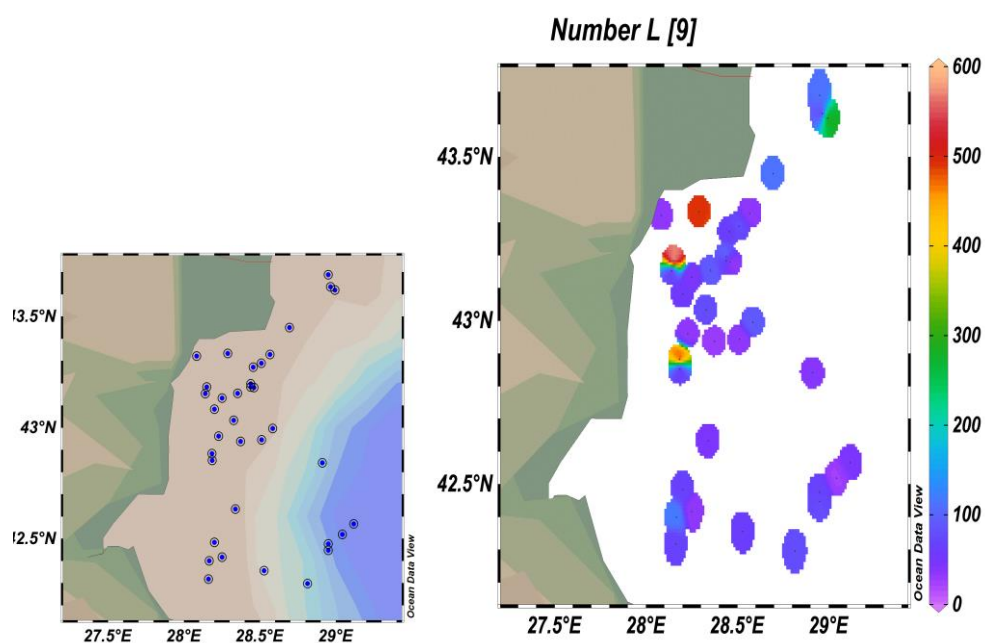


Figure 4.4.7. Sampling stations and spatial distribution of length class 9 cm of sprat in Bulgarian marine area, December 2015.

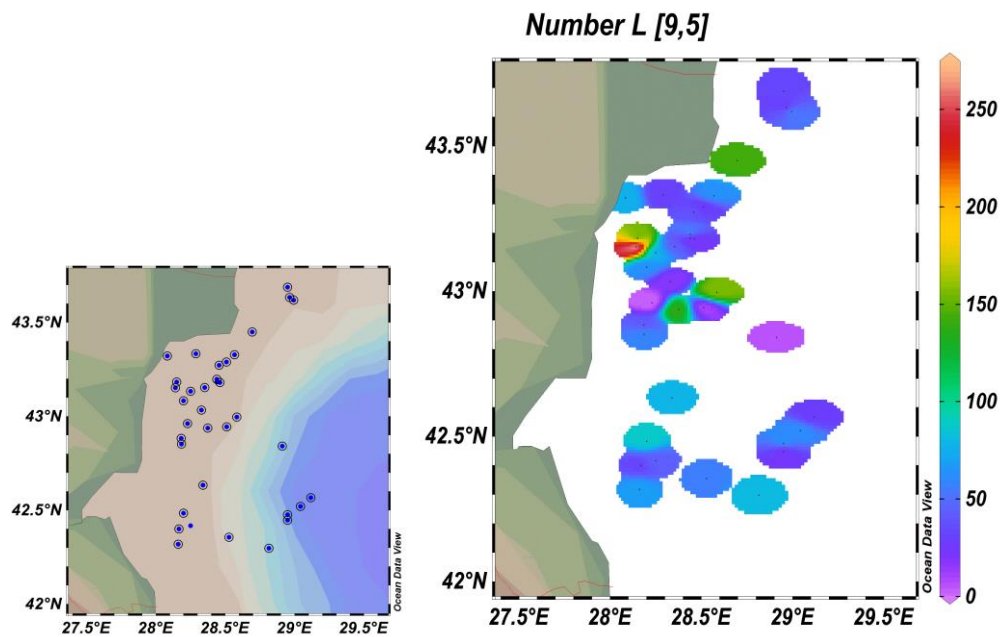


Figure 4.4.8. Sampling stations and spatial distribution of length class 9.5 cm of sprat in Bulgarian marine area, December 2015.

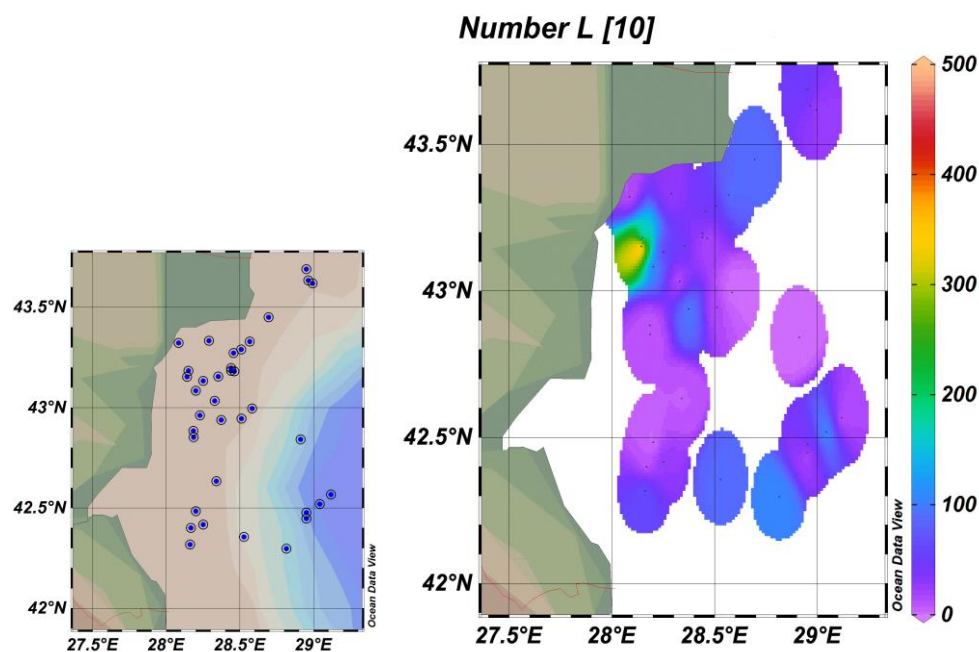


Figure 4.4.9. Sampling stations and spatial distribution of length class 10 cm of sprat in Bulgarian marine area, December 2015.

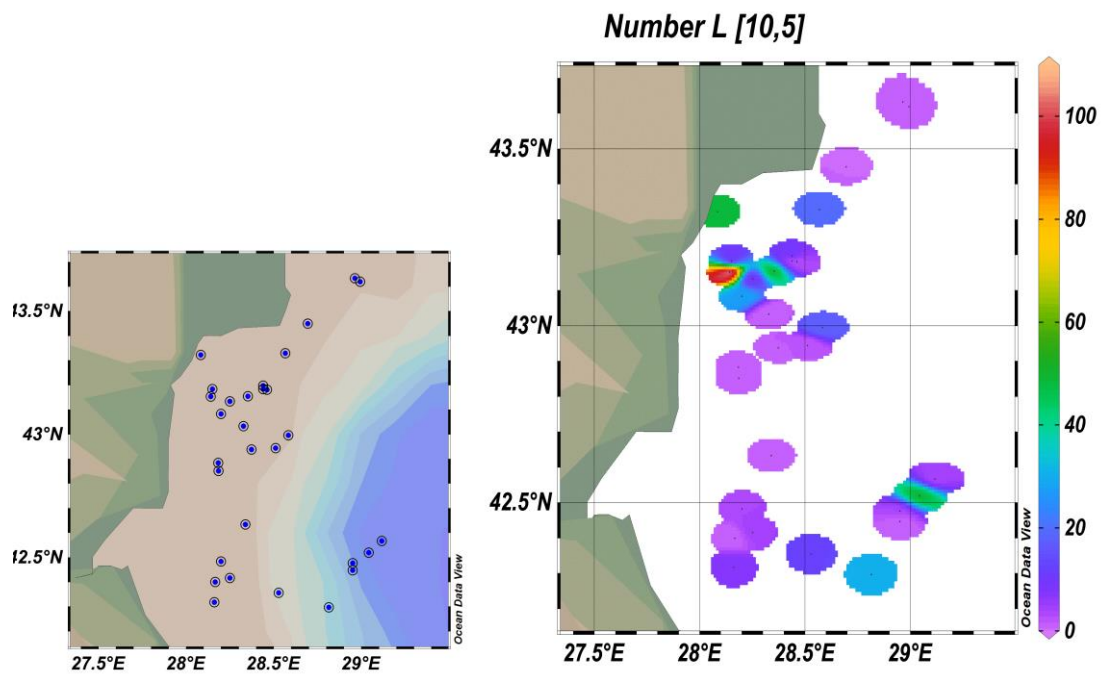


Figure 4.4.10. Sampling stations and spatial distribution of length class 10.5 cm of sprat in Bulgarian marine area, December 2015.

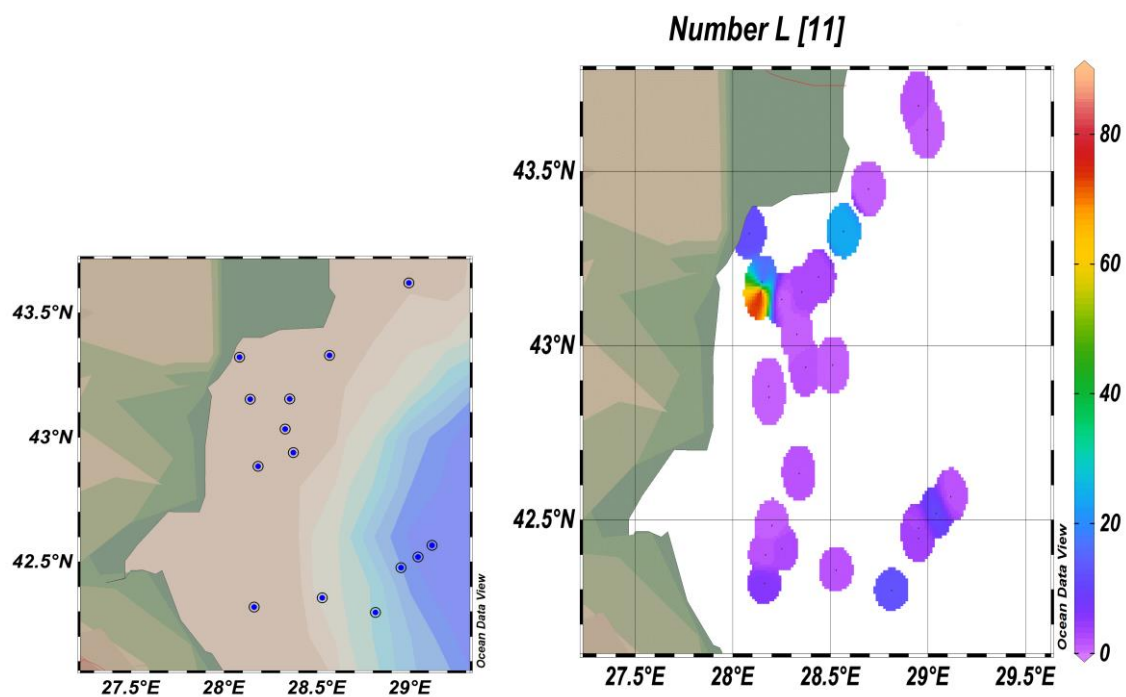


Figure 4.4.11. Sampling stations and spatial distribution of length class 11 cm of sprat in Bulgarian marine area, December 2015.

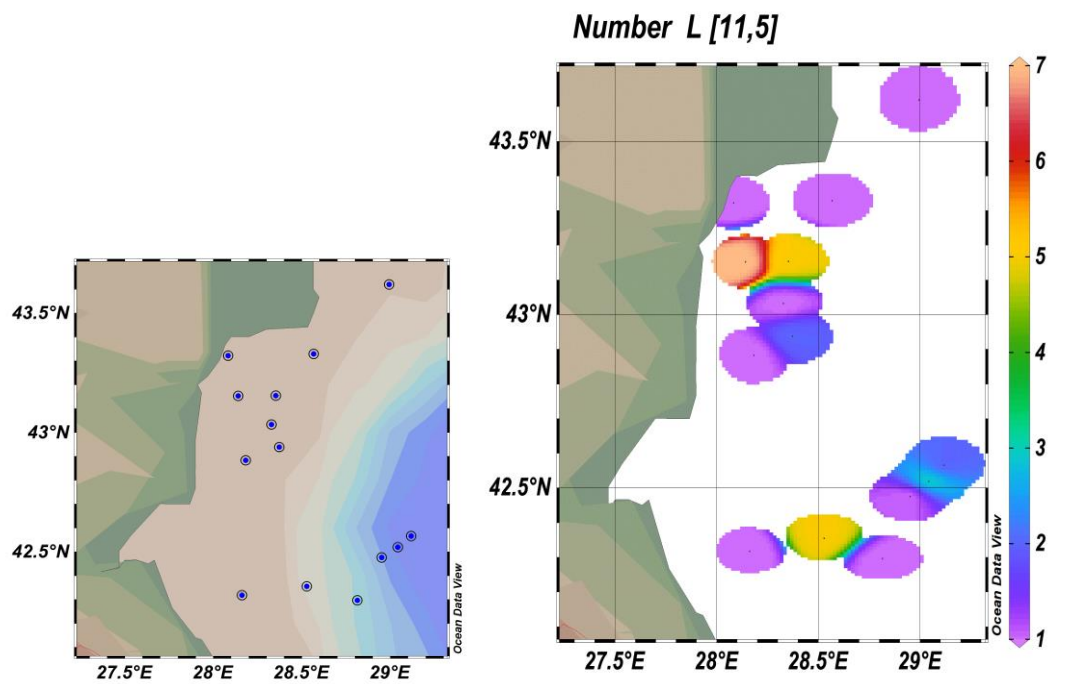


Figure 4.4.12. Sampling stations and spatial distribution of length class 11.5 cm of sprat in Bulgarian marine area, December 2015.

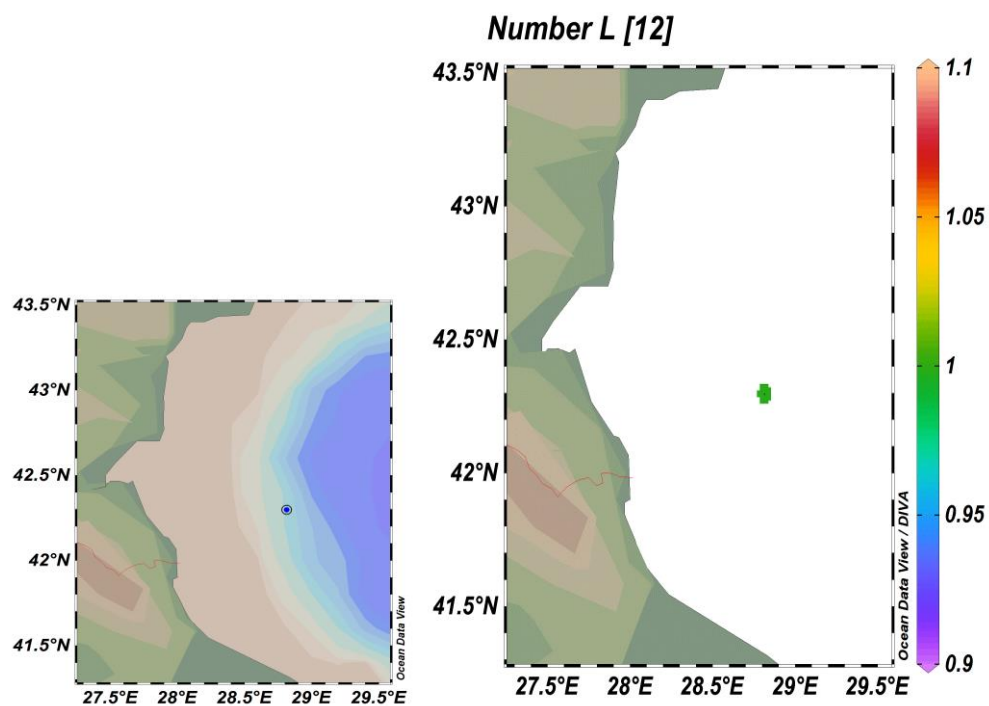


Figure 4.4.13. Sampling stations and spatial distribution of length class 12 cm of sprat in Bulgarian marine area, December 2015.

