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Non-Indigenous Species Dynamics in Time and Space within the Coastal Waters of The Netherlands

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Abstract: Information on temporal and spatial trends with regard to the introduction of non-indigenous species (NIS) is often sparsely available. These trends may potentially help improve the design and focus of monitoring programs, give insights into new pathways and hotspots, and facilitate horizon scanning. We provide an overview of 215 marine and brackish water NIS recorded in The Netherlands. Temporal trends over the most recent three decades for taxonomic groups, species origin, introduction vectors, and water systems were analysed. We attempt to explain the observed patterns and discuss factors that hamper their explanation. A shift in the region of origin from Pacific to W Atlantic can potentially be linked to legislation prohibiting Pacific oyster imports, whereas a subsequent shift backwards cannot. Case studies illustrate that NIS may not be first detected in the water systems where they were originally introduced. Additionally, it is shown that changes in allegedly native species' distribution or seasonal pattern should be linked to an introduced cryptic NIS instead. We also discuss the shortcomings of monitoring programs that were originally not focused on NIS, the importance of naturalists' observations, and the added value of a more recent network that is focused on NIS detection in the coastal waters of The Netherlands.

Keywords: marine invasive species; cryptic species; monitoring effort; origins; stepping stones; temporal trends; likely vectors; *Aurelia coerulea*; *Notocomplana koreana*; *Prosthiostomum wagurensis*; flatworms; NIS detection network; The Netherlands

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1. Introduction

Rapidly being able to detect marine non-indigenous species (NIS) after they have arrived in new water systems is becoming increasingly important to understand the underlying patterns of species' introductions, spread, and establishment. Some of these alien species become invasive, having significant impacts on the environment, including changes in food webs, the alteration of habitats, the displacement of native species, and socioeconomical losses [1–4].

On a global level, the Convention on Biological Diversity (CBD) deals with these NIS, aiming to protect and restore all ecosystems, while the International Maritime Organization (IMO) aims to reduce the risk of NIS specifically within the marine realm. At a European level, the EU Regulation 1143/2014 Invasive Alien Species (IAS) (number 7) is aimed at prevention, control, and eradication for those IAS of Union concern to mitigate (if feasible) their negative impact [5]. In the marine environment, some IAS actually form a global threat to marine biodiversity [6,7]. Within Europe, the aim of the Marine Strategy Framework Directive (MSFD) is ensuring the good environmental status of the seas and oceans [8]. This directive also includes provisions for preventing the introduction and spread of NIS. To achieve this, the D2 descriptor requires EU Member States to conduct risk assessments and monitor the presence and abundance of NIS. To support strategies dealing with marine NIS, Zenetos et al. [9] have presented a baseline study at European and subregional levels. To add to this European baseline study on a national level, countries such as France and Denmark have published reviews on marine NIS in their waters, evaluating and analysing their origins, pathways, and establishment [10,11]. In line with these studies, we present a review of all NIS that have been recorded over the years in the brackish to marine coastal waters of The Netherlands, including planktonic unicellular species and pathogens, such as viruses. Focusing on macrofauna and macroflora species that have their origin outside the NE Atlantic and that were first recorded in the last three decades, additional analyses were carried out. These analyses were based on taxonomic groups, species origins, water systems in which NIS were first detected, distribution vectors, and pathways. The observed temporal trends are discussed, identifying factors that may hamper their explanation, such as monitoring effort. During the last decade in particular, the detection of marine alien species within The Netherlands has become more organised, focusing on hotspots, pathways including stepping stones, and vectors. This was carried out for the EU Habitats and Birds Directives, for MSFD purposes, and for the EU Regulation 1143/2014 [5,8], as well as in support of, for example, the Management and Action Plan Alien Species (MAPAS) of the Trilateral Wadden Sea, a UNESCO world heritage site in The Netherlands, Germany, and Denmark [12]. Support was also provided for international guidelines and legislation (e.g., the Ballast Water Convention) developed by IMO, and more specifically, to protect nature values set for marine Natura 2000 waters to comply with the Habitats Directive [13,14]. The interpretation of the results of the analyses, the various NIS monitoring efforts, and the available methods and tools to detect coastal NIS in The Netherlands, including improved molecular analyses, are briefly discussed.

2. Materials and Methods

2.1. Analyses Based on NIS Recorded within the Coastal Waters of The Netherlands

Over the last ~20 years, the authors have kept up-to-date lists of NIS introduced into the coastal waters of The Netherlands based on sources they have verified. These lists have been used for various purposes, such as D2 calculations and risk assessments related to activities within Natura 2000 water systems within The Netherlands. Within the scope of the present study, all NIS that were recorded in the coastal waters of The Netherlands with a salinity of >~5 ppt are included in the final overview, as long as it is considered more than likely that they were introduced with human aid.

The more detailed trend analyses over time of, for example, the origins, pathways, and introduction sites were only performed with a selection of species, as not all NIS taxa are equally well-recognised and monitored. To deal with the higher uncertainty of the origin and year of introduction, the following NIS are excluded in the more detailed analyses: micro-organisms (<2 mm), such as unicellular planktonic species; pathogens, such as bacteria and viruses; and endo-parasites. These are obviously more difficult to detect than macro-organisms that can be seen with the naked eye. The pathways used by NIS that have their native region in NW European waters are often uncertain. If it is considered likely that a given NIS was introduced with human aid, it is included in the NIS list of The Netherlands. These species are excluded in the more detailed analyses, however, because of the possibility that they have reached The Netherlands by natural expansion.

