

JRC SCIENCE FOR POLICY REPORT

Marine Strategy Framework Directive Descriptor 2, Non-Indigenous Species

*Delivering solid recommendations for setting
threshold values for non-indigenous species
pressure on European seas*

2021

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Abstract

Marine Non-Indigenous Species (NIS) are animals and plants introduced accidentally or deliberately into the European seas, originating from other seas of the globe. About 800 marine non-indigenous species (NIS) currently occur in the European Union national marine waters, several of which have negative impacts on marine ecosystem services and biodiversity. Under the Marine Strategy Framework Directive (MSFD) Descriptor 2 (D2), EU Member States (MSs) need to consider NIS in their marine management strategies. The Descriptor D2 includes one primary criterion (D2C1: new NIS introductions), and two secondary criteria (D2C2 and D2C3). The D2 implementation is characterized by a number of issues and uncertainties which can be applicable to the Descriptor level (e.g. geographical unit of assessment, assessment period, phytoplanktonic, parasitic, oligohaline NIS, etc.), to the primary criterion D2C1 level (e.g. threshold values, cryptogenic, questionable species, etc), and to the secondary criteria D2C2 and D2C3. The current report tackles these issues and provides practical recommendations aiming at a smoother and more efficient implementation of D2 and its criteria at EU level. They constitute a solid operational output which can result in more comparable D2 assessments among MSs and MSFD regions/subregions. When it comes to the policy-side, the current report calls for a number of different categories of NIS to be reported in D2 assessments, pointing the need for the species to be labelled/categorised appropriately in the MSFD reporting by the MSs. These suggestions are proposed to be communicated to the MSFD Working Group of Good Environmental Status (GES) and subsequently to the Marine Strategy Coordination Group (MSCG) of MSFD. Moreover, they can serve as an input for revising the Art. 8 Guidelines.

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RO: National Institute Marine Research and Development “Grigore Antipa”, Constanta, Romania

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SI: Marine Biology Station Piran, National Institute of Biology, Piran, Slovenia

SPA: UNEP-MAP-Specially Protected Areas/Regional Activity Centre

UNEP: UNEP/MAP - Barcelona Convention Secretariat

Executive summary

There are currently about 800 marine non-indigenous species (NIS) in the European Union (EU) national marine waters, several of which have negative impacts on marine ecosystem services and biodiversity. To address the risks they pose, the Marine Strategy Framework Directive (MSFD) requires EU Member States (MSs) to consider NIS in their marine management strategies.

Policy context

NIS are treated as a distinct Descriptor (D2) of Good Environmental Status (GES) of the MSFD: "*Non-indigenous species introduced by human activities are at levels that do not adversely alter the ecosystem*". The Descriptor D2 includes one primary criterion (D2C1: new NIS introductions), and two secondary criteria, dealing with the abundance and spatial distribution of NIS (D2C2), and their adverse effects on particular indigenous species groups and broad habitat types (D2C3).

The MSFD Common Implementation Strategy (CIS) Working Group on GES has the mandate to develop common approaches for assessing the environmental status of marine waters and setting of environmental targets, in order to ensure coherence and consistency of GES across all marine regions/ subregions. The work relevant to D2 is carried by national appointed experts under the coordination of JRC.

Key conclusions

The current report provides practical recommendations aiming at a smoother and more efficient implementation of D2 and its criteria at EU level. They constitute a solid operational output which can result in D2 assessments with more comparable data among MSs and MSFD regions/subregions. In addition, they call for a number of different categories of NIS to be reported in D2 assessments, pointing the need for the species to be labelled/categorised appropriately in the MSFD reporting by the MSs. Finally, the establishment of a high-level group concerning D2 is proposed streamlining the EU level marine NIS work.

Main findings

The D2 implementation is characterized by a number of issues and uncertainties. These issues can be applicable to the Descriptor level (e.g. geographical unit of assessment, assessment period, phytoplanktonic, parasitic, oligohaline NIS, etc.), to the primary criterion D2C1 level (e.g. threshold values, cryptogenic, questionable species, etc), and to the secondary criteria D2C2 and D2C3. The current report tackles these issues in a consistent way as possible, trying to find the optimum balance between scientifically acceptable standards and practical solutions with a policy perspective.

In addition, information was gathered regarding the date of first record of each NIS in each MS and MSFD subregion, as well as its related pathway of introduction. This dataset can support the process towards the establishment of the threshold values for D2C1 (i.e. the number of new introductions of NIS per 6-year period which is set as the GES threshold).

Related and future JRC work

The recommendations of the current report are proposed to be communicated to the MSFD Working Group GES and subsequently to the Marine Strategy Coordination Group (MSCG) of MSFD. Moreover, they can serve as an input for revising the Art. 8 Guidelines.

Quick guide

Marine Non-Indigenous Species (NIS) are animals and plants introduced accidentally or deliberately into the European seas, originating from other seas of the globe. They constitute one of the most important threats to biodiversity, causing severe ecological and socio-economic impacts. Under the Marine Strategy Framework Directive (MSFD) Descriptor 2 (D2), MSs need to consider NIS in their marine management strategies. The D2 implementation is characterized by a number of issues (e.g. geographical unit of assessment, assessment period, threshold values, cryptogenic, species, etc.). The current report tackles these issues, and provides practical solutions aiming at a smoother and more efficient implementation of the MSFD D2 and its criteria at EU level. The recommendations of the current report are proposed to be communicated to the MSFD Working Group GES.

1 Introduction

1.1. Policy context

The Marine Strategy Framework Directive (MSFD; EU 2008, 2010, 2017) aims to achieve Good Environmental Status (GES) of the European Union (EU) marine waters and to protect the resource base upon which marine-related economic and social activities depend. The Directive enshrines in a legislative framework the ecosystem approach to the management of human activities having an impact on the marine environment, integrating the concepts of environmental protection and sustainable use.

The MSFD interlinks with the new EU Biodiversity Strategy for 2030 (EU 2020), which aims to strengthen the protection of marine ecosystems, including through the expansion of protected areas and the establishment of strictly protected areas for habitats and fish stocks recovery. It stresses the need for an ecosystem-based approach to the management of human activities at sea.

To help EU countries achieve GES, the MSFD sets out 11 Descriptors. In addition, the Directive follows an adaptive management approach, and thus it must be kept up-to-date and reviewed every 6 years.

The MSFD requires from EU Member States (MSs) detailed and coordinated input. In order to facilitate this work, MSs and the European Commission have set up an informal programme of coordination, the Common Implementation Strategy (CIS).

In the frame of the MSFD CIS, the Marine Strategy Coordination Group (MSCG) of MSFD, chaired by the European Commission, is responsible for overseeing the implementation of the Directive. The group is composed of representatives of EU MSs authorities responsible for the implementation of MSFD at national level, and stakeholder organizations.

A sub-group of the MSCG is the Working Group on Good Environmental Status (WG GES), which oversees technical work on issues related to the assessment and determination of GES, the related environmental targets and indicators, and the monitoring obligations. It provides a platform for expert exchange at EU level between the MSs, other countries and stakeholders including the related ongoing work of the Regional Sea Conventions (RSCs). The WG GES is there to advise the MSCG on matters related to GES. Participants to WG GES are nominated representatives of the MSs, other countries (in particular Candidate Countries), RSCs and other international organisations.

The MSFD Working Group on GES has the mandate (CIS work programme 2020-2022) to develop common approaches for assessing the environmental status of marine waters and setting of environmental targets in order to ensure coherence and consistency of GES across all marine regions/ subregions. The work relevant to D2 is carried by national appointed experts under the coordination of JRC.

1.2. The role of the Joint Research Centre in MSFD

When it comes to MSFD, the Joint Research Centre (JRC), the European Commission's in-house science service, aims to share harmonised marine policy and science information, and to provide the MSFD Common Implementation Strategy with up-to-date scientific knowledge. The JRC has established the MSFD Competence Centre (MCC) which responds to needs and requests identified by the MSFD WG GES and the MSCG.

The activities of the MCC are linked to the development and implementation process of the MSFD. The MCC also acts as a science-policy interface, and provides a platform for sharing knowledge and scientific expertise on different MSFD-related themes, including assessment methods, and monitoring and modelling tools. In this context, MCC activities seek to ensure that proper advice is given to policy developers for improved implementation of the MSFD.

The JRC is working toward the identification of methodological approaches to monitoring for MSFD Descriptors 1, 2, 5, 7, 8, 9 and 10, with a view to proposing standardised methods for monitoring and identifying aspects which need further harmonisation between MSs to improve the consistency of monitoring data.

1.3. Background on non-indigenous species

At least 800 marine non-indigenous species (NIS) currently occur in the EU national marine waters (Tsiamis et al., 2019), several of which exhibit invasive behavior and have negative impacts on marine ecosystem services

and biodiversity (Katsanevakis et al. 2014; Ojaveer et al. 2015). To address the risks they pose, the MSFD requires MSs to consider NIS in their marine management strategies with the aim to reach GES.

NIS are treated as a distinct Descriptor (D2) of GES in the context of the MSFD (EU 2017): *"Non-indigenous species introduced by human activities are at levels that do not adversely alter the ecosystem"*. The Descriptor D2 includes one primary criterion (D2C1), based on which *"The number of non-indigenous species which are newly introduced via human activity into the wild, per assessment period (6 years), measured from the reference year as reported for the initial assessment under Article 8(1) of Directive 2008/56/EC, is minimised and where possible reduced to zero. MSs shall establish the threshold value for the number of new introductions of non-indigenous species, through regional or subregional cooperation"*. There are also two secondary criteria for D2 (EU 2017), dealing with the abundance and spatial distribution of NIS, particularly of the invasive ones (criterion D2C2), and their adverse effects on particular indigenous species groups and broad habitat types (criterion D2C3).

