

Sentinels of Seabed (SoS) indicator: Assessing benthic habitats condition using typical and sensitive species

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ABSTRACT

Indicators are key tools used to assess the ecological status of the environment for ecosystem based management. Anthropogenic disturbances produce changes to habitat condition, which include modifications in species composition and their functions. Monitoring a group of sentinel species (from a taxonomic and functional point of view) provides useful insights into benthic habitat condition. Here, a new indicator, Sentinels of the Seabed (SoS) is proposed to assess state of benthic habitats using “sentinel” species (species which are characteristic of a habitat and sensitive to a given pressure). The selection of these sentinel species has two stages. First, a ‘typical species set’ is computed using intra-habitat similarity and frequency under reference conditions. Second, the ‘sentinel species set’ is generated by selecting the most sensitive species from the typical species set. This selection is made using specific indexes able to assess species sensitivity to a particular pressure. The SoS indicator method was tested on six case studies and two different pressure types (trawling disturbance and pollution), using data from otter trawl, box-corer and Remote Operate Vehicle images. In each scenario, the SoS indicator was compared to the Shannon-Wiener diversity index, Margalef index and total biomass, being the only metric, which showed the expected significant negative response to pressure in all cases. Our results shows that SoS was highly effective in assessing benthic habitats status under both physical and chemical pressures, regardless of the sampling gear, the habitat, or the case study, showing a great potential to be a useful tool in the management of marine ecosystems.

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1. Introduction

Coherent management of marine ecosystems requires a good understanding of the structure and functioning of their components, including how natural and anthropogenic disturbances affect their ecological status. The main component of seabed ecosystems are benthic habitats, and in particular biogenic habitats, since the structure and functions provided by the biocenosis are combined with those of the biotope (de la Torre et al., 2020). Anthropogenic disturbances can produce changes in habitats distribution and condition, including modifications in species composition as a function of differential species' responses to environmental changes (Pearson & Rosenberg, 1978; Villnäs, 2013, González-Irusta et al., 2018). Depending on their biological characteristics, some species can exert strong effects on ecosystem processes and therefore, variations in their natural abundance can cause alterations in the structure and function of the habitats (Smith et al., 2014). As a result of human impacts on biological communities, declines in biodiversity can occur, but also species shifts and replacements (Fariña et al., 2003), affecting not only α -diversity, but also β -diversity as well as functional diversity (Dauvin et al., 2012; de la Torre et al., 2020). Therefore, monitoring a group of key species (from taxonomic and functional points of view) can provide a useful tool for assessing the ecological status of habitats.

Given the vital role that those sensitive and key species play in species composition, the main European marine nature conservation directives require the monitoring of typical species composition as a method to evaluate human impacts on benthic habitats. The concept of "typical species" (TS) emerges from the Habitats Directive (HD, 92/43/EEC), which defines (together with other criteria) that the conservation status of a habitat is favourable if "the conservation status of its typical species is favourable" (Evans & Arvela, 2011). Although the HD uses the term "typical species", it neither provides a definition of this term nor provides a list of typical species per habitat type. The Marine Strategy Framework Directive (MSFD; Directive 2008/56/EC) aims to implement an integrated ecosystem-based approach to manage anthropogenic activities and achieve Good Environmental Status (GES) of the marine environment. Of the 11 Descriptors in which the MSFD bases the GES assessment, two are related to benthic habitats: Descriptor 1- Biodiversity and Descriptor 6- Seafloor integrity. Seafloor integrity addresses GES in a more operational way to assess environmental conditions and anthropogenic pressures. It aims to ensure that the sea-floor integrity is at a level that guarantees that ecosystems structure and functions are safeguarded and benthic ecosystems are not adversely affected. The MSFD revised decision on GES (2017/848/EU Commission Decision) (EU, 2017), explicitly mentions the proportion of "typical species" (D6C5 criterion) or "particularly sensitive or fragile species" (D6C3 and D6C5 criteria) adversely affected by physical pressures (D6C3; e.g. fishing, dredging) or by the combination of all pressures (D6C5; e.g. physical pressures, pollution, eutrophication, etc.). In the context of the MSFD, changes in the specific composition must therefore be determined by monitoring a group of species that meet the double requirement of 1) being characteristic or typical of that habitat, and 2) being sensitive to the pressures being evaluated.

Sentinel species are "species that, by its presence or its relative abundance, indicates possible imbalances in the surrounding environment or distortions in community functions" (Dauvin et al., 2012). A multi-specific approach is recommended in which sentinel species are selected considering the variety of their habitats and feeding strategies but are also chosen among key species. These key species interact with numerous components of the ecosystem and, if no ecological equivalents are present, their disturbance can cause modifications of the structure and the functioning of the ecosystem (i.e. Bellwood et al., 2003; Guillemot et al., 2011; Karlson et al., 2016).

The main objective of this paper is to determine the feasibility, applicability, and effectiveness of the Sentinel of the Seabed (SoS) indicator as a metric for measuring the status of benthic habitats based on

the relative abundance of sensitive and structural species (sentinel species). This objective responds to the need to establish a limited group of MSFD and HD common indicators that respond to a high number of pressures and their synergies (Elliott et al., 2018). Under the umbrella of MSFD and HD, several indicators have been proposed on the good environmental status of benthic habitats, but none of them deal with sentinel species. SoS indicator was developed and subsequently tested in six different case studies, using different combinations of habitat types, sampling methods for data acquisition, as well as different pressure types. The following secondary objectives of this paper are: (i) to determine the sensitivity of the habitat to the pressure, defining its environmental status or response to pressure, and (ii) to determine the extent of the habitat affected by the pressure, so that adversely and non-adversely affected areas can be modelled and mapped.

2. Materials and methods

2.1. Sentinels of the Seabed (SoS) indicator

The SoS indicator requires sentinel species to assess the environmental status of a particular habitat using their proportional abundance (measured as biomass or number) across a pressure gradient. In this work, the term "sentinel species" has been used to refer to those species which fulfil two conditions: 1) species that can be frequently found in the natural habitat and 2) species that are sensitive to the studied pressure. To define "frequent or typical species", two different metrics were applied, i) relative contribution of species to intra-habitat similarity between stations sampled in the target habitat within reference condition areas (no disturbance or very low disturbance) using the Similarity Percentages procedure (SIMPER; Clarke, 1993) and ii) relative frequency for each species within the target habitat under reference conditions.

This initial set of "frequent or typical species" is filtered by prioritizing species according to a SoS sensitivity index (species responses to the analysed pressure), avoiding, when possible, tolerant species (i.e. those whose abundance does not show a clear response to the pressure) and always avoiding opportunistic species (i.e. those whose abundance increases with the pressure). SoS sensitivity index is calculated from available classifications of sensitivity to a pressure or pressures group. The SoS sensitivity index is not a new index but an adaptation of currently existing indexes to the SoS methodology. In the case studies explored here, two different indexes were used, depending on the pressure type considered (Table 1), but any other sensitivity index for specific pressures can be used. For trawling disturbance we used the Benthic Sensitivity Index to Trawling Operations (BESITO, González-Irusta et al., 2018) which scores species with values ranging from 1 to 5 (Supplementary Table 1). Species with a BESITO score of 1 show an opportunistic response to trawling (their abundance increase with pressure). A BESITO score of 2 indicates a tolerant response to trawling (no response). Species with values >2 indicate a sensitive response to trawling (decrease in abundance with pressure) with increasing

Table 1
Indices used to assess species sensitivity per pressure type.

Pressure	Index	Score/ Groups	SOS sensitivity index	Response
Pollution	AMBI groups (Borja et al., 2000)	Groups IV, V	I	Opportunistic
		Groups II, III	II	Tolerant
		Groups I	III	Sensitive
Trawling disturbance	BESITO index (González-Irusta et al., 2018)	I	I	Opportunistic
		II	II	Tolerant
		III	III	
		IV	IV	Sensitive
		V	V	

sensitivity from 3 to 5. For pollution we have used the same groups used by the AMBI indicator (Borja et al., 2000), which classifies species as sensitive (Group I), tolerant or indifferent (Groups II and III) and opportunistic species (Groups IV and V). Here we analysed two different pressure sources: trawling impacts and chemical pollution. However, the SoS indicator is potentially able to assess other anthropogenic pressure such as other bottom fishing activities, if the species sensitivity to the pressure is known. Fig. 1 outlines the method to generate the list of sentinel species and can be applied through a publicly available R function (<https://github.com/Gonzalez-Irusta/SoS>) which uses part of the code applied in Fariols et al., (2015), but adapted and extended to the SoS characteristics.

