

Material and methods for biological sampling in the Bulgarian Black Sea area

Target species for sampling are Sprat (*Sprattus sprattus*), Turbot (*Scophthalmus maximus*), Anchovy (*Engraulis encrasicolus*), Piked dogfish (*Squalus acanthias*), Horse mackerel (*Trachurus mediterraneus*), from Group 1 species in GFCM-DCRF Annex A.1; Whiting (*Merlangius merlangus*) and Rapa whelk (*Rapana venosa*) from Group 2 species in GFCM-DCRF Annex A.2; Red mullet (*Mullus barbatus*) is chosen because the average landings for the reference period are 617 tonnes. Data collection method will be from landings and by observers on board. Métiers selected through the ranking procedure for the Black Sea were GNS_DEF_360-400_0_0, OTM_MPD_>=13_19_0_0 and FPO_DEF_0_0_0. Random draw from the vessel at the landing port which landed at the day in which the samples will be collected. Samples from sprat, red mullet, whiting, anchovy, piked dogfish, horse mackerel, will be collected from main landing ports directly from the landings of fishing vessels. For aging the otoliths will be extracted. In the case with red mullet scales will be used where is necessary. No aging for piked dogfish will be performed. Means and standard deviation of total length and weight will be estimated. The samples collection for the pelagic species will be carried out in order to cover the biggest landing ports the northern and southern part of the coast. Each sample will consist of a specific number of individuals. Length-weight relationships will be presented. The distribution of targeted species numbers, length and weight characteristics among ports and fishing vessels will be reported. Precision will be evaluated, based on the coefficient of variation, $CV = 0.20$. Stock-related biological variables will be collected from both onboard (during landings) and market sampling. Age: Age compositions are generally estimated from two-stage sampling where random length samples are taken and length-stratified age samples are used to construct an age-length-key.

Length: Length distributions are obtained from random samples. Weight: Individual weights are recorded for all fish that are aged. A length-weight relationship is fitted to estimate weight-at-length and weight-at-age is estimated from this using an age-length-key Sex: Sex-at-age is estimated using a sex-age-length-key Maturity: Maturity-at-age is estimated using a maturity-age-length-key or, if appropriate, a sex-maturity-age-length-key.

Landings of turbot occur from July to March. From April until June there is a catch ban during the reproduction season. The regional turbot landing distribution is about 75% for the northern part of the Black sea coast, and around 25% for the southern part of the coast, which determines that the samples will be collected more intensively in the northern part of the Black sea. The total length and weight data collection will be performed in 3 or 4 of the quarters depending on the ban and cover at least 4 ports. The total length and weight sampling will be based on the spring-summer collection on at least 100 individuals and in the autumn-winter collection on at least 300 individuals. The length and weight structure distribution and the weight-length relationship will be calculated separately for male and female individuals. Market sampling is an essential source of data for age-based finfish stock assessment. The age sampling data will be based on fish purchase twice a year –a total of 100 individuals will be investigated -50 in the first half of the year and 50 in the second half. Age distribution at total length and weight will be estimated for male and female separately as well as sex ratio at age and sex ratio at length. Additionally, other biological parameters such as fecundity at length and fecundity at age and GSI, % will be presented for females (at least 20 individuals) sampled close or during the breeding season (February-May) too.