The NIS list presented here includes all species that were probably introduced with the aid of humans in the coastal waters of The Netherlands. The more detailed analyses concentrate on macroflora and macrofauna only (>2 mm), excluding endo-parasites, while including only NIS that have a known origin outside of NW Europe. To illustrate trends from more recent years in particular, we compare the introductions of NIS in the last three decades, i.e., 1991–2000, 2001–2010, and 2011–2020. An accumulation graph illustrating the NIS recorded along the coasts of The Netherlands over the years, divided by taxon, includes a wider selection of species records, i.e., from 1900 up to 2021.

Where it concerns the coastal waters of The Netherlands, Figure 1 illustrates the main water systems where NIS were first recorded. This categorisation by water system is also used in the analyses illustrating the main sites of "first NIS records" in The Netherlands, which may provide information about the pathways that were used by the NIS concerned. Considering that NIS-focused monitoring has not been equally intensive in all these water systems, we discuss to what degree the sites of "first records" are likely to be the sites of first introduction, as well.

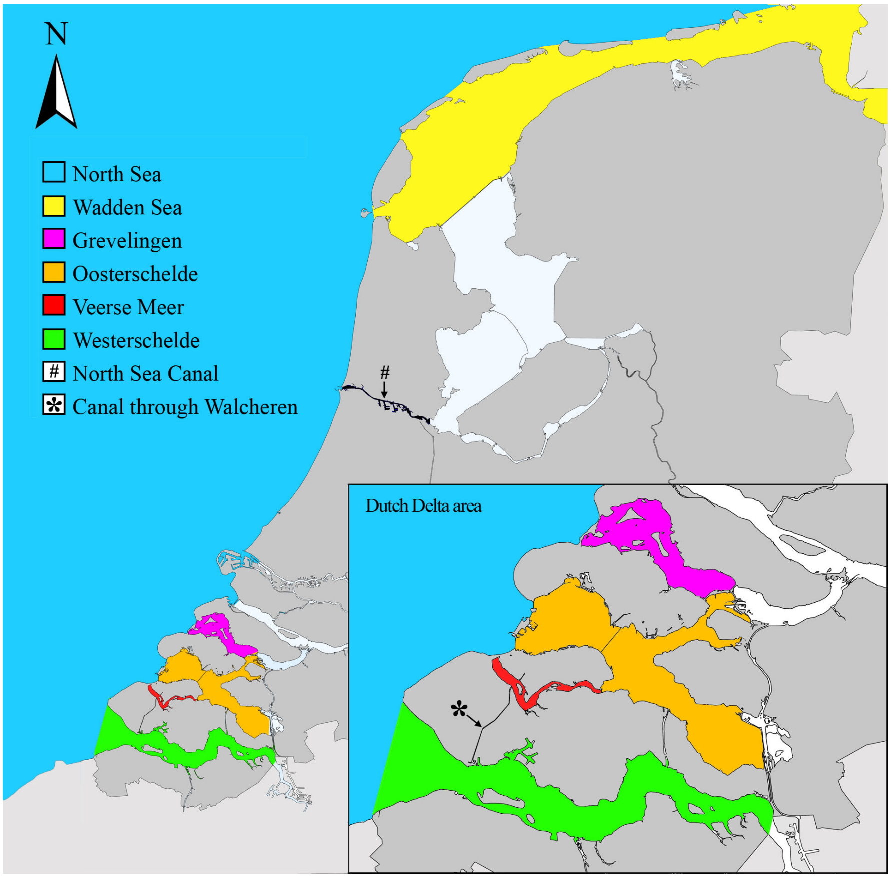


Figure 1. The coastal waters of The Netherlands where NIS were first detected. Water systems with at least in certain parts a salinity of >5 ppt are shown.

2.2. Pathways, Vectors, and Their Uncertainties

To assess the most likely pathways and vectors of NIS introduction remains difficult, as NIS are generally detected with a certain delay and not during or directly after introduction. Especially since NIS have been more intensively monitored along the coasts of The Netherlands in the last decade, the most likely pathways and vectors in the past, present, and potentially near future can be analysed as generalised trends. In the present study, this was achieved by focusing on the three most recent decades and a selection of macroflora and macrofauna species only. To study introduction pathways, these analyses included the origins of the species, the most likely vectors connected to the introduction, and the water systems where they were first recorded. The trends that appeared over time were discussed. Changes over time in the main pathways and vectors of introduction, as well as misguiding trends linked to monitoring effort, were considered. Taking these trends into consideration while trying to predict the main invasion pathways for the near future, three NIS that were first detected in 2020 and 2021 are discussed in more detail. After their discovery, these species turned out to have a much wider distribution in The Netherlands than first expected. Three case histories are presented to exemplify the nature of cryptic species and their sometimes unexpected pathways, which may not be directly linked to the first water systems in which they were recorded.

2.3. Marine Alien Species Detection Network

In 2006, the SETL project was started within marinas and ports all along the coasts of The Netherlands, with the specific aim of detecting alien fouling species [15]. Within this project, grey PVC plates of 14 × 14 cm were deployed, similar to those used by the Smithsonian Marine Invasions Laboratory along the whole Northern American coastline. Since then, new plates have been deployed and a selection of older plates (about 200) photographed and checked for species every season, i.e., once every 3 months. Initially, the SETL project was the only continuous NIS-focused monitoring program along the coasts of The Netherlands. From 2009 on, NIS monitoring in coastal waters intensified considerably. At that time, the first NIS-focused survey was conducted in the Dutch Wadden Sea, searching most habitats with a large variety of survey and sampling methods (Figure 2). It was the first NIS-focused baseline study in this UNESCO World Heritage site, resulting in the discovery of 11 species new to the Wadden Sea, 2 of which were new to The Netherlands [16]. Subsequently, similar surveys were conducted in the region in 2011, 2014, 2018, and 2022 [17–20]. While just over 50 NIS were known for the Dutch Wadden Sea before 2009, at present, close to 100 NIS have been recorded.

