



# INTERREG ITALY-CROATIA PROGRAMME 2021 – 2027

Deliverable 1.3.2  
Review of management  
strategies related to  
invasive species  
Final Version – February 2025

## ALIENA

### ALigning Efforts to control Non-indigenous species in the Adriatic sea

<b>WP 1</b>	Review of non-indigenous species and causes determining their invasiveness in Adriatic Sea
<b>Activity 1.3</b>	Review of existing management systems related to each target species already in place
<b>Deliverable 1.3.2</b>	Report on the identified target NIS for each pilot areas
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## Summary

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## 1. Introduction

The Convention on Biological Diversity (CBD) calls for the collection and dissemination of information on alien species that threaten ecosystems, habitats or species, to be used in the context of all prevention, introduction and mitigation activities. In Europe, the Marine Strategy Framework Directive (MSFD) also recognizes alien marine species as a major threat to European biodiversity and ecosystem health and requires Member States (MS) to take them into account when developing strategies to achieve Good Environmental Status (GES) in all European seas (Katsanevakis *et al.*, 2015). The MSFD makes explicit reference to non-indigenous species introduced by human activities as one of the 11 descriptors used to assess GES. NIS are treated as a separate descriptor (D2) of GES: "Non-indigenous species introduced by human activities are at levels that do not adversely alter the ecosystem". MSFD Descriptor 2 (D2) requires MS to consider NIS in their marine management strategies. The MSFD requires that "the number of non-indigenous species newly introduced into the wild by human activity per assessment period (6 years) shall be minimized and, where possible, reduced to zero" and also states that "Member States shall establish the threshold for the number of new introductions of non-indigenous species through regional or sub-regional cooperation". The latest report from the European Commission (Magliozzi *et al.*, 2024a) concerns the threshold methodology and value for the GES assessment of D2C1 "Newly introduced non-indigenous species". In this report, threshold scenarios for D2C1 are established based on the best available scientific knowledge and understanding, taking into account the policy requirements and implementation needs of the MSFD. Thresholds for D2C1 are provided for marine regions and sub-regions (e.g. Adriatic), which are recommended for use in the MSFD GES assessment (Magliozzi *et al.*, 2024a).

The most efficient (and cost-effective) way to deal with the threat of NIS to biodiversity and society is through a combination of prevention, early detection and rapid response to new invasions, with permanent management only as a last option (EEA, 2024). Efforts under the MSFD focus on limiting the environmental pressure of NIS by reducing the rate of their



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introduction and spread, and managing the pathways through which they move. A similar approach is also the main driver behind the IAS Regulation (Reg. EU No 1143/2014) on the prevention and management of the introduction and spread of invasive alien species. It requires MS to prevent the introduction and spread of regulated IAS (IAS of Union concern), enforce effective early detection and rapid eradication mechanisms for new introductions, and adopt management measures for species that are already widely distributed. The IAS Regulation thus supports Natura 2000 through the Habitats Directive (Dir. 92/43/EEC) and the Birds Directive (Dir. 2009/147/EC) by providing MS with a specific legal instrument to prevent the introduction and spread of IAS of Union concern and to mitigate their impact (EEA, 2024). Currently, only three marine species are on the list of IAS of Union concern: the fish *Plotosus lineatus* (Thunberg, 1787), *Morone americana* (Gmelin, 1789) and the brown seaweed *Rugulopteryx okamurae* (E.Y. Dawson) I.K. Hwang, W.J. Lee & H.S. Kim, 2009. Finally, the Chinese mitten crab *Eriocheir sinensis* can be considered as a marine, brackish and freshwater species. The European Green Deal and the Biodiversity Strategy for 2030 both underline the importance for the EU to put nature on a path to recovery by 2030 by better protecting and restoring healthy ecosystems. In particular, the Biodiversity Strategy commits to reducing the number of Red List species threatened by IAS by 50% (EEA, 2024). In addition, Regulation (EC) No 708/2007 on the use of alien and locally absent species in aquaculture applies to all aquatic species used in aquaculture and for stocking to supplement wild stocks, with the exception of species used solely for ornamental purposes (EEA, 2024).

Finally, the International Convention for the Control and Management of Ships' Ballast Water and Sediments (BWM), which entered into force globally on 8 September 2017, is a critical step in reducing the spread of NIS regionally and globally. This global response aims to prevent the potentially devastating effects of the spread of invasive species carried by ships' ballast water and sediments (EEA, 2024). Under the Convention, all ships engaged in international trade are required to manage their ballast water and sediments to a certain standard in accordance with a ship-specific ballast water management plan. All ships must also carry a ballast water record book and an international ballast water management certificate. A number of guidelines have been developed to facilitate the implementation of



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the Convention, which is administered by the International Maritime Organization (IMO) (<https://www.imo.org/en/OurWork/Environment/Pages/BWMConventionandGuidelines.aspx>).

Guidelines for Controlling Introduction Vectors - Management of the main introduction vectors, including:

- Navigation and ballast water: compliance with the International Maritime Organization (IMO) Ballast Water Management Convention.
- Biofouling on ship hulls: use of anti-fouling coatings and cleaning protocols.
- Aquaculture and trade: restrictions on the movement of potentially invasive species and quarantine measures.

Regulatory and Legislative Framework - Various international, regional, and national policies regulate the management of invasive species:

- **EU Regulation 1143/2014:** obligates member states to develop action plans and risk assessments for invasive species control.
- **International Maritime Organization (IMO) biofouling guidelines (2023):** best practices for ship cleaning and biosecurity measures.
- **Barcelona Convention (SPA/BD Protocol):** promotes regional cooperation in regulating intentional and accidental introductions.
- **National Marine Planning Framework (NMPF):** integrates NIS management into national environmental policies.

These integrated strategies underscore the importance of regional collaboration, scientific research, proactive management, and robust policy frameworks in combating invasive species in the Mediterranean Sea.

Main Challenges and Recommendations



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- Lack of enforcement of regulations: many regulations exist, but compliance monitoring is often insufficient.
- Data gaps and scientific uncertainties: limited knowledge on NIS distribution and ecological impacts.
- Limited resources: budgets for surveillance, monitoring, and rapid response are often inadequate.

#### Potential Solutions:

- Better cross-sector collaboration: involvement of industries, governments, and NGOs in NIS management.
- Strengthened monitoring programs: use of advanced technologies such as remote sensing, AI analysis, and citizen science.

The following section provides a list of existing management systems related to the target NIS for each pilot area identified on the basis of Activity 1.2 (see Deliverable D.1.2.1 for further details on species selection).



