WORKSHOP ON BETTER COORDINATED STOMACH SAMPLING (WKBECOSS)

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Contents

i Executive summary

The Workshop on Better Coordinated Stomach Sampling (WKBECOSS) reviewed existing stomach sampling programmes and associated guidelines. Programmes were categorized as i) stomach sampling for multispecies modelling, ii) stomach sampling for Marine Strategy Framework Directive (MSFD) indicator measurement and development, iii) stomach sampling for ecosystem modelling, iv) stomach sampling for process-related studies driven by fundamental research questions and v) long-term time series of stomach sampling to understand trophic impacts related to large-scale environmental change, such as global warming.

While sampling approaches to meet these different objectives may be linked, the demands for sampling intensity and stomach analyses are fundamentally different. Consequently, findings based on the different data that result will vary in temporal, spatial and organisational scale from studies on consumption rates to analyses of ecosystem connectivity and structure.

Stomach sampling was considered particularly relevant for providing inputs to multispecies models and Ecosystem Status (i.e. MSFD) indicators. For both purposes, detailed sampling best practices exist and, in general, are appropriate. However, a small amount of additional sampling, including of areas outside those strictly included in established surveys, diel and seasonal/monthly variation sampling, and of under-targeted species, would help assess whether current sampling programmes adequately account for predator trophic dynamics in currently evaluated areas.

Currently, few national collection programmes for stomach data are coordinated with others in the ICES region, and no common EU stomach sampling is ongoing. Stomach sampling for multispecies modelling, e.g. ICES Year of the Stomach, has been irregular and typically focussed on relatively few commercially important species, with the aim of estimating predator-prey selectivity. MSFD food web indicators, such as functional groups or mean trophic level, require information across a broader range of taxa, including rare and minor species not of commercial interest, because their aim is to provide evidence of change in structure and functioning within and across ecosystems. In the absence of coordinated international sampling, indicator development has often relied on data collated across projects, such as held on Fishbase and DAPSTOM (integrated database and portal for fish stomach records).

Stable isotope analyses are an additional tool to measure mean trophic level. They provide unique information in relation to stomach contents and genetics because they incorporate a longer-term view of predator diet, albeit with less resolved information on prey identity that can be challenging or impossible to interpret.

Genetic methods show promise in identifying quickly digested prey (e.g. jellyfish), resources of intermediate consumers, lower trophic levels and larvae whose prey may be small, not ingested whole and therefore not readily identifiable using traditional methods. Examples of consumers whose diets could be better resolved using genetic methods include planktivorous fishes, cephalopods and crustaceans. Information on their diets will be key to broadening our understanding of ecosystem structure and functioning. At present, these genetic methods do not provide information on cannibalism or age of prey, and are limited in estimating prey weight and size, and are thus not yet suitable for delivering the data needs for multi-species models and estimating mean trophic level.

ii Expert group information

Expert group name	Workshop on Better Coordinated Stomach Sampling (WKBECOSS)
Expert group cycle	Annual
Year cycle started	2019
Reporting year in cycle	1/1
Chair(s)	Izaskun Preciado, Spain
	Stefan Neuenfeldt, Denmark
Meeting venue(s) and dates	3-6 September, Santander, Spain (19 participants)

1 Terms of Reference

ToR a) Review, update and disseminate existing best practice guidelines for stomach sampling programmes (e.g. spatio-temporal information, sampling sizes, taxonomic resolution of food items, data compatibility with ICES stomach database).

ToR b) Present and discuss recent findings from fish diet studies, including those using stable isotope analysis, relevant for advancing regional stomach sampling schemes.

ToR c) Summarize specific input data needs of end users of fish diet data and define the end products for the data collection (multi-species models, MSFD indicators, etc.).

ToR d) Identify matches and mismatches between end users and current EU MAP (DCF) and national collection of diet data, and propose an Action Plan to improve regional stomach sampling schemes (involving species, methods, sampling design, databases etc.).

2 Term of Reference a) Review, update and disseminate existing best practice guidelines for SCA programmes (e.g. spatio-temporal information, sampling sizes, taxonomic resolution of food items, data compatibility with ICES stomach database)

2.1 Summary of presented SCA programmes

The EU Multi-Annual Programme (EU MAP) on Data Collection requests data on predator-prey relationships and planning for future data collection specific for each marine region, which means that pilot studies involving fish stomach sampling are needed. Currently there is no coordinated EU SCA program and thus the presented SCA programmes aren't nationally or even institutionally coordinated. Despite all these programmes having the goal to extend the knowledge on the diet of the target fish species, and the fact that all register certain information on the target species and analyse stomach contents, there are clear differences originating from the differences in purpose of the programmes and with end-users needs (Chapter 4). To realise the benefits of stomach sampling and other useful methodologies (i.e. stable isotope analyses, genetics) better coordination is needed.

A problem with non-coordinated programmes is that owing to even small differences in methodology it is challenging to combine these data in analysis. Furthermore, the lack of coordination leads to unbalanced sampling effort resulting in a lack of statistically sound sampling of all key species needed for food web characterisation and finally does not allow moving towards the Ecosystem Approach to Fisheries (EAF).

There is a single long running program collecting diet information of a large number of species, the SCA program run by IEO on the continental shelf of the Southern Bay of Biscay. This program provides interesting time-series of dietary development and it provides valuable information for MSFD indicators and ecosystem modelling. Next to this the data is used in process studies as well. The program samples stomachs based on size classes of predatory fish, determines empty/full stomachs, uses the gallbladder to determine regurgitation, prey is determined to the lowest taxonomic level and counted and when possible measured. Besides that, for quantification pur-poses the percentage of volume by each prey item is estimated.

All other presented programmes were based upon the Year(s) of the Stomach in the North Sea, or the EU 'Lot' project MARE/2012/02 in which internationally coordinated stomach sampling occurred in the North Sea and Baltic, or one-off pilot studies.

Poland, Sweden and Germany continued collecting stomachs following the MARE-2012 protocols. The main focus of these programmes is the multi-species modelling and in line with this end-user prey-species identification of most invertebrate species is done on larger groups (e.g. cnidarian, cephalopoda, Euphausiacea, etc.) rather than on the lowest taxonomic level.

Overall these programmes use very similar methods, which are largely in line with the detailed guidelines provided by the FishPi2 (see section 1.9). Discussion issues were:

- Determining regurgitation, with a slightly different interpretation of each other's methods;
- Feasibility of the gall bladder method, agreement on the use, and stages, however large subjectivity in stage determination in the field;
- Registration of empty, regurgitation and full stomachs, with a slightly different interpretation of each other's methods;
- Registration of digested items and otoliths/bones;
- Usefulness of the volumetric method;
- Spatial, seasonal, temporal requirements.

Despite these different methodologies, the data obtained are not essentially different (e.g. weight / volume of stomachs, prey number / volume, prey size), being useful for all possible end-users. The data of these programmes is stored in institutional databases. A data subset was submitted to the ICES-datacentre of inclusion in the ICES stomach database. It is clear that if coordinated stomach sampling becomes operational the ICES stomach database has to be updated and uploading and downloading facilities have to be improved.

Next to the ICES stomach database, CEFAS runs the DAPSTOM database (section 1.3) including current and historical data, from a variety of sources. The database is adjusted to enable the inclusion of data collected using the guidelines of the MARE-2012 and FishPi2 projects.

A major gap in all of the presented programmes and guidelines is the substantiation of the number of stomachs to be sampled. None of the programmes could provide a statistical substantiation of the numbers they currently collect. This is largely due to a lack of clear requirements by the end-users, but also because of the opportunistic nature of most programmes. This is still the case despite the effort of IEO (annex 4) to provide statistical support for their sampling design based on which they decided on the length classes to be sampled. In order to proceed in coordination of stomach programmes, which in most case means convincing countries/institutes to participate, this is a necessity. In the first place because the numbers to be collected determine the required budgets, but also determines if available budgets are sufficient and if not determine if a reduced program could still provide useful data for the end-users data needs. In the second place, there is an ethical aspect to this as mortality/pain is induced to animals for collecting the stomach data. In some of the countries, animals of which diet information is collected by dissection are considered experimental animals. This means the stomach programmes are treated similarly as for example medical experiments on dogs and monkeys, requiring extensive justification of their purpose and the numbers required and have to work on the three R's (Replace, Reduce, Refine, Russel, Burch & Hume, 1959).

Table 1.1. List of EU stomach sampling programmes presented in WKBECOSS showing the goal, area and methodology used

Program	Institute/Country	Goal	Area	Methodology
FishKOSM	Ireland/UK	Multi-species marine community models	Irish and Celtic Sea	ICES guidelines 18 species
Poland stomach sampling	NMFRI Poland	Stock assessment	Baltic Sea	MARE-2012 Cod Flounder
Sweden stomach sampling	Sweden	Stock assessment	Baltic Sea	MARE-2012 Cod

				Flounder
German Bight stomach sampling	TI Germany	Multi-species models	North Sea	MARE-2012 Whiting Cod, Turbot Grey gurnard
GSA 22 stomach sampling	Fisheries Research Institute of Kavala Greece		North Aegean Sea	WKSTCON Hake
French stomach sampling	IFREMER, France	Trophic questions, fundamental research	North Sea, Atlantic, Mediterranean Sea	Various protocols and methods
IEO stomach sampling	IEO, Spain	MSFD foodweb indicators	Southern Bay of Biscay	Volumetric method 24 species
Jellyfish consumption	Cefas, UK	Estimate Jelly fish consumption	Irish and Celtic sea	cnidarian-specific mtDNA primers and sequencing 38 species

2.2 FishKOSM stomach sampling program

FishKOSM (Fisheries Knowledge for Optimal Sustainable Management) is a large scale collaborative project between the Marine Institute (ROI), Agri-Food and Bioscience Institute (NI) and a number of universities. The project covering both the Irish and Celtic Seas (Figure 1. 1), aims to generate the knowledge needed to support an ecosystem approach to fisheries management aligned with the Common Agricultural Policy and Marine Strategy Framework Directive. At the core, FishKOSM proposes to build multi-species marine community models to calculate and evaluate maximum sustainable yield management targets. Multi-species models require predator diet data (fish stomach samples). FishKOSM carried out an extensive review of publically available diet data for the Irish and Celtic Seas. Analysis demonstrate that the majority of these data are limited to commercial species and most originate from historical sampling programmes carried out > 20 years ago. Gap analysis also identified a low level of spatial (ICES divisions) and temporal (quarters) resolution for many species. These data limitations prompted the development of a sampling programme to collect stomach contents for the key and wider species assemblage in the Irish Sea and Celtic Seas management area. Fieldwork in the marine environment is extremely costly if dedicated surveys are required. For this reason, the FishKOSM sampling programme has taken advantage of current ongoing research surveys. Both the Irish Sea and Celtic Sea are periodically surveyed over multiple quarters by project partners AFBI and Marine Institute. Fish species were selected for stomach collected due to their (1) commercial importance (2) important piscivore predator (including early life history stages, eggs, and larvae) and (3) ecosystem prevalence. A full list of target species is given in Table 1. 1. FishKOSM stomach sampling programme followed best research practices as outlined in the Manual for ICES stomach sampling projects in the North Sea and Baltic Sea (ICES 2010) and MARE/2012/02. To ensure comprehensive spatial sampling, the Irish Sea and Celtic Sea were split into sub-regions based on the current sub-region locations, bathymetry, species distribution and expert knowledge. FishKOSM aims to sample six stomachs per 5cm size group for each predator for each sub-region during multiple quarters when possible. Stomachs are taken from individuals already used to gather biological information. To ensure the diet data collected during this programme is available to the wider scientific community for the development of further ecosystem tools and facilitate eco-

system research in the area it will be uploaded to the stomach database maintained by ICES and DAPSTOM.

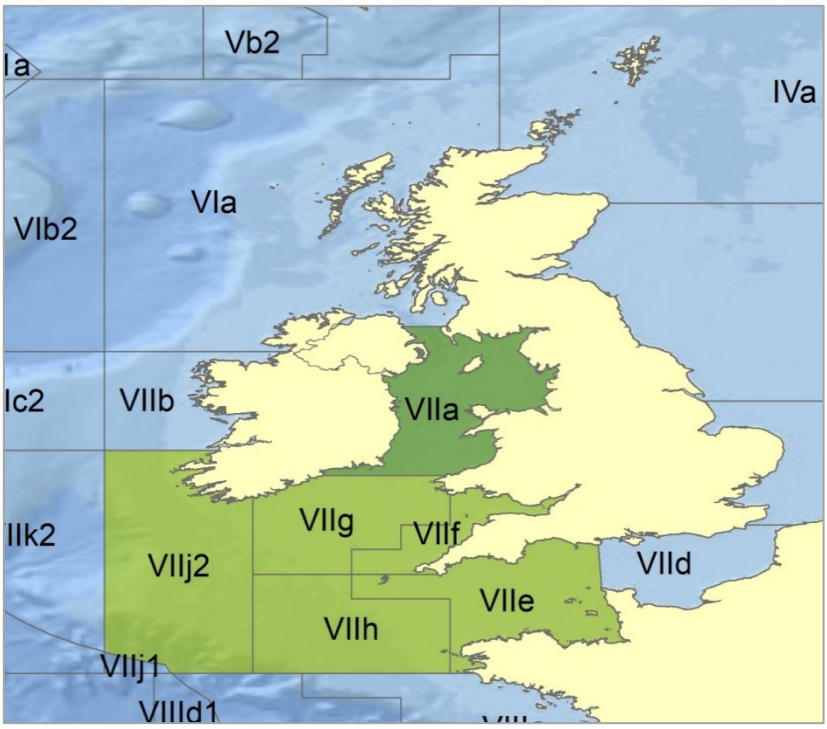

Figure 1.1. Study area of FishKOSM. Irish Sea ICES division VIIa.Celtic Sea ICES divisions VII e – j.

Table 1.1. List of target species in the Irish and Celtic Sea regions.

Species	Latin name	Irish Sea	Celtic Sea
Atlantic cod	*Gadus morhua*	*	*
Haddock	*Melanogrammus aeglefinus*	*	*
Whiting	*Merlangius merlangus*	*	*
European hake	*Merluccius merluccius*	*	*
Lesser spotted dogfish	*Scyliorhinus canicula*	*	*
Grey gurnard	*Eutrigla gurnardus*	*	*
Mackerel	*Scomber scombrus*	*	*
Atlantic herring	*Clupea harengus*	*	*
Sprat	*Sprattus sprattus*	*	*
Spurdog	*Squalus acanthias*	*	
Red gurnard	*Chelidonichthys cuculus*	*	
Tub gurnard	*Chelidonichthys lucerna*	*	
Thornback ray	*Raja clavata*	*	

Spotted ray	*Raja montagui*	*	
Black bellied angler	*Lophius budegassa*		*
White bellied angler	*Lophius piscatorius*		*
Boarfish	*Capros aper*		*
Poor cod > 15cm	*Trisopterus minutus*		*

Figure 1.2. Irish Sea Cod stomach containing *Nephrops norvegicus.*

2.3 Stomach sampling protocol NMFRI Poland

Cod (*Gadus morhua*) is one of the most important fish species in the Baltic Sea due to the both economic and ecological point of view. It is an important species in northern Europe fisheries. From the ecological point of view cod plays a role of top predator in the ecosystem of the Baltic Sea, as well as salmonid fish and marine mammals.

In 2012-2014 NMFRI participated in the project MARE/2012/02 (Study on stomach content of fish to support the assessment of good environmental status of marine food webs and the prediction of MSY after stock restoration) coordinated by DTU Aqua. The research material consisted of stomachs of Baltic cod and whiting. Over 13 000 of cod stomachs and 7000 of whiting have been analyzed. After the project, NMFRI continued collecting cod stomachs. Annually, Baltic cod stomachs are collected during the BITS survey in quarter 1 (February) and quarter 4 (November) in the Polish Exclusive Economic Zone, as a part of EU DCF. Samples of cod are collected: 5 stomachs per 1 cm length group per ICES subdivision 24, 25, 26 (cod 10-14 cm length frozen whole).The methodology of analyzing stomach content is the same as in MARE/2012/02 and based on the 'Manual for ICES Stomach sampling projects in the North Sea and Baltic Sea' (ICES, 2010). The detailed ichthyological analysis of each of the cod individual consists of measuring the total length, total weight, gutted weight, determination of sex and stage of development of

the gonads, the degree of stomach fullness(5 stage scale), gall bladder stage (4 stage scale). Otoliths are collected to determine the age of the fish. The samples of cod stomachs are frozen until analysis of food content. All preys are determined to the lowest possible taxonomic unit based on morphological characteristics, depending on the state of decomposition of the remains. Fish and invertebrate (crabs, shrimps, Isopod *Saduria entomon*) prey are measured and weighted individually. Other prey are counted and weighted. For each food item digestion stage were determined. A database is created.

NMFRI also cooperates with SLU Swedish University of Agricultural Sciences. Since 2016 the Baltic cod and flounder stomachs from Swedish commercial and research surveys have been analyzed. Due to decreasing condition of flounder in Puck Bay, in 2018 stomachs have been collected and analyzed. Research material contained over 500 flounder stomachs from commercial survey.

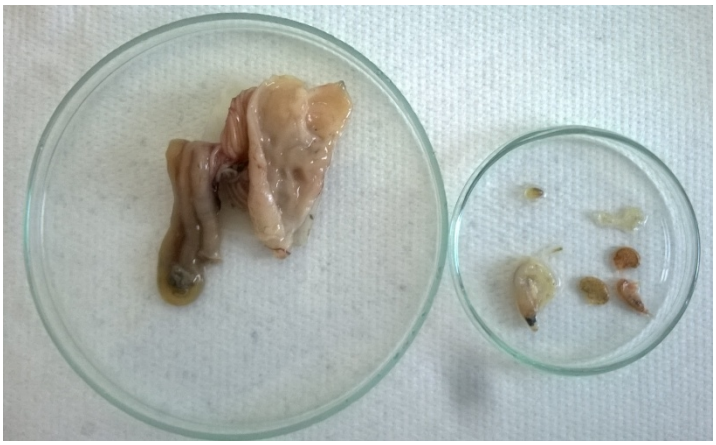

Figure 1.3. Digestive tract of cod (*Gadusmorhua*) and the food items (photo: J. Pawlak)

2.4 DAPSTOM – An Integrated Database and Portal for Fish Stomach Records

In recent years, considerable emphasis has been placed on finding 'ecosystem-based' approaches to fisheries management and multi-species models are seen as crucial for addressing this new agenda. However, there are currently very few long-term datasets within the European context available for parameterising such models. DAPSTOM (integrated database and portal for fish stomach records) is an ongoing UK initiative (supported by Defra and the EU) to digitise and make available fish stomach content records spanning the past 100 years. The online database, hosted by Cefas (the Centre for Environment, Fisheries & Aquaculture Science) contains information (256,354 records from 360,561 individual predator stomachs) on 204 predator species and can be searched by **predator name** or by **prey name** for given sea areas and years. CSV data files can be outputted containing all records from a particular query. A new version of the database (Version 5.5) was completed in August 2019. Records span the period 1836 to 2016 and in this latest version particular emphasis has been placed on adding data from Ireland and Northern Ireland. The DAPSTOM database includes records from fish ranging from 0.1cm total length (a herring larva) to 768 cm for a basking shark caught in 1947. A major achievement over the past year has been the construction of a new 'prey weights' table that allows the user to output diet composition in the form of percentage wet weight (biomass) as well as percentage numbers (figure 1). As part of the EU 'Lot' project MARE/2012/02 the DAPSTOM database was re-engineered, such that it can now be used to yield data in a similar format to that of the ICES 'Year of the Stomach' dataset. The relational database comprises ten interlinked tables (figure 2), some of

which were incorporated to 'correct' the taxonomy of species or to provide more information about sampling procedures. The database also includes a 'PROVENANCE' table providing a detailed description of the original source material (e.g. logbooks, folders of 'raw' data, or full citation for the paper/report etc.). Most of the data included in the database was collected by Cefas scientific staff over the past 100 years, but datasets have also been 'donated' by partner organisations (mostly universities) or have been digitised from published peer-reviewed papers or reports.

