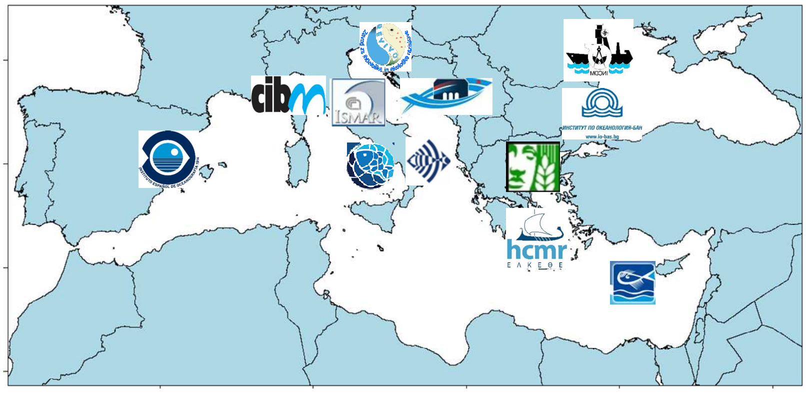

Call for Proposals MARE/2016/22 “Strengthening regional cooperation in the area of fisheries data collection”, Annex 1 “Biological data collection in EU waters” – Agreement Number – MARE/2016/22 – SI2.770115

Work Package 4 – REGIONAL SAMPLING PROGRAM FOR THE COLLECTION OF DATA ON FISHERIES IMPACTS ON THE ECOSYSTEM

Deliverable D. 4.1 - Updated protocols and guidelines for collection, processing and analysis of stomach contents

B. Guijarro (IEO), P. Sartor (CIBM), G. Daskalov (IBER -BAS), I. Georgieva (IBER-BAS), M. Panayotova (IO-BAS), V. Rajkov (IO-BAS)

Partners involved:
IEO, CIBM, COISPA, CNR-ISMAR, NISEA, HCMR, FRI, DFMR, IOF, IO-BAS, NIMRD, REVIVO.

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Contents

Executive summary

The main objective of Task 4.1 was to develop further the RSP-IE designed under the grant MARE/2014/19 Med&BS, in order to design and propose a RSP for 2019 adapted to the characteristics of the stock/fisheries object of regional monitoring, which were identified by the STREAM WP2. Task 4.1 was divided into 3 sub-Tasks:

- Sub-task 4.1.1 Data on stomach contents of fish.
- Sub-task 4.1.2 Data on co-occurrence and relative abundance of species/stocks.
- Sub-task 4.1.3 Data on incidental catch of non-target species, such as protected, endangered or threatened species (PET species).

This document is the Deliverable D4.1"Updated protocols and guidelines for collection, processing and analysis of stomach contents". Under Sub-Task 4.1.1, some revision of the methodologies developed by the MARE/2014/19 Med&BS project for the collection and analysis of fish stomach content data for selected stocks was applied, in particular concerning the selected stocks. In addition to the main stock proposed by MARE/2014/19 for stomach content data collection, namely European hake in Mediterranean and turbot in the Black Sea, additional stocks were proposed for this data collection in the new sampling program: anglerfish, *Lopius piscatorius* and *L. budegassa*, in the Mediterranean, Mediterranean horse mackerel, *Trachurus mediterraneus*, and sprat, *Sprattus sprattus*, in the Black Sea. As main criteria followed for the selection of the new species, we considered the species importance in terms of landings and commercial value, the trophic relationships (e.g. predator, prey) with European hake in the Mediterranean, and turbot in the Black Sea. This will allow increasing the overall knowledge on the stocks from an ecological point of view, and gathering information that could be used to evaluate the natural mortality.

The proposed methodology for the stomach content analysis is practically the same of that suggested by the MARE/2014/19 Med&BS project. As for the previous grant, the sampling scheme takes into account, for each species, factors such as size class, season (quarter), and type of sampling (e.g. experimental fishing and biological sampling on commercial fishery).

1. Introduction

In the recent decades, there has been a progressive change from the traditional approach to fishery assessment and management based on monospecific assessments (Caddy, 1993; Lleonart and Maynou, 2003), to an approach focused on the entire ecosystem (Browman and Stergiou, 2004; Pikitch et al., 2004). This approach is particularly relevant in multispecies fisheries, as the calculations for a single species are of limited value for management purposes (Caddy, 1993). The EAFM (Ecosystem Approach for Fisheries Management) requires different methodologies than the traditional ones, and the use of ecosystem indicators (e.g. Trenkel and Rochet, 2003; Nicholson and Jennings, 2004; Shin and Shannon, 2010), as well as the inclusion of ecological and economical models (e.g. Coll et al., 2006, 2008; Merino et al., 2007; Albouy et al., 2010; Sartor et al., 2014).
There are several ways to define and charcterise an ecosystem and its functioning. One widely used approach is based on the trophic level concept. The trophic level is the position that an organism occupies in a food chain. In aquatic ecosystems, this concept was introduced by assigning integer trophic levels to the individual numbers, to the biomass or to the biological production by its component species. Trophic relationships are fundamental to understand the biological interactions in animal communities and how they respond to human exploitation and thus, it has an application in ecological studies of predation, assessment of competition and optimal foraging. The evaluation of the degree of food resource partitioning, as well as the identification of the pool of food resources sustaining critical life cycle phases (e.g. recruitment), can provide useful elements to better evaluate and manage the stocks in an ecosystem context.
Stomach contents analysis is the primary method for qualitative estimation of dietary composition by investigating the prey items in the fish stomachs. The study of the feeding habits of fish based on the analysis of stomach content can provide important insights not only to assess food spectra at species level, but also to understand the prey-predator relationships, useful aspects to contribute to multispecies stock assessment (Rindorf et al., 2013; Casini et al., 2008, 2009) or to be included in ecological models as mentioned before. Stomach content data are also useful to evaluate the resource partitioning among the species inhabiting a particular habitat/fishing ground. At species level, the information on predator-prey relationship can also be helpful for a better evaluation of the natural mortality of the key exploited stocks.
There are different ways to assign a trophic level and to characterise the food spectrum of a given species; the stomach content analysis undoubtedly provides the main source of information, although important insight can proceed also from other approaches, as the Stable Isotope Analysis (SIA, Cresson et al., 2014) or the “metabarcoding”, a molecular method (Riccioni et al., 2015). These last two approaches can be useful to integrate and validate the results coming from the classical stomach content analysis. The three methods have pros and cons, and although stomach content analysis has been criticized for providing only a relative ‘snapshot’ of diet composition (Pinnegar and Polunin, 2000), for not capturing true interaction dynamics (Deb, 1997), and for neglecting possible dietary items that quickly break down (Polunin et al., 2001), some discrepancies have been detected when comparing fish trophic level based on isotopic or molecular methods, which calls for a careful interpretation of stable isotope values as direct indicators of trophic level (Cresson et al., 2014).
The approach based on stomach content analysis, although rather expensive in terms of time and expertise needed, represents a classical method in fishery ecology, therefore it is still the most appropriate method to be implemented for a wide spatio-temporal monitoring basis. As a matter of fact, data collection by means of stomach contents is the routine method applied to monitor the trophic spectra and the species trophic relationships in the ICES context, for several decades.

