OSPAR CEMP Guidelines

Common Indicator in Region IV: Change in average trophic level of marine predators (FW4)

(OSPAR Agreement 2018-08)[1]

This OSPAR biodiversity indicator is still in the early stages of implementation and as a result of iteration and learning, it is anticipated that there will be evolution of the methods and approaches documented in the CEMP guidelines. Version updates will be clearly indicated and be managed in a phased approach via ICG-COBAM through its expert groups and with the oversight and steer of BDC.

Contents

[1] – This document exists in English only

1 Introduction

“Changes in average trophic level of marine predators” is an indicator that reflects mainly the effects of fishing pressure on the structure of food webs. The indicator is based on biomass data and trophic level of species. The trophic level (TL) reflects the position of an organism in a food web, and energy is transferred from the lower TLs to the higher ones following the interconnections of organisms in the food web. TL is estimated using data from dietary analyses.

In February 2004, the Mean Trophic Level (MTL) indicator with a trophic level cut-off of 3.25 [Known as the Marine Trophic Index (MTI), as described by Pauly and Watson (2005)] was adopted by the Conference of the Parties to the Convention on Biological Diversity (CBD), as one of the eight indicators to monitor achievement of a significant reduction in the rate of biodiversity loss by 2010. MTI is currently calculated on landings data and is available at different geographic scales from the Sea Around Us website, hosted by the Fisheries Centre of the University of British Columbia (http://www.seaaroundus.org/). The MTL indicator is highly developed and currently applied globally across ecosystems (www.indiseas.org).

An important advantage of the MTL indicator is that the proposed concept is transferable across OSPAR regions. The MTL can be estimated on a regional/sub-regional scale using existing biomass data from landings and scientific surveys. As such, the indicator would be applicable in regions where comprehensive scientific surveys currently exist, *i.e* North Sea, Eastern English Channel, Celtic Seas, Bay of Biscay and Iberian coast (Figure 1). Currently the indicator is only agreed as OSPAR common indicator for OSPAR Region IV.

Fisheries have been demonstrated to have an especially marked impact on top predators, whose abundance can be severely depleted. The MTL indicator was developed to assess the impacts of fishing on food webs, it is thus specific (Pauly and Watson, 2005). It is easy-to-estimate and has been widely applied as a “large-scale“ indicator of the health of both fisheries and marine ecosystems, using the full range of ecosystem TLs, and for areas of different spatial scales in both data-rich (*e.g.* Canada, Iceland, North-Sea) and data-poor areas (*e.g.* Greece, Cuba, Portugal, India, Brazil, Uruguay) (Pauly *et al.*, 1998; Pauly and Watson 2005; Cury *et al.*, 2005).

The MTL has been traditionally calculated using biomass data from landings and TL of species available online. However, these TLs are mean values of worldwide estimations. A regionalisation of the TL estimation (*e.g.* using stomach contents and stable isotopes analysis) catches better the trophic interactions between local species. Regarding landings data, they do not reflect the ecosystem in many cases because of the biases caused by the various fishing strategies, changing markets and commercial needs. Conversely, data coming from standardised scientific surveys give a more comprehensive view of the whole ecosystem, but are usually limited to a specific season and hence only give a snap-shot of the communities at that given moment. Thus, both approaches are complementary and give a broader idea of the environmental status of a food web in a particular area.

2 Monitoring

2.1 Purpose

It is important for the MTL indicator calculation to use scientific survey data as they are most representative of ecosystem changes. Landings data however, have the advantage of having longer time series. Although fisheries catches have been criticised due to potential biases induced by changes occurring in the fishing strategies, both data sources provide useful and complementary information about changes in the ecosystem as a result of fishing (Branch et al., 2010; Shannon et al., 2014).

