BALMAS

Ballast water management system for Adriatic Sea protection

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BALMAS overview

The United Nations had recognized the transfer of harmful organisms and pathogens across natural barriers as one of the four greatest pressures to the world's oceans and seas, causing global environmental changes, and posing threat to human health, property and resources. Ballast water transfer by vessels was recognized as a prominent vector of such species, and was regulated by the International Convention for the Control and Management of Ship´s Ballast Water and Sediments, 2004 (BWM Convention). The BWM Convention sets the global standards on ballast water management (BWM) requirements, while recognizing that regional and local specifics have to be considered for its effective implementation. The Adriatic Sea is a unique and highly sensitive ecosystem. The economic development and social existence of the coastal states strongly depend on the clean and preserved Adriatic Sea. However, the Adriatic Sea is also a seaway mainly used by international shipping transporting goods to or from Europe as hinterland, with also intense local shipping. Increasing, serious concern is the introduction of harmful aquatic organisms and pathogens (HAOP) by ships' ballast water. By developing a joint Adriatic Ballast Water Management Decision Support System, Ballast Water Management Plan and Strategy, BALMAS will ensure uniform BWM requirements to ease shipping and at the same time to maximize environmental and economic protection of all sea users. The general BALMAS objective is to establish a common cross-border system, which will link all researchers, experts and responsible national authorities from Adriatic countries in order to avoid unwanted risks to the environment from the transfer of HAOP. This can be achieved through control and management of ships' ballast waters and sediments. Further, long-term effective ballast water management (BWM) in the Adriatic will be set at the cross-border level utilizing this project's related knowledge and technology.

Executive summary

The Marine Strategy Framework Directive (MSFD; Directive 2008/56/EC) was formally adopted by the European Union in June 2008 and requires that EU Member States take the necessary steps to achieve or maintain good environmental status (GES) by the year 2020. By applying an ecosystem-based approach to the management of human activities, the Directive is aimed at enabling a sustainable use of marine goods and services, while protecting the marine environment and preventing its deterioration. The present document focuses on art. 8 (Initial assessment), art. 9 (Determination of Good Environmental Status) and art. 10 (Establishment of environmental targets and associated indicators) of the Slovenian and Italian MSFD report to the European Commission, with reference to Descriptor 2, 'Non-indigenous species'.

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1. The Marine Strategy Framework Directive (MSFD)

The Marine Strategy Framework Directive (MSFD; Directive 2008/56/EC) is the environmental pillar of the Integrated Maritime European Policy. It was formally adopted by the European Union in June 2008 and requires that EU Member States take the necessary steps to achieve or maintain good environmental status (GES) by 2020. Marine strategies are being implemented to protect and preserve the marine environment, prevent its deterioration or where practicable restore marine ecosystems, and to prevent and reduce inputs that have a significant impact. For this purpose Member States should regularly, every six years from the year 2012, assess the environmental status (Art. 8), determine GES (Art. 9), and set environmental targets (Art. 10) for their marine waters. Monitoring programmes (Art. 11) should be established by 2014 and updated at least every six years. Programmes of measures (Art.13) should be set by 2015, become operational by 2016 and follow the six years cycle. Fig.1 shows the management cycle of the MSFD.

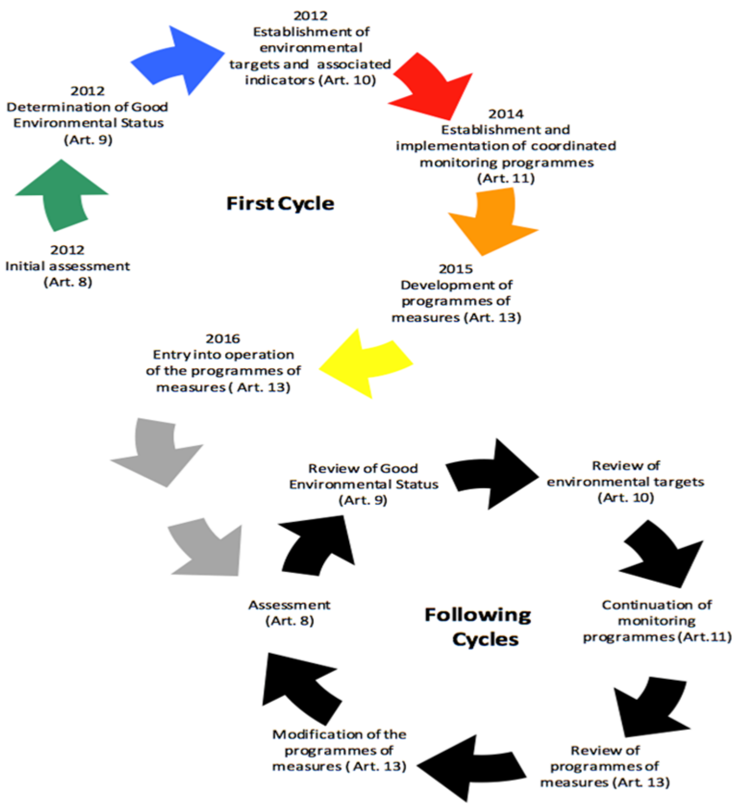

Figure 1 – The Marine Strategy Framework Directive (MSFD) management cycle.

