# REPORT

## Bottom Trawl Survey in the Bulgarian Black Sea area

**Agricultural Academy** 

Institute of Fish Resources (IFR, Varna) Institute of Fisheries and Aquaculture (IFA, Plovdiv)

March, 2015

The present study was conducted owing to the financial support from the European Commission under Council Regulation (EC) No.199/2008 and Commission Decision 2010/93/EU which intended to help Member States (MS) in producing Technical Report establishing a community framework for the collection, management and use of data in the fisheries sector and support for scientific advice regarding the common fisheries policy for National Agency for fisheries and Aquaculture in Bulgaria and contract No D - 262 / 19.08.2014.

The survey was carried out during the period 12-30 November 2014 in Bulgarian Black Sea area on board of trawler vessel "EGEO 2".

#### List of authors:



Institute of Fish Resources (IFR, Varna)

- Assistant, PhD student Feriha TSERKOVA
- Assoc. Prof. Daniela Petrova, PhD
- Assoc. Prof. Elitsa Petrova Pavlova, PhD
- Assoc. Prof. Stoyko Stoykov, PhD
- Assoc. Prof. Vesselina Mihneva, PhD



#### Institute of Fisheries and Aquaculture (IF), Plovdiv)

- Prof. Tania Hubenova, PhD
- Prof. Angel Zaikov, PhD
- Prof. Liliana Hadjinikolova, DSc.
- Assis.Prof. Doichin Terzieski, PhD
- Assis.Prof. Angelina Ivanova
- Assis.Prof. Maria Gevezova, PhD



#### Consultants: National Institute For Marine Research And Development"Grigore Antipa" Constanta, Romania

- Dr. Valodea MAXIMOV
- Dr. Gheorghe RADU



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# I. National Bottom Trawl Survey in autumn 2014

## 1. Biological result from National Bottom Trawl Survey in autumn 2014

In the period 12 - 30 November 2014, in the conformity with the contract objectives with NAFA Bulgaria/2014, in the framework of National Data Collection Program for Fisheries, a research team from Institute of Fisheries Resources and an observer from National Institute for Marine Research and Development "Grigore Antipa", Constanta, Romania, carried out a research demersal survey with trawler vessel "EGEO 2", in the marine area of the Bulgarian littoral, between Durankulak and Ahtopol, until near the 100 m isobaths.

The provided analysis is focused on the reference species *Psetta maxima* and is based on the whole survey data. The main analyses are related to estimations of biomass and density of *Psetta maxima* and its geographical distribution. Furthermore, analysis of the distribution and abundance of the by-catch species is presented for the whole investigated area.

The document includes series of tables and figures, related to the biomass/abundance indices and length frequency distributions of *Psetta maxima*, collected during the survey.

Overall, the demersal survey aimed to achieve the following activities:

- Sampling with demersal trawl;

- Qualitative and quantitative analysis of catch, biometric measurements, biodiversity establishment;

- Collection of material for determination of turbot age and sex structure;
- Collection of turbot stomachs to establish food composition and stomach fullness;
- Collection of genetic samples;
- Collection of materials for estimation of turbot fillet chemical composition;



## 2. Fishing vessel and fishing gear

Fishing survey was conducted with the fishing vessel "EGEO 2" (photo 1), with the following characteristics:



Photo 1. Fishing vessel "EGEO 2"

- Type of fishing vessel TAKA;
  - Total length 17.0 m;
    - Maximum width 6.0 m;
      - Year of construction 2007;
        - Engine power 530 kW;
          - Maximum Tonnage 300 t;
            - Net Tonnage 140 t;
              - Speed 9.5 Nd;
                - Number of crew 4 people
                  - Number of researchers 4 people

Fishing demersal trawl 22/27-34 was used during the survey (photo 2), with the following functional and technical parameters:







Photo 2, Demersal trawl 22/27-34

- Vertical opening - 2 m;

- Horizontal opening between otter board 9 m;
  - Horizontal opening between the wings 13 m;
    - Trawling speed 2.2 to 2.3 Nd;
      - Trawling time 60 min;
        - The volume of water filtered in the unit of time 50  $m^3/s$ .;



## 3. Material and Methods

The methodology and techniques, that have been used for collection, verification, processing, data analysis and for turbot stock assessment in 2014 are in compliance with the generally applied methodology during the previous turbot data collection programs in the Bulgarian Black Sea zone.

A standard fishing technique - demersal trawling was used throughout the current survey. Productive potential of stock is estimated, based on methods, accepted at regional/international level and applying standard methodologies.

The main target species for biomass assessment were turbot and dogfish.

The "swept area" method, based on demersal trawl sampling, was used. The sampling area is shown in Figure 1.

#### 3.1. Collected information from the turbot surveys

The data recorded at each haul includes:

- Depth, measured by the vessel's echo sounder;
  - GPS coordinates of start/end haul points;
    - Haul duration;
      - Abundance of the target species;
        - Weight of total catch;
          - Absolute and standard length, individual weight of the separate specimens;
            - Otholits collection for age determination;
            - Sex identification;
              - Stomachs;
                - The species composition of the by-catch;
                  - Discards;
                    - Chemical composition of turbot meat;

The turbot surveys provide additional information for calculation of the catch per unit effort (CPUE) (kg/hour) and the catch per unit area (CPUA) (kg/km<sup>2</sup>).

The final results are presented in maps and tables that include data on:

- Investigate surface area (km<sup>2</sup>);

- Average mass per unit area (t/km<sup>2</sup>);
  - Limits of variation of mass per unit area;
    - Total amounts of biomass (t);



- Indices of abundance (specimens/ km<sup>2</sup>);

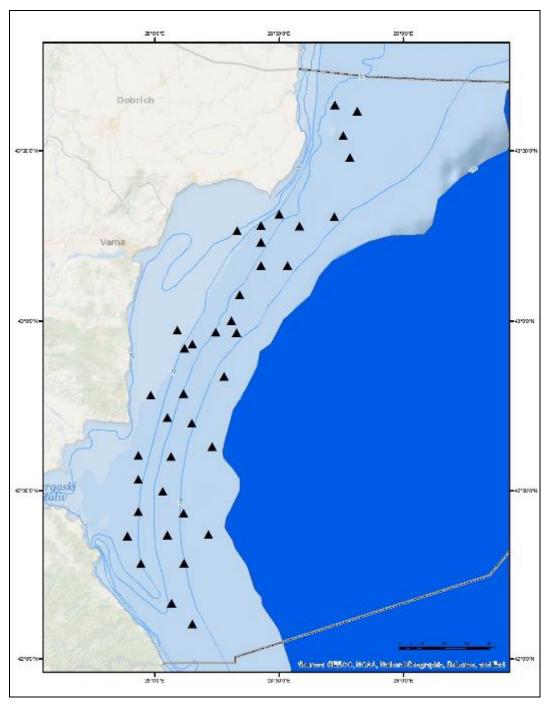


Fig. 1 Sectors of activity in the demersal survey November 2014

#### 3.2. Sampling design

To establish the abundance of the reference species *Psetta maxima* in front of the Bulgarian Black Sea coast, a standard methodology for stratified sampling was employed (Gulland, 1966; Sparre, Venema, 1998; Sabatella, Franquesa, 2004). To address the research objectives, the region was divided into four strata according to depth – Stratum 1 (15 - 30 m), Stratum 2 (31 – 50 m), Stratum 3 (50 – 75 m) and Stratum 4 (75 – 100 m).

To design the sampling points, the study area was partitioned into equal in size, not overlying fields, situated at depth between 15 - 100 m. The sampling was carried out at 38

randomly chosen fields (Fig. 1). Each field is a rectangle with sides 5' Lat  $\times$  5' Long and area around 62.58 km<sup>2</sup> (measured by application of GIS). Also, each row of rectangles is denoted by letters and numbers (Fig. 2).



Fig. 2 Rectangles used to design sampling points in the demersal survey, November 2014



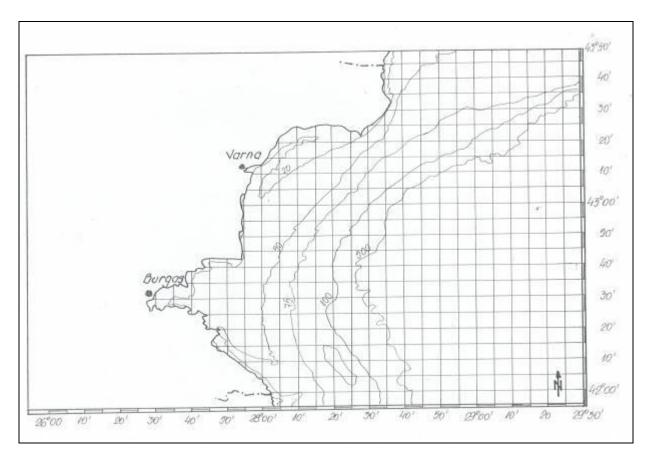


Fig. 3 Grid lines used for calculations of the area

To assess to indices of abundance and biomass of turbot, the study area was partitioned into 143 squares with sides 5' Lat. (5 Nm) – 25 Nm<sup>2</sup> (85.8569 km<sup>2</sup>) (Fig. 3). The seabed area covered during a single haul represents a basic measurement unit, deemed representative, since turbot do not aggregate in dense assemblages (Martino, Karapetkova, 1957). The duration of each haul was 60 min, with speed of 2.2 knots.

For each turbot specimen the absolute and standard length and individual weight were measured. The ratio of the specimens with length below the allowable fishing length and larger specimens was determined (Fig.6). Otoliths were collected for ageing, gender was identified with corresponding maturation stages, and stomachs were collected from part of the individuals for stomach content analysis. To study the chemical composition of turbot, meat fillets were taken during the survey.

#### 3.3. Laboratory analyses

The samples collected on board were processed in the laboratory for determination of age and food composition.

The age was established by otoliths reading under binocular microscope.

The food spectrum was determined by identification of taxonomic groups of food items and quantitative measurements.



#### Food composition

To determine the food composition a total of 57 stomachs were collected in 2014. The analysis includes determination of the total number of food components, wet weight and frequency of occurrence.

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

$$IRI = (CN + CW) * F$$

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

$$\% IRI_{i} = \frac{100 * IRI_{i}}{\sum_{i}^{n} IRI_{i}}$$

where: n - is the total number of food categories considered at a given taxonomic level.

The evaluation of the sex structure of catches comprised estimations of average, minimum and maximum total lengths (TL, cm) and weight of specimens from both genders, and analysis of the size classes distribution per genders. Also, the average, minimum and maximum values of gonadosomatic index (%) were determined using the formula GSI,  $\% = (ovary/testis weight/body weight) \times 100$ .

The samples for estimation of the biochemical composition of turbot meat were collected in fields L1, N1, M2, E11 - E13, C17-E19 in the period 12.11-30.11.2014, as the fish have been divided into 5 groups according to fields of catches (Table 8):

Table 8

Place and date of turbot catch

| Place of catch | L1         | N1         | M2         | E11-E13    | C 17-E19      |
|----------------|------------|------------|------------|------------|---------------|
| Date           | 12.11.2014 | 12.11.2014 | 12.11.2014 | 17.11.2014 | 19-29.11.2014 |

Samples for analysis of edible fish tissue were collected according to BSS 3419:1978 "Fish and fish products. Rules for sampling". Individuals of average size were selected, which for the respective group varied from 800 to 1600 g.

For individual samples, chemical meat composition was determined with respect to: water content (drying at 105 °C, 24h; Bulgarian State Standard – SR ISO 5984); protein content (Kjeldahl method; Bulgarian State Standard – SR ISO 5983 using a semi-automated DK 6 digester unit and UDK 132 distillation system, Velp Scientifica); fat content, % (by the method of Smidt-Boudzynski Ratzlaff); mineral content, % (by burning in a muffle furnace at 550 °C, BSS – SR ISO 6496).