In order to establish trophic relationships more accurately through regional TL estimations, data on species feeding habits is urgently needed. Currently, comprehensive datasets on the feeding ecology of many of the key species in marine food webs are insufficient, and this is especially true for species at lower TLs. The first requirement therefore, is further extensive data collection to fill these gaps in our knowledge of food web structure and connectivity (Carafa *et al.*, 2007; Moloney *et al.*, 2010; Rossberg *et al.*, 2011). Hence, we suggest that large-scale surveys should aim to collect more systematically dietary analyses data, both stomach content analyses (which give an accurate evaluation of the species that have been eaten) and stable isotope analyses (which give the trophic level based on a long feeding signature).

2.2 Quantitative Objectives

Data needed:

Annual biomass per species (fishes and invertebrates) per year and TL per species +/- SE (Standard Error).

Product delivered:

Time series of the MTL indicator with different scenarios (variation of compartment included and cut-offs applied) at the sub-regional level.

2.3 Monitoring Strategy

Data for the MTL indicator come from scientific demersal surveys from OSPAR Region IV (bright green area Figure 1 left panel) and IXa, VIIIc and VIIIab ICES areas (Figure 1 right panel) and landings data (national declarations available on the ICES website). Any extension of the geographic range of this indicator would be subject to a policy decision.

Figure 1. Maps showing OSPAR Regions (left) and ICES areas (right). This CEMP guideline applies only to OSPAR Region IV (Bay of Biscay and Iberian coast) which includes IXa, VIIIc and VIIIab ICES areas.

2.4 Sampling Strategy

The sampling design of scientific surveys is available at this link:

http://www.ices.dk/marine-data/data-portals/Pages/DATRAS-Docs.aspx

An example of a proposal of the resources that would be necessary for implementing diet studies on scientific surveys in order to estimate regional TL's species is presented below (Table 1).

An example of a sampling protocol for stomach content analyses on board oceanographic surveys is presented as an Annex 1.

Table 1: Example of a proposal of resources estimation with no financial costing because of disparities between regions.

Analysis	Protocol description	Staff	Expertise	Working time (month/year)	Equipment and analyses
Stomach contents	After defining a list of species for each zone, a total of 200 stomachs per species (taking into account size classes when relevant) should be sampled. Analysis of the samples should preferably be done on board, although storage at -20ºC is also acceptable until analysis in the laboratory.	2 technicians	- Species identification - Sampling - Samples treatment (dissection and species identification)	9	Freezer (-20ºC) to stock samples +Stereomicroscope (macrofauna) +Microscope (microfauna)
	PS: Working time is an estimation for 600 samples analysed.	1 Researcher	-Conception -Supervision -Valorisation	1	
Stable isotopes	After defining a list of species for each zone (taken into account depth strata), a total of 5 to 10 individuals per size class per species + baseline (POM and SOM* whenever possible; bivalvia or zooplankton is also acceptable) of the food web should be sampled. Stock the samples at -20ºC until analysis in the laboratory.	2 technicians	- Species identification - Sampling - Samples treatment (dissection, delipidation, lyophilisation, samples conditioning)	10	Freezer (-20ºC) to stock samples and material for samples preparation (tin, capsules, pill, bags, etc.) +Isotopic analysis platform
	PS: Working time is an estimation for 3000 samples analysed.	1 Researcher	-Conception -Supervision -Valorisation	1	

*POM: Particulate Organic Matter (water column), SOM: Sediment Organic Matter (sediment).

2.5 Quality assurance/ Quality Control

Bottom Trawl Surveys are part of the DCF directive. Protocols and data are available on the ICES DATRAS database website (see links below). DATRAS has an integrated quality check utility. All data, before entering the database, have to pass an extensive quality check. Despite this, errors and missing data arise, which are subsequently dealt with by the data submitters from the contributing countries as required. The MSFD quality assured groundfish survey monitoring and assessment data products have been built for the existing dataset in DATRAS to solve the remaining issues.

The official requirements for monitoring of landings established through catch statistics are found in the ICES dataset collections(see links below).The catch data are constantly updated with corrections and amendments that the countries provide after the submission deadlines. Data presented in the datasets have not been corrected for non-reported landings, where these may have occurred.

