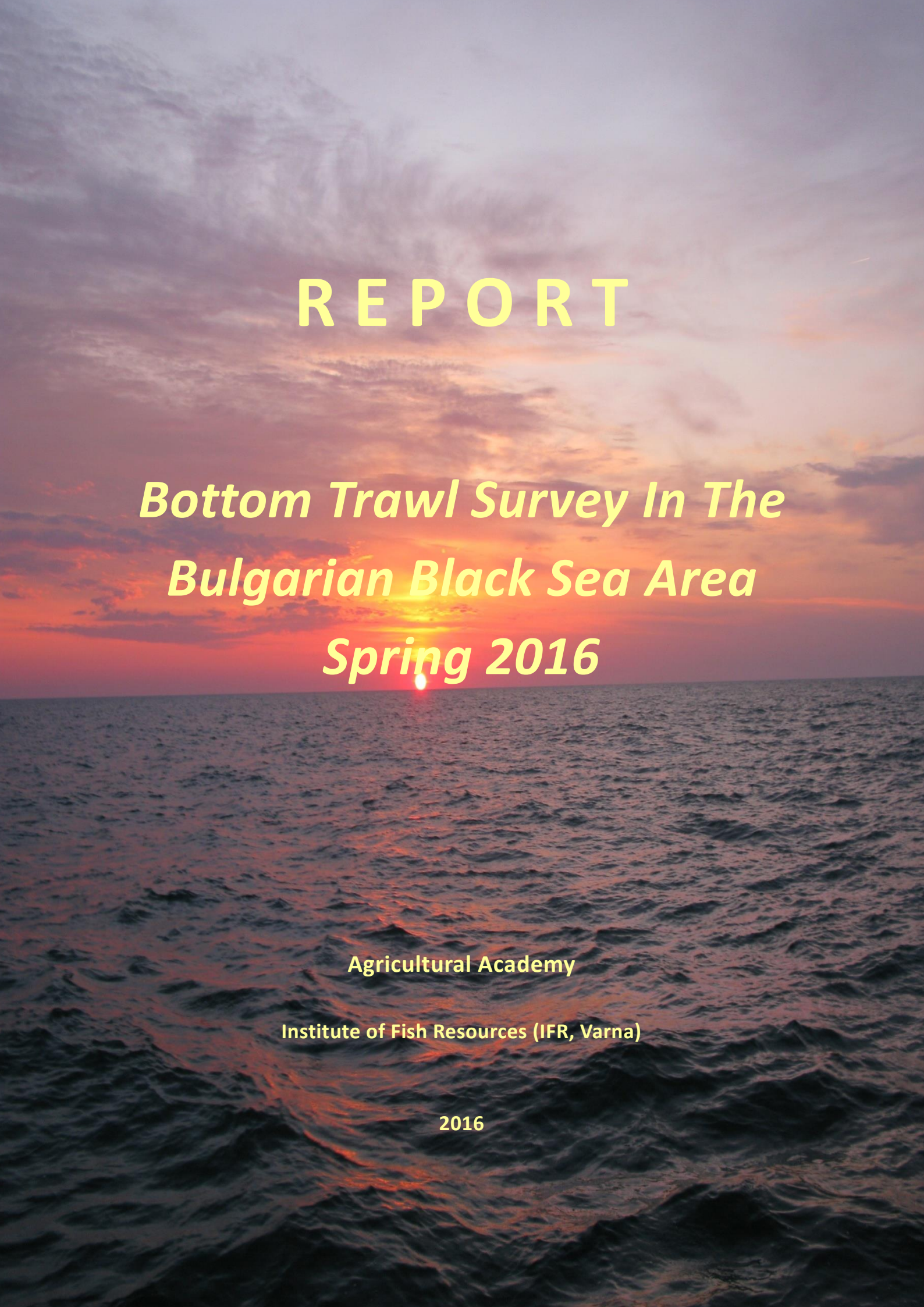

REPORT

Bottom Trawl Survey In The Bulgarian Black Sea Area Spring 2016

Agricultural Academy

Institute of Fish Resources (IFR, Varna)

2016

The present study was conducted by a team of specialists from the Institute of Fishery Resources (IFR) - Varna to the Agricultural Academy, and from the "Grigore Antipa" National Institute for Marine Research and Development (NIMRD), Constanta, Romania, under contract No. /D-46/14.05.2016between IFR - Varna and the National Agency for Fisheries and Aquaculture (NAFA) - Burgas, for turbot stock assessment in the Bulgarian Black Sea waters during the spring season of 2016.
The study was conducted owing to the financial support of the European Commission in compliance with Council Regulation No. 199/2008 and Commission Decision 2010/93/EU, aimed to help the member states for creating a common frame for the collection, management and use of data in the fisheries sector and support for scientific advice regarding the Common Fisheries Policy.
The study was performed in the period 19 – 31 May 2016 in the Bulgarian Black Sea waters on board the “EGEO 1” fishing vessel.

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National Bottom Trawl Survey – May 2016

1. Results from the National Bottom Trawl Survey in May 2016

In the period 19 - 31 May 2016, in compliance with a contract with NAFA - Burgas/D-46/14.05.2016**,** under the National programme for fisheries data collection, the research team from IFR - Varna conducted a demersal trawl survey with the fishing ship "EGEO 1" in the Bulgarian Black Sea waters - between Durankulak and Ahtopol, within the 100-meter isobath.

This report is based on the data collected during the trawl survey, which are indicative of the distribution and values of the relative biomass and abundance of the reference species *Scophthalmus maximus*. The analyses include an estimation of the biomass and density of the reference species by depth strata, and a study of size/age and sex structure of the stock. The turbot stock assessment is based on application of standard methods (methodology and software), analogous to those, used during the preceding trawl surveys of the species in the Black Sea.

This document contains a series of tables and figures that show the relative biomass distribution, as well as size/age and sex structure analysis of the reference species.

As a whole, the trawl survey in 2016 included the following activities:

- Bottom trawl sampling;
- Qualitative and quantitative analysis of the catch, identification of biological diversity, biometric measurements;
- Collection of material for turbot age determination;
- Sampling and analysis of stomach contents for identification of quantity and composition of the consumed food;

2. Fishing vessel and fishing gear

The trawl survey was conducted on board the fishing ship "EGEO 2" (picture 1) with the following parameters:

- Fishing vessel – TAKA;

- Fishing vessel length -19.90 m;
- Maximum width – 6.55 m;
- The fishing vessel year of built - 2005;
- Engine power – 420 kW;
- Maximum tonnage – 30.56 t;
- Net tonnage – 9.17 t;
- Speed – 8.0 Nd;
- Crew – 3 people;
- Research team – 3 people;

Picture 1 *Fishing ship "EGEO 1"*

During the study, a fishing bottom trawl 22/27-34 was applied (picture 2), with following functional and technical parameters:

- Trawl vertical opening – 2 m;
- Horizontal opening between the otter boards – 9 m;
- Effective part of wing spread – 13 m;
- Trawling speed – 2.2 - 2.6 Nd;
- Trawling duration – 60 min.

Picture 2 *Bottom trawl 22 / 27-34.*

3. Material and methods

The target species of this study was turbot - *Scophthalmus maximus,* as the by-catch species - the spiny dogfish (*Squalus acanthias)* and the thornback ray (*Raja clavata*) were also analysed.

The methodology and techniques that were used for collection, verification, processing, and data analysis and for overall turbot stock assessment in spring 2016 were in compliance with the generally applied methodology during the previous turbot data collection programs in the Bulgarian Black Sea zone.

The filed data were collected by standard techniques (bottom trawl) and methodology, which remained constant through the survey. The GPS system of the ship was connected to NAFA satellite system for monitoring of the fishing vessels (VMS) and the ship location was strictly controlled during the trawling.

Data analysis was performed by the "swept area method" and the obtained results can be reproduced and compared.

3.1 Information collected through the bottom trawling:

- ✓ Depth - measured with the echo-sounder;
- ✓ GPS coordinates of the trawling - starting and end points;
- ✓ Trawling duration;
- ✓ Abundance of fish species in the trawl;
- ✓ Weight of the total catch in the trawl;
- ✓ Absolute and standard length; weight of collected specimens;
- ✓ Collection of otoliths for age determination;
- ✓ Sex identification;
- ✓ By-catch species composition;
- ✓ Turbot stomachs;

Specimens with absolute length under the minimum, permitted by Fisheries and Agriculture Act (FAA, 2005), (below 45 cm), were immediately returned into the sea, after the measurements.

For turbot biomass calculations, data for catch per unit effort (CPUE) (kg/h) and catch per unit area (CPUA) (kg/km^2) were used.

The final results are presented in the form of maps and tables that include data for:

- ✓ Surface area (km^2);
- ✓ Average mass per unit area (t/km^2);
- ✓ Limits of variation of mass pet unit area;
- ✓ Total biomass (t.);
- ✓ Abundance index (individual/km^2);

3.2. Sampling scheme

To establish the abundance and biomass of the reference species *Scophthalmus maximus* off the Bulgarian Black Sea coast, a standard methodology for stratified sampling (Gulland, 1966; Sparre, Venema, 1998; Sabatella, Franquesa, 2004) was applied. The zones, where trawling was performed, are presented in Figure 1.

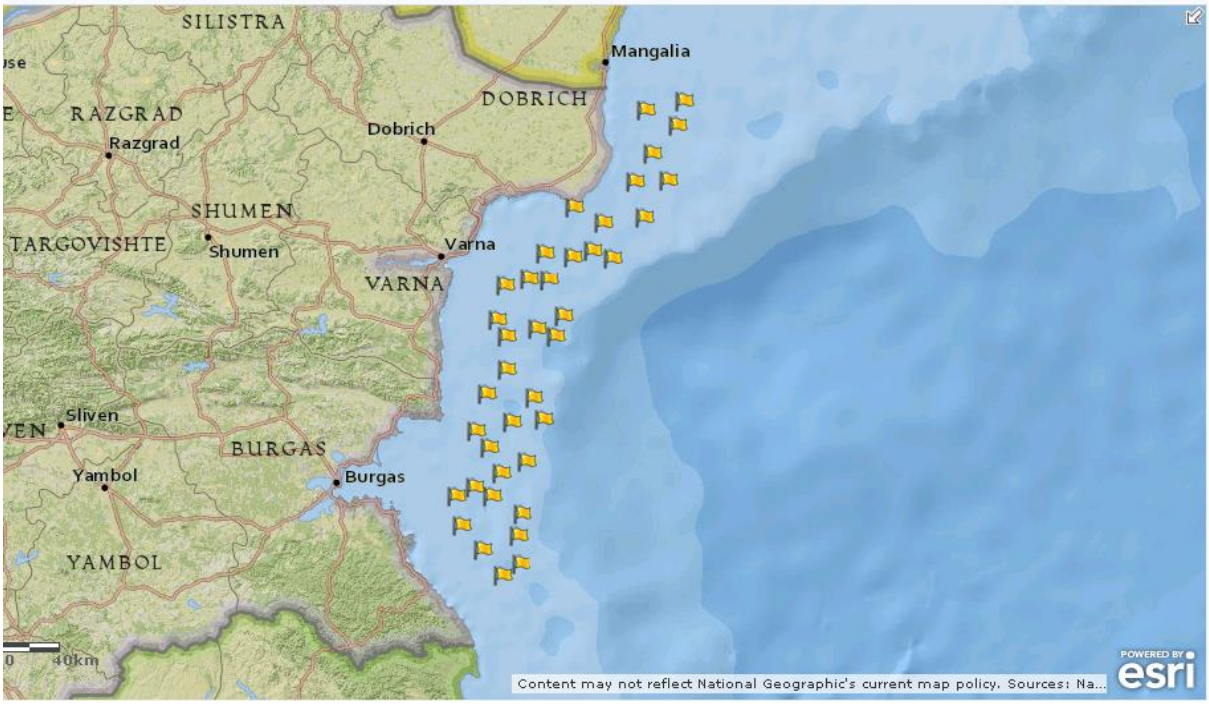


Fig.1 Map of the surveyed sectors.

The surveyed region off the Bulgarian coast was divided into four strata, depending on the depth – Stratum 1 (15 - 35 m), Stratum 2 (35 - 50 m), Stratum 3 (50 - 75 m) and Stratum 4 (75 - 100 m). For the purpose of turbot abundance and biomass assessment, the surveyed territory was divided into 143 squares, each of them with sides 5 x 5 Nm, area 25 Nm^2 (or 85.8569 m^2) (Fg.2).