The environmental status of the EU national marine waters in the context of the MSFD was assessed by the MSs as part of the reporting obligations linked to the MSFD initial assessment, for most MSs in 2012. In that context, lists of NIS were reported at national level by each MS. Analysis of these lists revealed important knowledge and data gaps, vague definitions, significant differences on the level of detail and focus of the approach followed by each MS, pointing to the need for common standards (Palialexis et al., 2014; Srebalienė et al., 2019).

Recently, Tsiamis et al. (2019) provided refined baseline inventories (hereafter referred to as baselines) of NIS per MS: marine NIS reported in each EU country and related MSFD marine subregion by the end of 2011. The inventories were based on the initial assessment of the MSFD and existing updated data, in collaboration with NIS experts appointed by the MSs. This work highlighted the uncertainty on the non-indigenous status of several species across EU marine waters, and the need for further harmonization and coherent implementation of MSFD D2, in relation to NIS baselines, monitoring, and thresholds.

The refined baselines constitute the basis for the assessment of the primary criterion D2C1, allowing for the determination of the number of new introductions subsequent to 2012 per MS and MSFD subregion. In addition, the refined inventories of NIS can support the process towards the establishment of the threshold values for D2C1 (i.e. the number of new introductions of NIS per 6-year period which is set as the GES threshold), through the information related with the time trends of the listed NIS introductions and their associated pathways.

During 2019-2020, MSs have, in response to their 2018 "reporting" obligations, reported on MSFD D2 information for the last 6-year MSFD reporting cycle, following the Article 17 requirements to update their Articles 8, 9 and 10 reports. By September 2020, 20 MSs had reported on D2 in electronic format, which was assessed by Tsiamis et al. (report under review). The latter assessment highlights gaps in setting threshold values for the D2 criteria, and a number of inconsistencies in D2 implementation, including the spatial and time coverage of D2 application among the MSs. The JRC's recent work on MSFD D2 aims to tackle these issues, and generally provide support in the implementation of the Descriptor.

1.4. Recent work of the JRC on non-indigenous species

The JRC initiated in 2019 an activity aiming to analyze the trends of new NIS introductions per MS and MSFD marine subregion and their associated pathways. The overall goal was to develop recommendations for setting threshold values for D2C1. Towards this goal, relevant information was gathered regarding the date of first record of each NIS in each MS and MSFD subregion, as well as its related pathway of introduction. Appointed national experts by the MSs and representatives of the RSCs were invited to participate in this task and the subsequent analysis, which included a related workshop. Other relevant Stakeholders were also invited to attend the workshop.

The NIS thresholds workshop was held online on 6-7 October 2020. In order to facilitate the workshop's deliberation and its effectiveness a related questionnaire, based on a preliminary analysis of the data previously gathered, was distributed among the NIS national experts' and to all Stakeholders prior to the workshop. The results of the questionnaires were summarized and discussed during the workshop.

1.5. Aim of the report

The task on NIS data gathering, the questionnaires and the workshop aimed to facilitate discussions at the national, regional and inter-regional level for making progress on the implementation of D2. A particular objective was to assess the primary criterion D2C1 and its threshold values for the number of new NIS introductions for each MS and MSFD subregion.

The final outcome achieved through the Workshop provides a number of recommendations for the overall implementation of D2. These recommendations aim to efficiently tackle D2 application issues in a consistent way among EU MSs and it is proposed to be reported to the MSFD Working Group GES and to MSCG. The target-audience of the report includes the members of these two groups (nominated representatives of the MSs), scientists working on MSFD D2 and generally on marine NIS, as well as policy-makers of the RSCs and relevant stakeholders dealing with marine NIS.

2 Work approach

2.1. Data gathering

The current activity aimed to analyse the trends of new NIS introductions per MS and MSFD marine subregion and their associated pathways. Towards this objective, relevant information was gathered by the JRC regarding the date of first record (= date of first observation in the field) of a NIS in each MS and MSFD subregion, as well as its related pathway of introduction.

The refined baseline inventories of NIS provided by Tsiamis et al. (2019) were used as the basis for this exercise. However, the following species were excluded from the exercise:

- cryptogenic species listed in each MS (see Tsiamis et al., 2019);
- questionable species listed in each MS (see Tsiamis et al., 2019);
- parasitic species.

For the rest of the species relevant information was gathered by JRC concerning:

- the date of first record of each NIS in each MS and MSFD marine subregion;
- the associated pathway(s) of introduction of each NIS in each MS and MSFD marine subregion.

Data sources used were AquaNIS (2020), EASIN (2020) and scientific literature. For NIS introduced before 1970 in a specific MS and/or MSFD subregion no information on associated pathways was gathered. Retrieved information on the possible pathways of introduction was aligned to the categorization scheme of CBD (2014). When the relevant information on a NIS pathway for a specific MSFD subregion was impossible to be found, then expert judgment was applied by the JRC or the pathway was assigned as "Unknown".

All relevant information gathered was summarized in excel files, one for each MS, including instructions (Annex 1) supporting the validation of the information by the national NIS experts of the MSFD. All appointed experts to the MSFD D2 Experts' Network (updated after request email of 22.10.2019 to Marine Strategy Coordination Group -MSCG-, in copy to Working Group GES) were then invited to check and validate the relevant information compiled (e-mails to relevant experts sent on 06.11.2019). In addition, the appointed experts had the opportunity to annotate the baseline inventories of Tsiamis et al. (2019) (NIS found up to 31/12/2011), which were also distributed in excel file. Finally, the experts were invited to check and validate relevant information compiled for new NIS introductions per MS and MSFD subregion that occurred after the baseline timeframe, i.e. between 2012-2017 (e-mails to relevant experts sent on 12.11.2020).

By the end of 2020, 21 MSs delivered the national files with validated information; these are provided in:

- ✓ Annex 2: information on refined NIS baselines (NIS up to 2011) per each Member State and national part of the MSFD subregion. NIS with dates of first record and associated pathways given per each Member State and national part of the MSFD subregion are also provided (also for NIS found up to 31/12/2017).
- ✓ Annex 3: NIS with dates of first record and associated pathways given at MSFD subregional level (for NIS found up to 31/12/2017).

2.2. Workshop questionnaire

In preparation, aiming to facilitate the Workshop's deliberation and its effectiveness, as well as to facilitate discussions at national, regional and inter-regional level, a Questionnaire (Annex 4) was distributed to all members of the D2 MSFD Experts' Network and to the representatives of the RSCs and relevant stakeholders. The topics included in the Questionnaire were largely based on the outcomes of the exercise on data gathering of NIS information, carried out in support of the process towards the establishment of the threshold values for D2C1.

In total, 21 MSs, 2 RSCs and one additional stakeholder (Coalition Clean Baltic) provided feedback. The aggregated results of the Questionnaires are depicted in Annex 5.

2.3. The Workshop

2.3.1. Time, place and agenda

The Workshop was held exclusively online on 6-7 October 2020. The agenda is provided below (Table 1).

Table 1. Workshop's agenda.

<i>Tuesday 6/10/2020</i>	
09:00	Welcome (JRC)
09:30	Background work on D2 (JRC)
09:45	Updates on MSFD (DG ENV)
10:00	<u>D2 criteria implementation</u> geographical and time scale application unicellular plankton and parasites
<i>11:30 - 12:00 Break</i>	
12:00	The EU IAS Regulation and marine NIS (DG ENV)
12:30	<u>D2C1 criterion implementation</u> NIS introduced through natural dispersal in criterion D2C1
<i>13:00 - 14:30 Break</i>	
14:30	<u>D2 criteria implementation</u> oligohaline NIS partly-native species in the same MSFD subregion <u>D2C1 criterion implementation</u> cryptogenic species questionable species extinct species
<i>16:00 - 16:30 Break</i>	
16:30	<u>D2C1 criterion implementation</u> inconsistencies in baselines of marine NIS
<i>17:30 End of Day 1</i>	

Wednesday 7/10/2020	
09:00	<u>RSCs related work on NIS</u> HELCOM UNEP-MAP OPSAR
<i>10:30 - 11:00 Break</i>	
11:00	<u>D2C1 criterion implementation</u> threshold values in criterion D2C1 monitoring bias
<i>13:00 - 14:00 Break</i>	
14:00	<u>Secondary Criteria</u> D2C2 D2C3
<i>15:00 - 15:30 Break</i>	
15:30	<u>AOB</u> use of citizen-science data pathways assignment uncertainty dates of first record of NIS uncertainty other
16:30	Sum-up and recommendations
<i>17:00 End of workshop</i>	

2.3.2. List of participants attended the Workshop

In total, there were 56 participants in the Workshop coming from:

- ✓ national NIS experts from 21 MSs;
- ✓ representatives from Regional Sea Conventions (HELCOM, UNEP-MAP, OSPAR);
- ✓ representatives from the European Commission (DG JRC, DG ENV);
- ✓ representative from European Environment Agency;
- ✓ representative from Coalition Clean Baltic.