The data collection method for Rapa whelk (*Rapana venosa*) will be from landings. The samples will be collected from fishing boats, that use beam trawls for Molluscs fishing. The fishing season takes place from late April until the end of September/early October. Random samples of *Rapana venosa* will be collected from the main ports for Rapa landings, according to the requirements for representativeness of the results and aims to characterize the species development during the active season. To establish the rapa whelk size-weight composition, between 20–60 samples (x 100 individuals each) will be collected; additionally, 10 samples x 100 individuals will be analyzed for estimation of the meat/shell ratio. The main sampling ports will include –Varna, Kavarna, and Nessebar (or another one), depending on the landings dynamics throughout the fishing season. Bulgaria will aim to collect samples from the northern and southern regions, in order to assess the differences in rapa whelk population parameters among the main fishing zones. To determine the specific biological parameters, the following metric characteristics will be used: length (L, cm), medium length (Ml, cm), weight (W, gr); The collection of the samples will be carried out every two months during the active fishing season (6 days per every two months' period); the data collection programme will involve minimum three ports with the largest recent landings -encompassing northern and southern coastal regions. The length and weight measurements will include all collected individuals (minimum 2000 individuals), and the ratio between meat/shell will be established for randomly chosen 10 samples (X100 individuals each). The length-weight relationship will be presented. The distribution of rapa whelk numbers, length and weight characteristics among ports and fishing vessels will be reported. Precision will be evaluated, based on the coefficient of variation, $CV = 0.20$. The precision of sampling program will be based on the requirements of following reports: “Sampling Calculation and Methodology for Fisheries Data” (WKSCMFD) (ICES 2004); SGPIDS report (ICES, 2011a), Report of the Study Group on Practical Implementation of Discard Sampling Plans (SGPIDS).

Biological monitoring of turbot landings in front of the Bulgarian Black Sea coast

1. Purpose

The objective of biological monitoring of turbot landings in front of the Bulgarian Black Sea coast is to collect biological data which will be used for analyzing the catches and also to form a database to track the structure of catches over the years. The collection of biological samples from turbot landings during the year except season of ban involves the following main tasks:

1. Data collection for landing ports, sampling vessels, number of samples collected, number of specimens examined, catch geographical data.
2. Determination of the size and weight structure of the landings of turbot.
3. Characteristics of the reproductive biology of turbot.

2. Materials and methods

2.1. Collection of biological data from landings

The biological data collection is performed during the year, except ban season at the Bulgarian Black Sea coastal zone.

2.1.1. Ports for collection of biological data

Biological data collection will be from ports where landings of turbot are permitted.

2.1.2. Vessels for sample collections

Biological data are collected from random vessels with turbot quota rights.

2.1.3. Number of collected samples

Biological data are collected from at least 10 landings.

2.1.4. Number of measured turbot

The total length and weight sampling will be based on the spring-summer collection on at least 100 individuals and in the autumn-winter collection on at least 300 individuals. The age sampling data will be based on fish purchase twice a year –a total of 100 individuals will be investigated

2.1.5. Geographical data of the fish catches

The coordinates and depths of the turbot catch places by vessels landing in the ports.

2.1.6. Determining the size and weight structure of the turbot landings

The measurements of the fish are made on board of the vessel immediately after docking at the port, on fresh ice-cooled fishes. The weight measurement is done with a precision of 0.1 g, while that of total and standard lengths with a precision of 0.1 cm.

2.1.7. Characteristics of the turbot's reproductive biology

50 turbot caught in the spring-summer will be purchased for the gender and age structure determination. On the basis of the collected gonads, the gender, the gonadosomatic index, and the maturity stage of the gonads are determined. From the same specimens, samples of otoliths will also be collected to determine the turbot's age. Correlations between the measured parameters are determined.

• Gender and sex ratio

The gender of 50 turbot caught during the spring-summer season will be determined and the ratio between females and males will be expressed.

• Gonadosomatic index (GSI, %)

The Gonadosomatic Index (GSI,%) will be determined in May, based on female individuals, and males. Will be calculated as a percentage of the body weight for each gender and individual separately, using the data from weight measures of the body and the gonads of female and male fish according to Wootton's formula (1998):

$$\text{GSI (\%)} = 100W_G \cdot W^{-1}, \text{ where:}$$

W_G - weight of the gonads, g

W - body weight, g

• Fecundity of female fish

The fecundity is determined on the basis of females caught in the spring-summer season. Data on the absolute and relative fertility will be presented.

• Determination of the maturity stage of the gonads

The gonads collected from the fish are dissected and fixed in formaldehyde for further processing to determine the degree of maturity of the testis and ovary.

