Methodologies for Biological sampling in the Bulgarian Black Sea area

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Version 2022

General description

Target species for sampling are Sprat (Sprattus sprattus), Turbot (Scophthalmus maximus), Anchovy (Engraulis encrasicholus), 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 2018-2020 are 490 tonnes. Data collection method will be from landings and by observers on board. 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 by quarters depending on the ban and cover at least 4 ports. The total length and weight sampling will be based on the studied individuals from landings of turbot in the first and second quarter and will be at least 100 specimens and in the third and fourth at least 300 specimens. The length and weight structure distribution and the weight-length relationship will be calculated separately for male and female individuals. Sampling of landings 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 vessels, 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 three months during the active fishing season (6 days per every months' period); the data collection programme will involve minimum four ports with the largest recent landings - encompassing northern and southern coastal regions. The length and weight measurements will include all collected individuals (minimum 1000 individuals), and the ratio between meet/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).

The study with scientific observers on board the fishing vessels of the Bulgarian fisheries' fleet aims the data collection for the bycatch of marine organisms by fishing activities. The data to be collected include the total catch of the target species, catches of other industrial species, composition, and quantities of the bycatches and discards of marine organisms. The dynamics of the main catches and bycatches quantities by months and seasons will be estimated, as well as bycatch species composition, bycatch rate, size, sex, and age structure.

The impact of commercial fishing will be assessed according to the indicator values of the bycatch rate for the observed fishing activities.

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

1. Aim and objectives

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:

- Collection of data from landings at port, vessels for sample collection, number of samples collected, number of measured turbot specimens, geographical data of turbot catch locations;
- Determination of size and weight structure of turbot landings;
- Characteristics of the reproductive biology of turbot;
- Determining the age of the turbot from the landings;
- Analysis of stomach contents and determination of the food spectrum of turbot.

2. Material and methods

2.1. Sampling procedures for collection of biological data from turbot landings

The method used in the monitoring programme is probability sampling. Probability sampling is based on Simple random sampling because is the most representative and straightforward probability sampling strategy, and each vessel involved in turbot fisheries has an equal and independent chance of being selected.

The collection of biological data from turbot landings is conducted each quarter of each year in the Bulgarian Black Sea coast.

The population is defined at the beginning of each year on the basis of provided quota rights for turbot which are established by the Executive agency for fisheries and aquaculture (EAFA). When the sample frame is identified the sampling unit is defined according to the hierarchical nature of the population. The sampling unit selected is the fishing trip which is representative of all trips carried out by commercial fishing vessels authorized to catch turbot during the period.

In order to ensure representative information and sampling for the entire fleet segmentation the list of vessels authorized to catch turbot is provided to the institute by the EAFA as well as the list of assigned landing points where turbot landings are allowed.

A field diary

The field diary is an official document of the team's activity, containing detailed records (data) of the field sampling carried out in situ (at the ports when the ships are docked). It is filled out consecutively as the data from the landings of the target turbot species is generated. It includes both qualitative and quantitative information, as well as descriptive and analytical information. The data is used in the research activity and in the preparation of the technical and scientific reports. The field register is filled out clearly and legibly by strictly entering the following information:

Data describing the catch

- date and time of sampling;
- port name;
- the name of the ship;
- catch coordinates (latitude and longitude);
- catch depth;
- the type of the used fishing net;

Data for measured turbot

The following measurements are listed in tabular form:

- number of fish (№);
- body weight (W, kg);
- standard length (SL, cm);
- total length (TL, cm);

A good field diary ensures the integrity of the data collected. It is used to create an electronic file containing a sequence of information, which is used for subsequent statistical processing of the data from the performed monitoring. Its consistency ensures the implementation of the set scientific tasks and activities necessary for the development and performance of the scientific research.

Collection, storage and transportation of the samples

The procedure is applied by all members of the research team who according to their responsibilities have the following obligations:

- Manipulation;
- Transportation;
- Labeling;
- Storage.

Turbots are purchased directly at the port after landing of the catch from the fishing vessels and in situ measurements of the total and standard length and the weight of the fish. The fish are placed individually in polyethylene bags for transportation. They are delivered to the laboratory by the team's experts with official transport, thus preserving their integrity during storage and transportation. Fish are taken for sample whole and not cut or divided into parts. The samples are stored during transport from the port to the laboratory in refrigerated bags at a temperature of +4°Cfor the shortest possible time. After receiving the samples (fish) in the laboratory, they are clearly and legibly labeled. The information on the label contains the following data: sample number, date and time of sampling, name of the turbot landing port.

2.1.1. Ports for collection of biological data

From the ports permitted for turbot landings, biological data is collected from Varna, Kavarna, Balchik, Byala, Shabla, Pomorie, Sozopol, Nesebar and Tsarevo ports. Covering the southern and northern parts of the Bulgarian Black sea coast representative data collection is additionally ensured.

2.1.2. Vessels for sample collection

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

2.1.3. Number of collected samples

Biological data are collected from at least 25 landings.

2.1.4. Number of measured turbots

The total length and weight sampling will be based on the studied individuals from landings of turbot in the first and second quarter and will be at least 100 specimens and in the third and fourth at least 300 specimens.

2.1.5. Geographical data of the fish catches

The data for coordinates and depths of the turbot catch places from vessels that landing in the ports are monitored.

2.2. 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.

The correlation between the length (L, cm) and the weight (W, g) is calculated, using LeCren equation (1951):

$$\mathbf{W = a.L^b},\ \text{where:}$$

- W - weight (g);
- L – total lenghth (TL, cm);
- a - constant;
- b - growth coefficient.

All measured individuals are represented in the length classes of 3 cm range.

The length-weight relationship for all species requires a large set of data in order to obtain good representation of the entire “size ranges” of species: from the smaller individuals to the larger ones. This helps to increase the accuracy of estimates.

2.3. Characteristics of the turbot’s reproductive biology

50 turbots caught in the second quarter and 50 turbots caught in the fourth quarter are purchased for the sex and age structure determination. On the basis of the collected gonads, the sex, the gonadosomatic index, and the maturity stage of the gonads are determined. From the same specimens, samples of otoliths will also collected to determine the turbot’s age. Correlations between the measured parameters are determined.