The energy content of turbot meat was estimated theoretically on the basis of the chemical composition using the following coefficients:  $17 \text{ kJ.g}^{-1}$  for proteins and carbohydrates and 37.0 kJ.g-1 for fat (Ordinance 23 /2001, Ministry of Health).

The fatty acid composition of meat triglycerides was analyzed by gas chromatography using a HP 5890 II gas chromatograph with flame ionisation detector and 60 m DB-23 capillary column.

The fat tocopherols composition and quality was directly assayed by gas-liquid chromatography (ISO 9936,1997).

3.4. Statistical analyses

#### Swept area method

The swept area method was used for estimation of turbot (*Psetta maxima*) exploitation biomass.

According to this method, the trawl swept a well defined path, the area of which is the length of the path, multiplied by the width of the trawl, called the "swept area" or the "effective path swept". The swept area can be estimated from equation:

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

where: a - swept area; V - velocity of the trawl over the ground when trawling; 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"; t - is the time spent trawling ; D - distance covered.

For the estimation of turbot biomass, the catch per unit of area (CPUA) is used:

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

where: Cw/t – catch in weight per unit of area, a/t – the area swept per hour.

The biomass for each stratum is obtained from equation:

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

where:  $\overline{C_{w/a}}$  - the mean catch per unit area of all hauls; A – the total area under investigation in stratum.

The variance of biomass estimated for each stratum is:

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

The total area of survey region, equals to the sum of all strata areas:

$$A = A1 + A2 + A3$$

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

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

where: Ca1- catch per unit area of stratum 1 and etc.; A1 – area of stratum 1 and etc.; A – total area of survey region.

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

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

where:  $\overline{Ca}(A)$ - mean catch for the entire survey area; A – total area of survey region.

#### Estimation of Maximum Sustainable Yield (MSY)

The Gulland's formula for virgin stocks is used:

$$MSY = 0.5*M*Bv$$

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

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.

$$MSY = 0.5 * Z * B$$

Where:  $\overline{B}$  - average 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:

$$MSY = 0.5 * (y + M * B)$$

where:

 $\mathbf{y}$  = the total catch in one year and,

 $\overline{B}$  = Average biomass in the same year.

#### Age and growth

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

$$L_{t} = L_{\infty} \left\{ 1 - \exp\left[-k(t - t_{0})\right] \right\}$$
$$W_{t} = W_{\infty} \left\{ 1 - \exp\left[-k(t - t_{0})\right] \right\}^{n}$$

where:

Lt, Wt are the length or weight of the fish at age **t** years;

 $L\infty$  ,  $W\infty$  - asymptotic length or weight;

k – curvature parameter;



to - the initial condition parameter.

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

$$W_t = qL_t^n$$

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:

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

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

where:

 $L\infty,\,W\infty$  and k – parameters in von Bertalanffy growth function;  $T^0C$  - average annual temperature of water.

#### Metoda Richter si Efanov (1976)

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

where:

 $t_{mat} = age at first maturation$ 

Metoda Lukashev (1970)

$$M=1-\left[\frac{1-e^{-k(t_{-t_o})}}{1-e^{-k(t_{+1}-t_o)}}\right]^3 \text{ where } t= \text{ age of mature fish}$$

Metoda Alverson - Cranev (1975)

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

 $t_{mb}$ = age at which biomass is maximum

### 4. Results

4.1 Abundance index and biomass

#### Introduction

A total of 38 hauls were carried out in November 2014 in Bulgarian waters with the RV "EGEO 2". All hauls were valid. For each haul, a qualitative and quantitative analysis of the catch

was accomplished. The biometric measurements included 91 turbot specimens and 22 dogfish specimens and 57 samples - for stomach content analysis.

During the survey, a total of 15 species were identified - 10 fish species, 2 crustaceans, 2 molluscs and 1 ascidian species.

Frequently observed were the following species: *Merlangius merlangus* (whiting), *Mytillus galloprovincialis* (Mediterranean mussel), *Aurelia aurita* (jellyfish), *Psetta maxima* (turbot), *Mullus barbatus* (red mullet), *Raja clavata* (Thornback ray). Other species, found during the cruise were: Squalus acanthias (picked dogfish), *Pomatomus saltatrix* (bluefish), *Crangon crangon* (common shrimp), *Trachinus draco* (greater weever), *Platichthys flesus* (european flounder), *Trachurus mediterraneus* (mediterranean horse mackerel), *Neogobius melanostomus* (Round goby) and *Anadara inaequivalvis* (anadara) (photo 3 and 4).



Photo 3. Fish and other species



**Overall comments.** During the survey were observed:

- Constant presence of turbot (*Psetta maxima*) during the demersal trawling at depths between 30-50 m and 50-75 m; obtained catches - at least 1-4 specimens per hauling, in a mixture with whiting specimens (*Merlangius merlangus euxinus*) 3-5% and mullet (*Mullus barbatus*). In the above mentioned two sectors were obtained the best turbot yields, ranging between 2.1 to 12.41 kg/haul.

- Rare presence of shark specimens (*Squalus acanthias*) in demersal trawls at depths 30-50 m and 50-75 m; captured and measured - 16 individuals (110-133 cm / 6.0 to 12.2 kg), of which 43.75 % females and 56.25 % males (photo 4).



Photo 4. Dogfish catches (Squalus acanthias)

- The catches from almost all demersal trawls included a mixture of species - whiting (*Merlangius merlangus euxinus*, 5 - 10.5%), mullet (*Mullus barbatus ponticus* 5%) and thornback ray (*Raja clavata* - over 20 specimens), that formed the catch basis. Along with these three species, in the catches appeared: mussels (*Mytilus galloprovincialis*), jellyfish (*Aurelia aurita*), sole (*Solea vulgaris*), large black scorpionfish (*Scopaena porcus*), various species of gobies (*Gobiidae*), single bluefish specimens (*Pomatomus saltatrix*), flounder (*Platichthys flesus luscus*) and shrimp (*Crangon crangon*).

#### Comments about Psetta maxima biomass by stratum

Taking into account that only two stations included depth < 30 m, for technical reasons statistical analysis was performed on stratum 15 – 50 m. Also, the strata - 50 – 75 m and 75 – 100 m have been analyzed. However, up to 30 m depth, biomasses at the two stations were - 181.129 kg/Km<sup>2</sup> and 39.622 kg/km<sup>2</sup>, abundance -113 specimens/km<sup>2</sup> and 38 specimens/km<sup>2</sup>.

#### Stratum 15 - 50 m

Generally, the biomass indices of turbot decreased from northern to southern direction and the abundance ranged between 0 -  $211 \text{ kg/km}^2$ , with average 83.7 kg/km<sup>2</sup> (Fig. 4, table 1). The



abundance indices oscillated between 0 - 113 specimens/km<sup>2</sup>, with average - 53 specimens/km<sup>2</sup> (Fig. 4, table 2).

#### Stratum 50 - 75 m

This stratum contains the largest number of sampling stations. Biomass has oscillated between 0 - 234.147 kg/km<sup>2</sup>, with average - 87.871 kg/km<sup>2</sup> (Fig. 4, table 1). Abundance was between 0- 170 specimens/km<sup>2</sup>, with average - 51 specimens/km<sup>2</sup> (Fig. 4, table 2).

#### Stratum 75 – 100 m

In this stratum, the biomass/ $\text{Km}^2$  reached value of 258.686 kg/km<sup>2</sup>, with average - 77.947 kg/km<sup>2</sup>(Fig.4, table 1). The average abundance was 26 specimens/km<sup>2</sup> (Fig. 4, table 2).

Table 1

| 15 - 50 n                   | 15 - 50 m |             | – 75 m     | 75-1        | 00 m     |
|-----------------------------|-----------|-------------|------------|-------------|----------|
| No. station t/sqKm          |           | No. station | t/sqKm     | No. station | t/sqKm   |
| 9                           | 0.181129  | 1           | 0.234147   | 5           | 0        |
| 17                          | 0.211317  | 2           | 0.116036   | 11          | 0.258486 |
| 18                          | 0.132073  | 3           | 0.134903   | 16          | 0.07547  |
| 19                          | 0.039622  | 4           | 0          | 20          | 0        |
| 21                          | 0.07547   | 6           | 0          | 24          | 0.05849  |
| 22                          | 0.071697  | 7           | 0.231128   | 25          | 0        |
| 27                          | 0         | 8           | 0.169808   | 29          | 0.231128 |
| 28                          | 0.077357  | 10          | 0.080187   | 34          | 0        |
| 31                          | 0         | 12          | 0.137733   |             |          |
| 32                          | 0.052829  | 13          | 0.076414   |             |          |
| 36                          | 0.079244  | 14          | 0.2911     |             |          |
|                             |           | 15          | 0          |             |          |
|                             |           | 23          | 0.047169   |             |          |
|                             |           | 26          | 0.024528   |             |          |
|                             |           | 30          | 0.037735   |             |          |
|                             |           | 33          | 0.022641   |             |          |
|                             |           | 35          | 0          |             |          |
|                             |           | 37          | 0.028301   |             |          |
|                             |           | 38          | 0.037735   |             |          |
| Total                       | 0.920738  | Total       | 1.669565   | Total       | 0.623574 |
| Average                     | 0.083703  | Average     | 0.08787184 | Average     | 0.077947 |
|                             |           |             |            |             |          |
| Variance                    | 0.00453   |             | 0.00805565 |             | 0.011535 |
| Standard deviation          | 0.067304  |             | 0.08975329 |             | 0.107402 |
| Relative standard deviation | 0.804071  |             | 1.02141126 |             | 1.37789  |
| Standard error              | 0.020293  |             | 0.02059082 |             | 0.037972 |

