

OSPAR CEMP Guidelines

Common Indicator in OSPAR Region IV, Change in average trophic level of marine predators (FW-4)

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¹ English only. Update 2023

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 (TL) of species. The TL reflects the position of an organism in a food web. Thus 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 and stable isotope analyses.

In February 2004, the Mean Trophic Level (MTL) indicator with a TL 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 surveys and 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 being highly developed and currently applied globally across ecosystems (www.indiseas.org). In addition, different scenarios using surveys and landings were also developed in the OSPAR Intermediate Assessment 2017 becoming a common indicator in OSPAR Region IV (Arroyo et al., 2019).

An important advantage of the MTL indicator is that the proposed concept is transferable across OSPAR regions and data for the calculation of the indicator (biomass of the species) are available online. The MTL can be estimated on a regional/sub-regional scale using existing biomass data from scientific surveys which are standardised in ICES DATRAS online database. 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). The trophic level of species should be regionally calculated using diet and stable isotopes analysis.

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 new approach presented here is the calculation of local MTL by haul by year in OSPAR Region IV, including data from three countries (France, Spain, Portugal).

The MTL has been traditionally calculated using biomass data from landings. However, due to the bias of landings caused by the multiple fishing strategies, changing markets and commercial needs we focus the assessment on standardise biomass data coming from scientific surveys giving a more comprehensive view of the whole ecosystem. They are usually limited to a specific season and hence only give a snapshot of the communities at that given moment. However, the availability of data throughout NE Atlantic and the standardisation of data make ICES DATRAS the most appropriate for this indicator. The trophic levels (TLs) of the species are regionally estimated using stomach contents and stable isotopes analysis. If local/regional TL values are not available, worldwide estimations can be downloaded in several online databases. However, a regionalisation of the TL estimation is strongly recommended since it captures better the trophic interactions in local networks.

2 Monitoring

2.1 Purpose and quantitative objectives

The purpose of this indicator is to assess the status of food webs using the biomass of the main benthodemersal species and their trophic level. It is important for the MTL indicator calculation to use scientific standardised survey data as they are most representative of ecosystem changes. Landings were analysed in OSPAR IA2017, however fisheries catches have been criticised due to potential biases induced by changes occurring in the fishing strategies. For this reason, landings and catches have not been taken into account in the present assessment.

In order to establish trophic relationships more accurately through regional TL estimations, data on species feeding habits is 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 latest OSPAR IA2017 report noted the need to collect more information on these data to fill the gaps in our knowledge of food web structure and connectivity (Carafa *et al.*, 2007; Moloney *et al.*, 2010; Rossberg *et al.*, 2011; Arroyo *et al.*, 2019). 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).

Data needed:

Data needed to calculate the MTL are: i) biomass of species by haul by year, and ii) trophic level of the species. Two ways of presenting the results are used in these guidelines:

- Temporal approach: the trend of the average MTL per haul per year is assessed across the historical series.
- Spatio-temporal approach: the average MTL per haul per year is assessed by square (0.2 x 0.2 grades)

The Trophic Level (TL) database per species ($\pm$ SE) should be reported by sub-division, whenever possible.

Product delivered:

- Time series of the MTL indicator in different scenarios (e.g. depth range, TL cut-offs) by sub-division.
- Maps showing mean Trophic Level (MTL) and trends by square.

2.2 Monitoring Strategy

Data for the MTL indicator come from scientific groundfish surveys from OSPAR Regions IV (Figure 1).

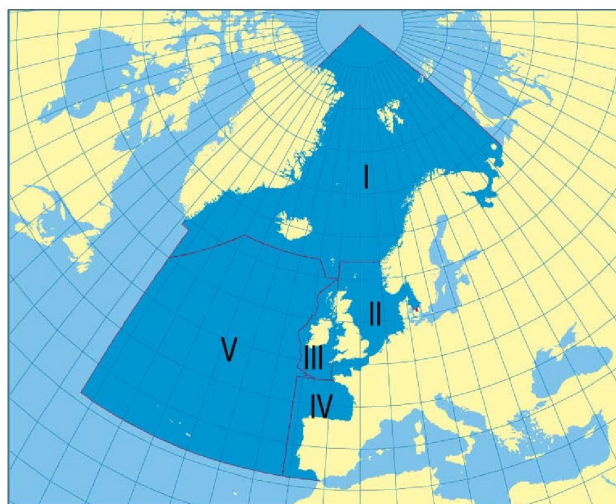


Figure 1. Map showing OSPAR Regions. These CEMP guidelines are applied to four sub-divisions within OSPAR Region IV.

The four sub-divisions taken into account within Region IV are:

- French continental shelf
- North Iberian continental shelf
- Portuguese continental shelf
- Gulf of Cadiz continental shelf

2.3 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 in Annex 1.