## 2. Pilot Area “The rocky coast areas of Apulia region”

### 2.1. List of the existing management systems related to target species already in place

#### 2.1.1. *Rugulopteryx okamurae* (E.Y.Dawson) I.K.Hwang, W.J.Lee & H.S.Kim, 2009

*Rugulopteryx okamurae* is a brown macroalga considered the fourth most harmful species among the 10 most invasive species reported in the Mediterranean (Tsirintanis *et al.*, 2022; Bellissimo *et al.*, 2023). As part of EU Regulation 1143/14, adopted to prevent and manage the introduction and spread of invasive alien species, *Rugulopteryx okamurae* is the only one macroalgae included in the list of EU relevance (Marletta *et al.*, 2024).

This species, native to the warm and temperate North-western Pacific Ocean, including Korea, Japan, China, Taiwan, and the Philippines (Hwang, 1994), ranges from tropical areas to the Gulf of California (Norris, 2010). It was reported for the first time in the Mediterranean in 2002 in Thau Lagoon (France), introduced by the aquaculture of the Japanese oyster *Magallana gigas* (Thunberg 1793). Since then, this species has spread in both the Mediterranean and Atlantic Ocean with a strong invasive behavior, particularly along the Strait of Gibraltar (Altamirano *et al.*, 2020). In detail, significant blooms were observed since 2015 in the northern coastal area of the Strait of Gibraltar (Tarifa-Gibraltar) (Afonso-Carrillo and Ocaña, 2016). It subsequently extended to the south of the Iberian Peninsula, reaching the Atlantic coast in 2018, the Canary Islands (<https://www3.gobiernodecanarias.org/cptss/sostenibilidad/biodiversidad/redexos/>)

Portugal (Liulea *et al.*, 2023), the Azores (Faria *et al.*, 2022a), Madeira Island (Bernal-Ibañez *et al.*, 2022), and the Mediterranean coast (Ruitton *et al.*, 2021).

*Rugulopteryx okamurae* was recently reported for the first time in Italian waters, in the Southern Adriatic Sea, off the coast of Bari, during April 2023 (Bottalico *et al.*, 2024) and along the northwestern coast of Sicily, in the Gulf of Palermo (Bellissimo *et al.*, 2024).



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The macroalga is characterized by a 10-20-cm-high dichotomous branched thallus with rhizoids in the basal parts. In invaded areas, it is usually present year-round as a sporophyte, maintaining and establishing new populations through fragmentation, vegetative propagules, and/or asexual monospores (Hwang *et al.*, 2009; Altamirano *et al.*, 2021; Navarro-Barranco *et al.*, 2019). Each propagule can generate a new clonal individual, whether the thallus is fixed to the substrate or free in the water column. The species typically expands as detached thalli, dispersed by currents or human vectors such as fishing or maritime transport, accumulating along shores or on the sea bottom to form new populations (Garcia-Lafuente *et al.*, 2023; Mateo-Ramirez *et al.*, 2023). *R. okamurae* has shown the ability to survive in adverse environmental conditions, contributing to its high colonization capacity. It exhibits maximum growth when temperatures exceed 15 °C. Its rapid proliferation has led to substantial ecological and socio-economic repercussions (Faria *et al.*, 2022b; García-Gómez *et al.*, 2020; Sempere-Valverde *et al.*, 2021).

The ability of *R. okamurae* to proliferate rapidly can produce a strong impact on native natural communities, such as seagrasses, reducing their extension and associated biodiversity as well as negative effects on both economic activity and tourism (Bottalico *et al.*, 2024). In invaded areas, this species can cover 80–100% of the seabed, causing the disappearance of many native macroalgae and invertebrate species. It forms dense mats that outcompete native marine flora and fauna, leading to a decline in local biodiversity (Garcia-Gomez *et al.*, 2021; Estevez *et al.*, 2022).

The overgrowth of this alga disrupts the natural balance of marine ecosystems, affecting food webs and habitat structures, with cascading effects on various marine organisms, from invertebrates to larger predators. Beyond ecological impacts, this species causes significant socioeconomic costs, particularly to fishing and tourism, with estimated losses of millions of euros. For example, economic impacts have been estimated at EUR  $0.8 \times 10^6$  in nine months in the fishery industry due to reduced captures and EUR  $0.4 \times 10^6$  in the same period due to the removal of drifted material from beaches (Altamirano *et al.*, 2021). Considering the reports known to date, the record of *R. okamurae* in the Adriatic Sea, the easternmost one in the Mediterranean basin, confirms an expanding trend of the invasive species. Maritime transport can be considered as potential primary introduction pathways,



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given that the port of Bari is crossed by numerous cruise and commercial routes (Bottalico *et al.*, 2024), but the trade of extra-Mediterranean fishery and aquaculture products have to be considered also (i.e. the Japanese oyster or other species). The rapid expansion of *R. okamurae* is raising concerns; thus, in order to safeguard native communities, it is crucial to keep monitoring this invasive species (Marletta *et al.*, 2024), using targeted and specific monitoring protocols for evaluating population dynamics and the effects on native communities (Bottalico *et al.*, 2024). For a comprehensive overview of the ecological and economic impacts of *R. okamurae* in Europe, the European Alien Species Information Network (EASIN) provides detailed insights into the associated consequences. However, there is a pressing need for coordinated monitoring, early detection, and management strategies to mitigate the adverse effects of *R. okamurae* on marine ecosystems and economies.

As regards the Apulian pilot area, to date it is noteworthy to report that the offices of the Puglia Region convened a coordination table on the subject in December 2023, and the Italian Ministry of the Environment in January 2024 proposed and prescribed to the Puglia Region to implement any eradication measures as made mandatory by art. 17 of EU Regulation 1143/14 and art.19 of Italian Legislative Decree 230/17.

### 2.1.2. *Codium fragile* subsp. *fragile* (Suringar) Hariot 1889

*Codium fragile* subsp. *fragile* is widely considered as a troublesome invader (DAISIE, 2009). This coastal and estuarine green alga is native to the Pacific Ocean (Japan, Korea) and has become invasive, spreading to the Northeast Pacific, the North Atlantic, Australia, and New Zealand (Dromgoole, 1975; Schmidt and Scheibling, 2005). It is now widely distributed throughout all three major oceans (Hubbard and Garbary, 2002; Provan *et al.* 2005, 2008; Schmidt and Scheibling, 2005). As regards the Mediterranean Sea, *Codium fragile* subsp. *fragile* was found widely distributed along the Adriatic coast (Falace *et al.*, 2010; Lipej *et al.*, 2012), especially on artificial reefs (Bulleri *et al.*, 2006) and in harbors.