3 Outcomes of the workshop

The discussion and eventual outcomes of the workshop were based on the Questionnaire's structure (Annex 4) and overall results (Annex 5), following the Workshop's agenda (Table 1). In more detail, the following scientific/technical topics related with D2 implementation were addressed:

Topic #1. Geographical unit for D2 implementation

Based on MSFD, "Descriptor 2, Methodological standards, Scale of assessment: Subdivisions of the region or subregion, divided where needed by national boundaries". Based on Tsiamis et al. (report under review), several MSs have applied D2 at: a) entire regional level (e.g. for FI: entire Baltic Sea), b) national part of a subregion (e.g. for DE: national part of the Great North Sea and Baltic Sea), c) subdivision of a national part of a subregion (e.g. for ES: north part of the national part of the Biscay Bay), and d) a combination of the above.

Workshop's outcome: Ideally, MSs should implement D2 in a consistent geographical unit, and the latter was agreed to be at the national part of the MSFD subregion (e.g. for PL it would be the Polish part of the Baltic Sea). However, the method followed in the national assessment needs to be coherent with that at subregional and regional levels to ensure coordination of efforts to tackle new introductions where needed. The Macaronesia subregion of PT can be an exception of the above, due to the sharp ecological and geographical differences between the Azores and Madeira. In the latter case, D2 can be implemented at the subdivision level (Azores, Madeira) of the national part of the MSFD subregion (PT part of Macaronesia).

Topic #2. Assessment period for D2 implementation

Based on MSFD, the assessment period covers a 6-year period measured from the reference year as reported for the initial assessment (EU 2017). For the majority of the MSs, the year of the initial assessment was 2012; thus, the subsequent assessment period would be the 6 years between 2012-2017. However, not all MSs reported the initial assessment in 2012. In the 2018 reporting, most MS reported as assessment period for D2 the period 2012-2017, while others the period 2011-2016, or even 2013-2018. In addition, when it comes to the new NIS introductions, it is argued that due to the time lags observed for reporting new NIS, there might be a need for additional available time between the year of the reporting of D2 and its assessment period.

Workshop's outcome: For consistency and harmonization reasons, the assessment period of D2 should be the same across MSs. The initial assessment of MSFD is the basis for determination of GES across all descriptors and the date of this assessment was for most MSs 2012. Logically, following the 6-year cycle, it was agreed that the next assessment should be due in 2018-2023. To be noted that in the 2018 MSFD reporting there had been long delays (e.g. several MSs reported by September 2020), giving extra time for MSs to finalize their reports and deal with the time lags of reporting new NIS. In time, all MSs would be able to streamline their reporting at the same timeframe: 2018-2023, 2024-2029, etc.

Topic #3. Unicellular plankton species in D2 criteria

Unicellular plankton species (phytoplankton) have high uncertainty regarding the native vs non-indigenous status in European seas. There have been scattered records across Europe, but no consistency in their treatment. In Tsiamis et al. (2019) baseline there was high variance of the number of phytoplanktonic species included in the inventories across the MSs, even between neighbouring countries, reporting either long lists of them or just a few or even none. Several countries chose not to include phytoplankton species in their NIS lists. Recently, Gomez (2019) and Gomez & Artigas (2019) argued that there is not enough evidence for tagging any phytoplankton species in Europe as non-indigenous. Tsiamis et al. (2019) suggested that unicellular plankton NIS should be treated with caution (e.g. flagged with high uncertainty) until further research clarifies their enigmatic status.

Workshop's outcome: There was a rather unanimous agreement for reporting phytoplankton NIS in D2 criteria, but not considering them when assessing against a GES threshold for the time being. The group agreed that there are large gaps in knowledge and relevant information, and that more work is needed on marine NIS of phytoplankton in Europe. A joined and coordinated revision of the current status of European NIS phytoplankton would be crucial and could contribute to D2 reporting obligations. To that end, the JRC invited the D2 NIS experts' Network to contact phytoplankton experts across Europe, aiming to set up a group of experts who could deliver this joined revision of phytoplankton NIS in European seas.

Topic #4. Parasitic species in D2 criteria

It has been argued that parasitic NIS should be omitted from MSFD D2 since from a legislative perspective they are managed under the Aquatic Animal Health Directive (2006/88/EC; EU, 2006) rather than the MSFD (Tsiamis et al. 2019). However, the Aquatic Animal Health Directive covers parasites on aquacultured animals, while there can be parasitic NIS found in non-aquacultured animals. Moreover, several MSs have included parasitic NIS in their D2 lists.

Workshop's outcome: The Aquatic Animal Health Directive is not enough to cover all parasitic NIS. Moreover, several parasitic NIS can have substantially high impact on the native communities. On the other hand, there are large gaps in knowledge and relevant information on parasitic NIS in European seas, taking into account also the lack of relevant experts and subsequently monitoring efforts needed to tackle this group. Overall, the group agreed that parasitic NIS should be reported in D2 criteria, but not considering them when assessing against a GES threshold, until further data comes to light. However, they might be considered for measuring GES at smaller scales (e.g. in a certain MSFD subregion) if available information is already sufficiently enough.

Topic #5. Oligohaline NIS in D2 criteria

Oligohaline (salinity between 0.5 and 5 g/l) waters (transitional waters) are under the realms of the Water Framework Directive (WFD). However, transitional waters were not defined in a consistent way across EU (McLusky & Elliott 2007). Tsiamis et al. (2019) baseline generally did not take into consideration oligohaline NIS. However, they were included by several MSs when these species have been also found in their coastal waters. These entail mostly the oligohaline (but also several freshwater) species found in the low saline Baltic Sea. Nevertheless, these species might be present in other MSs as well, but they were not listed in their inventories since they occur exclusively in their transitional and/or inland systems (e.g. *Corbicula fluminea*, *Cordylophora caspia*, *Elodea canadensis*, *Elodea nuttallii*).

Workshop's outcome: Overall, oligohaline species should be addressed in a fully consistent way at EU level. Transitional waters, under the realms of the WFD, were not defined in a consistent way across EU, as in some countries these were designated as coastal waters. This may explain why some freshwater species have been included in D2 assessments. Therefore, there is a need for better classification of transitional waters under MSFD vs WFD. The Workshop's participants concluded that oligohaline species should be included in the D2 assessments of a MS if the relevant species are found in coastal systems of the country. Moreover, under a broader scale, D2 assessments should include all NIS found in **coastal waters**, regardless their oligohaline/marine/freshwater status.

Topic #6. Partly-native species in the same MSFD subregion, in D2 criteria

Several species are native in some MSs while they are non-indigenous in other EU countries. These species are generally known as "partly native" (or partly alien) species. In exceptional cases, there are partly native species in the same MSFD subregion. For example, certain species are native in the Azores but non-indigenous in the Madeira and/or the Canary Islands (or vice versa), although all these areas belong to the same MSFD subregion (Macaronesia). This would result in a discrepancy when assessing the related information at the subregional level of the Macaronesia about the species status.

Workshop's outcome: The group agreed that for the sake of practicality this kind of NIS should be reported in D2 criteria but not considered when assessing against a GES threshold at the subregional scale.

Topic #7. NIS introduced from one infested area to another through natural dispersal in criterion D2C1

The primary criterion D2C1 measures "*The number of non-indigenous species which are newly introduced **via human activity** into the wild, per assessment period (6 years), ...*". It has been argued that NIS introduced exclusively through natural dispersal, from already infested areas of European seas, to other neighbouring areas (e.g. a NIS introduced from Lebanon to Cyprus through natural dispersal) should not be taken into consideration for defining GES based on D2C1. However, taken into consideration if there is evidence that the species is transferred also through human-mediated activities, such as shipping (Palialexis et al. 2015). Doing so, there

will be the possibility to manage such introductions and measure the effectiveness of management measures, which prevent NIS human-mediated introductions in a given assessment area. Several Lessepsian species fall under this category.

Workshop's outcome: The group made clear that these species are truly non-indigenous, and they should not be confused with range-expanding species or species introduced into Europe due to climate change. These NIS have been introduced into European seas through human-mediated activities. However, from already established populations in Europe they might be expanding through exclusively natural dispersal, from one European country to another. Nevertheless, it is challenging to prove that a NIS is secondarily spreading across Europe exclusively through natural dispersal and not also through other means (e.g. fouling, fishing nets, etc). The group agreed that these NIS should be reported in D2C1 application. However, there was a debate if these NIS should be also considered when assessing against a GES threshold based on D2C1. At the end, the Workshop's participants proposed that these NIS should be handled case-by-case for D2C1 based on pathways certainty, availability of data, and the impact caused by them. Moreover, it was highlighted that NIS included also in the Union concern list of the EU Invasive Alien Species Regulation 1143/2014 must be reported and considered for GES in D2C1 assessments. Finally, the group clarified that these NIS should be reported and considered for GES in the secondary criteria D2C2 and D2C3 if they are relevant.

Topic #8. Cryptogenic species in criterion D2C1

Cryptogenic species are species that cannot be demonstrably classified as native or non-indigenous in a particular region (Carlton 1996). A close category is the one of crypto-expanding species, i.e. those with no definite evidence of their native or non-indigenous status due to unclear mode of introduction from the native range: natural spread vs human mediated. Characteristic examples include *Palaemon elegans* in the Baltic and *Antithamnionella spirographidis* in the Mediterranean Sea. Due to the lack of data, it is common that NIS experts disagree on the status of cryptogenic species in a specific area. As a result, these species may be treated as non-indigenous in some countries, while in neighbouring countries they are reported as cryptogenic or even as native species. Moreover, the status of cryptogenic species can be altered in time, based on new available research data coming into light, thus changing their status. In the recent JRC data gathering on pathways and dates of first record of NIS in each MS and subregion, cryptogenic species were excluded. Similarly, cryptogenic species were not further analyzed in Tsiamis et al. (2019) baseline inventories, but they were simply listed in an annex.