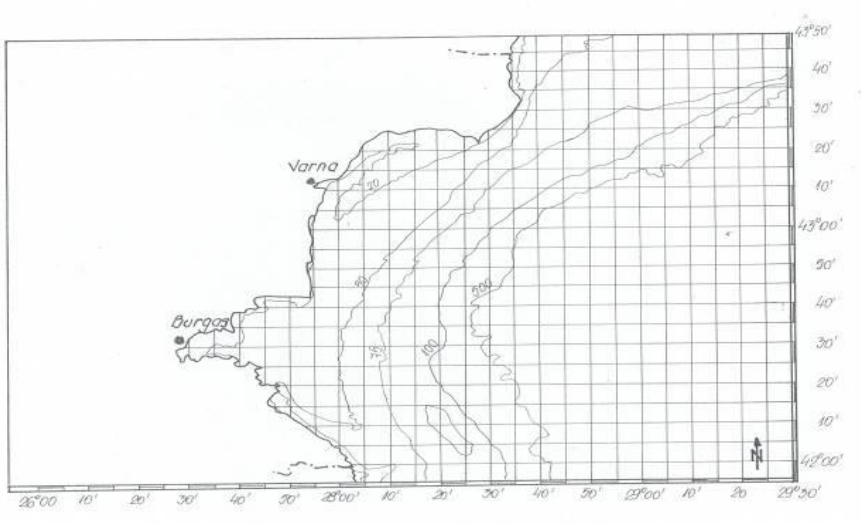


Fig.2. Grid lines used for calculations of the area.

The sampling was carried out at 39 randomly chosen fields (rectangles), situated at depth between 15-100 m. Each rectangle is with sides 5'Lat × 5'Long, while the total area is 62.58 km^2 (measured by GIS). Each field was marked with letters and digits for better distinction (Fig.3).

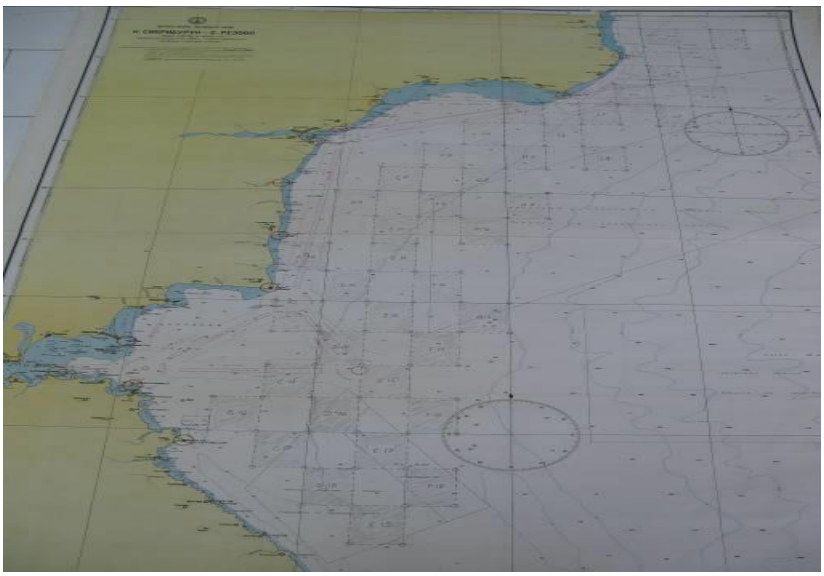


Fig.3 Map and scheme of the fields used in the design of the sampling.

The seabed area covered during a single haul represents a basic measurement unit, considered representative, as turbots do not aggregate in dense assemblages (Martino, Karapetkova, 1957). The duration of each hauls was 60 min. at trawling speed of 2.6 knots.

On the ship board, the absolute and standard length, as well as the individual weight of each specimen were measured in order to determine the size and weight structure of the turbot stock and to estimate the share of specimens with length below the allowable fishing length in the catches.

3.3. Laboratory analysis

After collecting the samples on shipboard, the age, maturity of the reproductive system, stomach food composition, and fillet chemical composition were determined in laboratory.

The turbot age was established by otoliths reading under binocular microscope.

To identify the food composition, a total of 53 stomachs were collected in spring 2016. The stomach content analysis included identification of the taxonomic composition and total number of food components, weight and frequency of occurrence of each food component. The index of relative importance (IRI) was used to determine the significance of food components in the trophic spectrum (Pinkas et.al., 1971):

$IRI = (C_N + C_W) * F$

C_N - percentage share of the food item i in total number; C_W - percentage share of the food item i in the total weight; F – frequency of occurrence.

IRI expressed as a percentage was calculated by the equation (Cortes, 1997):

$$\%IRI_i = \frac{100 * IRI_i}{\sum_i^n IRI_i}$$

n – total number of the taxonomic categories at a given taxonomic level.

3.4. Statistical method

<u>Swept areas method</u>

To determine the relative biomass of the reference species (*Scophthalmus maximus*), the “swept area method” was applied. According to this method, trawl sweeps a well-defined path, the area of which is the length of the path times the width of the trawl, called the "*swept area*" or the "effective path swept", thus the swept area can be estimated from equation:

a = D*hr*X2

D = V*t

V is the velocity of the trawl over the ground when trawling, t is the time spent trawling, hr is the length of the head-rope. X2 is that fraction of the head-rope length, hr, which is equal to the width of the path swept by the trawl, the "wing spread", hr*X2, D-distance covered.

To calculate turbot biomass, the catch per unit area(CPUA) was used:

$$\frac{C_{w/t}}{a/t} = \frac{Cw}{a} kg/km^2$$

Cw/t – catch in units of weight per trawling hour, a/t – area swept per trawling hour.

The biomass for each stratum was obtained from equation:

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

$\overline{C_{w/a}}$ - mean catch per unit of area for all trawl sweeps in the stratum, A – stratum area.

The variance of biomass estimated for each stratum is:

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

The total area of the surveyed region is equal to the sum of the areas of every stratum:

A = A1+A2+A3

The mean catch for the entire survey area was obtained from equation:

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

Ca1- catch per unit area in stratum 1; A1 – stratum 1 area, etc.; A – total water area.

The total biomass in the survey area is estimated by equation:

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

$\overline{Ca}(A)$ - mean weighted catch for the entire surveyed water area, A – total area surveyed.

Maximum sustainable yield

Gulland's formula for virgin stock is:

MSY = 0.5*M*Bv

M – coefficient of natural mortality, Bv- biomass of virgin stock.

A generalized version of Gulland was proposed by Cadima (in Troadec, 1971) for exploited fish stocks for which only limited data are available for stock assessment:

$$MSY = 0.5 * Z * \overline{B}$$

$\overline{B}$ - mean annual biomass, Z – total mortality.

Because Z = F + M and Y = F * $\overline{B}$, Cadima suggested that in the absence of data for Z, the equation can be rewritten:

$$MSY = 0.5 * (y + M * \overline{B})$$

y – total catch in one year, $\overline{B}$ - mean biomass in the same year.

Age and growth

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

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

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

Lt, Wt are the length or weight of the fish at age **t** years;L∞ , W∞ - asymptotic length or weight;k – curvature parameter;to - the initial condition parameter.

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

$$W_t = qL_t^{\,n}$$

q –constant in length-weight relationship;n – constant in length-weight relationship.

Natural mortality (M)

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

$$\log M = -0.0066 - 0.279 * \log L_\infty + 0.6543 * \log k + 0.4634 * \log T^\circ C$$

$$\log \mathrm{M} = -0.2107 - 0.0824 * \log \mathrm{W}_\infty + 0.6757 * \log \mathrm{k} + 0.4687 * \log \mathrm{T}^\circ \mathrm{C}$$

L∞, W∞ and k – parameters in von Bertalanffy's equation; TºC - the annual average temperature of the seawater in the horizons of habitation and reproduction of the species.

***Method of Richter si Efanov* (1976)**

$$M = \frac{1.521}{(t_{mat.50\%})^{0.720}} - 0.155$$

t_{mat} – age at first maturation;

***Method of Lukashev* (1970)**

$$M=1-\left[\frac{1-e^{-k(t-t_o)}}{1-e^{-k(t_{+1}-t_o}}\right]^3$$

t = age of mature fish

Method of Alverson-Carney (1975)

$$M=\frac{3.k}{e^{t_{mb}.k}-1}$$

t_{mb}= age at which biomass is maximal;

4. Results

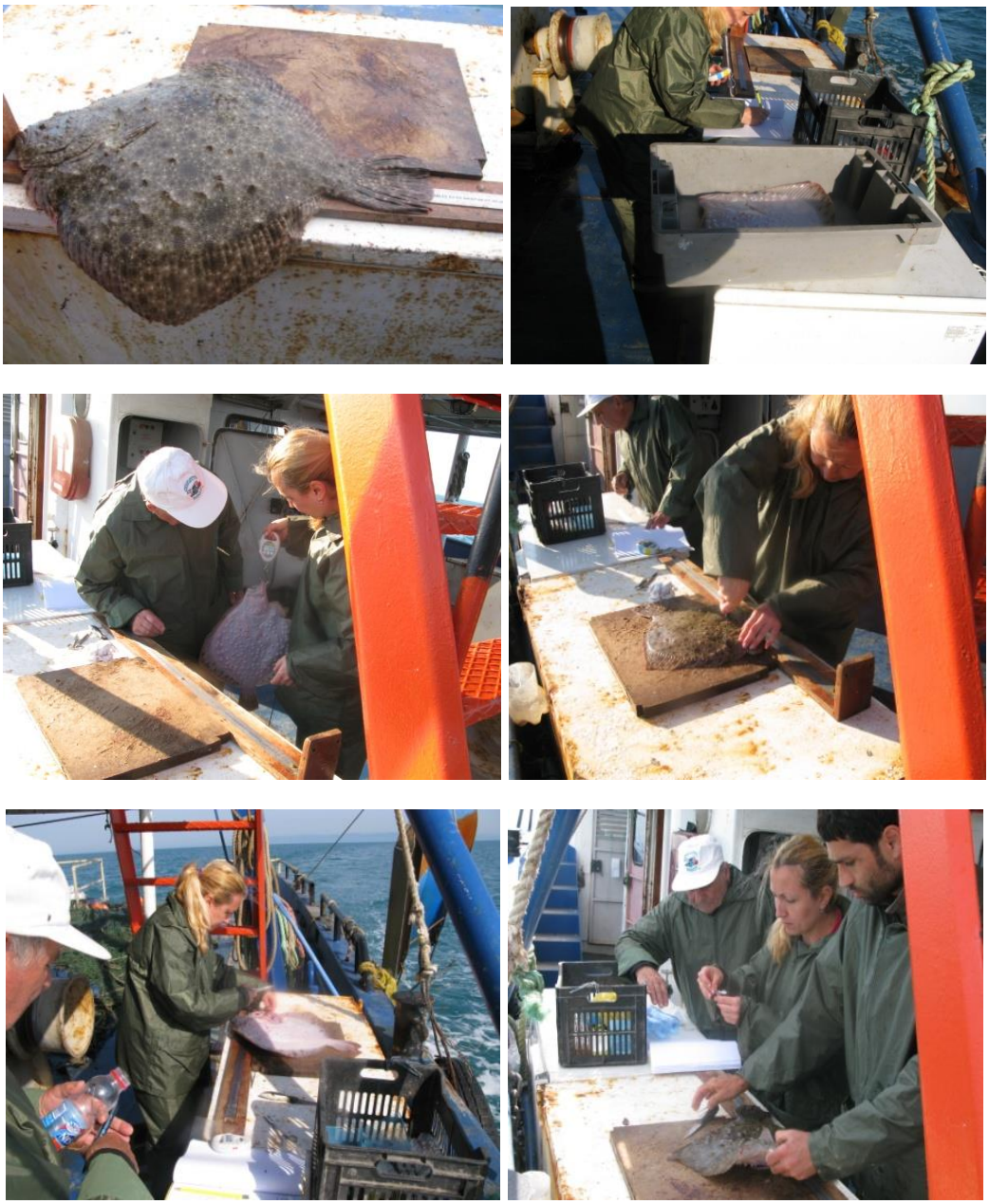
4.1. Population number and biomass

During the demersal trawl survey in V/2016 the following activities were carried out:

- 39 hauls were performed with a bottom trawl, with duration of 60 minutes for each trawl at depths between 15.0 m and 100.0 m, covering entirely the continental shelf of the Bulgarian Black Sea zone, between Durankulak and Ahtopol. For each haul, a qualitative and quantitative analysis of the catch was accomplished. (Picture 3);

- Biometric measurements of 97 turbots and 2 spiny dogfishes (Picture 4).

Picture 3 *Bottom trawling yield*



Picture 4 *Conducting biometric measurements and sampling for study of the stomach contents*

During the survey was established: constant presence of *Scophthalmus maximus* in almost all bottom trawls at a depth between 50–75 m and 75-85 m, with yield - at least 1-3 individuals per haul, mixed with specimens of whiting (*Merlangius merlangus euxinus*), red mullet (*Mullus barbatus*)and thornback ray (*Raja clavata*) (picture 5). The highest turbot catches, varying between 8.38 to 28.25 kg/trawl, were observed in seven sectors off the Bulgarian coast.

Picture 5 *Yield of turbot (Scophthalmus maximus) and associated species Merlangius merlangus (whiting), Raja clavata (thornback ray), Mesogobius batrachosephalus (goby), Platichthys flesus (European flounder) and other species.*

A total of 2 dogfish (*Squalus acanthias*) were caught and measured (76 - 105 cm /1.54 - 5.36 kg), of which - one female and one male (picture 6).