Whereas it remained highly uncertain in 2009 how long ago the newly discovered NIS had established themselves in the region, this was much clearer for the NIS that were discovered in the subsequent surveys in 2011, 2014, 2018, and 2022. Many of these species could be followed in their spread throughout the Wadden Sea over the years [19]. As a result, hotspots could be pinpointed, highlighting where in the Wadden Sea most NIS were introduced, or at least where they were first established. These prime sites are the islands Texel and Terschelling, where the majority of the new NIS tend to remain established for several years within the marinas and ports, before spreading to more natural, farther offshore sites, and the mainland ports and marinas. In addition to the surveys of the Wadden Sea, NIS-focused baseline studies were initiated from 2010 in other inland waters, such as the Oosterschelde, Westerschelde, and Grevelingen, and in 2020 and 2021, the Veerse Meer [21–24]. These studies were conducted for various reasons. For example, some were in accordance with national legislation [14] focused on minimising the risk of shellfish transports introducing alien species that may negatively impact the Natura 2000 values of the Wadden Sea and Oosterschelde, in the north and south of The Netherlands, respectively (Figure 1). In addition to the inland water systems, various baseline studies have been carried out since 2014 in ports, viz., in the main ports of Vlissingen, Rotterdam, IJmuiden, Den Helder, and Eemshaven [25–29]. These studies were primarily conducted following the OSPAR/HELCOM port survey protocol, as developed in 2013 [30] in support of evaluating the possibilities for exemptions to the Ballast Water Convention, which came into force in 2017 and will be effective in 2024. This OSPAR/HELCOM port survey protocol closely resembles the NIS survey protocol focused on all habitats (Figure 2) that was already used in The Netherlands for NIS baseline studies in waters, such as the UNESCO World Heritage site the Wadden Sea [16–20].

Although it has been acknowledged that, since around 2010, for various reasons, intensified NIS baseline studies were carried out along the whole Dutch coast, the surveys were not part of a particular common program. Therefore, in 2021, in an effort to reduce costs and improve efficiency, compatibility, and accessibility, the Marine Alien Species Detection Network was started by the Office for Risk Assessment and Research (BuRO) of The Netherlands Food and Consumer Product Safety Authority of the Ministry of Agriculture, Nature and Food Quality (NVWA) [24]. The annually monitored hotspots (see Figure 3 for the sites included in 2021) do not only include vector-related sites, such as ports and shellfish production areas, but also sites that may function as stepping stones, such as navigational buoys along the North Sea coast. To optimise species detection, monitoring is conducted qualitatively, with molecular analyses supporting identification based on morphology. As a result, the main hotspots and pathways of alien species introduction to The Netherlands could be identified with higher accuracy [24].

The potential impact of the intensified NIS monitoring in the last decade is considered and discussed in more detailed analyses, focused on the origins, pathways, and vectors in time and space within The Netherlands.

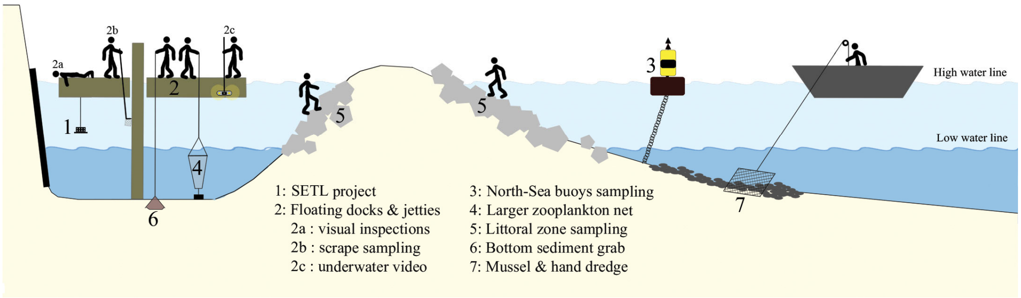


Figure 2. Main sampling methods used in the Marine Alien Species Detection Network of The Netherlands (from [24]).