- ***Sex and sex ratio***

The sex is determined of 50 turbots caught in the second quarter and 50 turbots caught in the fourth quarter. Based on that the ratio between females and males is calculated.

- ***Gonadosomatic index (GSI, %)***

The gonadosomic index (GSI,%) will be determined about50 turbots caught in the second quarter and 50 turbots caught in the fourth quarter, based on female individuals, and males. The fishes are dissected laterally to expose the internal organs. The gonads are then removed and weighed. The GSI is calculated as a percentage of the body weight for each sex 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):

$$\mathbf{GSI\ (\%) = 100WG \bullet W^{-1}}, \textbf{ where:}$$

WG -weight of the gonads, g

W - body weight, g

- ***Fecundity of female fish***

The fecundity is determined on the basis of females caught during the breeding season (February-May). Data on the absolute and relative fecundity, fecundity at length, fecundity at age and GSI, % are analysed. The sub sample method is applied to estimate the fecundity. Three different sections, each smaller than 1 g in weight is taken from the anterior, middle and posterior regions of ovary, respectively. Sections are weighed on digital balance Kern AEG (min-0.01 g; max-220 g; e=0.001 g). Ova from these three small sections of ovary are separated and counted. As it was over, the individual number of these sections is added and made a sum total of it. Then the fecundity of the collected specimen is calculated according to (Yelden & Avsar, 2000). Samples for determining the fecundity of turbot, which are taken and examined in the 2nd quarter of each year, are stored in 4% solution of formaldehyde in plastic containers for 4 years. Each sample is numbered and labeled. The eggs count is performed under a binocular stereo microscope (Ceti model).

- ***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. Maturity samples including ovaries and testes of 50 studied turbots in the 2nd quarter and 50 turbots in the 4th quarter are stored in 4% solution of formaldehyde in plastic containers for 4 years. All samples are numbered and labeled in the order in which the fish are dissected. A trinocular microscope model MAGNUM T (fine focusing knob minimum division 0.002 mm) is used to determine the degree of maturity.

2.4. Determination of fish age

50 turbots caught in the second quarter and 50 turbots caught in the fourth quarter are purchased for determination of fish age.

The age of the turbot is determined by the concentric circles (dark and light zones) of the otoliths corresponding to the growth periods. Each pair of these zones represents yearly increment (1-1+ year old). The otoliths (statolits) are situated in the middle ear of fish and they are balance organ. 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.

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

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

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

Lt, Wt 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:

$$\mathbf{Wt = q\, L_t^n}$$

q – constant in length-weight relationship;

n – constant in length-weight relationship.

2.5. Stomach content and food composition of turbot

In order to ensure the availability of data on stomach content_from commercial fishery, the sampling will be carried out during second and fourth quarter where full stomachs will be analysed. For this aim 50 turbots caught in the second quarter and 50 turbots caught in the fourth quarter are used for the analysis of stomach content. In regards to the sampling in last years, the work shows that 15-20 specimens are with full stomachs from the purchased 50. After doing the biometric measurements, each of the specimens is processed as follows: measurement of individual body length and weight, removal of the stomach (from the esophagus to the pyloric valve), weighing of full and empty stomach on digital balance Kern (min-0.5 g; max-600 g; e-0.1 g) to determine the biomass of gastric contents; identification of food components found in the stomach contents and weighing each taxonomic group on the same digital balance. The samples of the stomachs immediately after their removal are inserted in plastic containers in

4% solution of formaldehyde for further analysis. All samples are labeled and numbered in the order in which the fish are dissected. The study of the food array is performed by analysing the content and determining the type of food contained in the stomach, followed by determining the prey species. For more accurate identification, certain food components are placed in petri dishes and observed under binocular stereomicroscope (Ceti model). After the stomach contents analysis, the samples are again stored in plastic containers in 4% solution of formaldehyde following the same numbering for period of 4 years.

The index of stomach fullness, ISF (Hureau, 1969), expressed as a percentage (%), is used to analyze turbot stomach contents. This indicator measures the ratio of food weight to body weight. ISF (%) is calculated by the following formula:

$$\mathbf{ISF = (FW/W) * 100}, \text{ where}$$

ISF – index of stomach fullness;

FW – food weight;

W – body weight of the fish.

For each component of the stomach content, the percentage share in the total number (C_N), the percentage share in the total biomass (C_W) and the frequency of occurrence (F) are determined. The index of relative significance, IRI (Pinkas et al., 1971) is established for all species that are part of the food spectrum of turbot. IRI is calculated by the following formula:

$$\mathbf{IRI = (C_N + C_W) * F}, \text{ where}$$

IRI – index of relative significance;

C_N – percentage share in the total number;

C_W – percentage share in the total biomass;

F – frequency of occurrence.

IRI expressed as a percentage, is used to determine the significance of the food components (Cortes, 1997):

$$\mathbf{\%IRIi = 100 * IRIi / ni\sum IRIi}, \text{ where:}$$

IRIi – index of relative significance of each food component;

n – total number of taxonomic categories included in the food spectrum

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

1.INTRODUCTION

The benthic snail Rapana venosa was introduced into the Black Sea in the 1940s and within a few decades spread over the entire basin and in the Azov Sea (Global Invasive Species Database). R. venosa is a predator of epifaunal bivalves, and its proliferation appears a limiting factor of oysters and mussels' populations (CEISM 2000). The species is considered as one of the most unwelcome invaders worldwide and it is blamed for the decline of the native bivalve fauna in the Black Sea (ZOLOTAREV 1996, in HARDING 2003).

The industrial exploitation of Rapa whelk in the Black Sea has started first in Turkey during the 1980s, and currently Turkish catch varies between 6 and 8 thousand tons annually. Recently, all Black Sea countries target Rapana, with a total annual catch of 10-15 thousand tons. In Bulgaria, this fishery has commenced in the 1990s and Rapa whelk was originally caught by scuba divers (DASKALOV and RÄTZ, 2010), with catches around 90% of the total shellfish catch (FAO, 2000). Since 2012, the Rapana catch with beam trawl has been allowed in Bulgaria, regulated by changes in the Fishery and Aquacultures Act.