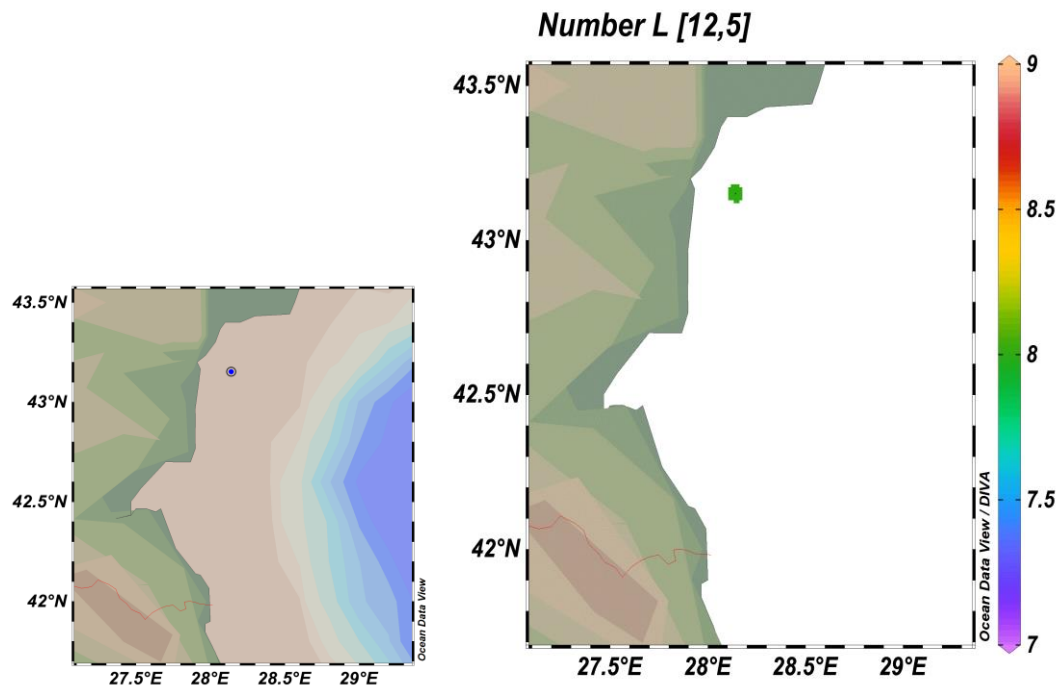


Figure 4.4.14. Sampling stations and spatial distribution of length class 12.5 cm of sprat in Bulgarian marine area, December 2015.

4.5. Age and growth of *S.sprattus*

The age structure was determined on the basis of direct otolith reading with binocular on reflected light. Analysis shows that the percentage of one year old fish is greatest in both areas. The difference between the two areas consists in the complete absence of 0+ and 4-4+ old individuals in the Romanian area (*Fig.4.5.1.A, B, Fig.4.5.2.*).

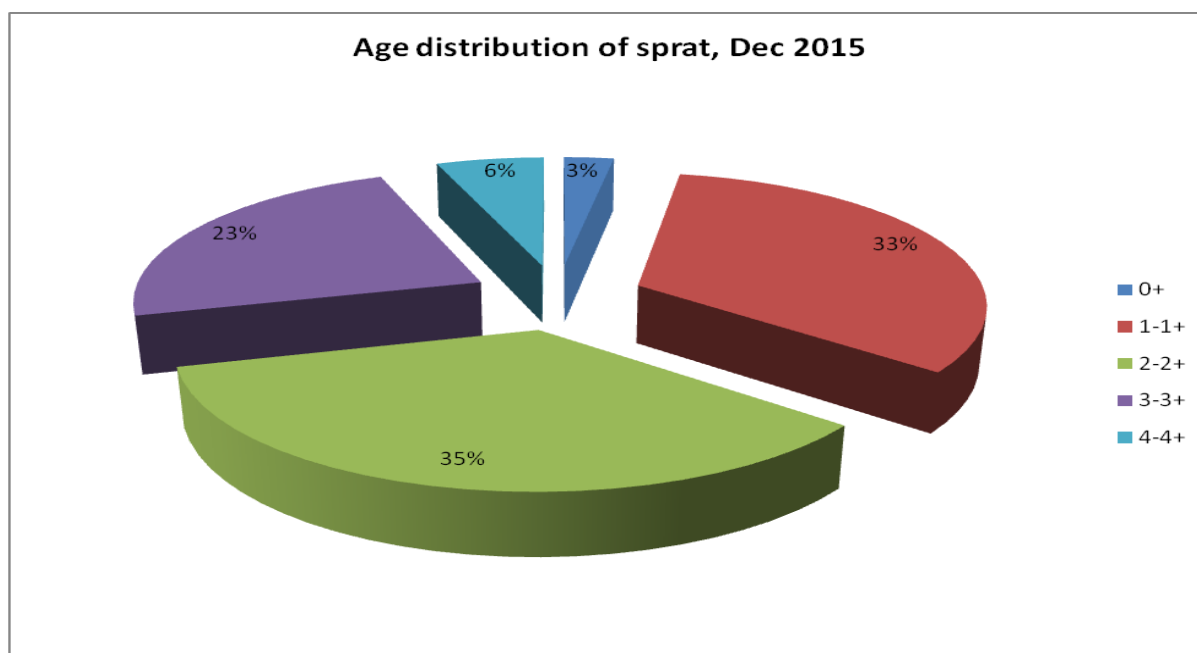
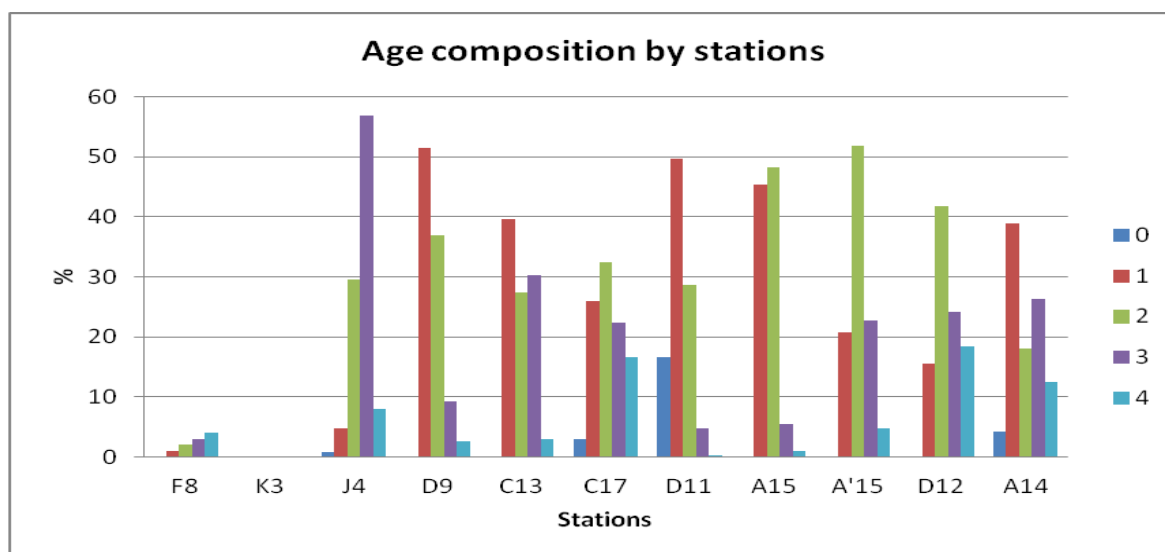


Figure 4.5.1. Share of age groups from A. Bulgarian marine area; B. Romanian marine area.



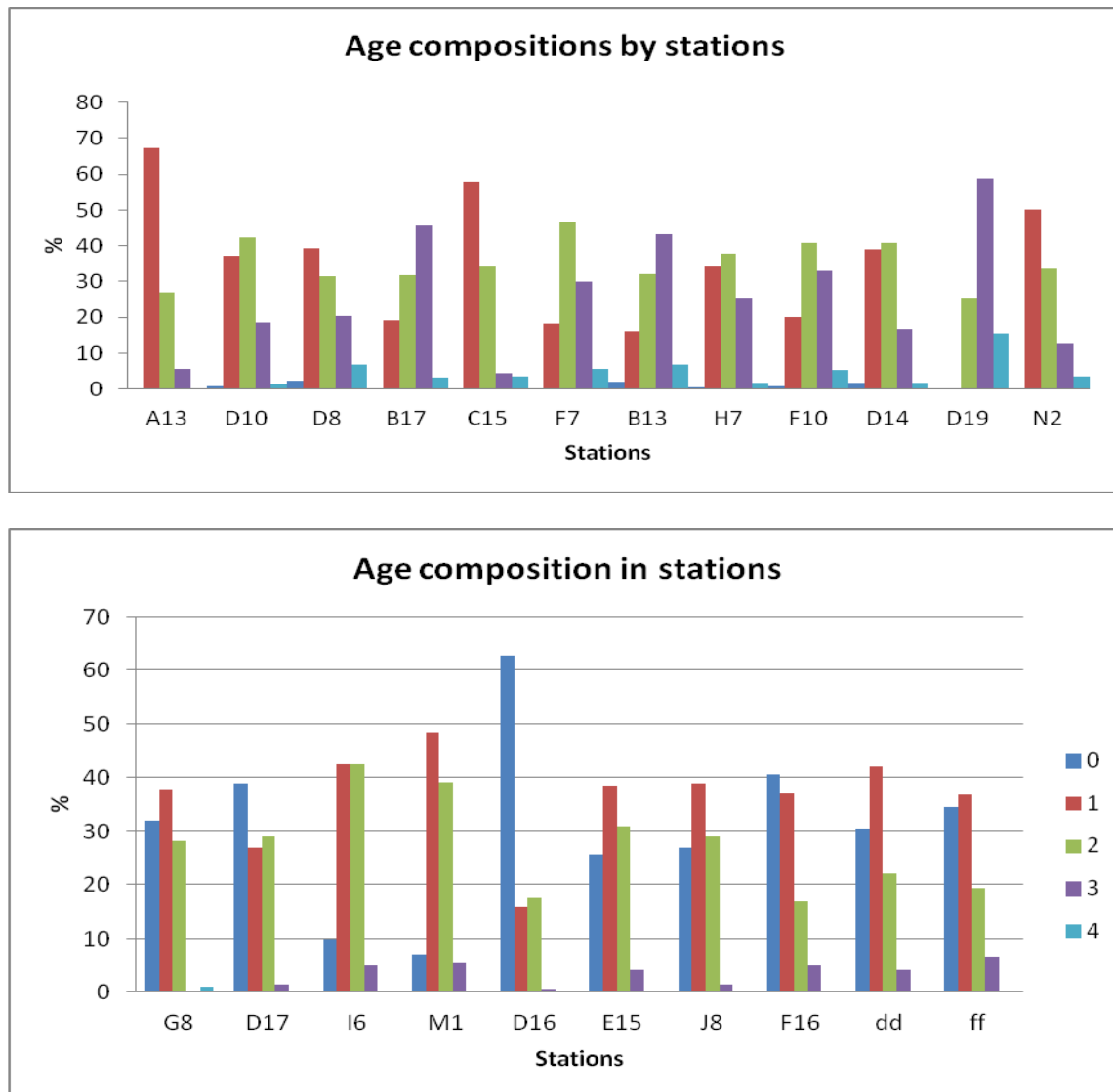


Figure 4.5.2. Age groups distribution in the investigated area.

4.6. Growth

To estimate the growth rate and population parameters of the sprat from Bulgarian and Romanian marine areas we used VBGF. The estimation of the asymptotic length, growth rate and related coefficients is presented on Table 4.6.1.

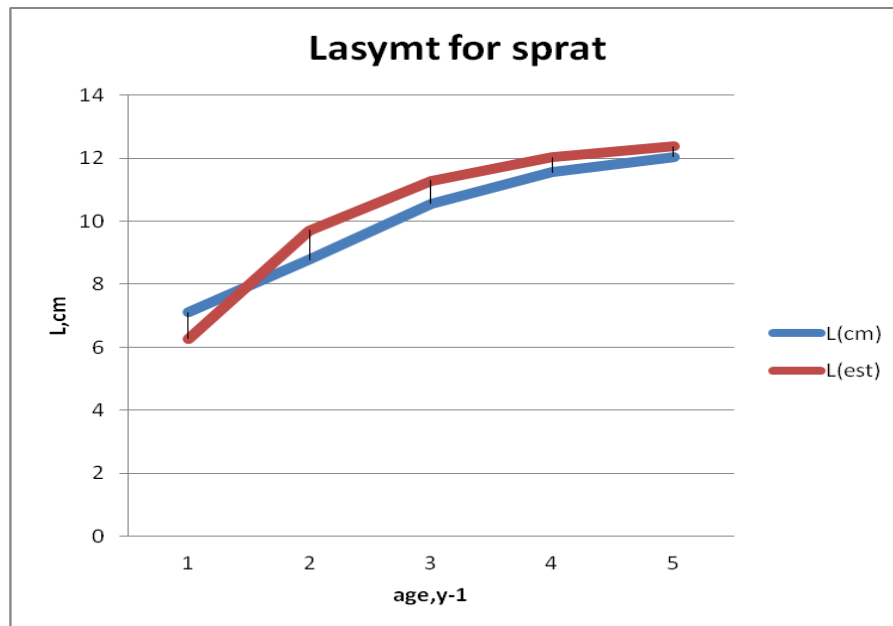


Fig.4.6.1. L asymptotic of sprat

Table 4.6.1. Values of parameters in VBGF.

$L_{\infty, \text{cm}} = 12.66$
$k = 0.77$
$t_0 = -0.11$
$q = 0.0008$
$n = 2.78$

Size growth

The asymptotic length reached 12.66cm and growth rate could be assessed as relatively high accounting 0.77 y^{-1} . The growth of sprat from present research is positive allometric ($n=2.78$) (Fig.4.6.1).

The important remark here is the fact that due to absence (or low share) of the oldest and individuals with large sizes, the VBGF accounts only the shorten size structure of sprat from present research. In this regard, the maximum or asymptotic length reached this value, which possibly not fully corresponds to the literature data about sprat size and marginal levels of length and growth rate. Hence, we could accept the growth analysis, as it is, reflecting only the present situation with large fish absence (low presence).

Somatic growth

The somatic growth of sprat from the present research shows that mean weight corresponding to the oldest age group is 7.21 g. The value corresponds to the marginal size class of 12.5 cm observed in the samples from the trawl survey in Bulgarian waters (*Fig.4.6.1.*).

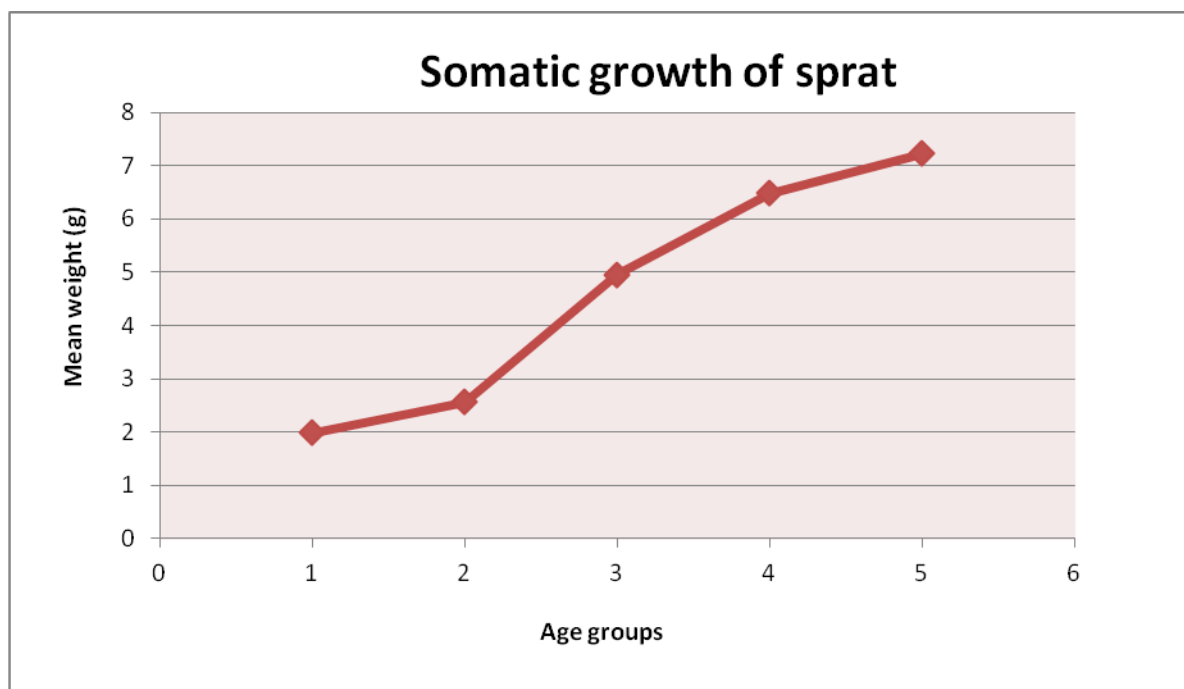


Figure 4.6.2. Somatic growth of sprat

Asymtotic somatic growth reached 11.41. The growth rate in weight is assessed as comparatively stable and high 0.44. This fact could possibly related to the fact that in December the species is in active gonad maturation and spawning with high batch fecundity.