Table 1. Proposal of a protocol for the analyses of diet compositions and stable isotope analyses to be implemented in groundfish surveys, including staff, expertise, working time and equipment needed. More details are described in Annex 1.

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.	2 technicians	• Species identification • Sampling • Samples treatment (dissection and species identification)	9	Freezer (-20°C) to stock samples
	Analysis of the samples should preferably be done on board, although storage at -20°C is also acceptable until analysis in the laboratory. <i>PS: Working time is an estimation for 600 samples analysed.</i>	1 Researcher	• Conception • Supervision • Valorisation	1	+Stereomicroscope (macrofauna) +Microscope (microfauna)

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/or SOM* whenever possible; bivalvia or zooplankton is also acceptable) of the food web should be sampled.	2 technicians	- Species identification - Sampling - Samples treatment (dissection, delipidation, lyophilisation, samples conditioning)	10	Freezer (-20°C) to stock samples and material for samples preparation (tin, capsules, pill, bags, etc.)
	Stock the samples at -20°C until analysis in the laboratory. <i>PS: Working time is an estimation for 3000 samples analysed.</i>	1 Researcher	- Conception - Supervision - Valorisation	1	+Isotopic analysis platform

2.4 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. Although the MSFD quality assured groundfish survey monitoring and assessment data products were built to solve the main problems, several issues still remain: length of time series, number of species reported by each country, different gears, vessels, quarters, etc.

Specific and relevant filter criteria should be agreed among countries and scientists leading different indicators so that the assessment is consistent across regions.

2.5 Data reporting, handling and management

Survey data are submitted to the Database of Trawl Surveys (DATRAS): <http://www.ices.dk/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/data/data-portals/Pages/DATRAS-Docs.aspx>

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

An excel file for annual TL regional estimation by sub-division 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

The TL estimated from stomach content analysis, stable isotope analysis or models can be calculated locally using data from each sampling programme or in the literature for regional and non-regional areas. Trophic Level values are also 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 are gathered into an excel file and should be populated by each country. TL database for OSPAR Region IV is uploaded on the OSPAR sharepoint (BDC/Foodweb EG/FW4 - Change in trophic level of predators in OSPAR Region IV/03_Data_Snapshot/TL_complete_Region IV).

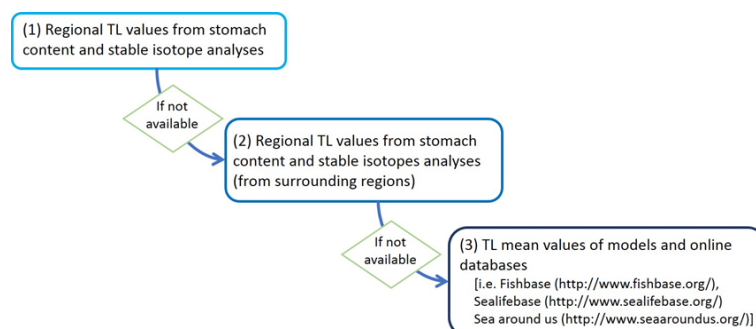
3.2 Preparation of data

In the present assessment, the indicator has been calculated starting in 2000 and using only data from the fourth quarter of the year (Q4) in order to keep consistency across the four sub-divisions under study. The differences in the DATRAS database uploaded by each country should be taken into account when calculating the indicator, as they may prevent comparisons across regions. In particular, the set of species reported are crucial for the comparability of the indicator among sub-divisions, as the inclusion

of some species or others (commercial versus non-commercial fish and cephalopods, and/or benthic invertebrates) can lead to erroneous results of the indicator or even to not being able to calculate it. In some OSPAR regions various countries are operating at the same time in the same area, using different vessels and gears. Filter criteria should be agreed among scientist leading indicators so that the assessment is consistent across regions. This agreement is strongly recommended. In OSPAR Region IV each country uses the same vessel and gear throughout the whole historical series.

The TL estimated from stomach content analysis, stable isotope analysis or models can be collected from the literature for regional and non-regional areas. Otherwise, TLs 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/>)]. For this assessment, information on TLs per species have been estimated by sub-division using local stomach contents and stable isotopes, except for Portuguese waters where TL coming from the North Iberian shelf has been applied in the calculations. The TL databases by sub-division were compiled into a single database and populated by each country.