2.6 Data reporting, handling and management

Survey data are submitted to the Database of Trawl Surveys (DATRAS):

http://www.ices.dk/marine-data/data-portals/Pages/DATRAS.aspx

The Reporting format is detailed online:

https://datras.ices.dk/Data_products/ReportingFormat.aspx

The metadata relating to the surveys are available here:

http://www.ices.dk/marine-data/data-portals/Pages/DATRAS-Docs.aspx

and here:

http://geo.ices.dk/geonetwork/srv/en/main.home?search=ices_datasets

The MSFD data product data and metadata can be found here under access conditions:

https://fishdata.sgworksite.org.uk/

For national survey databases, different institutions are in charge of the data where specific conditions apply (relative to the country).

Official landings data are submitted by countries to ICES, working in collaboration with the Statistical Office of the European Communities (EUROSTAT) and FAO.

http://www.ices.dk/marine-data/dataset-collections/Pages/Fish-catch-and-stock-assessment.aspx

An excel file for TL regional estimation on a (sub) regional level has to be delivered per regional assessment.

3 Assessment

3.1 Data acquisition

Survey data are downloaded directly from:

DATRAS in exchange format

https://datras.ices.dk/Data_products/Download/Download_Data_public.aspx

MSFD quality assured groundfish survey monitoring and assessment data products

https://fishdata.sgworksite.org.uk/

National databases

Landings data are downloaded from the ICES website (ICES Historical catch statistics 1950-2010 and Official catch statistics 2006-2013).

(http://www.ices.dk/marine-data/dataset-collections/Pages/Fish-catch-and-stock-assessment.aspx).

The TL estimated from stomach content analysis, stable isotope analysis or models can be collected in the literature for regional and non-regional areas. Otherwise, TL are available in online databases [i.e. Fishbase (http://www.fishbase.org/), Sealifebase (http://www.sealifebase.org/) and Sea around us (*http://www.seaaroundus.org/)*]. Information about the regional TL per species can be gathered into an excel file.

3.2 Preparation of data

Biomass data:

Defining depth range: select data in the depth range corresponding to the continental shelf of the studied sub-region (e.g.< 200 m depth for the Northern Bay of Biscay).

Annual biomass/species: aggregate data by summing biomass per species (or average in case of biomass per species per km²), in order to have one annual value per species of the period covered by the surveys/catch statistics.

Two parallel scenarios are applied considering the fact that bottom trawl surveys do not specifically target pelagic species and are designed to capture and evaluate bentho-demersal ones:

Including all species biomass data (i.e.bentho-demersal and pelagic species)

Excluding pelagic species

Excluding pelagic species allows increasing the signal of the indicator (i.e. significance of its trend) by excluding the high inter-annual biomass variability of the pelagic species.

Trophic level data:

A preliminary work is needed to collate sub-regional TL estimations per species. This work aims to improve the reliability of TL values as the diet of species (and hence the predator-prey relationships) may vary among regions. Sub-regional TL estimations are then more relevant to calculate the MTL of the sub-region. Three sources of TL have to be respectively prioritised to keep the more appropriate TL estimation for each species, as shown in the diagram:

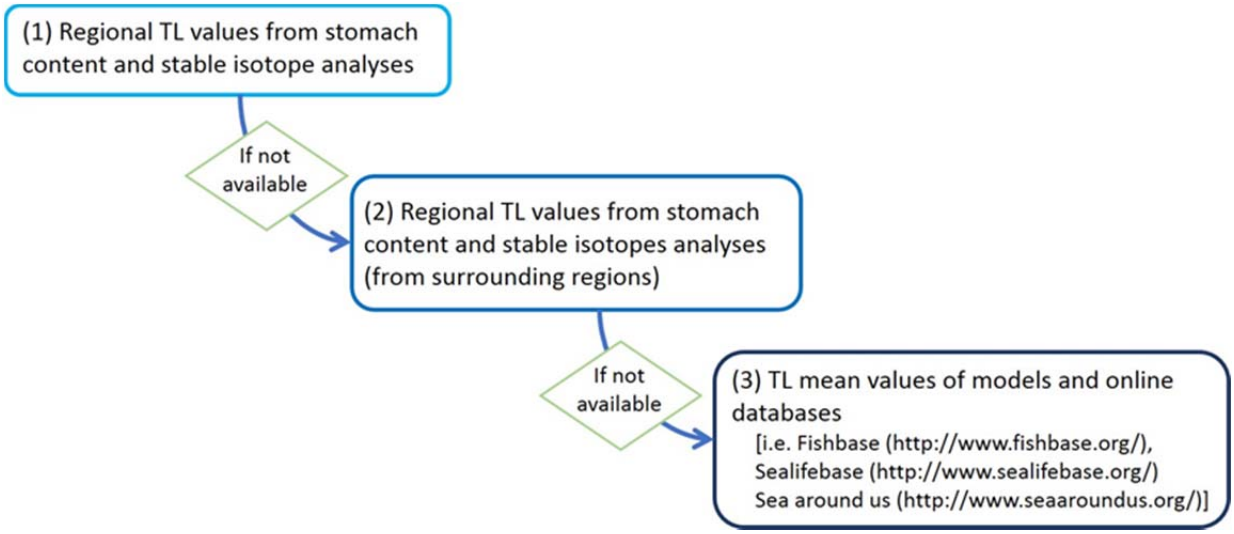

Figure 2: Trophic Level value selection according to source type.

However, as TLs are assigned at the species level, it seems not appropriate to assign TLs to large groups gathering species from low to high TL (*e.g*. Osteichthyes). High taxonomic ranks (i.e. phylum, sub-phylum, class, superorder, order, suborder and infraorder) need to be removed from the MTL computation. Only genus and family ranks have to be considered. For them, an average of TL values of all species present in the region has to be applied to calculate their TL.

Calculation of Mean Trophic Level indicator (MTL):

The Mean Trophic Level indicator for each year k can be calculated using the biomass and TL of species with the following formula:

$$MTL_k = \sum_i (TLi) \cdot (Yik) / \sum_i Yik$$

Formula 1: The Mean Trophic Level indicator formula

MTL_k refers to the MTL indicator for each year k

TL_i refers to the trophic level estimation of species (group) i

Y_{ik} refers to the biomass (Y) of species (group) i in year k, as included in landings data or in survey data.

While assessing this indicator, the group of species considered can be changed by applying a TL cut-off (e.g. 3.25). The purpose of these cut-offs is to exclude low TL species in order to focus on predators (i.e. high TL species) and detect changes in the upper food web structure.

The first TL cut-off (i.e. MTL_3.25) to be applied was described by Pauly and Watson (2005) and excluded all landed species under a TL of 3.25 (known as the Marine Trophic Index, MTI). The MTI corresponds to MTL_3.25. Another cut-off fixed at 4 can be applied to the data (i.e. MTL_4) to focus on higher predators.

Table 2: Summary of the different scenarios in the MTL indicator.

	Surveys		Landings	
	All sp.	Excluding pelagic sp.	All sp.	Excluding pelagic sp.
MTL_2.0	TL cut-off of 2.0 (Including all consumers into the analysis)			
MTL_3.25	TL cut-off of 3.25 (= MTI of Pauly & Watson 2005)			
MTL_4.0	TL cut-off of 4 (focus on higher predators)			

Uncertainty exists around each TL value estimated by diet studies, which is related to spatio-temporal variability and species ontogeny (Pinnegar *et al.*, 2002; Chassot *et al.*, 2008; Vinagre *et al.*, 2012). This uncertainty needs to be reported as a standard error for each TL value of a species.

In order to include uncertainty in the MTL model, a bootstrap methodology developed using the R software (R version 3.3.3) needs to be run. A random sampling will be applied on TL values and their standard error performing 500 MTL computations per studied year. The model will be then fitted as a mean value of the 500 MTL generated with an uncertainty related to its standard error. The uncertainty around the MTL model will be thus linked to the uncertainty of the TL estimations.