The SoS function follows six steps to obtain the list of sentinel species for each habitat (Fig. 1):

- Step 1:** To define reference conditions using only samples located in areas with no pressure. If insufficient samples are available in areas of no pressure, the reference condition can be extended to areas with low-pressure levels.
- Step 2:** The SoS function computes the ‘typical species set’, formed by species that explain 90% of the intra-habitat similarity within the samples under reference conditions and/or species present in >10% of the samples under reference conditions (with a minimum of 2 samples).
- Step 3:** All species with the highest SoS sensitivity index (5 using BESITO or 3 using AMBI groups, see table 1 for equivalences) are selected from the ‘typical species set’ to generate the ‘sentinel species set’. Species are firstly selected from the SIMPER analysis and secondly from the species exceeding the frequency threshold. If the

number of sentinel species is 10 or more after selecting all species with the highest sensitivity index from the SIMPER, the function stops. Otherwise, it starts selecting species above the frequency threshold in decreasing order of frequency. Again, the selection procedure stops after reach 10 sentinel species ($S \geq 10$). However, if this value is not reached, the function continues to step 4.

- Step 4, 5 and 6:** Procedure of step 3 is repeated, adding to the list of sentinel species already generated new species with a lower SoS sensitivity index in each new step (species with sensitivity 4 in step 4, 3 in step 5 and 2 in step 6). It is important to highlight that once the function reaches species of sensitivity 3, the threshold to stop the function decreases to 5. The reason is that species with a SoS sensitivity index lower than 3 are tolerant to the pressure instead of sensitive to it, therefore their inclusion has been limited only to habitats where sensitive species are not present or are very scarce. The function stops when the threshold for the number of sentinel species is reached or when all species with a sensitive index of 2 or higher presented in the typical species set have been included.

Once the list of sentinel species has been defined, its relative abundance (proportion) within each level of disturbance is computed and its evolution across the disturbance gradient analysed to assess the habitat status. It is important to highlight that although the minimum number of species is 10 (or 5 if there are not enough sentinel species with a sensitivity of 3 or higher), the final value of sentinel species is usually higher. This is because once the minimum number of species is reached, all species of the same sensitivity with the same values in terms of SIMPER or frequency will be included. So, for instance, if the value of 10 is reached after including the first species of sensitivity 3 based on

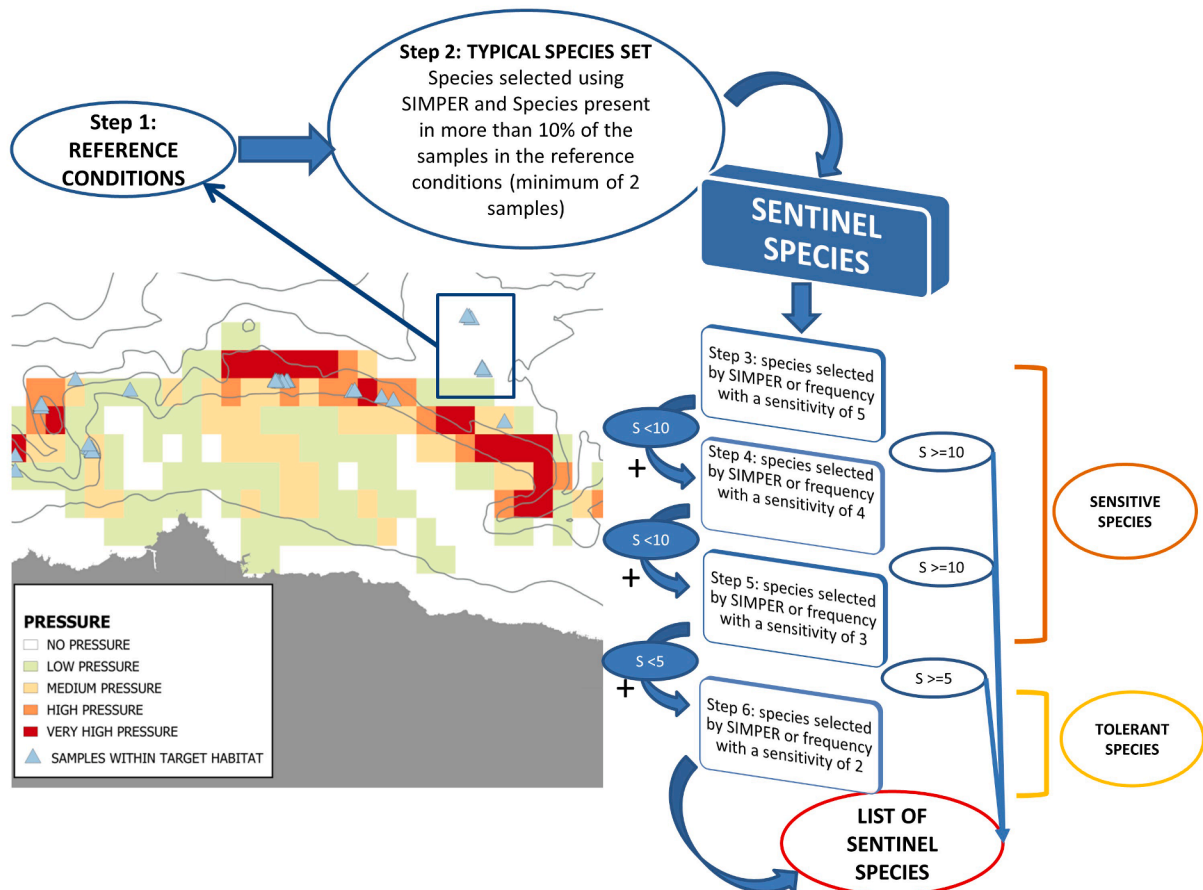


Fig. 1. Methodology used to determine the sentinel species list. Reference conditions are established in areas with no pressure effort (ideally) or low pressure (if no pressure areas are unavailable). S is the number of sentinel species already included in the set.

frequency, all other species of the typical species set with that sensitivity and frequency will be included as well (Fig. 1).

2.2. Case studies

In order to test the indicator, we analysed its performance in six different case studies (Fig. 2), located in three different areas of the Atlantic and one area of the Mediterranean: the north coast of Spain (case studies A1-A2 and B); south-west of England (case study C); Flemish cap (in the high seas, case study D); and the Seco de los Olivos seamount on the south-east coast of Spain, western Mediterranean (case study E). Two different pressure types (trawling disturbance and pollution), three different sampling methods (otter trawl, Remote Operate Vehicle - ROV-, and box-corer), and four different MSFD broad habitat types were included (Table 2). Most case studies analyse epibenthos with the exception of B and C which analyse endobenthos.

2.2.1. Case studies A1 and A2. Bottom trawl survey DEMERSALES

DEMERSALES is one of the surveys coordinated by the International Bottom Trawling Survey Working Group (IBTSWG) of the International Council for the Exploration of the Sea (ICES). Its design covers the northern Spanish shelf from the Portuguese border in the west to the French border in the east (Fig. 2). The survey has a random stratified (by depth and geographic strata) sampling according to the standard IBTS methodology for the western and southern areas (ICES, 2017). In each haul, all species caught are identified, counted, and weighed. These measures provide the biological data used to select the list of sentinel species for the assessed habitats. The DEMERSALES sampling strategy (random stratified) incorporates several MSFD broad habitats, including the two selected as case studies A1 and A2 (based on its extent) for testing the SoS indicator: 'offshore circalittoral sand' and 'upper bathyal sediment' respectively. The distribution of hauls between 2013 and 2019, carried out in these habitats is shown in Fig. 2. These years were selected to assure consistency between available fishing effort data (2010–2019) and biological data.