The data collection method for Rapa whelk (Rapana venosa) will be from landings. The samples will be collected from fishing vessels, 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 three months during the active fishing season (6 days per every months' period); the data collection programme will involve minimum four ports with the largest recent landings - encompassing northern and southern coastal regions. The length and weight measurements will include all collected individuals (minimum 1000 individuals), and the ratio between meet/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).

Based on a large catch of this species in the Bulgarian waters, scientific information on the Rapana landings at ports is needed, and the data collection has been performed on a quarterly basis throughout each year since 2018. The minimal amount, collected per year should include 1200 individuals, gathered from four ports – from the northern and southern regions of the Bulgarian Black Sea coast.

1.1. COLLECTED DATA

The study should allow the collection of several types of data:

1. Data about the fishing vessels' activity

• Fish expedition data

• Departure port

• Arrival port

• Fishing vessel name

• Vessel length (m)

2. Fishing gear

• Depth scale of the fishing activities

3. Basic biological data

• Total weight of the target species, landed at a port

• Number of collected individuals in the biological sample

• Total weight of the individuals (Total weight, weight with shell (TW, g))

• Shell length of the individuals (Shell length, SL, mm),

• Shell width of the individuals (Wd, mm)

• Aperture shell length of the individuals (Aperture length, AL, mm).

4. Additional biological data

• Ratio between sexes, sex maturity of collected individuals and gonadosomatic index (when applicable);

• Size and weight structure by sex, sex ratio to shell length and sex ratio to total weight;

The final results should be included in reports with tables and figures, including a full explanation of the collected data about:

• Landings of the target species at ports

• Biological parameters of Rapana venosa – lenght, weight, length-weight relationships, sex structure from the samples of the observed ports.

2. MATERIAL AND METHODOLOGY

2.1. SAMPLING SCHEME

Full explanation of the observation scheme should be presented (by the models given below).

Table

Vessels and ports, where biological samples were taken from Rapana venosa landings on quarterly basis

Date	Fishing vessel	Reg. No of fishing vessel	Technical specifications	Departure port	Arrival port	Fishing method

Table

Summarized data about the landings by days and ports by different types of vessels and different fishing methods on quarterly basis

Date	Landing port	Total daily landing of *R. venosa* at the port (kg/day)	Name of the fishing vessel	Landed quantity from the studied fishing vessel (kg)	Weight of the sample of 100 individuals *R. venosa* (kg)	Fishing technique

2.2. SAMPLE ANALYSIS

Random samples of R. venosa should be taken from the landings by ports with the purpose to monitor the dynamics and species characteristics during the active fishing season.

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

• "Report of the Workshop on Sampling and Calculation Methodology for Fisheries Data" (WKSCMFD) (ICES 2004): https://www.ices.dk/sites/pub/CM%20Doccuments/2004/ACFM/ACFM1204.pdf

• Report SGPIDS (ICES, 2011a): https://www.ices.dk/community/Documents/PGCCDBS/SGPIDS%202011.pdf

• Report of the Study Group on Practical Implementation of Discard Samples (SGPIDS) 2013: https://www.ices.dk/sites/pub/Publication%20Reports/Expert%20Group%20Report/acom/2013/SGPIDS/SGPIDS13.1.pdf

2.3. LABORATORY ANALYSIS

- For each individual, the following biometric parameters were measured – total weight of the individual (total weight, weight with shell, TW, g), body weight (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 was calculated;

- The sexual maturity of the collected individuals and the relationship between the sexes were determined, as well as the gonadosomatic index (if applicable);

- The length - weight structure by sex, the ratio of the sexes to the shell length and to the weight of the specimens were determined.

- Estimation of sex of Rapana venosa is based on the following paper: Bondarev, 2015, Sexual differentiation and variations in sexual characteristics of Rapana venosa (Valenciennes, 1846), International Journal of Marine Science, Vol.5, No.19 1-10 (doi: 10.5376/ijms.2015.05.0019; https://www.researchgate.net/publication/277553129_Sexual_differentiation_and_variations_sexual_characteristics_Rapana_venosa_Valenciennes_1846Sex

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.

2.4. ANALYTICAL METHODS

The morphometric relationships between the biological parameters - total weight (TW), shell length (SL), shell width (Wd), aperture length (AL) were analyzed on the basis of classical allometric models. The least squares method was used to estimate the linear - weight relationships (LWR), based on the following equation:

$W = a \times Lb$, where, W – weight; L – length; a, b – constants.

The XLSTAT software product was used to display the linear-weight histograms of the samples from the Rapana landings. The statistical data about the different length and weight classes, presented in the histograms, include lower and upper limits, frequency, relative frequency, and density.

2.5 DQA AND DQC

IFR-Varna apply internal rules for Data Quality Control (DQC) and Data Quality Assurance (DQA) for laboratory studies, including all steps of marine data collection and analysis - from sea expedition to final reporting. For example, all measurements should be included in protocols and checked by two different persons for mistakes (scientist and project leader). If any corrections are needed, they are presented in separate protocols, controlled by two scientists. http://dcf-bulgaria.bg/wp-content/uploads/2021/10/Guidelines_DQA_DQC_Bulgaria_FINAL_2021_Oct.pdf

2.6. REPORTING

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 Rapana by ports - Total weight (TW - weight with shell, TW, g), body weight (BW, g), % of BW from TW, shell length (shell length, SL, mm), shell width (Wd, mm) and aperture length (AL, mm) should be presented separately.

Table

Summarized statistics about the measured biological parameters - total weight (TW - weight with shell, TW, g), body weight (BW, g), % of BW from TW, shell length (shell length, SL, mm), shell width (Wd, mm) and aperture length (AL, mm) for the sample from the port (name), date.