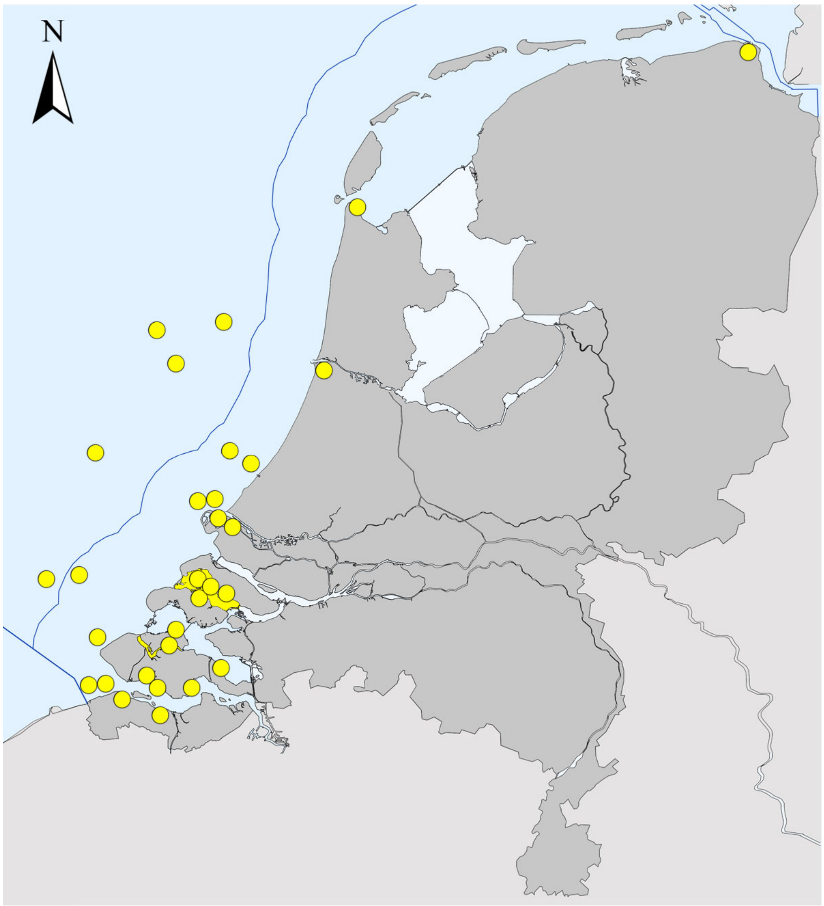


Figure 3. Sites searched for NIS within the Marine Alien Species Detection Network of The Netherlands in 2021, using the methods illustrated in Figure 2. In 2022, similar sites were included, with the addition of a few hundred sites in the north, which were sampled during a Wadden Sea survey focused on NIS [20]. At each site, the presence/absence of indigenous and non-indigenous species was assessed, based on morphology and, where deemed useful, molecular analyses. To ease the identification of NIS, increasing the chances of detection, taxon-specific collection, photography, fixation, and DNA analysis methods were used.

2.4. Further Data Collection

Observations by naturalists can be an important source of records for newly arrived NIS, especially when records are made and registered in a standardised and organised way. In The Netherlands, for example, the naturalist observations recorded by the ANEMOON Foundation come from a large number of moments and locations. Observations by scuba divers on hard substrata in particular have proven to be valuable. Professional monitoring of sublittoral hard substrata is time-consuming and expensive, while scuba diving is a popular activity by naturalists. The popularity of digital photography and sharing on social media enables the rapid dissemination of observations and the discussion of identifications. Naturalists often have a sophisticated knowledge of the morphology of local species. If they are aware of potential NIS, this facilitates (early) detection. Organisations such as the ANEMOON Foundation make observations available in an aggregated way [31]. There are obviously some downsides of naturalists' observations. Such observations have a bias towards larger and more easily recognisable groups, sometimes somewhat ignoring small and cryptic species, parasites, and infauna. Usually, no voucher specimens are taken, and there is mostly no secondary confirmation by molecular methods. Finally, another source of bias is that scuba diving mostly takes place in a certain selection of water systems only, increasing the chances of NIS being detected there.

Regarding the detection of marine alien species within ongoing monitoring programs that are not specifically focused on the detection of NIS, the MWTL (Monitoring Waterstaatkundige Toestand des Lands) program is the main long-term program on the water quality and biology of Dutch waters, e.g., for compliance with the European Marine Strategy Framework Directive 2008/56/EC (MSFD) and Water Framework Directive 2000/60/EC (WFD). A study in 2015 assessing the effectiveness of detecting alien species within the MWTL program indicated that, in total, only 24 marine alien species were detected within the period 1990–2010 [32]. In comparison, a total of 72 alien species were detected in 2021 alone within the Marine Alien Species Detection Network [24]. For the first detection of NIS, i.e., detection as new to the country, the setup of the MWTL program is clearly unsuitable. Of the 24 species detected in the MWTL program within the period 1990–2010, 21 species were already established several years or even decades before the start of the program [32]. Of the three remaining species, two were first detected by naturalists outside the MWTL program (this paper), and the remaining one, *Marphysa sanguinea* (Montagu, 1813), turned out to be a European species. Where it concerns other ongoing programs, such as the SIBES program focused on soft substrata in the Wadden Sea, and the surveys focused on WOT shellfish in the coastal zone, the same study concluded that these programs, without adjustments, were not clearly better equipped for the detection of alien species than the MWTL program. In more recent years, adjustments have been made to make these programs more suitable to detect NIS in the coastal waters of The Netherlands, e.g., by clearly labelling species as being an NIS or not.

3. Results and Discussion

3.1. Non-Indigenous Species Dynamics in Time and Space: Origins, Pathways, and Vectors

An overview of the 215 NIS that have been recorded in the coastal waters of The Netherlands with a salinity of >5 ppt is provided in Table S1. This list includes species assumed to have spread outside their native region with human aid. Whether they arrived in The Netherlands directly from their native region (primary introduction) or after an initial introduction elsewhere in NW Europe (by secondary distribution) is not discussed within the present article. While this list in Table S1 also includes, for example, unicellular species, endo-parasites, and viruses, for subsequent analyses in the following paragraphs, only macroflora and macrofauna >2 mm were selected, excluding endo-parasites and including only NIS that have a known origin outside of NW Europe.

3.1.1. NIS Taxa Recorded over Time in the Coastal Waters of The Netherlands

Over the years, a steadily increasing number of NIS has been reported for the coastal waters of The Netherlands, as may be concluded from Figure 4.