Picture 6 Yield of spiny dogfish (*Squalus acanthias*)

The basic part of the catch consisted of a mixture of species: whiting (*Merlangius merlangus euxinus*), red mullet (*Mullus barbatus ponticus*) and thornback ray (*Raja clavata* – over 14 individuals). The associated species were identified as Mediteranean mussel (*Mytilus galloprovincialis*),common jellyfish (*Aurelia aurita*), common sole (*Solea vulgaris*), black scorpionfish (*Scopaena porcus*), flounder (*Platichthys flesus luscus*),sea snail(*Rapana venosa*),goby fish (*Neogobius melanostomus*, *Mesogobius batrachocephalus*, *Gobius niger*)and prawn (*Crangon crangon*).

It should be noted, that in almost all sectors where the bottom trawling was conducted (including the coastal area), large amounts of marine litter was established (drums, boxes and various metal parts, trees, plastic bags, plastic bottles, gillnets for turbot, etc.) (Picture 7).

Picture 7 Marine debris identified during the bottom trawl in May 2016.

Comments on the biomass of Scophthalmus maximus in the Bulgarian waters by strata

Trawling at a depth of up to 30 m cover three stations, and due to their small number they are grouped together with the stations preformed up to 50 m, thus the statistical analysis was conducted for the stratum 15 - 50 m.

The biomass of the three shallow stations (at a depth up to 30 m) reached respectively 70.565 kg/km^2, 29.375 kg/km^2 and 0 kg/km^2, with abundance - 48 ind./km^2, 16 ind./km^2 and 0 ind./km^2. In shallow stations, the highest yield was found offthe Cape Kavarna, while in front of the Rakitnik area the turbot specimens were not found.

The information about the yields by stratum is given below:

Stratum 15 - 50 m

The relative turbot biomass varied between 0 and 140.267kg/km^2, 43.773kg/km^2 on average (Table 1, Fig. 4 and 5). The abundance indices varied between 0 and 80 individuals/km^2, on average - 25 ind./km^2 (Table 2, Fig. 7).

Stratum 50 -75 m

This stratum contained the highest number of individuals. The relative turbot biomass varied between 43.903 and 451.008kg/km^2, 104.299kg/km^2 on average (Table 1, Fig. 4 and 5). The abundance indices varied between 8 and 192 ind./km^2, with average value of 44 ind./km^2 (Table 2, Fig. 7).

Stratum 75 - 100 m

In this stratum, the relative biomass varied from 172.740 to 58.112 kg/km^2, on average 71.403kg/km^2 (Table 1, Fig. 4 and 5), and the average abundance was 53 ind./km^2 (Table 2, Fig.7).

Table 1

Turbot biomass by strata, May, 2016

15 - 50 m		*50 – 75 m*		*75-100 m*	
No. station	***t/sqKm***	***No. station***	***t/sqKm***	***No. station***	***t/sqKm***
7	0.070565	1	0.031132	5	0
12	0.060028	2	0.060970	9	0.133786
13	0	3	0.284813	10	0.068968
14	0.031132	4	0.043903	17	0.138096
15	0.017561	6	0.149112	18	0.071682
16	0.140267	8	0.451008	23	0
20	0.029375	11	0.069767	24	0
21	0.00894	19	0.095470	25	0
26	0.091319	22	0.060986	36	0.124686
27	0.11367	28	0.098024	37	0.172740
31	0.026502	29	0.010696	38	0.088765
32	0	30	0	39	0.058112
33	0.023468	35	0		
34	0				
Total	**0.612828**	**Total**	**1.355881**	**Total**	**0.856835**
Average	**0.04377**	**Average**	**0.104299**	**Average**	**0.071403**
Variance	0.002013		0.016615		0.003848
Standard deviation	0.044864		0.1289		0.06203
Relative standard deviation	1.024906		1.235877		0.868737
Standard error	0.011216		0.03575		0.020677

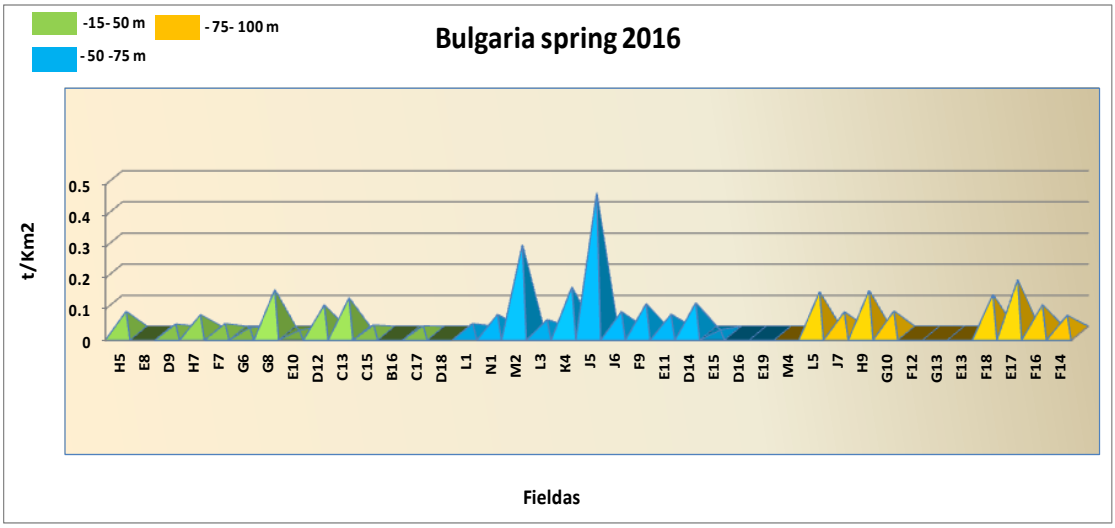


Fig.4 Relative biomass (t/km^2) of *S. maximus* by strata off the Black Sea coast, May 2016.

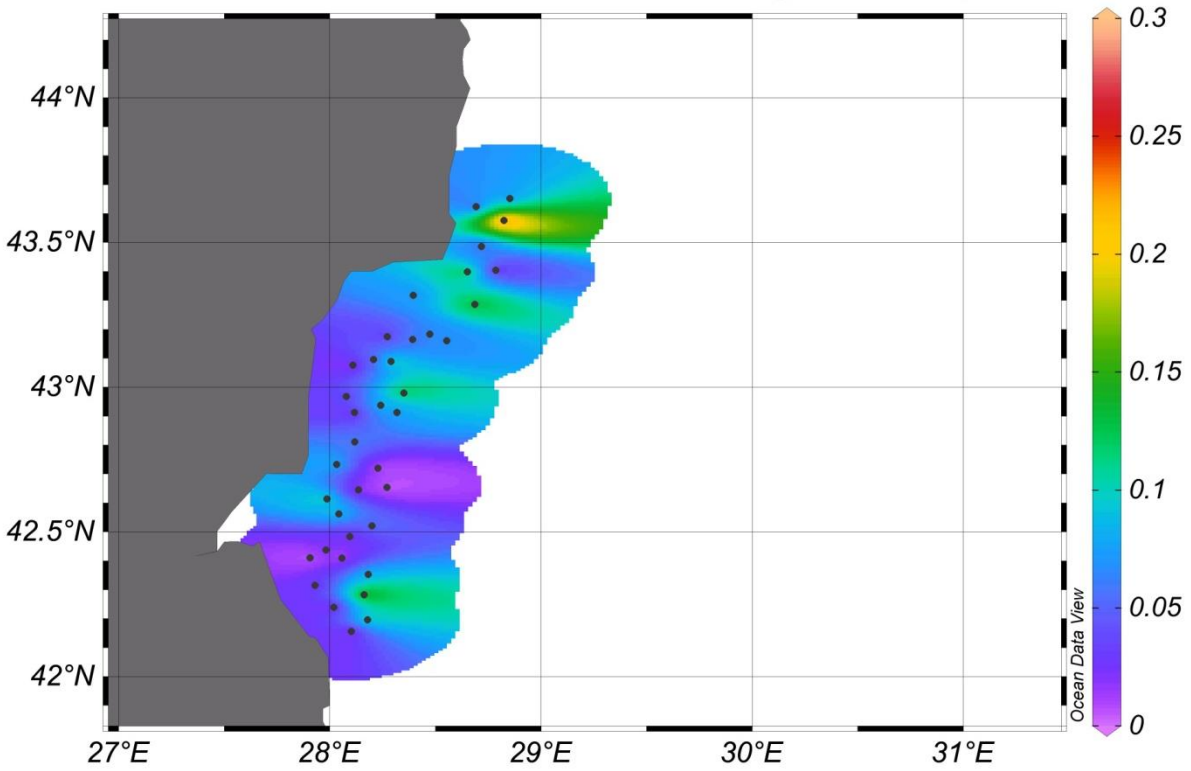


Fig.5 Distribution of the relative biomass *(t/km^2)* of *Scophthalmus maximus* off the Bulgarian Black Sea coast in May 2016 (The zones with high relative turbot biomass are shown in green and yellow).

High relative biomass was established in five sectors of the Bulgarian Black Sea zone, in the north direction between Shabla and Rusalka at a depth of 54 - 64.5 m (stations M2 and K4). The highest relative biomass was observed in front of Kaliakra (st. J5 and L5) at depths of 57.5 - 66.1 m and 82 m, respectively. Along the central part of the coast,

the relative turbot biomass was high in front of the River Kamchia and Shkorpilovtsi Beach (st. G8 and H9) at a depth of 41 - 80 m, and in south direction – in front of the Cape Maslen (E 17, depth of 77.3 - 76.7 m) and in front of Tsarevo (74-80 m). In the above mentioned sectors, the relative biomass of turbot varied between 113.670 kg/km^2 - 451.008 kg/km^2.

Table 2 shows the results of the study of turbot abundance in May 2016.

Table 2

Current abundance of S. maximus by strata in May 2016

15 - 50 м		*50 – 75 м*		*75-100 м*	
No. station	***No. Ind./km^2***	***No. station***	***No.ind./km^2***	***No. station***	***No.ind./km^2***
7	48	1	16	5	0
12	32	2	32	9	64
13	0	3	144	10	32
14	32	4	32	17	80
15	16	6	96	18	48
16	80	8	192	23	0
20	16	11	48	24	0
21	16	19	64	25	0
26	32	22	16	36	96
27	48	28	64	37	80
31	16	29	16	38	64
32	0	30	0	39	16
33	16	35	0		
34	0				
Total	**351**	*Total*	**718**	*Total*	**479**
Average	**25**	*Average*	**55**	*Average*	**53**
Variance	18.46487		55.81417		34.76408
Standard deviation	4.297077		7.470888		5.896107

Relative standard deviation	0.171282		0.143671		0.11080
Standard error	0.137265		0.254312		0.457627

4.2 Catch per unit effort (CPUE)

Catches from a total of 39 trawls are distributed as follows:

- 9 hauls (23.08 % of total no. hauls), catch 0 kg;
- 17 hauls (43.59% of total no. hauls), catch 0.0 – 4.99 kg per haul;
- 11 hauls (28.21% of total no. hauls), catch5.0 – 9.99 kg per haul;
- 1 hauls (2.56% of total no. hauls), catch10.0 – 14.99 kg per haul;
- 1 hauls (2.56% of total no. hauls), catch20.00 – 24.99 kg per haul;

Stratum < 30 m; 3 hauls:

- 1 haul, catch 0 kg;
- 2hauls, catch0 – 4.99 kg per haul;

Stratum 31 – 50 m; 11hauls:

- 2hauls, catch - 0
- 6 hauls, catch - 0.1 - 4.99kg per haul;
- 3 hauls, catch - 5.0 - 9.99kg per haul;

Stratum 50 – 75 m; 13 hauls:

- 2hauls, catch 0 kg per haul;
- 6hauls, catch 0.1 – 4.99 kg per haul;
- 3hauls, catch 5.0 – 9.99 kg per haul;
- 1haul, catch 15.0 – 19.99 kg per haul;
- 1haul, catch20.00 – 24.99 kg per haul;

Stratum 75 – 100 m; 12 hauls:

- 4hauls, catch 0 kg per haul;
- 3hauls, catch 0.1 – 4.99 kg per haul;
- 4hauls, catch 5.0 – 9.99 kg per haul;
- 1 haul, catch 10.0 – 14.99 kg per haul;

The CPUE distribution during the expedition off the Bulgarian coast in May 2016 is shown in Table 3 and Fig. 6.