To increase the knowledge on predator-prey relations is one of the aspects to be taken into account for the future EU Multiannual Programs. The general objective of the WP4 of the EU Project

STREAM is to design a Regional Sampling Program (RSP) for the collection of data on fisheries impacts on the ecosystem. In this WP, the Task 4.1 “Develop/refine methodologies for data collection and processing”, includes the Sub-task 4.1.1 “Data on stomach contents” and the present Deliverable, D4.1 “Updated protocols and guidelines for collection, processing and analysis of stomach contents”. This Sub-Task has been planned to build upon the experience of previous grants, such as FishPi and MARE/2014/19 Med&BS (Spedicato, 2016). One of the outcomes of the MARE/2014/19 Project was the proposal of a sampling plan for the collection and analysis of fish stomach content data for selected stocks, that of the European hake in north-western Mediterranean (GSAs 6, 7 and 9) and that of turbot in Black Sea (GSA 29). A detailed sampling design for the collection of the stomachs was also proposed, taking into account various factors for the appropriate stratification.
Starting from this proposal, the main objective of Sub Task 4.1.1 was to possibly refine the methodologies elaborated by MARE/2014/19, regarding both the investigated stocks and the sampling size and the sampling stratification. Finally, the outcomes of Sub Task 4.1.1 can be considered for the implementation of a multi-annual regional sampling plan on stomach contents in the region.

During the first months of the STREAM project, some activities of Sub-Task 4.1.1 have been performed in liaison with the Workshop on sampling, processing and analysing the stomach contents (WKSTCON) held in Palma de Mallorca (Spain), 24-27 April 2018. The WKSTCON represented an important chance to present the STREAM project to a wide audience of scientists working in the Mediterranean, and to create possible synergies among the actors involved in fisheries data collection and stomach content analysis.

2. Stocks selected for stomach content data collection

One of the outcomes of the MARE/2014/19 Med&BS Project was the proposal of the stocks to be sampled for stomach content analysis, following the criteria of the Call of Proposals, as well as the advices of Primary End Users, as STECF, GFCM and MEDAC. Two stocks were selected, the European hake, *Merluccius merluccius,* in the Mediterranean, and the turbot, *Psetta maxima,* in the Black Sea. The two species are among the most important resources, both in terms of production and value, exploited by demersal fisheries in the two areas and also play an important role in the demersal species assemblages. The standardised data collection of stomach contents of these two stocks will allow obtaining useful information to better understand their role in the ecosystems, as well as to know and monitor the pool of resources sustaining the two species in the different phases of their life cycle.

M. merluccius and *P. maxima* are proposed as the main targets for the data collection by means of stomach content analysis also for the new Regional Sampling Programmes. However, according to the discussions made in several fora (e.g. the Workshop of Palma de Mallorca, WKSTCON 2018, and the STREAM Plenary Meeting held in Bari in October 2018), additional species are proposed to be included in the data collection by means of stomach content analyses. As main criteria followed for the selection of the new species, we choose the species importance in terms of landings and commercial value, and the trophic relationship (e.g. predator, prey) with the target stocks (European hake and turbot). This will allow increasing the overall knowledge from the ecological point of view and also to increase biological information on the stocks that could be used to evaluate the natural mortality.

As regards Mediterranean, several potential candidates stocks were taken into account, such as blue whiting, *Micromesistius poutassou*, anglerfish, *Lophius* spp., conger eel, *Conger conger,* and blackmouth catshark, *Galeus melastomus.* After a wide discussion during the STREAM Plenary Meeting in Bari (4-5 October 2018), blackbellied anglerfish, *Lophius budegassa,* was proposed as the candidate stock to be included together with *M. merluccius* in the collection of stomach content data. It is a commercially important and piscivorous species, with European hake among its most important preys; *L. budegassa* is occurring in the same species assemblage of *M. merluccius,* although with notably lower density values. Therefore, in order to reach the expected sample size, it has been suggested to include also the congeneric species, monkfish *L. pisctorius,* in the stomach content data collection. The two species show very similar ecological characterisitcs. Therefore, the sampling plan proposed for the Mediterranean includes *Lophius* spp. other than *M. merluccius*.

The selection of the additional species for stomach data collection in the Black Sea was based on criteria related to trophic impacts on other commercial stocks, as well as on the importance for the fisheries and the ecosystem. In Table 2.1, the most important fish predators in the Black Sea, ranked by the amount of fish prey that they consume, are presented. Table 2.1 also shows data on biomass and fishery catches of each predator species. Predatory fishes are obviously priorities for the feeding studies, because of their impact on other fishes including valuable commercial stocks. The classification presented in Tab. 2.1 is based on the biomass of fish preys consumed by the most important (commercially and for the ecosystem) predatory fishes in the Black Sea. The estimation of fish consumption is based on the biomass of each stock, as estimated in recent stock assessments (STECF, 2017a) multiplied by the annual consumption rates (consumption/biomass, Demirel et al. 2019). As shown by Tab. 2.1, the largest amount of fish preys are consumed by the mid-sized pelagic predators: bonito, bluefish and Mediterranean horse mackerel. Bonito and bluefish are commercially and ecologically important, but currently they are not covered by the DCF. Because of their commercial and ecological (as predators) importance bonito and bluefish should be covered by future DCF. It should however be kept in mind, that bonito and bluefish are pelagic migratory species, which overwinter in the Marmara Sea, and their abundance in the EU Black Sea waters

vary from year to year, relative to the state of the stocks and migration behaviour. Between the demersal predators, most of prey fish biomass is consumed by the whiting, turbot and dogfish. Between them, turbot is the most important commercial stock in the Black Sea.
Finally, other than *P. maxima*, other two species were chosen for stomach analyses in the Black Sea: the Mediterranean horse mackerel, *Trachurus trachurus*, as an important predator (Table 2.1) as well as a prey species, and the sprat, *Sprattus sprattus*, as the most important prey species in the EU Black Sea waters. The proposed species are also of great commercial importance and are all already included in the current DCF.

Tab. 2.1. Consumption of fish prey, biomass and catches (in thousands tons) of the most abundant predatory fishes in the Black Sea (STECF, 2017a, Demirel et al., 2019).