4.7. Natural mortality

We used the formula incorporated asymptotic length and weight derived by Pauly (1980). The average habitat temperature in the bottom layers was 6.9°C:

From asymptotic length:

$$M = 0.7632$$

From asymptotic weight:

$$M = 0.582$$

In the present research we used natural mortality coefficient for sprat as equal to **0.95** (Ivanov and Beverton, 1985; Prodanov et al., 1997; Daskalov, 1998).

4.7. Sex ratio

Females prevailed with 49%, followed by males (48%). The juveniles presented with low percent (3%)

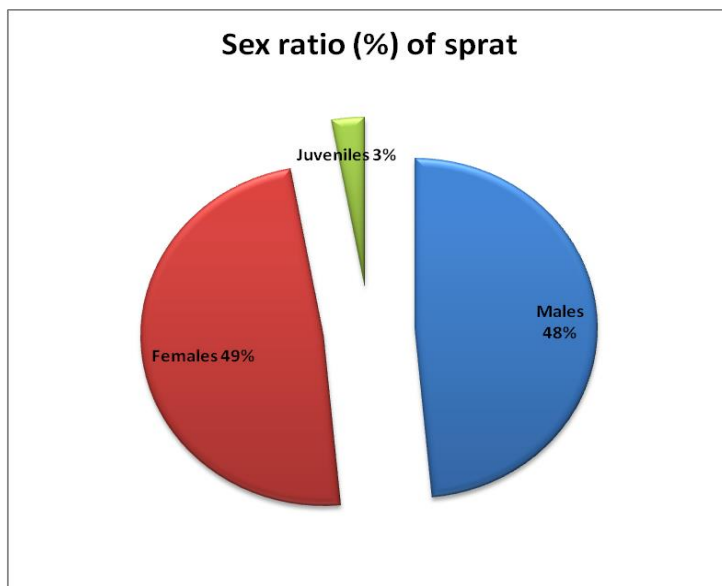


Figure 4.7.1. Sex ratio of sprat in December 2015.

4.8. Maturity

The sprat in active spawning during the present investigation. Most of the individuals are with III - IV stage of gonads. More detail analysis should be done in the active spawning period of the species (October-February).

Gonado somatic index

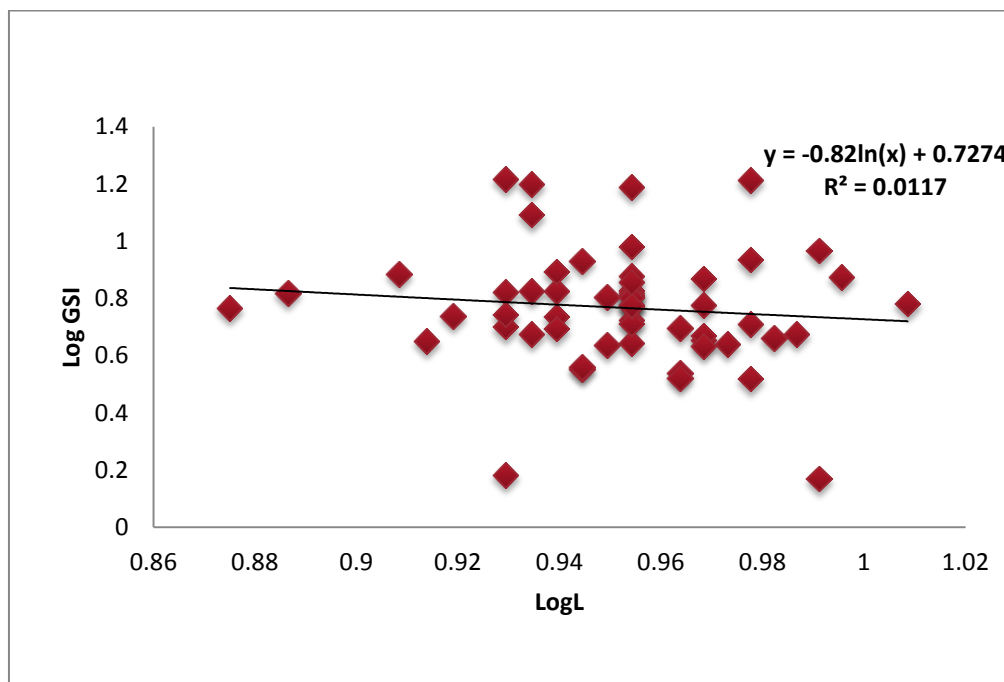
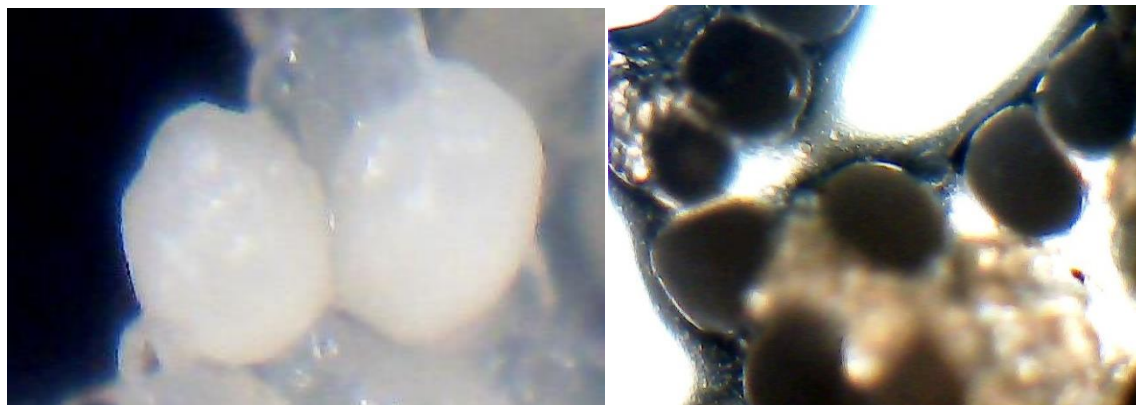


Figure 4.8.1. Gonado somatic index of sprat from present research (GSI,%)

The GSI(%) show that over 50% of females were active spawners. Very few specimens

Were with early maturation stages, so we can conclude that in December 2016, the active spawning have been initiated, even the SST was relatively high for the season.



Picture 4. Eggs of sprat

Fecundity

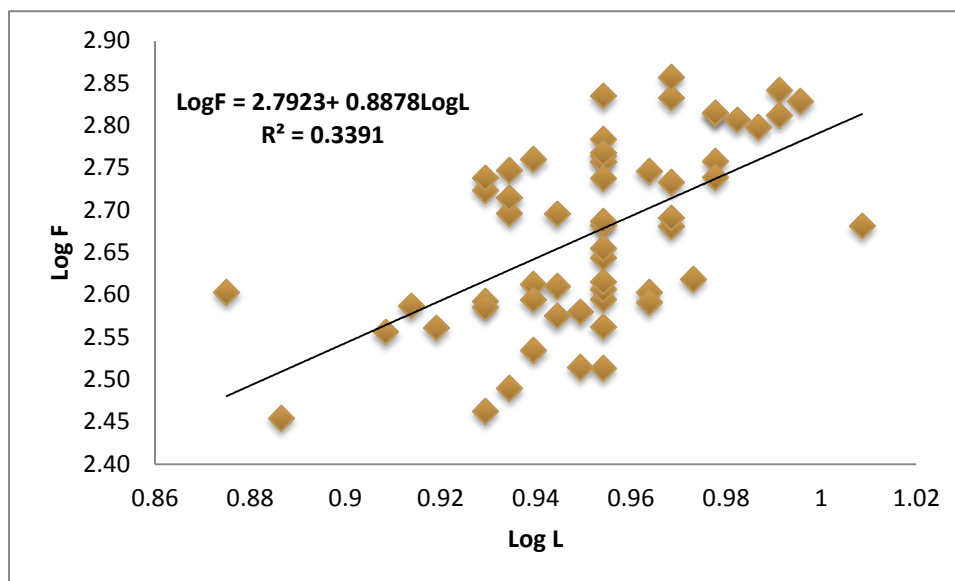


Figure 4.8.2. Batch fecundity (LogF) in relation to the length (LogL) of sprat in December, 2016

Fecundity of sprat correlate positive with sprat length ($R^2 = 0.34$), as the biggest length classes posses highest fecundity.

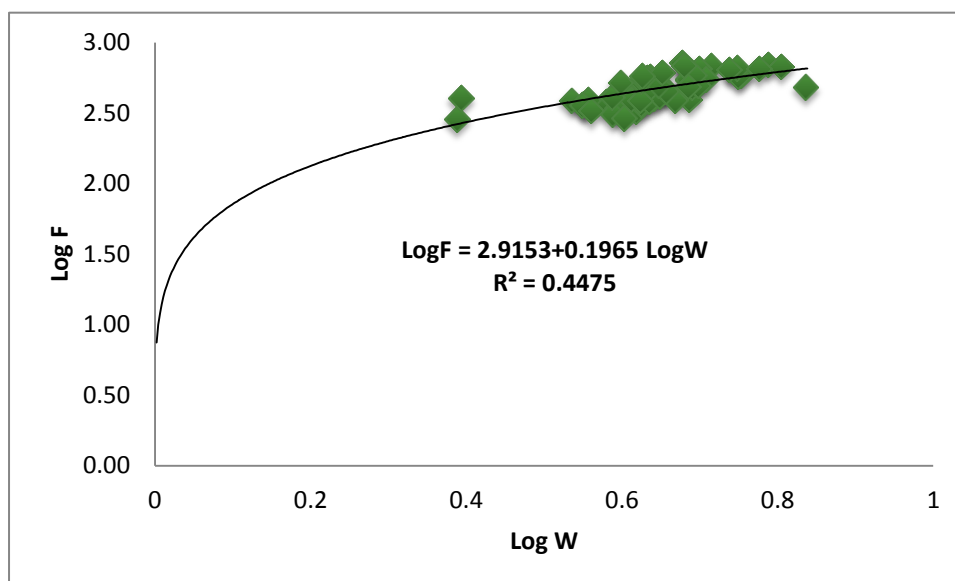


Figure 4.8.3. Batch fecundity (LogF) related to weight (LogW) of sprat in December, 2016

The correlation between fecundity and weight of sprat was much stronger (Fig.4.8.3.) ($R^2 = 0.4475$)

4.9. Food composition

Results

Zooplankton taxonomic structure, abundance and biomass

A total of 21 zooplankton species and taxa were identified in front of the Bulgarian coast distributed among to phyla Protozoa, Cnidaria, Myxozoa, Annelida, Arthropoda, Mollusca, Chaetognatha, Chordata. The class Crustacea was the most diverse (9 species and 1 taxa). The non-fodder part of the community was presented by *Noctiluca scintillans* and Jellyfish group: *Aurelia aurita*, *Pleurobrachia pileus*, *Beroe ovata*. The obtained results revealed maximum frequency of occurrence factor (100%) in zooplankton community structure for *Paracalanus parvus*, *Oicopleura dioica*, *N. scintillans* and larvae of Bivalvia (table 2).

Table 4.9.1. Frequency of occurrence of zooplankton species/taxa investigated in front of Bulgarian coast in December 2015

Species/taxa	FO%	FO* categories
Mesozooplankton		
<i>Calanus euxinus</i>	82	F
<i>Pseudocalanus elongatus</i>	91	F
<i>Paracalanus parvus</i>	100	A
<i>Acartia clausi</i>	91	F
<i>Centropages ponticus</i>	9	R
<i>Oithona similis</i>	91	F
<i>Oithona davisae</i>	82	F
<i>Pleopis polyphemoides</i>	36	U
<i>Penilia avirostris</i>	27	U
<i>Polychaeta larvae</i>	91	F
<i>Bivalvia veliger</i>	100	A
<i>Gastropoda veliger</i>	36	U
<i>Cirripedia larvae</i>	91	F
<i>Parasagitta setosa</i>	91	F
<i>Oikopleura dioica</i>	100	A
Pisces larvae	27	U
Pisces ova	73	C
<i>Noctiluca scintillans</i>	100	A
Jellyfish		

<i>Aurelia aurita</i>	36	U
<i>Pleurobrachia pileus</i>	45	O
<i>Beroe ovata</i>	64	C

***Frequency of occurrence categories:**

A- always collected within the sampling study area.

F- frequently collected on average in 80-99% of stations within study area.

C- commonly collected on average in 60-79% of stations within study area.

O- occasionally collected on average in 40-59% of stations within study area.

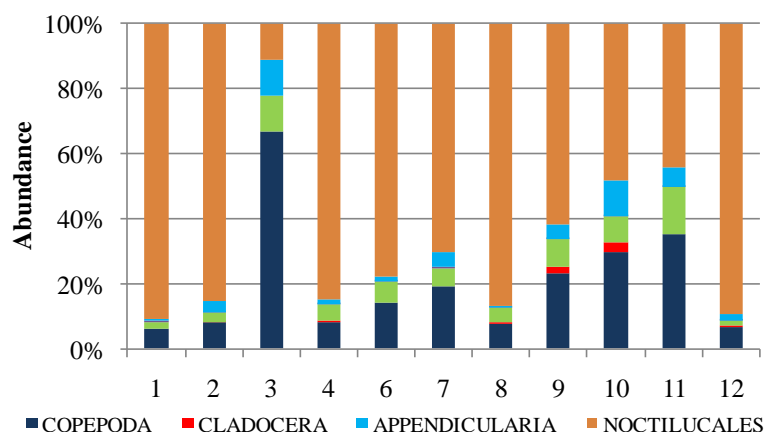
U- uncommonly collected on average in 20-39% of stations within study area

R- rarely collected on average in 1-19% of stations within study area.

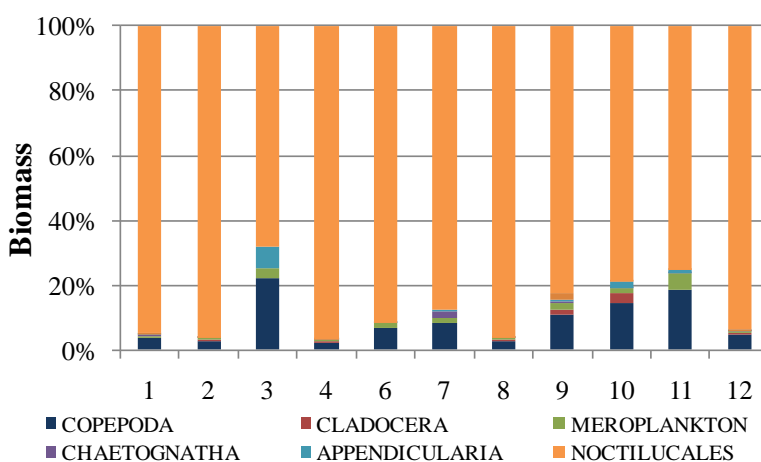
Calanus euxinus, *Acartia clausi*, *Pseudocalanus elongatus*, *Oithona similis*, *O.davisae*, *Parasagitta setosa*, Polychaeta larvae, Cirripedia larvae frequently occurred within sampling period (Table 4.9.1.). Rarely collected (under 19%) was only *Centropages ponticus*, but uncommonly collected on average in 20-39% were Cladocera (*Penilia avirostris*, *Pleopis polyphemoides*), Gastropoda veliger, Pisces larvae, *A. aurita*..