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The successful invasion of *C. fragile* subsp. *fragile* has been related to its wide physiological tolerance, reproductive mode (sexual, parthenogenetic, and vegetative), growth strategies, and grazing defense mechanisms (Falace *et al.*, 2010). The potential vectors of introduction and dispersal of *C. fragile* in the Mediterranean Sea have been recognized as fouling of ship hulls and importation of Asian shellfish (Zenetos *et al.*, 2012). The presence of man-made sheltered hard substrates in the vicinity of major commercial ports and the sources of eutrophication could increase the dispersal of invasive species on a regional and geographical scale (Bulleri *et al.*, 2006).

*Codium fragile*'s invasion can impact on productive marine habitats such as kelp and seagrass beds (Schmidt and Sheibling, 2006; Kelly *et al.*, 2011), potentially affecting biodiversity of coastal habitats if mismanaged (Bulleri *et al.*, 2006). Economic impacts of the species include interference with fishing gear and aquaculture, as well as with recreational use of the coastal areas, due to masses of the slimy weed washed up on beaches (Ramus 1971; Boudouresque *et al.*, 1994).

Due to the invasive features of this species, it is necessary to be always on one's guard; to date, any management strategies have been developed for this NIS in the Puglia Region.

### 2.1.3. *Ostreopsis cf. ovata* Y. Fukuyo 1981

*Ostreopsis cf. ovata* has been mentioned since the 1970s in the Mediterranean Sea, but it has become increasingly frequent with massive reported blooms in the last decades (Neves *et al.*, 2018). The species produces toxins, in particular palytoxins and new analogues (i.e. Ovatoxin-a) (Ciminiello *et al.* 2008; Guerrini *et al.*, 2008), causing detrimental effects on marine benthic communities (Pavaux *et al.*, 2019), and on human health through toxic aerosols (Gallitelli *et al.*, 2005; Brescianini *et al.*, 2006; Vila *et al.*, 2016), skin contact (Tubaro *et al.*, 2011) and contaminated seafood (Amzil *et al.*, 2012).

As regards Italian seas, the presence of the species is currently reported in almost all Italian coastal regions (ISPRA, 2024). Especially in the summer period, blooms of *Ostreopsis cf. ovata* have been recorded in Italian coastal areas with health implications also (Bottalico *et*



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*al.*, 2002; Di Turi *et al.*, 2003; Ungaro *et al.*, 2005; Barone and Prisinzano, 2006; Congestri *et al.*, 2006; Bianco *et al.*, 2007; Monti *et al.*, 2007; Totti *et al.*, 2007; Ungaro *et al.*, 2008; Ungaro *et al.*, 2010). For instance, in summer 2005, approximately 200 individuals along the Ligurian coast of Italy required medical attention due to symptoms such as respiratory irritation, general malaise, headache, low fever, eye irritation, and dermatitis (Gallitelli *et al.*, 2005; Durando *et al.*, 2007; Tichadou *et al.*, 2010; Pfannkuchen *et al.*, 2012; Berrdalet *et al.*, 2022; Vila *et al.*, 2016; Paradis *et al.*, 2024). Blooms have also been associated with mass mortalities of marine organisms, including sea urchins and fish, disrupting local marine ecosystems (Faimali *et al.*, 2012; Guidi-Guilvard *et al.*, 2012; Gorbi *et al.*, 2013; Neves *et al.*, 2018; Pavaxau *et al.*, 2020).

So, the interaction between the presence/ abundance of *O. cf. ovata* and the recreational use of beaches (bathing), together with the potential risk for fishery (nets manipulation) and shellfish farming (toxin contamination), was the main reason to carry out the monitoring of this NIS epi-benthic dinoflagellate along Italian coastal regions.

The presence of *O. cf. ovata* and other potentially toxic benthic microalgae is monitored through national surveillance programs carried out by the Regional Environmental Protection Agencies (ARPA) according to the Ministerial Decree of April 19, 2018. The ISTISAN Report 14/19 (Funari *et al.*, 2014) is the reference document for managing the presence of *O. cfr. ovata* in Italian marine-coastal environments. Monitoring, carried out during the bathing season, is divided into three phases: routine, alert, and emergency.

For the routine phase, ARPA Puglia monitors from June to September, fortnightly (increased in case of clear flowering), 20 sites distributed throughout the regional territory and representative of the coastal typology potentially affected by the presence of the species (Table 1).



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Monitoring of <i>Ostreopsis ovata</i>				
Site name	Latitude	Longitude	Municipality	Province
Tremiti (S. Domino - sotto il ristorante Il Pirata)	42.121200	15.496800	Isole Tremiti	Foggia
Loc. Pietra nera 30 mt dx canale	41.933500	15.928300	Vico del Gargano	Foggia
Porto di Vieste (100 mt dx)	41.888100	16.178500	Vieste	Foggia
Spiaggia Pugnochiuso	41.785400	16.188400	Vieste	Foggia
Spiaggia Baia delle Zagare	41.748300	16.146900	Mattinata	Foggia
Bisceglie (500 mt sud fogna citt.na)	41.241200	16.526500	Bisceglie	Barletta – Andria - Trani
Molfetta 1a cala	41.198400	16.615900	Molfetta	Bari
Giovinazzo (Riva del Sole)	41.173700	16.717100	Giovinazzo	Bari
Giovinazzo (200 mt lido Lucciola)	41.170400	16.733200	Bari	Bari
Bari (Lido Trullo)	41.108100	16.937500	Bari	Bari
Mola di Bari (ditta IOM - ex Sansolive)	41.044500	17.129400	Mola di Bari	Bari
Monopoli (Castello S. Stefano)	40.930500	17.333300	Monopoli	Bari
La Forcatella prima casa bianca	40.854800	17.445600	Fasano	Brindisi
Torre Canne (di fronte al Faro)	40.841900	17.471100	Fasano	Brindisi
Brindisi (Apani, lido S. Vincenzo)	40.696200	17.827800	Brindisi	Brindisi
Lecce - San Cataldo (vicino al Faro)	40.393400	18.305400	Lecce	Lecce
Porto Badisco - scalo di Enea	40.080100	18.484600	Otranto	Lecce
Ugento (ex scarico Ittica Ugento)	39.850100	18.171600	Ugento	Lecce
Torre Columena (spiaggia libera)	40.295700	17.745900	Manduria	Taranto
Taranto (stabilimento Baia d'Argento)	40.361400	17.330200	Leporano	Taranto

Table 1. Name, coordinates, municipality and province of the 20 sites monitored in Puglia Region for the presence of *Ostreopsis ovata*. In grey, the sites belonging to the Pilot area of the ALIENA Project.