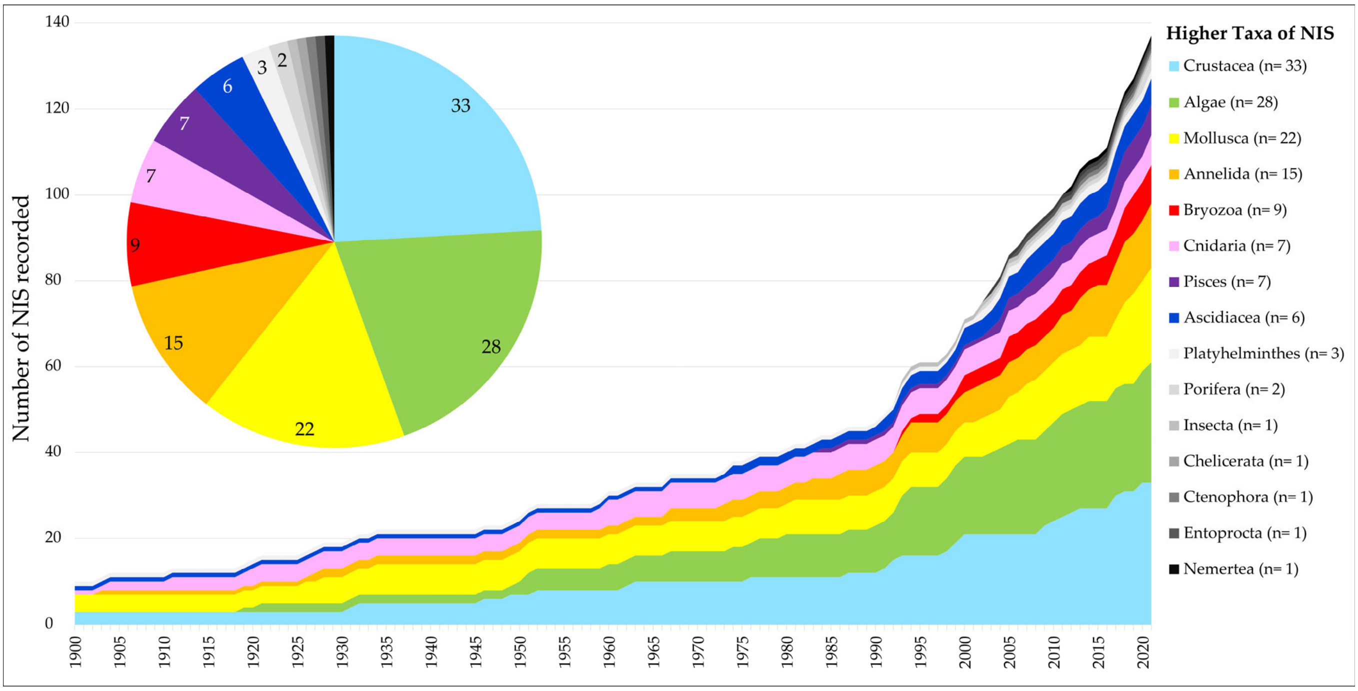


Figure 4. An accumulation graph illustrating the first record years of NIS that were probably introduced with human aid in the coastal waters of The Netherlands with a salinity of >5 ppt, from 1900 up to 2021, divided by taxon (after Table S1). Within this graph only macroflora and macrofauna species (>2 mm) are considered with a known origin outside of NW Europe, excluding endo-parasites. Taxa are ordered by the number of NIS recorded in total (see pie chart).

Several trends are visible:

1. Crustacea, followed by the (macro-)Algae and the Mollusca, form the main taxa of which NIS are recorded in the coastal waters.
2. The number of recorded NIS has shown a steady increase from the early 20th century up to the early 1990s. From the 1990s on, the number of new marine NIS that were recorded for The Netherlands on an annual basis increased. Although this increase is clear for NIS from various higher taxonomical groups, bryozoans seem to play an exceptional role. While no records of alien bryozoan species are known from before the 1990s, they have become an increasingly important group of NIS since the 1990s. This is also clear in Figure 5, illustrating that 6 out of the 10 alien bryozoan species recorded were first recorded in the last decade, i.e., between 2011 and 2020. Although this may partly be explained by a recently increased research effort regarding bryozoans, it may also be linked to hull fouling becoming the most important introduction vector of marine NIS in recent years, as is also discussed in paragraph 3.1.4. Whatever the underlying reason, the sudden increase in the annual rate of new NIS discoveries appears typical for the Greater North Sea area in general [9,11]. In other NE Atlantic regions, i.e., the Bay of Biscay–Iberian Shelf, Celtic Seas, and Macaronesia, no such increase was apparent from the 1990s on [9].
3. The main higher taxa of the NIS that were recorded as new to The Netherlands differed over the last three decades (Figure 5). These differences cannot easily be linked to research effort. Molluscs, for example, have always been a prominent part of various monitoring programs. It is, therefore, unclear why not even a single new non-indigenous molluscan species was recorded for The Netherlands during 1991–2000,

while in the following two decades, six and seven molluscan species, respectively, were recorded. Vice versa, there appears to have been a negative trend over the last three decades where it concerns the number of non-indigenous macro-algal species, with only two new NIS recorded in the most recent decade, i.e., 2011–2020 (Figure 5). One does have to be careful, however, with the interpretation of such trends, as in 2021 alone, two additional non-indigenous seaweed species were recorded: *Kapraunia schneideri* (Stuercke & Freshwater) Savoie & G.W. Saunders, 2019, and *Neopyropia yezoensis* (Ueda) L.-E. Yang & J. Brodie, 2020.