The DAPSTOM database (Version 5.5) includes information from sites all over the North East Atlantic. However, half (53%) of the records (52% of stomachs) relate to the North Sea, given that this has continued to be the main focus of survey work at Cefas/MAFF in Lowestoft for the past 115 years. Relatively large numbers of records have also been digitised for the Celtic Sea (9%), Irish Sea (10%) as well as the area around Spitzbergen (10%), where a dedicated survey vessel operated from 1949 to 1977 (see Townhill *et al.* 2015).

The DAPSTOM database has been cited in many peer-reviewed publications and has been used for many different purposes. In particular DAPSTOM has been used:

To study **temporal and spatial variability** in the diet of haddock (Tam *et al.* 2016), whiting (Lauerburg *et al.* 2018) and pelagic fish species in the north Atlantic (Pinnegar *et al.* 2015).

To determine the relative **contributions of pelagic and benthic pathways** to consumer production (Duffill-Telsnig *et al.* 2019; Silberberger *et al.* 2018),

To **parameterise Ecopath with Ecosim food-web models** (Bentley *et al.* 2019), and **multispecies fisheries assessment models** (Thorpe *et al.* 2015; Blanchard *et al.* 2014).

Alongside stable isotope analysis (Hinz *et al.* 2017) and fatty acid profiles (Meyer *et al.* 2019) in order to characterise the feeding behaviour

To define feeding guilds, the status of which can be tracked over time and space, as part of monitoring commitments under the EU Marine Strategy Framework Directive (see Thompson *et al.*, submitted).

To elucidate the **potential predators of particular prey items**, for example the Norway lobster *Nephrops norvegicus* (Sbragaglia *et al.* 2015).

To indicate the **incidence of microplastics or plastic litter** in fish stomachs (e.g. Lusher *et al.* 2013).

Data extraction routines have now been developed by external users (using github) in order to generate tailored outputs, directly by drawing on data 'scraped' from the Cefas web portal.

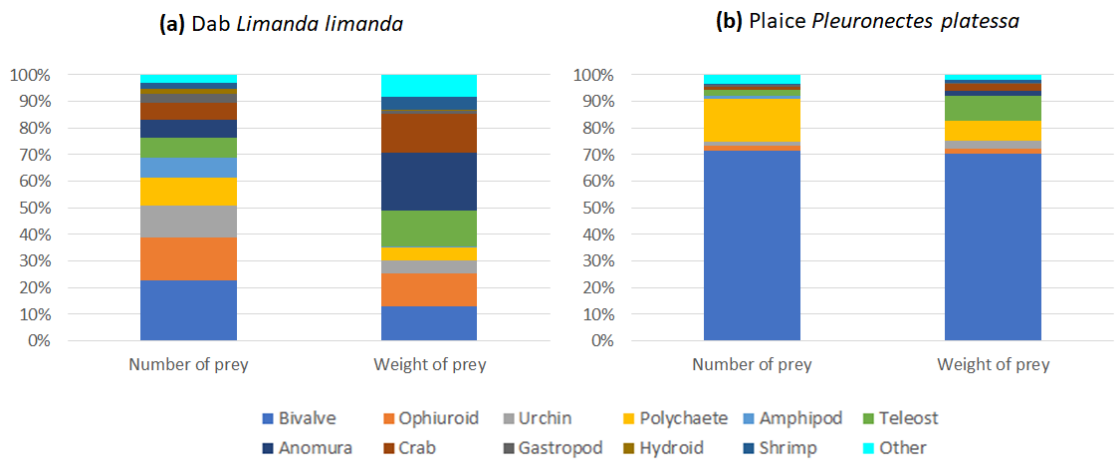

Figure 1.4. Diet composition of (a) dab *Limanda limanda* and (b) plaice *Pleuronectes platessa* based on the number and estimated biomass (wet weight) of prey items consumed.

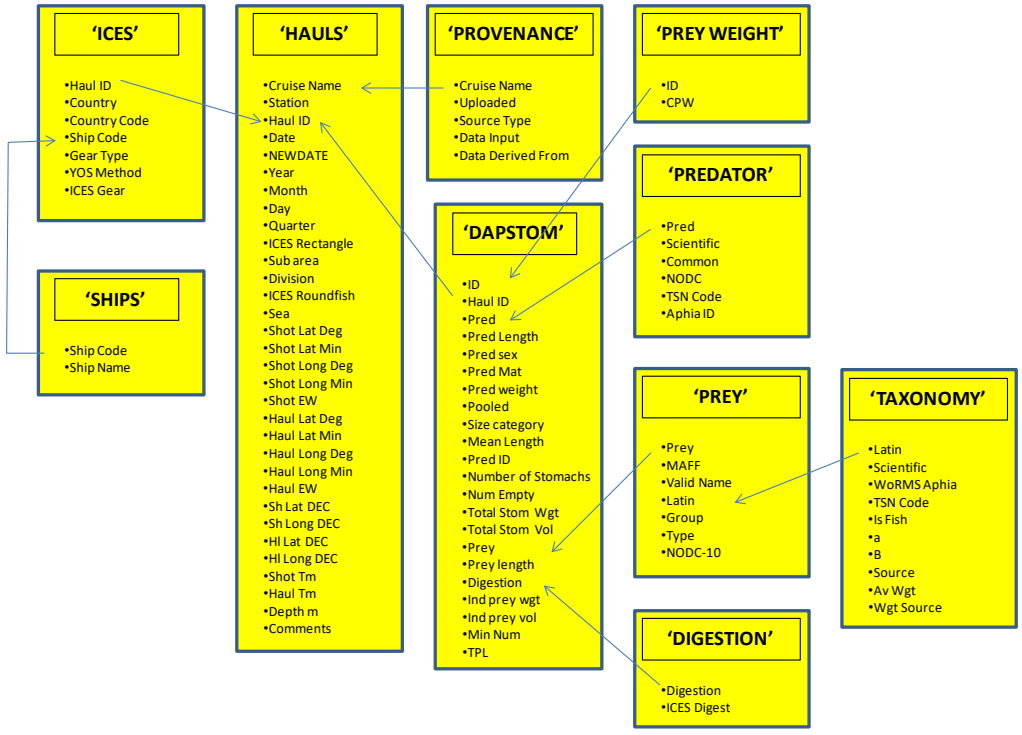

Figure 1.5. Structure of the revised DAPSTOM 5.5 relational database, including a list of the fields included.

A particular characteristic of the DAPSTOM database is that records are available over multiple decades throughout the 20th Century. In some cases it is possible to examine long-term changes in the prey selected by predators, and hence long-term shifts in the structure and functioning of ecosystems. For example, figure 3 shows that the importance of large bivalve molluscs (red) as a prey item for plaice, dab and haddock has declined dramatically since the early 20th Century on the Dogger Bank in the central North Sea, whereas sandeels (yellow) have increased in importance, as have polychaete worms (green) to plaice and crabs (orange) to haddock. The importance of sandeels has also increased in whiting and grey gurnard stomachs (not shown), and these changes reflect real changes that are known to have occurred in the availability of these prey items over the 100 year period. Benthic surveys conducted on the Dogger Bank in the 1950s and more recently have demonstrated that large slow-growing bivalve molluscs have largely

disappeared from this region in recent years, whereas fast-growing polychaetes and crabs have proliferated (Kröncke 1992; Callaway *et al.* 2007).

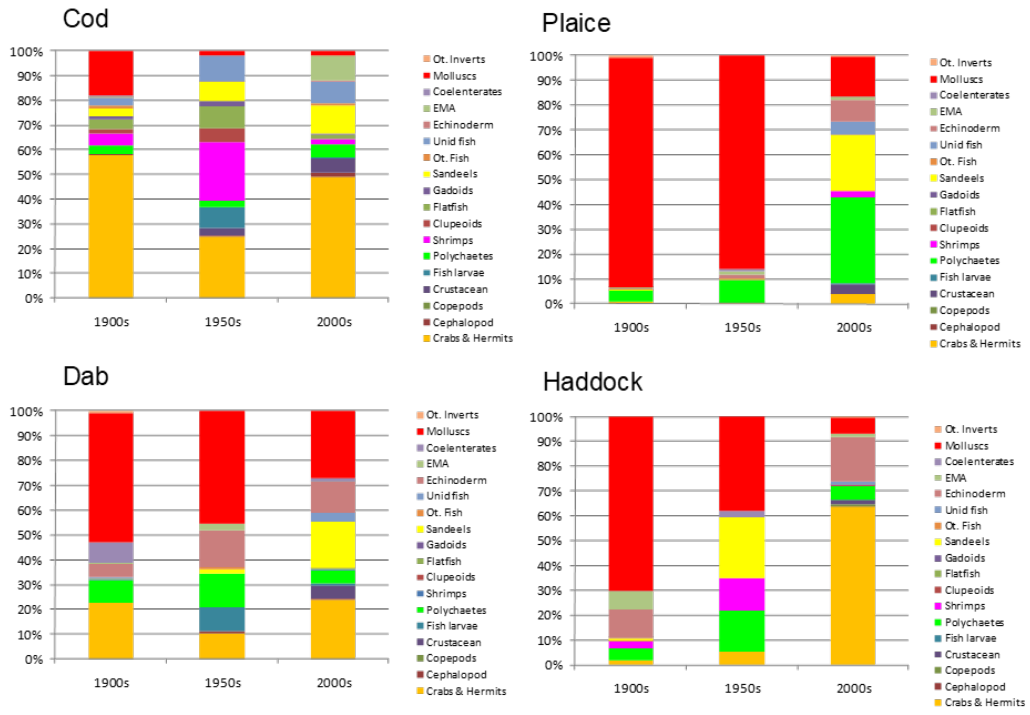

Figure 1.6. Changing diet composition of dab *Limanda limanda* sampled on the Dogger Bank in the North Sea throughout the 20th Century, based on prey number data included in the DAPSTOM database. 'EMA' comprises euphausids, mysids and amphipods.

2.5 German Bight stomach sampling program

Fundamental changes in the importance of natural versus fishing induced mortality are observed while moving towards MSY management targets. The comprehensive reduction of fishing mortality and successive recovery of fish stocks, especially of the larger predatory species, led to an increasing natural mortality as opposed to fishing mortality. Consequently, estimates of natural mortality become more important for stock assessments and forecasts. As part of the EU 'Lot' project MARE/2012/02a pilot study on stomach sampling in the North Sea and Baltic was able to prove, in cooperation with the ICES Working Group on Multi Species Stock Assessment Methods (ICES WGSAM), that cost-effective sampling of stomachs is possible during existing surveys. It was possible to analyse stomachs in a cost-effective manner with the help of national labs and/or external contractors. Results of the FishPi project (EU MARE/2014/19) conclude that opportunistic stomach sampling on existing DCF surveys is a promising way forward. However, missing regional coordination was identified a major problem by the project. The lack of coordination leads to unbalanced sampling effort resulting in a lack of statistically sound sampling of all key species needed for food web characterisation and finally does not allow moving towards the Ecosystem Approach to Fisheries (EAF).

Based on the lessons learned from the DG MARE pilot study and the FishPi project, Germany has decided to start a pilot study for establishing a regular sampling scheme for stomachs on its vessels during international and national surveys in close cooperation with ICES WGSAM, survey planning groups, regional coordination groups and international partner labs. The sampling was carried out based on the guidelines from ICES WGSAM (updated by the FishPi2 project) to ensure that data can be used for multi-species modelling, assessments and advice. It was decided

that rather than trying to sample all potential fish predators in the same year, applying a rolling scheme sampling each year 2-3 key fish predators should be sufficient to ensure a sufficient availability of time series data. In 2018, the pilot study started with sampling whiting (*Merlangius merlangus*) in the Greater German Bight. The samples were all taken during the 3rd quarter on the German part of the International Bottom Trawl survey (IBTS), the German Small-Scale Bottom Trawl Survey (GSBTS) and the near-shore operating Demersal Young Fish Survey (DYFS) (Figure 1.7). The GSBTS uses the same methodology as the IBTS; however, it applies a high-intensity sampling in certain 10 x 10 NM GSBTS-"boxes" to investigate small-scale variations in the abundance and feeding of predator species.

A total of approximately 3000 whiting (6 – 30 cm total length) were sampled on 176 stations. It was decided to sample at each station 2-3 whiting per 5 cm length class. The samples were immediately frozen and transferred to the lab after the completion of the surveys. In the lab, the stomachs are currently being analysed. Since this stomach sampling program is designed to produce results for the application in Multi-species stock assessment models, fish prey in the stomachs are being identified to the most detailed level possible (and weighed and length measured when possible), while the invertebrates are being identified to larger groups (e.g. cnidarian, cephalopoda, Euphausiacea, etc.). Exceptions are commercial species like brown shrimp *Crangon crangon*, edible crab *Cancer pagurus*, common whelk *Buccinum undatum*, etc., which are identified to species or lowest taxonomical level possible. Results of the whiting stomach samplings in 2018 are expected in the second half of 2020.

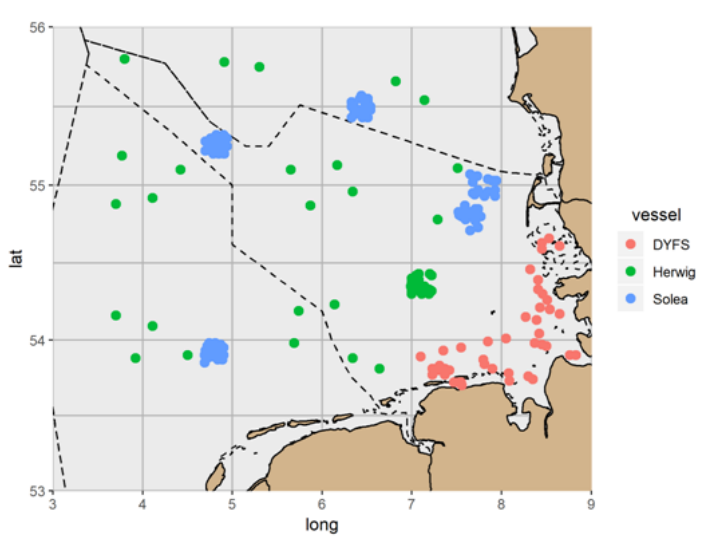

Figure 1.7. Overview of the locations of the whiting samples in the German Bight area during the 2018 stomach sampling pilot study.

2.6 Pilot study on stomach content analysis of *Merluccius merluccius* in the GSA 22 (Greece, North Aegean Sea, Mediterranean Sea)

Following the indication of the workshop organized by the Regional Coordination Group for the Mediterranean and Black Sea (RCG-Med&BS) on sampling, processing and analyzing the fish stomach contents (WKSTCON) in Palma de Mallorca (24-27 April 2018) the Fisheries Research Institute of Kavala (Greece) performed a pilot study on hake (*Merluccius merluccius*) in the GSA 22 during summer 2018. Samples were collected during the MEDITS survey and a total of 102

individuals were processed for stomach content analysis. The sampling stratification by size proposed during the WKSTON was impossible to realize because of the lack of larger individuals collected during the survey. Furthermore the large number of everted stomachs found during the lab analysis demonstrated that the personnel on board was not appropriately trained. Fish were the most important preys (79,3 IRI%) followed by decapoda (19.1 IRI%) and cephalopoda (1,7 IRI%). Anchovy was the most important prey in the stomach contents of hake cough up to 100 m depth. This first pilot study on the diet of hake was a very useful exercise to understand how we can, practically, incorporate diet analysis in the National Program. Samples were easily collected on board by the personnel employed for the MEDITS 2018 survey and the time needed wasn't excessive. Also, a more focused training can overcome the problems associated to the stomach eversion. Some aspect on sampling design should be redefined and discussed.

2.7 Summary of the stomach content samplings performed by Ifremer

Trophic questions have become central in the research topics of Ifremer, notably within the Biological Resources and Ecosystems department, as trophic interactions are key information needed for an integrated fisheries management. As Ifremer's labs is located all along French coastlines, studies investigate all surrounding marine areas, and are mostly hand-tailored to answer specific research questions, with no ongoing routine stomach content survey and no national coordination of stomach content analyses. Studies covered research questions at both assemblage (green boxes in Fig 1.8) and mono or multispecies levels (orange boxes). At assemblage level, projects involve stomach content analysis, in addition with C and N stable isotopes data, to address structure and functioning of fish assemblage and trophic fluxes within the assemblage. At multispecies level, studies using stomach content were mostly driven by specific questions, such as density dependence trophic limitation in nursery, or as a trophic effect originating changes in abundance or condition of commercial species. Most works are based on sampled collected during surveys, with fishes frozen onboard, then dissected and analyzed at laboratory. The organization of surveys and the amount of work at sea do not allow dissecting or analyzing stomach contents onboard. Collection of fish by professional fishermen was also used to resolve some limitation of the current survey scheme (*e.g.* sampling at seasons with no surveys, or for species hard to sample like tuna in the Mediterranean) but may require some methodological adaptations, notably to preserve fish after collection and to limit/stop digestion processes. Stomach contents are analyzed visually at lab with binocular magnifiers coupled with computers. Preys items are identified at the most accurate taxonomic level possible, *i.e.* usually at genus or species level for intact items, following a routine protocol based on degradation stage. Intact or lowly degraded preys are numbered, pictured, measured and weighed. Measurement made on degraded preys depends on the interest of the information. By example, otoliths are numbered, pictured and measured, as otolith number, shape and size are informative on prey number, species and size, while of non-countable items (e.g polychete bristle, remains of colonials animals or of bivalve shells) are just recorded in term of presence/absence. These data, in conjunction with other gathered from repositories such as DAPSTOM, are then used as input data in statistical tools, such as isotopic mixing models, specifically developed tools that combines stable isotope, stomach content and literature data, and in ecosystemic models (mostly EwE, OSMOSE and Atlantis).