During the 2017 assessment, a preliminary work was performed in to collate regional TL estimations per species for OSPAR Region IV. However, the use of TL databases by sub-division was recommended in order to capture local trophic interactions and regional differenced in the structure of the trophic networks. TL estimates by sub-division are then more relevant to calculate the MTL of such particular sub-division. We compiled the TL in three different steps, prioritising in each case local estimates over TL estimated at larger scale, and making use of the latter only when the former were unavailable, as shown in the following scheme:



To do this, we considered only taxons at high taxonomic resolution (species, genus or family), excluding taxa identified at low taxonomic resolution (i.e. phylum, sub-phylum, class, superorder, order, suborder and infraorder). Nevertheless, in terms of biomass, taxa identified with low taxonomic resolution were practically negligible.

Uncertainty exists for each TL value estimated by diet studies, which is related to the spatio-temporal variability of the diet and differences of diet throughout the 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.

Ecological and geographical scenarios

Different scenarios have been considered for the calculation of MTL and MTL changes across time. Firstly, the MTL of the four sub-divisions was calculated independently, using the same methodology (specified in the following section) but based on local TL and biomass data.

For each of the sub-divisions, three different bathymetric ranges have been considered, assessing independently MTL at the coastal (< 30 m depth), continental shelf (30-200 m depth) and continental slope (>200 m depth). These bathymetric ranges reflect the fact that communities on regional seas are commonly structure along the depth gradient, and the community inhabiting the coastal area is fundamentally different from that inhabiting the deeper area, although both can be summarised into an MTL value. Thus, the bathymetric segregation allowed to explore temporal changes in these communities independently, with the potential to identify divergent trends along the depth gradient. The coastal area (<30 m) did not have a sufficient sampling coverage in the Northwestern Iberian and Portuguese regional seas as to be included in this assessment, so in these two sub-divisions only the continental shelf and slope were assessed.

Furthermore, for each sub-division and depth range we consider three ecological cut-offs in the trophic structure of the community. MTL2 included all species in the community except for primary producers. MTL3.25 included only those species with $TL \geq 3.25$, which are essentially secondary consumers and top predators, here called meso + top predators. Lastly, MTL4 included only species in the top of the food web, here called top predators (Figure 2). Differentiating the ecological community in these thresholds allow identifying patterns affecting only top predators (as might be the case of the “fishing down” hypothesis) which might be obscured by the high biomass of low trophic level species otherwise.

Finally, considering the fact that bottom trawl surveys do not specifically target pelagic species but are designed to capture and evaluate benthic-demersal ones and also, that the biomass of pelagic species dominates the community showing a very high variability, we considered for each sub-division, bathymetric range and MTL cut-off the whole community but also the community excluding pelagic species. The combination of this geographic and ecological constraints resulted in 18 scenarios which are summarised in Table 1.

Table 2. Summary of 18 different scenarios in the MTL indicator by sub-division.

		Top predators (MTL_4.0)	Meso + top predators (MTL_3.25)	All community (MTL_2.0)
Coastal (depth < 30 m)	with pelagics	top predators	mesopredators + top predators	all consumers
	without pelagics	top predators (excluding pelagic species)	Mesopredators + Top predators (excluding pelagic species)	all consumers (excluding pelagic species)
Shelf (depth 30- 200 m)	with pelagics	top predators	mesopredators + top predators	all consumers
	without pelagics	top predators (excluding pelagic species)	Mesopredators + Top predators (excluding pelagic species)	all consumers (excluding pelagic species)
Deep (depth > 200 m)	with pelagics	top predators	mesopredators + top predators	all consumers
	without pelagics	top predators (excluding pelagic species)	Mesopredators + Top predators (excluding pelagic species)	all consumers (excluding pelagic species)

3.3 Calculation of the Mean Trophic Level

Before performing the calculations and in order to select a set of species that have been consistently well sampled through the historical series, cumulative curves were applied to the biomass database to make a selection of species. Filter criteria should be agreed among scientists so that the assessment is consistent across regions. The following criteria were finally decided, based on the inflection points of species frequency of occurrence by space and time, and their abundance distribution:

- species that appeared at least in 50% of the years
- species that appeared at least in 10% of the hauls each year
- species that displayed an abundance above the quantile 10

To calculate the MTL by haul, we weight the TL value of each species by the biomass of each species in each haul. The indicator is then calculated for the whole community (all community, MTL2.0), including only species with TL higher than 3.25 (meso + top predators, MTL3.25) and including only species with TL higher than 4 (top predators, MTL4.0) (Figure 2).