Zooplankton community structure was influenced by heterotrophic dinoflagellates *N.scintillans*. The species was presented with more than 80% at more stations with an exclusion of the station 3 which was differentiated with very low abundance and biomass - figure 1. Noctiluca is characterized as a dead-end of the food web (Yilmaz et al., 2005). No toxic effects are known, but the high ammonia content of the vacuole most probably irritates fish, which generally avoid the bloom areas (<http://www.imas.utas.edu.au/zooplankton/image-key/noctiluca-scintillans>).

Prevalence of Noctiluca reflected lower water quality and pelagic fish food supply respectively. Copepods were the most important species in the fodder zooplankton structure sharing 50-75% according to abundance and 58-78% in respect of biomass. Complementary key species/ecological groups of the plankton fauna were benthic larvae (22% in abundance and 12% in biomass), *Oikopleura dioica* (13% in abundance structure), Cladocera (3% and 6% respectively) and Chaetognatha (shared 4% in biomass) (fig.4.9.1.).



a)



b)

Figure 4.9.1. Zooplankton community structure (key ecological groups) by biomass presented in percent.

As a main group in the zooplankton community Copepoda dominated in the *S. sprattus* food diet. Totally seven species/taxa characterized Copepods community structure in late autumn 2015. *A.clausii* (33%), *P.parvus* (21%) and *P.elongatus* (16%) together shared 70 % in abundance structure (Fig. 2) while *A.clausii* (50%), *P.elongatus* (18%), *C.euxinus* (11%) prevailed in biomass structure. Copepods like *Oithona similis*, *Oithona davisae* *C. ponticus* could be considered as relevant (Fig.4.9.2).

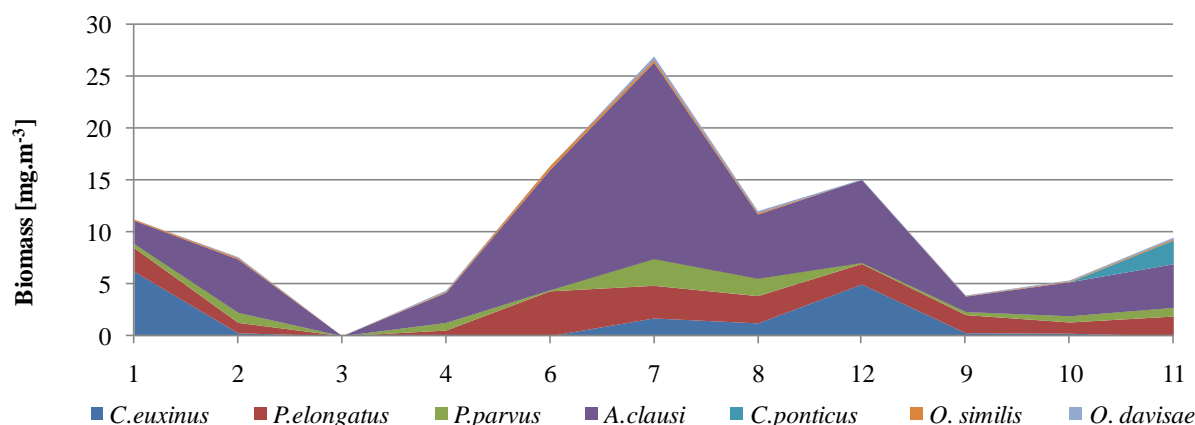


Figure 4.9.2.. Copepods distribution pattern in late autumn 2015 according to biomass.

The ecological significance of the opportunistic dinoflagellate *Noctiluca scintillans* (Myzozoa) and Jellyfish as food competitors of fish larvae and planktivorous fish species for the pelagic ecosystem is significant. Maximum of *N. scintillans* was registered at st.8 (7453 ind.m⁻³), situated to the north. The species was presented at the entire study area with average biomass of 208±136.73 mg.m⁻³. The spatial distribution of Jellyfish in the Western Black Sea exhibited patchiness: *B. ovata* and *P. pileus* were concentrated to the north in front of Varna Bay, while the medusa *A. aurita* occurred commonly at the south stations. Gelatinous species density varied from 0.2 to 2 ind.m⁻³ and biomass ranged from 0.02 to 64 g.m⁻³ (Fig.4.9.3.). It provides arguments that the negative impact of gelatinous species even at low concentrations could not be ignored as a key controlling factor for zooplankton community and sprat population development respectively taking into account the food competition.

Mesozooplankton standing stock showed high spatial heterogeneity and variability during the study area and period. Excluding the data of st.3 where quantities were extremely low, abundance and biomass demonstrated huge range almost equivalent for both metrics, 10 fold in density (max. 8579 ind.m⁻³ and min. 813 ind.m⁻³) and respective figure for biomass (13 folds) with maximum 465.56 mg.m⁻³ and minimum 36.54 mg.m⁻³ (Fig. 4.9.2).

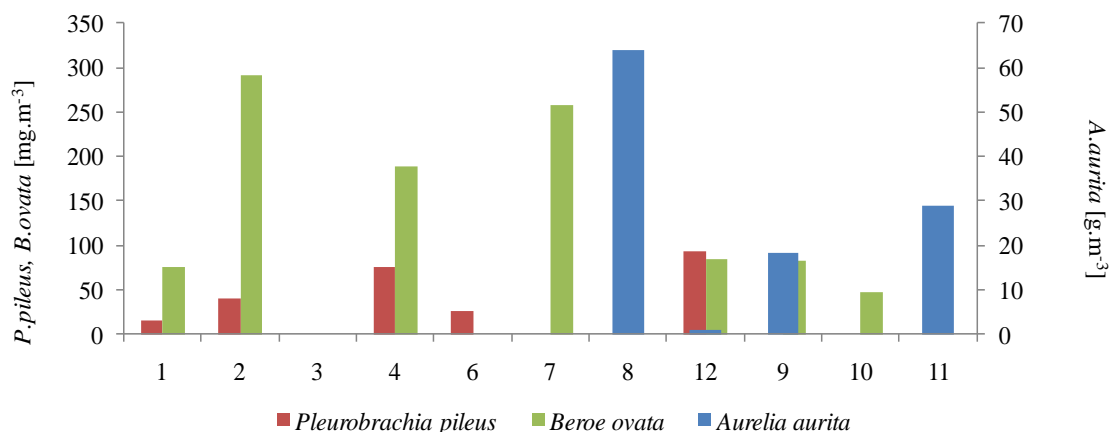


Figure 4.9.3. Biomass dynamic of gelatinous species during the late autumn 2015.

According to spatial distribution mesozooplankton abundance and biomass diminished 5-6 folds from the North toward the South (Fig. 4.9.3.). Zooplankton community at south stations was characterized by relative homogeneity.

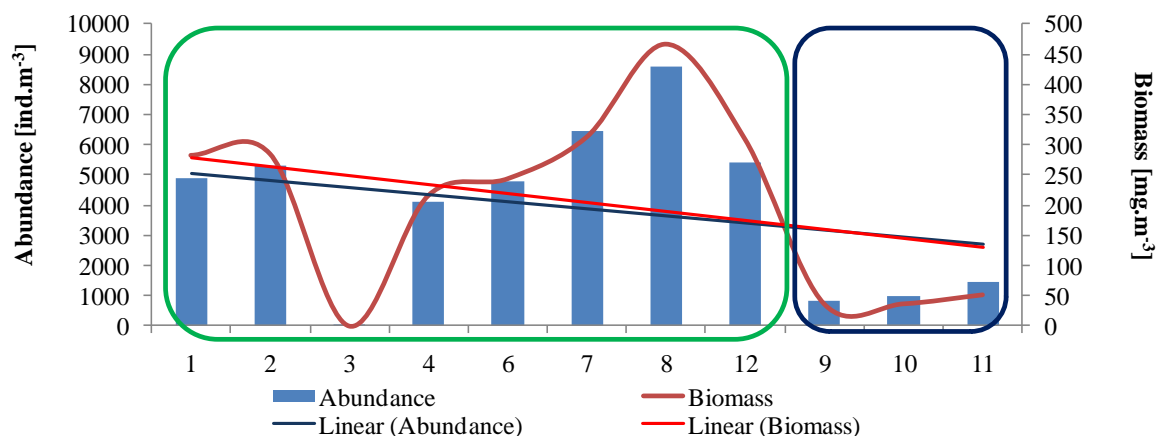


Figure 4.9.4. Variability of the zooplankton abundance [ind.m⁻³] and biomass [mg.m⁻³] by sampling stations and their gradient according to space distribution. Distribution of sampling stations along the Bulgarian Black Sea: North area – green box, South area – blue box.

No clear distribution pattern in zooplankton density and biomass was discerned. However, higher biomasses were recorded at area closed to the North: coastal (st. 6, 7, 8) – area closed between 19 to 35 m meter isobaths and shelf – area limited between 75-85m isobaths (st.12) (Table 4.9.2.). Zooplankton species *A. clausi* contributed mainly for the highest biomass at coastal stations while *C. euxunus* mainly and *P. setosa* for deeper waters. Generally, the fodder biomass in most stations was relatively homogenous distributed.

The average mesozooplankton biomass (ignoring *N. scintillans*) during the monitoring survey ($14.58 \pm 10.42 \text{ mg.m}^{-3}$) is about 9 times higher to those recorded in 2014 - $132.42 \pm 72.61 \text{ mg.m}^{-3}$ and approximately 7 folds more than in 2011 (Panayotova et al, 2014; Panayotova et al, 2011), which confirms poor ecological state of the zooplankton community and worse trophic conditions for the *Sprattus sprattus* population.

Table 4.9.2.. Percentage distribution of main zooplankton species/groups per stations along the Bulgarian coast according to biomass.

Станция	Copepoda	Cladocera	Meroplankton	Chaetognatha	Appendicularia	Ichthyoplankton	Mesozooplankton biomass [mg.m^{-3}]
1	74	0	8	8	2	8	15.208
2	66	10	14	0	9	1	11.539
3	69	0	11	0	20	0	0.008
4	57	17	18	1	5	1	7.669
6	78	0	18	2	2	0	20.950
7	68	0	9	19	4	0	39.444
8	66	13	13	5	2	1	18.387
9	61	11	10	3	3	12	6.427
10	69	14	8	0	8	1	7.823
11	74	0	21	0	4	1	12.862
12	75	8	3	4	3	6	20.014

Food composition of sprat

Weight – length dependence, Index of stomach fullness (ISF).

The average length of 132 measured sprat specimens reached 87.55 ± 5.19 (SD) mm; varying between 70 до 102 mm, respectively the mean weight was 4.18 ± 0.8 (SD) g, varying from 2.03 g to 9.52 g. The weight-length dependence during the investigated time period could be described by the following equation: $\text{Log WW (r)} = 2.2882 + 3.0821 * \text{Log L (cm)}$; ($r^2=0.87$, $p<0.00001$) (Fig. 4.9.5).

Measured body weights and lengths respectively and indexes of stomach fullness in 2015 were lower to those in 2011 (1.2 fold) which were almost similar to those measured in 2009 where correspondingly the mean length and weight were 87.42 mm and 3.97 g and higher than measured in 2010 ($L=82.76 \text{ mm}$ и $W=3.30 \text{ g}$).

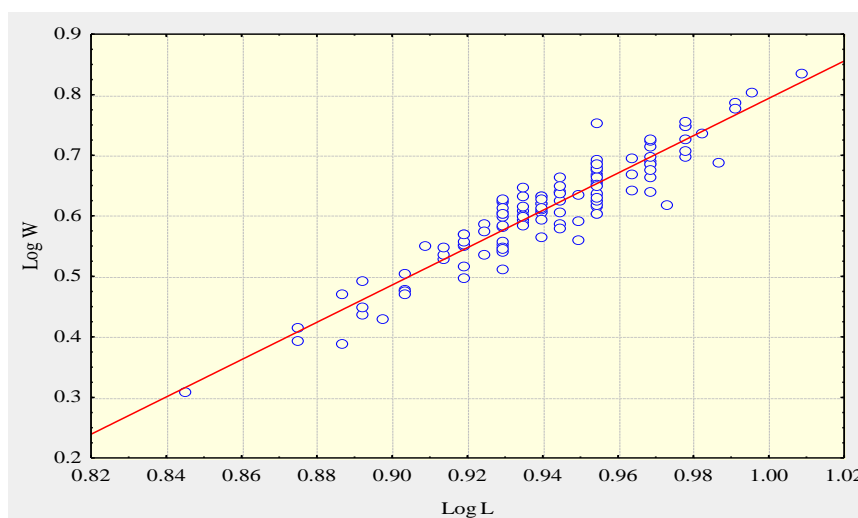


Figure 4.9.5. Relationship weight-length of sprat specimens in 2015.

The average value of index of stomach fullness reached 0.649 % of sprat body weight, equivalent with those registered in 2009 (0.6%) (Raykov et al., 2010) and about 2 times lower than in comparison with the estimated index in 2011 (Raykov et al., 2011). The highest mean value of the stomach fullness index $SFI = 4.47\%$ and 2.79 % has been estimated at stations 1 and 12, situated to the North between Galata and Emine, in the zone limited 70-80 meter isobaths. (Fig. 4.9.6). The same stations were characterized with the highest density and biomass of mesozooplankton where most probably planktivorous fish found good food resource.

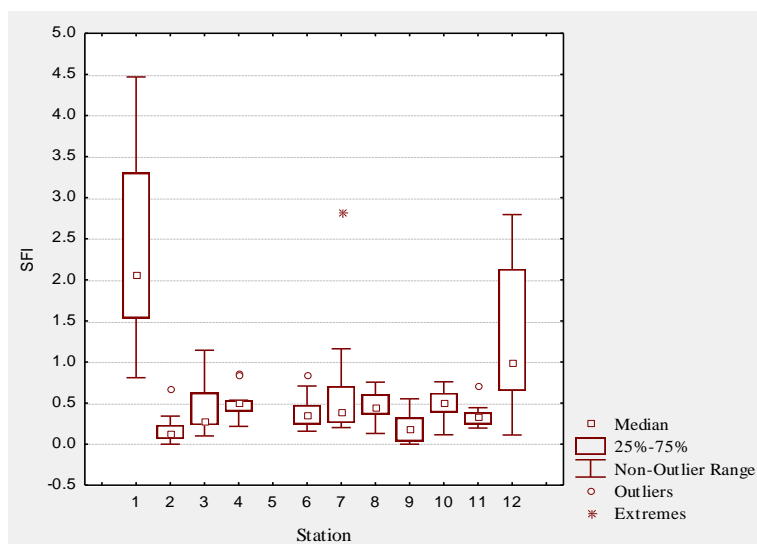


Figure 4.9. 6. Spatial distribution of sprat stomachs fullness (SFI) index in late autumn 2015.

The lowest index of SFI was registered at stations 9 (located to the south of Burgas Bay , 80-85 meter isobath) – about 0.02 % body sprat weight. It station was

nominated as a station with the lowest standing stock of mesozooplankton which reflected to the food availability and trophic state conditions (Fig. 4.9.6).

Non-linear dependence was found between SFI and sprat weight (with range 2.03 – 6.85 g). The most significant food amount, represented as a percent share from the fish body weight was estimated in a middle-sized sprat group with weight 3-6 g (Fig.4.9.7.)

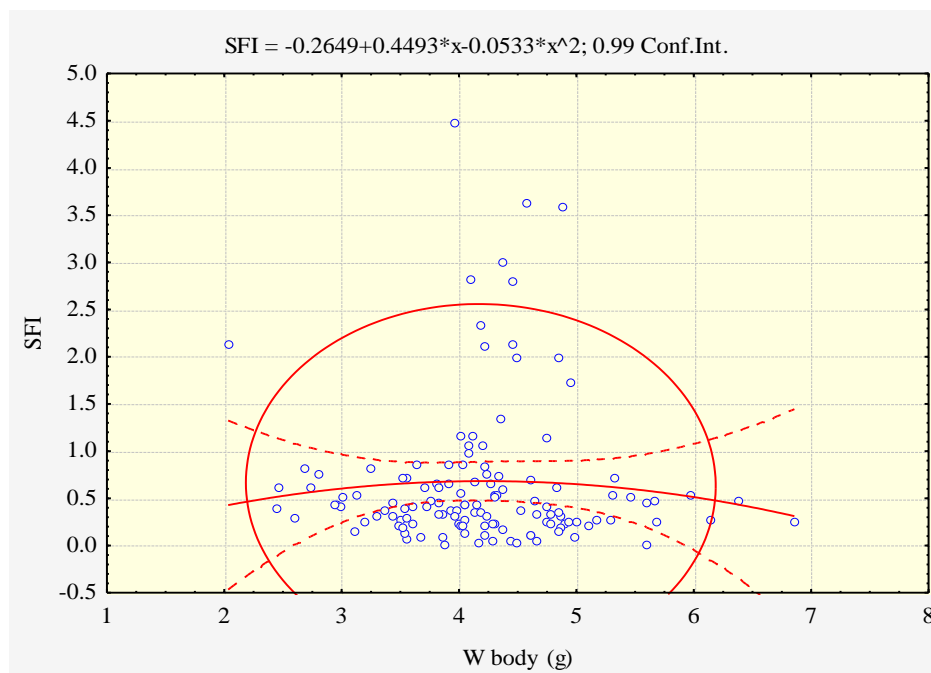


Figure 4.9.7. Contribution of the factor weight (W, g) to the fitted values of ISF. (With dotted and ellipse lines were estimated 99% confidence interval)..