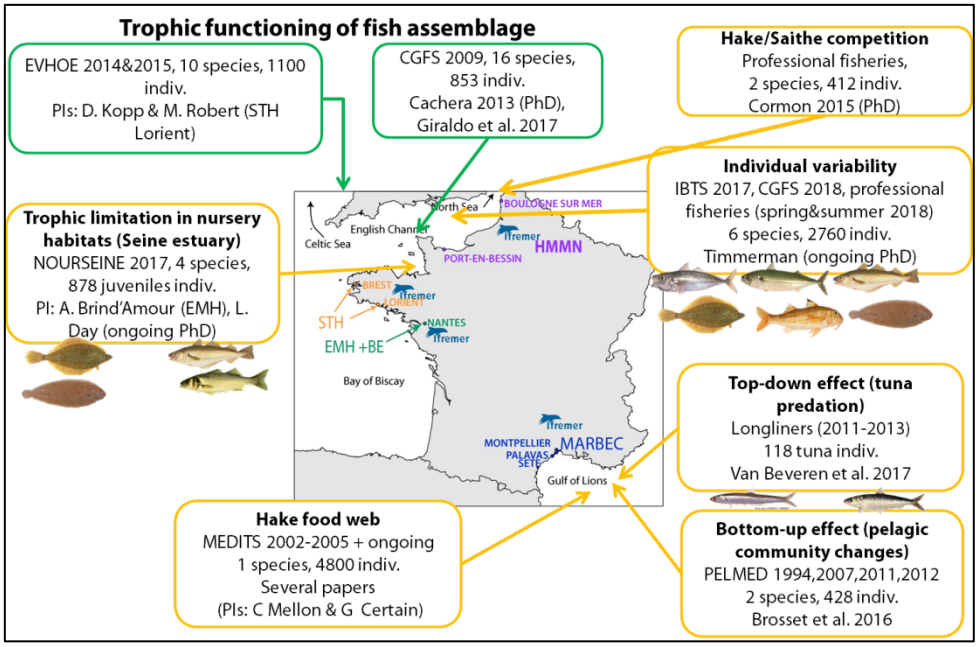

Figure 1.8. Graphical (and not exhaustive!) summary of some Ifremer research projects based on stomach content data. Projects covered questions at assemblage (green boxes) or mono/multispecies levels (orange boxes).

2.8 IEO stomach sampling program

The stomach content analysis protocol is based on the procedures and methodologies carried out every autumn within the Spanish IBTS otter trawl surveys ("Demersales") conducted in the continental shelf of the Southern Bay of Biscay (Cantabrian Sea). Stomach content analysis is a traditional methodology in food web analyses. The "Demersales" protocol is a well-established one which has been proved to reliably characterize some of the most abundant predators' diets in the area (Velasco *et al.*, 1998a, 1998b, Preciado *et al.*, 2008, 2009, 2015).

A set of 24 species have been consistently sampled following almost the same methodology along the entire time series, while a series of prospective diet analyses have also been performed for several predator species to acquire some knowledge on their feeding habits.

Data are collected during IBT surveys on soft bottoms of the Galician and Cantabrian Sea continental shelf. Sampling follows a randomly stratified design over five geographical sectors and three depth strata (a total of 15 sectors-strata), with some additional "special" tows outside these ranges following the same methodology (Figure 1.9).

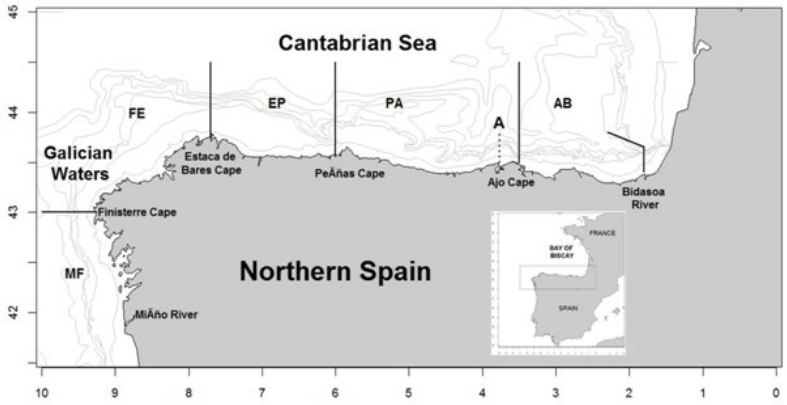

Figure 1.9. Area covered by the Demersales surveys in the Southern Bay of Biscay showing the 5 different sectors considered.

After each haul the catch is separated by species and weighted. All fish and invertebrates are identified at the lowest taxonomic level possible. All retrieved individuals from the total catch of each species (or a representative sample) are counted and measured.Ten individuals (if possible) from each caught predator species, are randomly set aside for stomach content analysis. Exceptionally, the species Merluccius merluccius, Lepidorhombus boscii and Lepidorhombus whiffiagonis are analysed by size range, examining 10 individuals by ontogenetic group. These ontogenetic groups are based on multivariate analyses conducted on the diet data matrices and are within the ranges9 - 17 cm, 18 - 34 cm, 35 - 69 cm and 70 - 90 cm, for M. merluccius (Velasco, 2007),11-17 cm, 18- 32 cm, and > 33 cm for L. whiffiagonis, and ≤ 15 cm, 16- 23 cm, 24 – 36, and 37 - 50 cm for L. boscii. In the case of Merluccius merluccius, and in order to prevent an overestimation of empty stomachs in the sample, the state of the gallbladder is used to determine whether regurgitation has taken place (Robb, 1992). When the gallbladder is empty, the stomach is considered as regurgitated. If not, the stomach is assigned as empty.All prey are separated and identified to the lowest possible taxonomic level and counted, when possible. Prey items (fish and decapod crustaceans) are measured whenever possible.

Quantitative diet estimates are obtained by measuring the stomach content volume using a trophometer (Olaso, 1990).The percentage of volume occupied by each prey in the stomach is estimated, and a "digestion state" degree is given to each prey item following the categorization: 1 = freshly ingested; 2 = partially digested (specimens can still be measured); 3 = highly digested (specimens cannot be measured).

All these data are recorded upon analysis on specifically designed data sheets and directly stored in a database onboard. More information on quality assurance can be found in Annex 5.

2.9 FishPi2_WP4: regional sampling plan for 2019 covering the collection of data on fisheries impacts on the ecosystems

The FishPi2 project was one of four projects funded under the call for proposals MARE/2016/22:Strengthening regional cooperation in the area of fisheries data collection. Annex 1, biological data collection in EU waters. The call was launched in May 2017 and the final report was submitted in July 2019 and the time series data used in the study were mainly from 2015 and 2016. The project has built on the work achieved in the FishPi project, further strengthening regional cooperation, and has provided some clear guidance on the implementation phase of

regional sampling. Work packages have specifically addressed the operation of putative Regional Coordination Groups (RCGs) (WP1); sets out scoping of regional fisheries (WP2); and proposes regional sampling plans for commercial fisheries (WP3). Other work packages have addressed stomach and incidental bycatch sampling (WP4); small scale and recreational fisheries sampling (WP5); and national and regional data quality (WP6). The project outcomes have been disseminated to the North Sea and Eastern Arctic, North Atlantic, and Baltic RCGs in 2018 (WP7). The feedback from these interactions led to a dissemination workshop with National Correspondents and DG MARE representatives in February 2019 (WP8). The project team established close links with other successful consortia and the STREAM project in particular, thus building both within region expertise and facilitating pan-regional cooperation.

The project's Summary Report and its Annexes can be found at the following URLs:

https://datacollection.jrc.ec.europa.eu/docs/regional-grants

https://www.masts.ac.uk/research/

An updated manual with best practices in stomach contents sampling based on the 'Manual for ICES Stomach sampling projects in the North Sea and Baltic Sea'(ICES, 2010) was produced (Annex 1 to this report). The protocol has been used in a trial sampling on the 2018 International Ecosystem Summer Survey in the North Seas for mackerel. To facilitate work on board the survey vessels, it is recommended to take stomach samples from fish already sampled for otoliths etc. up to the limit given in the manual. Species can be sampled in different years in a rolling scheme, ensuring that at least one species for which biological samples are taken (e.g. maturity and/or otoliths) and one species for which this is not the case (and which hence provides a greater increase in work load) is sampled every year and that a maximum of five years passes between the sampling of any one species.

The potential use of modern meta-genomic techniques to identify stomach contents to species was also investigated. This has the potential to drastically reduce the analysis costs, but currently works on a presence/absence basis only.

3 Term of Reference b) Present and discuss recent findings from other methodologies (Stable Isotope Analyses-SIA, DNA-metabarcoding etc..) and their usefulness for ecosystem modelling

A review of different approaches using stomach contents analyses (SCA), stable isotope analyses (SIA) and genetics was presented, showing the utility of these methodologies in ecosystem models. SCA provides an image of the trophic structure of the ecosystem providing a snapshot of the diet while SIA offers a medium-term view on the food assimilated during the recent past (i.e.: previous months) of a species. In this way, stable isotope techniques provide an important tool for answering general questions about trophic structure such as trophic position. The use of DNA techniques offers exclusive advantages compared to morphological analysis, given the high taxonomic resolution of prey items specially with heavily macerated or microscopic prey (Nielsen *et al.*, 2017). These approaches on their own generally does not provide the resolution required to track energy or material flow through a large number of specific food web pathways. To obtain the level of resolution required to discern complex trophic interactions, SCA, SIA and genetics must be used in conjunction. Such information will be key to broadening our understanding of ecosystem structure and functioning. Some examples of the three methodologies are given in this section.

3.1 Why do we need SCA data? A history from an Atlantis modeler perspective

The study focuses on the Bay of Biscay (BoB) ecosystem, a gulf of the Atlantic Ocean situated off the western coast of France and the northern coast of Spain.

This work aims at: 1) analyzing the spatial dynamics of the different human activities occurring in the Bay of Biscay ecosystem and how they interact, 2) exploring how different human activities affect the state of the components of the ecosystem, 3) analyzing the cumulative effects of the activities in certain areas, accounting also for the effects caused by natural stressors and climate change. So, to start with, we need to characterize the system, specify the food web ecology, analyze how the environmental variability affect the dynamics of lower trophic levels, couple hydrography with food web, etc.

An Atlantis model will be used to achieve the goals described in the previous paragraph, since we want and end-to-end modelling tool that help us move towards an integrated and qualitative assessment of the ecosystem for the first time in the Bay of Biscay.

Atlantis is a whole-of system 3D spatially explicit marine ecosystem modelling framework based on dynamically integrated complementary sub-models used to simulate physical and biogeochemical processes, ecology, human uses of marine coastal systems (primarily fisheries) and management. It was developed by CSIRO scientists Beth Fulton and Bec Gorton and it's now been applied to more than 30 locations around the world.

The Atlantis model – case study

As Atlantis is 3D model, the spatial resolution and the geographical boundaries have to be defined. A total of 36 boxes were identified, each of them containing a maximum of 5 vertical layers (0-50 m, 50-100 m, 100-200 m, 200-500 m and 500m+).

For defining the structure of the food web, information on functional groups coming from Moullec *et al.* (2017) was used, adding other relevant species or groups, such as, boarfish, *Mauroli-icus*, tunas and sharks.

The stomach contents in Atlantis

One of the primary ecological processes modelled is predation. It's determined by the user defined predator-prey interaction matrix that sets a maximum availability of each prey biomass available to a specific predator (ranging from 0 to 1). If the predator-prey matrix does not allow a specific interaction (the value is set to 0), predation will never occur. Four values are given for each predator-prey combination: juvenile-juvenile, juvenile-adult, adult-juvenile, adult-adult.

Diet information was taken from most recent literature available in the study area (Moullec *et al.* (2017)).

During the simulation, this diet matrix is modified by the spatial overlap in each cell of the model, biomass of the prey, habitat refuge for the prey, gape limitation of the predator, spawning time of the predator, possible effect of acidification or contaminants on the predator and prey, and availability of fisheries discards as food (Audzijonyte *et al.* (2019)).

Outputs from the model in relation to the stomach content information are in terms of proportional make up of a diet of each age class for each functional group. There is also an option to get the proportions for each cell (box and layer) of each age group for each functional group.

Having good quality stomach content data would be useful for a more accurate and realistic calibration of the model.

3.2 From co-occurrence patterns to trophic connections: insights from integrated ecosystem-based surveys

To evaluate the dynamics of the pelagic ecosystems, we need to better understand the trophic interactions between different pelagic components, including the interaction with different external stressors such as fishing activity and environmental changes. Within this context, we present the EPELECO research project (funded by the Spanish Ministry of Science, Innovation and University) based on a multidisciplinary approach that integrates the existing and innovative information derived from integrated ecosystem surveys to implement an ecosystem-based management of the Bay of Biscay pelagic ecosystem.

Integrated oceanographic surveys collect information on oceanographic conditions and 3D distribution of different pelagic species (plankton, fish) (Boyra *et al.* 2016) to advance our understanding on the distribution and abundance patterns of different megafauna species based on integrated ecosystem surveys of the Bay of Biscay. Different ecosystem components can be integrated (1) to understand the 3D environment of pelagic predators (Louzao *et al.* 2019) and (2) to identify interspecific associations of the pelagic predator-prey networks that can be applied for management purposes (Astarloa *et al.* 2019) such as (3) the assessment of marine protected areas networks (García-Barón *et al.* 2019), (4) the evaluation of the Good Environmental Status within the Marine Strategy Framework Directive (Saavedra *et al.* 2018) and (5) the study of physical transport of floating marine litter (Declerck et al 2019) to advance ecosystem-based management.

To advance towards the implementation of the ecosystem-based fisheries management (EBFM), it is necessary to obtain a detailed description of the biotic interactions (i.e. predator-prey relationships) structuring pelagic ecosystems. For that, it is necessary to obtain not only information on predator and prey distribution and abundance, but also on competition and predation processes that are needed to support several policies (e.g. Common Fisheries Policy, Marine Strategy Framework Directive) that envisage the implementation of an EBFM. With this overall objective

in mind, the EPELECO project will describe the biotic interactions through the pelagic food web of the Bay of Biscay by using a holistic framework to better understand the dynamics of pelagic species and thus provide appropriate information to decision makers.

3.3 Stomach sampling and molecular techniques

Molecular gut content analysis (the use of DNA to describe prey items within a stomach) offers unique advantages (and a few caveats) compared to morphological analysis. The principal advantages are that taxonomic expertise is not required during stomach dissection (recovered DNA is matched against a database) and is well suited to identifying heavily macerated or microscopic prey (Nielsen *et al.*, 2017). Furthermore, depending on the cost of scientist-time, molecular techniques are often more cost-effective when processing large numbers of stomachs.

Most molecular techniques rely on primers (synthetic sections of DNA designed to match a sequence in target DNA) to copy sections of DNA until there is a strong signal for analysis. Primers can be designed to target single species or taxa spanning many phyla. Adapting the type of primer used changes the type of ecological question to be answered:

- Single-taxa primers are well suited for identifying cryptic predation or resolving the identity of prey that cannot be resolved to level required for management. For example, predation of eggs and larvae significantly affects stock recruitment, yet many eggs cannot be identified to species level. Fox *et al.*(2012) used molecular probes to determine the incidence of plaice egg predation when morphological GCA was unable to do so. Similarly, single-taxa primers can be used to identify the presence and trophic role of rare or invasive species;
- Multiple-taxa primers can be used to resolve the role of functional groups. Lamb *et al.*(2017) used cnidarian-specific primers to uncover widespread jellyfish predation in the Irish Sea. Previously, due to the rapid rate of digestion (Arai *et al.*, 2003), jellyfish predation had been missed in many fish species by morphological gut contents analysis;
- Universal primers amplify a broad range of phyla (usually multiple phyla – although it must be noted no primer will sample all biodiversity, to achieve more-complete coverage multiple primers should be used). These can be used to characterise the diet of an organism: this is especially useful for microscopic prey which require painstaking microscope use. For instance (Walters *et al.*, 2019) used universal primers to characterise the diet of gelatinous zooplankton using metabarcoding.

Looking forward molecular GCA will harness the increasing power of sequencers and analytical approaches. The extreme sensitivity of the technique is such that the prey of prey (secondary predation) can also be detected (Nielsen *et al.*, 2017), but secondary predation cannot be conclusively demonstrated without direct observation via traditional stomach content analysis. Nevertheless, stomach samples from top predators could provide highly resolved taxonomic information of, e.g., resources at the base of the food web in a way that has not been achieved using traditional techniques. This could be leveraged to use top predators gut contents as a means of retrieving information about food webs. Similar techniques have already been applied to sample terrestrial biodiversity (Boyer *et al.*, 2015). New sequencing technologies now facilitate the molecular assessment of diet without the need for primers (Srivathsan *et al.*, 2016), this is significant because all DNA within the stomachs can be detected (not just that which primers have an affinity for). This may also allow quantitative data to be used, as copying DNA with primers, is thought to be one of the major sources of quantitative bias (Piñol *et al.*, 2018; Lamb *et al.*, 2019). Finally, as the technology is being miniaturised (e.g Oxford Nanopores MinION) facilitating onsite molecular analysis (Peel *et al.*, 2019): this could be particularly useful for rapidly identifying parasite or pathogen outbreaks in aquaculture.

3.4 How should DNA metabarcoding based food web data be integrated into marine ecosystem models?

Understanding predator-prey interactions is key for a successful ecosystem approach- based fisheries management. Yet, comprehensive datasets on fish diets are lacking, mostly due to the difficulty and high economic cost of taxonomically characterizing fish stomach contents, composed of preys of a broad range of organismal groups, which are often semi-digested and/or in early life stages. DNA metabarcoding has been shown as a promising tool for inventorying gut contents, but technical challenges, such as the presence of large amounts of predator DNA or the difficulty of relating number of reads to biomass, as well as the suitability of DNA metabarcoding data to feed ecosystem models have yet to be addressed. Here, we have presented the results of an experiment testing the efficiency of newly designed blocking primers to avoid amplification of predator DNA in metabarcoding-based analyses of stomach contents of European hake (*Merluccius merluccius*), European anchovy (*Engraulis encrasicolus*), European sardine (*Sardina pilchardus*), Atlantic horse mackerel (*Trachurus trachurus*) and Altantic mackerel (*Scomber scombrus*). Our results show that blocking primers are efficient in preventing large amounts of predator sequencing reads, but also reveal that they can affect the amount of preys detected and/or their relative proportions. We conclude evaluating the appropriateness and cost-effectiveness of DNA-based stomach content data for feeding multispecific or ecosystem models or for developing ecosystem indicators requires a clear understanding of the advantages and disadvantages of the methodology coupled with an evaluation of the assessment needs. In this sense, metabarcoding is advantageous with respect to visual inspection of stomach contents as i) it can accurate taxonomic classification even for semi-digested preys and early life stages, ii) it does not rely on taxonomic expertise, iii) it can be automatized to analyze hundreds of stomachs simultaneously, iv) it is less costly. However, metabarcoding is not suitable to i) detect cannibalism, ii) distinguish preys eaten by other preys as oppose to being eaten directly by the predator, iii) quantify number of individuals (although it can provide approximate biomass estimates), iv) distinguish among age classes of preys.