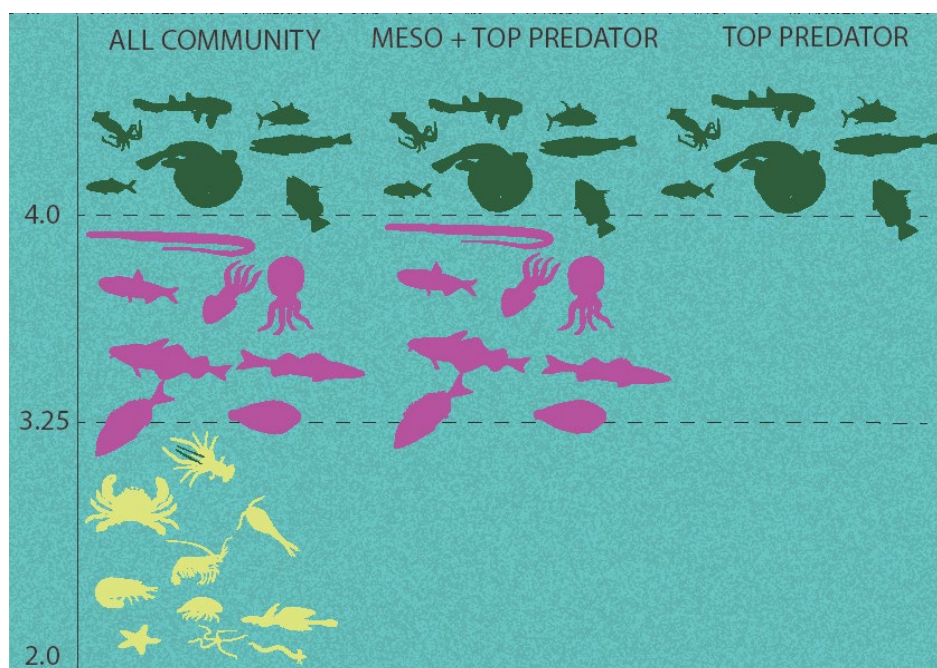


Figure 2. Schematic diagram showing the composition of species/taxa included in each TL cut-off: i) all community (MTL_2.0), ii) mesopredators and top predators (MTL_3.25), iii) top predators (MTL_4.0).

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

$$MTL_h = \sum_i (TL_i) * (Y_{ih}) / \sum_i (Y_{ih})$$

MTL_h refers to the MTL indicator by haul

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

Y_{ih} refers to the biomass (Y) of species (group) i in each haul h .

The resulting MTL values were explored to identify changes along the historical series in the different scenarios, by modelling the MTL as a function of year using Linear Models (LM) and/or Generalised Additive Models (GAM).

For the spatio-temporal approach, the calculation process follows the next steps for each of the scenarios (Figure 3):

- Step 1. Calculate the mean MTL on a 0.2 x 0.2 degrees over the whole time series.
- Step 2. For those cells having a sufficient number of data ($N > 10$) assess any linear trend in MTL by year, reporting the slope of the linear model and its p-value.
- Step 3. Plot these results spatially, with MTL and the slope of MTL represented by a colour scale, and any significant trend represented by the cell of interest framed in bold.

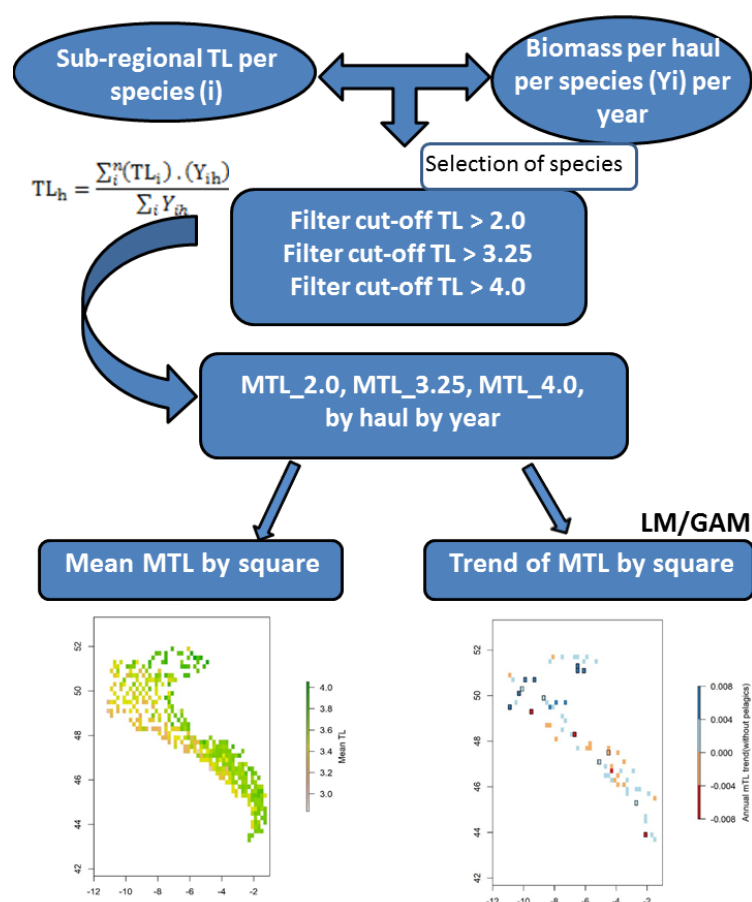


Figure 2. Testing framework for the calculation of the spatio-temporal approach: MTL by haul by year.