This phase includes the following conditions:

1. Cell density in the water column  $\leq 10,000$  cells/L, regardless of meteorological and marine conditions;
2. Cell density in the water column of 10,000-30,000 cells/L, coinciding with unfavorable weather conditions for bloom development over an extended period (7-10 days).



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Health risks under these conditions are negligible, and no actions are required. However, if potential effects are reported, necessary health prevention measures should be taken, and the activities outlined in the alert phase should be initiated.

The alert phase is triggered under the following conditions:

1. *O. cf. ovata* density in the water column of 10,000-30,000 cells/L, with favorable weather and marine conditions for bloom development over an extended period (7-10 days).
2. *O. cf. ovata* density in the water column of 30,000-100,000 cells/L, with unfavorable weather conditions for aerosol and/or seawater spray formation on the beach (strong winds and storms).

Under these conditions, bathers may develop symptoms, although at a low incidence and reversible within a few hours; so, in such a situation the water-based recreational activities may be discouraged. Adequate information should also be provided to professional and recreational fishermen as well as to the citizens about the risks associated with consuming seafood harvested in the affected bloom area. During the alert phase, the extent of the bloom-affected area is defined, the frequency of observations and monitoring is increased, and health authorities are informed. The Mayor, as the local health authority, upon receiving information from the Regional Environmental Protection Agency (ARPA) and the Local Health Authority (ASL), informs the citizens to prevent hazardous exposure through signage, bulletins, local information systems, brochures and ordinances. Moreover, the Mayor communicates the management measures implemented at this stage to the Italian Ministry of Health and the Ministry of Environment.

The emergency phase is triggered under the following conditions:

1. *O. cf. ovata* density in the water column >30,000 cells/L, with favorable weather and marine conditions for seawater spray formation and aerosol (strong onshore winds and storms).
2. *O. cf. ovata* density in the water column >100,000 cells/L, regardless of meteorological conditions.
3. Presence of surface dense material resulting from *O. cf. ovata* blooms.



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Under the conditions 2 and 3, the risk of developing systemic clinical symptoms due to involuntary or accidental ingestion of water can be significant, making it necessary to ban swimming and other recreational activities in the affected area. Additionally, it would be advisable to quantify palytoxins in aerosols. During the alert and emergency phases, public information is provided through national and local institutional portals (Ministry of Health, Regions, ASL, Municipalities), bulletins, signage, and beach information boards.

Overall, the management system for the invasive species *Ostreopsis cf. ovata* described above has been conducted over the years and has enabled the collection of a large amount of valuable data and information regarding this species and its bloom dynamics. These data have been crucial in understanding its ecological behavior, environmental triggers, and potential impacts on human health and marine ecosystems. The knowledge gained through these monitoring efforts is highly relevant, providing a solid scientific basis for developing and implementing effective management and mitigation strategies tailored to invasive marine species.



### 3. Pilot Area “The rocky shore in the Gulf of Trieste and 1 or 2 marine sites”

#### 3.1. List of the existing management systems related to target species already in place

##### 3.1.1. *Ostreopsis cf. ovata* Y. Fukuyo 1981

The non-indigenous benthic microalga *Ostreopsis cf. ovata* has been detected in the Mediterranean Sea since the 1970s. The first report in Italy dates back to 1989 along the Tyrrhenian coasts of Lazio. Since then, this microalga and its blooms have been increasingly recorded in Italy (Funari *et al.*, 2014). With rare exceptions, almost all Italian coastal regions have been affected by this phenomenon, making it necessary to implement a national-scale management system for this species.

The presence of *O. cf. ovata* and other potentially toxic benthic microalgae is monitored through national surveillance programs carried out by the Regional Environmental Protection Agencies (ARPA). Specifically, since 2010, coastal ARPAs have been monitoring potentially toxic microalgae in bathing waters in compliance with Article 3 of the Ministerial Decree of March 30, 2010 (Ministry of Health, 2010), subsequently amended by the Ministerial Decree of April 19, 2018. Following this amendment, the ISTISAN (Istituto Superiore di Sanità) Report 14/19 became the reference document for managing the presence of *O. cf. ovata* in Italian marine-coastal environments. The control activities outlined in these guidelines contribute to understanding the distribution and bloom dynamics of *O. cf. ovata*.

The *O. cf. ovata* surveillance plan includes activities such as monitoring the microalga in bathing waters and health surveillance in coastal areas. These activities aim to prevent



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health issues associated with *O. cf. ovata* exposure for beachgoers and coastal visitors and to minimize the occurrence of symptoms caused by contact with water affected by blooms. Therefore, these activities should enable early identification of the presence of *O. cf. ovata* in the marine environment and any suspected human cases related to exposure to this alga.

High-risk and medium-risk coastal areas, along with their respective sampling points, have been identified and monitored by individual regions. The selection of these points is based on the higher probability of *O. cf. ovata* occurring at high densities and on the greater frequency of bathers in these areas.

Monitoring is conducted during the bathing season and is divided into three phases: routine, alert, and emergency. The monitoring period and frequency of checks are adjusted according to the phase and the risk profile of the affected area.

The routine phase involves activities carried out from June to September with a biweekly frequency, intensified in case of bloom presence. This phase includes the following conditions:

1. Cell density in the water column  $\leq 10,000$  cells/L, regardless of meteorological and marine conditions;
2. Cell density in the water column of 10,000-30,000 cells/L, coinciding with unfavourable weather conditions for bloom development over an extended period (7-10 days).

Health risks under these conditions are negligible, and no actions are required. However, if potential effects are reported, necessary health prevention measures should be taken, and the activities outlined in the alert phase should be initiated.

The alert phase is triggered under the following conditions:



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1. *O. cf. ovata* density in the water column of 10,000-30,000 cells/L, with favourable weather and marine conditions for bloom development over an extended period (7-10 days).
2. *O. cf. ovata* density in the water column of 30,000-100,000 cells/L, with unfavourable weather conditions for aerosol and/or seawater spray formation on the beach (strong winds and storms).

Under these conditions, bathers may develop symptoms, although at a low incidence and reversible within a few hours. Adequate information should also be provided to professional and recreational fishermen about the risks associated with consuming seafood harvested in the affected bloom area, and water-based recreational activities may be discouraged.