3.5 Using molecular techniques to study fish predation in jellyfish

A very recent development is the emergence of molecular techniques to provide insights into the identity of fish stomach contents. Such methods are useful because they can yield information about species or life-stages (e.g. gelatinous plankton, fish eggs etc.) that are otherwise very difficult to detect from visual examination alone. Lamb *et al.* (2018, 2019b) used molecular techniques (cnidarian-specific mtDNA primers and sequencing) to examine fish stomachs from the Irish and Celtic sea (38 species, 1126 individual stomachs) to determine whether or not predators had eaten jellyfish, and this suggested that many more fish predators including mackerel, herring and whiting consume jellyfish than were previously appreciated (see figure 1). Researchers can either use specific primers to detect particular prey items (e.g. jellyfish), or they can use next-generation sequencing to provide a full inventory of all prey items in a particular stomach. These techniques are starting to become more frequently used and economically viable, sometimes alongside traditional gut content analysis. Molecular analysis typically yields data that would be equivalent to 'frequency of occurrence', but there is growing evidence that it can yield quantitative information that correlates (although admittedly only weakly) with % weight or % volume (Lamb *et al.* 2019). For now, such data are likely to be complimentary to the existing datasets and it may even be possible to accommodate such information in future releases of databases (such as DAPSTOM), or in ecosystem models in the not too distant future.

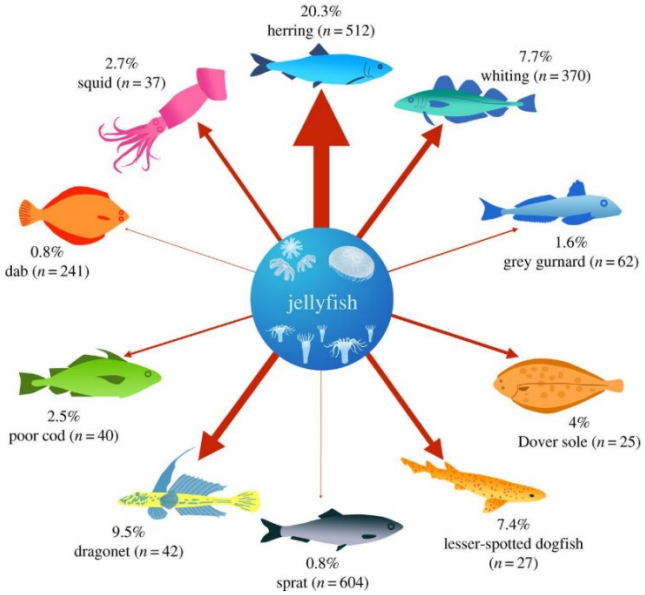

Figure 2.1. Species that feed on jellyfish in the Irish Sea validated using molecular sequencing. Thickness of arrow is representative of the percentage of stomachs jellyfish were detected in (also displayed as a percentage) across the years 2008–2009. Reported sample sizes (n) refer to the number of stomachs sampled from each species (from Lamb *et al.* 2018).

4 Terms of Reference c) Data needs of end users of SCA data and define the end products for the data collection (multi-species models, MSFD indicators, etc.)

Stomach sampling was considered to be particularly relevant for multispecies models and food web indicators (i.e. MSFD). Good practices exist for both final products, although some shortcomings and limitations were discussed: stomach sampling for multispecies modelling, e.g. ICES Year of the Stomach, has been irregular and typically focussed on relatively few commercially important species with the aim of estimating predator-prey selectivity. MSFD food web indicators, such as mean trophic level and trophic guild indicator, require information across a broader range of taxa, including non commercial species, to provide evidence of change in structure and functioning within and across ecosystems. This section gives examples of the use of SCA in multispecies models and MSFD indicators.

4.1 Stomach Content Analysis in multispecies modeling

In multispecies models, predation mortality of commercially important prey species and food preferences of their predators are based on stomach contents data by size. The food preference process has been size based because preference depends on size rather than age. Maximum likelihood technique is e.g. in SMS (Vinther and Lewy 2004) used to estimate parameters and to weight the various data sources. The likelihood function consists as a sum of four terms for observations of international catch at age, survey CPUE and stomach contents observation, and a stock-recruitment (penalty) function.

In a multispecies model including fish predation total mortality, total mortality Z is divided into three components, natural mortality exclusive predation (M1), predation mortality (M2) and fishing mortality (F):

$$Z(s, a, y, q)\ Z(s, a, y, q) = M1(s, a, q) + M\ 2(s, a, y, q) + F(s, a, y, q) \quad (3)$$

The indices s, a, y, q represent species, age, year and quarter. The quarterly separation is applied to account for seasonal changes in the predators´ feeding behaviour. Natural mortality is divided into two components, predation mortality caused by the predators included in the model (M2) and a residual natural mortality (M1). The residual mortality is assumed to be known and is given as input.

The predation mortality of a prey entity due to predation from a predator entity is calculated as function of the predator's food intake rate and the predator´s selectivity for the given prey. For this reason, stomach data that are collected to inform multispecies models of this type need to contain data on single prey items mass and length. They also need to resemble the seasonal changes in predator feeding behaviour. Finally, it is implicitly assumed that the stomach content data cover the complete distributional area of the predator, and that this distributional areas as well as the resulting predator-prey overlap are constant in time. SMS foresees the possibility to correct for changes in spatial overlap tough, but this would require regular sampling of predator and prey spatial distributions.

Hence, the observations considered for modelling predator food preference are the average proportions by weight in the stomach averaged over the entire North Sea and obtained from stomach samples. The observations for given prey and predator species, STOM (lprey, lpred,y,q), are grouped by size groups, lprey and lpred and are assumed to be stochastic variables subject to sampling and process variations.

4.2 Use of SCA in trophic indicator development

Food web and trophodynamic indicators are considered useful tools when trying to gain a holistic and broad vision of the state of ecosystems and the various compartments inhabiting them. Specifically, they have proved useful in broad Environmental Status assessments and monitoring schemes such as those envisioned within the MSFD. However, their acceptance as common indicators is still under surveillance, their increased use depending on their capacity to reflect the effects of specific pressures (i.e., fishing), and/or the reliability and availability of the data used to construct them. In this context, stomach content analyses (SCA) provide a valuable source of data for many trophic indicators.

At the IEO's Santander trophic group we have been working in trophic indicator development for the past years, using the stomach content historical series compiled during the "Demersales" IBTS surveys over the past 25 years.

Within OSPAR's indicator development framework, based on an ecosystem and regionally coordinated approach to management, we have collaborated with French colleagues in the development and testing of the FW4 (Mean trophic level of marine predators, Arroyo *et al.*, 2019) indicator, common in the Bay of Biscay, and in the first outline of the potential indices to be used within the FW9 (Ecological Network Analysis, ENA) indicator.

We used SCA to calculate regionally adjusted trophic levels for the main predator species, based on data compiled during the IBT surveys and used the same data to examine ontogenic variations in TL, and how these variations affected overall calculations in MTL trends.

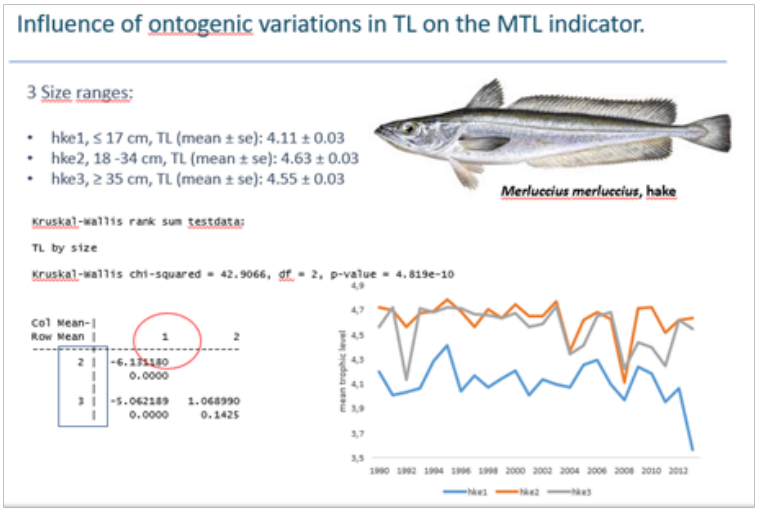

Figure 3.1. Influence of ontogenetic variations in trophic levels on the Mean Trophic Level indicator.

We also used SCA data to calculate the number of links between the main predator species and their potential prey items, as well as the interaction strength of these relationships and their evolution over time (both FW9, ENA indices, Arroyo *et al.*, 2017). These trends we compared with overall bentho-demersal species and functional group abundance and diversity trends, in order to obtain a picture of the effect of fisheries regulations in the food web.

4.3 Small scale spatial variations of trawling impact on food web structure

In the present work we explored the impact of bottom trawling on the structure of benthic and demersal communities and the spread of this impact through the food web. For this purpose nine ecological indicators were developed: 4 community indicators i) total biomass, ii) species richness, iii) Shannon diversity in biomass (H'_b), iv) Shannon diversity in number (H'_n) and 5 trophic indicators v) fullness index, vi) trophic richness, vii) trophic diversity in volume (H'_v), viii) trophic diversity in number (H'_n) and ix) mean Trophic Level of the community using a cut-off 2 (mTL_2). We also analysed the impact of bottom trawling on the biomass of 15 functional groups. Two types of data were used: biological data coming from the IBT survey Demersales (southern Bay of Biscay) and pressure data (Vessel Monitoring System).

Nine out of 15 functional groups showed significant changes, most of them with a decreasing trend with increasing fishing effort: benthic cephalopods, benthivorous fish, echinoderms, large demersal fish, rays and squids. Only 2 functional groups (benthic and deposit-feeder decapods) showed increased biomass in high trawled areas which could be attributed to the removal of large biomasses of their predators. The increase in deposit-feeders decapods (such as squat lobster or pagurid crabs) could also respond to an increase in prey availability, in the form of injured prey and carrion supplied by discards and left by otter trawls.

Significant decrease in total biomass and species richness of the community with increasing fishing effort was also detected. Regarding trophic indicators, we also found significant changes although with different responses. Trophic richness, trophic diversity and mTL declined significantly with increasing pressure. However, fullness index showed significant increase. We also computed changes in mTL between fishing and non-fishing scenario with a spatial resolution of 3 km x 3 km (Figure 3.2). We first produced a no-fishing scenario (where all VMS values where substituted by 0). Then the difference between both scenarios (fishing and non-fishing) was assessed by computing the percentage of change as follows: % Change in mTL= ΔmTL between scenarios/mTL in real scenario. Red areas in Figure 3.2 show 21% mTL lower values than those expected in a non-fishing scenario.

Using this approach we conclude that 1) biomass and richness of bentho-demersal communities decline with increasing fishing effort, 2) fishing impact spreads through the benthic-demersal food web and 3) mean Trophic Level (mTL) of the community response to fishing pressure. We also stress the need of small-scale spatial resolution when investigating fishing impact.

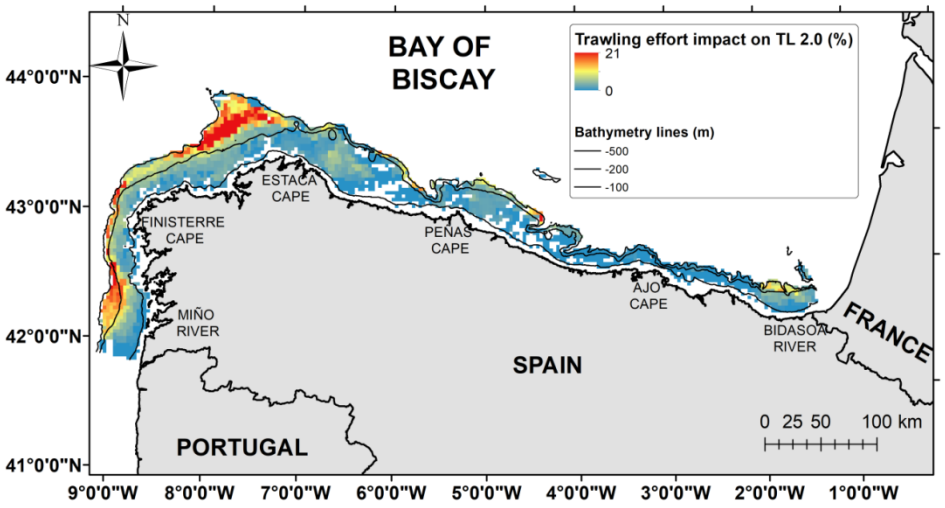

Figure 3.2. Map showing the decrease in mTL_2 of the community between fishing and non-fishing scenarios according to the predictions made by the Generalised Additive Model.

4.4 A feeding guild indicator to assess environmental change impacts on marine ecosystem structure and functioning

Summary

Integrating food web indicators into ecological status assessments is central to developing effective management measures. However, the substantial investment required to construct site-specific food webs has hampered the development of such indicators. Information from inventories of trophic interactions can be applied to infer food web structure and energy flow. We use data from 415,294 fish stomachs to demonstrate how feeding guilds (i.e. groupings based on diet and life stage) can be defined and then apply these to investigate changes in the distribution of fish biomass in the North Sea.

Seven distinct feeding guilds were evident in the trophic interaction data. Differences between guilds were related to predator size which positively correlated with piscivory and habitat, with pelagic, benthic and shallow-coastal foraging apparent. Guild biomasses were largely consistent through time at the North Sea-level and spatially aggregated at the regional-level with spatio-temporal change relating to changes in resource availability, temperature, fishing, and the biomass of other guilds. This suggests that fish biomass was partitioned across broad feeding and environmental niches, and changes over time were governed partly by guild carrying capacities, but also by a combination of covariates with contrasting patterns of change.

Feeding guilds could be developed as a food web indicator to assess Good Environmental Status of the North East Atlantic shelf system, and enable targeted management advice focused towards specific guilds and pressures in a given area. Such an approach could be readily extended to other marine ecosystems and biomes to establish its wider applicability.

Guild classification

Guilds are defined here as a group of predators that have many prey taxa in common, and whose prey differentiate it from other predator guilds. We pooled all observed feeding links for five size classes of each predator taxa (usually predator species; predator groupings are thus referred to as taxa-by-size-classes) across both space and time to produce an aggregated diet for each. We

pooled in this way because stomach contents analysis captures only a snapshot of a predator's diet, predators are typically gape-limited (i.e. body size is an important determinant of what prey are available to a predator), the developmental stage of fish is important for stock assessment, and fishing is known to disproportionately remove large fish from high trophic levels (Greenstreet *et al.*, 2011; Shephard *et al.*, 2012; Shin *et al.*, 2005). Taxa-by-size-class categories were defined as: <3 cm considered larvae (Lv); small juvenile fish (Js) of 3 cm to half of length at maturity; juvenile-medium fish (Jm) from half of length at maturity to length at maturity; medium fish (M) from length at maturity to half-length at infinity; and large fish (L) above half-length at infinity. Length at maturity and length at infinity were estimated for fish taxa using the R package Fishlife (Thorson, Munch, Cope, &Gao, 2017).

The following seven guilds were identified (Fig. 1):

1. A 'Generalist planktivore' guild containing forage fish such as herring (*Clupea harengus*), mackerel (*Scomber scombrus*), sandeel (Ammodytes), and Norway pout (*Trisopterus esmarkii*), and early life stages of the demersal taxa saithe (*Pollachius virens*) and whiting (*Merlangius merlangus*). Their diet consisted of high proportions of krill zooplankton (Euphausiidae, Calanidae and Temoridae), but also benthic dwelling amphipods (Hyperiidae) and sandeel (Table S2);

2. A 'Zooplanktivore' guild feeding mostly on planktonic arthropods (Clausocalanidae, Calanidae and Temoridae). This guild was almost exclusively larvae and/ or juvenile life-stages of taxa, such as dab (*Limanda limanda*), cod (*Gadus morhua*) and herring, but also included all size classes of sprat (*Sprattus sprattus*);

3. A 'Coastal benthivore' guild, whose diet was made up of benthic dwelling crabs (Porcellanidae and Portunidae), worms (Nereididae), and shrimp known to be both benthic and planktonic (Mysidae, Crangonidae and Gammaridae). This guild contained a range of size classes of many coastal dwelling taxa such seabass (*Dicentrarchus labrax*), flounder (*Platichthys flesus*) and shanny (*Lipophrys pholis*);

4. A 'Generalist benthivore' guild containing taxa such as rockling (*Ciliata mustela*, *Enchelyopus-cimbrius*), American plaice (*Hippoglossoides platessoides*) and lemon sole (*Microstomus kitt*), and preyed mostly on benthic prey (65.8%), but was more piscivorous (17.8%) and planktivorous (16.1%) than the other benthic guilds;

5. A 'Specialist benthivore' guild containing a mixture of size classes of dab, haddock (*Melanogrammusaeglefinus*), European plaice (*Pleuronectes platessa*) and sole (*Soleaosolea*), which consumed the highest proportion of benthic prey (88.3%), with sandeel, clams (Semelidae, Pharidae) and trumpet worms (Pectinariidae) most frequently encountered;

6. A 'Zoobenthivore' guild, containing bentho-demersal predators such as juvenile to medium sized rays (*Raja brachyura*, *Raja clavata*, *Leucoraja naevus*, and *Raja montagui*) gurnard (*Chelidonichthyscuculus* and *Chelidonichthyslucerna*) and poor cod (*Trisopterusminutus*). This guild consumed relatively high proportions of benthic dwelling shrimp and crabs (72.3%; e.g. Crangonidae, Pandalidae and Portunidae) but also fish (15%) and planktonic prey (11.7%);

7. A 'Piscivore' guild containing many commercially valuable taxa such as cod, hake (*Merluccius merluccius*), whiting and Turbot (*Psetta maxima*), among apex predators such as tope (G*aleorhinus galeus*), blue shark (*Prionace glauca*) and Starry ray (*Amblyraja radiata*), but also smaller taxa such lesser weever fish (*Echiichthys vipera*) and Grey gurnard (*Eutrigla gurnardus*). This guild was the most piscivorous (57.9%, e.g. Gadidae, Ammodytidae and Clupeidae), with important contributions of shrimp also (e.g. Crangonidae and Euphausiidae).