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For this first cycle of MSFD, the initial assessment (Art. 8) has been based on available data and information. An analysis of the features or characteristics of, and pressures and impacts on, the marine waters has been performed, identifying the predominant pressures and impacts. Furthermore, an economic and social analysis of the use of marine waters and of the cost of degradation of the marine environment has been performed.

On the basis of such analyses, Member States have determined for their marine waters a set of characteristics for good environmental status (Art. 9 of MSFD).

The Commission Decision 2010/477/EU sets out the approach to be adopted for the determination of GES, by outlining a total of 26 criteria and 56 associated indicators for the 11 descriptors under the MSFD (Annex I).

The 11 descriptors for Good Environmental Status (GES) are the following:

1. Biodiversity
2. **Non-indigenous species**
3. Commercially exploited fish
4. Marine food webs
5. Human-induced eutrophication
6. Sea floor integrity
7. Hydrographical conditions
8. Concentration of contaminants
9. Contaminants in fish & seafood
10. Marine litter
11. Energy

In accordance with Article 10 of the MSFD, Member States have identified environmental targets and their associated indicators (Annex IV), which are operational tools to lay down the basis for the preparation of the monitoring programmes and of the programmes of measures. Environmental targets are management objectives for devising national, regional and EU measures that are required under art.13 in order to achieve or maintain GES; they are primarily related to pressure, impact and response indicators.

2. Descriptor 2 (D-2)

Descriptor 2 focuses on the identification and assessment of pathways and vectors that are responsible for spreading non-indigenous species (NIS) as a result of human activities. The Commission Decision 2010/477/EU provides a definition of Descriptor 2 and of its associated criteria and indicators:

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Descriptor 2: "Non-indigenous species introduced by human activities are at levels that do not adversely alter the ecosystem".

Criterion 2.1. Abundance and state characterisation of non-indigenous species, in particular invasive species

— Trends in abundance, temporal occurrence and spatial distribution in the wild 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 (Indicator 2.1.1);

Criterion 2.2. Environmental impact of invasive non-indigenous species

— Ratio between invasive non-indigenous species and native species in some well-studied taxonomic groups (e.g. fish, macroalgae, molluscs) that may provide a measure of change in species composition (e.g. further to the displacement of native species) (Indicator 2.2.1)

— Impacts of non-indigenous invasive species at the level of species, habitats and ecosystem, where feasible (Indicator 2.2.2)

The identification and assessment of pathways and vectors as part of human activities that are responsible for the spreading of non-indigenous species is a prerequisite to prevent their impacts wherever they can adversely affect native ecosystems.

Unfortunately, there still is a very limited knowledge about the effects of the non-indigenous species on the environment and additional long-time series of field data are required to properly assess the impacts of NIS on native species and habitats.

The present report specifically focuses on Art. 8, 9 and 10 of the EC reporting on Descriptor 2 from Member States Italy and Slovenia.

3. Initial Assessment for D-2

3.1 Introduction of non-indigenous species

Non-indigenous species (NIS) are considered as one of the key causes of biodiversity changes worldwide and their impacts are sometimes disastrous and usually irreversible. The annual number of records of new species world-wide since 1850 has paralleled the developments of trade (shipping) and aquaculture. As an example, in ICES member countries a new introduction forming a new population beyond its natural range occurs approximately every nine weeks (Minchin et al. 2005). Non-indigenous species received key attention by the United Nations as they are seen as one of the top four anthropogenic threats to the world´s oceans.

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The introduction of species to habitats outside their native ranges is a growing problem due to the unexpected impacts these species might cause on indigenous species and ecosystems. Nowadays, it is quite impossible to predict how a species will behave when it is introduced into a new environment. Because of this unpredictability every effort should be made to prevent or at least to monitor the introduction of species from an ecosystem into another (Verlaque, 2001).

The most ancient vector of species introduction is probably the transportation on the ships' hulls (Ribera and Boudouresque, 1995), since the use of ballast waters started only sometime in the second part of the 19th century (David, in prep.). Ostenfeld (1908) reported the first possible occurrence of a non-indigenous species attributed to being introduced with ships' ballast water to the North Sea in 1903. Gollasch et al. (in prep.) identified that it was not until seventy years later that the first biological samples from a ballast water sampling study were undertaken (Medcof, 1975), and followed by several others in different regions around the world (e.g., Carlton 1986; Williams et al. 1988; Locke et al. 1991; Hallegraeff and Bolch 1992; Gollasch 1996; Hamer et al. 2000; Gollasch et al. 2000a, 2000b, 2002; Murphy et al. 2002; David et al. 2007; McCollin et al. 2008; Briski et al. 2010, 2011).