	TW, g	BW, g	% BW from TW	SL, mm	Wd, mm	AL, mm
Mean						
Standard Error						
Median						
Mode						
Standard Deviation						
Sample Variance						
Kurtosis						
Skewness						
Range						
Minimum						
Maximum						
Sum						
Count						
Confidence Level (95.0%)						

Sex structure data:

The sex ratio and statistics about - total weight of the individuals, shell length (SL, mm) and total weight (TW, g), shell width (Wd, mm), and aperture length (aperture length, AL, mm) by sex should be given by the models below:

Table

Summarized statistics of the biological parameters - total weight of the individuals, shell length (SL, mm) and total weight (TW, g) by sex in the sample from port (name), Date

	SL, mm		TW, g	
	Females	Males	Females	Males
Mean				
Standard Error				
Median				
Mode				
Standard Deviation				
Sample Variance				
Kurtosis				
Skewness				
Range				
Minimum				
Maximum				
Sum				
Count				
Confidence Level (95.0%)				

Table

Summarized statistic of the biological parameters - shell width (Wd, mm) and aperture length (aperture length, AL, mm) by sex in the sample from port (Name), (date)

	Wd, mm		Al, mm	
	Females	Males	Females	Males
Mean				
Standard Error				
Median				
Mode				
Standard Deviation				
Sample Variance				
Kurtosis				
Skewness				
Range				
Minimum				
Maximum				
Sum				
Count				
Confidence Level (95.0%)				

The summarized data about the length, weight and sex structure should be included in the final analysis.

3.REFERENCES

1. ICES, 2004/ACFM:12: Advisory Committee on Fishery Management Report of the Workshop on Sampling and Calculation Methodology for Fisheries Data (WKSCMFD), 26–30 January 2004 Nantes, France, 242 pp, https://www.ices.dk/sites/pub/CM%20Doccuments/2004/ACFM/ACFM1204.pdf

2. ICES, 2011. Report of the Study Group on Practical Implementation of Discard Sampling Plans (SGPIDS), 27 June - 1 July 2011, ICES Headquarters, Denmark. ICES CM 2011/ACOM: 50. 116 pp

https://www.ices.dk/community/Documents/PGCCDBS/SGPIDS%202011.pdf

3. ICES. 2013. Report of the Study Group on Practical Implementation of Discard Sampling Plans (SGPIDS), 24 June – 28 June 2013, Lysekil, Sweden. ICES CM 2013/ACOM:56. 142pp

https://www.ices.dk/sites/pub/Publication%20Reports/Expert%20Group%20Report/acom/2013/SGPIDS/SGPIDS13.1.pdf

4. Bondarev, 2015, Sexual differentiation and variations in sexual characteristics of Rapana venosa (Valenciennes, 1846), International Journal of Marine Science, Vol.5, No.19 1-10 (doi: 10.5376/ijms.2015.05.0019; https://www.researchgate.net/publication/277553129_Sexual_differentiation_and_variations_sexual_characteristics_Rapana_venosa_Valenciennes_1846Sex

Methodology for Biological monitoring of sprat (Sprattus sprattus), Horse macherel (Trachurus mediterraneus ponticus), red mullet (Mullus barbatus), Anchovy (Engraulis encarsicolus), Whiting (Merlangius merlangus), and Picked dogfish (Squalus acanthias) landings

The purpose of this study is to collect and analyze the dynamics of length, weight, sex, maturity and fecundity and age distribution, as well as to determine the condition of the observed species using the so-called condition factor (Ricker, 1975). Biological information on a species is collected every quarter and thus analyzed and compared with previous periods and can then be used to assess growth parameters. These indicators are very important for species with a short life cycle. Long-term information is also crucial for the assessment of fish stocks, fisheries management, and thedecision-making process in general.

1 Sampling

1.1 Geographic area coverage

Data of analysis are collected directly from landing ports (Fig.1) main landing sites of Bulgarian active fisheries.

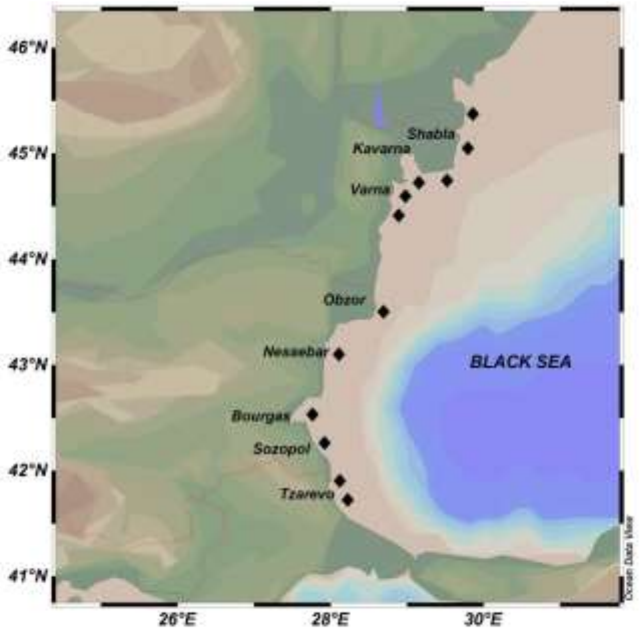

Figure 1 Map of sampling points

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 are obtained from long line fishery.

1.3 Laboratory description and laboratory analyses

Description of the biological laboratories at IO-BAS, Varna and photos of the laboratories are available at following link: http://io-bas.bg/en/biological-laboratories/.

Analytical scales for measuring hard structures and individual body weight:

- Rapid and efficient operation thanks to the graphic display. Simple plain text user guidance in the display, following languages available: DE, EN, FR, IT, ES, PT
- KERN ALJ-160-4NM: Ioniser to neutralise electrostatic charge for fixed installation in the analytical balance. This it makes for convenient handling as you no longer need a separate device.

Simply enable the ioniser fan at the push of a button. Suitable for all models in this range. See accessories

- Adjusting program CAL for quick setting of the balance accuracy, external test weights at an additional price
- Short stabilisation time: Steady weight values within approx. 4 sec under laboratory conditions (on all models with readout [d] = 0,1 mg), 10 | 6 s (on all models with readout [d] = 0,01 mg)
- Weighing with tolerance range (checkweighing): Input of an upper/lower limit value. A visual and audible signal assists with portion division, dispensing or grading
- Dosage aid: High stability mode and other filter settings can be selected
- Internal memory for complete recipes with name and target value of the recipe ingredients. User guidance through display
- Large glass draught shield with 3 sliding doors for easy access to the items being weighed
- Compact size, practical for small spaces
- Protective working cover included with delivery

6inch 150mm Electronic Digital Caliper Ruler Carbon Fiber Vernier:

Made of strong carbon fiber composites and aluminum, lightweight and durable. Four way measurement: inside diameter, outside diameter, depth, and step to zero setting at any position. Can be used for measuring bearings, fasteners, mechanical parts, tire tread depth and more.