Workshop's outcome: The group agreed that cryptogenic (including also crypto-expanding) species should be reported in D2C1 assessments but not considered when assessing against a GES threshold due to their high uncertainty. This approach should be applied in a coherent way across all EU MSs. In the light of new available evidence, these species might change their status in the future to "non-indigenous". If that will be the case, they will be considered when measuring GES in criterion D2C1.

Topic #9. Questionable species in criterion D2C1

Questionable species are those with unresolved taxonomic status or new NIS entries not verified by experts (e.g. records coming from citizen-science but not yet validated by experts, or records in technical reports without providing the necessary taxonomic evidence). In the recent JRC exercise on pathways and dates of first records of NIS in each MS and subregion, questionable species were excluded. Similarly, questionable species were not further analyzed in Tsiamis et al. (2019) baseline, but they were simply listed in an annex.

Workshop's outcome: There was a unanimous agreement to report questionable species in D2C1, but not considering them when assessing against a GES threshold based on D2C1. In the light of new available evidence, these species might change their status in the future to "non-indigenous". If that will be the case, they will be considered when measuring GES in criterion D2C1.

Topic #10. Extinct species in criterion D2C1

Several NIS have been reported in MSs several decades ago (even in the 19th century or before) but never recorded again in the wild in these MSs, and thus are considered as possibly extinct, presuming that these NIS did not survive in the new environment. However, it is difficult to prove if a NIS has been truly extinct from a marine area or MS due to monitoring difficulties and the continuum of the marine environment. In case a presumably extinct NIS is reported during the last assessment period from the same or adjacent area that was

originally reported in a MS, then it is questionable whether this record corresponds to a new introduction or to the "extinct" population that was apparently overlooked in previous years.

Workshop's outcome: There was a unanimous agreement that these species should be examined case-by-case whether they correspond to a new introduction in the area or not. The workshop's participants highlighted that these species should be investigated in terms of: a) dates of old records, b) continuity of records, c) body size of the species, d) difficulty in taxonomic identification, e) area's conditions and characteristics, f) monitoring effort and its continuity, and g) possible pathway of introduction.

Topic #11. Threshold values in criterion D2C1

Based on D2C1 *"The number of non-indigenous species which are newly introduced via human activity into the wild, per assessment period (6 years), is minimised and where possible reduced to zero. MSs shall establish the threshold value for the number of new introductions of non-indigenous species, through regional or subregional cooperation"*. HELCOM (2018) has set the threshold value for D2C1 = zero new NIS in the assessment period (e.g. 6 years). OSPAR (2018) highlights that the relative change of the number of new NIS introductions seen over subsequent assessment periods (e.g. 6 years) can facilitate the specification of threshold values; however, OSPAR has not yet concluded in specific values. UNEP-MAP and the Black Sea Commission have not started yet the related work on setting threshold value for D2C1.

Workshop's outcome: The workshop's participants agreed that the most suitable approach for setting threshold values for D2C1 is to adopt the percentage reduction of new NIS, meaning that: a) the threshold is a quantitative measure, i.e. specific number of new NIS introductions during the assessment period for a specific MSFD region/subregion, and b) the number of new NIS introductions is defined based on a specific percentage reduction of new NIS occurred in this MSFD region/subregion compared to the average number of new NIS introductions that occurred in the previous 6-years cycle periods in the same area. A practical hypothetical example is displayed in Box 1.

Box 1. A practical hypothetical example on setting threshold values for criterion D2C1 (number of new NIS) based on the percentage reduction approach.

The percentage reduction is agreed to be 50% for a certain subregion, and during the last three 6-years cycle periods (18 years in total) the new NIS introductions occurred in that subregion were 30 taxa (thus the average number is 10 new NIS per one 6-year cycle). The threshold value should be the 50% reduction of the average number of new NIS during the selected 6-years cycle periods: 50% of 10 new NIS = 5 new NIS.

In conclusion, the threshold value is set at the number of 5 new NIS introductions for that subregion.

The group highlighted that:

- The exact value of percentage reduction should be decided at regional and/or subregional scale, based on the pathways pressure and level of monitoring coverage of each region/subregion. For example, for the Baltic Sea countries the percentage reduction could be 100% (thus, the threshold value of new NIS should be zero, such as in the case of the HELCOM approach).
- The number of the previous 6-years cycle periods, which will serve as the basis for defining the percentage reduction of new NIS should be ideally long, e.g. starting from 1970s. However, the exact number of the previous 6-years cycle periods should be decided at regional and/or subregional scale, based on the history of monitoring and pathways intensity in each region/subregion. Consequently, for MSFD regions/subregions that have not been efficiently monitored in terms of NIS during the previous decades, a shorter time span of 6-years cycle periods should be preferred.

The group finally remarked that the percentage reduction could be adjusted at regional/subregional scale in order to serve short-term and mid-term goals for D2C1.

Topic #12. Monitoring of marine NIS for D2

The monitoring of marine NIS differs across MSs. Only three countries have a monitoring scheme on marine NIS applied at fully national level (Figure 1). On the other hand, the majority of countries have a monitoring strategy

on marine NIS applied only at hot-spot areas of the country or they do not have a dedicated strategy on NIS at all. Hot-spot areas for NIS monitoring include mainly ports and aquaculture units (Figure 2). The focus of NIS monitoring include mainly the detection of new NIS and the measurement of abundance/coverage/biomass of established and/or invasive NIS (Figure 3). NIS-related data come mainly from various research projects and at a lesser extent from MSFD Descriptor 1 monitoring (Figure 4).

Figure 1. Monitoring strategy on marine NIS in EU Member States.

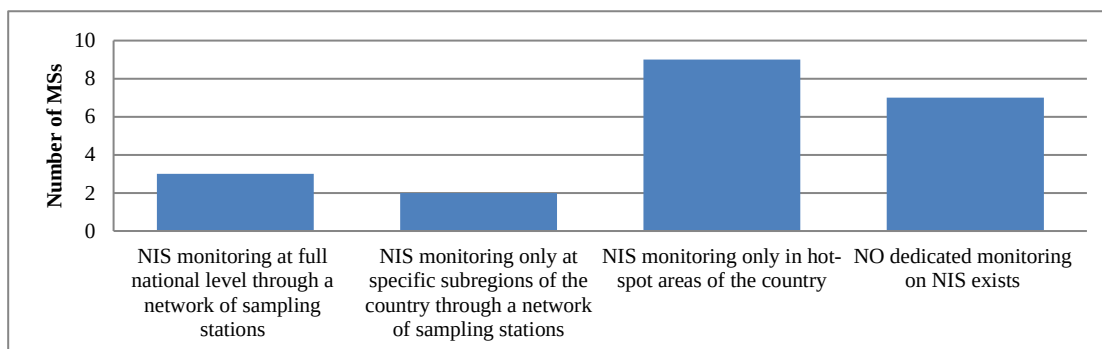


Figure 2. Hot-spot areas for marine NIS monitoring in EU Member States.

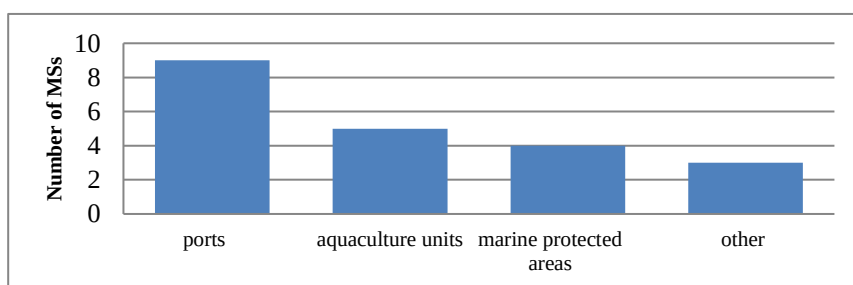


Figure 3. Focus of marine NIS monitoring in EU Member States.

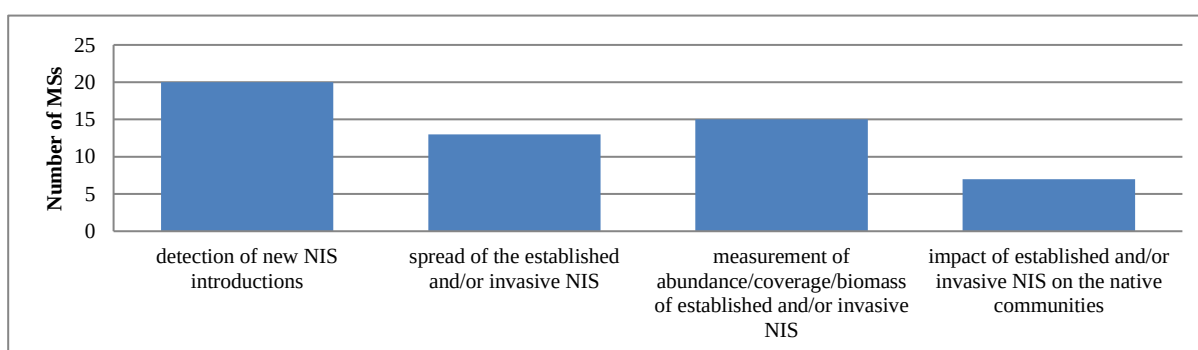
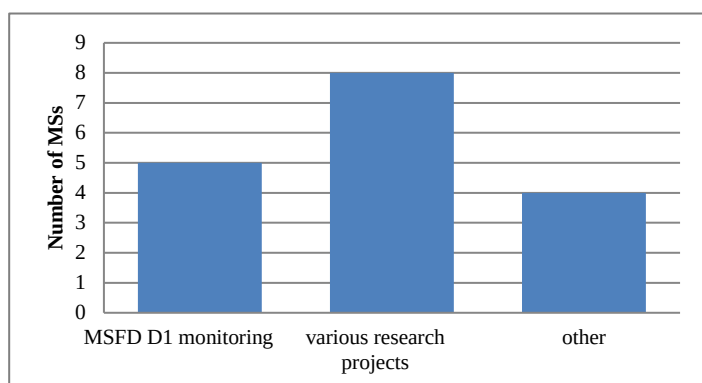


Figure 4. Sources of marine NIS data in EU Member States.