• Determination of fish age

The age of the turbot is determined by the concentric circles (zones) of the otoliths corresponding to the growth periods. Otoliths are taken out through the gills of the fish, without opening the skull, carefully removing each pair without damages. Otoliths are cleaned and stored until observation is done using a binocular stereo microscope with appropriate light.

Biological monitoring of *Rapana Venosa* landings in front of the Bulgarian Black Sea coast

1.1 DATA COLLECTION

Types of data:

1. Data about the fishing vessels' activity: Fish expedition data, Departure port, Arrival port, Fishing vessel name, Vessel type, Vessel length (m).
2. Used fishing gear and Depth scale of the fishing activities
3. Main biological data: Total weight of the target species, landed at a port, Number of collected individuals in the biological sample, Total weight of the sample (Total weight – shell weight (TW, g)), Shell length (Shell length, SL, mm), Shell width (Wd, mm), Aperture shell length (Aperture length, AL, mm).
4. Additional biological data: Total weight without shell (Body weight – shell weight, BW,g); Ratio between genders, gender maturity of collected individuals and gonadosomatic index (when applicable); Ratio between gender maturity and shell length, gender to shell length ration and gender to total weight ratio; Landings of the target species at ports; Biological parameters of rapana – size, weight, linear-weight ratios, gender structure from the samples;

2. MATERIAL AND METHODOLOGY

2.1 SAMPLING SCHEME

The collection of the biological samples (based on 1000 individuals) carried out by taking samples from landings from different ports along the northern and southern territorial waters of Black Sea. The main task is to assess the differences between the population parameters in the main fishing areas.

Fishing by beam trawls and scuba diving technique will be monitored.

2.2 SAMPLE ANALYSIS

Random samples of *R. venosa* will be collected at the landing ports in the northern and southern territorial waters. The main task is to assess the development of the species during the active fishing season.

2.3 LABORATORY ANALYSIS

The size, weight and sex classes of *R.venosa* will be analyzed by using an analysis technique for the weight, size (shell length) and sex structures of the researched individuals from different landings.

- For each individual, the following biometric parameters will be measured – total weight of the individual (total weight - weight with shell, TW, g), body weight of the individual (body weight – weight w/o shell, BW, g), shell length (shell length, SL, mm), shell width (Wd, mm) and aperture length (aperture length, AL, mm);
- The ratio between the different biometric parameters is calculated in relation to each of the parameters;
- The sex maturity is analyzed, as well as the ratio between the sexes, GSI (if applicable);

- The ratio between the sex and the length is calculated and the ratio between the sex to the total weight of the individuals.

2.4 METHODOLOGY FOR ANALYSIS

The morphometric relationships between the biological parameters – total weight (TW), body weight (BW), shell length (SL), shell width (Wd), aperture length (AL) – are analyzed by allometric models. The derived results are processed by using the least squares method and the following equations:

$$\text{Log } W = \text{Log } a + b * \text{Log } L$$

Where, W – weight; L – size; a, b – constants.

Natural logarithm (ln) is used as a second way of calculating the parameters a,b of the L-W ratio:

$$TW(g) = a \cdot SL(mm)^b, \text{ followed by anti-logarithm so } a = \ln q \text{ and } q = \exp^a.$$

XLSTAT software is used to make the linear-weight histograms of the samples from the landed catch, as well as for the data processing. The statistical data about the different classes, presented in the histograms, include – lower and upper limits of the classes, frequency, relative frequency and density.

The accuracy of the program for sample collection is based on the following documents:

- " Report of the Workshop on Sampling and Calculation Methodology for Fisheries Data" (WKSCMFD) (ICES 2004);
- Report SGPIDS (ICES, 2011a);
- Report of the Study Group on Practical Implementation of Discard Samples (SGPIDS).

