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Agenda Item 7: Status of implementation of the Ecosystem Approach (EcAp) Roadmap

7.1. Implementation of the second phase (2019-2021) of the Integrated Monitoring and Assessment Programme (IMAP - Biodiversity and non-indigenous species) in the framework of the EcAp Roadmap

Implementation of the second phase (2019-2021) of the Integrated Monitoring and Assessment Programme (IMAP - Biodiversity and non-indigenous species) in the framework of the EcAp Roadmap

Appendix E: Monitoring and Assessment Scales, Assessment Criteria and Thresholds Values for the IMAP Common Indicator 6 related to Non-Indigenous Species

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EXECUTIVE SUMMARY

To address the risk NIS pose on marine ecosystems, the Contracting Parties to the Barcelona Convention have updated the Action Plan concerning species introduction and invasive species in the Mediterranean Sea and updated/developed their national monitoring programmes based on the Integrated Monitoring and Assessment Programme (IMAP) Common Indicators. With regards to Non-Indigenous Species (i.e. Ecological Objective 2 or EO2), the Common Indicator 6 (CI6) i assesses "Trends in abundance, temporal occurrence, and spatial distribution of non-indigenous species". The national implementation and harmonization of IMAP across all Mediterranean countries requires the elaboration of a number of parameters, namely monitoring and assessment scales as well as assessment elements (i.e. assessment criteria, thresholds and baseline values).

This report aims to develop monitoring and assessment scales as well as assessment criteria and to make recommendations for establishing threshold values for CI6, based on the available data for the non-indigenous species in the Mediterranean. In order to facilitate discussions and ensure input from all the Contracting Parties (CPs), a questionnaire addressing these issues was sent to 10 non-EU CPs (Albania, Algeria, Egypt, Israel, Lebanon, Libya, Montenegro, Morocco, Tunisia and Turkey), complementing similar work carried out in the framework of Marine Strategy Framework Directive (MSFD) reporting for the 8 EU CPs (Croatia, Cyprus, France, Greece, Italy, Malta, Slovenia and Spain). The results and recommendations presented herein integrate responses by national experts from all these 18 CPs and make use of data derived from recent (2017-2020) publications and the Hellenic Centre for Marine Research (HCMR) offline data base.

Assessment Criteria

Assessment criteria for preparing validated check lists of NIS to be used for assessing GES include a) taxa (all taxa or excluding phytoplankton, parasites); b) species to be considered in trends indicator (extinct species, cryptogenic species, crypto-expanding, questionable species); c) pathways to be considered (all pathways or excluding unaided expanding species, e.g. Lessepsian immigrants).

The views of the national experts were generally in good agreement and the majority of them proposed that **partly native species, NIS introduced through natural dispersal, unicellular marine algae, parasites, extinct and freshwater species** should be reported in NIS lists but considered in CI6 assessments on a case-by-case basis.

Spatial and Temporal Scales of Monitoring and Assessment

Broad Geographic Units: Assessment of threshold values based on the trends indicator (CI6) calculated to date can be achieved at the basin and country level, although it is more meaningful to be assessed at the subregional level (i.e. EcAp subregional units) and, accordingly, at the national part of a subregion for each country, e.g **Greece:** EMED, CMED, ADRIA; **Italy:** WMED, CMED, ADRIA, **Tunisia**: WMED, CMED. It is recommended that the geographic borders of the Mediterranean EcAp subregions should be fully harmonized, during the review of the EcAp roadmap and IMAP phases, with those proposed by EU countries and adopted by the European Environment Agency (i.e. MSFD delimitations).

Broad Temporal Scales: For consistency and harmonization reasons, it is recommended that the assessment period of CI6 should be the same across all Mediterranean countries and follow the assessment and reporting 6-year periods already established for EU countries under the MSFD. Specifically, the next assessment should cover the 2018-2023 period, such that the reference year to set national NIS baselines should be 2017 at the earliest, taking also into account reporting lags. Trends in new marine NIS introductions are consistently increasing throughout the Mediterranean and, in many countries, this is the result of increased scientific effort, (bringing to light species already widely established in the region). Therefore, for some countries even 2017 as the reference year, may be premature.

Finer scales for NIS monitoring

At basin scale, there are no established standard protocols for the monitoring of NIS. However, guidelines for monitoring NIS in the Mediterranean were developed and endorsed by the CPs to the Barcelona Convention in 2019 under the framework of the EcAp/IMAP (UNEP/MED WG.467/16 (2019) "Monitoring Protocols for IMAP Common Indicators related to Biodiversity and Non-Indigenous species").

Responses to the questionnaires revealed that the majority of countries do not have a dedicated strategy but have a monitoring strategy including marine NIS applied either at hot-spot areas of the country (i.e. ports, aquaculture units, marine protected areas) or in specific subregions through a related network of sampling stations. Targets of NIS monitoring include mainly the detection of new NIS and the measurement of abundance/coverage/biomass of established and/or invasive NIS, while only a small number of countries monitor the impact of established/invasive NIS on the native communities.

The **IMAP Common Indicator Guidance** propose more intense monitoring effort at "hot-spots" and "steppingstone areas" for non-indigenous species introductions, e.g. sampling at least once a year at ports and their wider area and once every two years in smaller harbours, marinas, and aquaculture sites. Importantly, the same sites should be surveyed each monitoring period, to avoid biases potentially caused by differences among sites.

<u>Threshold Values</u>

Currently, threshold values for the number of new introductions of non-indigenous species have not been set neither at the EU or the Mediterranean level. Ongoing work in the framework of the MSFD (Tsiamis et al., 2021b) has concluded that the most suitable approach for setting threshold values for D2C1 is to adopt the percentage reduction of new NIS and 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.

Preliminary analysis of the available data for the Mediterranean between 1970-2017 for the purposes of this report demonstrated that there is a significant increase in the rate of new NIS entering all EcAp subregions after 2000 (presumably as a result of increased scientific effort) and that this parameter is significantly different between EcAp subregions. Consequently, the initial recommendations are that i) the threshold values for CI6 in the Mediterranean need to be set at subregional level and not at regional level and ii) we need to consider data only after 2000s in order to establish today's threshold values. Furthermore, for Mediterranean region/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, e.g. 3 years.

Conclusively, threshold values should be established separately for each of the Mediterranean subregions and should be sought by examining the data of the last two decades, if not an even more recent time period. At the same time, a consensus needs to be reached about which species groups will be included in the calculations and how their environmental impact will be taken into account. These are decisions that will determine the definition of GES for EO2 and will affect the management obligations of Contracting Parties to the Barcelona Convention. As such, it is proposed that further work takes into account the contribution of regional experts not only from the fields of taxonomy, monitoring and assessment but also conservation and management and last, but not least, ecologists with strong statistical/mathematical background.

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INTRODUCTION

1. There are currently about 1000 marine non-indigenous species (NIS) in the Mediterranean marine waters, two thirds of which have established viable populations (Zenetos & Galanidi, 2020). A subset of the established species exhibits invasive behaviour and have negative impacts on marine ecosystem services and biodiversity (Streftaris & Zenetos, 2006, Galil, 2007, Katsanevakis et al. 2014; 2016; Korpinen et al., 2019). Cumulative impacts of invasive NIS (CIMPAL; Katsanevakis et al., 2016) were estimated on the basis of the distributions of invasive species and ecosystems, and both the reported magnitude of ecological impacts and the strength of such evidence.

2. To address the risk NIS pose on marine ecosystems, the Contracting Parties to the Barcelona Convention have updated the Action Plan concerning species introduction and invasive species in the Mediterranean Sea (Decision IG.22/12 of the CoP 19) and updated/developed their national monitoring programmes based on the Integrated Monitoring and Assessment Programme (IMAP) Common Indicators per each cluster namely Biodiversity and Non-indigenous species (NIS), Pollution and Marine Litter, and Coast and Hydrography.

3. The project "Towards achieving the Good Environmental Status of the Mediterranean Sea and Coast through an Ecologically Representative and Efficiently Managed and Monitored Network of Marine Protected Areas" (hereinafter IMAP-MPA project) aims to support the national implementation of IMAP, and for the delivery of reliable data for IMAP common indicators on three clusters: (i) biodiversity and NIS, (ii) pollution and marine litter (iii) and coast and hydrography. The IMAP-MPA project will also enable the development and implementation of integrated monitoring programmes at the sub-regional level which address the same above-mentioned IMAP clusters, and particularly in areas which are known to be under human activity pressure. This project also includes another important aspect which is the elaboration of monitoring and assessment scales as well as assessment elements (i.e. assessment criteria, thresholds and baseline values) per each IMAP cluster with a focus on biodiversity and hydrography.

4. This report aims to develop monitoring and assessment scales as well as assessment criteria and to establish threshold values based on the available data for the non-indigenous species IMAP common indicator 6 (CI6) under the Ecological Objective 2 (EO2). CI6 requires "Trends in abundance, temporal occurrence, and spatial distribution of non-indigenous species", particularly invasive, non-indigenous species, notably in risk areas, in relation to the main vectors and pathways of spreading of such species in the water column and seabed, as appropriate. To date the only measurement for assessing the Good Environmental Status (GES) is the number of new NIS per 6 years.

5. The environmental status of marine waters of European Union (EU) Mediterranean countries in the context of the Marine Strategy Framework Directive (MSFD) was assessed by the Member States (MSs) as part of the reporting obligations linked to the MSFD initial assessment, for most MSs in 2012 (Palialexis et al., 2014) taking 2011 as reference year for baseline. Updates of the baseline NIS check lists (Tsiamis et al., 2019) that were reported and validated my Member States are provided in Tsiamis et al. (2021b).

