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Working Group on Introductions and Transfers of Marine Organisms (WGITMO)

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WORKING GROUP ON INTRODUCTIONS AND TRANSFERS OF MARINE ORGANISMS (WGITMO)

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ICES International Council for the Exploration of the Sea
CIEM Conseil International pour l'Exploration de la Mer

Contents

i Executive summary ii
ii Expert group information iii
1 Introduction 1
2 ToR A: National Reports 1
2.1 AquaNIS Database 2
2.2 Historical Perspective on Introductions of Non-Native Species 3
2.3 National Report Overview 2019–2021 4
3 ToR B: Climate Change Impact on Spread of NIS 10
4 ToR C: Biofouling as a Vector for NIS 12
5 ToR D: Indicators of Impacts of NIS on Marine Environments 15
6 ToR E: Molecular Approaches for NIS 19
7 ToR F: Marine Debris as a Vector for NIS 21
8 ToR G: Aquaculture as a Vector for NIS 21
9 Other Activities and Next Steps 23
Annex 1: List of participants 24
Annex 2: WGITMO resolution 33
Annex 3: WGITMO National Reports 37

i Executive summary

The goal of the ICES Working Group on the Introduction and Transfers of Marine Organisms (WGITMO) is to contribute to the ICES vision and mission by addressing specific science objectives related to the introduction, spread and impacts of non-indigenous marine species (NIS). The expert working group provides information and advice on the impacts of human activities (e.g. pressures caused by invasive species, litter, aquaculture, biofouling) on marine ecosystems including the Arctic. The Terms of Reference include annual national reporting on NIS detections and research, climate change impact on the spread of NIS, the risk and impacts of NIS, vectors of spread of NIS including biofouling, marine debris and aquaculture.

This report summarizes the key findings and outcomes from the 2020–2022 term. A joint meeting in (2020) with WGBOSV [Ballast Water and Other Ship Vectors] and WGHABD [Harmful Algal Bloom Dynamics] was held to discuss scientific issues of joint interest including climate change in the Arctic environment, and early detection of and response to species through the use of innovative molecular tools. The discussion on molecular tools was used by the groups to develop the framework for a targeted Molecular Tools workshop in conjunction with at the International Conference for Marine Bioinvasions (ICMB) in May 2023.

National activities were presented and discussed from over 20 countries each year. Common themes included, new species introduction and monitoring, recent activities on traditional and innovative methods for detection and surveillance (including molecular tools). Science highlights include the submission of the ICES biofouling viewpoint to the International Maritime Organization (IMO) regarding biofouling guidelines, development and publication of a global scale NIS screening tool, and publication of a global review of NIS trends over 50 years. The Information system on aquatic non-indigenous and cryptogenic species AquaNIS continues as the main repository of new NIS data. The expert group will continue to work collaboratively with PICES, CIESM and other ICES working groups and international organizations focussing on impacts, innovative research tools, and vectors of NIS as it relates to human activities and pressures on marine ecosystems.

ii Expert group information

Expert group name	Working Group on Introduction and Transfers of Marine Organisms (WGITMO)
Expert group cycle	Multiannual Fixed Term
Year cycle started	2020
Reporting year in cycle	3/3
Chair	Cynthia McKenzie, Canada
Meeting venue(s) and dates	Gdynia, Poland, 4–6 March 2020 (60 participants)
	Online meeting, 1–3 March 2021 (64 participants)
	Online meeting, 4–6 May 2022 (63 participants)

1 Introduction

This report summarizes the work of the International Council for the Exploration of the Seas Working Group on the Introduction and Transfers of Marine Organisms (WGITMO) during the latest three-year term (2020–2022). Specifically, the progress made toward each of the seven terms of reference (ToRs) is detailed in the following sections. After that, the next steps for the group reviewed: a new Chair has been elected, and new ToRs have been proposed for the next term (2023–2025). In the annexes, the participants in the group meetings are listed (Annex 1). Next, the ToRs are briefly summarized in a table, which includes each ToR's links to the ICES Science Plan (Annex 2). Finally, national reports for the 2019–2021 period are provided (Annex 3).

The expert group now has at least one member from each ICES country as well as several chair-invited experts from CIESM regional countries participating and contributing to this working group. The group is a good mix of long-term and medium-term contributors who are leaders in this field, as well as new members who are early career scientists.

The virtual meetings (2021, 2022) allowed for a high participation (64 participants) which included ICES country members and chair invited members participating in their first WGITMO meetings. However, it is important to note that following discussion regarding future meetings (virtual/in person/hybrid), members strongly supported inperson and/or hybrid meetings as the opportunity for networking or in-depth strategic planning was not possible during the shorter, half-day, virtual meetings. This was particularly true for our newer Terms of Reference on marine debris and aquaculture as vectors for NIS introduction and spread. The members were concerned that committed engagement, collaborations and WG productivity would be impacted if only virtual meetings were held. The next meeting of the working group will be held in Athens, Greece in March 2023 and will be a hybrid meeting.

2 ToR A: National Reports

Advance research, develop collaborations and address surveillance and knowledge gaps in issues related to the introduction and transfer of marine organisms, through annual reviews of national/ international activities and responding to advice requests

National activities were presented and discussed from over 20 countries each year. Common themes included, new species introduction and monitoring, recent activities on traditional and innovative methods for detection and surveillance (including molecular tools). Several opportunities for collaboration were advanced. Timely accessability of national reports was discussed (2020) with regards to the new ICES report guidelines. Publication and report posibilities were explored (Canada, Italy, Denmark, US) following the 2020 meeting in Poland and it was recommended that in years with no report, the national reports would be available on the open ICES WGITMO weblink. At the end of each three year cycle, the national reports for the three year period will be added to the ICES report as an Appendix. The value of these national reports for NIS are a unique long term record (almost 50 years in some cases) and provide added knowledge for current and future studies on the introduction, spread, vectors and impacts and

are a critical deliverable for this ToR. A three year overview by nation is provided in Section 2.3 with the full reports for 2019–2021 attached in Appendix 3.

Ecosystem overview (EO) NIS updates had been completed for the Bay of Biscay- Iberian Coast (BoB-IC) and the Celtic Seas (CS). An EO NIS update was recently provided for the North Sea by WGITMO and WGBOSV).

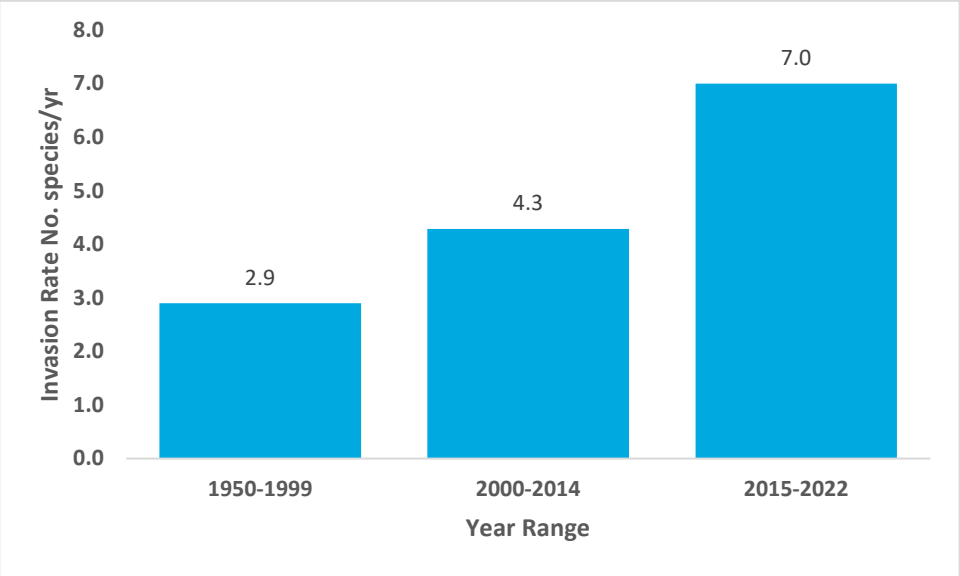

Figure 1. Rate of NIS introductions in the ICES North Sea Ecosystem. Data obtained from AquaNIS (accessed on 14 October 2022 by C.H. McKenzie; pers. comm. S. Olenin).

2.1 AquaNIS Database

The Information system on aquatic non-indigenous and cryptogenic species AquaNIS continues as the main repository of new NIS data with each country updating the database annually. AquaNIS (www.corpi.ku.lt/databases/aquanis) provides a unique opportunity to reveal data on new arrivals of introduced species at different geographical scales, i.e. recorded for the first time in: a particular recipient region (country coast) within the Large Marine Ecosystem (LME), a country (including coasts of the same country in different LMEs), a particular LME and, a larger biogeographical region, including, e.g. two or more neighbouring LMEs.

Two papers should be noted in particular as they demonstrate the use of the AquaNIS database and collaborations among WGITMO and WGBOSV members for a global review of trends in NIS over 50 years (Bailey *et al.* 2020, see Section 2.2) and a global scale screening tool for NIS (Vilizzi *et al.* 2021, see Section 5 ToR D).

Publications using AquaNIS data in 2019 and 2020 (AquaNIS. Editorial Board, 2022. Information system on Aquatic Non-Indigenous and Cryptogenic Species. World Wide Web electronic publication. www.corpi.ku.lt/databases/aquanis. Version 2.36+):

1. Casties, I. & Briski, E. (2019). Life history traits of aquatic non-indigenous species: freshwater vs. marine habitats. Aquatic Invasions, 14(4), 566–581. **View online**
2. Menéndez Teleña, D. (2019). Contaminación biológica de especies invasoras por agua de lastre e incrustaciones en el Puerto de Gijón. **View online**
3. Rey, A. (2019). From port to ballast water: application of ADN metabarcoding of ship-borne biodiversity. **View online**

4. Strubbe, D., White, R., Edelaar, P., Rahbek, C. & Shwartz, A. (2019). Advancing impact assessments of non-native species: strategies for strengthening the evidence-base. NeoBiota, 51, 41–64. **View online**
5. Bojko, J., Burgess, A.L., Baker, A. G. & Orr, C.H. (2020). Invasive non-native crustacean symbionts: diversity and impact. Journal of Invertebrate Pathology, article 107482. **View online**
6. Duarte, S., Vieira, P.E. & Costa, F.O. (2020). Assessment of species gaps in DNA barcode libraries of non-indigenous species (NIS) occurring in European coastal regions. Metabarcoding and Metagenomics, 4, article e55162. **View online**
7. Ramos-Esplá, A., Micael, J., Halldórsson, H.P., & Gíslason, S. 2020. Iceland: a laboratory for non-indigenous ascidians. BioInvasions Records 9 (3): 450-460. **View online**
8. Azevedo, J., Antunes, J.T., Machado, A.M., Vasconcelos, V., Leão, P.N. & Froufe, E. (2020). Monitoring of biofouling communities in a Portuguese port using a combined morphological and metabarcoding approach. Scientific Reports (Nature Publisher Group), 10(1), Article 13461. **View online**
9. Obst, M., Exter, K., Allcock, A. L., Arvanitidis, C., Axberg, A., Bustamante, M., Cancio, I., Carreira-Flores, D., Diego Carreira-Flores, Chatzinikolaou, E, Chatzigeorgiou, G., Chrismas, NM., Clark, M.S., Comtet, T., Dailianis, T., Davies, N. Deneudt, K., Diaz de Cerio, O., Fortič, A., Gerovasileiou, V., Hablützel, P.I., Keklikoglou, K., Kotoulas, G., Lasota, R., Leite, B.R., Loisel, S., Lévêque, L., Levy, L., Malachowicz, M., Mavrič, B., Meyer, C., Mortelmans, J., Norkko, J., Pade, N., Power, A.M., Ramšak, A., Reiss, H., Solbakken, J., Staehr, R.A., Sundberg, R., Thyrring, J., Troncoso, J.S., **Viard**, F., Wenne, R., Yperifanou, E.I., Zbawicka, M. & Pavloudi, C. 2020. A marine biodiversity observation network for genetic monitoring of hard-bottom communities (ARMS-MBON). Frontiers in Marine Science, 7, article 572680. **View online**
10. Shalovenkov, N.N. 2020. Tendencies of Invasion of Alien Zoobenthic Species into the Black Sea. Russian Journal of Biological Invasions, 11(2), 164–171. **View online**
11. Tsiamis, K., Azzurro, E., Bariche, M., Çinar, M.E., Crocetta, F., De Clerck, O., **Galil**, B., Gómez, F., Hoffman, R., **Jensen**, K.R., Kamburska,L., Langeneck, J., Langer, M.R., Levitt-Barmats, Y., Lezzi, M., **Marchini**, A., **Occhipinti-Ambrogi**, A., **Ojaveer**, H., Piraino, S., ShenkarMaria Yankova, N., **Zenetos**, A., Žuljević, A. & Cardoso, A.C. 2020. Prioritizing marine invasive alien species in the European Union through horizon scanning. Aquatic Conservation: Marine and Freshwater Ecosystems, 30(4), 794–845. **View online**

2.2 Historical Perspective on Introductions of Non-Native Species

In a project led by WGBOSV and in collaboration with the WGITMO, the temporal and spatial trends in the introduction of aquatic, non-native species were analyzed over a 50-year period (1965–2015). In total, >2200 records of detection across 49 aquatic ecosystems were analyzed. The 29 co-authors concluded that, on average, a new detection occurred every 8.4 days. Given that many introductions are not reported, this value is surely an underestimate, and the authors highlighted the urgent need for standardized detection methods to be employed over long-time scales. The results of this study can be used to inform management decisions. Limited resources must be allocated to reduce the transport, release, and spread of non-native species, which occur via multiple pathways. Additionally, this 50-year perspective provides an important lens through which the effects of regulatory measures can be assessed over time.

Bailey, S.A., Brown, L., Campbell, M.L., Canning-Clode, J., Carlton, J.T., Castro, N., Chainho, P., … Curd, A., Darling, J., Galil, B.S., … Marchini, A., McKenzie, C.H., Occhipinti-Ambrogi, A., Ojaveer, H., ..Ruiz, G.M.…Therriault, T.W. & Zhan, A. 2020. Trends in the detection of aquatic non-indigenous species across global marine, estuarine and freshwater ecosystems: A 50-year perspective. Diversity and Distributions, 26, 1780–1797. https://doi.org/10.1111/ddi.13167.

2.3 National Report Overview 2019–2021

New NIS detected in **Canada** include *Hemigrapsus sanguineaus* Asian Shore Crab, locations in St. Mary's Bay to Shelburne, Nova Scotia in 2020 and *Schizoporella japonica* Ortmann, bryozoan from Japan, reported for the first time in Magdalen Islands, Québec, in 2021. Other marine species that have already invaded Canadian waters continue to spread, including European green crab (*Carcinus maenas*), vase tunicate (*Ciona intestinalis*), carpet tunicate (*Didemnum vexillum)*, golden star tunicate (*Botryllus schlosseri*), violet tunicate (*Botrylloïdes violaceus*), clubbed tunicate (*Styela clava*), Japanese skeleton shrimp (*Caprella mutica*), coffin box (*Membranipora membranacea*), and oyster thief (*Codium fragile* subsup. *fragile*). Canada's Ocean Protection Plan includes policies, funding and activities to address the issue of derelict vessels which have been found to be a source of pollution and of biofouling including NIS. Transport Canada's program applies to larger commercial vessels and Fisheries and Oceans Canada (DFO) Small Craft Harbour (SCH) program applies to smaller fishing and recreational vessels. The goal is to remove these vessels as part of Canada's commitment to protecting the ocean environment. Fisheries and Oceans Canada has added AIS Core Management to its activities and each region has several personnel working on areas of AIS management including working on control licences for green crab to work with groups including Indigenous groups to form citizen science teams to monitor for AIS in remote locations.

A new database for registering all biodiversity in **Denmark**, including invasive alien species is now available. The NW Atlantic Sea slug (*Haminella solitaria*) was first observed with egg masses in Roskilde Fjord in the summer of 2020. The established population of *Hemigrapsus takanoi* in Dybsø Fjord, a lagoon connected to the southern part of the Great Belt, boomed during the summer of 2020. Species specific DNA barcodes were identified for several crab species. The amphipod, tiger shrimp (*Gammarus tigrinus*), has been documented in Danish waters for the first time at the Baltic Island Bornholm. The tanaid *Sinelobus vanhaareni* has been observed for the first time in Denmark in the harbour of Copenhagen. The Danish Environmental Protection Agency is now permanently established in Odense.

Finland collected the needed information for the first reporting of the EU IAS Regulation and reported presence/absence of the EU listed species in Finland in June 2019. The only aquatic species that was on the reported species list is Chinese mitten crab (*Eriocheir sinensis*), which is observed only few times a year. Two new non-indigenous species were observed in 2021 on the Finnish coastline in the Baltic Sea. *Rangia cuneata* clam and the Japanese cumacean (*Nippoleucon hinumensis*) were found 2021 the first time in Finland close to port areas in the Gulf of Finland. The round goby (*Neogobius melanostomus*) that was first recorded in Finland in 2005, has spread to almost entire Finnish coastal waters. The northernmost observation was made in 2019 in Oulu, Bothnian Bay. Also the tanaid (*Sinelobus vanhaareni*) has been spreading and increasing in abundance during the past years (first observation in 2016). A proposal on non-native species monitoring framework was completed and submitted to HELCOM providing monitoring guidelines for multiple methods to complement the NIS monitoring programme in COMPLETE project. Finland conducted NIS sampling events on three coastal marinas in 2018 (Southwestern Finland), while creating biofouling survey protocol for leisure boats and marinas. The protocol was further tested in a study on different anti-fouling paints on a sailing boat hull which was completed in 2020 (tasks of the COMPLETE project).

Twenty-six new introductions, all unintentional, were reported for the country or for new maritime regions within **France**. They include one macrophyte (*Halophila stipulacea*), three algae (*Lophocladia lallemandii, Ulva ohnoi*, *Symphiocladia tanakae*), six peracarids (*Aoroides longimerus & A. semicurvatus, Ericthonius didymus*, *Ianiropsis serricaudis, Paranthura japonica, Synidotea laticauda*), one amphipod *(Caprella scaura),* one pycnogonid (*Ammothea hilgendorfi),*one bryozoan *(Schizoporella japonica),* five annelids *(Bispira polyomman Marphysa victori, Polydora colonia, Terebella banksyi, Thelepus japonicus),* two molluscs *(Arcuatula senhousia, Lamprohaminoea ovalis),* two fish (*Cynoscion regalis*, *Holocentrus adscensionis)* and three tunicates (*Botrylloides diegensis, Didemnum pseudovexillum, Styela clava*), as well as a novel mussel lineage (resulting from admixture between native mussels and the introduced Mediterranean mussel in North Atlantic commercial ports). The blue crab *Callinectes sapidus* is undergoing a rapid population expansion in French Mediterranean coastal lagoons, leading to the adoption of a regional action plan in March 2022. The first review of NIS in the French overseas territories was carried out by the IUCN French committee. They reported 61 NIS, a third of which are ascidians, with the highest number (31) found in French Polynesia. In the absence of dedicated monitoring, these numbers are probably underestimated, as is likely to be the case in continental France. Several research programs targeted pathogens and diseases, notably the protist *Haplosporidium costale* responsible for an important die-off of the *Crassostrea gigas* oyster in one locality, and a transmissible cancer in mussels (for which a new strain has been identified, shared by the blue mussel and the Chilean mussel suggesting international shipping as pathways). Other research projects aimed to 1) examine the relationships between MPAs and invasive species, 2) analyze biofouling by NIS in ports and 3) develop molecular tools to support surveys and monitoring – including with eDNA and metabarcoding, tracing back introduction routes, and analyzing impacts on native species (hybridization). Following a national risk analysis exercise, eleven marine IAS have been shortlisted for inclusion in two different levels of national regulation.

The unintentional introduction of non-indigenous species (NIS) is an ongoing process. According to national **Germany** surveys new NIS were found each year (Table 1). Further reports from neighbouring countries documented NIS, which were not yet known from German coastal waters. Owing to the possibility of spread into German open waters, this information could be highly relevant for the implementation of national early warning and prevention measures.

Table 1. New unintentionally introduced species along the German North and Baltic Seas coasts.

Year	North Sea	Baltic Sea	Total
2019	5	3	8
2020	2	1	3
2021	-	-	
Total	7	4	11

We included those species with first record before 2019 for which their occurrence became known in the reporting period 2019–2021.

Regular and NIS targeted monitoring programmes occur along the German coast line, now with more than 20 sampling sites. Activities took place in form of extended rapid assessments (e-RAS) at hot spots like harbours, marinas and aquaculture sites with a sampling frequency of at least one event annually. In addition, some ports have been sampled according to the HELCOM/OSPAR Joint Harmonized Procedure sampling protocol and selected marinas were sampled with settlement plates. New findings in this reporting period[1] in the North Sea include *Schizoporella japonica* (Bryozoa), an unknown polychaete (Ampharetidae), *Hypereteone* cf. *lighti* (Polychata), bryozoan *Pacificincola perforate*, nudibranch *Corambe obscura*, amphipod *Aoroides semicurvatus*, Bacillariophyceae *Plagiolemma distortum* sp. nov., hermit crab *Pagurus longicarpus*. Along the Baltic costs these species were found: brown algae *Fucus distichus* (listed as F. edentatus), *Moerisia inkermanica* (Hydrozoa), cumacean *Nippoleucon hinumensis*, gammarid

Echinogammarus ischnus and two earlier recorded species occurred in new areas with eastwards spread, namely, the polychaete *Ficopomatus enigmaticus* and the oyster *Magallana gigas*.

Intentional living species introductions remain at similar levels as in the last years and include predominantly sturgeons, salmonid species, rainbow trouts, carps, Pacific oyster (*Crassostrea gigas*; both seed mussels and adults), scallops (*Mytilus* sp.), American lobster (*Homarus americanus*) and other decapods, cephalopods as well as the red alga (*Palmaria palmata*). Imports are predominantly from Ireland, United Kingdom and the Netherlands. In addition, a substantial amount of various living ornamental fish was imported. In some cases, the identification of species and whole samples follow with genetical methods (eDNA and metabarcoding). DNA studies may in the future reveal more introduced species. As an example, DNA analysis revealed presence of Japanese mitten crab sequences in specimens morphologically identified as Chinese mitten crab where were collected in Holland, Germany and Poland between 2009 and 2015. The German individual was collected inland in the Rhine River, and may not have necessarily migrated successfully to the North Sea for reproduction (Hayer *et al*. 2019).

In the years 2019–2021 twelve introduced species were reported in **Iceland**, seven newly established and five previous sightings. In total six of those twelve species were listed in 2021. Majority of the introduced species have first been reported at the S or W part of the country, and the seven newly established species in the period 2019–2021 were all reported at the southwest coast. The number of confirmed NIS in Iceland is now 32. Updated information on the colonization by NIS in selected locations and/or environments (like marinas and harbours) is provided. An expert group (ISAT) on non-indigenous species was formally established in Iceland in 2020 and has met several times in 2020 and 2021. NIS targeted monitoring program is now operated along the Icelandic coast. The program started in 2018 and focuses on hot spots like harbours and marinas. In total there are eight monitoring sites with fixed equipment, additionally other sites are also visited yearly in the form of rapid assessments survey (RAS).

The **Israeli** coastline, 190 km long, is the world's most invaded marine ecosystem due to its vicinity to the Suez Canal. Update of a recent review (Galil *et al*. 2020) increased the recorded number of multicellular introduced species to 465 species (Table 1 in Appendix 3 - Israel National Report). The extraordinarily large number of records highlights the role of the southern Levant as a "hotspot", a beachhead and dispersal hub for secondary spread. The present report lists 72 NIS (4 vertebrates, 34 invertebrates, 1 seagrass and 33 macrophytes) newly described from the Israeli coast in 2019–2021.

Seventeen new introduced species are reported from **Italian** coasts. These include: three algae, one foraminiferan; two polychaetes, five molluscs, one crustacean, two tunicates, and three fish species. In addition, five cryptogenic foraminiferan species have been recorded from the Sicily Straits, one polychaete from the River Po Delta and two molluscs from Sicily and Tuscany. Significant ecological studies on various already known NIS have been published. Updated information on the colonization by NIS in selected locations and/or environments (like marinas and harbours) is provided. Citizen science initiatives, involving local population and operators have contributed to a wider knowledge of the distribution of NIS.

No new arrivals were recorded in **Lithuania** in the period from 2019 to 2021. All previous introductions, such as common rangia *Rangia cuneata*, the killer shrimp *Dikerogammarus villosus* and the round goby (*Neogobius melanostomus*) were first recorded in other Baltic Sea countries and then in Lithuania.

In **Poland**, there were two new sightings of non-indigenous species – a copepod *Eurytemora carolleeae* native to Atlantic coast of North America was recorded in 2019 in Szczecin Lagoon, whereas racer goby (*Babka gymnotrachelus*), which is native to the Ponto-Caspian region, was noted in 2021 in the Gulf of Gdańsk. In 2020, there was also record of non-native microsporidian

parasite (*Hepatospora eriocheir*), which infested Chinese mitten crab (*Eriochier sinensis*) from Vistula Lagoon. In 2020 Poland finally ratified BWM Convention, but in 2021 implemented Act on Alien Species. For the first time, studies of the presence of non-indigenous species on the hulls of recreational boats were conducted, the results of which indicate a high potential for introduction and spreading of these species within Polish as well as Baltic Sea waters.

In **Portugal**, 211 marine and brackish non-indigenous species (NIS) are currently recorded for the estuarine and coastal aquatic systems. There were 34 new additions to the list of previous reports and there were 33 new NIS for mainland Portugal, 12 new NIS for the Madeira archipelago and one for the Azores archipelago. These new records included 2 Rhodophyta, 2 Ochrophyta, 1 Tracheophyta, 1 Nematoda, 1 Porifera, 4 Cnidaria, 6 Annelida, 6 Mollusca, 10 Arthropoda and 9 Chordata species. These new records included species that have been overlooked and were added after a thorough revision of scientific articles, thesis and other literature after validation by expert judgement. A list of cryptogenic species was also created, with 26 species listed. The most recent records included the algae (*Rugulopteryx okamurae*), in São Miguel (Azores) and Lagos (Algarve) and the amphipod (*Grandidierella japonica*) in Ria Formosa (Algarve). The blue crab (*Callinectes sapidus*) and the weakfish, which were initially detected in the Tagus estuary, appear to be expanding southwards with large populations registered in the Algarve, whereas the slipper limpet (*Crepidula fornicata*), firstly detected in Ria de Aveiro is currently expanding southwards, with a well-established population observed in the Tagus estuary. Decree-Law no. 92/2019 was published in 2019, to carry out the implementation of Regulation (EU) No. 1143/2014 on the prevention and management of the introduction and spread of invasive alien species at national level.

Two new species were discovered in **Sweden** during 2019: the comb jellyfish (*Beroe ovate*) at the station Släggö, North Sea and the Atlantic rock crab (*Cancer irroratus*) in the southern Kattegat. During 2020, three new species were sighted at the Swedish west coast: the shell-boring annelid (*Polydora websteri*), the stalked jellyfish (*Craterolophus convolvulus*), and non-native tanaid (*Sinelobus vanhaareni*). There has been no report indicating the occurrence of new species in Sweden since January 2021. Following the European ARMS programme and using Environmental DNA Metabarcoding, five new species were detected in the Swedish west coast during 2018-2020 including: *Cribrilina mutabilis* (Bryozoa), *Rhyssoplax olivacea* (Mollusca), *Spio decorate* (Annelida), *Spio symphyta* (Annelida), *Limapontia senestra* (Mollusca). The round goby (*Neogobius melanostomus*) continues to expand its range in the Baltic Sea every year. In 2019, round goby was detected in Östhammar (the Archipelago Sea), which shows that the species is spreading northwards on the Swedish coast. On 19 September 2019, one male round goby was caught in Rökan, Instön, which is located 25 km from the Gothenburg harbour. This is the first confirmed round goby observation in truly saline water (23–25 psu), although two other citizen science observations in the Skagerrak (2015 and 2018) indicate even higher salinity conditions around 30 psu. In the summer of 2020, a large number of round goby was found in Gävle municipality, which has been the northernmost finding in Sweden to date. Round goby was also caught in the relatively high numbers in Södermanland County (the southeast coast of Sweden), Gävleborg County, and eastern Gotland's coastal waters during 2021. The occurrence of all different development stages, from juveniles to adults and ovigerous females of the Harris mud crab (*Rhithropanopeus harrisii*), indicates that this species has been established in the invaded area during 2019-2020. *R. harrisii* is now common in Blekinge County (the south of Sweden) but is also found in the area around Nynäshamn in Stockholm County in 2021. In 2020, colonies of the Australian tubeworm (*Ficopomatus enigmaticus*) were recorded as common in the Lomma leisure marina in the Baltic Proper. No observations were made on the American lobsters (*Homarus americanus*) during 2019–2021. The two Asian shore crabs (*Hemigrapsus sanguineus* and *H. takanoi*) are found in increasing numbers on the Swedish west coast. Both observations of juveniles of the two species and egg bearing females of *H. takanoi* are a strong indications of reproduction on the west coast in 2019. During

2020–2021, there were many reports of *H. sanguineus* and *H. takanoi* along the west coast down to the Sound in all stages and even ovigerous females. The Chinese mitten crab (*Eriocheir sinensis*) is sporadically found along the coast of eastern Sweden and up to the archipelago of Piteå in the Bay of Bothnia. In 2020, most reports were from the southern part of the Stockholm archipelago. During 2021, several findings have also been made on *E. sinensis* along the Baltic coast (from Stockholm to Blekinge) as well as in the Halland County (the western coast of Sweden) during 2021. *Eriocheir sinensis* was also observed for the first time in Gävleborg County in 2021. During summer 2019, five individuals of pink salmon (*Oncorhynchus gorbuscha*) were observed in the fish counter at the Herting power plant in Halland County. Several individuals of the dark false mussel (*Mytilopsis leucophaeata*) were found at two sites in Singö, the north of Stockholm County in 2019. Some large specimens of *M. leucophaeata* were observed for the first time in Galtfjärden (Östhammar manucipality) in 2020. The Japanese wireweed (*Sargassum muticum*) is seemed to have continued stable occurrence at Ringhals nuclear power plant. A large number of old (5+–6+) North American wedge clam (*Rangia cuneate^)* were found in Stockholm County. Large quantities of the *R. cuneata* were also found in Smältevik (southern Sweden) in 2021. Since 2016, the Pacific oyster (*Magallana gigas*) has been expanding its range southwards. There is a gradual increase in the occurrence of Pacific oysters in the Halland County in 2021. *Magallana gigas* infested with *Polydora websteri* in two places on Orust (Västra Götaland County) was found in November 2021.

Three new marine species were reported or identified in 2020 in the **United Kingdom**, the Atlantic croaker (*Micropogonius undulatus*), an Asian bivalve (*Theora lubrica*), and a polychaete (*Paleanotus chrysolepis*). The amphipod, *Caprella scaura*, was also discovered in 2020, with records from the same site subsequently backdated to 2009. One new species was recorded in 2019, the polychaete (*Lepidasthenia brunnea*), and another was first reported in 2019 from 2017 records, the freshwater/brackish amphipod (*Crangonyx floridanus*). The polychaete (*Bispira polyomma*) was recorded in 2021, as was a specimen provisionally identified as rosy barb (*Pethia conchonius*, formerly *Puntius conchonius*). The leathery sea squirt (*Styela clava*) was recorded in Scapa Flow, Orkney in December 2020. This is new location for *S. clava* in Scotland and the northern most record of the species in the UK (Want & Kakkonen 2021). The anticipated invasion of UK rivers by pink salmon, as a repeat of that observed in 2017, did not occur. In 2019, relatively few specimens were reported (33), and these almost entirely in Scottish rivers (21 fish), with four pinks in England, one in Wales and nine in Northern Ireland. In 2020, no specimens of pink salmon were reported for England, Northern Ireland and Wales, though this lack of reports could either be a true reflection of pink salmon incursions or the result of reduced surveillance due to Covid-19 restrictions on travel. In 2021, no pink salmon were reported for Wales, but 26 pink salmon were reported for England, mainly in the northeast. However, one pink salmon was a trapped in the River Tamar, which is the most southwestern report for England ever. The greatest number of pinks salmon reported in 2021 was in Scotland, where about 169 pink salmon were captured from 29 different river basins, though these records are an underestimate of the number of pink salmon present. The first tentative report (based on a published photo) of a lionfish (*Pterois* sp.), captured Wednesday 22 September 2021 by an angler off of Chesil Beach, Dorset, was announced on various internet sites (e.g. Chao-Fong 2021). Following release (and a journal publication about) of a multi-lingual version of the Aquatic Species Invasiveness Screening Kit (AS-ISK), described in Copp *et al*. (2021), the global trial of the AS-ISK, a contribution to the WGITMO ToR, reached its conclusion during 2020, with the journal article submitted and published online in May and then definitively in September 2021 (Vilizzi *et al.* 2021).

In the **United States of America** (US) report on 2019, 10 new introductions or substantial range expansions were reported in the US, along with three additional taxa that have been observed in US waters but suspected not yet to have established populations. No new intentional introductions or pathogens were reported during this reporting period. In the 2020 report, three new

introductions or substantial range expansions were reported within the US, along with four additional taxa observed in US waters not yet suspected to have established populations. In the 2021 report, six unintentional introductions or range expansions were reported and there were three sightings not yet known to be established. Unfortunately, 2019 saw the discontinuation of the Invasive Species Advisory Committee, a high-level non-federal stakeholder group providing advice to the National Invasive Species Council on all aspects of invasive species research, management, and policy. The US Environmental Protection Agency (USEPA) continues to work toward development of ballast water discharge standards within the framework of the 2018 Vessel Incidental Discharge Act (VIDA), as well as outreach to improve sharing of ballast water reporting data. VIDA requires Coast Guard, EPA, and Aquatic Nuisance Species Task Force to develop an intergovernmental response framework for aquatic invasive species. In addition to establishing a new regulatory framework for vessel discharges, VIDA also established the Great Lakes/Lake Champlain Invasive Species Program, to be administered by the USEPA. This Program represent a major research and monitoring effort aimed at detecting introduction and spread of ANS into or within these waters and assisting with and prioritizing management and response actions including monitoring vectors likely contributing to ANS concerns. EPA hosted a series of virtual outreach meetings related to ballast water discharge in Fall 2021. A final policy letter was published by the Coast Guard on 24 March 24 2022 describing the process to accept alternate test methods for ballast water management systems that render organisms non-viable.

3 ToR B: Climate Change Impact on Spread of NIS

Evaluate the impact climate change may have on the introduction and spread of non-indigenous marine organisms, including in Arctic environments

Evaluating the impact climate may have on non-indigenous species in Arctic environments and their detection continued to be a focus of this ToR during this three-year period. Adding to the significant progress in a comprehensive list of introduced species to the Arctic, knowledge gaps on vectors and harmful species identified from the previous period (2017–2019) were address during several of the meetings, particularly during the 2020 meeting shared with WGBOSV and WGHABD. Three presentations, one from the United States and two from Canada, on non-indigenous (NIS) and harmful algal (HA) species recently detected in Alaska and the Canadian Arctic provided on the joint day with WGHABD and WGBOSV as a shared issue of concern. The presentations highlighted the unexpectedly large number of HA in the region as well as the importance of innovative detection techniques in these remote locations for both NIS and HA. At least five peer-reviewed papers have been published relating to Arctic NIS and HA and their detection and risk (screening tools and modelling) under this ToR and acknowledge the contribution of the WGITMO, WGBOSV and WGHABD special session (Chan *et al*. 2019; Dhifallah *et al.* 2022; Goldsmit *et al.* 2020, 2021; McKenzie *et al.* 2021). Building on this research, a study which aims to predict habitat suitability of native and their analogue non-native species in the Canadian Arctic was presented and discussed (2022). Both WGITMO and WGHABD requested (2020) that in the future each group share information on reported NIS or HA. These presentations were organized to lead into discussions on molecular tools (ToR e) used by all three groups considered during the joint day session (see ToR e below). Information on the spread of the invasive colonial tunicate (*Didemnum vexillum*) in Norwegian coastal waters was provided (2022) and highlighted the use of molecular tools to detect this high-impact species which was the subject of a 2017 WGITMO ICES Cooperative Research Report (McKenzie *et al.* 2017, CRR No. 335).

The Group discussed the need to continue to identify and monitor activities of the Arctic Council under the ARIAS strategy and contribute to the Stratiegic Initiative on Climate Change Impacts on Marine Ecosystems (SICCME) created by ICES and PICES, with a view to providing advice or scientific information in the near future. A presentation on "Screening tools and modelling to predict NIS risk in the Canadian Arctic New CAFF/PAME project -marine Invasive Alien Species in Arctic waters" (2022. Canada) addressed the Artic Council's Arctic Invasive Alien Species Strategy objectives. Owing to the rapidly changing climate and exponential growth of human activities in the Arctic, the Expert Group identified the need to continue to assess risks of species introductions and mitigation strategies, to serve as guidance for future research and management efforts related to NIS in the Arctic.

The political situation (Russia-Ukraine conflict) will likely impede the Arctic cooperation for a considerable period, but hopefully an UN-acceptable peace will again allow cooperation with Russia, in the medium range future.

The use of DNA-based techniques is expected to increase rapidly, as many of the important prerequisites the methods have improved (lower price for sequencing, larger suits of methods, simpler and more dedicated instruments and utensils, larger sequence libraries for important organism-groups, etc).

Publications relating to climate change impacts on spread of NIS, presented and discussed at WGITMO meetings and during the intersession period include (ICES members given in bold):

1. Chan, F.T., Stanislawczyk, K., Sneekes, A.C., Dvoretsky, A., **Gollasch, S.**, **Minchin, D.**, David, M.,… *et al.* 2019. Climate change opens new frontiers for marine species in the Arctic: Current trends and future invasion risks. Global Change Biology, 25, 25–38. https://doi.org/10.1111/gcb.14469
2. Goldsmit, J., **McKindsey, C.W.**, Shlegel, R.W., Stewart, D.B., Archambault, P. & **Howland, K.L**. 2020. What and where? Predicting invasion hotspots in the Arctic marine realm. Global Change Biology, 26, 4752–4771 https://doi.org/10.1111/gcb.15159
3. Goldsmit, J., **McKindsey, C.W.**, Stewart, B. & **Howland, K.L**. 2021. Screening for high-risk marine invaders in the Hudson Bay Region, Canadian Arctic. Frontiers in Ecology and Evolution, 9, article 627497. https://doi.org/10.3389/fevo.2021.627497
4. Dhifallah, F., Rochon, A., **Simard, N**., **McKindsey, C.W**., Gosselin, M. & **Howland, K.L**. 2022. Dinoflagellate communities in high-risk Canadian Arctic ports. Estuarine, Coastal and Shelf Science, 266, 1–16
5. **McKenzie, C.H**., S.S. Bates, J.L. Martin, N. Haigh, **K.L. Howland**, N.I. Lewis, A. Locke, A. Pena, M. Poulin, A. Rochon, W.A. Rourke, M.G. Scarratt, M. Starr, M. & T. Wells. 2021. Three decades of Canadian marine harmful algal events: Phytoplankton and phycotoxins of concern to human and ecosystem health. Harmful Algae, 102, article 101852. https://doi.org/10.1016/j.hal.2020.101852
6. Casties, I. & Briski, E. 2019. Life history traits of aquatic non-indigenous species: freshwater vs. marine habitats. Aquatic Invasions, 14(4), 566–581. **View online**
7. Menéndez Teleña, D. 2019. Contaminación biológica de especies invasoras por agua de lastre e incrustaciones en el Puerto de Gijón. MSc. Thesis, Universidad de Oviedo, 103 pp. **View online**
8. Rey, A. 2019). From port to ballast water: application of ADN metabarcoding of shipborne biodiversity. PhD. Thesis, Universidad de Oviedo, 207 pp. **View online**
9. Strubbe, D., White, R., Edelaar, P., Rahbek, C. & Shwartz, A. 2019. Advancing impact assessments of non-native species: strategies for strengthening the evidence-base. NeoBiota, (51), 41–64. **View online**
10. Bojko, J., Burgess, A.L., Baker, A.G. & Orr, C.H. 2020. Invasive non-native crustacean symbionts: diversity and impact. Journal of Invertebrate Pathology, 186, article 107482. **View online**
11. Duarte, S., Vieira, P.E. & Costa, F.O. 2020. Assessment of species gaps in DNA barcode libraries of non-indigenous species (NIS) occurring in European coastal regions. Metabarcoding and Metagenomics, 4, article e55162. **View online**
12. Azevedo, J., Antunes, J.T., Machado, A.M., Vasconcelos, V., Leão, P.N. & Froufe, E. 2020. Monitoring of biofouling communities in a Portuguese port using a combined morphological and metabarcoding approach. Scientific Reports (Nature Publisher Group), 10(1), Article 13461. **View online**
13. Obst, M., Exter, K., Allcock, A.L., Arvanitidis, C., Axberg, A., Bustamante, M., ... & Pavloudi, C. 2020. A marine biodiversity observation network for genetic monitoring of hard-bottom communities (ARMS-MBON). Frontiers in Marine Science, 7, article 572680. **View online**
14. Shalovenkov, N.N. 2020. Tendencies of Invasion of Alien Zoobenthic Species into the Black Sea. Russian Journal of Biological Invasions, 11(2), 164-171. **View online**
15. Tsiamis, K., Azzurro, E., Bariche, M., Çinar, M.E., Crocetta, F., De Clerck, O., ... & Cardoso, A.C. 2020. Prioritizing marine invasive alien species in the European Union through horizon scanning. Aquatic Conservation: Marine and Freshwater Ecosystems, 30(4), 794-845. **View online**

4 ToR C: Biofouling as a Vector for NIS

Investigate biofouling as a vector for the introduction and transfer of aquatic organisms on vessels and artificial hard structures, their pressure and impact on the ecosystem with a comparison of prevention or selective mitigation methodologies

The WGITMO with the WGBOSV provided submissions (papers) to inform and progress the discussions at the International Maritime Organization (IMO) on the topic of biofouling (comments on the IMO Biofouling Guidelines) based on a biofouling viewpoint (Galil *et al.* 2019, ICES VIEWPOINT, ICES 2019a, 2019b). Several WGITMO members continue to serve as part of a Correspondence Group on the review of the IMO Biofouling Guidelines (2011) led by KLD in Norway.

During the joint day with WGBOSV in 2020, information was provided on the UNDP-GEF- IMO GloFouling Partnerships project[2], and research projects in the Baltic (Project COMPLETE[3]). This was followed by COMPLETE plus project[3], which ended in December 2021, during which draft guidance documents on biofouling management and a risk assessment for in-water cleaning of ships in the Baltic Sea region was developed (Engels *et al.* 2021). This risk assessment bases on an extensive literature study and on the input of a stakeholder group that consisted ofadministrations and ports, who are the main bodies responsible for IWC, as well as transport agencies, EPAs, scientific institutes and diving companies from all interested Baltic Sea countries. The HELCOM/OSPAR JTG Ballast & Biofouling 2-2022 agreed that the risk assessment sufficiently covers the fundamental and relevant issues of a risk assessment for in-water cleaning and that it will be practically tested in the HELCOM and OSPAR area in cooperation with in-water cleaning companies in the respective country. The results will be shared at the next meeting. Following the joint day, WGITMO also reviewed and discussed presentations on biofouling on vessels and structures in marinas (Poland, the Netherlands, France) and standardized methods for assessment (Greece, Canada, United Kingdom). One product of the COMPLETE project was the proposal for a regional Biofouling Management Roadmap[4], covering biofouling management from a holistic perspective by considering species introduction and spread but also input of biocides and microplastics from AFS and in-water cleaning operations, as well as waste management. This Roadmap has been accepted by HELCOM as one basis for the regional implementation of the IMO Biofouling Guidelines and Guidance. In addition, Germany was active in the Correspondence Group top review the Biofouling Guidelines and a result report was submitted to IMO PPR 9 (to be held remotely in April 2022). The most recent meeting of the OSPAR JTG BALLAST & BIOFOULING was held in October 2022 and, amongst other things, the Meeting noted the progress and process in reviewing the IMO Biofouling Guidelines.

Information on GESAMP WG 44 on Biofouling Management was provided (2021, Germany) and members of WGITMO were invited to contribute to the GESAMP WG 44 with relevant research results. The first meeting of the group was held from 19 to 21 October 2020, using virtual tools. Linkages continue with WGBOSV, IMO GloFouling Partnership, and other biofouling focused groups to develop and improve biofouling management guidelines and practices, and to support the evaluation of the international guidelines at the IMO.

IMO Regulatory updates on biofouling were provided on the shared day with WGBOSV by the IMO each year. Also, during the shared day in 2022, technical considerations for development of policy and approvals for in-water cleaning of ship biofouling (United States) were given. In addition, the possible impact of shipping related supply change issues in biofouling, which

occurred due to the Covid pandemic may have increased species introductions and were presented and discussed (United States) as immediate issues of concern under this ToR.

Research on recreational vessels and biofouling discussed at WGITMO meetings was published (Pelletier-Rousseau *et al.* 2019) and a publication on the survival and recovery of fouling mussels between varying environmental conditions were presented (Riley e*t al*. 2022). A presentation and further discussions also addressed concerns on biofouling management with respect to extended ship lay-ups originating from trade disruptions due to the recent Covid pandemic (United States). Establishing the Smithsonian protocols for biofouling monitoring in the Mediterranean Sea was presented and discussed (Tamburini *et al.* 2021). In 2020, an In-Port Inspection & Cleaning Conference (PortPIC) was held in Germany to address aquatic invasive species, diver operations in ports, next-generation antifouling technologies, operator perspective on cleaning, performance-based cleaning, regulations and guidelines, robotic cleaning and inspection (Bertram 2020). This conference was followed by annual PortPIC events.

Publications relating to biofouling as a vector for NIS, presented and discussed at WGITMO meetings and during the intersession period include (ICES memebrs given in bold):

1. Bertram, V. 2020. Proceedings of the 1st Port In-Water Cleaning Conference (PortPIC'20), Hamburg, 14–15 September 2020. http://data.hullpic.info/PortPIC2020_Hamburg.pdf
2. **Galil, B.S**., **McKenzie, C., Bailey, S**., Campbell M., Davidson, I., Drake, L., Hewitt, C., **Occhipinti-Ambrogi, A**. & Piola, P. 2019. ICES Viewpoint background document: Evaluating and mitigating introduction of marine non-native species via vessel biofouling. ICES Ad Hoc Report 2019. 17 pp. (WGITMO; WGBOSV) https://doi.org/10.17895/ices.pub.4680
3. ICES VIEWPOINT on *Evaluating and Mitigating Introduction of Marine Non-native Species via Vessel Fouling*, was reviewed and produced jointly with WGBOSV. vp. 2019.01 https://doi.org/10.17895/ices.advice.4687
4. ICES. 2019a. Additional actions to minimize biofouling introductions. Submitted by ICES to PPR 7 as document PPR 7/7. International Maritime Organization. pp. 1–3. (EG: WGITMO; WGBOSV; SG: HAPISG).
5. ICES. 2019b. References to inform additional actions to evaluate and minimize biofouling introductions. Submitted by ICES to PPR 7 as document PPR 7/INF.2. International Maritime Organization. pp. 1–23. (EG: WGITMO; WGBOSV**;** SG: HAPISG).
6. Engels, A., Heibeck, N. & **Broeg, K.** 2021. Risk assessment on in-water cleaning (IWC) of ships in the Baltic Sea Region. COMPLETE PLUS report, 30 pp.
7. Pelletier-Rousseau, M., **Bernier, R**., Clarke Murray, C., Drolet, D., Lacoursière-Roussel, A., Locke, A., Martin, J.L., **McKenzie, C.H**., **McKindsey, C.W**., **Therriault, T.W**. & **Simard, N**. 2019. Assessment of recreational boating as a vector for marine non-indigenous species on the Atlantic Coast of Canada. Biological Invasions, 21, 2447–2470. (WGITMO) https://doi.org//10.1007/s10530-019-01991-1
8. Oliveira, D.R. & Granhag, L. 2020. Ship hull in-water cleaning and its effects on fouling-control coatings, Biofouling, 36(3), 332–350, https://doi.org/10.1080/08927014.2020.1762079, 2021-05-05.
9. **Outinen, O.,** Puntila-Dodd, R., Barda, I., Brzana, R., **Hegele-Drywa, J**., Kalnina, M., Kostanda, M., Lindqvist, A., **Minchin, D**., **Normant-Saremba, M**., Ścibik, M., **Strake, S.**, Vuolamo, J. & **Lehtiniemi M**. 2021. The role of marinas in the establishment and spread of non-indigenous species in Baltic Sea fouling communities. Biofouling, 37, (9–10), 984–997.

10. Riley, C., Drolet, D., Goldsmit, J., Hill, J.M**., Howland, K.L**, Lavoie, M-F, **McKenzie, C.H.**, **Simard, N**. and **McKindsey, C.W**. 2022. Experimental analysis of survival and recover of ship fouling mussels during transit between marine and freshwaters. Frontiers in Marine Science, 8, article 808007. https://doi.org/10.3389/fmars.2021.808007
11. Tamburri, M.N., Davidson, I.C, First, MR, Scianni, C., Newcomer, K., Inglis, G.J., Georgiades, E.T., Barnes, J.M. & **Ruiz, G.M.** 2020. In-Water Cleaning and Capture to Remove Ship Biofouling: An Initial Evaluation of Efficacy and Environmental Safety. Frontiers in Marine Science, 7, 437. https://doi.org/10.3389/fmars.2020.00437.
12. Tamburini, M., Keppel, E., **Marchini, A**., Repetto, M.F., **Ruiz, G.M**., Ferrario J. & **Occhipinti-Ambrogi, A.** 2021. Monitoring non-indigenous species in port habitats: first application of a standardized North American protocol in the Mediterranean Sea. Frontiers in Marine Science, 8, article 700730. https://doi.org/10.3389/fmars.2021.700730
13. Watermann, BT., **Broeg, K**., Krutwa, A. & Heibeck, N. 2021. Guide on best practices of biofouling management in the Baltic Sea. www.balticcomplete.com/attachments/article/321/Guide%20on%20best%20practices%20of%20biofouling%20management%20in%20the%20Baltic%20Sea_20210308.pdf

[2] www.glofouling.imo.org/

[3] https://balticcomplete.com/

[4]https:/balticcomplete.com/publications/project-reports/320-proposal-for-a-regional-baltic-biofouling-management-roadmap

5 ToR D: Indicators of Impacts of NIS on Marine Environments

Advance knowledge base to further develop indicators to evaluate the status and impacts of non-indigenous species in marine environments

Building on several tools developed by WGITMO expert members, AS-ISK and CMIST (Copp *et al.* 2021; Goldsmit *et al.* 2021), there has been increasing use of these tools to access the risk of risks of impacts posed by NIS in many marine environments (Goldsmit *et al.* 2021; Killi *et al.* 2021; Roy *et al.* 2019). Use of the AS-ISK includes applications as far afield as sea-cage mariculture in Bolivia (2018), brackish wetlands of eastern Iran (Moghaddas *et al.* 2021), the coastal waters of South Korea (Uyan *et al.* 2020), the Arabian/Persian Gulf region (Clarke *et al.* 2010), Vietnam (Ruykys *et al.* 2021), and the Caribbean islands of Grenada, and St Vincent and the Grenadines (Tidbury *et al.* 2021). A primary publication led by several WGITMO members, which applied the AIS-ISK tool globally to brackish, fresh and marine species, has been published (Vilizzi *et al.* 2021). Further development of the AS-ISK continues so as to facilitate wider use, with 32 languages now provided for assessors to use for their risk screening applications (Copp *et al.* 2021). Following its application in the Eastern Mediterranean (Bilge *et al.* 2019; Tarkan *et al.* 2021), plans for of the AS-ISK to be used in the Levantine Sea (Israel) were provided (United Kingdom/Poland, 2020). Several of these publications are contributions to this WGITMO ToR.

The Canadian Marine Invasive Screening Tool (CMIST) has been used to predict NIS risk for a CAFF/PAME project in the Canadian Arctic (Goldsmit *et al.* 2020, 2021), and to develop a national watchlist framework for AIS species in Canada (2021). Applications of the CMIST include a comparison of risk-screening tools and full risk assessment schemes (Srėbalienė *et al.* 2019), the assessment of invasion risk posed by *Didemnum vexillum* to Atlantic Canada (2018) as well as non-native species transported by tsunami marine debris (2018). A presentation (2021) assessing the ecosystem impacts of marine NIS based on the absolute effect size was provided (Estonia) and discussed. A primary publication on this subject has been published by members of the working group and acknowledges the discussions at the WGITMO meetings (Ojaveer *et al.* 2021).

A target species selection criterion for risk assessment-based exemptions for ballast water management was developed and published by members of the WGITMO expert group (Gollasch *et al.* 2020). The trends in pathways of marine NIS for EEA indicators was presented (2022, Finland) with sections of this report used for the North Sea Ecosystem Overview.

Publications and presentations relating to the application of these tools reviewed, tested and discussed at WGITMO meetings and during the intersession period include (ICES members given in bold):

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Presentations on the ITMO-initiated global trials of the Aquatic Species Invasiveness Screening Kit (AS-ISK) were given at the following symposia and invited university lectures: the *2nd National Scientific and Technical Conference on Operation and Protection of Flowing Waters* (*PotamON 2019*) – *Biocenosis of flowing waters in the context of hydrological changes* (Łukęcin, Poland, 25–27 September 2019), the *22nd International Conference on Aquatic Invasive Species*, ICAIS (Oostende, Belgium, 18–22 April 2022), and the Marine Research Institute, Klaipeda University, Lithuania (invited lecture, 22 November 2021).

6 ToR E: Molecular Approaches for NIS

Evaluate the development and utilization of DNA- and RNA-based molecular approaches to provide science-based tools for strategic planning, policy development, and operational processes regarding non-indigenous species and biological invasions (including detection and monitoring, reconstruction of patterns and vectors of introduction and spread, assessment of establishment and impact risk, and application for invasive species control)

The 2020 meeting (Gdynia, Poland- the last face to face meeting since the Covid pandemic) provided an opportunity for three ICES working groups (WGITMO, WGBOSV [Ballast Water and Other Ship Vectors] and WGHABD [Harmful Algal Bloom Dynamics]) to collaborate on a shared day to discuss scientific issues of joint concern. These issues included climate change, particularly as it relates to the Arctic environment (see ToR B above), and detection/monitoring of and response to non-indigenous species through the use of innovative molecular tools. The session expanded on Darling *et al*. (2017). This publication provides recommendations for future development of genetic tools for assessment and management of NIS in marine systems, within the context of the explicit requirements of the MSFD (Marine Framework Strategy Directive) although applicable to other similar policies.

The afternoon of the shared day was used to review needs and uses of various molecular tools, with a presentation by each working group on the status and future of this technology. Presentations were delivered by members of WGITMO (Canada, United States), WGBOSV (United States), and WGHABD (Germany) to highlight issues with molecular tools as they relate to the three working groups' requirements for early detection, monitoring and other operational processes. This information was used by the groups to develop Terms of Reference for a targeted Molecular Tools workshop organized by members of the working group in conjunction with the International Conference for Marine Bioinvasions (ICMB) originally scheduled for May 2021 (Annapolis, Maryland, USA) but later cancelled (due to Covid restrictions).

Subsequent shared session with WGBOSV were used to review the framework and planning for the workshop and included presentations on the presence and detection of NIS (2021, France) and the potential of DNA based methods for the identification of NIS in Port of Rostock and for biofouling on recreational boats (Germany). It should be noted that there has been considerable overlap with other ToR, particularly with identifying Arctic and other NIS and HA under the climate change ToR B and Biofouling detection and mitigation ToR C. Other presentations were given on the topics of: regional connectivity of non-native species, detecting rapid differentiation in marine invaders, challenges and possibilities of genome-editing technology, using metabarcoding to assess the risk of non-native species introductions via ballast water, the persistence and extinction of eDNA signals, and differences in detecting organisms in marinas using traditional methods vs. metabarcoding.The widespread use of various molecular tools in the National reports, highlights the increasing importance of these innovative tools for a wide variety of goals and has become invaluable in most research programs. These reports also led the working group to support further work to clarify the conditions of use of these tools to report novel NIS, and provide recommendations for their reliable use. In particular, guidance on standardization for comparable methods and appropriate molecular tool use for reporting, policy decisions and regulation including validation of new species detection have been identified as important contributions the workshop and expert group could contribute to this ICES Science priority issue.

Terms of Reference and focus of the workshop have been modified based on substantial advancement in this technology in the last few years and recognition of multiple international efforts aimed at developing frameworks for adoption of similar methods in a variety of environmental management contexts, with the aim to develop guidance for future implementation of these tools. Plans are proceeding to convene a workshop on molecular tools for biological invasions at the 11th International Conference on Marine Bioinvasions (ICMB XI www.marinebioinvasions.info/) to be held in May 2023 in Baltimore, Maryland, USA.

An ICES theme session entitled "Integration of molecular tools for biodiversity, risk assessment, ecosystem advice within a changing climate" has been approved by the ICES Science Committee for the 2023 Annual Science Conference in September in Bilbao, Spain. This is a joint session convened and co-chaired by members of WGHABD, WGITMO and WGPME [Working Group on Phytoplankton and Microbial Ecology].

A peer-reviewed paper that benefitted from discussions on the joint day with WGBOSV was published. Using field samples, the utility of using high throughput sequence metabarcoding to characterize ballast water communities was demonstrated, and differences in the effort required to estimate diversity across receiving ports was revealed.

Reference

Darling, J.A., Galil, B.S., Carvalho, G.R., Rius, M., Viard, F. Piraino, S. 2017. Recommendations for developing and applying genetic tools to assess and manage biological invasions in marine ecosystems. Marine Policy 85: 54-64

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7 ToR F: Marine Debris as a Vector for NIS

Investigate the role of human-produced marine debris as a vector and facilitator for the introduction and spread of non-indigenous species (NIS). Advance research and identify knowledge gaps on marine debris-NIS interactions (eg. Marine debris as a facilitator for jellyfish blooms)

A framework for the review of the status of knowledge of this new ToR was developed during the 2020 meeting in Poland. Presentations on biofouling paints and microplastic release (Finland) and NIS in marine debris from Madeira (Portugal) were provided (2021) and discussed. In 2022 the results of a NIS and marine plastic litter study in the United Kingdom as presented and discussed (United Kingdom) which led to the formation of a WGITMO sub-group of experts targeted on this ICES Science priority (marine debris) ToR. A review conducted by this sub-group (led by Portugal, Spain, France, United Kingdom) of the knowledge gaps and research activities on marine debris-NIS interactions is underway intersessionally and will be discussed with a resulting research strategy at the next WGITMO meeting in Athens. A manuscript highlighting results of a pilot study conducted in Madeira Island designed to examine the role of marine debris as a vector for NIS introductions is in its final stages and is expected to be submitted in 2022. In addition, the WGITMO sub-group also developed a protocol for opportunistic sampling of NIS colonizing marine debris. This protocol was shared with all WGITMO members. Finally, several meetings were held with other groups and organizations, namely the ICES WG on marine litter and colleagues from Chile and USA, to develop an online database of marine debris and NIS.