During the alert phase, the extent of the bloom-affected area is defined, the frequency of observations and monitoring is increased, and health authorities are informed. The Mayor, as the local health authority, upon receiving information from the Regional Environmental Protection Agency (ARPA) and the Local Health Authority (ASL), informs the public to prevent hazardous exposure through signage, bulletins, local information systems, brochures and ordinances. Moreover, the Mayor communicates the management measures implemented at this stage to the Ministry of Health and the Ministry of Environment and Energy Security (MASE).

The emergency phase is triggered under the following conditions:

1. *O. cf. ovata* density in the water column >30,000 cells/L, with favourable weather and marine conditions for aerosol and seawater spray formation (strong onshore winds and storms).
2. *O. cf. ovata* density in the water column >100,000 cells/L, regardless of meteorological conditions.
3. Presence of dense surface material resulting from *O. cf. ovata* blooms.



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Under these conditions, the risk of developing systemic clinical symptoms due to involuntary or accidental ingestion of water can be significant, making it necessary to ban swimming and other recreational activities in the affected area. Additionally, it would be advisable to quantify palytoxins in aerosols.

During the alert and emergency phases, public information is provided through various national and local institutional portals (Ministry of Health, Regions, ASL, Municipalities), bulletins, signage, and beach information boards.

### **3.1.2. *Mnemiopsis leidyi* A. Agassiz, 1865**

*Mnemiopsis leidyi* is an IAS sensu IUCN and is considered one of the world's worst invaders. However, it is not listed as an IAS of Union concern sensu Reg. EU No 1143/2014 and, therefore, no management systems are currently in place for this species. Moreover, it is not considered an IAS of regional concern, despite its significant impact on small-scale fisheries in the Friuli Venezia Giulia Region (Tirelli, 2019).

### **3.1.3. *Rugulopteryx okamurae* (E.Y. Dawson) I.K. Hwang, W.J. Lee & H.S. Kim, 2009**

*Rugulopteryx okamurae* is an IAS sensu IUCN and is included among the IAS of Union concern on the basis of Commission Implementing Regulation (EU) 2022/1203 of 12 July 2022 amending Implementing Regulation (EU) 2016/1141 to update the list of invasive alien species of Union concern.

National legislation has been adapted to Reg. EU No. 1143/2014 by Italian Legislative Decree No. 230 of 15 December 2017, which entered into force on 14 February 2018. This Decree-Law entrusts the Regions with a number of tasks related to the control of IAS, including the enforcement of bans and related penalties, the obligation to apply rapid eradication measures (i.e. the complete and permanent eradication of small populations of



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IAS of EU concern), and the identification of management measures for species found to be widespread on national territory or in inland or marine territorial waters. In the Friuli-Venezia Giulia Region, the Regional Strategy for the Control of Invasive Alien Species for the period 2021-2026 (updated by DGR 6 June 2022, n. 811) defines the regional organization in the field of IAS, the known situation of IAS in the Region and the objectives, general criteria and priorities for action. The regional strategy is a dynamic tool that needs to be updated on the basis of new data on the presence of IAS of EU and regional concern in the FVG. For this reason, the Friuli-Venezia Giulia Region has set up a surveillance system consisting of more than 100 regional staff, trained and operational in the territory, with the task of monitoring IAS (Bolognini *et al.*, 2024).

In the future, *R. okamurae* should be considered at the level of the Regional surveillance system as a possible newcomers IAS of Union concern through a preventive surveillance system.



## 4. Pilot Area “The western Istrian coast & Northern Adriatic: several sites on rocky shore and RV001 station 1 nm from coast”

### 4.1. List of the existing management systems related to target species already in place

#### 4.1.1. *Ostreopsis cf. ovata* Y. Fukuyo 1981

*Ostreopsis cf. ovata* is an epiphytic dinoflagellate that inhabits benthic macroalgae or substrata. In the coastal areas of the eastern northern Adriatic, where brown algae are widespread, the genus *Ostreopsis* can occur in large numbers, especially in shallow coastal sites along western Istrian peninsula (Pfannkuchen *et al.*, 2012, Kužat, 2021). Due to its epiphytic and benthic lifestyle, *Ostreopsis* is difficult to detect during regular monitoring activities for phytoplankton community, as the usual phytoplankton sampling with nets or a niskin bottles is not entirely suitable for this genus. The first identification of *Ostreopsis cf. ovata* in Croatian waters was made in 1984 in Kaštela Bay in the central Adriatic (Marasović 1990) and later has been found on the Istrian peninsula by Pfannkuchen *et al.* (2012), Kužat *et al.* (2021). There is no national monitoring dealing with this species.

It is likely that the species remained undetected, usually there were no visible blooms but just occasionally some biofilm of floating aggregations of *Ostreopsis* cells (Pfannkuchen *et al.*, 2012) can be seen on the surface. Cases of respiratory problems and skin irritation in humans associated with massive blooms of *O. cf. ovata* in Croatian waters were first reported in the northern Adriatic in 2010 (Pfannkuchen *et al.*, 2012) but often they are mistaken by allergies and flu. Still, an extensive approach to *Ostreopsis* monitoring and research is missing for the eastern Adriatic Sea. In study from Kužat *et al.* 2021. detailed information about the distribution and genetic identification of the genus *Ostreopsis* along the Istrian coastal waters were given. Bloom periods were observed at the end of summer



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with higher abundances recorded at the northern stations compared to the south Adriatic (Figure 1). All sampled *Ostreopsis* populations were determined to be the species *Ostreopsis* cf. *ovata* by light microscopy and DNA based analysis.

Furthermore, all populations along the north-eastern (NE) Adriatic coast share the ITS1-5.8S-ITS2-28S genotype, identical with most of the populations recorded around the Mediterranean.

The spread of *O. cf. ovata* along the eastern Adriatic coast recorded in Kužat *et al.* 2021 paper indicates high potential of *Ostreopsis* blooms to form with even small scale and localized favorable conditions possibly allowing blooms with harmful effects on human health. During that period (in 2016), the abundance of *O. cf. ovata* reached  $6.5 \times 10^5$  cells  $g^{-1}$  macroalgae substrate on the stations in front Rovinj and Poreč (Figure 1- red).