6. During 2018-2020, EU MSs among which eight Mediterranean countries, have, in response to their 2018 "reporting" obligations, reported on MSFD Descriptor 2 (D2) information for the last 6-year MSFD reporting cycle, following the Article 17 requirements of updating Articles 8, 9 and 10. A number of inconsistencies in D2 implementation, including the spatial and time coverage of D2 application among the MSs was highlighted by Palialexis et al. (2014) and Tsiamis et al. (2021a).

7. In order to facilitate the discussions towards the establishment of threshold values for D2 criterion 1 (D2C1)/EO2 CI6 at national, regional and inter-regional level, a questionnaire was distributed by the European Commission's Joint Research Centre (JRC) to all EU members and to the representatives of the Regional Seas Conventions and relevant stakeholders. A similar, less extensive questionnaire (see Annex and Table 1) was circulated to national NIS experts from 10 non-EU Contracting Parties (CPs) to the Barcelona Convention (namely Albania, Algeria, Egypt, Israel, Lebanon, Libya, Montenegro, Morocco, Tunisia and Turkey). The topics presented and discussed herein are based largely on the results of the aforementioned questionnaires, the resulting report for the Mediterranean EU countries (Tsiamis et al., 2021b), and data derived from recent (2017-2020) publications and the HCMR offline data base.

Table 1: Queries addressed to national experts

<table>
<tr><td rowspan="2">Species in baseline lists</td><td>Unicellular plankton species</td><td>Parasitic species</td><td>NIS introduced through natural dispersal</td><td>Cryptogenic species</td><td>Questionable species</td><td>Extinct species</td></tr>
<tr><td colspan="6">To tick
✔ Species reported and considered when measuring GES based on CI6
✔ Species reported but not considered when measuring GES based on CI6
✔ Decision should be made species-by-species, based on the available data
✔ Other</td></tr>
<tr><td rowspan="2">Monitoring schemes</td><td>at full national level</td><td>only in specific subregions of the country</td><td colspan="2">only in hotspot areas of the country
to tick
✔ ports
✔ aquaculture units
✔ marine protected areas
✔ other</td><td>NO dedicated monitoring</td></tr>
<tr><td colspan="5">Monitoring efforts include
To tick
✔ the detection of new NIS introductions
✔ the spread of the established and/or invasive NIS
✔ the measurement of abundance/coverage/ biomass of established and/or invasive NIS
✔ the impact of established and/or invasive NIS on the native communities</td></tr>
</table>

8. This report is articulated in 3 parts, namely:

A. Assessment criteria towards preparing the baseline check lists for evaluating CI6;

B. Scales of monitoring and assessment which examines spatial and temporal scales for monitoring;
C. Thresholds values of the IMAP CI6 which examines potential thresholds under different scenarios towards EO2.

A. ASSESSMENT CRITERIA

Scope: Develop the assessment criteria for the IMAP CI6

9. Assessment criteria for preparing validated check lists of NIS to be used for assessing GES include a) taxa (all taxa or excluding phytoplankton, parasites); b) species to be considered in trends indicator (extinct species, cryptogenic species, crypto-expanding, questionable species); c) pathways to be considered (all pathways or excluding unaided expanding species, e.g. Lessepsian immigrants). With regards to the temporal scales of assessment (yearly or every 6 years) it is discussed in section B.

10. The discussions on the assessment criteria are based on the responses to the questionnaires as described earlier. The results are presented in Figure 1 and summarised in Table 3.

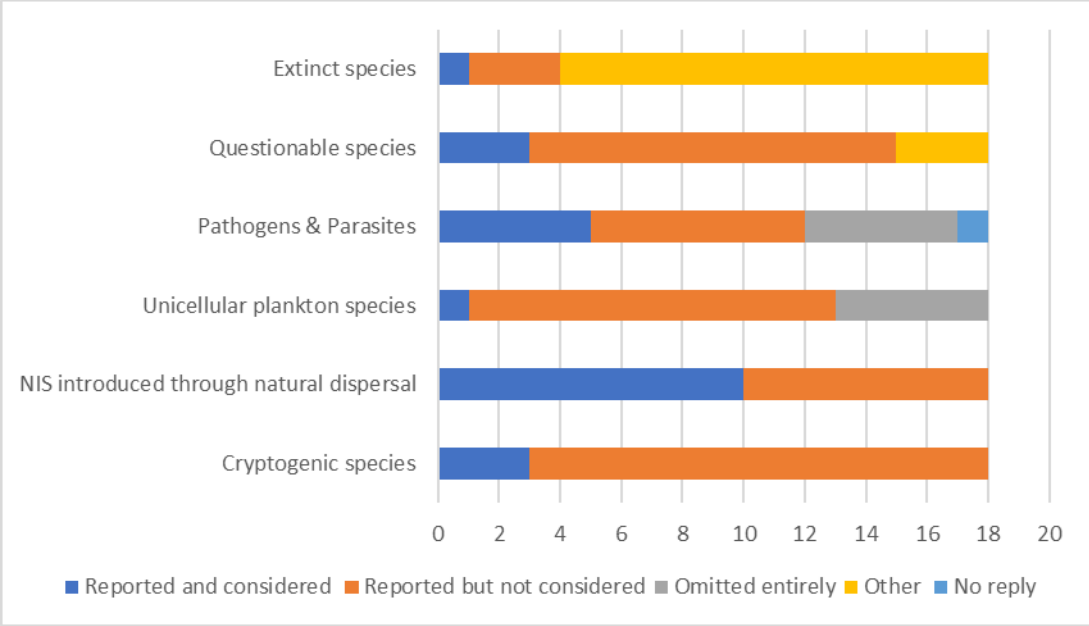

Figure 1: Responses to questionnaires by 18 Mediterranean countries

A.1. Cryptogenic species

11. Species with no definite evidence of their native or introduced status according to Carlton (1996) such is the case for some species witnessed in the old times (e.g. prior to 1800). Characteristic examples include the shipworm Teredo navalis Linnaeus, 1758, one of the earliest invasive species in the Mediterranean and most harmful marine invaders worldwide. It is not clear, whether it invaded Europe from South East Asia or whether it originated in Europe and invaded the rest of the world from there. Often NIS experts disagree on the status of a 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; such is the case of the ragged sea hare Bursatella leachii Blainville, 1817, a well-established species in the Mediterranean that is reported as cryptogenic in Libya and Italy but non-indigenous in Greece and Cyprus. Moreover, the status of cryptogenic can be altered in time, based on new available research data coming into light, thus changing their status. A good example is that of the annelid Chaetozone corona Berkeley & Berkeley, 1941: the species was initially reported as cryptogenic in the Mediterranean Sea (Çinar & Ergen, 2007), but it was later reported from the eastern Atlantic coast as NIS that was introduced by shipping from the East Coast of the USA (Le Garrec et al., 2017). Therefore, the species can be considered as an established NIS in the Mediterranean Sea. Cryptogenic species were not analyzed in Tsiamis et al. (2019) baseline inventories, but they were simply listed in an annex.

12. According to the questionnaires, with the exception of Algeria and Montenegro, the national experts of the CPs to the Barcelona Convention agreed that they should be reported but not considered in assessing CI6 (Figure 1). Israel suggested that they are reported separately from NIS, pending proof (taxonomic identity, status), while Lebanon suggested that in the case where the species has a significant impact, it is better to give an idea of this positive or negative impact.

13. <u>Suggestion</u>. *As the status of cryptogenic species may change in the future to NIS with new data coming to light, they should be included in NIS lists but not considered in assessing GES under CI6 unless proven to be NIS.*

A.2. Crypto-expanding species

14. Crypto-expanding species are those with no definite evidence of their native or non-indigenous status due to unclear mode of introduction from the native range (natural spread or human mediated) (Zenetos et al., 2020a). Such species in the past were classified either as alien with high degree of uncertainty with regards to their mode of introduction, or as cryptogenic or as range expanding. In the case of certain introduction that the origin is known but the pathway is dubious, it is best to assign a species to the crypto-expanding category. The term fits best species of Atlantic origin with a disjunct distribution. A good example is that of the fangtooth moray Enchelycore anatina (Lowe, 1838) that appeared in Israel in the 1970s and has established in the eastern Mediterranean, while it spread to the central Mediterranean in the 2010s but is still absent from the western Mediterranean. Another typical example is that of the nimble spray crab Percnon gibbesi (H. Milne Edwards, 1853), one of the most recent invasive species in the Mediterranean that was hitherto reported as alien. Yet, because of the high uncertainty regarding its introduction pathway (vessels, aquarium escapee, range expansion), Italy and other countries have changed its status to "cryptogenic". Indeed, sensu lato and based on Carlton 1996 the species also falls under the term cryptogenic. However, the term crypto-expanding fits better as it specifies the cause of the cryptogenic uncertainty. There are many other cases of east Atlantic species that due to their rarity, we cannot eliminate the possibility that they have been introduced in recent years by human interference, e.g. the tropical African hermit crab Pagurus mbizi (Forest, 1955) that was reported from the Alboran Sea (García Raso et al., 2014).

15. <u>Suggestion</u>. *The status of crypto-expanding species may change in the future to NIS with new data coming to light and so they should be included in NIS lists but not considered in assessing GES.*

A.3. Range expanding and vagrant species

16. By definition, natural shifts in distribution ranges (e.g. due to climate change or dispersal by ocean currents) do not qualify a species as a NIS. This category concerns:

a) Atlanto-Mediterranean species: There are many species in the Mediterranean NIS check lists, of Atlantic origin, that have expanded their geographic range via natural dispersal; and
b) Vagrant species: The term 'vagrant' has been used for large species belonging to the offshore nekton (mainly perciform fishes, sharks, large cephalopods and marine mammals) recorded occasionally as isolated animals.

17. Essl et al. (2019) proposed the term "neonative" for those taxa that have expanded geographically beyond their native range and that now have established populations, whose presence is due to human-induced changes of the biophysical environment, but not as a result of direct movement by human agency, intentional or unintentional, or to the creation of dispersal corridors such as canals, roads, pipelines, or tunnels.