Species composition and index of relative importance (IRI) of different mesozooplankton species in the sprat diet.

In 90% of the investigated sprat gut content the detected mesozooplankton prey species were in good condition allowing to be quantitatively and qualitatively identified. In 8 % of all events only food remains were found, which could not be analyzed due to food digestion process and in 2% of spat specimens only empty stomachs were observed. The maximum prey number of items identified per fish stomach reached 385 ind/stomach, recorded at station 4 in front of c. Kaliakra (50m isobaths). The estimated average prey number was 82 ± 69 ind/stomach. The mean prey density was 1.5 fold lower than those registered in the period 2010-2011 (110 and 116 ind/stomach) and two folds higher in comparison with the period 2007-2009 (~ 43 ind/stomach) (Raykov et al 2007, 2008, 2009,2010,2011). Minimum prey number (<10 ind/stomach) was established at station 9 (situated to the south of

Burgas Bay). Higher prey number (> 100 ind/stomach) was recorded at stations 1, 3, 4, 7 (located around c.Kaliakra and c.Galata), while the lower prey number (between 9-100 ind/stomach) was associated to all other stations (Fig. 4.9.8). In December 2015 sprat found good food base in water strip among 20–50 and 70-80 meter isobaths mainly along the Northern part of Bulgarian coast and shelf.

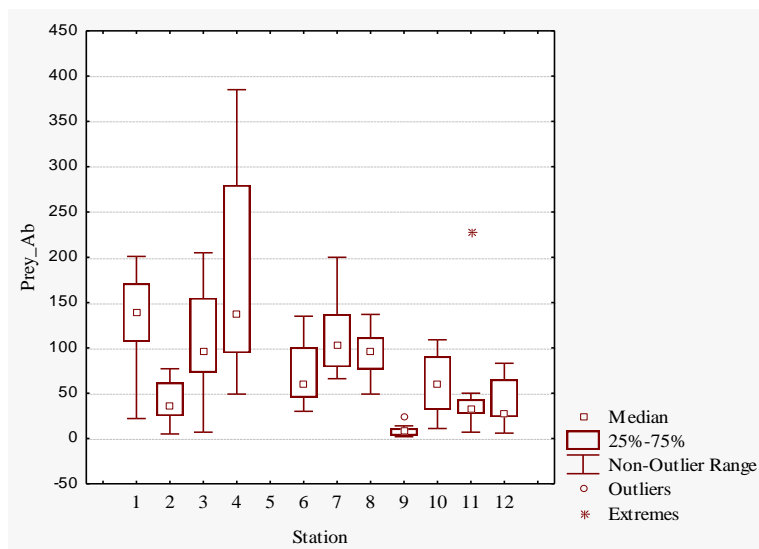


Figure 4.9.8. Prey abundance distribution in December 2015.

A total of 15 species and taxa identified in analyzed zooplankton samples collected simultaneously with trawl survey were found in the sprat diet. Copepoda representatives *C. euxinus*, *A. clausi*, *P. elongatus*, *P. parvus*, *O. similis*, *O. davisae* with their larvae stages were well presented in the sprat diet. Benthic larvae of Bivalvia, Cirripedia (nauplii and cypris), Polychaeta and Decapoda, and species *Oikopleura dioica* (Appendicularia), *Parasagitta setosa* (Chaetognatha) were identified in sprat gut content as well.

The indices of relative importance (IRI) of the main mesozooplankton species in sprat food spectrum with their percent share regarding to abundance and biomass, as well as their frequency of occurrence in sprat samples are presented in Table 4. During the study period the sprat food was predominated by copepods *P. parvus*, *A. clausi*, *C. euxinus* with their larvae stages. Bivalvia larvae ranked second in sprat food spectrum.

Table 4.9.10. The sprat food composition (the main zooplankton species/taxa composition), Index of relative importance.

species/taxa	N (% of the total abundance)	B (% of the total biomass)	FO (frequency of occurrence)	IRI (index of relative importance)
<i>Acartia clausi</i>	22.4	40.70	100	6312.91
<i>Calanus euxinus</i>	12.9	20.22	73	2409.94
<i>Paracalanus parvus</i>	21.2	12.93	100	3413.71
<i>Pseudocalanus elongatus</i>	2.7	2.29	100	498.06
<i>Oithona similis</i>	0.0	0.00	9	0.16
<i>Oithona davisae</i>	0.3	0.06	55	20.28
Copepoda sp.	4.2	7.98	64	774.33
Copepoda ova	0.9	0.02	18	16.58
Polychaeta larvae	0.0	0.00	18	0.35
<i>Lamellibranchia veliger</i>	24.1	8.22	100	3235.94
Cirripedia nauplii+cypris	3.8	2.11	100	590.72
Decapoda megalopa	0.01	0.00	9	0.06
<i>Oicopleura dioica</i>	3.8	0.48	73	309.90
Pisces ova	0.1	0.87	45	43.69
<i>Parasagitta setosa</i>	3.4	4.09	27	203.22

According to the distribution pattern of IRI and importance of zooplankton species in the sprat diet not clear distribution pattern relevant to depth were distinguished. (Table 4.9.5). The most important species/taxa in the sprat food diet as *A. clausi*, *P. parvus*, Bivalvia larvae were well presented at coastal and shelf waters but with preference to 20 – 50 m isobaths. *C. euxinus* prevailed in integrated deeper sites between 70-90 m isobaths

Table 4.9.11. IRI distribution pattern of important zooplankton species/taxa in sprat diet at stations.

Вид/таксон	1	2	3	4	6	7	8	9	10	11	12
	75 м	46 м	41 м	48 м	28 м	19 м	33 м	84 м	59 м	37 м	85 м
<i>Acartia clausi</i>	10.1	263.40	6306.77	8305.83	8027.54	14935.41	8667.24	39.80	6196.63	12872.07	8.96
<i>Calanus euxinus</i>	17618.2	86.79	29.38	60.86		40.29	87.54		1746.28		8564.29
<i>Paracalanus parvus</i>	4.3	1202.32	6207.18	7985.08	5305.22	2191.96	2280.82	2070.74	6518.99	240.37	4.27
<i>Pseudocalanus elongatus</i>	299.9	1851.38	24.51	3.89	2.64	1.20	6.10	30.56	224.23	12.70	487.76
<i>Oithona similis</i>										1.61	
<i>Oithona davisae</i>			40.93	53.16	1.15			10.70	1.26	26.23	
Copepoda sp.	8.22	5908.18	1622.93	9.54	279.38			2928.29		3.12	
Copepoda ova	0.5	498.48									
Polychaeta larvae			0.82	0.97							
Lamellibranchia veliger	42.4	5097.99	3347.64	2479.09	4331.06	1761.05	7242.72	7918.81	608.95	2236.45	59.20
Cirripedia larvae	187.6	192.09	208.32	429.67	1045.02	177.86	693.50	414.02	947.06	388.36	290.53
Decapoda megalopa	0.6										
<i>Oicopleura dioica</i>			117.17	189.77	432.87	72.29	195.01	50.58	155.26	2025.11	
<i>Pisces ova</i>	2.1	48.83	8.31		13.95					14.87	
<i>Parasagitta setosa</i>	49.8					14.64					4976.87

5. Predictions and possibilities for exploitation

Equilibrium state of the sprat stock

Equilibrium yield and related biomasses of sprat from Bulgarian and Romanian Black Sea waters have been presented graphically on Fig.5.1.1. On first graphic Equilibrium Yield with Confidence intervals (uncertainties) showed very low values at CI_{med} and $CI_{2.5\%}$. Y/R with $CI_{97.5\%}$ reached its maxima and corresponded to fishing mortality at around 1.16 then plateau of the curve follows and F_{max} determination became impossible.

It is evident that levels above $F=0.8$ would lead to stock collapse. Sustainable levels of fishing mortality are those around $F=0.5$, which would correspond to the catch level of 12.5 thous.tons of sprat in Bulgarian and Romanian marine waters.

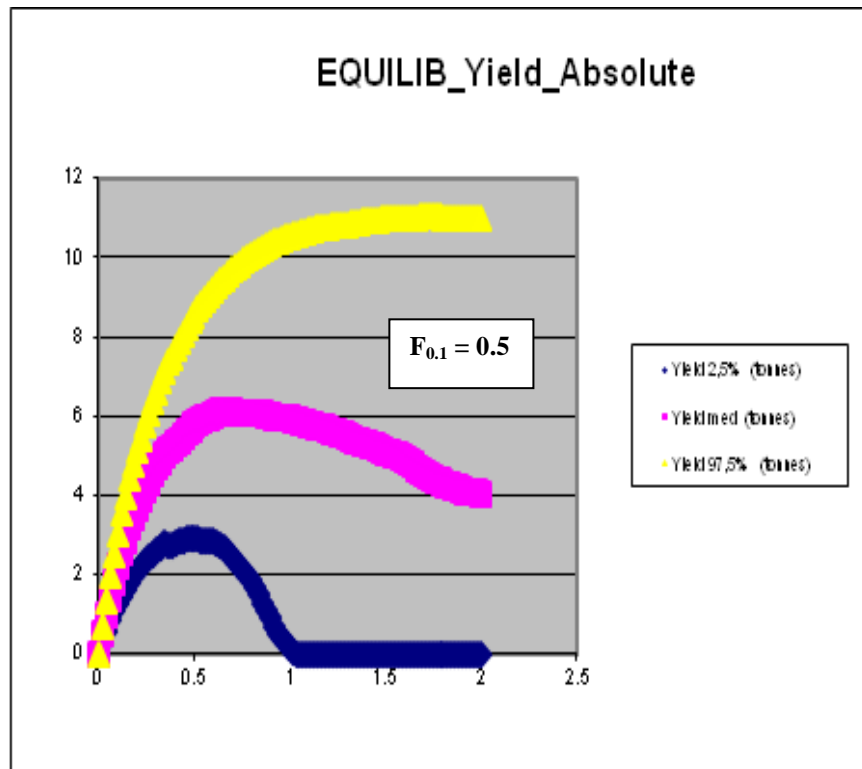


Figure 5.1.1. Equilibrium yield with CI. Optimal level of fishing mortality and corresponding catch of sprat from Bulgarian and Romanian waters.

Spawning stock biomass, fishable biomass and total biomass followed similar trend of steep decrease, since only values with $CI_{97.5\%}$ have relatively high values at corresponding lowest fishing mortality. Hence, with increasing fishing mortality all tested biomasses (Fig.5.1.2, Fig.5.1.3, and Fig.5.1.4.) followed diminishing trend, as after $F=0.8$ (at $CI_{2.5\%}$) and after 1.16 (at CI_{med}) the sprat stock would decrease below unsustainable levels – Fig.5.1.1.

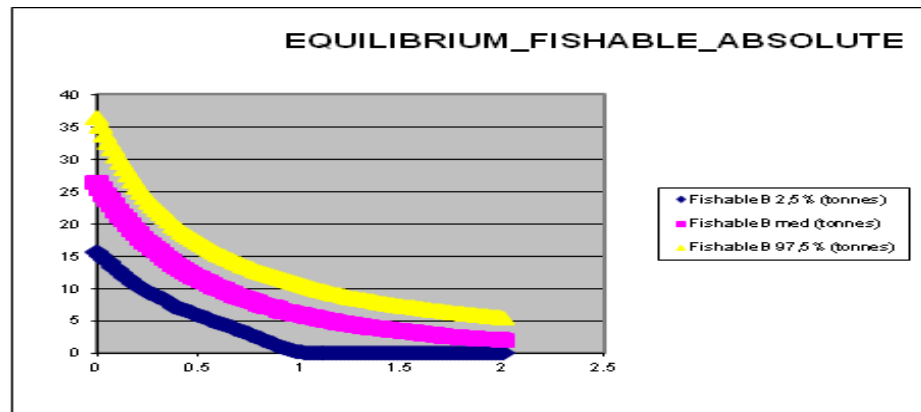


Figure 5.1.2. Equilibrium Fishable Biomass

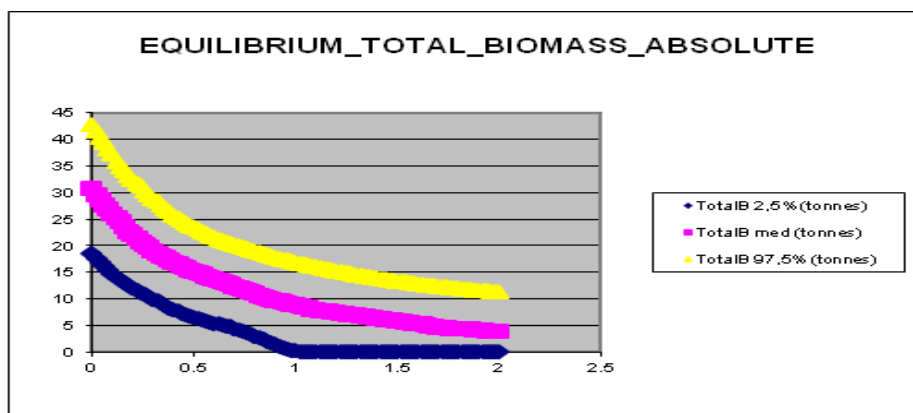


Figure 5.1.3. Equilibrium Total Biomass

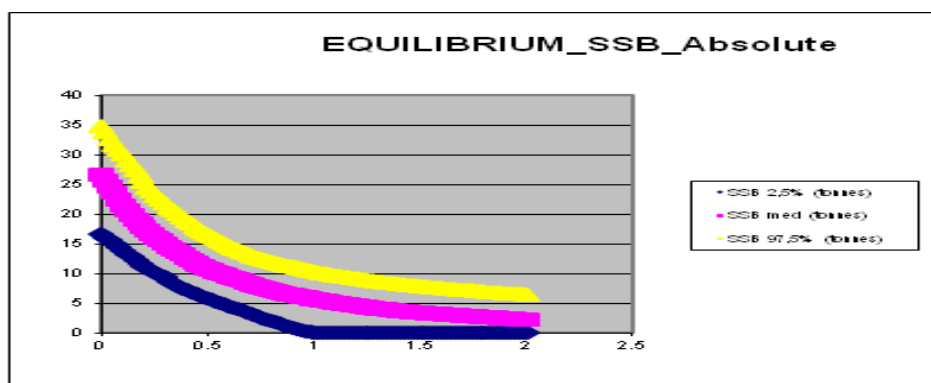


Figure 5.1.4. Equilibrium SSB

Recruitment is highly affected by fishing mortality and after $F = 0.5$ drops very steeply – Fig. 5.1.5.