3.3 Assessment criteria

The assessment of the indicator is currently based on its trend analysis.

Reference valuescan be determined later on in some regions where data-series exist. The MTL has to be known from a past state of the ecosystem assumed to be not impacted (or moderately impacted) by fishing at the ecosystem scale. However, most historical standardised data series beginin periods (80's - 90's decades) when ecosystems were already overexploited. Alternatively, reference values can be simulated and the MTL estimated, using trophic models such as Ecopath with Ecosim (Christensen and Pauly 1992; Walter *et al.*, 1997) or EcoTroph (Gascuel 2005;Gascuel and Pauly 2009).

With the current available knowledge of experts, reference values can be discussed for an ecosystem over the studied period (for instance with the NEAT TOOL), under the precautionary principle. Reference values can be based on the most recent years if they are reflecting a recovering ecosystem that may experience further improvement (increase of the MTL indicator). The 10^{th} and 90^{th}percentiles of this period can be used to define a reference interval as well as the period when the MTL indicator would be at its lowest values, *i.e.* reflecting a period where impacts due to fishing were stronger than in the current situation, and hence in a less-desirable state. A precautionary approach should be applied and the reference values

should be updated in each new OSPAR cycle based on experts' knowledge on the evolution of their ecosystem. It is worth noting that the reference values that are discussed do not represent any agreement of reference values by European Member States within OSPAR.

3.4 Spatial Analysis and / or trend analysis

Analysis at the sub-regional level, example: southern and northern Bay of Biscay (part of Region IV).

Trend analysis: evolution of the MTL indicator under different scenarios taking into account inter-annual biomass variability and TL estimations variability. Statistical tests can be applied to detect significant trends.

3.5 Presentation of assessment results

A time series of the MTL indicator for each scenario over the period studied for each sub-region will be presented with reference values/interval when available.

A confidence interval representing the uncertainty around the TL estimations will also be added.

4 Change Management

Responsibility for this CEMP guideline and follow up of indicator assessments falls under the OSPAR Biodiversity Committee, the work is undertaken by the expert group for food webs which provides input to ICG-COBAM.

5 References:

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Annex 1

Example of a protocol for stomach content analyses

PROTOCOL FOR STOMACH CONTENT ANALYSIS WITHIN IBT SURVEYS

Santander IEO Trophic Ecology Team

1. Sampling protocol

The herewith described 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. 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 well-established and has been proved to reliably characterize some of the most abundant predators' diets in the area (Velasco and Olaso, 1998; 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 (Appendix 1).

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).

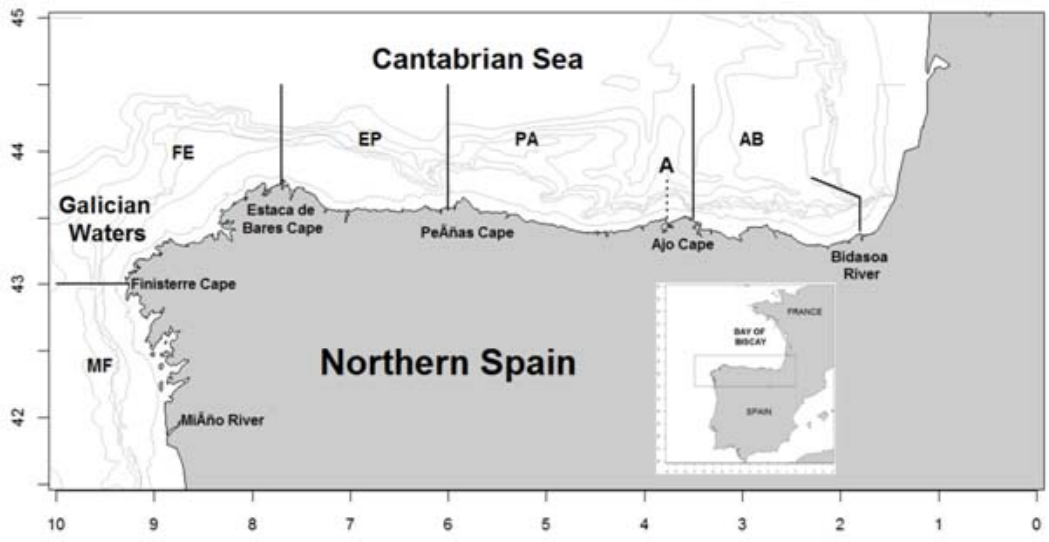


Figure 1. Area covered by the Demersales surveys in the Southern Bay of Biscay showing the 5 different sectors considered.