8 ToR G: Aquaculture as a Vector for NIS

Investigate best practices to minimize the role of aquaculture as a vector for the introduction and transfer of non- indigenous aquatic organisms. This would include both non-indigenous species targeted for aquaculture and hitchhikers (biofouling and interstitial, parasites and pathogens). Impacts of non-indigenous species on aquaculture and on ecosystems will be addressed

A broad overview of the status of Aquaculture as a vector for NIS was provided (2020, Portugal) and led to a discussion outlining the planned approach for a review paper(s) on the status of aquaculture, the transfer of NIS and best practices and knowledge gaps with updates to current practices. Bearing in mind that a significant increase in aquaculture is expected to meet the growing needs of food supply, the implementation of prevention and mitigation measures is even more urgent and requires an update of knowledge in this area. A global view of the species currently cultivated worldwide is needed and the biological diversity translocated through the importation of species for aquaculture, including pathogens, parasites and others. Other research areas relevant to this issue are the analysis of the risk of the various species and vectors of introduction, gaps in current regulations, the establishment of early detection systems and testing the effectiveness of measures to remove and control populations of non-indigenous species. The translocation of shellfish species between different areas also involves the risk of transfer or accidentally introduced NIS with it, even when the translocated species are native to both areas.

This problem has been overlooked and guidance on good practice concerning inspection, disinfection, quarantine or destruction of introduced organisms and transfer material is strongly needed. . Information on a review of mitigation measures to reduce risk of AIS (aquatic invasive species) introductions through aquaculture transfers was presented (2021, Canada) and will provide formal science advice for managers and regulators on this issue in late 2022.

A journal special issue on NIS and Aquaculture (led by Portugal, Canada) is being organized with WGITMO members contributing to the issue. A survey on the interest of the WGITMO members to participate in a journal special issue on NIS and aquaculture was conducted among the 2022 WGITMO meeting participants. Nearly 62% of the respondants declared that they were willing to contribute with a manuscript t be published in that special issue and 77% declared that they were interested to contribute to a review article on aquaculture and non indigenous species. These numbers indicated a broad interest of WGITMO members to participate in this proposal, with 81% of the respondants considering that the special issue should be published in an open acess journal

9 Other Activities and Next Steps

Revised Terms of Reference have been developed for 2022–2025 by the working group to address the many scientific questions regarding the introduction and transfer of marine organisms. A new chair has been elected, Dr. João Canning-Clode (Portugal), for the next three-year term and will be leading the expert group in the development and deliverables of the new Terms of Reference. There are still several issues that the working group feels uniquely qualified to address as reflected in the ToRs. Further, the group is a good mix of long-term and medium-term contributors who are leaders in this field, as well as new members who are early career scientists.

The virtual meetings allowed for a high participation (64 participants) which included and chair invited members participating in their first WGITMO meetings. However, it is important to note that following discussion regarding future meetings (virtual/in person/hybrid), members strongly supported in person and/or hybrid meetings as the opportunity for networking or in-depth strategic planning was not possible during the virtual meetings. The members were concerned that committed engagement, collaborations and WG productivity would be impacted if only virtual meetings were held. Therefore, the next WGITMO meeting to be held in Athens, Greece will be a hybrid meeting.

In recognition of the passing of Dr. Carl J. Sindermann, a previous chair of WGITMO (1979–1990), and distinguished researcher on parasitology, fisheries and marine biology, the first "Dr. Carl Sindermann Memorial Presentation on Parasitology" was presented at the 2020 meeting in Poland.

Plans are proceeding to convene a workshop on molecular tools for biological invasions at the 11th International Conference on Marine Bioinvasions (ICMB XI www.marinebioinvasions.info/) to be held in May 2023 in Baltimore, Maryland, USA

An ICES Theme session entitled "Integration of molecular tools for biodiversity, risk assessment, ecosystem advice within a changing climate" has been approved by the ICES Science Committee for the 2023 Annual Science Conference in September in Bilbao, Spain. This is a joint session convened and co-chaired by members of WGHABD, WGITMO and WGPME [Working Group on Phytoplankton and Microbial Ecology].

Members of the working group also participated in the Fourth ICES PICES Early Career Scientist Conference in St. John's NL Canada, July 2022. PICES and ICES have a long history of collaborating on many important marine issues, including non-indigenous marine species. Understanding marine invasions is complex but PICES, ICES and CIESM have worked well together (and with other organizations) in the past and there are many avenues for continued collaboration on this topic. Discussions continue on activities and collaborations between ICES, PICES and CIESM with active participation of PICES and CIESM representatives at the WGITMO meetings.

Annex 1: List of participants

Virtual meeting 2022

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Virtual meeting 2021

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**Shared day WGBOSV

Gdynia, Poland, 2020: Shared meeting with WGBOSV and WGHABD

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Annex 2: WGITMO resolution

The **Working Group on Introductions and Transfers of Marine Organisms** (WGITMO), chaired by Cynthia McKenzie, Canada, will work on ToRs and generate deliverables as listed in the Table below.

	Meeting dates	Venue	Reporting details	Comments (change in Chair, etc.)
Year 2020	4–6 March	Gdynia, Poland		Joint meetings with WGBOSV and WGHABD
Year 2021	1–3 March	Online meeting		
Year 2022	4–6 May	Online meeting	Final report by 15 June to SCICOM	Meeting in association with WGBOSV

ToR descriptors

ToR	Description	Background	Science plan codes	Duration	Expected Deliverables
a	Advance research, develop collaborations and address surveillance and knowledge gaps in issues related to the introduction and transfer of marine organisms, through annual reviews of national/international activities and responding to advice requests.	Data, information and knowledge collated and synthesised ensures timely update of AquaNIS as well as national and international databases as appropriate. This information will be used as an underlying information source for other ToRs, responding to incoming advice requests as well as organising collaboration with other international science organisations (e.g. PICES and CIESM).	2.1, 2.4, 3.3	3 years	Annual reports to ICES. Further develop and advance AquaNIS database, and populate it with new data. Respond to incoming advice requests as requested.
b	Evaluate the impact climate change may have on the introduction and spread of non-indigenous marine organisms, including Arctic environments.	This work will be carried out jointly with WGBOSV. Contributes to SICCME and ICES high-priority action areas 'Arctic research'.	2.5, 2.2, 3.6	3 years	Primary publication on the Arctic environment and the spread of non-indigenous species.
c	Investigate biofouling as a vector for the introduction and transfer of aquatic organisms on vessels and artificial hard structures, their pressure and impact on the ecosystem with a comparison of prevention or selective	Biofouling has been increasing recognized as an important vector in the introduction and transfer of aquatic organisms. Elements of this work will be carried out jointly with WGBOSV as a comparison vector in invasion pathways. Biofouling is an increasing concern for aquaculture,	2.7, 2.1, 6.4	3 years	Input on the general applicability of preventive measures and selective mitigation technologies through a technical paper or manuscript submitted to a peer-reviewed scientific journal.

	mitigation methodologies.	energy installations, and coastal development as stressors on coastal environments.			Input to IMO Biofouling guidelines.
d	Advance knowledge base to further develop indicators to evaluate the status and impact of non-indigenous species in marine environments	The aim is to develop a wider knowledge-base to more effectively address several legislative acts related to introductions of non-native species, such as EU IAS Regulation and EU MSFD (D2). Specifically, WGITMO aims to improve/develop metrics and critically evaluate the underlying uncertainties, including the on-going global trial of the Aquatic Species Invasiveness Screening Kit (AS-ISK) and a comparison of AS-ISK and the Canadian Marine Invasive Species Tool (CMIST).	2.2, 2.7, 6.1	3 years	At least one manuscript to be submitted to a peer-reviewed scientific journal.
e	Evaluate the development and utilization of DNA- and RNA-based molecular approaches to provide science-based tools for strategic planning, policy development, and operational processes regarding non-native species and biological invasions (including detection and monitoring, reconstruction of patterns and vectors of introduction and spread, assessment of establishment and impact risk, and application for invasive species control)	Molecular (DNA-based and RNA-based) approaches have been increasingly used in the past decades to uncover cryptic introduced species, understand underlying processes of population establishment and spread, and detect novel introductions and monitor existing ones. Recent innovations have increased the power of these approaches to understand invasion risk and offer possibilities for novel biotechnological solutions for control or eradication of invasive populations. With the advent of recent technologies, it is timely to assess and evaluate their potential applications as well as their limitations.	2.5, 1.6, 4.4	3 years	Input on the effective utilization of these methods for international and national policies and regulations through meeting participation, group correspondence, and/or development of technical reports or peer-reviewed papers.
f	Investigate the role of human-produced marine debris as a vector and facilitator for the introduction and spread of non-indigenous species (NIS). Advance research and identify knowledge gaps on marine debris-	The accumulation of debris in the ocean is severely affecting ocean and coastal ecosystems, as its ingestion and entanglement directly impacts marine organisms. Furthermore, recent research indicates that marine debris is both a growing vector for the	2.5, 2.6, 2.1	3 years	Review paper on NIS introduced to European waters via marine debris

	NIS interactions (eg. Marine debris as a facilitator for jellyfish blooms).	introduction of non-indigenous species (NIS), with transoceanic rafting already likely to intensify species invasions worldwide and a potential facilitator of marine diseases. Develop collaborations with other working groups (HELCOM-TGML; OSPAR ICG-ML, ICES-WGML, MSFD-ML; PICES; CIESM)			
g	Investigate best practices to minimize the role of aquaculture as a vector for the introduction and transfer of non- indigenous aquatic organisms. This would include both non-indigenous species targeted for aquaculture and hitchhikers (biofouling and interstitial, parasites and pathogens). Impacts of non-indigenous species on aquaculture and on ecosystems will be addressed.	Aquaculture has been recognized as an important vector in the introduction and transfer of aquatic organisms. ENSARS provided some baseline information on aquaculture risk analysis, including development and global testing of ENSARS' derivative, the AS-ISK. There are important social and economic impacts (positive and negative) of introductions related to aquaculture. Linkages with aquaculture working groups, and WGPDMO will be sought as well as a close collaboration with WGECON.	2.1,2.2, 5.6	3 years	Input on the general applicability of preventive measures (good practice codes) and selective mitigation technologies through technical guidance and/or a peer-reviewed paper.

Summary of the Work Plan

Year 1	Work on all ToRs with special focus on a, c, e, f, g
Year 2	Work on all ToRs with special focus on a, b, d, e, f
Year 3	Report on All ToRs

Supporting information

Priority	The work of the Group forms the scientific basis for essential advice related to the introduction and transfer of marine organisms, particularly non-indigenous species. Consequently these activities are considered to have a very high priority.
Resource requirements	The research programmes which provide the main input to this group are already underway, and resources are already committed. The additional resources required to undertake additional activities in the framework of this group are negligible.
Participants	The Group is normally attended by some 40-50 members and guests.
Secretariat facilities	None.
Financial	No financial implications.
Linkages to ACOM and groups under ACOM	The group will serve as primary respondents to incoming advice requests on various issues relating to introduction and transfer of marine organisms, including non-indigenous species.

Linkages to other committees or groups	There is a very close working relationship with the Working Group on Ballast Water and Other Ship Vectors (WGBOSV). In addition to relevance to the Working Group on Harmful Algal Bloom Dynamics (WGHABD), Biodiversity Science (WGBIODIV), and aquaculture focused working groups, WGITMO also contributes to Integrated Ecosystem Assessment EG's. Anticipate building linkages with the Working Group on Integrated Morphological and Molecular Techniques (WGIMT) during the next three years under these ToRs. Potential linkages with WGML, WGECON, WGPDMO.
Linkages to other organizations	PICES, CIESM, IMO, HELCOM, OSPAR

Annex 3: WGITMO National Reports 2019–2021

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Overview

NEW 2021

Schizoporella japonica Ortmann, bryozoan from Japan, reported for the first time in Magdalen Islands, Qc.

NEW 2020

Hemigrapsus sanguineaus Asian Shore Crab, locations in St. Mary's Bay to Shelburne, Nova Scotia.

SPREAD

Other marine species that have already invaded Canadian waters continue to spread, including European green crab (*Carcinus maenas*), vase tunicate (*Ciona intestinalis*), carpet tunicate (*Didemnum vexillum)*, golden star tunicate (*Botryllus schlosseri*), violet tunicate (*Botrylloïdes violaceus*), clubbed tunicate (*Styela clava*), Japanese skeleton shrimp (*Caprella mutica*), coffin box (*Membranipora membranacea*), and oyster thief (*Codium fragile* subsup. *fragile*).

Canada's Ocean Protection Plan includes policies, funding and activities to address the issue of derelict vessels which have been found to be a source of pollution and of biofouling including Non-Indigenous Species (NIS). Transport Canada's program applies to larger commercial vessels and Fisheries and Oceans Canada (DFO) Small Craft Harbour (SCH) program applies to smaller fishing and recreational vessels. The goal is to remove these vessels as part of Canada's commitment to protecting the ocean environment.
Fisheries and Oceans Canada has added AIS Core Management to its activities and each region has several personnel working on areas of AIS management including working on control licences for green crab to work with groups including Indigenous groups to form citizen science teams to monitor for AIS in remote locations.

1. Regulations:
Fisheries and Oceans Canada has developed regulations to manage the threat of aquatic invasive species (AIS). The Aquatic Invasive Species Regulations for the *Fisheries Act* is now in force in Canada effective June 17, 2015. (http://gazette.gc.ca/rp-pr/p2/2015/2015-06-17/html/sor-dors121-eng.php). The AIS Core Management group has been created to manage these regulations.

2. Intentional Introductions:

Prior to December 31, 2015, Fisheries and Oceans Canada, along with the provinces and territories, managed disease, genetic, and ecological risks associated with aquatic animal movements through a variety of federal, provincial, and territorial regulations under the National Code on Introductions and Transfers of Aquatic Organisms. However, disease risk is now managed by the Canadian Food Inspection Agency (CFIA) through the National Aquatic Animal Health Program under the Health of Animals Regulations. For details on the intentional introductions by province for 2020, see

www.dfo-mpo.gc.ca/aquaculture/management-gestion/rep-rap-eng.htm

3. Unintentional Introductions:

New Sightings – 2021

Schizoporella japonica Ortmann non-indigenous bryozoan from Japan, introduced and established to the Pacific Coast, reported for the first time on the Canadian Atlantic coast in the Magdalen Islands, Qc.

New Sightings- 2020

Hemigrapsus sanguineaus Asian Shore Crab, locations in St. Mary's Bay to Shelburne, Nova Scotia.

There were no sightings of new marine AIS reported in 2019

Spread of established AIS species-

Established and Spreading

Diplosoma listerianum was found for the first time in Nova Scotia in Lunenburg Harbour in 2012. It was not observed again until 2016 where it was found at 9 locations in SW NB and 1 location in SW NS; after-which it spread rapidly to 14 locations throughout SW NB and 5 locations within SW NS by 2018. *Diplosoma listerianum* subsequently disappeared from SW NB in 2019 following a cold winter, but continues to persist in SW NS at 3 locations (Digby Neck, Digby, and Wedgeport) where it is now considered as established. In addition to these 3 locations, *Diplosoma listerianum* also spread to 5 additional locations within Nova Scotia during 2019. *Diplosom listerianum* was first found in Quebec in Magdalen Islands (Havre-Aubert marina) in 2008 and then in 2011. It was not observed again until 2017, but was also found in 2019 and 2020, all at the same location.

Didemnum vexillum, confirmed for the first time in 2013 in Atlantic Canada in Minas Basin, in the upper Bay of Fundy, was also confirmed in 2017 by genetic analysis of samples collected within the Bay of Fundy (near Minas Channel) from colony attached to small rock.

Carcinus maenas is established in several areas along the shores of Prince Edward Island (PEI) and the Gulf of St. Lawrence (GSL) coast of New Brunswick (NB) and Nova Scotia (NS), and continues to be reported in new areas along these shores. The northern limit of its distribution along the eastern coast of NB remains Pokemouche harbour. Increased abundances observed in 2016-17 in several recently invaded bays of eastern NB and PEI, were followed by significant decreases in 2018-2019 and slight increases in 2021 (no data for 2020, no trapping in NB and PEI due to pandemic restrictions). Significant decreases in green crab were found in 2020 on the west coast of NL, however, green crab continues to spread in Placentia Bay, Fortune Bay on the south coast, which is an area of high lobster productivity. In 2019 DFO received reports of the presence of green crab in St. Mary's Bay and confirmed its presence and spread there following a focussed survey of the bay in 2019, 2020 and 2021. Abundances of green crab in Magdalen Islands, Quebec have decreased since 2013 with only one crab found in 2019, one in 2020 and no crab reported in 2021. Cold winters or control efforts are potential factors that could explain this important drop.

Ciona intestinalis is now well established on the eastern shore of Nova Scotia, in Chedabucto Bay, Cape Breton, along the south and southwest shores of mainland Nova Scotia and in SW New Brunswick. In Newfoundland and Labrador *C. intestinalis*is found in isolated areas of the Burin Peninsula where control efforts are focussed (2019, 2020) and it has since expanded on the south coast into Fortune Bay (2020, 2021). This species is also well established along the eastern shore of Prince Edward Island and was confirmed on the southern shore in 2019 (Borden) and the northern shore in 2020 (St. Peters Bay and Southwest River) and 2021 (Alberton). It is sporadically distributed along the GSL shore of Nova Scotia where increasing occurrences have been observed (new report in 2019: Mabou). *Ciona intestinalis* is very abundant in one harbour (Cap-aux-Meules) in the Magdalen Islands, where control efforts have been put in place to minimize dispersal risks into aquacultures sites. In 2021, *C. intestinalis* was detected in the marina of Havre-aux-Maisons (close to aquaculture sites), first with eDNA samples and then confirmed by divers.

Botryllus schlosseri is now present in most bays and harbours along the south and SW coast of mainland Nova Scotia, as well as in coastal Cape Breton and the Bras D'Or lakes, the GSL shore of Nova Scotia, Prince Edward Island and the Magdalen Islands. It is well established in SW New Brunswick and continues to spread along the eastern shore of the province, where the northern limit has been extended to Miscou Island (first occurrence in 2017). In 2019, two new detections of *B. schlosseri* were reported in PEI (Egmont Bay and Basin Head (Marine Protected Area)), while new detections in 2021 included several areas along the eastern coast of NB (Bathurst, Bouctouche and Cape Tormentine) and one report in NS (Mabou). It was detected for the first time in Gaspésie, Quebec on collector plates in 2012 but was never observed in that area since that time. In Newfoundland, *B. schlosseri* has been found in many coves throughout Placentia Bay. It has also been found in isolated areas along the south coast of Newfoundland, including Fortune Bay, Hermitage Bay, and since 2013 has been found on the southwest coast of the Island. It is present in only one harbour (Long Pond) in Conception Bay.

Botrylloides violaceus is well established and continued to spread to new locations in SW New Brunswick, while its occurrence in the NE portion of the province remains limited. *B. violaceus* has established in most bays on the northern shore of Prince Edward Island and several bays along the eastern and southern shores of the island (confirmed in Pinette in 2019; Wood Islands in 2020; Cumberland Cove and Victoria in 2021) and was reported at two new locations along the GSL coast of NS (Barrachois Harbour and Mabou) in 2021. *Botrylloides violaceus* is also present in the Magdalen Islands and along the Atlantic and GSL coasts of Nova Scotia. In Belleoram, Newfoundland, where *B. violaceus* was originally detected in that province, abundances had decreased and rarely detected in 2019 and not detected in 2020. Isolated populations have been discovered throughout Newfoundland, including the west coast (Codroy), Placentia Bay (Arnold's Cove), and Conception Bay (Long Pond).

Styela clava, first reported in Prince Edward Island in 1998, is mostly restricted to the eastern shore and a few bays on the northern and southern shores of this province, with a recent NW range expansion following several detections in Cascumpec Bay in 2021. *Styela clava* was reported for the first time in Nova Scotia in 2012 at a few locations within Halifax Harbour and at Lunenburg harbour. In 2013, it was found at all these sites and in addition recorded at several sites within Chedabucto Bay and this species appears to be established within all these areas as they have been found at the same locations every year including 2017. *Styela clava* was reported for the first time (2002) in the Magdalen Islands, Qc, at the commercial port of Cap-aux-Meules. It has still not been detected in Newfoundland and Labrador.

Caprella mutica is well established on the Atlantic coast of Canada since the 1990's. In 2016, *C. mutica* was observed for the first time in the Bay of Sept-Îles located on the West Coast of the GSL. It has been reported at several locations within the southern GSL, mostly along the shores of PEI and northern NB (confirmed in Foxley and Cape Egmont, PEI in 2019, and Bathurst (NB)

and Nine Mile Creek (PEI) in 2021). *C. mutica* was found for the second time in Bay of Sept-Îles in 2018, and now is considered to be established on the north coast of the GSL. *Caprella mutica* is considered established in several locations in Newfoundland and Labrador including Notre Dame Bay, Placentia Bay, Fortune Bay, and most recently (2019) Trinity Bay.

Membranipora membranacea is well established on the Atlantic coast of Canada since the 1990's. In Quebec, this invasive bryozoan is found in the Magdalen Islands, Gaspe Peninsula and on the West coast of the GSL. In 2018 *M. membranacea* was detected for the first time in the northern part of the GSL (close to the Labrador border). Low densities have been detected in eastern and western PEI, the GSL and Atlantic coasts of Nova Scotia, and SW New Brunswick. *Membranipora membranacea* is well established in Newfoundland and has been found in most coastal areas throughout the province, including southern Labrador.

Codium fragile subsp. *fragile* is established along the shores of the Northumberland Strait and in Malpeque Bay (northern shore of PEI) as well as in the Magdalen Islands. On the eastern shore of NB, confirmed reports extend as far north as Lamèque, where it was first reported in 2002. In Newfoundland, it has been observed attached in Notre Dame Bay (near Pilley's Island) and within Fortune Bay (near Little Harbour East). A focussed survey was conducted in Notre Dame Bay in 2019 and it had not spread from the original location. In Nova Scotia, *C. fragile* was reported for the first time in 1991 and spread along coasts since that time.

4. Pathogens

None reported.

5. Research and Monitoring Programs This should also include sections on **Planned Research**, **Research Needs** and **Research Gaps**.

Research Needs

- Research is needed to develop better management practices for biofouling, such as remotely-operated devices for risk assessment and cleaning with particle retention.
- A baseline data set for Canadian Arctic plankton and benthos by marine ecoregions has been collated, but species records for different taxa should be incorporated into a consistent, standardized database format and ideally published/archived in a way that they can be made publicly available.

Research Gaps

- DNA barcode reference libraries to catalogue biodiversity of lower trophic level taxa and provide a basis for the use of new genetic tools for the detection of changes in biodiversity and detection of new species.
- Risk assessment for recreational boating as a vector of AIS to Arctic region
- Population genetics studies of cryptogenic species found in port surveys, to better understand origins (native versus introduced)

Research and Monitoring projects and programs

1) Fisheries and Oceans National AIS Monitoring Program – Atlantic Canada Zone (2005–ongoing)

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Fisheries and Oceans Canada (DFO) has included a monitoring component in its Aquatic Invasive species (AIS) framework since 2005. The Atlantic Zone (Nova Scotia, New Brunswick, Newfoundland and Labrador, Prince Edward Island, Quebec) Monitoring program has been conducting surveys, rapid response, and outreach projects for non-native and invasive species since that time using standardized methods and protocols. Using a combination of harbour surveys (settling plates, video and SCUBA) for detection and spread of biofouling organisms and trapping for invasive green crab, a baseline distribution of AIS in the Atlantic zone has been compiled. Ongoing surveys and monitoring projects are aimed at prevention and early detection, mitigation strategies and providing advice for management of AIS in Canada. Data collected from these surveys are included in the ICES AQUANIS database.

2) In-transit survival and post-arrival performance of hull fouling aquatic invasive species (2017–2021)

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Ships may act as vectors of introduction for aquatic invasive species (AIS) through hull fouling. However, there is limited understanding of the survival of fouling species following short-term in-transit changes in environmental conditions. Normally, last port-of-call (LPoC) information is used to assess the relative risk of introduction of AIS. However, species present in hull fouling communities may be very different from those in the LPoC given that organisms may have accumulated over time through voyages to multiple destinations subjected to a variety of environmental conditions. Further, it is typically assumed that individuals from populations of a given hull fouling species across a range of latitudes have similar probabilities for survival in novel receiving environments, although studies of other organisms show that physiological performance and capacity for adaptation may vary substantially depending on population of origin. This study used a two-step approach for studying the environmental tolerances for survival and post-arrival performance of fouling organisms. The first will evaluate AIS survival and recovery following exposure to short-term changes in salinity and temperature simulating pathways with transitions between marine and freshwater and back to marine environments, or vice-versa. The second will evaluate the performance and acclimation capacity of fouling AIS populations from different latitudes to the full range of expected temperatures in Canadian waters (temperate-polar). The results of this project will provide a better understanding on fouling vectors and the response of species to changes in environmental conditions. This can contribute to the development of more complete and realistic hull fouling risk assessments.

3) Developing a risk-based pathway analysis to prevent the spread of the high impact invasive solitary vase tunicate, *Ciona intestinalis* (2019–2022)

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The objectives of this three-year project are to document vectors and pathways associated with small harbours and marinas (< 25 m vessels) for potential regional and zonal spread of invasive species, using the vase tunicate as a proxy for other biofouling organisms. The study will develop and evaluate effective prevention and early AIS detection strategies and best management practices that may be useful to stakeholders, regulators and policymakers with regard to small habours and marinas. A decisions tree type guidance document will be developed and tested for a preventative approach to the introduction of AIS and rapid response events as part of harbour management protocols

4) Development of community-based program for monitoring and early detection of aquatic invasive species in the Canadian Arctic – preparing for increased shipping related to resource development and climate change (2015–2019)

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Increased shipping in the Canadian Arctic associated with resource development and climate warming will inevitably result in unwanted species introductions. Preventative measures, such as ballast water exchange and treatment and reduction of vessel fouling, are key components for management of aquatic invasive species (AIS). However, these measures are not 100% effective. Thus, in addition to prevention, management should focus on strategies for monitoring and early detection, especially where AIS have not yet established or population levels are still low, as in the Canadian Arctic. Monitoring improves the likelihood of detecting invasions at early stages when there is a greater chance for successful eradication, containment, or to prepare to adapt to the presence of a new species. Through this project we are developing a foundation for the development of a monitoring and early detection system in the Canadian Arctic. This includes the following elements and is extending past research efforts by DFO and the Canadian Aquatic Invasive Species Network (CAISN): 1) Identification and ranking of key ship-mediated AIS for early detection and monitoring, and geographic locations with highest probability for establishment via Environmental Niche Modelling; 2) Development and trial of genetic early detection methodologies (e.g., environmental or eDNA) for AIS in high risk ports; 3) Establishment of a community based monitoring network/capacity; and 4) development of generic Risk Assessment models based on detailed shipping information

5) Climate change-driven range expansion of an invasive marine invertebrate (2020–2024)

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This project evaluates the ecology of an invasive gastropod - the common periwinkle *Littorina littorea.* The snail has moved about 250 km upstream in the St Lawrence estuary over the past 20 years or so and any work will inform about its basic ecology and perhaps let us better understand its expansion in the context of global change. The first project (MSc student Shao) evaluates the movement and habitat associations of *L. littorea* in an intertidal muddy boulder field. Most work to date has focused on rocky coasts and this work will provide basic understanding of the snail's ecology in a heretofore unstudied habitat. The second project (PhD candidate Giuseppe Garlaschè) comparises the snails physiology from across its distribution in both its native (Europe) and invaded (eastern North America) ranges. Risk assessments take the best available information available for a species and that is often from a single population. This project will inform on the logic of this approach given the results of genetic, transcriptomics, and physiological work.

6) Science Advice on "clean, drain, dry and decontaminate" protocols to prevent the introduction and spread of AIS (2019–2021)

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To prevent the introduction and spread of AIS, many government and non-government organizations operate Clean, Drain, Dry (CDD) programs. CDD requires watercraft operators to undertake cleaning, draining, and drying steps to reduce the likelihood of transporting AIS on their equipment. In situations where there is a higher risk that AIS could be transported, an additional decontamination step may be applied (CDD+D). To date, a comprehensive evaluation of the effectiveness of CDD+D protocols used in Canada on marine and freshwater AIS has not been conducted. The need for a fulsome review is compounded by the fact that a wide variety of methods are endorsed and used by different organizations without national consistency. This project aims to: 1) review and characterize existing freshwater and marine CDD+D protocols used in AIS management across Canada or abroad, including specific decontamination methods and; 2) assess the effectiveness of existing CDD+D protocols and methods at reducing the propagule pressure of marine and freshwater AIS[1] along the overland transportation pathway. The scope of this project will be limited to watercraft, trailers, and related equipment and gear that move from water to land before entering a new waterbody, excluding those that remain in the water. Following this work, common elements across protocols could be identified by AIS management programs to derive best management practices for CDD+D in Canada.

7) Science Advice on Trapping methods for the invasive European Green crab in (2019–2022)

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European green crab (*Carcinus maenas*) is a voracious aquatic invasive species (AIS) that poses a serious threat to Canada's marine and estuarine ecosystems on the Atlantic and Pacific coasts. They prey on commercial and recreational shellfish, compete with commercial fisheries, and destroy ecologically and biologically-significant habitat for native species. Fisheries and Oceans Canada (DFO) has acquired substantial knowledge on European green crab, particularly regarding trapping as a form of physical removal to control their spread. Knowledge acquired includes information on species life history and biology, population dynamics, gear types, and, in some cases, Catch per Unit Effort (CPUE) of trapping gear (e.g., Fukui traps, fyke nets), as well as bycatch, control measures, and mitigation strategies. However, much of this knowledge has yet to be captured formally in a comprehensive review that can be applied to AIS management.

DFO's AIS National Core Program has requested science advice because trapping of European green crab is critical for early detection, determining impacts on native species and habitat, and control efforts to prevent ecosystem degradation and commercial fishery loss. In order to translate DFO's scientific knowledge into management action, information on various removal techniques and strategies must be incorporated into decision-making and be adaptable to different situations, such as variation in habitat, gear type, and trapping goals, balanced with operational capacity.

8) Biofouling as a vector for NIS Introductions in (2020–2022)

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Biofouling has been identified as a vector for the introduction of aquatic nonindigenous species (NIS) that may pose a threat to Canadian marine and freshwater ecosystems. Transport Canada has requested science advice from DFO to inform the development of commercial ship biofouling management policies that will better protect Canadian marine and aquatic ecosystems against aquatic invasive species. A study is being conducted using a multistage mechanistic model to assess the probability of introduction of NIS into Canada based on one year of commercial shipping arrival data. This assessment will evaluate the relative risks of NIS establishment (arrival, survival, and final establishment) into different regions of Canada, via both the hull and niche areas of the ship. Empirical biofouling and environmental data were used to inform the model, as well as estimates of biological processes with variability introduced to simulate the potential risk of NIS establishments via biofouling.

9) Science advice on mitigation measures/treatments to reduce the risk of spreading invasive species in Aquaculture (2021–2022)

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This project aims to review and characterize existing methods to mitigate the risk of spreading marine invertebrate and macroalgae AIS during shellfish/macroalgae introductions and transfers (or movements). More specifically, we will assess the effectiveness of existing methods at killing/removing marine invertebrate and macroalgae AIS

and the impacts on shellfish and macroalgae species survival. This work will be based on a literature review and limited to marine invertebrate and macroalgae AIS that have the potential to be transported on shellfish/macroalgal cultured species during movements (e.g., aquaculture transfers). Representative species from various functional and taxonomical groups (e.g. bivalves, tunicates, crustaceans) will be selected according to their presence (or their expected arrival) in Canadian marine environments and data availability. Effects of treatment methods on shellfish products (e.g., mussels, oysters, scallops) and macroalgae will be assessed for species cultured on Atlantic and Pacific coasts. Recommendations will serve to establish standardized conditions of license for DFO Introduction and Transfer committees with regard to Canadian AIS mitigation, for shellfish and macroalgal species that may be vectors of marine AIS.

10) Arctic Marine Ecosystem Resilience and Resistance to Environmental Changes (2019–2023)

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This project integrates community-led monitoring and traditional ecological knowledge data with physiological threshold experiments and ecological modelling to generate data on projected future suitable habitat and interactions between ecologically and commercially important species and potential future invasive species in the Hudson Bay region of the Canadian Arctic. The work includes collection of comprehensive baseline ecosystem data across the Hudson Bay region using community-led monitoring approaches developed for tracking changes in Arctic coastal diversity. This is complemented with environmental niche modelling of current and future climate scenarios to predict how suitable habitat for species of interest may change, and experimental methods with multi-omics analyses to test the physiological response of key species to environmental stressors, such as temperature and salinity.

6. Meetings

2021

No conferences

2020

No conferences

2019

- National Aquatic Invasive Species Monitoring Meeting Ottawa, ON February, 2019
- Northeastern Aquatic Nuisance Species (NEANS) Panel spring meeting, Springfield, Massachusetts, United States, 3–5 June, 2019
- International Conference on Aquatic Invasive Species, October 27–31 2019, Montréal Canada
- Northeastern Aquatic Nuisance Species (NEANS) Panel fall meeting, Delmar, New York, United States, 10–11 December 2019

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Denmark

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Highlights

A new database for registering all biodiversity in Denmark, including invasive alien species. The NW Atlantic sea slug (*Haminella solitaria*) was first observed with egg masses in Roskilde Fjord in the summer of 2020. The established population of *Hemigrapsus takanoi* in Dybsø Fjord, a lagoon connected to the southern part of the Great Belt, boomed during the summer of 2020. Species specific DNA barcodes were identified for several crab species.

The amphipod (*Gammarus tigrinus*) has been documented in Danish waters for the first time at the Baltic island Bornholm. The tanaid (*Sinelobus vanhaareni*) has been observed for the first time in Denmark in the harbour of Copenhagen. The Danish Environmental Protection Agency is now permanently established in Odense.

Content

1. Regulations

After a highly publicized scandal in November 2020 about closing down all minkfarms, the Ministry of Environment was again separated from the Ministry of Food, Agriculture, and Fisheries. However, legislation and regulation related to mariculture remains in the Ministry of Environment.

The website and database for reporting sightings of invasive species has been transferred to a new database and website (Arter: https://arter.dk/dashboard). However, you need to know the name of the species you want to find because this database contains all species found in Denmark, native as well as non-indigenous, terrestrial, freshwater and marine).

Work on implementation of MSFD phase II has continued, and in 2020 the report on the monitoring programme 2021–2026 has been published (Miljøstyrelsen, 2020). For Descriptor 2 (non-indigenous species) monitoring is based on the existing national monitoring programme for marine habitats (NOVANA) and some additional water samples to be analyzed for species specific DNA. For this a technical report on methodology has been published (Knudsen *et al.* 2020b). Another method for sampling and analyzing eDNA has also been developed (Nielsen 2020). It should be noted that none of the non-indigenous species reported in recent years have been detected by the national monitoring system. They have been observed by "concerned citizens" (e.g. small-scale fishers, SCUBA divers), EIA reports from off-shore construction projects, student theses, etc. The monitoring report states that the monitoring will not detect new species, but may detect dispersal and increased abundance of existing species. It can be mentioned that the report from 2019 (part 1) is now available in an English version (https://mim.dk/media/216849/danish_marine_strategy_ii_uk.pdf).

Implementation of the EU directive on Invasive species is also progressing with *Hemigrapsus sanguineus*, *Perna viridis* and *Rapana venosa* on the list of proposed new species to be included. The former two species were also among the species prioritized by a horizon scanning excersise (Tsiamis *et al.* 2020). The Danish government is in general against including marine species on the list because eradication and containment are not possible, and prevention is difficult and expensive. A decision is expected medio 2021; delayed (draft dated Nov. 2021 available at https://ec.europa.eu/info/law/better-regulation/).

The major part of the Danish Environmental Protection Agency (EPA) moved to Odense during the spring and early summer of 2019. Many new staff had been hired to replace experienced staff who did not wish to move. This has somewhat slowed down progress of many areas of work. Furthermore, there was a change of government after a parliamentary election in June 2019. This caused some reorganizations in ministries and agencies. Thus, the Fisheries department was transferred from the Ministry of Foreign Affairs back to the Ministry of Environment and Food.

The second update of the EU list of alien species of union concern has been approved and entered into force in July 2019, and the amended list was made publicly available on the website of the Danish EPA on 12 August 2019 (https://mst.dk/media/177675/oversigt-arter-paa-eulisten-til-hjemmeside-opdateret-august-2019_1.pdf). The updated list still contains only one tropical marine fish and the Chinese mitten crab. The former Danish government (before June 2019) stated that it does not wish to place marine species on the EU list because management measures are not possible or too expensive. The present government has not made any comments to this. The list for the third update contains some marine species, but risk assessments have not yet been completed.

The second phase of implementing the EU Marine Strategy Framework Directive (MSFD) was initiated in 2019 (Consolidation Act 1161 of 25 November 2019) (all laws, consolidated acts, executive orders, etc. can be downloaded from https://www.retsinformation.dk/). Prior to this, a report on the marine strategy containing status and impacts on the marine environment, as well as establishment of environmental targets for the various descriptors of the MSFD (Miljø- og Fødevareministeriet, 2019a). For Descriptor 2 (NIS) they conclude that the existing monitoring program does not capture new introduced species and they do not expect good environmental status in 2020. There is a general lack of data, and they recommend targeted collection of data from leisure boats and marinas concerning fouling organisms. The inclusion of eDNA analyses for capturing NIS in the monitoring program is still in a preliminary phase (Hansen & Høgslund 2019; Winding *et al.* 2019).

Concerning mariculture the Danish Environmental Protection Agency organized a thorough investigation of all existing operators and their compliance with legislation and regulations (Miljø- og Fødevareministeriet 2019b). This was in response to several cases of illegal activities in

previous years. No new permits have been issued and no existing operator has been permitted to expand or change their production during 2019. The report concluded that there is a general lack of documentation, a lack of compliance with habitat regulations and a lack of transparency of who is responsible for what – both for operators and authorities.

For the Ballast Water Convention and hull fouling, collaboration within the HELCOM/OSPAR framework has continued. An Executive Order (BEK 1000 of 18 September 2019) on treatment of ballast water and sediments from ships' ballast tanks has been approved by the minister of Environment and Food. It replaces BEK 968 of 24 July 2017.

2. Intentional

Fisheries statistics on imports and exports are still available only as "dynamic tables" at https://dwp.fiskeristyrelsen.dk/udenrigshandelsrapport/ . However, they appear to be much less detailed than before, and the most recent data are from 2019. Hence it has not been possible to separate import of live lobsters and/or oysters.

Although the import of live American lobster remains high, there are no reports of catching them in the wild. For escapes of fish from mariculture, see below. There have been no reported cases of illegal import of fishes for mariculture in 2019.

Fisheries statistics are no longer published as annual reports, but only available as "dynamic tables" on the website https://fiskeristyrelsen.dk/fiskeristatistik/.

3. Summary of sightings

Un-intentional, new species sightings:

Haminella solitaria. This Cephalaspidean gastropod was first recorded from the northern part of Roskilde Fjord in 2020. It re-occurred from July through September 2021 in the same locality. It has also been found in several localities in the Belt Sea (Norsminde Fjord (south of Aarhus), Gyldensteen Lagoon (north coast of Fyn) and Helnæs (southwest coast of Fyn) in September 2021. A photographic record of egg masses from the Limfjord (Lendrup Lagoon) shows that it apparently arrived here in 2018 (Jensen 2022).

Austrominius modestus. A paper has been published on the further spread of this barnacle in Danish waters from 2010 to 2020 (Glenner *et al.* 2021). Permanent populations are established at several localities along the northwestern coast of Jylland as well as the northern Kattegat coast and the Limfjord. These authors also model the predicted future expansion in northwestern Europe. It will be restricted by low salinity in the Baltic and by temperature in Arctic waters.

In 2019 the first occurrence of the amphipod *Gammarus tigrinus* Sexton, 1939, was recorded in the scientific literature. It has been found at two locations on the east coast of Bornholm in 2018, but was not published until 2019 (Rewicz *et al.* 2019). Ferries are suggested to be the most likely vector.

A student at the Natural History Museum of Denmark has collected the tanaid *Sinelobus vanhaareni* Bamber, 2014, in the harbour of Copenhagen during the summer of 2019. The ID has been confirmed by the Crustacea specialist at the museum, Dr Jørgen Olesen. At this time it is unknown if there is an established population. This is the first record from Danish waters. It has been found previously along the German Baltic coast and most recently in the Gulf of Gdansk (Brzana *et al.* 2019).

Unintentional, species previously recorded, but new sightings:

There has been a bloom of the toxic dinoflagellate *Karenia mikimotoi* in the Limfjord in March 2020 (EPA: https://mst.dk/service/nyheder/nyhedsarkiv/2020/mar/giftig-alge-i-limfjorden/). I am not sure whether this species is non-indigenous, cryptogenic or maybe native.

Mnemiopsis leidyi – new records 2020: Throughout Danish waters. Highlight: Extremely high salinity levels and temperatures in the SW Baltic Sea during winter (Jan.-March 2020) correlated with *M. leidyi* being present throughout the year. (Jaspers *et al.* 2021).

The amphipod *Caprella mutica* was recorded from the Limfjord on artificial boulder reef structures (Dahl *et al.* 2020). The species appears to be associated with artificial structures in most of its introduced area.

Hemigrapsus takanoi and *H. sanguineus*

The population of *H. takanoi* in Dybsø Fjord (Southern Great Belt) seems to be exploding during the summer of 2020 (see https://sn.dk/Naestved/Rekordmange-invasive-krabber-forsker-er-i-gang-med-kortlaegning/artikel/942454 , and S. and M. Mikkelsen, pers. comm.), and specimens have been used for identifying species specific DNA sequences for eDNA analysis (Knudsen *et al.* 2020a).

In July 2020, several specimens of *Hemigrapsus*, preliminarily identified as *H. sanguineus* were observed in Nissum Fjord on the North Sea coast of Denmark. The brief video-sequence seems to show both *H. sanguineus* (with striped legs) and *H. takanoi* (dark brown with patch of setae on claws) (see www.tvmidtvest.dk/nyheder/02-07-2020/1930/1930-02-jul-2020?clip=ca98f75c-5918-4b4b-8e75-a83f7a6e8c07). This is a northward extension from previous occurrences in the Wadden Sea.

Other records from 2020 (from Arter database):

H. takanoi: 1 specimen, 1 July 2020, 56.02N 10.27E; 12 specimens, 6 Oct. 2020, 55.08N 11.76E; 1 specimen, 7 Oct. 2020, 55.4N 8.71E; 6 specimens, 11 Nov. 2020, 54.87N 10.51E.

H. sanguineus: 1 specimen, 16 Aug. 2020, 56.08N 12.54E; 5 specimens, 6 Oct. 2020, 55.08N 11.76E.

Rhithropanopeus harrisii – new records 2020:

Few specimens, 13 July 2020, Kastrup Lystbådehavn; 1 specimen, 30 July 2020, Vordingborg.

Eriocheir sinensis – new records 2020:

1 specimen, 26 June 2020, Nissum Fjord (www.hof-storaa.dk/forside/nyheder/kinesisk-uld-haandskrabbe); 1 specimen captured in net, Aug. 2020, Sorthat Rev, Bornholm (https://m.tv2bornholm.dk/artikel/pighvar-rurer-og-kaempe-krabbe); 1 specimen, 16 October, 54.83N 11.57E; 1 specimen, 22 October, 55.43N 12.17E.

Pacific oyster (*Magallana gigas*):

New records for 2020 – mostly from localities along the Kattegat coast and into northern Belt Sea. In the Wadden Sea Pacific oysters are now forming dense reefs, and the permission for two licensed fishers to commercially collect (by hand) in specified areas has been extended. Because the Wadden Sea is protected under several directives, the fishers have been complaining about the bureaucracy, but apparently they still make a profit.

One mass escape of rainbow trout, *Oncorhynchus mykiss*, has been reported from a mariculture facility at the harbour of Grenå (Kattegat coast) in April 2020 (Carl 2020)

Neogobius melanostomus: new records 2020–2021.

Apparently, the distribution range has not expanded in 2020, but abundance in some localities has increased, in other localities decreased (Carl 2020).

Mnemiopsis leidyi A. Agassiz, 1865, continues to be abundant in Danish waters through summer months (when people go to the beach).

During April through July 2019, several specimens of *Hemigrapsus takanoi* Asakura & Watanabe, 2005, were found in Dybsø Fjord some of which were egg-bearing females. Also several *Rhithropanopeus harrisii* (Gould, 1841), were collected. Identification has been confirmed by photos and preserved specimens collected by S. & M. Mikkelsen and sent to the Natural History Museum of Denmark. Some of the *H. takanoi* specimens have been sent to NIVA Denmark for DNA-analysis. The occurrence of egg-bearing females means that *H. takanoi* is now established in the Danish part of the Baltic Sea. Dybsø Fjord is an enclosed lagoon-like estuary with a narrow connection to the Baltic Sea south of the Great Belt. Another record, confirmed by photo, is from July 2019 in Guldborgsund, the strait between the islands of Falster and Lolland. Here, *H. takanoi* was found with *Rhithropanopeus harrisii*, which has been recorded from this locality before. Further records of *H. takanoi* have been reported to the web-site of the Danish EPA (https://invasive-arter.dk/Menu.aspx) in June, July and August (when people go to the beach): at Als in the westernmost part of the Baltic, where they also occurred in 2018, and at the island Langeland, plus an unconfirmed record from the narrow strait between the islands of Falster and Møn (all in the Baltic Sea).

There have also been some sightings (recorded on the web-site of the EPA – see URL above) of *Hemigrapsus sanguineus* (De Haan 1835) during the summer of 2019, mostly from the Baltic Sea, but one record from July 2019 from Ebeltoft, at the border between Kattegat and the Belt Sea, and a second record from near Helsingør in the northern Sound (southern Kattegat) in October 2019 stand out as expansions of the previously observed range (www.oresundsakvariet.ku.dk/nyheder/2019/invasiv-krabbe-fundet-i-oeresund/). None of these records mentions egg-bearing females, so it is uncertain if this species is established in the Danish part of the Baltic Sea and Kattegat.

Some cases of mass escapes of rainbow trout (*Oncorhynchus mykiss*) (Walbaum, 1792) have been reported in Newsletters from anglers' associations, e.g. Nyborg Fjord in April 2019 and Lillebælt in December 2019.

The round goby (*Neogobius melanostomus*) (Pallas, 1814), continues its spread through Danish waters. In 2019, it was found in Odense Fjord (Belt Sea) (Kuhn & Christoffersen 2019).

4. Pathogens

Potential fish pathogens (*Vibrio* and *Mycoplasma* species) have been found as members of the mucus associated microbiota in non-indigenous comb jellyfish as well as native bloom forming jellyfish in the SW Baltic Sea (Jaspers *et al.* 2020a).

5. Research and monitoring

A research project has determined population density and other population parameters for the two *Hemigrapsus* spp. in the Danish part of the Wadden Sea (Vogensen *et al.* 2020).

The population in Løgstør Bredning (Limfjord) has been estimated to be 1200 t at depths below 3 m (Nielsen *et al.* 2020), less than half of the estimated population in 2019 (see report from last year).

Another report from Løgstør Bredning describes establishment of flora and fauna on artificial boulder reef structures (Dahl *et al.* 2020). Several alien species, both macroalgae and benthic invertebrates were found. Except for the amphipod *Caprella mutica*, the alien species have been recorded in the Limfjord previously.

A study from Odense Fjord (Quintana & Kristensen 2020) demonstrated that bioturbation by *Marenzelleria viridis* decreased the concentration of H_2S in porewater significantly more than the native *Hediste diversicolor*.

Several studies on behavior, ecology and physiology of round goby have been published (Behrens *et al.* 2020; Brauer *et al.* 2020; Schwartzbach *et al.* 2020).

In 2020, several collaborative international studies on management of non-indigenous and invasive species were published. Bailey *et al.* (2020) analyzed a huge amount of data to describe trends in number of new introductions and pathways over a 50 year period. Tsiamis *et al.* (2020) published the results of a horizon scanning exercise covering European and Mediterranean coastal seas, resulting in a prioritized list of high-impact invasive species. Obst *et al.* (2020) published a paper on establishing a network for molecular monitoring of hard bottom communities. The two Danish stations included (Læsø and Limfjord) are included in the NOVANA monitoring programme.

The Danish Environmental Protection Agency has initiated a project to develop teaching materials about invasive species for 7th–9th grade school children.

A cookbook has been published in collaboration between the EPA and the cooking school in Copenhagen. It features short descriptions of the species, their biology and spread, and then some recipes; the only marine species included are *Crassostrea gigas* and *Neogobius melanostomus* (Dalsgård *et al.* 2019).

In relation to the EU Water Framework Directive (WFD), a research project has investigated the invasive seaweed (*Sargassum muticum*) on other vegetation (Stæhr *et al.* 2019). They concluded that *S. muticum* has a significant effect on macroalgal composition, decreasing especially other large brown algal species. They found no negative effects on eelgrass.

The bacterial composition of ectodermis and gastrodermis of warty comb jelly (*Mnemiopsis leidyi*) has been compared for native and invasive populations of the comb jelly (Jaspers *et al.* 2019). Also, a dataset on biodiversity and abundance of gelatinous macro zooplankton, including *M. leidyi* from the North Sea in August 2018 has been published (Gawinski *et al.* 2019).

The population of Pacific oysters (*Crassostrea gigas*) in the Danish Wadden Sea has been estimated to about 72,000 t. Pacific oysters often occur mixed with blue mussels. In some places there are oyster reefs with many mussels on and between the oysters, in other places there are extensive mussel mounds with small oysters attached (Nielsen *et al.* 2019a).

There are two reports assessing the population of Pacific oysters in the Limfjord, one from Nissum Bredning, the westernmost part of the fjord, where an estimated population of 90 t was found in the areas where blue mussels are fished (Nielsen *et al.* 2018). The second assessment area was in Løgstør Bredning in the central part of the Limfjord, where a population of about 2450 t was estimated at depths > 3 m (Nielsen *et al.* 2019b).

There is also a Nordic report on harvesting Pacific oysters. This report summarizes knowledge about Pacific oysters in Nordic waters and presents suggestions for commercial exploitation (Mortensen *et al.* 2019b). In connection with this research, a Policy Brief has also been published (Mortensen *et al.* 2019a).

Movements in the sediment caused by activities of permanently buried *Mya arenaria* from the northernmost tip of Fyn, Funen island (Camillini *et al.* 2019).

A paper analyzing the genetic composition on the alien barnacle (*Amphibalanus improvises*) has been overlooked in the previous report (Wrange *et al*. 2016).

Acoustic telemetry has been used to study behavior and seasonal migrations of *Neogobius melanostomus* in Karrebæk and Dybsø Fjords (Christoffersen *et al.* 2019).

In connection with the implementation of the MSFD, a study on cumulative effects of human stressors has been published in which spatial modeling is applied (Andersen *et al.* 2020). The

model showed that NIS ranked second in impacts if climate anomalies were not included, and third when climate anomalies were included.

Also, in connection with the implementation of the MSFD, a provisional EU-wide consolidated list of NIS has been assembled by experts from 16 EU countries (Tsiamis *et al.* 2019).

Future planned research:

The methodology for eDNA will be further developed; primers for more species will be developed.

The EPA has proposed monitoring of fouling organisms on leisure boats and in marinas should be included in national monitoring, but they are still seeking funding for a preliminary study.

The final report of MONIS 4 on the baseline of NIS in 16 Danish harbours has still not been published, but research results have been presented at meetings.

Knowledge gaps:

With the rapid expansion of various small crab species (*Hemigrapsus* spp., *Rhithropanopeus harrisii*) it would be nice to know whether the Baltic invasion is from the same genetic strains as the North Sea populations, and whether the Baltic is being invaded through Skagerrak, the Limfjord or maybe the Kiel Canal.

As mentioned several times over the past years, jelly plankton should be included in monitoring. *Beroe ovata* was reported from the Belt Sea (Baltic) in 2014, but has not been reported since – probably because nobody looked.

6. Meetings

The advisory group on invasive species have only held one meeting in 2019 due to the relocation of the Environmental Protection Agency from Copenhagen to Odense. The meeting was held on 8 May 2019 in Odense

Danish Society for Marine Biology held a meeting on 27 February 2019 with presentations on monitoring of non-indigenous species by eDNA, night-diving and fouling plates.

A Marine Science symposium in January 2019 at University of Southern Denmark in Odense featured some presentations and posters on NIS.

Jysk Naturhistorisk Forening held a meeting on invasive species 3 October 2019. This was related to the EU list and the problems of implementing the control measurements.

An ICES Shellfish Symposium: Shellfish – Resources and Invaders of the North was held in Tromsø, Norway 5–7 November 2019. Staff from Danish Shellfish Center presented data on expansion of *Crassostrea gigas* in Denmark (Freitas *et al*. 2019).

The advisory group on invasive species had one meeting on 4 March 2020 in Odense. Progress of the third update of the EU list was presented. Other projects concerned with invasive species were presented.

Advisory group on IAS of EU importance has held a virtual meetings (11 March 2021) on the progress of implementation and update of species lists. Also, the transfer of citizen records/ observations from the platform at the Environmental Protection Agency to the new platform "Arter" [species] (https://arter.dk/landing-page) was discussed. Furthermore, there has been a "horizon scan" exercise risk assessing a long list of species not yet found in Denmark, followed by a consensus conference on 30 November 2021 (also virtual), which unfortunately did not reach final consensus, so they are still working on it.

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Summary 2019–2021

Finland collected the needed information for the first reporting of the EU IAS Regulation and reported presence/absence of the EU listed species in Finland in June 2019. The only aquatic species that was on the reported species list is Chinese mitten crab (*Eriocheir sinensis*), which is observed only few times a year. Two new non-indigenous species were observed in 2021 on the Finnish coastline in the Baltic Sea. *Rangia cuneata* clam and the Japanese cumacean (*Nippoleucon hinumensis*) were found 2021 the first time in Finland close to port areas in the Gulf of Finland. The round goby (*Neogobius melanostomus*) that was first recorded in Finland in 2005, has spread to almost entire Finnish coastal waters. The northernmost observation was made in 2019 in Oulu, Bothnian Bay. Also, the tanaid (*Sinelobus vanhaareni*) has been spreading and increasing in abundance during the past years (first observation in 2016). A proposal on non-native species monitoring framework was completed and submitted to HELCOM providing monitoring guidelines for multiple methods to complement the NIS monitoring programme in COMPLETE project. Finland conducted NIS sampling events on three coastal marinas in 2018 (Southwestern Finland), while creating biofouling survey protocol for leisure boats and marinas. The protocol was further tested in a study on different anti-fouling paints on a sailing boat hull which was completed in 2020 (tasks of the COMPLETE project).

Overview:

Highlights of the 2021 report

Two new non-indigenous species were observed in 2021 in the in the Gulf of Finland, the Baltic Sea. *Rangia cuneata* clam was found in May 2021 the first time in Finland from the sea area close to Loviisa harbor in the eastern Gulf of Finland and the cumacean crustacean (*Nippoleucon hinumensis*) was found in late summer close to Kotka port. The number of pink salmon (*Oncorhynchus gorbuscha*) ascending to the rivers in northern Finland was higher than ever before in 2021.

Content

1. Regulations

Finland has been taking part to the expert groups of EU invasive species issues related to the implementation of the EU IAS Regulation (2014), to MSFD Non Indigenous Species expert network and to EU MSFD D2 Core Group. The first reporting to the Commission was completed in June 2019. Finland is active also in the joint HELCOM-OSPAR Task Group BALLAST & BIOFOULING) where countries around Baltic Sea and North East Atlantic work to develop a common framework on the specific issues of exemptions for the BWMC and towards harmonized biofouling strategy, for the Baltic Sea (managed by HELCOM) and for the North East Atlantic (OSPAR) region.

2. Intentional

Deliberate releases into the Baltic Sea continued (including rivers draining into the Baltic) for fisheries and fish stock enhancement purposes in 2019–2021 with newly hatched and older salmon (*Salmo salar*), sea trout (*Salmo trutta* m. *trutta*), eel (*Anguilla anguilla*), pikeperch (*Sander lucioperca*) and whitefish (*Coregonus lavaretus*).

3. Summary of sighting

Unintentional:

Two new non-indigenous species were observed in 2021 on the Finnish coastline in the Baltic Sea. *Rangia cuneata* clam was found in May 2021 the first time in Finland from the sea area close to Loviisa harbor in the eastern Gulf of Finland. More clams were found and reported along the Gulf of Finland and even from the northern sea areas in the Bothnian Sea close to Olkiluoto nuclear power plant where tens of very small individuals have been observed indicating reproduction. After spreading to Finland and Latvia in 2021, *Rangia* has been observed in all Baltic Sea countries after its first record in 2010 in the Kaliningrad area. *Rangia* is a brackish water species indicating that it has potential to spread and establish in all sub-regions of the Baltic although reproduction may be limited on the northernmost and easternmost coasts due to very low salinity.

The other new non-indigenous species observed in 2021 in Finland is the cumacean *Nippoleucon hinumensis* (~5 mm in size) found in late summer close to Kotka port in the Gulf of Finland. The only other Baltic country where the species has been previously found is Germany (2019). *N. hinumensis* is also a brackish water species having potential to spread further in the Baltic Sea area and to establish permanent populations in all sub-regions. It is probable that the specimens found in Finland where transported by shipping as all observations so far have been close to ports (also found close to the large cargo and oil port Sköldvik in Porvoo in 2021). It is a benthic crustacean living in the soft sediments and can be found in routine benthos monitoring.

Previous sightings:

General information

Since 2017 pink salmon (*Oncorhynchus gorbuscha*) numbers have been increasing strongly in the north Atlantic. The number of pink salmon ascending to the rivers in northern Finland in 2021 was higher than ever before.

4. Pathogens

5. Research and Monitoring Programs

1) **Non-indigenous species monitoring in ports -project (2022–2024)**

Maiju Lehtiniemi (Finnish Environment Institute)

Contact: maiju.lehtiniemi@syke.fi

The purpose of the Ministry of the Environment funded project is to sample non-indigenous species from all habitats from the ports in Finland to gather data and information for the HELCOM HOLAS, EU MSFD and IMO BWMC. The project will use the port survey protocol developed in HELCOM/OSPAR cooperation to sample 6 largest international ports in Finland (Baltic Sea). The data will be reported to HELCOM Joint Harmonized Procedure port database and new NIS observations to AquaNIS to provide open access to the collected data.