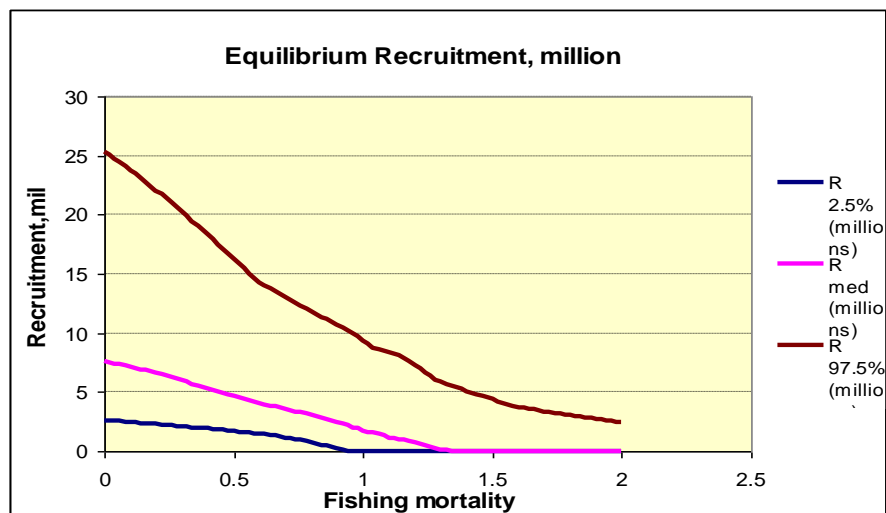
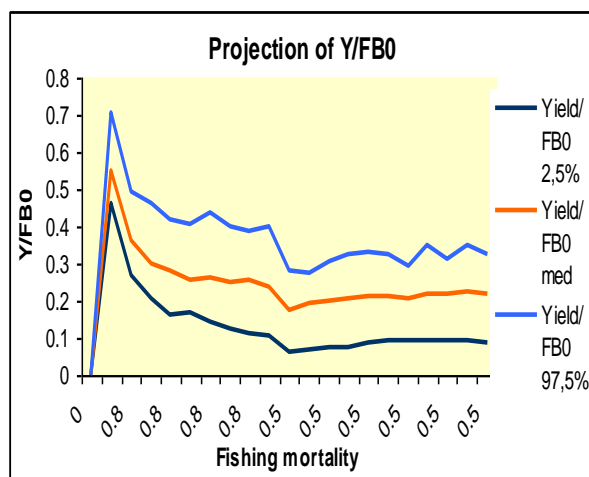
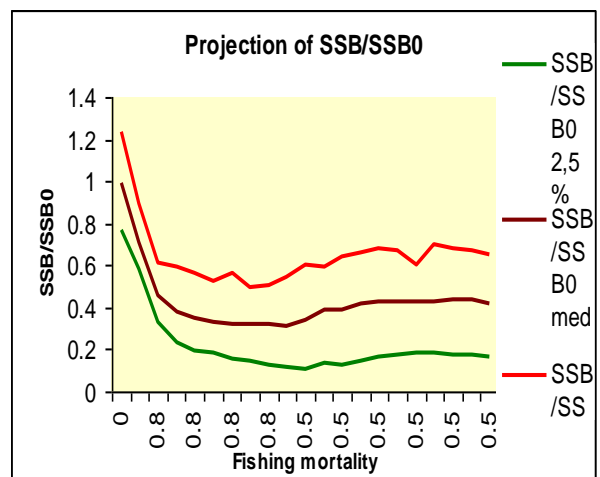


Fig.5.1.5. Equilibrium Recruitment

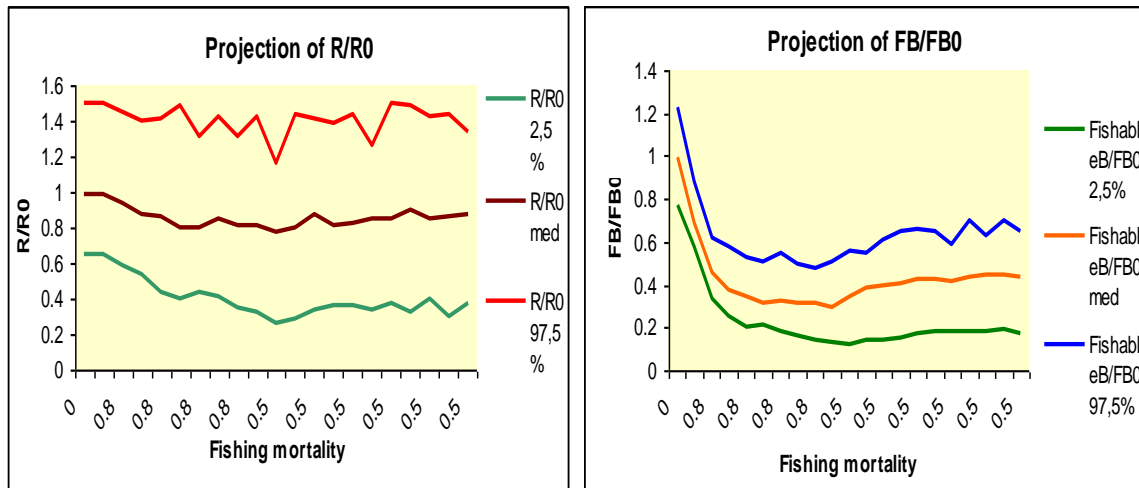
6. Prediction model of the stock parameters in relation with fishing mortality variation for 10 years



A)

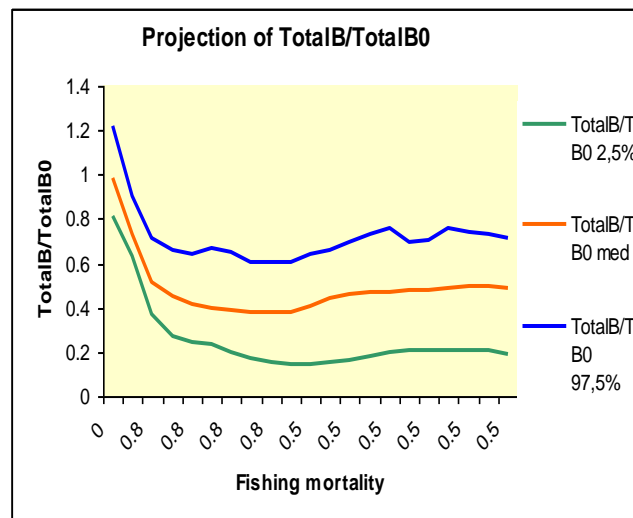


B)



C)

D)



E)

Figure. 6.1. Predictions of the stock parameters of sprat, related to fishing mortality
Unexploited state

The relative yield (Y/F_0) at very low levels of the fishing mortality is high during the first projected year (Fig.6.1. A). At $F = 0.8$, in the second projected year fall of the relative yield was detected up to the levels of $F = 0.5$ (Fig. 6.1, A). After the fifth projected year, in all tested confidence intervals, plateau of the relation Y/F_0 was observed. Similar is the case with the relative (SSB/SSB_0) (Fig. 6.1, B), and even slight increase at CI 97.5% and SSB/SSB_0 med, after change of fishing mortality (from $F = 0.8$ to $F = 0.5$). Recruitment (Fig. 6.1, C) is stable, none influenced by the

changes in fishing mortality. Fishable and total biomass, represented as relation with the biomass at unexploited state, show similar trends with those of relative SSB (Fig. 6.1, D, E).

variations.

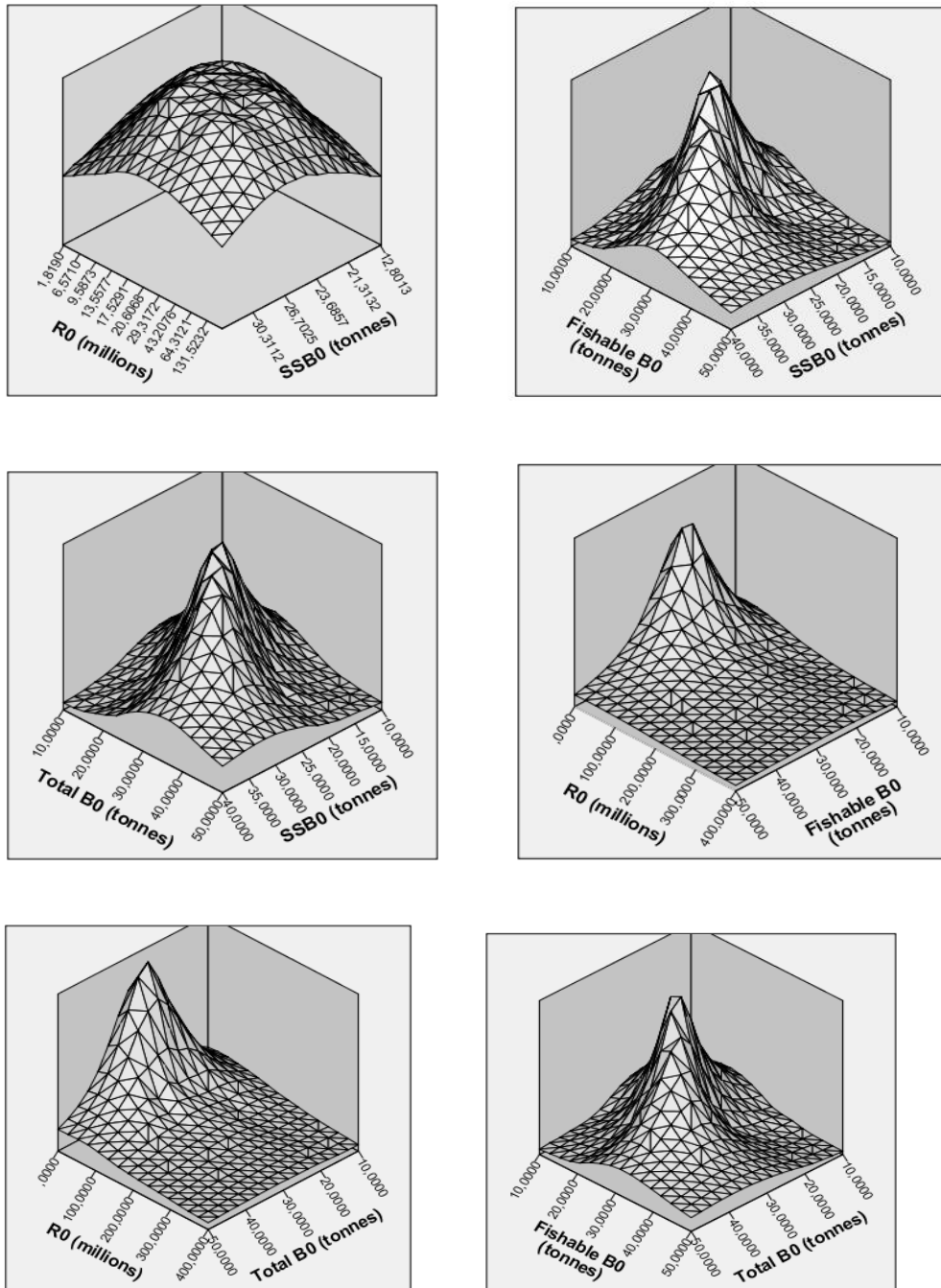


Figure. 6.2. Unexploited state

7. Maximum sustainable yield

Maximum sustainable yield (MSY) by countries, according to the method of Gulland (1970), was calculated for both exploitation biomasses – in Bulgarian and in Romanian waters. In the present research we used the natural mortality coefficient $M=0.95$ (Ivanov and Beverton, 1985; Prodanov et al., 1997; Daskalov, 1998). The results obtained are given on Tabl.7.1.

Table.7.1. Biomass (tons) by countries and MSY.

Country	Biomass	MSY (t)	
	(t)	Gulland	BH steepness, $F_{0.1}$
Bulgaria	44281.961	41 035	11 500

The estimated MSYs represent the maximum potential yield, including official landings, regulated by quota, as well as misreporting or illegal catches and by-catch in other fisheries. The calculated exploitation biomasses and equilibrium levels (MSYs) should not be regarded as absolute value for possibly future yields in view of the fact that the methods have some uncertainties and the share of misreported and illegal catches and the by-catch during fisheries on other target species has been still unknown. In such cases, special approaches has been applied, as use of $2/3$ MSY (Caddy and Mahon, 1995).

II. Biological monitoring of European sprat (*S. sprattus*) landings.

II.1. Objectives

The Black Sea sprat (*Sprattus sprattus* L.) is a key species in the Black Sea ecosystem. Sprat is a marine pelagic schooling species. In the daytime it keeps to deeper water and in the night moves near the surface. It forms big schools and undertakes seasonal movements between foraging (inshore) and spawning (open

sea) areas (Ivanov and Beverton 1985). Adults tend to remain under the seasonal thermocline, penetrating above its only during the spring and autumn homothermia. Juveniles are distributed in a larger area near the surface. Sexual maturity is attained at the age of 1 year and length of 7 cm (Sampson et al., 2014).

Multi annual biological monitoring on the landings provides the so called “Fishery dependant” information. The aim of this study is to collect and to analyze dynamics in length, weight and age distribution as well as to determinate condition of the monitored species using the so-called Condition factor (Ricker, 1975). Biological information on a given species collected each month thus analyzed and compared for previous periods could be used then for estimation of growth parameters. These indicators are with very high importance of short lived species. Robust and informative long-term information is of crucial importance for fisheries stock assessment, fisheries management and decision making process as a whole.

II.2. Sampling

II.2.1.1. Geographic area coverage

Data of present analysis were collected directly from landing ports of Nessebar and Sozopol – two main landing sites of Bulgarian sprat active fisheries. The catches and landings of sprat were realized in the zone in front of Sozopol, Nessebar and Cape Emine at depths of 40-60 m. Mean depth of the operating vessels were 45 m.

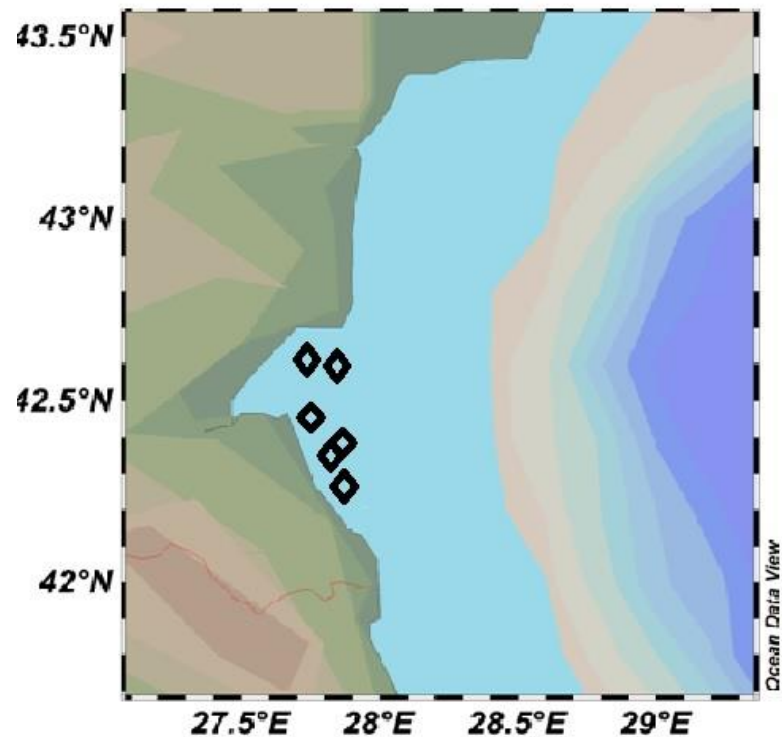


Figure 2.1.1.1. Map of sampling points with corresponding samples of sprat.

II.2.1.2. Sampling period

Two samples were collected in November (Nessebar), four samples in December (Sozopol). All samples originated from active fishery with trawlers and using mid-water trawls (OTM). Table II.2.1.2.1.). The collected samples in November 2015 from landing site of Sozopol (4 samples) consisted of 1388 individuals and collected samples in December (2 samples from Nessebar) consisted of 638 sprat individuals. The total numbers of sprat individuals in random samples were 2026.

Table.2.1.2.1. Sample sites or biological monitoring of sprat in November – December, 2015.

Landing site	Coordinates	Number of samples
Nessebar	<u>42°39'N 27°44'E</u>	2
Sozopol	<u>42°417'N 27°7'E</u>	4

II.2.1.3. Statistical analysis of data

All samples tend to be collected in accordance with the variation statistics from significant landings in terms of quantity where is possible. Random sampling theory was followed when taking the sample. The catches of sprat of described period for sampling were scarce due to “shift” in target species (e.g. Bluefish *Pomatomus saltatrix*) and lack of significant schools formations in the period of present research. The samples were processed in laboratory conditions. Length was measured to the nearest cm, as Total length was taken into consideration only. The weight was measured to the nearest gram (0.1g). The age determination were conducted under binocular microscope , in reflected light, magnification X10. Thus, the yearly annulus were detected as hyaline and opaque zones, shifting active growing with period of growth stagnation. Condition factor, also known as Fulton’s index was estimated following the procedure proposed by Ricker, (1975):

$$K = \frac{W}{L^3} * 1000$$

For all the samples “Age-Length” (weight) Keys were created. Thus, the mean values of length, weight and condition factor were resulted. The share (in %) of individuals per age groups and length groups were reflected in the analysis as well. Distribution fitting using statistical routine for histogram of the mean representation with corresponding analysis of cumulative distribution were presented and discussed.

Statistics estimated on the input data and computed using the estimated parameters of the Normal distribution was conducted using XL Stat Software.

Q–Q plot ("Q" stands for quintile) is a probability plot, which is a graphical method for comparing two probability distributions by plotting their quantiles against each other.