Shipping is considered to be the principal pathway worldwide, by which species are spread. The prime vectors involving shipping are ballast water and sediments accumulated at the tank bottom as well as hull fouling, where also free-living (non-fouling) species were found (Gollasch et al., 2002). According to expert estimates, 3000 to 4000 different species are moved each day around the globe by ships (Carlton and Geller 1993; Gollasch 1996). More recent estimates indicate that the number of species in transit with ships is most probably in the range of 7000 every day (Carlton 2001) and this does not take into account the transfer of microorganisms such as bacteria and pathogens. While even the general estimates vary greatly, the scale of the phenomenon of species transfer is exceptionally large and it was concluded that each vessel has the potential to introduce a species (Gollasch 1996). The living organisms found during ballast water sampling studies included viruses, bacteria (comprising human pathogens), fungi, protozoa, algae (unicellular phytoplankton algae and macroalgae), invertebrates and fish (e.g., Williams 1988; Hay 1990; Hallegraeff and Bolch 1992; Gollasch

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1996; Macdonald and Davidson, 1998; Olenin et al., 2000; Gollasch et al., 2002; Murphy et al. 2002; David et al. 2007; Briski et al. 2010, 2011). Crustaceans, molluscs and polychaetes, as well as algae, were the dominant groups found in samples and consisted of more than 1000 identified species (Gollasch et al. 2002). Ballast water is the least selective means of transportation of species from the ecological and taxonomic points of view, and it is a vector that has no equivalent on land (Carlton and Geller, 1993). The survival time in ballast water for some species may exceed 18 days (Salt, 1992), so that many of these organisms are still alive after their intercontinental voyage at the time of deballasting.

Aquaculture is also playing an important role in intentional transfer of species. Some economically important non-indigenous species were introduced intentionally for aquaculture purposes from the Indo-Pacific region, with a consequent accidental introduction of accompanying species (Zibrowius, 1994; Ribera and Boudouresque, 1995; Gollasch and Leppäkoski, 1999).

Canals serve as shortcuts of major trade routes to save fuel and time to vessels. Aquatic organisms can cross canals by natural dispersal, by autochthonous active or passive larval or adult movements, and when transported by vessels (Minchin et al. 2006).

In different world regions the importance of species introduction vectors varies. Nevertheless, in all the regions considered the most important three vectors are (possibly in changing order): ballast water, hull fouling, and aquaculture, so that shipping is considered to be the worldwide principal pathway by which species are spread. The identification of the vector for each species is extremely challenging as several species may be related to more than one vector and vectors overlap, which makes many of them indistinctive (Figure 2).

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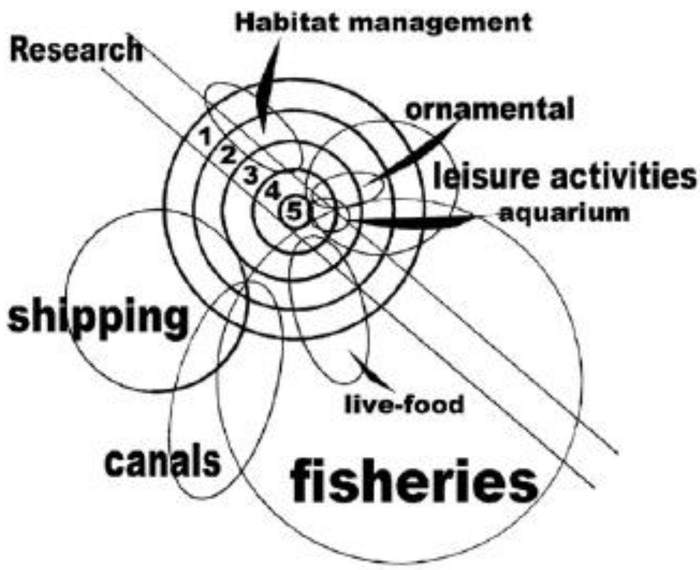

Figure 2 - Overlap of different vectors of species introduction (Minchin, 2007).

Further, ocean currents can move species and under certain rare hydrodynamic conditions, with perturbations in the strength and direction of flow, species can be moved beyond their normal geographic range, perhaps also as a result of climate alterations and changes in local environmental conditions. These natural phenomena result in changes to local species richness and may only appear on a temporary basis within a region, being known as rare guests, or vagrants. Such natural appearances, especially on the fringing ranges of a species where their ability to survive is just possible, are a normal part of nature´s biodiversity and are often seen as an advantage (Hoppe 2002; Weber and Frieß, 2003; Reid and Valdés 2011). Natural introductions, like in the Mediterranean the natural invasion of species through the Strait of Gibraltar, are frequently the result of local changes in environmental conditions, so that a species normally occurring outside the considered area can extend its range and move into it. These types of introductions do not appear to be harmful, while in contrast are the human-assisted species movements which can cause irreversible negative impacts (Boudouresque et al. 1994; Buttermore et al. 1994; GESAMP 1997; Ivanov et al., 2000; Leppäkoski et al 2002; Hewitt et al. 2004).