- 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 *Merlucciusmerluccius*,*Lepidorhombusboscii*and *Lepidorhombuswhiffiagonis* 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 following ranges: 9 - 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 *Merlucciusmerluccius*, 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.

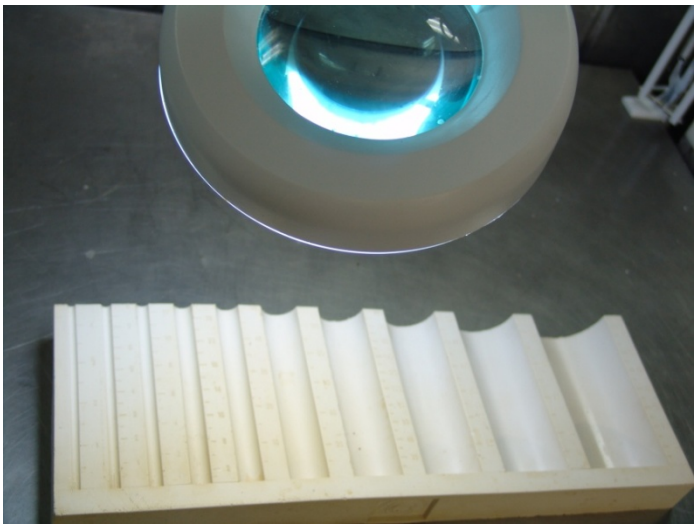


Figure 3. Trophometer used during Demersales surveys for stomach content analyses.

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.

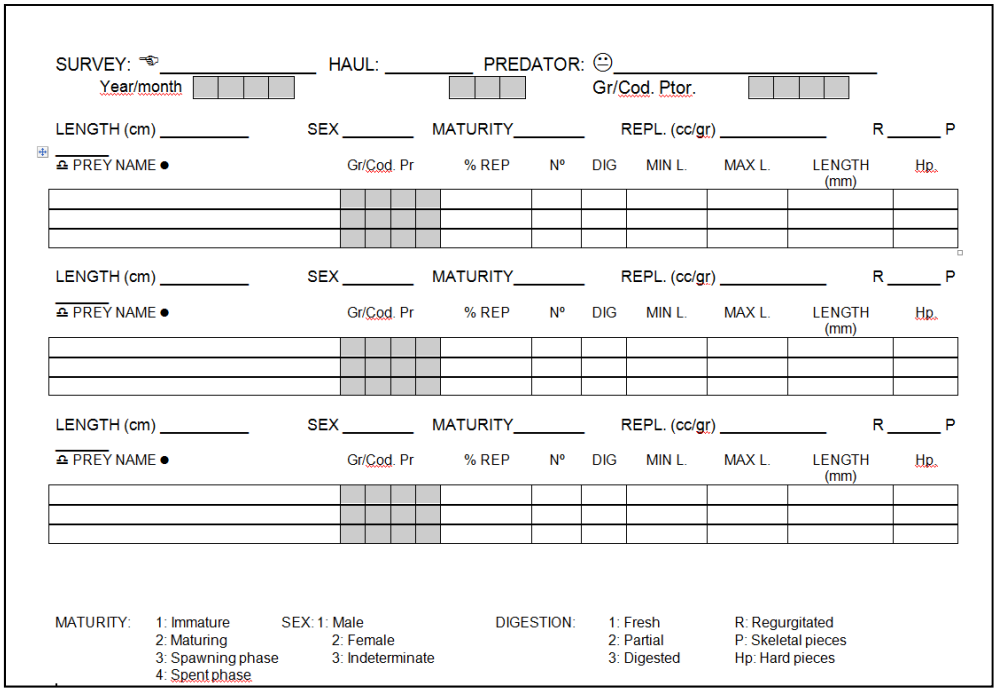


Figure 4. Data sheet used during *Demersales* surveys to record stomach content analyses data.