A grid size of 0.2 x 0.2 degrees was selected as a compromise to spatially integrate hauls data information from the bottom trawl surveys while keeping discrete cells over each of the depth ranges, particularly in the narrow continental shelves of north Iberian and Portuguese sub-divisions.

3.4 Assessment criteria

The assessment of the MTL is based on its trend analysis. To summarise the results of different scenarios and show a comprehensive message to the scientific and non-scientific community, the results of the trend analyses were binned into categories after McQuatter-Gollop et al., 2022. Colours are the result of the linear models showing increase/no-trend/decrease of the indicator. Trends were considered to be increasing or decreasing only when linear models were significant (*p<0.05, **p<0.01, ***p<0.001). The resulting categories are shown in Table 2. Please note that the present assessment shows changes in the indicator starting in 2000 when the bentho-demersal ecosystems were already overexploited, so the values at the start of the series do not represent ecological target values. Any positive trend in MTL is therefore considered as a sign of recovery, while a non-significant trend can be considered as a sign of the high resilience characterising systems enduring high and sustained impacts.

Table 3. Biodiversity status categories and colours used for the interpretation of the results. Trend analysis (linear model and significant tests) and expert judgement are used to set colours based on the categories proposed by McQuatter-Gollop et al., 2022.

Biodiversity status categories	
	Indicator value is below assessment threshold, or change in indicator represents a declining state, or indicator change is linked to increasing impact of anthropogenic pressures (including climate change), or indicator shows no change but state is considered unsatisfactory
	No assessment threshold and/or unclear if change represents declining or improving state, or indicator shows no change but uncertain if state represented is satisfactory
	Indicator value is above assessment threshold, or indicator represents improving state, or indicator shows no change but state is satisfactory
	Indicator was not assessed in a region due to lack of data, lack of expert resource, or lack of policy support.

3.5 Presentation of assessment results

3.5.1 Temporal approach

Time series of MTL by haul for each scenario over the period studied for each sub-division are performed. Confidence intervals representing the uncertainty in the LMs and GAMs are also included.

3.5.2 Spatio-temporal approach

Maps showing average MTL and trends in the time series in each square and scenario over the period studied. Significant/non-significant trend analyses are also reported.

References

Arroyo N.L., Safi G., Vouriot, P., López-López L., Niquil N., Le Loc'h F., Hattab T., Preciado I. 2019. Towards coherent GES assessments at sub-regional level: signs of fisheries expansion processes in the Bay of Biscay using an OSPAR food web indicator, the mean trophic level. ICES Journal of Marine Science, doi:10.1093/icesjms/fsz023

- Carafa, R., Dueri, S., Zaldívar, J.M. 2007. Linking terrestrial and aquatic ecosystems: Complexity, persistence and biodiversity in European food webs. In: EUR 22914 EN, Joint Research Centre.
- Chassot, E., Rouyer, T., Trenkel, V.M., and Gascuel, D. 2008. Investigating trophic-level variability in Celtic Sea fish predators. *Journal of Fish Biology* 73: 763–781.
- Christensen, V., and Pauly, D. 1992. The ECOPATH II—a software for balancing steady-state ecosystem models and calculating network characteristics. *Ecological Modelling*, 61: 169–185.
- Cury, P. M., Shannon, L. J., Roux, J-P, Daskalov, G. M., Jarre, A., Moloney, C. L., and Pauly, D. 2005. Trophodynamic indicators for an ecosystem approach to fisheries. *ICES Journal of Marine Science*, 62: 430-442.
- Gascuel, D. 2005. The trophic-level based model: a theoretical approach of fishing effects on marine ecosystems. *Ecological Modelling*, 189: 315–332.
- Gascuel, D., and Pauly, D. 2009. EcoTroph: modelling marine ecosystem functioning and impact of fishing. *Ecological Modelling*, 220: 2885–2898.
- Moloney, C.L., St John, M.A., Denman, K.L., Karl, D.M., Koster, F.W., Sundby, S., Wilson, R.P. 2010. Weaving marine food webs from end to end under global change. *Journal of Marine Systems* 84, 106-116.
- Pauly, D., Christensen, V., Dalsgaard, J., Froese, R., and Torres, F. C. 1998. Fishing down marine food webs. *Science*, 279: 860–863.
- Pauly, D., and Watson, R. 2005. Background and interpretation of the Marine Trophic Index as a measure of biodiversity. *Philosophical Transactions of the Royal Society of London, Series B*, 360: 415–423.
- Pinnegar, J.K., Jennings, S., Brien, C.M.O., and Polunin, N.V.C. 2002. Long-term changes in the trophic level of the Celtic Sea fish community and fish market price distribution. *Journal of Applied Ecology* 39: 377-390.
- Preciado I., Arroyo N.L., González-Irusta J.M., López-López, Punzón A., Muñoz I., Serrano A. 2019. Small-scale spatial variations of trawling impact on food web structure. *Ecological indicators* 98: 442-452.
- Rossberg, A. G., Farnsworth, K. D., Satoh, K., Pinnegar, J. K. 2011. Universal power-law diet partitioning by marine fish and squid with surprising stability-diversity implications. *Proc. R. Soc. B* 278 (1712), 1617–1625.
- Samhuri, J. F., Levin, P. S., & Ainsworth, C. H. 2010. Identifying thresholds for ecosystem-based management. *PLoS One*, 5(1), e8907.
- Vinagre, C., Salgado, J., Mendonca, V., Cabral, H., and Costa, M. 2012. Isotopes reveal fluctuation in trophic levels of estuarine organisms, in space and time. *Journal of Sea Research* 72:49-54.