#### Turbot biomass by stratum in November 2014

National Bottom Trawl Survey 2014



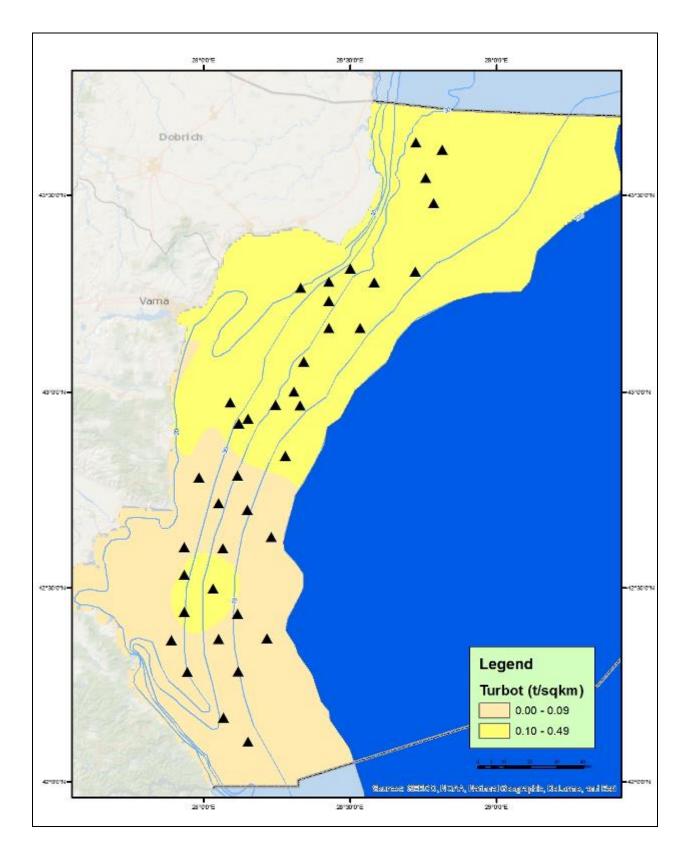


Fig. 4 Turbot distribution off the Bulgarian coast in November 2014



| 15 - 50 m          |                                    | 5              | 0 – 75 m                           | 75             | 5-100 m                            |
|--------------------|------------------------------------|----------------|------------------------------------|----------------|------------------------------------|
| No. station        | No.<br>individuals/km <sup>2</sup> | No.<br>station | No.<br>individuals/km <sup>2</sup> | No.<br>station | No.<br>individuals/km <sup>2</sup> |
| 9                  | 113                                | 1              | 170                                | 5              | 0                                  |
| 17                 | 113                                | 2              | 57                                 | 11             | 75                                 |
| 18                 | 94                                 | 3              | 38                                 | 16             | 57                                 |
| 19                 | 38                                 | 4              | 0                                  | 20             | 0                                  |
| 21                 | 57                                 | 6              | 0                                  | 24             | 19                                 |
| 22                 | 38                                 | 7              | 113                                | 25             | 0                                  |
| 27                 | 0                                  | 8              | 113                                | 29             | 57                                 |
| 28                 | 38                                 | 10             | 94                                 | 34             | 0                                  |
| 31                 | 0                                  | 12             | 19                                 |                |                                    |
| 32                 | 57                                 | 13             | 57                                 |                |                                    |
| 36                 | 38                                 | 14             | 129                                |                |                                    |
|                    |                                    | 15             | 0                                  |                |                                    |
|                    |                                    | 23             | 19                                 |                |                                    |
|                    |                                    | 26             | 38                                 |                |                                    |
|                    |                                    | 30             | 38                                 |                |                                    |
|                    |                                    | 33             | 19                                 |                |                                    |
|                    |                                    | 35             | 0                                  |                |                                    |
|                    |                                    | 37             | 38                                 |                |                                    |
|                    |                                    | 38             | 38                                 |                |                                    |
| Total              | 585                                | Total          | <i>9</i> 78                        | Total          | 208                                |
| Average            | 53                                 | Average        | 51                                 | Average        | 26                                 |
|                    |                                    |                |                                    |                |                                    |
| Variance           | 26.55372241                        |                | 45.265654                          |                | 34.18357488                        |
| Standard deviation | 5.15303041                         |                | 6.72797547                         |                | 5.84667212                         |
| Relative standard  |                                    |                |                                    |                |                                    |
| deviation          | 0.09691195                         |                | 0.12938414                         |                | 0.224872005                        |
| Standard error     | 0.212869724                        |                | 0.21491729                         |                | 0.405393772                        |

Turbot abundance by stratum in November 2014

The total turbot biomass in the Bulgarian area amounted at **980 tons** (table 4). The **abundance** was estimated at **540 143 individuals** (table 4), of which, more than 53%, were below the minimum length allowed in fishing (< 45cm).

#### 4.2 Catch per unit effort (CPUE)

During the expedition were achieved:

- 38 hauls with demersal trawl, trawling duration 60 minutes, on depths between 22.0 m and 90.0 m, covering almost entirely the continental shelf of the Bulgarian Black Sea coast, between Durankulak and Ahtopol. Table 3 represents the distribution of CPUE during the demersal trawl survey in November 2014.



| N⁰  | Field | Starting coordinates |             | Depth | Speed | Trawl<br>ing |    | Catch |
|-----|-------|----------------------|-------------|-------|-------|--------------|----|-------|
|     |       |                      | ^           | (m)   | (Nm)  | time         |    | urbot |
|     |       | Ф                    | λ           |       |       | (min)        | N⁰ | Kg    |
| 1.  | L1    | 43°38'057"           | 28°43'600"  | 52-58 | 2.2   | 60           | 9  | 12.41 |
| 2.  | N1    | 43°37'000"           | 28°49'000"  | 64-67 | 2.2   | 60           | 3  | 6.15  |
| 3.  | M2    | 43°32'666"           | 28°45'550"  | 61-68 | 2.2   | 60           | 2  | 7.15  |
| 4.  | L3    | 43°28'900"           | 28°47'170"  | 68-65 | 2.2   | 60           | 0  | 0     |
| 5.  | L5    | 43°18'439"           | 28°43'399"  | 86-79 | 2.2   | 60           | 0  | 0     |
| 6.  | J5    | 43°16'825"           | 28°35'016"  | 66-50 | 2.2   | 60           | 0  | 0     |
| 7.  | K5    | 43°18'919"           | 28°30'200'' | 55-50 | 2.2   | 60           | 6  | 12.25 |
| 8.  | H5    | 43°16'920"           | 28°25'719"  | 52-28 | 2.2   | 60           | 6  | 9.0   |
| 9.  | G6    | 43°16'000"           | 28°20'000'' | 26-27 | 2.2   | 60           | 6  | 10.10 |
| 10. | J6    | 43°14'000"           | 28°25'700'' | 40-78 | 2.2   | 60           | 5  | 4.25  |
| 11. | J7    | 43°09'900"           | 28°32'100"  | 82-85 | 2.2   | 60           | 4  | 13.7  |
| 12. | H7    | 43°09'900"           | 28°25'700'' | 53-50 | 2.2   | 60           | 1  | 7.3   |
| 13. | G8    | 43°04'800"           | 28°20'580"  | 50-63 | 2.2   | 60           | 3  | 4.0   |
| 14. | G9    | 43°00'200"           | 28°18'600"  | 61-60 | 2.2   | 35           | 4  | 9.0   |
| 15. | F9    | 42°58'200"           | 28°14'780"  | 40-61 | 2.2   | 60           | 0  | 0     |
| 16. | H9    | 42°58'107"           | 28°19'900"  | 79-80 | 2.2   | 60           | 3  | 4.1   |
| 17. | E9    | 42°56'070"           | 28°09'150"  | 36-30 | 2.2   | 60           | 6  | 11.2  |
| 18. | D9    | 42°58'500"           | 28°05'600'' | 29-22 | 2.2   | 60           | 5  | 7.0   |
| 19. | E10   | 42°55'300"           | 28°07'270'' | 42-56 | 2.2   | 60           | 2  | 2.1   |
| 20. | G10   | 42°50'300"           | 28°16'900'' | 82-74 | 2.2   | 60           | 0  | 0     |
| 21. | E11   | 42°47'300"           | 28°07'080'' | 53-42 | 2.2   | 60           | 3  | 4.0   |
| 22. | C11   | 42°47'000"           | 27°59'130"  | 33-42 | 2.2   | 60           | 2  | 3.8   |
| 23. | D12   | 42°43'107"           | 28°03'200'' | 49-65 | 2.2   | 60           | 1  | 2.5   |
| 24. | F12   | 42°42'080"           | 28°09'120"  | 73-87 | 2.2   | 60           | 1  | 3.1   |
| 25. | G13   | 42°37'900"           | 28°14'000'' | 90-80 | 2.2   | 60           | 0  | 0     |
| 26. | E13   | 42°36'120"           | 28°04'100"  | 60-50 | 2.2   | 60           | 2  | 1.3   |
| 27. | C13   | 42°36'300"           | 27°56'150"  | 43-42 | 2.2   | 60           | 0  | 0     |
| 28. | C14   | 42°32'100"           | 27°56°170"  | 47-55 | 2.2   | 60           | 2  | 4.1   |
| 29. | F14   | 43°32'07"            | 28°12'430"  | 80-76 | 2.2   | 60           | 3  | 10.7  |
| 30. | E15   | 42°26'070"           | 28°07'100"  | 67-52 | 2.2   | 60           | 2  | 2.0   |
| 31. | C15   | 42°26'300"           | 27°56'160"  | 43-37 | 2.2   | 60           | 0  | 0     |
| 32. | B16   | 42°22'000"           | 27°53'500"  | 36-46 | 2.2   | 60           | 3  | 1.3   |
| 33. | D16   | 42°22'200"           | 28°03'200'' | 58-72 | 2.2   | 60           | 1  | 1.2   |
| 34. | F16   | 42°22'260"           | 28°13'060'' | 86-78 | 2.2   | 60           | 0  | 0     |
| 35. | E17   | 42°17'150"           | 28°07'140"  | 65-52 | 2.2   | 60           | 0  | 0     |
| 36. | C17   | 42°17'100"           | 27°56'800'' | 41-44 | 2.2   | 60           | 2  | 4.2   |
| 37. | E19   | 42°06'300"           | 28°09'130"  | 59-56 | 2.2   | 60           | 2  | 1.4   |
| 38. | D18   | 42°10'030"           | 28°04'130"  | 54-44 | 2.2   | 60           | 2  | 2.05  |

TOTAL 38 hauls - 10 hauls (26, 32%, of total no. hauls), catch 0 kg;

15 hauls (39, 47%), catch 0.1 – 4.99 kg per haul;

8 hauls (21, 06%), catch 5.0 – 9.99 kg per haul;

5 hauls (13, 15%), catch  $10.0-14.99\ kg$  per haul;

Sector < 30 m - 2 hauls;

Sector 31 – 50 m- 9 hauls;

Sector 50 – 75 m - 19 hauls;

Sector 75 – 100 m - 8 hauls;

4.3 Catch per unit area (CPUA)

After processing, the data for abundance and biomass were obtained (table 4):

Table 4

| No.     |       | No.               |           |
|---------|-------|-------------------|-----------|
| Station | Field | individulals/sqKm | t/sqKm    |
| 1       | L1    | 170               | 0.2341468 |
| 2       | N1    | 57                | 0.1160357 |
| 3       | M2    | 38                | 0.1349032 |
| 4       | L3    | 0                 | 0         |
| 5       | L5    | 0                 | 0         |
| 6       | J5    | 0                 | 0         |
| 7       | K5    | 113               | 0.2311279 |
| 8       | H5    | 113               | 0.1698083 |
| 9       | G6    | 113               | 0.1811288 |
| 10      | J6    | 94                | 0.0801872 |
| 11      | J7    | 75                | 0.2584859 |
| 12      | H7    | 19                | 0.1377334 |
| 13      | G8    | 57                | 0.0764137 |
| 14      | G9    | 129               | 0.2910999 |
| 15      | F9    | 0                 | 0         |
| 16      | H9    | 57                | 0.0754703 |
| 17      | E9    | 113               | 0.211317  |
| 18      | D9    | 94                | 0.1320731 |
| 19      | E10   | 38                | 0.0396219 |
| 20      | G10   | 0                 | 0         |
| 21      | E11   | 57                | 0.0754703 |
| 22      | C11   | 38                | 0.0716968 |
| 23      | D12   | 19                | 0.047169  |
| 24      | F12   | 19                | 0.0584895 |
| 25      | G13   | 0                 | 0         |
| 26      | E13   | 38                | 0.0245279 |
| 27      | C13   | 0                 | 0         |
| 28      | C14   | 38                | 0.0773571 |
| 29      | F14   | 57                | 0.2311279 |
| 30      | E15   | 38                | 0.0377352 |

Abundance and biomass obtained in the Bulgarian area in November 2014



| 31 | C15  | 0                      | 0         |
|----|------|------------------------|-----------|
| 32 | B16  | 57                     | 0.0528292 |
| 33 | D16  | 19                     | 0.0226411 |
| 34 | F16  | 0                      | 0         |
| 35 | E17  | 0                      | 0         |
| 36 | C17  | 38                     | 0.0792439 |
| 37 | E19  | 38                     | 0.0283014 |
| 38 | D18  | 38                     | 0.0377352 |
| Тс | otal | 1771                   | 3.2138778 |
|    |      | 47                     | 0.0845757 |
|    |      | 540 143<br>individuals | 980 tons  |

|                             | No.<br>individulals/sqKm | t/sqKm   |
|-----------------------------|--------------------------|----------|
| Variance                    | 40.70159                 | 0.007341 |
| Standard deviation          | 6.37978                  | 0.085679 |
| Relative standard deviation | 0.138691                 | 0.026659 |
| Standard error              | 0.151599                 | 0.013899 |

#### 4.4 Size structure of turbot (Psetta maxima)

#### Length frequency

The length frequency distribution of turbot population in front of the Bulgarian Black Sea area in November 2014 included size classes from 26.0 to 66.0 cm (with weights between 200 g and 7300 g) (photo 5). From the total of 91 turbot specimens, caught and measured, 6 belong to the size class < 30 cm (5.59%), the size of 25 specimens ranged between 30-40 cm (27.47%), 35 specimens ranged between 40-50 cm size (38.46%), 22 specimens were in the range 51-60 cm (24.18%), and 3 - between 61-70 cm (3.30%).