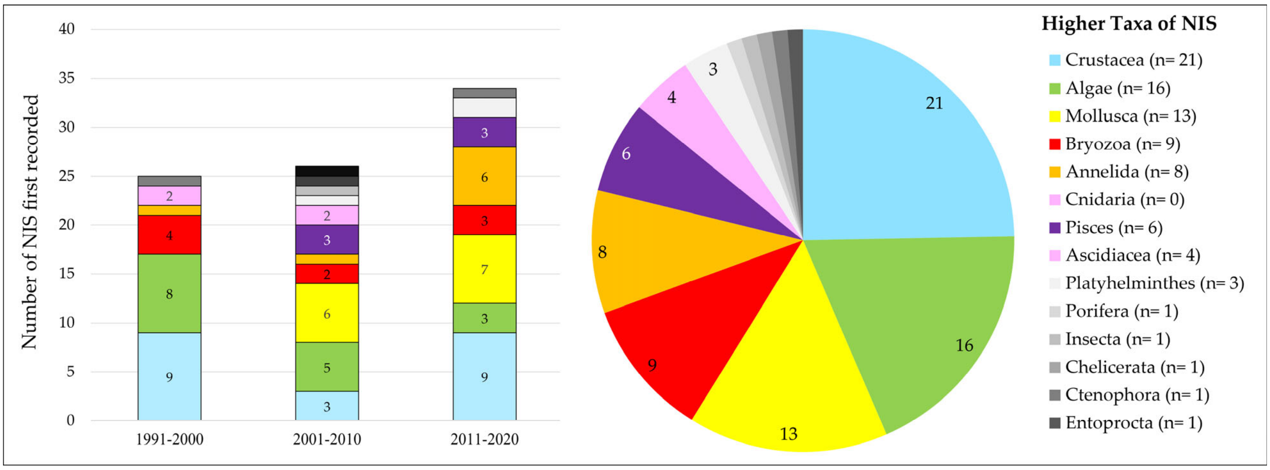


Figure 5. NIS taxa recorded for the first time in 1991 to 2020 in the coastal waters of The Netherlands (after Table S1). Within these graphs, only macroflora and macrofauna species (>2 mm) are considered with a known origin outside of NW Europe, excluding endo-parasites. Bar chart: NIS taxa first discovered in the decades 1991–2000, 2001–2010, and 2011–2020. Pie chart: NIS taxa first recorded in the period 1991 to 2020. Taxa are ordered by the number of NIS recorded in total (see pie chart).

3.1.2. NIS Origins over Time

Over the years, NIS have been arriving in The Netherlands from various seas and oceans worldwide. From where NIS are introduced appears to vary strongly over time, as is visible in Figure 6. Based on this figure, several conclusions are possible:

1. The marine NIS that have been recorded in the coastal waters of The Netherlands originate from various regions worldwide, with the most significant areas being the Indo-Pacific region (blue in Figure 6), directly followed by the West Atlantic coast (greenish in Figure 6).
2. A relatively small number of NIS originate from the Mediterranean and the Ponto-Caspian region. Although this may suggest that NIS are not often introduced from these seas, one should consider that the Mediterranean in particular can also function as a stepping stone for NIS primarily introduced there from the Pacific. For example, the cryptic moon jellyfish *Aurelia coerulea* von Lendenfeld, 1884, originates from the Pacific. It was recently found to be established in The Netherlands [23] and was most likely introduced by secondary spread from the Mediterranean Sea, where the species is known to be well-established [33]. Concerning the relatively low number of species from the Ponto-Caspian, it must be considered that most species coming from those seas prefer salinities lower than 5 ppt, and are, therefore, not included in the present study.
3. Three decades ago, i.e., between 1991 and 2000, close to eighty percent of all newly recorded NIS originated from the Indo-Pacific. This is often linked [34] to the imports of the Pacific oyster *Magallana gigas* (Thunberg, 1793) directly from the Pacific, for example, by plane, in the 1970s and 1980s. Such Pacific oyster imports have no longer been allowed in most European countries since the 1990s, however, acknowledging

the risk of NIS introductions. This may explain why a much lower percentage (about 50%) of all NIS that were first recorded in 2001–2010 originate from the Pacific.

4. The relative decrease in the number of species originating from the Pacific in 2001–2010 does not explain why, in this decade, the absolute number of NIS originating from the W Atlantic had more than doubled.
5. Finally, looking at the origins of species in the most recent decade, 2011–2020, it is surprising to see that the number of NIS that originate from the Pacific has increased again to about the same number as in 1991–2000. As Pacific oyster imports from other continents are no longer allowed, a different pathway (maybe hull fouling) and, thereby, vector must have been responsible for this increase.