2) **Completing management options in the Baltic Sea Region to reduce risk of invasive species introduction by shipping, COMPLETE (2017–2020)**

Miina Karjalainen, Kotka Maritime Research Association Contact:miina.karjalainen@merikotka.fi

Maiju Lehtiniemi, Finnish Environment Institute: Contact: maiju.lehtiniemi@syke.fi

The project was led by Kotka Maritime Research Association, Finland and funded by INTERREG Baltic Sea Region Programme. There were 12 project partners from seven Baltic Sea countries. Finland was represented by five partner institutes (Kotka Maritime Research Association, Finnish Environment Institute, University of Helsinki: Dept of Environmental Sciences, Keep Archipelago Tidy and Kymenlaakso University of Applied Sciences). This project tackled several knowledge gaps: the need to take into account rights and obligations of involved stakeholders; approaches for NIS monitoring and surveillance for EU Marine Strategy Framework Directive (MSFD) and Ballast Water Management Convention (BWMC); risk assessment based exemptions from ballast water management requirements; legal aspects; regional cooperation and information exchange. Project results provided comprehensive knowledge for decision making to understand the different antifouling practices in maritime and leisure traffic. Based on this knowledge, recommendations were compiled which enable the development of harmonized biofouling management strategies for the entire Baltic Sea region. The project aimed at developing a roadmap for a harmonized approach by involving all relevant stakeholders from the beginning of the project. SYKE led the work package 2 in the project where the aim was to develop and test new methods for NIS monitoring that could be taken into routine use in the Baltic Sea countries and to complete the development of the harmonized monitoring program for the Baltic Sea area.

3) **BONUS BLUEWEBS (2017–2020)**

Laura Uusitalo, Finnish Environment Institute: Contact: laura.uusitalo@syke.fi

BLUEWEBS was designed to deliver an assessment of the consequences of simultaneously achieving the good environmental status and providing blue growth (i.e. the capability of Baltic Sea food webs to sustainably produce ecosystem goods and services). Global climate change will likely result in novel climates, leading to combinations of physical oceanographic conditions never encountered before in the Baltic Sea. These will affect ecosystems in addition to the regional anthropogenic impacts of eutrophication, fisheries exploitation, invasions of non-indigenous species and accumulation of hazardous substances. These cumulative impacts have already

(and will likely continue in the future) cause novel food webs that significantly differ in structure and function from historical predecessors.

4) **Practical implementation of the COMPLETE project outputs and tools (COMPLETE PLUS) (2021)**

Miina Karjalainen, Kotka Maritime Research Association Contact: miina.karjalainen@merikotka.fi

Maiju Lehtiniemi, Finnish Environment Institute: Contact: maiju.lehtiniemi@syke.fi

Main focus in the COMPLETE PLUS project was improvements of the HELCOM/OSPAR risk assessment tool for exemptions via updated Baltic Sea target species list and operationalization of the Early Warning System for the Baltic Sea HAOPs via AquaNIS database.

5) **FIN-HAVI (2019–2020)**

Erja Huusela, Natural Resources Institute Finland, contact: erja.huusela@luke.fi

Creating a national management plan for invasive alien species in Finland.

6) **EU-HAVI3 (2020–2021)**

Erja Huusela, Natural Resources Institute Finland, contact: erja.huusela@luke.fi

Distribution, pathways and management measures of the second updated list of EUs invasive alien species

6. Meetings

- Several national meetings (of the board on invasive species issues, the expert group on development of national NIS legislation)

- project COMPLETE-PLUS meetings

- COMPLETE final conference in February 2021

- HELCOM/OSPAR JTG Ballast&Biofouling meetings in December 2019, 2020, 2021.

- EU MSFD D2 Core Group meetings in autumn 2021

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France

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Audience: (ICES, Member Countries & Observers, and Scientists)

Overview:

A total of 26 new introductions, all unintentional, were reported for the country or for new maritime regions within France. They include one macrophyte (*Halophila stipulacea*), three algae (*Lophocladia lallemandii, Ulva ohnoi*, *Symphiocladia tanakae*), six peracarids (*Aoroides longimerus & A. semicurvatus, Ericthonius didymus*, *Ianiropsis serricaudis, Paranthura japonica, Synidotea laticauda*), one amphipod *(Caprella scaura),* one pycnogonid (*Ammothea hilgendorfi),*one bryozoan *(Schizoporella japonica),* five annelids *(Bispira polyomman Marphysa victori, Polydora colonia, Terebella banksyi, Thelepus japonicus),* two molluscs *(Arcuatula senhousia, Lamprohaminoea ovalis),* two fish (*Cynoscion regalis*, *Holocentrus adscensionis)* and three tunicates (*Botrylloides diegensis, Didemnum pseudovexillum, Styela clava*), as well as a novel mussel lineage (resulting from admixture between native mussels and the introduced Mediterranean mussel in North Atlantic commercial ports). The blue crab *Callinectes sapidus* is undergoing a rapid population expansion in French

Mediterranean coastal lagoons, leading to the adoption of a regional action plan in March 2022. The first review of NIS in the French overseas territories was carried out by the IUCN French committee. They reported 61 NIS, a third of which are ascidians, with the highest number (31) found in French Polynesia. In the absence of dedicated monitoring, these numbers are probably underestimated, as is likely to be the case in continental France. Several research programs targeted pathogens and diseases, notably the protist *Haplosporidium costale* responsible for an important die-off of the *Crassostrea gigas* oyster in one locality, and transmissible cancer in mussels (for which a new strain had been identified, shared by the blue mussel and the Chilean mussel suggesting international shipping as pathways). Other research projects aimed to 1) examine the relationships between MPAs and invasive species, 2) analyze biofouling by NIS in ports and 3) develop molecular tools to support surveys and monitoring –including with eDNA and metabarcoding, tracing back introduction routes, and analyzing impacts on native species (hybridization). Following a national risk analysis exercise, eleven marine IAS have been shortlisted for inclusion in two different levels of national regulation.

Content

1. Regulations

Throughout 2019, the EU regulation on the prevention and management of the introduction and spread of IAS (EU 1143/2014) has been rolled out across Frances' five overseas departments. Through a series of decrees, the islands of la Réunion, Mayotte, Guadeloupe, Martinique and French Guiana are gradually banning all activities involving non-indigenous flora and fauna. By the end of 2022, all remaining overseas French territories, including Saint-Pierre and Miquelon, will be covered.

As part of the implementation of the second cycle (2014–2020) of the EU Marine Strategy Framework Directive (MSFD), the monitoring programs for France's four sub-regions have been revised and gradually implemented by regional authorities since February 2019. During a national workshop held in December 2019, it was agreed to focus monitoring efforts on a) hard substrates and 'high-risk' areas and b) the 'D2C2' criteria, which aims to survey established NIS (abundance and spatial distribution), based on a list of 68 priority species (drawn up in 2018) considered as being already or potentially invasive.

Eleven marine Invasive Alien Species (IAS) have been shortlisted for inclusion in two different levels of national regulation following a risk analysis. "Level 1" species (*Callinectes sapidus, Hemigrapsus sanguineus, Brachidontes pharaonis and Didemnum vexillum*), where their release in the wild will be regulated. "Level 2" is the transposal of EU regulation 1143/2014 into national law. If accepted, then seven species (*Arcuatula senhousa, Lagocephalus sceleratus, Mnemiopsis leidyi, Pterois miles, Pterois volitans, Rapana venosa* and *Rugulopteryx okumurae*) will be submitted to the EU for inclusion in the European IAS list.

In March 2021, a regional action plan targeting *Callinectes sapidus* was adopted by the Occitanie region of the French Mediterranean. All three French Mediterranean regions (Occitanie, PACA and Corsica) are pooling resources and knowledge to face this rapidly proliferating species.

On 15 March 2022, a national action plan was adopted, extending from 2022 to 2030, to prevent the introduction and propagation of terrestrial and aquatic IAS (www.ecologie.gouv.fr/sites/default/files/20220315_EEE_VDEF.pdf). This plan aims to strengthen the coordination and mobilisation of actors and increase the coordination of human health, animal health and environmental health policies in a "One Health" approach.

A coordinated action from the French National Assembly focused on the impacts of invasive flora (including marine plants and algae) to develop new management options. A report was published on 21 July 2021 by two deputies (Nadia Essayan and Patrice Perrot) on behalf of the Commission for sustainable development and national planning.

In February 2022, the FRB (Fondation de Recherche Biodiversité) coordinated the French external review of the chapters of the IPBES thematic assessment of IAS.

2. Summary of sightings

Important note: the sightings reported below are for mainland France only. However, the first review of NIS in the French overseas territories was carried out by the IUCN French committee. A total of 61 NIS were reported, although it is difficult to determine the year of first observation in most cases. We therefore did not fill the table below with overseas NIS but recommend to read the following reports for further information: IUCN Comité Français (2019). "*Espèces exotiques envahissantes marines : risques et défis pour les écosystèmes marins et littoraux des collectivités françaises d'outre-mer. Etat des lieux et recommandations. Paris, France, 100p.* » https://especes-envahissantes-outremer.fr/wp-content/uploads/2019/12/publication-eee-marines-outre-mer-2019.pdf. For Saint-Pierre and Miquelon, an annual report on NIS is published by the local government (DTAM, 2019–2021). There is a decrease in the number of trapped green crabs (*Carcinus maenas*), and the rapid expansion of the bryozoan *Membranipora membranacea* is also of note.

2.1 Intentional

A recent report by "FranceAgriMer" published key fishing and aquaculture figures. In 2019, 108M € worth of "oysters" (although the distinction between *Crassostrea gigas* and *Ostrea edulis* is not made, the latter is very limited in landings, i.e. <2000t) were exported from France (corresponding to ≈21 760 tonnes) (FranceAgriMer, 2020).

2.2 Unintentional

New sightings (2019–2021): new country records or new sub-region records

Molluscs –Mytilus spp.

Using a dataset of 4279 mussels genotyped at 77 SNPs, Simon *et al.* (2020) documented a very recent (<50 years) introduction of the Mediterranean mussel *Mytilus galloprovincialis* into several Atlantic harbours. In each case, the introduced species shows extensive admixture with the local native *Mytilus* background (*i.e.*, in all cases *M. edulis*, with one exception in the port in Brest where the native mussel is the Atlantic lineage of *M. galloprovincialis*). Interestingly, these "dock mussels" are so far confined to harbours, with sharp transitions at the port entrance.

Tunicates -*Botrylloides diegensis* and *Botrylloides* spp.

Based on COI sequencing of >750 colonies of *Botrylloides* spp. including reference samples identified by taxonomist experts, and sequences retrieved from BOLD and Genbank, Viard *et al.* (2019) showed database errors particularly regarding *B. diegensis* (introduced), which can thus be mistaken as *B. leachii* (native). This work provides evidences for new reports of *B. diegensis* in Italy (Venice), Spain (Delta of Ebro and Catalan coast) and France (Thau Lagoon). In addition, a particular unicolor morph of *B. diegensis* can easily be mistaken with another introduced botryllid (*B. violaceus*). It is thus likely that the distribution of *B. diegensis* had been underestimated in European seas. The authors point the need to revise previous reports of *B. leachii*, as it may have been confused with *B. diegensis*. In the same study, colonies belonging to a new lineage of *Botrylloides* sp. have also been identified in the English Channel (in the UK only, so far). This lineage could be either a divergent lineage of *B. israeliense* (newly described in the Mediterranean Sea) or a sister species (possibly undescribed, and maybe introduced) of the latter.

Putative New species

Through research on the well-known invasive species *Didemnum vexillum*, Spanish researchers discovered specimens sampled in Brittany (France) belonging to a very divergent lineage although morphologically similar to *D. vexillum*. The French and Spanish researchers pursued their research on this lineage, and found both molecular and morphological evidences supporting the

description of a novel species, named *Didemnum pseudovexillum* (because of its high external similarity with *D. vexillum*). This novel species had been found in ports in Brittany (Channel) and Spain (Mediterranean Sea), suggesting that it might be an introduced species in European waters, and should be considered as a cryptogenic species (Turon *et al.* 2020).

Taxon	Phylum	References	MSFD sub-region, location	First country record?	Year of observation
Ammothea hilgendorfi	Arthropoda - Pycnogonida	Le Roux *et al.* 2020	Bay of Biscay (BoB) – Ria d'Etel	Yes	2019
Aoroides longimerus & A. semicurvatus	Arthropoda - Crustacea	Dauvin *et al.* 2020	EC - Normandy	No – previously in BoB (2009)	2019
Arcuatula senhousia	Mollusca	Massé *et al.* 2020	EC (Dunkerque) & BoB (La Rochelle	No – previously in BoB. First record for French EC	2018
Bispira polyomma	Annelida	Droual *et al. in prep*	Celtic Seas – port of Brest	Yes	2021
Botrylloides diegensis	Chordata - tunicates	Viard *et al.* 2019	W. Med	No – previously in BoB (2007) and EC (2005)	2012
Caprella scaura	Arthropoda - Crustacea	MSFD surveillance, Massé *et al.* 2020	Bay of Biscay – Arcachon marina	No – previously in EC	2021
Cynoscion regalis	Chordata - scianidae	Iglesias *et al.* in prep	Bay of Biscay	Yes	2021
Didemnum pseudovexillum	Chordata - tunicates	Turon *et al.* 2020	EC - Roscoff	Yes - cryptogenic	2015
Ericthonius didymus	Arthropoda - Crustacea	Gouillieux *et al.* 2020	Bay of Biscay – Arcachon Bay	No – previously in W. Med (2015)	2013
Halophila stipulacea	Macrophyte	Thibaut *et al.* 2022	W. Med - Cannes	Yes	2021
Holocentrus adscensionis	Chordata - squirrelfish	Iglesias *et al.* 2021	W. Med	Yes	2019
Ianiropsis serricaudis	Arthropoda - Crustacea	Raoux *et al.* 2020	EC – le Havre	No - previously in Bob	2019

				(Arcachon) – 2013	
Incisocalliope aestuarius	Arthropoda - Crustacea	Bachelet *et al*. 2020	Bay of Biscay – Gironde Estuary	Yes	2015
Lamprohaminoea ovalis	Mollusca	Bourjon & Sittler 2022	W. Med (Corsica, near Bastia)	Yes	2021
Lophocladia lallemandii	Rhodophyta	Boudouresque *et al*. 2022	W. Med (Port Cros national park)	Yes	2021
Marphysa victori	Annelida	Lavesque *et al*. 2020a	Bay of Biscay – Arcachon Bay	Yes	2016
Paranthura japonica	Arthropoda - Crustacea	Pezy *et al*. 2020	EC (Le Havre & Cherbourg)	No – previously in Bob (Arcachon) – 2013	2019
Polydora colonia	Annelida	Gouillieux *et al*. 2022	Bay of Biscay – Arcachon Bay & Hossegor lake	Yes	2018
Schizoporella japonica	Bryozoa	Droual *et al. in prep*	Celtic Seas – port of Brest	Yes	2021
Styela plicata	Chordata - tunicates	MSFD surveillance, Massé *et al*. in prep ; Viard and Levêque – MarEEE project (i-site MUSE)	Bay of Biscay – Arcachon marina & la Rochelle marina	No – previously in EC	2020
Synidotea laticauda	Arthropoda - Crustacea	Massé *et al*. 2019	EC (Seine Estuary)	No -Previously in BoB – Gironde Estuary (1993)	2017
Symphiocladia tanakae	Rhodophyta	Verlaque & Breton 2019	EC – ports of le Havre and Antifer	No – 1st European observation was in Thau lagoon	2008
Terebella banksyi sp. nov.	Annelida	Lavesque *et al*. 2021	Bay of Biscay – Arcachon Bay	Yes – new species	2017

Thelepus japonicus	Annelida	Lavesque *et al.* 2020b	Bay of Biscay – Arcachon Bay and EC – Saint-Vaast-la-Hougue	Yes – first European record	2018
Ulva ohnoi	Chlorophyta	Verlaque & Breton 2019	EC – ports of le Havre and Antifer	No – previously observed in Sète port	2015

Previous sightings (2019–2021): new records within the same sub-region

Taxon	Phylum	References	MSFD sub-region, location	Year of most recent observation	Comments
Boccardia proboscidea	Annelida	Gully & Cochu 2019	EC Northern Brittany (Trégor)	2018	Previously near Belgium border (Wimereux)
Callinectes sapidus	Arthropoda - Crustacea	Unpublished observations	W. Med	2020	Rapid population expansion
Carcinus maenas	Arthropoda - Crustacea	DTAL 2019	Saint Pierre et Miquelon (grand étang de Miquelon)	2019	~60 individuals, including berried females
Gracilupurpura rostrata	Mollusca	Le Garrec, 2020	EC Northern Brittany (Penvénan)	2017	Previously reported in Morlaix (2007) and Saint-Brieuc (2009)
Kyphosus sectatrix	Chordata	Iglesias *et al*. in prep	W. Med, Marseille & Narbonne	2020	Previously reported in St Tropez (2006)
Mnemiopsis leidyi	Cnidaria	Nowaczyk *et al.* in prep	Bay of Biscay	2021	Clearly established along 500km of coastline
Mytilus spp. (dock mussel)	Mollusca	Simon *et al.* 2020	EC & Bay of Biscay	2015-2017	Previously in Cherbourg (2003)
Pseudodiaptomus marinus	Arthropoda - Crustacea	Unpublished observations	EC	2019	Paluel power station - abundant

Rapana venosa	Gastropoda	Unpublished observations	Bay of Biscay	2020	~40 individuals collected by fishermen in the French pertuis
Rugulopteryx okamurae	Ochrophyta	Ruitton *et al.* 2021	W. Med, Marseille	2018	Rapid spread through Calanques national park
Ulva australis	Chlorophyta	Sauriau *et al.* 2021	Bay of Biscay (Concarneau)	2019	Suggested origin: Kobe (Japan) and/or south-eastern Korea

Not Seen Species Yet

A newly established population of the fouling polychaete *Spirobranchus cf. tetraceros* is reported from the western Mediterranean (Valencia Port). Despite previous intensive surveys, this is the first record for the taxon in the Iberian Peninsula (Palero *et al.* 2020). The compound ascidian *Symplegma brakenhielmi* is rapidly spreading across the Mediterranean and was observed in the NE of Sardinia in 2018 (Ramos-Epla *et al.* 2020). The probability this species will be, or is already, present in Corsica is therefore highly likely.

3. Pathogens

Protists: On 8 June 2019, after a massive mortality event in the farmed *Crassostrea gigas* oysters from the Ifremer Bouin marine station, the parasite *Haplosporidium costale* was detected, supposedly for the first time in France. This parasite is known to cause low mortality of *C. virginica* along the east coast of North America. As part of a risk prevention strategy, all oyster batches originating from Bouin were destroyed. A retroactive analysis revealed this parasite as already being present in the wild in the UK and in France, as early as 2009. qPCR tests on 730 wild individual oyster revealed a greater percentage of contaminated individuals in Atlantic shellfish farming areas. On-going research is focusing on genome sequencing of the parasite and understanding what caused the sudden oyster mortality (Arzul *et al.* 2021). An international seminar was help in spring 2021 (cf. Events §). A newly described Haplosporidian endoparasite (*Haplosporidium pinnae*) is the most probable cause of a mass mortality event devastating the populations of the endemic bivalve *Pinna nobilis* in the Mediterranean Sea since 2016 (Cabanellas-Reboredo *et al.* 2019), that has spread from the west to the east Mediterranean. Although the origin of this parasite is unknown, there is speculation it too may have been introduced through human activities.

Transmissible cancers: Transmissible cancers are rogue cell lineages that spread between individuals (i.e., disseminated neoplasia). Such a transmissible cancer had been identified in bay mussels (*M. trossulus*) in the Northern hemisphere by the team led Michael Metzger at the Pacific Northwest Research Institute in Seattle, Washington. Tumour cells with genetic markers characteristic of bay mussels, although revealing a second strain of this disseminated neoplasia, had been then identified in both the Chilean mussel (*M. chilensis*) and the blue mussel (*M. edulis*) (Yonemitsu *et al.* 2019). The transmissible cancer thus spread to South American and European mussel species — probably via international shipping vessels, the researchers suggest. Since 2014, the blue mussel (*Mytilus edulis*) has experienced mass die-offs in France. After showing initial signs of being caused by a transmissible cancer, the most probable cause of the mass

mortality event is likely to be a pathogen and/or non-transmissible tumour (Burioli *et al.* 2019, Charles *et al.* 2020).

4. Research and Monitoring Programs

The programs have been ordered by Term of Reference and year (start of program).

4.1 ToR c - Biofouling

4.1.1 EMBIMANOR (ENrichissement de la BIodiversité MArine Littorale en NORmandie) (2017–2021)

Jean-Claude Dauvin, University of Caen: jean-claude.dauvin@unicaen.fr

This EMBIMANOR project aimed to understand the evolution in biodiversity of the Normandy coastline and marinas and encompassed two actions targeting non-native species. The first is a monitoring of the crab populations of asian origin *Hemigrapsus sanguineus* and *Hemigrapsus takanoi,* which was jointly carried out by researchers and environmental NGOs. The second action studied the fixed hard-substrate fauna present in marinas and tested the hypothesis that the ports of Normandy with high numbers of cross-Channel and international traffic (Cherbourg, Ouistreham, Le Havre et Dieppe) are the main pathway of introduction of non-native (but rarely invasive) species. The chosen protocol is similar to that develop by the Interreg project Marinexus: 40 alveolated polyethylene plates were immersed at 1.5m depth in 17 marinas between Granville and Tréport. Plates were removed and analysed after 3, 6, 9, 12 and 24 months, in order to view the entire colonisation sequence. Species which colonised the plates will be identified to the lowest taxonomic level possible. The protocol was maintained during 2021 in five marinas encompassing different environmental and maritime traffic conditions: Granville, Cherbourg, Ouistreham, Le Havre and Dieppe.

4.1.2 COECODIGUE (2021–2023)

Amelia Curd : amelia.curd@ifremer.fr

The port of Brest has invested massively in renewable ocean energy infrastructure. 2022 will see the completion of a **new 14 hectare polder** that will be used for reception, pre-construction and transportation of offshore wind turbine components. A long-arched dam of 870 m extent retains the new polder land. This steeply sloping rock revetment is made up of large granite boulders. With the **target effect of enhancing biodiversity,** local stakeholders decided to place **100 artificial pools (ecoblocks)** within the revetment at three different intertidal levels. These ecoblocks are large hollow 8m^3 cement cubes able to retain 0.5m^3 of water. By **combining drone imagery, quadrat still imagery, rapid assessment surveys and DNA-based molecular approaches**, the project aims to answer three main questions: 1) Do the ecoblocks support benthic communities comparable to both the adjacent boulders and to the subtidal part of the rock revetment? 2) Are the ecoblocks characterised by depauperate communities, dominated by opportunistic and **invasive alien species**? 3) What are the **temporal dynamics** of these communities? Can they reach a stable state in a port area, and if so, when?

4.1.3 MAREEE (2020–2023)

Frédérique Viard, ISE-M: frederique.viard@umontpellier.fr

Funded by the i-site MUSE (Univ Montpellier) the MarEEE project is dedicated to the study of NIS that are contributing to biofouling in ports by combining ecology, evolutionary biology and socio-economical studies. Steered by three laboratories, ISEM, MARBEC and CEEM, it aims to feed knowledge fronts (e.g. the emergence of an "Urban

Marine Science") and to support public environmental policies, through three questions: 1) what is the importance of biotic homogenization in port habitats, and with what contribution from NIS (studies done with eDNA and metabarcoding); 2) Does hybridization between NIS and their native congeners cause evolutionary novelties and contribute to stop the expansion of NIS (a question examined by means of population genomics applied to two abundant foulers in ports namely *Ciona* species and dock mussels of the *Mytilus* genus); 3) what are the perceptions and acceptability of the costs associated with port development, according to users and stakeholders, and how are these modified by the presence and impact of NIS?

See also 4.3.2- AquaNIS2.0 project

4.2 ToR d – Advance knowledge base to further develop indicators

4.2.1 Réseau Alien Corse (2015–present)

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Since 2015, the dedicated '*Caulerpa* alert network' in Corsica has expanded to monitor a broader list of non-native species. In the first half of 2019, 32 NIS were reported around the island, mainly via SCUBA-diving club observations (Viel *et al.* 2019). In 2020 and 2021, no new species were reported, however a sharp increase in *C. sapidus* numbers, together with unconfirmed sightings of *Pterois miles*, are of note.

4.2.2 PAVIS (Assessing the relationships between marine protected areas and invasive species) (2016–2018)

Sylvaine Giakoumi, ECOSEAS, CNRS, University of Nice Sophia-Antipolis : sylvaine.giakoumi@unice.fr

The PAVIS ANR project investigated the following hypotheses: 1) whether MPAs influence the expansion of invasive species and mitigate their effects on native assemblages, 2) whether the ecological effects of such species could alter, reduce, or nullify ecosystem responses to protection in MPAs, and 3) whether local economic activities, such as artisanal fisheries, carried out in MPAs and adjacent areas, have been impacted by the presence of invasive fish. The main findings from this project are presented in Giakoumi e*t al.* (2019a and 2019b), with a key result being that MPAs had no impact on non-indigenous fish (Giakoumi *et al.* 2019a).

4.2.3 MSFD D2 monitoring programme (2019–present)

Centre d'expertises et de données Patrimoine Naturel, Cécile Massé : masse@mnhn.fr

A national monitoring program targeting NIS started in 2019. It targets both high-risk areas of introduction and marine protected areas. In 2021, six ports, three shellfish farming areas and two marine protected areas were monitored.

4.2.4 RESEAU ALIEN OCCITANIE (CPIE THAU) (2021–present)

Esther Emmanuelli & Florian Martel; CPIE Bassin de Thau: f.martel@cpiebassindethau.fr **or** e.emmanuelli@cpiebassindethau.fr

This project was initiated in 2021 and is coordinated by the CPIE Bassin de Thau (an association aiming to develop initiatives for the ecological transition of the territory, through networking with members working in various and complementary fields such as Environmental Education and Sustainable Development (EEDD), biodiversity, scientific mediation, or fisheries). The ALIEN Occitanie network aims to deploy a network to improve knowledge on non-indigenous marine species of the Occitan maritime coast,

through a citizen watch. This is a first participatory science project, co-designed and involving many structures of the Occitanie Sea Sentinel network, institutional and academic partners. The ALIEN Occitanie network is part of a national context (Marine Strategy Framework Directive) and part of a regional context to meet the roadmap of the Regional Strategy for Biodiversity. The main objectives of the project are : 1) to improve the knowledge on marine, coastal and lagoon NIS; 2) to federate and mobilize a large panel of citizens "sentinels" to deploy a real observation force of these species,3) to organize the reporting to better capitalize and exploit the data; 4) to test monitoring protocols that could be replicated over time and in other territories; 5) to raise public awareness of this issue and make known the existing systems for monitoring NIS; 6) to constitute a network of actors, on a regional scale, to start a dynamic in favor of NIS monitoring.

http://thau-infos.fr/index.php/environnement/122828-reseau-alien-occitanie-2

4.2.5 Nea Panacea (2021–2023)

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NEA PANACEA (North-East Atlantic project on biodiversity and eutrophication assessment integration and creation of effective measures) is an EU-funded project in which eight partners from five OSPAR Contracting Parties (Germany, France, the United Kingdom, Spain and the Netherlands) collaborate to deliver biodiversity assessments for OSPAR's Quality Status Report (QSR) 2023. Its focus lies on pelagic habitats, benthic habitats, food webs and marine birds' assessments. Other biodiversity groups are also associated through workshops, notably the NIS expert group. These assessments can be used by EU member states in the North East Atlantic region to inform their reporting to the EU for the MSFD. The project works on the development of new biodiversity indicators as well as on the improvement of existing ones, i.e. data flow, indicator operability, and expansion of geographical coverage or the development of threshold values. In addition, the best ways to integrate multiple indicators to deliver a single integrated assessment of a specific ecosystem component are explored. https://www.ospar.org/about/projects/nea-panacea

4.3 ToR e – Molecular approaches

4.3.1 IDEALG (2011–2020)

Philippe Potin, CNRS Roscoff Biological Station: potin@sb-roscoff.fr
The IDEALG project aims to develop sustainable aquaculture of seaweeds in Brittany. Several studies were carried out on *Undaria pinnatifida*, an edible seaweed native to Asia and cultivated in Brittany for more than 40 years. Studies based on the use of surveys and DNA-based analyses (Guzinski *et al.* 2018; Salamon *et al.* 2020) showed that 1) populations in the wild are self-sustaining; 2) marinas are the primary vectors of spread in natural habitats; 3) that novel marinas are quickly colonized by this species. More specifically, Salamon *et al.* (2020) carried out, over three years, field surveys (>20 000 individuals geo-localized) and genetic analyses (10 microsatellite loci, N = 890 individuals) of *Undaria pinnatifida* to study the real-time colonization dynamics of a newly-built marina in Brittany (France). They showed a remarkable snow-ball effect over time, with local density reaching locally up to 50 individuals per m^2 after two years. Genetic assignment showed that the founders mainly came from neighbouring populations (established in natural rocky reefs). Spill-over effects (i.e., spread from marinas to the wild)

have been previously documented to explain the expansion of this seaweed. The authors here showed that the on-going ocean sprawl also offers a perfect arena for spill-back events (*i.e.,* spread from natural habitats to artificial structures), highlighting the need for careful surveillance of newly built infrastructures. Lastly, in collaboration with researchers from France, South Korea and New Zealand, a genome-wide analysis of natural, cultivated and introduced populations of *U. pinnatifida* was carried out. It showed that both introduction and domestication leave contrasted footprints at the genome level (Graf *et al.* 2020).

4.3.2. AQUANIS 2.0 (2016–2021)

Frédérique Viard, CNRS Roscoff Biological Station : viard@sb-roscoff.fr

Supported by the Fondation TOTAL, the AQUANIS 2.0 project was conducted at the Station Biologique of Roscoff. It aimed to develop methods based on environmental DNA studies to detect, monitor and study non-indigenous species in marine coastal habitats, with a particular focus on biofouling assemblages in marinas/ports. This project also continued to support surveys of NIS in marinas from Brittany (e.g. Rapid Assessment surveys in 2019, repeating previous surveys carried out in 2013 and 2016). Regarding DNA-based tools, a first published study (Couton *et al.* 2019), based on the metabarcoding of larval samples, showed the efficiency of the approach to detect NIS in the wild (12 were recovered). Also as part of the PhD work of Marjorie Couton within this project, an investigation of *Botrylloides* species established under the pontoons of ten marinas from north-western France was carried out. In this work (Couton *et al.* 2021), DNA was extracted from ethanol (ebDNA) after various storage times and from a bulk of preserved colonies (bulkDNA), and processed using High-Throughput Sequencing (HTS). The study showed that these HTS-data can jointly and accurately depict genetic and taxonomic diversity. In addition, the effectiveness of water-eDNA-metabarcoding to identify biofouling NIS in the same ten marinas was assessed (Couton *et al.* under revision). Water-eDNA-metabarcoding detected two thirds of the NIS that were observed in the field, failing to detect important NIS, but also allowing the detection of additional NIS from neighboring micro-habitats. Finally, marina communities were described using metabarcoding. This work (Couton *et al.* in prep) showed that, despite potential transport via recreational boating, marinas exhibit different assemblages and a strong spatio-temporal structure.

4.3.3 ASSEMBLE + - JRA1 (2018–2021)

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As part of the AssemblePlus project (www.assembleplus.eu/), coordinated by EMBRC-France, Autonomous Reef Monitoring Structures (ARMS) were deployed across a network of 20 observatories in European coastal waters and in the polar regions. This action was coordinated as part of JRA1 'Genomic observatories' by Mathias Obst (Sweden). The Station Biologique of Roscoff is one of the 17 research institutes involved in this network. One of the initial scientific goals of the network is to report novel NIS and track the spread of already known NIS in European continental waters, which is achieved with short-term (3 months) deployments. Pilot studies were carried out in 2019, and materials scraped from ARMS were prepared for High-Throughput Sequencing by HCMR, with the aim to make taxonomic assignment through metabarcoding. The results of the 30-month pilot phase are presented in Obst *et al.* (2020).

4.3.4 ROME (Réseau d'Observatoires pour la recherche en Microbiologie Environnementale intégrée (2019–2025)

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The ROME project aims to develop an integrated observatory of the microbiology of mainland France's coastal waters. Its purpose is to develop new approaches and data (-omics data) for scientific research. Batch environmental DNA and RNA analyses are carried out, with a view to assess whether these tools can be used for "routine" monitoring purposes. In 2019, four sampling sites were designated and the sampling protocol defined; fieldwork started in 2020.

4.3.5 RAPSODI (2022–2023)

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The RAPSODI project focuses on two NIS that could threaten shellfish farming on the Atlantic coast in France, the first of which is the veined rapa whelk *Rapana venosa.* Since one individual was reported in the Pertuis Breton (off the coast of the île de Ré) area of the Bay of Biscay in November 2019, there have been dozens of reports in the area. The second NIS is a newly described polycladida worm, *Idiostylochus nov. sp*, already observed for two years in the Arcachon basin. High abundances of this worm may have been associated with mussel mortalities. The objectives are (1) to raise awareness among local maritime actors about these NIS, (2) develop and validate two protocols to detect these NIS with molecular tools from eDNA samples and then (3) analyse samples collected *in situ*. As a first step, the two NIS will be targeted with a qPCR approach, but possibly later the survey will expand to a metabarcoding approach. Only two species are targeted in this project but other NISs may also be a concern for shellfish farming in France.

Additional Information

1) Research on Climate Change Impacts (ToR b)

Chefaoui *et al.* (2019) used an ENM to predict the future range of the invasive seaweed (*Sargassum muticum*) under different scenarios of climatic change. Under the most severe scenario (RCP 8.6), the ENM predicted northward expansion with putative substantial ecological consequences for subarctic coastal ecosystems. However, after filtering to take into account thermal constraints on the reproductive phenology, they showed a reduced northward expansion, as compared to a non-filtered ENM. In particular, the total range area was expected to increase by 61.75% by 2100, but only by 1.63% when the reproductive temperature window was considered. Although this study points to substantial changes under climate change, it also shows the need to better integrate phenology and physiological constraints in ENMs.

A review paper by Drobetsov *et al.* (2019) provides an overview of knowledge about the effects of climate change, in particular the effect of elevated temperature and ocean acidification on microfouling communities and bioactive molecules. The authors point out that the existing studies suggest different categories of impact such as changes in the production of bioactive compounds or composition of biofouling communities.

2) Research on Biofouling – Marinas & ports (ToR c)

As part of a long-standing collaboration between French and Chilean researchers, a set of experiments was set-up to examine ecological processes at play in ports, and the role played by NIS in these artificial habitats. In a first study, settlement plates were deployed upon two types of artificial habitats (floating and non-floating hard substrates) at a total of ten ports, associated either with local or international shipping traffic, along the Central Chilean coastline. After colonization periods of three and 13 months, fouling sessile assemblages were examined. A total of 78 taxa were identified across all sites and sampling times, among which 25 were non-indigenous or cryptogenic. While shipping traffic categories had no discernable effect on the assemblages,

there were strong differences between the two habitats. These differences were driven by non-indigenous species which contributed 10 times more to the assemblages in floating habitat than in their non-floating counterparts (Leclerc *et al.* 2020). More attention should be paid to the type of habitats, regardless of the intensity of ship traffic.

In a second study, using an exclusion experiment to examine predator effect on the early establishment (4 months) of new assemblages on settlement panels, Leclerc *et al.* (2019) showed that the community structure was significantly influenced by the exclusion treatments. Altogether, predators reduced the abundance of most NIS and cryptogenic species, some of them being only observed when the two categories of predators were excluded. Similar findings were obtained when examining more mature assemblages (26 months). This study shows an effective consumptive biotic resistance.

Another novel experiment was carried out to examine the effects of physical disturbances (through the removal of dominant native habitat-builders) on both the recolonization of disturbed patches and colonization of novel substrates on pier pilings, in a Chilean port. Opportunistic taxa, including NIS, were observed at early successional stage (three months). The richness of cryptogenics and NIS upon settlement plates increased significantly with the severity of disturbance experienced by the surrounding assemblages. However, community structure on the pilings converged across treatments after 10 months, and interestingly the recovery of the habitat-builders occurred at the expense of cryptogenic and non-indigenous taxa (Leclerc *et al.* 2021).

3) Molecular approaches to provide science-based tools for strategic planning, policy development, and operational processes (ToR e)

See also IDEALG and AquaNIS2.0 projects

Based on microsatellite markers, Le Cam *et al.* (2020) did not detect any genetic variation over >1200 individuals sampled from 46 locations over the Pacific-Atlantic introduction range of the invasive seaweed, Japanese wireweed (*Sargassum muticum*). Thanks to high-throughput genotyping (ddRAD-sequencing), they then confirmed severe founder events in both the Pacific and Atlantic introduction ranges. These markers also revealed two unexpected additional distinct genetic origins of introductions, and suggest that conversely to scenarios based on historical records, southern rather than northern NE Pacific populations could have seeded most of the European populations. Altogether, this study exemplifies the usefulness of new sequencing technologies to uncover introduction routes, and also underline the need for extreme caution in interpreting neutral genetic diversity as a proxy for invasive potential.

As part of a collaboration between researchers from France and New Zealand, planktonic and biofouling communities, including NIS, were surveyed in four locations (two marinas, one port and one pristine) in Tahiti using a multi-gene approach (18S rRNA and COI). Detection of NIS based on selected lists of globally invasive species revealed a wide diversity of potentially invasive taxa, especially in the more anthropogenically

impacted sites. The study highlights the vulnerability of remote islands to shipping-mediated incursions (Pearman *et al.* 2020).

The PhD work of Marjorie Couton, defended on October 2020 (co-supervision: Thierry Comtet & Frédérique Viard), Sorbonne Université) was carried out under the umbrella of the project AquaNIS2.0 (2017–2021; Coord F. Viard) at the Station Biologique of Roscoff. This PhD thesis, entitled "High-throughput sequencing techniques for the detection and survey of marine non-indigenous species: a valuable addition to traditional methods?" was dedicated to the use of DNA-based molecular approaches for NIS monitoring and biological invasions studies. This thesis was focused on marine benthic sessile organisms in biofouling communities in marinas along the coast of Brittany (France). In a first study, High-Throughput-Sequencing (HTS) of

environmental DNA obtained in ten marinas around Brittany over two seasons showed that, as expected, HTS-based techniques provided information on a much larger taxonomic coverage and detected numerous NIS. They however failed to identify important target NIS. False positives were also observed, which might be a severe limitation of the approach for NIS early detection. In another study, NIS presence in the wild was examined. Interestingly and unexpectedly, most NIS observed in the marina were also present in natural habitats although not reported with traditional approaches. Then, the reproductive output (period/intensity) of several benthic NIS was assessed by applying a metabarcoding approach on zooplankton samples from a time-series. Metabarcoding was efficient in detecting NIS whatever their life-cycle, and in inferring reproductive period for species with long-lived larvae. Finally, the benefit of using HTS approaches to analyze jointly taxonomic and genetic diversity of NIS was examined, using the invasive colonial tunicates as a case study. Altogether this work showed that HTS-based and metabarcoding are effective methods for NIS surveys and studies, if precautions can be taken to circumvent the reported uncertainties of the method.

Molecular approaches were also shown to be powerful to detect novel lineages and impact of introduced species on native ones by means of hybridization and introgression, which might have consequences in terms of management (see for a review Viard *et al.* 2019). For instance, a genome-wide survey showed introgression of genes from the invasive species *Ciona robusta* into the genome of the native species *Ciona intestinalis* in their contact zone (Le Moan *et al.* 2021). Interestingly this introgression is limited to a very short regions of a single chromosome, which might, with other evidences, suggest that this introgression is adaptive. A very different outcomes of secondary contacts between native and invasive species was observed in the *Mytilus* genus; here a genome-wide admixture between the introduced *Mytilus galloprovincialis* and the blue mussels was documented (Simon *et al.* 2020). These two case studies are further examined as part of the MarEEE Project.

5. Meetings

2019–2021

- Franco-English exchanges on IAS Biosecurity in Aquatic Environments (Concarneau, France, 16 May 2019). The event brought together some 50 participants from the UK, Belgium, France and Ireland, for two days co-organised by the British LIFE RAPID project and the French Centre de ressources espèces exotiques envahissantes. Exchanging information on experience and good practice between these countries helped to identify potential collaborations and make recommendations. In September 2020 a summary of the exchange was published in FR/ENG.
- "Ballast Water and Invasive Species: the IMO BWMC status, questions ad solutions". European Maritime Day (Lisbon, Portugal, 16–17 May 2019), organized by the Interreg Atlantic Blue Ports project.
- Biological Invasions: how can scientists respond to scientific denialism and social unawareness? CNRS Conference organized ahead of the 7th IPBES Session, (Paris, France, 25–26 April 2019)
- National MSFD 'Descriptor D2' monitoring workshops – (Paris, France, 3 December 2019 & 14 October 2021)
- International online seminar on parasites of the *Haplosporidium* genus (19 March 2021)
- NIS Training for environmental stakeholders (Sète, France, 17–20 September 2020). Entitled "from prevention to monitoring of non-indigenous species: key knowledge and challenges for management". The four-day training session aimed to provide a common scientific and regulatory knowledge base, to share experiences and to give an overview of the local and national NIS networks. 16 participants attended from different environmental management bodies, from all French seaboards as well as the Caribbean overseas

territories. The full program and presentation media are available online on the CDR EEE website.

- *Callinectes sapidus* French Mediterranean regional action plan was presented in a webinar on 28 January 2021. After reaching the Italian and Spanish coasts of the Western Mediterranean Sea, the American blue crab *Callinectes sapidus* is now regularly observed in the lagoons along the French Mediterranean coast (Labrune *et al.* 2019) and in Corsica. Its strong invasion capacities will probably lead to important changes in the structure and composition of French lagoon biota. Regional authorities in the French Mediterranean are pooling resources and knowledge to tackle the invasion through the adoption of a regional action plan (see Regulations). A conference regrouping all French Mediterranean regions was held on 14 March 2022 to coordinate regional efforts to prevent the spread of *C. sapidus*.
- IUCN World Conservation Congress with a dedicated workshop on Marine Non-indigenous Species (Marseille, France, 3–10 September 2021)
- In September 2021, a thematic stream session (Stopping the tide: Best practices and solutions to tackle marine invasive alien species (IAS)), co-organized by the IUCN French committee and the IUCN secretariat, focused on the management of key pathways of introduction. Using concrete case studies from around the world and by sharing ideas and lessons learned, this session will identified current challenges, areas for priority action and collaboration, present best practices, and tools to support decision-making that effectively manage the major pathways of introduction of IAS. This event brought together 200 participants from the diverse community of stakeholders associated with the use of marine waters, including environmental protection, tourism and recreation, education, transport. The session outputs are a list of ranked priority actions and partnership opportunities for future projects that will address the identified issues. The session involved IMO, World Sailing as well as French organizations (Ministry of ecology, UMS PatriNat, Ifremer,) and marine protected area managers. For more information on this session: https://www.iucncongress2020.org/programme/official-programme/session-43188
- Webinar "Le potentiel des méthodes basées sur l'ADN environnemental pour la surveillance et l'évaluation de la biodiversité marine dans le cadre des politiques publiques » – 25 novembre 2021- organisers : UMS patrinat (OFB/MNHN/CNRS) This workshop intended to support national efforts to develop an optimized eDNA monitoring strategy for the marine environment, covering as much as possible the various components of marine biodiversity in conjunction with public policies. About fifty people attended, including experts, scientists, managers and project leaders. The speakers were invited to report on their field experiences or their work involving eDNA in aquatic environments - both freshwater and marine - by focusing their analyses on two main areas of reflection: 1) The interest of eDNA for major biocenotic components, 2) the interest and feasibility of a shared eDNA monitoring system.
- Webinar "Marine environmental DNA workshop: Defining best practices for oceanic eDNA studies from coastal to deep sea ecosystems, from micro- to macro-organisms and from past to present times" 26–28 April 2021– Organisers Ifremer, Fondation Tara Océan and EMBL This workshop gathered around 50 international scientists interested in the future application of eDNA-based studies in the marine environment with the objective of identifying priority research questions as well as conceptual, methodological and technological limitations and generating a roadmap towards overcoming these. Five sessions structured the workshop: 1) Best practices/State-of-the-art, 2) Sampling methodologies, 3) Analyses methodologies, 4) Distinguishing DNA in living and from dead organisms, 5) Session 5: Examples of applications and flagship projects. The latest

one included presentation and discussion regarding application of eDNA to pathogens and invasive species.

Future meetings

- Planned for 2022: National workshop with French experts, plus local workshops to work on the MSFD D2 monitoring program strategy.

- A second NIS training session for environmental stakeholders will be held in Arcachon in October 2022.

OSPAR Regional Sea Convention (North-East Atlantic):

- The NIS-Expert Group was reactivated by a new lead (Peter Staehr, Aarhus Univ., Denmark) in mid-2021. OSPAR and HELCOM agreed end of 2021 to create and coordinate a Joint Expert Group on NIS (JEG-NIS), which met for the first time on the 18/02/2022. The main driver for the next two years works is the production of the North-East Atlantic Quality Status Report (to be published in 2023), notably the assessment of the "NIS3 indicator" (Number of new introductions of NIS per assessment period), related methodological standards and the thematic assessment (Drivers-Activities-Pressures-State-Impacts-Responses).
  For both groups, French members are: Laurent Guérin, Cécile Massé (French Biodiversity Agency) and Frédérique Viard (CNRS).

Barcelona Regional Sea Convention (UNEP-MAP, Mediterranean Sea):

- The online working group on NIS was reactivated early 2021 by the UNEP-MAP Secretariat. Further meetings will take place in 2022. As for other Regional Seas, the main driver for ongoing work is the production of the Mediterranean Sea Quality Status Report (to be published in 2023), notably the assessment of the NIS indicator (New introduction of NIS per assessment period) and related methodological standards.

Other international meetings:

22nd International Conference on Aquatic invasive species (ICAIS) 19–23 April 2022 (Ostend, Belgium).

Nine presentations by French participants on marine NIS were delivered throughout the conference. The final program can be viewed here: https://icais.org/preliminary-program/

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Overview

The unintentional introduction of non-indigenous species (NIS) is an ongoing process. According to national surveys new NIS were found each year (Table 1). Further reports from neighboring countries documented NIS which were not yet known from German coastal waters. Owing to the possibility of spread into German open waters, this information could be highly relevant for the implementation of national early warning and prevention measures.

Table 1. New unintentionally introduced species along the German North and Baltic Seas coasts.

Year	North Sea	Baltic Sea	Total
2019	5	3	8
2020	2	1	3
2021	-	-	
Total	7	4	11

We included those species with first record before 2019 for which their occurrence became known in the reporting period 2019–2021.

Regular and NIS targeted monitoring programmes occur along the German coast line, now with more than 20 sampling sites. Activities took place in form of extended rapid assessments (e-RAS) at hot spots like harbours, marinas and aquaculture sites with a sampling frequency of at least one event annually. In addition, some ports have been sampled according to the HELCOM/OSPAR Joint Harmonized Procedure sampling protocol and selected marinas were sampled with settlement plates.

Intentional living species introductions remain at similar levels as in the last years and include predominantly sturgeons, salmonid species, rainbow trouts, carps, *Crassostrea gigas* (seed mussels and adults), scallops, *Mytilus* sp., *Homarus americanus* and other decapods, and cephalopods

as well as the red alga *Palmaria palmata*. Imports are predominantly from Ireland, United Kingdom and the Netherlands. In addition, a substantial amount of various living ornamental fish was imported.

In some cases, the identification of species and whole samples follow with genetical methods (eDNA and metabarcoding). DNA studies may in the future reveal more introduced species. As an example, DNA analysis revealed that Japanese mitten crab (*Eriocheir japonica)* individuals were collected in Holland, Germany and Poland between 2009 and 2015. The German individual was collected inland in the Rhine River, and may not have necessarily migrated successfully to the North Sea for reproduction (Hayer *et al* 2019).

For activities where shipping stands in the focus, please consult the WGBOSV report.

Content

1. Regulations

No new German regulations, but see below for an update on the EU Regulation 1143/2014 on Invasive Alien Species.

2. Intentional

Synthesis of introductions

Intentional living species introductions remain at similar levels as in the last years and include predominantly sturgeons, salmonid species, rainbow trouts, carps, *Crassostrea gigas (*seed mussels and adults*)*, scallops, *Mytilus* sp., *Homarus americanus* and other decapods, and cephalopods, as well as the red alga *Palmaria palmata*. Imports are predominantly from Ireland, United Kingdom and the Netherlands. In addition, 170 tonnes of various living ornamental fish were imported (FactFish 2020).

Statistics in Germany are not precise enough to deliver more meaningful results in WGITMO needs. The newest German trade statistics dataset is for 2020 and lists that 89% of the fish and fisheries products (includes invertebrates and algae) was imported to Germany from EU and non-EU countries. However, it is not indicated how much of this is live imports (FIZ 2021).

3. Summary of sightings

Unintentional:

During the last reporting period (2016-2018), the annual number of newly reported species in the North Sea (3.7 species) was almost three times higher than of those in the Baltic Sea (1.3 species). In this reporting period 2.3 new species were annually found in the North Sea and 1.3 in the Baltic Sea. It should be noted that no new species was reported in 2021, but this report was prepared before the sampling season began.

The most up-to-date lists of alien species in German coastal and marine waters may be found at https://www.neobiota-plattform.de/english/news/ and in AquaNIS: www.corpi.ku.lt/databases/index.php/aquanis/. As species findings are frequently published with some delay, we include here also those species found in 2018 when the relevant publication came out in 2019 or later. However, an overlap with the earlier three-year reporting period cannot fully be avoided.

North Sea

- *Schizoporella japonica* (Bryozoa) was found in October 2018 on fouling plates in the harbor of Hoernum (Sylt Island, North Sea) (Lackschewitz & Buschbaum **2019**).
- An unknown polychaete (Ampharetidae) was found in November 2018 near the locks of the Kiel Canal, Brunsbüttel (North Sea). The taxonomic analysis for identification is on-going (Lackschewitz **2019** pers. comm.).

- Five individuals of *Hypereteone* cf. *lighti* were found in August **2019**, at Trischendamm. However, the taxonomy is not yet confirmed (Hoffmann pers. comm.) (so that this species is not yet registered in AquaNIS).
- Two colonies of the bryozoan *Pacificincola perforate* were found in offshore samples taken in the German Bight in **2019**. This species is also known from The Netherlands with findings in 2005 (IFAÖ pers. comm.).
- The nudibranch *Corambe obscura* was found in offshore samples taken **2019** in the North Sea. The species is known to occur in The Netherlands, France and the Black Sea (IFAÖ pers. comm.).
- About one hundred amphipods *Aoroides semicurvatus* were found on an oyster reef near Sylt Island in **2019**. This species is known to be introduced to the French Atlantic coast since 2009, in the Dutch Oosterschelde (2017) and along the Normandy coast (2019) (pers. comm. Nadarzinski).
- The Bacillariophyceae *Plagiolemma distortum* sp. nov. was recorded in 2018 samples from the Jade-Weser-Port (Schanz *et al.* **2020**). This new diatom species was formally described by Nezan *et al.* (2018). Before its taxonomic identification it was named "pringle" (after the potato crisps) due to its characteristic shape. Investigating plankton photos it was in retrospect found with the earliest record documented by an image from the English Channel in 1992. Further material confirmed records in the North Sea (The Netherlands and Germany - with many records near or in ports) since 2001 and after 2010 in the eastern and western English Channel (Kraberg *et al.* 2018). As a result, this species occurred in Europe long before it was described as new species. As this newly described species has a characteristic shape it is assumed that this would not have been overlooked in earlier samples. However, due to its uncertain introduction status it is registered as cryptogenic.
- A new Hermit crab, *Pagurus longicarpus*, was found in February **2020** in the Meldorf Bight (North Sea). This is likely belonging to the Diogenidae. Taxonomists currently work on the species identification (Neumann *et al.* submitted).

Baltic Sea

- In the Port of Kiel, at three sampling sites, *Fucus distichus* (listed as *F. edentatus*) was found (Schanz *et al*. **2018**). This species is widely distributed in the northern hemisphere along Pacific and Atlantic shores, including the North Sea (www.algaebase.org), but this seems to be the first record in the Baltic Sea (AquaNIS, last checked February 2021).
- *Moerisia inkermanica* (Hydrozoa) found in summer 2018 in Vitter Bodden and Greifswalder Bodden (Baltic Sea) (Sven Dahlke, Knut Weidemann & Markus Otto via Kai Hoppe **2019** pers. comm.). This Ponto-Caspian hydrozoan was also recorded in The Netherlands, but only in 1959 and never since.
- The cumacean *Nippoleucon hinumensis* was found in Stralsund and Rostock in **2019**. This is a new record for Europe. Its native area is the Northwest Pacific and it was previously introduced to the west coast of North America where it was first reported from Coos Bay, Oregon in 1977. Its non-native range now stretches from Puget Sound, Washington to Port Hueneme, California. (IFAÖ pers. comm., Fofonoff *et al.* 2018).
- During sampling events in the Port of Kamminke, which is located near the Polish border, *Echinogammarus ischnus* was found. This species record is a range expansion as it is known from German waters since the 1940s and since 1980s in Baltic Sea watersheds in waters with salinity <1 PSU (Zettler & Zettler **2020**b).
- In the scope of HELCOM/OSPAR Port Survey in Rostock a mass occurrence of *Ficopomatus enigmaticus* agg. was observed in 2020. Especially the habitat collectors and settlement plates on the sampling site named “Stadthafen” were fully covered with the tubeworm. On the sampling site marina “Warnemünde” with a salinity of 11,4 PSU a single individual of

Magallana gigas was found in a scratch sample, this represents the easternmost record in the Baltic Sea (IFAÖ 2022 "Assessment of Non-Indigenous Species (NIS) in the Port of Rostock", not yet published).

Previous Sightings

- DNA analysis revealed that Japanese mitten crab (*Eriocheir japonica*) individuals were collected in Holland, Germany and Poland between 2009 and 2015. The German individual was collected inland in the Rhine River, and may not have necessarily migrated successfully to the North Sea for reproduction (Hayer *et al* 2019).
- In Thedinghausen, near Bremen on the Weser River, hundreds of Chinese mitten crabs (*Eriocheir sinensis*) were reported as crossing streets and warning signs were put up to alert car drivers (DPA 2019, various newspaper publications).
- Please note also additional information on the taxonomic unclarity of *Sinelobus vanhaareni* "vs" *S. stanfordi*. The German *Sinelobus* specimens found in several North Sea ports are all *Sinelobus vanhaareni*, i.e., not *S. stanfordi*, neither *Sinelobus cf. vanhaareni* (Lackschewitz pers. comm.). Taxonomic analysis further revealed that there is no *S. stanfordi* in Europe until there is further evidence proving otherwise (van Haaren pers. comm.).
- Additional findings of *Hemimysis anomala* in Stralsund 2019 confirmed its presence in the Baltic (IFAÖ pers. comm.).
- The polychaete *Pileolaria berkeleyana*, recorded since 2013 on Helgoland Island, was in 2019 also found in Hörnum (Sylt Island) (Lackschewitz pers. comm.).
- *Ficopomatus enigmaticus* spreads eastwards in the Baltic and was in 2020 found up to Rostock (Michael Zettler pers. comm.) and pleasure boat owners are concerned (https://www.ndr.de/nachrichten/mecklenburg-vorpommern/Australischer-Roehrenwurm-breitet-sich-in-MV-aus,roehrenwurm102.html).

Not Seen Species Yet

We last searched AquaNIS and other resources (see reference list) for new introduction events since 2015 in our neighboring countries The Netherlands, Denmark and Poland. For earlier findings in our neighboring countries, please see relevant national reports.

Baltic Sea

Seven new introduction events are included in AquaNIS along the **Danish** Baltic coast since 2015. Four of those, all found in 2017, are not yet known from German part of the Baltic waters:

- *Caprella mutica*, this amphipod was (so far) only found in the Baltic at Danish shores, it was first recorded in the German part of the North Sea in 2004,
- *Crepidula fornicata*, the slipper limpet (Gastropoda) is known from the German part of the North Sea since 1934. Its Baltic occurrence is limited to Denmark,
- *Hemigrapsus sanguineus*, this decapod has been found in the German part of the North Sea since 2006. In the Baltic it is only reported from Denmark and Sweden,
- *Polydora aggregata*, this Danish record is the only record in Europe.

In total four introduction events are included in AquaNIS for **Poland** since 2015. The species not yet known from the German Baltic coastal waters are:

- the decapod *Callinectes sapidus* found as single specimen in 2018 in Polish waters. *Callinectes sapidus* was not yet in the German part of the Baltic Sea, (but found in Germany on the North Sea shores),

- the amphipod *Chelicorophium robustum* was not found elsewhere in the Baltic, but was found in Poland in 2018 (but found in Germany in the rivers Main and Rhine which drain into the North Sea).

North Sea

Two new introduction events are included in AquaNIS along the **Danish** North Sea coast since 2015. Both species were found in 2017, but were not yet recorded from the German North Sea coasts:

- the tube-building polychaete *Polydora cornuta*, next to Denmark it was only found in Portugal,
- *Oncorhynchus mykiss*, not recorded from the German North Sea coasts, but this fish is known from the German Baltic coast since long ago. However, findings are single records with no self-sustaining populations, so that these records represent artificial releases and fish farm escapees.

When comparing the recent new NIS/CS records of **The Netherlands** we note that the following were not (yet) found in Germany:

- *Desdemona ornata,* found in the Oosterschelde in 2015
- *Pseudopolydora paucibranchiata,* in the Oosterschelde in 2015
- *Caprella scaura,* in the Westerschelde in 2016
- *Ceramium sungminbooi* was described as new to science in 2016. It has been present in the Wadden Sea for much longer however. Based on material of *Ceramium cimbricum*, collected during our 2014 survey, it was confirmed that it was already widespread in the Wadden Sea at that time.
- *Polysiphonia senticulosa* was found in 1993. As it is a known winter species, it may have been missed during previous alien species focussed surveys in the Wadden Sea, which were all done in summer. It is therefore unclear since when it has been present in the Wadden Sea.
- the bryozoan *Biflustra grandicella* was found in the Westerschelde in 2016 as only European record
- the goby *Tridentiger barbatus,* Oosterschelde in 2016 as only European record
- another goby *Gobiosoma bosc* in the North Sea Canal in 2017
- 2018 record of the alga *Ulvaria splendens.* This species is probably native to western Europe so that a range expansion may also be considered
- *Caulacanthus okamurae* is an alien algal species from the NW Pacific, which is already known in the Netherlands from records in 2018, but occurs nowhere else in Europe
- in 2019 the bivalve *Yoldia limatula* was recorded. However, its taxonomy needs to be confirmed. It was found in a Westerschelde bottom sample. This is the first record for Europe.