3.2 History of non- indigenous species in Slovenian marine waters

In the late 1990 a study prepared an overview about the introduction and potential impacts of potentially toxic algae and NIS in the Northern Adriatic Sea (David 1999; David 2000). As a follow-up a ballast water sampling study was conducted in the Port of Koper in 2003 which confirmed suspects that potentially harmful algae and NIS are being discharged with ballast waters in the Slovenian Sea (David et al. 2007). Despite the long-standing tradition in marine research in the Northern Adriatic, non-indigenous species in the Gulf of Trieste received less attention within published papers in comparison with neighbouring areas (Lipej et al., 2012). Few papers are dealing with first records of alien species in the area (Lipej et al., 2008b; Shiganova et al., 2009; Poloniato et al., 2010), other papers published lists of non-indigenous species (Orlando-Bonaca, 2001, 2010; Krmac, 2009, Lipej et al., 2012), while records of alien mollusca from the whole Gulf of Trieste were recently reviewed (Crocetta, 2011; Crocetta and Turolla, 2011). Non-indigenous species were also mentioned in works about biotic globalisation as a whole (Lipej et al*.,* 2009a, 2009b). By 2012, 15 non-indigenous species were confirmed in Slovenian coastal waters (Lipej et al., 2012; Orlando-Bonaca et al., 2012a, 2012b), some of which are reported in Figure 3. The number of NIS in Slovenian waters is expected to increase in the future. The possibility of new introductions from the Venice Lagoon, considered to be rich in invasive species, is very high (Mizzan, 1999). In the whole Adriatic region 190 non-indigenous species were registered by Zenetos et al. (2010).

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a) *Codium fragile* subsp. *fragile* (photo: L. Lipej)

b) Red algae *Asparagopsis armata* (phase *Falkenbergia rufolanosa*) (photo: M. Orlando-Bonaca)

c) Gastropod species *Bursatella leachi* (photo: M. Orlando-Bonaca)

d) Japanese Oyster (*Crassostrea gigas*) (photo: N. Bettoso)

e) Sessile polychaete *Ficopomatomus enigmaticus* (photo: T. Makovec).

f) Gastropod species *Rapana venosa* (photo: T. Makovec)

Figure 3: a.) – f.) Non- indigenous species representatives in Slovenian Sea.

The majority of non- indigenous species has appeared only sporadically but some of the species were able to establish reproductive populations in the area of introduction. Already established species are the green alga *Codium fragile* subsp. *fragile,* the red alga *Asparagopsis armata*, the crustacean *Balanus trigonus*, seven molluscs (*Bursatella leachi, Rapana venosa,*

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Crassostrea gigas, Venerupis philippinarum, *Anadara kagoshimensis, Anadara transversa, Arquatula senhousia*), and the sessile polychaete *Ficopomatomus enigmaticus* (Table 1).

Table 1- List of the recorded non-indigenous species in Slovenian waters and their status, origin, year of record, vector and reference (from Lipej et al., 2012).

Status: E – established, C – casual and R – rejected.

Pathway/vector: BW –ballast waters, LM – lessepsian migration, MA –mariculture, NS – natural spreading, BC – biocontrol, FO – fouling.

HIGHER TAXA	SPECIES	STATUS	ORIGIN	YEAR	PATHWAY/VECTOR	REFERENCE
Phyto-plankton	*Ceratoperidinium yeye*	R	Indo-Pacific	2003	Range expansion/NS?	France & Mozetič (2008)
Algae	*Asparagopsis armata*	E	Australia	1991	MA	Orlando-Bonaca (2001)
Algae	*Bonnemaissonia hamifera*	C	Pacific ocean	1995	MA	Orlando-Bonaca (2001)
Algae	*Codium fragile* subsp. *fragile*	E	Pacific ocean	1992	Shipping/BW	Munda (1992)
Cnidaria-Siphonophora	*Muggiaea atlantica*	R	Atlantic ocean	2001	Range expansion/NS?	Miloš & Malej (2005)
Ctenophora	*Mnemiopsis leydyi*	C	Black Sea	2007	Shipping/BW	Shiganova & Malej (2009)
Ctenophora	*Beroe ovata*	C	Black Sea	2007	Shipping/BW	Shiganova & Malej (2009)
Crustacea	*Balanus trigonus*	E	Tropical seas	2005	Shipping/FO	Mavrič et al. (2010)
Mollusca	*Bursatella leachi*	E	Red Sea	1999	Corridor/LM	Lipej et al. (2008a)
Mollusca	*Rapana venosa*	E	Japan	1983?	Shipping/BW	De Min & Vio. (1998)

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Mollusca	*Venerupis philippinarum*	E	Indo-Pacific	1993	MA	Lipej (1994)
Mollusca	*Anadara kagoshimensis*	E	Indo-Pacific	1996	Shipping/ BW	De Min & Vio (1997)
Mollusca	*Anadara transversa*	E	Indian ocean	2003	Shipping/ BW	Crocetta (2011)
Mollusca	*Crassostrea gigas*	E	Japan	?	MA	De Min & Vio (1998)
Mollusca	*Arquatula senhousia*	E	Indo-Pacific	2005	MA	Mavrič et al., (2010)
Polychaeta	*Ficopomatomus enigmaticus*	E	Southern hemisphere	1994	Shipping/ FO	Lipej et al. (2009)
Fish	*Gambusia holbrooki*	R	SE USA	<1930	BC	Leiner et al. (1995)
Fish	*Terapon theraps*	C	Indo-Pacific	2007	LM	Lipej et al. (2008b)