Trawling effort was computed as the mean fishing effort of the four

previous years, including the year when the biological samples were taken. For instance, for biological data sampled in 2013, we used the mean fishing effort from 2009 to 2013. These fishing effort maps were derived using vessel GPS locations from the Vessel Monitoring Systems (VMS) and logbook data (gear information). Both were provided by the Spanish Ministry of Agriculture, Food and Environment for the period 2010–2019. Gear and GPS location data were linked using ship code and trip date fields. VMS pings not related to fishing activity were removed using speed and other criteria (González-Irusta et al., 2018). To obtain the spatial distribution of swept area, hauls were assigned to individual fishing trips and VMS pings were interpolated to obtain the fishing track of each haul using the cubic-hermite spline interpolation (Hintzen et al., 2010). To compute the swept area the two kinds of trawl gears used by the fishing fleet in the study area (otter trawl and twin trawl) were considered. According to Castro et al. (2007) we used a 20 m width gear for otter trawls and 65 m for twin trawls (the information about gear type was also obtained from the logbooks). The mean annual swept area for each cell (km^2) was converted into the number of times each cell was trawled by dividing the mean annual swept area by the cell area (using the recommended ICES cell size of 0.05×0.05 degrees). Finally, the continuous values of swept area were converted into categories, mainly for graphical purposes; 'very low effort (≤ 0.6), low effort (0.61–1.54), medium effort (1.55–2.12), high effort (2.13–3) and very high effort (>3). The hauls undertaken in each target habitat and in areas with 'very low effort' were selected to compute the typical species set and then the relative abundance of these species at the different levels of disturbance was calculated following the methodology previously described.

2.2.2. Case study B. Ría de Vigo

The Ría de Vigo is an estuary located in northwestern Spain (Fig. 2). The effect of chemical pollution on the proportion of sentinel species of the target habitat (infralittoral mud) was analysed through 20 box-corer samples distributed across six different locations of Ría de Vigo. Biological communities were sampled at each station using a modified BOUMA box-corer with a sampling area of 0.0175 m^2 . Samples were

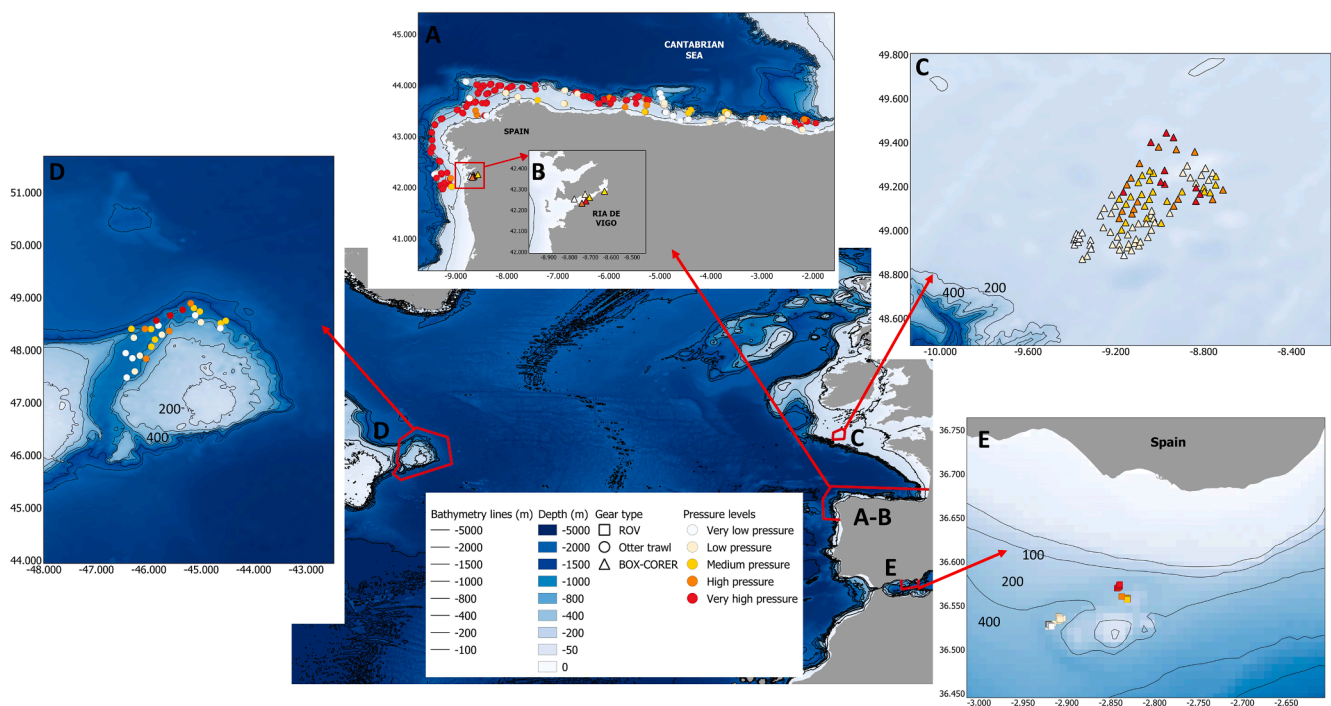


Fig. 2. Location, sample distribution and gear type of the case studies: A) North coast of Spain (Galicia and Cantabrian Sea) including two different MSFD broad habitats, Offshore circalittoral sand (A1) and Upper bathyal sediment (A2), B) Ría de Vigo (Galicia, NW Spain), C) South west Deeps (Celtic Sea), D) Flemish Cap (NW Atlantic), and E) Seco de los Olivos seamount (Mediterranean Sea).

Table 2

Case studies summary. SAMP (sampler type), PRES (assessed pressure), ASSIG. (percentage of total biomass with sensitivity score assigned).

CASE STUDY	MSFD BROAD HABITAT	METRIC	SAMP.	PRES.	ASSIG. (%)
A1) DEMERSALES circalittoral	Offshore circalittoral sand	Biomass (kg/km ²)	Otter trawl	Trawling disturbance	74%
A2) DEMERSALES bathyal	Upper bathyal sediment	Biomass (kg/km ²)	Otter trawl	Trawling disturbance	74%
B) Ría de Vigo	Infralittoral mud	Density (ind/km ²)	Box-Corer	Chemical pollution	91.57%
C) South-west of England	Offshore circalittoral sand	Biomass (kg/km ²)	Box-Corer	Trawling disturbance	82.19%
D) Flemish Cap	Mid-bathyal sediment	Biomass (kg/km ²)	Otter trawl	Trawling disturbance	100%
E) Seco de los Olivios	Upper bathyal sediment	Density (ind/km ²)	ROV	Trawling disturbance	100%

sieved on board using a 0.5 mm sieve. In addition, particle size, organic matter, heavy metals and other pollutants were also quantified. Details on the precise methods used can be consulted in [Beiras et al. \(2012\)](#). The 20 stations were located on muddy grounds (in the same MSFD broad habitat: infralittoral mud) at depths from 4 to 20 m. In order to group all pollutants into a single pressure value we used the CPI index that combines the different pollutants in one unique metric ([Bellás et al., 2011; Beiras et al., 2012](#)). CPI values in the 20 box corers ranged from -1.84 to 7 and were pooled in 5 levels of pressure: no pollution (CPI ≤ 0), low pollution (0–1), medium pollution (1.1–2.5), high pollution (2.6–4) and very high pollution (>4.1).

2.2.3. Case study C. South West Deep West marine conservation zone

This case study is located in the marine conservation zone of the South West Deep, an area sited in the south-west of England ([Fig. 2](#)). The effect of trawling disturbance on the proportion of sentinel species of the target MSFD broad habitat ‘offshore circalittoral sand’ was analysed using 101 different box-corer samples distributed across a gradient of trawling effort across a narrow depth range, from 130 to 172 m depth. Biological communities were sampled using a mini Hamon grab, with a sampling area of 0.1 m². Samples were sieved on board, preserved and classified in the laboratory to the highest possible taxonomic level. More details about the precise methods applied can be consulted in [Dove et al. \(2015\)](#). Trawling effort distribution in the area was computed as described in [Eigaard et al. \(2016\)](#) and pooled in five categories, using the same categories applied in the case studies A1 and A2. Since no-effort areas did not occur, samples from the low effort areas were used to select the list of sentinel species.