18. As the term is rather complicated, we suggest the use of the term Range expansion. Range expanding species were initially included in the first annotated list of alien species in the Mediterranean Zenetos et al (2005, 2008) but were subsequently excluded (Zenetos et al., 2012). Table 2 includes 35 taxa classified as range expanding, and six as vagrant species in Zenetos et al. (2012). To these, some additional species are included while 2 species have been re-assigned to other categories. In particular, Fistularia petimba reported from Spain was considered as range expanding until it was discovered in Israel (Stern et al., 2017) where it is considered a Lessepsian immigrant. In contrast, Halavi's guitarfish (Glaucostegus halavi), which was reported from Egypt by Tortonese (1951), is added to the list as a very old record of a vagrant species.

19. In recent publications addressing NIS, range expanding species are listed as introduced and/or newcomers but are not considered as NIS (Evans et al., 2015; Grimes et at., 2018). In the Marine Mediterranean Invasive Alien Species (MAMIAS) Database, range expanding species are included but clearly classified as such.

20. *Suggestions. Range expanding species should not be included in NIS lists for assessing GES.*

Table 2: Range expanding and vagrant species in the Mediterranean. In bold, recent changes

group	Species	Zenetos et al. 2012		
Crustacea	***Cancer bellianus***		range expansion	2012, Spain: Carrido & Pena-Rivas in Mytilineou et al., 2016
Crustacea	*Scaphocalanus amplius*	range expansion	range expansion	
Crustacea	*Scaphocalanus brevirostris*	range expansion	range expansion	
Crustacea	*Scolecithrix valens*	range expansion	range expansion	
Crustacea	*Sphaeroma venustissimum*	range expansion	range expansion	
Crustacea	*Synalpheus tumidomanus africanus*	range expansion	range expansion	
Echinodermata	***Coronaster briareus***		range expansion	2015, Malta: Evans et al, 2016
Echinodermata	***Luidia atlantidea***		range expansion	2013, Spain: Gallardo-Roldan et al., 2015
Elasmobranchii	*Carcharhinus altimus*	vagrant	vagrant	
Elasmobranchii	*Carcharhinus falciformis*	vagrant	vagrant	
Elasmobranchii	*Galeocerdo cuvier*	vagrant	vagrant	
Elasmobranchii	***Glaucostegus halavi***		vagrant	Egypt: Tortonese, 1951
Elasmobranchii	*Isurus paucus*	vagrant	vagrant	
Elasmobranchii	***Mobula japanica***		vagrant	2014, Tunisia: Capape, 2015
Elasmobranchii	*Rhizoprionodon acutus*	vagrant	vagrant	
Elasmobranchii	*Sphyrna mokarran*	vagrant	vagrant	
Macroalgae	*Osmundea oederi*	range expansion	range expansion	
Miscellanea	*Clytia mccradyi*	range expansion	range expansion	
Miscellanea	*Eirene viridula*	range expansion	range expansion	
Osteichthyes	*Acanthurus monroviae*	range expansion	range expansion	
Osteichthyes	*Aluterus monoceros*	range expansion	range expansion	
Osteichthyes	*Anarhichas lupus*	range expansion	range expansion	
Osteichthyes	*Beryx splendens*	range expansion	range expansion	
Osteichthyes	*Cephalopholis taeniops*	range expansion	range expansion	
Osteichthyes	*Dicologlossa hexophthalma*	range expansion	range expansion	
Osteichthyes	*Diodon hystrix*	range expansion	range expansion	
Osteichthyes	*Diplodus bellottii*	range expansion	range expansion	
Osteichthyes	*Enchelycore anatina*	range expansion	Crypto-expanding	
Osteichthyes	*Fistularia petimba*	range expansion	ALIEN	
Osteichthyes	*Gephyroberyx darwini*	range expansion	range expansion	
Osteichthyes	*Gymnammodytes semisquamatus*	range expansion	range expansion	
Osteichthyes	*Halosaurus ovenii*	range expansion	range expansion	
Osteichthyes	*Kyphosus incisor*	range expansion	range expansion	
Osteichthyes	*Microchirus boscanion*	range expansion	range expansion	
Osteichthyes	*Pagellus bellottii*	range expansion	range expansion	
Osteichthyes	*Pisodonophis semicinctus*	range expansion	range expansion	
Osteichthyes	*Scorpaena stephanica*	range expansion	range expansion	
Osteichthyes	*Seriola carpenteri*	range expansion	range expansion	
Osteichthyes	*Seriola fasciata*	range expansion	range expansion	
Osteichthyes	*Seriola rivoliana*	range expansion	range expansion	
Osteichthyes	*Solea senegalensis*	range expansion	range expansion	
Osteichthyes	*Sphoeroides marmoratus*	range expansion	range expansion	
Osteichthyes	*Sphoeroides pachygaster*	range expansion	range expansion	
Osteichthyes	*Synaptura lusitanica*	range expansion	range expansion	
Osteichthyes	*Syngnathus rostellatus*	range expansion	range expansion	
Osteichthyes	***Taractes rubescens***		range expansion	2014, Italy: Fiorentino et al. in Karachle et al., 2016
Osteichthyes	*Trachyscorpia cristulata echinata*	range expansion	range expansion	
Osteichthyes	***Zenopsis conchifer***		range expansion	2004, Tunisia Ben Souissi et al, 2007

A.4. Partly-native species

21. Several species are native in a Mediterranean country while they are non-indigenous in other Mediterranean countries. A typical example is that of the macroalgae *Fucus spiralis* Linnaeus. At the frontiers of its native range (Morocco and southern Spain) it is considered as native (marginal dispersal), but is alien in France (Verlaque et al., 2015). Two molluscan species, are considered as partly alien in the Mediterranean:

- *Gibbula albida* (Gmelin, 1791) has been considered a native species to the Adriatic Sea, but an alien in the western Mediterranean Sea due to recent introductions into the Ebro Delta (Spain) and the French Mediterranean lagoons (see Zenetos et al., 2010). Molecular data is necessary to elucidate whether past and current western Mediterranean distributions of *G. albida* are due to human activities.
- *Siphonaria pectinata* (Linnaeus, 1758) is native to the South Mediterranean from the Strait of Gibraltar, the African coastline up to Algeria and the Spanish coastline up to Murcia/Valencia area. While the species was considered as alien in Croatia and Greece, the known historical range of *S. pectinata* sensu stricto in the Mediterranean basin is unclear and widely debated (Crocetta, 2016). However, in the absence of past sightings, there is a general agreement in considering that the Greek and Croatian records are the result of a human induced introduction.

22. Other species falling into this category are zooplanktonic species such as the colonial jellyfish *Muggiaea atlantica* (Cunningham, 1892). Since the mid-1980s, *M. atlantica* has progressively colonized the Western Mediterranean (Riera et al.,1986) and Adriatic where it was initially considered as alien (Kršinic & Njire, 2001); However, its presence is probably in response to hydrological variability that occurred under the forcing of large-scale climate oscillations (Licandro et al., 2012).

23. Suggestion: *Partly native NIS should be reported under CI6 but be considered case by case when measuring GES at the subregional scale.*

A5. NIS introduced through natural dispersal / Lessepsian species

24. For most species introduced via the Suez Canal, there is some uncertainty as to the vector of their introduction in the Mediterranean. We call Lessepsian those Red Sea species that have invaded the Mediterranean. In the first area, they were detected / reported the assigned pathway to them is Corridor. When they spread to neighbouring countries / seas, the most appropriate pathway is "unaided". This applies well to fishes. However, in many cases there is no evidence that the species is exclusively transferred unaided and not through human-mediated activities, such as shipping (Palialexis et al. 2014).

25. At pan-European scale, Tsiamis et al. (2021b) suggested that these NIS should be reported in D2C1 application. However, there was a debate if these NIS should be also considered when measuring GES based on D2C1. This debate is more intense within Mediterranean countries (Figure 1). Apart from Cyprus, all Levantine countries suggested that they are included and considered, arguing that they are NIS and require management as such.

26. Suggestion*: Unaided NIS should be handled case-by-case for CI6 based on pathways certainty, availability of data, and the impact caused by them. For example, for fish species that are exclusively*

transferred unaided (true Lessepsian immigrants), such data should be omitted. However, NIS that are included in the Union concern list of the EU Invasive Alien Species Regulation 1143/2014 such as the striped eel catfish Plotosus lineatus ((Thunberg, 1787) and the pufferfish Lagocephalus sceleratus (Gmelin, 1789) (candidate for inclusion in 2021), must be reported and considered for GES in CI6 assessments. It was further suggested that a list of Lessepsian fish among the invasive ones with documented considerable impact on biodiversity be prepared and agreed by the countries for inclusion in assessing GES.

6. Unicellular plankton species

27. The introduction of marine microalgae in the Mediterranean Sea is hard to document. The list of Indo-Pacific taxa in the Mediterranean (Lakkis & Zeidane, 2004) is full of dubious or poorly known species. Zenetos et al (2005) compiled an extensive list of phytoplanktonic species (alien, cryptogenic and questionable) which in subsequent updates was removed (Zenetos et al., 2010). Phytoplankton invasions go totally unobserved in the case of rare species, which are a conspicuous part of the phytoplankton biodiversity in all seas. In addition, to prove that a species is an alien requires very sound background knowledge of the species of a given area. Unfortunately, the diversity of marine microalgae is scarcely known in wide areas of the Mediterranean Sea, e.g. the southern shores, where only a few sites have been investigated, or the offshore waters, where studies are limited to occasional sampling during cruises. Even in the northern Mediterranean waters the knowledge of the distribution of these unicellular organisms in a given area is far from being exhaustive (Zenetos et al., 2010).