Predatory fishes	Biomass of predatory fishes	Catches of predatory fishes	Total biomass of fish preys consumed by each predator
Bonito	98.1	15.2	166.8
Bluefish	29.1	6.3	56.9
Horse mackerel	34.5	15.5	32.7
Whiting	19.1	9.1	29.9
Turbot	4.8	1.6	20.8
Dogfish	1.6	0.4	4.7

2.1 Mediterranean Sea

European hake, *Merluccius merluccius* (Fig. 2.1.1), is an eurhybatic species and an important component of the demersal assemblages of the continental shelf and upper slope in Mediterranean Sea (see the Deliverable D.4.2). The European hake occurs in the Mediterranean and Eastern Atlantic, from Norway and Iceland coasts to Mauritania coasts. In the Black Sea, it lives along the Southern coasts only (Lloris et al., 2003; Colloca et al., 2016). In the Mediterranean, the highest abundance occurs between 50 and 400 m depth. Juveniles migrate from their nurseries towards shallower depths when they reach size of 13-14 cm Total Length; maturing specimens (15-30 cm TL) concentrate between 50 and 120 m depth, whereas larger size specimens, adults over 30 cm TL cm show a wider bathymetric distribution (Bartolino et al., 2008). The reproductive period of the species extends almost all the year round, although seasonal peaks are present according to the different areas; males mature at a lower size (around 20 cm TL) compared to females (30-35 cm TL) (Arneri and Morales-Nin, 2000; Vrgoč et al., 2004; Sbrana et al., 2007; Recasens et al., 2008; Donnaloia et al., 2012, among others).
Spatially and temporally stable nursery areas have been identified in many areas of the Mediterranean (Jukic and Arneri, 1984; Abella et al., 2008; Manfredi et al., 2009; Lembo 2010; Murenu et al., 2010; Garofalo et al., 2011).
In the Mediterranean, *M. merluccius* is one of the most important demersal resources for trawl fisheries, but also for the small scale fisheries using gillnets and bottom longlines (Martin et al., 1999). Trawlers mostly exploit specimens below 30 cm TL, whereas artisanal vessels target larger specimens (Sartor et al., 1996; Abella et al., 1997). As shown by the results of the assessments carried out in last years in the context of GFCM and STECF, the stocks of European hake in the Mediterranean are suffering a "chronical" overexploitation, mostly due to the high fishing pressure produced by trawling on the first age classes (FAO, 2016; STECF, 2017a). The Minimum Conservation Reference Size in EU Mediterranean waters is 20 cm TL (Reg. EU 1967/2006).

Fig. 2.1.1. European hake, *Merluccius merluccius*

The diet of European hake has been widely studied in both Atlantic and Mediterranean waters. In the Mediterranean, where the diet of this species has been widely investigated, European hake preys on a wide spectrum of organisms (from small crustaceans to medium sized fish), with sharp ontogenetic changes in its diet (Bozzano et al., 1997; Carpenteri et al., 2004; 2005; 2008, Carrozzi et al., 2019; Cartes et al., 2009; Fanelli et al., 2018; Sartor et al., 2003; Stagioni et al., 2011; Sinopoli et al., 2012). The daily food consumption was estimated as 5.0–5.9% of body wet weight (Carpentieri et al., 2008).
The main switches in the diet occur when juveniles migrate from nurseries towards shallower depths and after the achievement of sexual maturity (Carpentieri *et al*., 2004). Recruits in nurseries (specimens lower than 15 cm TL) feed mostly upon euphausiids and mysids. A key role, as food resource for the juveniles, is played by the Euphausids *Meganychtiyphanes norvegica* and *Nychtiphanes couchii*, and by the mysid *Lophogater typicus*, has been evidenced in several areas (Bozzano et al., 1997; Carpentieri et al., 2004; 2005; Sartor et al., 2003; Stagioni et al., 2011).
Before the transition to the complete ichthyophagous phase (at around 30-35 cm TL) hake shows more generalized feeding habits where crustaceans decapods (such as *Processa* spp., *Solenocera membranacea*, *Chlorotocus crassicornis*), benthic (Gobiidae, Callionymidae) and necktonic fish (anchovy, sardine) dominate the diet, whereas cephalopods have a lower incidence. The cannibalism has been observed in several areas; it increases with hake size, achieving a proportion of about 15-20% in the weight of preys, as regards the diet of specimens over 30 cm TL and it seems to be positively correlated to the density of hake recruits (STECF, 2008). The largest hake specimens feed on a variety of fish preys, that vary according to the habitat, from small pelagics (e.g. anchovy and sardine) and Myctophids, to benthic and demersal fishes (e.g. *Trisopterus capelanus*, *Cepola macrophtalma*, *Gaidropsaurus biscayensis*, *Trachurus trachurus*).
A predominance of fishes during winter and a greater diversification of preys in summer has been noticed as well.
Therefore, the studies carried out on European hake diet have evidenced a change in the food spectrum according to three main size groups, corresponding to different life cycle stages: i) recruits, preying mostly on small crustaceans and small benthic fishes, ii) post-recruits, preying on larger fish and crustaceans decapods and iii) adults, preying mostly on active swimming fishes. However, some differences in the size range of each group can depending on the area analysed (Table 2.1.1).
Daily migrations (Froglia, 1973; Orsi-Relini et al., 1989; Bozzano et al., 2005) and changes of diet as a function of temporal/spatial prey availability have also been reported (Cartes et al., 2004), with daily movements often related to diel feeding rhythms. Diet has shown differences among seasons

(Bozzano et al., 2005), although in the Balearic Islands these changes are restricted to recruits (Cartes et al., 2009).

Table 2.1.1. Examples of ontogenic changes in the diet of European hake in different Mediterranean areas.