Features:

Made of strong carbon fiber composites and aluminum
Two way measurement, internal and external
Linear capacitive measuring system
Can be reset to zero setting at any position
With easy to read large LCD display
Minimum scale to read is 1.0mm/0.01inch
An ideal tool for a broad range of industrial and automotive applications

Specifications:

Measuring range: 0-150mm or 0-6inch
Measuring decimals: 0.1mm or 0.01inches
Repeat measurement offset: 0.1mm or 0.01inch
Maximum measuring speed: 1.5m/Sec or 60inch/Sec
Display type: LCD screen
Powered by: LR44, AG13, SR44 1.5V battery (included)
Total size: 9.4x3x0.6in or 245x77x15mm
LCD display size: 1.6x0.5in 40x14mm
Weight: 1.75oz or 50g

CX31 Upright Microscope+ digital camera USB3.0

Technical specifications:

Observation Method	Brightfield		✓
	Darkfield		✓
Focus	Focusing Mechanism	Stage Focus	✓
	Coarse Handle Stroke		• 25 mm
	Coarse Handle Stroke per Rotation		• 36.8 mm
	Features		•Stage height movement by roller guide (rack & pinion) •Upper limit stopper •Tension adjustment on coarse focus adjustment knob
Stage	Manual	Manual Stages with Right-Hand Control	• Built-in • X: 76 mm, Y: 50 mm
Condenser	Manual	Abbe Conenser	NA 1.25/ W.D. - (4X–100X) (Built-in)
Observation Tubes	Widefield (FN 22)	Binocular	✓
		Tilting Binocular	✓
	Tube Inclination Angle		• 30°
	Interpupillary Distance Adjustment		• 48–75 mm
Dimensions (W × D × H)			233 (W) x 367.5 (D) x 411 (H) mm
Weight			8 kg (Standard Configuration)

- Kern CH 50K100 Hanging Scales 50kg

- Tweezers;
- Measuring rulers;
- Filter paper;
- Laboratory glassware.

The samples collected onboard are processed in the laboratory for determination of age and food composition. The age is established in otoliths under binocular microscope. The food spectrum was determined by separation of the stomach contents into taxonomic groups identified to the lowest possible level.

Preservation of fish samples:

1. Cooling is one of the method used to preserve fish samples;
2. Freezing samples - on board and subsequently placed in freezer at institute laboratories.

Freezing and cooling led to different effects on morphological characters. In the case of freezing, a degradation in colour from goldish-brown to grey-blackish is visible in every case, while the body shape is unaffected overall, except for the belly being less elevated, soft and pliable after defrosting.

Otolith preparation and analysis

Sagittal otoliths are removed, as were the large pieces of remaining tissue, using tweezers, before being placed in water filled eppendorfs to soak overnight. If tissue still remained after this, otoliths were either left to soak in eppendorfs filled with a 1% solution of potassium hydroxide overnight or a 3% solution of potassium hydroxide for 5 h before being washed in water. Otoliths were then dried overnight before being photographed using the Olympus Trinocular Stereomicrscope at 6.3× magnification with an attached Olympus DP25 camera (Japan) equipped with the imaging system cell^a. An image is taken of the interior and exterior of both the left and right otoliths. Using the same imaging software, measurements (μm) on the exterior side are taken of otolith length – the longest distance between the most anterior and posterior points - (OL) and otolith width – the longest distance between the ventral and dorsal edges - (OW), with the measurements for OL and OW perpendicular to each other. Otoliths then can be weighed to the nearest 0.001 g – otolith mass – (OM)

1.4 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 ichtyometer, and total fresh weight was measured using an electronic analytical balance (W, ±1g 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.

Determination of individual growth parameters is of crucial importance, especially for key species, not only for the proper analysis of length, weight and age structure but also for the proper stock management – definition of minimal length presented in catches and recommendations for the selectivity of the fishing gears and etc.

The mathematical expression of von Bertalanffy growth model gives the length (L) as a function of age t: $L(t) = L_\infty[1 - exp(-K(t - t_0))]$, where: t is the age of individual, L_∞ - is the asymptotic length, to which a given biological species increase in length during their lifespan, K – curvature parameter, which reflects the speed of approaching the asymptotic length, t_0 – initial condition parameter (determines the moment, when the length of the studied specie is equal to 0).

LWR model is widely applied in analysis of the of marine living resources as it represents the condition of the stocks. LWR analysis results can be implemented to provide weight estimates on the base of length measurements and vice versa; as well as comparison of growth parameters of one and the same species spread in different geographical areas.

The model assumes that with the increase of the total length the species also increase in weight, which determines the following functional relationship between the two parameters W and L, which is nonlinear:

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.

Batch fecundity can vary considerably during the short spawning season, low at the beginning, peaking during high spawning season and declining again towards the end.Annual egg production is the product of the number of batches spawned per year and the average number of eggs spawned per batch. Batch fee undi t Y. 0f sprat was'. determined as'Hydrated Oocyte Method'. (HUNTER et ale 1985). Only hydrated females are used. After sampling their body cavity is opened and they are preserved in a buffered formalin solution (HUNTER 1985). The ovary free female weight ind the ovary weight are determined. Three tissue samples of - 50 mg are removed from different parts of the ovary and their exact are determined. Under binocuklar number of hydrated oocytes, in each of the three subsamples is determined. Hydrated oocytes can easily be separated from all other types of oocytes because of their large size their translucent appearance and their wrinkled surface which is due to formalin preservation. Batch fecundity is estimated based, on the average number of hydrated oocytes per unit weight of the three subsamples. Gonadosomatic Index (GSI) is also determined. GSI is calculated as: $GSI = \frac{GW}{SW} X\ 100$, where, GW is gonads weight and SW is somatic weight (represents the BW without GW).