28. Most of the recent checklists on Mediterranean NIS have excluded unicellular taxa (Zenetos et al., 2017; Galil et al, 2018) because the origin of many unicellular taxa is in doubt and subject to revisions. Recently, Gomez (2019) argued that most diatoms and dinoflagellates reported in the literature as NIS are in fact examples of marginal dispersal associated with climatic events instead of species introductions from remote areas. He concluded that the number of non-indigenous phytoplankton species in European Seas has been excessively inflated.

29. In response to the questionnaire, five countries proposed omission of unicellular plankton species until molecular-based evidence clarifies taxonomic and biogeographical identity. Apart from Turkey that suggested full consideration of phytoplankton, all other countries proposed that they are reported but not considered in assessing GES (Figure 1).

30. Suggestion: *It is proposed that unicellular plankton NIS should be treated with caution (e.g. flagged with high uncertainty) until further research clarifies their enigmatic status. Therefore, their consideration in assessing GES should be decided on a case-by-case basis.*

A.7. Pathogens and parasites

31. Pathogens and parasites have been included in Mediterranean NIS lists both at basin level (Zenetos et al., 2008) and at country level (e.g. Libya: Shakman et al., 2019; Tunisia: Ounifi-Ben Amor et al., 2016; Israel: Galil et al., 2020). The Aquatic Animal Health Directive (2006/88/EC; EU, 2006) covers pathogens and parasites on marine farmed animals, but in the Mediterranean and in particular the eastern and central subregions, the vast majority of the alien parasites are platyhelminthes, all reported as fish parasites, that have co-invaded the Mediterranean through the Suez Canal on Red Sea immigrant hosts. Parasitic NIS may have a substantially high impact on the native communities. El-Rashidy & Boxshall (2009) provided evidence of alien parasites switching to native hosts.

32. The responses to the questionnaires varied (Figure 1) but the majority suggested omission or inclusion in the list but not to be considered in measuring GES. Five countries (EL, TR, IL, LY, AL) suggested that they are included and considered. Israel argued that parasites are important ecologically and economically and as such they ought to be reported.

33. Suggestion*: Parasites and pests NIS should be reported under CI6, but considered when measuring GES case by case - excluding parasites and pathogens that fall under the Animal Health Directive, e.g. those transferred with oysters, mussels.*

A.8. Questionable species

34. Questionable species are those species with unresolved taxonomic status: species complexes, or non-validated NIS entries coming from citizen-science, or records not supported by morphological studies and lack reference material, and which in most cases are likely to be misidentifications of native species; or records showing discrepancies in morphology and/or ecology that might suggest the occurrence of an overlooked undescribed native species. Many polychaete species fall in this category. Questionable species were not further analyzed in Tsiamis et al. (2019) baseline, but they were simply listed in an annex.

35. Questionable records are included in MAMIAS and in many Mediterranean NIS checklists (Langeneck et al., 2020; Stulpinaite et al., 2020). According to Tsiamis et al. (2021b), there was a unanimous agreement to report questionable species, but not consider them when measuring GES. Greece, Cyprus and Algeria suggested inclusion, but the majority of the national experts suggested that they should be listed but not considered until their status is resolved, or omitted from NIS lists (Figure 1). Lebanon suggested that in the case where the non-indigenous species has a significant impact, it is better to give an idea of this positive or negative impact.

36. Suggestion*: As the status of questionable species may change in the future to NIS with new data coming to light, they should be included in NIS lists but not considered in assessing GES until the status of a particular species is fully resolved.*

A.9. Extinct species

37. In the Mediterranean Science Commission (CIESM) atlas series, alien species recorded before 1920 (of Indo-Pacific origin) or 1950 (of Tropical Atlantic origin) were excluded as extinct. In an ongoing review, any species reported only once before 1970 is removed from NIS lists. Moreover, for mollusca, any record based on empty shells reported only once before 2010 is excluded (Zenetos et al. in preparation). However, all extinct and excluded species are marked as such with low confidence level.

38. Tsiamis et al (2021b) agreed that these species should be investigated in terms of: a) dates of old records, b) continuity of records, c) size of the species, d) difficulty on taxonomic identification, e) area's conditions and characteristics, f) monitoring effort and its continuity, and g) possible pathway of introduction, e.g. very old records of species released from aquaria should be excluded.

39. According to the questionnaire responses, most countries (14/18) suggested that the decision should be made species by species depending on taxon, research effort, regional data, etc.

40. Suggestion*: In agreement with Tsiamis et al (2021b), the majority of the national experts proposed to include such species in the reports, on a case by case based on the available data* (Figure 1).

A.10. Freshwater species

41. In the first EU baseline inventory for D2 (Tsiamis et al., 2019), freshwater species were not considered although they were included by several MSs when these species have been also found in their coastal waters. Examples of freshwater species reported from Mediterranean lagoons are the Chinese mitten crab *Eriocheir sinensis* H. Milne Edwards, 1853, the red swamp crayfish *Procambarus clarkii* (Girard, 1852) and the Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758). Although these species live in freshwater ecosystems, they can withstand brackish waters and inhabit estuarine habitats. *Eriocheir sinensis*, which was reported from France and Italy, was to date missing from marine aliens check lists until it reappeared in the Adriatic (Crocetta et al., 2020). An undetected population already thriving in the area is suspected, as the Adriatic Sea could be a new perfect house for this invader. *Procambarus clarkii*, which is included along with *E. sinensis* in the list of species of Union concern pursuant to Regulation (EUR-lex, 2016), is present in a Mediterranean coastal lagoon in the Albufera Natural Park, Valencia, Spain since 1976 and continues to be present for four decades (Martín-Torrijos et al., 2021). *Oreochromis niloticus* is present in the coastal lagoon of Italy (Azzurro & Cerri, 2021) and Turkey (Innal, 2020).

42. Suggestion. *CI6 assessments should include all NIS found regardless of their marine/freshwater status provided they are found in coastal systems of the country.*

Table 3:Summary of responses to the questionnaire

Species categories	**To be reported**	**To be considered for the assessment**
Cryptogenic	YES	NO
Crypto-expanding	YES	NO
Range-expanding	NO	NO
Partly native	YES	Per case
NIS introduced through natural dispersal	Case by case	Per case
Questionable	YES	NO
Unicellular marine algae	YES	Per case
Parasites	YES	Per case

Extinct species	Case by case	Per case
Freshwater	YES	Per case

43. From the above categories, it is suggested that **partly native species, NIS introduced through natural dispersal, unicellular marine algae, parasites, extinct and freshwater species** should be considered in CI6 assessments on a case-by-case basis.

B. SCALES OF MONITORING & ASSESSMENT

Scope:

- **Revise the existing scale of monitoring and further work on developing adequate scales of monitoring for the IMAP CI related to NIS**
- **Develop scale of assessment**

B1. Geographical unit for implementation of CI6

44. Assessment of threshold values based on the trends indicator (CI6) calculated to date can be achieved at the basin and country level, although it is more meaningful to be assessed at the national part of a subregion, e.g **Greece:** EMED, CMED, ADRIA; **Italy:** WMED, CMED, ADRIA, **Tunisia**: WMED, CMED. For borders between subregions see Figures 2 ~~and 3~~.

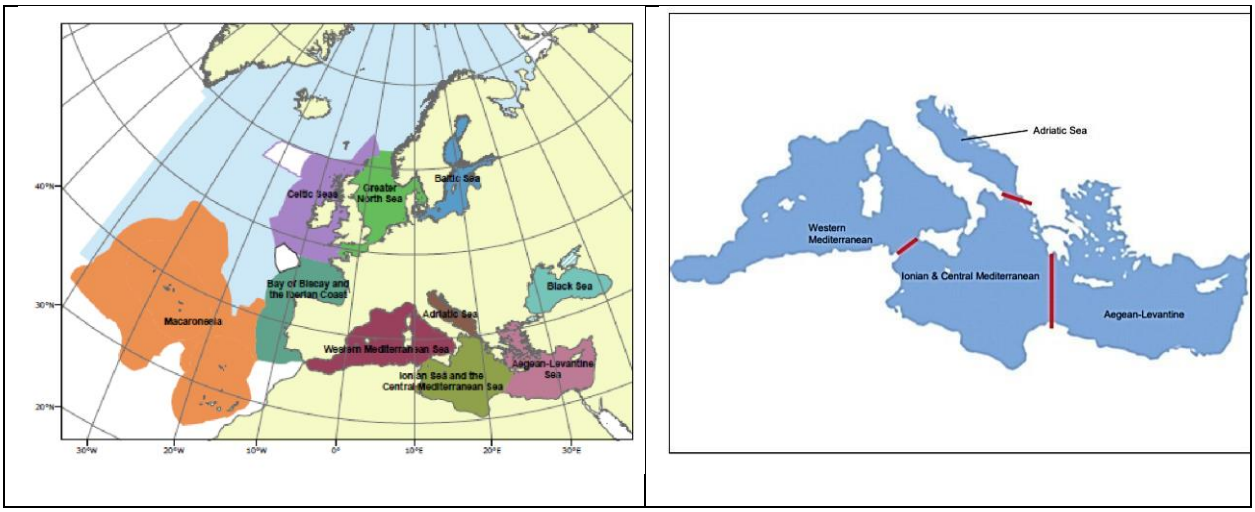

Figure 2. left: Representation of the marine regions and subregions of MSFD Article 4. (from Jensen et al., 2017) -right: EcAp subregions for the Mediterranean adopted by the CPs of the Barcelona Convention (Decision IG.20/4, 2012)[1]

45. Suggestion*: The geographic borders of the Mediterranean EcAp subregions be fully harmonized with those proposed by EU countries and adopted by the European Environment Agency (Jensen et al., 2017). (Figures 2 ~~and 3~~).*

B.2. Monitoring of marine NIS

46. The monitoring of NIS generally should start on a localized scale, such as risk areas and "steppingstone areas" for non-indigenous species introductions. Such areas include ports and their surrounding areas, docks, marinas, aquaculture installations, heated power plant effluents sites, offshore

[1] Disclaimer: The designations employed and the presentation of material in this publication do not imply the expression of any opinion whatsoever on the part of the Secretariat of the United Nations concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries."

structures. Areas of special interest such as marine protected areas, lagoons etc. may be selected on a case-by-case basis, depending on the proximity to non-indigenous species introduction risk areas . The selection of the monitoring sites should therefore be based on a previous analysis of the most likely "entry" points of introductions and risk areas expected to contain elevated numbers of alien species. (Integrated Monitoring and Assessment Programme of the Mediterranean Sea and Coast and Related Assessment Criteria, UN Environment/MAP Athens, Greece,2017).