Annex 1. Protocol for stomach content analysis within IBT surveys

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.

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 tows outside these ranges (in shallower and deeper areas) following the same methodology (Figure 1).

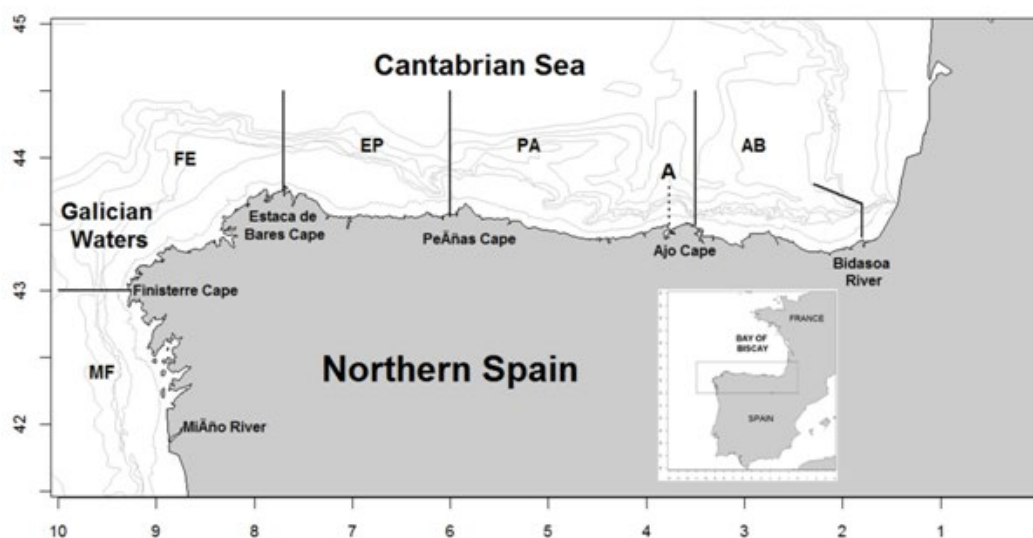


Figure 1. Area covered by the Demersals surveys in the Southern Bay of Biscay showing the 5 different sectors considered.

- 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 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 *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, whenever 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 the stomach content 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.

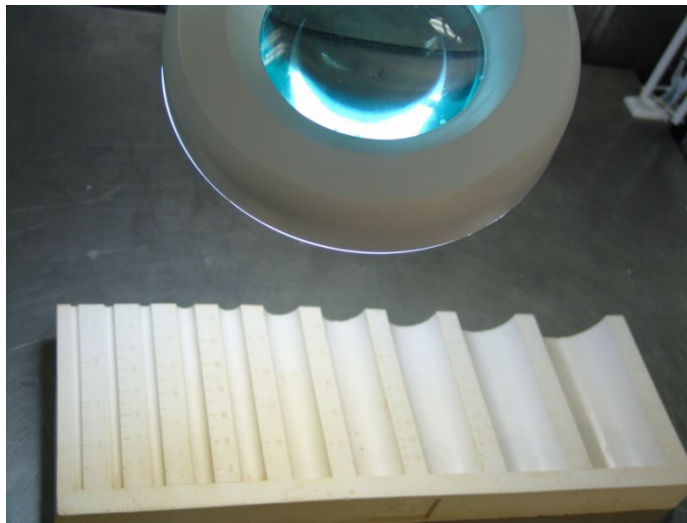


Figure 3. Trophometer used during Demersales surveys for stomach content analyses.

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 p_j 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.