Table 3

Results achieved in the survey of the month in May 2016

№	Field	Starting Coordinates		Depth (m)	Speed (Nm)	Trawling time (min)	Catch turbot	
		ϕ	λ				№	Kg
1	L1	43º37'150"	28º41'371"	49.6-52.1	2.6	60	**1**	**1.95**
2	N1	43º39'089"	28º51'153"	56.3-63.6	2.6	60	**2**	**3.819**
3	M2	43º34'574"	28º49'483"	64.5-65.0	2.6	60	**9**	**17.84**
4	L3	43º29'213"	28º43'021"	64.5-69.6	2.6	60	**2**	**2.75**
5	M4	43º24'426"	28º47'067"	75.9-79.0	2.6	60	**0**	**0**
6	K4	43º23'880"	28º39'260"	65.4-54.6	2.6	60	**6**	**9.34**
7	H5	43º19'003"	28º23'645"	18.9-30.0	2.6	60	**3**	**4.42**
8	J5	43º16'293"	28º30'940"	57.5-66.1	2.6	60	**12**	**28.25**
9	L5	43º17'185"	28º41'220"	82.7-85.4	2.6	60	**4**	**8.38**
10	J7	43º09'626"	28º33'192"	84.7-76.0	2.6	60	**2**	**4.32**
11	J6	43º10'908"	28º28'674"	66.0-56.0	2.6	60	**3**	**4.37**
12	H7	43º09'879"	28º23'670"	50.0-42.0	2.6	60	**2**	**3.76**
13	E8	43º04'577"	28º06'296"	24.0-29.5	2.6	60	**0**	**0**
14	F7	43º05'615"	28º12'169"	31.1-30.5	2.6	60	**2**	**1.95**
15	G6	43º10'462"	28º16'213"	30.9-38.5	2.6	60	**1**	**1.1**
16	G8	43º05'000"	28º17'266"	41.1-57.0	2.6	60	**5**	**8.786**
17	H9	42º59'000"	28º21'000"	76.7-80.0	2.6	60	**5**	**8.65**
18	G10	42º54'741"	28º18'968"	80.7-71.0	2.6	60	**3**	**4.49**
19	F9	42º56'113"	28º14'541"	62.0-45.4	2.6	60	**4**	**5.98**
20	D9	42º58'170"	28º04'588"	28.0-28.5	2.6	60	**1**	**1.84**
21	E10	42º54'751"	28º06'994"	36.1-44.0	2.6	60	**1**	**0.56**
22	E11	42º48'603"	28º07'046"	48.9-62.0	2.6	60	**1**	**3.82**
23	F12	42º43'363"	28º13'562"	80.2-88.0	2.6	60	**0**	**0**
24	G13	42º39'351"	28º16'210"	90.0-89.0	2.6	60	**0**	**0**
25	E13	42º38'648"	28º08'264"	70.0-58.0	2.6	60	**0**	**0**
26	D12	42º43'609"	28º01'530"	39.0-42.7	2.6	60	**2**	**5.72**
27	C13	42º39'000"	27º59'562"	40.7-42.1	2.6	60	**3**	**7.12**
28	D14	42º33'687"	28º02'517"	54.5-59.0	2.6	60	**4**	**6.14**
29	E15	42º29'187"	28º05'642"	67.5-66.0	2.6	60	**1**	**0.67**
30	D16	42º24'580"	28º03'429"	59.3-49.0	2.6	60	**0**	**0**
31	C15	42º26'216"	27º59'100"	46.7-38.7	2.6	60	**1**	**1.66**
32	B16	42º24'632"	27º54'323"	37.0-36.4	2.6	60	**0**	**0**
33	C17	42º19'006"	27º55'795"	37.3-38.0	2.6	60	**1**	**1.47**
34	D18	42º14'444"	28º01'035"	48.0-52.0	2.6	60	**0**	**0**
35	E19	42º09'356"	28º06'079"	56.7-64.7	2.6	60	**0**	**0**

36	F18	42°11'678"	28°10'667"	74.7-80.0	2.6	60	**6**	**7.81**
37	E17	42°16′697"	28°09'764"	77.3-76.7	2.6	60	**5**	**10.82**
38	F16	42°21′069″	28°10′784″	82.0-83.3	2.6	60	**4**	**5.76**
39	F14	42°31'205″	28°11′993″	85.3-84.0	2.6	60	**1**	**3.64**

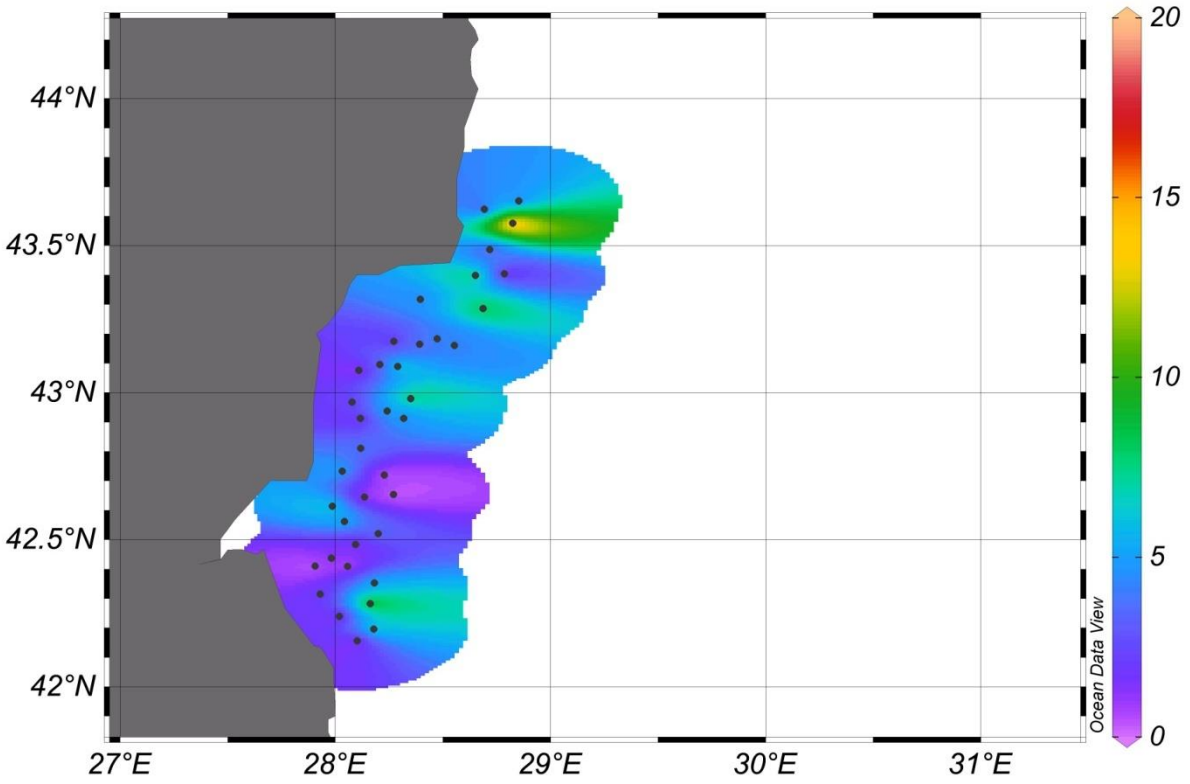


Fig. 6 Distribution of catch per unit effort (CPUE)

4.3. Catch per unit area (CPUA)

The turbot abundance and biomass per unit area are presented at Table 4.

Table 4

Turbot abundance and biomass observed in the Bulgarian waters in May 2016

No. Station	*Field*	*No. individulals/sqKm*	*t/sqKm*
1	L1	16	0.031132
2	N1	32	0.06097
3	M2	144	0.284813
4	L3	32	0.043903

5	M4	0	0
6	K4	96	0.149112
7	H5	48	0.070565
8	J5	192	0.451008
9	L5	64	0.133786
10	J7	32	0.068968
11	J6	48	0.069767
12	H7	32	0.060028
13	E8	0	0
14	F7	32	0.031132
15	G6	16	0.017561
16	G8	80	0.140267
17	H9	80	0.138096
18	G10	48	0.071682
19	F9	64	0.09547
20	D9	16	0.029375
21	E10	16	0.00894
22	E11	16	0.060986
23	F12	0	0
24	G13	0	0
25	E13	0	0
26	D12	32	0.091319
27	C13	48	0.11367
28	D14	64	0.098024
29	E15	16	0.010696
30	D16	0	0

31	C15	16	0.026502
32	B16	0	0
33	C17	16	0.023468
34	D18	0	0
35	E19	0	0
36	F18	96	0.124686
37	E17	80	0.17274
38	F16	64	0.091958
39	F14	16	0.058112
Total		1549	2.828738
Average		40	0.072532
Total in the Bulgarian area		**460 237** Ind.	**840.692** tonnes

	№ ind./км²	*t/км²*
Variance	1754.173	0.007703
Standard deviation	41.88285	0.087765
Relative standard deviation	1.054784	1.210019
Standard error	6.706623	0.014054

The calculated current turbot biomass in the Bulgarian Black Sea waters amounted to **840.692** tons (Table 4). The turbot abundance in the surveyed area amounted to **460 237** individuals (Table 4).

4.4. Size structure

The information about the size structure of turbot population in the Bulgarian Black Sea waters in May 2016 was based on biometric measurements (absolute and standard length and individual weight) of 97 fish specimens (Picture 4).

The absolute length of the measured individuals varied between 29.50 and 73.00 cm, and their weight - between 390 and 5980 g. The total catch of turbot was 177.185 kg. Out of the total number of 97 individuals, one was of size under 30 cm (1.03 %), 11 were of sizes between 30 - 40 cm (11.34%), 55 individuals - between 40-50 cm (56.70%), 27 - in the range between 51-60 cm (27.84%) and 3 individuals - between 61-73 cm (3.09%) (Fig. 7).

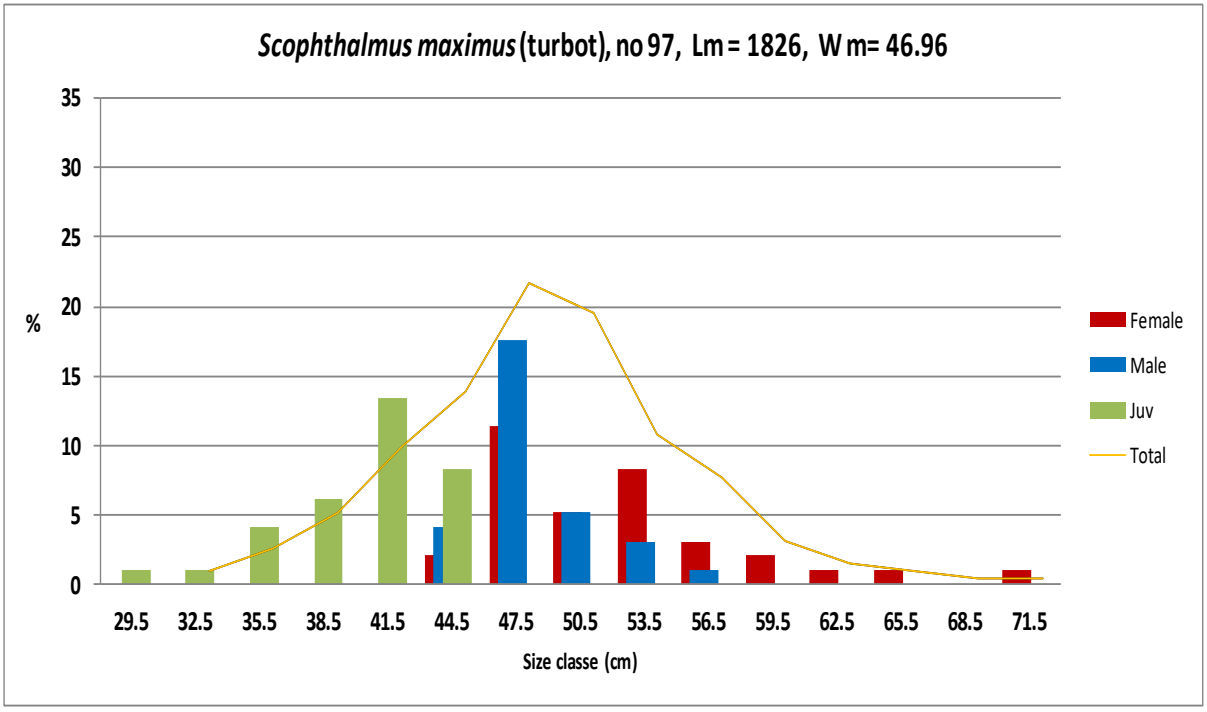


Fig.7 Length structure of *Scophthalmus maximus.*

Sexually mature individuals dominated in the total catch - 65.98% /64 specimens/, while juveniles were 33 specimens or 34.02 %. With a percent share of 38.02% /46 specimens/ the females dominated over the males - 19.00% /23 specimens/.

The size structure was analysed in compliance with the national regulations, setting out the minimum permissible length of the individuals for fishing purpose. The individuals with absolute length under 45 cm are marked as undersized, and those with length > 45 cm - as standard.

Fig. 8 shows the total turbot abundance (ind./km^2) and the distribution of the ratio between the undersized individuals and those of standard length.