47. With the application of the risk-based approach, it is possible to obtain an overview of the NIS present at a large spatial scope while only monitoring a relatively small number of locations. While Rapid assessment protocols (Pedersen et al., 2003; Ashton et al., 2006) target all fouling macroinvertebrate taxa, "rapid assessment surveys" target a predefined list of species, involve an onsite team of experts, and generally last an hour (Katsanevakis et al., 2011). As the most effective monitoring method, a Rapid Assessment Survey (RAS) is suggested to be carried out in risk areas (e.g. ports and their surrounding areas, docks, marinas, aquaculture installations, heated power plant effluents sites, offshore structures).

48. The IMAP Common Indicator Guidance Factsheets (Biodiversity and Fisheries) propose Monitoring at "hot-spots" and "steppingstone areas" for NIS introductions would typically involve more intense monitoring effort, e.g. sampling at least once a year at ports and their wider area and once every two years in smaller harbours, marinas, and aquaculture sites.

49. For the estimation of Common Indicator 6, it is important that the same sites are surveyed each monitoring period, otherwise the estimation of the trend might be biased by differences among sites. Standard monitoring methods traditionally being used for marine biological surveys, including, but not limited, to plankton, benthic and fouling studies described in relevant guidelines and manuals are suggested for studying NIS.

50. At basin scale, monitoring protocols of the IMAP CI6 on NIS in the Mediterranean were developed and endorsed by the CPs to the Barcelona Convention in 2019 under the framework of the EcAp/IMAP process (UNEP/MED WG.467/16 (2019) "Monitoring Protocols for IMAP Common Indicators related to Biodiversity and Non-Indigenous species").

51. In some EU Mediterranean countries, monitoring protocols are used in implementing EU policies such as the Ballast Water Management Convention, the EU Water Framework Directive, and the Marine Strategy Framework Directive. These methods may be useful for the estimation of CI 6. The EU Project BALMAS has provided guidelines for the monitoring of NIS in ballast water (David & Gollasch, 2015). An international standardized monitoring protocol for sessile fouling species, developed by the Smithsonian Environmental Research Center (SERC), the SERC protocol, was employed for the first time in La Spezia, Mediterranean Sea (Tamburini et al., 2019). The second Summer School on "Monitoring marine alien species in ports with the SERC protocol", organized by the University of Pavia (Italy) and the Smithsonian Environmental Research Center (USA), has been scheduled in Pavia (Italy), June28-July 2,2021 (http://aliensummerschool.unipv.it).

52. The compilation of citizen scientists' input, validated by taxonomic experts, can be useful to assess the geographical ranges of established species or to early record of new species. Recent developments in citizen science (CS) provide an opportunity to improve data flow and knowledge on NIS. At the same time advances in technology, particularly on-line recording and smartphone apps, along with the development of social media (Table 4), have increased connectivity while new and innovative analysis techniques are emerging to ensure appropriate management, visualization, interpretation and use and sharing of the data (Roy et al., 2018).

Table 4. Citizen Science networks/ FaceBook groups in the Mediterranean, focusing / including NIS, that are active at county or basin level.

Citizen science name	Manager	Geographic coverage	Link
Oddfish	FB group	Mediterranean	https://www.facebook.com/groups/1714585748824288/
Is it Alien to you? Share it!!!	NGO	Greece and Cyprus	https://www.facebook.com/groups/104915386661854/
Mediterranean Marine Life	FB group	Mediterranean	https://www.facebook.com/groups/396314800533875/
البحر اللبناني—Sea Lebanon	FB group	Lebanon	https://www.facebook.com/groups/109615625861815/
Marine Life and Biodiversity in	FB group	Lebanon	https://www.facebook.com/groups/351425191625456/
Invasive Species in Albanian Coast	NGO	Albania	https://www.facebook.com/groups/1377118565724588/
AlienFish	NGO	Italy	https://www.facebook.com/alienfish/?ref=br_rs
Marine Biology in Libya	NGO	Libya	https://www.facebook.com/MarineBiologyinlibya/
Aliens in the Sea	Project	Italy	https://www.facebook.com/Progetto-Aliens-in-the-sea-
Spot the Alien	FB group	Malta	https://www.facebook.com/aliensmalta/
Ellenic Network on Aquatic	Network	Greece	https://elnais.hcmr.gr/
Seawatchers	Web Based Platform	Mediterranean	https://www.observadoresdelmar.es/
MedMIS	IUCN	Mediterranean	http://www.iucn-medmis.org/?c=About/show
Opisthobranchia	Network	Mediterranean	https://opistobranquis.info/en/
Hellenic Conches	Malacologists	Greece	https://www.facebook.com/groups/helleniconches/
i-naturalist	Web Based Platform	Global	https://www.inaturalist.org/

53. The monitoring on marine NIS differs across Mediterranean countries. According to questionnaires, to date, only one Mediterranean country has a monitoring scheme on marine NIS applied at fully national level (Table 5), while five countries have no monitoring running or at least not implemented yet. In Algeria for example, the network of observing areas and sampling stations has been identified in the

Algerian monitoring programme but not implemented yet. In Tunisia, the Ministry of Environment had established "The strategy and an action plan for the prevention, management and control of invasive alien species in Tunisia" since 2018, but the implementation may take some time. However, individual initiatives are conducted in hotspot areas (lagoons, ports, marinas and MPA's).

54. On the other hand, the majority of countries do not have a dedicated strategy but have a monitoring strategy including marine NIS applied either at risk areas of the country or in specific subregions through a related network of sampling stations, e.g. Saronikos Gulf in Greece. Hot-spot areas for NIS monitoring include mainly ports and marine protected areas (Table 6). NIS related data in the majority of countries, where no monitoring is in place, come mainly from various research projects. In EU countries, NIS data is derived from monitoring under the WFD and/or the MSFD.

Table 5. Monitoring strategy on marine NIS in the Mediterranean Sea

	CY	EL	ES	FR	IT	HR	MT	SI	EG	LY	LB	DZ	MA	IL	TN	AL	ME	TR
NIS monitoring at full national level through a network of sampling stations						1												
NIS monitoring only at specific subregions of the country through a network of sampling stations										1	1							
NIS monitoring only in hot-spot areas of the country	1	1	1	1	1		1	1	1			1						1
NO dedicated monitoring on NIS exists													1	1	1	1	1	

Table 6. Hot-spot areas for marine NIS monitoring in the Mediterranean Sea. (NA= no monitoring)

	CY	EL	ES	FR	IT	HR	MT	SI	EG	LY	LB	DZ	MA	IL	TN	AL	ME	TR
Ports		1	1	1	1		1	1	1	1		1						
Aquaculture units				1	1			1										
Marine protected areas	1		1				1		1	1		1						1
Other			1					1	1			1	NA	NA	NA	NA	NA	

55. Targets 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).

56. Many countries study NIS (coverage, impact) through the study of specific habitats, e.g. Morocco under the monitoring of some key habitats such as coralligenous and seagrass beds; Tunisia by monitoring algae and phanerogams and lately fauna. Studies are often conducted in the framework of MSc and PhD theses.

Figure 3. Targets of marine NIS monitoring in the Mediterranean as reported by the countries. Israel and Montenegro did not reply as they stated there is no monitoring in place To be revised with new ME data

B3. Assessment period for CI6 implementation

57. Based on EU (2017) assessment criteria for D2, the assessment period covers a 6-years period measured from the reference year as reported for the initial assessment (2011, reported in 2012). However, not all EU countries reported in 2012 for the 2006-2011 period; nor in 2018 for the 2012-2017 period (Tsiamis et al., 2021a). Considering the time lags in reporting NIS, which vary a lot (Figure 4) among Mediterranean countries and taxonomic groups (Zenetos et al., 2019), a baseline for IMAP CI6 should be covered sufficiently (be representative of the NIS status by **2017).** EU MSs have agreed that the next assessment should cover the 2018-2023 period. For consistency and harmonization reasons, the assessment period of CI6 should be the same across Mediterranean countries.