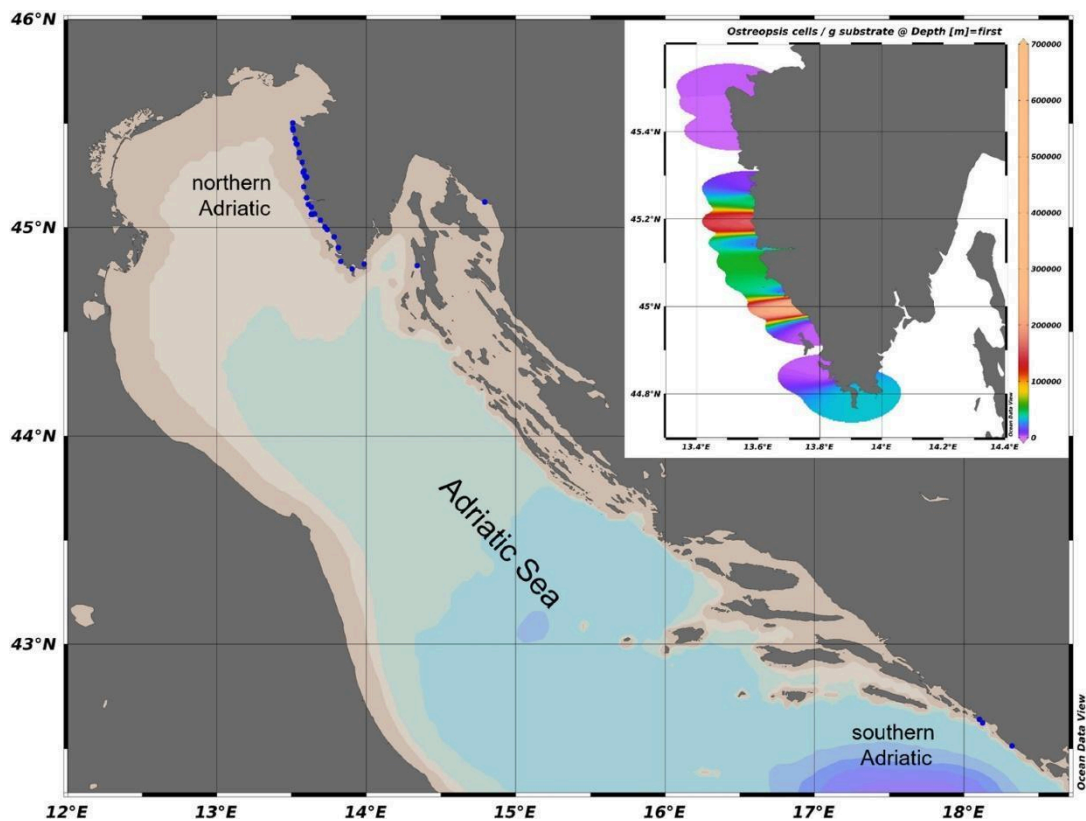


Figure 1. from Kužat *et al.* 2021. g. 1. Map of the investigated area (Adriatic Sea). Blue dots show sampling sites. An inset depicts the eastern coast of the northern Adriatic in more detail with a color coded representation of *O. cf. ovata* abundances. Stations



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are numbered 1–31 from north to south along the coastline.

Observed occurrences and abundances indicate that the *O. cf. ovata* bloom intensity is not evenly distributed with large scale dimensions, but is rather highly structured with a marked local (small scale) abundance variability. Geographical distance between some northern Adriatic stations was just a few kilometers and the abundances variability were pronounced. This might reflect the small-scale heterogeneity of ecological conditions along the eastern Adriatic rocky coastline. Previous studies in the Adriatic Sea mostly referred to sporadic sampling of *Ostreopsis* and therefore there is no national or regional monitoring program for this toxic dinoflagellate. Furthermore, there is no register number of harmful events associated with this species. There are no alerts or emergency systems in Croatian waters that can inform the public about the possible occurrence of this species although in last 15 years toxic *Ostreopsis* blooms were recorded multiple times with very high abundances.

### 4.1.2. *Mnemiopsis leidyi* A. Agassiz, 1865

*Mnemiopsis leidyi* is a lobate ctenophore belonging to the phylum Ctenophora, commonly known as the sea walnut or warty comb jelly. Its native range extends along the eastern coasts of North and South America, particularly in temperate and subtropical estuarine waters and bays, where it thrives across a broad range of temperatures and salinities. However, *M. leidyi* has proven to be a highly successful invasive species, expanding its range into the Mediterranean and adjacent seas, as well as the North and Baltic Seas (Shiganova *et al.*, 2019; Shiganova *et al.*, 2020).

To date, *M. leidyi* has also been observed in coastal waters of Italy (Boero *et al.*, 2009), Spain (Fuentes *et al.*, 2010), France (Delpy *et al.*, 2016), and the northern Adriatic Sea (Shiganova & Malej, 2009).

*M. leidyi* is an IAS sensu IUCN and is considered one of the world's worst invaders. However, it is not listed as an IAS of Union concern sensu Reg. EU No 1143/2014 and, therefore, no management systems are currently in place for this species. Moreover, it is





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not considered an IAS of regional concern, despite its significant impact on small-scale fisheries along the west Istrian coast (Budiša *et al.*, 2021; Hamer *et al.*, 2022).



## 5. Pilot Area “Several sites in Kaštela Bay and Šibenik Bay area”

### 5.1. List of the existing management systems related to target species already in place

#### 5.1.1. *Ostreopsis cf. ovata* Y. Fukuyo 1981

*Ostreopsis cf. ovata* is an epiphytic dinoflagellate that inhabits benthic red and brown macroalgae (genera *Hypnea*, *Spyridia*, *Sargassum*). In the coastal areas of the eastern central Adriatic, where brown algae are widespread, the genus *Ostreopsis* can occur in large numbers, especially in shallow areas such as Kaštela Bay (Marasović, 1990). Due to its epiphytic and benthic lifestyle, *Ostreopsis* is difficult to detect during regular microscopic analyses of the phytoplankton community, as the usual phytoplankton sampling with nets or a plankton sampler is not entirely suitable for this genus. The first identification of *Ostreopsis cf. ovata* in Croatian waters was made in 1984 in Kaštela Bay in the central Adriatic (Marasović 1990).

It is likely that the species remained undetected thereafter, as there were no visible blooms and nor negative effects on human health were reported. Cases of respiratory problems and skin irritation in humans associated with massive blooms of *O. cf. ovata* in Croatian waters were first reported in the northern Adriatic Sea in 2010 (Pfannkuchen *et al.*, 2012).