Biological monitoring of sprat (*Sprattus sprattus*), Horse mackerel (*Trachurus mediterraneus ponticus*), red mullet (*Mullus barbatus*), Anchovy (*Engraulis encarsicolus*) Bluefish (*Pomatomus saltatrix*), Whiting (*Merlangius merlangus*) Picked dogfish (*Squalus acanthias*) landings

I.2 Sampling

I.2.1.1 Geographic area coverage

Data of present analysis were collected directly from landing ports (see Fig.1) main landing sites of Bulgarian active fisheries. The catches and landings were realized in the EEZ of Bulgaria in depths of 40 to 80m. Mean depth of the operating vessels were 45 m.

Picked dogfish samples were originated from Romanian EEZ.

I.2.1.2 Sampling period

All samples originated from active fishery with trawlers and using mid-water trawls (OTM). The fishery using mid water trawls is been carried out whole year round in suitable meteorological conditions. Samples from Picked dogfish were obtained from long line fishery in winter period (December-February, Romanian EEZ).

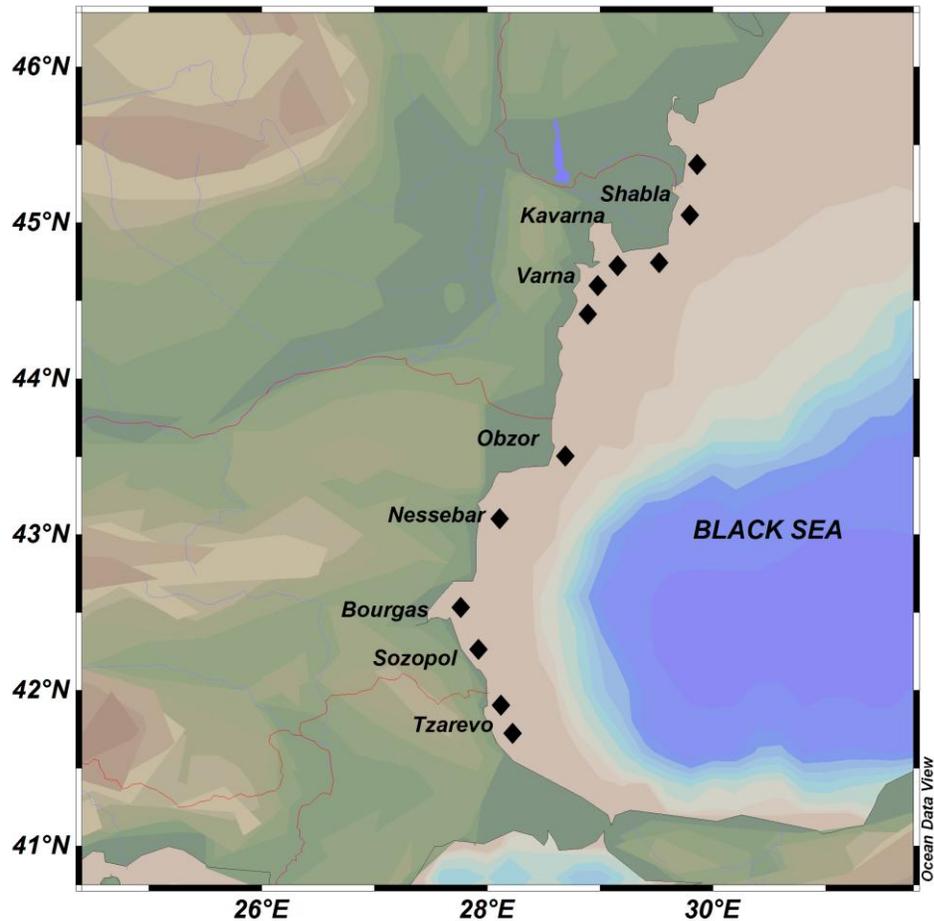


Figure 2.1.1.1 Map of sampling points

I.2.1.3 Statistical analysis of data

All samples tend to be collected in accordance with the variation statistics from significant landings in terms of quantity where is possible. Random sampling theory was followed when taking the sample. The samples were processed in laboratory conditions. Total length (TL, ± 0.5 cm precision) was measured using an ichthyometer, and total fresh weight was measured using an electronic analytical balance (W, ± 1 g precision). The study used otoliths to determine age, which was determined from otolith rings. Otoliths were removed and dried in the laboratory and stored in labeled envelopes. Age was determined by microscope Olympus CX 31RTSF-6 and recorded. Thus, the yearly annulus was detected as hyaline and opaque zones, shifting active growing with period