2.2.4. Case study D. Flemish Cap

This case study was conducted in the Flemish Cap area, a high-seas zone off the Canadian coast ([Fig. 2](#)). The effect of trawling disturbance on the proportion of sentinel species of the target MSFD broad habitat ‘mid bathyal sediments’ located at depths ranging from 600 to 1300 m was analysed. The trawling impact on the target habitat was analysed using data from the 2007 EU Flemish Cap bottom-trawl research survey ([Durán Muñoz, et al., 2020](#)), using standardised sets of a Lofoten bottom trawl (with a swept area of ≈0.04 km² each) following a depth-stratified sampling design. For more information about the sampling area or method see [Murillo et al., \(2016, 2020\)](#). After filtering out hauls located in the depth range of the selected MSFD broad habitat (600–1300 m), and removing 4 hauls located in the south side of the bank, 26 hauls distributed across a trawling gradient were analysed; 6 of them were located in no pressure areas (0 pings by km²), 5 in low pressure (0.1–0.15 pings by km²), 8 in medium pressure (0.16–0.5 pings by km²), 4 in high pressure (0.6–2 pings by km²) and 3 in very high pressure (>2.1 pings by km²).

2.2.5. Case study E. Seco de los Olivios seamount

This case study is located in the Site of Community Importance (SCI) of the European marine Natura 2000 network ‘Sur de Almería - Seco de los Olivios’, in southern Spain ([Fig. 2](#)). The effect of trawling disturbance on the proportion of sentinel species of the target MSFD broad habitat ‘upper bathyal sediment’ was analysed using data from VMS for the period 2010–2012 with the same categories and methods explained in

the previous case studies. Species data were obtained from three ROV (Seaeye Falcon & FalconDR) surveys conducted by OCEANA on board the Oceana Ranger between 2010 and 2012 (for more information about the sampling area or method see [de la Torre et al., 2018; De la Torre et al., 2019; de la Torre et al., 2020](#)). The sampling unit consisted of 1-minute continuous movement ROV tracks at a speed of 0.2–0.4 knots, covering an average distance of 13 m (mean = 13.16 ± 5.74 SD). The final data set selected for analysis was composed by 86 samples located in the target MSFD broad habitat (upper bathyal sediment) across a trawling effort gradient. To select the ‘typical species set’, we used samples located in those areas with the lowest trawling effort and with the presence of the biological habitat EUNIS ‘A6.514- facies of compact muds with *Isidella elongata*’. To compare this typical set with samples located in areas exposed to higher levels of pressure, we used the rest of the samples which corresponded with environmentally similar areas but without the presence of the biogenic habitat. Trawling effort was calculated using the same methodology and threshold for the trawling levels described for the case study A.

2.3. Comparison of SoS with other indicators

To analyse the capacity of SoS to assess habitat status across a pressure gradient in comparison with other popular methods in the field, the correlation between the proportion of sentinel species and the pressure (trawling effort or pollution) was measured using the Spearman’s rank-order correlation. These results were compared with the values obtained for three other indexes; i) Shannon-Wiener diversity index ([Shannon, 1948](#)), ii) Margalef richness index ([Margalef, 1958](#)), replaced by species richness in the Flemish Cap case study (due to the unavailability of densities) iii) Community biomass when possible and as community density otherwise, which has been recently highlighted as a powerful tool to measure the impact of trawling on benthic habitats ([Hiddink et al., 2020](#)).

2.4. Determining habitat sensitivity with the SoS indicator

To estimate habitat sensitivity to a given pressure we used three different approaches. The first approach consisted of computing the weighted (by its abundance) mean sensitivity index (BESITO, AMBI, other) value of the sentinel species (S_{ss}) for the samples under reference condition, using the following formula:

$$S_{ss} = \frac{\sum (A_{ssi} \cdot B_{ssi})}{\sum A_{ssi}}$$

where A_{ssi} is the importance (measured as biomass or density) of the sentinel species i and B_{ssi} is the SoS sensitivity index for that species. The second approach was equivalent to the first, but using all species present in the reference condition sample instead of only sentinel species. In the third approach we used the evolution of the indicator across the pressure gradient for each habitat to compute habitats sensitivity. In this approach, the observed development in SoS values across the pressure gradient was compared with five theoretical models (see [supplementary Fig. 1](#)) using a new R function developed for this purpose (<https://github.com/Gonzalez-Irusta/SoS>). The theoretical models were generated

based on the pressure-state relationships described in Elliott et al. (2018) and they represent five different possible responses to a pressure, from a sensitivity of 1 (not sensitive) to 5 (very sensitive). The function assigns a value from 1 to 5 to each habitat depending on the SoS indicator response to the pressure for that specific habitat, after checking to which of the five theoretical models this response curve better adjust (computed after comparing all the sum of squares and choose the lowest). This calculation is repeated 1000 times using bootstrapping obtaining the mean sensitivity of each habitat and its standard deviation based on the type of response observed in the SoS indicator.

2.5. Using the SoS indicator to assess the extent of the habitat affected by a physical pressure (MSFD criterion D6C3)

The SoS indicator can be directly applied to assess D6C3 by converting pressure maps into percentage of sentinel species using correlative approaches. For example, the SoS indicator was used to assess the environmental status of the MSFD broad habitat ‘upper bathyal sediment’ in the north coast of Spain (case study A2, see below). The correlation between the proportion of sentinel species and trawling effort was analysed using General Additive Models (GAMs). Since the response data was a proportion, they were analysed using a binomial GAM with Logit as link function (Zuur et al., 2009). The statistical models were then applied to the GIS layers of trawling effort (supplementary Fig. 2),