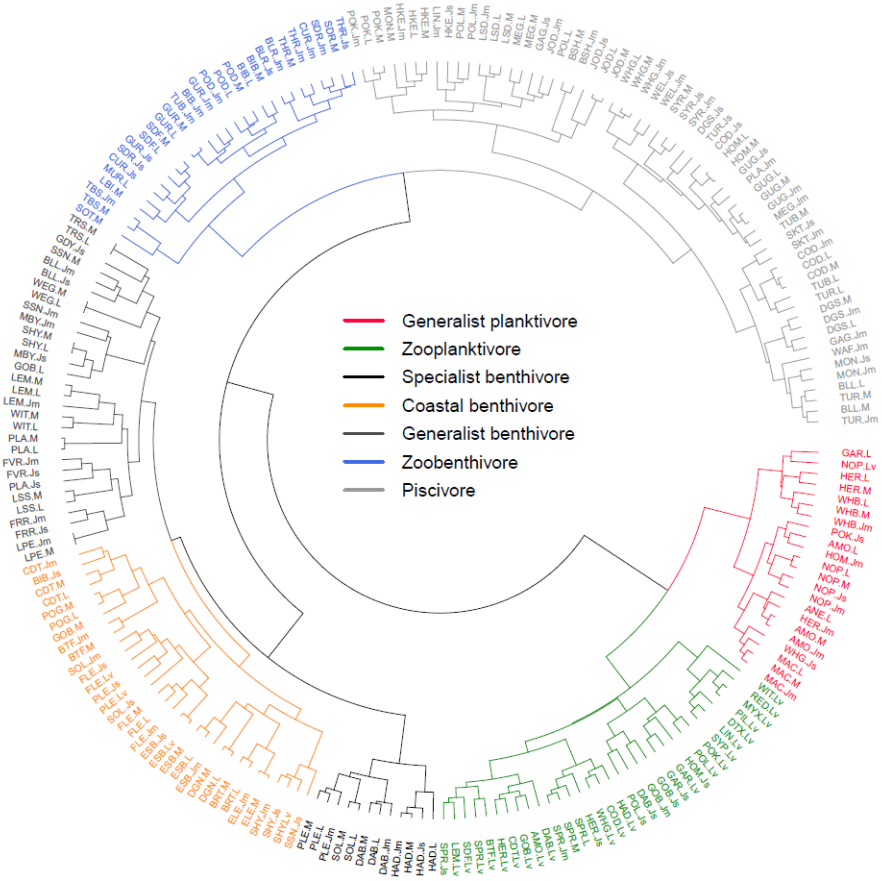

Figure 3.3. Dendrogram showing seven feeding guild clusters (letter codes represent taxa and size category, e.g. COD.L = large cod, see Table S1) based on prey families present in stomach contents.

Guilds were widely distributed but their biomass was spatially aggregated within the North Sea (Fig. 2). The Piscivore and Zoobenthivore guilds aggregated in the west, Specialist and Generalist benthivore guilds in the north, the Coastal benthivore and Zooplanktivore guilds in the south, and Generalist planktivores were more patchy aggregating around Dogger bank and in the north, among other areas.

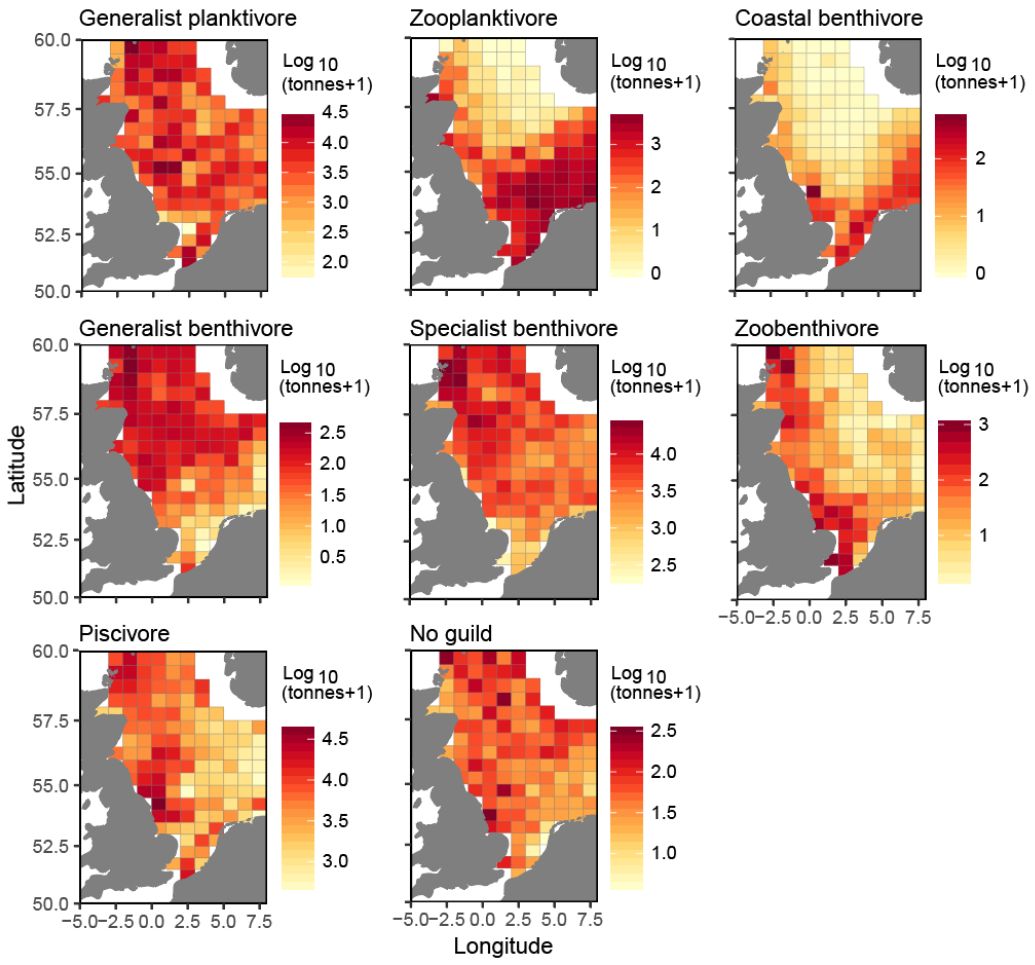

Figure 3.4. Mean feeding guild biomass distribution between 1985 and 2014 across ICES statistical rectangles in the North Sea.

Looking ahead

Much stomach sampling effort to date has focused on commercially valuable species and size classes which means many predators have little or no data. Our indicator work would benefit if those poorly resolved diets were targeted (Table 1). That doesn't exclude targeting other species and sizes, but rather there are diverse needs for feeding information that need to be considered.

Feeding interactions could be further resolved by inferring from similar predators where species-level data are sparse (e.g. following Gray *et al.*, 2015), but also via predictive modelling (Link, 2004; Petchey *et al.*, 2008) and future targeted stomach content sampling (e.g., under-sampled species-size-class feeding interactions, Table 1) using conventional and emerging molecular techniques (e.g. see Pompanon *et al.*, 2012).Information on interactions that have so far been difficult to detect, e.g. on jellyfish that are quickly digested (Lamb *et al.* 2018, 2019b), could further refine our understanding of feeding guilds.

Table 3.1. Species and size classes with limited diet information across ICES and DAPSTOM databases. Priority species are highlighted in green. Entries with 0 data show species and/ or size classes that were caught in the Greater North Sea otter trawl data in quarter 1 (i.e. the International Bottom Trawl Survey) that have no available stomach contents data. Entries with relatively high values (>30) are those whose prey taxonomy was not resolved to family level.

Taxa	Common	Code	Size class	Size category (cm)	N. guts
Raja undulata	Undulate ray	UNR	Jm	35 to 69	1

Raja montagui	Spotted ray	SDR	L	69+	11
Raja microocellata	Painted ray	PTR	Jm	40 to 80	2
Raja fullonica	Shagreen ray	SHR	Jm	29 to 57	13
Raja fullonica	Shagreen ray	SHR	Js	3 to 29	2
Raja fullonica	Shagreen ray	SHR	L	70+	5
Raja fullonica	Shagreen ray	SHR	M	57 to 70	6
Raja clavata	Thornback ray (roker)	THR	L	98+	4
Raja circularis	Sandy ray	SAR	Js	3 to 29	3
Raja circularis	Sandy ray	SAR	M	57 to 70	1
Raja brachyura	Blonde ray	BLR	M	91 to 110	0
Molva molva	Common ling	LIN	Js	3 to 42	24
Molva molva	Common ling	LIN	L	116+	1
Molva molva	Common ling	LIN	M	84 to 116	15
Lophius piscatorius	Anglerfish (monk)	MON	L	96+	15
Lophius budegassa	Black-bellied anglerfish	WAF	Js	3 to 17	17
Lophius budegassa	Black-bellied anglerfish	WAF	L	61+	3
Lophius budegassa	Black-bellied anglerfish	WAF	M	35 to 61	20
Lophiidae	Angler fishes	ANF	Jm	14 to 27	5
Lophiidae	Angler fishes	ANF	L	44+	1
Lophiidae	Angler fishes	ANF	M	27 to 44	8
Anguilla anguilla	European eel	ELE	Js	3 to 17	2
Anguilla anguilla	European eel	ELE	L	53+	5
Anarhichas lupus	Catfish (wolffish)	CAA	Jm	33 to 66	19
Anarhichas lupus	Catfish (wolffish)	CAA	Js	3 to 33	2
Anarhichas lupus	Catfish (wolffish)	CAA	L	96+	8
Anarhichas lupus	Catfish (wolffish)	CAA	M	66 to 96	22
Dipturus batis	Common skate	SKT	L	185+	0
Dipturus batis	Common skate	SKT	M	135 to 185	8
Dasyatis pastinaca	Sting ray	SGR	L	99+	1
Spondyliosoma cantharus	Black seabream	BKS	L	34+	4

Spondyliosoma cantharus	Black seabream	BKS	M	23 to 34	2
Conger conger	European conger eel	COE	Jm	19 to 39	2
Conger conger	European conger eel	COE	L	57+	18
Conger conger	European conger eel	COE	M	39 to 57	2
Capros aper	Boar fish	BOF	Jm	4 to 9	24
Capros aper	Boar fish	BOF	Js	3 to 4	4
Capros aper	Boar fish	BOF	L	11+	17
Capros aper	Boar fish	BOF	M	9 to 11	22
Trisopterus minutus	Poor cod	POD	Js	3 to 7	7
Trisopterus minutus	Poor cod	POD	Lv	<3	3
Scyliorhinus canicula	Lesser spotted dogfish	LSD	Js	3 to 24	28
Scomber scombrus	(European) mackerel	MAC	Js	3 to 14	5
Myliobatis aquila	Eagle ray	EGR	L	83+	1
Mustelus mustelus	Smooth hound	SMH	Jm	46 to 91	11
Mustelus mustelus	Smooth hound	SMH	M	91 to 124	4
Mustelus asterias	Starry smooth hound	SDS	Jm	36 to 71	10
Mustelus asterias	Starry smooth hound	SDS	Js	3 to 36	1
Mustelus asterias	Starry smooth hound	SDS	L	93+	4
Mustelus asterias	Starry smooth hound	SDS	M	71 to 93	7
Lepidorhombus whiffiagonis	Megrim	MEG	Js	3 to 10	2
Lepidorhombus boscii	Four spot megrim	LBI	Jm	8 to 16	11
Lepidorhombus boscii	Four spot megrim	LBI	L	27+	21
Hippoglossus hippoglossus	Halibut	HAL	Jm	49 to 97	9
Hippoglossus hippoglossus	Halibut	HAL	Js	3 to 49	4
Gadiculus argenteus	Silvery pout	SYP	Jm	5 to 10	7
Gadiculus argenteus	Silvery pout	SYP	Js	3 to 5	0
Gadiculus argenteus	Silvery pout	SYP	L	13+	0
Gadiculus argenteus	Silvery pout	SYP	M	10 to 13	22
Amblyraja radiata	Starry ray	SYR	L	72+	1
Alosa fallax	Twaite shad	TAS	L	40+	1

Alosa alosa	Allis shad	AAS	M	37 to 52	3
Scophthalmus rhombus	Brill	BLL	Lv	<3	8
Zoarces viviparus	Eelpout/viviparous blenny	ELP	Js	3 to 9	2
Zoarces viviparus	Eelpout/viviparous blenny	ELP	L	27+	24
Zoarces viviparus	Eelpout/viviparous blenny	ELP	M	19 to 27	24
Trisopterus luscus	Whiting-pout (bib)	BIB	Lv	<3	7
Trigloporus lastoviza	Streaked gurnard	GUS	Jm	13 to 26	7
Trigloporus lastoviza	Streaked gurnard	GUS	Js	3 to 13	8
Trigloporus lastoviza	Streaked gurnard	GUS	M	26 to 32	1
Trachurus trachurus	Horse-mackerel (scad)	HOM	Lv	<3	27
Trachurus mediterraneus	Mediterranean scad	HMM	L	29+	5
Trachinus draco	Greater weever fish	WEG	Jm	11 to 21	0
Trachinus draco	Greater weever fish	WEG	Js	3 to 11	0
Thunnus thynnus	Blue-fin tunny	BFT	Js	3 to 88	1
Thunnus thynnus	Blue-fin tunny	BFT	L	263+	2
Taurulus bubalis	Sea scorpion	SSN	L	15+	3
Taurulus bubalis	Sea scorpion	SSN	Lv	<3	28
Syngnathus rostellatus	Nilsson's pipefish	NPF	Jm	6 to 12	4
Syngnathus rostellatus	Nilsson's pipefish	NPF	Js	3 to 6	3
Syngnathus acus	Great pipefish	GPF	Jm	6 to 12	2
Syngnathus acus	Great pipefish	GPF	L	16+	25
Syngnathus acus	Great pipefish	GPF	Lv	<3	1
Syngnathus acus	Great pipefish	GPF	M	12 to 16	24
Syngnathidae	Pipe-fishes/seahorses	PFX	Jm	5 to 11	1
Symphodus melops	Corkwing	CWG	Js	3 to 6	20
Symphodus melops	Corkwing	CWG	Lv	<3	4
Symphodus melops	Corkwing	CWG	M	11 to 17	1
Squatina squatina	Angelshark (monkfish)	ALS	Jm	44 to 89	3
Squatina squatina	Angelshark (monkfish)	ALS	L	112+	4
Squatina squatina	Angelshark (monkfish)	ALS	M	89 to 112	3

Spinachia spinachia	Sea stickleback	SSS	Jm	6 to 11	3
Spinachia spinachia	Sea stickleback	SSS	L	14+	4
Spinachia spinachia	Sea stickleback	SSS	M	11 to 14	5
Somniosus microcephalus	Greenland shark	GSK	L	98+	3
Sebastes mentella	Rose fish	REB	L	37+	1
Sebastes marinus	Norway haddock	REG	Jm	16 to 31	9
Sebastes marinus	Norway haddock	REG	L	39+	1
Sebastes marinus	Norway haddock	REG	M	31 to 39	9
Sebastes	Redfishes	RED	Jm	13 to 26	1
Sebastes	Redfishes	RED	M	26 to 32	1
Scyliorhinus stellaris	Nurse hound	DGN	Jm	30 to 60	2
Scomber scombrus	(European) mackerel	MAC	Lv	<3	10
Sardina pilchardus	Pilchard	PIL	Jm	7 to 14	12
Sardina pilchardus	Pilchard	PIL	Js	3 to 7	24
Sardina pilchardus	Pilchard	PIL	L	17+	23
Sardina pilchardus	Pilchard	PIL	M	14 to 17	0
Sarda sarda	Bonito	BON	M	37 to 58	1
Salmo trutta	Sea trout (brown trout)	TRS	Jm	12 to 24	11
Salmo salar	N.atlantic salmon	SAL	Js	3 to 32	1
Reinhardtius hippoglossoides	Greenland halibut	GLH	Jm	32 to 65	8
Regalecus glesne	Ribbon fish	RNF	L	43+	1
Raniceps raninus	Lesser forkbeard	LFB	Lv	<3	1
Psetta maxima	Turbot	TUR	Lv	<3	3
Phycis blennoides	Greater forkbeard	GFB	Jm	13 to 27	3
Phycis blennoides	Greater forkbeard	GFB	L	38+	2
Phycis blennoides	Greater forkbeard	GFB	M	27 to 38	4
Pholis gunnellus	Butter fish	BTF	Js	3 to 7	17
Pholis gunnellus	Butter fish	BTF	L	19+	2
Perca fluviatilis	European perch	FPE	Jm	8 to 16	1
Pegusa lascaris	Sand sole	SOS	Jm	10 to 19	13

Pegusa lascaris	Sand sole	SOS	Lv	<3	1
Parablennius gattorugine	Tompot blenny	TBY	Jm	5 to 10	17
Parablennius gattorugine	Tompot blenny	TBY	Js	3 to 5	18
Parablennius gattorugine	Tompot blenny	TBY	Lv	<3	1
Osmerus eperlanus	Smelt(sparling)	SME	Jm	9 to 17	1
Osmerus eperlanus	Smelt(sparling)	SME	M	17 to 20	2
Nerophis ophidion	Straight-nosed pipefish	SNP	L	13+	1
Nerophis lumbriciformis	Worm pipefish	WPF	Lv	<3	1
Nerophis lumbriciformis	Worm pipefish	WPF	M	10 to 14	220
Myoxocephalus scorpius	Bullrout	BRT	Jm	7 to 15	7
Myoxocephalus scorpius	Bullrout	BRT	Js	3 to 7	0
Mullus surmuletus	Red mullet	MUR	Jm	8 to 16	8
Mullus surmuletus	Red mullet	MUR	Js	3 to 8	2
Mullus surmuletus	Red mullet	MUR	M	16 to 24	24
Mugilidae	Grey mullets	MUL	Jm	11 to 21	5
Microstomus kitt	Lemon sole	LEM	Js	3 to 13	139
Micromesistius poutassou	Blue whiting	WHB	Js	3 to 12	1
Microchirus variegatus	Thickback sole	TBS	Js	3 to 7	22
Microchirus variegatus	Thickback sole	TBS	L	17+	18
Merluccius merluccius	European hake	HKE	Lv	<3	1
Maurolicus muelleri	Pearlside	PLS	L	6+	3
Maurolicus muelleri	Pearlside	PLS	M	5 to 6	1
Mallotus villosus	Capelin	CAP	Jm	8 to 16	10
Mallotus villosus	Capelin	CAP	M	16 to 18	7
Malacocephalus laevis	Soft headed rattail	SRT	Jm	16 to 31	2
Malacocephalus laevis	Soft headed rattail	SRT	L	41+	1
Malacocephalus laevis	Soft headed rattail	SRT	M	31 to 41	1
Macrorhamphosus scolopax	Snipe-fish	SNI	L	13+	1
Lumpenus lampretaeformis	Snake blenny	SBY	Jm	13 to 26	3
Lumpenus lampretaeformis	Snake blenny	SBY	M	26 to 36	22