Area	Size groups (Total length)	Diet composition (main food items)	Reference
Western Mediterranean (Gulf of Lions) - GSA 7	<14.5 cm	Small crustaceans (Euphausiids, Mysids), small benthic fishes (e.g. Gobidae)	Bozzano et al. (1997)
	14.5-39.5 cm	Decapod crustaceans, small fishes (sardine, anchovy)	
	>40 cm	Necktonic Fishes	
Western Mediterranean (Balearic Islands) - GSA 5	<18 cm	Small crustaceans (Euphausiids, Mysids), small benthic fishes	Cartes et al. (2009)
	18-21.9	small fish (sardine, anchovy)	
	>22 cm	Nektonic fish, crustacens	
Central Mediterranean (central Tyrrhenian Sea) GSA 9	>16 cm	Small crustaceans (Mysids)	Carpentieri et al. (2005)
	16-35.9 cm	Decapod crustaceans (Pandalidae, small fish (e.g. Gobidae, Callyonimidae)	
	>36 cm	Fishes (sardine, anchovy, small Gadidae) cephalopods, Decapod crustaceans	

Conversely, as concerns the role played by *M. merluccius* as a prey, less information is available. Hake juveniles, due to their important densities, especially in some areas, undoubtedly constitute an important food resource for the piscivorous species. The presence of hakes in the stomach contents of anglerfish, *Lophius piscatorius* and *L. budegassa*, have been widely documented in several Mediterranean areas (Stagioni et al., 2013; López et al., 2016; Ainouche and Nouar, 2018). There are several studies reporting a constant and abundant presence of hakes of different size in the stomachs of cetaceans, such as the bottlenose dolphin, *Tursiops truncates*, and the striped dolphin, *Stenella coeruleoalba* (Wurtz and Marrale, 1993; Voliani and Volpi, 1990; Blanco et al., 2001; Scuderi et al., 2011).

Blackbellied anglerfish, *Lophius budegassa*, and monkfish, *Lophius piscatorius* (Fig. 2.1.2), are two important demersal species distributed in the Mediterranean Sea, which are commercially exploited due to their economic value (Fariña et al., 2008; Colmenero et al., 2013; Gangitano 2015a; 2015b). Both species are characterised by dorso-ventrally compressed morphology, a wide mouth, and the presence of an illicium, a modified first dorsal ray which serves as a lure (Fariña et al., 2008). Both species have a wide geographic distribution that includes the North-eastern Atlantic Ocean from the Barents Sea to the Strait of Gibraltar, the Mediterranean and the Black Sea (Relini et al., 1999; Velasco et al., 2008; Gangitano 2015a; 2015b). Their bathymetric distribution is also large, between the continental shelf and the upper slope down to 1000 m depth. They are benthic species and live on sandy and muddy bottoms. Both species are exploited mainly by the bottom trawl fishery, caught together with other species including *M. merluccius*, *Mullus barbatus*, *Helycolenus dactylopterus* and *Phycis blennoides* (Ungaro et al., 2002; Gristina et al., 2006) and represent an important portion of the commercial value of the catch.
An assessment of the exploitation status of *L. budegassa* was performed in the Strait of Sicily (GSA 15 and 16) using length distribution of commercial landings in 2009-2010 and data collected from MEDITS trawl surveys (2002-2011) carried out in the Strait of Sicily (Gancitano et al., 2013a). The

results obtained showed an overfishing condition. At present, no stock assessments are available for *L. piscatorius* in the Mediterranean; evaluations on the exploitation status of this species have been performed in Atlantic, Faroe Islands (Ofstad et al., 2013), showing that the species is suffering the excessive fishing pressure. Nowadays, no specific management measures for the two *Lophius* *s*peces are applied in Mediterranean. No minimum landing size has been established for the two species in accordance with EU Reg. No. 1967/2006.

Fig. 2.1.2. Monkfish, *Lophius piscatorius* (left) and blackbellied anglerfish, *Lophius budegassa* (right).

Both anglerfish species are opportunistic, non-selective feeders displaying a common feeding strategy called “sit and wait”, as they do not prey actively, but attract preys by moving the *illicium* (Laurenson and Priede, 2005). Although available information shows that bony fish constitute the principal prey category for both species, the studies carried out for these species have evidenced a change in the food spectrum according to size (Table 2.1.2). Thus, in the case of *L. budegassa*, available studies show that small individuals prey on smaller fish species and large individuals on larger species, such as *M. merluccius*, as well as crustaceans. Although the information on the diet of *L. piscatorius* in the Mediterranean is scarce, also this species shows an ontogenic change, which has also been described at genus level worldwide (Fariña et al., 2008).

Table 2.1.2 - Ontogenic changes in the diet of *L. budegassa* and *L. piscatorius* in some Mediterranean areas.

Lophius budegassa			
Area	Size groups (total length)	Diet composition (main food items)	Reference
Tunisian coast	<40 cm	Carangidae and Penaeidae	Negzaoui-Garali *et al.*, 2008
	>40 cm	Carangidae, Argentinidae, Merlucciidae	
Adriatic Sea	<15 cm	Small fish (*Lesuerigobius friesii*, *Gaidropsarus biscayensis*, *Callionymus maculatus*)	Stagioni *et al.*, 2013
	>15 cm	*M. merluccius*, *M. poutassou*, *E.*	

		encrasicolus, *M. barbatus*, crustaceans	
Catalan Sea	<30 cm	Small fish (*Gobius niger*, *Lesuerigoibus friesii*, *Trisopterus minutus*)	López *et al.*, 2016
	>30 cm	*Merluccius merluccius*, *Gadiculus argenteus*, *Ophidion barbatum*, crustaceans.	
Algerian coast	<24 cm	Small fish (*Gadiculus argenteus*, *Leuseurigobius suerii*)	Ainouche and Nouar, 2018
	>24 cm	Large fish (*Trachurus trachurus*, *Mullus barbatus*, *Merluccius merluccius*) and crustaceans (*Parapenaeus longirostris* and *Aristeus antennatus*)	
Lophius piscatorius			
Catalan Sea	<30 cm	*Ophidion barbatum*, *Cepola macrophthalma*, *Merluccius merluccius*, crustaceans	López *et al.*, 2016
	>30 cm	*Cepola macrophthalma*, *Gadiculus argenteus*	

2.2 Black Sea

Turbot, *Psetta maxima*, (Fig. 2.2.1) is a large, broad-bodied left-eyed demersal flatfish that belongs to the family Scophthalmidae. Its geographical range extends from Icelandic seas to the Mediterranean, including the Sea of Marmara, and the Black Sea (Blanquer et al., 1992). The turbot is one of the most important commercial fish species in the Black Sea, where it is heavily fished using otter trawls, gillnets, beach seines, and trammel nets (Aydin and Sahin, 2011).