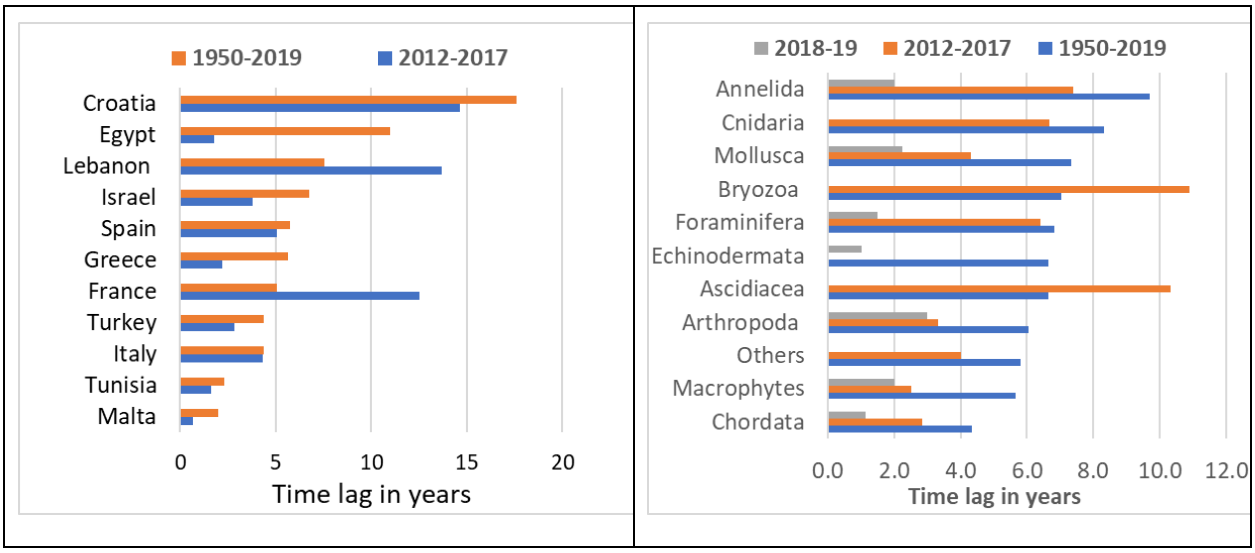

Figure 4. Average time lags in reporting of NIS in association with the country (left) of their first collection in the Mediterranean and their main taxonomic groups (right). Source: Zenetos et al., 2019

58. CI6 assessments are missing from non-EU Mediterranean countries but trends in new introductions can be deducted from recent publications [Algeria (Grimes et al., 2018; Bensari et al., 2020; Bakalem et al., 2020, Libya (Shakman et al., 2019); Montenegro (Petović et al., 2019; Pešić et al., 2020); Israel (Galil et al., 2020)], and updates. Figure 5 depicts the cumulative number of NIS in Libya and Algeria, while Figure 6 shows the trends in new NIS as required by CI6.

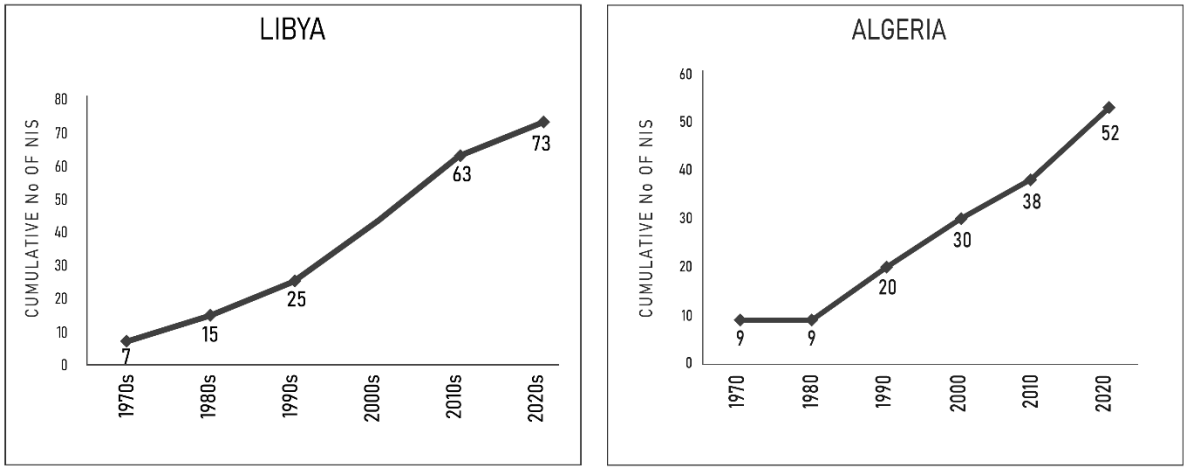

Figure 5. Cumulative trend in NIS reported for Libya (Shakman et al., 2019) and Algeria (Grimes et al., 2018; Bensari et al., 2020; Bakalem et al., 2020).

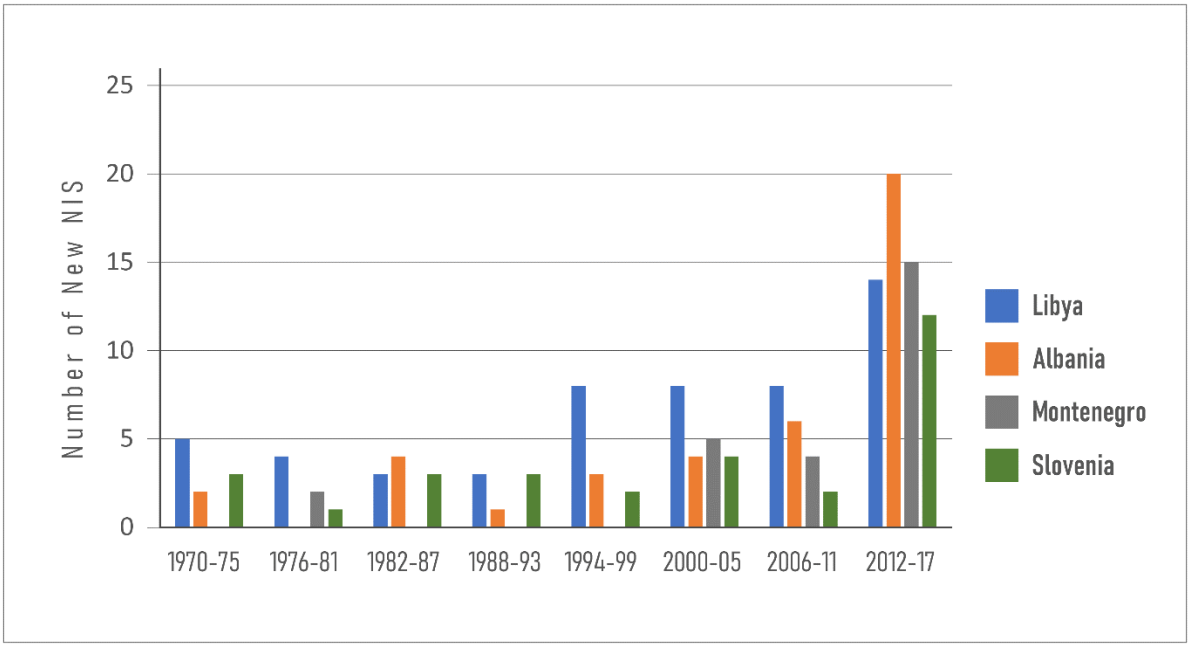

Figure 6. Trends in new marine NIS per 6 year since 1970 (source: HCMR database)

59. The high number of new NIS in all countries is clearly the result of increased scientific effort. In Slovenia, Montenegro and Albania for example, approximately half of NIS detected until 2017 resulted from the BALMAS project (Ballast water management system for Adriatic Sea protection) that run in the period November 2013 – March 2016 (Petović et al., 2019; Spagnolo et al., 2019). On the other hand, recent research in Tripoli harbour (Libya) and the contribution of citizen scientists (Mannino et al., 2021) has revealed more than 13 new NIS in the last 3 years (2018-2020) some of which, such as gastropods *Cerithium scabridum* Philippi, 1848 and *Diodora ruppellii* (G.B. Sowerby, 1835) are among the older Mediterranean invaders (known since 1883 and 1939 respectively) that were presumably undetected (Rizgalla et al., 2019a,b). Therefore, for some countries **even 2017 as the reference year, appears to be premature**.

60. Considering all pathways, it is clear that the rate of new introductions differs significantly among subregions and is increasing with time (Zenetos et al., 2012). However, as reported by Zenetos (2019), this increase does not necessarily imply increasing introduction but rather increasing scientific effort.

61. Suggestion: *For harmonization of assessments between EU and non-EU countries, it is proposed to keep the main assessing periods as proposed for EU (Tsiamis et al., 2021b) but take 2017 as baseline (reference year).*

Summarizing Indicator: Number of New NIS

Scale of monitoring and assessment	
Geographic	Country and subregional revel
Reference year	At least 2017 as baseline
Frequency of reporting	Every six years

C. THRESHOLDS VALUES OF THE IMAP EO2/CI6 "NON-INDIGENOUS SPECIES"

Scope: Develop the thresholds values for IMAP CI related to NIS

62. In order to define threshold values, validated check lists of NIS are needed. EU has prepared such validated lists considering all the aforementioned criteria as much as possible (Tsiamis et al., 2019; 2021b). The information on dates of first introductions and pathways of NIS can be used for establishing thresholds for D2/CI6 by analyzing time trends of new NIS introductions. At IMAP level, baseline lists validated by local experts are under preparation. In preparing these lists both published and grey literature were considered.

63. According to the Commission Decision (EU) 2017/848 of 17 May 2017 "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 **minimized and where possible reduced to zero**". Moreover "Member States shall **establish the threshold value** for the number of new introductions of non-indigenous species, through regional or subregional cooperation".

64. As stated by Tsiamis et al. (2021b), for establishing the threshold values, the percentage reduction of new NIS can be used. 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. 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 the 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.

65. At Mediterranean level, according to the description of IMAP CI6 'Trend in spatial distribution' is defined as the interannual change of the total marine 'area' occupied by a non-indigenous species. To estimate Common Indicator 6, a trend analysis (time series analysis) of the available monitoring data needs to be performed, aiming to extract the underlying pattern, which may be hidden by noise. A formal regression analysis is the recommended approach to estimate such trends. This can be done by a simple linear regression analysis or by more complicated modelling tools (when rich datasets are available), such as generalized linear or additive models.