Dedicated software

Fisheries assessment software (Length Frequency Distribution Analysis [LFDA], Catch Effort Data Analysis [CEDA], Yield, and ParFish) developed by MRAG Ltd. are used. MATLAB and RStudio are used as well.

1.5 Estimation procedures

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

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.

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.

1.6 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.

1.7 Description of sampling:

Sprat (*Sprattus sprattus*)			
Area	Biological variable	Origin	Number of individuals
Black Sea	Length	Landings	1250
Black Sea	Age	Landings	1250
Black Sea	Weight	Landings	1250
Black Sea	Sex	Landings	250
Black Sea	Maturity	Landings	500
Black Sea	Fecundity	Landings	500

Horse mackerel (Trachurus mediterraneus)			
Area	Biological variable	Origin	Number of individuals
Black Sea	Length	Landings	1500
Black Sea	Age	Landings	500
Black Sea	Weight	Landings	1500
Black Sea	Sex	Landings	250
Black Sea	Maturity	Landings	250
Black Sea	Fecundity	Landings	100

Anchovy (Engraulis encrasicolus)			
Area	Biological variable	Origin	Number of individuals
Black Sea	Length	Landings	500
Black Sea	Age	Landings	250
Black Sea	Weight	Landings	500
Black Sea	Sex	Landings	250
Black Sea	Maturity	Landings	250
Black Sea	Fecundity	Landings	100

Whiting (Merlangius merlangus)			
Area	Biological variable	Origin	Number of individuals
Black Sea	Length	Landings	250
Black Sea	Age	Landings	250
Black Sea	Weight	Landings	250
Black Sea	Sex	Landings	100
Black Sea	Maturity	Landings	100
Black Sea	Fecundity	Landings	100

Red mullet (Mullus barbatus)			
Area	Biological variable	Origin	Number of individuals
Black Sea	Length	Landings	500
Black Sea	Age	Landings	500
Black Sea	Weight	Landings	500
Black Sea	Sex	Landings	250
Black Sea	Maturity	Landings	250
Black Sea	Fecundity	Landings	100

Picked dogfish (Squalus acanthias)			
Area	Biological variable	Origin	Number of individuals
Black Sea	Length	Landings	100
Black Sea	Weight	Landings	100
Black Sea	Sex	Landings	30
Black Sea	Maturity	Landings	30

Methodology for Monitoring of fisheries and for biological sampling by scientific observers on board

The study with scientific observers onboards the fishing vessels of the Bulgarian fisheries' fleet aims the data collection for the total catch of the target species, catches of other commercial species, composition, and quantities of the bycatches and discards of marine organisms by following types of fishing activities:

(1) turbot fishing with gillnets;
(2) pelagic and bottom species fishing with pelagic trawl;
(3) Rapana fishing with beam trawl;
(4) pelagic and bottom species fishing with polyvalent active and passive gears.

The impact of commercial fishing on marine organisms will be assessed according to the bycatch rate, estimated for the all observed fishing activities.

The scientific observations of vessels of the Bulgarian fishing fleet should cover 60 commercial fishing days:

- 20 days on fishing vessels with gillnets (GNS),
- 10 days on vessels with pelagic trawls (OTM),
- 20 days on vessels with beam trawls (TBB) and
- 10 days - on polyvalent vessels (PMP).

Assessment of the relative risk of bycatch

The data collected by scientific observers on board of four types of fishing vessels (gillnets fishery; pelagic trawlers; beam trawlers; fishery with polyvalent gears) is based on the recommendations of FA0 (2019a, b)*:

1. Data about the fishing vessels' activity:
    - Fishing expedition data
    - Departure port
    - Arrival port
    - Fishing vessel name
    - Vessel type
    - Vessel length (m)
2. Fishing gears:
    - Total number of fishing efforts per expedition
    - Depth scale of the fishing activities
3. Basic biological data:
    - Total catch weight (target catch + bycatch)
    - Target catch weight
    - Weight of the bycatch of marine organisms
    - Length structure of catch and bycatch species
4. Additional biological data:
    - Total weight of other industrial species
    - Total weight of the bycatch of industrial species
    - Length structure of the other industrial species landed at the port

- Length structure of the other species in the bycatch, landed at the port
- Data about sex and age structure of the catches and bycatches

The gathered data allow analysis and assessment of the relative risk of bycatch for the different gear types/metiers.

*FAO. 2019a. Monitoring discards in Mediterranean and Black Sea fisheries: Methodology for data collection. FAO Fisheries and Aquaculture Technical Paper No. 639. Rome. http://www.fao.org/3/ca4914en/ca4914en.pdf available also at http://dcf-bulgaria.bg/wp-content/uploads/2021/10/Monitoring-discards-in-Mediterranean-and-Black-Sea-fisheries.pdf.

FAO. 2019b. Monitoring the incidental catch of vulnerable species in Mediterranean and Black Sea fisheries: Methodology for data collection. FAO Fisheries and Aquaculture Technical Paper No. 640. Rome, FAO http://www.fao.org/3/ca4991en/CA4991EN.pdf available also at http://dcf-bulgaria.bg/wp-content/uploads/2021/10/Monitoring-incidental-catch-of-vulnerable-species-in-the-Mediterranean-and-the-Black-Sea.pdf.

Calculation of the observation effort

Based on FAO (2019a): Monitoring the incidental catch of vulnerable species in Mediterranean and Black Sea fisheries: Methodology for data collection. FAO Fisheries and Aquaculture Technical Paper No. 640. Rome, FAO http://www.fao.org/3/ca4991en/CA4991EN.pdf it has been suggested applying the following methodology for the calculations of bycatch rate of vulnerable species (Table 1 and formulae below).

Table 1 Variables for the estimation of the total number of presented specimens.

Variable	Description
N	Sum of the number of individuals of each species caught in each sampled fishing trip (ni) ($N = \Sigma nii$)
D	Number of sampled fishing trips
F	Total number of fishing trips carried out during the reference year by the analysed fleet segment

The bycatch rate (T, per species and fleet segment) is estimated as: $T = N/D$.