4. Pathogens

Sightings/records

No new findings were reported since last reporting period.

5. Research and Monitoring Programs

Project Title: "Completing management options in the Baltic Sea Region to reduce risk of invasive species introduction by shipping" (COMPLETE)

Contact:
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This project was funded by the Interreg Baltic Sea Region Programme and ran from October 2017 to March 2021.

Short description: In the COMPLETE project 12 project partners from seven Baltic Sea countries, HELCOM and several associated associations addresses two major sources of harmful organism introduction by shipping: ballast water and biofouling. COMPLETE is tackling several gaps in ballast water and biofouling knowledge and resulted in the development of operational frameworks and actual tools, e.g. a proposal for a Roadmap on Biofouling Management; effective risk assessment procedures and tools as basis for ballast water management exemptions; an early warning system for NIS introductions; an integrated regional NIS monitoring system and surveillance for compliance control with ballast water management standards. The target groups are national ministries and agencies of transport and environment; ship owners and their associations; cleaning companies; Baltic Sea ports and coastal municipalities; shipyards; marinas and boating associations; as well as HELCOM and its contracting parties. For an update on the project achievements, please visit https://www.balticcomplete.com/. In short, COMPLETE work harmonized the monitoring approaches of non-indigenous species, developed a proposal for a regional biofouling management strategy, contributed to the IMO process of the review of the Biofouling Guidelines, and harmonized the implementation of the Ballast Water Management Convention. The latter was supported by a practical workshop on ballast water sampling and analysis, conducted in May 2019 in Hamburg, Germany. In 2020, criteria for the identification of target species (TS) were agreed and published (Gollasch *et al.* 2020). TS are used in the IMO adopted species-specific risk assessment under the IMO BWM Convention to address selective BWM requirements for granting exemptions from Ballast Water Management. The end of project conference was held online in early February 2020. The successful project COMPLETE was followed by an extension stage project COMPLETE PLUS.

Project Title: "Practical implementation of the COMPLETE project outputs and tools" (COMPLETE PLUS)

Contact: Katja Broeg, Federal Maritime and Hydrographic Agency (BSH), Katja.Broeg@bsh.de
This project received co-financing from the Interreg Baltic Sea Region Programme. The project began April 2021 and ended in December 2021.

Short description: COMPLETE PLUS was based on the work of COMPLETE and aimed to ensure that COMPLETE project outputs will be operationalized to ensure their sustainable use by all relevant actors and stakeholders. This has been achieved by outreach and training of all main target groups (i.e., shipping companies, environmental and maritime authorities, ports, contracting parties of HELCOM) in the practical implementation and real-world application of COMPLETE outputs. A central part is the elaboration of specific mechanisms and pathways for the long-term maintenance of these tools and their further sustainable use through coordination between relevant end-users. The first working package aimed to ensure the sustainable use of the COMPLETE products for ballast water management. This has been achieved by, e.g., the operationality-testing of the Early Warning System (EWS), which was developed within the COMPLETE project. Furthermore, the Target Species list was updated based on the new criteria developed within the COMPLETE project and an operational regional data flow has been developed to ensure the accessibility of all non-indigenous species (NIS) data to be directly available for the HELCOM NIS core indicator. The second working package addressed the practical implementation of the Biofouling Roadmap in the Baltic Sea Region by, e.g., compiling research results of the COMPLETE project in presentations and coming papers to guide ship operators in selecting the best practice biofouling management. Additionally, several online stakeholder workshops/meetings have been organized for potential end-users of the biofouling management toolkit and a risk assessment procedure as a basis for granting permissions of in-water cleaning

(IWC) has been developed, together with administrations and authorities in the Baltic Sea Region.

Project Title: Rapid-assessment of non-native species in German Coastal Waters including further development of the trend indicator
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Short description: The German alien species monitoring programmes continue with several sampling stations in ports along the Baltic and North Seas as presented last year. The samplings are conducted annually between August and October with a focus on benthos and to a lesser degree on plankton. Recent monitoring activities in Germany filled geographical gaps in the network of coastal monitoring stations. The monitoring activities took place in form of extended rapid assessments (e-RAS) at hot spots like harbours, marinas and aquaculture as routine monitoring programme as Germany considers it important to perform NIS monitoring frequently (at least once a year). This allows for a higher probability of early NIS detection. Since a yearly survey of all hot spots following the HELCOM/OSPAR Join Harmonized Procedure (JHP) sampling approach would be too cumbersome and expensive, e-RAS has been chosen as a cheaper but still adequate alternative. The NIS monitoring programme of the German North Sea and Baltic coast included 17 locations. Results of the rapid assessments indicate that the rate of newly recorded NIS is lower in the Baltic Sea compared to the North Sea. In 2019 and 2020 additional Baltic ports were sampled using e-RAS, i.e., Wismar-Wendorf, Rostock-Schmarl, Stralsund-Dänholm, Oderhaff-Kamminke, Sassnitz-Mukran and Sassnitz-Stadthafen. At certain locations, in addition fouling plates and lines were installed to document the presence of certain species (Zettler & Zettler 2020a, b).

Project Title: eDNA and metabarcoding
Contact:
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A project is ongoing analysing Dogger Bank and German Bight samples with eDNA and metabarcoding approaches for non-indigenous species. First results indicate the presence of non-indigenous species which have not been found during the monitoring campaigns. Further analysis suggests that, e.g., *Hemigrapsus penicillatus* and *H. takanoi* should be considered as one species (Martinez pers. comm.).

Project Title: Marine NIS fast response concept
Contact: Manuela Krakau, Umweltbundesamt, manuela.krakau@uba.de
In 2020, AquaEcology presented a first draft of a marine fast response concept, which was commissioned by the German Environment Agency. In those draft documents, an overview of current literature to fast response considerations in water environments worldwide was presented

as well as examples of a decision support tool for several marine NIS introduction events. Germany aims to evolve this process to incorporate it in an early warning system.

Project Title: Begleitende Untersuchung zur Experience Building Phase (BU-EBP)
Contact:
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Short description: The project, focusing on development and testing of sampling devices and instruments for analyses and their practical application by water police and port state control started in 2018 and is launched as another contribution to the IMO Experience Building Phase. This effort will be related to the earlier reported, and completed BSH project entitled "Ballast Water Test Quality Assurance (BAQUA)". The project BAQUA, i.e., the development of a prototype of a round robin test facility to compare sampling and analysis of various test facilities or methods, has been finalised in 2017. Both projects are considered as part of the experience building phase of the Ballast Water Management Convention to support its proper implementation.

Project Title: BMDV Network of experts
Contact:
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Short description: The German Federal Ministry of Digital Infrastructure and Transport (BMDV) initiated a new format of departmental research. Their objective is to address urgent transport-related questions through innovations in the areas of adapting to climate change, environmental protection and risk management. In the scope of the sub-project "Development of practical and preventive strategies to control and minimize impairments from non-native species", critical pathways of non-indigenous species concerning the German transport sector are identified. The main objective is to prevent the introduction of NIS or control established non-native species under consideration of costs and benefits. Further tasks are, e.g., expanding the knowledge base for future exemptions from ballast water management requirements, identification of species introduction hot spots and introduction vectors as well as an improvement of the German authority network regarding NIS. Focus will be on early detection and early warning methods in conjunction with monitoring data quality assurance and management concepts. A first phase primarily focused on monitoring in harbors (Port Surveys) and the relationship between species introductions via international transport and the secondary spread by inland waterway transportation and recreational boating considering both, ballast water and biofouling, the latter with a focus on pleasure boats. Approval of genetic methods for early warning and practical usage of the national Neobiota-Information System complete the goals of the project.

a) Sub-Project Title: Hamburg and Kiel Port sampling
   Short description: The ports of Hamburg and Kiel have been surveyed for non-indigenous species according to the HELCOM/OSPAR Joint Harmonized Procedure port survey protocol (Schanz *et al* 2018). In the Port of Hamburg overall, 179 species were found, of these 16 NIS occurred. In the Port of Kiel, 205 species were found, of which 13 NIS species were identified. The "only" new NIS reported for Germany was Fucus distichus (listed as F. edentatus) found in Kiel.
b) Sub-Project Title: Port of Cuxhaven and Jade-Weser-Port sampling
   Short description: The ports of Port of Cuxhaven and the Jade-Weser-Port were surveyed for non-indigenous species according to the HELCOM/OSPAR Joint Harmonized Procedure port survey protocol (Schanz *et al*. 2020). In the Port of Cuxhaven 246 species were found, of which 24 were identified as NIS. In the Jade-Weser-Port 272 distinct species were found, including 28 NIS. One species listed as NIS in the Jade-Weser-Port is a species new to science (see Table 1).

c) Sub-Project Title: Port of Rostock (HELCOM/OSPAR Joint Harmonized Procedure Port Survey Protocol) in combination with the application of genetic methods
Short description: The port of Rostock was surveyed for non-indigenous species according to the HELCOM/OSPAR Joint Harmonized Procedure port survey protocol.
The salinity within the sampling area varies between 8 PSU and 13,6 PSU. In total, 38 NIS (12%) were identified. For the whole collected samples, Crustacea were the most represented NIS taxonomic group (27) followed by Chromista (10). Most species (20) were found on hard substrate. In summer 2021 the survey in Port of Rostock was repeated. This time only scratch and plankton samples were conducted.
Additionally, eDNA and metabarcoding methods were applied in parallel for the determination of NIS.

d) Sub-Project Title: Biofouling Survey on Recreational Boats
Short description: Recreational boat sampling continued in selected marinas in the German part Baltic Seas and waterways in 2017/2018. In total 34 NIS were found which represents 22,4 % of all found 152 taxa, clearly indicating the importance of this species introduction vector. The most commonly found NIS were *Amphibalanus improvisus, Sinelobus sp. nov., Cordylophora caspia, Chelicorophium curvispinum, Dikerogammarus villosus* and *Dreissena rostriformis bugensis* (Zabrocki *et al* 2021).
In 2020/2021 biofouling sampling on recreational boats started at the North and Baltic Sea. The taxonomic and microbiological analysis are still ongoing.

Research Needs and **Research Gaps**

A comparison of performance test results of ballast water management systems (BWMS) is difficult as various test teams are using different test methods and approaches. Therefore, a kind of a ring test is planned inviting relevant researchers to take and process samples of a water body spiked with a known concentration of beads and/or organisms (see BU-EBP project above). We look forward to see the different results when applying all these different methods for ballast water sampling and sample processing.

We reported earlier on the EU Regulation 1143/2014 on Invasive Alien Species. This regulation comprises the compilation of lists of "critical species" as species of Union-wide concern, species of regional concern, and species of national concern. In the beginning, only one marine (catadromous) species, i.e., the Chinese mitten crab, was added to the list. Based on the IAS Regulation each Member State needs to report the presence and distribution of the species on this list also providing options for management and/or eradication. However, some species on the lists are so widely distributed that eradication efforts are meaningless. Only species which are non-indigenous in the entire EU can be added to the list. Species which are suggested for addition will be reviewed by an expert forum. We see a possible research gap to, in detail, identify species selection criteria for these lists. It was agreed that the list should be reviewed and the addition of species will be considered in a biannual rhythm. The progress of these updates may be followed here: https://ec.europa.eu/environment/nature/invasivealien/list/index_en.htm.

Germany completed its reporting requirement of invasive NIS in EU Regulation 1143/2014 for the 1st reporting period (Nigmann & Nehring 2020) and continued afterwards to cover the species added 2019 to that list (Nehring & Skowronek 2020), which includes now 66 terrestrial and aquatic invasive NIS. The second update of the Union list entered into force on 15 August 2019. The third update was initiated in the end of 2021 and 30 species were considered for addition of which most are not occurring in Germany. This update will hopefully appear in spring 2022. Since June 2021 Germany has the first action plan to address EU Regulation 1143/2014 in place. This plan covers a wide range of NIS introduction vectors including botanical gardens, hobby

aquariums, zoos, decorative plants (garden ponds), ballast water, biofouling, canal migrations, fisheries and angling.

6. Meetings (selection)

GlobalTestNet - A formal group of test organizations involved in certification tests of BWMS was established in 2010 under the umbrella of GloBallast Partnership to facilitate increased standardization and harmonization of test procedures and information exchange. As biofouling became more and more into focus, the members voted in 2017 in favour of expanding the network beyond BWMS testing, and into biofouling to further support the shipping industry and its numerous stakeholders in managing the risk of bio-invasions and harmful species introductions into aquatic environments. Meetings are held approximately annually, with the most recent one in February 2020. The minutes of the meetings were published on the homepage http://www.globaltestnet.org/home.

In September 2020 the 1st In-Port Inspection & Cleaning Conference (PortPIC) was held in Hamburg. Topic to address include aquatic invasive species, diver operations in ports, next-generation antifouling technologies, operator perspective on cleaning, performance-based cleaning, regulations and guidelines, robotic cleaning and inspection. (http://data.hullpic.info/PortPIC2020_Hamburg.pdf).

Also, in September 2020 was an online meeting regarding MSFD Descriptor 2 (D2) aimed to facilitate discussions at national, regional and inter-regional level to support the implementation of D2. The meeting was hosted by Konstantinos Tsiamis and Ana-Cristina Cardoso (EU Joint Research Council (JRC)). In focus was the criterion D2C1 (new NIS records) and its threshold values for the number of new NIS introductions for each Member State and MSFD sub-region to reach Good Environmental Status (GES). The workshop addressed 18 topics of quite diverse character. The final report of this workshop is still in the making. However, we have seen a draft in which also relatively new terminology was mentioned. The German participants in the workshop felt that new terms, such as, “neo-natives”, “pseudo-indigenous” and “crypto-spreading” may not be helpful as the benefit of these new termini remain unclear and this may generate more confusion. These new terms are still under debate in the scientific community and not yet robust and clear enough to be applied in assessment and management. Thus, the science behind these new terms is not yet sufficiently mature for applications. Also, these new terms seem not to fit into the MSFD D2 context as they are not mentioned in the Directive. This statement was further agreed with Sergej Olenin (Lithuania) and Henn Ojaveer (Estonia) and then sent to the workshop coordinator, who agreed not to include these terminology discussions in the meeting report to avoid creating unnecessary confusion, but to revisit this at a future meeting on marine NIS. This is mentioned here to alert other European NIS experts that such a debate on NIS-related terminology may be coming up soon.

At a meeting of the OPSAR NIS EG the proposal of a Joint Working Group on NIS (JWGNIS) was discussed. A JWGNIS with HELCOM will serve as a good platform for exchanging knowledge on the status of NIS, including clarifying definitions on e.g. cryptogenic or NIS. The clear advantage for countries which have waters in both OSPAR and HELCOM, is that we establish a JWGNIS as this will hopefully help resolve differences in assessments and definitions etc. in the overlapping waters.

The 15th International Scientific Wadden Sea Symposium was held in Büsum, Germany in 2021 and had climate change and NIS related aspects as a focus subject.

Future meetings

To develop and coordinate German monitoring efforts and other non-indigenous species related aspects, meetings of the national neobiota expert working group (Meeresschutz der Bund/Länder-Arbeitsgemeinschaft Nord- und Ostsee (BLANO)) are anticipated, at least twice a year.

Germany is actively contributing to ballast water and other maritime transport related panels and meetings at IMO, HELCOM, OSPAR, TWSC and regional as well as international meetings related to the implementation of the EU-MSFD.

The HELCOM/OSPAR JTG BALLAST changed its Terms of Reference in 2020. The Joint Task group continues its work on the practical implementation of the OSPAR/HELCOM Joint Harmonised Procedure on the issue of exemptions in accordance with Regulation A-4 1.4 BWMC, but also considers the issues related to compliance control and enforcement of the BWMC and work toward further harmonization of implementing the BWMC. In addition, the group will consider the ships' and recreational crafts' biofouling issues at regional level. In this context, the Meeting also agreed that the name of the Group should be abbreviated as JTG BALLAST & BIOFOULING to reflect that the Terms of Reference now incorporate biofouling in addition to ballast water.

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Iceland

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Overview

In the years 2019–2021 twelve introduced species were reported in Iceland, seven newly established and five previous sightings. In total six of those twelve species were listed in 2021. Majority of the introduced species have first been reported at the S or W part of the country, and the seven newly established species in the period 2019–2021 were all reported at the southwest coast. The number of confirmed NIS in Iceland is now 32. Updated information on the colonization by NIS in selected locations and/or environments (like marinas and harbours) is provided. An expert group (ISAT) on non-indigenous species was formally established in Iceland in 2020 and has met several times in 2020 and 2021.

NIS targeted monitoring program is now operated along the Icelandic coast. The program started in 2018 and focuses on hot spots like harbours, marinas and aquaculture sites. In total there are eight monitoring sites with fixed equipment, additionally other sites are also visited yearly in the form of rapid assessments survey (RAS).

Content

1. **Regulations**

   No new Icelandic regulations.

2. **Intentional**

Two new intentional introductions were reported in the period 2019–2021, for research purpose in closed land-based aquacultures. *Litopenaeus vannamei* in 2019 and *Seriola dumerili* in 2021 (Jónsson 2021).

The Icelandic Food and Veterinary Authority (MAST) allowed introduction of four species for aquacultures in the period 2019–2021 (Jónsson 2019, 2020, 2021). Information about intentional introductions of aquatic species can be found in the annual reports of the Icelandic Food and Veterinary Authority under the heading *Eldisfiskur*. The reports are in Icelandic:

https://www.mast.is/is/um-mast/utgefid-efni/skyrslur#eldisfiskur-og-skel

3. Summary of sighting

Unintentional: Twelve species were reported in Iceland, seven newly established and five were added to the list after a literature review. Six ascidians, one mollusc and five macroalgae species were reported in Iceland in the period 2019–2021.

New Sightings

- *Ciona* cf. *robusta* Hoshino and Tokioka, 1967; first observed on floating dock in Sandgerði harbour (N64.036500 W-22.718833) in 2018. Has not been reported elsewhere (Ramos-Esplá *et al.* 2020).
- *Diplosoma listerianum* (Milne Edwards, 1841) first observed on floating dock in Sandgerði harbour (N 64.036500 W-22.718833). Has not been reported elsewhere (Ramos-Esplá *et al.* 2020).
- *Botryllus schlosseri* (Pallas, 1766) was first observed in 2011 in front of Vogar (N63.983044 W-22.435241) on *Mytilus edulis* in a long-lines aquaculture facility, at approximately 3m depth (Ramos-Esplá 2016, 2020). Was observed on floating dock in the harbours of Sandgerði and Grindavík for the first time in 2018 (Ramos-Esplá *et al.* 2020).
- *Ascidiella aspersa* (Müller, 1776), first observed on floating dock in Sandgerði harbour (N64.036500 W-22.718833) in 2018. Was also observed on floating dock in the harbour of Hafnarfjörður in 2018 (Ramos-Esplá *et al.* 2020).
- *Botrylloides violaceus* Oka, 1927, only been observed on floating dock in the harbour of Hafnarfjörður (N 64.063362 W-21.961941). First record in 2018 (Ramos-Esplá *et al.* 2020).
- *Molgula manhattensis* (De Kay, 1843), observed on floating dock in Sandgerði harbour in 2018 (N64.036500 W-22.718833), also observed in Reykjavík harbour the same year (Ramos-Esplá *et al.* 2020).
- *Ensis terranovensis* (Vierna & Martínez-Lage, 2012), first observed on a sandy beach in Reykjavík (N64.16950 W-21.74510) in 2020. Has now been observed elsewhere in SW-Iceland (Gunnarsson *et al*. in review).

Previous Sightings

- *Ulva rigida* C.Agardh, 1823, first reported in Vestmannaeyjar archipelago in S-Iceland in 1972 (Caram and Jónsson 1972). The species has since been found at the W and E coast and is considered an NIS (Micael *et al.* 2021).
- *Fucus distichus* subsp. *evanescens* (C.Agardh) H.T.Powell, 1957. First reported in 1976 (Munda 1976). The species is widespread now and considered an NIS (Micael *et al.* 2021).
- *Desmarestia ligulata* (Stackhouse) J.V.Lamouroux, 1813. First reported in S-Iceland in 1967 (Jónsson 1967, Munda 1972). The species has currently a scarce distribution in Iceland. It is considered an NIS (Micael *et al.* 2021).
- *Pyropia leucosticta* (Thuret) Neefus & J. Brodie, 2011. First reported in Dýraförður NW-Iceland in 1979 (Munda 1979). Has a current distribution clockwise from S to NW–Iceland. It is considered an NIS (Micael *et al.* 2021).

- *Pyropia njordii* Mols-Mortensen, J. Brodie & Neefus, 2012. First reported in SE-Iceland in 2005 (Gunnarsson & Nielsen 2016). It is considered an NIS (Micael *et al.* 2021).

4. Pathogens

No new pathogens have been reported for Iceland.

5. Research and Monitoring Programs

No governmental research or monitoring programs are ongoing on marine alien species in Icelandic waters.

Project Title: CRAB-ICE

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Short description: Research and monitoring project that started in 2007. The focus of the project has been on the alien crab species *Cancer irroratus*. Research have e.g. been done on its genetic variation (Gíslason *et al.* 2013), moulting, and density (Gíslason *et al.* 2017) and population dynamics (Gíslason *et al.* 2020). Annually both larvae and crab abundance are monitored at several sampling stations in Faxaflói, SW-Iceland, and information about its distribution along the coastline is updated. The project was started on the initiative of the University of Iceland, it has been carried out in collaboration by the SINRC and the University of Iceland's Research Centre in Suðurnes. It is not a governmental project and not funded specially, so far it has only been financed with funding from competitive funds.

Project title: BF-ICE

Contact: Sindri Gíslason, Southwest Iceland Nature Research Centre (SINRC), sindri@natturustofa.is.

Short description: Research and monitoring program on biofouling in Icelandic harbours and marinas. The program started in 2018 and its aim is to monitor selected floating docs in selected marinas around the Iceland for NIS. The project has so far revealed six new NIS in Iceland (Ramos-Esplá *et al*. 2020), the distribution and density of *Ciona intestinalis* (Micael *et al.* 2020), and the invasive behaviour of the cryptogenic species *Rhizoclonium riparium* (Micael *et al.* 2020). The project was started on the initiative of the SINRC, is not a governmental project and not funded specially. So far, the project has only been financed with funding from competitive funds.

Project title: ENSIS-ICE

Contact: Sindri Gíslason, Southwest Iceland Nature Research Centre (SINRC), sindri@natturustofa.is.

Short description: Research and monitoring project that started in 2020. The focus of the project is on the alien mollusc species *Ensis terranovensis*. Ongoing research are on the species distribution, density, origin and population genetics. SINRC is leading the monitoring and research of the species in Iceland, in collaboration with the Natural History Museum of Iceland, the Marine Research Institute, and MATÍS.

Project title: European flounder in Iceland

Contact: Theresa Henke, University of Iceland, Research Centre of the Westfjords, thh183@hi.is.

Short description: The University Research Centre in the Westfjords has an ongoing research project on the origin, distribution, and impact of the European flounder (*Platichthys flesus*).

Publication on the niche overlap of the species with the native European plaice came out in 2020 (Henke *et al.* 2020).

Research Needs and **Research Gaps**

More financial support and genetic work is needed. Information on genetic diversity and demography is absent for most species (samples for all reference samples of ascidians stored at SINCR). The use of e-DNA would be beneficial as monitoring method to use in harbours.

6. Meetings

ÍSÁT: Icelandic expert group on non-indigenous species was formally established in May 2020. The group consists of specialist that have worked with non-indigenous species for years in Iceland, both in aquatic and terrestrial environments. The group had two online meetings in 2020 and two in 2021.

Future meetings

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Þórðardóttir, G.B. & Guðbergsson, G. 2021. Lax- og silungsveiði 2020. Marine and Freshwater Institute. National report. (In Icelandic with English abstract).

Þórðardóttir, G.B. & Guðbergsson, G. 2020. Lax- og silungsveiði 2019. Marine and Freshwater Institute. National report. (In Icelandic with English abstract).

Confirmed non-indigenous species in Icelandic waters in 2021.

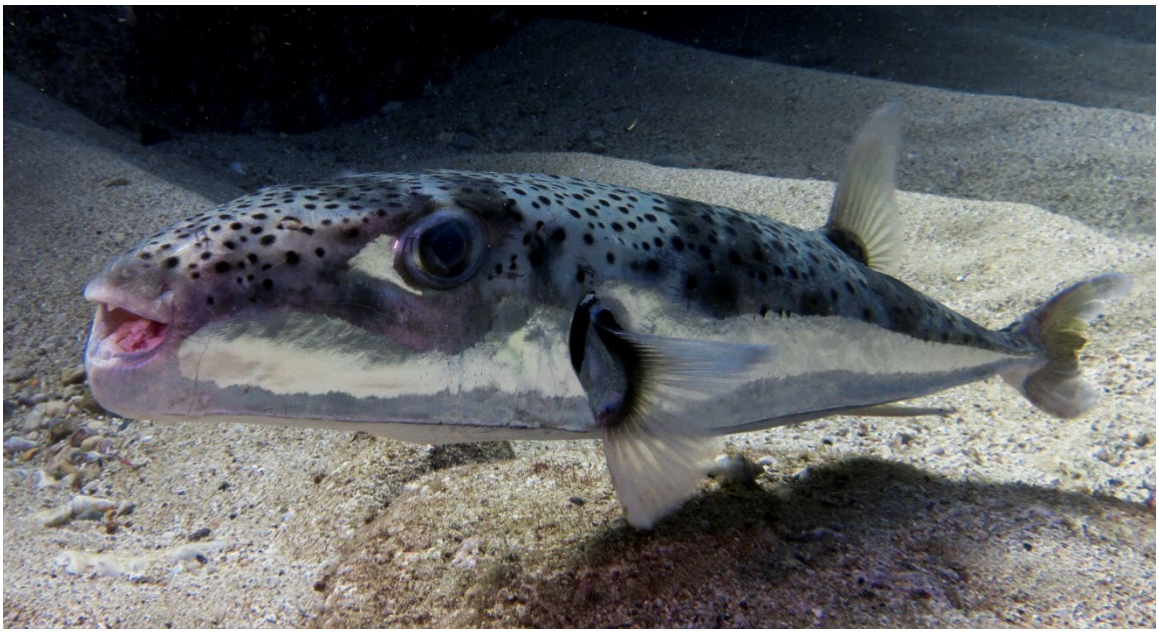
Taxa	*First recorded*	*Invasive*	*References*
Phytoplankton			
Heterosigma akashiwo	1987, SW-Iceland	*No*	Þórarinsdottir & Þórðardóttir 1997
Mediopyxis helysia	2007, W-Iceland	*No*	Gunnarsson et al. 2011
Stephanopyxis turris	1997, SW-Iceland	*No*	Gunnarsson et al. 2011
Macroalgae			
Bonnemaisonia hamifera	1978, NW-Iceland	*No*	Munda, 1978
Codium fragile subsp. *fragile*	1974, SW-Iceland	*No*	Jónsson & Gunnarsson 1975
Desmarestia ligulata	1967, S-Iceland		Jónsson 1967, Munda 1972, Micael et al. 2021
Fucus disticus sp. *evanescens*	1976, W-Iceland	*Potentially*	Munda 1976, Micael et al. 2021
Fucus serratus	1900, SW-Iceland	*Potentially*	Jónsson 1903
Pyropia leucosticta	1979, NW-Iceland	*Potentially*	Munda 1979, Micael et al. 2021
Pyropia njordii	2005, SE-Iceland	*Potentially*	Gunnarsson & Nielsen 2016, Micael et al. 2021
Ulva rigida	1972, S-Iceland	*Potentially*	Caram & Jónsson 1972, Micael et al. 2021
Crustacea			
Cancer irroratus	2006, SW-Iceland	*Potentially*	Gíslason et al. 2014
Crangon crangon	2003, SW-Iceland	*Potentially*	Gunnarsson et al. 2007
Homarus americanus	1965, SW-Iceland	*No*	Skúladóttir 1968
Orchestia gammarellus	1971, SW-Iceland	*No*	Ingólfsson 1974
Praunus flexuosus	1970, SW-Iceland	*No*	Ástþórsson 1987
Mollusca			
Cerastoderma edule	1948, SW-Iceland	*No*	Óskarsson 1982
Ensis terranovensis	2021, SW-Iceland	*Potentially*	Gunnarsson et al. in prep
Mya arenaria	1958, SW-Iceland	*No*	Óskarsson 1982
Tunicata			
Ciona intestinalis	2007, SW-Iceland	*Potentially*	Svavarsson & Dungal 2008
Ascidiella aspersa	2018, SW-Iceland	*Potentially*	Ramos-Esplá et al. 2020
Botrylloides violaceus	2018, SW-Iceland	*Potentially*	Ramos-Esplá et al. 2020
Botryllus schlosseri	2015, SW-Iceland	*Potentially*	Ramos-Esplá et al. 2020
Ciona cf. *robusta*	2018, SW-Iceland	*Potentially*	Ramos-Esplá et al. 2020
Diplosoma listerianum	2018, SW-Iceland	*Potentially*	Ramos-Esplá et al. 2020
Molgula manhattensis	2018, SW-Iceland	*Potentially*	Ramos-Esplá et al. 2020
Cnidarian			
Gonionemus vertens	2008, SW-Iceland	*?*	Gíslason 2015
Rhizogeton nudus	2010, SW-Iceland	*?*	Schuchert, 2001
Fish			
Platichthys flesus	1999, SW-Iceland	*Potentially*	Jónsson et al. 2001
Taurulus bubalis	2005, SW-Iceland	*No*	Pálsson 2007
Oncorhynchus gorbuscha	1960 (2017*), W-Iceland	*Potentially*	Þórðardóttir & Guðbergsson 2017
Oncorhynchus mykiss	1983, SW-Iceland	*Potentially*	Jónsson 1983

* Spawning

Israel

Prepared by Bella S. Galil, Steinhardt Museum of Natural History, Tel Aviv University, Israel

Note: This report does not reflect an official position or knowledge of the relevant Israeli Government bodies.



The lethally poisonous *Lagocephalus sceleratus* (Gmelin, 1789) was recently accorded the status of a '**Protected Nature Value**' in Israel's Mediterranean waters. Photo: O. Klein.

The Israeli coastline, 190 km long, is the world's most invaded marine ecosystem due to its vicinity to the Suez Canal. Update of a recent review (Galil *et alet al.* 2020) **increased the recorded number of multicellular introduced species to 465 species** (Table 1). The extraordinarily large number of records highlights the role of the southern Levant as a "hotspot", a beachhead and dispersal hub for secondary spread. **The present report lists 72 NIS (4 vertebrates, 34 invertebrates, 1 seagrass and 33 macrophytes) newly described from the Israeli coast in 2019-2021**.

Content

1. Regulations: An update on new regulations and policies (including, aquaculture and vector management)

At the instigation of the Nature and Parks Authority, all invertebrates in marine nature reserves along the Mediterranean coast of Israel were declared 'Protected Nature Values' in 2005, in addition to all Coelenterata, Echinodermata and Mollusca (excepting cephalopods) in the waters under national Israeli jurisdiction – not excepting NIS.

Recently (17 December 2019) Israel's Minister of Environmental Protection amended regulations concerning 'Protected Nature Values' (https://www.gov.il/he/departments/news/protected_species_list, viewed 15 February 2021). Invertebrates – including hundreds of NIS – in marine nature reserves remain protected, despite manifest negative impacts on the native communities. In addition, all Cnidaria (excepting scyphozoans), Crustacea (excepting Scyllaridae), Echinodermata and Mollusca (excepting cephalopods) in the waters under national Israeli jurisdiction were confirmed 'Protected Nature Values' – including NIS. Even the lethally poisonous puffer

Lagocephalus sceleratus was accorded the status of a 'Protected Nature Value' in Israel's Mediterranean waters.

2. Intentional introductions

3. Unintentional introductions

New Records 2019–2021

CHLOROPHYTA

Bryopsis pennata J.V.Lamouroux 1809 Bryopsidaceae

Caulerpa denticulata Decaisne, 1841 Caulerpaceae

Caulerpa racemosa *var.* ***lamourouxii*** *f.* ***requienii*** (Montagne) Weber Bosse 1913 Caulerpaceae

Caulerpa taxifolia *var.* ***distichophylla*** (Sonder) Verlaque, Hui an & Procaccini 2012 Caulerpaceae

Cladophora patentiramea (Montagne) Kützing, 1849 Cladophoraceae

Codium taylorii P.C.Silva 1960 Codiaceae

Derbesia boergesenii (M.O.P.Iyengar & Ramanathan) Mayhoub, 1976 Derbesiaceae

Derbesia rhizophora Yamada, 1961 Derbesiaceae

Neomeris annulata Dickie, 1874 Dasycladaceae

Parvocaulis parvulus (Solms-Laubach) S.Berger, Fettweiss, Gleissberg, Liddle, U.Richter, Sawitzky & Zuccarello, 2003 Polyphysaceae

Ulva ohnoi M.Hiraoka & S.Shimada, 2004 Ulvaceae

PHAEOPHYTA

Dictyota acutiloba J.Agardh, 1848 Dictyotaceae

Lobophora lessepsiana C.W.Vieira, 2019 Dictyotaceae

RHODOPHYTA

Anotrichium okamurae Baldock, 1976 Wrangeliaceae

Asparagopsis taxiformis (Delile) Trevisan, 1845 Bonnemaisoniaceae

Botryocladia madagascariensis G.Feldmann, 1945 Rhodymeniaceae

Chondria coerulescens (J.Agardh) Sauvageau, 1897 Rhodomelaceae

Chondria curvilineata Collins & Hervey, 1917 Rhodomelaceae

Diplothamnion jolyi Hoek, 1978

Gayliella fimbriata (Setchell & N.L.Gardner) T.O.Cho & S.M.Boo in T.O.Cho *et alet al*. 2008 Ceramiaceae

Gracilaria viridis A.Sfriso, M.A.Wolf, K.Sciuto, M.Morabito, C.Andreoli & I.Moro, 2013 Gracilariaceae

Grateloupia turuturu Yamada, 1941 Halymeniaceae

Herposiphonia parca Setchell, 1926 Rhodomelaceae

Hypnea cornuta (Kützing) J.Agardh, 1851 Cystocloniaceae

Laurencia caduciramulosa Masuda & S.Kawaguchi, 1997 Rhodomelaceae

Laurencia minuta Garbary & Guiry 1990 Rhodomelaceae

Lophocladia lallemandii (Montagne) F.Schmitz, 1893 Rhodomelaceae

Melanothamnus harveyi (Bailey) Díaz-Tapia & Maggs, 2017 Rhodomelaceae

Pyropia koreana (M.S.Hwang & I.K.Lee) M.S.Hwang, H.G.Choi Y.S.Oh & I.K.Lee, 2011 Bangiaceae

Solieria dura (Zanardini) F.Schmitz 1895 Solieriaceae

Spyridia aculeata (C.Agardh ex Decaisne) Kützing, 1843 Callithamniaceae

Vertebrata fucoides (Hudson) Kuntze, 1891 Rhodomelaceae

Womersleyella setacea (Hollenberg) R.E.Norris, 1992 Rhodomelaceae

Galil, B.S., Mienis, H.K., Hoffman, R. & Goren, M., 2020. Non-indigenous species along the Israeli Mediterranean coast: tally, policy, outlook. Hydrobiologia, 848, 2011-2029. .

Katsanevakis, S., *et al*. 2020. Unpublished Mediterranean records of marine alien and cryptogenic species. BioInvasions Records, 9(2), 165-182.

Tsiamis, K., *et al*. 2020. Prioritizing marine invasive alien species in the European Union through horizon scanning. Aquatic Conservation: Marine and Freshwater Ecosystems, 30(4), 794-845.

Vieira, C., Aharonov, A., Paz, G., Engelen, A.H., Tsiamis, K., Einav, R. & De Clerck, O., 2019. Diversity and origin of the genus *Lobophora* in the Mediterranean Sea including the description of two new species. Phycologia, 58(2), 163-168.

TRACHEOPHYTA

Halophila stipulacea **(Forsskål) Ascherson, 1867 Hydrocharitaceae**

Hoffman, R., 2021. Ecological significance of the late occurrence of *Halophila stipulacea* (Hydrocharitaceae, Tracheophyta) on the southern Levantine Mediterranean shores of Israel. Oceanography and Fisheries, 14(1), 555877. https://doi.org/0.19080/OFOAJ.2021.14.555877

SCYPHOZOA

Chrysaora pseudoocellata **Mutlu, Çağatay, Olguner & Yilmaz, 2020 Pelagiidae**

Douek, J., Rinkevich, B., Gevili, R. & Galil, B.S. 2020. First record of a non-native jellyfish (Scyphozoa: Pelagiidae: *Chrysaora*) in the easternmost Mediterranean Sea. BioInvasion Records, 9(3), 482-489.

CRUSTACEA

Micippa thalia (Herbst, 1803) Majidae

Oithona davisae (Ferrari and Orsi, 1984) Oithonidae

Panulirus longipes longipes (A. Milne Edwards, 1868) Palinuridae

Paracaprella pusilla Mayer, 1890 Caprellidae

Urocaridella pulchella Yokes & Galil, 2006 Palaemonidae

Galil, B.S., Mienis, H.K., Hoffman, R. and Goren, M., 2020. Non-indigenous species along the Israeli Mediterranean coast: tally, policy, outlook. *Hydrobiologia*, , 848, 2011-2029.

Katsanevakis, S., *et al.* 2020. Unpublished Mediterranean records of marine alien and cryptogenic species. BioInvasions Records, 9(2), 165-182.

Lo Brutto, S., Iaciofano, D., Guerra García, J.M., Lubinevsky, H. & Galil, B.S., 2019. Desalination effluents and the establishment of the non-indigenous skeleton shrimp *Paracaprella pusilla* Mayer, 1890 in the south-eastern Mediterranean. BioInvasions Records, 8(3), 661-669.

Spanier, E. & Friedmann, E., 2019. The collection of an exuvia identified as *Panulirus longipes longipes* (A. Milne-Edwards, 1868) from off Haifa, Israel. Mediterranean Marine Science, 20(1), 227-229.

Velasquez, X., Morov, A., Terbiyik Kurt, T., Meron, D. & Guy-Haim, T. 2021. Two-way bioinvasion: tracking the neritic non-native cyclopoid copepods *Dioithona oculata* and *Oithona davisae* (Oithonidae) in the Eastern Mediterranean Sea. Mediterranean Marine Science, 22(3), 586-602. https://doi.org/10.12681/mms.26036

MOLLUSCA

Chavania erythraea (Issel, 1869) Lucinidae

Circulus octoliratus (Carpenter, 1856) Vitrinellidae

Conradia eutornisca **(Melvill, 1918) Conradiidae**

Conus fumigatus Hwass in Bruguière, 1792 Conidae

Coriophora lessepsiana Albano, Bakker & Sabelli, 2021

Cycloscala hyalina (G.B. Sowerby II, 1844) Epitoniidae

Cylichna collyra Melvill, 1906 Cylichnidae

Dikoleps micalii **Agamennone, Sbrana, Nardi, Siragusa & Germanà, 2020 Skeneidae**

Epitonium (Parviscala) vaillanti (Jousseaume, 1912) Epitoniidae

Ervilia scaliola Issel, 1869 Semelidae

Eunaticina papilla (Gmelin, 1791) Naticidae

Hemiliostraca clandestina (Mifsud & Ovalis, 2019) Eulimidae

Joculator problematicus **Albano & Steger, 2021 Cerithiopsidae**

Laevichlamys superficialis (Forsskål in Niebuhr, 1775) Pectenidae

Lioberus ligneus (Reeve, 1858) Mytilidae

Melanella orientalis Agamennone, Micali & Siragusa, 2020 Eulimidae

Mnestia girardi (Audouin, 1826) Mnestiidae

Muculus coenobitus (Vaillant, 1865) Mytilidae

Nerita sanguinolenta Menke, 1829 Neritidae

Oscilla virginiae **Peñas, Rolán & Sabelli, 2020 Pyramidellidae**

Parthenina cossmanni (Hornung & Mermod, 1924) Pyramidellidae

Parthenina typica (Laseron, 1959) Pyramidellidae

Pegophysema philippiana (Reeve, 1850) Lucinidae

Perna perna (Linnaeus, 1758) Mytilidae

Pyrgulina craticulata (Issel, 1869) Pyramidellidae

Turbonilla cangeyrani Ovalis & Mifsud, 2017 Pyramidellidae

Turbonilla funiculata de Folin, 1868 Pyramidellidae

Varicorbula erythraeensis **(**H. Adams, 1871) Corbulidae

Albano, P.G., Steger, J., Bakker, P.A., Bogi, C., Bošnjak, M., Guy-Haim, T., Huseyinoglu, M.F., LaFollette, P.I., Lubinevsky, H., Mulas, M. & Stockinger, M. 2021. Numerous new records of tropical non-indigenous species in the Eastern Mediterranean highlight the challenges of their recognition and identification. ZooKeys, 1010, 1–95, https://doi.org/10.3897/zookeys.1010.58759

Bonfitto, A., Bogi, C. & Lubinevsky, H. 2021. First record of *Epitonium* (*Parviscala*) *vaillanti* (Jousseaume, 1912) (Mollusca: Gastropoda: Epitoniidae) in the Mediterranean Sea. BioInvasions Records, 10 (3), 612–622, https://doi.org/10.3391/bir.2021.10.3.11

Douek, J., Paz, G., Gayer. K., Mendelson, M., Rinkevich, B. & Galil, B.S. 2021. An outbreak of *Perna perna* (Linnaeus, 1758) (Mollusca, Bivalvia, Mytilidae) in the Eastern Mediterranean. BioInvasions Records, 10(1), 136–148, https://doi.org/10.3391/bir.2021.10.1.15

Edelman-Furstenberg, Y., Koral, H., Abramovich, S. & Mienis, H.K., 2020. A new occurrence off the coast of Israel of the mon-indigenous gastropod *Circulus octoliratus* (Carpenter, 1856). Triton, 39, 10-12.

Malki, Y., Korkos, K. & Mienis, H.K., 2019. A first record of *Laevichlamys superficialis* from the Mediterranean coast of Israel. Triton, 40, 1-3.

Mienis, H.K. 2019. A first record of *Anodontia philippiana* from the Mediterranean Sea off Israel. Triton, 38, 4-5.

Rabi, C., Rilov, G., Morov, A.R. & Guy-Haim, T., 2020. First record of the Red Sea gastropod *Nerita sanguinolenta* Menke, 1829 (Gastropoda: Cycloneritida: Neritidae) from the Israeli Mediterranean coast. BioInvasions Records, 9(3), 496-503.

Scaperrotta, M., Bartolini, S. & Bogi, C. 2019. Accrescimenti. Stadi di accrescimento dei molluschi marini del Mediterraneo. Volume X. L'Informatore Piceno, 212 pp.

Schechter, H.C. & Mienis, H.K. 2020. A first record of *Eunaticina linnaeana* from the Mediterranean coast of Israel (Gastropoda, Naticidae). Triton, 40, 4-5.

ACTINOPTERYGII

Cryptocentrus steinhardti Goren and Stern, 2021 Gobiidae

Istiblennius meleagris (Valenciennes, 1836) Blenniidae

Lutjanus argentimaculatus (Forsskål, 1775) Lutjanidae

Ostracion cubicus Linnaeus, 1758 Ostraciidae

Goren, M. & Stern, N., 2021. *Cryptocentrus steinhardti* (Actinopterygii; Gobiidae): a new species of shrimp-goby, and a new invasive to the Mediterranean Sea. PeerJ, 9, e12136.

Katsanevakis, S., *et al.* **2020**. Unpublished Mediterranean records of marine alien and cryptogenic species. BioInvasions Records, 9(2), 165-182.

Rothman, S.B., Gayer, K. & Stern, N. 2020. A long-distance traveler: the peacock rock-skipper *Istiblennius meleagris* (Valenciennes, 1836) on the Mediterranean intertidal reefs. Biological Invasions, 22(8), 2401-2408.

Sonin, O., Edelist, D. & Golani, D. 2019. The occurrence of the Lessepsian migrant *Lutjanus argentimaculatus* in the Mediterranean, (Actinopterygii: Perciformes: Lutjanidae) first record from the coast of Israel. Acta Adriatica: International journal of Marine Sciences, 60(1), 99-102.

ASCIDIACEA

Didemnum perlucidum Monniot F., 1983 Didemnidae

Novak, L.& Shenkar, N., 2020. Occurrence of *Didemnum perlucidum* Monniot F., 1983 on artificial substrates along the Mediterranean coast of Israel. Mediterranean Marine Science, 21(2), 386-392.

4. Pathogens

Viral encephalopathy and retinopathy (VER), a disease caused by the marine nervous necrosis virus (NNV), is recognised as one of the main infectious threats for marine fish. The prevalence of NNV in indigenous Mediterranean species (*Sardinella aurita, Lithognathus mormyrus*) and NIS (*Nemipterus randalli, Saurida lessepsianus*) was surveyed using molecular methods. In *N. randalli*, a recently established invasive species, the prevalence was significantly higher than in both indigenous species. The authors suggest that the susceptibility of an introduced fish species to locally acquired viral pathogens such as NVV may be important, in terms of both its successful establishment in its newly adopted environment and its role as a reservoir 'host' in the new area.

Lampert, Y., Berzak, R., Davidovich, N., Diamant, A., Stern, N., Scheinin, A.P., Tchernov, D. & Morick, D. 2020. Indigenous versus Lessepsian hosts: Nervous Necrosis Virus (NNV) in eastern Mediterranean sea fish. Viruses, 12(4), 430.

5. Research and Monitoring programs

No nationally funded program focused on monitoring marine NIS. Most publications result from serendipitous finds. The recently published list of marine NIS along the Mediterranean coast of Israel was compiled by curators of the Steinhardt Museum of Natural History, Tel Aviv University.

Douek, J., Harbuzov, Z., Galil, B.S. & Rinkevich, B. 2020. Developing novel microsatellite markers by NGS technology for *Rhopilema nomadica*, an invasive jellyfish. Molecular Biology Reports, 47, 4821-4825.

Galil, B.S., Mienis, H.K., Hoffman, R. and Goren, M., 2020. Non-indigenous species along the Israeli Mediterranean coast: tally, policy, outlook. Hydrobiologia, 848, 2011-2029.

Ivkić, A., Steger, J., Galil, B.S. & Albano, P.G. 2019. The potential of large rafting objects to spread Lessepsian invaders: the case of a detached buoy. *Biological Invasions*, 21(6), 1887-1893.

CODA

Not on the shelf alone

In the 1960s the maximum depth of the Suez Canal was 15.5 m, and since has been increased to 24 m (https://www.suezcanal.gov.eg/English/About/SuezCanal/Pages/CanalCharacteristics.aspx).

It thus has been assumed that populations of the NIS introduced through the canal into the Mediterranean will be restricted to the upper shelf. Indeed, until the 1970s the Erythraean NIS were mostly confined to habitats shallower than 50 m. The post-millenial excavations increased the canal's cross-section and hence water volume and through-current velocities, and likely raised propagule pressure—including epipelagic larvae/juveniles of deeper living biota. The increasing sea water temperature facilitated establishment. Already in 2008–2012, surveys of bottom communities off the southern Mediterranean coast of Israel, recovered Erythraean NIS from the deeper portions of the shelf and beyond the shelf break, the lesser swimming crab, *Charybdis longicollis*, was collected at 100, 120 and 250 m. A survey along the 200 m isobath in 2017 collected 3 Erythrean fish: the crocodile toothfish *Champsodon nudivittis*, Golani's round herring *Etrumeus golanii*, and the burrowing goby *Trypauchen vagina*.

The descent of Erythraean NIS into the upper slope revealed that thermal niche estimations based on the species' native environment likely underestimated their thermal tolerance, and thus their bathymetric and geographic expansion.

Galil, B.S., Danovaro, R., Rothman, S.B.S., Gevili, R. & Goren, M. 2019. Invasive biota in the deep-sea Mediterranean: an emerging issue in marine conservation and management. Biological Invasions, 21(2), 281-288.

Innocenti, G., Stasolla, G., Goren, M., Stern, N., Levitt-Barmats, Y., Diamant, A. & Galil, B., 2017. Going down together: invasive host, Charybdis longicollis (Leene, 1938) (Decapoda: Brachyura: Portunidae) and invasive parasite, Heterosaccus dollfusi Boschma, 1960 (Cirripedia: Rhizocephala: Sacculinidae) on the upper slope off the Mediterranean coast of Israel. Marine Biology Research, 13(2), 229-236.

No replacement

Comparison of native molluscan richness in living and death assemblages revealed that only 12% and 5% of species present in the death assemblages were live-collected on the Israeli shallow subtidal soft and hard substrates, respectively. This is the largest regional-scale diversity loss in the oceans documented to date. Assemblages in the intertidal, more tolerant to climatic extremes, and in the cooler mesophotic zone show approximately 50% of the historical native richness. Importantly, approximately 60% of the recorded shallow subtidal native species do not reach reproductive size, making the shallow shelf a demographic sink. As climate warms, it is likely native biodiversity collapse will intensify, counteracted only by Erythraean NIS.

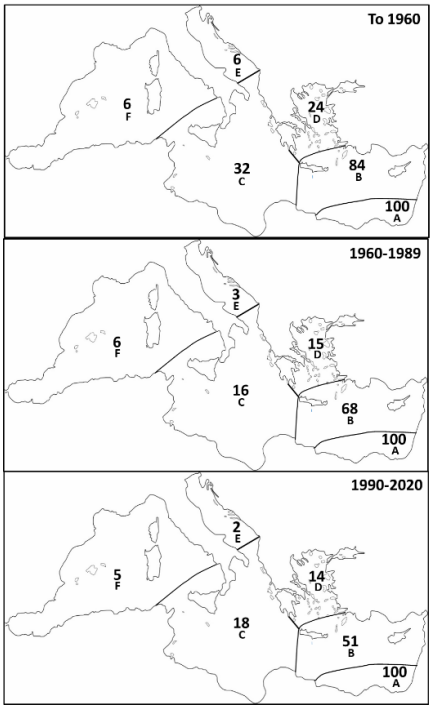
Trait information compiled for all molluscan species sampled in living and death assemblages on the Israeli shelf. Comparison of the abundance-weighted trait composition, and functional diversity of native and non-indigenous assemblage components revealed they consistently differed both in living and death assemblages, as well as between living non-indigenous and historical native assemblages. The rising NIS dominance has considerably altered the functional properties of shallow-water molluscan assemblages, and cannot functionally replace lost native species.

Albano, P.G., Steger, J., Bošnjak, M., Dunne, B., Guifarro, Z., Turapova, E., Hua, Q., Kaufman, D.S., Rilov, G. & Zuschin, M. 2021. Native biodiversity collapse in the Eastern Mediterranean. Proceedings of the Royal Society B: Biological Sciences, *288*, 20202469. https://doi.org/10.1098/rspb.2020.2469

Steger, J., Bošnjak, M., Belmaker, J., Galil, B.S., Zuschin, M. & Albano, P.G. 2022. Non-indigenous molluscs in the Eastern Mediterranean have distinct traits and cannot replace historic ecosystem functioning. Global Ecology and Biogeography, 31(1), 89-102.

What happens in the Levant, doesn't stay in the Levant

Spread of Erythraean NIS (molluscs, fish, crustaceans) throughout the Mediterranean Sea, first recorded in the SE Levant prior to 1960, 1960–1989, 1990–2020, shown as percentage of records in the SE Levant Sea.



Italy

Overview

Seventeen new introduced species are reported from Italian coasts. These include: three algae, one foraminiferan; two polychaetes, five molluscs, one crustacean, two tunicates, and three fish species. In addition, five cryptogenic foraminiferan species have been recorded from the Sicily Straits, one polychaete from the Po river Delta and two molluscs rom Sicily and Tuscany. Significant ecological studies on various already known NIS have been published. Updated information on the colonization by NIS in selected locations and/or environments (like marinas and harbours) is provided. Citizen science initiatives, involving local population and operators have contributed to a wider knowledge of the distribution of NIS.

1. Regulations

The EU regulation on the prevention and management of the introduction and spread of invasive alien species (IAS) is based on the core concept of "IAS of EU concern": species to be targeted for action. Besides the existing, and continuously being updated, European list, Italy is developing its own national list. Occhipinti-Ambrogi *et al.* (2019) describe the process of development of the list of marine IAS of Italian concern and show the highest priority marine IAS.

2. Intentional introduction

No new intentional introductions have been reported.

3. Summary of sightings

Unintentional introduction

List of New Sightings 2019-21

(* = Cryptogenic species)

ALGAE

Acanthosiphonia echinata (Harvey) A.M. Savoie & G.W. Saunders

Caulacanthus okamurae Yamada, 1933

Pachymeniopsis gargiuloi S.Y.Kim, Manghisi, Morabito & S.M.Boo, 2014

FORAMINIFERA

Amphisteginalobifera Larsen, 1976
Amphistegina lessonii * d'Orbigny, 1826
Amphisorus hemprichii * Ehrenberg, 1839
Peneroplis arietinus * (Batsch, 1791) (Reported as *Coscinospira arietina*)
Coscinospira hemprichii * Ehrenberg, 1839
Nautilus orbiculus * Forsskål in Niebuhr, 1775

POLYCHAETA

Dorvillea similis (Crossland, 1924)

Pseudopolydora paucibranchiata (Okuda, 1937)
Streblospio eridani * n. sp., Munari *et alet al.* 2020

MOLLUSCA

Favorinus ghanensis Edmunds, 1968
Isognomon legumen (Gmelin,1791)
Malleus regula (Forsskål in Niebuhr, 1775)
Mitrella psilla (Duclos, 1846)

Odetta zekiergeni * Öztürk, 2013
Okenia picoensis * Paz-Sedano, Ortigosa & Pola, 2017
Teredo bartschi Clapp, 1923

MALACOSTRACA

Parametopella cypris (Holmes 1905)

TUNICATA

Aplidium accarense (Millar,1953)
Polyclinum constellatum Savigny, 1816

ACTINOPTERYGII

Bregmaceros nectabanus Whitley, 1941
Neogobius melanostomus (Pallas 1811)
Siganus fuscescens (Houttuyn, 1782)

Algae

A small red alga of the family Rhodomelaceae, *Acanthosiphonia echinata* (syn. *Polysiphonia echinata*), was recorded from the Lagoon of Venice in 2018 (Wolf *et alet al.* 2020). The species is considered native to the West American coast and is distributed also in South Eastern Asia; it was found in two stations (Pellestrina Island, Venice, Italy: 45.237778N; 12.300833E; 45.329722; 12.318056E) during March to October 2018. Specimens were identified using the DNA barcoding method and represent the first finding for the Mediterranean Sea. One of the possible introduction vectors of this species in the Mediterranean Sea is transport by ship via the Western Atlantic-Mediterranean-Indonesia route, but this pathway may also be associated with algal aquaculture shipments from Indonesia.

The first Italian finding of the red alga *Caulacanthus okamurae* was reported in the Ionian and Adriatic Seas by Petrocelli *et al.* (2020b). Stable populations were detected in some areas of the Mar Piccolo of Taranto (40.4794444N; 17.228056E) and in the whole lagoon of Venice (45.44275N, 12.343361E), forming dense patches of low turf that reach high biomasses. Specimens were identified through molecular analyses based on the plastid ribulose-1,5-bisphosphate carboxylase/oxygenase (rbcL) marker. The specimens from the two Italian locations show the same origin of specimens from South Korea and Atlantic Spain. The species was described from Japan and is considered native from the Indo-Western Pacific. It has been introduced to the Atlantic coasts and was recorded before in two French Mediterranean localities.

A previously overlooked record of a cryptic species of Rhodophyta, *Pachymeniopsis gargiuloi,* described as a new species by Kim *et al.* (2014), as distinct from *Pachymeniopsis lanceolata*, has been reconsidered and added to the Italian list of introduced species. The Italian material examined in the species description was collected on both sides of the Messina Strait - from the towns of Messina and Reggio Calabria - and bears close resemblance with material from Korea, where the species is a component of the native algal flora of Northeast Asia. Previous records from Sicily, under the names of *Grateloupia cuneifolia* (Giaccone, 1969) and *Grateloupia doryphora* (De Masi and Gargiulo, 1982) indicate that the introduction may have occurred since some times. The most likely scenario is that the Italian populations were introduced from Korea (and possibly Japan), in relation with the import of oysters for aquaculture.

Invertebrates

Polychaetes

Six specimens of the Erythrean polychaete *Dorvillea similis*, known until now only from the Mediterranean Eastern basin, were collected in fouling assemblages sampled with a scraping net at 1 m depth on concrete docks in the marina of Capraia Island, Tyrrhenian Sea (43.0513N; 9.8367E) on 14 May 2019 (Langeneck and Tempesti 2019).

Pseudopolydora paucibranchiata, originally described for the Northern Pacific Ocean, since 2010 is currently known from the Eastern Mediterranean Sea, as well as from the Atlantic European coast. In Italy, the species was officially reported in Ischia Island by Gambi *et al.* (2016), who pointed out that material previously identified in Ischia harbour as *Pseudopolydora antennata* actually was *P. paucibranchiata*. Thanks to the paper by Radashevsky *et al.* (2020), the presence of the species in the Southern Tyrrhenian Sea is confirmed by molecular analyses at least since 1977; it was also found in the coastal lagoon of San Teodoro in Sardinia, where it was detected in 2015 and probably arrived through oyster farming (Langeneck *et alet al.* 2020).

Molluscs

During a survey of four marinas in the Gulf of La Spezia (Ligurian Sea), Mioni *et al.* (2021) recorded for the first time in Italy the heterobranch mollusc *Favorinus ghanensis*, originary from west Africa. It was found at Fezzano (44.080197N; 9.828649E).

Mitrella psilla is a Western African gastropod, occurring from Angola to Mauritania, only recorded in the Mediterranean Sea from the Gulf of Tunis. 213 living specimens were collected (Nappo *et alet al.* 2019) from 1 to 7 meters in depth, in the Civitavecchia harbor, Central Tyrrhenian Sea (42.0880556N; 11.7880555E), on 14 December 2016.

The occurrence of the bivalve mollusc *Isognomon legumen* has been ascertained only recently in the Mediterranean Sea; Giacobbe and Renda (2019) report the first record of this taxon from Italian seas and the westernmost locality in the Mediterranean Sea. Six *I. legumen* specimens from the Strait of Messina (38.259819N; 15.628871E) were collected on 9 September 2019, from a scraping of about 0.25m^2 of vegetated rocky surface dominated by *Lithophyllum* algae at 1m depth.