Topic #13. Monitoring bias in criterion D2C1

It is generally accepted that as monitoring efforts increase in an area, more new NIS are detected. On the other hand, with low monitoring effort fewer new NIS are detected and GES can be achieved more easily, regardless of the actual situation. As a result, differences in monitoring of NIS introductions among MSs introduce a significant bias, affecting the assessment of D2C1 and in turn whether GES has been achieved against a specific threshold value.

Workshop's outcome: There is a need for dedicated and well-defined monitoring on marine NIS, which should be harmonized at least at MSFD subregional level. In addition, there needs to be a safeguard on a minimum monitoring effort. The group highlighted the below recommendations:

- Ensure harmonization of monitoring protocols and methods. Data obtained should be comparable across MSs, at least of the same MSFD subregion. Share and exchange of information on the current monitoring protocols.
- The monitoring should be systematic in **space, time** and across **taxonomic groups**:
 - ❖ space: priority should be given to hotspot areas of marine NIS (e.g. ports, aquaculture units and marine protected areas);
 - ❖ time: temporal aspect of sampling and frequency (related to life histories and ecology of target groups) should be harmonized across MSs;
 - ❖ taxonomic groups: enhance the taxonomic capacity and number of related experts; promote the establishment of regional/subregional networks of experts to increase taxonomic knowledge on difficult taxonomic groups, including inconspicuous taxa that remain understudied (e.g. parasites, phytoplankton).
- Arrange training programmes for monitoring techniques, protocols and species taxonomy. Link with universities that through MSc and PhD projects can increase human resources (students) working on NIS.
- Consider e-DNA analysis as a supplementary tool in monitoring techniques.
- Citizen scientists through social networks (divers, recreational fishers, amateur conchologists) can provide data on new NIS as well as on the distribution of invasive NIS. However, there is the need for quality control.
- Align with other research projects working on marine biodiversity.
- Keep NIS databases updated, harmonized and checked by experts, since these can constitute valuable working tools for D2C1.

Topic #14. Inconsistencies in baselines of marine NIS (Tsiamis et al. 2019)

Tsiamis et al. (2019) published the refined baseline inventories of NIS per MS and MSFD subregion, in collaboration with NIS experts appointed by the MSs. The refined baseline inventories constitute a milestone

for the D2C1 implementation, providing an improved basis for reporting new NIS introductions. However, after the publication of the baselines, several inconsistencies were reported by the MSs (MSCG meeting 2019).

Workshop's outcome: These inconsistencies were successfully addressed during the work process of the current report (data gathering exercise, questionnaire, workshop), in collaboration with the NIS experts appointed by the MSs (see Annex 2). The latter Annex contains the most updated and verified (by the NIS experts) baseline information for each MS. The baselines for each MS were merged at subregional level by the JRC (Annex 3). Still, there were a few inconsistencies of NIS observed, depicted in Table 2. These inconsistencies need to be addressed at subregional scale with the contribution of relevant taxonomic experts.

Table 2. Inconsistencies of NIS that needed to be addressed in terms of the non-indigenous/cryptogenic status in specific MSFD subregions.

MSFD subregion	Non-indigenous species with observed inconsistency
Ionian - Central Mediterranean:	<i>Bursatella leachii</i> : treated as cryptogenic by IT, MT / as non-indigenous by EL
Bay of Biscay and the Iberian Coast	<i>Amphibalanus amphitrite</i> : treated as cryptogenic by ES / as non-indigenous by FR, PT
Greater North Sea	<i>Fucus distichus</i> subsp. <i>evanescens</i> : treated as cryptogenic by DK / as non-indigenous by SE <i>Hydroides elegans</i> : treated as cryptogenic by NL / as non-indigenous by FR <i>Pleurosigma simonsenii</i> : treated as cryptogenic by DE / as non-indigenous by NL
Baltic Sea	<i>Fucus distichus</i> subsp. <i>evanescens</i> : treated as cryptogenic by DK, DE / as non-indigenous by SE <i>Mya arenaria</i> : treated as cryptogenic by DE, DK, EE, FI, LT, LV, SE / as non-indigenous by PL

Topic #15. Inconsistencies of marine NIS in future D2 reporting

There are high chances that inconsistencies of NIS at MSFD subregion level such as the ones of Topic #14 (i.e. certain species considered as NIS in several MSs but as cryptogenic or even native in other MS of the **same** MSFD subregion) would also occur in the future D2 reporting cycles, due to the high uncertainty of several species concerning their native or non-indigenous status.

Workshop's outcome: The uncertainty of these NIS should be tackled through sharing of data and the use of available sources and databases (e.g. Aquanis, EASIN, ESENIAS, MAMIAS, etc.). Moreover, taxonomic expertise should be explored through bringing together experts at subregional scale to address inconsistencies of marine NIS case-by-case and propose a solution by consensus. Still, this might not be enough for all problematic NIS, since experts might not reach a consensus for all such NIS. In this case, these NIS should be reported in D2 assessments in the next reporting cycles of D2, but they should not be considered when assessing against a GES threshold.

Topic #16. Secondary criterion D2C2

The criterion D2C2 concerns the "abundance and spatial distribution of established non-indigenous species, particularly of invasive species, contributing significantly to adverse effects on particular species groups or broad habitat types". The information provided on D2C2 by the MSs in the 2018 reporting was scarce (Tsiamis et al. report under review). The related parameters included the distribution, abundance and biomass of established NIS, as well as changes in their number of species, but in most cases no related quantified values were provided by the MSs. NIS included in the Regulation 1143/2014 on Invasive Alien Species must feed the D2C2 reporting. Similarly, species treated in the Aquaculture Regulation 708/2007 could also feed D2C2.

Workshop's outcome: The group agreed that MSs should gather more data on invasive NIS and apply D2C2. The application of this criterion would benefit from specified assessment methods and guidelines in order to clarify

how D2C2 could be assessed in a more efficient way. Moreover, the role of this criterion as a stepping stone for the application of D2C3 was highlighted.

Topic #17. Secondary criterion D2C3

The criterion D2C3 concerns the "*proportion of the species group or spatial extent of the broad habitat type which is adversely altered due to non-indigenous species, particularly invasive non-indigenous species*". The information provided by D2C3 should contribute to assessments of D1 and D6 of the MSFD. The MSs provided scarce information on D2C3 in the 2018 reporting (Tsiamis et al., report under review). For coherence with targets of the EU Biodiversity Strategy 2030, threatened groups of species of the IUCN Red List should be taken into consideration for the application of D2C3.

Workshop's outcome: The group agreed that MSs should gather more data on invasive NIS and apply D2C3. As in the case of D2C2, the application of D2C3 would benefit from specified assessment methods and guidelines in order to clarify how this criterion could be assessed in a more efficient way. References to the methods proposed such as the Biopollution Index (BPL; Olenin et al. 2007) and the CIMPAL index (Katsanevakis et al. 2016) were made. The workshop's participants highlighted the difficulties in assessment of this criterion, especially when it comes to the lack of related data. However, the group also flagged the importance of this criterion (together with D2C2) on trying to tackle invasive established NIS that have already a tremendous negative impact on the European seas. In addition, it was highlighted that the comprehensive assessment of D2 should take in consideration D2C3, i.e. GES related to NIS cannot be achieved when D1 or D6 are below GES due to NIS.

Topic #18. Other issues

Uncertainty of NIS information

The group highlighted the uncertainty of information related with pathways and the date of first record of a NIS into a marine area. To that end, related sources should be shared and exchanged. Moreover, NIS databases need to be updated, harmonized and checked by experts. For several NIS, expert judgment must be applied. The latter would benefit from groups of experts formed at regional/subregional scale.

When it comes to pathways of introduction, it was agreed to follow the terminology of CBD (2014), as revised by Pergl et al. (2020).

4 Conclusions

The MSFD Descriptor 2 implementation is characterized by a number of issues and uncertainties. These issues were tackled by the NIS expert group in a consistent way as possible, trying to find the optimum balance between scientifically acceptable standards and practical solutions with a policy perspective. The primary criterion D2C1 is of particular importance, since it is used to ensure consistency across the Union, and it is based on solid scientific data (new NIS introductions). The secondary criteria could also be important, since through them invasive NIS with tremendous impacts to the European seas, placing at risk marine species and habitats, can be addressed.