Liparis liparis	Sea snail	SSL	Lv	<3	3
Leucoraja naevus	Cuckoo ray	CUR	M	59 to 71	27
Lepadogaster lepadogaster	Shore clingfish	SCF	Jm	3 to 5	47
Lepadogaster lepadogaster	Shore clingfish	SCF	M	5 to 7	47
Lampris guttatus	Opah (moon-fish)	OPA	L	43+	2
Lamna nasus	Porbeagle shark	POR	Jm	92 to 185	3
Lamna nasus	Porbeagle shark	POR	M	185 to 243	3
Labrus mixtus	Cuckoo wrasse	CUW	Js	3 to 9	2
Labrus mixtus	Cuckoo wrasse	CUW	L	27+	1
Labrus mixtus	Cuckoo wrasse	CUW	Lv	<3	2
Labrus bergylta	Ballan wrasse	BNW	Js	3 to 13	2
Labrus bergylta	Ballan wrasse	BNW	L	37+	1
Labrus bergylta	Ballan wrasse	BNW	Lv	<3	2
Hippoglossoides platessoides	American plaice (Ir dab)	PLA	Lv	<3	20
Hexanchus griseus	Six-gilled shark	SGS	L	139+	1
Heptranchias perlo	Sharpnose seven-gill shark	SVS	Jm	52 to 104	1
Helicolenus dactylopterus	Blue-mouth redfish	RBM	Jm	12 to 24	15
Helicolenus dactylopterus	Blue-mouth redfish	RBM	Js	3 to 12	5
Helicolenus dactylopterus	Blue-mouth redfish	RBM	L	30+	1
Glyptocephalus cynoglossus	Witch	WIT	Jm	13 to 27	10
Glyptocephalus cynoglossus	Witch	WIT	Js	3 to 13	2
Gasterosteus aculeatus	Three-spined stickleback	TSS	Jm	2 to 5	26
Gasterosteus aculeatus	Three-spined stickleback	TSS	L	6+	5
Gasterosteus aculeatus	Three-spined stickleback	TSS	M	5 to 6	26
Galeus melastomus	Blackmouthed dogfish	DBM	Jm	38 to 76	2
Galeus melastomus	Blackmouthed dogfish	DBM	Js	3 to 38	5
Galeorhinus galeus	Tope shark	GAG	L	153+	0
Galeorhinus galeus	Tope shark	GAG	M	131 to 153	10
Gaidropsarus vulgaris	Three-bearded rockling	TBR	Jm	14 to 28	1
Gaidropsarus vulgaris	Three-bearded rockling	TBR	Js	3 to 14	1

Gaidropsarus vulgaris	Three-bearded rockling	TBR	L	37+	2
Gaidropsarus vulgaris	Three-bearded rockling	TBR	Lv	<3	8
Gaidropsarus vulgaris	Three-bearded rockling	TBR	M	28 to 37	4
Gaidropsarus mediterraneus	Shore rockling	SRR	Js	3 to 10	2
Gadidae	Codlike fishes	GAD	Lv	<3	2
Eutrigla gurnardus	Grey gurnard	GUG	Lv	<3	17
Engraulis encrasicolus	European anchovy	ANE	Jm	5 to 10	0
Engraulis encrasicolus	European anchovy	ANE	Js	3 to 5	0
Engraulis encrasicolus	European anchovy	ANE	M	10 to 14	3
Enchelyopus cimbrius	Four-bearded rockling	FRR	L	30+	3
Enchelyopus cimbrius	Four-bearded rockling	FRR	M	24 to 30	18
Echinorhinus brucus	Spiny shark	SYS	L	98+	1
Echiichthys vipera	Lesser weever fish	WEL	Lv	<3	3
Echiichthys vipera	Lesser weever fish	WEL	M	21 to 30	2
Dalatias licha	Darkie charlie	DCH	Jm	39 to 78	8
Dalatias licha	Darkie charlie	DCH	Js	3 to 39	8
Cyclopterus lumpus	Lumpsucker	LUM	Jm	15 to 30	28
Cyclopterus lumpus	Lumpsucker	LUM	Js	3 to 15	8
Cyclopterus lumpus	Lumpsucker	LUM	L	41+	2
Cyclopterus lumpus	Lumpsucker	LUM	Lv	<3	3
Cyclopterus lumpus	Lumpsucker	LUM	M	30 to 41	1
Ctenolabrus rupestris	Goldsinny	GDY	Jm	5 to 10	1
Ctenolabrus rupestris	Goldsinny	GDY	Lv	<3	2
Ctenolabrus rupestris	Goldsinny	GDY	M	10 to 14	2
Cottus gobio	Bullhead	BUL	L	6+	3
Cottunculus microps	Polar sculpin	CTM	M	20 to 27	1
Coryphoblennius galerita	Montague's blenny	MBY	Lv	<3	58
Ciliata septentrionalis	Northern rockling	NNR	Js	3 to 19	4
Ciliata mustela	Five-bearded rockling	FVR	Lv	<3	17
Chelon labrosus	Thick lipped mullet	MTL	Jm	13 to 25	2

Chelon labrosus	Thick lipped mullet	MTL	Js	3 to 13	4
Chelidonichthys lucerna	Tub gurnard	TUB	Js	3 to 12	9
Cetorhinus maximus	Basking shark	BSK	Jm	246 to 491	1
Cetorhinus maximus	Basking shark	BSK	L	695+	1
Cepola macrophthalma	Red bandfish	RPF	L	43+	10
Cepola macrophthalma	Red bandfish	RPF	M	24 to 43	1
Callionymus maculatus	Spotted dragonet	SDT	Jm	8 to 15	27
Callionymus lyra	Common dragonet	CDT	Js	3 to 7	9
Callionymidae	Dragonets	DTX	M	18 to 25	1
Buglossidium luteum	Solenette	SOT	Jm	4 to 8	8
Buglossidium luteum	Solenette	SOT	Js	3 to 4	0
Buglossidium luteum	Solenette	SOT	L	10+	259
Brosme brosme	Tusk	USK	Jm	25 to 49	4
Brosme brosme	Tusk	USK	M	49 to 64	4
Brama brama	Rays bream (pomfret)	POA	L	47+	5
Blicca bjoerkna	Silver bream	FSB	Jm	9 to 18	1
Blennius ocellaris	Butterfly blenny	BBY	Js	3 to 6	2
Blennius ocellaris	Butterfly blenny	BBY	Lv	<3	1
Belone belone	Garfish	GAR	Jm	11 to 22	0
Belone belone	Garfish	GAR	M	22 to 32	0
Atherina presbyter	Sand smelt	SMT	Jm	4 to 8	12
Atherina presbyter	Sand smelt	SMT	M	8 to 11	12
Aspitrigla obscura	Long-finned gurnard	GUL	Jm	12 to 24	13
Aspitrigla obscura	Long-finned gurnard	GUL	M	24 to 33	5
Arnoglossus laterna	Scald fish	SDF	Jm	4 to 7	24
Arnoglossus laterna	Scald fish	SDF	Js	3 to 4	1
Arnoglossus imperialis	Imperial scaldfish	ISF	L	14+	8
Arnoglossus imperialis	Imperial scaldfish	ISF	M	10 to 14	4
Argentinidae	Argentines	ARG	Jm	8 to 16	2
Argentinidae	Argentines	ARG	L	19+	1

Argentinidae	Argentines	ARG	M	16 to 19	8
Argentina sphyraena	Lsr silver smelt	LSS	Jm	8 to 16	11
Argentina sphyraena	Lsr silver smelt	LSS	Js	3 to 8	1
Argentina silus	Gt silver smelt	GSS	M	33 to 38	2
Ammodytidae	Sandeel	AMO	Js	3 to 8	0
Alopias vulpinus	Thresher shark	ATH	Js	3 to 149	1
Alopias vulpinus	Thresher shark	ATH	L	395+	2
Agonus cataphractus	Pogge (armed bullhead)	POG	Jm	4 to 8	8
Agonus cataphractus	Pogge (armed bullhead)	POG	Js	3 to 4	0

5 Term of reference d) Discussion on matches and mismatches between and user needs and national collection diet data for EU MAP (DCF) and MSFD

Stomach sampling is needed for a variety of research and management issues. The commonality between all of them is that they rely on observations of the focal area or region. However, different sampling schemes vary substantially in spatial and temporal scale of the sampling as well in the forms of analyses of stomach contents. While some of this variation might be minimized by focussed coordination of different sampling activities, some variation has to be accepted, because the objectives of stomach sampling vary substantially (Table 4.1).

Table 4.1 List of categories and objectives/applications with different forms of stomach sampling as examples. Depending on the objective/research a specific sampling programme will fit better than another. Observations state the link between the stomach sampling and end-users.

Categories	Objectives/applications	Specific Sampling	Observations
1. Multispecies models	Stock assessment (natural mortality and growth)	North Sea/Baltic Sea stomach programs (Year of the Stomach, MARE-2012, FishKosm, German Small Scale boxes)	Stomach sampling is being used to feed in MS models
2. Food Web indicators	Ecosystem status (MSFD)	Volumetric/gravimetric data; stable isotopes; drawing stomach contents data together from across projects (e.g. DAPSTOM); DNA metabarcoding for biodiversity	Stomach sampling is being used to develop MSFD indicators
3. Ecosystem models	Impact assessments, large-scale functioning	No specific sampling	Currently diet matrices feeding most of these models are based on bibliography. Stomach sampling is strongly recommended.
4. Process studies	Fundamental research questions, specific management-related questions	Hyslop, 1980; Cortés, 1996; Baker *et al.*, 2014	Stomach sampling in this category could be useful to the other categories.
5. Track species, plastic and climate over long-term series	Fundamental research questions, specific management-related questions, developments over time	DAPSTOM	Stomach sampling in this category could be useful to the others.

The discussions were centred on stomach sampling for MSFD-Descriptor 4 indicators and for multispecies models, because these are the two sampling categories where there is most activity and also most demand. The two sampling schemes are not fundamentally different. The volumetric/gravimetric method applied in the indicator related sampling has shown to yield very reliable biomass estimates for ingested prey types (Velasco & Olaso, 1998a, 1998b, Preciado *et al.*, 2008, 2009, 2015). Other methodologies, based on prey observation, measurement and/or weighing also revealed powerful, notably to calculated several simple numerical indices (e.g percent by number, by weigh or by occurrence) or more complex indices (e.g Index of Relative Importance, IRI) that combine simple measurement to overcome the caveats of one indicator, like the overrepresentation by number of preys living in group such as zooplankton, or by mass of large preys (Hyslop, 1980; Cortés, 1996; Baker *et al.* 2014). These indices can be finally used to plot graphical representation of the diet, like "Costello plots", that inform on the relative importance of preys in the diet of the predators (Costello, 1990). If length measurements of multispecies relevant specimen are added, these methods can also be used for the multispecies-related sampling. Discrepancies between both sampling schemes are mainly due to the species under study: whereas multispecies modelling is mainly focused on commercial species, ecosystem modelling and food web indicators require knowledge on as much ecosystem compartments as possible.

Multi-species models used in the advice process are coordinated by ICES (WGSAM) and part of the EU regulations that determine the fisheries quota. Getting the share of the quota requires participation of the individual countries, making internationally coordinated sampling simple as has occurred in the past (ICES Year of the Stomach). However, currently collection of stomach data is not a mandatory part of the Data Collection Framework (DCF). As a result countries/institutes are collecting data in an opportunistic manner.

Food web indicator development is driven by OSPAR, however each country is required to develop its own national sampling program within the MSFD, without a strong incentive to participate in the OSPAR coordinated developments. As a result, a small number of countries have developed national stomach sampling programs, although coordinated stomach sampling is strongly recommended, for the regional indicator development.

Not surprisingly as most participants are actively working on stomach content data WKBECOSS agrees that existing stomach sampling programmes are worthy to maintain, although many scientific and management questions could benefit from additional coordinated sampling. WKBECOSS suggests therefore developing an Action Plan. This Action Plan should contain the preferred and minimal needs for the specific questions asked by the MS-models and indicators. Based upon these needs a coordinated sampling design including clear guidelines - such as an adjusted version of the FishPi2 proposal that recognizes the need for dietary information from a broader range of taxa including rare and minor species not of commercial interest- could be developed based upon which the share of the workload and the costs could be estimated.

The request for determining the minimum requirements resulted in discussions that this would lead to minimalistic sampling programs. However, showing the minimum requirements of each question could also show that with limited additional effort/funding multiple questions could be answered, promoting multiple use of the same data. This is likely to help determining the coordinated sampling program, and is necessary in convincing current countries/institutes reluctant to invest in stomach sampling.

The Action Plan should be a combined effort of WGBIOP, WGSAM, OSPAR Food web expert group and the survey coordination groups (IBTSWG, WGBIFS, WGBEAM, WGIPS, etc.),in order to combine the data needs with the survey possibilities.

Currently, the existing stomach content data are not archived centrally. ICES holds the North Sea and Baltic Sea multispecies modelling relating stomach content data, and also offers a link to the DAPSTOM database. Indicator related data are currently stored only in national laboratories. WKBECOSS concluded that it would be desirable to have also these data stored in an open source database. However, the format of the data differs from the current standard ICES transfer data format, and it would be a significant task, to include the existing indicator data into one unified data base. The challenge is aggravated by the different formats of the data, resulting from nationally and not regionally coordinated sampling schemes. In conclusion, including all available stomach data in a common data base and managing these data will demand significant resources.

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Annex 2: Recommendations

WKBECOSS recommends the sampling protocol for predator stomachs proposed by the FishPi2 project to be considered by WGBIOP. The sampling protocol is especially useful to support multispecies modelling. The first year of the sampling is used as a pilot study to verify the proposed costs for the stomach analyses. Furthermore, during the first year additional stomachs outside the routine surveys should be collected especially on hard substrates to investigate, if the survey-based stomach data are representative for the whole predator population in a given eco-region.

WKBECOSS recommends that WGSAM discusses the prioritization of species and regions for the sampling programme.

WKBECOSS recommends that WGEAWESS discusses the utility of stomach content analyses (including genetics) and data needs to improve ecosystem assessment and/or detect changes in ecosystem functioning.

Annex 3: IEO Protocol for stomach sampling

1. Sampling protocol

The herewith described stomach content analysis protocol has been produced by the Santander IEO Trophic Ecology Team. It is based on the procedures and methodologies carried out every autumn within the Spanish IBTS otter trawl surveys ("Demersales") conducted in the continental shelf of the Southern Bay of Biscay (Cantabrian Sea).Stomach content analysis is a traditional methodology in food web analyses. However, studies using this technique hardly ever explain their sampling protocol or assess whether a sufficient number of samples has been analysed to characterize the diet of the species under study (Ferry and Caillet, 1996). The "Demersales" protocol is a well-established one which has been proved to reliably characterize some of the most abundant predators' diets in the area (Velasco *et al.*, 1998b, Velasco, 2007).

A set of 24 species have been consistently sampled following the same methodology along the entire time series, while a series of prospective diet analyses have also been performed for several predator species to acquire some knowledge on their feeding habits.

The sampling strategy is summarized in the following points:

- Data are collected during IBT surveys on soft bottoms of the Galician and Cantabrian Sea continental shelf.
- Sampling follows a randomly stratified design over five geographical sectors and three depth strata (a total of 15 sectors-strata), with some additional "special" tows outside these ranges following the same methodology (Figure 1).

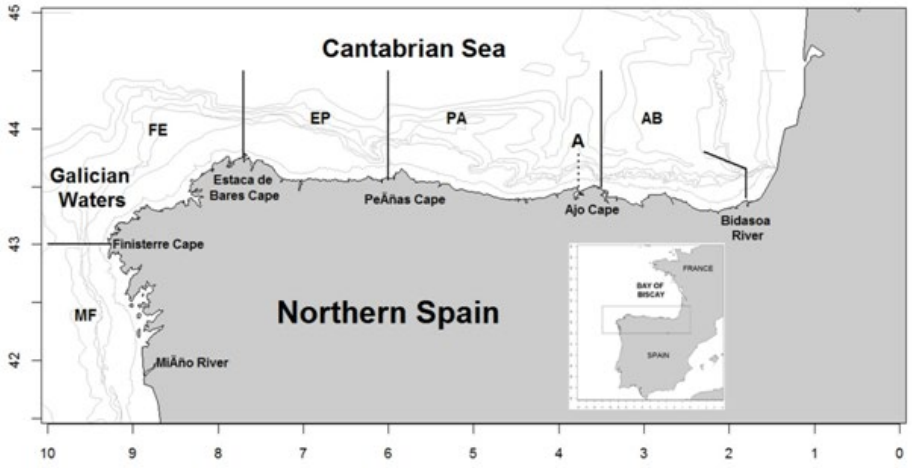


Figure 1. Area covered by the Demersales surveys in the Southern Bay of Biscay showing the 5 different sectors considered.

- The sampling gear is a baka otter trawl with 20mm mesh size at the codend, which is towed during 30 min. at an approximate speed of 3 knots.
- After each haul the catch is separated by species and weighted. All fish and invertebrates are identified at the lowest taxonomic level possible.
- All retrieved individuals from the total catch of each species (or a representative sample) are counted and measured.
- Ten individuals (if possible) from each caught predator species, are randomly set aside for stomach content analysis. Exceptionally, the species *Merluccius merluccius*, *Lepidorhombus boscii* and *Lepidorhombus whiffiagonis* are analysed by size range, examining 10

individuals by ontogenetic group. These ontogenetic groups are based on multivariate analyses conducted on the diet data matrices and are within the ranges9 - 17 cm, 18 - 34 cm, 35 - 69 cm and 70 - 90 cm, for *M. merluccius* (Velasco, 2007),11-17 cm, 18- 32 cm, and > 33 cm for *L. whiffiagonis*, and ≤ 15 cm, 16- 23 cm, 24 – 36, and 37 - 50 cm for *L. boscii*.

- In the case of *Merluccius merluccius*, and in order to prevent an overestimation of empty stomachs in the sample, the state of the gallbladder is used to determine whether regurgitation has taken place (Robb, 1992). When the gallbladder is empty, the stomach is considered as regurgitated. If not, the stomach is assigned as empty.
- All prey are separated and identified to the lowest possible taxonomic level and counted, when possible.
- A "digestion state" degree is given to each prey item following the categorization: 1 = freshly ingested; 2 = partially digested (specimens can still be measured); 3 = highly digested (specimens cannot be measured) (Figure 2)

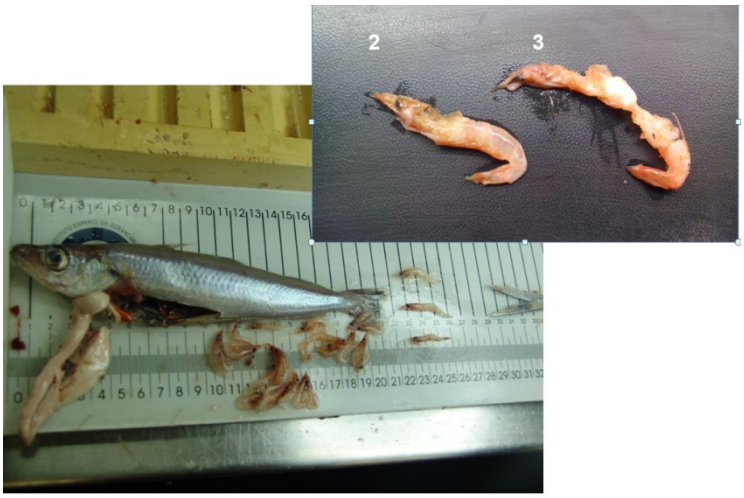


Figure 2. Specimen of blue whiting showing prey extracted from stomach contents and a couple of shrimps in digestion states "2" (partially digested) and "3" (completely digested).

- Whenever possible, prey items (fish and decapod crustaceans) are measured.
- Quantitative diet estimates are obtained by measuring the stomach content volume using a trophometer (Olaso, 1990, Figure 3).
- The percentage of volume occupied by each prey in the stomach is estimated.
- All these data are recorded upon analysis on specifically designed data sheets (Figure 4) and directly stored in a database onboard.