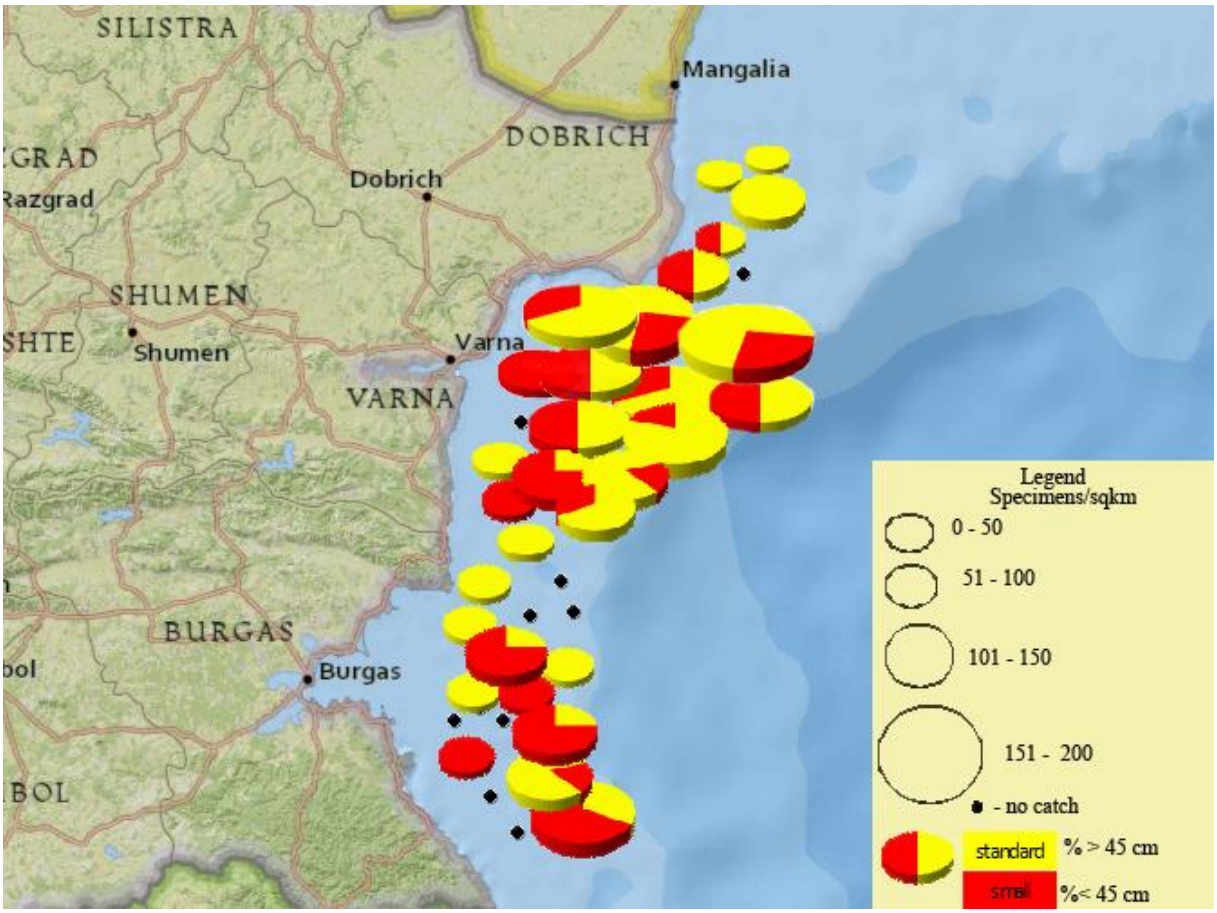


Fig. 8 Distribution of the *Scophthalmus maximus* abundance (individuals/km^2) and ratio between the undersized individuals and those with standard length.

The relative turbot biomass by mean size classes is presented in Fig.9 and show high biomass of two groups - 47.5 cm and 53.5 cm.

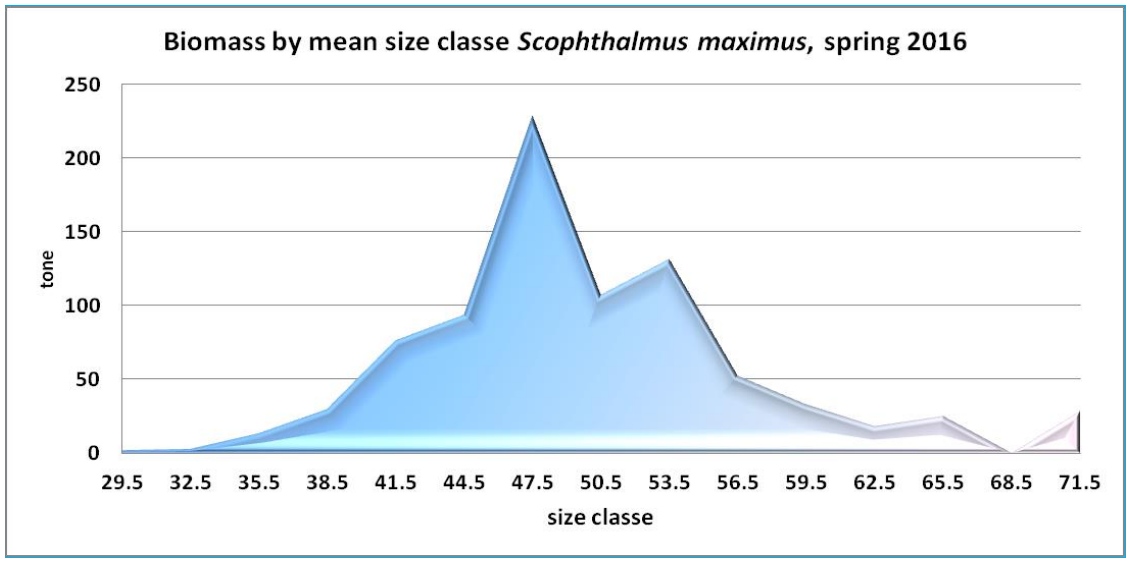


Fig.9 Biomass by mean size classes of *S. maximus.*

4.5. Age structure

Age structure

The turbot age composition was determined based on an analysis of 64 pairs of otoliths.

The turbot age structure included 1 to 9 - years classes, with domination of the 3 (43.30%) and 2 (24.74%) - years (68.04 % in total), followed by 5 - year class – 14.43% (Fig. 10). In the spring season of 2016, the share of the replenishment of 2 - year class was 24.74%. Although having small frequency of occurrence, specimens from 6 to 9 - years classes were also registered in the yield.

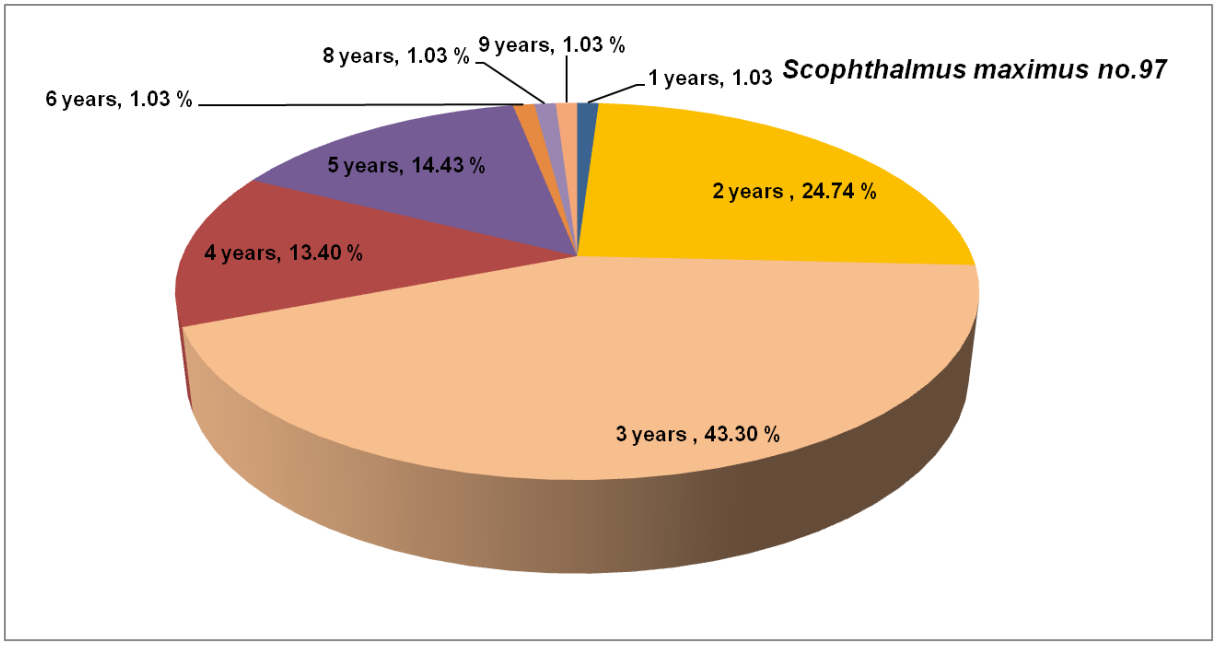


Fig.10 Age structure of turbot in May 2016.

The distribution of the age composition of *Scophthalmus maximus* in the surveyed areas is shown in Fig.11.

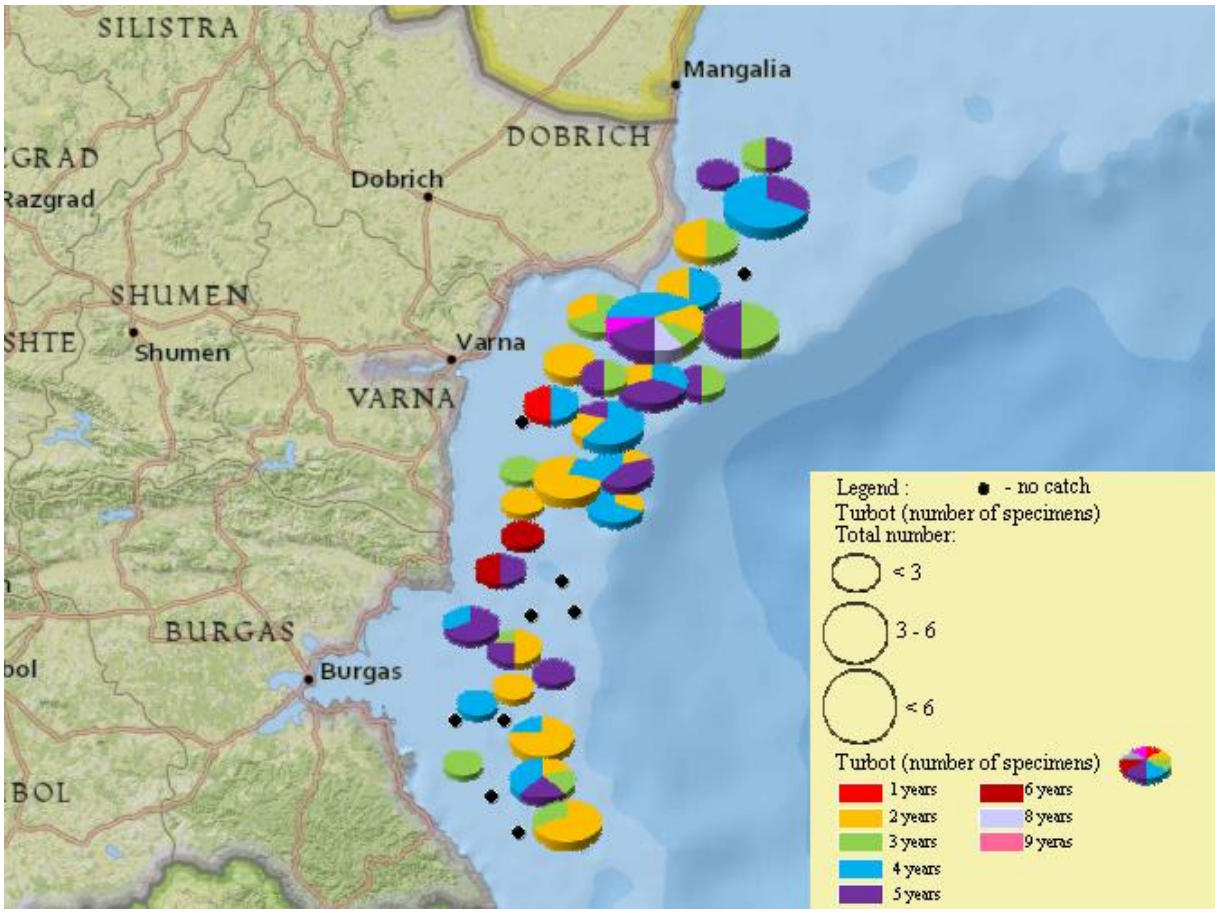


Fig. 11 Age structure of *Scophthalmus maximus* in the spring of 2016.

4.6. Biological parameters of *S. maximus*

To identify the turbot growth rate in the Bulgarian Black Sea waters in spring 2016, the data about the average length and weight by age groups for the two sexes were combined.

The calculated values of the parameters in von Bertalanffy's and L-W equation are as follows:

a = 0.0164
b = 3.000
q = -1.784
L∞ = 76.84
k = 0.314
to = - 0.41

The turbot length-weight relationship, based on the spring survey data is shown in Fig.12.

