Methodology for demersal research in the Bulgarian Black Sea waters

The methodology and techniques used for data collection, verification, processing and analysis for the general assessment of demersal stocks (turbot, whiting) are, generally, follow the methodology applied during previous data collection programs for the Bulgarian Black Sea area.

The target species of the demersal survey was turbot (*Scophthalmus maximus*), and the by-catch species - the spiny dogfish (*Squalus acanthias),* the thornback ray (*Raja clavata*) and the European flounder (*Platichthys flesus*) were also measured and analysed.

The methodology and techniques, used for data collection, verification, processing and analysis and for complete turbot stock assessment were following the generally applied methodology in the Bulgarian Black Sea zone.

The field data were collected by standard techniques - bottom trawl that remained constant through the surveys. The GPS system of the ship was connected to EAFA satellite system for monitoring of the fishing vessels (VMS) and the ship location was strictly controlled during the trawling.

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

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 for stomach content analysis;

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

- Survey area (km^2);
- Catch per unit effort (kg/trawl)
- Catch per unit area (t/km^2);
- Abundance index (individual/km^2);
- Limits of variation of CPUA;
- Total biomass (t.);
- Abundance (ind);

During the studies, a fishing bottom trawl 32/27-34 was applied, with following functional and technical parameters:

- Trawl vertical opening - 2 m;
- Effective part of Headrope - 13 m;
- Effective part of Footrope - 15 m;
- Trawling speed - 2.2 - 2.6 Nd;
- Trawling duration - 60 min.
- Mesh size – 80/80 mm;

2. Sampling scheme

To establish the abundance and biomass of the reference species *S. maximus* off the Bulgarian Black Sea coast, a standard methodology for stratified sampling (Gulland, 1966; Sparre, Venema, 1998; Sabatella, Franquesa, 2004) was applied.

The surveyed region 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 assessment of turbot abundance and biomass, 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)(Fig.1). The sampling was carried out at 40 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),(Fig.2). Each field was marked with letters and digits for better distinction.

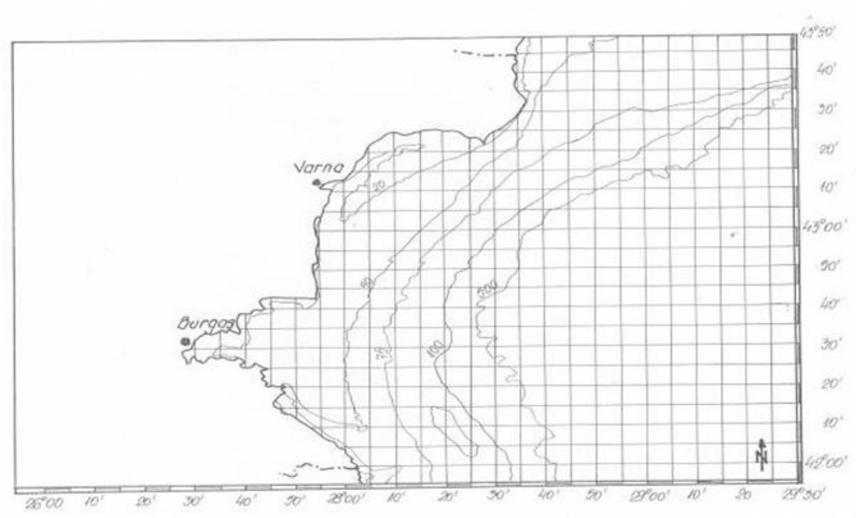

Fig.1. Grid lines used for calculations of the area.

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).

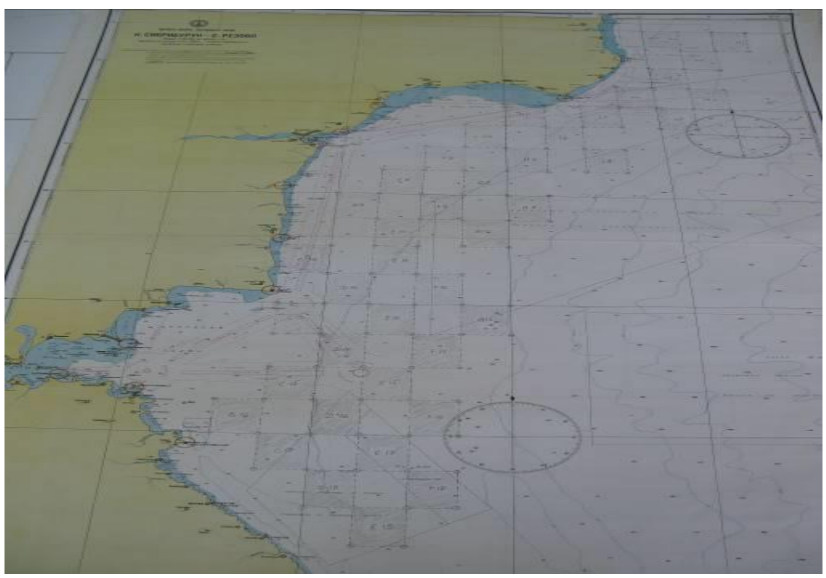

Fig.2. Map and scheme of the fields used in the design of the sampling,

The duration of each hauls was 60 min. at trawling speed of 2.3-2.5 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. Laboratory analyses