of growth stagnation. Sections from the other otoliths were judged illegible and were excluded from this study. In order to check the accuracy of the age readings in the present study, an ageing intercalibration exercise was carried out between the authors. Age readings were compared using a signed rank statistical test. We found consistent agreement between readers with low average percentage error (APE) values.

The condition factor was obtained from Fulton's equation (Ricker, 1975): where W is total weight (g) and L is length (cm) cubed, multiplied by 100 to represent values as percentages.

$$K = \frac{W}{L^3} * 100$$

The condition factor 'K' was computed for each age groups separately for different months. For all the samples "Age-Length" (Weight) Keys were created. Thus, the mean values of length, weight and condition factor were resulted. The share (in %) of individuals per age groups and length groups were reflected in the analysis as well.

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

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

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

Estimation procedures

Age: Age compositions are generally estimated from two-stage sampling where random length samples are taken and length-stratified age samples are used to construct an age-length-key.

Length: Length distributions are obtained from random samples.

Weight: Individual weights are recorded for all fish that are aged. A length-weight relationship is fitted to estimate weight-at-length and weight-at-age is estimated from this using an age-length-key

Sex: Sex-at-age is estimated using a sex-age-length-key

Maturity: Maturity-at-age is estimated using a maturity-age-length-key or, if appropriate, a sex-maturity-age-length-key.

The estimation of the biological parameters will be made using analytical methods.

Data quality evaluation

According to the Commission Decision 2010/93/EU, Chapter III, section B2.4, the stock-related variables should be estimated with a precision level 3 - for the stocks that can be aged.

Sample sizes were calculated for selected list of species. CVs for maturity-at-age and size-at-age for all sampled species will be calculated annually at the GSA level.

Description of samplings:

<i>Sprat (Sprattus sprattus)</i>			
Black Sea	Length @age	market	Market: 1090
Black Sea	Weight @length	market	Market: 3682
Black Sea	Weight @age	market	Market: 1090
Black Sea	Sex-ratio @length	market	Market: 3682
Black Sea	Sex-ratio @age	market	Market: 1090

<i>Horse mackerel (Trachurus mediterraneus)</i>			
Black Sea	Length @age	market	Market: 2070
Black Sea	Weight @length	market	Market: 3751
Black Sea	Weight @age	market	Market: 2070
Black Sea	Sex-ratio @length	market	Market: 3751
Black Sea	Sex-ratio @age	market	Market: 2070

<i>Anchovy (Engraulis encrasicolus)</i>			
Black Sea	Length @age	market	Market: 2007
Black Sea	Weight @length	market	Market: 2959
Black Sea	Weight @age	market	Market: 2007
Black Sea	Sex-ratio @length	market	Market: 2959

Black Sea	Sex-ratio @age	market	Market: 2007
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<i>Whiting (Merlangius merlangus)</i>			
Black Sea	Length @age	market	Market: 1630
Black Sea	Weight @length	market	Market: 2824
Black Sea	Weight @age	market	Market: 1630
Black Sea	Sex-ratio @length	market	Market: 2824
Black Sea	Sex-ratio @age	market	Market:1630

<i>Red mullet (Mullus barbatus)</i>			
Black Sea	Length @age	market	Market: 1840
Black Sea	Weight @length	market	Market: 3445
Black Sea	Weight @age	market	Market: 1840
Black Sea	Sex-ratio @length	market	Market: 3445
Black Sea	Sex-ratio @age	market	Market: 1840

<i>picked dogfish (Squalus acanthias)</i>			
Black Sea	Length @	survey	84
Black Sea	Weight @	survey	84
Black Sea	Sex-ratio @length	survey	84