Fig. 2.2.1. Turbot, *Psetta maxima*, (Photo by Valodia Maximov)

Turbot is a slow growing, and long living species (Stoyanov et al., 1963), with maximum longevity of 10-12 years along the Bulgarian, Romanian and Turkish coasts (Stoyanov et al., 1963; Karapetkova and Zivkov, 2006), and 17-23 years along the Russian and Ukrainian coasts (STECF, 2015). Turbot reaches a maximum total length of 85-87 cm TL, and weight of 12-15 kg (Stoyanov et al., 1963; Karapetkova and Zivkov, 2006). The length at first maturity is estimated of about 41-51 cm TL that correspond to age 3-5 years, based on research in Bulgaria (Stoyanov et.al., 1963; Karapetkova and Zivkov, 2006) and 31-37 cm TL that corresponds to age of 2-3 years in Romania based on samples from scientific surveys (STECF, 2015). In Turkish waters, length at first maturity is estimated at 20-25 cm TL (Eryilmaz and Dalyan, 2015). The Minimum Conservation Reference Size for this species in EU Black Sea waters is 45 cm TL (Maximov et al., 2013) and was introduced in Bulgaria under Fisheries and Aquaculture Act (FAA, 2001).
The stock is subject to EU regulations fixing the fishing opportunities. A multiannual management plan for turbot fisheries in GSA29 was agreed during the 41st Annual Meeting of the General Fisheries Commission for the Mediterranean (GFCM), October 2017. The agreement was based on the best available scientific advice and the principles of the reformed Common Fisheries Policy (CFP). The stock exploited by Bulgaria and Romania is shared with non-EU countries, such as Turkey, Ukraine, Georgia and the Russian Federation. So far, no TACs had been decided at regional level between EU and non-EU countries, and every year since 2008, the European Union had been fixing autonomous quotas for turbot in order to ensure that the CFP rules were applied (Regulation (EU) 2018/2058 fixing for 2019 the fishing opportunities for certain fish stocks and groups of fish stocks in the Black Sea). Supporting the development of multiannual management plans in the Black Sea are management measures such as minimum standards for bottom-set gillnet fisheries for turbot in the Black Sea (Recommendation GFCM/37/2013/2 on the establishment of a set of minimum standards for bottom-set gillnet fisheries for turbot and conservation of cetaceans in the Black Sea) and measures adopted recently to prevent, deter and eliminate IUU fishing in turbot fisheries in the Black Sea (Recommendation GFCM/39/2015/3 on the establishment of a set of measures to prevent, deter and eliminate illegal, unreported and unregulated fishing in turbot fisheries in the Black Sea). The turbot stock in the Black Sea was assessed in 2017 by state-space assessment model (SAM); the current F (0.82) is larger than FMSY (0.26), which indicates that turbot in GSA 29 is being fished above FMSY (STECF, 2017b).
Due to its importance to aquaculture, numerous studies regarding the use of different types of food in cultivated turbot are available (e.g. Bonaldo et al., 2015; Sevgili et al., 2015; Kroeckel et al., 2013 and references cited therein). However, information on the diet of wild turbot is rather scarce. Age 0 individuals of turbot avoid non-motile organisms (such as gastropods and bivalves) and prey on crustaceans and polychaetes on west of Ireland nursey grounds (Haynes et al., 2011). In the Black Sea, small fish with lengths between 2.0 – 20.0 cm (age groups 0+ - 1+) feed on polychaets, crustaceans and fish, mainly gobiids (Karapetkova, 1962). Adult turbot prey on fish (e.g. *Merlangius merlangius* and Gobiidae), followed by small Crustaceans, like the shrimp *Crangon crangon* and mollusks (Panayotova and Todorova, 2008; Karapetkova, 1962; Bulgurkov, 1965) Totoiu et al. (2014) have found small quantities of bivalves and decapods in turbot diet in Romanian waters. To our knowledge, no studies of ontogenetic changes and few studies on seasonality (Karapetkova, 1962) have been performed until now.

The Black Sea population of the Mediterranean horse mackerel, *Trachurus mediterraneus*, Fig. 2.2.2, is distributed across the Black, Azov and Marmara seas (Stoyanov et al.1963, Karapetkova and Jivkov 2006). Its wintering areas are situated along the coasts of the Crimea, Caucasus, Anatolia and parts of the Marmara Sea (Ivanov and Beverton 1985). In spring, the horse mackerel appears in the Bulgarian coastal waters usually in mid-May. By mid-June, the majority of the schools migrate toward the northwestern part of the Black Sea for feeding and spawning. The autumn migration spans from mid-September to mid-December when the horse mackerel moves southward, towards its wintering grounds (Stoyanov et al. 1963). Mediterranean horse mackerel is a species with maximum longevity of 10-12 years reported in the past (Stoyanov et al., 1963), but nowadays the age of most of the individuals in the Black Sea population does not exceed 6 years. The horse mackerel matures at the age of 1-2 years in spring-summer (May-August), when the main feeding and growth season also takes place. It spawns in the upper layers, mainly in the open part of the sea as well as near the coast (Stoyanov et al. 1963; STEFC 2017b).

Fig. 2.2.2. Mediterranean horse mackerel, *Trachurus mediterraneus*

The horse mackerel is a species of great commercial importance for the Black Sea fishery (Shlyakhov and Daskalov 2008). The catches of Black sea horse mackerel are realized by active (pelagic trawl and seine) and passive fishing gears (trap nets, beach seine, STEFC 2017b). The horse mackerel of age 1-3 years generally prevails in the commercial catches. About 90% of the total horse mackerel catch is taken by Turkey (STEFC 2017). After the 1950, the horse mackerel catches have been gradually increasing, reaching a maximum level of about 140 thousand tons in the late 1980s (Prodanov et al. 1997). During 1990-2010 the catches have decreased down to about 10 thousand tons. In recent years, the reported Black Sea catches fluctuate between 10,229 and 25,367 tons (STEFC 2017b). The minimum landing size of horse mackerel in EU Black Sea waters (Bulgaria and Romania) is 12 cm total length (STEFC 2017b; FAA 2017). In the Black Sea the status of the horse mackerel stock is considered in a state of overexploitation (STEFC 2017b).
The feeding biology of *T. mediterraneus* in the Black Sea has been investigated by several authors (e.g. Fortunatova, 1948; Briskina, 1954; Stoyanov et al.1963, Stikov 1978, Zlatev 1986, Yankova et al. 2008, Georgieva et al. submitted). The horse mackerel occupies a position in the middle of the trophic pyramid (trophic level 3.5), that makes it important for the functioning of the ecosystem. Depending on the season the diet of horse mackerel is dominated by benthic crustaceans such as Mysidacea, Caridea, Amphipoda in spring and summer, and zooplankton and fishes (sprat, anchovy) in autumn **(**Fortunatova, 1948; Briskina, 1954; Stoianov et al. 1963, Yankova et al. 2008; Georgieva et al. submitted). To our knowledge, only two studies have reported on ontogenetic changes in horse mackerel diet (Yankova et al. 2008; Georgieva et al. submitted).