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.

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

The parameter theta 1 (ϕ_1) represents the value of the prey pool that is obtained when x →∞. 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: *Callionymuslyra, Chelidonichtyscuculus, Conger conger, Eutriglagurnardus, Galeusmelastomus, Helicolenusdactylopterus, Lepidorhombusboscii, Lepidorhombuswhiffiagonis, Merlucciusmerluccius, Micromesistiuspoutassou, Mullussurmuletus, Pagellusacarne, Raja clavata, Raja montagui, Scomberscombrus, Scyliorhinuscanicula, Trisopterusluscus* and *Trisopterusminutus.*

Table 1. List of 67 fish species subject to diet analyses in Demersales IBT survey. We indicate if the diet has been analysed following the above mentioned methodology and/or if prospective diet determination has been performed. Relative abundance of each species is given as a discrete category: Very low = not annually found, Low = annually found in abundances that do not allow diet determination, Medium = annually found in abundances that allow diet determination only some years, High = annually found in abundances that allow diet determination every year.

Species	Prospective sampling	Consistent Sampling	Relative abundance
Acantholabruspalloni	X		Very low
Aphanopus carbo		1990-	Very low
Arnoglossusimperialis	X		High
Arnoglossuslaterna	X		High
Boopsboops	X		High
Callionymuslyra		1990-	High
Cepolarubescens	X		High
Chelidonichthyscuculus		1990-	High
Chelidonichthyslucerna	X	1993-	High
Chelidonichthys obscurus		1990-	High
Conger conger		1990-	High
Deaniacalcea		1990-	Very low
Deaniaprofundorum		2009-	Low
Diploduscervinus	X		Very low
Diplodussargus	X		Very low
Diplodus vulgaris	X		Very low
Etmopterusspinax	X	1993-	Medium
Eutriglagurnardus		1990-	High
Gaidropsarusmacrophtalmus	X	1993-	Medium
Galeusatlanticus		2009-	Low
Galeusmelastomus	X	1993-	High
Helicolenusdactylopterus	X	1998-	High
Hoplostetusmediterraneus	X	2009-	Medium
Labrusmixtus	X		Very low
Lepidion eques	X		Low

Lepidopuscaudatus	X		Very low
Lepidorhombusboscii		1990-	High
Lepidorhombuswhiffiagonis		1990-	High
Lepidotriglacavillone /dieuzedei	X	2001-	Medium
Leucorajacircularis		1990-	Very low
Leucorajanaevus		1990-	Medium
Lithognathusmormyrus		1992-	Very low
Lophiusbudegassa		1990-	High
Lophiuspiscatorius		1990-	High
Malacocephaluslaevis	X		Medium
Merlucciusmerluccius		1990-	High
Microchirusvariegatus	X		High
Micromesistiuspoutassou		1990-	High
Molvamacrophthalma	X	1999-	Medium
Mora moro	X		Very low
Mullussurmuletus		1990-	High
Notacanthusbonaparte	X		Very low
Pagellusacarne		1990-	High
Pagellusbogaraveo		1990-	Very low
Pagelluserythrinus		1990-	Medium
Pagruspagrus	X		Very low
Phycisblennoides		1990-	High
Raja clavata		1990-	High
Raja montagui		1990-	High
Scomberscombrus		2000-	High
Scorpaenaloppei	X	1999-	Medium
Scorpaenanotata	X		Very low
Scorpaenascrofa	X	1999-	Low
Scyliorhinuscanicula		1990-	High
Scyliorhinusstellaris	X		Low
Scymnodonringens		1995-	Very low
Serranuscabrilla	X		Low
Solealascaris	X		Low
Soleasolea	X		Medium
Spondyliosomacantharus	X	1996-	Low
Trachinusdraco	X	2001-	Medium
Trachurustrachurus	X		High
Trachyscorpiacristulata		1999-	Very low
Triglalyra	X	1993-	High
Trisopterusluscus	X	1990-	High
Trisopterusminutus	X	1993-	High
Zeus faber	X	1992-	High