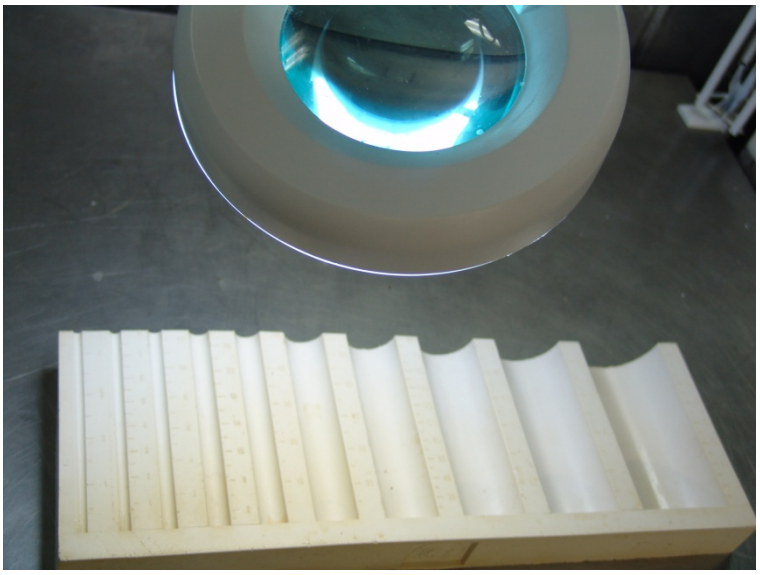


Figure 3. Trophometer used during Demersales surveys for stomach content analyses.

2. Diet metrics

The **percentage of vacuity** is annually calculated dividing the number of individuals of a given species with empty stomachs by the total number of individuals of that species. Niche breadth is computed using the Levins' standardized **niche breadth**, which measures the uniformity of prey contribution to the predator diet (Levins, 1968; Krebs, 1988) following the formula:

$$B_A = \frac{\frac{1}{\sum p_j^2} - 1}{n - 1}$$

Where pj is the fraction of items in the diet belonging to food category j, and n is the total number of possible food categories. The index is *maximum* when all resources contribute equally to the diet, meaning that the species has the broadest possible niche. The index varies between 0-1 and can be compared among different predator species.

The **trophic richness** measures the different number of prey species which can be found in a single stomach. We provide mean trophic richness for each predator, computed as the annual average of individual trophic richness.

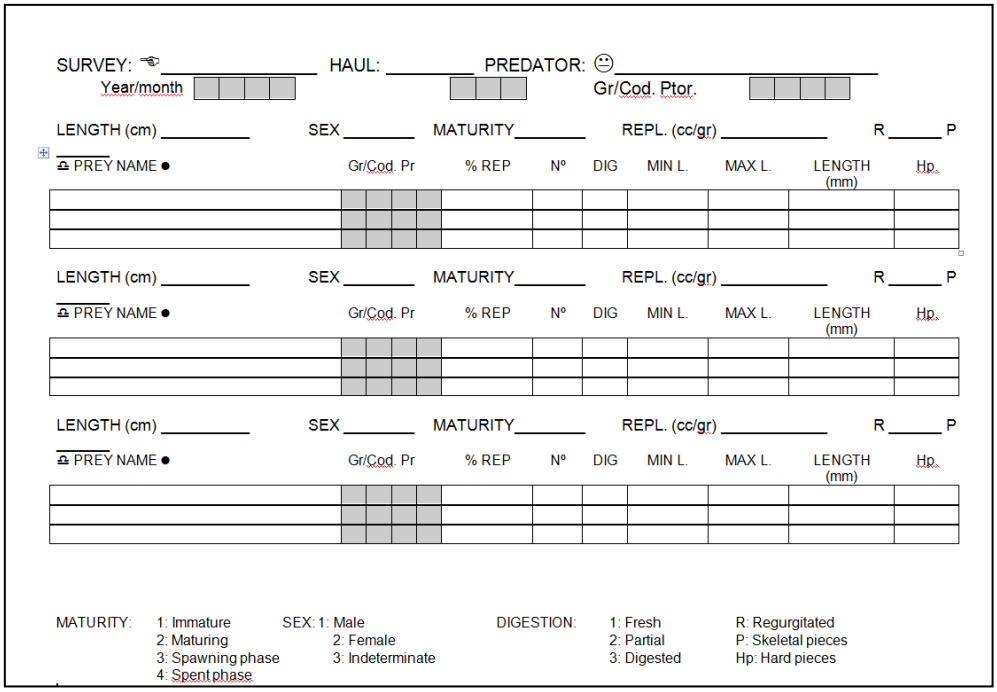


Figure 4. Data sheet used during *Demersales* surveys to record stomach content analyses data.

3. Quality assurance

The proposed quality assurance protocol stems from the analyses performed within López-López's PhD thesis.

In order to determine whether a sufficient amount of stomachs is being analysed during Demersales surveys, cumulative curves were performed annually for each species, between 1990 and 2012, running 999 permutations of the original data (R library vegan: function specaccum). Thereafter, the empirical curve was adjusted through minimum squares to a non-linear asymptotic model (R library base: function nls; *Formula 1*) to determine the upper limit of the asymptote, and thus, the prey species pool. Originally, a minimum number of 20 predators per species and year was set to perform the analysis, as below this threshold the automatic routine used to adjust the observed values to the asymptotic curve rarely converged.

$$m(x,\phi) = \phi_1 + (\phi_2 - \phi_1)exp[-exp(\phi_3)x] \quad Formula\ 1$$

The parameter theta 1 (ϕ_1) represents the value of the prey pool that is obtained when x $\rightarrow\infty$. To estimate the diet with confidence, we consider 90% of the asymptote is acceptable and calculate the corresponding x value.

These quality assurance analyses have been conducted on all species whose stomach contents are analysed during Demersales surveys, the result being that the diet of 19 species has been adequately characterized along the time series using the above mentioned methodology. These species are: *Callionymus lyra, Chelidonichtys cuculus, Conger conger, Eutrigla gurnardus, Galeus melastomus, Helicolenus dactylopterus, Lepidorhombus boscii, Lepidorhombus whiffiagonis, Merluccius merluccius, Micromesistius poutassou, Mullus surmuletus, Pagellus acarne, Raja clavata, Raja montagui, Scomber scombrus, Scyliorhinus canicula, Trisopterus luscus* and *Trisopterus minutus.*

The following examples show the different degrees of acceptability obtained for the various ontegenetic stages of hake (*M. merluccius*). First, a small description of each ontogenetic stage's habitat and/or feeding habits is given, followed by general trophic metrics such as thepercentage of

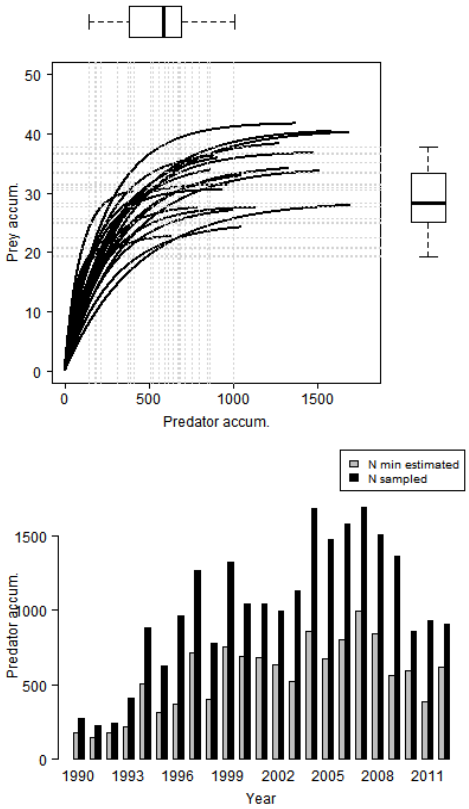
vacuity, niche breadth and trophic richness. Afterwards, the sampling strategy is evaluated giving the range of prey species annually identified along the time series and the maximum number of prey which remain unidentified. We also provide the range of specimens of each predator category that should be analyzed to achieve an adequate annual description of the diet.

The accompanying figures summarize these results: the upper panel combines all the prey accumulation curves, and summarizes, in the lateral boxplots, the annual minimum number of predators needed for determining the diet (x- axis) and the annual prey pool identified with this predator minimum (y- axis).These x and y values correspond to 90% of the annual asymptotic maximum. The lower panel, compares the number of predators annually analyzed with the minimum number necessary to determine diet confidently using a barplot, thus providing a time series overview.

Examples:

M. merluccius 9 - 17 cm

The ontogenetic group of juvenile *Merluccius merluccius* is mainly found at its nursery areas during autumn in the Northwestern Iberia Sea Shelf (Sanchez and Gil, 2000; Preciado *et al.*, 2015). It feeds mainly on euphausiids, small benthic-pelagic shrimps and small fish (Velasco and Olaso, 1998; Velasco, 2007).



Mean stomach vacuity was 55%.The mean species' niche breadth was 0.20 while prey richness averaged 1.12 prey/stomach.

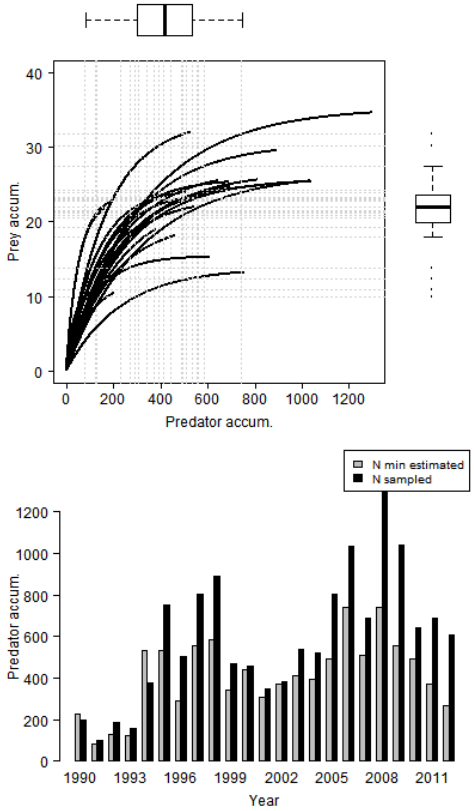
The sampling strategy identified annually100% of the prey pool indicating that all prey were identified along the time series.

The number of stomach samples necessary to reach a 90% precision in the diet varied between 144 and 996. The sampling design generally sufficed to characterize the annual diet of this ontogenetic stage.

Merluccius merluccius 18 - 34 cm

The pre-adults of *Merluccius merluccius* feed mainly on *Micromesistius poutassou* showing as well the highest rate of cannibalism of this species (Velasco and Olaso, 1998; Velasco, 2007; Preciado *et al.*, 2015; Lopez-Lopez *et al.*, 2015).

Mean stomach vacuity was 58%.The mean species' niche breadth was 0.08 while prey richness averaged 1.10 prey/stomach.



The sampling strategy identified 98- 100% of the prey pool annually, indicating that all prey were identified along the time series.

The number of stomach samples necessary to reach a 90% precision in the diet varied between 81 and 743. The sampling design generally sufficed to characterize the annual diet of this group.

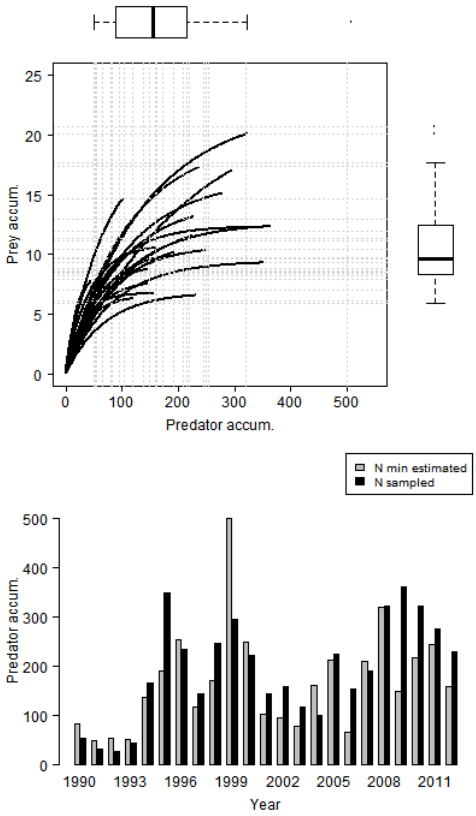
Merluccius merluccius 35 - 69 cm

Adults of *Merluccius merluccius* fed mainly on *Micromesistius poutassou* with an important portion of pelagic fish in their diet (Velasco and Olaso, 1998; Velasco, 2007; Lopez-Lopez *et al.*, 2015).

Mean stomach vacuity was 68%. Mean species' niche breadth was 0.25 while prey richness averaged 1.09 prey/stomach.

The sampling strategy identified annually 81- 100% of the prey pool indicating that up to 4 prey remained unidentified along the time series.

The number of stomach samples necessary to reach a 90% precision in the diet varied between 50 and 500. The sampling design did not suffice to characterize the diet of this group on an annual basis.



Merluccius merluccius 70 - 90 cm

The ontogenetic group comprised by the largest *Merluccius merluccius* did not have enough observations to conduct the analyses: only 71 individuals were caught along the time series (82% vacuity).

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Appendix 1.List of the predator fish species subject to diet analyses. We indicate if the diet has been analysed following the above mentioned methodology and/or if prospective diet determination has been performed. The abundance categories indicate if the species is not annually found (Very low), if it is annually found in abundances that do not allow diet determination (Low), if it is annually found in abundances that allow diet determination only some years (Medium), or if it is found in abundances that allow diet characterization every year (High).

Species	Prospective sampling	Consistent Sampling	Relative abundance
Acantholabrus palloni	X		Very low
Aphanopus carbo		1990-	Very low
Arnoglossus imperialis	X		High
Arnoglossus laterna	X		High
Boops boops	X		High
Callionymus lyra		1990-	High
Cepola rubescens	X		High
Chelidonichthys cuculus		1990-	High
Chelidonichthys lucerna	X	1993-	High

Chelidonichthys obscurus		1990-	High
Conger conger		1990-	High
Deania calcea		1990-	Very low
Deania profundorum		2009-	Low
Diplodus cervinus	X		Very low
Diplodus sargus	X		Very low
Diplodus vulgaris	X		Very low
Etmopterus spinax	X	1993-	Medium
Eutrigla gurnardus		1990-	High
Gaidropsarus macrophtalmus	X	1993-	Medium
Galeus atlanticus		2009-	Low
Galeus melastomus	X	1993-	High
Helicolenus dactylopterus	X	1998-	High
Hoplostetus mediterraneus	X	2009-	Medium
Labrus mixtus	X		Very low
Lepidion eques	X		Low
Lepidopus caudatus	X		Very low
Lepidorhombus boscii		1990-	High
Lepidorhombus whiffiagonis		1990-	High
Lepidotrigla cavillone /dieuzedei	X	2001-	Medium
Leucoraja circularis		1990-	Very low
Leucoraja naevus		1990-	Medium
Lithognathus mormyrus		1992-	Very low
Species	Prospective sampling	Consistent Sampling	Relative abundance
Lophius budegassa		1990-	High
Lophius piscatorius		1990-	High
Malacocephalus laevis	X		Medium
Merluccius merluccius		1990-	High
Microchirus variegatus	X		High
Micromesistius poutassou		1990-	High

Molva macrophthalma	X	1999-	Medium
Mora moro	X		Very low
Mullus surmuletus		1990-	High
Notacanthus bonaparte	X		Very low
Pagellus acarne		1990-	High
Pagellus bogaraveo		1990-	Very low
Pagellus erythrinus		1990-	Medium
Pagrus pagrus	X		Very low
Phycis blennoides		1990-	High
Raja clavata		1990-	High
Raja montagui		1990-	High
Scomber scombrus		2000-	High
Scorpaena loppei	X	1999-	Medium
Scorpaena notata	X		Very low
Scorpaena scrofa	X	1999-	Low
Scyliorhinus canicula		1990-	High
Scyliorhinus stellaris	X		Low
Scymnodon ringens		1995-	Very low
Serranus cabrilla	X		Low
Solea lascaris	X		Low
Solea solea	X		Medium
Spondyliosoma cantharus	X	1996-	Low
Trachinus draco	X	2001-	Medium
Trachurus trachurus	X		High
Trachyscorpia cristulata		1999-	Very low
Trigla lyra	X	1993-	High
Trisopterus luscus	X	1990-	High
Trisopterus minutus	X	1993-	High
Zeus faber	X	1992-	High

Annex 4: Manual for stomach sampling. FishPi2

(Please find the manual on the page below)

Annex 4.2
Annex 4.2.1.- Manual for stomach sampling

Prioritised predator fish species

Based on the priorities defined in 'D4.1 Report on ecosystem components and species for which information would be particularly important to obtain', a list of species have been identified for either regular sampling or one-off sampling events. These species are included in a 2 to 5 year sampling programme for each area. At the end of this programme, the species included should be reviewed and species discovered to be less relevant based on the updated information obtained can be removed from the sampling programme.

Baltic Sea incl. Kattegat: cod and whiting

Bay of Biscay: tuna, hake, monkfish, rays, megrim, sea bass, blue whiting, mackerel and horse mackerel

Irish Sea: cod, whiting, gurnards, haddock

North Sea and Skagerrak: whiting, horse mackerel, saithe, cod, mackerel, hake, grey gurnard, halibut, haddock, starry ray, monkfish, plaice, turbot, megrim

Species can be sampled in different years in a rolling scheme, ensuring that at least one species for which biological samples are taken (e.g. maturity and/or otoliths) and one species for which this is not the case (and which hence provides a greater increase in work load) is sampled every year and that a maximum of 5 years passes between the sampling of any one species. For example, the stomach sampling scheme could be:

Survey area	Year	Species sampled for biology	Species not sampled for biology
North Sea IBTS	1	Whiting and monkfish	Megrim
	2	Horsemackerel	Starry ray
	3	Saithe (Q1 and Q3) and mackerel (Q3 only)	Gurnard
	4	Cod and plaice	Halibut
	5	Haddock and hake	Turbot
North Sea IESSNS	1	Mackerel	
	2	Horsemackerel	

Irish Sea	1	Whiting	Grey gurnard
	2	Cod and haddock	
Baltic Sea	1	Cod	
	2	Whiting	
Bay of Biscay	1	Hake	Tuna
	2	Blue whiting and monkfish	rays
	3	Horsemackerel	Megrim
	4	Mackerel	Sea bass

In addition to these species, it should be considered to sample rays and sharks to derive estimates of the proportion of commercial fish in the diet of the most abundant elasmobranchs.

Sampling level

- Five stomachs should be sampled per 5-cm length group of each predator species from on average every fifth haul resulting in a total number of stomachs sampled per 5-cm group by a country in a given survey, which is equal to the number of hauls performed by a given country. A wide geographical coverage of samples should be obtained whenever possible and no more than two specimens should be taken per cm group and square.