3.3 Initial Assessment of Environmental Status for D-2 (Slovenia)

Based on the scarce and non-continuous information on the presence of non-indigenous species in the Slovenian sea, it has been difficult to reliably assess the number of NIS species and their abundance in the 2012 MSFD initial assessment for D-2. There is a lack of a systematic and continuous monitoring programme, with the exception of the Neobiota project (monitoring only in the period 2010-2011). Without a proper monitoring program, it is difficult to assess the population growth and colonization trends of NIS already present in the area. Moreover, there are no data available regarding possible impacts that NIS may have on the autochthonous species and ecosystems. Basic information related to species richness, abundance, population growth, feeding patterns and habitat preferences are of crucial importance for the understanding of possible NIS impacts and success rates of their invasion. When more detailed and continuous data will be available for the Slovenian waters, the comparison and exchange of information with nearby areas (Italian and Croatian waters) will be possible. At the moment, the lack of a monitoring programme results in low confidence of the available data.

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However, based on all currently available data, the Environmental Status of Slovenian marine waters according to D-2 was preliminarily estimated as *Good*.

At least 15 NIS were recorded, but due to the lack of a monitoring programme and scientific projects, the number of NIS could be considered as underestimated. Most NIS recorded in Slovenian coastal waters were reported only for the last two decades.

3.4 Impacts of non- indigenous species in Slovenian waters

For indicators 2.2.1 (Ratio between invasive non-indigenous species and native species in some well-studied taxonomic groups) and 2.2.2 (Impacts of non-indigenous invasive species at the level of species, habitats and ecosystem, where feasible) Slovenia could not define the GES. The available information regarding NIS occurrence in Slovenian waters do not comprise any data about ecological and biological parameters of those species. Therefore, there is a huge gap regarding the ratio between autochthonous and allochtonous species in the area. Indicator 2.2.1. resulted to be not practical as it requires a very high sampling effort. Therefore, it was proposed that only indicator 2.2.2 should be developed with new appropriate data collected according to a defined monitoring procedure.

According to the available data and expert knowledge, non-indigenous species currently have no significant negative effects on native species and habitats in Slovenian marine waters. The Environmental Status in the whole Slovenian sea can be nowadays considered as *Good* according to Indicator 2.1.1 (Trends in abundance), while it was not possible to define the GES according to Indicators 2.2.1 (Ratio between invasive non- indigenous species and native species) and 2.2.2 (Impacts of non- indigenous invasive species) due to the lack of appropriate data. Therefore, it is very important to establish a monitoring programme for established and common NIS in the Slovenian sea.

3.5 History of non- indigenous species in the Italian Northern Adriatic Sea (from Ancona to Trieste)

A total of 62 non indigenous species (NIS) belonging to 13 main taxonomic groups (microalgae, macrophytes, cnidarians, molluscs, polychaetes, bryozoans, decapod crustaceans, copepods, amphipods, isopods, pycnogonids, ascidians, fishes) were recorded in the assessment area selected for the subregion Adriatic Sea, which extends from Ancona to Trieste (Tab. 2); 32 of

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these NIS (52%) are considered to be invasive according to the definition of IAS1 in the MSFD Task Group 2 report (Olenin et al. 2010), though in some cases the species do not show invasive characters in Italian seas (6 IAS have been recorded only once). The cumulative number of NIS shows a gradual and relatively slow increase until the decade 1972-1982, when the increase becomes faster (Fig. 4).

Table 2. List of non indigenous species recorded in the Northern Italian Adriatic Sea (from Ancona to Trieste).

Microalgae: *Ostreopsis cf. ovata*; *Pseudo-nitzschia multistriata*

Macrophytes: *Aglaothamnion feldmanniae, Antithamnion hubbsii, Bonnemaisonia hamifera, Codium fragile ssp. fragile, Grateloupia turuturu, Heterosiphonia japonica f. nipponica, Hypnea flexicaulis, Leathesia difformis, Lomentaria hakodatensis, Neosiphonia harveyi, Polysiphonia morrowii, Sargassum muticum, Scytosiphon dotyi, Ulva pertusa, Undaria pinnatifida*

Cnidarians: *Cordylophora caspia, Diadumene cincta, Ectopleura dumortieri, Garveia franciscana, Gonionemus vertens, Helgicirrha schulzei*

Polychaetes: *Desdemona ornata, Hydroides dianthus*

Molluscs (non cephalopods): *Anadara inaequivalvis, Anadara transversa, Arcuatula senhousia, Bursatella leachii ,Cerithium scabridum, Crassostrea gigas, Haminoea japonica, Limnoperna securis, Mercenaria mercenaria, Mya arenaria, Pinctada imbricata radiata, Polycera hedgpethi, Rapana venosa, Saccostrea cucullata, Saccostrea glomerata, Venerupis philippinarum*

Cephalopods: *Tremoctopus gracilis*

Amphipods: *Caprella scaura, Elasmopus pectenicrus*

Copepods: *Acartia tonsa, Paracartia grani*

Decapods: *Callinectes danae, Callinectes sapidus, Charybdis lucifera, Dyspanopeus sayi, Eriocheir sinensis, Rhithropanopeus harrisii, Scyllarus caparti*

Isopods: *Paracerceis sculpta*

Pycnogonids: *Ammothea hilgendorfi*

[1] Invasive alien species (IAS) are a subset of established NIS which have spread, are spreading or have demonstrated their potential to spread elsewhere, and have an adverse effect on biological diversity, ecosystem functioning, socio-economic values and/or human health in invaded regions. Species of unknown origin which can not be ascribed as being native or alien are termed cryptogenic species. They also may demonstrate invasive characteristics and should be included in IAS assessments.