Photo 5 Measurements of the total, standard length and weight of turbot specimens (P.maxima)

The analysis of length-weight structure of turbot catches has highlighted the presence of mature specimens and a relative homogeneity of herds. The dominant classes were two - 37.0 - 43.0 cm / 1000 - 1590 g and 49.0 - 55.0 cm / 2000 - 3100 g (Fig. 4). The average body length was 44.27 cm and the average weight - 1.76 kg.

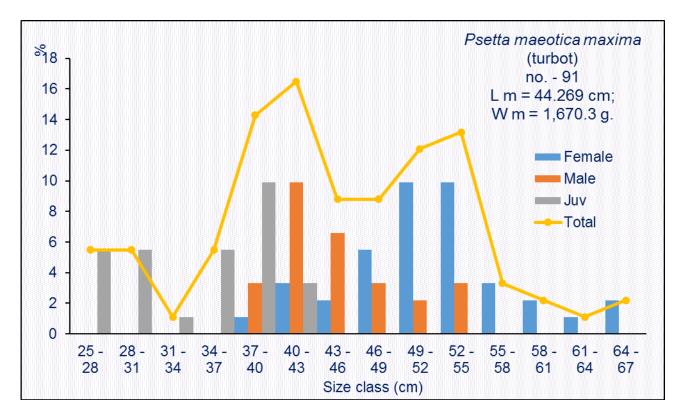
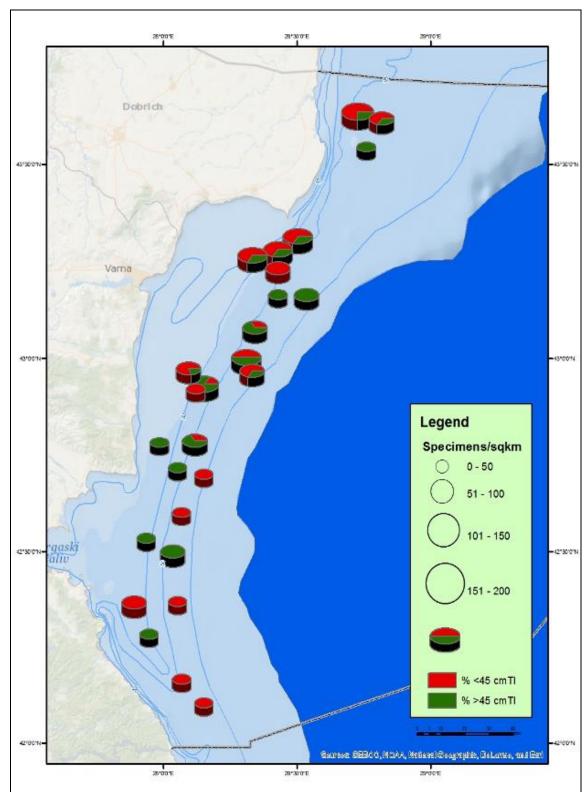


Fig. 5 Length structure of turbot

The size structure of catches was analyzed in agreement with the Bulgarian low regulations (Fisheries and Agriculture Act, 2005), determining the minimum allowable total length for turbot fishing. Thus, the individuals with absolute length < 45 cm are indicated as small and those > 45 cm – as standard. The distribution of turbot size groups off the Bulgarian coast in November 2014 is represented on a map (Fig. 6), where the diameter of circles reflects the total abundance of turbot,



caught in the respective area, while the colours - green/red appoint the abundance of small and standard size specimens.

*Fig. 6* Number of turbot specimens/Km<sup>2</sup> and ratio between the specimens with small and standard sizes.

#### 4.5 Age structure of turbot (Psetta maxima)

The age composition of turbot was determined on the basis of 35 couples of otoliths. The age structure encompassed  $2^+$  -  $6^+$  years classes, while catches were dominated by  $3^+(62.6\%)$  -  $4^+$  (20.9 %) - years' classes (total 83.5%) (Fig.7). In 2014, the share of recruitment - $2^+$  years classes, has increased to 28.28% in comparison with the results from autumn survey in 2013.

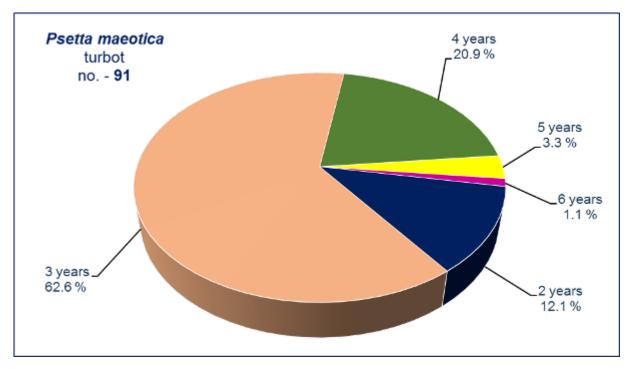


Fig. 7 Turbot age structure in November 2014

The distribution of age classes along the investigated fields is presented on Fig. 8.



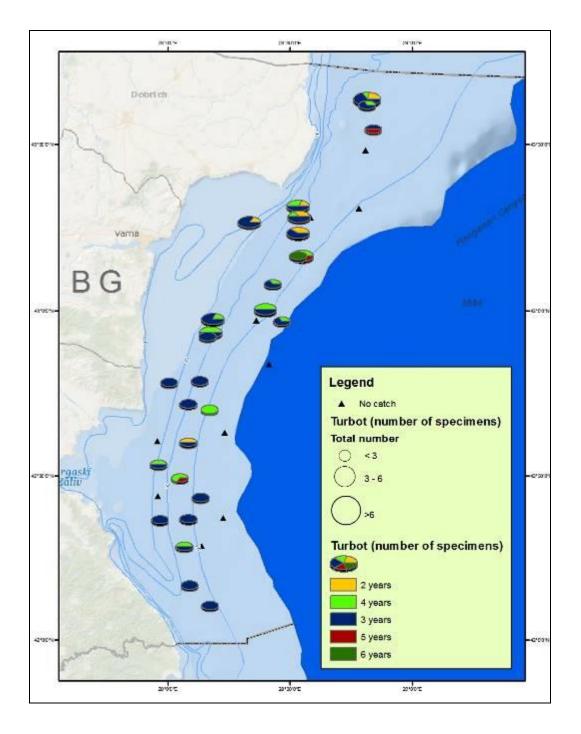


Fig. 8 Age structure of Psetta maxima in November 2014.



#### 4.6 Biological parameters

For estimation of turbot growth rate, data for average length and weight by age groups for both genders, were used (due to the small number of collected specimens). The calculated values of the parameters in von Bertalanffy growth function were:

b = 3.185544403 q = -2.074745846 a = 0.008418  $L_{\infty} = 69.47$  k = 0.362to = -0.476

The natural mortality coefficient of turbot in Bulgarian Black Sea area was calculated according to Pauly's empirical equation (1980), which describes the natural mortality as a function of k,  $L_{\infty}$ ,  $W_{\infty}$  and mean habitat temperature (T). For calculation of M using Pauly's formula, the temperature was chosen between 12 and 15 degrees Celsius, as that of the breeding period. Total for both genders M = 0.490 - 0.54

 $L_{\infty}$  = Lt max/0.95 – empirical formula

 $\mathbf{k} = 1/(t_2-t_1)*ln(\mathbf{L}_{\infty}-\mathbf{L}_1)/(\mathbf{L}_{\infty}-\mathbf{L}_2)$  from age composition

 $\mathbf{t_0} = \mathbf{t_1} + 1/k*\ln(1-L_1/L_\infty)$ 

4.7 Maturity of turbot (Psetta maxima)

#### Sex ratio

Out of all 38 catch fields at 18 no female turbots have been caught, in 26 localities - no males were found, while in 9 - neither female and male specimens were observed. (Table 5).



Photo 6. Determine the development stages of gonads and diet composition



|                                    | n  | female fish   | male fish     |
|------------------------------------|----|---------------|---------------|
| total number of fields of catch    | 38 |               |               |
| number of fields without:          |    | 18            | 26            |
| female/male fish                   |    |               |               |
| total number of caught fish        | 91 | 40            | 24            |
| average weight of female/male fish |    | 2,556 (n=40)  | 1,597 (n=24)  |
| maximum weight                     |    | 7300          | 3000          |
| minimum weight                     |    | 900           | 1000          |
| GSI, %                             |    | 1.4501 (n=30) | 0.3566 (n=16) |
| max                                |    | 2,97          | 0.836         |
| min                                |    | 0.33          | 0.149         |

Turbot catches: female/male presence, weight, and GSI in November 2014

The sex structure of turbot catches is represented on Fig. 9. In the Bulgarian Black Sea waters the juvenile turbot specimens represent 30.80% of all collected individuals, the females formed 40.7 %, whereas the percent share of males was lowest - 28.6 %.

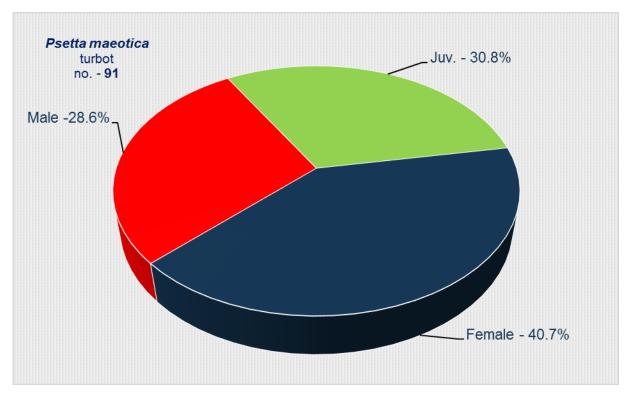


Fig. 9 Sex structure of turbot catches in November 2014.

The sex structure of turbot in the investigated area is presented on Fig. 10.



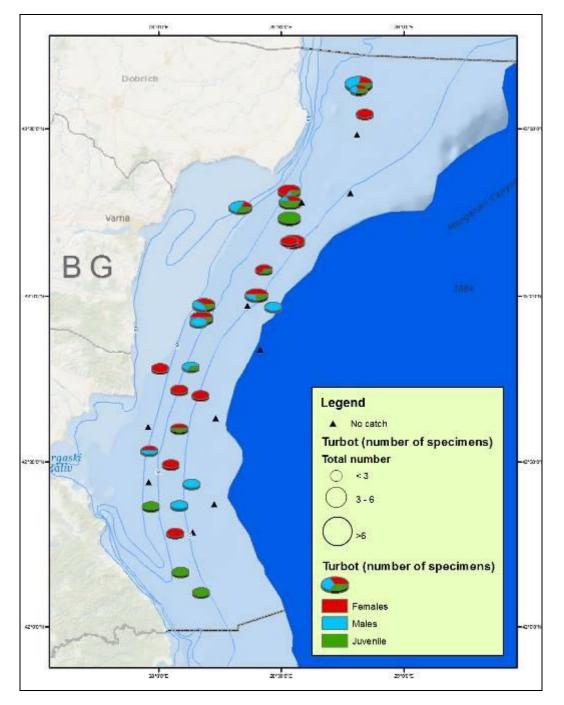


Fig.10 Sex structure of Psetta maxima in November 2014.