- Stomachs should be selected randomly within 5-cm groups, but can be taken from fish sampled for maturity and age determination. The stomachs are frozen individually in plastic bags together with a label describing the sampled fish. Only predators larger than 15 cm should be sampled as fish below this size are generally not piscivorous.

- Data are recorded in the ICES exchange format on the labels used for year, quarter, ship and haul consistent with those used for haul information uploaded to DATRAS (Table 2 and 3). This assures accessibility of further haul details if necessary.

The optimal sampling level depends on the overall survey effort, predator feeding strategies, species composition, and distribution overlap between predator and import prey in the specific sea areas. For example, expanding on the concept of intra-haul correlation (Bogstad et al. 1995), more stomachs from predators that are specialists at the individual level (e.g., cod, Hüssy et al. 2016) should be sampled in each haul as compared to species that are specialists at the population level (e.g., saithe).

It is therefore recommended that data obtained in the first 5 year period is reviewed to determine the needed level of future sampling.

Selection of stomachs at sea

The selection of stomachs should be based on the following stomach classification:

1. **Everted stomach**. Some fish have everted stomachs due to the pressure difference between trawling depth and the surface of the sea. Since it not known whether these stomachs contained food or not, such ones **should not be sampled**.

2. **Stomach showing evidence of regurgitatio**n. Some fish have regurgitated all or part of their stomach contents and these stomachs **should not be sampled**. The number of such stomachs encountered during the examination **must however be recorded** to ensure that the proportion of feeding fish in the sample is accurately defined. In practice, it is often difficult to tell whether regurgitation has taken place, except in situations of prey remains in mouth or pharynx. However if the stomach is flaccid or its wall is thin but contains no or little prey remains, experimental work by Robb (1992) indicates that the size of the gall bladder is a useful indicator of the recent feeding history of the fish. A large densely-coloured gall bladder indicates that the stomach has been empty for some time and has not recently lost its content by regurgitation. The criteria are summarized in Table 1 and should be applied when classifying a stomach as either being truly empty or originating from a fish that shows signs of regurgitation.

3. **Non-everted stomach showing no evidence of regurgitation** – with or without contents – **should be sampled**. It should be noted that not all feeding fish have significantly distended stomachs, i.e. feeding does not necessarily mean full.

4. **Empty stomach** is included in the category **Stomach of a fish showing no evidence of regurgitation**. Remember also to check and record the status of the gall bladder of a sampled fish with a seemingly empty stomach (Table 1).

The stomachs sampled at sea should thus originate from feeding fish showing no evidence of regurgitation (category 3) and from non-feeding fish (empty stomachs; category 4). The sampling should continue until at least one stomach classified in one of these two categories is obtained.

Protocol for stomach sampling at sea

1. Collect predators according to the sampling scheme elaborated for each sea area and predator species.
2. Do not sample everted stomachs.
3. Check the individual predators for evidence of regurgitation according to the categorization described above. Do not sample stomachs showing evidence of regurgitation, but remember to record them.
4. Sample the other (valid) stomachs (with and without contents) and avoid loss of prey remains when cutting the oesophagus during removal of the stomach from the fish.
5. Bag the stomachs individually (also empty stomachs) and preserve them by freezing as quickly as possible after removal from the fish. Each bag should contain a label giving the information listed in Table 2.
6. Record further relevant data including the number of regurgitated stomachs using the data exchange format in Table 3.
7. Send the frozen stomachs to the species coordinator upon arrival (Table 4).

It is recommended that the predator species are recorded using WORMS' AphiaID codes (http://www.marinespecies.org/aphia.php).

Laboratory analysis of stomach contents

The stomachs are analysed individually. They are thawed and cut open with scissors after which the contents may be carefully separated using water from a spray bottle in a 200–300 µm sieve. By use of water: remove the prey from the sieve, place it on moistened paper towel and gently dab it with another moistened paper towel to get rid of excess water.

Fish are identified to species or lowest possible taxonomical level possible and weighed individually. When possible, the total length is measured to the nearest mm below. Alternatively, for more digested fish, standard length or reduced standard length is measured – or estimated if still recognizable (Table 8). Be careful to completely unfolding the prey so that the length is not underestimated. Eggs are recorded as having the length 0. The digestive stage of the fish is recorded (Table 5) and pristine fish prey with intact and glistening bodies are categorized as eaten in the trawl and can be left out of the analysis later on to avoid bias introduced by feeding during the catch process.

Invertebrates are generally identified to the taxonomical levels shown in Table 6. The exceptions are the commercial species Norway lobster *Nephrops norvegicus*, northern prawn *Pandalus borealis*, Baltic prawn *Palaemon adspersus*, brown shrimp *Crangon crangon*, edible crab *Cancer pagurus*, common whelk *Buccinum undatum*, king scallop *Pecten maximus*, and queen scallop *Aequipecten opercularis* together with the isopod *Saduria entomon*, sea mouse *Aphrodita aculeata*, and hermit crab *Pagurus bernhardus*, which are identified to species or lowest taxonomical level possible. The latter prey are weighed individually and the other individually or by group as convenient. Invertebrates are measured to nearest mm below according to Table 8. The digestive stages of crabs and shrimps/prawns are recorded to avoid excessively biased estimates of diet composition and food consumption rates (Table 5).

Detached prey remains are handled as follows when water separation is used. If possible, separate the materials into identifiable categories in the sieve. Then, extract water from the materials by use of moistened paper towel to underside of the sieve; use tweezers to *lift* the materials from the sieve; get rid of excess water like it is done for the prey. Detached, prey remains that cannot be assigned to any particular prey are recorded as unidentified.

Notice that it is highly important to identify all prey items including detached materials at the lowest possible taxonomic level to avoid excessive bias arising in the subsequent data analysis. It is therefore not recommended to open the stomach and identify the contents aboard. Dispatch all stomachs to the species coordinator. In addition, it is recommended to use the water separation method described here to avoid dry out smaller amount of materials. This is particularly important for materials originating from small predators that generally in total contain small amounts of prey. Also, do not use alcohol to defrost the stomach contents as it accelerates the drying-up process.

Stomachs with no content and without evidence of regurgitation are classified as empty.

Stomachs with only indigestible remains (polychaete bristles, mollusc shells and opercula, chitin remains from crustacean exoskeletons, fish bones, otoliths etc.) are also categorized as empty to avoid bias when estimating diet composition and food consumption rates by use of a gastric evacuation rate model to stomach content information. For the same reason, indigestible prey remains with no attached organic materials, and that cannot be allocated to identified prey in stomachs with other prey remains, are excepted.

All data obtained from the laboratory processing of sampled stomachs are recorded in the exchange data format (Table 3) and submitted to the ICES database.

All prey species are recorded using WoRMS' AphiaID codes (http://www.marinespecies.org/aphia.php).

Potential use of modern meta-genomic techniques to identify stomach contents to species

Next generation sequencing (NGS) DNA barcoding can potentially be used to underpin stomach content analysis the food web models. While the method will not be able to replace stomach content analysis completely where the prey length distribution is required, it can potentially make screening of important predators more efficient, provide measurements of the relative importance of different prey types in the diet and improve the accuracy of the identification of species in the stomach content. In comparison to more traditional approaches, DNA barcoding allows both for a more accurate snapshot of predator diet and for species-level prey identification. Another advantage of DNA barcoding is that the methodology can be used both for comparative/control reasons (i.e. against previously known/suspected diets) for "*de novo*" diet description.

The application of DNA barcoding in diet studies has gained a major boost with the development of next-generation sequencing (NGS) methodologies. It is now readily feasible to identify even uncommon prey from multiple predators to family, genus and ultimately to species level in a single sequencing run while keeping the ability to individually trace back each prey to the particular samples from which it came. Technical issues related to prey identification from DNA in stomach contents linked to both uncertainty about taxonomic diversity (e.g. number of species) expected in the sample and often poor DNA quality (e.g. resulting from partially digested food) have now been largely overcome through the use of species specific universal DNA primers. The use multiple-universal markers (e.g. 16S, CO1) can amplify and resolve (i.e. identify) species across a broad range of taxa (i.e. Furthermore, the use of multiple-universal markers also allows for a broader taxonomic resolution. These universal markers are designed to target small fragments sizes, thus making amplification of degraded DNA more reliable.

Predator-prey interactions among key commercial species in the Celtic Sea and Irish Sea (e.g. cod, haddock, whiting, hake, plaice, sole, herring, sprat, mackerel, gurnard and blue whiting) is currently in progress. These species are being investigated as models to develop and implement an accurate genomics based approach, based on NGS DNA barcoding that can be reliably used to describe these species food chain interactions. Generated data can be compared with information derived from conventional approaches (e.g. field observation and stomach content analysis). The multi-species approach will also allow for an assessment of the potential effect of secondary consumption (i.e. the identification of the prey within the prey, which can potentially lead to misleading results).

The methodological approach for NGS DNA barcoding follows that described by Bowser et al. (2013) with some modifications to allow for the use of alternative sequencing platforms. |Universal primers (accounting for the species under investigation) targeting small (~130-300bp) regions of the mitochondrial DNA genes 16S and cytochrome *c* oxidase subunit 1 (CO1) fragments can be employed.

Sequencing of PCR products can be carried out through pooled massively parallel sequencing. To allow recovery of specific sample identification from sequencing data a DNA identifier tag can be linked to existing custom universal primers. To account for overrepresentation of host (i.e. predator) DNA from the sequencing analysis, which can potentially reduce detection signal of prey, species specific blocking primers can be employed (Vestheim & Jarman 2008). Following quantification and pooling, libraries can be sequenced using e.g. an Illumina MiSeq platform. After filtering for length and quality control, resulting sequences would be demultiplexed based on the DNA tags and separated by amplicon type (i.e. 16S or CO1). The bioinformatics analysis can be implemented using e.g. jMOTU (Jones et al. 2011) and BOLD (Ratnasingham & Hebert 2007).

The main advantage of these approaches is the ability to analyse a huge number of samples quickly and accurately. Compared to the estimated number per day that can be analysed manually of 30-100 stomachs, metagenomic approaches can perform many thousands of analyses, and at relatively low unit cost e.g. €5-10. There will still be some sample handling time in preparing the stomach samples for analysis, e.g. homogenising the sample, but this would be minimal.

The main disadvantage currently is that metagenomic techniques are only able to identify presence/ absence of each species. However, this makes the method ideal for screening for potential priority predators for more detailed sampling and for species determination.

Cost of sampling and laboratory processing stomachs

When fish selected for biology (maturity, age etc.) sampling are used for collection of stomach as well, it takes less than a minute to remove the stomach, fill-in the label and bag the stomach with label if all other information on the fish already has been acquired and recorded. When the fish is used exclusively for stomach sampling, the time spent is accordingly longer because weighing and length measuring of the fish, opening of the body cavity, and basic data recording is needed. The entire procedure may then take up to five minutes per stomach. In total, this corresponds to a maximum number of 1 to 7 minutes per 5 cm group per haul. Assuming that most predators are in the length range 15-50 cm, this corresponds to 7 to 49 minutes per haul if all species and length groups are caught in the haul (corresponding to 7 length groups sampled for each species). Generally, this will not be the case and hence the time allocation at sea will be less.

The subsequent processing of stomachs in the laboratory is more time consuming. Skilled manpower with a good taxonomic knowledge should be able to work up 30–100 stomachs per person-day depending on the size of the predator and the stomach content composition. Generally, stomach contents from smaller predator individuals and predators that prey on relatively small prey items (e.g. mackerel or haddock) are more time consuming, as are stomachs containing a large proportion of invertebrates. This is because it takes more time to disentangle and identify the different prey.

However, the suggested, coarse categorization of invertebrate prey help reduce the overall time consumption.

With a sampled number of 7 for each species and haul, the maximum cost in days of working up all stomachs are given in the table below.

NUMBER OF HAULS	NUMBER OF STOMACHS IF 3 SPECIES ARE SAMPLED	NUMBER OF DAYS USED TO ANALYSE STOMACHS AT 30 STOMACHS PER DAY
1	21	0.7
100	2100	70
200	4200	140
400	8400	280

Tables

Table 1. Condition of gall bladder, bile and hindgut, which can be used to differentiate between empty and regurgitated stomachs (from Robb 1992)

Gall bladder	Bile colour	Hindgut	State
Shrunken, empty or with a small amount of bile	Pale	Contains large amounts of bile and digested food material	Feeding*
Elongate	Pale green to light emerald green	Contains some bile and digested food material	Feeding*
Elongate	Dark green	Empty or contains some food particles	Empty
Round	Dark blue	Empty	Empty

*NB: If fish satisfying these criteria are found without food in their stomach, they should be classified as regurgitated

Table 2. Label to be included in each stomach bag

Cruise/survey	
Ship	
Haul number	
Species	
Total body length (mm)	
Sample ID	

To speed up the sampling process, the number of information lines has been reduced (as compared to earlier applied versions). Information on cruise and ship be pre-printed.

Table 3. ICES data exchange format for stomach data (http://ices.dk/marine-data/data-portals/Pages/Fish-stomach.aspx)

Field	Description
Dataset	Dataset name
RecordType	SS for single stomach
Country	Country that collected the data
Ship	Vessel that collected the data
Latitude	Data sampling position – latitude
Longitude	Data sampling position – longitude

Estimated_Lat_Long	Flag whether the sampling position based on the reported area
ICES_StatRec	ICES statistical rectangle
ICES_AreaCode	ICES area code
Year	YYYY
Month	MM
Day	DD
Time	Sampling time: HHMM
Station	Station reference
Haul	Haul number
Sampling_Method	Predator sampling method code (see Table 7)
Depth	Sampling depth
Temperature	° C
SampleNo(FishID)	Predator reference code – Fish ID unique for country, year, quarter and ship
ICES_SampleID	ICES predator reference
Predator_AphiaID	Predator WoRMS AphiaID
Predator_LatinName	Predator taxon Latin Name
Predator_Weight(mean)	(Mean) predator weight
Predator_Age(mean)	(Mean) predator age
Predator_Lengh(mean)	(Mean) predator length
Predator_LowerLengthBound	Predator's length lower bound
Predator_UpperLengthBound	Predator's length upper bound
Predator_CPUE	Predator catch per hour
GallBladder_stage(class)	Gall bladder stage
Stomach_METFP	Method of stomach preservation
Stomach_TotalNo	Total number of stomachs in the pool. Should always be 1.
Stomach_WithFood	Number of stomachs with food. Can be 0 or 1.
Stomach_Regurgitated	Number of stomachs regurgitated. Can be 0 or 1.

Stomach_WithSkeletalRemains	Number of stomachs with skeletal remains. Can be 0 or 1.
Stomach_Empty	Number of empty stomachs. Can be 0 or 1.
Stomach_ContentWgt	Stomach content weight
Stomach_EmptyWgt	Stomach empty weight (This field is in historical data but no longer considered necessary)
Stomach fullness	Stomach fullness (This field is in historical data but no longer considered necessary)
Stomach_Item	Stomach item name
ICES_ItemID	ICES stomach item ID
Prey_AphiaID	Prey WoRMS AphiaID (see Table 6)
Prey_LatinName	Prey taxon Latin Name
Prey_IdentMet	Prey identification method
Prey_DigestionStage	Prey digestion stage (see Table 5)
Prey_TotalNo	Total number of preys
Prey_Weight	Prey weight in grams
Prey_LengthIdentifier	Prey length identifier (see Table 8)
Prey_Length	Prey length in cm
Prey_LowerLengthBound	Prey length lower bound
Prey_UpperLengthBound	Prey length upper bound
Prey_MinNo	Minimum number of preys (This field is in historical data but no longer considered necessary)
Remarks	Any relevant comments

Table 4. List of species (by survey) coordinators

Predator / sea area	Coordinator
…	…
…	…
…	…
…	…
…	…
…	…

Table 5. Digestive stages of fish, crab, and shrimp/prawn

Prey type	Stage 1	Stage 2	Stage 3	Stage 4
Fish	Shiny body surface - probably with scales. Clear eyes	Intact body, which however may be discoloured	Body cavity opened. Parts of the head region may be digested	**a.** Nothing or only some of the body cavity left **b.** Tail muscle mass 'triangle' left **c.** Spine with little muscle mass **d.** Only spine / bones / otoliths left

Crab*	Carapace intact. Some appendages might be detached	Carapace cracked enabling the digestive fluids to work on the inner parts	N/A	N/A
Shrimp/prawn*	Entire body intact. Some appendages might be detached	Cephalothorax detached from the abdominal part	N/A	N/A

*Important for application of gastric evacuation model to data for estimation of diet composition and food ration (Andersen et al. 2016)

Table 6. Invertebrate groups and the corresponding AphiaID codes

Taxonomic level	Prey group	Code
Phylum	Ctenophora	1248
Phylum	Cnidaria	1267
Phylum	Annelida	882
Species	Aphrodita aculeata (sea mouse)	231869
Phyllum	Mollusca	51
Class	Gastropoda	101
Species	Buccinum undatum (common whelk)	138878
Class	Bivalvia	105

Species	Aequipecten opercularis (queen scallop)	140687
Species	*Pecten maximus* (king scallop)	140712
Class	Cephalopoda	11707
Phyllum	Echinodermata	1806
Phyllum	Arthropoda	1065
Subphyllum	Crustacea	1066
Order	Mysida	149668
Order	Euphausiacea	1128
Order	Isopoda	1131
Species	Saduria entomon	293511
Order	Amphipoda	1135
Order	Decapoda	1130
Infraorder	Caridea	106674
Family	Crangonidae	106782
Species	*Crangon crangon* (brown shrimp)	107552
Family	Palaemonidae	106788
Species	Palaemon adspersus (Baltic prawn)	107613
Species	*Pandalus borealis* (northern prawn)	107649
Infraorder	Astacidea	106672
Species	Nephrops norvegicus (Norway lobster)	107254
Infraorder	Brachyura	106673
Species	*Cancer pagurus* (edible crab)	107276
Infraorder	Anomura	106671
Species	Pagurus bernhardus (hermit crab)	107232
	Other invertebrates	9990

Plastic	9991
Litter other than plastic	9992

Table 7. Sampling method codes

Description of fishing gear	Code
Demersal trawl or seine	DEM
Pelagic trawl or seine	PEL
Demersal hook and line	DHL
Pelagic hook and line	PHL
Demersal gill net	DGN
Pelagic gill net	PGN

Table 8. Length measurement by prey type

Prey group	Length measured	Code
Vertebrata	Total length from snout to end of tail fin	TL
	Standard length from snout to basis of tail fin	SL
	Reduced standard length: from first vertebra to basis of tail fin (i.e. the length of the vertebral column).	RL
Crustacea	Total length of small crustaceans like mysids, krill and amphipods and intact nephrops, shrimps, prawns and *Saduria entomon*.	TL
	Length from bases of eye stalks or rostrum to uropods or carapace length in the case of advanced digestion stage of nephrops, shrimps and prawns.	CL
	Carapace width of crabs	CW
	Pleotelson length of *Saduria entomon* in the case of advanced digestion stage.	PL
Cephalopoda	Mantle length	ML
	Beak length in the case of advanced digestion stage	BL
Others	Total length of complete specimens	TL

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