66. The Indicator units were defined in the Guidance factsheet of CI6 as follow:

- 'Trends in abundance': absolute value and % change per assessment period
- 'Trends in temporal occurrence': number and % change in new introductions or number and % change in the total number of alien species per assessment period
- 'Trends in spatial distribution': absolute value and % change in the total marine surface area occupied or absolute value and % change in the length of the occupied coastline (in the case of shallow-water species that are present only in the coastal zone)

67. Time trends analyses can support establishing suitable thresholds for CI6 per marine subregion. The number of new NIS at subregional scale in the Mediterranean after 1970 is presented in Table 7. At first sight the highest number of NIS were detected in the 2000-2005 period. The period 2018-2020 was not considered in the analyses as the time lags between detecting and reporting a new NIS may skew the true invasion pattern (Zenetos et al., 2019).

Table 7. Number of New NIS in the EcAp subregions after 1970 (cryptogenic, crypto-expanding, questionable species, Parasites and Lessepsian fish excluded). Note: the figures are provisional. They need to be updated after validation of the national checklists (work in progress in the framework of the elaboration of a baseline of NIS in the Mediterranean)

	WMED	CMED	ADRIA	EMED
1970-75	11	13	6	25
1976-81	32	15	8	21
1982-87	29	8	10	29
1988-93	23	18	13	44
1994-99	27	18	17	74
2000-05	**37**	**30**	**26**	**78**
2006-11	30	31	33	57
2012-17	39	53	31	71
2018-20	8	9	6	31

68. As a first step, a linear regression analysis was performed for the period 2000 to 2020 at basin level (Figure 7). However, the results are inconclusive.

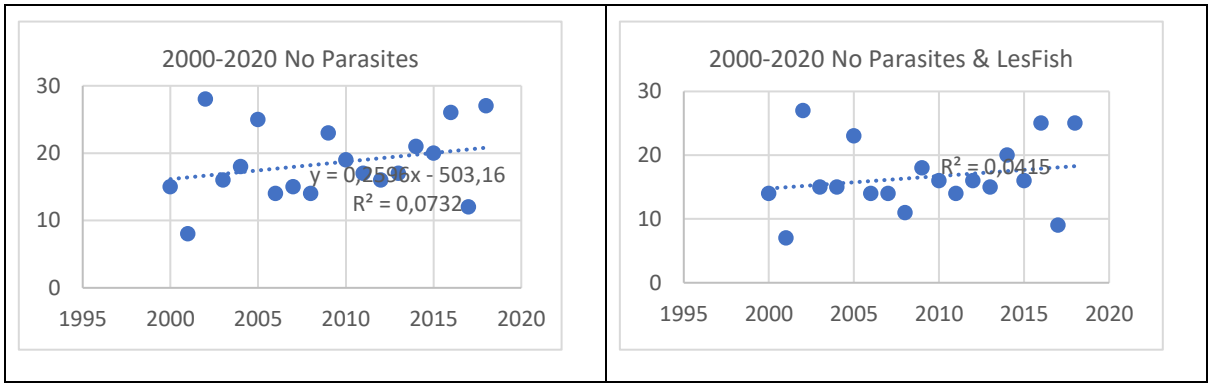

Figure 7. Number of NIS introduced (no cryptogenic, crypto-expanding, questionable species) in the Mediterranean yearly: left: excluding all parasites, right=excluding parasites and Lessepsian fish

69. Regression analysis of trends per subregion (Figure 8) depicts the variation in the rate of new NIS introductions, which ranges from 2.54 species per 6-year period in the WMED to 8.08 species per 6-year period in the EMED. A linear fit was deemed statistically acceptable based on a number of diagnostics (residual errors are normally distributed according to the Anderson-Darling test and independent according to the Durbin-Watson test), however there is still the indication of a non-linear pattern, both in the data used for the regression and in the residuals' plots. Nevertheless, the linear fit is provided as a first indication of the rate of new NIS introductions per EcAp subregion and how these rates differ between areas. Further analysis with a richer dataset is required to better elucidate these patterns.

Figure 8. Trend in NIS introductions per 6-year cycle at EcAp subregions level (data in Table 7)

70. Analysis of Variance was performed on the yearly average number of new introductions to compare the values by ~~MSFD~~ EcAp area before and after the year 2000. The choice to split the data set at that particular point in time was made based on a first visual inspection of the data which indicates that an increase in the number of new introductions took place (or was reported in the literature) between 1994 and 2005.

71. The analysis was also repeated between two different time periods (1970-1993 and 1994-2017 – not shown here) with an equal number of observations per period, it resulted however in a much larger, and significant, interaction term between area and time (MS=11.529, F=4.99, p=0.008), due to the different behaviour of the response variable between the west and the east Mediterranean.

72. The analysis was performed on the raw (untransformed data) as they met the statistical requirements of normality and homogeneity of variance. Both the factors subregion (WMED, CMED, ADRIA, EMED) and time period were significant (see Table 8) but there was no significant interaction between the two factors, meaning that the number of new NIS varied in a similar way before and after 2000 for all the ~~MSFD~~ EcAp areas. More specifically, the analysis demonstrates that there is a significant increase in the rate of new NIS entering all EcAp subregions after 2000 and that this parameter is significantly different between EcAp subregions. Consequently, **the threshold values for CI6 in the Mediterranean need to be set at subregional level and not at regional level.**

Table 8. Results of the Analysis of Variance with yearly average of new NIS introductions per 6-year period as the response and EcAp subregions & Time period as the fixed factors. The levels of the two

factors were a) for EcAp subregions: WMED, CMED, ADRIA, EMED and b) for Time: before 2000 (five 6-year periods, i.e. 1970-75, 1976-81, 1982-87, 1988-93, 1994-99) and after 2000 (three 6-year periods, i.e. 2000-05, 2006-11, 2012-17).

Source	df	Ads SS	Adj MS	F value	p value
Ecap subregions	3	223.02	77.764	23.81	<0.001
Time	1	137.42	137.42	42.12	<0.001
EcAp subregions *Time	3	16.65	5.552	1.7	0.193
Error	24	78.3	3.262		
Total	31	455.49			

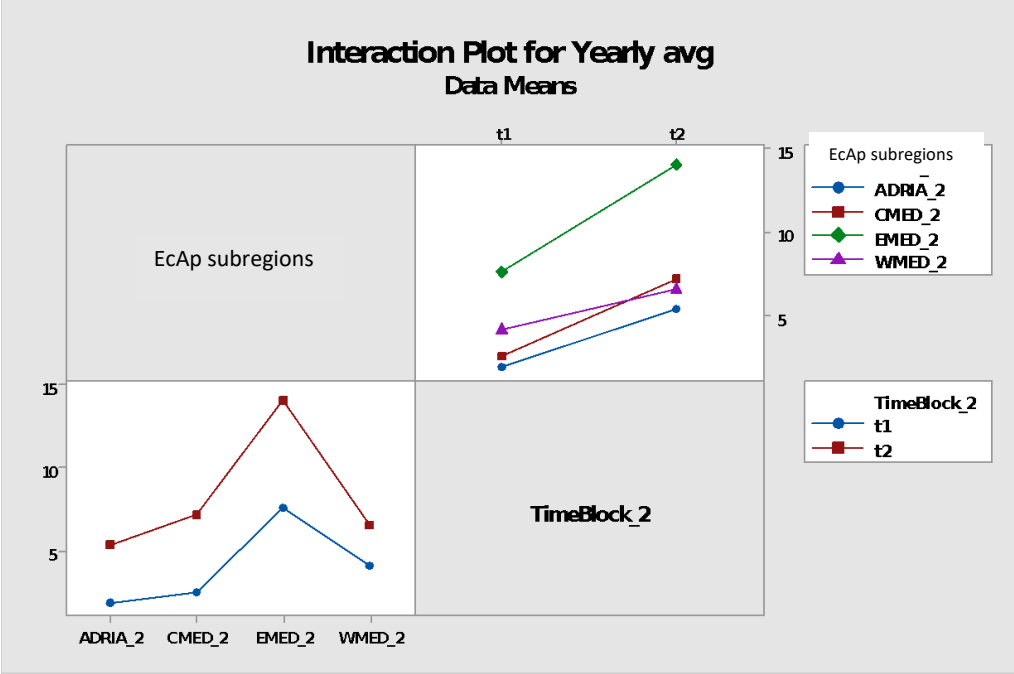

Figure 9. Interaction plot illustrating the main effects of the two separate factors (EcAp subregions and Time period) and the lack of an interaction between them.

73. The data was also analysed separately per EcAp subregion, with one-way ANOVA and time as the single factor (levels as above). In all EcA subregions, there is a clear increase in the rate of new NIS introductions after 2000, which was statistically significant in every subregion (see Table 9). This is graphically illustrated in Figure 11, where it can be seen that the number of new NIS entering/being reported per year at the sub-regional level after 2000 has roughly doubled for 3 out of 4 subregions, compared with before 2000, and is 1.5 times higher in the West Mediterranean.

74. In conclusion, we need to consider data only after 2000s in order to establish today's threshold values.

Table 9. Summary of results for the separate one-way ANOVAs for each EcAp subregions, comparing the yearly average number of new NIS introductions before and after the year 2000.

