1. Methodology for demersal research in the Romanian 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 Romanian Black Sea area.

The submitted data were collected by standard techniques (bottom trawl) and methodology, which remained constant in the survey evaluation. The vessel's GPS system was connected to the NAFA satellite system for fishing vessel monitoring system (VMS) and the vessel's location was strictly controlled during trawling activities. The data analysis was performed by the "swept area method" and the results obtained can be reproduced and compared.

1.1 Information collected through bottom trawl activities:

* GPS coordinates from the start and of trawling;
* The depth measured with the vessel acoustic probe;
* The mean trawling speed (Nd);
* Time / trawling duration;
* The total catch;
* The abundance of the target species;
* Total catch weight;
* The absolute and standard length, individual weight;
* The otoliths extraction for age determination;
* The sex determination;
* The composition of by-catch species;
* The discharged catches;
* The stomach content (turbot).

The specimens with absolute length below the minimum, specified in the Ministerial Order no. 432/2008 regarding the minimum individual dimensions of living aquatic resources in the public domain of the state, by species, which can be captured from the aquatic environment (MADR 2008), (below 45 cm), are returned to the sea, after being measured.

All the data collected from demersal research expeditions were entered in the program developed by COISPA, Biondex Script (version 2.0) running in program R version 3.6.0, 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 1.4) 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 NIMRD database, as well as in a special module created within NAFA Romania.

The survey was conducted with the research vessel "*Steaua de Mare 1*" (ROU – P – 040254 CT), belonging to the National Institute for Marine Research and Development "*Grigore Antipa*" Constanța, having the following characteristics:

* Type of fishing vessel: B-410
* Total length: 25,71 m;
* Maximum width: 7,22 m
* Maximum
* The engine power: 420 kw (570 HP)
* Maximum tonnage: 134 t
* Net tonnage: 40 t
* The speed: 7-10 Nd
* The crew number: 7 people
* Researchers number: 6 people
* The year of construction: 1981

During the demersal expeditions, the bottom trawl 22 / 27-34 was used, with a horizontal opening of 13 m, the average speed of the vessel was 1.8-2.1 Nd in the spring season expedition and 1.9-2.2 Nd in the autumn expedition, the trawling time being standardized at 60 minutes.

2. Methodology for pelagic research in the Romanian Black Sea waters

The methodology and techniques used for data collection, verification, processing and analysis for the general assessment of pelagic stocks (*sprat*, *picked dogfish*, *jellyfish*) are, generally, follow the methodology applied during previous data collection programs for the Romanian Black Sea area.

The submitted data were collected by standard techniques (*pelagic trawl*) and methodology, which remained constant by sampling. The vessel's GPS system was connected to the NAFA satellite system for fishing vessel monitoring system (VMS) and the vessel's location was strictly controlled during trawling activities. The data analysis was performed by the "*swept area method*" and the results obtained can be reproduced and compared.

2.1 Information collected through pelagic trawl activities:

* GPS coordinates from the start and of trawling;
* The depth measured with the vessel acoustic probe;
* The mean trawling speed (Nd);
* Time / trawling duration;
* The total catch;
* The abundance of the target species;
* Total catch weight;
* The absolute and standard length, individual weight;
* The otoliths extraction for age determination;
* The sex determination;
* The composition of by-catch species;
* The discharged catches.

During the pelagic expeditions, the pelagic trawl 50 / 357-74 was used, with a horizontal opening of 22 m, the average speed of the vessel was 2.2-2.5 Nd in the spring season expedition and 2.1-2.4 Nd in the autumn expedition, the trawling time being standardized at 30 minutes.

Pelagic expeditions for the assessment of sprat agglomerations provide additional information for the calculation of the catch effort per unit CPUE (kg/hour) and of catch per unit area CPUA (kg/m2) in the researched areas. The collected data are stored in the NIMRD database, as well as in a special module created within NAFA Romania.

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

The results obtained are presented like maps and tables include data on:

* the surface of the researched square (Km^2, m^2);
* average mass per unit area (g/m^2, t/Km^2);
* mass limits variation per unit area;
* total biomass values (t).

The survey was conducted with the research vessel "Steaua de Mare 1" (ROU – P – 040254 CT), belonging to the National Institute for Marine Research and Development "*Grigore Antipa*" Constanța.

3. *The sampling scheme*

In order to establish the abundance and biomass of pelagic and demersal species, at the Romanian Black Sea coast, the standard methodology for stratified sampling was applied (Gulland, 1966; Sparre, Venema, 1998; Sabatella, Franquesa, 2004).

The analyzed area at Romanian Black Sea coast was divided into four layers, depending on the depth: *Stratum* 1 (0-30 m), *Stratum* 2 (30-50 m) and *Stratum* 3 (50-75 m). Also, to assess the abundance and biomass of pelagic and demersal species, the analyzed area was divided into 57 squares, each with sides of 10 x 10 Mm, surface of 100 Nm^2 (or 2000 – 2500 Mm^2).

On board of the vessel, the absolute and standard length, as well as the individual weight, were measured to determine the size and weight structure of the turbot stock and to estimate the share of specimens with a length below that allowed in the fisheries legislation.

4. Laboratory analysis

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

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

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

$IRI = (C_N + C_W) * F$

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

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

$$\% IRI\ i = -\frac{100 * IRI}{\sum_i^n IRI\ i}$$

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

Statistical method

4.1 Swept areas method

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

$$a = D * hr * X2,$$

$$D = V * t$$

V is the velocity of the trawl over the ground when trawling,

t is the time spent trawling,

hr is the length of the head-rope.

X2 is that fraction of the head-rope length, hr, which is equal to the width of the path swept by the trawl, the "wing spread", hr*X2,

D - distance covered.

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

$$\frac{C\,w/t}{a/t} = \frac{Cw}{a}\ kg/Km^2$$

$C_{w\,/\,t}$ – cath in units of weight per trawling hour;

a / t – area swept per trawling hour

The biomass for each stratum was obtained from equation:

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

$C_{w\,/\,a}$ – men cath per unit of area for all trawl sweeps in the stratum,

A – stratum area

The variance of biomass estimated for each stratum is:

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

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:

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

Ca1- catch per unit area in stratum 1;

A1 – stratum 1 area,

A2 – stratum 2 area;

A3 – stratum 3 area; ….. etc.;

A – total water area.

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

$$B = Ca\,(A) * A$$

Ca (A) - mean weighted catch for the entire surveyed water area;

A – total area surveyed

4.2 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:

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

$\overline{B}$ - 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:

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

$\overline{y}$ – total catch in one year,

$\overline{B}$ - mean biomass in the same year.

4.3 Age and growth

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

$$L_t = L_\infty \{1 - e^{[-k(t - t_0)]}\}$$

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

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;

to - the initial condition parameter.

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

$$W_t = q\, L_t^{\,n}$$

q – constant in length-weight relationship;

n – constant in length-weight relationship.

4.4 Natural mortality (M)

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

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

$$\log M = -0.2107 - 0.0824 * \log W_\infty + 0.6757 * \log k + 0.4687 * \log T^oC$$

$$\ln M = -0.0152 - 0.279 * \ln L_\infty + 0.6543 * \ln k + 0.463 * \ln T^0$$

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 - Efanov (1976)

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

tmat – age at first maturation;

Method of Lukashev (1970)

$$M = 1 - \left[\frac{1 - e^{-k(t-to)}}{1 - e^{-k(t-t0)}}\right]^{3}$$

t = age of mature fish