The average weight of females was 2556 g, while the average total length (TL,cm) was 49.42 cm and standard length (SL,cm) - 40.46 cm. The maximal weight of female specimen was 7300 g, whereas the minimal weight was 900 g.

The analysis of the dominating size classes, showed that for females, the greatest share (20%) has been formed by the large size class - 51-54 cm, followed by the size class 47-51 cm – 17.5 %, and size class 44-47 cm – 12.5% (Fig. 11). Female specimens with TL 44-51 cm comprised 50% of all caught females. The upper size classes > 57 cm (up to 66 cm) included only females specimens and this class comprised 15% of the size structure. The results show expressed sex dimorphism as regards the body size, and superior numbers of large size classes of females.

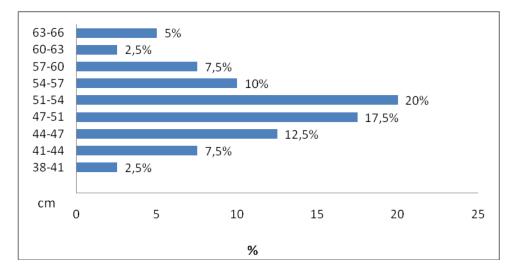


Fig.11 Percentage distribution of female fish by body size class (TL, cm)

Almost half of the male turbots (41.7%) belong to the size class 41- 44 cm, followed by size classes 44-47 cm, and 47-51 cm - with the similar percentage shares - 16.7 % (Fig.12). Three quarters of all studied males belong to the size group - 41-51 cm.

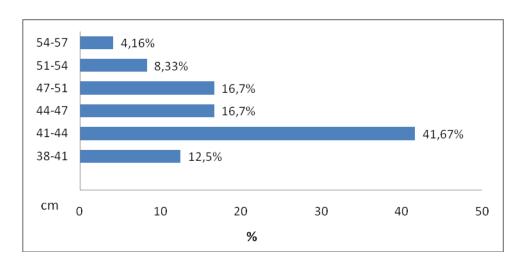


Fig.12 Percentage distribution of male fish by body size class (TL, cm)

#### Maturation stages

The ovary and testis were in the second degree of maturity, with predominance of previtellogenic oocytes and spermatids fractions. The gonadosomatic index (GSI, %) of females was 1.45% on the average, with maximum and minimum values - 2.97 and 0.33%, respectively (Fig.13). The average GSI,% of male turbots was 0.36%, with maximum/minimum values - 0.84 and 0.15%. GSI variation was markedly pronounced in females vs males, which could be attributed to the relatively consistent weight structure of the collected male individuals (3000-1000 g) in comparison to that of females (7300-900 g).

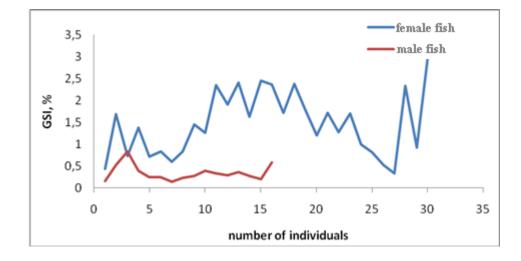


Fig. 13 Dynamics of GSI (%) in female and male turbots caught in December 2014

#### 4.8 Food diet of turbot (Psetta maxima)

The previous investigations of turbot in the Black Sea have been focused on the species biology and stock assessments and encompassed a few data of its diet composition (Bulgurkov, K., 1965, Ivanov, L., M. Karapetkova, 1979, Marinov, K., M. Karapetkova, 1957, Zengin, M., 2005). Turbot is referred to predatory fishes, as its ration consists of other fish species, crustaceans, polychaetes and molluscs.

For estimation of the turbot diet composition, a total of 58 stomachs were collected and investigated in 2014. The fish stomachs were preserved in a concentrated formaldehyde solution. The food spectrum analysis included identification of the main taxonomic groups and measurements of quantitative parameters - frequency of occurrence, and percent composition by abundance and biomass (more data in the section **Materials and Methodology**). The fish specimens with an advanced stage of food items decomposition were marked and quantified. From all 57 investigated fish specimens, 40% were found with empty stomachs.

During the survey the qualitative composition of the turbot diet included *Pisces, Crustacea, Mollusca* and *Polychaeta*. Nematodes were also found in the stomach contents of turbot, forming a group of parasites (diseases indicator). The quantitative parameters, measured during this study are presented at Table 6 and 7, and Fig 14 and 15.

Table 6

| Species                               | C <sub>N</sub> | C <sub>w</sub> | F     | IRI     |
|---------------------------------------|----------------|----------------|-------|---------|
| Pisces                                | 70,88          | 89,92          | 88,24 | 5574,47 |
| Merlangius merlangus (Linnaeus, 1758) | 46,83          | 61,74          | 58,82 | 4926,60 |
| Mullus barbatus Linnaeus, 1758        | 20,25          | 23,32          | 20,59 | 593,19  |
| Sprattus sprattus (Linnaeus, 1758)    | 1,27           | 0,47           | 2,94  | 8,37    |
| Gobiidae sp.                          | 2,53           | 4,39           | 5,88  | 46,31   |
| Crustacea                             | 22,79          | 9,06           | 35,29 | 1654,54 |
| Crangon crangon (Linnaeus, 1758)      | 1,27           | 0,01           | 2,94  | 119,02  |

The qualitative and quantitative composition of the turbot food specrum in November 2014.

National Bottom Trawl Survey 2014



| Liocarcinus holsatus (Fabricius, 1798)  | 20,25 | 8,55 | 29,41 | 1530,08 |
|---|-------|------|-------|---------|
| Rhithropanopeus harrisii (Gould, 1841)  |       | 0,5  | 2,94  | 5,44    |
| Mollusca                                |       | 1,01 | 8,82  | 30,79   |
| Mytilus galloprovincialis Lamarck, 1819 | 1,27  | 0,23 | 2,94  | 13,21   |
| Chamelea gallina (Linnaeus, 1758)       | 1,27  | 0,31 | 2,94  | 8,16    |
| Anadara inaequivalvis (Bruguière, 1789) | 1,27  | 0,47 | 2,94  | 9,42    |
| Polychaeta g. sp.                       | 2,52  | 0,01 | 2,94  | 7,11    |

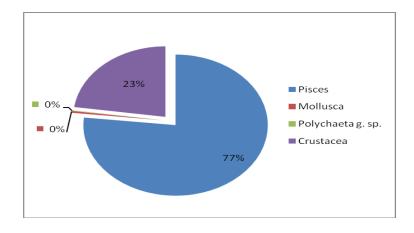


Fig. 14 Food spectrum (IRI): percentage of the main taxonomic groups in the turbot stomach content

The group of fishes has formed the highest percentage in the turbot diet - 77%, followed by crustaceans - 23%, while the groups of molluscs and polychaets represented minimal shares - 0,42 and 0,10% (Fig.14). The most important components in the turbot food were whiting (IRI = 4926.60) and mullet (IRI = 593.19) and the crustacean species *L. holsatus* (IRI = 1530.08), i.e. during the autumn season the turbot diet has been significantly limited and consisted of few components.

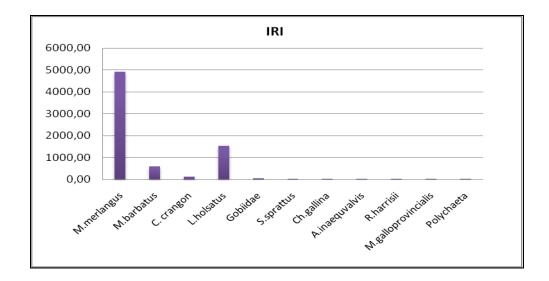


Fig. 15 Indexes of relative importance (IRI) by food items, found in turbot stomach content.

| Area | L (cm) | S./full (gr) | S./empty (gr) | S.contents (gr) | ISF    |
|------|--------|--------------|---------------|-----------------|--------|
| L1/1 | 50     | 0            | 0             | 0               | 0      |
| L1/4 | 46,5   | 0            | 0             | 0               | 0      |
| L1/5 | 39,5   | 0            | 0             | 0               | 0      |
| L1/6 | 45     | 0            | 0             | 0               | 0      |
| L1/7 | 52,5   | 35,5463      | 25,9144       | 9,6319          | 0,4281 |
| L1/8 | 40     | 43,031       | 24,9385       | 18,0925         | 1,1308 |
| L1/9 | 41,5   | 81,7402      | 38,2649       | 43,4753         | 2,8984 |
| H9/2 | 52     | 0            | 0             | 0               | 0      |
| H9/3 | 43     | 0            | 0             | 0               | 0      |
| H9/4 | 58,5   | 0            | 0             | 0               | 0      |
| H9/5 | 43,5   | 28,2421      | 25,6293       | 2,6128          | 0,0933 |
| H9/6 | 51     | 35,034       | 5,4769        | 29,5571         | 1,0748 |
| H9/7 | 37     | 0            | 0             | 0               | 0      |
| N1/2 | 59,5   | 93,709       | 78,5383       | 15,1707         | 0,3700 |
| N1/3 | 43     | 41,079       | 33,5438       | 7,5352          | 0,6028 |
| M2/1 | 56     | 45,9898      | 22,7678       | 23,222          | 0,4788 |
| M2/2 | 49,5   | 106,0513     | 41,9395       | 58,1118         | 2,5266 |
| K5/2 | 37,8   | 32,4828      | 27,9093       | 4,5736          | 0,2613 |
| K5/3 | 42     | 100,0513     | 41,9395       | 58,1118         | 2,9056 |
| K5/4 | 52,5   | 93,6068      | 89,9106       | 3,692           | 0,1119 |
| K5/5 | 42,5   | 46,111       | 15,6136       | 30,4974         | 1,6943 |
| K5/6 | 54,5   | 0            | 0             | 0               | 0      |
| H5/1 | 42,5   | 0            | 0             | 0               |        |
| H5/2 | 37,5   | 79,0348      | 14,334        | 64,7008         | 8,0876 |
| H5/3 | 49,5   | 37,1366      | 24,5662       | 12,574          | 0,5988 |
| H5/4 | 51,5   | 66,551       | 39,7          | 26,851          | 0,8950 |
| H5/5 | 34     | 0            | 0             | 0               |        |
| G6/2 | 46,5   | 27,1798      | 22,2274       | 4,9519          | 0,2476 |
| G6/3 | 44     | 0            | 0             | 0               | 0      |
| G6/4 | 47     | 0            | 0             | 0               | 0      |
| G6/5 | 43,5   | 0            | 0             | 0               | 0      |
| H7   | 65     | 84,3529      | 48,6226       | 35,7303         | 0,4895 |
| G8/1 | 40,5   | 0            | 0             | 0               | 0,0000 |
| G8/2 | 46     | 30,1258      | 20,316        | 9,8098          | 0,8175 |
| G8/3 | 55     | 0            | 0             | 0               | 0      |
| G9/1 | 38,7   | 36,58        | 22,5645       | 14,0155         | 1,4016 |
| G9/2 | 52     | 80,6454      | 42,608        | 38,0374         | 1,2679 |
| G9/3 | 43     | 0            | 0             | 0               | 0      |

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



| G9/4  | 53   | 0       | 0       | 0       | 0      |
|-------|------|---------|---------|---------|--------|
| F9/1  | 47,5 | 0       | 0       | 0       | 0      |
| F9/3  | 46,5 | 0       | 0       | 0       | 0      |
| F9/5  | 58,5 | 0       | 0       | 0       | 0      |
| E9    | 47,5 | 28,5544 | 18,8506 | 9,038   | 0,6952 |
| E10/1 | 44   | 20,9607 | 15,199  | 5,7617  | 0,5238 |
| E10/2 | 48   | 21,344  | 18,8515 | 4,925   | 0,2463 |
| E11   | 42,8 | 35,9843 | 17,9833 | 18,001  | 1,8001 |
| E13   | 43   | 34,6711 | 14,795  | 19,8761 | 1,8069 |
| C11/1 | 50   | 46,5041 | 33,3968 | 13,1073 | 0,6554 |
| C11/2 | 49   | 34,8297 | 32,1438 | 2,6859  | 0,1492 |
| C14   | 46,5 | 58,7941 | 37,8945 | 20,8996 | 0,9087 |
| F14/2 | 57   | 72,6315 | 42,8123 | 29,8192 | 0,7455 |
| F14/3 | 51   | 76,751  | 25,3454 | 51,4056 | 2,5703 |
| E15/1 | 39   | 44,5015 | 11,0552 | 33,4463 | 3,3446 |
| E15/2 | 41,5 | 0       | 0       | 0       | 0      |
| B16   | 38   | 24,5138 | 22,062  | 2,4518  | 0,2724 |
| D16   | 41   | 0       | 0       | 0       |        |
| C17/1 | 47   | 23,4445 | 19,5504 | 3,8941  | 0,1947 |
| C17/2 | 53   | 0       | 0       | 0       | 0      |

The box-plots diagrams of the weight of turbot specimens, included into the stomach content analysis, and the stomach fullness index (SFI) are presented at Fig. 16, 17, 18, 19. Currently, the evaluation of SFI values and comparison with the previous studies is a difficult task, due to scarce available information, but this study can support future assessments of the turbot diet dynamics.