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Ascidians: *Botrylloides violaceus, Didemnum vexillum, Perophora viridis*

Bryozoans: *Celleporella carolinensis, Tricellaria inopinata*

Fishes: *Epinephelus coioides, Siganus luridus*

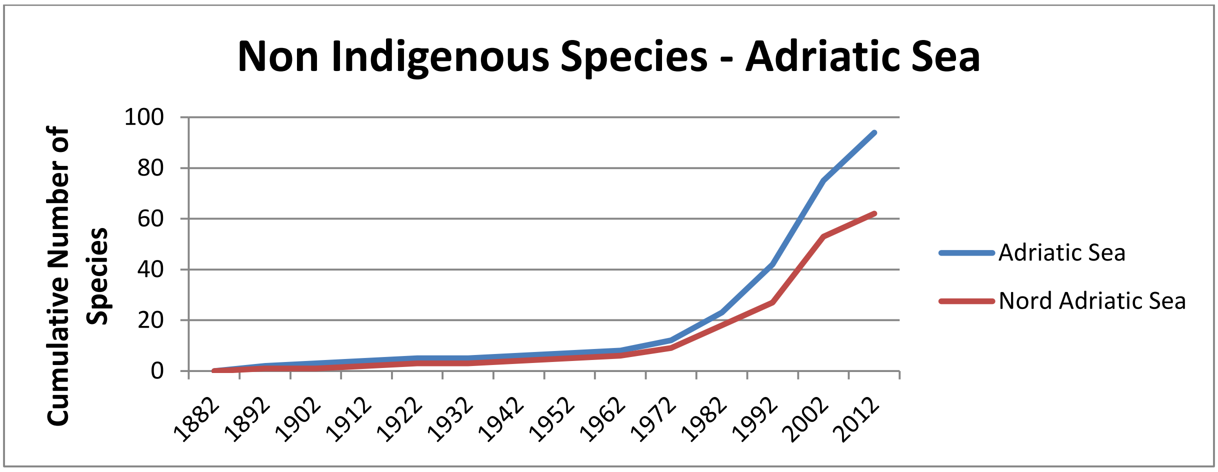

Figure 4. Trend of non indigenous species in the whole Italian Adriatic Sea (blue line) and in the Northern Italian Adriatic Sea (from Ancona to Trieste) (red line).

Spatio-temporal distribution of *Ruditapes philippinarum*, *Arcuatula senhousia* and *Dispanopeus sayi* in the Po delta area is the following: *R. philippinarum* has an increasing trend in abundance (northern Adriatic areas have become one of the greatest clam production areas in the world); *A. senhousia* had an impressive increase in abundance from the mid '90s to the beginning of the year 2000, after which the populations have stabilized; *D sayi* has become one of the commonest crabs in the Po delta area. As far as the taxonomic group of Bryozoa is concerned, the ratio between non-indigenous and indigenous species in the Lagoon of Venice is low, however the presence of *Tricellaria inopinata* is overwhelmingly abundant in comparison to the other species. Conversely, the presence of *Celleporella carolinensis* is limited to a few sampling localities and always with very low abundance, suggesting that its occurrence could be negligible in terms of possible impacts on native species. In the same area, *Rapana venosa* is present with low abundances (5 ind/10^4 m^2 on sandy bottom, 34 ind/ 100 m^2 on rocky bottom) and no impact has been detected so far. In the case of the potentially toxic benthic dinoflagellate *Ostreopsis cf. ovata*, the species was not recorded in the Adriatic until 2002, but surveys of microphytobenthos were rare before that date. Presently, this

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species is widely distributed along the rocky shores all across the Adriatic Sea, while it is not found in Veneto and Emilia Romagna, possibly because of lack of proper substrates. Since the first records of *O. cf. ovata* in Apulia, Marche and Venezia Giulia, the abundance of this species has been high and up to 10^5-10^6 cells/g macroalga, with maxima abundances generally in late September-October. Over the last ten years no clear trend has been reported in terms of expansion or increased abundance. The isopod NIS *Paracerceis sculpta* has been recorded since 1981 in the Venice lagoon, while a more recent record (2004) is from the touristic harbour of Ravenna.