The current report summarizes and provides recommendations on a number of issues on D2 implementation, provided in Table 3.

Table 3. Recommendations of the NIS expert group on MSFD Descriptor 2 implementation and its criteria.

Level of issue	D2 issues	Recommendations
At Descriptor Level	Geographical unit	Applied at the national part of the MSFD subregion, with the exception of the Macaronesia subregion of PT.
	Assessment period	For all MSs and MSFD subregions the next assessment period should be due in 2018-2023.
	Phytoplankton NIS	Should be reported in D2 criteria, but not considering them when assessing against a GES threshold for the time being.
	Parasitic NIS	Should be reported in D2 criteria, but not considering them when assessing against a GES threshold until further data is available. They might be considered for measuring GES at smaller scales (e.g. in a certain MSFD subregion) if available information is already sufficiently enough.
	Oligohaline NIS	D2 assessments should include all NIS found in coastal waters, regardless their oligohaline/marine/freshwater status.
	Partly-native NIS in the same MSFD subregion	Should be reported in D2 criteria, but not considering them when assessing against a GES threshold at the relevant subregional scale.
	Inconsistencies of marine NIS	Group of experts at subregional scale should propose a consensus case-by-case for these NIS. In case a consensus is not reached, these NIS should be reported in D2 criteria, but not considering them when assessing against a GES threshold.
At criterion D2C1 level	NIS introduced from one infested area to another through natural dispersal	Should be reported in D2C1 criterion. Considering them for GES should be examined case-by-case, based on pathways' certainty, availability of data, and the impact caused by them. NIS included in the Union concern list of the EU Regulation 1143/2014 must be considered for GES in D2C1.
	Cryptogenic species	Cryptogenic (and crypto-expanding) species should be reported in D2C1, but not considering them when assessing against a GES threshold due to their high uncertainty.
	Questionable species	Should be reported in D2C1, but not considering them when assessing against a GES threshold.
	Extinct species	Should be examined case-by-case whether they correspond to a new introduction in an area (and thus addressed in D2C1) or have been overlooked.
	Threshold values	Adopt the percentage reduction of new NIS compared to the average number of new NIS introductions that occurred in the previous 6-years cycle periods. The dataset gathered (Annexes 2 and 3) facilitates the application of the adopted method and the discussion on setting threshold values. This process is proposed to be guided and handled by a European high-level group concerning D2, consisting of representatives of RSCs and the JRC (see also below). This group can decide on:

		➤ the exact value of percentage reduction at regional and/or subregional scale; ➤ the number of the previous 6-years cycle periods, which will serve as the basis for defining the percentage reduction of new NIS, at regional and/or subregional scale.
	Monitoring bias	Need for a minimum, dedicated and well-defined monitoring on marine NIS, which should be harmonized at least at MSFD subregional level (more details can be found in page 17).
At criterion D2C2 level		MSs should gather more data on invasive NIS and apply D2C2. The criterion D2C2 acts as a stepping stone for the application of D2C3.
At criterion D2C3 level		MSs should gather more data on invasive NIS and apply D2C3. The Biopollution Index and the CIMPAL index are mentioned. The importance of this criterion was flagged on trying to tackle invasive established NIS that have already a tremendous impact on the European seas.
Other issues	Introduction pathways terminology	Follow the terminology of CBD (2014), as revised by Pergl et al. (2020).

The above recommendations provide practical solutions aiming at a smoother and more efficient implementation of the MSFD D2 and its criteria at EU level. They constitute a solid operational output which can result in D2 assessments with more comparable data among MSs and MSFD regions/subregions.

When it comes to the policy-side, the experts' group call for a number of different categories of NIS to be reported in D2 assessments, pointing the need for the species to be labelled/categorised appropriately in the MSFD reporting by the MSs. It is proposed that these suggestions, together with the practical recommendations on D2 implementation (Table 3), are communicated to the MSFD Working Group GES and subsequently to the MSCG Group of MSFD. Moreover, they can serve as an input for revising the Art. 8 Guidelines.

Finally, the NIS expert group proposed for the establishment of a high-level group concerning D2, consisting of representatives of RSCs and the JRC, streamlining the EU level marine NIS work. The group's aims and responsibilities should include:

1. promote sharing of knowledge and exchange of information regarding NIS data, monitoring methodologies and approaches;
2. coordinate NIS strategies and avoid duplication of work;
3. contribute on setting threshold values at regional/subregional based on the percentage reduction approach;
4. resolve NIS inconsistencies among MSFD subregions and regions.

The establishment of the high-level group is expected to take place in early 2021.

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List of abbreviations and definitions

AQUANIS Information system on Aquatic Non-Indigenous and cryptogenic species

BPL	Biopollution Level index
CBD	Convention of Biological Diversity
CCB	Coalition Clean Baltic
CIMPAL	Cumulative Impacts of invasive Alien species
DG ENV	DG Environment
DG JRC	DG Joint Research Centre
EASIN	European Alien Species Information Network
EEA	European Environmental Agency
EU	European Union
GES	Good Environmental Status
HELCOM	Helsinki Commission (Baltic Marine Environment Protection Commission)
IAS	Invasive Alien Species
MS	Member State
MSCG	Marine Strategy Coordination Group
MSFD	Marine Strategy Framework Directive
NIS	Non-Indigenous Species
OSPAR	Oslo and Paris conventions (for the Protection of the Marine Environment of the North- East Atlantic)
RSC	Regional Sea Convention
UNEP	United Nations Environment Programme
WFD	Water Framework Directive

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Annexes

Annex 1. Instructions supporting the validation by the national NIS experts of the D2 MSFD Expert Network, on the information gathered by the JRC through the "Data Gathering" phase (see Section 2.1) including: the NIS baselines inventories, the date of first record (= date of first observation in the field) of each NIS in each MS and MSFD subregion, as well as its related pathway of introduction. These instructions were sent to the experts via email emails on 06.11.2019.

Each excel file corresponds to the information relevant to a Member State.
Excel Sheet #2. Refined baseline inventory of non-indigenous, cryptogenic and questionable species for each MS and relevant MSFD marine subregion, based on Tsiamis et al. (2019). For several MSs, specific amendments were recently proposed by MS authorities and/or experts applied to the inventory list of Tsiamis et al. (2019); these are highlighted with color: • excel cells highlighted in red: species proposed for exclusion from the baseline inventory of Tsiamis et al. (2019); • excel cells highlighted in green: species proposed for addition in the baseline inventory of Tsiamis et al. (2019). Note: Parasite species are also excluded from this analysis.
Experts contribution: experts are invited to check and validate the above changes (if applicable). They are also welcomed to propose additional changes / revisions based on the most recent scientific findings. They should highlight these changes with yellow coloration and add their remarks in the excel column "Remarks by appointed experts (if applicable)".
Excel Sheet #3. Information on the date of first introduction and associated pathway for each NIS in the specific MSFD subregion and country. The source of the relevant information is also provided as well as related comments. When associated information was impossible to be located the corresponding cell was highlighted in blue color. Note: The information on date of first introduction and associated pathway(s) in a specific MSFD subregion was retrieved only for non-indigenous species, but not for cryptogenic and questionable species which were excluded from the analysis. In addition, the associated pathway(s) information was retrieved only for NIS introduced after 1970, and not for species introduced before 1970 (the latter are highlighted in grey color and they were also excluded from the analysis when it comes to pathways).
Experts contribution:
1) experts are invited to check and validate the relevant information provided for the dates of first introduction and associated pathways. They should highlight any changes/additions with yellow coloration. Experts are also welcomed to add comments/remarks in the excel column "Remarks by appointed experts (if applicable)".
2) experts are invited to provide a certainty score to each assigned pathway for every NIS, based on the scheme proposed by Tsiamis et al. (2018):
A) High certainty (to be marked as P_HIGH): there is direct evidence of a pathway; this is the case e.g. for most intentional introductions (e.g. Release in Nature: fishery in the wild - including game fishing) and in many cases of NIS entering through natural spread when there is direct evidence of a gradual expansion.
B) Medium certainty (to be marked P_MEDIUM): a likely pathway can be inferred; the NIS appears for the first time in a locality where a pathway is known to operate. This applies to many species introduced e.g. by shipping ballast (Transport - Stowaway: ship/boat ballast water) or as aquaculture contaminants (Transport - Contaminant: contaminant on animals - except parasites, species transported by host/vector). In many cases inference is based on known examples of introductions elsewhere for the same species, the biology and ecology of the species, the habitats and locales it occupies in both the native and introduced range, and its pattern of dispersal (if known), e.g. for a fouling species frequently recorded in/near ports, Transport - Stowaway: ship/boat hull fouling has been assumed to be the most probable pathway.
C) Low certainty (to be marked as P_LOW): the NIS cannot be convincingly ascribed to a single pathway; usually, two or more possible pathways can be inferred. Inference is based on the activities in the locality where the NIS was found and may include evidence on similarly behaving species reported elsewhere.

Annex 2. Information on refined NIS baselines (NIS up to 2011) per each Member State and national part of the MSFD subregion. NIS with dates of first record and associated pathways given per each Member State and national part of the MSFD subregion are also provided (for NIS found up to 2017).

Due to the high number of excel sheets, the information is directly provided through a web-link in the EASIN website (<https://easin.jrc.ec.europa.eu/>). The related information is also available on request by the JRC D2 team (JRC-EASIN@ec.europa.eu).