The first detailed description of this species in the central Adriatic was by Ninčević *et al.* (2019). Species was confirmed by light and electron microscopy in conjunction with molecular and toxicological analyses. Although the article describes the occurrence of *Ostreopsis* only in Kaštela Bay, in the same summer period in 2015, complaints of respiratory and skin symptoms were reported from beaches along the coast of the city of Split, indicating a bloom of *O. cf. ovata*. During that period (in 2015), the abundance of *O. cf. ovata* reached  $2.9 \times 10^4$  cells  $L^{-1}$  in seawater, while on macroalgae it was estimated to be up to  $2.67 \times 10^6$  cells  $g^{-1}$  macroalgae fresh weight and  $14.4 \times 10^6$  cells  $g^{-1}$  macroalgae dry



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weight. The toxin profile of the *Ostreopsis* species present in Kaštela Bay, determined by indirect immunoenzymatic sandwich assay (ELISA) and LC-HRMS, showed a total toxin content of 5,6 pg PLTXeq per cell<sup>-1</sup> and by LC-HRMS 6.3 pg of the sum of OVTX and PLTX per *Ostreopsis* cell, with OVTX-a (3.6 pg per cell<sup>-1</sup>) being predominant (Ninčević Gladan *et al.* 2019).

Previous studies in the central Adriatic mostly referred to sporadic occurrences of *Ostreopsis* and therefore there is no national or regional monitoring program for this toxic dinoflagellate. Furthermore, there is no register recording the number of harmful events associated with this species.

Toxic phytoplankton species are regularly monitored on a weekly basis in the mussel farming areas of the central Adriatic (Narodne novine 13/2013, Croatian Ministry of Agriculture, Forestry and Fisheries, 2024), and as part of this monitoring we have found *Ostreopsis* only sporadically. In recent years, for example, it occurred in low abundance in Kaštela Bay in 2021-2024 (abundance 160 cells /L and 3600 cells /L), as well as in the Šibenik Bay area in 2017 (1110 cells/m<sup>2</sup>) and in 2021 (80 cells /L).

There are no alerts or emergency systems in Croatian waters that can inform the public about the possible occurrence of this species.

### 5.1.2. *Pseudodiaptomus marinus* Sato, 1913

The marine calanoid copepod *Pseudodiaptomus marinus* Sato, 1913 was first identified from zooplankton collected from the west coast of Hokkaido, Japan (Sato, 1913). Since then, it has been reported from various marine regions of Japan, China and the coastal waters of the northwestern Pacific Ocean (De Olazabal and Tirelli, 2011). In the Adriatic Sea, this alien planktonic copepod was first detected in 2007 on the Italian coast near Rimini, and in 2009 it was discovered near the port of Monfalcone in the Gulf of Trieste, most likely introduced by ballast water (De Olazabal and Tirelli, 2011). Although the first records of *P. marinus*



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were limited to the northern Adriatic (e.g. Bay of Trieste, Port of Koper, Venice Lagoon) (de Olazabal and Tirelli, 2011; Lučić *et al.*, 2015; Vidjak *et al.*, 2019), its presence was soon detected further south.

In the Croatian Adriatic, *P. marinus* was first observed in Šibenik Bay in the Krka River estuary in 2015, whereupon several new locations were soon confirmed along the east coast, all closely associated with port activities: in 2018 it was detected in the port of Ploče (Neretva river estuary) and in 2022 in the eastern part of Kaštela Bay near the local cargo port (Uttieri *et al.*, 2023). Recent DNA metabarcoding analyzes revealed new distribution areas of *P. marinus* extending from the northern to the southern Croatian Adriatic coast (ports of Pula, Zadar, Dubrovnik and the city harbor of Split). (Lin *et al.*, 2022; Uttieri *et al.*, 2023). Studies of the ecology of *P. marinus* in Croatian waters revealed common ecological characteristics of this species, including preference for harbor areas, related to ballast water discharge, nocturnal migration to the water column while staying close to the bottom during the day, and evidence of rapid population establishment (Vidjak *et al.*, 2020). So far, no adverse impact on local zooplankton has been detected, however, as this copepod is spreading at an unprecedented rate in European and neighboring waters, the final consequences of its invasion are still uncertain (Uttieri *et al.*, 2023). *P. marinus* is characterized by the combination of several biological and behavioral traits (e.g. euryhaline and eurythermal nature, plasticity in feeding and habitat occupation) that could potentially enhance its invasive behavior under the right conditions.

At European level and under the auspices of ICES, the working group WGEUROBUS (Towards a EUROpean OBservatory of the non-indigenous calanoid copepod *Pseudodiaptomus marinus*) was established in 2018, which has successfully created a European network of institutions and researchers working on the various aspects of the biology and ecology of *P. marinus*. One of the results are regularly updated distribution maps of *P. marinus* in European waters (Uttieri *et al.*, 2020, 2023) (Figure 2). *P. marinus* is currently present in 7 of the 10 European marine subregions identified under the MSFD (2008/56/EC), making it one of the most widespread non-indigenous copepod species (Tsiamis *et al.* 2019).



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As ballast water remains the most likely primary vector, followed by secondary spread to nearby coastal areas, measures to prevent the uncontrolled and untreated discharge of ballast water are of paramount importance for containing the spread of *P. marinus* (and other ballast water-mediated non-indigenous species). In general, management options for planktonic species are very limited and eradication techniques are often not possible (Hayes *et al.*, 2005). To date, there are no proposals for specific management of *P. marinus* and all efforts are currently focused on ballast water management under the Ballast Water Convention Management, which entered into force in 2017 (BWCM). In the Croatian Adriatic, the occurrence and abundance of *P. marinus* is monitored under Descriptor 2 (Non-indigenous species) of the Marine Strategy Framework Directive (2008/56/EC), and targeted monitoring is conducted in two areas where its presence has been confirmed: Šibenik Bay and the Neretva River estuary. To improve the monitoring strategy, a molecular approach (DNA metabarcoding) has been proposed for regular use (Uttieri *et al.*, 2020). According to current knowledge, this species does not pose a health risk. However, the ecological risks are not sufficiently known.