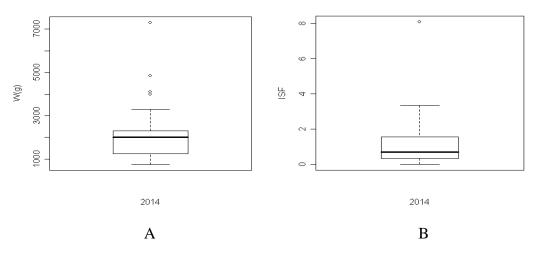


Fig. 16 Box plot of the weight of turbot (included in the analysis of the stomach contents) (A) and the index of fullness of stomachs (ISF) in 2015 (shown are: median, range of values: 25-75% minimum and maximum value) (B).



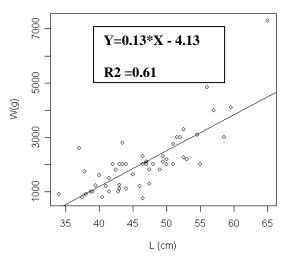


Fig.17 Length - weight relationship of turbot specimens, included into the stomach content analysis in November 2014.

Distribution of the indices of stomach fullness by stations and weight of turbot (included in the stomach contents analysis ) by stations are presented in Fig .18 and 19.

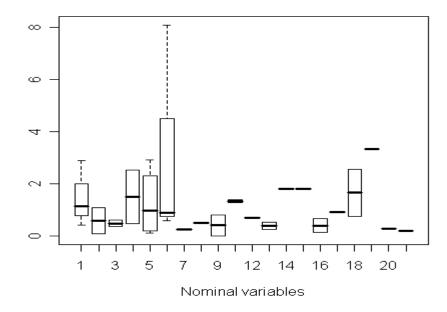


Fig. 18 Distribution of the ISF of turbot by stations (the coordinates of the stations are given in Table 3).

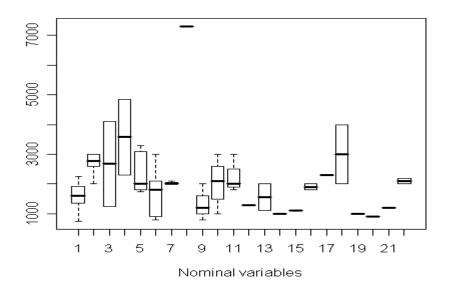


Fig.19 Distribution of the weight of turbot, included in the study for determination of stomach contents, by stations.

The food analysis showed a limited variety of turbot diet during the investigated period. The database has been created and future monitoring would help to clarify the trophic dynamics of turbot.

#### 4.9 Predictions and possibilities for exploitation

Calculation of the MSY (Maximum Sustainable Yield) was realized by the Gulland's formula for virgin stocks.

#### MSY = 0.5\*M\*Bv

The coefficient of natural mortality (M) was calculated using Pauly's empirical formula (1979, 1980). In the calculations was used the average value for M = 0.2, because turbot is a species with relatively long cycle life. In our calculations the value of M was higher because most specimens were young. Taking into account the values obtained for MSY of **98 tons** in the Bulgarian part, but taking into account the precautionary approach too, we believe that TAC for turbot of 50-60 tons is relative to a good quantity.

4.10 Meat biochemical composition of turbot, caught in the autumn of 2014 (November).

#### 1. Chemical composition and energy value of turbot meat

The values of water, protein, fat and mineral contents of meat form studied groups of turbot are presented in Table 9 The water content varied from 78.23 % (M2) to 80.37 % (C17). The differences between groups were lower than 3%. Data showed that the water was the principal component of the chemical composition of studied groups of turbot. The analysis of this parameter is important as it is relevant for the organoleptic properties of meat.



Absolute protein content values varied between 16.87 % (M2) and 19.32 % (C17). The studied groups of fish were distinguished with high relative proportion of proteins in the dry matter: from 85.94 to 89.49%.

The fat content of fish meat from the studied groups varied within a very narrow range (1.29%-2.08%). On the basis of data for the fat content and the classification of Kyosev and Dragoev (2009), turbots caught from the studied places in November could be classified as meager fish (with meat fat content < 2%). The higher water content and low lipid content of turbot meat are indices for deficiency in available nutrients. Taking into consideration that during the study period, turbots begin their migration to more shallow waters in order to get fatter, it is anticipated that the spring values of these parameters would be considerably higher.

The amount of minerals in turbot meat ranges between 0.84 and 1.00 %. Energy values of turbot meat from the studied places varied from  $351.91 \text{ kJ}.100\text{g}^{-1}$  to  $384.68 \text{ kJ}.100\text{g}^{-1}$ .

Table 9

| Place of catch                         | L1     | N1     | M2     | E11-E13 | C 17-E19 |
|--|--------|--------|--------|---------|----------|
| water                                  | 78.56  | 79.26  | 78.30  | 80.03   | 80.37    |
| protein                                | 18.88  | 18.56  | 19.32  | 16.99   | 19.87    |
| fat                                    | 1.69   | 1.29   | 1.52   | 2.08    | 1.76     |
| ash                                    | 0.84   | 0.89   | 0.86   | 0.90    | 1.00     |
| energy (total), kJ.100 g <sup>-1</sup> | 383.49 | 363.25 | 384.68 | 365.79  | 351.91   |

Chemical composition (%) and energy value (kJ.100 g<sup>-1</sup>) of turbot meat caught in November 2014

#### 2. Fatty acids composition and quantity in turbot meat fat.

The fatty acid composition of turbot meat lipids is presented in Table 10.

Table 10

|                | Places of catch |            |            |  |
|----------------|-----------------|------------|------------|--|
| Fatty acids ,% | L1/N1/M2        | E11 – E 13 | C17 – E 19 |  |
| C 12:0         | -               | 0.1        | 0.1        |  |
| C 14:0         | 5.8             | 5.2        | 3.9        |  |
| C 15:0         | 0.6             | 0.6        | 0.5        |  |
| C 16:0         | 24.8            | 30.4       | 31.5       |  |
| C 16:1         | 8.9             | 8.4        | 6.9        |  |
| C 17:0         | 0.1             | 0.2        | 0.2        |  |
| C 18:0         | 2.5             | 2.9        | 3.0        |  |
| C 18:1 (n-9)   | 26.7            | 15.2       | 15.4       |  |
| C 18:1 (n-7)   | 1.8             | 16.7       | 17.8       |  |
| C 18:2 (n-6)   | 1.7             | 0.9        | 0.6        |  |
| C 18:3 (n-6)   | 0.5             | 0.2        | 0.2        |  |
| C 18:3 (n-3)   | 0.4             | -          | -          |  |
| C 20:1         | 3.3             | 4.7        | 4.1        |  |
| C 20:2 (n-6)   | 0.1             | -          | -          |  |
| C 20:5 (n-3)   | 2.4             | 1.9        | 2.0        |  |
| C 22:0         | 4.2             | 2.5        | 1.9        |  |
| C 22:1         | 0.8             | -          | -          |  |

Individual fatty acid composition of turbot caught in November 2014



| C 22:6 (n-3)       | 15.3 | 10.1 | 11.9 |
|--------------------|------|------|------|
| C 24:0             | 0.1  | -    | -    |
| Saturated FA       | 38.1 | 41.9 | 41.1 |
| Unsaturated FA     | 61.9 | 58.1 | 58.9 |
| Monounsaturated FA | 41.5 | 45.0 | 44.2 |
| Polyunsaturated FA | 20.4 | 13.1 | 14.7 |
| <u>Σ</u> ω-6       | 2.3  | 1.1  | 0.8  |
| <u>Σ</u> ω-3       | 18.1 | 12.0 | 13.9 |
| ω-6/ω-3 ratio      | 0.13 | 0.09 | 0.06 |

The fish from the L1/N1/M2 fields exhibited a lower SFA level (by 7.3 %) and higher content of unsaturated fatty acids (by 5.8%) compared to other studied groups. The following distribution of fatty acid groups was found out: MUFA>SFA>PUFA.

In the SFA group, the distribution of the individual fatty acid profile was as followed: palmitic acid (C16:0)> myristic acid (C14:0) > stearic acid (C18:0). In the MUFA group, predominating fatty acids were C18:1  $\pi$ 7 (in two groups of fish), oleic acid (C18:1  $\pi$ 9) and palmitoleic acid (C16:1). A higher content of oleic acid (C18:1  $\pi$ 9) was observed for the group caught in fields L1/N1/M2 compared to the other groups (by 73%). The distribution of individual polyunsaturated FA showed that the eicosapentaenoic acid proportions (C20:5 $\pi$ 3) were similar, from 1.9 to 2.4%. Docosahexaenoic acid (C22:6 $\pi$ 3) was the predominant one, and its content for fish caught from fields E11-E13 and C17- E19 was comparable - 10.1-11.9%, while being by 51% higher for the group of fish from L1/N1/M2 fields. The higher levels of specific mono- and polyunsaturated fatty acids of fish meat from the L1/N1/M2 fields were most probably due to the type and abundance of available feed in the different places of catch.

The amount of omega-6 fatty acids is largely determined by the linoleic (C18:2  $\omega$ -6) and linolenic (C18:3  $\omega$ -6) acid concentrations, whose proportions were within the range 0.2-1.7%.

Data from Table 10 demonstrate that the biologically important omega-6/omega-3 ratios of fish caught in the studied catch locations varied within a broad range – from 0.06 to 0.13. The fish from fields L1/N1/M2 were superior to those from other places with respect to this ratio. The large differences in values (more than twice) were mainly associated to differences in abundance of feed and its specifics in studied localities.

#### 3. Tocopherols composition and quantity in turbot meat fat.

The average tocopherol levels and the structure of main tocopherol classes are summarised in Table 11. It shows that tocopherol concentrations varied within a rather large range between 25 mg.kg-1 and 94 mg.kg-1. In the field E11- E13, the turbot meat fat contained only  $\alpha$ -tocopherols while in fields L1/N1/M2 – only -tocopherols.



#### Table 11

#### Tocopherols composition and quantity

|                                   | C17 – E 19 | E11 – E 13 | L1/N1/M2 |  |
|-----------------------------------|------------|------------|----------|--|
| Tocopherols, mg/kg                | -          | 94.0       | 25       |  |
| Individual tocopherol composition |            |            |          |  |
| α-tocopherol,%                    | -          | 100.0      | -        |  |
| δ-tocopherol, %                   | -          | -          | 100      |  |

#### <u>Remarks</u>

It should be noted, that almost at all sectors, where demersal trawling was conducted, (including the coastal zone) the presence of large amounts of marine litter was observed (drums, boxes and various metal parts, scrap trees, plastic bags, plastic bottles, turbot gill nets of relon /monofilament, etc.).