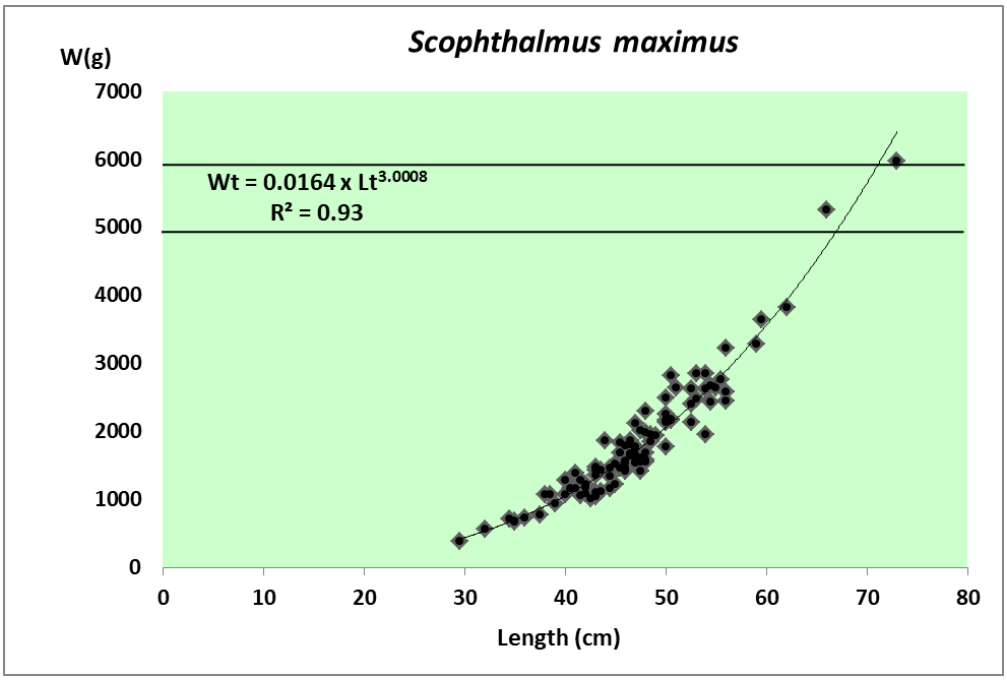


Fig.12 *Scophthalmus maximus*: Length-weight relationships in May 2016.

The coefficient of natural mortality (M) was calculated according to Pauly's empirical formula (1980), describing the natural mortality as a function of k, L∞, W∞ and water temperature (T) at the bottom layer.

L_∞ = Lt max/0.95

k = $1/(t_2-t_1)*\ln(\mathbf{L_\infty-L_1})/(\mathbf{L_\infty-L_2})$

log $(-t_0)$ = -0.3922 – 0.2752*logL_∞- 1.038*logk

Taking into account, that the water temperature was 12 °C during the study, the coefficient of natural mortality (M) for both sexes is 0.44.

4.7. Sex structure

Sex ratio

The results of the turbot sex structure analysis in spring 2016 are shown in Fig.13. The total share of sexually immature individuals comprised 34.02 % of the total yield, the female individuals formed 35.05 %, and the share of males was 30.93 %.

From a total of 39 fields, studied off the Bulgarian coast in May 2016, female specimens were not identified in 8 fields, in 7 fields - males were not estimated, and in 3 fields - only young forms were found, while adult specimens were absent (Fig.14).

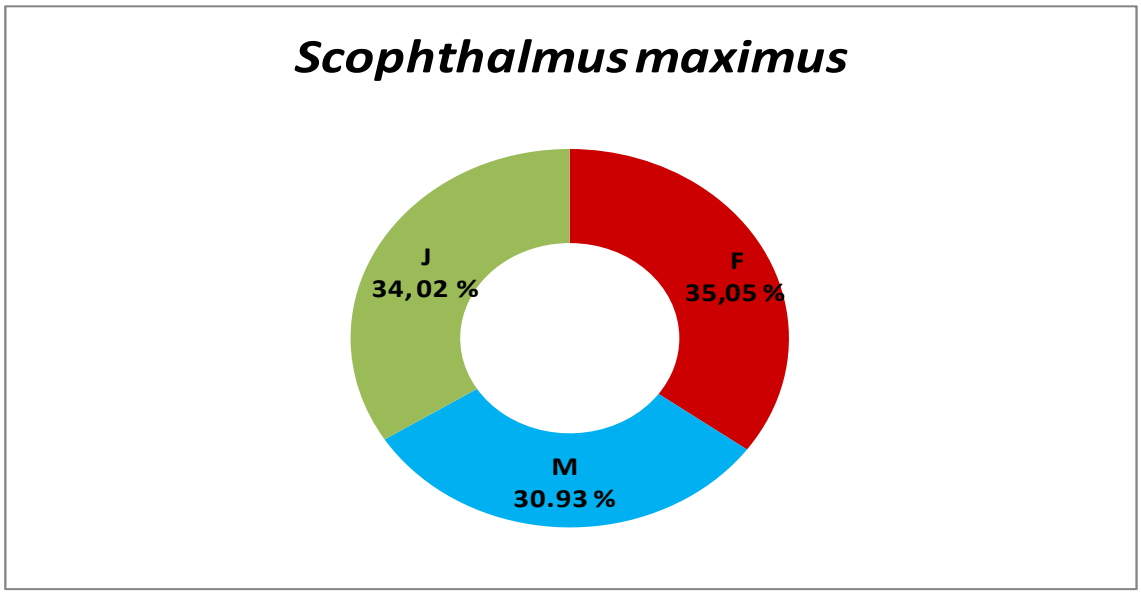


Fig.13 Sex structure of turbot in May 2016.

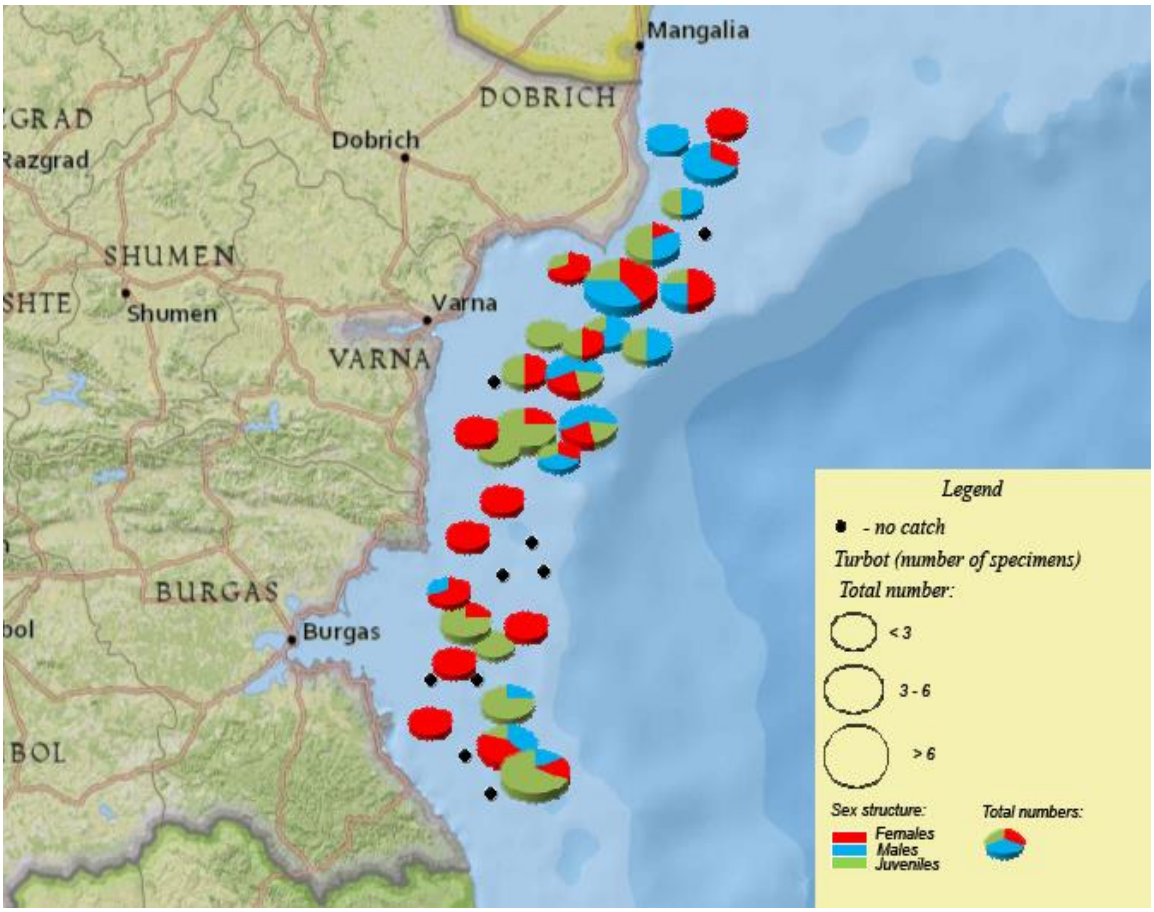
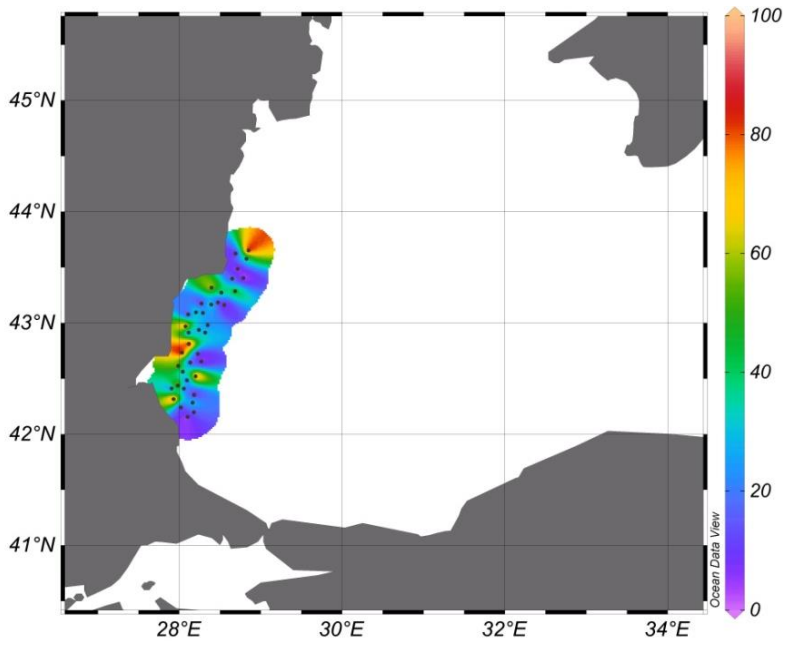
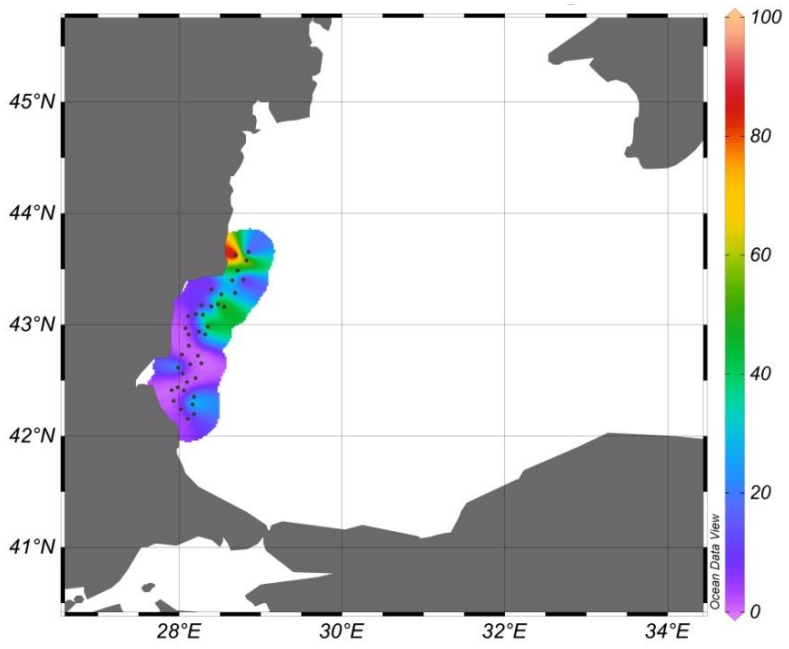


Fig.14 Sex structure of *Scophthalmus maximus* in May 2016: distribution by stations.