3.6. Impacts of non-indigenous species in Italian waters

The impact of *Rapana venosa* predation on bivalves was estimated by an experimental study (Savini and Occhipinti-Ambrogi, 2006). A caging experiment in natural environment was performed during the summer of 2002 in Cesenatico (Emilia-Romagna, Italy) in order to estimate consumption rates and prey preference of *R. venosa*. The prey items chosen were the Mediterranean mussel *Mytilus galloprovincialis*, the introduced carpet clam *Tapes philippinarum* and the Indo-Pacific invasive clam *Anadara inaequivalvis*. Results showed an average consumption of about 1 bivalve prey per day (or 1.2 g wet weight per day). Predation was species- and size-selective towards small specimens of *A. inaequivalvis*; consumption of the two commercial species was lower. These results might reduce the concern about the economical impact on the local bivalve fishery due to the presence of the predatory gastropod. On the other hand, selective predation might probably alter local community structure, influencing competition amongst filter feeder/suspension feeder bivalve species and causing long-term ecological impact.

The impact from the mollusc *Ruditapes philippinarum* was assessed in the Po Delta area (Basso et al. 2004, Sgro et al. 2004, Sgro et al. 2005). On sediments, proteins represent the main biochemical class of organic compounds (44.5% of the biopolymeric fraction). Sediment resuspension increased with increasing *R. philippinarum* abundance: at 25 cm s^{-1} at the lowest clams density (71 clams m^{-2}), there was more than a 4–fold increase in erosion rate (from 6 mg m^{-2} s^{-1} to 27 mg m^{-2} s^{-1}); at 129 clams m^{-2} the increase was more than 6-fold (from 6 mg m^{-2} s^{-1} to 40 mg m^{-2} s^{-1}) and at the highest *R. philippinarum* density (206 clams m^{-2}) it was about

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27-fold (from 6 mg m^{-2} s^{-1} to 156 mg m^{-2} s^{-1}). Clearance rate (CR) is associated with food concentration. CR increases with an increasing concentration of food and reaches a maximum at about 32000 cell ml^{-1}, in Spring , and 65500 cell ml^{-1}, in Summer. On the biota, diversity and number of species are reduced due to high abundance of clams.

The impact of *Ostreopsis* was reported as production of high amount of mucous material and benthic animal kills (Mangialajo et al., 2011). No quantitative assessment is available in terms of the impact of the blooms on the benthic systems.

3.7. Vectors of non-indigenous species and identified gaps in Italian waters

In the assessed area (form Ancona to Trieste) aquaculture is considered a propagation vector for 33% of NIS. The introduction of NIS through aquaculture is mainly due to unintentional introductions caused by movements and transfer of seeds and commercial products of shellfish. *Ruditapes philippinarum* and *Crassostrea gigas* are the only species introduced intentionally in this area. The propagation of *Rapana venosa* egg masses takes place through marine farming, as associated organism during restocking of aquaculture production (Reporting Sheet 8B10 Italy MSFD). Also in the case of *Ostreopsis* cells, the transfer with aquaculture products is suspected (Zingone pers. comm. in Reporting Sheet 8B10 Italy MSFD).

Maritime traffic is considered a propagation vector for 39% of NIS. Maritime traffic transfers planktonic larvae of the mollusc *Rapana venosa* in ballast waters (Reporting Sheet 8B10 Italy MSFD), while the most likely vector of introduction of *Celleporella carolinensis* into the Lagoon of Venice is the hull fouling on ships (Reporting Sheet 8B10 Italy MSFD).

Main identified gaps within Descriptor 2 are the low availability of data on abundance and on impacts of NIS and the very poor information on the main vectors of introduction. These gaps may be filled through i) monitoring activities aimed at evaluating the real settlement of non indigenous species, in particular the invasive ones, and their impact, through a standardized methodology; ii) monitoring in marine protected areas where human impact is absent or limited; iii) surveillance actions in areas with high probability of introduction, such as harbors and aquaculture sites, and iv) control actions on ballast water and fouling, and aquaria trade. Italy has adopted the Council Regulation (EC) 708/2007 concerning the use of alien and locally absent species in aquaculture, which will allow a better control of target species introductions due to NIS farming. However, contribution of non voluntary introductions to NIS pressure should be better understood by improving the assessment of farming-associated risks of introduction. Other gaps to be filled are the application of the UNEP-MAP Mediterranean Strategy for the management of ships' ballast waters and sediments, within the framework of the Barcelona Convention, and the ratification of the International Convention for the Control and Management of Ships' Ballast Waters and Sediments (IMO, 2004).

4. Determination of Good Environmental Status (GES) for D2

4.1 Slovenia

Slovenia has defined GES at the following criterion and indicator level:

Criterion 2.1 Abundance and state characterisation of non-indigenous species, in particular invasive species.

Indicator 2.1.1 Trends in abundance, temporal occurrence and spatial distribution in the wild 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.

Based on the available data it can be concluded that 11 NIS can be considered as established in the Slovenian waters, while others were found only sporadically. Monitoring surveys will be carried out in the whole Slovenian marine area, but more frequently in areas with a high probability of introduction and in areas of ecological interests. The risk of introduction of NIS should be minimal, pathways and vectors must be constantly controlled according to a defined monitoring procedure.

4.2 Italy

Italy has defined GES at the following criteria level:

2.1 Abundance and state characterisation of non-indigenous species, in particular invasive species. GES is considered the status when there is no increase of the indicator value (abundance and frequency of occurrence of invasive species) with respect to a threshold value that will be defined on ad hoc monitoring surveys basis. Monitoring surveys will be carried out in selected areas with a high probability of introduction and in areas of ecological interests.