During the expeditions, the weather conditions were characterized by dominant wind from S and SE, with force - oscillating between the 2-4° Bf at the coast and 5-6° Bf - in the open sea. The waves ranged from 2-3 °Bf in the coastal area and 3-4 °Bf offshore.

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

Based on the results from the survey for turbot stock assessment, carried out in November 2014, the following conclusions and recommendation were made:

▶ The exploited biomass of turbot in the Bulgarian Black Sea area was assessed at **980 tones**.

► The size structure of turbot catches in front of the Bulgarian Black Sea coast included size classes from 28.5 to 65.5 cm. The average turbot weight was estimated at 1.760 kg.

Age composition of catches in Bulgarian area encompassed  $2^+$  to  $6^+$  - years old individuals, predominantly by  $3^+$  (62.6%) and  $4^+$  (20.9%) years.

► The established ratio between female, male and juvenile individuals in the catches during the study in the Bulgarian area was 34.38% : 34.79% : 23.91%.

► The recommended MSY for Bulgaria should not exceed **98 tons**.

The most important food items for turbot are fishes (whiting and mullet) with IRI = 5574,47, followed by crustaceans IRI = 1654,54, however the turbot diet showed limited variety during the investigated autumn season.

► The higher water content and low lipid content of turbot meat are indices for deficiency in available nutrients. Taking into consideration that during the study period, turbots begin their migration to more shallow waters for feeding, it is anticipated that the spring values of these parameters would be considerably higher.

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# II. Biological monitoring of turbot landings at the Bulgarian Black Sea shore in December 2014

# 1. Purpose

The purpose of biological monitoring of turbot catches at the Bulgarian Black Sea shore was to build up a database for analysis of the catches structure over the years. The main tasks of the study included measurements of body weight, absolute and standard body length of turbots from landing operations in order to determine the size structure of catches and to analyse the results.

# 2. Material and methods

### 2.1 Collection of biological data from landing operations

The biological data collection was performed in December 2014 in the northern Bulgarian Black Sea coastal zone.

### 2.2Ports for collection of biological data

From ports where landings of turbot are permitted, the Kavarna, Balchik and Varna ports were used for biological data collection.

### 2.3 Vessels for sample collections

Out of 156 ships, authorised for turbot catch in 2014, 20 vessels were used for biological data from catches as outlined in Table 1.

Table 1.

Ports and vessels used for monitoring and biological data collection during landings of turbot

#### caught in December 2014.

| Catch No. | Port Kavarna     |  |
|-----------|------------------|--|
|           | Ship             |  |
| 1         | KB 5562 Gulliver |  |
| 2         | BH 4601          |  |
| 3         | BH 4321 Gondola  |  |



| 4        | BH 8190 Sveti Nikolay   |  |
|----------|---|--|
| 5        | BH 7180 Sigma   |  |
| 6        | BH 4496 Amber   |  |
| 7        | KB 6275 Delfin  |  |
| 8        | KB 6241 Hera  |  |
| 9        | BH 7041 Akula 1   |  |
| 10       | KB 5642 Puldin  |  |
| 11       | KB 6231 Ivana   |  |
| 12       | KB 6262 Hishtnik  |  |
|          | Port Balchik  |  |
|          | Ship  |  |
| 13       | BH 2998   |  |
| 14       | BH 8042 Elektra   |  |
|          |   |  |
| 15       | BH 440 Reke Irina   |  |
| 15<br>16 |   |  |
|          | BH 440 Reke Irina   |  |
| 16       | BH 440 Reke Irina<br>BH 8112                                  |  |
| 16       | BH 440 Reke Irina   BH 8112   BH 8114                         |  |
| 16       | BH 440 Reke IrinaBH 8112BH 8114Port Varna                     |  |
| 16<br>17 | BH 440 Reke Irina<br>BH 8112<br>BH 8114<br>Port Varna<br>Ship |  |

# 2.4 Number of collected samples

Biological data were collected from 20 catches unloaded from the vessels on the three ports – Kavarna, Balchik and Varna.

### 2.5 Number of measured turbots

The total number of fish used for biological data collection was 600, provided that the minimum required, according to the contract with the Executive Agency for Fisheries and Aquaculture was 150.

### 2.6 Coordinates and depth of the turbot catch place

The coordinates and depth of places of catch for ships that landed on port Kavarna are shown in Table 2. The catches were from the aquatory between latitudes  $43^0$  12' N and  $43^0$  45' N and longitudes  $28^0$  19' E and  $29^0$ 11' E. The depth of places where fish were caught varied from 60 to 67

m. For the other 8 vessels, no data for the places of catch and their depth were available, but they were similar to aforementioned ones.

Table 2.

| N⁰   | Port Kavarna          | Coordinates of catch place | Depth of    |
|------|-----------------------|----------------------------|-------------|
| улов |                       |                            | catch place |
|      | Ship                  |                            |             |
| 1    | KB 5562 Gulliver      | 4335831-02835556           | 60 m        |
| 2    | BH 4601               | 4333583-0283559            | 66 m        |
| 3    | BH 4321 Gondola       | 4340956-02858320           | 62 m        |
| 4    | BH 8190 Sveti Nikolay | 4334817-02854015           | 64-65 m     |
|      |                       | 4335890-02855502           |             |
|      |                       | 4326781-02843261           |             |
| 5    | BH 7180 Sigma         | 4333089-02853146           | 65 m        |
| 6    | BH 4496 Amber         | 4345200-02819300           | 62.5-60 m   |
| 7    | KB 6275 Delfin        | 4338930-02913456           | 73 m        |
| 8    | KB 6241 Hera          | 4319286-02823461           | 60 m        |
| 9    | BH 7041 Akula 1       | 431200-0282400             | 67 m        |
| 10   | KB 5642 Puldin        | 431200-0282500             | 60-65 m     |
| 11   | KB 6231 Ivana         | 4338370-02911020           | 63 m        |
| 12   | KB 6262 Hishtnik      | 4341800-02839070           | 65          |

#### Coordinates and depth of the turbot catch place

### 2.7Data processing

The measurements of fish were made on the board of ship immediately after docking of ships at the port, on fresh ice-cooled subjects.



The weight measurement was done with a precision of 0.1 g, while that of total and standard lengths – with a precision of 0.1 cm.

UPP - Rapas



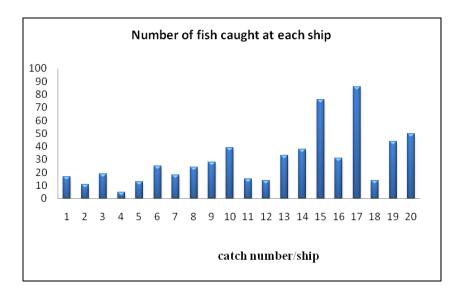


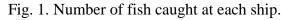
The data processing, graphs and tables were elaborated by means of statistical software.

# 3. Results

## 3.1. Number of fish caught from each ship

The total number of fish, used for biological data collection, was 600. The number of fish caught from each vessel and their percentage distribution is presented on **Fig. 1** and **Fig. 2**. Thirty turbots were caught from each ship on the average, with maximum number of 86 and minimum – 5 fish. Eight vessels or 40% of all 20 have caught between 10 and 20 fish; 4 ships (20%) - between 30 and 40 fish; 2 ships (15%) – between 40 and 50 fish. Catches of <5 turbots, 70-80 and 80-90 turbots were each registered in one vessel.







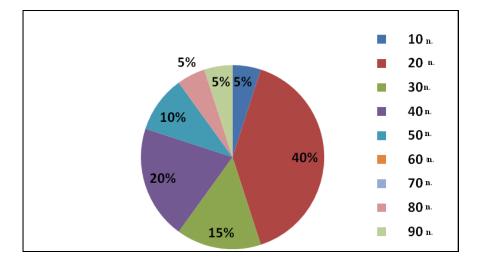


Fig. 2. Percentage distribution of catches from each of ships, %

### 3.2 Weight structure of catches

The average, maximum and minimum weights of the landings of turbots are depicted on Fig. 3. The average weight of measured turbots was 2.63 kg, and the maximum-minimum range: 7.0-1.25 kg.



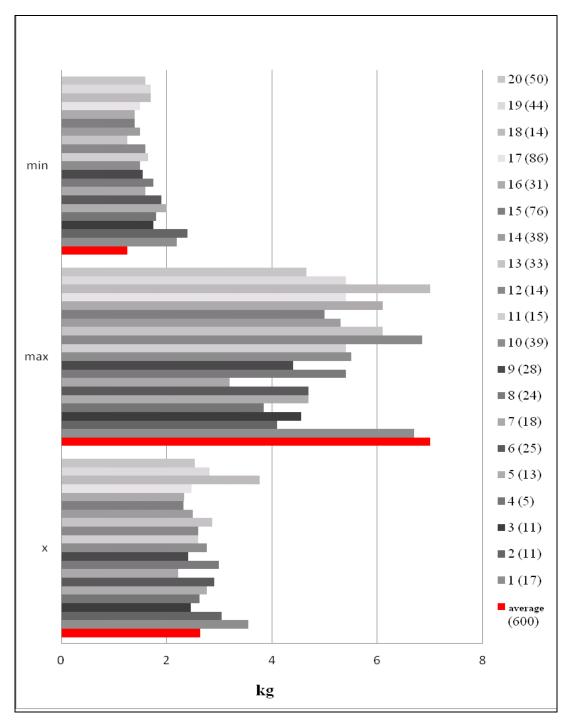


Fig. 3. Average, minimum and maximum weights (W, kg) of turbots from 20 landings. The number of fish from each ship is given in brackets.

Fig. 4 presents the distribution of average, minimum and maximum weights of measured fish. Average weights of turbots from the different catches varied between 3.8 and 2.21 kg; maximum weights – between 7.0 and 3.2 kg, and minimum ones: between 2.4 and 1.25 kg.

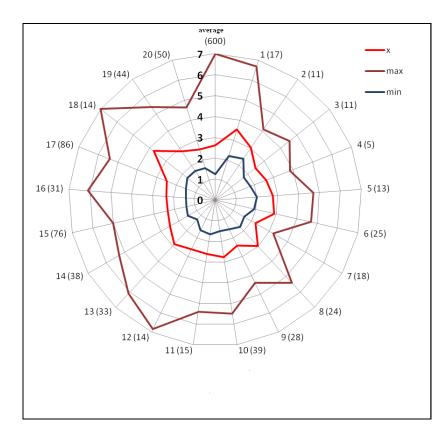


Fig. 4. Distribution of variations in average, minimum and maximum weights (W, kg) of turbots from 20 landings. The number of fish from each ship is given in brackets.

Fig. 5 acquaints with the percentage distribution of the different weight groups for all 600 weighed turbots. The highest share was that of the weight group from 2.0 to 3.0 kg - 44%, followed by the weight group from 1.25 to 2.0 kg - 31%. The share of the weight group between 3.0 and 4.0 kg was 16%, that of the group from 4.0 to 5.0 kg - 6%; that of the group weighing from 5.0 to 6.0 kg - 2% and that of the group from 6.0 to 7.0 kg - only 1%. The graph shows that turbots weighing between 1.25 and 3.0 kg comprised 75% of all weighed fish.

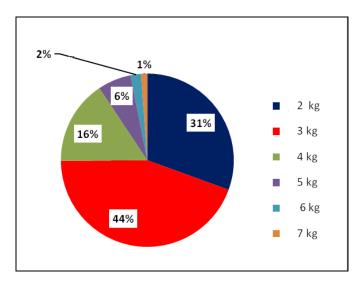


Fig. 5. Proportions of the different weight groups of turbots



When the first weight group (from 1.25 to 2 kg) was divided into two subgroups: from 1.25 to 2.0 kg and from 2.0 to 3.0 kg, it becomes obvious that only 5% of turbots weighed from 1.25 to 1.5 kg, while those weighing between 1.5 and 2.0 kg were 95% (Fig. 6).