SURVEY: 📄		HAUL: 📄		PREDATOR: 😊			
Year/month				Gr/Cod. Ptor.			
LENGTH (cm)	SEX	MATURITY	REPL. (cc/gr)	R P			
PREY NAME ●	Gr/Cod. Pr	% REP	Nº	DIG	MIN L.	MAX L.	LENGTH (mm) Hp.
LENGTH (cm)	SEX	MATURITY	REPL. (cc/gr)	R P			
PREY NAME ●	Gr/Cod. Pr	% REP	Nº	DIG	MIN L.	MAX L.	LENGTH (mm) Hp.
LENGTH (cm)	SEX	MATURITY	REPL. (cc/gr)	R P			
PREY NAME ●	Gr/Cod. Pr	% REP	Nº	DIG	MIN L.	MAX L.	LENGTH (mm) Hp.

MATURITY: 1: Immature SEX: 1: Male DIGESTION: 1: Fresh R: Regurgitated

 2: Maturing 2: Female 2: Partial P: Skeletal pieces

 3: Spawning phase 3: Indeterminate 3: Digested Hp: Hard pieces

 4: Spent phase

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.

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

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 were analysed during *Demersales* surveys. The result showed that the diet of 19 species was adequately characterized along the time series using the above mentioned methodology. These species were: *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*, *Scombers combrus*, *Scyliorhinus canicula*, *Trisopterus luscus* and *Trisopterus minutus*.

Table 1. List of 67 fish species subjected 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
<i>Acantholabrus palloni</i>	X		Very low
<i>Aphanopus carbo</i>		1990-	Very low
<i>Arnoglossus imperialis</i>	X		High
<i>Arnoglossus laterna</i>	X		High
<i>Boops boops</i>	X		High
<i>Callionymus lyra</i>		1990-	High
<i>Cepola rubescens</i>	X		High
<i>Chelidonichthys cuculus</i>		1990-	High
<i>Chelidonichthys lucerna</i>	X	1993-	High
<i>Chelidonichthys obscurus</i>		1990-	High
<i>Conger conger</i>		1990-	High
<i>Deania calcea</i>		1990-	Very low
<i>Deania profundorum</i>		2009-	Low
<i>Diplodus cervinus</i>	X		Very low
<i>Diplodus sargus</i>	X		Very low
<i>Diplodus vulgaris</i>	X		Very low
<i>Etmopterus spinax</i>	X	1993-	Medium
<i>Eutrigla gurnardus</i>		1990-	High
<i>Gaidropsarus macroptalmus</i>	X	1993-	Medium
<i>Galeus atlanticus</i>		2009-	Low
<i>Galeus melastomus</i>	X	1993-	High
<i>Helicolenus dactylopterus</i>	X	1998-	High
<i>Hoplostetus mediterraneus</i>	X	2009-	Medium
<i>Labrus mixtus</i>	X		Very low
<i>Lepidion eques</i>	X		Low
<i>Lepidopus caudatus</i>	X		Very low
<i>Lepidorhombus boscii</i>		1990-	High
<i>Lepidorhombus whiffiagonis</i>		1990-	High
<i>Lepidotrigla cavillone /dieuzedei</i>	X	2001-	Medium
<i>Leucoraja circularis</i>		1990-	Very low
<i>Leucoraja naevus</i>		1990-	Medium
<i>Lithognathus mormyrus</i>		1992-	Very low
<i>Lophius budegassa</i>		1990-	High
<i>Lophius piscatorius</i>		1990-	High
<i>Malacocephalus laevis</i>	X		Medium
<i>Merluccius merluccius</i>		1990-	High
<i>Microchirus variegatus</i>	X		High
<i>Micromesistius poutassou</i>		1990-	High

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

EXAMPLES

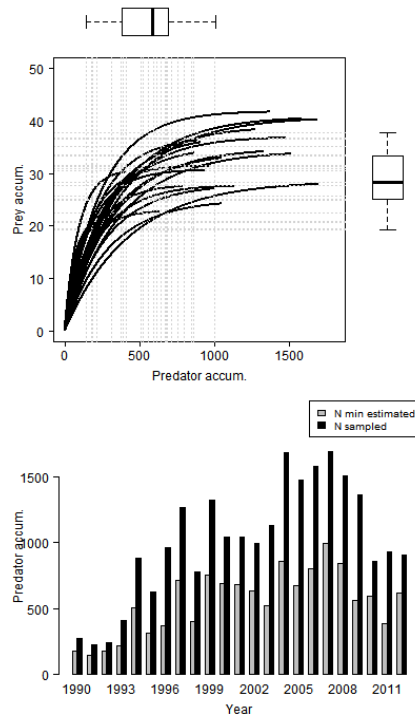
The following examples show the different degrees of acceptability obtained for the various ontogenetic stages of hake (*M. merluccius*). First, a small description of each ontogenetic stage's habitat and/or feeding habits is given, followed by general trophic metrics such as the percentage of vacuity,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