From the bycatch rate, then it is possible to compute the estimation of individuals caught (I) by that fleet as: $I = T \cdot F$

According to the requirements of FAO (2019b): "Monitoring discards in Mediterranean and Black Sea fisheries: methodology for data collection", and of Pérez et al (2019) * the proportion of bycatch might be derived as the bycatch weight (kg) is divided by the total catch weight (landed + bycatch) according to the formulae:

Bycatch (%) = bycatch/ (bycatch + catch) x 100.

The above-mentioned methods will be used for assessment of the bycatch rate of PETs and assessment of the bycatch rate, presented as % TC, that will be used as an indicator of the impact of the different fishing techniques on marine species.

* Pérez Roda, M.A. (ed.), Gilman, E., Huntington, T., Kennelly, S.J., Suuronen, P., Chaloupka, M. and Medley, P.2019. A third assessment of global marine fisheries discards. FAO Fisheries and Aquaculture Technical Paper No. 633. Rome, FAO. 78 pp. http://www.fao.org/3/CA2905EN/ca2905en.pdf available also at http://dcf-bulgaria.bg/wp-content/uploads/2021/10/A-third-assessment-of-global-marine-fisheries-discards.pdf.

Sampling design and protocol

Fishing days will be considered as a main unit, used in the observations.

To ensure that data collected provide representative information and sampling for all planned fleet segments distribution of fishing days that will be observed is planned and descriobed in the short description above. The primary sampling units commercial fishing days have an equal and independent probability of being observed.

Simple random sampling without replacement (SRSWOR) is planned. http://home.iitk.ac.in/~shalab/sampling/chapter2-sampling-simple-random-sampling.pdf

The sampling design and protocol follow the methodologies bellow:

- FAO (2019a): "Monitoring discards in Mediterranean and Black Sea fisheries: methodology for data collection" http://www.fao.org/3/ca4914en/ca4914en.pdf.
- FAO (2019b): "Monitoring the incidental catch of vulnerable species in Mediterranean and Black Sea fisheries: Methodology for data collection" http://www.fao.org/3/ca4991en/CA4991EN.pdf

The collected data should include the total catch of the target species, catches of other industrial species, composition and quantities of the bycatches of marine organisms. The dynamics of the main catches and bycatches quantities by months and/or seasons will be estimated, as well as bycatch species composition, bycatch rate, size, sex and age structure.

The impact of commercial fishing will be assessed according to the indicator values of the bycatch rate for the observed fishing activities.

The refusal rate is recorded.

Additional information on observer protocols can be found at:

- FAO (2019a): "Monitoring discards in Mediterranean and Black Sea fisheries: methodology for data collection" http://www.fao.org/3/ca4914en/ca4914en.pdf
- FAO (2019b): "Monitoring the incidental catch of vulnerable species in Mediterranean and Black Sea fisheries: Methodology for data collection" http://www.fao.org/3/ca4991en/CA4991EN.pdf
- FAO (2021): Technical guidelines for scientific surveys in the Mediterranean and the Black Sea; Carpentieri, P.; Bonanno, A.; Scarcella, G. http://www.fao.org/3/ca8870en/CA8870EN.pdf available also at http://dcf-bulgaria.bg/wp-content/uploads/2021/10/TECHNI1.pdf.
- West Africa Regional Scientific Observer Training Manual, Version 1.0, July 15, 2010, Compiled by K.S. Dietrich for ICCAT Fisheries Capacity Building Fund in collaboration with Teresa Turk, Kate Wynne and Manjula Tiwari, 318 p. http://www.kimdietrich.com/WAfr-Manual/WAfr_obs_manual_V1.0_final.pdf.

Sampling scheme

The scheme covers Black Sea waters.

Scientific observers are dispatched on a random principle to fishing vessels using the following gears: (1) gill nets, (2) pelagic trawls, (3) beam trawls for Rapana, (4) polyvalent gears.

The planned activities for the study are based on the following information:

- the activity of the fishing vessel for a past period of time, in order to identify vessels with significant contribution to the relevant fishing activities;

- forecasted monthly activity for all types of fishing activities;

- the fishing day is considered the main unit used in the observations (Stratoudakis, Fryer and Cook, 1998).

The target catch of the observed gillnets will be turbot (Scophthalmus maximus). The research on pelagic fishery will be mainly focused on the catches of sprat (Sprattus sprattus) and horse mackerel (Trachurus mediterraneus). The Rapana catches will be observed by beam-trawlers and polyvalent vessels. The polyvalent vessels observations will include also fishery for horse mackerel (Trachurus mediterraneus) and/or red mullet (Mullus barbatus).

Thus, the target catches of the observed fishing vessels in the Bulgarian Black Sea waters will be:

GNS - Scophthalmus maximus

OTM - Sprattus sprattus, Trachurus mediterraneus

TBB - Rapana venosa

PMP - Rapana venosa, Trachurus mediterraneus, and/or Mullus barbatus.

Sampling frames

To select the representative fishing vessels (fishing vessels, using the same gear type for more than 50 % of the time at sea during a year), the following previous information will be needed in collaboration with NAFA-Bulgaria:

- the activity of the fishing vessel for a past period of time, in order to identify vessels with significant contribution to the relevant fishing activities;

- forecasted monthly activity for all types of fishing activities;

- the fishing day is considered the main unit used in the observations.

All parts of the target population have equal chances of being observed and there is no part of the population which is unreachable.

Laboratory analyses

Part of the available measuring instruments and softwares used in IFR (responsible for observers on board program) are:

Precision scale Kern KB 360-3N, 360 g, Precision: 0.001 g,
Precision scale Kern KB 3600-2N, 3600 g, Precision: 0.01 g
Scales with hook Kern HDB 5K5N, 5 kg
Scales with hook Kern HDB 10K10N 10 kg

LAB13_10447197- Stereo microscope Leica EZ 4,
Stereo microscope OLIMPUS SZ51,
Stereo microscope OLIMPUS SZ61,
Statistical program for fish parameters calculation:
The Catch Effort Data Analysis package (CEDA)
The Length Frequency Distribution Analysis (LFDA)
Yield Version 1.0
FiSAT (FiSAT II),
PRIMER,
XLSTAT.