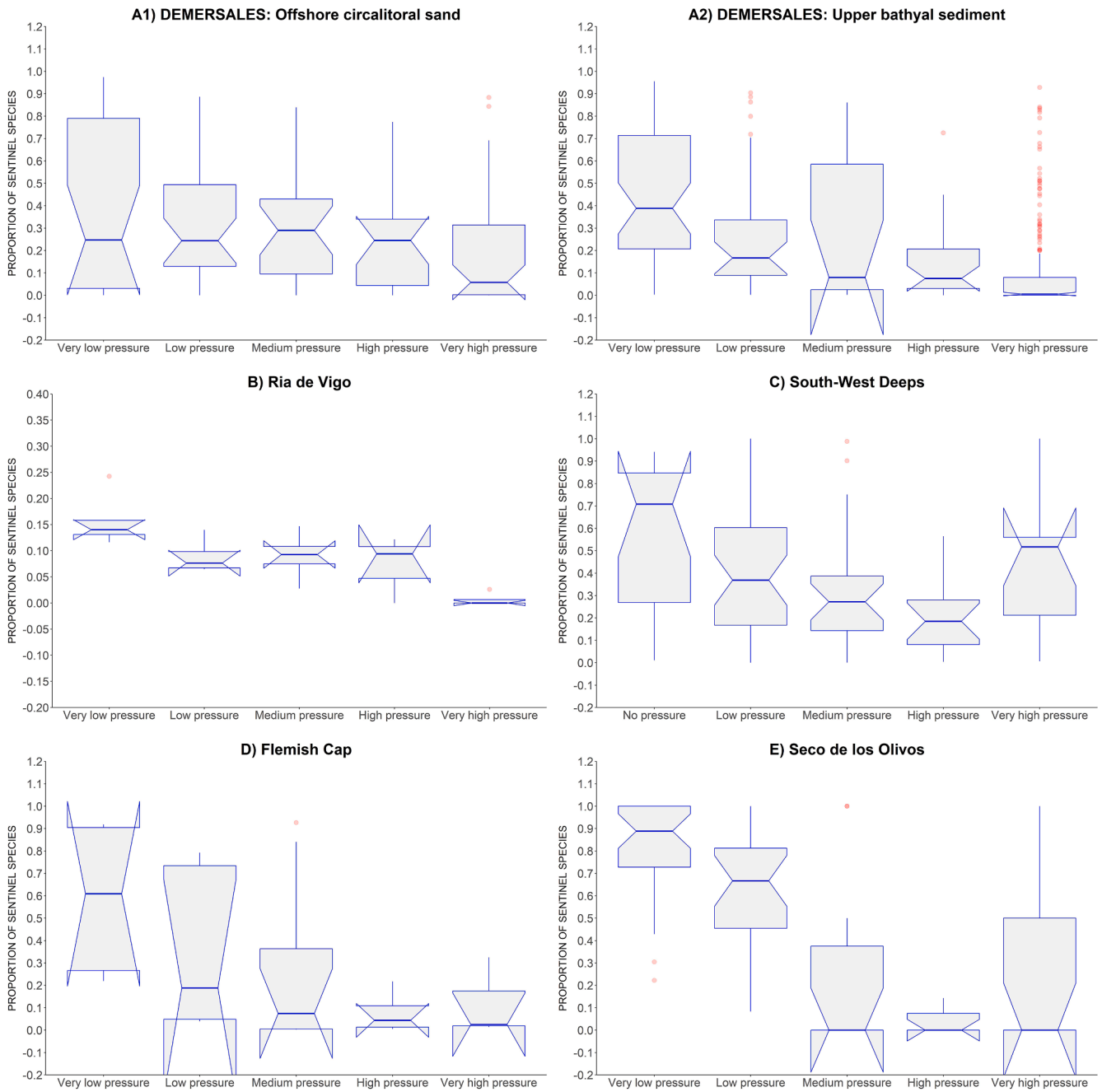


Fig. 3. Proportion of sentinel species across the pressure gradient by case study A1) DEMERSALES offshore circalittoral sand, A2) DEMERSALES upper bathyal sediment, B) Ría de Vigo, infralittoral mud (Pollution), C) UK Waters, offshore circalittoral sand, D) Flemish Cap, mid bathyal sediment, and E) Seco de los Olivos seamount, upper bathyal sediment. The boxes represent the interquartile range (IQR), the line is the median and the notches are its confidence interval. The lines of the whiskers extend 1.5 IQR and outliers are identified as points beyond the whiskers.

after masking them to the extent of the 'upper bathyal sediment' polygons (supplementary Fig. 3), to generate a geographical prediction of the proportion of sentinel species. Finally, these values were converted into no adversely affected areas (i.e. areas with reference conditions) using a specific threshold for each habitat. We used an arbitrary threshold of 0.25 based on the observed relationship between trawling effort and the proportion of sentinel species only for demonstration purposes (the threshold could be any other value). If the predicted proportion of sentinel species (after removing the standard error) was higher than the threshold, the area was considered to be no adversely affected (No AA). On the other hand, if the predicted proportion of sentinel species was lower than the threshold (after adding the standard error) the area was considered to be adversely affected (AA). If after modifying the proportion of sentinel species with the standard error prediction the result (regarding the threshold) varied, the areas were classified as uncertain.

3. Results

3.1. Performance of SoS indicator to trawling and pollution pressures

The SoS indicator was tested in six different case studies in four MSFD broad habitat types against two different pressure types (trawling effort and pollution) using three different sampling gears (otter trawl, box-corer and ROV images). Not surprisingly, the sentinel species list was quite different between habitats. Some species were, however, recurrently present in several habitats (Table 3). For instance, the sea pen *Funiculina quadrangularis* was selected in three of the four habitats

Table 3
Final sentinel species set for each case study / habitat.

CASE STUDY	HABITAT	SENTINEL SPECIES
Case study A1: DEMERSALES circalittoral	Offshore circalittoral sand	<i>Actinauge richardi</i> , <i>Anseropoda placenta</i> <i>Funiculina quadrangularis</i> , <i>Gracilechinus acutus</i> , <i>Lytocarpia myriophyllum</i> , <i>Ophiothrix fragilis</i> , <i>Parastichopus regalis</i> , <i>Phakellia ventilabrum</i> , <i>Spatangus purpureus</i>
Case study A2: DEMERSALES circalittoral	Upper bathyal sediment	<i>Acanella arbuscula</i> , <i>Actinauge richardi</i> , <i>Araeosoma fenestratum</i> , <i>Asconema setubalense</i> , <i>Pheronema carpenteri</i> , <i>Funiculina quadrangularis</i> , <i>Gracilechinus acutus</i> , <i>Hymenodiscus coronata</i> , <i>Kophoblemnon stelliferum</i> , <i>Nymphaster arenatus</i> , <i>Parastichopus regalis</i> , <i>Parastichopus tremulus</i>
Case study B: Ría de Vigo	Infralittoral mud	<i>Ampelisca</i> sp., <i>Atylus</i> sp., <i>Calyptraea chinensis</i> , <i>Atylus</i> sp., <i>Chamelea striatula</i> , <i>Euclymene oerstedii</i> , <i>Eudorella truncatula</i> , <i>Lumbrineris scopa</i> , <i>Metaphoxus fultoni</i> , <i>Musculus costulatus</i> , <i>Nucula</i> sp.
Case study C: South-West Deeps, West Marine Conservation Zone	Offshore circalittoral sand	<i>Aglaophamus agilis</i> , <i>Cerianthus lloydii</i> , <i>Echinocyamus pusillus</i> , <i>Galathowenia oculata</i> , <i>Glycera oxycephala</i> , <i>Notomastus</i> sp., <i>Phoronis</i> sp., <i>Pista cristata</i> , <i>Polycirrus</i> sp., <i>Scolecopsis bonnieri</i>
Case study D: Flemish Cap	Mid bathyal sediment	<i>Actinoscyphia saginata</i> , <i>Anthoptilum grandiflorum</i> , <i>Balticina finmarchica</i> , <i>Duva florida</i> , <i>Funiculina quadrangularis</i> , <i>Heteropolytus sol</i> , <i>Mycale lingua</i> , <i>Phelliactis</i> sp., <i>Stryphmus fortis</i> , <i>Thenea</i> sp.
Case study E: Seco de los Olivos bank	Upper bathyal sediment	<i>Caryophyllia smithii</i> var. <i>clavus</i> , <i>Funiculina quadrangularis</i> , <i>Isidella elongata</i> , <i>Kophoblemnon stelliferum</i> , <i>Parastichopus regalis</i> , <i>Pennatula phosphorea</i>

and in four of the six case studies, including Flemish Cap, Seco de los Olivos and the two DEMERSALES case studies. Other frequent sentinel species were the holothuroidea *Parastichopus tremulus* and the sea pen *Kophoblemnon stelliferum*, both present in three case studies (Seco de los Olivos and DEMERSALES case studies). In general, sentinel species were specific to each study area and most of the species were selected as sentinel species only for one case study. The exception was the two MSFD broad habitats analysed in the DEMERSALES case study, which showed a higher level of similarity.

The taxonomic composition of the sentinel species list is obviously different between the epibenthic and endobenthic case studies. The former are dominated in increasing order of frequency by Anthozoa (Pennatulacea, Alcyonacea and Actiniaria), Porifera (predominantly Demospongia, but also Hexactinellida), and Echinodermata (Echinoidea, Asteroidea, Holothuroidea). Endobenthic case studies list is dominated by Polychaeta in case C and by Polychaeta and Mollusca (Gastropoda, Bivalvia) in case B.

Indicator results showed a decreasing proportion of sentinel species with increasing pressure values, as conceptually was expected in all case studies against both pressures, although the clarity and intensity of this response was highly variable among case studies (Fig. 3).

Case studies based on otter trawl data (A1, A2, B and D) showed similar initial and final values in the proportion of sentinel species (proportions from 0.35 to 0.6 of total biomass in no pressure areas and values close to zero in the high-pressure areas) but with slightly different trends. Case study A2 (DEMERSALES: upper bathyal sediment) and the case study D (Flemish Cap) showed an acute decrease in the proportion of sentinel species with a reduction under values lower than 0.1 at medium pressure levels, whereas the case study A1 (DEMERSALES: offshore circalittoral sand) showed a less severe reduction for intermediate pressure levels (values higher than 0.2). Case study B (Ría de Vigo) which tested the impact of chemical pollution on the proportion of sentinel species also showed a clear decrease with pressure, although the initial proportion values of the sentinel species were much lower than in other case studies (<0.2). Case study C (South-West Deeps) showed a less clear negative trend, with an acute decrease from low to high pressure and then an increase in the highest pressure level. Finally, case study E (Seco de los Olivos) also showed a clear decrease in the proportion of the sentinel species, from the initial values higher than 0.9 to values of 0 for medium pressure values although with increasing variability for higher levels.