AREA	DF	F	p	R-sq
WMED	1	7.93	0.003	56.9
CMED	1	16.8	0.006	73.7
ADRIA	1	43.5	0.001	87.9
EMED	1	9.1	0.024	60.2

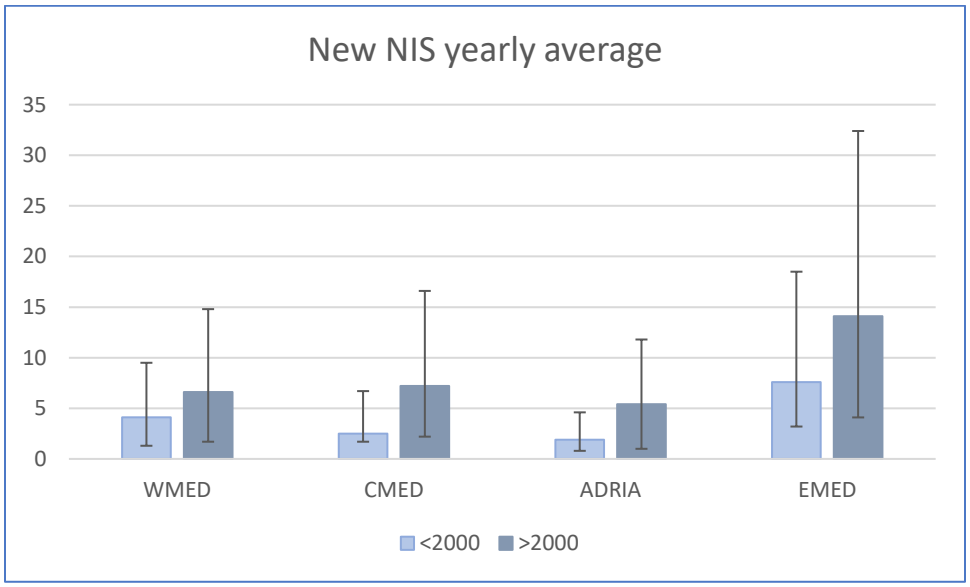

Figure 10. Yearly average number of new NIS introductions per EcAp subregion before and after the year 2000. Error bars represent 95% Confidence Intervals.

75. Trends in new NIS on 6-years cycle at national level against trends at sub-regional level were tested (Table 10) for three countries in each basin.

- ✔ For the WMED, trends were based on data provided for: Algeria (Grimes et al., 2018; Bensari et al., 2020); Morocco (MAMIAS database), and western Italy (Servello et al., 2019; Tsiamis et al., 2021b).
- ✔ For the Central Mediterranean, South Tunisia (Sghaier et al., 2017; Ounifi-Ben Amor et al., 2016; Chebaane et al., 2019); Malta (Evans et al, 2015; Tsiamis et al., 2021) and Libya (Shakman et al., 2019; Rizgalla et al., 2019a,b).

- ✔ For the Adriatic, Slovenia (Tsiamis et al., 2021), Albania (GEF ADRIATIC PROJECT) and Montenegro (Petović et al., 2019; Pešić et al., 2020)
- ✔ For the Eastern Mediterranean, Cyprus (Tsiamis et al., 2021), Greece (Zenetos et al., 2020b) and Israel (Galil et al., 2020)

76. Data in the aforementioned countries were cleaned for cryptogenic, crypto-expanding, range expanding and questionable species. Parasites, oligohaline species were not considered.

Table 10. Year Average (Yravg) of new NIS at subregional and country/region level.

Basin scale Yravg >2000	WMED=6.6	CMED=7.2	ADRIA=5.4	EMED=14.1
Country level	Algeria=0.8 Morocco=0.4 W. Italy =4.1	S. Tunisia=3.8 Malta=2.9 Libya=1.9	Slovenia=1 Montenegro=1.3 Albania=1.7	Israel=9.4 Cyprus=4.1 E. Greece=4.9

77. On looking at Table 10, it is clear that, even excluding the influx of Lessepsian NIS into the Mediterranean, which is considered a major threat for the basin, the yearly number of new NIS per country is by far lower than the average value calculated at basin scale. This would lead to the assumption that the Mediterranean coastal areas have a good GES, based on NIS. However, this assumption is contradicting the increasing trend observed in figure 8. Any trends observed are an artefact affected by a monitoring bias, which appears to be the main factor influencing the number of new NIS introductions reported both by EU and Non-EU Mediterranean countries. This was highlighted for EU countries (Zenetos, 2019; Servello et al., 2019; Zenetos et al., 2020b) but is even more evident in non-EU countries where recent research projects have attributed to a burst of new NIS, e.g. the BALMAS and GEF Adriatic Projects for Montenegro and Albania.

78. Tsiamis et al. (2021b) 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, and b) the number of new NIS introductions is defined based on a specific percentage reduction of new NIS compared to the average number of new NIS introductions that occurred in the previous 6-years cycle periods.

79. 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.

80. For the Mediterranean, some threshold values are only indicative.

81. For Mediterranean region/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, e.g. 3 years. Moreover, dedicated monitoring of marine NIS should be established and be constant in space, time and

across taxonomic groups. Prioritization should be given to hot-spot areas of new NIS introductions, such as ports, aquaculture units and marine protected areas. This should be a prerequisite for applying the CI6 of IMAP properly, at both national and subnational level.

82. The current work is a first exploration of the available data and the concepts that will need to underpin the formulation of the threshold for CI6. While the baseline data is still being validated, further statistical analysis will be necessary to elucidate the patterns of NIS introductions in the Mediterranean such that more robust suggestions can be made both at the temporal and at the sub-regional scale**.**

83. Some initial conclusions are that thresholds should be established separately for each of the Mediterranean subregions and should be sought by examining the data of the last two decades, if not an even more recent time period. At the same time, a consensus needs to be reached about which species groups will be included in the calculations and how their environmental impact will be taken into account. These are decisions that will determine the definition of GES for EO2 and will affect the management obligations of Contracting Parties to the Barcelona Convention. As such, it is proposed that further work takes into account the contribution of regional experts not only from the fields of taxonomy, monitoring and assessment but also conservation and management and last, but not least, ecologists with strong statistical/mathematical background.

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Annex

Elaboration of the scales of monitoring and assessment, assessment criteria and thresholds values of the IMAP EO2/CI6 regarding NIS in the context of the EcAp process of the Barcelona Convention

Questionnaire

Experts on marine NIS are invited to fill-in the questionnaire below, which has largely a multiple-choice format.

Definition of NIS

Non-indigenous species (NIS; synonyms: alien, exotic, non-native, allochthonous) are species, subspecies or lower taxa introduced outside of their natural range (past or present) and outside of their natural dispersal potential. This includes any part, gamete or propagule of such species that might survive and subsequently reproduce. Their presence in the given region is due to intentional or unintentional introduction resulting from human activities.

Natural shifts in distribution ranges (e.g. due to climate change or dispersal by ocean currents) do not qualify a species as a NIS. **However, secondary introductions of NIS from the area(s) of their first arrival could occur without human involvement due to spread by natural means.** In the latter case, the species should be still considered as NIS.

Species that appear in a new area as the result of a natural dispersal coming from an area that the species is considered as native, with the facilitation of the availability of new substrate (e.g. artificial reef), are not qualified to be considered as NIS.

Non-indigenous species can include also very old introductions, that occurred even before 1492.

Question #1: **Unicellular plankton species in E02/CI6**

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, even between neighboring countries, reporting either long lists of them or just a few. More recently, Gomez (2019) argued that there is not enough evidence for tagging any plankton species in Europe as non-indigenous. For the implementation of **CI6**, unicellular planktonic NIS species should be (put a "X" in the appropriate answer):

a) reported and considered when measuring GES based on CI6 (...)	b) reported but **not** considered when measuring GES based on CI6 ()	c) omitted entirely from CI6 assessments (...)	d) other (...)

Question #2: **Parasitic species in E02/CI6**

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).. However, several countries have included parasitic NIS in their **CI6**, lists. For the implementation of **CI6**, parasitic NIS species should be (put a "X" in the appropriate answer):

a) reported and considered when measuring GES based on CI6 (…)	b) reported but **not** considered when measuring GES based on CI6 (…)	c) omitted entirely from CI6 assessments (...)	d) other (...)

Question #3: **NIS introduced through natural dispersal in criterion CI6**

The primary criterion CI6 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 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 **CI6**, unless there is evidence that the species is transferred also through human-mediated activities, Several Lessespian species fall under this category. For the implementation of **CI6**, NIS that have been introduced into country **exclusively** through natural dispersal should be (put a "X" in the appropriate answer):

a) reported and considered when measuring GES based on CI6 (…)	b) reported but **not** considered when measuring GES based on CI6 (...)	c) other (…)

Question #4: **Cryptogenic species in criterion CI6**

Cryptogenic species are those with no definite evidence of their native or non-indigenous status (due to unknown origin natural spread *vs* human mediated). Characteristic example is *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. For the implementation of **CI6**, 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 CI6 (...)	b) reported but **not** considered when measuring GES based on CI6 (…)	c) other (...)

Question #5: **Questionable species in criterion CI6**

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 introductions of NIS in each country 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 **CI6**, 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 CI6 (…)	b) reported but **not** considered when measuring GES based on CI6 (…)	c) other (...)

Question #6: **Extinct species in criterion CI6**

Several NIS have been reported in a country several decades ago (even in the 19th century or before) but never recorded again in the wild in these countries, 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 country 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 country, then (put a "X" in the appropriate answer):

a) it should be considered as a new introduction and measured in CI6C1 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 (...)

Question #7: **Monitoring of marine NIS for CI6**

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	ports (…)	relevant data on marine NIS come from	IMAP biodiversity monitoring (...)

<table>
<tr><td></td><td></td><td>choices can be marked):</td><td>aquaculture units (...)</td><td>(multiple choices can be marked):</td><td>various research projects (...)</td></tr>
<tr><td></td><td></td><td></td><td>marine protected areas (...)</td><td></td><td rowspan="3">other (...)</td></tr>
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<tr><td></td><td></td><td></td><td>other (...)</td><td></td></tr>
<tr><td colspan="6">Monitoring efforts on marine NIS in your country include (put a "X" in the appropriate answer; multiple choices can be marked):</td></tr>
<tr><td>a) the detection of new NIS introductions (…)</td><td colspan="2">b) the spread of the established and/or invasive NIS (…)</td><td colspan="2">c) the measurement of abundance/coverage/biomass of established and/or invasive NIS (...)</td><td>d) the impact of established and/or invasive NIS on the native communities (...)</td></tr>
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