The coefficient of variation (CV) is defined as the ratio of the standard deviation σ to the mean μ :

$$c_v = \frac{\sigma}{\mu}$$

The coefficient of variation is useful because the standard deviation of data must always be understood in the context of the mean of the data. In contrast, the actual value of the CV is independent of the unit in which the measurement has been taken, so it is a dimensionless number. For comparison between data sets with different units or widely different means, one should use the coefficient of variation instead of the standard deviation.

II.3. Results

II.3.1. Landings statistics in 2015

Official statistics of landings in the period of interest was presented on Table 3.1.1. It is visible that the catch in November accounted of 81.879 tons In December - 204.819 tons. The unfavorable hydrometeorological conditions were main prerequisite for low registered catches in this period of the year.

Table 3.1.1. Landings statistics for December 2015.

November	December
81879kg	204819kg

II.3.2. Length structure of landings

Length frequency of sprat in Nov-Dec 2015 is shown on Fig.3.2.1. In November and December three modal distributions were observed as length classes 8-8,5-9.0 cm predominated. The individuals at 2-2+ prevail with high percent, followed by 3-3+ age old in November.

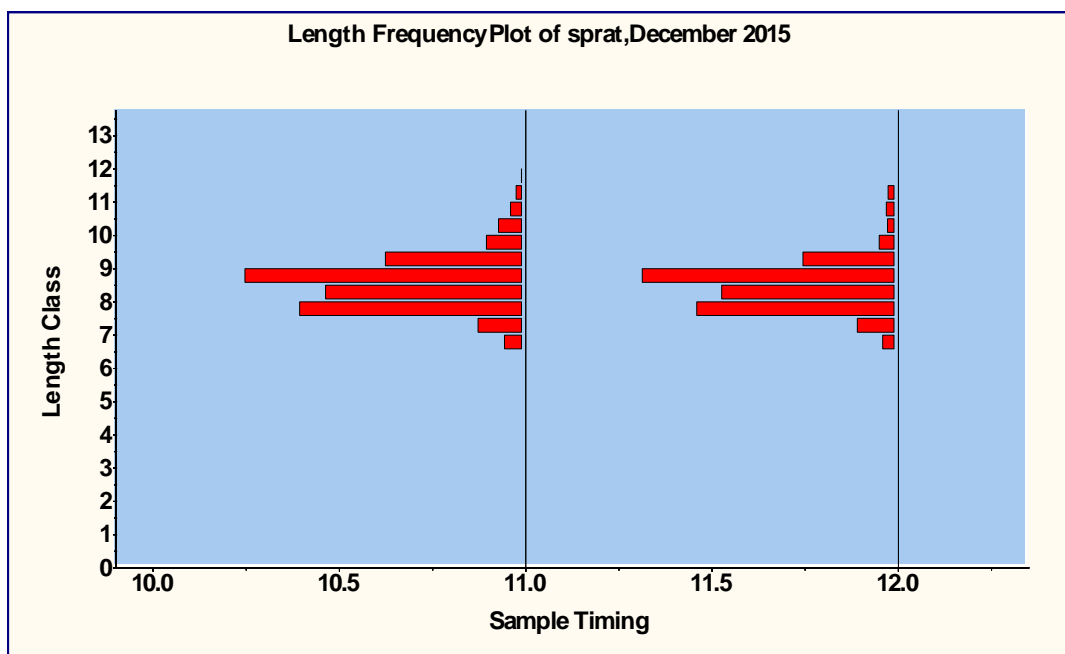
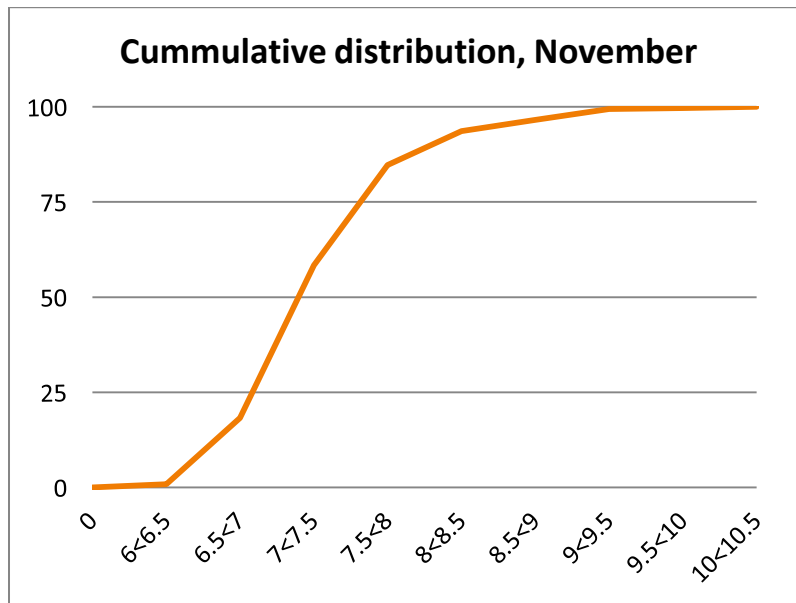
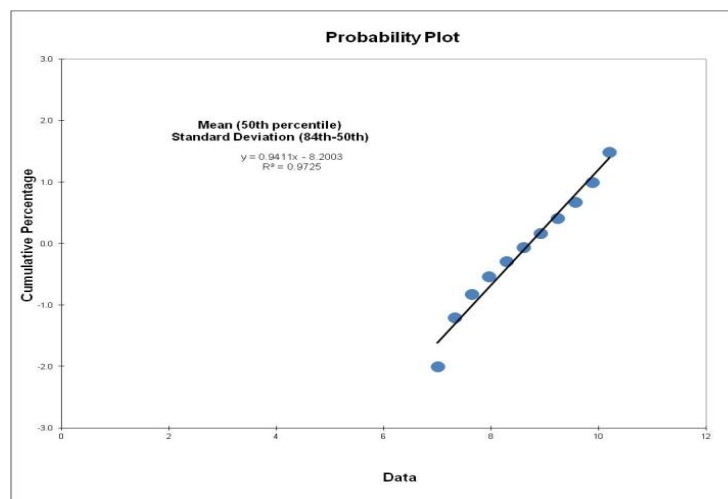


Figure 3.2.1. Length frequencies of sprat landings in November – December, 2015



a.



b.

Figure 2.1.3.1. (a) cumulative distributions (Nov, 2015) (c) Q-Q probability plot

The risk to reject the null hypothesis (H_0) was tested by Kolmogorov-Smirnov test:

Table 2.1.4. Kolmogorov-Smirnov test (November, 2015)

D	0,149
p-value	0,973

alpha 0,05

In Q-Q probability plot we observe that the circles all lie quite close to the line; close enough to say these data come from a normal distribution. (Figure 2.1.3.1.c). The trend is indeed slight - so slight that we still call these data normal.

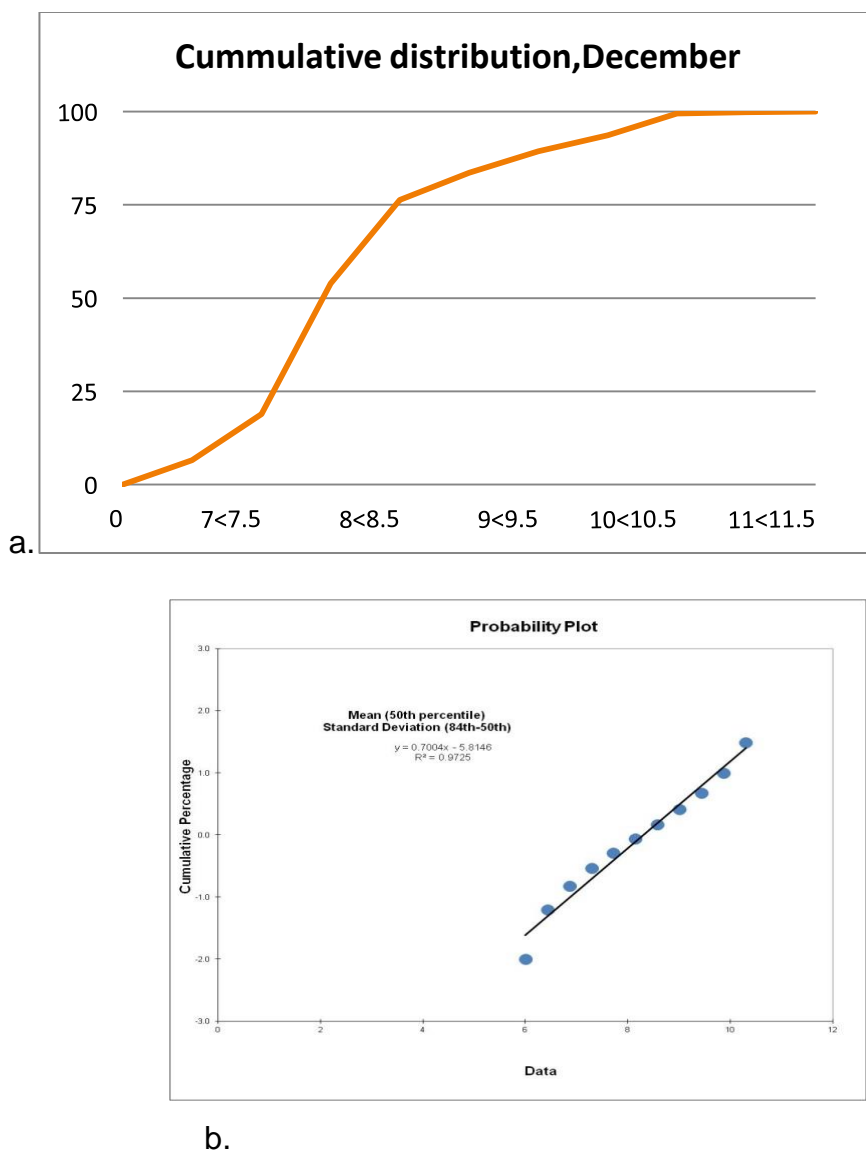


Figure 2.1.3.2. (a) cumulative distributions (December. 2015) (b) Q-Q probability plot.

In Q-Q probability plot we observe that the circles all lie quite close to the line; close enough to say these data come from a normal distribution. (Figure 2.1.3.2.c). The trend is indeed slight - so slight that we still call these data normal.

Table 2.1.6. Kolmogorov-Smirnov test (December, 2015)

D	0,147
p-value	0,712
alpha	0,05

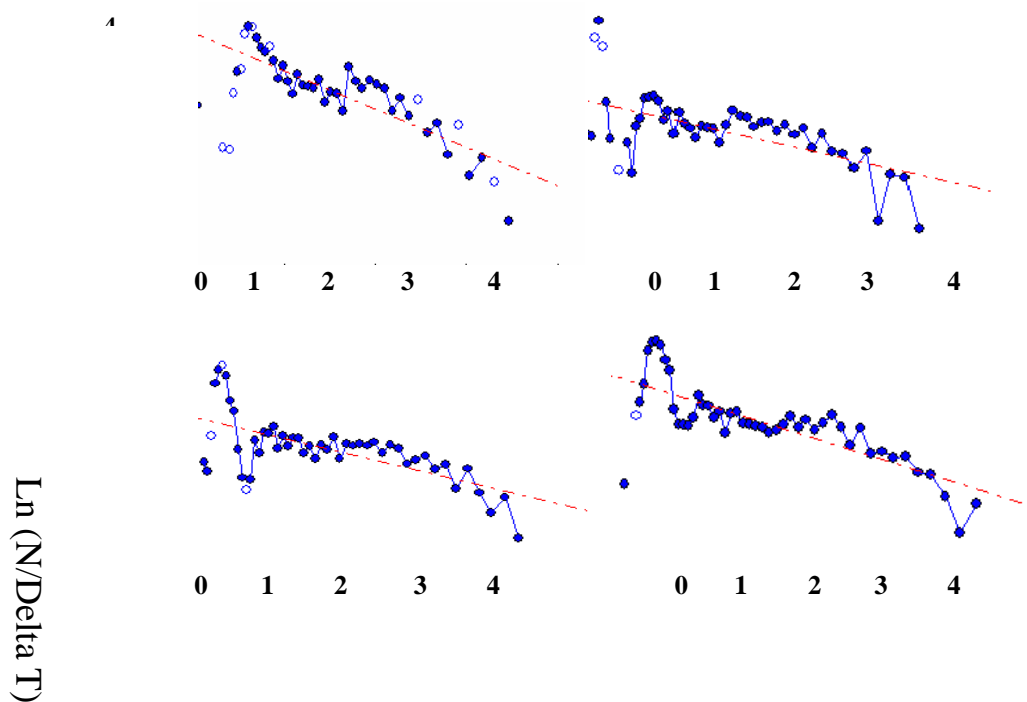
c.

Figure 2.1.3.3. (a) cumulative distributions (October, 2014).(b). Q-Q probability plot.

In Q-Q probability plot from October samples we observe that the circles all lie quite close to the line, almost stay in one line. Here, we definitely can say taht these data come from a normal distribution. (Figure 2.1.3.3.c).

The estimation of the total mortality using length-converted catch curve resulted mean 1.46 as the total mortality varies from 1.26 to 1.66

Distributions	1	2	3	4	5	6	7	8	9	10
Z	1.35	1.40	1.51	1.45	1.29	1.26	1.31	1.66	1.34	1.54



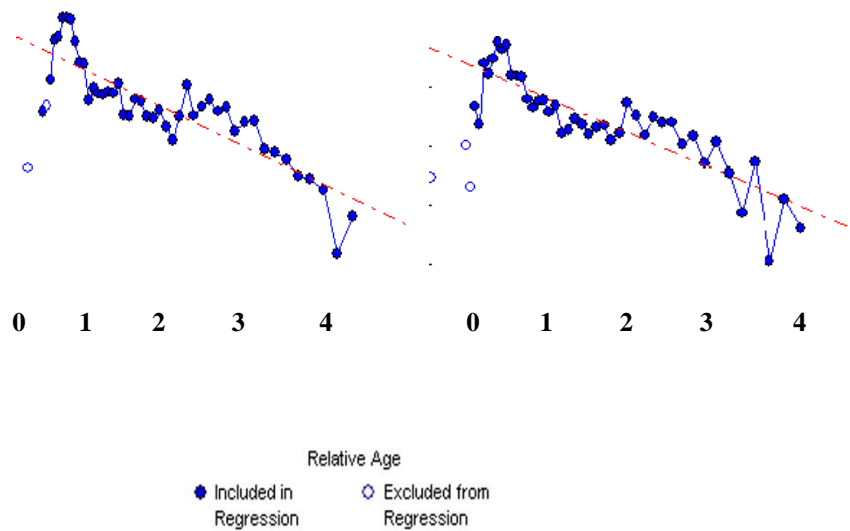


Figure.2.1.3.4. Length-converted catch curve of sprat from present research

II.3.3. Age structure of landings

It is evident (Fig.3.3. 1.) that 2-2+ age class present in the landings with very high percent (over 45), followed by age 3-3+ aged individuals. Oldest registered in the samples belong to 4-4+ years old, with very low share in the landings. Recruitment (0+) presented with 5% from the total.

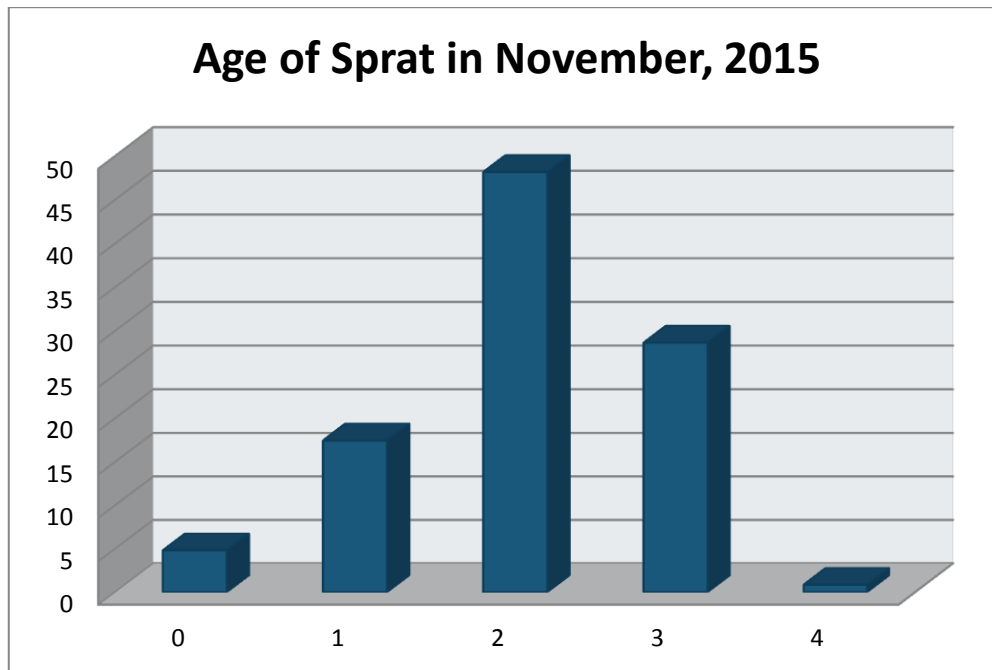


Figure 3.3.1. Age distribution of Sprat in November, 2016

In December the age distribution shifted as three age classes (1-3+) have almost equal share in the landings. The recruitment was detected but with lower share in December 2015. Older 4-4+ in December slightly increased in comparison with November 2015.