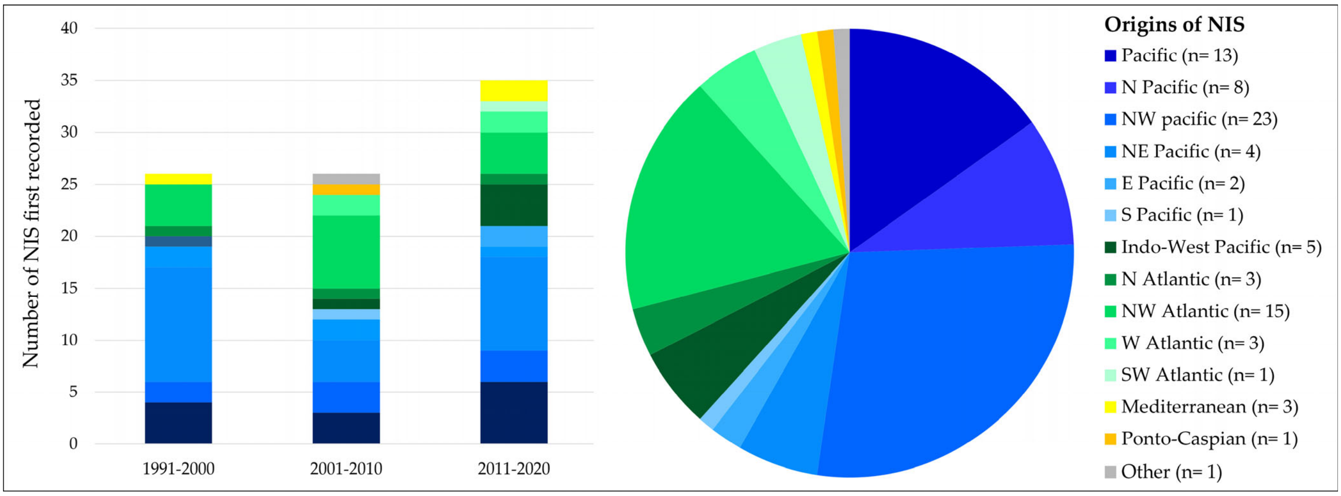


Figure 6. Origins of NIS recorded for the first time in 1991 to 2020 in the coastal waters of The Netherlands (after Table S1). Within these graphs, only macroflora and macrofauna species (>2 mm) are considered with a known origin outside of NW Europe, excluding endo-parasites. Bar chart: Origins of NIS in the decades 1991–2000, 2001–2010, and 2011–2020. Pie chart: Origins of NIS first recorded in the period 1991 to 2020. NIS originating from the Pacific are all represented by shades of blue in the figures, while those from the Atlantic are represented by shades of green. Species assumed to be native to only a region within an ocean, e.g., the northeast of the Pacific, are included in that category only in the figures, and are therefore excluded in the count for the whole ocean, e.g., the darkest blue-coloured "Pacific" category.

The reasons for the explanations of trends in NIS origins mentioned above not being as clearcut as we would like are at least twofold. First, a number of NIS were directly introduced to The Netherlands from other parts of the world (primary introduction), while others were first introduced to other countries in the NE Atlantic, with subsequent spread to The Netherlands, sometimes by different vectors or natural dispersal (secondary introduction). Therefore, a region-wide analysis of NIS arrivals could potentially provide a clearer picture of trends in origin (as well as the taxonomic group and water body of first introduction). However, as national NIS data are very unequal with respect to 'completeness', spatial coverage, taxonomic emphasis, and 'uncertainty' in vectors, a Europe-wide analysis has its own drawbacks. A second difficulty in analysing trends in NIS data is the stochastic character of the introduction process. The establishment of new NIS depends on many chances, among them, the chance of the uptake of a species by a vector, the chance of survival during transport, and the chances of release, survival, and reproduction in the receptor region. This may result in fluctuations in the numbers of new NIS originating from different areas, merely caused by chance processes. Especially if the numbers are low, this may cause relatively high differences between years or decades.

To conclude, when studying introduction pathways and vectors of marine NIS, for example, to develop management options and horizon scanning possibilities, one needs to consider that the marine NIS that have been recorded over time strongly vary in their origins. Thus, further studies should focus on explaining the more than doubling of the NIS originating from the W Atlantic in 2001–2010 and the sudden increase in NIS originating from the Indo-Pacific in 2011–2020. Finding an explanation for these trends will ease horizon scanning initiatives predicting where NIS may come from in the near future.

3.1.3. Water Systems Where NIS Were First Recorded

Over the years, new NIS for The Netherlands have been recorded in all of the main water systems (Figures 1 and 7). Where most NIS are first recorded varies strongly over time, as is visible in Figure 7. Based on this figure, several conclusions are possible:

1. The Oosterschelde, a Natura 2000 area, is the area in which most NIS were recorded for the first time in The Netherlands (Figure 7). However, the absolute number of new NIS recorded in the Oosterschelde has declined over the last 3 decades, from 14 in 1991–2000 to 9 in 2011–2020 (Figure 7). The relatively high number of NIS recorded in this water system may be due to a combination of causes:
    a. The Oosterschelde is the most popular Dutch area for scuba divers who are actively scoring the species present. These "citizen science" records, which also include NIS, are recorded by the ANEMOON Foundation and represent an important part of all new NIS recorded [31].
    b. Within the Oosterschelde, there are many marinas for pleasure crafts, which may act as introduction vectors for NIS.
    c. The Oosterschelde is an important area for shellfish transports, which may function as an introduction vector for NIS.
    d. Other water systems in The Netherlands are either brackish, have less hard substrata, and/or are in general less diverse in terms of micro-habitats, offering newcomers fewer niches to settle in.
2. Monitoring effort may explain in large part the trends over time visible in Figure 7. This becomes especially clear in the last period "2011–2020", during which seven NIS new to The Netherlands were recorded in the Wadden Sea and Veerse Meer. This high number of new NIS records is directly linked to the NIS-focused surveys that were conducted in these waters since 2009 [16–20,23]. As these were the first NIS-focused surveys there, they resulted in the detection of a relatively high number of new NIS, for which it was not always clear how long they had already been established there.
3. Concerning the number of new NIS recorded within the North Sea Canal (Figures 1 and 7), it should be considered that more NIS than shown here, new to The Netherlands, were recorded. This concerns NIS that established themselves in parts of the canal with salinities <5 ppt, e.g., *Laonome xeprovala* Bick & Bastrop, 2018 (see [35]). As the present study focuses on species occurring in waters with salinities of >5 ppt, they were not included.