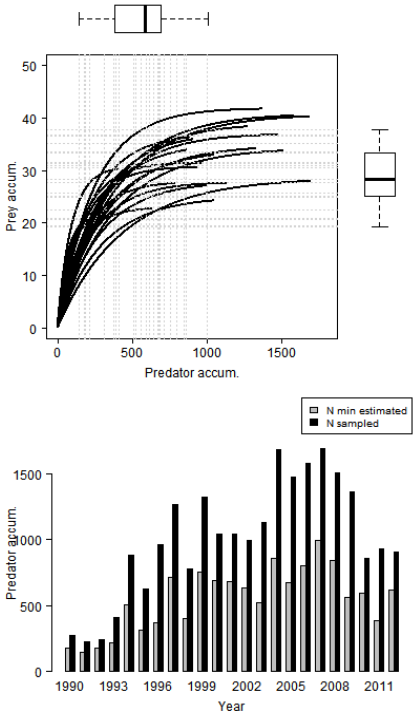
EXAMPLES

The following examples show the different degrees of acceptability obtained for the various ontogenetic 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 the percentage 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.

***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 (Sánchez 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.

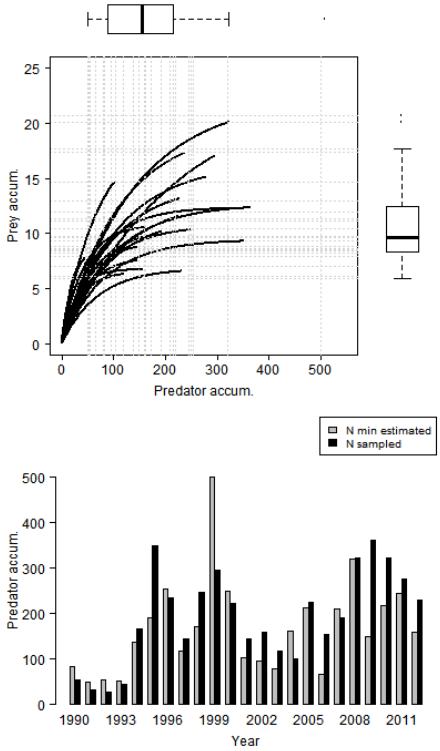
The sampling strategy identified annually100% of the prey pool indicating that all prey were identified along the time series.

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.

***Merlucciusmerluccius*(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.



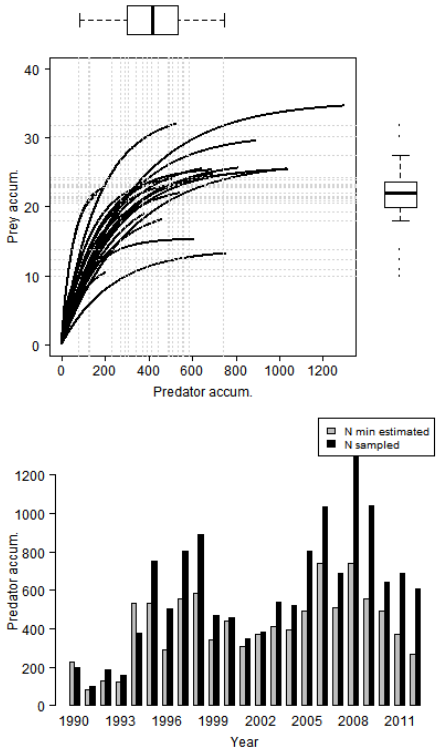
The sampling strategy identified 98- 100% of the prey pool annually, indicating that all prey were identified along the time series.

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;López-López 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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