GES is considered the status when there is no increase of impact based on an ad-hoc assessment carried out in selected areas with a high probability of introduction and in areas of ecological interests.

Italy has proposed to develop the *indicator 2.1.1* (*Trends in abundance, temporal occurrence and spatial distribution in the wild 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*) and the indicator 2.2.2 (*Impacts of non-indigenous invasive species at the level of species, habitats and ecosystem, where feasible*) by 2018 through *ad hoc* monitoring, considering the current data gaps.

The indicator *2.2.1* (*Ratio between invasive non-indigenous species and native species in some well-studied taxonomic groups (e.g. fish, macroalgae, molluscs) that may provide a measure of change in species composition (e.g. further to the displacement of native species) are related to non- indigenous impact assessment goal)* is deemed not practical as it requires an extremely high sampling effort compared to the outcome indication of the impacts and there is no evidence of impacts related to this within the Adriatic subregion.

Determination of GES for D2 will be based on *ad hoc* monitoring activities carried out in selected areas with a high probability of introduction (e.g. ports or aquaculture farms) and in areas of ecological interests.

For the indicator 2.1.1, a threshold value will be established, based on the results of monitoring surveys. Investigations will be carried out on benthic macrofauna and macroflora (possibly fish fauna), of infralittoral hard and mobile substrate, with standard sampling technique (scraping, grab, box corer, etc.). For some specific cases, such as invasive species of megabenthos, there could be other techniques of investigation (e.g. Local Ecological Knowledge approach). At least two marine areas with a high probability of introduction will be identified (adjacent to port areas and / or sites for aquaculture), and at least two marine areas of control (e.g. MPA, Natura 2000 sites). In each area identified at least two monitoring sites are planned with annual sampling frequency for macrofauna and semi-annual for macroflora.

The indicator 2.2.2 will be expressed based on the Biopollution Index (BPI) according to the method of Olenin et al., 2007. Investigations will be the same as for the indicator 2.1.1 on benthic macrofauna and macroflora (eventually ichthyofauna) of hard and/or mobile infralittoral substrata, with standard sampling techniques plus eventual ad hoc methods. Sampling will be carried out in the same areas and sites as for the indicator 2.1.1 with the same monitoring plan. As for the assessment of impacts within the definition of BPI, manipulation activities of selected species might be required, to be implemented only twice (once per year over a 2 years period).

5. Environmental Targets for D2

5.1 Slovenia

Environmental Targets for non-indigenous species are related to all Slovenian marine waters.

The first proposed *ad interim* target is to evaluate correctly the impact of non-indigenous species on native organisms and habitats. Right now it is not possible, due to lack of data about ecological and biological parameters of established NIS. After this first step, the final environmental target will be defined (threshold, trends).

Associated Indicator:

The target is associated with three indicators: 2.1.1 Trends in abundance, temporal occurrence and spatial distribution in the wild 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; 2.2.1 Ratio between invasive non-indigenous species and native species in some well-studied taxonomic groups (e.g. fish, macroalgae, mollusks) that may provide a measure of change in species composition (e.g. further to the displacement of native species); and 2.2.2 Impacts of non-indigenous invasive species at the level of species, habitats and ecosystem, where feasible. The risk of introduction of NIS should be minimal, pathways and vectors must be constantly controlled according to a defined monitoring procedure.

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5.2 Italy

Italy has set one operative environmental target for Descriptor 2, with one associated indicator.

The target proposed is the development of an early warning system in areas at high risk of NIS introduction (ports, aquaculture sites). The system should guarantee a rapid identification of undesired introduced species and an associated risk evaluation followed by rapid alert of competent authorities The associated indicator should be the territorial coverage of the early warning system, with a threshold value of 50% of the sites at high risk of introduction of NIS due to human activities.

The development of the indicator requires preliminary mapping of the sites at high introduction risk, like ports with high maritime traffics, ballast waters discharge areas, aquaculture sites. A protocol for rapid taxonomic species identification and associated risk evaluation will be set. Alert system by relevant authorities will be designed.

6. Common aspects between Slovenian and Italian MSFD reports for D-2

Initial assessment

Within the initial assessment of MSFD, both Slovenia and Italy produced a NIS list which is the starting point of the implementation of the Directive for Descriptor 2. A smaller number of NIS is reported in Slovenian waters than in Italian ones.

The main pathways/vectors identified in both countries are shipping (specifically ballast waters in Slovenia) and aquaculture.

GES determination

Both countries defined GES only at criteria level, choosing the same indicators (2.1.1 and 2.2.2) and rejecting the indicator 2.2.1 as it was considered to be non practical.

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Environmental targets

The targets proposed by Slovenia and Italy for Descriptor 2 result rather different. Slovenia proposed one environmental target, with indicators to be developed, based on control of vectors/pathways and vulnerable areas as well as of high invasive already established species and their impacts.

Italy proposed one environmental target with associated indicator, based on an early warning system in areas at high risk of introduction.

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