Table 2.2.1. Ontogenic changes in the diet of *Trachurus mediterraneus* in the Black Sea

Area	Size group Total Length	Main food items in the diet	Reference
Bulgarian Black Sea	<10 cm	Zooplankton (Copepoda; Decapoda larvae, Gastropoda larvae, Cirripedia larvae)	Georgieva et al. submitted
	>10 cm	Benthic invertebrates (Crustacea, Polychaeta), Pisces (Clupeidae, Engraulidae)	Georgieva et al. submitted; Yankova et al. 2008

The sprat, *Sprattus sprattus*, (Fig. 2.2.3) is a small pelagic planktivorous fish. It forms one of the most abundant stocks in the Black Sea, that has a great importance for the commercial fisheries, as well as for the marine predators (including other commercial fish such as whiting and turbot) and the ecosystem as a whole (Daskalov et al 2008, Shlyakhov and Daskalov 2008). Together with the anchovy, sprat is one of the most abundant, planktivorous, pelagic species. The level of its stocks depends on the conditions of the environment mainly and on the fishing effort. The changes in the environment due to anthropogenic influence affect the dry land as well as the world ocean. The level of the sea pollution and its "self-purifying" ability are completely different. There is a clear indication of changes in the nature equilibrium in the corresponding ecological niches. The greatest impact in the world ocean is played by commercial fisheries, which directly devastate a significant part of the given species populations. As a result of this, some of the species stocks are declined or depleted.

Fig. 2.2.3. Sprat, *Sprattus sprattus*

The long-term (Yankova et al., 2008; Mihneva et al., 2015; Raykov et al., 2019) studies on food composition and feeding patterns of sprat have been based on analysis of stomach content composition of samples collected in front of Bulgarian Black Sea coast in 2007-2018 under Reg. EU 199/2008 and Reg. (EU) 2017/1004 of the European Parliament and of the Council. The above mentioned studies encompassed also analyses of the zooplankton species composition and biomass in the marine environment, as these pelagic organisms form the main food source of planktivorous fish species.

Eight zooplankton species/groups have been identified in the stomach contents of the studied sprat specimens during November 2018: – copepods such as *Calanus euxlinus, Pseudocalanus elongates, Paracalanus parvus,* and *Acartia clausi;* from meroplankton were detected only Decapoda larvae; class Chaetognatha was represented by *Parasagitta setosa*, and class Appendicularia - by Oicopleura dioica. The sprat food spectrum was dominated by the cold-water copepod *Calanus euxinus*, followed by *Parasagitta setosa*, *Acartia clausi*, *Oicopleura dioica, Ps. elongatus* and Decapoda larvae. The cold-water zooplankton dominated in the sprat diet by frequency of occurrence, as well as by abundance and biomass. Parasitic Nematoda were found in 10% of the studied fish specimens.

3. Sampling methodology and guidelines

3.1 Rationale

During the WKSTCON (Palma de Mallorca, April 2018), different methodological approaches for the study of trophic ecology were discussed, highlighting their pros and cons in terms of robustness of results, costs and capability of implementation for a long term monitoring activity, such as that needed for the Regional Sampling Program of Data Collection. A similar summary was already performed during the MARE/2014/19 Project, and it is available in the Deliverable 3.3 of that Project.

As reported in the Introduction, the classical method based on stomach content analysis still remains the most valid approach for the implementation a coordinated and a long term program of data collection. Therefore, this method is proposed also for the next Regional Sampling Program. This methods is based on the taxonomic identification of the prey items present in the stomach contents; the role of each prey item is then evaluated according to its frequency of occurrence, numerical or weight/volumetric importance.

It is important also to consider that, as shown by the abundant literature available, both for Mediterranean and Black Seas, there is wide experience and expertise (both in Ecology and in Taxonomy) in the field of trophic ecology based on stomach content analysis.

3.2 Proposed sampling protocol

The proposed sampling protocol is the same as the one proposed by the Project MARE/2014/19 Med&BS; the validity of this method was also underlined by the review carried out during the Workshop WKSTCON, where this method was agreed at Mediterranean and Black Sea level, and considering the preliminary results of pilot studies carried out under national work plans (e.g. Italy).
The individuals sampled for the analysis of their stomach contents should be sampled at sea and, preferably, analysed later in the laboratory. The recommended method for preservation is frozen. If there is any alteration to this protocol, it should be taken into account. The preservation can be done for: i) the entire specimen or ii) only the stomach.
Individuals without everted stomachs should be prioritized.
For the individuals to be sampled, it is necessary to register the general information of the sampling (i.e., haul, date and time of the haul, position, depth).
The following steps should be followed for each individual:

1. Measure the size, as **Total Length** (to the lowest half cm).
2. **Open** the fish's body carefully with a knife or scissors to avoid cutting internal organs.
3. Identify the different organs and **determine sex** (male, female or indeterminate) and **maturity stage** (following the usual procedures, according to the reference maturity scales in use, e.g. A.A. V.V., 2017).
4. **Remove the stomach.** Identify if it is in one of the following states. Any of these states should be include in the scoreboard, as this information is important in order to calculate some of the dietary indices.
    - 4.1. Full: Stomach with some content. If the contents are only hard structures or a mass of unidentified species, this should be annotated.
    - 4.2. Empty: Stomach without any content, but gallbladder with content. This means that there was nothing in the stomach when the fish was caught.

4.3. Regurgitated: Stomach without any content and gallbladder without content. This means that there was some content in the stomach when the fish was caught, but it was expelled due to stress of the catch.

5. For full stomachs, estimate its content quantitatively (volume or/and weight). For empty and regurgitated stomachs, no further steps should be taken.

6. **Classify** the stomach content according to the categories decided (major prey Taxa and, when possible, at species level for Teleostea, Crustacea and Cephalopoda).

7. For **each prey** category identified:

7.1. Estimate the percentage in volume or/and weight.

7.2. Estimate the state of digestion (1: intact prey, 2: partially- digested prey or 3: well-digested prey).

7.3. When possible, estimate the number of preyed individuals.

7.4. When possible, for those preys (Teleostea, Crustacea and Cephalopoda) in which the state of digestion allows it (i.e. entire preys), measure the size of each individual. If more than a prey is identified, measure the smaller (minimum length) and larger (maximum length) individuals. Type of length: Teleostea (total or standard length), Crustacea (carapace or total length), Cephalopoda (mantle length).

A proposal of scoreboard for stomach content data collection and analysis is included in Annex I, with an example of filled scoreboard with a detailed description.

3.3 Estimation of dietary indices

For each prey item (species of major taxon preyed by the investigated species), the following dietary indices are proposed to measure feeding intensity and to evaluate the trophic spectrum of each species:

1) **Frequency of occurrence** (%F), percentage of stomachs with a specific type of prey in relation to the total number of stomachs containing food;

2) **Numerical** (%N) and **volumetric** (%V) **composition**, expressed as the percentage contribution of each prey to the whole content, in number or volume respectively;

3) **Index of relative importance** (IRI), IRI = %F(%N+%V), standardized following %IRI=(IRI/ΣIRI)x100 (Cortes, 1997);

4) **Index of relative importance prey-specific** (%PSIRI = % Fpi(%Npi+ %WPi) 0·5) (Brown et al., 2012).