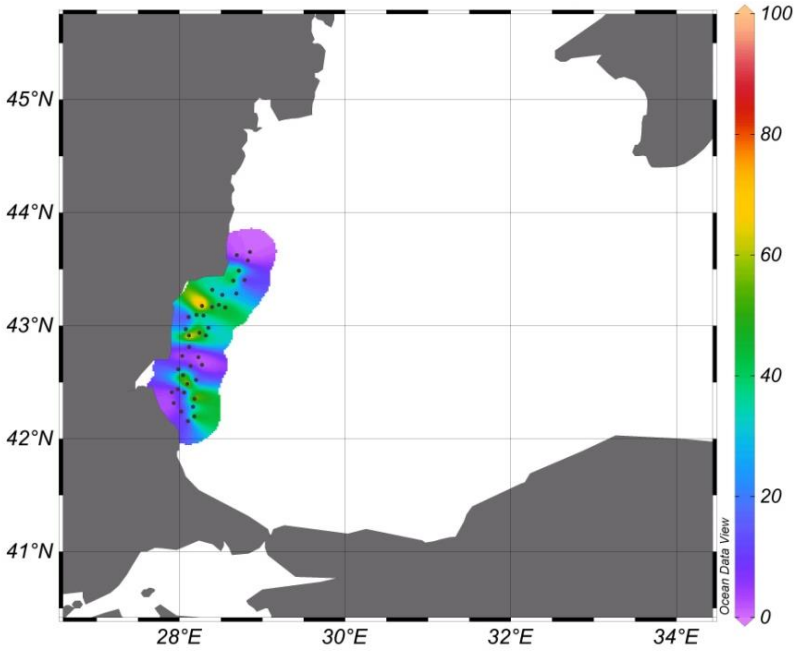
The percentage distribution of turbot yields by sex, as well as the share of young individuals are shown in Fig. 15.



(A)



(B)



(C)

Fig. 15 Percentage distribution by sex and juvenile stages of *S. maximus* in May 2016: (A) females, (B) males, (C) juvenile stages.

The males, as well as the sexually immature forms were found at depths 55 - 75 m, whereas the females were observed from 20 to 80 m depth due to intensive turbot breeding in the spring. The juveniles were concentrated in the section Varna and Byala, off the Bourgas Bay and in the area between Primorsko and Ahtopol (Fig.15 C). The females were established mainly in the regions between Durankulak - Cape Kaliakra, Byala - Pomorie and Primorsko (Fig.15 A), and high concentration of males was identified in the region of Krapets - Shabla and Varna - Kamchia (Фиг.15 B).

The average weight of females was 2524.4 g, with average absolute length TL -52.16 cm and standard length SL - 40.54cm. The maximum and minimum weight of females were 5980 g - 1460 g, respectively.

In females, the length distribution analysis showed the maximum percentage of the class 46-49 cm - 32.35% (of abundance), followed by size class 52-55 cm - 23.53% (Fig.15). Therefore, from all studied female specimens, those of the length class - 46-55 cm comprised 76.47 %. In the interval of largest length classes - over 61 cm and up to 73 cm - all studied specimens were females and this group constituted 8.82 % of the total abundance of females. The results demonstrate well expressed sexual dimorphism with regard to the body

length and superior number of large size classes in females compared to males (Fig.16/17).

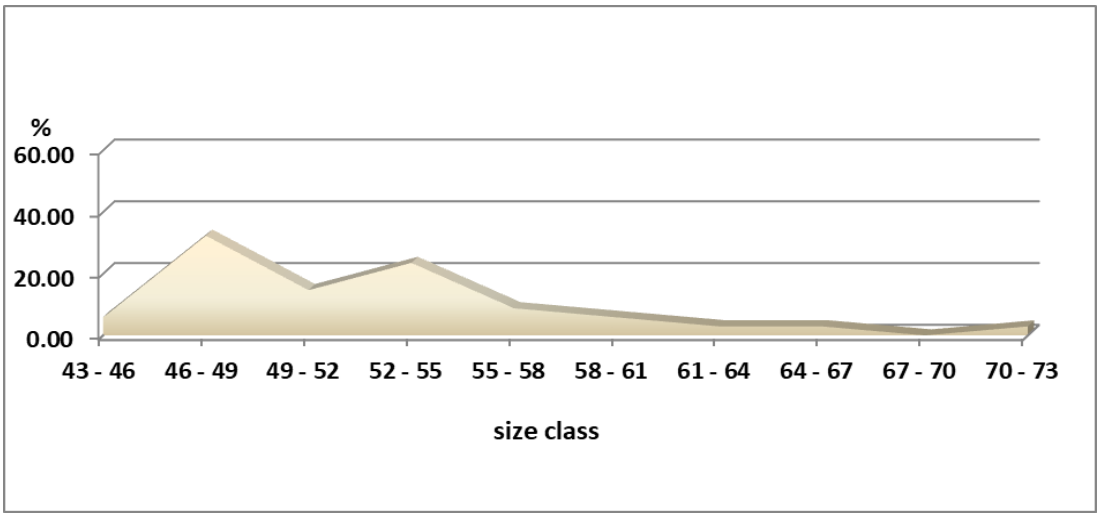


Fig.16 Female specimens: Percentage distribution by length classes.

For males, the most significant proportion of total abundance - 56.67 % belonged to the length class 46-49 cm, followed by size classes 49-52 cm, 52-55 cm and 55-58 cm (Fig.17).

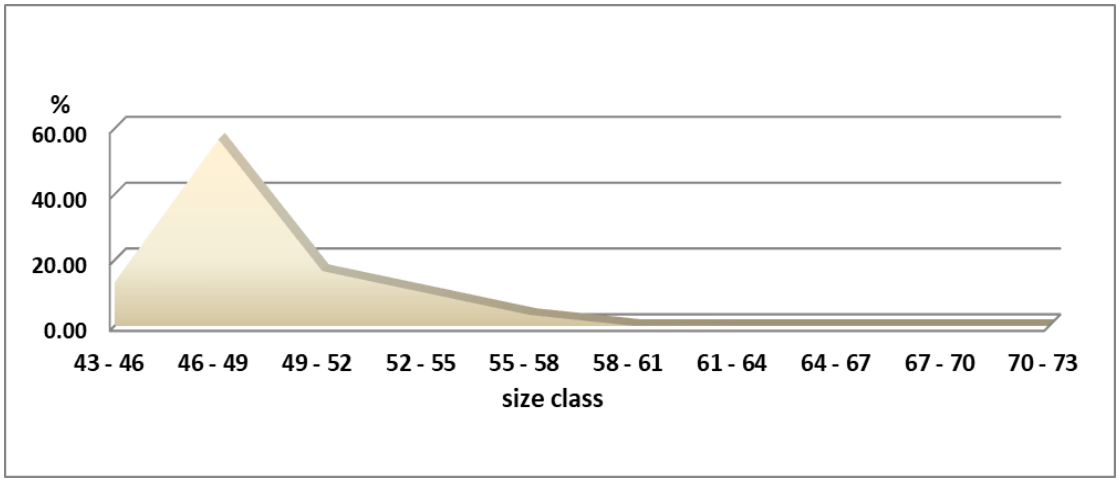


Fig.17 Male specimens: Percentage distribution by length classes.

4.8. Food composition

In May 2016, a total of 53 fish stomachs were investigated, from them - 66 % were empty. The results are presented at Table 5.

Table 5

Surveyed area, length, weight of specimens, and weight of stomachs (full, empty), and of stomach contents, index of stomach fullness (ISF).

Area	L (cm)	W (gr)	St./full(gr)	St./empty (gr)	St. contents. (gr)	ISF
E17/2	46,5	1670	16,1773	0	0	0
E17/3	50	2490	20,1988	0	0	0
E17/4	52,5	2630	32,5544	21,5054	11,049	0,420114
E17/5	53	2850	57,9825	32,6933	25,2892	0,88734
E11/1	62	3820	52,971	0	0	0
J5/1	54,5	2670	61,426	56,465	4,961	0,185805
J5/2	73	5980	169,5	124,5	45	0,752508
J5/3	46,5	1650	19,251	0	0	0
J5/4	46,5	1680	36,941	18,764	18,177	1,081964
J5/8	48	1590	19,1906	0	0	0
J5/9	66	5260	82,4228	61,9365	20,4863	0,389473
J5/10	53	2480	24,9793	0	0	0
J5/11	48	1550	16,8286	0	0	0
J5/12	47,5	1560	18,3174	0	0	0
J6/2	47	1540	33,0475	15,751	17,2965	1,123149
J6/3	50	1770	17,6173	0	0	0
D9/1	45,5	1840	32,8055	21,4635	11,342	0,616413
G8/1	45	1520	16,3755	0	0	0
G8/2	55	2650	35,6851	0	0	0
G8/4	47	1560	19,4272	0	0	0
C13/1	50	2130	23,3643	20,1948	3,1695	0,148803
C13/2	56	2580	33,0182	25,291	7,7272	0,299504
C13/3	52,5	2410	23,6244	0	0	0
C15/1	47	1660	21,0825	0	0	0
C17/1	45,5	1470	15,6972	0	0	0
F14/1	59,5	3640	35,5205	0	0	0
F9/4	48	1990	25,163	0	0	0
F7/2	46	1560	14,4841	0	0	0
F18/3	46,5	1810	19,2458	0	0	0

F18/6	46	1790	15,3419	0	0	0
H9/2	46,5	1660	15,37	0	0	0
H9/3	47	1740	13,585	0	0	0
H9/4	52,5	2130	19,346	0	0	0
H9/5	50,5	2180	26,4448	0	0	0
H5/1	46,5	1870	44,3898	0	0	0
H5/2	46	1540	41,984	37,1039	4,8801	0,31689
G10/2	49	1940	25,7421	19,5837	6,1584	0,317443
G10/3	47	1770	24,6635	20,095	4,5685	0,258107
D12/1	54,5	2430	28,9034	20,507	8,3964	0,345531
D12/2	59	3290	34,427	0	0	0
D14/4	54	2630	27,2828	0	0	0
F16/4	50	2170	85,811	34,0818	51,7292	2,383834
L5/1	45	1230	28,3963	16,3638	12,0325	0,978252
L5/2	56	3220	83,7333	41,9585	41,7748	1,297354
L5/4	55,5	2770	97,408	51,108	46,3	1,67148
K4/4	48	2300	16,8782	0	0	0
K4/6	47,5	1420	11,9114	0	0	0
L3/2	47,5	2020	15,0494	0	0	0
L1/1	54	1950	40,6003	0	0	0
H7/2	51	2640	37,6868	0	0	0
J7/2	56	2450	28,8889	0	0	0
M2/1	50,5	2830	12,5933	0	0	0
M2/9	46	1460	56,5937	0	0	0

The box-plot diagrams of the weight of turbot specimens, included into the stomach content analysis, and the stomach fullness index (ISF) are presented at Fig. 18. During the survey a low mean level of the ISF was estimated - 0,25 % from the turbot body weight.

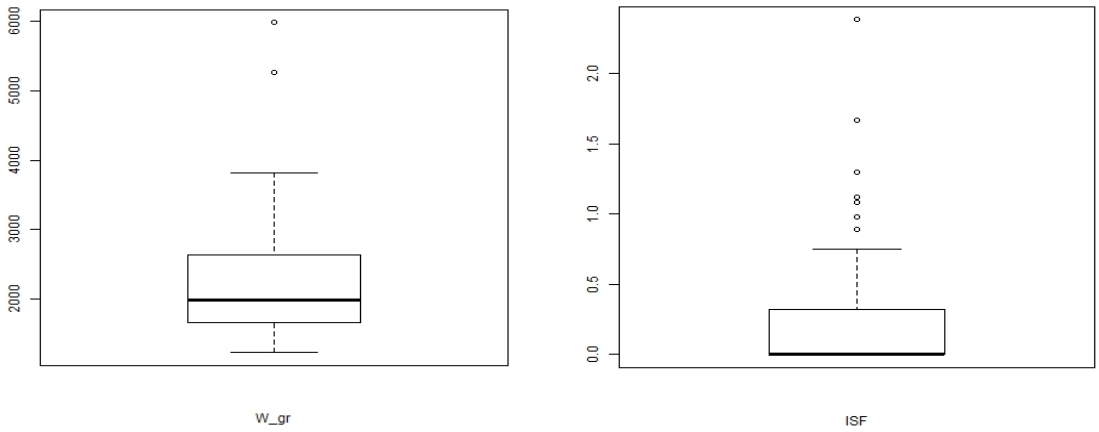


Fig. 18 Box plot: Weight of turbot specimens included in stomach content analysis (A) and index of stomach fullness (ISF) (B) (shown are: median; range of values: 25-75%; minimum and maximum value).

The qualitative composition of the turbot diet included *Pisces, Mollusca* and *Crustacea* (Table 6). Nematodes were also found in the stomach contents of turbot, forming a group of parasites (diseases indicator).

Table 6

The qualitative and quantitative composition of the turbot food spectrum in May 2016. (C_N - percentage share of different food items in total number; C_W - percentage share of different food items in the total weight; F – frequency of occurrence).

Species	C_N	C_W	F	IRI
Pisces	**66,78**	**70,07**	**56,61**	**2163,23**
Merlangius merlangus (Linnaeus, 1758)	64,93	67,84	54,72	2161,45
Squalus acanthias, Linnaeus, 1758	1,85	2,23	1,89	1,78
Mollusca	**10,56**	**7,52**	**20,75**	**18,21**
Mytilus galloprovincialis, Lamarck, 1819	4,58	1,84	16,98	8,38
Cerastoderma edule (Linnaeus, 1758)	5,98	5,68	3,77	9,83
Nassarius reticulatus (Linnaeus, 1758)	0,42	0,19	1,89	0,09
Crustacea	**11,11**	**9,53**	**9,43**	**96,75**
Liocarcinus holsatus (Fabricius, 1798)	11,11	11,11	9,43	96,75
Other	**11,55**	**11,30**	**3,77**	**17,74**

The turbot feeds mainly on fish species, as the percentage contribution of this component in abundance comprised 67 % of the total abundance, and respectively 70 % from the total biomass. Second place was taken from the group of Crustaceans – 11 % - by abundance and 10 % of food biomass (Table 6).