The correlation of the SoS indicator with the pressure was tested using the Spearman coefficient (Table 4). The correlation was negative and statistically significant in all cases (p-value < 0.05) although the intensity of this correlation varied between case studies and habitats, ranging from rho values of -0.76 (case study B, Ría de Vigo) to values of -0.22 (case study C, South-West Deeps). In addition to the SoS indicator, three other metrics frequently used to measure the impact of anthropogenic pressures on benthic habitats were tested (Supplementary Fig. 4 and Table 4): Shannon-Wiener diversity index, Margalef index (except for the case study D, Flemish Cap were species richness was used) and total biomass (or total density when biomass was not available). Total abundance showed a negative and statistically significant correlation with pressure in 4 of the 6 case studies, but it also showed a positive and significant correlation in two of them (A2-DEMERSALES: upper bathyal sediment and C- South-West Deeps). Shannon diversity index and Margalef index showed a negative and significant correlation in 4 of the 6 case studies, although the trend was less clear than for SoS or total abundance (Supplementary Fig. 4). Furthermore, Shannon diversity index also showed a significant and positive correlation with pressure in case study C, South-West Deeps.

3.2. Habitat sensitivity

The six combinations of habitat and case study showed pressure-state curves (Fig. 4) that can be pooled in three theoretical models

Table 4
Correlation values of the four tested metrics for each case study: proportion of sentinel species, total biomass (or total density when biomass not available), Shannon-Wiener index and Margalef index (replaced by species richness in Flemish Cap).

CASE STUDY/HABITAT	VARIABLE	rho	p-value
A1) DEMERSALES: Offshore Circalitoral Sand	Proportion of sentinel species	-0.24	0.006
	Total biomass(kg/km ²)	-0.25	0.003
	Shannon index	0.00	0.984
	Margalef index	0.09	0.293
A2) DEMERSALES: Upper Bathyal Sediment	Proportion of sentinel species	-0.58	<0.001
	Total biomass (kg/km ²)	0.10	0.061
	Shannon index	-0.44	<0.001
	Margalef index	-0.49	<0.001
B) Ría de Vigo: Infralitoral Mud	Proportion of sentinel species	-0.76	<0.001
	Total density (ind/km ²)	-0.72	<0.001
	Shannon index	-0.72	0.001
	Margalef index	-0.76	<0.001
C) South-West Deeps: Offshore Circalitoral Sand	Proportion of sentinel species	-0.22	0.036
	Total biomass (kg/km ²)	0.25	0.029
	Shannon index	0.22	0.026
	Margalef index	0.13	0.204
D) Flemish Cap: Mid Bathyal Sediment	Proportion of sentinel species	-0.49	0.011
	Total biomass (kg/km ²)	-0.60	0.001
	Species Richness	-0.61	0.001
	Shannon index	-0.46	0.018
E) Seco de los Olivos: Upper Bathyal Sediment	Proportion of sentinel species	-0.55	<0.001
	Total density(ind/km ²)	-0.66	<0.001
	Margalef index	-0.15	0.160
	Shannon index	-0.31	0.003

(supplementary Fig. 1). Flemish Cap showed the most sensitive response, with a sharp decrease in the proportion of sentinel species after the start of the pressure, a feature of the theoretical models for highly sensitive habitats (sensitivity 4). The theoretical model for medium sensitivity (sensitivity 3) was the most frequent with up to 3 habitat types (both DEMERSALES and the Ría de Vigo case studies) showing a quasi-linear response to pressure, although with different slopes also affecting their sensitivity. Flemish Cap and Seco de los Olivos showed a pressure-state curve clearly linked to the theoretical model for sensitive habitats (sensitivity higher than 3). Finally, South-West Deeps showed a trend that cannot be easily assimilated to any theoretical model. These different responses were used to compute numerical values of sensitivity using the sensitivity function developed as part of the SoS indicator frame. Habitat sensitivity was also computed using two

other different methods based on fauna composition and SoS sensitivity values under reference conditions (Fig. 4). All three methods showed a good agreement with each other, with Seco de los Olivos and Flemish Cap showing the highest sensitivity values in the three methods and the South-West Deeps the lowest in all methods. The Ría de Vigo case study was not included in this comparison since the range of SoS sensitivity index values for pollution (based on the AMBI groups) only has 3 values.

3.3. Extent of the habitat affected by a physical pressure (criterion D6C3)

The relationship between trawling effort and the proportion of sentinel species for the case study of DEMERSALES ‘upper bathyal sediment’ was analysed using a binomial GAM. The model explained 24.3% of the total deviance, with a correlation between predicted and observed values of 0.58 (Spearman correlation, p-value < 0.001). The proportion of sentinel species showed a negative and statistically significant (p-value < 0.001) relationship with trawling effort. The shape of this relationship is shown in Fig. 4. Both variables showed an inverse relationship, with a negative sharp slope at the lowest values of effort which tail off when reaching higher values of fishing effort. The observed correlation was used to calculate areas not adversely affected within the ‘upper bathyal sediment’ habitat (Fig. 5).

From the 13110 km² seabed areas with upper bathyal sediment, 7061 km² (53.9 %) showed higher values in the proportion of sentinel species than the threshold and were therefore classified as being not adversely affected (Table 5).

4. Discussion

The SoS indicator was highly effective in assessing changes in species composition of benthic habitats under both physical and chemical pressures, regardless of the sampling gear, the habitat or the case study. The indicator showed the expected response (decrease in the proportion of sentinel species with increasing pressure values) with differences in significance and intensity between combinations of pressure, habitat, sampling gear and biogeographic zone. The key process of this indicator, and one of its main strengths, relies on the methodology proposed to obtain the set of sentinel species. Its efficiency as indicator is directly connected to the sensitivity of the selected set of sentinel species to the considered pressure.

The sentinel species lists obtained in the six case studies were quite different between areas and habitats, with a low overlapping in the

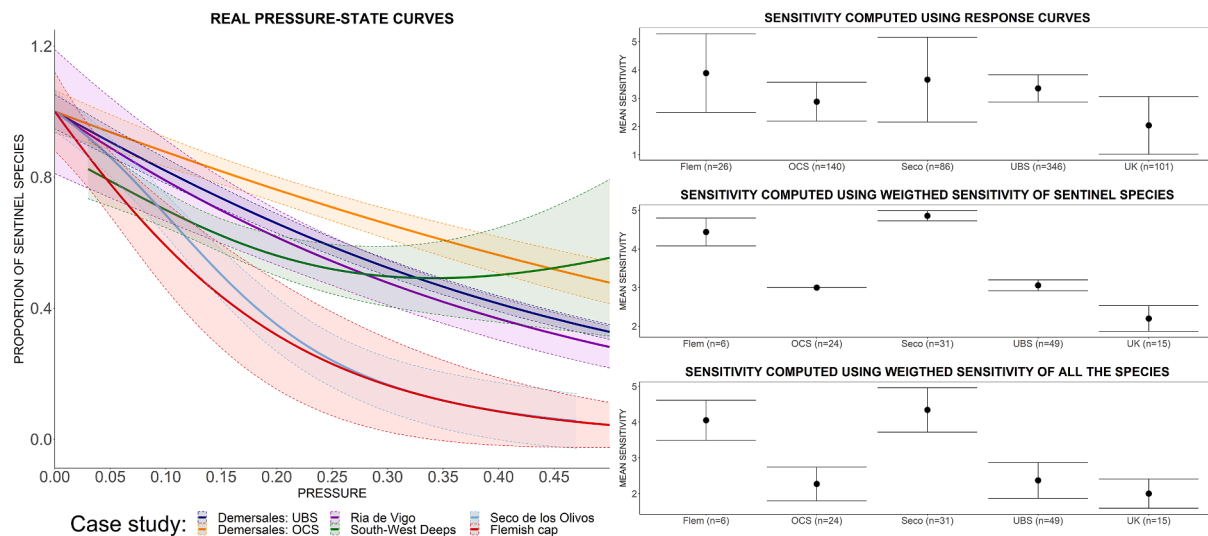


Fig. 4. Response curves and associated standard error (transparent shade) for each case study showing the relationship between the proportion of sensitive species and the studied pressure (left panel). Sensitivity values and standard deviation for each case study and method (right panel). N is the number of samples for each case study, including all samples in the method base on the curves and only samples in reference condition for the other two methods.