5) **Vacuity index (v),** calculated as the percentage of empty stomachs divided by the sum of the full and empty stomachs, or its reverse, **repletion index (R),** calculated as the percentage of full stomachs divided by the sum of the full and empty stomachs. Everted stomachs are not considered by this index.

6) **Gastro-somatic index** (Ga.SI), Ga.SI=100(total stomach content weight/total fish weight) (Desai 1970)

7) **Diet breadth**, following Levin's standardized index:

$$B_i = \frac{1}{n-1} \cdot \left(\frac{1}{\Sigma_j p_{ij}^2} - 1\right),$$

Where p_{ij} is the proportion of diet of predator i that is made up of prey j and n is the number of prey categories. This index ranges from 0 to 1, low values indicating diets dominated by a few prey items (specialists predators) and higher values indicating generalist diets (Krebs, 1999);

8) **Species diversity** in both prey number (H'_n) and prey volume (H'_v), calculated using the classical Shannon-Wiener index:

$$H' = -\sum_{i=1}^{S} p_i \log_2 p_i$$

Where *S* is the number of species, p_i the proportion of species *i* (in number of H'_n and in volume for H'_v) in relation to total abundance/volume individuals (that is relative abundance or volume of the species).
Prey categories with frequency of occurrence lower than 3% and unidentifiable remains should be excluded from these indices.

3.4 Proposal of sampling plan

Two possible sources of data shall be considered in the collection of stomachs of the selected Mediterranean and Black Sea species: **experimental bottom trawl surveys** (like MEDITS survey in the Mediterranean) and **biological sampling** (sampling from commercial fishing). In the first case, the sampling would not take into account seasonality but the second would. Sampling can be performed from fresh or frozen individuals, depending on the possibilities, as explained in the Chapter 3.2.
The sampling of stomachs has been planned taking into account the following criteria (strata), which are known to influence the diet of the investigated species:

- **Size class**: for European hake, three different groups should be considered (see Table 2.1.1): i) juveniles (<20 cm TL, which would be part of the discarded fraction, in the case of sampling from commercial catches); ii) sub-adults (20-35 cm TL) and iii) adults (>35 cm LT).
For the anglerfish, *Lophius* spp., two different groups should be considered: i) small (<30 cm TL, which would be part of the discarded fraction in the case of sampling from commercial catches) and ii) large (>30 cm TL) individuals.
As concerns turbot, three size groups have been proposed, taking into account both the length at first maturity and the Minimum Conservation Reference Size (Karapetkova, 1962): i) juveniles (<20 cm TL), ii) discarded adults (20-45 cm TL) and iii) adults taken by the commercial fisheries (>45 cm TL).
For the Mediterranean horse mackerel, two size groups should be considered for the sampling of stomachs: i) juveniles <10 cm TL, and ii) adults >10 cm TL.
Concerning sprat, 2 size groups should be regarded: i) <8 cm TL and ii) >8cm TL.
Seasonality: This stratum will only refer to the samplings from commercial catches and, as explained before, quarter should be the time interval.
- **Sample size**: The proposal on the number of individuals to be sampled for stomach content analysis is reported in the tables below.
The proposed numbers correspond to **full stomachs to be sampled**. For the moment, no estimations of the optimal sample size is available; the samples sizes proposed for each species are the product of a first evaluation based on various aspects: sample size from previous study, heterogeneity of the diet of each species, availability of the samples, including the information on the stomach repletion.

Hake (Table 3.4.1):

- *Biological sampling (commercial fishery)*: In order to take into account seasonality, sampling should be carried out by quarter and 30 full stomachs by quarter and length group shall be analysed by each GSA.
- *Experimental trawl surveys*: 20 full stomachs by length group should be analysed by GSA.

Total number of stomachs to sample per GSA/year: 420. This number is lower than that (690) proposed by the Project MARE/2014/19 Med&BS, due that the results of the Pilot Studies (e.g. those performed in the Italian GSAs) highlighted the difficulty to reach the expected samples size, especially for the first and last size class.

Anglerfish (*Lophius* spp., Table 3.4.2):

- *Biological sampling (commercial fishery)*: In order to take into account seasonality, sampling should be carried out by quarter and 12 full stomachs by quarter and length group shall be analysed by GSA.
- *Experimental trawl surveys*: 12 full stomachs by length group should be analysed by GSA.

Total number of stomachs to sample per GSA/year: 120.

Turbot (Table 3.4.3):

- *Commercial fishery*: In order to take into account seasonality, sampling should be carried out by quarter and 30 full stomachs by quarter and length group shall be analysed. It should be noted, that the number of juveniles in commercial catches is zero or very low. Discarded adults could be found in sprat fisheries using OTM trawls, in Rapana fisheries using beam trawls and in turbot gillnets fisheries, but in low quantities.
- *Surveys*: A total of 15/30 individuals by length group should be analysed. The number of juveniles in survey catches is usually very low.

Total number of stomachs to sample in GSA 29: 315

Horse mackerel (Table 3.4.4.):

- *Commercial fishery*: In order to take into account seasonality, sampling should be carried out by quarter and 450 full stomachs should be analysed.
- *Surveys*: A total of 100 individuals should be analysed.

Total number of stomachs to sample in GSA 29: 550.

Sprat (Table 3.4.5):

- *Commercial fishery*: In order to take into account seasonality, sampling should be carried out quarterly and 200 full stomachs (total for all size categories) shall be analysed per quarter.
- *Surveys:* 100 individuals in total should be analysed.

Total number of stomachs to sample in GSA 29: 900.

It is worth noting that this sampling program could be revised in the future, according of the results of the analysis of the stomachs content; this statement is valid especially for the sample size. Therefore, the sampling strategy and the sampling effort could be refined in order to minimize the variability of the estimates of the food spectra and to properly consider all the factors influencing the variations in diet composition.

- **Precision level in prey identification**: It is recommended to classify the preys to the lowest taxonomic level, possibly at species level. However, in order to have the most standardized and comparable information possible, in addition to the list of prey with the most detailed classification level, prey should be grouped into major Taxa, not necessarily at species level.

The proposed grouping categories are the following taxa: Polychaeta, Sipuncula, Gastropoda, Bivalvia, Cephalopoda, Euphusiacea, Amphipoda, Isopoda, Mysidacea, Crustacea Reptantia, Crustacea Natantia and Teleostea. For the stomach contents of sprat in Black Sea, also the following categories should be taken into account: Copepoda, Cladocera, Chaetognatha, and Lamellibranchia veliger.