The most important component in the turbot food was whiting *(Merlangius merlangus)*, that formed high index of relative importance IRI = 2161,45. The decapod species *Liocarcinus holsatus* took the second place with IRI = 96,75, but the share of crustaceans was significantly lower than those, formed by the group of fishes (Fig 19).

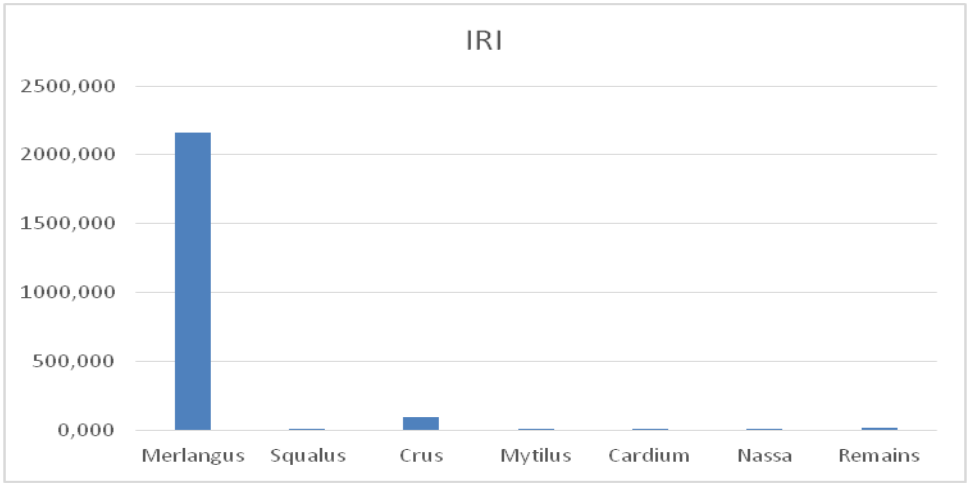


Fig.19 Indexes of relative importance (IRI) by food items.

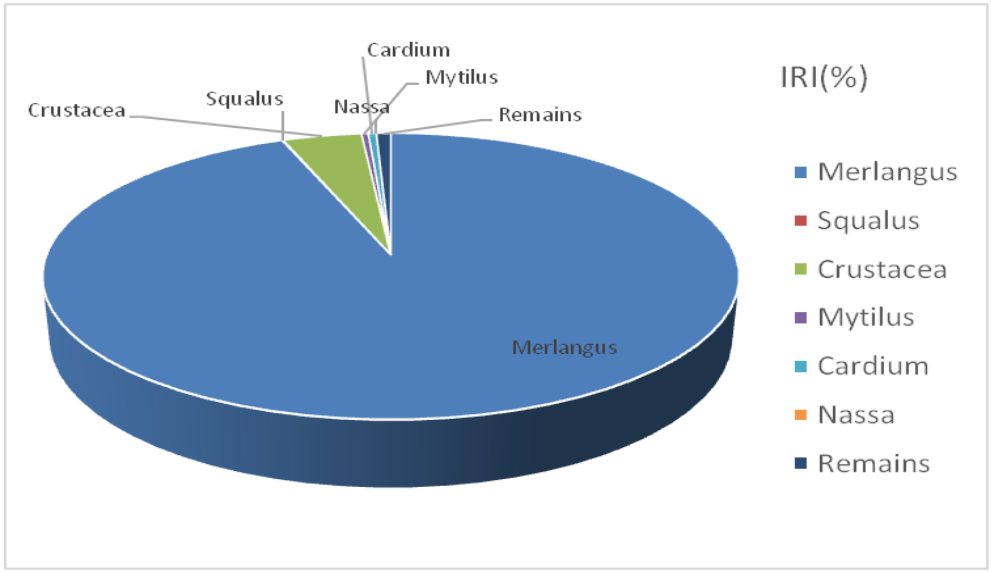


Fig. 20 Food spectrum (IRI %): percentage of the main taxonomic groups in the turbot stomach content.

According to percent composition by IRI, the species *Merlangius merlangus* (with share 94,14 %) dominated the turbot diet in May 2016 (Fig, 20).

4.9. Forecasts and opportunities for exploitation

The estimation of maximum sustainable yield (MSY) was realised using Gulland's formula for virgin stocks.

$$MSY = 0.5*M*Bv$$

The coefficient of natural mortality (M) is calculated by Pauly's empirical formula (1979, 1980).

By MSY value of **84.069**, it is assumed that the total allowable catch (TAC) of turbot in the Bulgarian Black Sea waters could comprise 50 tons as a relatively acceptable quantity.

Observed other particular problems

During the expedition in V/2016, the wind direction Southeast, East and North dominated, and its power varied between Beaufort 2-4º along the coast and Beaufort 5-6º in the open sea. Sea state - Beaufort 2-3º in the coastal zone, and Beaufort 3-4º in the open sea. The conditions in the field were normal for conducting the research activities for estimation of turbot stocks in the Bulgarian Black Sea waters.

During the survey, in several geographical points, were seen dolphins - moving and jumping over water (photo 8).

Photo 8. Dolphins observed during the expedition

5. Conclusions and recommendations

In accordance with the collected information and obtained results from the trawl survey in May 2016, the following conclusions and recommendations can be made:

- The turbot biomass in the Bulgarian Black Sea waters was assessed at **840.692** tons. The turbot abundance in the surveyed area was estimated as **460 237** individuals.
- The recommended MSY (maximum sustainable yield) for Bulgaria should not exceed **84** t., it is assumed that the total allowable catch (TAC) of turbot in the Bulgarian Black Sea waters could comprise **50** tons as a relatively acceptable quantity.
- The size structure of the turbot population in the Bulgarian Black Sea zone included length classes from 29.5 cm to 73 cm, with weight between 390 g and 5980 g. The average turbot weight was estimated as 1826.65 g. In the turbot length structure, the undersized individuals, with length < 45 cm, formed 34.02% from the total number, while those of standard length made up 65.98 %.
- The age composition of the population included age classes from 1+ to 9+- years of age, with domination of the **3 (43.30%) and 2+ (24.74%)** – year classes.
- The established ratio between female, male and sexually immature individuals in the yield was **35.05%: 30.93%: 34.02%.**
- The most important component in the turbot diet was whiting, that formed high index of relative importance (IRI, %) – 94,14 %. In spring 2016, the turbot diet diversity was lower in comparison with the data from autumn seasons of 2014 and 2015.

6. References

Alverson, D. L., 1971. Manual of methods for fisheries resource survey and appraisal. Part 1. Survey and charting of fisheries resources. *FAO Fish.Tech.Pap.*, (102): 80 p.

Bertalanffy, L. Von, 1934. Untersuchungen über die Gesetzlichkeiten des Wachstums. 1. Allgemeine Grundlangen des Theorie. *Roux'Arch.Entwicklungs-mech.Org.*, 131: 613-653.

Beverton, R. J. H., S. J. Holt, 1956. A review of methods for estimating mortality rates in exploited fish populations, with special reference to sources of bias in catch sampling. *Rapp.P.-V.Réun.CIEM*, 140:67-83.

Beverton, R. J. H., S. J. Holt, 1957. On the dynamics of exploited fish populations. *Fish.Invest.Minist.Agric.Fish.Food.G.B. (2 Sea Fish.)*, 19: 533 p.

Beverton, R. J. H., S. J. Holt, 1966. Manual of methods for fish stock assessment. Part 2. Tables of yield functions. Manuel sur les méthodes d'évalution des stocks ichtyologiques. Partie 2. Tables de fonctions de rendement. Manual de métodos para la evaluacion de los stocks de peces. Parte 2. Tablas de funciones de rendimiento. *FAO Fish.Tech.Pap./FAO Doc.Tech.Pêches/FAO Doc Téc.Pesca*, (38) Rev. 1:67 p.

Bulgurkov, K., 1965. On the distribution of food and industrial turbot (Rhombus maeoticus Pallas) in the southern area of the Bulgarian Black Sea coast. Izvestia. the Research Institute of Fisheries and Oceanography stapanstvo - Varna, point VI, pp. 99-109.

Cadima, E.L., 2003. Fish stock assessment manual. *FAO Fisheries Technical Paper.* No. 393. Rome, 161p.

Gulland, J.A., 1966. Manual of sampling and statistical methods for fisheries biology. Part I: Sampling methods. FAO Manuals in Fisheries Science No. 3, Rome.

Gulland, J.A., 1969. Manual of Methods for Fish Stock Assessment - Part 1. Fish Population Analysis. *FAO Manuals in Fisheries Science No.4:*154p.

Ivanov, L. S., M. Karapetkova, 1979. Dynamics of stocks of turbot (Scophthalamus maeoticus Pallas) from Bulgarian Black Sea shelf and measures for rational exploitation. I. Growth and mortality; II. Stocks and reproduction. Hydrobiology, 9, 3-14 and 15-28.

Kyosev and Dragoev 2009. Technology of fish and fish products. Publisher HVP, Sofia.

Martino, K., M. Karapetkova, 1957. Distribution of turbot during the first months of 1955. Scientific annals of Research Institute of Fisheries and fish industry. – Varna, vol.I, Publ. Zemizdat, Sofia, 45-51 pp.

Pauly, D., 1980. On the interrelationships between natural mortality, growth parameters and mean environmental temperature in 175 fish stocks. *J.Cons.Int .Explor. Mer,* 39:175-192.

Petrova D., E. Pavlova, S. Stoykov, V. Mihneva, D. Gerdjikov, F. Tserkova, S. Valchev T. Hubenova, A. Zaikov, L. Hadjinikolova, A. Ivanova, M. Gevezova, G. Rusenov, V. Maximov, G. Radu., 2016. Stock assessment of turbot (*Scophthalmus maximus*) by swept area method in front of Bulgarian Black Sea coast during autumn – winter 2015. Project report for the National Agency of Fisheries and Aquaculture of Bulgaria to National Data Collection program for 2015, 66 pp.

Ricker, W. E., 1975. Computation and interpretation of biological statistics of fish populations. *Bull.Fish.Res.Board Can.*, (191):382 p.

Rikhter, J. A., V. N., Efanov, 1976. On one of the approaches to estimation of natural mortality of fish population. *ICNAF* 76/VI/8, 12p.

Sabatella E., R., Franquesa, 2004. Manual for fisheries sampling surveys: Methodologies for estimation of socio-economic indicators in the Mediterranean Sea. General Fisheries Commission for the Mediterranean. Studies and Reviews, No.73, FAO Rome, ISBN 1020-7236, 38 pp.

Sparre P., S. C. Venema, 1998. Introduction to tropical fish stock assessment. Part I: Manual. FAO Fisheries Technical Paper, 306/1, rev.2, DANIDA, Rome FAO. 407p. ISBN 92-5-103996-8.

Sparre, P., and Venema, S.C., 1992. Introduction to tropical fish stock assessment. Part1. *FAO Fisheries Technical Paper* No 306.1, Rome, 376 p.

Troadec, J. P., 1977. Méthodes semi-quantitatives d'évaluation. *FAO circ. Pêches*, (701) :131-141.

Tserkova, F., D. Petrova, E. Pavlova, S. Stoykov, V. Mihneva, T. Hubenova, A. Zaikov, L. Hadjinikolova, D. Terziyski, A. Ivanova, M. Gevezova, V. Maximov, G. Radu., 2015. "Stock assessment of turbot (*Psetta maxima*) by swept area method in front of Bulgarian Black Sea coast during autumn – winter 2014". Project report for the National Agency of Fisheries and AquacultureProject report for the National Agency of Fisheries and Aquaculture of Bulgaria to National Data Collection program for 2014, 56 pp.

Tserkova, F., D. Petrova, E. Pavlova, S. Stoykov, V. Mihneva, V. Maximov, G. Radu., 2015. Abundance of Turbot (*Psetta maxima* L.) along the Bulgarian Black Sea Coast in

Autumn 2014.Ozhan, E.(Editor),2015, *Proceedings of Twelfth International Conference on the Mediterranean Coastal Enviroment, MEDCOAST, 15,* 06-10 October 2015, Varna, Bulgaria, MEDCOAST, Mediterranean Coastal Foundation, Dalyan, Mugla, Turkey, vol 1, 419-430 p. ISBN : 978-605-85652-4-1.

Walford, L.A., 1946. A new graphic method of describing the growth of animals. Biol. Bull. Mar. Biol. Lab. Woods. Hole, 90:141-147.

Zengin, M., 2005. Report of the Assessment Methodologies for the Turbot Stock in the Black Sea; Proposals for Standardized Methodology and Implementation at the Regional Level. AG FOMR, BSC.