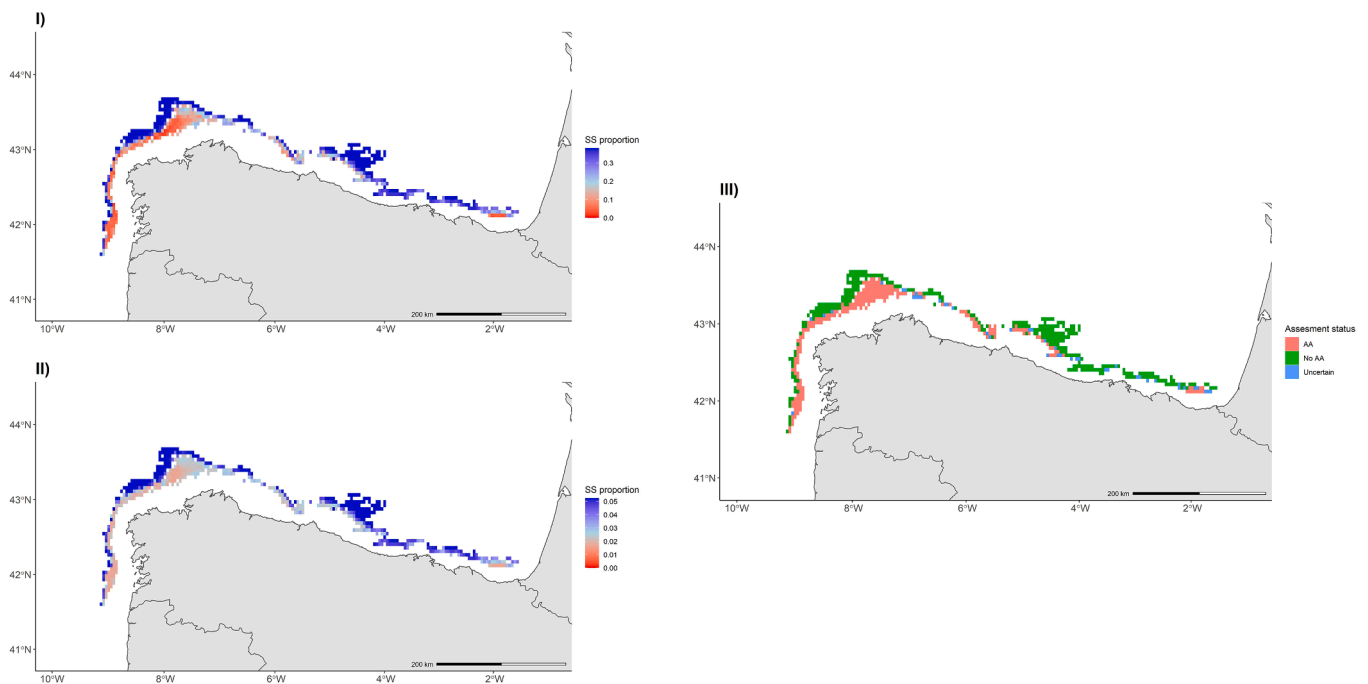


Fig. 5. I) Prediction of sentinel species based on the response curve for DEMERSALES Upper bathyal sediment (see Fig. 4). II) Standard error associated with the prediction. III) Final assessment status for the Upper bathyal sediment based on the prediction of sentinel species and the quality threshold of 0.25 (arbitrary selection only used for demonstration purposes). If the predicted proportion of sentinel species (I) minus the standard error (II) was higher than the threshold the area was considered to be no adversely affected (No AA). If the predicted proportion of sentinel species (I) minus the standard error (II) was lower than the threshold the area was considered to be adversely affected (AA). Otherwise the result was uncertain.

Table 5

Summary of assessment statistics. AA = Adversely Affected.

Total area of upper bathyal sediment (km ²)	Environmental assessment status	Area by category (km ²)	Proportion of total area by category (%)
13,110	No AA	7061	53.86
	AA	5102	38.92
	Uncertain status	947	7.22

sentinel species set between case studies. This can indicate that sentinel species are not ubiquitous, but rather species highly adapted to regional and local environmental conditions. Sensitivities of sentinel species sets for the different case studies range from the most sensitive group of species (within Flemish Cap and Seco de los Olivivos) to the least sensitive of South West Deeps. As expected, the gradient in the SoS response follows the same trend, with both (Flemish Cap and Seco de los Olivivos) having the most intense response to trawling and the South West Deeps showing the weakest. The higher sensitivity of the sentinel species (and therefore of the habitat, especially in habitats with low inertia of disturbance such as those analysed in this work) depends on two factors: 1) degree of sensitivity of the habitat in its pressure state 0 (inertia of disturbance of the system, van Denderen et al., 2015), which is reflected in the number of species with high sensitivity in those areas without pressure at the current period; and 2) efficiency of the sampler towards the most sensitive species to pressure. The first factor can probably explain the high number of sensitive species in the sentinel species sets in case D and E (Flemish Cap and Seco de los Olivivos epibenthos). The second factor probably explains the low number of species in case C (South West Deeps endobenthos). For South-West Deeps, it is clear that endobenthos (from box-core sampling) is not as efficient in evaluating trawling disturbance as epibenthos. However, the endobenthic biota does respond clearly to pollution using an appropriate index such as AMBI, as described in existing literature (e.g. Muxika et al., 2005; Borja et al., 2011). In general, endobenthic species exhibit certain biological

traits (such as short life cycles, small size, or burrowing behaviour) that make them less sensitive to trawling impacts than epibenthos (Jennings & Kaiser, 1998; Thrush & Dayton, 2002). Therefore, it is not surprising that the indicator response was less clear in the case study C (South-West Deeps, sampled using box-corer) than in the other case studies which analyse trawling impacts using data from otter trawl or images from ROV. Despite these limitations, SoS was the only indicator of the five tested indices able to find a negative and significant correlation with trawling effort as well in this case study.

From the evaluation of pollution effects on benthos, there is considerable consensus on the use of a small number of indicators, notably based on the AMBI index (Borja et al., 2000). On the contrary, there are numerous approaches to assess the impact of physical pressures (e.g. trawling). Here, the efficiency of the SoS indicator is compared with the total biomass, and taxonomic species richness and diversity for trawling effects assessment. In our results, SoS appears to be more sensitive to trawling pressure than the other indicators tested here (e.g. species richness, diversity and total biomass). It is well established (e.g. Mackey & Currie, 2001; Laure et al., 2009; Sheil, 2016) that relationships between disturbances and species richness or diversity are often not significant or not linear. In the same area as case study D, Flemish Cap, Murillo et al. (2020) found significant non-linear relationships between diversity and trawling. A hump-shaped pattern usually describes the relationship between diversity and disturbance as predicted by the *Intermediate Disturbance Hypothesis* (IDH; Connell, 1978). This hypothesis predicts maximum diversity at intermediate levels of disturbance, where competitive exclusion is prevented, enabling coexistence of both early colonisers and later-colonising, competitively superior species. The model predicts that species richness will be maximised at intermediate disturbance frequency, or intensity, both within and across patches which are disturbed at different times (Laure et al., 2009). Furthermore, metrics based on species richness are highly sensitive to sample size and the sampler used. Margalef's index was one of the first attempts to compensate for the effects of sample size by dividing the number of species in a sample by the natural

log of the number of organisms collected. Van Loon et al. (2018) found that Margalef diversity was a better performing benthic index than species richness and Shannon diversity for the anthropogenic pressures bottom fishing and organic enrichment. Despite the attempt to correct for sample size, Margalef index remains strongly influenced by sampling effort (Gamito, 2010). These biases lead us to consider that taxonomic species richness and diversity are less reliable indicators of disturbances than functional diversity. The latter may also explain their low performance detected in our results compared to the SoS indicator.