However, in the case of Teleostea, Crustacea and Cephalopoda, when the level of digestion would allow the identification, it is strongly suggested to provide the information at the lowest taxonomic level, possibly the Species level.

Table 3.4.1. European Hake in Mediterranean; proposal on number of full stomachs to be sampled by type of sampling, quarter and size class, for each GSA.

Biological sampling (landing and discard)	**Full stomachs to sample**			
Quarter	Juveniles	Sub-adults	Adults	Total
I	30	30	30	90
II	30	30	30	90
III	30	30	30	90
IV	30	30	30	90
Total biological sampling	**120**	**120**	**120**	**360**
Experimental trawl survey (MEDITS)	**Full stomachs to sample**			
	20	20	20	60
Total survey	**20**	**20**	**20**	**60**
Total	**140**	**140**	**140**	**420**

Table 3.4.2. Anglerfish (*Lophius* spp.) in Mediterranean; proposal on number of full stomachs to be sampled by type of sampling, quarter and size class, for each GSA

Biological sampling (landing and discard)	**Full stomachs to sample**		
Quarter	Small	Large	Total
I	12	12	24
II	12	12	24
III	12	12	24
IV	12	12	24
Total biological sampling	**48**	**48**	**96**
Experimental trawl survey (MEDITS)	**Full stomachs to sample**		
	12	12	24
Total survey	**12**	**12**	**24**
Total	**60**	**60**	**120**

Table 3.4.3. Turbot in Black Sea; proposal on number of full stomachs to be sampled by type of sampling, quarter and size class.

Biological sampling (landing and discard)	**Full stomachs to sample**			
Quarter	Juveniles	Discarded adults*, **	Commercialized adults	Total
I	0	30	30	60
II	0	30	30	60
III	0	30	30	60
IV	0	30	30	60
Total biological sampling	**0**	**120**	**120**	**240**
Bottom trawl survey	**Full stomachs to sample**			
	15	30	30	75
Total survey	**15**	**30**	**30**	**75**
Total	**15**	**150**	**150**	**315**

*In Romania, according to the legislation, commercial fishing of turbot is carried out only with gillnets (selective gears) and no discards or < 45 cm individuals are reported. Consequently, in Romanian waters is very unlikely to achieve the target value for discarded adults (30 ind. x 4 seasons). ** In Bulgaria, only in the case the annual turbot quota is reached and the catch should be partially discarded or in the case the adults in the catch are under the minimum allowed size (45 cm). The proposed sampling intensity is based on the previous observations and cannot be guaranteed due to high uncertainty whether the above mentioned two cases will happen.

Table 3.4.4. Mediterranean horse mackerel in Black Sea; proposal on number of full stomachs to be sampled by type of sampling, quarter and size class.

Biological sampling (landing and discard)	**Full stomachs to sample**		
Quarter	Juveniles	Adults	Total
I	0	0	0
II	30	150	180
III	15	75	90
IV	30	150	180
Total biological sampling	**75**	**375**	**450**
Research survey	**Full stomachs to sample**		
	30	70	100
Total survey	**30**	**70**	**100**
Total	**105**	**445**	**550**

*The number of the samples was determined based on previous observations on horse mackerel feeding but also depends on the availability of the species in front of the Bulgarian coast. The presence of horse mackerel along the Bulgarian coast is conditional to the species migration (to/from Marmara Sea) and depends on the season. Hence, the appearance of horse mackerel in the Bulgarian Black Sea waters is sporadic – fact that cannot always guarantee the exact number of the proposed sampling. **Horse mackerel, is present in Romanian waters only at the end of May (for about 2 weeks); in summer it is missing, and it returns in autumn (starting in September). Reported catches are very small (30 tons), only from pound nets. Thus, likely it will be possible to not achieve the target value in summer (quarter III).

Table 3.4.5. Sprat in Black Sea; proposal on number of full stomachs to be sample by type of sampling, quarter and size class.

Biological sampling (landing and discard)	**Full stomachs to sample**	Total
Quarter		
I	200	200
II	200	200
III	200	200
IV	200	200
Total biological sampling	**800**	**800**
Research survey	Full stomachs to sample	
	100	100
Total survey	**100**	**100**
Total	**900**	**900**

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Annex I. Example of scoreboard for stomach content sampling

Proposed scoreboard for stomach content sampling

SURVEY: ______________________________ HAUL: __________ DATE: ____________________________

RESPONSIBLE: ____________________________________

CODE PREDATOR: ____________________ PREDADOR: _____________________________________

N _______ LENGHT (mm) __________ SEX _______ MATURITY ________ STOMACH STATE ____________ REPL. (cc/g) ____________

PREY NAME	% REP	N	DIG	MIN L.	MAX L.	L (mm)	HP

N _______ LENGHT (mm) __________ SEX _______ MATURITY ________ STOMACH STATE _____________ REPL. (cc) ____________

PREY NAME	% REP	N	DIG	MIN L.	MAX L.	L (mm)	HP

N _______ LENGHT (mm) __________ SEX _______ MATURITY ________ STOMACH STATE _____________ REPL. (cc) ____________

PREY NAME	% REP	N	DIG	MIN L.	MAX L.	L (mm)	HP

N _______ LENGHT (mm) __________ SEX _______ MATURITY ________ STOMACH STATE _____________ REPL. (cc) ____________

PREY NAME	% REP	N	DIG	MIN L.	MAX L.	L (mm)	HP

N _______ LENGHT (mm) __________ SEX _______ MATURITY ________ STOMACH STATE _____________ REPL. (cc) ____________

PREY NAME	% REP	N	DIG	MIN L.	MAX L.	L (mm)	HP

N _______ LENGHT (mm) __________ SEX _______ MATURITY ________ STOMACH STATE _____________ REPL. (cc) ____________

PREY NAME	% REP	N	DIG	MIN L.	MAX L.	L (mm)	HP

N _______ LENGHT (mm) __________ SEX _______ MATURITY ________ STOMACH STATE _____________ REPL. (cc) ____________

PREY NAME	% REP	N	DIG	MIN L.	MAX L.	L (mm)	HP

SEX

M: Male
F: Femal
I: Indeterminate

STOMACH STATE

EM: Empty
EV: Everted
FU: Full

DIGESTION (DIG)

1: Intact
2: Partially digested
3: Well digested

REPL. (cc/g): Repletion (total volume in cubic centimetres, or weight in grams)
% REP: Percentage of repletion by prey
N: number of preys for each species
MIN L., MAX L: minimum and maximum length in mm (when more than 1 individual of each species)
L (mm): length in mm
HP: put a cross if only hard parts (otoliths, peaks, chelipeds,...)

Example of the proposed scoreboard for stomach content sampling