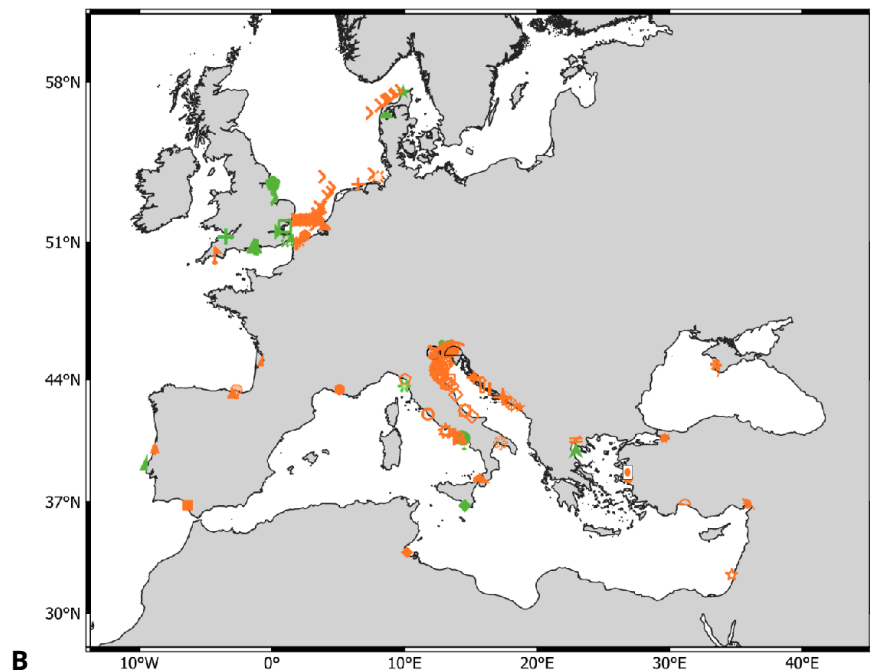
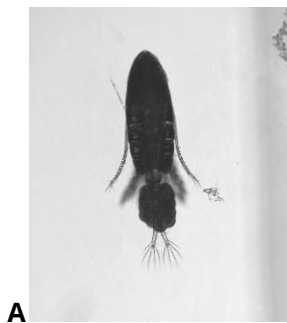


Figure 2. A-Pseudodiptomus marinus (egg-carrying female); B- Updated distribution map of P. marinus in European waters (from Uttieri et al., 2023; at <https://doi.org/10.3390/jmse11061238>)

## 6. Conclusion

Cross-border cooperation is crucial for achieving the objectives of European Union (EU) policies, as cooperation and decision-making between different governments and international and non-governmental organizations is essential to address issues that transcend national borders, such as the problem of IAS. Indeed, all EU Member States (MS) have been affected by IAS to varying degrees and have taken different approaches to address the problem (European Commission, 2021). However, many of these actions have been fragmented and uncoordinated (Caffrey *et al.*, 2014).

Monitoring and management of IAS varies between MS and is carried out at different scales, from local to regional (Magliozzi *et al.*, 2024b and references therein). Indeed, active monitoring of IAS requires considerable time and resources, often leading to delays or failure to achieve containment targets (Haubrock *et al.*, 2021). Therefore, cooperation and coordination between regional, national and supranational authorities, both in the monitoring and management phases, is crucial for the implementation of the IAS regime, but transnational cooperation and coordination is often difficult due to language barriers, different organizational contexts, differences in sampling designs and protocols, and capacity to cooperate (Magliozzi *et al.*, 2024b and references therein).

Magliozzi *et al.* (2024b), discussing transnational cooperation in the EU and ways to address IAS, proposed the following recommendations:

- Emphasise the need to use existing information platforms such as EASIN;



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- Stress the importance of organising regular workshops to update priorities and management practices;
- Stress the importance of organising permanent working groups on IAS management methodologies to ensure harmonised approaches;
- Exchange standardised protocols for monitoring and management of IAS;
- Stress the need to conduct follow-up surveys to evaluate monitoring and management efforts;
- Develop pilot studies in local areas within the EU;
- Highlight the importance of information campaigns to raise awareness among stakeholders.

In this context, the ALIENA project is fully in line with some of the above recommendations. In particular, the definition of common standardized protocols for the monitoring and management of the target NIS for each pilot area is one of the main objectives of the project, together with their dissemination to stakeholders.

The review of the management strategy already in place for each target species can be summarized as follows:

***Ostreopsis cf. ovata***: there are no warning or emergency systems in Croatian waters to inform the public of the possible occurrence of this species, although several toxic *Ostreopsis* blooms with very high abundances have been recorded in the last 15 years. Conversely, in Italy the presence of *O. cf. ovata* is monitored by national surveillance programs carried out by the Regional Environmental Protection Agencies (ARPA). The surveillance, carried out during the bathing season, is divided into three phases: routine, alert and emergency. During the alert and emergency phases, the public is informed through national and local institutional portals (Ministry of Health, Regions, Local Health Authorities, Municipalities), bulletins, signage and beach information boards. *O. cf. ovata* has been identified as a target species in all pilot areas of the ALIENA project and this will



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be the opportunity to establish a common approach to the management of this IAS in the Adriatic Sea.

***Mnemiopsis leidyi***: it is one of the world's worst invaders, but is not listed as an IAS of Union concern and therefore no management systems are currently in place for this planktonic species. Nevertheless, it is massively abundant, especially in the northernmost part of the Adriatic (e.g. western Istria and the Gulf of Trieste), where it has a significant impact on fisheries.

***Rugulopteryx okamurae***: it is considered the fourth most damaging species among the 10 most invasive species reported in the Mediterranean. As part of the EU Regulation 1143/2014, adopted to prevent and manage the introduction and spread of invasive alien species, *R. okamurae* is the only macroalgae included in the list of EU relevance. Among the pilot areas, it has so far only been detected along the rocky coast of Puglia, where the Italian Ministry of the Environment in January 2024 proposed and prescribed to Apulia Region to implement eradication measures, as required by Art. 17 of EU Regulation 1143/14 and art.19 of Italian Legislative Decree 230/17. *R. okamurae* was also identified as a target species for the WP2 activities in the pilot area 2 (Gulf of Trieste), as this species could be a possible newcomer mainly due to the presence of the port of Trieste.

***Codium fragile subsp. fragile***: it is widely regarded as a troublesome invader. Economic impacts of the species include interference with fishing gear and aquaculture, as well as recreational use of coastal areas. To date, no management strategies have been developed for this NIS in the Puglia Region or in the other pilot areas.

***Pseudodiaptomus marinus***: in the Croatian Adriatic, the occurrence and abundance of this species is monitored under Descriptor 2 (Non-indigenous species) of the Marine Strategy Framework Directive (2008/56/EC), and targeted monitoring is conducted in two areas where its presence has been confirmed: Šibenik Bay and the Neretva River estuary. To date, there are no proposals for specific management of *P. marinus* and all efforts are



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currently focused on ballast water management under the Ballast Water Convention Management, which entered into force in 2017 (BWCM). In any case, management options for planktonic species are very limited and eradication techniques are often not possible.



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