***Merluccius 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.

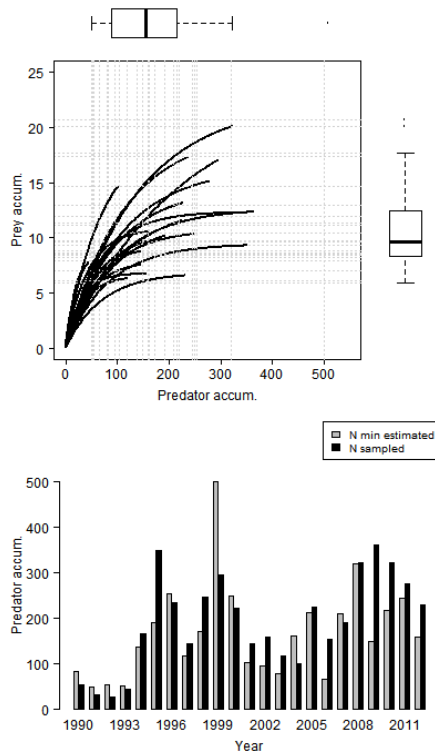
The sampling strategy identified annually 100% of the prey pool indicating that all prey were identified along the time series.

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; López-López et al., 2015).

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



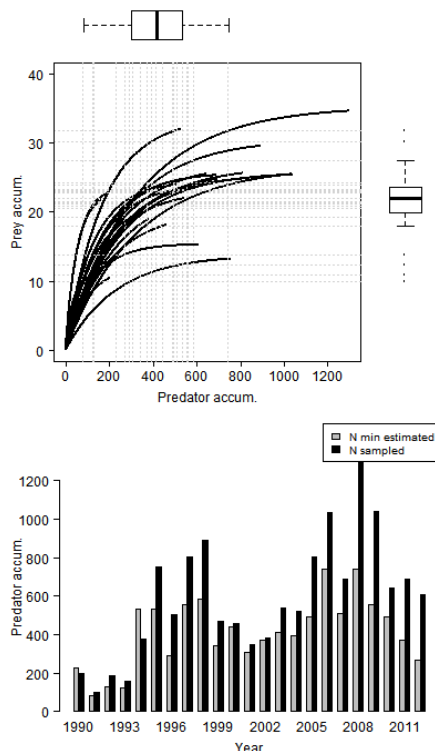
The sampling strategy identified 98- 100% of the prey pool annually, indicating that all prey were identified along the time series.

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).

REFERENCES

- Ferry LA, Cailliet GM 1996. Sample size and diet analysis: are we characterizing and comparing diet properly? In: Feeding ecology and nutrition in fish. 71- 80 pp
- Krebs C.J. 1988. Ecological methodology. Harper Collins Publishers Inc., New York, NY.
- López-López L, Preciado I, Villamor B, Velasco F, Iglesias M, Nogueira E, Gutierrez-Zabala JL, Olaso I 2012. Is juvenile anchovy a feeding resource for the demersal community in the Bay of Biscay? On the availability of pelagic prey to demersal predators. ICES J Mar Sci 69: 1394– 1402
- López-López L. 2017. Trophic structure and functioning of the marine food webs on the North-Atlantic continental shelf of the Iberian Peninsula: Implications of benthic-pelagic coupling. Universidad de Oviedo. FPI-IEO 2011-04 grant.
- Olaso I. 1990. Distribución y abundancia del megabentos invertebrado en fondos de la plataforma cantábrica. Publicaciones Especiales Instituto Español de Oceanografía n°5 pp 128

- Preciado I., Punzón, A., Velasco, F. 2015. Spatio-temporal variability in the cannibalistic behaviour of European hake *Merluccius merluccius*: The influence of recruit abundance and prey availability J. Fish Biol 86: 1319- 1334
- Robb A. P. 1992. Changes in the gall bladder of whiting (*Merlangius merlangus*) in relation to recent feeding history. ICES J. mar. Sci., 49: 41-436
- Sánchez F, Gil J 2000. Hydrographic mesoscale structures and Poleward Current as a determinant of hake (*Merluccius merluccius*) recruitment in southern Bay of Biscay. ICES J Mar Sci 57: 152– 170
- Velasco F., Olaso, I 1998. European Hake *Merluccius merluccius* (L., 1758) feeding in the Cantabrian Sea: seasonal, bathymetric and length variations. Fish Res 38: 33–44
- Velasco F. 2007. Alimentación de la merluza europea (*M. merluccius* L.) en el mar Cantábrico. PhD thesis, Universidad Complutense de Madrid.