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

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

To identify the composition of the food stomachs were collected. The stomach content analyses included: Identification of the taxonomic composition at possible lowest taxonomic level; Nematodes were also found in the stomach contents of turbot, forming a group of parasites (diseases indicator); Total number and weight of food components; F, Frequency of occurrence of each food component; N %, Percentage share of the food item in total number; B %, Percentage share of the food item in total weight; The index of relative importance (IRI) was used to determine the significance of food components in the trophic spectrum (Pinkas et.al., 1971). IRI expressed as a percentage was calculated by the equation (Cortes, 1997).

The index of relative importance (IRI) was used to determine the significance of each food component 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

To ensure accurate measurements, the laboratory equipment (Fig.1) should be kept in good condition, scales should be regularly calibrated and checked (preferably yearly by a qualified technician).

Fig. 1 Laboratory equipment

The laboratory protocols for each sample include a full description of all measurements. All biological data, produced in a laboratory, should be completely documented and should be traceable back to its origin. The necessary documentation should contain a description of sampling equipment and procedures, reference to standard operating procedures (SOP) for sample handling and analytical procedures involved. Data files should be kept on several laptops and to be updated synchronously.

Summarized statistics (Mean values, Standard Error, Median, Mode, Standard Deviation, Sample Variance, Kurtosis, Skewness, Range, Minimum, Maximum, Confidence Level, 95.0%) about the measured biological parameters of the stomach by fields - Total weight (TW - weight, g), body weight, % of BW from TW, should be presented separately.

Table 1

Full description of the collected data and some statistical parameters, General statistical data for the measured parameters in stomach content analysis and diet spectrum of turbot by species and group are given:

	L (cm)	*W (kg)*	*gr*	*ST/full/, gr*	*ST/empty, gr*	*ST/cont,gr*	*ISF*
Mean							
Standard Error							
Median							
Mode							
Standard Deviation							
Kurtosis							
Skewness							
Range							
Minimum							
Maximum							
Sum							
Count							

Species	CN	CB	F	IRI	IRI%

4. Statistical methods

Swept areas method

To determine the relative biomass of the reference species *S. 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 - the time spent trawling, hr - 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.

CPUE (Catch per unit effort) - is calculated by dividing the trawl catch by the fishing hours (kilograms/hour):

CPUE = yield/effort

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.

TAC - total allowable catch, Prediction models

***Beverton and Holt yield per recruit model* (1957):**

$$Y/R = F*\exp[-M*(Tc-Tr)]*W_{\infty} * \left[\frac{1}{Z} - \frac{3S}{Z+K} + \frac{3S^2}{Z+2K} - \frac{S^3}{Z+3K}\right]$$

S = exp [-K (Tc – t_0)], K = von Bertalanffy growth parameter, t0 = von Bertalanffy growth parameter, Tc = age at first capture, Tr = age at recruitment, W = asymptotic body weight, F = fishing mortality, M = natural mortality, Z = F + M, total mortality.

To evaluate the exploitation ratio, the formulae of ***Pauly (1983)*** was used: E = F / Z; E - exploitation ratio, F - fishing mortality, Z - total mortality;

***Jones' Length-Based Cohort Analysis* (1981)**

Jones' length-based cohort analysis:

$$\exp\left(\frac{M}{2} * \Delta t\right) = \exp\left[\frac{M}{2} * \frac{1}{K} * \ln\left(\frac{L\infty - L1}{L\infty - L2}\right)\right] = \exp\left[\ln\left(\frac{L\infty - L1}{L\infty - L2}\right)^{M/2K}\right] = \left[\frac{L\infty - L1}{L\infty - L2}\right]^{M/2K}$$

Age and growth

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

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

L_t, W_t are the length or weight of the fish at age **t** years; L∞, W∞ - asymptotic length or weight; k – curvature parameter; t_o - 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.

Stock exploitation (E)

is determined by Pauly (1983): E = F/Z,
where Z - total mortality, and F - fishing mortality.

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