The accuracy of length measurement of the investigated specimens will be to the nearest 0.1 cm, and for weight - to the nearest 0.01 g. To ensure accurate measurements, the laboratory equipment (Fig.2) should be kept in good condition, scales and microscopes should be regularly calibrated and checked (preferably yearly by a qualified technician).

The laboratory protocols for each sample include a full description of all measurements. IFR-Varna keeps the protocols from the observations in xls-files. 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 quality for biological sampling methodologies in the Bulgarian Black Sea area

The quality of fisheries data,according to Cochran 1977; FAO, 1999; Kolding & Ubal Giordano, 2002; Pennington et al., 2002; Vigneau & Mahevas, 2004; ICES, 2008, 2010e, is related to the precision and accuracy of measurements made on fisheries variables. It is subject to many factors, including:

- data type,
- nature of fisheries and economic,
- social or ecological values of fisheries.

All collected data contain some level of bias and random variation. Improvements needed in precision and accuracy often require greater relative expenditures on sampling and analysis. Data quality must be ensured in all data collection phases: survey design, field and laboratory methods, data processing and transmission. Errors deriving from different sources have different statistical characteristics: errors linked to direct measurement of fishery variables (e.g. length, weight), or to well-designed sampling programmes, are likely to be random and small. In some cases, errors associated with fisheries data can be non-random and biased, a characteristic of low-quality data. In the region, the definition of such indicators is in progress and needs to be agreed upon by all countries in order to establish control routines. A routine quality validation/control is performed at both internal (before preparation of data sets) and external level (by GFCM Secretariat and JRC) to quantify the accuracy and precision level and, if problems are identified, they are solved before datasets are assembled respectively in a timely manner during the data call.

Internal validation

The design and implementation of statistical sampling schemes is placed under the responsibility of the country, according to the planned activities in the national work plan. In order to meet fisheries data requirements in existing EU requirements and GFCM recommendations/decisions, it may be necessary to improve, standardize and possibly modify current national data collection systems and these modifications are done as revisions of the national work plan and submission in the end of each year, if needed. A more efficient use of sampling resources may require the definition of best practices for sampling design and data analysis, with the aim to obtain accurate variable estimates with minimum bias and uncertainty levels. It is fundamental that field collection, laboratory techniques and data processing are applied consistently and correctly. The integrity of the data are also maintained and documented, from sample collection to entry in the data record.

Ensuring the quality of scientific data from the biological monitoring is supported by protocols from data collection, processing and storage.

External validation

External quality validation/control of the submitted data sets is made from the relevant international bodies. In case of Bulgaria the main end users to which data is provided through different data calls are European Commission - DG MARE (JRC) and GFCM.

Every year, after the Mediterranean and Black Sea DCF Data call, JRC is performing a check of the provided data. The report includes data on surveys, landings at length and biological parameters. The lists of checked data from biological monitoring of landings and surveys are provided below:

Biological monitoring:

1. Fisheries catch data (including discards and biological parameters at age)

2. Fisheries landings at length data

3. Fisheries discards at length data

Surveys:

1. Maturity ogives at Length

2. Maturity ogives at Age

3. Growth parameters

4. Sex ratio at age

5. Sex ratio at length

6. Age length key

7. Medits haul data, in accordance to MEDITS instruction manual Version 9, 2017. (In this table the data on BTSBS is provided)

8. Medits catch by haul data (In this table the data on BTSBS is provided)

9. Medits length and biological parameters by haul data (In this table the data on BTSBS is provided)

10. Annual scientific survey ABUNDANCE by length and sex of pelagic and demersal species

11. Annual scientific survey BIOMASS by length and sex of pelagic and demersal species

12. Annual scientific survey ABUNDANCE and BIOMASS by age and sex of pelagic and demersal species

In case any discrepancies are spotted in any of the data sets, two independent scientists and one expert from the EAFA are checking the data and provide an explanation or edit the information.

The Data Collection Reference Framework (DCRF) is a GFCM instrument in support of the collection of fisheries data necessary to improve the formulation of sound scientific advice by relevant GFCM subsidiary bodies in the Mediterranean and the Black Sea. The validation performed by the GFCM Secretariat to assess the quality of data transmitted by the countries is well described in Chapter 8 - Data quality on page 83-84 of GFCM – DCRF - manual - http://dcf-bulgaria.bg/wp-content/uploads/2021/10/GFCM-DCRF-manual-2018-v.21.1.pdf.

Within the DCRF platform fisheries, data quality indicators are implemented. The implementation of fisheries quality indicators - Timeliness, Completeness, Conformity (with preliminary thresholds for different data variables), Stability (with threshold equals to 10%), and Consistency to assess the data submitted by countries through the DCRF online platform has been identified by SAC, WGBS, and CoC as a crucial step to consolidate the use of data for scientific advice.

The national data is entered into the country-specific DCRF-Excel tools annually generated on the DCRF online platform. The DCRF-Excel tools consist of preliminary quality checks (completeness and conformity) to support the country in assessing the status of their data before proceeding with the submission. Data quality checks are then performed after the official submissions made by the country.

The countries have a possibility to modify the data transmitted by submitting a request to the GFCM Secretariat and specifying the rationale and the type of changes to be applied to the data. Stock assessment forms input data are managed differently in the context of concerned GFCM Stock Assessment Working Groups.

After transmission of data sets by the country through the DCRF online Platform into a special staging area of the DCRF database where data quality checks are then triggered. Data processing is made by R-based package developed by the GFCM secretariat to specifically address data quality assessments. The use of the RStudio online environment set up over the last few years by the Secretariat for data processing purposes was the natural choice since R is of widespread use in the scientific community for data quality and analytics purposes, thus enhancing the reproducibility and transparency of data processing tasks, regardless of purpose; and not only.

The information processed during the data processing is endowed with data row-level pass/fail status. The data quality R package takes care of compiling data check results into a format that can be conveniently and timely consulted by countries. The data quality reports are provided to the country in the section of the DCRF platform where a list represents the set of available reports as soon as they are published to the platform.