Annex 3. NIS with dates of first record and associated pathways given at MSFD subregional level (for NIS found up to 2017).

Due to the high number of excel sheets, the information is directly provided through a web-link in the EASIN website (<https://easin.jrc.ec.europa.eu/>). The related information is also available on request by the JRC D2 team (JRC-EASIN@ec.europa.eu).

Annex 4. The Questionnaire distributed (30.07.2020) to all members of the D2 MSFD Experts' Network and to the representatives of the RSCs and relevant stakeholders, aiming to facilitate the Workshop (6-7.10.2010) process and its effectiveness, as well as to facilitate discussions at national, regional and inter-regional level.

Question #1: Geographical unit for D2 implementation

Based on Tsiamis et al. (report under review), several MSs have applied D2 at: a) regional level (e.g. entire Great North Sea), b) subregional level (e.g. DE part of the Great North Sea for Germany), c) subdivision of a subregion (e.g. north part of Biscay Bay of Spain), and d) a combination of the above. Ideally, MSs should implement D2 in a consistent geographical unit, and the latter should be at (put a "X" in the appropriate answer):

a) entire regional level (...)	b) national part of a subregion (...)	c) subdivision of a national part of a subregion (...)	d) a combination of the previous (...)
comment (optional):	comment (optional):	comment (optional):	comment:

Question #2: Assessment period for D2 implementation

Based on MSFD the assessment period covers a 6-years period measured from the reference year as reported for the initial assessment (EU 2017). For the majority of the MSs, the year of the initial assessment was 2012; thus the assessment period would be the 6 years between 2012-2017. However, not all MSs reported the initial assessment in 2012. In the 2018 reporting, several MSs reported as assessment period for D2 the period 2011-2016, while others the period 2012-2017, or even 2013-2018. In addition, when it comes to the new NIS introductions, it is argued that due to the time lags observed for reporting new NIS, there is a need for additional available time between the year of the reporting of D2 and its assessment period. For consistency and harmonization reasons, the assessment period should be the same across MSs, at least at regional scale. For the next MSFD reporting due in 2024, the assessment period for D2 should be (taking also into account the time lags in reporting new NIS introductions) (put a "X" in the appropriate answer):

a) 2018-2023 (...)	b) 2017-2022 (...)	c) 2016-2021 (...)
comment (optional):	comment (optional):	comment (optional):

Question #3: Unicellular plankton species in D2 criteria

Unicellular plankton species have high uncertainty regarding the native vs non-indigenous status in European seas. There have been scattered records across Europe, but no consistency in their treatment. In Tsiamis et al. (2019) baseline there was high variance of the number of planktonic species included in the inventories across the MSs, even between neighboring countries, reporting either long lists of them or just a few. Several countries chose not to include plankton species in their NIS lists. More recently, Gomez (2019) argued that there is not enough evidence for tagging any plankton species in Europe as non-indigenous. Tsiamis et al. (2019) suggested that unicellular plankton NIS should be treated with caution (e.g. flagged with high uncertainty) until further research clarifies their enigmatic status. For the implementation of D2, unicellular planktonic NIS species should be (put a "X" in the appropriate answer):

a) reported and considered when measuring GES based on D2 criteria (...)	b) reported but not considered when measuring GES based on D2 criteria (...)	c) omitted entirely from D2 assessments (...)	d) other (...)
comment (optional):	comment (optional):	comment (optional):	comment:

Question #4: Parasitic species in D2 criteria

In Tsiamis et al. (2019) baseline parasitic NIS were omitted since from a legislative perspective they are managed under the Aquatic Animal Health Directive (2006/88/EC; EU, 2006) rather than the MSFD. However, several MSs have included parasitic NIS in their D2 lists. For the implementation of D2 parasitic NIS species should be (put a "X" in the appropriate answer):			
a) reported and considered when measuring GES based on D2 criteria (...)	b) reported but not considered when measuring GES based on D2 criteria (...)	c) omitted entirely from D2 assessments (...)	d) other (...)
comment (optional):	comment (optional):	comment (optional):	comment:

Question #5: Oligohaline NIS in D2 criteria Oligohaline (salinity between 0.5 and 5/1000) waters (transitional waters) are under the realms of the Water Framework Directive (WFD). However, transitional waters were not defined in a consistent way across EU. Tsiamis et al. (2019) baseline generally did not take into consideration oligohaline NIS. However, they were included by several MSs when these species have been also found in their marine coastal waters. These entail mostly the oligohaline (but also several freshwater) species found in the low saline Baltic Sea. Nevertheless, these species might be present in other MSs as well, but they were not listed in their inventories since they occur exclusively in their transitional and inland systems (e.g. <i>Corbicula fluminea</i> , <i>Cordylophora caspia</i> , <i>Elodea canadensis</i> , <i>Elodea nuttallii</i>). In overall, oligohaline species should be addressed in a fully consistent way at EU level. For MSFD D2, oligohaline species should be (put a "X" in the appropriate answer):			
a) generally included in D2 assessments (...)	b) included in D2 assessments of a MS only if these species are found also in marine coastal waters of the country (e.g. Baltic Sea waters) (...)	c) excluded from D2 assessments (...)	d) other (...)
comment (optional):	comment (optional):	comment (optional):	comment:

Question #6: Partly-native species in the same MSFD subregion, in D2 criteria Several species are native in some MSs while they are non-indigenous in other EU countries. These species are generally known as "partly native" (or partly alien) species. In exceptional cases, there are partly native species in the same MSFD subregion. For example, <i>Haliclona simulans</i> is considered as non-indigenous in NL, but as native in the rest of the Great North Sea countries. This would result in a discrepancy when assessing the related information at the subregional level of the Great North Sea about the species status. Therefore, at subregional level, partly native species of the same MSFD subregion , such as <i>Haliclona simulans</i> , should be (put a "X" in the appropriate answer):			
a) reported and considered when measuring GES at subregion scale (...)	b) reported but not considered when measuring GES at subregion scale (...)	c) omitted from D2 assessments at subregional level (...)	d) other (...)
comment (optional):	comment (optional):	comment (optional):	comment:

Question #7: NIS introduced through natural dispersal in criterion D2C1 The primary criterion D2C1 measures "The number of non-indigenous species which are newly introduced via human activity into the wild, per assessment period (6 years), ...". It has been argued that NIS introduced exclusively through natural dispersal from already infested areas of European seas to other neighboring areas (e.g. a NIS introduced from Lebanon to Cyprus through natural dispersal) should not be taken into consideration for defining GES based on D2C1, unless there is evidence that the species is transferred also through human-mediated activities, such as shipping (Palialexis et al. 2015). Doing so, there will be the			
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possibility to measure the effectiveness of management measures, which prevent NIS human-mediated introductions in a given assessment area. Several Lessepsian species fall under this category. For the implementation of D2C1, NIS that have been introduced into a MS or MSFD subregion exclusively through natural dispersal should be (put a "X" in the appropriate answer):		
a) reported and considered when measuring GES based on D2C1 (...)	b) reported but not considered when measuring GES based on D2C1 (...)	c) other (...)
comment (optional):	comment (optional):	comment:

Question #8: Cryptogenic species in criterion D2C1

Cryptogenic species are species that cannot be demonstrably classified as native or non-indigenous in a particular region (Carlton 1996). Usually, there is high uncertainty for the origin, biogeography and their pathway. Characteristic examples include *Palaemon elegans* in the Baltic and *Antithamnionella spirographidis* in the Mediterranean Sea. Due to the lack of enough data, it is not uncommon that NIS experts disagree on the status of cryptogenic species in a specific area. As a result, these species may be treated as non-indigenous in some countries, while in neighboring countries they are reported as cryptogenic or even as native species. Moreover, the status of cryptogenic species can be altered in time, based on new available research data coming into light, thus changing their status. In the recent JRC exercise on pathways and dates of first introductions of NIS in each MS and subregion, cryptogenic species were excluded. Similarly, cryptogenic species were not further analyzed in Tsiamis et al. (2019) baseline, but they were simply listed in an annex. For the implementation of D2C1, species that are considered by the NIS experts as cryptogenic should be (put a "X" in the appropriate answer):

a) reported and considered when measuring GES based on D2C1 (...)	b) reported but not considered when measuring GES based on D2C1 (...)	c) other (...)
comment (optional):	comment (optional):	comment:

Question #9: Questionable species in criterion D2C1

Questionable species are those with unresolved taxonomic status or new NIS entries not verified by experts (e.g. records coming from citizen-science but not yet validated by experts, or records in technical reports without providing the necessary taxonomic evidence). These species are also known as "data-deficient", following Essl et al. (2018). In the recent JRC exercise on pathways and dates of first introductions of NIS in each MS and subregion, questionable species were excluded. Similarly, questionable species were not further analyzed in Tsiamis et al. (2019) baseline, but they were simply listed in an annex. For the implementation of D2C1, species that are considered by the NIS experts as questionable should be (put a "X" in the appropriate answer):

a) reported and considered when measuring GES based on D2C1 (...)	b) reported but not considered when measuring GES based on D2C1 (...)	c) other (...)
comment (optional):	comment (optional):	comment:

Question #10: Extinct species in criterion D2C1

Several NIS have been reported in MSs several decades ago (even in the 19th century or before) but never recorded again in the wild in these MSs, and thus are considered as extinct; presumably that the NIS did not survive in its new environment. However, it is difficult to prove if a NIS has been truly extinct from a marine area or MS due to monitoring difficulties and the continuum of the marine environment. When a presumably extinct NIS is reported during the last assessment period from the same or adjacent area that was originally reported in a MS, then (put a "X" in the appropriate answer):

a) it should be considered as a new introduction and measured in D2C1 assessment (...)	b) it should not be considered as a new introduction, the species should had been overlooked	c) the decision should be made species-by-species, based on the available data (...)	d) other (...)
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	(...)		
comment (optional):	comment (optional):	comment (optional):	comment:

Question #11: Threshold values in criterion D2C1

Based on D2C1 "The number of non-indigenous species which are newly introduced via human activity into the wild, per assessment period (6 years), ..., is minimised and where possible reduced to zero. Member States shall establish the threshold value for the number of new introductions of non-indigenous species, through regional or subregional cooperation". HELCOM (2018) has set the threshold value for D2C1= zero new NIS. OSPAR (2018) highlights that the relative change of the number of new NIS introductions seen over subsequent assessment periods (e.g. 6 years) can facilitate the specification of threshold values; however, OSPAR has not yet concluded in specific values. UNEP-MAP and the Black Sea Committee have not started yet the related work on setting threshold value for D2C1. In overall, the threshold value for D2C1 could be:

A) zero new NIS introductions; such as in the case of HELCOM.