Several studies using total biomass as an indicator of trawling disturbance have been carried out in recent years (Queirós et al., 2006; Hiddink et al., 2006, 2020; Sciberras et al., 2018). In our results, total biomass showed a positive and significant correlation in 2 of the 6 case studies, and a significant negative correlation in the other 4. This fact can be explained by changes in the composition of species throughout a process of succession. Under conditions of medium or high disturbance there may be a high proportion of species with an opportunistic response (González-Irusta et al., 2018, Clare et al., 2021), less affected by disturbance. These opportunistic species also benefit from reduced levels of competition (Sheil, 2016; Castorani & Baskett, 2020) and predation (van Denderen et al., 2013; Hiddink et al., 2016). Scavenging species favour increased food availability through fishing discards (Ramsay et al., 1998; Shephard et al., 2014). The increase of these trawling-benefited species may mask the changes produced in sensitive species (González-Irusta et al., 2018), especially under specific environmental conditions (e.g. under climate change scenarios: Clare et al., 2021). Therefore, the use of total biomass as an indicator of the effects of trawling must be accompanied by functional information on the sensitivity of the groups that contribute to that biomass (e.g. longevity, Rijnsdorp et al., 2018) as well as information on the broader consequences for community structure and ecosystem functioning. As a result, interspecific relationships modifications may allow new species to populate the habitat. This mechanism may explain the significant decreases in the SoS indicator at medium and high levels of disturbance, since the set of species used in its calculation are effectively structural species. For example, species selected here in the epibenthic case studies have a good representation of engineering sessile filter-feeder species with a high degree of fragility, mostly sponges and corals. Sponge species (e.g. *Asconema*, *Pheronema*, *Phakellia*, *Mycale*) bamboo corals (e.g. *Acanella*, *Isidella*) and seapens (*Balticina*, *Funiculina*) are considered as indicators of Vulnerable Marine Ecosystems (e.g. Maldonado et al., 2017; Morato et al., 2018; Burgos et al., 2020), highlighting the importance of these species/taxa.

Pressure-state curves of each case study were fitted to 3 theoretical models using a function that relates real response curves to theoretical curves, allowing a sensitivity value to be assigned to each habitat. Habitat sensitivity is one of the key tools for assessing the effect of pressures on ecological status. Habitat sensitivity can be a consequence of the sum of the sensitivities of all species that make up the habitat, but it can also be considered that the contribution of a selected group of more sensitive species is greater and therefore they are the ones that determine the final value (Leonardsson et al., 2015, de la Torre et al., 2022). The most paradigmatic example of this latter option is biogenic habitats made up of a single or few engineering species. In this case, the sensitivity of these species to pressure is obviously critical (e.g. *Lophelia* reefs, Fosså et al., 2002; *Sabellaria* reefs, Desroy et al., 2011; *Laminaria* forests, Tegner & Dayton, 2000; *Posidonia* meadows, Boudouresque et al., 2009). The most sensitive response model (e.g. Flemish Cap) showed a sharp decrease in the proportion of sentinel species at low pressure levels, a feature which is characteristic of theoretical models for highly sensitive habitats. The no-pressure reference conditions in this model are related to the dominance of sensitive species (fragile, long-lived, larger, sessile with great vertical development, etc.; de Juan & Demestre, 2012; González-Irusta et al., 2018). These populations can be related to advanced stages of ecological succession, with mature communities (Pearson & Rosenberg, 1978; Simpson & Watling, 2006; de la

Torre et al., 2020). Therefore, low pressure levels cause a serious decrease in condition (in this case proportion of sensitive species, SoS). The least sensitive models (e.g. both cases study for offshore circalittoral sands) have already disturbed reference conditions (high disturbance inertia), with intermediate or low successional stage communities, characterized by less sensitive and more resistant species, an effect exacerbated by the use of endobenthic species in the analysis.

In the present study, the method based on pressure-state curves was compared with two other potential models to assess habitat sensitivity. Ideally, the method based on the pressure-state curves is preferable since it evaluates the sensitivity of each habitat across a pressure gradient and not only at reference conditions. However, in the real world, managers may need to assess habitat sensitivity with limited information, causing difficulty in understanding pressure-state relationships. Since the three methods used to compute habitat sensitivity showed a good agreement with each other, they can be used interchangeably (limited by data availability) to assign a sensitivity value to each habitat for each pressure, responding to the majority of criteria and indicators listed under European nature conservation directives.

The six case studies were used to develop and test the SoS indicator, with the case study A2 (Upper Bathyal Sediments in the north coast of Spain) being the one chosen to test a complete application of the SoS method from habitat and pressure distribution to the final GES assessment. The final product provides a map of the SoS indicator (percentage of sentinel species) that enables, through the identification of quality thresholds, areas of GES compliance and non-compliance to be mapped. Pressure-state response curves also provide information about the quality thresholds. The inflexion point at which the loss of sensitive species increases significantly (significant drop in the SoS value) can be used as a reference level or threshold for that habitat and that pressure (at a regional / sub-regional scale). Identifying thresholds is the main challenge in developing MSFD indicators nowadays (Lambert et al., 2017; Elliott et al., 2018). The SoS indicator developed here contributes to the evaluation of benthic habitats under the D6C3 criterion through the OSPAR indicators BH1 (“Typical species composition”) and BH3 (“Area of physical damage”), which requires sensitivity input and quality and pressure thresholds. Future advances in the application of this indicator will be aimed to respond to the MSFD D6C5 criterion, which is, the effect of all the pressures on the habitat condition together with other indicators (such as BH2). For this purpose, progress should be made on multi-pressure sensitivity indices, on the additive or overlapping effects of all pressures on benthic habitat condition (e.g. proportion of sensitive species, SoS). Despite the fact that trawling and pollution are the two main pressures that affect benthic habitats at North-East Atlantic regional scale, it is important to improve our understanding of the species sensitivity to other pressures.

The MSFD descriptors identify other pressures such as eutrophication, marine litter and other fishing activities. Among the latter, the need to evaluate longline fishing disturbance on vulnerable benthic hard-bottom habitats and species is noteworthy (e.g. Durán Muñoz et al., 2011; Brewin et al., 2020). In coastal areas, the development of an index of eutrophication effects on the biological traits of infralittoral species is needed (Pearson & Rosenberg, 1978; Cognetti, 2001; Conley et al., 2009). The development of sensitivity indices to longline, gillnet, seine, dredges, and also to eutrophication or hydrographical alterations will allow SoS to be applied in a multi-pressure context.

5. Conclusions

The establishment of environmental directives (MSFD, Habitats Directive) by the European Union requires the development of indicators to assess and to monitor the effect of human pressures on the marine environment. Sentinels of Seabed (SoS) indicator is based on the change in “sentinel species” relative abundance of each habitat affected for a given pressure by monitoring a group of sensitive and structural species that can be frequently found in each natural habitat and that

show the highest levels of sensitivity to the pressure. SoS has shown a very sensitive performance to all combinations of habitat, pressure and sampling gear. In addition, by comparing pressure-state curves using SoS indicator with 5 theoretical pressure-state response curves, a sensitivity value of the habitat can be determined. Finally, the proportion of sentinel species across the habitat extent was also mapped, enabling the area adversely affected to be computed. Using appropriate quality thresholds, these maps can be converted into binary maps showing Good Ecological Status compliance and non-compliance areas. The SoS indicator is therefore an important tool in determining the GES of benthic habitats.

CRedit authorship contribution statement

A. Serrano: Conceptualization, Validation, Methodology, Writing – original draft. **A. de la Torriente:** Conceptualization, Methodology, Writing – review & editing. **A. Punzón:** Methodology, Writing – review & editing. **M. Blanco:** Data curation, Resources, Writing – review & editing. **J. Bellas:** Formal analysis, Writing – review & editing. **P. Durán-Muñoz:** Formal analysis, Writing – review & editing. **F.J. Murillo:** Formal analysis, Writing – review & editing. **M. Sacau:** Formal analysis, Writing – review & editing. **A. García-Alegre:** Formal analysis, Writing – review & editing. **A. Antolín:** Resources, Data curation. **S. Elliott:** Formal analysis, Writing – review & editing. **L. Guerin:** Writing – review & editing. **C. Vina-Herbón:** Writing – review & editing. **S. Marra:** Formal analysis, Writing – review & editing. **J.M. González-Irusta:** Conceptualization, Methodology, Software, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecolind.2022.108979>.

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