The well-established Mediterranean alien bivalve *Malleus regula,* native to the Western Indian Ocean, Persian Gulf, and Red Sea, is first reported from Italy, based on field observations carried out since 2016 in Mar Piccolo, a semi-enclosed marine basin in the Gulf of Taranto (Ionian Sea). In particular, during a visual survey in August 2018, several specimens of *M. regula* were noticed on the poles of mussel farms and rocky substrata at about 2m depth in the first inlet of Mar Piccolo (40.484809N; 17.249412E). A single individual was collected and deposited (Prato and Rubino, 2019).

After almost a century of permanence in the Mediterranean, the warm water species *Teredo bartschi* was detected for the first time in the Lagoon of Venice in October 2013 (Tagliapietra *et alet al.* 2021). A thorough analysis of discoveries, synonyms, museum collections and grey literature, has allowed the Authors to estimate that the species entered the Mediterranean through the Suez Canal since at least 1935. It has progressively adapted to colder climates up to overwintering at water temperatures of only a few degrees above zero, which are typical of the Lagoon of Venice. Here, thanks to retrospective analyses, the species has been present for over ten years, and has now become invasive, forming stable and abundant populations.

Crustaceans

The stenothoid amphipod *Parametopella cypris*, known from the US East coast, was recorded with a few specimens from experimental recruitment panels deployed in the Pialassa Baiona, a microtidal lagoon, connected to the port of Ravenna (Northern Adriatic) (Desiderato *et alet al.* 2018). It is the first record of this genus for the Mediterranean.

Tunicates

Polyclinum constellatum is a colonial ascidian with a pantropical distribution, originally described from the Maritius Islands (South Africa), recently introduced into the Eastern Mediterranean Sea (reported along Egyptian and Turkish coasts in 2016 and 2018, respectively). Montesanto *et al.* (2022) report for the first time its presence along the coasts of Greece and Italy (Eastern and Central Mediterranean Sea, respectively), using an integrated approach combining morphological and molecular analysis. Ten colonies of *P. constellatum* were collected from artificial substrata at 1 m depth in the harbour of Taranto, Ionian Sea (40.478966N; 17.225649E) in November 2018.

The aplousobranch ascidian *Aplidium accarense*, first described on the western coast of Africa, has been recently reported along Catalan coasts and in the Tyrrhenian Seas (X. Turon personal communication on data by López-Legentil *et alet al.* 2015 – Lake Fusaro) in harbours and aquaculture farms. Montesanto *et al.* (2021) found several colonies of *A. accarense* in the Mar Piccolo of Taranto (Italy, Ionian Sea). They provide a detailed morphological description of the species and its molecular characterization with an extended COI (Cytochrome Oxidase subunit I) sequence. A detailed comparisons of the specimens found in Taranto with *A. accarense* sampled in other areas of the world is added, in order to highlight the intra-species variability.

Fish

Two specimens of the round goby, *Neogobius melanostomus*, were found in the northernmost branch of the river Po Delta in 2012 (Busatto *et alet al*. 2016), but this sighting had been overlooked until now. No further findings have been reported yet. The two fishes was captured on 8 May 2012 during a sampling campaign using electrofishing in the Po di Levante near Porto Viro (45.0383333N;12.195E), in the Veneto Region.

Two individuals of the smallscale codlet *Bregmaceros nectabanus* were recorded by bottom trawl off Mola di Bari (Southern Adriatic Sea) at 100 m depth on a muddy bottom on 19 December 2019 (Dulčić *et alet al*. 2020). This finding represents the first record from the Adriatic Sea of this Red Sea species, already reported from the Central and Eastern Mediterranean Sea. The present record extends its known distribution northward. Further findings of the species were reported in the southeast Adriatic (Bello, pers. comm.)

The occurrence of *Siganus fuscescens* was documented for the first time in the Mediterranean Sea (Azzurro and Tiralongo, 2020). A single individual was captured on 1 March, 2020, within the harbor of Gioia Tauro, southern Tyrrhenian Sea (38.44428N; 15.90459E).

Cryptogenic species

The study of Guastella *et al*. (2019), besides giving an update of the fora-NIS *Amphistegina lobifera* in the Sicily Channel, also provides new information on the current distribution of other Indo-Pacific foraminiferal species, considered as cryptogenic, namely *Amphistegina lessonii, Amphisorus hemprichii, Coscinospira arietina, Coscinospira hemprichii* and *Sorites orbiculus,* that were also recorded from the same area.

A new species of spionid polychaete, *Streblospio eridani* n. sp. was described by Munari *et al*. (2020) along the Italian coasts of the Northern Adriatic Sea. It was recorded in high density (> 21000 ind. m^{-2}) from the shallow lagoon of the Sacca di Goro (Po River Delta) (44.798253N, 12.343667E) in October 2017 and with lower abundance from the marine soft bottoms of the Lido di Dante (Emilia Romagna) (44.386133N, 12.321567E) between 2016 and 2017. The phylogenetic reconstruction, based on the mitochondrial cytochrome c oxidase subunit I (COI) gene, showed that the Adriatic taxon is clearly distinct from the other species of the genus and forms a well-supported clade with sequence of a *Streblospio* sp. from India. Since there is no evidence that the native origin of this species is actually in the Indian Ocean, we regard this species as cryptogenic, waiting for additional evidence to determine its origin.

A specimen of the Goniodorid nudibranch *Okenia picoensis* was found by Lombardo and Marletta (2021a) in the site of "Acque Fredde" (Santa Tecla, Acireale, SE Sicily), at a depth of 21.9 m on a small rocky wall covered by a turf of red algae, bryozoans, and sponges. An *Okenia* sp. was recorded by Macali in the coastal Lake of Sabaudia (Central Tyrrhenian Sea) in 2011 and was found by molecular analysis to be similar to *O. picoensis* by Paz-Sedano *et al*. (2017). Based on its distribution in Mediterranean Sea and on the opinion of other Authors, the species has been classified as cryptogenic.

The gastropod *Odetta zekiergeni* was found in two harbours (Leghorn – Tuscany - and Olbia – Sardinia) surveyed by Tempesti *et al*. (2022). It was described only recently from the Eastern Mediterranean (Alsancak harbour, Izmir Bay, Aegean Sea) and observed only in port environments, suggesting a case of new introduction, but not knowing its native origin, for the moment it has to be considered cryptogenic.

Previous Sightings

Algae and higher plants

A review by Zingone *et al*. (2021) covers the Mediterranean distribution of marine, toxin-producing microalgae and the cases of toxin-related harmful events, including direct impact on human health or natural resources and indirect impact to the aquaculture industry. In addition, harmful algal blooms (HABs), causing discolorations, anoxia or other damages, and the trends of HABs in Adriatic Sea, which is considered a HAB hotspot, are reviewed. Some of the toxic species have been suspected to be nonindigenous: the main possible NIS in the Mediterranean Sea are *Pseudo-nitzschia multistriata*, *Alexandrium pacificum* and *Ostreopsis* cf. *ovata*.

HABs along the Emilia Romagna coast (from Goro to Cattolica) were reviewed by Pompei *et al*. (2018), for the period 1975-2017, including the identification of species and of the produced toxins (yessotoxins produced by Dynophiceae and others responsible for Diarrhetic Shellfish Poisoning – DSP). The only case of saxitoxin contamination (responsible for Paralytic Shellfish Poisoning – PSP) was recorded in 1994. Alien species were listed in a paper on HAB formations in the Taranto Gulf: *Pseudo-nitzschia multistriata*, *Akashiwo sanguinea* and *Ostreopsis* cf. *ovata* (Caroppo *et alet al*. 2019).

A new record of the microalga *Chrysopheum taylorii* along the coast of Ortona was added in August 2016 (Grechi *et alet al*. 2018), in 3 out of 6 sampled stations. This represents the second location in the Adriatic Sea after the Tremiti Island. Manipulative experiments were carried out at the Tavolara-Punta Coda Cavallo Marine Protected Area (NE Sardinia) to study the interactive effect of mucilage produced by *Chrysopheum taylorii* on encrusting coralline algae (Caronni *et alet al*. 2018) and to investigate the role of the grazer *Cerithium vulgatum* (Mollusca, Gastropoda) during blooms of the invasive microalga (Caronni *et alet al*. 2019).

In the summer of 2015, in a small artificial lagoon in western Sicily (locality Tonnarella, near Mazara del Vallo) a red tide episode was observed (Cangini *et alet al*. 2018), due to a bloom of *Bysmatrum subsalsum* (Dinophyceae). This species is known from tropical and sub-tropical regions and only recently recorded in the Mediterranean Sea. No biotoxins were isolated.

Piazzi *et al*. (2020) compared native and nonnative seaweed community structure after the disturbance to a rocky subtidal macroalgal assemblage in the Mediterranean Sea (Italy) caused by a shipwreck (Costa Concordia). They used traditional abundance measures, in addition to the ALien Biotic IndEX (ALEX), at the disturbed site and at two reference sites. In January 2012, the ship Costa Concordia collided with a submerged natural rocky reef near Giglio Island, west Mediterranean, off Tuscany. In July 2014, the wreck was extracted and the remaining parbuckling structures were removed by spring 2017. In each area, three independent samples were collected at two times (July 2017 and November 2017), in order to obtain species with different seasonal life cycles. Overall, a total of four NIS were found: the Chlorophyta *Caulerpa cylindracea*

and the Rhodophyta *Asparagopsis* spp. (sporophyte stadium), *Acrothamnion preissii* and *Womersleyella setacea*. The nonnative macroalgae *C. cylindracea* and the grouped non-indigenous filamentous species (*W. setacea* and *A. preissii*) contributed to moderate levels of cover at the sites, particularly in comparison to most native taxa. In this study, the decline in NIS cover at the disturbed site to undisturbed levels suggests a quick recovery of the native algal assemblage.

Piazzi *et al*. (2021) studied the role of depth and habitat in the spread of macroalgal NIS. A multifactorial sampling design was employed to compare the abundance of NIS macroalgae over a one-year period across different substrates and depths within Capo Carbonara MPA (South-Eastern Sardinia). The ecological quality of macroalgal assemblages in relation to invasion was assessed through the ALien Biotic IndEX (ALEX). *Caulerpa cylindracea*, *Acrothamnion preissii*, *Womersleyella setacea* and *Falkenbergia* sp. were overall quite low in abundance and, accordingly, the ALEX index had generally high values. These four NIS will not demonstrate further expansion (at the habitat scale) in the MPA: this general result might be due to the specific habitat resistance of the pristine protected area to invasions.

A revision of algal taxa in the Lagoon of Venice (Sfriso *et alet al*. 2018) revealed 31 introduced species: a number that has been growing steadily in time. On the basis of macrophyte distribution assessed over five years, the total NIS standing crop was approximately 147 ktonnes (corresponding to one third of the total algal biomass measured in spring 2014). The most abundant species are *Gracilaria vermiculophylla* (66 ktonnes), *Agardhiella subulata* (37 ktonnes), and *Hypnea flexicaulis* (28 ktonnes). These species grow mainly free-floating and colonise soft substrata. Other two invasive species (*Sargassum muticum* and *Undaria pinnatifida*) grow attached to hard substrata and show a significantly lower biomass. Sfriso *et al*. (2020) observe a positive effect on the environment of the massive growth of *Agarophyton vermiculophillum* (recorded for the first time, as *Gracilaria vermiculophylla,* in May 2008), that replaces Ulvaceae in confined areas, avoiding or reducing rapid biomass collapses and triggering of hypo/an-oxic crises.

Rindi *et al*. (2020) investigated the macroalgal flora of a coastal area subjected to urban influences (Conero Riviera, Adriatic Sea, close to Ancona) comparing the contemporary flora with historical data. Besides the disappearance of 25–30 species, the main change that took place in recent decades in the Riviera del Conero is the introduction of several NIS, many of which are probably coming from the Lagoon of Venice. These are *Aglaothamnion feldmanniae, Antithamnion hubbsii, Codium fragile, Grateloupia turuturu, Hypnea spinella, Lomentaria hakodatensis, Melanothamnus japonicus, Polysiphonia morrowii* and *Sargassum muticum*. In particular, *Melanothamnus japonicus* (recorded for the first time in Venice in 2016) is nowadays one of the most common and widespread seaweed in the area. Yet, due to its cryptic morphology, its presence went unnoticed until recently, when the species was identified using DNA sequence data.

Alien macroalgae present in coralligenous deep habitats of the Marine Protected Area (MPA) Isole Ciclopi, along the Ionian coast of Sicily, were surveyed by Remotely Operated Vehicle (ROV) videos and destructive samples analysed in the laboratory (Costanzo *et alet al*. 2021). Five alien species were identified: *Caulerpa cylindracea*, *Antithamnion amphigeneum*, *Asparagopsis armata*, *Bonnemaisonia hamifera*, and *Lophocladia lallemandii*. Since *A. amphigeneum* was previously reported only in the western Mediterranean and Adriatic Sea, the present report is the first of this species in the central Mediterranean.

Morri *et al*. (2019) tested the assumption that NIS expanding into different habitats reduce the dissimilarity among the recipient communities. They used a simulation experiment, analyzing a comprehensive database (78 species × 229 samples) collected between 2012 and 2017 in the marine protected area of Portofino (NW Italy), where the green alga (*Caulerpa cylindracea*) exhibits high substratum cover at depths between 1 m and 45 m. Apparently *C. cylindracea* did not replace any native species, most of them having already disappeared or got rarer due to climatic and local human impacts during the 1980–90s ecosystem shift at Portofino reefs; the simulation,

however, evidenced that *C. cylindracea* is cause of homogenization in the recipient ecosystem. In particular, the depth gradient was better defined, with a greater multivariate dispersion (considered a measure of β diversity), when the invader was excluded from the analysis.

Mannino *et al*. (2019) updated the current distribution of *Caulerpa taxifolia* var. *distichophylla* in the Mediterranean Sea, based on relevant scientific publications, grey literature and personal observations. The species was found over a wide range of environmental conditions (depth, light and substratum), suggesting a broad ecological plasticity of this alga making it a potential threat for the Mediterranean benthic communities.

Mannino and Balistreri (2019a, 2019b) reported first observations on the effects of *Caulerpa cylindracea* on the communities living along the coasts of the Island of Favignana (Egadi Islands MPA, Western Sicily). According to the study performed in the Northern part of the island, at Cala San Giuseppe (37.935278N; 12.334028E), *C. cylindracea* may have negative effects on the habitat where it settles in two different ways: a) affecting the structure of the native algal community that presents a low diversity, and b) favouring the settlement of other alien species, such as the sabellid polychaete *Branchiomma bairdi*.

A manipulative experiment was conducted (Blasi *et alet al*. 2019) along the Northeastern Sardinian coast in order to investigate the interaction between the native seaweeds *Caulerpa prolifera* and its allochtonus invasive congeneric invasive *Caulerpa taxifolia*. The results suggest that the settlement of *C. taxifolia* on the substratum does not have a significant negative effect on the presence of *C. prolifera*. Balestri *et al*. (2021) found that biotic resistance and propagule pressure co-regulate the invasion success of *C. cylindracea* against the native *Caulerpa prolifera* in a mesocosm experiment. Defence mechanisms against native generalist grazers such as sea urchins were demonstrated by laboratory tests in Sicilian strains of *C. cylindracea* and *C. taxifolia* var. *distichophylla* (Vega Fernández *et alet al*. 2019).

Rizzo *et al*. (2020) investigated the biochemical composition of sediments along with the abundance and composition of meiofaunal assemblages in sediments colonized and not-colonized by the seaweed *Caulerpa cylindracea* under different regimes of sediment deposition. The results show that the presence of the invasive alga *C. cylindracea* could alter quantity, biochemical composition, and nutritional quality of organic detritus and influence the overall functioning of the benthic system, but also that the observed effects could be context-dependent. Sinopoli *et al*. (2020) highlighted that *C. cylindracea* was the only algal species affecting significantly the macrozoobenthos communities in Lampedusa (Pelagie Islands – Sicily).

The role of habitat degradation on the spread of the alien green alga *Caulerpa cylindracea* was reported by Casoli *et al*. (2021), by comparing observations achieved through a multi-year assessment on three Mediterranean habitats, namely *Posidonia oceanica* meadows, *Phyllophora crispa* turf, and coralligenous reefs. Dead matte areas of *P. oceanica* represented the most vulnerable habitat among those analyzed, whereas coralligenous reefs were less susceptible to the invasion both in impacted and healthy habitats.

The red alga *Grateloupia turuturu*, present in Mar Piccolo of Taranto since 2007 and monitored for ten years (2008-2018), has been showing a progressive reduction (Petrocelli *et alet al*. 2020a).

The new species *Hypnea corona* was described by Huisman *et al*. (2021) to accommodate specimens from Australia, New Zealand, Italy, and Japan that were previously included in the *Hypnea cornuta* complex, but were shown by recent molecular studies to represent a species-level clade. The Mediterranean record of *H. corona* is based on specimens from the Mar Piccolo of Taranto, where it has been found since 2000.

Tiberti *et al*. (2021) assessed the extent of the macroalga *Lophocladia lallemandii* in a MPA of the Ischia island (Tyrrhenian Sea), its presence over time, variability in cover, and reproductive output in order to document the cycle of colonization, as well as to describe the associated flora and fauna. The alga is distributed all over the Mediterranean and is known from Ischia since 2009.

A study on the invasive alga *Asparagopsis taxiformis* was performed to understand to what extent invasive seaweeds can modify local biodiversity. Mancuso *et al*. (2021) compared the molluscan assemblage associated with *A. taxiformis* with that of the native *Ericaria brachycarpa* on two shallow rocky shores of the Favignana island (Egadi Islands MPA, Sicily, Italy). They also explored if variation in the molluscan assemblage diversity was related to the substrate attributes (biomass, and thallus, canopy, and interstitial volumes). Results showed that *A. taxiformis* harboured lower diversity and simplertrophic structure of the molluscan assemblage compared to *E. brachycarpa*.

In another paper Mancuso *et al*. (2022) compared the structure of the epifaunal community (amphipods, molluscs and annelids) associated with three plausible alternative states of the transition between the native *E. brachycarpa* and the invasive *A. taxiformis*. In particular, they characterized and compared the biomass and the diversity (richness, evenness, structure and composition) of the epifauna associated with the fronds of homogenous and mixed stands of the algae. The results confirm that the invasive seaweed exhibits a less diverse and abundant epifaunal component compared to the native seaweed, depending on their respective structural complexity. The results also suggest that the presence of *A. taxiformis* affects the epifaunal assemblages associated with *E. brachycarpa* in mixed stands.

The alien seagrass *Halophila stipulacea* has been a well-known Red Sea NIS for 120 years in the Mediterranean. In Italy it was first recorded in 1995 (Vulcano island, Sicily, Acunto *et al.* 1997) and has since then reached its northern limit at Cape Palinuro, Tyrrhenian Sea (some 110 nautical miles northwards). A study of two populations in the Palinuro harbor in 2018/2019 has revealed some ecological adaptations of this species related to the recently warming climatic conditions of the area (Di Genio *et al.* 2021). Pica *et al*. (2021) reported several established and flowering patches of *Halophila stipulacea* in north-east Sardinia (two sites: Razza di Juncu and Golfo Aranci), representing the north-westernmost point of its distribution in the Mediterranean Sea.

Invertebrates

Foraminifera

The benthic foraminiferal species *Amphistegina lobifera*, native to the Red Sea, was reported (Guastella *et al.* 2018; 2019) from the southern coast of Sicily; it was very abundant on the soft bottom sediments and as epiphyte on algae. The sampling period was August-November 2017 in 12 sites (from Marzameni to Capo Passero and Pozzallo; and in the small islands of Pantelleria and Favignana). *Amphistegina lobifera*, which has an invasive behaviour in the Eastern Mediterranean, was already known for Italy from a previous record in the Pelagian islands (Caruso and Cosentino, 2014), but the present findings extend its distribution range in the Central Mediterranean Sea, and update the predicted species distribution models for the years 2040–2050 and 2090–2100, indicating that the sea warming trend will facilitate north-westward migration of amphisteginids.

Coelenterata

The upside-down jellyfish *Cassiopea* sp. is a benthic scyphozoan, considered a non-indigenous invasive species in the Mediterranean, forming large blooms in eutrophic areas. The taxonomy of the genus *Cassiopea* is extremely difficult because morphological/meristic characters used are variable within the same species, overlapping among different species. The first documented record of *Cassiopea* in northern Sicily dates back to 2014 and has since resulted in an abundant population. An analysis of the main morphological characters of the sampled *Cassiopea* jellyfish from Palermo harbour (Tyrrhenian Sea 38°07.22′N 13°22.09′E) was carried out and subsequently, molecular analyses were performed by using CO1 barcode (Maggio *et alet al*. 2019). *Cassiopea* specimens were found to belong to *andromeda* species. Moreover, high values of sequence

divergence were found between Mediterranean *Cassiopea* and the other *C. andromeda* from the Red Sea, Hawaii and Florida. Different hypotheses on the origin of the Palermo population have been discussed. The population established in the marina of Cala (Palermo) was studied for the years 2017–2018 by Cillari *et al*. (2022) through visual sampling, GIS-based statistical and stable isotope analyses. De Rinaldis *et al*. (2021) studied the biochemical composition of *C. andromeda* from specimens collected in 2017 from the same marina.

Polychaeta

Giangrande *et al*. (2018) proposed the exploitation of polychaete filter-feeder biomass derived from integrated multi-trophic aquaculture (IMTA) in various sectors such as sport fishing, ornamental aquarium and feed production. The NIS *Branchiomma boholense* and *B. luctuosum* are reared in the "Maricoltura" plant at Taranto.

Langenek *et al*. (2020) published a review of alien polychaetes in Italy. Their work provided a revised checklist, based on the examination of newly collected and deposited material, on the critical analysis of published and grey literature, and whenever possible on the re-examination of historical material. Of the 86 polychaete species reported as NIS in Italian waters, 25 were confirmed as alien, and three as cryptogenic, while the remaining 58 species were excluded because native, misidentified or still under study.

Mollusca

In the Lesina and Varano coastal lagoons (Apulia, Central Adriatic Sea), during the period 2000-2016, nine species of Bivalve molluscs have been recorded, of which four are NIS: *Arcuatula senhousia, Anadara transversa, A. inaequivalvis, Ruditapes philippinarum* (Scirocco *et alet al*. 2018).

The nudibranch *Polycera hedgepeti* was recorded from the coast of the Salento peninsula, between the Adriatic and the Ionian Sea, during a survey that yielded a total of 160 Heterobranchia species in the area (Furfaro *et alet al*. 2020).

An underwater visual census with scuba diving was carried out at three stations (Santa Tecla, Santa Maria La Scala and Catania - central-eastern coast of Sicily) in 2017–2020 to collect data on seasonality of *Lamprohaminoea ovalis* (Lombardo and Marletta, 2021b).

Crustacea

Vidjak *et al*. (2019) reported several species of NIS zooplankton from North Adriatic port environments, including *Oithona davisae* from Venice port. *Acartia tonsa* was present in Bari, Ancona, Venice and Trieste, *Pseudodiaptomus marinus* in Venice, Trieste and Koper, *Paracartia grani* in Ancona and Bari. The small cyclopoid copepod *Oithona davisae* was also recorded from the brackisk water lagoons of Ganzirri and Faro (Sicily) (Zagami *et alet al*. 2018).

Pansera *et al*. (2021) studied an annual cycle and distribution of *Oithona davisae* in the Lagoon of Venice, considering the coexistence patterns with the congeneric resident *O. nana*. Zooplankton samplings were carried out monthly from August 2016 to July 2017 at five Long-Term Ecological Research LTER stations. *Oithona davisae* showed a persistent occurrence throughout the year with the highest abundances in the warm season and in the inner areas, while the congeneric *O. nana* was more abundant near the inlets of the Lagoon. The coexistence mechanism between the two species was described by Niche Apportionment Models.

The calanoid *Parvocalanus crassirostris* is a free living copepod recently (2014) found in the eastern Adriatic Sea. It was collected in the Italian waters of the Gulf of Trieste during a plankton survey on 9 November 2016 (Goruppi and Tirelli, 2021).

The American amphipod *Stenothoe georgiana* was known from the coasts of Spain, and the western and southern coasts of Italy, plus a single record from Slovenia. Martinez-Laiz *et al*. (2020) analysed additional samples from Italy and Spain and reported the species as occurring in the Lagoon of Venice.

Currently, the Pacific isopod *Paranthura japonica* represents one of the most common species in Mediterranean marinas (Ulman *et alet al*. 2019b): a study on its biology and ecology in a newly invaded site (Marina di Ragusa, Sicily) was performed by Scribano *et al*. (2021), focusing on population dynamics and biological traits, using artificial sponge substrate for site investigation. The population of *P. japonica* was the most abundant of the other NIS, surprisingly overtaking in abundance even *Caprella scaura*, whose exceptional invasive potential is well documented in the Mediterranean and European coasts.

The geographic distribution and population dynamics of *Procambarus clarkii* were studied for the first time in inland waters in the eastern Po Valley and its Delta, an area for which such information was absent Mistri *et al*. (2019). *Procambarus clarkii*, in summer 2017, was present in all sampling sites, 12 geographically distant water bodies representative of an area of 3,000 km^2. Population dynamics was studied on a total of 773 crayfish, 377 males and 396 females from 2017 to 2018, collected during the sampling sessions in Gramicia, a canal running through the town of Ferrara.

The northern brown shrimp *Penaeus aztecus*, first caught in the Tyrrhenian Sea in 2014 (Cruscanti *et alet al*. 2015) and along the Sicilian coasts of Italy in 2015 and 2018 (Scannella *et alet al*. 2017; Pipitone and Lombardo, 2019), further enlarged its distribution along the Adriatic coast (Zava *et alet al*. 2018) and it is now consistently present in the Gulf of Taranto, where it constitutes an economic valuable resource for the local fish market (Ferdinando Rubino pers. comm.). Specimens of *Penaeus aztecus* were sampled from commercial trawlers and trammel nets from various areas of Sicily, Calabria and Apulia (Ionian and South Adriatic Sea) (Donnaloia *et alet al*. 2019), from the Tyrrhenian and Ligurian Sea (Ligas *et alet al*. 2019) and Sardinia (Mulas *et alet al*. 2019).

A flourishing population of the Massawan mantis shrimp, *Erugosquilla massavensis*, an Erythraean species, was recorded (Gianguzza *et alet al*. 2019) off Sicily, Italy, one year after the very first specimen was collected off the eastern coast of the island (Corsini-Foka *et alet al*. 2017). The species is already established as a minor, albeit valuable, fishery resource. Once its population increases, however, it may compete with the native Mediterranean spot-tail mantis shrimp, *Squilla mantis*.

The first record of *Palaemon macrodactylus* in the Lagoon of Venice occurred in 2012, between 2014 and 2020, Redolfi Bristol *et al*. (2021) collected more than 5000 specimens from stations along the inner edge of the Lagoon.

Large new information was gathered on the blue crab *Callinectes sapidus*, whose native distribution in the western Atlantic extends from Nova Scotia to Argentina. Introduced to Europe at the beginning of the 20th century, it is currently recorded almost ubiquitously in the Mediterranean and in the Black Sea. The capture of a male specimen of blue crab in the coastal waters of Matzaccara, Sardinia, 39°11′N; 8°43′E, was reported with morphometric data by Piras *et al*. (2019). The ongoing expansion of *C. sapidus* in marine, transitional and freshwater sites in Sardinia in the period 2017–2018 (adding this species to the seven non-indigenous decapod species reported from the island in the last decades) was provided by Culurgioni *et al*. (2020). An overview of the occurrence, abundance, and ecological impact of *C. sapidus* in southern European waters had been given by Mancinelli *et al*. (2017a,b); additionally, they present a pragmatic assessment of its management scenarios, explicitly considering the dual nature of *C. sapidus* as both an invasive species and a fishery resource. The trophic role and feeding flexibility in invaded benthic food webs have been addressed by Mancinelli *et al*. (2017c): they conducted field samplings in winter and summer in five coastal systems of the Apulia region (SE Italy), three located on the Ionian Sea (Mar Piccolo, Torre Colimena, and Spunderati) and two on the Adriatic Sea (Acquatina and Alimini Grande). Captured blue crabs were weighed and had their $d^{13}C$ and $d^{15}N$ isotopic signatures measured; their trophic level (TL) was estimated using the mussel *Mytilus galloprovincialis* as isotopic baseline. Additional records of the blue crab *Callinectes sapidus* were reported in the Strait of Sicily (Falsone *et alet al*. 2020), from an unusually deep locality off the small Island of Favignana (Aeolian archipelago, Sicily) (Sercia and Innocenti 2020), in two localities of the

Latium coasts nearby Latina by recreational fishermen with fishline, including a female bringing eggs (Martina Gaglioti, pers. comm.), and by trawling off Termoli (Molise Central Adriatic Sea) in February 2020 (Ludovica Di Rienzo, pers. comm), thus testifying that the species is perfectly acclimated and rapidly enlarging its distribution along the Italian coasts. Two post-spawning females of *C. sapidus* were described at Lido di Pomposa, in Northern Adriatic coastal waters (Munari and Mistri, 2019). Tiralongo *et al*. (2021a) studied *C. sapidus,* by fishing campaigns carried out in July–October 2020 in Latium and Campania (central Tyrrhenian Sea). They also summarize previous records from Italy. Di Muri *et al*. (2022) assembled a dataset of available georeferenced and individual-based isotopic values ($\delta^{13}C$ and $\delta^{15}N$) of *C. sapidus* and its potential animal prey in Mediterranean waters, with the aim of understanding how the trophic ecology of the blue crab shapes benthic food webs in invaded ecosystems, a crucial step for an accurate assessment of its impact. The geographic scope of the data span from westernmost records located in Spain, the northernmost in Croatia, the easternmost in Turkey, and the southernmost in Greece. The majority of records lie in Italy.

The Indo-Pacific blue swimming crab, *Portunus segnis,* entered the Mediterranean Sea a few decades ago through the Suez Canal, and more recently (2014) reached Tunisia, where it started to increase enormously in number (Hamida *et alet al*. 2019). *Portunus segnis* was recorded in 2019 in the Island of Lampedusa. An alert communicate was issued by ISPRA, the Italian Institute for the Protection of the Environment (http://www.isprambiente.gov.it/it/evidenza/ispra/no-homepage/il-granchio-blu-del-mar-rosso-arriva-a-lampedusa).

The mud crab *Dyspanopeus sayi*, native to the western Atlantic, was recorded for the first time in a Sardinian lagoon. The first three specimens of this NIS crab were collected in the central area of the Santa Gilla (Cagliari) lagoon in December 2013. Occurrence of the species was also recorded in December 2018 (102 specimens). Its presence in the Santa Gilla lagoon might be related to the import of mussels for aquaculture purposes (Cabiddu *et alet al*. 2020).

The Pycnogonid *Ammothea hilgendorfi* was recorded for the first time in Venice in 1983; since then, the presence of this species has no longer been reported neither in the Venice lagoon nor in other sites of the Mediterranean Sea. However, this species is actually present and quite widespread in the Venice lagoon, as indicated by a number of individuals caught between 1991 and 2017, totaling 29 specimens (Mizzan, 2018).

Tunicata

Colonies of the non-indigenous colonial ascidian *Symplegma brakenhielmi* were collected (Mastrototaro *et alet al*. 2019) in 2014 and 2018, along the North-eastern Sardinia coasts (Olbia). Further colonies were observed in 2016 in the Mar Piccolo basin (Gulf of Taranto). Synergistic analyses of morphological and molecular type are provided.

During surveys performed in 2018, 2020 and 2021 on artificial substrata within the semi-enclosed basin of the Mar Piccolo of Taranto (Italy, Ionian Sea), Montesanto *et al*. (2021) besides finding several colonies of the ascidian *Aplidium accarense*, a new sighting for Italy, noted that the sampled substrates were almost totally covered by other NIS, such as *Polyandrocarpa zorritensis* and *Distaplia bermudensis*. *Distaplia bermudensis*, a colonial ascidian originally described from Bermuda, was first reported in the Mediterranean Sea within the Gulf of Taranto in 2000: a new finding of this species in the Tyrrhenian Sea, collected from rocky substrata within the brackish Miseno Lake (Gulf of Naples, Tyrrhenian Sea), was reported by Montesanto and Mastrototaro (2021) in September 2020.

Chimerism occurs frequently in *Didemnum vexillum:* in colonial species, natural chimerism implies the presence of zooids with different genotypes within the same colony. A colony fusion experiment was carried out at the Venetian Lagoon, where *D. vexillum* is widely distributed (Casso *et alet al*. 2019). Manipulation was done in the laboratory and 31% of intercolony pairs fused. The Authors concluded that chimerism and colony fusion may be important factors to enhance genetic diversity and promote successful expansion of *D. vexillum*.

Various invertebrates

The SW Atlantic sponge *Paraleucilla magna*, the Red Sea polychaete *Branchiomma luctuosum*, and the presence of the range-expanding, amphi-American and amphi-Atlantic crab *Percnon gibbesi* were confirmed in the area of the Gulf of Genoa (Bianchi *et alet al*. 2018).

Mancini *et al*. (2021) recorded the presence of six NIS associated with marine litter, through demersal trawl nets in the coastal sector of Civitavecchia (northern Tyrrhenian sea) at 50–120 m of depth. The species found were the polychaete serpulids *Hydroides elegans* and *H. dirampha*, the bivalve mollusc *Magallana gigas* and the bryozoan *Celleporaria brunnea*. In particular, two bryozoan species, previously unreported in Italian waters, *Conopeum reticulum* and *Alcyonidium* cfr. *gelatinosum* were recorded.

Fish

A single adult specimen of *Synagrops japonicus* was collected by trawling during a scientific campaign off the Tuscany coast (Northern Tyrrhenian Sea) (Serena *et alet al*. 2020). This finding confirms, after about 28 years the presence of *S. japonicus* recorded for the first time in the same portion of the sea of the north-western Mediterranean (Orsi Relini, 1990).

Abudefduf saxatilis is an Atlantic representative of the Pomacentridae family; in the Western Mediterranean Basin, the species was spotted only twice; a single individual of the non-native damselfish was observed by Alvito and Grech (2021) at a depth of 18 m, in the Gulf of Cagliari (Sardinia).

Notes on pseudo-indigenous species

Several taxonomically challenging invertebrate taxa that were known to occur in Italy since a long time (but not recognised as introduced) were submitted to taxonomic analyses, using morphological and/or genetic approaches, and their identity and introduced status was clarified. Therefore, several new introduced species, which were already present but overlooked (pseudo-indigenous species) have to be added to the Italian list of NIS.

The amphipod *Ericthonius didymus* was described as a new native species in 2013 from the Venice lagoon (Krapp-Schickel, 2013). The species was subsequently found in Port Camargue, France (Ulman *et alet al*. 2017, reported as *E.* cf. *pugnax*), in the port of Sao Miguel, Azores, Portugal and in Arcachon Bay, France (Gouilleux *et alet al*. 2020). Specimens from all these localities were compared with material of two indo-pacific species (*Erichtonius pugnax*, *E. convexus*) from Japan and of the cosmopolitan *E. brasiliensis* from Sicily, using an integrative taxonomic approach (morphological + genetic). Results indicate that *E. didymus* is a valid species, but its genetic similarity to the Pacific congeneric species suggests a non-european native origin. Furthermore, European findings in sites of oyster farming and/or recreational boating suggest a human-mediated introduction.

The amphipod *Jassa marmorata* was originally described from the Atlantic coasts of USA and is common along the European Atlantic, Mediterranean (for Italy see Scinto *et alet al*. 2007) and Black Sea coasts, where it had long been perceived as a native species. Analyses of CO1 sequences of *J. marmorata* specimens from various world regions strongly suggest a native origin in the NW-Atlantic, and human-mediated introductions elsewhere, via shipping.

The congeneric species *Jassa slatteryi* was first reported from the Tyrrhenian coast of Italy by Bonifazi *et al*. (2018) as a cryptogenic species. CO1 sequences indicate an introduction history for *J. slatteryi* in the Mediterranean Sea, although the native origin is less clear, being likely located in the Northern Pacific Ocean (which side is not known; Beermann *et alet al*. 2020). Given the very high morphological similarity between juveniles of *J. marmorata* and *J. slatteryi*, it is possible that the latter is more widespread than currently assumed.

The ascidian *Botrylloides pizoni* was described as a new species from Taranto, Ionian Sea, by Brunetti and Mastrototaro (2012) and mentioned in the 2013 Italian National Report as a case of

uncertain origin. Genetic analyses (Rocha *et al*., 2019) revealed that it is the same species of *Botrylloides giganteus*, known from Senegal, South Africa, Mozambique, Brazil, California, Galapagos and New Zealand. Therefore *B. pizoni* is considered a junior synonym of *B. giganteus*. We now consider this species as introduced to Italy, due to the remarkably small genetic variation within mithochondrial genome, combined with the finding in a hotspot of introductions (Taranto).

The ascidian *Botryllus schlosseri*, commonly occurring along European coasts, was recognised as a species complex, including distinct clades with possibly different biogeographic origins (Reem *et alet al*. 2017). Brunetti *et al*. (2020) analysed in particular 'clade E' of *B. schlosseri* and identified a new species, *B. gaiae*, reporting it from Venice Lagoon (N-Adriatic Sea), Barletta (S. Adriatic), Taranto (Ionian Sea) and Carrara (North Tyrrhenian Sea). According to Nydam *et al*. (2017), the clade E (= *B. gaiae*) has its origin from the Southern English Channel. The findings in introduction hotspots such as Venice and Taranto support the scenario of human-mediated introduction. Clade A of *B. schlosseri*, the most common clade in Europe, is instead to be considered cryptogenic. There is evidence from genetic analyses that Clade A originated in the Pacific, but it has also had a long evolutionary history in the Mediterranean and has colonized the English Channel and the Bay of Biscay several times. The *B. schlosseri* species complex either originated in the Pacific Ocean and diversified after Clade A arrived in European waters, or originated in Europe, with ancestors of Clade A dispersing to the Pacific Ocean (Nydam *et alet al*. 2017).

Botryllus and *Botrylloides* colonial ascidians are widely studied for their potential invasiveness, however the morphological description and discrimination of these species is very problematic, leading to frequent specimen misidentifications. The difficulties of morphological species description and identification of Botryllinae highlighted the need for new discriminant characters to complement the classical morphological ones. To facilitate species discrimination and detection of cryptic/new species, Salonna *et al*. (2021) developed new barcoding primers for the amplification of a COI fragment of about 860 bp (860-COI), which is an extension of the common Folmer's barcode region. They considered 177 worldwide-sampled botryllid colonies. Combined with morphological analyses, 860-COI allowed not only discriminating known species, but also identifying undescribed and cryptic species, resurrecting old species currently in synonymy, and proposing the assignment of clade D of the model organism *Botryllus schlosseri* to *Botryllus renierii*.

Notes on Range expanding species

Evidence of predation of the crab *Percnon gibbesi*, an Atlantic range expanding species, by the rock goby *Gobius paganellus* was found by Tiralongo *et al*. (2021b). The diet of the native *G. paganellus* was studied analyzing the stomach content of 162 specimens collected in the Ionian Sea (south-east coast of Sicily, central Mediterranean Sea), along 2 km of the coastline between the localities of Avola and Noto.

Not seen species yet

Ferrario *et al*. (2019a) reported on fouling species in the harbor of Piran (Slovenia); among others, they found two NIS, whose presence has been firstly reported in the Mediterranean (including the Italian Tyrrhenian coast) only very recently: *Stenothoe georgiana* and *Watersipora arcuata*. The record in the Northern Adriatic Sea notably extends their colonization range and suggests these species may further spread elsewhere in the Adriatic Sea.

The alien gastropod *Biuve fulvipunctata* was reported for the first time from Croatia, also representing the first record for the Adriatic Sea (Petani and Crocetta, 2019). The species had been recorded only once in Sicily (under the name of *Chelidonura fulvipunctata,* Malaquias *et alet al*. 2016) but never before along the Italian coasts of the Adriatic Sea.

In Italian waters 27 out of 98 Mediterranean alien Ctenophores and Cnidarian are present. Fifteen other NIS belonging to these taxa, some of them with high invasive potential, should be

regarded as good candidates to become future immigrants of the Italian waters according to a Horizon scanning exercise by Gravili and Rossi (2021). *Macrorhynchia philippina*, *Oulastrea crispata*, *Eucheilota paradoxica* are the three species that deserve most attention.

In a study by Stasolla *et al*. (2021), 20 species of non-native marine crustacean decapods and barnacles, of which eleven extant and nine 'horizon' were screened for their potential invasiveness in the Mediterranean Sea. The potential invasiveness was assessed by theAquatic Species Invasiveness Screening Kit (AS-ISK). The highest risk species not yet recorded in Italy are *Charybdis (Goniohellenus) longicollis, Hemigrapsus sanguineus, Gonioinfradens paucidentatus.*

4. Pathogens

Mass mortality events across the Mediterranean Sea of the iconic bivalve species *Pinna nobilis* have been attributed to the protozoan *Haplosporidium pinnae* (Cercozoa: Haplosporidiidae). It was firstly detected (and described as a new species) in infected specimens from the Balearic Sea, where a recent arrival of this species was suggested by the scarce variability detected in the SSU rDNA sequence (Catanese *et alet al*. 2018). Haplosporidan parasites were known to infect species of bivalves, gastropods, crustacean, worms, ascidians, but were never reported from members of the Pinnoidea Superfamily. In the early summer of 2018, a mass mortality event (MME) of *P. nobilis* was recorded in the Gulf of Taranto (Southern Italy, Ionian Sea), for which *H. pinnae* was again identified as the causative agent (Panarese *et alet al*. 2019). The authors hypothesize that anthropic activity, such as maritime transport, ballast waters and trade of living bivalves may have enhanced the dispersal of the protozoan. Afterwards, other mass mortality events were recorded from Greece, Turkey, Croatia, and *Halosporidium pinnae* was always found to be the causative agent (Özalp *et alet al*. 2020; Čižmek *et alet al*. 2020). On the other hand, mortality of *P. nobilis* observed in the western coasts of Italy were not related to the haplosporidian parasite, but to a mycobacteriosis (Carella *et alet al*. 2019). Further research is urgently needed on the modality of transmission, distribution and source of these pathogens.

5. Research and Monitoring Programs

*Some **reviews and studies** have been published by various Italian Authors on different aspects of introduced species and biological invasions, focusing on the Italian experience:*

A literature survey was performed (Cardeccia *et alet al*. 2018) in order to check the occurrence of marine and brackish-water **non-indigenous species in each Italian region**. The updated (2017) total count is of 613 regional records, referred to 205 NIS.

A collaborative paper (Giakoumi *et alet al*. 2019) was prepared to guide the evaluation of **management options** for marine invaders at an early stage of invasion when reducing managers' response time is crucial. It could also guide decision-making in subsequent invasion stages, without requiring detailed species-specific information. Expert knowledge was elicited to prioritize 11 management actions for controlling 12 model species, distinguished by differences in dispersion capacity, distribution in the area to be managed, and taxonomic identity. Unlike previous studies, the aim was not to prioritize the invasive species for which management should be applied but to prioritize management actions for groups of invasive species that share similar traits.

A re-examination of marine non-indigenous species (NIS) reported in Italian Seas until December 2018, was compiled (Servello *et alet al*. 2019) in order to comply with the requirements of **Descriptor D2 of the Marine Strategy Framework Directive**, focusing on establishment success, year of first record, origin, potential invasiveness, and likely pathways, in particular.

A review of the **seaweed distribution** along the Italian seas included an update on the presence and abundance of NIS (Petrocelli and Cecere, 2019). Analysis of the published records in scientific literature concerning non-indigenous macrophytes in ports, marinas and transitional waters have been published by Orlando-Bonaca *et al*. (2019) for the Adriatic Sea, and for the

Central Mediterranean (Orlando-Bonaca *et alet al*. 2021), covering 7 Adriatic and 11 Ionian Sea Italian sites.

Tempesti *et al*. (2020b) produced a literature review as a knowledge baseline on NIS in **Mediterranean ports**. NIS distribution in Mediterranean ports showed a high degree of heterogeneity in terms of studies across the whole basin, with a limited knowledge on both specific taxa and geographical areas, as well as a generally low proportion of investigated ports.

Occhipinti-Ambrogi (2021) reviewed **biopollution** literature on ecosystem deterioration induced by non-indigenous species in coastal sites, the assessment procedures proposed, and risk assessment and management strategies, comparing them with the experience gained for chemical pollution. Mediterranean Sea case studies are presented and management measures besides those already established in international treaties and national legislations, are advocated to prevent the disruption of the dynamic ecological equilibria in the receiving environment and to control the direct adverse effects of alien species.

Slišković *et al*. (2021) provided an updated list of NIS introduced into Adriatic Sea by **shipping** as pathway, the most likely vectors of introduction, country of first detection, year, origin, establishing success and invasive characteristics. In total, 127 NIS were identified as the most probably introduced by maritime shipping into the Adriatic Sea. Rhodophyta, Arthropoda and Annelida represent almost 50% of all the listed species. The highest number of NIS introduced likely by shipping was identified for the period from 2001 to 2010. More than 73% of NIS listed for the first time in the Adriatic Sea were recorded in Italy. Half of listed NIS (50%) were probably introduced by boat hull fouling.

Gentilucci *et al*. (2021) aimed to assess whether there are any relationships between temperature variations and changes in the marine fauna. Specimens were collected from 2005 to 2019 every year from mid-April to mid-June in the Gaeta Gulf (Tyrrhenian Sea), photographed, identified and analyzed. All samples were barcoded using DNA isolation and PCR amplification and sequencing and then aligned and compared with GenBank sequences. Under an SST increase of 0.8 °C in 24 years, three NIS of fish were recorded (since 2009): *Fistularia commersonii*, *Arothron diadematus* and *Sphoeroides pachygaster* (sporadic, but interesting due to its tetradotoxin in the filet).

Based on data from current literature, Haubrock *et al*. (2021) synthesised and described, for the first time, the costs of invasions on the Italian economy.

*Some **monitoring surveys** were performed, and some are underway, to continue observations on marine NIS in Italy:*

In the framework of the **BALMAS** (Ballast Water Management System for Adriatic Sea Protection) project, carried out jointly by Italian, Slovenian and Croatian teams, the results of the zooplankton surveys have been published by Vidjak *et al*. (2019).

In the **Lagoon of Venice**, the Natural History Museum surveys the alien species benthic populations, reporting interesting observations on their fluctuations (L. Mizzan, personal communication): among crabs, *Callinectes sapidus* is caught regularly but no juveniles or small individuals are reported; *Dyspanopeus sayi* is similarly stable, with moderate densities in all areas of the Lagoon, whereas *Rhithropanopeus harrisii* is rare in a few areas of low salinity in Northern part of the Lagoon. *Paracerceis sculpta* and *Xenostrobus* cfr. *securis* are well established. Within the phylum Mollusca, *Arcuatula senhousia* is less abundant but still present. *Bursatella leachii* is present only during the summer; while less frequent are *Rapana venosa* and *Anadara transversa* (*Anadara inaequivalvis* has almost disappeared). The assessment of the taxonomic and biogeographic status of the ascidian *Clavelina oblonga* or *flegrea*, invasive since a few years ago, is still under study. The ctenophore *Mnemiopsis leidyi* is a stable component of macrozooplankton in the Lagoon and gives

rise to large summer blooms: interestingly, in winter the specimens are double in size compared to summer ones.

The presence of NIS has been updated also in the **Sacca di Goro**, an enclosed bay in the Po River delta (Infantini *et alet al*. 2018). During 3 sampling campaigns (January 2015, June and October 2017) 16 NIS have been recorded out of a total of 93 taxa. Four species have been found in all of the three sampling occasions: *Arcuatula senhousia, Ruditapes philippinarum, Ficopomatus enigmaticus, Grandidierella japonica*.

A comprehensive compilation of recent data on the **Genoa Gulf** was complemented by regular monitoring by snorkelling at two sites near Genoa city (Lido and Quarto) between 2009 and 2015: a total of 20 southern species (11 NIS and 9 Warm Water Natives) were found (Bianchi *et alet al*. 2018). The monitoring exercise with a standardized protocol for 7 years in Genoa allowed inventorying 18 southern species, 8 Warm Water Native and 10 NIS, in relation with the recorded temperature data. NIS were found on artificial substrates and/or in degraded habitats, confirming their greater susceptibility to invasion. Global warming is facilitating the poleward range expansion of plant and animal species. In the Mediterranean Sea, the concurrent temperature increase and abundance of (sub)tropical NIS is leading to the so-called 'tropicalization', which is dramatically evident in the south-eastern sectors of the basin. At the same time, the colder northwestern sectors of the basin have been said to undergo a process of 'meridionalization', that is the establishment of warm-water native species previously restricted to the southern sectors.

In the framework of a project funded by the **Tuscany** Region (Tyrrhenian Sea), Serena *et al*. (2016) compiled a summary of all nonnative species (alien, cryptogenic and range extending species) that have been recorded along the coast.

Macrozoobenthic fouling assemblages were studied (Tempesti *et alet al*. 2020a) in the **port area of Leghorn**, focusing on the occurrence of non-indigenous species (NIS). Sampling was carried out at ten sites characterised by different anthropic impacts related to their use destination. Among the 262 species identified, 26 were alien or cryptogenic, 17 of which were new records for the study area, confirming the role of the port of Leghorn as a hotspot of NIS introduction. The distribution of NIS showed a clear segregation depending on sampling sites; however, some species were more widespread than others. Even though the majority of transoceanic maritime traffic from and to Leghorn pertains to the commercial harbour, the touristic harbour hosted the highest number of NIS, possibly because of secondary spread from other Mediterranean ports. The ALien Biotic IndEX (ALEX) identified all sites as high or good environmental status, but the large number of NIS detected suggests caution about their impact and further spread. Fouling communities were studied by Tempesti *et al*. (2022) in **three port systems** of Northern Tyrrhenian Sea (Livorno - Tuscany, Olbia - Sardinia – and Bastia - Corsica), for each port system two harbour types (large port and recreational marina nearby) were sampled. Among the 431 taxa identified, 42 were alien or cryptogenic, 4 of which were new records for the study area. High fouling variability was detected within port environments and between different marinas. NIS showed the highest occurrence in large ports, in which the touristic harbour generally hosted the greatest amount. Widely distributed species in the Mediterranean, such as *Hydroides dirampha*, *Paranthura japonica, Watersipora subtorquata* and *Styela plicata* were the most widespread in the study area. Four NIS that represent first records for the study area were recorded, namely the pycnogonid *Ammothea hilgendorfi* (Livorno and Bastia), the gastropod *Odetta zekiergeni* (Livorno and Olbia), the polychaetes *Lumbrineris* cf. *perkinsi* (Bastia) and *Syllis hyllebergi* (Macinaggio, near Bastia).

Gambi *et al*. (2019) updated the list of NIS from the **island of Ischia** (off Naples, Tyrrhenian Sea) presenting seven additional alien species, together with a taxonomic emendation (*Branchiomma boholensis* instead of *B. bairdi*). Among the seven species, the red alga *Lophocladia lallemandi,* first recorded in 2009 showed massive coverage, densely populating the Northern part of the Island.

The phytobenthic assemblages of the Mar Piccolo of **Taranto** (Ionian Sea), a lagoon like semi-enclosed coastal basin, have been analysed (Petrocelli *et alet al*. 2019), thanks to the availability of quantitative long-term datasets describing changes occurred in the structure of the community over about thirty years. The total number of taxa and the dominant taxa differed over the years. Thirteen non-indigenous species in total were found, their number varied over the years, reaching its highest value in 2017. By surveying artificial substrates, both static and floating, in an inner area of the Mar Grande di Taranto, Giangrande *et al*. (2021) found 10 NIS out of 82 fouling species. NIS were not different from those found in a previous (2014) study in the same area with experimental panels, but showed differences in their colonization according with the nature of the substratum. Factors affecting the establishment of introduced species could be compared, such as exposure time, organic load, dominance of native invertebrates or algae. In the framework of EU structural funds, the Project "M.I.A. RETE NATURA 2000 - Monitoraggi Innovativi Ambientali" has been established, allowing to continue the long-term seasonal monitoring of marine fanerogams and alien species in the Mar Piccolo of Taranto (A. Petrocelli personal comm.).

Mannino and Balistreri (2019b, 2021) analysed eight Mediterranean **MPAs**, located in **Sicily** and its surrounding smaller islands - a strategic geographic position within the Mediterranean basin. All the analysed MPAs were affected by the presence of Invasive Alien Species, showing evidence of the vulnerability of MPAs to biological invasions. Valuable habitats such as vermetid reef and coralligenous formations have been impacted by alien species.

The results of a large-scale study on **recreational boating** as vector of spreading in the Mediterranean Sea were published in three distinct papers (Martinez-Laiz *et alet al*. 2019; Ulman *et alet al*. 2019a,b). The work involved the analysis of biofouling in 50 Mediterranean marinas spanning seven countries from Spain to Turkey, and from about 600 boat hulls, also interviewing their owners. The surveyed marinas had between 2 and 27 NIS each, hence their role as 'hotspots of introduction' is comparable to the role of larger commercial harbours. NIS richness was related to sea surface temperature, number of berths, proximity to Suez Canal, aquaculture sites or commercial harbours, absence of pontoons, biogeographic sector and climate type (Ulman *et alet al*. 2019a). Interestingly, 71% of sampled hulls, including those that had recently been cleaned professionally, hosted from 1 to 11 NIS. Boats with high NIS richness strongly correlated to home marinas with high NIS richness. The surveyed boaters travelled considerably (on average, 67 travel days per year and 7.5 visited marinas per year), showing high potential for spreading NIS (Ulman *et alet al*. 2019b). The levels of awareness of Mediterranean boaters regarding the issue of marine bioinvasions was often low (Martinez-Laiz *et alet al*. 2019). The most recorded NIS were the serpulid polychaete *Hydroides elegans*, the bryozoan *Amathia verticillata* and the caprellid amphipod *Caprella scaura*. Cryptogenic species were also frequent, such as the bryozoan *Bugula neritina,* and the amphipod *Monocorophium acherusicum* (Ferrario *et alet al*. 2019b).

A **standardized protocol** developed by the Smithsonian Environmental Research Center (SERC) was followed by Tamburini *et al*. (2019; 2021). A three-year monitoring survey (2018–2020) was conducted in the Gulf of La Spezia (Ligurian Sea), with the deployment of a total of 50 PVC panels per year in five different sites (a commercial harbor, three marinas and a site in the proximity of a shellfish farm). A total of 79 taxa were identified, including 11 NIS, ranging from zero to seven NIS for each panel. In comparison with previous surveys, new NIS arrivals were observed in the Gulf of La Spezia: *Botrylloides* cf. *niger*, *Branchiomma* sp., *Branchiomma luctuosum*, *Paraleucilla magna*, and *Watersipora arcuata*. Higher values of mean richness and percent cover of NIS were measured in two marinas and in the commercial harbor. Among years, richness of NIS was relatively stable at each monitoring site. The structure of the fouling was influenced more by native and cryptogenic species than by NIS. The application of the SERC method across the Mediterranean is recommended.

Citizen science initiatives, involving local population and operators have contributed to a wider knowledge of the distribution of NIS:

Italy, at the center of the Mediterranean Sea, hosts a high diversity of fishes, but to a certain extent, this richness remains hidden or poorly known because of rare, cryptic or recently introduced species, that are hardly to detect with the traditional sampling approaches. The **local ecological knowledge** (LEK) of small-scale and recreational fishers was accessed in order to reconstruct the dynamics of fish perceived as "new" or increasing in different fishing areas of the Mediterranean (Azzurro *et alet al*. 2019). Over 500 fishers across 95 locations and nine different countries were interviewed, and semiquantitative information on yearly changes in species abundance was collected. Overall, 75 species were mentioned by the respondents, mostly warm-adapted species of both native and exotic origin. Interviews were realized between 2009 and 2016 by local researchers in local languages (Albanian, Arabic, Croatian, Greek, Italian, Montenegrin, and Turkish). The LEK protocol is currently applied in other Mediterranean countries, such as Libya, Spain, and France and adopted by five Mediterranean marine protected areas generating new data.

Results obtained in 2018 from the **AlienFish project**– monitoring and study of rare and non-indigenous fish species in Italian waters collected through citizen science – were reported by Tiralongo *et al*. (2019). Overall, 36 observations of 21 fish species belonging to 17 families were provided from the central and southern sectors of Italian seas. Nonindigenous fishes were sighted in 5 locations out of 18 sites: *Fistularia commersonii* (in Sabaudia), *Lagocephalus sceleratus* (in Tropea), *Siganus luridus* (in Catania and Avola) and *Stephanolepis diaspros* (in Avola) were reported. In particular, *S. diaspros* and *S. luridus* were recorded for the first time along the Ionian coast of Sicily. A targeted study within the AlienFish project on *Fistularia commersonii* reported it as established along the southeastern coast of Sicily, Ionian Sea (Tiralongo *et alet al*. 2018). Tiralongo *et al*. (2020) gained complementary knowledge, engaging Italian sea users, especially fishers and underwater photographers, to share their observations. Results obtained during 2019 provided 124 new records distributed in 40 species. Few observations (~5%), however, concern non-indigenous taxa. Maggio *et al*. (2022) described a NIS monitoring exercise involving citizens, through Local Ecological Knowledge (LEK) data collection in the Pelagie Islands Marine Protected Area (Sicily) interviewing fishers and dive masters, particularly focusing on the blue crab *Portunus segnis* and the subsequent sampling and validation of the species occurrence. Other easily recognizable species reported species were *Fistularia commersonii*, *Siganus rivulatus* and *Percnon gibbesi.*

Citizen science observations collected through the app **avvistAPP**, a smartphone application, were also active since 2019, starting from the Gulf of Trieste, but gradually extending to various parts of the Italian coastline. In summer 2019, *Mnemiopsis leidyi* was observed and reported by avvistAPP along the Apulian coast (southern Adriatic), in several localities (Tirelli and Goruppi 2019). Overall 530 sightings of the invasive ctenophore were sent through avvistAPP together with marine jellyfish and large vertebrates in the following years (Tirelli *et alet al*. 2021). The comb jellyfish, *M. leidyi*, is distributed all along the Adriatic coast, from the Lagoon of Venice, where is a stable component of the zooplankton, to Bari. Costa Crociere Foundation has partnered with OLPA (Osservatorio Ligure Pesca e Ambiente), to run the project of citizen science "Guardiani della Costa", for schools, comprising alien species detection (Bernat *et alet al*. 2019).