B) could be specified based on the number of new NIS introductions during the last 6 years (e.g. 10 new NIS), and this could be the threshold for new NIS in the next D2C1 assessment.

C) same as above (B), but based on the number of new NIS introductions during a longer time span; e.g. for the last 30 years and conclude in an average per 6 years (e.g. if 100 new NIS during the last 30 years / 5 assessment periods of 6 years = 20 new NIS per 6 years). Therefore, the threshold value could be equal to 20 new NIS for the next assessment of D2C1.

In your opinion, among the above options, the threshold value for D2C1 should be (put a "X" in the appropriate answer):

option (A)	option (B)	option (C)	other
(...)	(...)	(...)	(...)
comment (optional):	comment (optional):	comment (optional):	comment:

Question #12: Monitoring of marine NIS for D2

For your country, is there a dedicated monitoring scheme for marine NIS? (put a "X" in the appropriate answer):

a) YES , at full national level through a related network of sampling stations (...)	b) YES , but only in specific subregions of the country through a related network of sampling stations (...)	c) YES , but only in hotspot areas of the country (...)	d) NO dedicated monitoring on NIS exists (...)
		hotspot areas include (multiple choices can be marked):	relevant data on marine NIS come from (multiple choices can be marked):
		ports (...)	MSFD D1 monitoring (...)
		aquaculture units (...)	various research projects (...)
		marine protected areas (...)	other (...)

			other (...)	
Monitoring efforts on marine NIS in your country include (put a "X" in the appropriate answer; multiple choices can be marked):				
a) the detection of new NIS introductions (...)	b) the spread of the established and/or invasive NIS (...)	c) the measurement of abundance/coverage/biomass of established and/or invasive NIS (...)	d) the impact of established and/or invasive NIS on the native communities (...)	

Question #13: Monitoring bias in criterion D2C1

It is generally accepted that as monitoring efforts increase in an area more new NIS are detected. As a result, differences in monitoring of NIS introductions among MSs introduce a significant bias, affecting the assessment of D2C1 and in turn whether GES has been achieved against a specific threshold value. Therefore, there is a need for dedicated and well-defined monitoring on marine NIS, which should be harmonized within and across regions. The monitoring should be systematic in space, time and across taxonomic groups, including inconspicuous taxa that remain understudied. The endorsement of similar monitoring schemes of species groups is essential (Tsiamis et al. 2019). Priority should be given to hotspot areas of marine NIS (e.g. ports), species and habitats protected areas Citizen science could be also used in monitoring new NIS introductions.

Do you agree with above? In your opinion, what is the priority for tackling the monitoring bias?

Please comment here:

Question #14: Inconsistencies in baselines of marine NIS (Tsiamis et al. 2019)

Tsiamis et al. (2019) published refined baseline inventories of NIS per MS and MSFD subregion, in collaboration with NIS experts appointed by the MSs. The refined baseline inventories constitute a milestone for the D2C1 implementation, providing an improved basis for reporting new NIS introductions. However, after the publication of the baselines, several inconsistencies were reported by the MSs (MSCG meeting 2019). These inconsistencies were successfully addressed in the ongoing exercise of the JRC on gathering information on dates and pathways of first introduction of NIS in each MS, in collaboration again with NIS experts appointed by the MSs (see excel sheets in Annex). The latter excels contain the most updated and verified by the experts baseline information of each MS. The baselines of each MS were merged at subregional level by the JRC (July 2020, see excel sheet # 1 in Annex). During the merging, the following inconsistencies were observed for the Greater North Sea:

Inconsistencies in species status in Greater North Sea	Please specify the status of the species for Greater North Sea (put a "X" in the appropriate column):		
	non-indigenous	cryptogenic	other -add comment-
<i>Alexandrium minutum</i> : treated as cryptogenic by DK, FR, DE / as non-indigenous by SE			
<i>Coscinodiscus wailesii</i> : treated as cryptogenic by BE, DK, FR, DE, NL, UK / as non-indigenous by SE			
<i>Fucus distichus</i> subsp. <i>evanescens</i> : treated as cryptogenic by DK, UK / as non-indigenous by SE			

<i>Hydroides elegans</i> : treated as cryptogenic by NL / as non-indigenous by FR, UK			
<i>Platorchestia platensis</i> : treated as cryptogenic by DE / as non-indigenous by SE			
<i>Pleurosigma simonsenii</i> : treated as cryptogenic by DE / as non-indigenous by NL			

Question #15: Inconsistencies of marine NIS in future D2 reporting

There are high chances that inconsistencies of NIS at subregion level such as the above of Question #14 (i.e. certain species considered as NIS in several MSs but as cryptogenic or even native in other MS of the **same** MSFD subregion) would also occur in the future D2 reporting cycles, due to the high uncertainty of several species concerning their native or non-indigenous status. In the next reporting cycles of D2, these species should be treated at MSFD subregion level as follows (put a "X" in the appropriate answer):

a) reported and considered when measuring GES at subregion level (...)	b) reported but not considered when measuring GES at subregion level, flagged with high uncertainty (...)	c) should not be reported at subregion level (...)	d) other (...)
comment (optional):	comment (optional):	comment (optional):	comment:

Question #16: Secondary criterion D2C2

The criterion D2C2 concerns the "abundance and spatial distribution of established non-indigenous species, particularly of invasive species, contributing significantly to adverse effects on particular species groups or broad habitat types". The information provided on D2C2 by the MSs in the 2018 reporting was scarce (Tsiamis et al. report under review). The related parameters included the distribution, abundance and biomass of established NIS, as well as changes in their number of species, but in most cases no related values were provided by the MSs. Quantification values of specified established NIS have been set only by RO, concerning the average concentration (biomass/water) of the species *Mnemiopsis leidyi* (Tsiamis et al. report under review). NIS included in the Aquaculture Regulation 708/2007 and in the Regulation 1143/2014 on Invasive Alien Species could feed the D2C2 reporting. In your opinion, regarding D2C2 (put a "X" in the appropriate answer):

a) MSs should gather more data on invasive NIS and apply D2C2 (...)	b) D2C2 should be neglected; focus exclusively on prevention of new NIS introductions (...)	c) D2C2 should be revised in a future revision of the MSFD D2, by... (...)	d) other (...)
comment (optional):	comment (optional):	comment:	comment:

Question #17: Secondary criterion D2C3

The criterion D2C3 concerns the "proportion of the species group or spatial extent of the broad habitat type which is adversely altered due to non-indigenous species, particularly invasive non-indigenous species". The information provided by D2C3 should contribute to assessments of D1 and D6 of the MSFD. The MSs provided scarce information on D2C3 in the 2018 reporting; only EE provided related data, referring to the Biopollution level (BPL; Olenin et al. 2007) and the relative biomass/abundance of NIS in native communities (Tsiamis et al., report under review). Associated threshold values have been set by EE: for BPL ≤ 1, and for the contribution of NIS in the native communities this should not be significantly higher than the average value from previous assessment period. For coherence with targets of the EU Biodiversity Strategy 2030, threatened species of the IUCN Red List should be taken into consideration for the application of D2C3. In addition, improved reporting for D2C3 could include species groups and broad habitat types of D1 of MSFD. When relevant, an explicit link between D2C2 and D2C3 should be made, to enhance the understanding of NIS impacts. In your opinion, regarding D2C3 (put a "X" in the appropriate answer):

a) MSs should gather more data on impacts of invasive NIS and apply D2C3 (...)	b) D2C3 should be neglected; focus exclusively on prevention of new NIS introductions (...)	c) D2C3 should be revised in a future revision of the MSFD D2, by... (...)	d) other (...)
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comment (optional):	comment (optional):	comment:	comment:
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Question #18: Any Other Business Please write here any comment or topic you would like to raise in the NIS workshop of 6-7 October:
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Annex 5. The results of the Workshop's Questionnaire (see Annex 4), which includes the feedback of 21 MSs, 2 RSCs and one additional stakeholder (Coalition Clean Baltic).

Due to the high number of excel sheets, the information is directly provided through a web-link in the EASIN website (<https://easin.jrc.ec.europa.eu/>). The related information is also available on request by the JRC D2 team (JRC-EASIN@ec.europa.eu).

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