The division of the second weight group (2.0 to 3.0 kg) into subgroups of 2.0-2.5 kg and 2.5-3.0 kg demonstrates that the major part of fish (59%) weighed between 2.0 and 2.5 kg (Fig. 7).

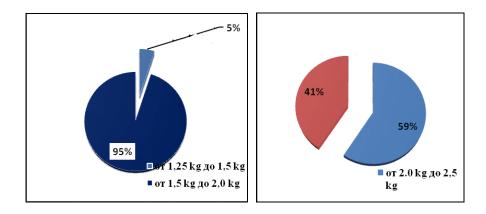
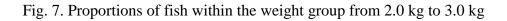


Fig. 6. Proportions of fish within the weight group from 1.25 to 2.0 kg



Summing up the results from the detailisation of the first two weight groups, it could be seen that turbots with body weight from 1.5 to 2.5 kg were more than one half -56 % of the entire group of 600 fish (Fig. 8).

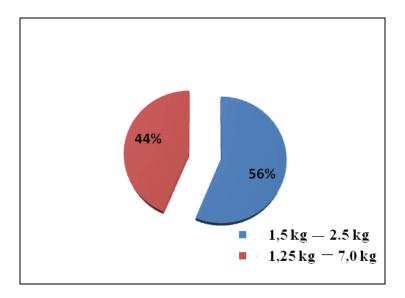


Fig. 8. Proportions of the weight group from 1.5 to 2.5 kg vs the entire sample of 600 fish

MPP - Bapard

#### 3.3 Body size structure (total and standard body length) of turbot catches

The average, minimum and maximum total body lengths (TL, cm) of turbots from the landings are presented on Fig. 9. The average total length of measured fish was 52.8 cm, the maximum was 77.0 cm, while the minimum -45 cm.

Fig. 10 presents the distribution of average, minimum and maximum total body lengths of all measured subjects. The average total lengths of fish varied from 60.6 and 51.1 cm among the catches; the maximum – between 77.0 and 56.0 cm, and the minimum – between 50.5 and 45.0 cm.

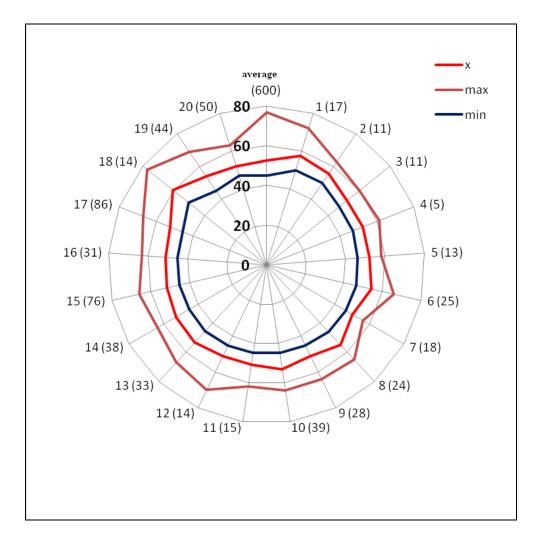


Fig. 10. Distribution of variations in average, minimum and maximum total body lenghts (TL, cm) of turbots from 20 landings. The number of fish from each ship is given in brackets.

The shares of the different size groups (at 3 cm-intervals) showed that three size groups were the most frequently encountered: 45-48 cm; 48-51 cm and 51-54 cm (Fig. 11).

The most numerous were the group of turbots with total body length (TL, cm) within 45-48 cm - 24.6%, followed with an insignificant difference by the 48-51 cm (23.3%), and 51-54 cm (20.1%) groups. Turbots with total body length from 45 to 54 cm comprised 68% of the entire

sample, whereas those with TL 45-51 cm were almost one half from all measured fish (47%). Fish with total body length of 45 cm, which is the minimum allowance for catch, were only 2.8% of all turbots, but one-quarter of all studied fish had TL within the range 45-48 cm.

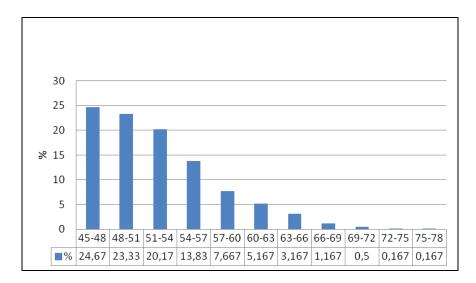


Fig. 11. Proportions of the different body size groups (TL, cm) at 3 cm-intervals of turbots from 20 landings, December 2014.

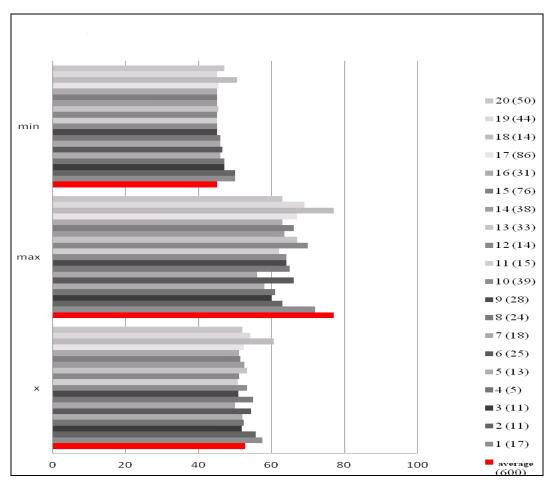


Fig. 9. Average, minimum and maximum total body lenghts (TL, cm) of turbots from 20 unloadings. The number of fish from each ship is given in brackets.



The average, minimum and maximum standard body lengths (SL, cm) of studied turbots are presented on Fig. 12. The average standard length was 43.4 cm, while and maximum and minimum ones: 61.0 cm and 35.5 cm, respectively.

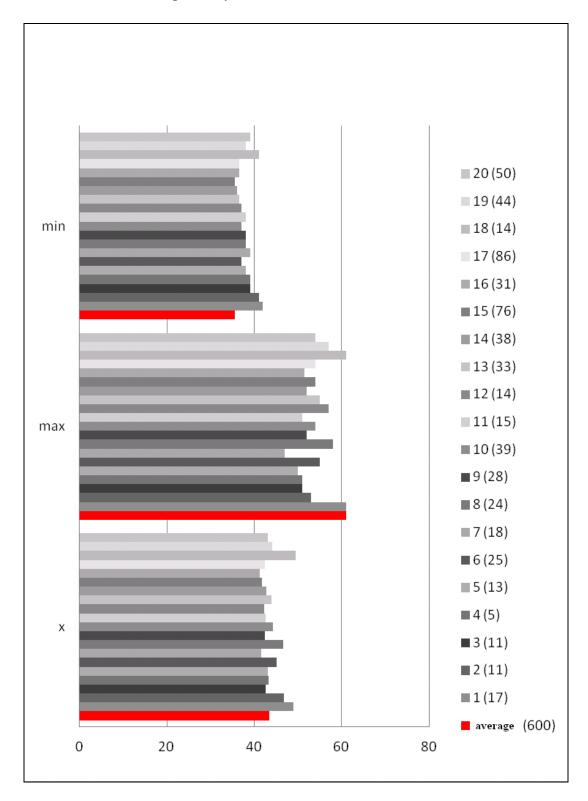


Fig. 12. Average, minimum and maximum standard body lenghts (SL, cm) of turbots from 20 unloadings. The number of fish from each ship is given in brackets.

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Fig. 13 depicts the pattern of distribution of average, minimum and maximum standard body lengths (SL, cm) of turbots among the catches. The average SL of fish from the 20 catches varied between 49.5 and 41.7 cm; the maximum one: from 61.0 and 51.0 cm, whereas the minimum: between 42.0 and 35.5 cm.

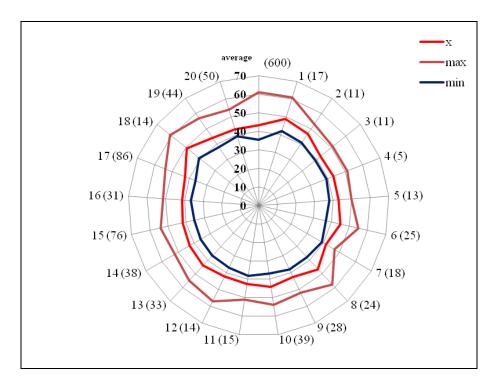


Fig. 13. Distribution of variations in average, minimum and maximum standard body lenghts (SL, cm) of turbots from 20 landings. The number of fish from each ship is given in brackets.

# 4. Conclusions and recommendations

On the basis of results from the biological monitoring of turbot landings at the Bulgarian Black Sea shore in December 2014, the following conclusions and recommendations could be made:

- The weight structure analysis of studied turbots specimens (600 fish, from 20 catches) showed that the average fish weight was 2.63 kg, with maximal value of 7.0 kg, and minimal – 1.25 kg.
- 2. The highest percentage within the weight structure was that of the weight group from 2.0 to 3.0 kg 44%, followed by the weight group from 1.25 to 2.0 kg 31%. The group from 3.0 to 4.0 kg comprised 16% of all fish, the group from 4.0 to 5.0 kg: 6% of the sample; the group from 5.0 до 6.0 kg: 2% of all turbots and the group from 6.0 to 7.0 kg only 1% of fish.
- Turbots weighing 1.25 to 3.0 kg were 75% of all weighed subjects, and those weighing from 1.5 to 2.5 kg more than half of the sample (56%).

- 4. The average total length (TL, cm) of measured fish was 52.8 cm, with maximal and minimal values of 77.0 cm and 45 cm, respectively.
- 5. The most numerous were three of all size groups: 45-48 cm; 48-51 cm and 51-54 cm, corresponding to 24.6%, 23.3% and 20.1% of the sample respectively. Fish with total length within 45-54 cm comprised 68% of all measured subjects, whereas those within 45-51 cm: almost half of the sample 47%. Fish with total length of 45 cm, which is the minimum allowed for catch were only 2.8%, but those with length in the near range of 45-48 cm comprised one quarter of the sample.
- 6. In general, the analysis of data obtained from 600 turbots with average weight of 2.63 kg or total biomass of 1 578 kg allowed assuming that a substantial part of catches consisted of fish with relatively low weight. Future monitoring studies would support or reject this suggestion.
- 7. According to the EC legislation, the mesh size for turbot nets must be at least 400 mm, which, according to professional opinions in the branch should prevent catching specimens weighing less than 2.3-2.5 kg The results from the present study showed that turbots weighing from 1.25 to 2.0 kg were one-third (31%) of the entire sample whereas those weighing from 1.5 to 2.5 kg more than half of the sample (56%). We suggest that the low weight of turbots with body size around the minimum allowed (45 cm) could be due to the poorer body condition of fish during the study period, as also supported by the results from turbot meat biochemical analysis.
- 8. We recommend continuation of the biological monitoring on turbot landings at the Bulgarian Black Sea shore in the future in order to obtain a more complete and more objective image of catch structure over the years, which is a parameter for the population structure of the species.
- 9. Spring catches could be also included in the analysis, as they are considerably bigger than those in the autumn. This would permit to perform a comparative analysis of data from both periods.
- 10. A better representatively of the data would be achieved on the basis of more catches analysis or through prolongation of the monitoring period, especially during the spring months.

We like to express our gratitude to NAFA - Sofia, AA - Sofia and fishing branch sector for their cooperate during activities as per Contract  $N_{\rm D}$  D - 262 / 19.08.2014.

National Bottom Trawl Survey 2014