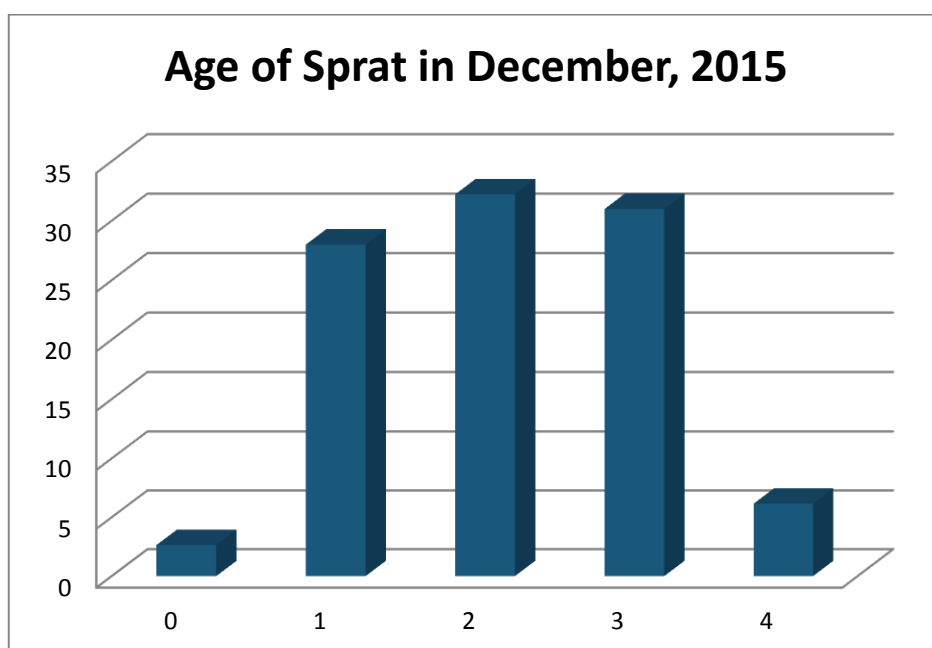


Figure 3.3.2. Age distribution of Sprat in December 2015.

II.3.4. Condition factor

In November 2015 condition of the sprat was low as only 4-4+years old individuals' show over 0.6. This is probably due to the high water temperature, lack of fodder cold water zooplankton and low consumption of the sprat in the summer period (Fig.3.4.1.).

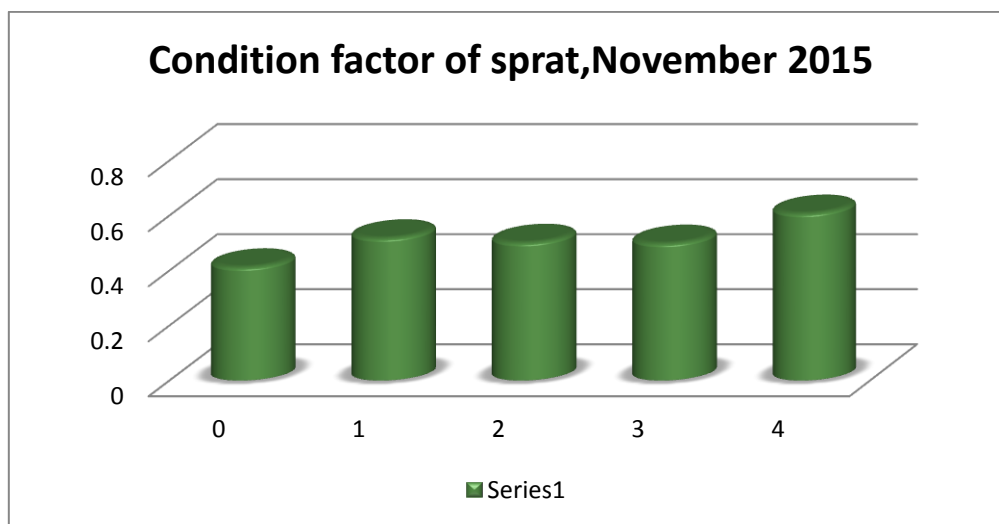


Figure 3.4.1. Condition factor of sprat in November 2015.

In December 2015 condition factor increased in all of the age groups with exception the oldest (4-4+). This fact could be related to active maturation, batch spawning and increased individual weight of females (Fig.3.4.2.).

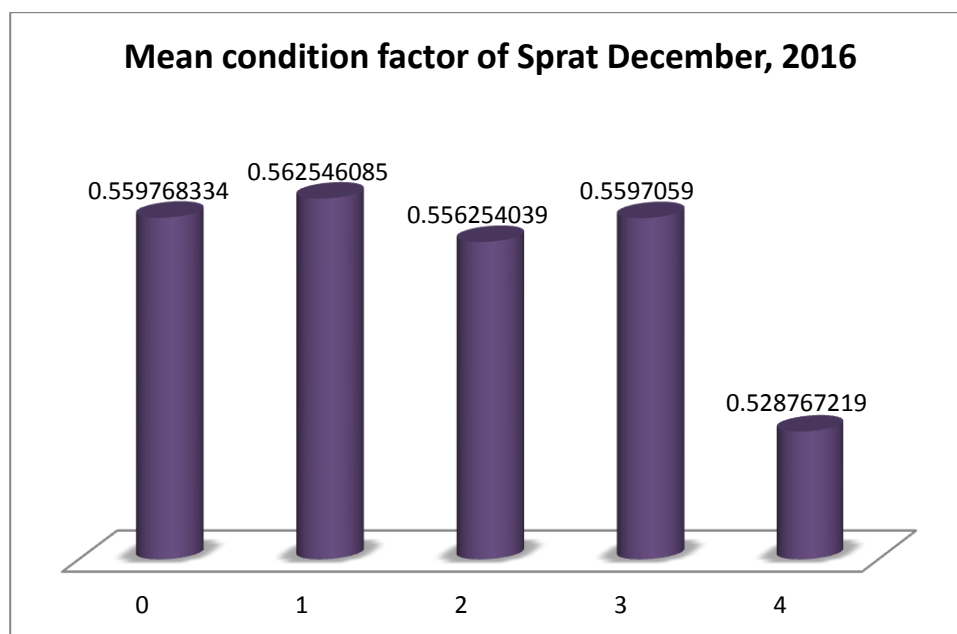


Figure 3.4.2. Condition factor of sprat in December 2015

II.3.5. Catch numbers and biomass by age and length

Monthly catches (in tons) together with mean weights of sprat were used to derive the monthly catch numbers. The share (%) by age groups and catch numbers were used to create catch-at-age matrix for selected months by age groups (Table 3.5.1.):

Table 3.5.1. Catch at age (10^{-6}) matrix and biomass (kg) of sprat for selected months

Age	nov	dec
0		11.34523
1	48.78543	38.11458
2	46.9876	40.01244
3	3.23649	9.78123
4	0.99048	0.74652
Catch-at-age (millions)	nov	dec
0		56.39275
1	92.9989	0.04574
2	0.051686	0.017005
3	0.001363	0.004362
4	0.000478	0.000369
catch numb	93.05245	56.46023

Monthly catches (in tons) together with mean weights of sprat were used to derive the monthly catch numbers. The share (%) by length groups and catch numbers were used to create catch at length matrix for selected months by age groups (Table 3.5.2.):

Table 3.5.2. Catch at length (10^{-6}) matrix and biomass (kg) of sprat for selected months

Catch-at-length (millions)		
Length group (cm)	Nov	Dec
6		2.73383742
6.5	4.022261	3.3369888
7	18.32569	10.256666
7.5	37.01617	26.32566
8	32.22458	10.5558996
8.5	38.3656	21.96501
9	23.37478	14.40447
9.5	13.63375	6.8896654
10	10.18623	9.256422
10.5	6.8245	3.25698
11	1.105645	2.88295582
11.5	1.025665	1.055899

II.3.6. Coefficient of variation of length

Table 3.6.1. Length Coefficient of variation of sprat samples (Nov-Dec)

Coefficient of variation	November	December
1st sample	CV = 0.18	
2nd sample	CV = 0.17	
3rd sample	CV = 0.05	
4rd sample	CV = 0.18	
5 th sample		CV = 0.14
6th sample		CV=0.14

The dimensionless expressions (Table 3.6.1) of CVs show relatively low magnitude of standard deviation around mean. The variability was in limits of 0.05 - 0.18 and could be estimated as low. This means that the random sampling of sprat in

months of interest was conducted according to the variation statistics and correctly reflected the general population at this time of the year.

8. Conclusions and recommendations

- ✓ Total number of identified species in Bulgarian waters was 17, from which 13 fish, 1 crustacean, 2 mollusk, 1 macro zooplankton;
- ✓ 36 random samples were collected from the catch (from 36 stations), as the total number of sprat individuals were 12 427.
- ✓ The total surveyed area in Bulgarian part was 9136.6 km² and total estimated biomass was 44 282 t;
- ✓ The densest sprat aggregations were detected in the shallowest stratum 50-75, (905.1 – 18103 kg.km⁻², CI (95%) = 3272.6. In contrast to the previous surveys the CUPA of *M.merlangius* was low.
- ✓ The rest of species (by catch) were presented with low percentage
- ✓ Are for biomass assessment (extrapolated) was 9136 kg.km⁻²
- ✓ The biomass of the sprat agglomerations for the surveyed area was estimated at about of 11 063 tons, extrapolated for the shelf from shore, at **44 282** tons;
- ✓ December is not the best month for biomass estimation due to the fact that sprat is batch spawning species (cold water species).The majority of population is in active maturation, not feeding actively. The fodder zooplankton in this period was dispersed, the sun activity was low and SST was relatively high, not favourite conditions for sprat aggregations.
- ✓ The highest sprat biomass aggregations were in 50-75 m isobaths.The densest aggregations were localized in front of Bourgas Bay predominantly, since in northern direction the indices were relatively low;

- ✓ The size composition comprised of length classes (TL, cm) from 6.5 cm up to 12.5 cm in the samples from Bulgarian marine area,
- ✓ The prevailing length classes registered in Bulgarian waters had tree modal distribution (8.0-8.5 cm; 8.5-9.0 cm; 9.0-9.5 cm);
- ✓ In Bulgarian waters, the size distribution has been characterized by tree modal distribution as prevailing class was TL = 8.0 cm, followed by class 8.5 cm,
- ✓ Analysis shows that the percentage of one and two years old fish was high.
- ✓ The asymptotic length reached 12.66 cm and growth rate could be assessed as relatively high accounting 0.77 y^{-1} . The growth of sprat from present research is positive allometric ($n=2.78$);
- ✓ In the present research we used natural mortality coefficient for sprat as equal to 0.95;
- ✓ Levels above $F = 0.8$ would lead to stock collapse. Sustainable levels of fishing mortality are those around $F=0.5$, which would correspond to the catch level of around 8.5 thous.tons of sprat in Bulgarian marine waters;
- ✓ Zooplankton structure was strongly influenced by the dominance of heterotrophic dinoflagellate *N. scintillans*. The species was presented at almost all stations with more of 80%. The dominance of Noctiluca reflects the poor quality of the food resource for the sprat and worsened trophic conditions for the sprat population. Copepods prevailed in the fodder zooplankton structure with 50-75% in abundance and 58-78% in biomass;
- ✓ The average mesozooplankton biomass (ignoring *N. scintillans*) was approximately 9 and 7 folds lower in comparison with those registered in 2014 and 2011 respectively which reflects the worsening trophic conditions for the species *Sprattus sprattus*;
- ✓ No clear distribution pattern in zooplankton density and biomass from coastal to open sea was discerned. However, two areas could be distinguished between 19-35 m isobaths and between 75-80 m isobaths characterized with higher fodder zooplankton density. The mesozooplankton density and biomass decreased in direction from the North towards South, where the

community was homogenous presented despite the difference in the stations depth;

- ✓ In December 2015 sprat found good food resources in water strip among 20–50 and 70-80 meter isobaths mainly to the Northern part of the Bulgarian coast and shelf (between c. Kaliakra and c. Emine). The sprat diet was predominated by copepods *A. clausi*, *P. parvus*, *C. euxinus* and larvae of *Bivalvia*.
- ✓ The average value of index of stomach fullness reached 0.649% of sprat body weight, equivalent to those registered in 2009 and almost 2 times lower than in 2011. It has increased two folds in comparison with the estimated index in 2009 (0.6%). The most significant food amount was estimated in a middle-sized sprat group with weight 3-6 g.
- ✓ The relative yield (Y/F_0) at very low levels of the fishing mortality is high during the first projected year. At $F = 0.8$, in the second projected year fall of the relative yield was detected up to the levels of $F = 0.5$;
- ✓ Sprat is a fast growing species with highly cycling nature of its recruitment and parental stock biomass dependant on the anthropogenic impacts different from fishing, as well as fishing press and dynamics in the environmental factors. Hence, when studied the continuity of the research on population parameter dynamics is of high importance. In studied months the observed length, weight and age structure were stable. The condition factor is high in the beginning of the spawning period and gonad maturation (IV-V; VI; III gonad stages were observed). To analyze and to make stronger recommendations regarding the sustainability of exploitation and measures for rational utilization of the marine living resources at least 4 samples per month should be collected from different depths and processed. New indicators as lipid content, otolith chemistry should be introduced when biological characteristics are studied.
- ✓ We strongly recommend the landings and catches sampling in all months, due to the fact that sprat fisheries is conducted whole year round with active and passive fishing gears.

- ✓ The calculated exploitation biomasses and equilibrium levels (MSYs) should not be regarded as absolute value for possibly future yields in view of the fact that the methods have some uncertainties and the share of misreported and illegal catches and the by-catch during fisheries on other target species has been still unknown. In such cases, special approaches has been applied, as use of 2/3 MSY (Caddy and Mahon, 1995).

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ANNEX I

CPUE kg.h⁻¹ and CPUAkg.km⁻² Bulgarian marine areas

station	CPUEkg/h	CPUAkg/km2
1	105	1221.885008
2	100	1205.260722
3	400	4821.042888
4	462.857143	5386.268606
5	300	3491.100022
6	400	4654.80003
7	440	5120.280033
8	280	3374.730022
9	266.666667	3214.028592
10	300	3491.100022
11	280	3258.360021
12	60	698.2200045
13	280	3258.360021
14	400	4654.80003
15	240	2892.625733
16	650	7564.050048
17	680	7913.160051
18	2400	28926.25733
19	400	4821.042888
20	400	4821.042888
21	180	2169.4693
22	380	4422.060028
23	150	1745.550011
24	120	1446.312866
25	342.857143	3989.828597
26	800	9309.60006
27	720	8378.640054
28	900	10473.30007
29	50	602.630361
30	130	1566.838939
31	500	6026.30361
32	200	2410.521444
33	500	5818.500037
34	250	3013.151805
35	240	2892.625733
36	600	6982.200045

ANNEX II

Species composition from Bulgarian area

[illegible]

ANNEX III

Market sampling, Discards and Surveys indicator targets and results in 2015 (Bulgarian part)

Black Sea	Length @age	market, discards, surveys	2,50%	Market: 2026 Discard: - Survey: 12 427 1250
Black Sea	Weight @length	market, discards, surveys	2,50%	Market: 2026 Discard: - Survey: 12 427 5000
Black Sea	Weight @age	market, discards, surveys	2,50%	Market: 2026 Discard: - Survey: 12 427 1250
Black Sea	Maturity @length	surveys	2,50%	5000 140
Black Sea	Maturity @age	surveys	2,50%	5000 140
Black Sea	Sex-ratio @length	market, surveys	2,50%	Market: 250 Survey: 250 125
Black Sea	Sex-ratio @age	market, surveys	2,50%	Market: 250 Survey: 250 500