A citizen science campaign in the harbor of Palermo (Sicily) was carried out in May 2017 (Lo Brutto *et alet al*. 2018) to collect Amphipods from fouling assemblages. Among the 325 Amphipod specimens, belonging to 5 species, *Elasmopus rapax*, *Erichtonius brasiliensis* and *Monocorophium acherusicum* are considered cryptogenic while *Caprella scaura* and *Stenothoe georgiana* are NIS. Azzurro and Cerri (2021) tested a new participatory method to map the distribution of three invasive species (*Callinectes sapidus*, *Procambarus clarkii* and *Oreochromis niloticus*) in the coastal lagoon of Lesina (Southern Adriatic). Supporting the citizen science activities in the Marine protected

area of Tavolara-Punta Coda Cavallo (Olbia, Sardinia), Ponti *et al*. (2021) have maintained a page in the web portal ReefScience where alien species observations are regularly annotated, with particular reference to *Caulerpa cylindracea*, *C. taxifolia* and *Rapana venosa* distribution.

A fund raising campaign organized by the University of Pavia, entitled "A brick against alien species", has been continued through dissemination events that have raised the awareness and promoted knowledge about non-indigenous marine species among the general public (Ferrario *et alet al*. 2021).

6. Meetings

The Joint Research Center of the European Commission (Ispra, Varese) organised in October 2018 a horizon scanning workshop for marine IAS, attended by several experts from Europe and neighbouring countries, who contributed to identify "top priority" species to be considered for inclusion in the EU Regulation on IAS (1143/2014). The exercise resulted in the identification of 26 top-priority species (Tsiamis *et al.*, 2020).

Data on the spread of marine alien and cryptogenic species after the first record are often overlooked and dispersed, and tracking the history of invasions is more and more cumbersome, especially in the Mediterranean Sea. A collaborative initiative to collect and harmonize unpublished available data after the first sighting was launched by an international team, including many Italian specialists. A large dataset comprising 5376 records was created. It includes records of 239 alien or cryptogenic taxa (192 Animalia, 24 Plantae, 23 Chromista) from 19 countries surrounding the Mediterranean Sea (Katsanevakis *et alet al*. 2020).

The University of Pavia, in collaboration with the Smithsonian Environmental Research Center (SERC, USA) organised in September 2019 and July 2021 two editions of a summer school entitled 'Monitoring marine alien species in ports with the SERC protocol'. This summer school, attended by professionals, public/private employees, researchers, master/PhD students, instructed to monitor ports in the Mediterranean Sea, using the method conceived and successfully applied for over 25 years in the United States by the SERC. Moreover, experts of different taxonomic groups provided lectures and laboratory activities on the identification of the most common fouling species of port habitats, with a focus on cryptogenic and alien invertebrate species.

In an abstract of the 1st DNAQUA International Conference Pinna *et al*. (2021) announced a study about the use of molecular tools (DNA barcoding and metabarcoding), combined with environmental DNA (eDNA) extracted from environmental samples like water or sediment, evaluating the gaps of this innovative approach for the identification of species and biodiversity in the Mediterranean.

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It has been prepared according with the guidelines for ICES WGITMO National Reports; it updates the Italian status up to 2021.

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Lithuania

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Overview:

No new arrivals were recorded in the period from 2019 to 2021. All previous introductions, such as common rangia *Rangia cuneata*, the killer shrimp *Dikerogammarus villosus* and the round goby *Neogobius melanostomus* were first recorded in other Baltic Sea countries and then in Lithuania.

Content:

1. Regulations: An update on new regulations and policies (including, aquaculture and vector management)

2. Intentional:

No new intentional introductions recorded.

3. Unintentional:

No new unintentional introductions recorded (Figure 1).

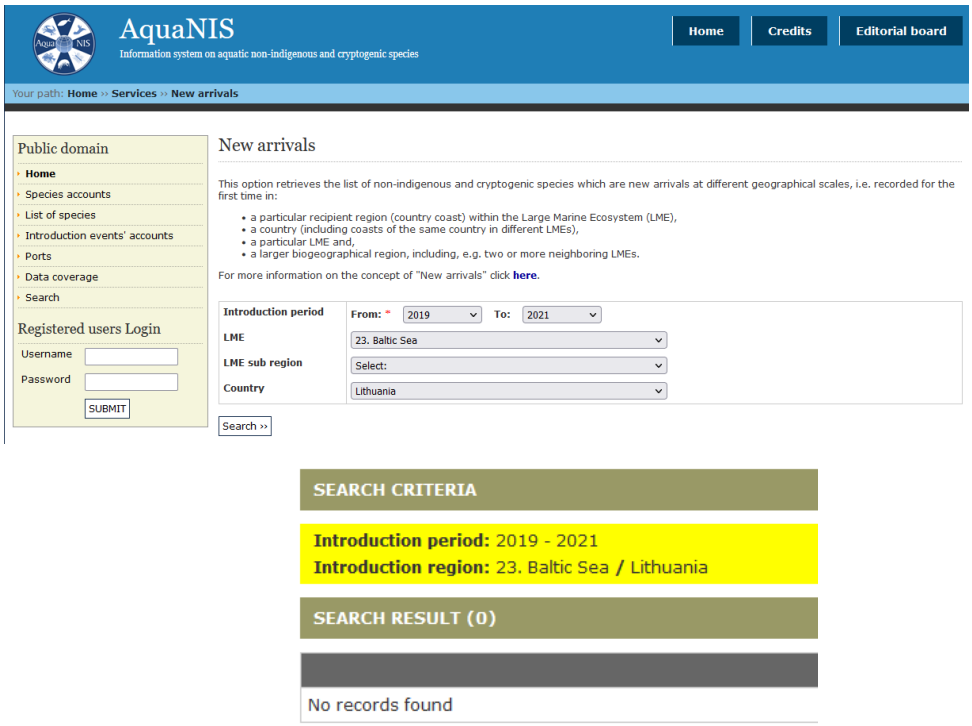

Figure 1. The Information system on aquatic non-indigenous and cryptogenic species - AquaNIS (www.corpi.ku.lt/databases/aquanis) provides a unique opportunity to reveal data provides a unique opportunity to obtain data on new arrivals of introduced species at different geographical scales, i.e. recorded for the first time in: a particular recipient region (country coast) within the Large Marine Ecosystem (LME), a country (including coasts of the same country in different LMEs), a particular LME and, a larger biogeographical region, including, e.g. two or more neighboring LMEs. The print screen shows the query ("New arrivals in Lithuania in 2019–2021) - at the top, and the result (below).

Species which previously established in the Lithuanian waters continue to spread. For example, *Dikerogammarus villosus*, an amphipod of Ponto-Caspian origin, by 2016 had invaded Russian (Kaliningrad region), Lithuanian and Latvian waters. Its rapid east and northward expansion is very likely due to vessel transport, which would account for the "jump" dispersal. Other vectors

facilitating further spread are almost certainly acting at a local scale such as overland transportation of vessels, movements of diving gear, drifting mats of algae, macrophytes and flotsam, as well as natural spread (Minchin *et alet al*. 2019).

The invasive round goby *Neogobius melanostomus* has established a viable population within 9 years of its first introduction to Lithuanian coastal waters (SE Baltic Sea). During its expansion phase, abundances increased 23-fold, which led to the near complete eradication of its main prey, the blue mussel, at < 20 m depth. The round goby population showed a stabilizing trend after blue mussel biomass was depleted; however, their abundance has not declined. The round goby feeds efficiently on newly settled mollusks, causing a severe constraint for blue mussel recovery. Changes in blue mussel availability and size structure induced a dietary shift in wintering long-tailed duck towards fish prey (Skabeikis *et alet al*. 2019; Rakauskas *et alet al*. 2020).

4. Pathogens

No data on alien pathogens in the Lithuanian coastal waters

5. Research and Monitoring Programs

- A DNA barcode reference library for nearly 60% of all known Ponto-Caspian amphipod species is compiled by Lithuanian, Polish, Canadian, Russian and other researchers (Copilaş-Ciocianu *et alet al*. 2022)
- Mitochondrial and nuclear genetic markers were used to explore the diversity of the invasive New Zealand mudsnail *Potamopyrgus antipodarum* in 19 locations in Lithuania, Poland, Ukraine and Finland (Butkus *et alet al*. 2020)

6. Meetings

Several online courses (e.g. on October 2020; April 2021; February 2022) were arranged by the AquaNIS team (Marine Research Institute, Klaipeda University) for those who are using AquaNIS for research and management purposes. The courses provided hands-on training in data entry procedures (registration of new species, introduction events, biological traits of species, library, etc.) and data extraction options (basic search, advanced search, species similarity calculation, etc. Instructions were provided upon submission of "bulk" data, such as complete lists of non-native species recorded in a particular country or region. Also, a recently developed AquaNIS-based functional module Early Warning System on Harmful Aquatic Organisms and Pathogens was presented (Figure 2). A special session (Zoom videoconference) was organized to introduce AquaNIS and encourage Canadian, Japanese and Korean members of the NIS PICES Expert Group to collaborate (27 September 2021 from 16:00-19:00 Pacific Daylight Time, Tuesday 28 September 2021 in Asia). AquaNIS goals, objectives and opportunities were presented by S. Olenin.

Figure 2. An example of a course on the use of the Information system on aquatic non-indigenous and cryptogenic species AquaNIS.

April 13, 2021		
Detailed program	**Timing**	**Duration**
Introduction to AquaNIS, staff, credits	14:00 – 14:10	10 min
Basic blocks presentation **(Species, Introduction events, geography)**	14:10 – 14:15	5 min

Data retrieval options **basic search** - species account 15 min. - introduction event 15 min.	14:15 – 14:40	30 min.
advanced search - recipient region 10 min. - data representation, Excel file 5 min. - data comparison 10 min.	14:40 – 15:05	25 min.
Interactive exercise 5 min. (advanced search: find NIS number in the Baltic Sea; North Sea and etc.; export data)		
Species similarity calculation	15:10 – 15:15	5 min.
Services and tools - New arrivals - Most widespread species - Last update for a recipient region (LME 23. Baltic sea)	15:15 – 15:25	10 min.
Early warning species (EWS) - The EWS Concept - List of Target Species - Warning signal - Regionally harmonized early warning system concept	15:25 – 15:40	15 min.
QA/ Discussion	15:40 – 16:00	20 min.

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Poland

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Overview:

There were two new sightings of non-indigenous species – a copepod *Eurytemora carolleeae* native to Atlantic coast of North America was recorded in 2019 in Szczecin Lagoon, whereas racer goby *Babka gymnotrachelus* native to Ponto-Caspian region was noted in 2021 in the Gulf of Gdańsk. In 2020 there was also record of non-native microsporidian parasite *Hepatospora eriocheir* which infested Chinese mitten crab *Eriochier sinensis* from Vistula Lagoon.

In 2020 Poland finally ratified the BWM Convention, but in 2021 implemented Act on Alien Species.

For the first time, studies of the presence of non-indigenous species on the hulls of recreational boats were conducted, the results of which indicate a high potential for introduction and spreading of these species within Polish as well as Baltic Sea waters.

Content:

1. Regulations

In July 2019 the Council of Ministers adopted a draft Act on the ratification of the BWM Convention. The multi-stage process of adopting the Act by the Polish Parliament lasted until November, when the consent to ratify the BWM Convention expressed in the Act was signed by the President of the Republic of Poland. On 26 August 2020, Poland ratified BWM Convention.

In March 2019, Ministry of the Environment submitted to the legislative process a draft of new Act on Alien Species, which defines the legal framework for preventing the spread of invasive alien species in nature, referring to (1) the Regulation No 1143/2014 of the European Parliament and of the Council of 22 October 2014 on the prevention and management of the introduction and spread of invasive alien species and (2) Commission Directive (EU) 2018/350 of 8 March 2018 amending Directive 2001/18/EC of the European Parliament and of the Council as regards the environmental risk assessment of genetically modified organisms. Basically, the Act covers guidelines on reporting the records of invasive alien species in the environment and emergency measures, prohibitions and permits, controls or criminal penalties. It also specifies the entities competent to undertake remedial actions against invasive alien species that pose a threat to the Union, which in the case of marine organisms are Maritime Offices and the Chief Inspectors of Sea Fisheries. On 19 August 2021, the President of the Republic of Poland signed the Act on Alien Species.

On 25 February 2021, the Regulation of the Minister of Infrastructure on adopting the update of the set of environmental objectives and indicators for marine waters was issued. Within the scope of its regulation, this ordination implements Marine Strategy Framework Directive adopted on 17 June 2008. Within anthropogenic pressures on the marine environment, maritime transport and its significance to introduction of non-indigenous species was taken into account when developing a proposal to update the set of environmental objectives for the pressure characteristics. Environmental objectives for all assessment criteria within descriptor D2 (Non-indigenous species introduced by human activities are at levels that do not adversely alter the ecosystems) included in Commission Decision (EU) 2017/848 of 17 May 2017 were defined at a general level: D2C1 - limiting the pressure related to the introduction of non-indigenous species and limiting the possibility of the spread of newly introduced non-indigenous species from the sites of their

primary introduction, D2C2 - minimizing new introductions of non-indigenous species and scale of their spreading to reduce their negative impact on the structure and functioning of the ecosystem and D2C3 - limiting the impact of invasive species to a level guaranteeing only a minimum or no appreciable negative environmental impact.

2. Intentional

In 2019-2021 there were deliberate releases of four migratory fish species to the wild (species and numbers are given in the table below), either directly to the marine environment or rivers discharging to the Baltic Sea (Dr. inż. Tomasz Czerwiński, Inland Sea Fisheries Institute in Olsztyn, pers. comm.). The releases were carried out within the "Restocking of Polish sea areas " task, where fish material is released to: the upper and lower Vistula inflows, central Oder tributaries, Pomeranian rivers and Puck Bay basin. However, it should be noted that these have been not the only deliberate releases of migratory fish species to the Polish rivers. Above mentioned restocking, but on a smaller scale, was also supported by the deliberate releases of fish by The Polish Angling Association, which additionally restore fish stocks in fishing districts, as well as rivers dis-charging to the Baltic Sea.

Species	**Deliberate releases (ind.)**		
	2019	**2020**	**2021**
Salmo salar	543 000	735 000	856 160
Salmo trutta trutta	3 410 000	2 776 990	2 722 650
Coregonus lavaretus	47 000	40 380	11 110
Vimba vimba	70 000	147 000	103 900
TOTAL	**4 070 000**	**3 699 370**	**3 693 821**

3. Summary of sighting

Unintentional

There were two new sightings of non-indigenous species:

- in 2019 a North-American calanoid copepod, *Eurytemora carolleeae* was recorded in Szczecin Lagoon, at few sites (Sługocki *et alet al*. 2021); it is however highly probable that this species has been already introduced earlier to Polish waters (Dr. Łukasz Sługocki, Department of Hydrobiology, University of Szczecin, pers. comm.);

- in 2021 a Ponto-Caspian racer goby *Babka gymnotrachelus* was recorded in the Gulf of Gdańsk during the survey of ports: Gdańsk, Gdynia, Puck and Hel (Dr. Bartosz Witalis, National Marine Fisheries Research Institute in Gdynia, Poland, pers. comm.); this fish has been recorded in few Polish rivers since 1995 where it was probably introduced via inland Bug-Prypec canal.

Previous Sightings

A tanaid *Sinelobus vanhaareni* (Crustacea), found previously only in the Gulf of Gdańsk (eastern Polish coast) and in Port of Świnoujście (western Polish coast), in 2019 has been also recorded in other regions, i.e. in Vistula Lagoon (the most eastern part of the Polish coast) and in the marina in Kołobrzeg (middle /open Polish sea coast) (Outinnen *et alet al*. 2021).

A talitrid *Platorchestia platensis* (Crustacea), has extended its range of occurrence beyond the area of Puck Bay and nowadays it also is recorded in the Gulf of Gdansk (Tykarska *et alet al*. 2019).

Estuarine shrimp *Palaemon longirostris* has been recorded in September 2020 at a new site located in Puck Bay, an inner part of the Gulf of Gdańsk (Dr. Adam Woźniczka, National Marine Fisheries Research Institute in Gdynia, Poland, pers. comm.).

A shrimp *Palaemon macrodactylus* has been recorded in 2021 at new site located in Port of Gdynia (Dr. Bartosz Witalis, National Marine Fisheries Research Institute in Gdynia, Poland, pers. comm.).

A clam *Rangia cuneata* became in 2021 very abundant at the western Polish coast, i.e. in Szczecin Lagoon and Pomeranian Bay, from mouth of Swina River to the eastern coast along Wolin National Park (Dr. Adam Woźniczka, National Marine Fisheries Research Institute in Gdynia, Poland, pers. comm.).

4. Pathogens

In 2020 a microsporidian parasite *Hepatospora eriocheir* was recorded in Chinese mitten crabs *Eriochier sinensis* from Vistula Lagoon (Magdalena Stachnik, National Reference Laboratory for Fish, Shellfish and Crustacean Diseases, National Veterinary Research Institute, pers. comm.).

5. Research and Monitoring Programs

In 2019–2021 national biological monitoring of the Polish Marine Areas was carried out under coordination of Chief Inspectorate for Environmental Protection. Monitoring included marine internal and territorial waters as well as the Polish Exclusive Economic Zone. Samples of phytoplankton and zooplankton were taken 5-6 times in a year while zoobenthos once a year. Additionally, samples of ichthyofauna were also collected in transitional waters (according to EU Water Framework Directive). One of the tasks of monitoring is to detect new non-indigenous species as well as to determine distribution and spreading of selected, already introduced ones. Analysis of samples collected in 2021 is still ongoing.

In 2019, studies on non-indigenous species (NIS) in four Polish marinas were carried out, based on the 'Biofouling assessment protocol for leisure boats and marinas' developed under COMPLETE project. Samples were collected using fouling sets deployed horizontally during vegetation season (May-October) as well as by scraping from vertical surfaces. In total 16 non-indigenous and cryptogenic species have been found. Among them, crustaceans (9 species) and bivalves (4 species) dominated. The high proportion of NIS in biofouling (36% of all taxa found) may indicate the role of marinas in their spread. The results of these studies were published jointly with data from Finland and Latvia (Outinen *et alet al*. 2021). One of the numerous species found in marinas in 2019 was *Sinelobus vanhaareni*. High abundances allowed for further studies on the population structure and reproduction of this species which were carried out in 2020 and 2021. The research results are currently being prepared for publication.

In 2019 studies on of most vulnerable areas for new introductions and spread of non-indigenous species in the Baltic Sea region (e.g. ports and marinas, hull cleaning facilities, natural reserves, offshore wind farms and drilling units, artificial habitats, seawater discharge sites and power plants) were conducted under COMPLETE project. As the result, GIS maps were created (https://complete.ug.edu.pl/en/participation/).

In 2019 and 2020 post-season assessment of recreational craft hull fouling was conducted in five marinas located in the Gulf of Gdańsk, based on the 'Biofouling assessment protocol for leisure boats and marinas' developed under COMPLETE project. Among 119 all examined boats examined dominated sailboats of a medium length (5-10 m) with fiberglass hulls covered with antifouling paint. Almost 70% hulls of boats were characterized by fouling rank 2 (light fouling), whereas only 2% by fouling rank 5 (very heavy fouling). Taxonomic analyses of samples scraped

from the hulls showed the presence 11 of non-indigenous and cryptogenic taxa, the most numerous of which were crustaceans (7 species). In the year of the study, the tested boats traveled both in territorial waters and visited other countries in the Baltic Sea region and beyond. Based on these studies, it can be concluded that recreational boating has a very high potential for introduction and spreading NIS in the Baltic Sea region. The results of these studies will be published jointly with data from Finland and Germany (Normant-Saremba *et alet al*. in prep.).

In 2019–2021 studies on the Chinese mitten crab *Eriocheir sinensis* in the eastern sites of the Polish part of Vistula Lagoon were carried out in order to monitor population status of this species, which is on the list of invasive alien species of Union concern pursuant to Regulation (EU) No 1143/2014 of the European Parliament and of the Council. About 350 individuals were collected in 2019, 200 in 2020, whereas in 2021 there were fewer crabs - only ca. 100 individuals (in 2020 and 2021 not so many fishing boats were in use compared to 2019). In addition to examining the sex structure, morphometry and condition, the collected crabs were used for shell diseases and mercury content analyses, as well as for studies on the morphological and haplotype differentiation of mitten crabs (genus *Eriocheir*) from Central-Northern Europe. The latter research was carried out in cooperation with Dr. Christine Ewers-Saucedo from Zoological Museum of the Christian-Albrechts-Universität in Kiel (Germany). Results indicates the persistence of Japanese mitten crab mitochondrial DNA in Europe for over 20 years and its introgression into Chinese mitten crabs (Homberger *et al.* 2022)).

In June-July 2021 four Polish ports: Gdańsk, Gdynia, Puck and Hel has been surveyed in regard to non-indigenous species under research studies carried out by National Marine Fisheries Research Institute in Gdynia, Poland (Dr. Bartosz Witalis, pers. comm.). During the research, various trap types used for sampling of mobile epifauna were tested, both commercial (e.g. Fukui baited trap) and own design (habitat collectors or light traps). The presence of a new species, *Babka gymnotrachelus*, has been detected in all four ports, whereas *Palaemon macrodactylus* has been found at new locality, i.e. in Port of Gdynia.

Planned Research

- Further surveys of non-indigenous species in ports Gdynia, Hel, Ustka and Świnoujście.
- Studies on population structure, biology and ecology of recently introduced non-indigenous species, like *Sinelobus vanhaareni*, *Palaemon macrodactylus*, *Rangia cuneata*, *Dreissena bugensis rostriformis*, *Eriocheir japonica*.
- Continuation of research on the role of biofouling in the spread of non-indigenous species.

Research Gaps

- Secondary spread, population status and risk assessment for targeted non-indigenous species.
- Population genetic of species identified as cryptogenic in the Baltic Sea region.
- DNA barcode reference library for NIS.

6. Meetings

145th Meeting of the National Section for the Protection of the Marine Environment (MEPC) operating at the Center for IMO, at the Polish Register of Shipping S.A., Gdańsk, Poland, 25 April 2019.

146th Meeting of the National Section for the Protection of the Marine Environment (MEPC) operating at the Center for IMO, at the Polish Register of Shipping S.A., Gdańsk, Poland, 26 June 2019.

Seminar "Ballast water and ship hulls - harmonized procedures for the Baltic Sea to reduce the risk of introduction of invasive species through shipping", national meeting with stakeholders in the frame of COMPLETE project, University of Gdańsk, Gdynia, 12 September 2019.

147th Meeting of the National Section for the Protection of the Marine Environment (MEPC) operating at the Center for IMO, at the Polish Register of Shipping S.A., Gdańsk, Poland, 27 February 2020.

148th Meeting of the National Section for the Protection of the Marine Environment (MEPC) operating at the Center for IMO, at the Polish Register of Shipping S.A., Gdańsk, Poland, 20 May 2021.

149th Meeting of the National Section for the Protection of the Marine Environment (MEPC) operating at the Center for IMO, at the Polish Register of Shipping S.A., Gdańsk, Poland, 14 October 2021.

Future meetings

55th European Marine Biology Symposium, 19–23 September 2022, Gdańsk, Poland (https://embs55.ug.edu.pl/)

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Portugal

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Overview 2022

A list of 211 marine and brackish non-indigenous species (NIS) is currently recorded for the Portuguese estuarine and coastal aquatic systems. There were 34 new additions to the list of previous reports and there were 33 new NIS for mainland Portugal, 12 new NIS for the Madeira archipelago and one for the Azores archipelago. These new records included 2 Rhodophyta, 2 Ochrophyta, 1 Tracheophyta, 1 Nematoda, 1 Porifera, 4 Cnidaria, 6 Annelida, 6 Mollusca, 10 Arthropoda and 9 Chordata species. These new records included species that have been overlooked and were added after a thorough revision of scientific articles, thesis and other literature after validation by expert judgement. A list of cryptogenic species was also created, with 26 species listed. The most recent records included the algae *Rugulopteryx okamurae*, in São Miguel (Azores) and Lagos (Algarve) and the amphipod *Grandidierella japonica* in Ria Formosa (Algarve). The blue crab (*Callinectes sapidus*) and the weakfish, which were initially detected in the Tagus estuary, appear to be expanding southwards with large populations registered in the Algarve, while the slipper limpet (*Crepidula fornicata*), firstly detected in Ria de Aveiro is currently

expanding southwards, with a well-established population observed in the Tagus estuary. Decree-Law no. 92/2019 was published in 2019, to carry out the implementation of Regulation (EU) No. 1143/2014 on the prevention and management of the introduction and spread of invasive alien species at national level.

Content

1. Regulations

Decree-Law no. 92/2019, of 10th July, establishes the legal framework applicable to the control, keeping, introduction into the wild and restocking of non-indigenous species and ensures the implementation in the national legal order, of Regulation (EU) No. 1143/2014 of the European Parliament and of the Council of 22 October 2014 on the prevention and management of the introduction and spread of invasive alien species. It revokes Decree-Law no. 565/99, of 21 December, which was in force for almost 20 years. Like the previous regulation, Decree-Law no. 92/2019 exempts from its scope harmful aquatic organisms and pathogens from ships ballast water and sediments, subjected to separate legislation (implementation of the Ballast Water Convention).

Portugal carried out a comprehensive analysis of the pathways of unintentional introduction and spread of invasive non-indigenous species and identified those which require priority action ('priority pathways') based on the number of species or on the potential adverse effects caused by the species entering the national territory through those pathways. Eleven priority pathways were identified:

- Aquaculture
- Contaminant on animals (excluding parasites and species transported by host and vector)
- Pet / aquarium / terrarium species
- Horticulture
- Ornamental (other than horticulture)
- Contaminant nursery material
- Contaminant on plants (excluding parasites and species transported by host and vector)
- Transportation of habitat material (soil, vegetation, wood…)
- Ship/boat hull fouling
- Vehicles (car, train, …)
- Natural dispersal (of invasive non-indigenous species that have been introduced through other pathways)

Seven action plans have been prepared to tackle the priority pathways which are currently under discussion with stakeholders and public entities to be involved in the implementation of the plans before formal approval by the Portuguese government:

- Aquaculture + Contaminant on animals
- Pet / aquarium / terrarium species
- Horticulture + Ornamental
- Contaminant nursery material + Contaminant on plants + Transportation of habitat material
- Ship/boat hull fouling
- Vehicles
- Natural dispersal

Funding has been granted in 2021 for the preparation of an Action Plan for the control of three aquatic invasive species in the south and southwestern coasts of mainland Portugal: Black Sea jellyfish, *Blackfordia virginica*, weakfish, *Cynoscion regalis*, and Atlantic blue crab, *Callinetes sapidus*.

In 2021 the national action plan for the control of the red swamp crayfish *Procambarus clarkii* was approved and published for mainland Portugal (Resolução do Conselho de Ministros n.º 133/2021).

Funding has been approved in 2022 for the preparation of an Action Plan for the management/control of the Manila clam, *Ruditapes phillipinarum*.

2. Intentional introductions

Information available for introductions in Portuguese estuarine and coastal waters is insufficient to distinguish between intentional and unintentional introductions.

3. Summary of sightings

Phaeophyta

Rugulopteryx okamurae

Rugulopteryx okamurae (Ochrophyta, Phaeophyceae) grows in the shallow subtidal and can be present all year-round as dormant rhizoidal bases. Macroscopically very similar to *Dictyota dichotoma*, individuals of *R. okamurae* are characterized by a membranous and erect dichotomously branched thallus with smooth margins of 10-20 cm high, attached by rhizoids restricted to the basal parts of the thallus. The fronds grow into a compressed flabellum and in dense groups, and intraspecific variation is known to occur. Originating in the Pacific Ocean (e.g., Philippines, China, Korea and Japan), *Rugulopteryx okamurae* was first identified in European waters in 2005. In 2015/16 massive expansion was observed across much of the Strait of Gibraltar and the coast of Andalusia (Spain), where it occurs abundantly.

This species was first detected in the Portuguese territory in early 2019 on the south coast of São Miguel Island, Azores archipelago, and the identification was confirmed by molecular and morphological data. It has invaded extensively the south coast of São Miguel and has also been detected in Faial and Terceira islands.

In 2021 it was also detected in continental Portugal, on the western Algarve coast, from Lagos to Sagres, where large beach casts were reported (R. Santos, CCMar-UAlgarve, pers. comm.). In the spring-summer of 2022, it was detected further North in Praia do Queimado, Porto Covo, Alentejo coast, and as beach cast in Ponto do Adoche, Troia peninsula, probably originating from rocky reefs in the Arrábida coast nearby (Setubal), in the southwest coast of Portugal.

The new 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 now includes *Rugulopteryx okamurae*.

Bryozoa

Schizoporella errata

Schizoporella errata is a heavily encrusting cheilostome bryozoan. First reported in Madeira in 2013, it was classified as a cryptogenic species (Ramalhosa *et al.*, 2019) and changed its status to NIS (Castro *et alet al*. 2022).

Mollusca

Crepidula fornicata

The common slipper shell was firstly recorded in 2009, in mainland Portugal, in Ria de Aveiro (Galante-Oliveira *et alet al*. 2013) and in the Mondego estuary (Callapez & Matos, 2020), but in the most recent years has colonized the Óbidos coastal lagoon and the Tagus estuary, registering high abundances. In 2021 it was also recorded in the Sado estuary (G. Calado, com. pess.). It might have been introduced by fouling in recreational and commercial boats, but its dispersal

might have been favoured by shellfish translocation, since the Óbidos coastal lagoon has only local boating activities.

Arthropoda

Callinectes sapidus

Several new records in 2019 and 2020 in the western Portuguese coast may show a northern expansion of the species (Encarnação *et al*. 2022). New historical records were retrieved from the Vasco da Gama Aquarium and the National Museum of Natural History and Science (Encarnação *et al*. 2022), showing the Atlantic blue crab was firstly detected in Portugal in 1967 in the Tagus estuary, eleven years before the first known scientific publication reporting the species in mainland Portugal by Gaudêncio and Guerra (1979).

Chordata

Ascidia curvata

Ascidia curvata is native to the West Atlantic Ocean where it has a widespread distribution and occurs in many islands (Rocha *et alet al*. 2005). It also occurs in Bermuda (Berrill, 1932) and is believed to be introduced in south Brazil (Bonnet and Rocha, 2011). The current rarity and association with artificial habitats in the Madeira Archipelago suggest it is a NIS. It was found for the first time in Funchal, the main port of Madeira in 2014 (Ramalhosa *et al*. 2021).

Distaplia bermudensis

The tunicate *Distaplia bermudensis* is native to the West Atlantic (Van Name, 1945) with reports of introduction in the Balearic Islands (Pérès, 1957) and Italy, since 2000 (Mastrototaro and Brunetti, 2006). It is a non-indigenous species in Madeira Archipelago, currently found on artificial substrates only. It was first encountered in Madeira Archipelago in 2006 (Canning-Clode *et alet al*. 2013) and in Porto Santo in 2015 (Ramalhosa *et al*. 2021).

Styela plicata

Styela plicata is a well-known fouling species usually found in marinas, ports and bivalve cultures in tropical and subtropical waters (Lambert and Lambert, 1998; Rocha *et alet al*. 2009). The rarity of the species in Madeira archipelago suggests that it is a NIS that does not yet have established populations. This species was found living in both natural and artificial substrates in Madeira Island in 1993 and 2016, respectively (Ramalhosa *et al*. 2021).

Symplegma rubra

The colonial ascidian *Symplegma rubra* was found in Madeira Island on settlement plates in marinas in 2014 (Ramalhosa *et al*. 2021). This species was initially identified as cryptogenic (Ramalhosa *et alet al*. 2019) but recent research changed its population status to NIS (Castro *et alet al*. 2022).

Cynoscion regalis

The NEMA citizen science campaign continued to register scattered records of *Cynoscion regalis* in the southern coast of Portugal during 2021, without any evident increase in abundance. Still, several mature females were recorded in the Guadiana estuary during spring, showing the usage of this ecosystem as a potential spawning ground in south Portugal.

Table 1. List of new registers of NIS in Portuguese waters in 2020-2022, updates and revision of previous records. Population status as: E – Established, NE – Not Established, and U – Undetermined.

Taxa	Year of first record	Location of first record	Possible introduction vector	Population Status	References
Algae – Rhodophyta					
Gymnophycus hapsiphorus Huisman & Kraft	2011	Madeira	Unknown	U	Ferreira *et al* 2018; Castro *et al* 2022
Melanothamnus harveyi (Bailey) Díaz-Tapia & Maggs, 2017	2003	Ancora	Ballast water	E	Araujo *et alet al*. 2009
Pyropia suborbiculata (Kjellman) J.E.Sutherland, H.G.Choi, M.S.Hwang & W.A.Nelson, 2011	2010	Faro Aljezur	Ballast water	E	Vergés *et alet al*. 2013
Algae – Ochrophyta					
Scytosiphon dotyi M.J.Wynne, 1969	2016	Magoito Beach	Fouling	NE	Berecibar, 2016
Rugulopteryx okamurae I.K. Hwang, W.J. Lee & H.S. Kim	2019	São Miguel Island, Azores	Ballast water; Fouling	E	Faria *et alet al*. 2019
Rugulopteryx okamurae I.K. Hwang, W.J. Lee & H.S. Kim	2021	Algarve, Lagos	Ballast water; Fouling	E	Liulea, 2021
Tracheophyta - Poales					
Spartina densiflora Brongn.	1986	Guadiana Estuary	Contaminant	E	Lousã, M.F., 1986
Nematoda					
Anguillicola crassus Kuwahara, Niimi & Itagaki, 1974	1988	Mondego estuary	Parasites on animals	Established	Domingos, I., 2002
Porifera					
Paraleucilla magna Klautau, Monteiro & Borojevic, 2004	2008	Sagres	Fouling	E	Guardiola, M., Frotscher, J. & Uriz, M. J., 2016
Bryozoa					
Beania maxilladentata Ramalho, Muricy & Taylor, 2010	2013	Madeira	Fouling	E	Souto *et al* 2015

Schizoporella errata (Waters, 1878)	1897	Madeira / Porto Santo	Fouling	E	Norman 1909, Ramalhosa *et al* 2019, Castro *et al* 2022
Cnidaria					
Diadumene lineata (Verrill, 1869)	2002	Tagus Estuary	Fouling/Aquaculture	E	Sousa, 2016
Ectopleura crocea (Agassiz, 1862)	2016	Matosinhos	Fouling	E	Ramos-Esplá *et alet al*. 2016; Azevedo *et alet al*. 2020
Macrorhynchia philippina Kirchenpauer, 1872	1976/88	Madeira	Fouling	E	Agis *et al* 2001, Moura *et al* 2012; Castro *et al* 2022
Annelida – Polychaeta					
Branchiomma luctuosum (Grube, 1870)	2015	Ria Formosa	Fouling	E	Gonçalves, J., Afonso, C., Oliveira, F. *et alet al*. 2019
Goniadella gracilis (Verrill, 1873)	1986	Tagus Estuary	Ballast water	E	Ferreira, M. A., da Fonseca, L. C., & Andrade, F., 2004
Hydroides elegans (Haswell, 1883)	1984	Albufeira lagoon	Fouling	E	Quintino, V. M., 1991
Isolda pulchella Müller in Grube, 1858	1994	Aveiro	Fouling/Ballast water	E	Ravara, A. & Moreira, M. H., 2013
Prionospio aluta Maciolek, 1985	1994	Aveiro	Ballast water	E	Ravara, A. & Moreira, M. H., 2013
Lumbrineris crassicephala (Hartman, 1965)	1994	Aveiro	Ballast water	U	Ravara, A. & Moreira, M. H., 2013
Euchone incolor Hartman, 1965	1971	Aveiro	Fouling/Ballast water	U	João Carlos Gil, 2011
Metasychis gotoi (Izuka, 1902)	1972	Esposende	Fouling/Ballast water	E	Amoureux, L., 1987 cited in João Carlos Gil, 2011
Mollusca					
Anteaeolidiella lurana (Ev. Marcus & Er. Marcus, 1967)	2018	Ria Formosa	Fouling	NE	Pérez-García, P., Carmona, L., Calado, G. *et alet al*. 2019
Antiopella cristata (Delle Chiaje, 1841)	1996	Madeira	Unknown	U	Wirtz 1998
Aplus assimilis (Reeve, 1846)	2015	Faro	Fouling	U	Sofia Tristancho-Ruiz, 2015

Ensis leei M. Huber, 2015	2018	Tagus Estuary	Ballast water	U	Project COCKLES, 2018
Mytilus galloprovincialis Lamarck, 1819	2009	Madeira	Unknown	U	Segers *et al* 2009; Castro *et al* 2022
Tritia neritea (Linnaeus, 1758)	2010	Ria Formosa	Fouling/Ballast water	E	Gonçalves, J., Afonso, C., Oliveira, F. *et alet al*. 2019
Arthropoda – Cyclopoida					
Myicola ostreae Hoshina & Sugiura, 1953	2004	Ria Formosa	Parasites on animals	E	Batista *et alet al*. 2009
Arthropoda – Balanomorpha					
Amphibalanus eburneus (Gould, 1841)	2015	Ria Formosa	Fouling	E	Sofia Tristancho-Ruiz, 2015
Arthropoda – Cumacea					
Eocuma dimorphum Fage, 1928	2007	Alcobaça (Pataias)	Ballast water	U	Sampaio, L., Mamede, R., Ricardo, F. *et alet al*. 2016
Arthropoda – Amphipoda					
Ampithoe valida Smith, 1873	1985	Ria de Aveiro	Fouling	E	Moreira *et alet al*. 1993
Grandidierella japonica Stephensen, 1938	2019	Ria Formosa -	Aquaculture	NE	Rafael Vieira, 2019
Stenothoe georgiana Bynum & Fox, 1977	2011	Sines Marina	Fouling	U	Martinez-Laiz *et alet al*. 2020
Arthropoda – Isopoda					
Paracerceis sculpta (Holmes, 1904)	2017	Ria Formosa	Aquaculture	E	Martinez-Laiz, G., Ros, M. & Guerra-Garcia, J. M., 2018
Synidotea laticauda Benedict, 1897	2009	Guadiana Estuary	Fouling/Ballast water	E	Nuño *et alet al*. 2018
Arthropoda – Decapoda					
Afropinnotheres monodi R.B. Manning, 1993	2016	Guadiana estuary	Parasites on animals	E	Pérez-Miguel *et alet al*. 2019
Jassa marmorata Holmes, 1905	1991	Porto Santo	Unknown	U	Cancela da Fonseca *et al* 1995; Fofonoff *et al* 2018; Castro *et al* 2022

Chordata – Tunicata					
Ascidia curvata (Traustedt, 1882)	2014	Madeira	Fouling	U	Ramalhosa *et al* 2021
Botrylloides violaceus Oka, 1927	2007	Madeira	Fouling	U	Canning-Clode *et al* 2013; Ferrario *et al* 2020; Castro *et al* 2022
Clavelina oblonga Herdman, 1880	2013	Porto Santo	Fouling	E	Wirtz and Ramalhosa 2020; Ramalhosa *et al* 2021
Didemnum perlucidum Monniot F., 1983	2006	Madeira	Fouling	U	Canning-Clode *et al* 2013 ; Castro *et al* 2022
Didemnum vexillum Kott, 2002	2015	Ria Formosa	Fouling/Aquaculture	E	Sofia Tristancho-Ruiz, 2015
Distaplia bermudensis Van Name, 1902	2006/2015	Madeira / Porto Santo	Fouling	U	Canning-Clode *et al* 2013 ; Ramalhosa *et al* 2021 ; Castro *et al* 2022
Styela plicata (Lesueur, 1823)	1992/3	Madeira	Fouling	U	Ramalhosa *et al* 2019; 2021
Symplegma rubra Monniot C., 1972	2014	Madeira	Fouling	E	Ramalhosa *et al* 2021 ; Castro *et al* 2022

Table 2. List of cryptogenic species recorded in Portuguese waters. Population status as: E – Established, NE – Not Established, and U – Undetermined.

Taxa	Year of first record	Location of first record	Possible introduction vector	Population Status	References
Myzozoa – Dinophyceae					
Gymnodinium microreticulatum C.J.S.Bolch, Negri & G.M.Hallegraeff, 1999	1860	Portuguese coast	Ballast water	U	Amorim, A., Dale, B., Godinho, R. *et alet al*. 2001
Gymnodinium nolleri M.Ellegaard & Ø.Moestrup, 1999	2002	Douro estuary	Ballast water	U	Ribeiro, S., Amorim, A., Andersen, T. J. *et alet al*. 2012
Chlorophyta					

Ulvaria obscura (Kützing) P.Gayral ex C.Bliding, 1969	1961	Portinho da Arrábida	1850	E	Ardré, F., 1961
Bryozoa					
Amathia verticillata Della Chiaje, 1828	1937	Berlengas	Fouling/Ballast water	E	Reverter-Gil, O., Souto, J. & Fernández-Pulpeiro, E. (2014).
Bugula neritina (Linnaeus, 1758)	1984	Ria Formosa	Fouling/Ballast water	E	Dexter, D. M., 1992
Bugulina fulva (Ryland, 1960)	1974	Setúbal	Fouling/Ballast water	E	Saldanha, L., 1974 ; Souto, J., 2016
Bugulina stolonifera Ryland, 1960	1977	S Portuguese coast	Fouling	E	Dexter, D. M., 1992
Cradoscrupocellaria bertholletii (Audouin, 1826)	1909	Madeira	Fouling	E	Normam 1909; Ramalhosa *et al*. 2019; Castro *et al*. 2022
Crisularia plumosa (Pallas, 1766)	1937	Portuguese coast	Fouling	U	Nobre, A., 1937
Membranipora membranacea (Linnaeus, 1767)	1903	Douro estuary	Fouling	E	Nobre, A., 1903
Reptadeonella violacea (Johnston, 1847)	1944	Setúbal	Fouling	E	Rosas, M., 1944
Watersipora souleorum Vieira, Spencer Jones & Taylor, 2014	2004	Ria Formosa	Fouling	U	Reverter-Gil, O. & Souto, J., 2019
Virididentula dentata (Lamouroux, 1816)	1909/2013	Madeira; Porto Santo	Fouling	E	Normam 1909; Canning-Clode *et al*. 2013; Ramalhosa *et al*. 2017, 2021; Castro *et al*. 2022
Annelida – Polychaeta					
Paraprionospio coora Wilson, 1990	1981	Odemira	Fouling	E	João Carlos Gil, 2011
Percnon gibbesi (H. Milne Edwards, 1853)	1931	Mira estuary	Ballast water	U	Nobre, A., 1931

Polydora cornuta Bosc, 1802	1989	Mondego estuary	Fouling/Ballast water	E	Pardal, M. A., Caldeira, A. M. & Marques, J. C., 1992
Mollusca – Bivalvia					
Lyrodus pedicellatus (Quatrefages, 1849)	1998	Ria Formosa	Fouling	E	Brown, C. J., 1998
Teredo bartschi Clapp, 1923	2002	Ria Formosa	Organic packing material, in particular wood packaging/Fouling	U	Borges, L., Sivrikaya, H. & Cragg, S., 2014
Teredo navalis Linnaeus, 1758	1916	Figueira da Foz	Organic packing material, in particular wood packaging/Fouling	E	Hidalgo, J. G., 1917
Mollusca – Gastropoda					
Hexaplex trunculus (Linnaeus, 1758)	1994	Madeira/ Porto Santo/ Desertas a	Unknown	E	Houart & Abreu 1994; Chainho *et al*. 2015; Castro *et al*. 2022
Arthropoda – Malacostraca					
Diamysis bahirensis (G.O. Sars, 1877)	1995	Ria de Aveiro	Fouling	U	Cunha, M. R., Sorbe, J. C. & Moreira, M. H., 1999
Limnoria quadripunctata Holthuis, 1949	1990	Tagus estuary	Organic packing material, in particular wood packaging/Fouling	E	Borges, L. M., Merckelbach, L. M. & Cragg, S. M., 2014
Limnoria tripunctata Menzies, 1951	1960	Tagus estuary	Organic packing material, in particular wood packaging/Fouling	E	Franco, S. E., 1975
Chordata – Tunicata					
Botrylloides leachii (Savigny, 1816)	2001	Portimão	Aquaculture/Fouling	E	Boaventura, D. F. & Dos Santos, P., 2007
Botryllus schlosseri (Pallas, 1766)	2006;2013	Madeira; Porto Santo	Fouling	E	Canning-Clode *et al*. 2013; Ramalhosa *et*

					al. 2021; Castro *et al*. 2022
Ciona intestinalis (Linnaeus, 1767)	1992/1993	Madeira	Fouling	U	Ramalhosa *et al*. 2021

4. Research and Monitoring Programs

Planned Research:

2021 – 2022. Development of an Integrated Management Model as a Support Tool for the Governance of the Sado Estuary (GI4SADO) (Local PI: Ana Mata).

2022 – Plan for the management/control of the Manila clam, *Ruditapes phillipinarum*. Portuguese Institute for Nature Conservation and Forests.

2022 – MONIS - Proposal for "Execution of activity 2.2.1 – Monitoring, characterization and analysis programs of the spatial distribution of non-indigenous species and their impacts on marine protected areas, within the scope of the project PLASMAR + MAC2/1.1a/347" (DRAM/GRA) Contract.

2021–2022. ATLAZUL (Poctep/Interreg 0755_ATLAZUL_6_E) – Impulso da Aliança Litoral Atlântica para o Crescimiento Azul. Secretaría General de Acción Exterior (SGAE) de la Consejería de la Presidencia, Administración Pública e Interior de la Junta de Andalucía (Spain).

Objective: Identify challenges, opportunities and generate networks that promote the specialization of the transboundary area in blue growth, as well as develop innovations within the scope of blue growth policies focused on new solutions with digital support for the problem of territorial metabolism and the improvement of terrestrial and marine knowledge. POCTEP project (Transfrontier Interreg Spain-Portugal) focused on Priority A1 – Improvement of research and innovation (R+I) infrastructures and the ability to develop excellence in R+I, and the promotion of centers of competence, especially those of European interest.

Contribute of ECOREACH in the NIS domain: 1) Two sampling campaigns in the Guadiana estuary to update the state of fisheries resources, with special focus on NIS; 2) Evaluate the potential of NIS in the blue economy of Algarve in areas such as tourism, fisheries, gastronomy; 3) Acoustic telemetry tagging program with the invasive Atlantic blue carb *Callinectes sapidus* in the Guadiana estuary.

2021–2022 - ALFCORAZUL (Fundo Ambiental, application #48) – Plano de Ação para o controlo de três espécies invasoras aquáticas em Portugal Continental – alforreca negra *Blackfordia virginica*, corvinata americana *Cynoscion regalis* e caranguejo azul *Callinectes sapidus.* University of Algarve.

Objective: The project aims to deliver an Action Plan for the control in mainland Portugal of three aquatic invasive species: *Blackfordia virginica*, *Cynoscion regalis*, *Callinetes sapidus*. This plan is divided in two parts. One aims at using water discharges from dams to control the populations of jellyfish and increment environmental disturbance to diminish the probably for non-indigenous species to settle or disperse. We have meet with Agência Portuguesa do Ambiente, Instituto de Conservação da Natureza e Florestas and Empresa de Desenvolvimento e Infra-estruturas do Alqueva. The other goal aims at using invasive species as new fishing resources to be used as a

source of food and income for local fishing communities in southern Portugal, many of which facing poverty conditions associated with the disappearance of native fishing resources. This goal intends to transform the threat of some aquatic invasive species into an opportunity to control their populations through their professional or recreational fishing and human consumption. The plan will be accompanied by a request to include the weakfish C. regalis and the blue carb C. sapidus in the Portuguese list of invasive species, along with changes in national law regarding the capture of these species.

2020–2023 - The project HOTMIC (JPI Ocean) aims to map the distribution of microplastics, in water, sediments and biota from the coastal waters to the open ocean gyre and deep sea. In order to understand the degradation of plastics in the field, and to see if there are any differences in the fouling community of different plastic types, experiments were performed in the field, and the number of non-indigenous species will be assessed.

2022–2023 - *Marine Strategy Framework Directive* - In the scope of the Marine Strategy Framework Directive (MSFD), the reporting to the European Commission of the monitoring programmes is a formal requirement (Article 11). In the beginning of this year, the Monitoring Programme for Descriptor 2 – non-indigenous species, was submitted for public consultation. Three sub-programmes were designed:

1) Monitoring of non-indigenous species in marine protected areas.
2) Monitoring of non-indigenous species in the hotspots of introduction (ports, marinas and aquaculture facilities).
3) Monitoring of non-indigenous species belonging to specific taxonomic groups (phytoplankton, zooplankton, benthos and nekton).

Currently, Portugal is finishing the Programme of Measures, that covers both existing and new measures designed to achieve or maintain the Good Environmental Status for each MSFD Descriptor (requirement defined in Articles 13 and 14).

2019–2021 - The project RAGES – Risk-Based Approaches to Good Environmental Status, aimed at developing a risk-based approach to support coordinated regional and sub-regional implementation of the Marine Strategy Framework Directive including the new decision on Good Environmental Status, by providing a replicable, transparent and standardised environmental decision support process based on international best practices in risk assessment and management. The final deliverables were concluded, which included: (i) a compilation of the existing spatial information on non-indigenous species introduction, spread and impacts in the NE Atlantic subregions Bay of Biscay and the Iberian Coast, and Macaronesia (Bartilotti *et alet al*. 2020a, b), and (ii) a preliminary risk assessment using a pilot Horizon-Scanning exercise was conducted in order to provide a ranked list of NIS that should be of high priority for risk assessment (Bartilotti *et alet al*. 2020c; Hollatz *et alet al*. 2021a, b). All deliverables are available at https://www.msfd.eu/rages/outputs.html.

Research Needs:

The monitoring programme designed for NIS in the scope of the MSFD will be fundamental for data acquisition considering new arrivals and abundances, as well as adverse effects of NIS.

5. Meetings

- Hollatz, C., Bartilotti, C., Tuaty-Guerra, M., Lobo-Arteaga, J., Brignon, J-M., Chapon, V., Cardoso, I., Gizzi, F., Monteiro, J., Macedo, J., Gaudêncio, M.J., Caning-Clode, J., and Carreira, G. Application of the risk-based approach to non-indigenous species in the Bay of Biscay and the

Iberian Coast, and in Macaronesia. XVII International Symposium on Oceanography of the Bay of Biscay (ISOBAY 17), 1 – 4 June 2021, Oviedo, Spain.

- Online event organized by IPMA ("To discover our ocean", 8 June 2021), in the European Maritime Day 2021, with a presentation about non-indigenous species in the Portuguese coast (title: "There are intruders in our ocean!")

- Workshop about the Lisboa canyon (22 June 2021), where the non-indigenous species were topic for two talks ("Noah's Arks of the 21st century: ballast waters as an introduction vector for non-indigenous species", and "No to immigration… of biodiversity").

- The 14th Iberian Meeting on Halmful Algal and marine biotoxins, "XIV Reunião Ibérica sobre Microalgas Nocivas e Biotoxinas Marinhas", 1to 3 June 2022, IPMA, Lisboa, Portugal.

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Sweden

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Overview

Two new species were discovered in Sweden during 2019: the comb jellyfish *Beroe ovata* at the station Släggö, North Sea and the Atlantic rock crab *Cancer irroratus* in the southern Kattegat. During 2020, three new species were sighted at the Swedish west coast: the shell-boring annelid *Polydora websteri*, the stalked jellyfish *Craterolophus convolvulus*, and non-native tanaid *Sinelobus vanhaareni*. There has been no report indicating the occurrence of new species in Sweden since January 2021. Following the European ARMS programme and using Environmental DNA Metabarcoding, five new species were detected in the Swedish west coast during 2018-2020 including: *Cribrilina mutabilis* (Bryozoa), *Rhyssoplax olivacea* (Mollusca), *Spio decorate* (Annelida), *Spio symphyta* (Annelida), *Limapontia senestra* (Mollusca).

The round goby *Neogobius melanostomus* continues to expand its range in the Baltic Sea every year. In 2019, round goby was detected in Östhammar (the Archipelago Sea), which shows that the species is spreading northwards on the Swedish coast. On 19 September 2019, one individual of round goby (male) was caught in Rökan, Instön, which is located 25 km from the Gothenburg harbour. **This is the first confirmed round goby observation in truly saline water (23-25 psu), although two other citizen science observations in the Skagerrak (2015 and 2018) indicate even higher salinity conditions around 30 psu. In summer of 2020, a large quantity of round goby was found in Gävle municipality, which has been the northernmost finding in Sweden to date. Round goby was also caught in the relatively high number in Södermanland County (the southeast coast of Sweden), Gävleborg County, and eastern Gotland's coastal waters during 2021.**

The occurrence of all different development stages, from juveniles to adults and ovigerous females of the Harris mud crab *Rhithropanopeus harrisii,* indicates that this species has been established in the invaded area during 2019-2020. *R. harrisii* is now common in Blekinge County (the south of Sweden) but is also found in the area around Nynäshamn in Stockholm County in 2021. In 2020, colonies of the Australian tubeworm *Ficopomatus enigmaticus* was recorded as common in Lomma leisure marina in the Baltic Proper. No observations were made on the American lobsters *Homarus americanus* during 2019-2021. The two Asian shore crabs *Hemigrapsus sanguineus* and *H. takanoi* are found in increasing numbers on the Swedish west coast. Both observations of juveniles of the two species and egg bearing females of *H takanoi* are a strong indications of reproduction on the west coast in 2019. During 2020-2021, there were many reports of *H. sanguineus* and *H. takanoi* along the west coast down to the Sound in all stages and even ovigerous females. The Chinese mitten crab *Eriocheir sinensis* is sporadically found along the coast of eastern Sweden and up to the archipelago of Piteå in the Bay of Bothnia. In 2020, most reports were from the

southern part of the Stockholm archipelago. During 2021, several findings have also been made on *E. sinensis* along the Baltic coast (from Stockholm to Blekinge) as well as in the Halland County (the western coast of Sweden) during 2021. *E. sinensis* was also observed for the first time in Gävleborg County in 2021.

During summer 2019, five individuals of pink salmon (*Oncorhynchus gorbuscha*) were observed in the fish counter at the Herting power plant in Halland County. Several individuals of the dark false mussel (*Mytilopsis leucophaeata*) were found at two sites in Singö, the north of Stockholm County in 2019. Some large specimens of *M. leucophaeata* were observed for the first time in Galtfjärden (Östhammar manucipality) in 2020. The Japanese wireweed (*Sargassum muticum*) is seemed to have continued stable occurrence at Ringhals nuclear power plant. A large number of old (5^{+}-6^{+}) North American wedge clam *Rangia cuneate* were found in Stockholm County. Large quantities of the *R. cuneata* were also found in Smältevik (southern Sweden) in 2021. Since 2016, the Pacific oyster (*Magallana gigas*) has been expanding its range southwards. There is a gradual increase in the occurrence of Pacific oysters in the Halland County in 2021. *M. gigas* infested with *Polydora websteri* in two places on Orust (Västra Götaland County) was found in November 2021.

Content

1. Regulations

Several new activities have been undertaken by the Swedish Agency for Marine and Water Management (SwAM):

- Updating on web information and factsheets on alien species.
- National and local information meetings and workshops on alien species.
- Updating on web information on American lobsters.
- Knowledge compilation of round goby.
- Information campaign for the public – to report and collect live American lobster for genetic analysis and disease screening.
- A new national monitoring program on marine non-indigenous species started by SwAM in 2019 after many years of development and evaluation. The program is developed according to Marine Strategy Framework Directive (MSFD) to assess the scale of the pressure and impacts of marine non-indigenous descriptor D2. The methodology is based on the HELCOM guidelines for non-indigenous species monitoring by extended Rapid Assessment Survey (eRAS). 20 hotspot areas will be investigated the next coming years in Swedish coastal waters.
- The Swedish Species Information Centre has risk-classified 1033 alien plants and animals (freshwater, marine and terrestrial) on behalf of SwAM and the Swedish Environmental Protection Agency during 2017-2018. A report was published in January 2019. Twenty aquatic species from the two categories with the highest-risk (High and Severe) were further analysed for their current impact on ecosystem services. Results were published in December 2019.
- Member states shall, according to article 13 of the regulation (EU) no 1143/2014, establish and implement an action plan to address the priority pathways of unintentional introduction and spread of invasive alien species of union concern covering (at least) their own territory. SwAM and the Swedish Environmental Protection Agency (SEPA) have, in collaboration, compiled this action plan which was reported to the EU in June 2019. The action plan is based on an analysis of prioritized pathways for the unintentional introduction of invasive alien species which was conducted by the Swedish Biodiversity Centre at the Swedish University of Agricultural Science (SLU) in 2019. The results of the analysis showed 12 highly prioritized pathways, through which at least three species could spread that were classified as highly invasive (high rate of invasion and high potential damage to biodiversity). Additional information was used in the determination of prioritized pathways for

aquatic species within this action plan. Two additional pathways were included based on this, bringing the total to 14 prioritized pathways. The measures in this action plan includes, among others, communication and information to the general public, especially through non-profit organizations and to other bodies through trade organizations. Developing or sharing best praxis for operators in different fields can also be effective measures for reducing the risk of unintentional introduction and spread of invasive alien species.

- The Swedish Transport Agency and the Danish Environmental Protections Agency decided in autumn 2020 to initiate a same risk area (SRA) in the Sound, between Denmark and Sweden (11th Meeting of the Joint HELCOM/OSPAR Task Group on Ballast Water Management Convention Exemptions, document JTG-Ballast 20/08/01: https://portal.helcom.fi/meetings/TG%20BALLAST%2011-2020-763/MeetingDocuments/0801_Designation%20of%20%C3%96resund%20as%20a%20Same%20Risk%20Area%20(SRA).pdf). It is based on a validated risk model by the Danish National Institute of Aquatic Resources (Hansen *et al*. 2018). When delineating the area, Swedish and Danish authorities have adopted a conservative approach based on the connectivity and natural dispersal of the relevant species. In general, exemptions may be granted to a ship or ships on a voyage or voyages between specified ports or locations; or to a ship which operates exclusively between specified ports or locations. Within the designated SRA, an exemption has to be applied by a ship owner and decided by both Swedish and Danish authorities. It will be valid for a maximum of five years and certain field investigation will be required during the exemption period.
- Since 1 January 2021, SwAM is the agency responsible for supervisory guidance for aquatic invasive alien species. This follows from chapter 3 paragraph 5 in the governmental ordinance on environmental supervision. This means that SwAM provides guidance for aquatic species to the responsible supervisory authorities – the county administrative boards. SEPA continues with the same task for terrestrial species.
- In 2021, the Swedish and Danish authorities have respectively granted exemptions for five ferries to treatment of ballast water, according to regulation A-4. The exemption only applies to the condition that the ships operate only between the ports of Helsingborg (Sweden) and Helsingör (Denmark).

2. Intentional

No information

3. Summary of sighting

Unintentional:

New Sightings

The comb jellyfish *Beroe ovata* was sighted at the station Släggö (N 58°15.50', E 11°26.00') in the North Sea (https://www.artportalen.se/Sighting/81863021) on 23 April 2019. The Atlantic rock crab *Cancer irroratus* was discovered in crayfish trap at a depth of 27 m in North of Kullen, outside Skäldeviken in the southern Kattegat (N 56°22'0.0", E 12°26'0.0"E) on 17 April 2019 (https://www.artportalen.se/Sighting/81887473). The species has planktonic larvae, so it is likely that the rock crab could reach the coast of Sweden through ballast water from vessels. Young small crabs can also settle on ship's hulls and be transferred to new marine area. It is also likely that adult animals could come from trade in live crab and be released, since Swedish trade with Iceland brings in live crabs (https://www.havochvatten.se/hav/fiske--fritid/arter/arter-och-naturtyper/stenkrabba.html).

Sightings of shell-damage of the invasive alien Pacific oyster (*M. gigas*) outside Lysekil on the Swedish west coast (Kalvhagen, Bohuslän, Västra Götaland County) (58°14'8.5"N 11°23'53.8"E) in September 2020 initiated a study by the IVL Swedish Environmental Research Institute, to reveal the cause. The presence of the shell-boring annelid *Polydora websteri* (Polychaeta, Spionidae), a new species for the region, was established. Studies on the flat oyster *Ostrea edulis* have so far not revealed any infection with this Polychaeta, confirmed by investigations by IVL. In addition, flat oysters collected and analysed genetically (18s rRNA och cytochrome oxidase I (COI) from Fjällbacka (c. 40 km north) by the National Veterinary Institute. No infections by *P. websteri* could be revealed, but only by indigenous species of polychaetes.

The stalked jellyfish *Craterolophus convolvulus* was sighted for the first time in Väderöarna (outside Fjällbacka) (58°32.284'N 11°1.315'E) at the Swedish west coast in April 2020. It was about 4 cm long and was discovered during diving at a depth of 6 m while attached to oak tongs *Halidrys siliquosa*.

On 28 November 2020, the first report of non-native tanaid *Sinelobus vanhaareni* Bamber, 2014 (Crustacea, Tanaidacea) was made at the beach in Långedrag in Gothenburg (57°40'11.2"N 11°51'4.3"E). The species was verified by the Gothenburg Natural History Museum (Kennet Lundin, pers. comm.).

There has been no report indicating the occurrence of new species in Sweden since January 2021.

Following the European ARMS programme (ARMS-MBON) (http://www.assembleplus.eu/research/ARMS-MBON, see below 5. Research and Monitoring Programs) and using environmental DNA metabarcoding, five new species were detected in the Swedish west coast during 2018-2020 (Sundberg *et al*. 2022; Matthias Obst, Rasmus Martaeng, and Erland Lettevall pers. comm.) including:

1) *Cribrilina mutabilis* (Bryozoa) (abundance: 82367 individuals; first investigation year: 2018; coordinates: 58.876330, 11.111884) 2) *Rhyssoplax olivacea* (Mollusca) (12 individuals, 2018, 58.876330, 11.111884) 3) *Spio decorate* (Annelida) (25 individuals, 2018-2019, 58.859877, 11.080491) 4) *Spio symphyta* (Annelida) (7 individuals, 2018-2019, 58.859877, 11.080491) 5) *Limapontia senestra* (Mollusca) (1 individual, 2019-2020, 58.876330, 11.111884).

In the aforementioned sites and their surrounding areas, total number of collected samples were 15291 *Cribrilina mutabilis* during 2019-2020, 2646 *Spio symphyta* in 2020, and 89 *Limapontia senestra* in 2020 (Sundberg *et al*. 2022).

Previous Sightings

An occurrence of the toxic clinging jellyfish *Gonionemus vertens* was assumed to be the cause of burning of the skin for swimmers in Swedish west coast in July 2018 **(**Björn Källström, pers. comm.). It has been suggested that the outbreak at the Swedish west coast is linked to the exceptionally warm summer of 2018 following either a climate-driven range shift or a direct introduction to the area via shipping activity (Govindarajan *et al.* 2019). An analysis of mitochondrial COI sequences demonstrated that the Swedish medusa belong to the same clade as highly toxic populations previously found in the Sea of Japan and the northwestern Atlantic (Govindarajan *et al.* 2019). Govindarajan *et al*. (2019) speculated that the toxic clinging jellyfish belong to a species other than *G. vertens* and that there has been a cryptic invasion of this species (*Gonionemus* sp.).

Range expansions

Round goby (*Neogobius melanostomus*) has slowly been expanding its range in the Baltic Sea now commonly occurring from the site of first introduction in Karlskrona eastwards along the coast

through Kalmar strait and up to Oskarshamn then the inner part of Bråviken and the northernmost findings at Muskö, south of Stockholm as well as in Östhammar, Uppsala County. The occurrence of individuals larger than 15 cm in the catch collected from Hargshamn on the Uppland coast during summer 2019 indicated an established stock of the round goby in this area. The previous northernmost finds in Sweden have been made in Nynäshamn. On 19 September 2019, one individual of round goby (male) was caught in Rökan, Instön (57°53.397'N 11°39.941'E) which is located 25 km from the Gothenburg harbor (salinity in the surface water: 23-25 psu; Björn Källström, pers. comm.) (www.artportalen.se/Sighting/82182166). This is the first confirmed round goby observation in truly saline water (23-25 psu), although two other citizen science observations in the Skagerrak (2015 and 2018) indicate even higher salinity conditions around 30 psu.

During the fishery survey in summer of 2020, a large quantity of round goby was found in Gävle municipality (for example Furuskär (near Furuviksparken) (60°39.483'N, 17°20.439'E) (Mikael Ekman, Tove Brolin, Ulrika Tollerz Bratteby). This is the northernmost finding in Sweden to date. In Södermanland County, the southeast coast of Sweden, about 300 specimens of the round goby were registered through environmental monitoring in 2021 (My Peterson, pers. comm.). This county was invaded by round goby in 2018 when only two individuals were found. Similarly, a high occurrence of round goby has been reported in Gävleborg County (**around Furuskär, Svartstensholmen and Klövstensharen.**) in 2021 (Linda Calamnius, pers. comm.). In August 2021, a high number of round goby was caught at the fisheries surveys in Asköfjärden and Östra Gotland's coastal waters (Anders Adill, pers. comm.)

During 2019, the findings of the Harris mud crab, *Rhithropanopeus harrisii,* have been reported for a wider area than before, first more or less the whole coast of Blekinge county and then westward in Öresund and into the channels of Malmö (Matz Berggren, Martin Stålhammar, pers. comm.). The occurrence of all different development stages, from juveniles to adults and ovigerous females of this species indicates that *R. harrisii* has been established in those areas. According to the National monitoring program on non-indigenous species (2019), *R. harrisii* was spread to Sweden for the first time (in 2014) to the sea drainage basin Arkona Sea and the Southern Sound (Bergkvist *et al*. 2021). Earlier observation were in the West Gotland Basin (2014) and Bornholm and Hanöbukten Basin (2017). *R. harrisii* is now common in Blekinge but is also found in the area around Nynäskamn in 2021 (Matz Berggren, pers. comm.).

In summer 2020, *R. harrisii* was observed abundant as both adults and juveniles in the archipelago in Karlskrona, the Baltic proper. This species was first appeared in Sweden in 2014 (in Oxelösund in northern part of the Baltic proper). *R. harrisii* was also observed in Ystad (c. 140 km SW Karlskrona) and Nynäshamn (c 50 km NE Oxelsund) in summer 2020.

In 2020, colonies of the Australian tubeworm (*Ficopomatus enigmaticus*) was recorded as common in a new locality, Lomma leisure marina in 2020, the Sound in the Baltic Proper. The first finding of this species in Sweden was in 2013 in Limhamn leisure marina of Malmö (c. 12 km SW).

No observations were made on the American lobsters *Homarus americanus* during 2019 and 2020 (Mats Ulmestrand, pers. comm.). Hence, a total of 38 American lobsters has been observed in Sweden since 2008. The two Asian shore crabs *Hemigrapsus sanguineus* and *H. takanoi* are still found in an increasing number on the Swedish west coast (Matz Berggren, pers. comm.). In 2019, a huge number of *H. sanguineus* was found in the Stenungsund area Västra Götaland County (Matz Berggren, Björn Källström, pers. comm.). Many juveniles of *H. takanoi* were observed occasionally in the Koster area (north part of the Swedish west coast down to Öresund (only one finding) (Matz Berggren, pers. comm.). Occurrence of ovigerous females of *H. takanoi* in the Stenungsund area (five from 11 females found on 13 Sep 2019) indicted that his species is reproducing in the Swedish west coast Björn Källström, pers. comm.).

In 2020, there were many reports of *H. sanguineus* and *H. takanoi* in the 8-fjord area (around Orust and Tjörn) and along the west coast down to the Sound in all stages and even ovigerous females (Björn Källström, Matz Berggren, pers. comm.). Both males and females of *H. sanguineus* and *H. takanoi* were also observed in Ljungskile (Torunn Skau, pers. comm) during 2020. Furthermore, five individuals of *H. sanguineus* were found in Ringhals cooling water discharge (Jakob Looström, pers. comm.). During 2021, both *H. sanguineus* and *H. takanoi* have continued to be common with all sizes along the west coast (Matz Berggren, pers. comm.).

The Chinese mitten crab, *Eriocheir sinensis* is sporadically found along the coast of eastern Sweden and up to the archipelago of Piteå in the Bay of Bothnia (Matz Berggren, pers. comm.). In 2019, although a finding of this crab was reported from inside Lake Mälaren, there was no reports for this species in Lake Vänern where the species has often been reported in the previous years (Matz Berggren, pers. comm.). Perhaps the fishermen do not consider it as a non-indigenous species and do not report it any longer (Matz Berggren, pers. comm.). In 2020, there were many reports about observation of *E. sinensis* from Medelpad and the Uppland coast down to Blekinge / Skåne and further up to Halland, also into Lake Mälaren as water intake to Västerås thermal power plant (Matz Berggren, pers. comm.). Most reports are from the southern part of the Stockholm archipelago. A total of 24 reports, but not all are reported, interestingly none from Lake Vänern (Matz Berggren, pers. comm.). *E. sinensis* is also seemed to be more common in Karlskrona archipelago in 2020 (Karl Lundström, pers. comm.). In 2021, several findings have also been made on *E. sinensis* along the Baltic coast (Stockholm to Blekinge) and inside Lake Vänern (Matz Berggren, pers. comm.). The Chinese mitten crab was also observed for the first time in Gävleborg County (outside Furuskär) (Linda Calamnius, pers. comm.). There is an increasing report about the occurrence of *E. sinensis* as well as other two Asian shore crabs (*H. sanguineus* and *H. takanoi*) in the Halland County (the western coast of Sweden) during 2021 (Frida Sundqvist, pers. comm.)

Pink salmon *Oncorhynchus gorbuscha* was observed for the first time in river Ljusnan (Hälsingland) in 1974. Since then, stray individuals of this species have occasionally been found in Sweden. The fish is probably originating from stocking in the Arctic Ocean. During summer 2019, five individuals of pink salmon were observed in the fish counter at Herting (Ätran), in southeast Falkenberg (Halland County) (Björn Fagerholm, pers. comm.). In this fish counter, 18 individuals of pink salmon were previously observed in 2017 (Björn Fagerholm, pers. comm.).

Several individuals of the dark false mussel (*Mytilopsis leucophaeata*; ≤ 5 mm shell length) were found at two sites in Singö, the north of Stockholm County (Enholmen and Höggrundet) (Chiara D'Agata, pers. comm.) in 2019. Moreover, one individual of *Gammarus* (CF) *tigrinus* was reported in the Askö area, Södermanland County) (Chiara D'Agata, pers. comm.). The Japanese wireweed (*Sargassum muticum*) is seemed to have continued stable occurrence at the Ringhals nuclear power plant (Karin Svanfeldt, pers. comm.). Some specimens of *M. leucophaeata* (the largest one with 20 mm shell length) were observed for the first time in Galtfjärden (Östhammars manucipality) (Per Holliland, pers. comm.) in 2020.

Since 2016, the Pacific oyster *M. gigas* has been expanding its range southwards. Under a school project (see below), 30 individuals of *M. gigas* were found on the sandy beach in Båtvik (Västra Götaland County) on 27 August 2019. There has also been a gradual increase in the occurrence of Pacific oysters in the Halland County (Frida Sundqvist, pers. comm.). The Pacific oyster infested with *Polydora websteri* in two places on Orust (Västra Götaland County) was found on 27 November 2021 (Kennet Lundin, pers. comm.). It was partly in Stigfjorden by the bathing place at Rossö, partly just north of Mollösund (Orust Municipality) (Kennet Lundin, pers. comm.)

During 2019 the dinoflagellate *Dinophysis tripos* (possibly harmful) has been more frequent in Swedish waters (Marie Johansen, pers. comm.). One single cell of the dinoflagellate *D. caudata*, which is a tropical-subtropical species and occurs in the southern Europe, was also found during

fall 2019 (Marie Johansen, pers. comm.). Nevertheless, these two plankton species, are most probably transported by water currents and not by humans, so these are not NIS for Sweden. In 2020, some number of invasive algae including *Dasya baillouviana*, *Dasysiphonia japonica* and *Bonnemaisonia hamifera* as well as pacific oyster were reported in Ringhals power plant. Moreover, a large number of old (5^{+}-6^{+}) North American wedge clam *Rangia cuneate* were found in Brandalssund, and in a bay even closer to Södertälje (Stockholm County) (Lena Kautsky, pers. com). *R. cuneata* was discovered in Svensksundsviken, a marine reserve in Bråviken, the Baltic Sea, during 2016. In a joint project between Linnaeus University and Klamar County, large quantities of the *R. cuneata* were found in Smältevik (southern Sweden) in 2021 (Helén Wåhlstrand Persson, pers. comm.). In autumn 2021, shells of *R. cuneata* were found in Raggarö and in Slätön-Medholma nature reserve, north of Norrtälje (Per Holliland, pers. comm.). This mussel was also found in Bolviken, Norrtälje municipality, and reported in high abundance in Brandalsund (Lena Kautsky, pers. comm.).

There is risk to overlook the two coastal shrimps, which were found in neighboring waters (*Palaemon macrodactylus* and *Palaemon tigrinus*) of eastern Denmark and Baltic coasts of Germany, in Sweden due to their similarity to Swedish indigenous species (*P. adspersus* and *P. elegans*) (Matz Berggren, pers. comm.). Public awareness about these two species can be increased.

In 2021, the Norrbotten County Administrative Board carried out inventories of 95 marine coastal sites with the aim of mapping the spread of the western waterweed (*Elodea nuttallii*), and noting any other invasive alien species (Lena Bondestad & Sara Byrsten, pers. comm.). The western waterweed were reported at several new premises in Norrbotten County, and at some of these premises the species was noted in a very large distribution and in a high degree of coverage, which has rarely been the case before (Lena Bondestad & Sara Byrsten, pers. comm.). The prevalence of the American waterweed (*E. canadensis*) has been noted to vary greatly from year to year in some places in the Norrbotten County (Lena Bondestad & Sara Byrsten, pers. comm.).

4. Pathogens

The shell-boring annelid *P. websteri* infested the pacific oyster in Swedish west coast in 2020. There is a non-validated report on oyster plague (Ostronpest) in Halland County (Frida Sundqvist, pers. comm.).

5. Research and Monitoring Programs

1) **From a research grant call in 2020 on research and development on eradication and management of invasive alien species**, SEPA, SwAM, The Swedish Transport Administration and the Swedish Research Council for Sustainable Development has provided funding for eight project sharing 33 million SEK during 2021–2024. Some of the granted projects are on marine species and management (Forskning om invasiva främmande arter - Naturvårdsverket (naturvardsverket.se)).

2) **Round goby – turning risk to resource (2021-2024)**

The aim of the project is to unite stakeholder needs and scientific practice to tackle the challenges to ecosystem services caused by the invasive round goby. The purpose is to quantify the impact of round goby in Swedish waters, develop and test new methods to decrease their negative effect by reducing dispersal and reducing their population, and to investigate if there is potential to capitalize on this species for a fishery.

3) **Dynamic management of the invasive Pacific oyster in Sweden (2021-2024)**

The ambition of the project is to facilitate the management of marine invasive alien species with a commercial value in order to contribute to the fulfillment of both ecological and socio-economic goals in accordance with the principles of circular economy and sustainability. Pacific oyster is

used here in a case study to develop a dynamic management model based on a zoning of the Swedish coast with specific management goals and measures in each zone.

4) **Action plan for invasive species in an aquatic environment (2021-2024)**

The project will use two invasive model species in a well-defined geographical area to be able to develop a high degree of detailed local and species knowledge. The project aims both to develop descriptions and advice for cost-effective practical field methods for the eradication and control of invasive alien species for stakeholders and purchasers, as well as to develop a new methodology to facilitate responsible authorities to improve the work of managing marine invasive alien species in Swedish coastal areas.

5) **Predators and biodiversity as biological control of the invasive round goby on the Swedish west coast (2021-2024)**

The project will help protect the marine coastal environments from being colonized by the invasive round goby. The project investigates whether predators such as cod and eel through predation can limit the distribution of round goby on the west coast. The project also looks at whether round goby lacks niche space in areas with high biodiversity. If these two ecological processes prove to limit the rate of invasion, conservation of both predators and biodiversity can be used as ecological "biocontrol" to limit the spread of the species. The results from the study can in turn be used to highlight the value of protecting both predators and biodiversity.

6) The **projekt "Nya arter"** is financed by County of Västra Götaland involving schools along the west coast of Sweden. In the project, there are 15 schools, about 35 teachers and 1500 students who learn more about invasive alien species and conduct their own field research. Following alien species are in focus (and investigated with different methods: *Ficopomatus enigmaticus, Austrominius modestus, Watersipora subtorquata, Caprella mutica* (panels), *H. takanoi, H. sanguineus* (cages) *M. gigas* (collection) and *N. melanostomus* (fishing with cage and angling). This project was run in 2017-2019.

In 2020, this project received funding from SwAM and the County Administrative Board of Västra Götaland to be further developed and operated during the period 2020-2023. During this period, the school project will be expanded to cover the entire coast of Sweden. It will involve new municipalities with interested teachers and students in primary and secondary schools as well as new participating researchers and additional "selected" marine invasive alien species that are found, or may be suspected to appear, in various areas along the coast will be added to the project. The reporting of findings in the school project will take place directly in the new version of SwAM's Rappen 2 (Björn Källström, pers. comm.). (Rappen see below 5. Research and Monitoring Programs.)

7) **Round goby in Blekinge County and Kalmar County (granted in 2019):** the purpose of the project is to investigate the seasonal distribution, movement patterns and habitat utilization of round goby in the area. Environmental toxin analysis will be performed and the importance of round as prey, predator and competitor (including behavioral interactions with native species) will be investigated.
Some investigations are underway to monitor the importance of round goby in the southern Baltic Sea as a prey species for natural predators and partly as a predator on other species (Karl Lundström, pers. comm.).

8) **The Goby fishing – a new resource**, here, Kalmar municipality is project coordinator who together with partners will investigate the potential for commercial fishing on the round goby (Martin Stålhammar, pers. comm.).

9) **The Swedish surveillance system on early detection of NIS consist of several monitoring program in marine environment**. The national monitoring program on marine NIS was launched by SwAM in 2019. It consists of 20 sampling sites in harbours, marinas and water ways of the Baltic Sea and the North Sea. In 2020 a niche model was made on hotspots for introduction of IAS, in order to identifying optimal monitoring stations in marine waters. The model is based on the calculation of the total invasion risk for 34 target marine and brackish IAS relevant for Swedish waters. On the basis on the results SwAM has pointed out 20 monitoring stations in the Baltic Prober and the North Sea. Similar niche modelling of limnic IAS will continue in order to identify further monitoring stations in the Bothnian Bay (see Bergkvist *et al*. 2020a)

The methods used in the national monitoring program on NIS in harbors, marinas and water ways using methods are based on the Helcom and Opsar port survey protocol of the Joint Harmonized Procedure for BWMC (HELCOM-OSPAR-Joint-Harmonized-Procedure-for-BWMC-A-4-exemptions_2020.pdf) and Helcom Guidelines for non-indigenous species monitoring by extended Rapid Assessment Survey (eRAS) Guidelines-for-monitoring-of-non-indigenous-species-by-eRAS.pdf (helcom.fi). The evaluation of the methods and results from the 2019 year's monitoring are presented and published in 2020–2021 (see Bergkvist *et al*. 2020b)

10) **Sweden has updated the monitoring programs for MSFD in 2020.** In total 14 monitoring strategies are described in a report. In addition, 48 online sheets describe the individual monitoring programs. For Descriptor 2 Non-indigenous species, in total three monitoring programs are running: The national monitoring program (as described above), citizen science and recipient control program of nuclear power plants and pulp mills. More information is available at:

SwAM's web site on monitoring of non-indigenous species: Främmande arter - Miljöövervakning - Övervakning och uppföljning - Havs- och vattenmyndigheten (havochvatten.se).

Sweden's report to the EU Commission: Uppdaterade övervakningsprogram för Nordsjön och Östersjön - Havsmiljöförvaltning - Planering, förvaltning och samverkan - Havs- och vattenmyndigheten (havochvatten.se).

An important part of the surveillance system for early detection and rapid response to tackle IAS is citizen science. In 2015, the web app Rappen was launched to facilitate reporting of alien species for citizen science. In late 2020, the second version was developed (www.havochvatten.se/Rappen). Beside improved interface and more user friendly the new app is directly integrated with Sweden's largest database for Species Observation System (SLU Swedish Species Information Centre) which enable faster and safer validation and publication of species findings. Rappen 2 was released in March 2021.

11) **ARMS-MBON**

The European ARMS programme (ARMS-MBON) is a network of Autonomous Reef Monitoring Structures (ARMS) placed in the vicinity of marine stations, ports, marinas, and Long-Term Ecological Research (LTER) sites distributed over Europe and polar regions. The aim of ARMS-MBON is to assess the status of, and changes in, hard-bottom communities of near-coast environments, using genetic methods supplemented with image analysis and visual inspection methods. ARMS-MBON is part of GEO BONs Marine Biodiversity Observation Network (MBON). The project is primarily funded through the Joint Research Activity (JRA1) of ASSEMBLE Plus, with financial contributions from the Swedish Agency for Marine and Water Management, the Interreg project GEANS and with considerable in-kind contributions by the participating institutes. The project is an activity of the Genomic observatories network with relations to the European Marine Biological Resource Center (EMBRC). A genetic observation network was established with 8 stations along the Swedish west coast plus an additional two in Denmark (Limfjord, Laesö) (Matthias Obst. pers. Comm.).

(http://www.assembleplus.eu/research/ARMS-MBON)

12) **IAS Hotspot Model**

The scripts in the repo are designed to use species distribution models (SDM) to identify potential high-risk areas for the introduction, establishment and spread of invasive arteries in Swedish water. The SDM workflow is based on a modeling workflows developed by University of Gothenburg (Leidenberger *et al*. 2015; Laugen *et al*. 2015; Stelzer *et al*. 2013; Karlsson *et al*. 2019) and was further adapted to model a large number of known invasive species present in the region but not yet established in Sweden.

13) **Round goby - mapping and method development to prevent / limit continued spread along the Baltic coast**. A joint project between - a joint project Uppsala and Gävleborg County administrative boards" in 2021 (Linda Calamnius, pers. comm.), which has received wide attention by different Swedish media.

6. Meetings

1) **Flora and Fauna Conservation Conference (SLU, April 2019):** The theme of this conference was "Nature in transformation - alien species and nature conservation". The risk with alien species in Swedish nature was discussed.

2) **The Pacific oyster – a new Nordic food resource and a basis for tourism (Gothenburg Feb 2019):** The workshop had 44 participants from Sweden, Norway and Denmark (both researchers, industry and authorities) and was about the management of Pacific oysters (*M. gigas*).

A report (https://www.norden.org/no/publication/hosting-av-stillehavsosters) and a policy brief (https://www.norden.org/en/publication/policy-brief-pacific-oyster-new-nordic-food-resource-and-basis-tourism) was published. At this workshop, mapping of the Pacific Ocean's distribution in Koster National Park and around Tjärnö, as well as around Orust were produced. Further mapping will be carried out in the Grebebstad–Lysekil area.

3) **Sweden participated in meetings at EU-level** namely the Invasive Alien Species Expert Group (IASEG) and the Invasive Alien Species Working Group (2020). These meetings covered amongst other topics news regarding the European Alien Species Information Network (EASIN) and the new EU Biodiversity Strategy for 2030 and, more specifically, the target relating to Invasive Alien Species (IAS). In November 2020 Sweden participated in the European Commission's Joint Research Centre (JRC) meeting which dealt with non-indigenous species thresholds workshop in the context of the Common Implementation Strategy of the MSFD. There was no meeting of the Committee on IAS.

4) **Together with SEPA, SwAM organized two webinars in 2019 and 2020** (one per year) to inform the county administrative boards of the ongoing work to prevent and manage invasive alien species, both aquatic and terrestrial.

5) **SwAM and SEPA created an information campaign** to increase public awareness of invasive alien species and how to prevent proliferation. The campaign was launched during both summer 2019 and 2020. IUCN, other NGOs and agencies participated. In addition, SwAM and SEPA conducted for the third year in a row a national information initiative on IAS together with 60 organisations such as county administration boards, municipalities and NGO:s . The theme this year was “More dangerous than you think” and was primarily a social media campaign. It ran from May to September 2021.

6) **In 12 May and 10 November 2021 SwAM and SEPA conducted meetings** of the national cooperative network on the work against invasive alien species. The network involves authorities with specific responsibilities under the national regulation on IAS. Their task is to inform

each other about the current state of IAS work, on new legislation, as well as on important cases and decisions in principle in their respective areas of responsibility.

7) **On 10 January 2021 was an online workshop by HELCOM** on the Early Warning System on findings of harmful aquatic organisms and pathogens in the Baltic Sea and its implementation in the AquaNIS information system". It was organised in the frame of the EU INTERREG Baltic Sea Region project COMPLETE (2017–2020). At this workshop the concept of the early warning system (EWS), its technical implementation and practical experience of its use will be presented. Joint HELCOM/OSPAR TG Ballast meeting parties agreed on the need for such a workshop for national focal points, i.e. institutions, responsible for the detection of HAOP and non-indigenous species, port authorities and other interested stakeholders. The Swedish partner of COMPLETE was the Chalmers University of Technology. Participators in the workshop were SwAM and Swedish Metrological and Hydrological Institute (SMHI).

8) **The Department of Aquatic Resources at SLU had some meeting** with reference group for round goby (2021).¨

9) **There have been some seminars** such as IAS Norden, IAS Law and Combating IAS in 2021 (Kristin Dahlgren, Chatarina Karlsson, pers. comm.).

10) **On 16 September 2021 a national workshop was conducted** on how to implement an early warning system (EWS) based on the project COMPLETE's frameworks for the Baltic Sea area. This proposed EWS on HAOP Harmful Aquatic Organisms and Pathogens (HAOP) findings should send calls for preventive measures to all relevant authorities in the HELCOM countries and international shipping in the Baltic Sea area.

The workshop clarified the different roles and responsibilities of the national authorities and agencies, as well stated the useful great national competence of this matter. Further, SwAM and the Swedish Transport Agency have to discuss and foresees the role of the Swedish detection, reporting and data provision, expertise as well as focal point for warning calls and actions. How the Swedish EWS HAOP will be implemented will be a later issue. The workshop was arranged by SwAM, the Swedish Transport Agency and the Danish Environmental Protections Agency and the SLU Swedish Species Information Centre. In total we gathered 12 national authorities as in addition to the organizers participated the National Food Agency, the County Administrative Board of Västra Götaland and Västerbotten county respectively, SMHI, the National Veterinary Institute, the Swedish Coast Guard, the Swedish Maritime Administration, and the Chalmers University of Technology.

11) **SEPA and SwAM held a national webinar** on IAS for the County Administrative Boards on 4–5 November 2020 (Linda Backlund, pers. comm.).

12) **Coastal water network IAS in 2021** – a collaboration between county administrative boards – held a meeting which will be continued in 2022 **(**Frida Sundqvist, **My Peterson, pers. comm.).**

13) Halland County Administrative Board has participated in a reference group in the DynamO project for Pacific oysters, which is run by the IVL Swedish Environmental Institute, in 2021, and this work will also continue in 2022. The board will also participate in a reference group for a research project on round goby at the University of Gothenburg in 2022 (Frida Sundqvist, **pers. comm.).**

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Overview

2019–2021: Three new marine species were reported or identified in 2020, the Atlantic croaker *Micropogonius undulatus*, an Asian bivalve *Theora lubrica*, and a polychaete *Paleanotus chrysolepis.* The amphipod *Caprella scaura* was also discovered in 2020, with records from the same site subsequently backdated to 2009. One new species was recorded in 2019, the polychaete *Lepidasthenia brunnea*, and another was first reported in 2019 from 2017 records, the freshwater/brackish amphipod *Crangonyx floridanus*. The polychaete *Bispira polyomma* was recorded in 2021, as was a specimen provisionally identified as rosy barb (*Pethia conchonius*, formerly *Puntius conchonius*). The leathery sea squirt *Styela clava* was recorded in Scapa Flow, Orkney in December 2020. This is new location for *S. clava* in Scotland and the northern most record of the species in the UK (Want & Kakkonen 2021).

The anticipated invasion of UK rivers by pink salmon *Oncorhynchus gorbuscha*, as a repeat of that observed in 2017, did not occur. In 2019, relatively few specimens were reported (33), and these almost entirely in Scottish rivers (21 fish), with four pinks in England, one in Wales and nine in Northern Ireland. In 2020, no specimens of pink salmon were reported for England, Northern Ireland and Wales, though this lack of reports could either be a true reflection of pink salmon incursions or the result of reduced surveillance due to Covid-19 restrictions on travel. In 2021, no pink salmon were reported for Wales, but 26 pink salmon were reported for England, mainly in the northeast. However, one pink salmon was a trapped in the River Tamar, which is the most western report for England ever. The greatest number of pinks salmon reported in 2021 was in Scotland, where about 169 pink salmon were captured from 29 different river basins, though these records are an underestimate of the number of pink salmon present. The first tentative report (based on a published photo) of a lionfish (*Pterois* sp.), captured Wednesday 22 September 2021 by an angler off of Chesil Beach, Dorset, was announced on various internet sites (e.g. Chao-Fong 2021).

Following release (and a journal publication about) of a multi-lingual version of the Aquatic Species Invasiveness Screening Kit (AS-ISK), described in Copp *et al*. (2021), the global trial of the AS-ISK, a contribution to the WGITMO ToR, reached its conclusion during 2020, with the journal article submitted and published online in May and then definitively in September 2021 (Vilizzi *et al.* 2021).

Content

1. Regulations

2019–2021: The UK government launched a consultation in 2019 on how best to manage invasive non-native species that threaten native wildlife. Under EU law, management measures must be put in place for widespread invasive alien species. The eight-week consultation asked for views on ways to manage populations of species of concern of which the aquatic species listed were mainly inhabitants of freshwater environments, i.e. Nuttall's waterweed (*Elodea nuttallii*), floating pennywort (*Hydrocotyle ranunculoides*), curly waterweed (*Lagarosiphon major*), parrot's feather (*Myriophyllum aquaticum*), signal crayfish (*Pacifastacus leniusculus*) and all sub-species of slider terrapins (*Trachemys scripta*), and Egyptian goose (*Alopochen aegypticus*). However, one marine/brackish water inhabitant was listed: Chinese mitten crab (*Eriocheir sinensis*). This consultation was followed by the UK government's enactment of the statutory instrument, the Invasive Alien Species (Enforcement and Permitting) Order 2019, which came into force on 1 December 2019 and thus aligned UK and EU legislation.

In Northern Ireland, the Invasive Alien Species (Enforcement and Permitting) Order (Northern Ireland) 2019 ("the Enforcement Order") came into force on 1 December 2019 (www.legislation.gov.uk/nisr/2019/159/made). The consultation and letters can be found at: www.daera-ni.gov.uk/consultations/consultation-management-measures-widely-spread-invasive-alien-species-ias-northern-ireland.

2. Intentional introductions

Synthesis of introductions

Fish

2019–2021: One silver dollar fish (*Metynnis argenteus*) and two jaguar cichlids (*Parachromis managuensis*) were recovered from the River Ness during September 2020. These were most likely fish discarded by aquarists. All three specimens were dead on discovery. Two live gold spot common pleco (*Pterygoplichthys joselimaianus*) were captured by electrofishing in the River Kelvin (Glasgow) during August 2021. These were also most probably unwanted specimens released by an aquarist. In October 2021, a specimen provisionally identified as rosy barb (*Pethia conchonius*; formerly *Puntius conchonius*), was captured during a fisheries survey of a pond in Golders Hill Park, London (Latitude = 51.567401, Longitude = –0.192269).

Invertebrates 2019–2021: None reported.*

Plants 2019–2021: None reported.*

*Note: Travel restrictions due to the Covid-19 pandemic may have affected species reporting.

3. Summary of sightings

Unintentional introductions:

Species	Location	Date	Note
polychaete (*Syllis garciai*)	Offshore Overfalls MCZ	24/01/19	New location for the UK

scale worm (*Lepidasthenia brunnea*)	Greater Haig Fras MCZ	09/06/19	Mediterranean species new to UK
crangonyctid amphipod (*Crangonyx floridanus*)	Lake Windermere in Cumbria, and Smestow Brook, West Midlands	Sept. 2017, July 2018, Sept. 2018	New record for UK first reported in 2019 by Mauvisseau *et al.* 2019
dwarf crab/ Harris mud crab (*Rhithropanopeus harrisii*)	Tidal section of the River Thames	October 2019	First record for England (but previously recorded in Wales)
slipper limpet (*Crepidula fornicata*)	Numerous locations on the South Coast, from Kent to Devon and the Channel Islands: Ices rectangles: 26E7 / 27E8 / 27E9 / 29E6 / 30E6 / 30E7 / 30 E8 / 30E9 / 30F0 / 30F1 / 31F1 29E7, 29E8, 31E5	2019 2020	Very high numbers occurring in beam trawls (23–176 limpets per trawl) throughout the English Channel
leathery sea squirt (*Styela clava*)	English Channel from Sussex to Southampton; Channel Islands; South-west Wales ICES rectangle: 27E7 / 27E8 / 28F0 / 29E8 / 29F0 / 30E8 / 30E9 / 30F0 / 32E5 Scapa Flow, Orkney Islands, Scotland 26E7, 28E7, 30E7, 31E5	2019 18/12/2020 2020	Further record for Sussex where the species is rare New location for Scotland
orange tipped sea squirt (*Corella euymota*)	Shetland Islands, Scotland	2020	Observations from natural habitat
polychaete (*Paleanotus chrysolepis*)	Newlyn, Cornwall	10 March 2020	First report for the UK

New sightings: new country records or new sub-region records

Fish

2019–2021: An Atlantic croaker (*Micropogonius undulatus*) was caught 4 miles SE off of Plymouth, Cornwall/Devon on 7 October 2020. This young individual was 285 mm in length (assumed to be total length), and identified by experts when found at the Plymouth fish market. The first report of the species Europe, along the Belgium coast in 1998 and 2001 (Stevens *et al.* 2004), suggested that ballast water was the likely introduction vector for this North American species.

A report was received in March 2020 of specimens of gibel carp (*Carassius gibelio*) being present in at least one water body in southern England (Copp & Sayer 2020). This species, which occurs in both brackish and fresh waters, has apparently been present in that water body for a few years but remained unreported until 2020. The first tentative report of a lionfish (*Pterois* sp.), captured Wednesday 22 September 2021 by an angler off of Chesil Beach, Dorset, was reported on various internet sites (e.g. Chao-Fong 2021).

Invertebrates

2019–2021: In 2020, the first UK records were published for the Asian bivalve (*Theora lubrica*). This mussel was first found in a survey by the APEM consultancy in Lowestoft Harbour, Suffolk, in 2018 (Taylor 2020; Worsfold *et al.* 2020). The second UK record, and first for Scotland, was of a single shell found at Granton Harbour, Edinburgh, in May 2020. (Notton 2020).

The polychaete worm **(***Paleanotus chrysolepis*), first reported for the UK at Newlyn (Cornwall) in March 2020, has a widely-scattered global distribution, which includes the Mediterranean and Red seas, the Indian Ocean, Australia, New Zealand and South Africa (www.cmar.csiro.au).

The skeleton shrimp (*Caprella scaura*) was added to the list of UK non-natives by Martínez-Laiz *et al.* (2021). First noticed in Sovereign Harbour Marina, Eastbourne, in August–September 2020, the amphipods' presence at the same site was backdated to October 2009 and August 2014 from material preserved during previous surveys.

The sabellid polychaete (*Bispira polyomma*) was recorded from two adjacent marinas in Plymouth, SW England, in summer/autumn 2021 (J. Bishop, MBA, confirmed by Teresa Darbyshire, National Museum of Wales). To date the species has been recorded, as a presumed non-native arrival, from the Netherlands and Belgium.

An amphipod of the genus *Ptilohyale* was discovered in a seaweed holdfast that washed ashore at Newlyn, Cornwall in August 2020. This represents a new non-native taxon for the UK (as there are no native species in that genus). However, because of damage to key features of the specimen, it could not be identified to species level. The most likely candidate is *P. littoralis*, a Pacific species recorded from France and Normandy. Photos of this specimen are available at: www.aphotomarine.com/amphipod_ptilohyale_sp_20-08-20.html

In 2021, the presence of the Uruguayan mud snail (*Heleobia charruana*) in UK waters was first established, although it was first collected in the Thames Estuary in 2003. Identification of samples from the Thames has only recently been confirmed by morphological and molecular methods. There are now records from a 40 km stretch of the tidal Thames, where in places it is the dominant macrofaunal species (Van Haaren *et al.* 2021).

First reports from the UK were published in 2019 from freshwater sites for the amphipod (*Crangonyx floridanus*), which also occurs in brackish waters elsewhere in its non-native range (e.g. California). Mauvisseau *et al.* (2019) report its discovery at two locations separated by over 200 km: Lake Windermere in Cumbria, and Smestow Brook in the West Midlands. Both sites are well connected to the UK river and canal network, so further spread, possibly into lower reaches of

rivers and into estuaries, is considered likely. Records in the UK's biodiversity database, the NBN Gateway, from Environment Agency surveys in 2019 and 2020 suggest that this species as now widespread across southern England. *Crangonyx floridanus* was also recorded during 2021 in Allt Loraich – a stream within the catchment of the River Spean (western Scotland).

Four individuals of the dwarf crab, a.k.a. Harris mud crab (*Rhithropanopeus harrisii*), were observed at one location in the tidal River Thames (Central London) in October 2019, as part of a baseline survey for development (Jarvis & Clark 2021). This appears to represent a second population in the UK, in addition to that first found in Cardiff Dock, South Wales in 1996.

Cefas' Marine Protected Area (MPA) surveys have identified new records for polychaete worms normally restricted to warmer Mediterranean waters, with 2019 being the first UK record of the Mediterranean polychaete (*Lepidasthenia brunnea*). This small scale worm originally described off South Africa, but known from the Mediterranean was recovered in samples from the Greater Haig Fras MCZ, west of Cornwall. Surveys by the MPA continued to record range extensions and new records for benthic invertebrates with the polychaete (*Syllis garciai*), which is native to the Western Mediterranean found in the Offshore Overfalls MCZ in Southeast England. Additional records of *S. garciai* were found around the Southwest Coast of England and southern Irish Sea in 2020, but the record from Southeast England remains exceptional. Cefas' integrated monitoring surveys recorded a large number of specimens of the slipper limpet (*Crepidula fornicata*) and the leathery sea squirt (*Styela clava*) in the western Channel in 2019 and again at the same locations in 2020, but there was a marked expansion in positive records among many more locations in the Western Channel. Provisional records of new taxa or range expansions seen in the MPA and in the macrofauna data of the integrated monitoring surveys, which have not been confirmed due to delays imposed by Covid-19 restrictions so cannot be included here.

The bryozoan (*Schizoporella japonica*) was found in Ballycastle Marina (Co. Antrim) in Sept. 2021 by D. Diamond (DAERA) and B. Picton (NMNI). This is the first record for Northern Ireland.

Plants

2019–2021:

Red seaweed (*Anthithamnion nipponicum*) was recorded in Scapa Flow, Orkney in 2020. This is the first known record of this species in the UK. The specimen was recorded in a scrape sample collected from a navigation buoy during the 2020 non-native species monitoring surveys by Orkney Islands Council Harbour Authority. For further information, contact Jenni Kakkonen (jenni.kakkonen@orkney.gov.uk)

In 2020, the first record of attached, established Japanese wireweed (*Sargassum muticum*) was made in Orkney Islands, Scotland. Several plants were observed growing in shallow pools on the West Coast of Orkney. A survey in 2021 confirmed this record and further records of *S. muticum* were made in several locations along the west and north-west coastline in 2021. Samples were collected in 2021 and sent to Marine Scotland Science (MSS) for their molecular research on the genetic diversity of *S. muticum* in Scotland. For further information, contact Jenni Kakkonen (jenni.kakkonen@orkney.gov.uk)

The first UK record of the red alga (*Antithamnion hubbsii*) was reported from Portland, Dorset in 2020 (Baldock *et al.* 2020).

The presence of two species of red algae, *Polysiphonia morrowii and P. delicata*, in the UK were reported for the first time in 2020 (Piñeiro-Corbeiro *et al.* 2020), using molecular methods of identification. The identification of *P. morrowii,* which is considered native to eastern Asia, was based on specimens collected from a large population in a yacht marina in Plymouth, Devon in April 2017. *Polysiphonia delicata* (of unknown native range) was identified from a drift specimen collected at Studland, Dorset in May 2017.

Significant new records of previously-recorded species

(new records within the same sub-region)

Fish

2019–2021: Various species and hybrids of sturgeon, including sterlet (*Acipenser ruthenus*) and Siberian sturgeon (*Acipenser baerii*), are regularly caught by rod-and-line anglers in the UK, most notably in England. Under UK law, these species should be present in managed, consented fisheries only, but captures of escapee non-native sturgeons are reported in the angling press (including social media and internet sites). Following the invasion of rivers of Great Britain in 2017 by pink salmon (*Oncorhynchus gorbuscha*), also known as 'humpback salmon', specimens were reported in small numbers in the UK (England, Scotland, Wales) and the Republic of Ireland (www.wildtrout.org/assets/img/general/Pacific-Pink-Salmon-Update-2019-Final-002.pdf). These include a few specimens captured in July 2019 in the lower River Dee and in the River Kyle of Sutherland (Scotland). Also, in Scotland, no pink salmon were captured from the River Ness in 2019, but a live specimen was recorded on underwater cameras on 26 August 2019. And, a dead male was found by an angler on the banks of the Ness at Whin Park on 11 September 2019 – the carcass was sent to MSS. The first known record of pink salmon on the River Findhorn was a dead specimen found at Gordon's Pool on the lower Findhorn on 26 August 2019, by local angler – the fish was male, length 53 cm and weight 0.7 kg (1.5lb). During 2019, the Fisheries Management Scotland reported pink salmon to have been recorded in a number of Scottish rivers, including the rivers Awe, Dee, Deveron, Esk, Irvine, Kyle of Sutherland, Polla, Spey and Tweed. The numbers recorded were much lower than observed in 2017 (www.fnlft.org.uk/2019/08/pink-salmon-in-findhorn/). To assess the extent of pink salmon incursions of UK rivers in 2019, a UK-wide survey was using eDNA detection methods was undertaken in August and September 2019, and in 2021 (details here below under 'Research and Monitoring Programmes').

No additional pink salmon records were received in 2020 for any part of the U.K. There may be two explanations for this. First, the pink salmon has odd-year and even-year cohorts, and the recent rise in pink salmon is based on an 'odd-year' stock, which is established in northern Europe (mainly Norway). Therefore, few pink salmon would be expected to arrive in 2020, though occasional sightings have been recorded in even years, e.g. 2018 (ICES-WGITMO 2020). Second, the Covid-19 pandemic incited lockdown and travel restrictions, which reduced angling effort for Atlantic salmon (*Salmo salar*), and limited surveillance by furloughed fisheries management staff, so it is possible that pink salmon could have gone undetected in UK freshwaters during 2020. In 2021, no pink salmon were reported for Wales, but 26 pink salmon were reported for England, mainly in the northeast. However, one pink salmon was a trapped in the River Tamar, which is the most western report for England ever. In Scotland, about 169 pink salmon were captured in 2021 from 29 different river basins and two coastal locations, with the vast majority from the rivers Thurso, Tweed and Dee (Aberdeenshire), Spey and Oykel, in descending order. Reports of 1–4 specimens were received for the other 24 river and two coastal locations.

In Northern Ireland, there are sporadic escapes of rainbow trout (*Oncorhynchus mykiss*) from several fish farms, however the numbers picked up in the River Strule catchment in particular have greatly decreased.

A single lionfish (*Pterois sp.)* was reported to have been caught off Chesil beach in Dorset in September 2021 by an angler, with photographs of the specimen posted on social media. The Association of Inshore Fisheries and Conservation Authorities has reported further likely catches of this species in Lyme Bay, Dorset in 2021 but none were recorded or reported.

Invertebrates

2019–2021: The following records from Northern Ireland were received in 2019:

Species	**Location** (in N. Ireland)	**Date**	**Note** (geo-coordinates)
Austrominius modestus	Strangford North	June 2019	various locations at or near Lat./Long. 54.560133/–5.629225
			54.54464/–5.69878
	Belfast Lough	Sept. 2021	54.247362/–5.855012
	St Johns Point		54.511179/–5.550844
	Carrickfergus marina		
	Inner Dundrum Bay		
Asterocarpa humilis	Rathlin Island	Sept. 2021	55.262103/–6.186052
	Strangford Lough		54.44994415/–5.632422155
	Carrickfergus		54.71093612001578/–5.812189572
Botrylloides violaceous	Strangford Lough	Sept. 2020	54.489862/–5.644173
	Belfast Outer	Sept. 2021	54.71093612001578/–5.812189572
	Belfast Harbour		55.206621/–6.239503
Caprella mutica	Belfast Outer	July 2019	54.664611/–5.671717
	Carrickfergus marina	June–Sept. 2021	55.262103/–6.186052
	Rathlin Island		54.44994415/–5.632422155
	Strangford Lough		54.483042/–5.64782
	Belfast Harbour		55.206621/–6.239503
Corella eumyota	Strangford North	May 2019	54.531732/–5.56146
	Larne lough	Sept. 2020	54.818095/–5.775367
Crassostrea (Magallana) gigas	Strangford North	June 2019	55.14738301/–7.160736241
	Larne lough	July 2020	54.823495/–5.752932
Cymbovula acicularis	North Channel	June 2019	55.20816981/–6.114486094
Dugesia japonica	Carlingford	May 2019	54.04004735/–6.172953192
Dreissena polymorpha	River Articlave at Ardina Bridge, Bann Brook at Quilley Road Bridge, Lower Bann at Portglenone, River Sillees at Drumkeen New Bridge and at Carr Bridge	March, April & Sept. 2018	IC 789 350, IH 230 413, IH 130 471, IC 975 038, IH 120 497, IC 794 335, IC 975 038
	Finn River at Wattle Bridge		

		12/04/2021	IH 425 203
Perophora japonica	Strangford Lough	Aug. 2020	54.490242/–5.648692
Schizoporella japonica	Ballycastle	Sept. 2021	55.206621/–6.239503
Styela clava	Belfast Outer Belfast Harbour Larne Lough	July 2019 Aug. 2020	54.664611/–5.671717 55.206621/–6.239503 54.818095/–5.775367
Tricellaria inopinata	Carrickfergus marina Belfast Harbour	Sept. 2021	54.71093612001578/–5.812189572 54.605047573379885/–5.914084536

Survey work has revealed the Asian clam (*Corbicula fluminea*) to be abundant in Cardiff Bay, with an estimated density of 110 clams per m^2. This represents the second record for Wales, following a previous record from Port Talbot Docks in 2006, where it is thought to have then died out when the site was flooded with sea water (Willing, 2020). First detected in Northern Ireland in the tidal section of the River Foyle in March 2016, the Asian clam has since spread into some of its inflowing tributaries (River Foyle (main channel and backwater), Burn Dennett, Glenmornan, Swilly Burn). Further surveys of these sites are planned for 2021 (Covid 19 restrictions permitting) along the following water courses: River Foyle (main channel and backwater), Burn Dennett, Glenmornan, Swilly Burn. For further information, contact Mark McCauley (mark.mccauley@loughs-agency.org).

Although not yet *recorded in Northern Ireland,* the quagga mussel, *Dreissena rostriformis bugensis, has been detected in early summer 2021 in loughs Ree and Derg (Republic of Ireland), as well as the Shannon River that connects these two lakes.* The Shannon-Erne waterway is a potential dispersal route into Northern Ireland

(http://invasivespeciesireland.com/news/first-discovery-of-population-of-quagga-mussel-in-ireland/)

Only three reports were received in 2019 for the American lobster (*Homarus americanus*) being found in UK waters: one adult in Cornwall and two juveniles in Northumberland and Scotland, the latter of which underwent molecular testing (in addition to the visual identification). However, owing to the preservatives used, the assessment was inconclusive. In 2020, there was one report of American lobster, in December off the coast of Sussex. An awareness campaign was launched across the fishing industry in early 2020, and again in 2021, to ensure that fishers are aware of the issues around American lobsters, and to encourage them to report all possible sightings.

In September 2020 in the County of Dorset, a live specimen of the Asian shore crab *Hemigrapsus sanguineus* was photographed in rock pools at Osmington, and dead specimens were observed at Portland Harbour within traps. This suggests possible new introductions or a significant range expansion, given the considerable distance from previous records (in Kent and Wales). The brush-clawed shore crab (*Hemigrapsus takanoi*) has spread north from Kent and Essex, where first seen in 2014 (Ashelby *et al.* 2017), to become abundant at some sites in Suffolk (e.g. in the Orwell Estuary, where found to be abundant in November 2020; P.I. Davison pers. obs.).

A baseline survey in the Thames in October 2019 found the trumpet tube worm *Ficopomatus enigmaticus* in the Thames, which represents a new expansion of this species in terms of known sightings. On 28 September 2020, two specimens of Chinese mitten crab *Eriocheir sinensis* were

found in Morecambe Bay by a fisher using fyke nets (www.nw-ifca.gov.uk/news/press-release-morecambe-bay-chinese-mitten-crab/). The species had not previously been recorded in Morecambe Bay. On both occasions, these crabs were caught on the way out of the estuaries – this is consistent with their known life cycle, whereby adults migrate into brackish and marine waters to breed during the autumn. The time of year and movement pattern now confirms they are migrating to marine estuary waters to breed. This may be a recent development or may reflect lack of surveying in autumn periods in the marine environment.

The clinging jellyfish (*Gonionemus vertens*), rarely recorded in the UK, was reported from Brightlingsea, Essex, in June 2019 (D. Shillito and R. Tabor, personal communication to John Bishop, MBA). Based on photographs, Dr A. Govindarajan (Woods Hole Oceanographic Institution, Mass., USA) considered the specimens to be most likely referable to the very toxic form of *G. vertens*, which probably deserves recognition as a separate species. The species was not reported again at this site during summer 2020 (R. Tabor, personal communication to John Bishop, MBA).

The common shore crab (*Brachynotus sexdentatus*) was reported from a single occurrence in Swansea Docks during surveys in 2018–2019 (Holmes & Callaway 2021). However, the specimen was not retained or photographed, so it has not been possible to verify the record, which would be the first since 1988 at the species' only known location in the UK.

Review of previous records of *Crepidula fornicata*: In 2019, removal of Scottish *Crepidula fornicata* records from the NBN (see map below):

There are three remaining records for *C. fornicata* in Scotland and these have been queried and the request has been made to have them removed:

1. (Kyle of Loch Alsh) Record has been proved to be a mis-identification of a juvenile species – the type specimen held at the National Museums Scotland was re-examined and found NOT to be *C. fornicata.*
2. (Clyde) Record is a transcription error in the geographic reference and the record is part of a sample set of a survey in the south coast of England where the recorder has recorded *C. fornicata* on the same day as the Clyde record!
3. (Firth of Forth) Record is from a MCS biological survey communications with the recorder confirmed this is a mistaken record. This is a regularly dived area and there is no *C. fornicata* found at this site.

All other records have been checked and removed, therefore meaning that this species has not yet been found in Scotland.

Atlantic blue crab (*Callinectes sapidus*): a single specimen was recorded in the Regents Canal, London submitted to iRecord in June 2021 and verified by the Marine Biological Association.

Plants

2019–2021: The following records were received from Northern Ireland in 2019–2021:

Species	**Marine locations** (in N. Ireland)	**Date**	**Notes** (geo coordinates)
Bonnemaisonia hamifera	North Coast	June 2019	55.210401/–6.655203
	Rathlin Island	June–Sept. 2021	55.22021292/–6.542962559
	Strangford Lough		55.195666/–6.693442
	Portstewart		54.434662/–5.544622
	Ballycastle		55.262103/–6.186052
	Ards Peninsula		55.206621/–6.239503

		August 2020	
Caulacanthus okumurae	North Channel Foyle* Rathlin Island* Strangford north * Ards Peninsula* Belfast Lough* Portstewart	June & July 2019 June–Sept. 2021	55.20816981/–6.114486094 and 55.15643441/–7.126876165 55.18903/–6.711561 54.628743/–5.530368 55.285101/–6.18812 55.290564/–6.194236 55.262103/–6.186052
Codium fragile atlanticum	North Channel Carrickfergus marina Rathlin Island	June 2019 Sept. 2021 Sept. 2021	55.20816981/–6.114486094 54.71093612001578/–5.812189572
Codium fragile fragile	North Channel* Carrickfergus marina Rathlin Island* Lough Foyle (ROI) Giants Causeway Portstewart*	June 2019 June–Sept. 2021	55.20816981/–6.114486094 54.710936/–5.812189572 55.28510/–6.18812 55.188894/–7.030695 55.18903/–6.711561 55.195666/–6.693442
Colpomenia peregrina	Strangford North & South, Dundrum Outer, Foyle, & Carlingford Carrickfergus marina and Harbour Ards Peninsula	May–July 2019 June–Sept. 2021	various locations, including 54.531732/–5.56146
Crepidula fornicata	Belfast Lough	August 2020	54.718202/–5.796232
Gracilaria vermiculophylla	Foyle & Carlingford* Strangford Lough South* Inner Dundrum Bay*	May & July 2019 June–Sept. 2021	55.14738301/–7.160736241 54.341452/–5.513267 54.263529/–5.834108
Neosiphonia harveyi	Belfast Inner & Foyle Ballycastle Portstewart North Channel	May & July 2019 June–Sept. 2021	Various locations including 54.67921296/–5.883474873 and 55.15643441/–7.126876165
Sargassum muticum	Belfast Lough, Belfast Inner, Carlingford, Strangford North & South, Foyle, North Coast & Dundrum Outer Rathlin Island	May–Sept. 2019 June–Sept. 2021	various locations, including 54.718285/–5.796194

	Ards Peninsula		
Undaria pinnatifida	Belfast Outer	July 2019 Sept. 2021	54.710721/–5.812562
Asparagopsis armata	Carlingford Lough (Narrow Water Castle) Strangford North	March 2021 Sept. 2021	54.111092/–6.2798859 54.44994415/–5.632422155
Grateloupia turuturu	Carrickfergus marina	Sept. 2020 Sept. 2021	54.71093612/–5.812189572
Dasysiphonia japonica	Strangford Lough* Lough Foyle* Ards Peninsula*	Sept 2020 Sept 2021	54.48593209/–5.54278381 55.188894/–7.030695 54.434662/–5.544622

*For exact coordinates at this location, contact Susan McCambridge (susan.mcCambridge@daera-ni.gov.uk)

Species	**Freshwater locations** (in N. Ireland)	**Date**	**Notes** (geo coordinates)
Impatiens glandulifera	Lough Neagh	August 2018	IH 98860 86554, IJ 09810 77819, IJ 00504 63241, IH 90121 66139, IH 95021 85031, IH 95865 87204, IJ 11727 87944, IH 95731 73307, IH 97692 64353, IJ 08141 86448
			Various
	Sub catchments of the River Foyle	June to Sept. 2018	C384005, H293940, H352964, H353907, H373900, H364875, H315845, H438873, H576875, H614848, H535859, H392766, H625530, H766933, H774953, H816982, H 49300 86713
	and various riverine locations	June to Sept. 2019	J087 896, J144 866, J442 712, J456 699
			IH 931 911, IH 888 952, IH 875 971, IJ 403 869, IJ 324 690, IJ 234 604, IJ 217 615, IH 180 639, IH

		September 2020 May – October 2021	219 652, IC 898 228, IC 477 193, IJ 324 690
Fallopia (syn. *Reynoutria*) *japonica*	Subcatchments of the River Foyle and various riverine locations	June to Sept. 2018 July 2018 & June–July 2019 September 2020 May – November 2021	Various C384005, H353907, H315845, H450886, G942518, H 84856 91471, IC 668 229, IC 683 098, IC 473 171, IC 474 175, IC 474 135 C679 203 IH 866 933, IH 888 952, IJ 523 823, IC 475 183, IC 47335 17351, IC 473 171, IC 477 193, IH 315 845
Mimulus sp.	Lough Neagh River Mourne at Victoria Bridge, Douglas Burn (Goyle) at Douglas Bridge Kilroot River at Kilroot	August 2018 July 2019 26/08/2021	IJ 09810 77819, IH 90121 66139, IJ 11727 87944, IH 95731 73307, IH 97692 64353 H353907, H373900 IJ 444 898
Azolla sp.	Lough Neagh Kilroot River at Kilroot	August 2018	IH 90121 66139
Elodea nuttallii	Lough Moor Lough Neagh	July 2018 August 2018	IH 44871 98164, IH 44540 98294, IH 44698 98427, IH 45067 98361 IH 96668 77751

	Quoile River at Quoile Bridge	17/05/2021	IC 488 465
Elodea canadensis	Lake Tullynawood and loughs Neagh, Lattone & Yoan	July & August 2018	IH 96668 77751, IH 00286 45042, IG 99877 45407, IH 86240 29185, IH 85884 29352, IH 25168 42323, IH 25303 42421, IH 25461 42195, IH 25334 42066
	River Blackstaff (south down) at Tullymurry Bridge, River Connswater	March & April 2018	IJ 429 411, IJ 374 732, IJ 364 735 IH 888 952; IJ 081 285
	Grange Water and Newry River	27/07/2021 & 9/06/2021	
Elodea nuttallii	Lough Moor	July 2018	IH 44871 98164, IH 44540 98294, IH 44698 98427, IH 45067 98361
Heracleum mantegazzianum	Sub-catchments of the River Foyle	June to Sept. 2018	Various
	Loughs Neagh & Moor	July & August 2018	IJ 08141 86448, IH 45067 98361
	Six Mile Water at Castle Farm Bridge (Antrim), River Faughan above Gorticross, River Temp	July–Sept. 2018	IJ 144 868, IC 474 175, IH 339 454
	River Mourne at Victoria Bridge, Douglas Burn (Goyle) at Douglas Bridge, River Colebrooke at Pollboy Bridge, River Owenkillew at Trinamadden Bridge	June & July 2019	H353907, H373900, H445434, H 49300 86713
	Mayobridge River		J14213 31041

	Various river locations	September 2020 April – August 2021	IH 853 919, IH 931 911, IJ 444 898, IJ 403 869, IJ 14213 31041
Lysichiton americanus	Ditch near Tempo River, Tattenweir Bridge	12/04/2021	H 36445 49118

In addition to the above, the Loughs Agency has some records for 2018 of Himalayan balsam (*Impatiens glandulifera*) and Japanese knotweed (*Fallopia* [syn. *Reynoutria*] *japonica*) in the sub-catchments of the River Foyle (Roe, Faughan, Burn Dennett, Glenmornan, Finn and Derg) and giant hogweed (*Heracleum mantegazzianum*) in the River Glenmornan; these have been identified and mapped to a GIS layer. For further information, contact Mark McCauley (mark.mccauley@loughs-agency.org).

Species Not Yet Seen

Fish

2019–2021: Ponto-Caspian fish species, most notable are the gobies, have not arrived in the UK, which might be expected given the arrival of Ponto-Caspian invertebrate species in past years. Similarly, the naked goby (*Gobiosoma bosc*), which has been reported for transitional waters in Belgium, Germany and The Netherlands prior to 2019, has not yet been found in the UK. Risk screening and environmental habitat suitability modelling were initiated by ITMO members and several international collaborators to assess the potential risk of this species to transitional waters of the North Sea region, risk screening and environmental habitat suitability modelling A journal publication is expected to result from this initiative, with the provisional title of: Invasion risks of naked goby, *Gobiosoma bosc*, to the North Sea transitional waters. For further information, contact Gordon H. Copp (gordon.copp@cefas.gov.uk) or Jennifer A. Dodd (j.dodd@napier.ac.uk).

Invertebrates

2019–2021: The dwarf surf clam (*Mulinia lateralis*) has been reported in large numbers in the Dutch and German sections of the Wadden Sea and in the Westerschelde estuary, Netherlands, these being the species' first recorded occurrences outside its western Atlantic native range (Craeymeersch *et al.* 2019; Klunder *et al.* 2019). Published records date back to 2017 and 2018. The species should therefore be regarded as a potential arrival in the UK.

A new ascidian, *Didemnum pseudovexillum*, has been described by Turon *et al.* (2020) based on a specimen collected from a marina in Brittany – this species was found to be co-existing with carpet sea squirt *D. vexillum*. These two species are indistinguishable externally but can be distinguished on the basis of DNA sequence information and from details of the tunic spicules and larvae. The current closest record is Brittany (France), so this new *Didemnum* species might be encountered in the UK – this should be taken into account when identifying specimens assumed to be *D. vexillum*.

Another species that could potentially arrive in the UK is the Pacific barnacle (*Balanus glandula*), which is widespread and invasive elsewhere in the world. This species has been reported for Belgium (Kerckhof *et al.* 2018) as well as The Netherlands (Faasse 2018).

The number of potential future arrivals to UK waters is highlighted in the supplementary information provided by Pezy *et al.* (2021), who reported 60 non-native species of invertebrates and

plants from Normandy waters that have not yet been reported from the English coast of the English Channel.

Plants

2019–2021: None reported.

4. Pathogens

2019–2021: A reoccurrence of oyster herpes virus (OHV-1) in farmed *Crassostrea gigas* was reported. New confirmed designations of koi herpes virus (KHV) on recreational coarse fisheries and in Japanese koi carp at ornamental facilities were also reported.

Further Information on finfish disease surveillance and compliance for the UK and designations for England and Wales is provided at: www.gov.uk/government/publications/fish-health-inspectorate-reports-2019

5. Research and Monitoring Programmes

2019–2021: In Scotland, validation of real time PCR assay for the detection of *Didemnum vexillum* in environmental samples (water/sediment) was completed by Marine Scotland Science (MSS) and a peer-reviewed paper has been published (Matejusova *et al.* 2021). A pilot eDNA-based routine monitoring programme was launched in 2019, and repeated to lesser extent in 2021, in the wider inner seas off the west coast of Scotland, also including Loch Linnhe and Loch Etive; as well as the Clyde area including Firth of Clyde, Loch Fyne and Sound of Bute, targeting artificial marine structures such as harbours, ferry terminals, recreational marinas and/or aquaculture related installations and infrastructures. The main aim of this pilot is to assess the spread of *D. vexillum* in these hotspot areas using eDNA, however there is an intention to utilise environmental samples collected to screen for presence of additional NNS according to the Scottish Non-Indigenous Species Strategy Priority Species List in future. Spread of *Didemnum vexillum* continues to be geographically limited. For further information, contact Iveta Matejusova (iveta.matejusova@gov.scot).

Monitoring of *D. vexillum* in Northern Ireland is also ongoing, with four samples collected in 2018 from the Ballydorn Lightship on Strangford Lough having been preserved in 100% ethanol and sent to Iveta Matejusova (MSS) for genetic testing. Results received in March 2019 for these samples indicate that the *D. vexillum* colonies sequenced were haplotype 1, thereby resembling the Kent haplotype described in Graham *et al.* (2015). A second batch of samples were sent to Aberystwyth University in 2019 to assess heterozygosity as a predictor of invasiveness (Bock *et al.* 2018). Following sightings of *Crepidula fornicata* at Carlingford, which were confirmed genetically in 2017 by Swansea University, the Loughs Agency has been maintaining a watching brief, but there have been no further confirmed observations.

In 2020, DAERA's Marine Assessment Team contributed samples to the Galway Mayo Institute of Technology project 'Marine Invasive Species Ireland', which aims to set up novel tools and develop eDNA protocols for detection, surveillance and mapping of invasive species in Irish Coastal waters.

Since 2018, MSS has investigated the potential of DNA-based monitoring to assess biofouling communities associated with the commercial harbours and recreational marinas. In collaboration with the Orkney Harbour Authority and Solway Firth Partnership, Marine Scotland Science investigates the use of settlement panels to capture biofouling and carries out comparisons between traditional rapid assessments followed by morphology-based identification of biofouling and high throughput sequencing (HTS) outputs. The HTS data for both 18S and COI markers have been analysed for panels collected in 2018 and a peer reviewed paper will be published in

2022. The HTS data collected from the bulk settlement panel material will be compared to HTS data, being currently generated from water samples collected alongside of the settlement panels. Contact: (iveta.matejusova@gov.scot).

A PhD project started in October 2019 to investigate the potential uses of eDNA to monitor marine invasive species in relation to the native European oyster (*Ostrea edulis*) restoration initiatives in the Dornoch Firth (Scotland). This is a collaborative project between MSS, the University of Aberdeen and Heriot Watt University Edinburgh and will develop and support robust biosecurity protocols relevant to the restoration project, using second and third generation sequencing approaches. Pacific oyster real-time PCR assay was designed and showing to be highly sensitive in laboratory experiments and so far had been tested also on water samples collected in a couple of Pacific oyster farms. For further information, contact Iveta Majejusova (iveta.matejusova@gov.scot).

The Harbour Authority, Orkney Islands Council, is continuing with its annual marine non-native species monitoring programme, which began in 2013. The programme recorded eighteen NNS in 2019, nineteen NNS in 2020 and eleven NNS in 2021. The NNS recorded during 2019-2021 were bryozoans (*Bugulina simplex, Fenestrulina delicia, Schizoporella japonica* and *Tricellaria inopinata*), ascidians (*Asterocarpa humilis, Botrylloides violaceus* [tentative ID] and *Corella eumyota*), seaweeds (*Antithamnion nipponicum,* harpoon weed *Asparagopsis armata, Bonnemaisonia hamifera, Codium fragile, Colpomenia peregrina* and *Dasysiphonia japonica*), crustacean (*Caprella mutica*), gastropod (*Potamopyrgus antipodarum*), bivalve (*Mya arenaria*), polychaete (*Boccardia proboscidea*) and marine splash midge (*Telmatogeton japonicus*). The monitoring programme continues annually with surveys for each year starting in June and ending in October. For further information, contact Jenni Kakkonen (jenni.kakkonen@orkney.gov.uk).

The NAFC Marine Centre UHI continued its Shetland wide non-native species monitoring programme (established 2012) with monitoring focusing on five locations. In addition, public records of NNS are encouraged. Monitoring work for 2020 was suspended, however public records of the orange-tipped sea squirt *Corella eumyota* were received from a cobble beach within Scalloway Harbour. This species is present within the Harbour on man-made infrastructure (detected by the NAFC Marine Centre UHI in 2015), these records within the Harbour indicate a transition to the natural environment.

Published SEPA Classification results report that *Didemnum vexillum* has been confirmed for another water body. However, this water body was already downgraded for non-native species as it already had *Styela clava* present. Details available at: www.sepa.org.uk/environment/water/aquatic-classification/.

The anticipated invasion of UK rivers, in particular those of Scotland and northern England, by the diadromous salmonid fish, pink salmon (*Oncorhynchus gorbuscha*), following the elevated numbers reported in 2017, did not occur. Relatively few specimens were reported, and these almost entirely in Scottish rivers. An eDNA survey of GB rivers for pink salmon was undertaken in August and September 2019, involving a collaboration of scientists from Cefas, the Game & Wildlife Conservation Trust, Queen Mary University (London), Marine Scotland Science, Scottish Natural Heritage, the Environment Agency (England) and the University of Gdańsk (Poland) (see: www.gwct.org.uk). This study was complemented by studies, using stable isotope analysis, to assess the impact of decaying carcasses on the invaded water course's food web. The eDNA analysis revealed a detectability issue using a primer taken from the literature, so the existing samples will require re-analysis with an alternative primer before results of the eDNA survey can be reported. This additional laboratory work is expected to be completed in 2022, once Covid-19 restrictions are lifted.

Marine Scotland Science had been using eDNA-based surveillance for monitoring of pink salmon in several Scottish rivers in 2019 and 2021. The real-time PCR assay (Gargan *et al.* 2021) has been applied and is demonstrating high sensitivity, with pink salmon detected in Scottish rivers in both 2019 and 2021. A new pilot project on the River Dee began in 2021 to investigate how far upstream of the river pink salmon migrate (and be detected by eDNA).

Stable isotope analysis of the foodweb study samples, which was planned for spring of 2020, was impossible due to Covid-19 restrictions, so these samples are expected to be analysed in 2021, once access to the laboratories at Queen Mary University is possible. In following to the above-mentioned work, an application submitted to the Marie Skłodowska-Curie Programme in 2020 has been awarded to Dr Michał Skóra (University of Gdańsk) due to begin in May 2021. This postdoctoral fellowship (PinkSIES), which aims to determine the impact of pink salmon on native salmonids both at sea and in recently invaded rivers, will be hosted by Queen Mary University (London) and Cefas (Lowestoft) and with the participation of Dr John Armstrong (Marine Scotland Science), Dr Rasmus Lauridsen (Game & Wildlife Conservation Trust, UK) and Dr Eva Thorstad (Norwegian Institute for Nature Research, Norway) on the fellowship's advisory/steering committee.

In 2019, a new project on diadromous species was initiated in the Interreg Atlantic Region 'Assessing and enhancing ecosystem services provided by diadromous fish in a climate change context' (DiadES). Led by colleagues at the French *Institut national de recherche en sciences et technologies pour l'environnement et l'agriculture* (Irstea; now known as INRAe), this project involves two UK partners (Cefas, the University of Plymouth) and their associates (Angling Trust, Rivers Trust) as well as partners from the Republic of Ireland, Portugal and Spain. The DiadES project aims to assess and enhance ecosystem services provided by diadromous fishes and lampreys in the Atlantic Area, and in parallel, the conservation status of these species, by explicitly considering in their management expected impacts of climate change on their distributions, with the potential impacts of non-native pink salmon incursions is a related concern. Data compiled for this project include existing data from partner countries as well as new data, including the analysis of water samples for eDNA and the microchemistry analysis of tissue samples to inform species distribution databases. For further information contact Tea Bašić (tea.basic@cefas.gov.uk) or Lynda Rodwell (lynda.rodwell@plymouth.ac.uk).

A multilingual version of the Aquatic Species Invasiveness Screening Kit (AS-ISK) was released in late 2020 (available for free download at: www.cefas.co.uk/nns/tools/) followed later that year by a journal article in which this multilingual decision-support tool is presented (Copp *et al.* 2021). The global trial of the AS-ISK reached its conclusion during 2020, the resulting journal article published in definitive form in September 2021 (Vilizzi *et al.* 2021). Co-coordinated by scientists from Cefas and the University of Lodz (Łódź, Poland) and involving several WGITMO members, this study involves risk screenings of marine, brackish and freshwater species around the globe. The outcome of this initiative, which involves several WGITMO members, is a contribution to the WGITMO ToR "Advance knowledge base to further develop indicators to evaluate the status and impact of non-indigenous species in marine environments". The resulting multi-author manuscript was published in 2021 (Vilizzi *et al.* 2021). For further information contact Gordon H. Copp (gordon.copp@cefas.gov.uk) and Lorenzo Vilizzi (lorenzo.vilizzi@gmail.com).

During 2019, Cefas contributed further to the development of a list for the EU Regulation on the management of invasive alien species (Roy *et al.* 2019a), resulting in the revision of assessments of western mosquitofish (*Gambusia affinis*) (Aislabie *et al.* 2019a), eastern mosquitofish (*Gambusia holbrooki*) (Aislabie *et al.* 2019b), and the completion of risk assessments on black bullhead (*Ameiurus melas*) (Aislabie *et al.* 2019c), brown bullhead (*Ameiurus nebulosus*) (Aislabie *et al.* 2019d), and northern snakehead (*Channa argus*) (Aislabie *et al.* 2019e). Risk screenings of non-native species during 2019 included jellyfishes in the Mediterranean (Killi *et al.* 2019) as well as marine and

brackish species for the Arabian/Persian Gulf and Sea of Oman (Clarke *et al.* 2019). For further information contact Gordon H. Copp (gordon.copp@cefas.gov.uk) or Hannah J. Tidbury (hannah.tidbury@cefas.gov.uk).

GBNNSS Risk Assessments completed in 2019 include species-specific assessments of sea walnut *Mnemiopsis leidyi* and pink salmon *Oncorhynchus gorbuscha* as well as a collective assessment of Ponto-Caspian gobies. For further information, refer to the risk assessments made available by the GB Non-Native Species Secretariat: www.nonnativespecies.org

An R&D project at Cefas, funded by the UK Department for Environment, Food & Rural Affairs (Defra), on Pacific oyster (*Magallana* [*Crassostrea*] *gigas*) population dynamics commenced in 2019. The project spans two financial years (2019/2020 and 2020/2021) and aims to: (1) use a modelling approach to provide insight into the impacts of temperature and external recruitment on oyster populations dynamics, and (2) integrate management into the model to examine the relative merit of different approaches. Where possible and appropriate, this project draws insight from the 2017 EMFF project on pacific oyster control in the southwest. Further project outputs will, where possible, be linked to the particle tracking study of the Pacific Oyster Control (Southwest) Project described here below.

A Defra-funded Cefas R&D project on the application of particle tracking modelling to NNS risk assessment and management commenced in 2019. Particle tracking modelling was combined with habitat suitability assessment to assess the risk of introduction and spread of NNS by the natural dispersal pathway. The work focussed on the Pacific oyster, however the approach can be broadly applied to non-native species with a pelagic phase. A journal article presenting the results of this study is due to be published in early 2021 (Wood et *al.* 2021).

In 2020, the Department of Agriculture, Environment and Rural Affairs (DAERA) has uploaded Angling Pathway Action plan and the Recreational Boating Action Plans to the GB Non-native Species website in December 2020 (www.nonnativespecies.org/index.cfm?pageid=586) for stakeholder consultation.

In March 2019, the Defra Centre of Excellence for DNA Methods was launched. This brings together UK government agencies with shared interests in developing molecular methods, with non-native species detection recognized as one of the group's priorities. One of the first set of projects launched by this virtual centre was to develop monitoring protocols for high-risk marine invasive NNS, led by Cefas, Natural England and Marine Science Scotland. A workshop was held at Cefas, Weymouth on 26 November 2019, which invited stakeholders from across the UK to contribute to a review of the current status of monitoring for invasive NNS at high-risk sites and provide recommendations for standardised protocols, incorporating molecular methods, and further research moving forwards. Cefas, Natural England and Marine Science Scotland are currently working on developing targeted PCR assays for particular high-risk species, including the Asian date mussel (*Arcuatula senhousia*) and the tubeworm (*Ficopomatus enigmaticus*) and compass sea squirt (*Asterocarpa humilis*), which will allow verification of methods across a range of species' ecologies and field conditions. For further information, contact Phil I. Davison (phil.davison@cefas.gov.uk) or Iveta Matejusova (iveta.matejusova@gov.scot).

Amongst the various initiatives to develop eDNA methodology (e.g. Davison *et al.* 2019; Holman *et al.* 2019), a project to improve eDNA monitoring by filling sequencing gaps for North Sea invertebrates was launched in 2019, as an element of the Interreg GEANS programme. Cefas are the UK partners, providing specimens for sequencing that will be led by the Senckenberg Institute. Non-native species for the North Sea region, including horizon species, are included in this project. For further information, contact Phil I. Davison or see the website: https://northsearegion.eu/geans/.

A PhD project on the Asian date mussel (*Arcuatula. senhousia*) began in October 2019. The project aims to include assessment of distribution, population dynamics, dispersal potential and impacts of the species in order to inform risk assessment and management. The PhD candidate is based at the University of Portsmouth, with supervisors from Cefas-Weymouth and APEM. For further information, contact Hannah J. Tidbury (hannah.tidbury@cefas.gov.uk).

Pathway action plans, led by the GBNNSS, have been drafted for angling and recreational boating pathways and are due to be completed in early 2020. Additionally a species pathway action plan for *Didemnum vexillum*, which is being led by Scottish Government under the Marine Pathways Action Group, was finalised in 2020. This included an action point to establish biosecurity protocols in relation to aquaculture. A working group, which was created and met in November 2020, will take forward in 2021 this action plan, including an action point to establish cross-jurisdictional biosecurity protocols in relation to aquaculture within the British-Irish Council (BIC) administrations. For further information, contact the chair of the working group Bernadette Moloughney (bernadette.moloughney@gov.scot).

Cefas and Marine Scotland Science have input into the UK Marine Strategy Part 2 consultation, which involved a review of marine NNS, and the provision of information on the current UK marine NNS monitoring programme and its gaps and limitations. The consultation took place in 2020. Details are to be published by Defra in 2021. UK MS part 3 consultation on programme of measures is currently in progress.

Marine Strategy Regulations (including Water Framework Regulations and Habitat regulations) monitoring features cross-cutting, UK-wide programmes that contain a NNS element. Intertidal and sub-tidal surveys using multiple methods, collecting data on a range of macro and microalgal and mobile and non-mobile animal species in coastal and transitional waters. Grab samples monitor benthic infauna and smaller epifauna (organisms living attached to the surface of the seabed or other organisms), whereas the observations collect data on larger animal and algal species. Intertidal surveys are carried out in MPAs, with macroalgal surveys in all coastal water bodies providing data on invertebrate and macroalgal species present. Marina Surveys have been completed with a focus on all invasive species and the detection of current and new species.

Grab sampling of benthic infauna and smaller epifauna are implemented across Northern Ireland at dredge and disposal sites.

A non-native species horizon scanning procedure for the UK's overseas territories (UKOTs), led by Dr Helen Roy (Centre for Ecology & Hydrology), was undertaken in December 2019 (see Roy *et al.* 2019b). For England, an updated horizon list across freshwater and marine (and terrestrial) environments was published during 2020 (Harrower *et al.* 2020). For Scotland, a horizon scanning exercise following the same methodology as the UKOTs and led by Helen Roy (Centre for Ecology and Hydrology) was conducted in 2021. The final report is due to be published shortly.

A Defra-funded R&D project at Cefas, 'Towards a cost-effective Non-Indigenous Species monitoring programme', began in October 2020. This project aims to review UK marine NNS monitoring, taking into consideration key legislative drivers of marine NNS management in UK waters. Gaps in the existing approach are highlighted and in light of marine biodiversity and conservation goals recommendations for potential improvement to address these are highlighted, including comment on practicalities.

The RAPID LIFE project, which is coordinated by the Animal and Plant Health Agency (APHA) in England with partners Natural England and the Bristol Clifton West of England Zoological Society, ended in July 2020 (https://ec.europa.eu/environment/life/project/Projects/index.cfm?fuseaction=search.dspPage&n_proj_id=6300). This project has produced Regional Invasive Species Management Plans (www.nonnativespecies.org/index.cfm?sectionid=139) as well as a biosecurity toolkit and training packs. Natural England hopes to continue work to develop

estuary-wide biosecurity plans in 2022 following on from the work of the RAPID LIFE project. The development of a biosecurity plan for Fal and Helford SAC is currently in progress.

Over the past five years there has been increasing concern around the spread of wild Pacific oysters in Marine Protected Areas. In Devon and Cornwall, the abundance of Pacific oyster (*Magallana* [*Crassostrea*] *gigas*) within intertidal Marine Protected Areas has led to some sites being reported as being in unfavourable condition. Natural England, in partnership with South Devon Area of Outstanding Natural Beauty Estuaries Partnership and Cornwall Wildlife Trust, with funding from the European Maritime and Fisheries Fund, undertook an investigation into monitoring and control measures for Pacific oysters within Marine Protected Areas.

Between 2017 and 2020, volunteers led by Cornwall Wildlife Trust and South Devon AONB Estuaries Partnership, conducted surveys around Cornwall and South Devon, to record the density of Pacific oyster populations and to test the effectiveness of culling as a method of controlling population expansion. The report of this project is now published - Partnership led strategy to monitor and manage the spread of Pacific oyster populations in south Devon and Cornwall (NERR100) http://nepubprod.appspot.com/publication/4889256448491520?_sm_au_=iVV0VntPSTv88vWHcf4HfK3t7C6f4

The project 'Mapping Invasive Alien Species in intertidal habitats within Natura 2000 sites in the Solent', which Natural England and the Marine Biological Association carried out, funded by the European Maritime Fisheries Fund, was completed in 2019. A new survey protocol was developed for that initiative in order to replicate rapid assessment surveys in marinas to shoreline environments. Rapid assessment surveys of 14 'Clusters' of three sites, one marina/harbour site, one nearby shore and one more distant shore, were completed in the Solent, recording alien species (AS) and native species (NS) from target lists. Report publication in progress. For further information, contact Jessica Taylor (jessica.taylor@naturalengland.org.uk).

Natural England is a partner in the new LIFE WADER project, which covers Marine Protected areas in Northumberland - Water And Disturbance Environmental Restoration on the Northumbrian coast https://webgate.ec.europa.eu/life/publicWebsite/project/details/5689. This project launched in October 2021 and will run to 2026. It is focusing on reversing the decline of habitats and species across the project area using best practice methods and will include improved IAS control through better surveillance, detection and removal. For further information, contact Catherine Scott (catherine.scott@naturalengland.org.uk)

6. Meetings

2019–2021:

CCFFR 2019 – Canadian Conference for Fisheries Research (London, Ontario, Canada; 4–6 January 2019) (www.uwo.ca/sci/ccffr_scl2019/).

ICZEGAR 2019 – International Conference on the Zoology and Ecology of Greece and the Aegean Region (Thessaloniki, Greece; 27–30 June 2019) (http://14iczegar.bio.auth.gr/).

ICAIS 2019 – International Conference on Aquatic Invasive Species (Montréal, Canada; 27–31 October 2019) (www.icais.org/).

CCFFR 2020 – Canadian Conference for Fisheries Research "*Aquatic Science in Changing Habitats*" (Halifax, Nova Scotia, Canada; 2–5 January 2020) (http://ccffr2020.acadiau.ca/home.html).

12th Global Summit on Aquaculture & Fisheries, Hong Kong, 30–31 March 2020 (https://aquaculture.global-summit.com/events-list/aquatic-invasive-species)

*RAPID LIFE End of Project Conference (Bristol Zoo, Bristol, England, 6 June 2020)

*ICMIS 2020 – International Conference on Marine Invasive Species (Berlin, Germany, 23–24 July 2020) (https://waset.org/marine-invasive-species-conference-in-july-2020-in-berlin)

*ICMISM 2020 – International Conference on Marine Invasive Species Management (Venice, Italy; 12–13 November 2020)_(https://waset.org/marine-invasive-species-management-conference-in-november-2020-in-venice).

Meetings in 2021

CCFFR 2021 – (online virtual) Canadian Conference for Fisheries Research "*Justice, Equity, Diversity, and Inclusion in Aquatic Sciences and Management*", including a Symposium on "*The future of invasive fish biology*: *detection and control through fundamental science and emerging technologies* "; 15–19 February 2021) (http://ccffr-scl2021.acadiau.ca/home.html).

*ICBF 2021 – 14th International Congress on the Biology of Fish, including a session on "*The Physiology and Control of Invasive Species*" (Montpellier, France; 6–9 July 2021

A list of Aquatic Invasive Species meetings is available at: https://aquaculture.global-summit.com/events-list/aquatic-invasive-species

Meetings in 2022

CCFFR-SCL 2022 "*Aquatic Systems Stewardship: Crisis, Change, and Cooperation*" (Vancouver, British Columbia, 24–27 February 2022) (http://ccffr-scl2022.acadiau.ca/home.html)

ICES Annual Science Conference 2022, including the session "*Steering shipping impact prevention towards holistic marine management*" (Dublin, Ireland, 19–22 September 2022) (www.ices.dk/events/asc/ASC2022/Pages/default.aspx)

International Conference on Aquatic Invasive Species (ICAIS), "*Global Climate Change Amplifies Aquatic Invasive Species Impacts*" (Oostende, Belgium, 18–22 April 2022) (https://icais.org/)

Fourth ICES PICES Early Career Scientist Conference, "*Ocean sciences for the future we want* " (St. Johns, Newfoundland, Canada, 18–21 May 2022) (www.ices.dk/events/symposia/ecsc4/Pages/default.aspx)

International Congress on the Biology of Fishes (ICBF-2022), including the session "*The Physiology and Control of Invasive Fishes*" (Montpellier, France, 28 June to 1 July 2022) (https://icbf-congress.com/en/programme)

NEOBIOTA 2022 – 12th International Conference on Biological Invasions, "*Biological Invasions in a Changing World*" (Tartu, Estonia, 13–16 September 2022) (www.elus.ee/index.php/en/neobiota-tartu-2022/)

* Meetings after 1 March 2020 most likely were cancelled, postponed or converted to online virtual meetings due to Covid-19 related restrictions on movements and meetings.

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Three-year report compiled by: April Blakeslee

Overview:

Regulations: Unfortunately, 2019 saw the discontinuation of the Invasive Species Advisory Committee, a high-level non-federal stakeholder group providing advice to the National Invasive Species Council on all aspects of invasive species research, management, and policy. The US Environmental Protection Agency (USEPA) continues to work toward development of ballast water discharge standards within the framework of the 2018 Vessel Incidental Discharge Act (VIDA), as well as outreach to improve sharing of ballast water reporting data. VIDA requires Coast Guard, EPA, and Aquatic Nuisance Species Task Force to develop an intergovernmental response framework for aquatic invasive species. In addition to establishing a new regulatory framework for vessel discharges, VIDA also established the Great Lakes/Lake Champlain Invasive Species Program, to be administered by the USEPA. This Program represent a major research and monitoring effort aimed at detecting introduction and spread of ANS into or within these waters and assisting with and prioritizing management and response actions including monitoring vectors likely contributing to ANS concerns. EPA hosted a series of virtual outreach meetings related to ballast water discharge in Fall 2021. A final policy letter was published by the Coast Guard on 24 March 2022 describing the process to accept alternate test methods for ballast water management systems that render organisms non-viable.

New Introductions / Range Expansions: In the 2019 report, 10 new introductions or substantial range expansions were reported in the US, along with 3 additional taxa that have been observed in US waters but suspected not yet to have established populations. No new intentional introductions or pathogens were reported. In the 2020 report, 3 new introductions or substantial range expansions were reported within the US, along with 4 additional taxa observed in US waters not yet suspected to have established populations. No new intentional introductions or pathogens were reported. In the 2021 report, six unintentional introductions or range expansions were reported and there were three sightings not yet known to be established. No new intentional introductions or pathogens were reported.

Content:

1. Regulations

On December 4, 2018, President Trump signed into law the Vessel Incidental Discharge Act (VIDA). That law establishes a new framework for the regulation of vessel incidental discharges under Clean Water Act (CWA) Section 312(p). VIDA requires EPA to develop national performance standards for those discharges within two years of enactment and the U.S. Coast Guard (USCG) to publish implementing regulations (i.e., monitoring, reporting, recordkeeping, equipment design requirements, etc.) to ensure, monitor, and enforce compliance with those standards two years thereafter. VIDA calls for these new standards to be technology-based and generally at least as stringent as existing VGP requirements. The EPA published draft standards for more

than 20 different vessel discharges, including ballast water, for public comment on 26 October 2020 (85 FR 67818).

In 2019, the Invasive Species Advisory Committee (ISAC), a non-federal stakeholder group advising the National Invasive Species Council (NISC), was placed on administratively inactive status and its charter was not renewed for administrative and financial reasons. In its absence, the Council and its staff continue to engage with these non-federal entities by participating in conferences, stakeholder meetings, and field activities. The implementation of priority activities contained in the NISC Annual Work Plan includes efforts to engage with relevant constituencies and experts as appropriate. Council staff and member agencies are open to identifying additional opportunities for interaction.

The Coast Guard continues to implement provisions of VIDA, which includes updating both Coast Guard and EPA regulations on non-accidental releases of invasive species (by ballast water and hull fouling) and other waste streams by vessels. EPA published a Notice of Proposed Rulemaking for discharge standards in October 2020. EPA hosted a series of virtual outreach meetings with states, tribal governments, and stakeholders who previously commented, which took place in Fall 2021. These discussions informed EPA's preparation of its Final Rule, or a Supplemental NPRM for some, or all, of its discharges, a decision on which is now expected in late 2022. In parallel, Coast Guard is developing complementary regulations to implement compliance and enforcement of EPA standards, which are due two years after EPA's Final Rule.

In addition to regulatory efforts, the Coast Guard continues other VIDA efforts, such as its state outreach to improve sharing of ballast water reporting data. A final policy letter was published March 24, 2022, that describes the process to accept alternate test methods for ballast water management systems that render organisms non-viable. VIDA requires Coast Guard, EPA, and Aquatic Nuisance Species Task Force to develop an intergovernmental response framework for aquatic invasive species. The framework will include a risk assessment and response framework and best management practices, but these efforts are still at the planning stage. Lastly, the Coast Guard continues to work with Smithsonian, EPA, Navy and Transportation partners on various research projects.

Summary of highlights

2. Intentional

There are no known new intentional introductions in US waters over the 3-year period.

3. Unintentional

New unintentional introductions (including significant range expansions)

2019 and 2020:

***Annelida: Polychaeta: Spionidae Polydora onagawaensis Abe, Nishitani & Endo* 2013**. Polydora onagawaensis is a shell-boring polychaete of the family Spionidae. It is native to the Northwest Pacific from Bohai Bay, China, and Miyagi Prefecture, south to Shanghai. In 2018, in a survey of shell-boring polychaetes at ten eastern oyster (*Crassostrea virginica*) farms from Buzzards Bay, Massachusetts to Mount Desert Island, Maine, the widespread cryptogenic shell-boring polychaete Polydora websteri was found at all farms, but at five farms in Maine, from Casco Bay northward, an unidentified shell-boring polychaete was found. Morphological features of the worms and DNA sequencing by PCR-RFLP (restriction fragment length polymorphism) of mitochondrial COI DNA matched Polydora onagawaensis, its first occurrences outside the Northwest Pacific. (Rawson and Rice 2018, Silverbrand 2019). The likeliest vector for introduction of this polychaete (*Polydora onagawaensis*) was unsuccessful plantings of Pacific Oysters (*C. gigas* = *Magallana gigas*) in 1949 and the 1970s (Shatkin *et al.* 1979). Shell-boring spionids can riddle the shells of oysters, weakening the shells, and creating mud-blisters, decreasing the quality of

oysters as food. The abundance and impacts of these polychaetes are exacerbated when oysters are cultured (Rawson *et al.* 2018; Simon & Sato-Okoshi 2015).

Annelida: Polychaeta: Spionidae: Polydora websteri Hartman 1942 (CRYPTOGENIC) The shell-boring spionid polychaete (*Polydora websteri*) was described from Milford, Connecticut, on Long Island Sound, where it infested the shells of oysters held in suspended trays (Loosanoff & Engel 1943). Shell-boring polychaetes weaken the shell and deposit sediment on the mantle, causing the oyster to create mud-blisters, making the oysters less edible. Polydora websteri had been previously confused with the European *P. ciliata*, which is not a shell-borer. *Polydora websteri* has been found from Maine to Alabama on the Atlantic Coast Washington State, Hawaii, Australia, New Zealand, China, and Ukraine. (Simon & Sato-Okoshi 2015; Rice *et al.* 2018; Lopes 2019; Silverbrand 2019). This polychaete shows a high degree of genetic homogeneity worldwide, with little variation and high connectivity among populations. Rice *et al.* (2018) suggest that *P. websteri* may have been native to the Northwest Pacific, and widely introduced with Pacific oysters, but indicate the need for further genetic sampling. Lopes *et al.* (2019) treat *P. websteri* as cryptogenic in Washington State, based on the tentative nature of Rice *et al.*'s (2018) conclusions. We also will consider this species cryptogenic in the Northwest Atlantic, pending more conclusive genetic information.

Bryozoa: Cheilostomata: *Cribrilina mutabilis* Ito, Onishi, and Dick 2015. *Cribrilina mutabilis* was described from the Akkeshi-ko estuary in northern Japan in 2014 but was found at about the same time off Kristineberg on the west coast of Sweden (Ostrovsky, cited by Ito *et al.* 2015) and off Norway. This bryozoan is probably native to Northwest Pacific, including China and Russia (Trott & Enterline 2019). On September 12, 2018, it was found growing on eelgrass (*Zostera marina*) around Clapboard, Mackworth, and Hog Islands in Casco Bay, near the harbor of Portland, Maine. Eelgrass is the most frequent substrate for this organism, but it has also been found on kelp (*Laminaria* sp.) and rockweed (*Fucus* spp.). Portland receives much shipping from Europe, and ship fouling is the likeliest vector for *C. mutabilis. Cribrilina mutabilis* is of concern, as a fouler of eelgrass, a critical component of coastal habitats and should be monitored (Trott & Enterline 2019).

Crustacea; Amphipoda: Grandidierella japonica Stephensen, 1938

Grandidierella japonica was originally described from Abashiri, Hokkaido, Japan. Its native range is from the southern Sea of Okhotsk to the East China Sea (Chapman & Dorman 1975). It is established on the West Coast, from British Columbia to northern Mexico (Pilgrim *et al.* 2013) and in Europe from England and Sweden to Italy (Marchini *et al.* 2013; Berggren 2015). In 2013 it was collected near the western end of the Connecticut shore of Long Island Sound at Stratford and Greenwich Points and by 2018 it was found at 14 stations along most of the coast of Connecticut. In 2018, *G. japonica* was also collected in Casco Bay, off Portland, Maine. This amphipod was found on a wide range of habitats, including rocky, muddy, and sandy substrates, and in eelgrass (*Zostera marina*) beds (Trott *et al.* 2020). Since this amphipod is established north and south of Cape Cod, in the Acadian and Virginian provinces, it is likely to have a wider range in the Northwest Atlantic.

Crustacea: Decapoda: Eriocheir sinensis H. Milne Edwards 1853 (Chinese Mitten Crab)

A single specimen of the Chinese mitten crab (*Eriocheir sinensis*), a male of 51.9 mm carapace width, was collected in New Haven Harbor Connecticut, Long Island Sound in April 2018 (Hudson *et al.* 2019). This may represent an independent introduction of crabs illegally imported as seafood, or dispersal from a population established in the Hudson River estuary (Schmidt *et al*. 2009; USGS Nonindigenous Aquatic Species Program 2020). Collections of single juvenile mitten crabs in the Mianus River, Connecticut, a Long Island Sound tributary, in 2012 and 2014 could represent natural or human-caused dispersal from the Hudson River (Hudson *et al.* 2019).

Crustacea; Mysida: Deltamysis holmquistae Bowman and Orsi 1993

Deltamysis holmquistae is a mysid known from fresh to marine waters. It was first described from the Sacramento-San Joaquin Delta, but appears to be conspecific with *Kochimysis pillai*, described from the Cochin backwater, India (the extent of its range in Asia is unknown). Its relatively recent (1977) appearance and its taxonomic uniqueness are strongly suggestive of introduction. In a recent Zootaxa paper, *D. holmquistae* was reported from Jacksonville, the Indian River, a canal in Fort Lauderdale, (all in Florida) and Freeport, Texas (Scripter *et al.* 2020). In 2014–2018 it was collected in four estuaries of the East and Gulf Coast, from Florida to Texas. It is associated with marshes and submerged vegetation and has been collected at 0.5 to 32.5 PSU. In general, collections of this mysid are rare and sporadic.

Chordata: Ascidiacea: Ciona intestinalis Linnaeus 1767

The sea vase tunicate (*Ciona intestinalis*) was formerly considered a cosmopolitan species, with a wide native and introduced distribution in the Atlantic and Pacific Oceans. Genetic sequencing and morphological observations revealed that *C. intestinalis* was actually several species, two of which, 'A' and 'B', were widely distributed. Species 'B', which is found along the coastlines of the North Atlantic, is the original *C. intestinalis* (Caputi *et al.* 2007; Nydam & Harrison 2007; Zhan *et al.* 2010). Historical records and recent genetic evidence (Hudson *et al.* 2019) support native status in European waters, an early introduction in coastal New England (Couthouy 1838; Agassiz 1859, cited by Gould 1870; Verrill 1880) and a more recent one in Atlantic Canada (2004, in the Brudenell River estuary, Prince Edward Island (2003, Ramsay *et al*. 2008). Hudson *et al.* (2020) found evidence that Atlantic Canada populations contain a combination of English Channel and Swedish shallow-water genotypes. They suggest that hybridization between English Channel and Swedish populations resulted in a population well-adapted to colonization of artificial structures in shallow water (Hudson *et al.* 2020). Further genetic sampling is needed in US waters to determine the origin of their populations of *C. intestinalis*. In US waters, *C. intestinalis*, is common south to Long Island Sound (Osman & Whitlach 1995). One complication in its invasion history in the Northwest Atlantic is the presence of some early records in deep waters off Nova Scotia ('east of Halifax', 232m, USNM 3471) and the Gulf of St. Lawrence (Van Name 1912; Carver *et al.* 2006). There may be genetic differentiation between deep (possibly native) and shallow-water populations, as was found for Sweden (Johannesson *et al.* 2018). *Ciona intestinalis* has had negative economic impacts on shellfish aquaculture in Atlantic Canada where it reduces the growth rates of cultured mussels and fouls ropes and equipment. It is also a formidable competitor, quickly occupying space and potentially displacing native fouling species (Carver *et al.* 2006; Ramsay *et al.* 2008).

Chlorophyta: Caulerpales: Codium simulans Setchell & N.L.Gardner 1924

The green seaweed (*Codium simulans*) was described from San Marcos Island in the Gulf of California, and ranges from Santa Cruz Island, California, south to Guerero Mexico, and has also been reported from Robinson Crusoe Island, Chile (Setchell & Gardner 1924; Norris 2010). Molecular analysis indicates that this eastern Pacific seaweed has been introduced to subtropical western Atlantic waters and has been unrecognized and misclassified. The earliest collection from US Atlantic waters was from St. George Island, Franklin County, Florida, in Apalachicola Bay (1972, Barcode 00231308, US National Museum of Natural History Botanical Collections 2017, initially as *C. isthmocladium* ssp. *clavatum* (Schneider *et al*. 2019)). A specimen originally identified as *C. decorticatum*, from the Indian River Lagoon, Florida, clustered next to sequences of *C. simulans*, and is considered conspecific (Schneider *et al.* 2019). In a genetic analysis, *Codium* previously identified as *Codium isthmocladium* ssp. *clavatum* from Bermuda were found to match *C. simulans*. Historical evidence suggests that this alga was established in Bermuda by the early 20th century. Solid ballast was considered the likeliest vector for transport of this seaweed, because it is unlikely to survive cold waters in the Straits of Magellan (Schneider *et al.* 2019).

Chordata: Osteichthyes: Pomacentridae Neopomacentrus cyanomos Bleeker 1856 (Regal Demoiselle).

The regal damselfish (*Neopomacentrus cyanomos*) has a broad native range in the Indo-Pacific from the Red Sea and Japan, to Madagascar, and New Caledonia. Its habitats include coral reefs and reef slopes, and harbors. It reaches lengths of 100 mm and is strictly marine (Froese & Pauly 2018). It was first discovered outside its native range in 2013 on coral reefs south of Veracruz, Mexico. Since then it has been recorded at multiple locations at sites in the northern Gulf of Mexico off Louisiana, Alabama, Florida, and Texas (2016–2019; USGS-NAS 2020). In July 2019, N. *cyanomos* was video recorded from various sites on the western side of Trinidad. These locations are 2050 miles southeast of the other known sightings in the northern Gulf of Mexico. Most probably, these fish were hitchhikers on oil platforms transported to Trinidad from the Indo-Pacific (Robertson & Kingon 2019).

Phaeophyta: Ectocarpales Colpomena peregrina Sauvageau 1927.

This brown seaweed, oyster thief and bladder weed (*Colpomena peregrina*) is native to the North Pacific and was first recorded in the Atlantic in 1960 in Halifax county, Nova Scotia. It has been extending its range southward through the Gulf of Maine, reaching the north shore of Cape Cod (Sandwich, Massachusetts) by 2012 (Green *et al.* 2011). In 2012 it was collected south of Cape Cod in Buzzards Bay, in the town of Bourne, Massachusetts (Macroalgal Herbarium Portal 2018, cited by Green-Gavrielidis *et al*. 2019), and in 2017 in several locations in Rhode Island in Narragansett Bay and Rhode Island Sound (Creen-Gavrielidis *et al.* 2019).

2021:

Annelida: Polychaeta: Spionidae Pseudopolydora achaeta (Radashevsky and Hsieh 2000)

Pseudopolydora achaeta is an infaunal polychaete of the family Spionidae. It is native to the Northwest Pacific from Pacific Russia to southern Taiwan (Radashevsky & Hsieh 2000). *P. achaeta* was collected in 2017 at several locations in intertidal silt and mud in the Indian River Lagoon, Florida (Bogantes *et al.* 2021). *Pseudopolydora achaeta* is also established in Parana, Brazil (Lana *et al.* 2006).

Annelida: Polychaeta: Spionidae Pseudopolydora paucibranchiata (Okuda 1937)

Pseudopolydora paucibranchiata is native to Northwest Pacific from Sakhalin, Russia to Hong Kong (Radashevsky 1993; Wu & Lu 2007) and is widely introduced around the world (Radashevsky *et al.* 2020). In 2017, it was collected at several locations in the Indian River Lagoon, its first record in the Western Atlantic (Bogantes *et al.* 2021).

Annelida: Polychaeta: Spionidae Pseudopolydora rosebelae (Radashevsky and Migotto 2009)

Pseudopolydora rosebelae was found burrowing in Thalassia beds in the Indian River Lagoon in 2017, its first reported occurrence outside Brazil. While this is possibly an introduction, it could also be part of a continuous distribution in the Western Atlantic (Bogantes *et al.* 2021).

Arthropoda: Crustacea: Decapoda: Lysmata vittata (Stimpson 1860)

The peppermint shrimp (*Lysmata vittata*), a.k.a.Indian lined shrimp and red-striped shrimp, is one of ≈48 named species within Lysmata, which includes obligate cleaner shrimps with distinctive coloration, and many of the species differ in small details of the accessory branch of the antennule (Aguilar *et al.* 2022). *Lysmata vittata* was described from Hong Kong and has been collected from Pacific Russia and the Red Sea to South Africa and Australia. It is reported as an introduction to Caribbean Panama and Brazil, Mediterranean Egypt, New Zealand, and recently the eastern US (Pachelle *et al.* 2020*et al*; Abdelsalam 2018; Aguilar *et al*. 2022). An extensive morphological and DNA survey identified numerous specimens of *L. vittata* from Chesapeake Bay and nearby Atlantic coastal bays, and compared Chesapeake specimens with those from Australia, Brazil, Hong Kong, Panama, Thailand, and New Zealand. Introduced US and New

Zealand populations were identified as *Lysmata vittata*, *seusu stricto*, which is native to the northwestern Pacific. Other introduced populations (Brazil, Caribbean Panama, Mediterranean Egypt) belong to the L. vittate/rauli complex and consist of one or more unresolved species (Aguilat *et al.* 2022). In Chesapeake Bay, *L. vittata* occurs in the lower Bay south of Tangier Island, and in Burton's Bay on the Atlantic shore, where it has been collected at temperatures ranging 4–27 °C, and 19–34 PSU (Aguilar *et al*. 2022). Specimens of L. vittata have also been collected from Little Egg Harbor, New Jersey and Long Island Sound, near Norwalk, Connecticut (Robert Aguilar, personal communication; James Carlton, personal communication). (Edited by Robert Aguilar)

Chordata: Osteichthyes: Chaenopsidae: Protemblemaria punctata Cervigón, 1966 (Warthead Blenny).

The bony fish, warthead blenny (*Protemblemaria punctata*), is endemic to reefs and mangroves off the northeast coast of Venezuela. It inhabits empty mollusk shells and feeds on benthic crustaceans, gastropods, bivalves, and zooplankton. The first individuals were observed June 2019 in the Gulf of Mexico in shallow water off Manatee County, Florida. Mode of introduction is unknown, but possibly as a hitchhiker on towed oil/gas platforms or ships. Literature review and discussions with colleagues indicate the species has been documented in the southern Gulf of Mexico off Veracruz and Trinidad. Additional reports of multiple individuals from 2019, 2020 and 2021 in Manatee County indicate that is has likely become established there (USGS Nonindigenous Aquatic Species Program 2021). Because of its cryptic nature, it is possible the species is more widespread in the Gulf of Mexico. Reports continued in 2021 from Manatee County, strengthening the assertion that this species is established. FL Department of Environmental Protection is making plans to do some surveys to determine if the distribution is wider than presently known.

Chordata: Osteichthyes: Pomacentridae: Neopomacentrus cyanomos (Bleeker, 1856); (Regal Damsel).

The Indo-Pacific regal damsel (*Neopomacentrus cyanomos*) was first seen in the southern Gulf of Mexico in 2013, the northeastern Gulf of Mexico in 2017, and the Florida Keys in 2020. In 2021, regal damsel continued to expand its range. A credible report of this species from Aruba has been received, although there are no vouchers or photos. This species may be more widespread than presently known, and as reported sightings are confirmed over time the distribution is expected to extend up and down both coasts of the Florida peninsula.

Sightings of species not yet known to have established

2019:

Chordata: Osteichthyes Heniochus acuminatus Linnaeus, 1758 (pennant coralfish).

The Pennant coralfish (*Heniochus acuminatus*) is widespread through the Indo-Pacific. It is usually found alone or in small groups and feeds primarily on zooplankton and benthic invertebrates. One specimen was observed on 17 September 2019 at Spud barge/Upsidedown barge off Palm Beach County, Florida. The introduction has been attributed to aquarium release. One previous sighting of the same species was made off the Florida Atlantic coast in 2016 (USGS NAS 2020).

Chordata: Osteichthyes Zebrasoma veliferum Bloch, 1758 (sailfin tang).

The sailfin tang (*Zebrasoma veliferum*) fish is distributed in the Indo-Pacific from Indonesia, Micronesia, Hawaii and from southwest Japan to the Great Barrier Reef and New Caldonia. It inhabits lagoon and seaward reefs and feeds on fleshy green and red algae. One specimen was observed on 28 June 2019 at Fishbowl/the Trench off Palm Beach County, Florida. The introduction has been attributed to aquarium release, with 14 previous sightings of the species having been made off the Florida Atlantic coast between 2000–2017 (USGS NAS 2020).

Crustacea: Decapoda: Metacarcinus magister Dana 1852 (Dungeness Crab)

The dungeness crab (*Metacarcinus magister*) is native from the Bering Sea to Point Conception, California. It is a large, edible crab, the subject of a male-only fishery. On 26 July 2017, a male dungeness crab was caught in a lobster trap near Norwalk, Connecticut, in Long Island Sound. Another was caught in Cape Cod Bay, Massachusetts, in November, 2018 (Hudson *et al.* 2019). Earlier catches of single male Dungeness Crabs were made in 2006 and 2009, near Cape Ann, Massachusetts (Prybot 2010). Since only male crabs are usually caught and sold, establishment of this species in the Atlantic is unlikely.

2020:

Chordata: Osteichthyes: Acanthuridae: Paracanthurus hepatus Linnaeus, 1766 (palette surgeonfish).

The palette surgeonfish (*Paracanthurus hepatus*) is native to the Indo-Pacific. It inhabits the exposed edges of outer reefs in flowing currents 2–4 m deep and feeds on zooplankton and benthic algae. One individual was observed several times in February 2020 at Phil Foster Park, Palm Beach County, Florida. This is the first record of this species in Florida waters (USGS Nonindigenous Aquatic Species Program 2021). The individual was seen multiple times over a few days, but appeared to be in poor health. After a few days, the fish was no longer seen even though extensive surveys were done for its presence. (Pam Schofield)

Chordata: Osteichthyes Heniochus acuminatus Linnaeus, 1758 (pennant coralfish).

The pennant coralfish (*Heniochus acuminatus*) is widespread through the Indo-Pacific. It is usually found alone or in small groups and feeds primarily on zooplankton and benthic invertebrates. One specimen was observed on 17 September 2019 at Spud barge/Upsidedown barge off Palm Beach County, Florida. The introduction has been attributed to aquarium release. One previous sighting of the same species was made off the Florida Atlantic coast in 2016 (USGS NAS 2020).

Chordata: Osteichthyes Zebrasoma veliferum Bloch, 1758 (sailfin tang).

The sailfin tang (*Zebrasoma veliferum*) is distributed in the Indo-Pacific from Indonesia, Micronesia, Hawaii and from southwest Japan to the Great Barrier Reef and New Caldonia. It inhabits lagoon and seaward reefs and feeds on fleshy green and red algae. One specimen was observed on28 June 2019 at Fishbowl/the Trench off Palm Beach County, Florida. The introduction has been attributed to aquarium release, with 14 previous sightings of the species having been made off the Florida Atlantic coast between 2000–2017 (USGS NAS 2020).

Crustacea: Decapoda: Metacarcinus magister Dana 1852 (Dungeness Crab).

The dungeness crab (*Metacarcinus magister*) is native from the Bering Sea to Point Conception, California. It is a large, edible crab, the subject of a male-only fishery. On 26 July 2017, a male dungeness crab was caught in a lobster trap near Norwalk, Connecticut, in Long Island Sound. Another was caught in Cape Cod Bay, Massachusetts, in November, 2018 (D. Hudson *et al.* 2019). Earlier catches of single male Dungeness Crabs were made in 2006 and 2009, near Cape Ann, Massachusetts (Prybot 2010). Since only male crabs are usually caught and sold, establishment of this species in the Atlantic is unlikely.

2021:

Arthropoda: Crustacea: Decapoda: Macrobrachium nipponense (East Asian River Prawn)

The East Asian river prawn (*Macrobrachium nipponense*) is native to fresh and brackish waters from northern China to Japan, Taiwan, and Vietnam. Adult males can reach 36 mm, and females up to 76 mm. This shrimp is extensively cultured for food in Asia. This shrimp frequently enters brackish water, but it can complete its life cycle in fresh water (Holthuis 1980). *Macrobrachium*

nipponense was collected in 2014 in a freshwater retention pond in Morehead City, North Carolina, adjacent to Bogue Sound. Populations are considered established in the White Oak River, a Bogue Sound tributary near Beaufort, where they have been collected from 2014 to 2018. Two specimens were collected in the Cape Fear River, North Carolina in 2017 and 2019. In South Carolina, this shrimp was collected at four locations in coastal ponds and creeks in the vicinity of Charleston and Pawley Island. One specimen of Macrobrachium nipponense was collected in the St. Johns River, near Jacksonville, Florida in 2017. Possible vectors include aquaculture, aquarium release and ballast water. The latter is supported by the frequency of records near major seaports (USGS Nonindigenous Aquatic Species Program 2021).

Chordata: Osteichthyes: Balistidae: Odonus niger Rupell 1836 (Red-toothed Triggerfish).

The red-toothed triggerfish (*Odonus niger*) is native to the Indo-Pacific from the Red Sea and Japan to the Great Barrier Reef and Society Islands (Froese and Pauly 2021). One specimen was caught near the Blue Heron Bridge, on the Intracoastal Waterway near Riviera Beach, Florida, on 9 May 2021. A few days after the sighting, the USGS Early Detection/Rapid Response team captured it alive. The specimen is now at the Phillip and Patricia Frost Museum of Science in a display featuring non-native marine fishes removed through the ED/RR program. For more info on the specimen: https://nas.er.usgs.gov/queries/SpecimenViewer.aspx?SpecimenID=1657507. For more info on Frost Science's Exhibit: https://www.frostscience.org/marine-exotic-species-removal/. For more info on theUSGS ED/RR program: www.usgs.gov/centers/wetland-and-aquatic-research-center/science/early-detection-and-rapid-response-removal

Chordata: Osteichthyes: Teraponidae: Terapon jarbua (Jarbua Terapon; Crescent Grunter)

The jarbua terapon or crescent grunter (*Terapon jarbua*) is native to the Indo-Pacific from the Red Sea and southern Japan to Australia. *Terapon jarbua* enters brackish and fresh water and occurs infrequently in the aquarium trade (Froese & Pauly 2021). One specimen was captured in a tidal pond on Dauphin Island, Alabama in 2020 (USGS Nonindigenous Aquatic Species Program 2021).

4. Pathogens

Sightings/records

No new pathogens to report.

5. Research and monitoring programs

The 2018 Vessel Incidental Discharge Act (VIDA), discussed above, also establishes two non-regulatory programs designed to address invasive species concerns from commercial vessel discharges. The first, the Coastal Aquatic Invasive Species Mitigation Grant Program, to be administered by the Secretary of Commerce and the National Fish and Wildlife Foundation, is designed to award grants to improve the understanding, prevention, mitigation of, and response to, aquatic invasive species in the coastal zone and the Exclusive Economic Zone; to support the prevention and mitigation of impacts from aquatic invasive species in the coastal zone; and to support the restoration of Pacific Island habitats, marine, estuarine, and Great Lakes environments in the coastal zone and the Exclusive Economic Zone that are impacted by aquatic invasive species. The second, the Great Lakes and Lake Champlain Invasive Species Program, to be administered by the EPA's Great Lakes National Program Office, in collaboration and consultation with other federal agencies, is intended to, among other things, monitor for the introduction and spread of ANS into or within these waters; assist with and prioritize management and response actions including monitoring vectors likely contributing to ANS concerns; develop ballast water management systems available for use by commercial vessels; and facilitate meaningful federal and state implementation of the VIDA regulatory framework. These programs are both currently

under development and although substantial funding has been authorized in the legislation it is uncertain what level of appropriation they will receive in coming federal budgets.

EPA partnered with the Maritime Administration and allocated $5 million from another funding source for initial funding to the Great Waters Research Collaborative (GWRC) at the Lake Superior Research Institute at the University of Wisconsin-Superior to develop and implement a five-year ballast water research and development plan with a primary focus of the plan to address one of the stated purposes of GLLCISP to develop, achieve type approval for, and pilot shipboard or land-based ballast water management systems installed on, or available for use by, commercial vessels operating solely within the Great Lakes and Lake Champlain Systems to prevent the spread of aquatic nuisance species populations within the Great Lakes and Lake Champlain Systems. A copy of the plan is publicly available for comment on the GWRC homepage. Activities commenced in October 2020 and address five research areas:

- Identification of Methods/Alternatives and Assessment of Cost
- Development of a Great Lakes Relevant BWMS Testing Protocol
- Accelerating Development of Emerging Ballast Water Treatment Technologies
- Development of Ballast Water Indicative Monitoring Methods
- Assessing the Risk of Aquatic Nuisance Species Transfer from Ballast Water Discharge

The Great Lakes Invasives Sentinel Site Network (GLISSNet) was created in 2021 for efficient early detection of aquatic non-indigenous invertebrates using systematic sampling and standardized protocols. GLISSNet is coordinated by the Marine Invasions Lab at Smithsonian Environmental Research Center and includes partners from the Environmental Protection Agency (EPA), the Lake Superior Research Institute (LSRI) at the University of Wisconsin Superior, Moss Landing Marine Laboratories, Fisheries and Ocean Canada, and the US Coast Guard. The program extensively surveys zooplankton, benthic habitats, and ballast water using a combination of morphological and genetic methods. In 2021, 50 different sites were surveyed in the St Louis River Estuary (SLRE) near Duluth, MN. This will be expanded to other sites within the Great Lakes, allowing assessment of spatial invasion patterns. This sentinel site parallels other monitoring programs established by SERC in coastal ports and provides a unique resource for investigating broadly interesting ecological questions on community and invasion dynamics.

The first-ever "Mid-Atlantic Coast Rapid Assessment Survey for Marine Bioinvasions" (led by Drs. Amy Fowler, April Blakeslee, Jim Carlton, and Judy Pederson) had been planned for 29 July to 7 August 2022, to survey marinas on the outer coast and outer bays from Eastern Shore, VA to Sandy Hook, NJ. However, unfortunately this RAD had to be postponed to June 2022 due to COVID illness and a personal emergency among the leaders of the initiative. In June 2022, the RAS will collect samples for taxonomic and genetic barcoding of fouling species located at the marinas. To date, no comprehensive RAS has been performed in the region. The RAS will utilize past methodologies for Northeast US RASs led by Pederson and Carlton. Participants of the RAS will include numerous taxonomic experts coming from multiple locations around the US. The RAS will provide valuable baseline information for future research in a vital region where there is a mix of leading and trailing edges of species, including those that are non-indigenous.

6. Meetings

Past meetings: No significant meetings to report in the past three years.

***Future meetings*:** The 11th International Conference on Marine Bioinvasions has been delayed due to the ongoing global pandemic but is scheduled to be held in 2023 in Baltimore, Maryland (https://marinebioinvasions.info/).

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