Although it is often assumed that the water system in which a NIS is first recorded is also the site where a species was first introduced, the present analyses (Figure 7) indicate that monitoring effort should always be considered. Although the high number of NIS first recorded in the Oosterschelde probably correctly indicates that many NIS are first introduced there, one should consider that scuba diving is much more popular in these waters than in the Westerschelde and Wadden Sea, for example. NIS being introduced into the latter two water systems may first be missed, being recorded by scuba divers after they have distributed themselves secondarily to the Oosterschelde. As NIS monitoring effort in water systems other than the Oosterschelde has intensified within the last decade, new NIS are now detected in a more even spread over The Netherlands. As a result, the period 2011–2020 column in Figure 7 probably provides a more reliable indication of the main sites where NIS were first introduced in the coastal waters of The Netherlands than the columns representing the prior two decades.

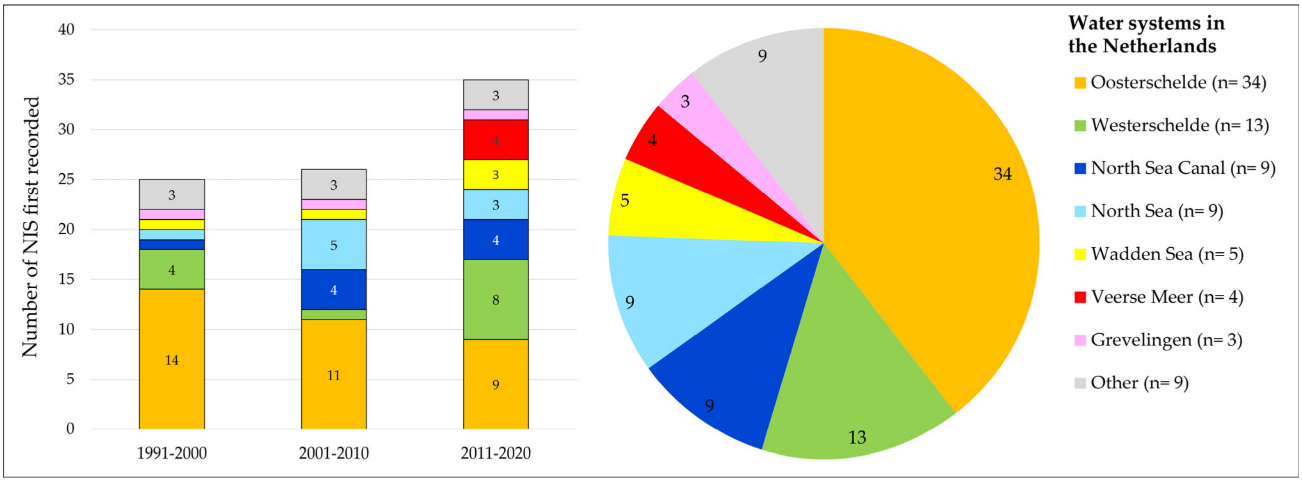


Figure 7. Water systems in The Netherlands (Figure 1) where NIS were first recorded from 1991 to 2020 in coastal waters (after Table S1). Within these graphs, only macroflora and macrofauna species (>2 mm) are considered with a known origin outside of NW Europe, excluding endo-parasites. Bar chart: Water systems where NIS were recorded for the first time in the decades 1991–2000, 2001–2010, and 2011–2020. Pie chart: Water systems where NIS were recorded for the first time in the period 1991 to 2020. Water systems are ordered by the total number of NIS recorded (as specified in the pie chart) in these systems.

3.1.4. Likely Vectors with Which NIS Were First Introduced

As shown in Figure 8, the likely vectors with which NIS were introduced have varied as much over the last three decades as the origins and the water systems where NIS were first detected (Figures 6 and 7). This is understandable, as the vector with which a NIS is introduced is often assumed to be linked to the origin of the species and the water system where it was first recorded. Taking this into account, based on Figure 8, several conclusions are possible:

1. During the last three decades, i.e., between 1991 and 2020, but also before that time [34], hull fouling has generally been assumed to be the main vector connected to the introduction of marine NIS into the coastal waters of The Netherlands.
2. After hull fouling, ballast water, fisheries, and aquaculture (mainly shellfish transports) have most frequently been linked to introductions, as well as the "natural distribution" of NIS that were introduced elsewhere in NW Europe and secondarily reached the Dutch coast by natural dispersal.
3. Over time, not all vectors were assumed to play an equally important role concerning the introduction of species. Ballast water, for example, is assumed to be responsible for the introduction of 15 NIS in 2001 to 2020, while none of the NIS first recorded in 1991–2000 were assumed to have arrived by this vector.
4. The number of NIS introduced by hull fouling appears to have almost tripled in 2011–2020 in comparison to 2001–2010, while the number of species assumed to be introduced by fisheries and aquaculture decreased slightly.

It is highly uncertain to what degree the trends in the numbers of NIS assumed to have been introduced by the various vectors are reliable. Varying NIS monitoring effort across water systems, for example, can give a wrong impression about the actual site where the species was first introduced. It should also be questioned whether an NIS may have arrived by primary introduction from its region of origin, or secondarily from elsewhere in Europe. The reliability in assessing likely vectors may be increased by taking such aspects routinely into consideration. This is beyond the scope of the present article, however, and will be the focus of future research. Here, primary and secondary vectors are assessed separately, and an "uncertainty score" is linked to either direct sightings of the NIS on the vector concerned (e.g., in a hull fouling community), in a region connected to the vector (e.g., in a port), and/or only in a habitat, which may indicate the potential of an NIS being transported with the vector concerned (e.g., in fouling communities).

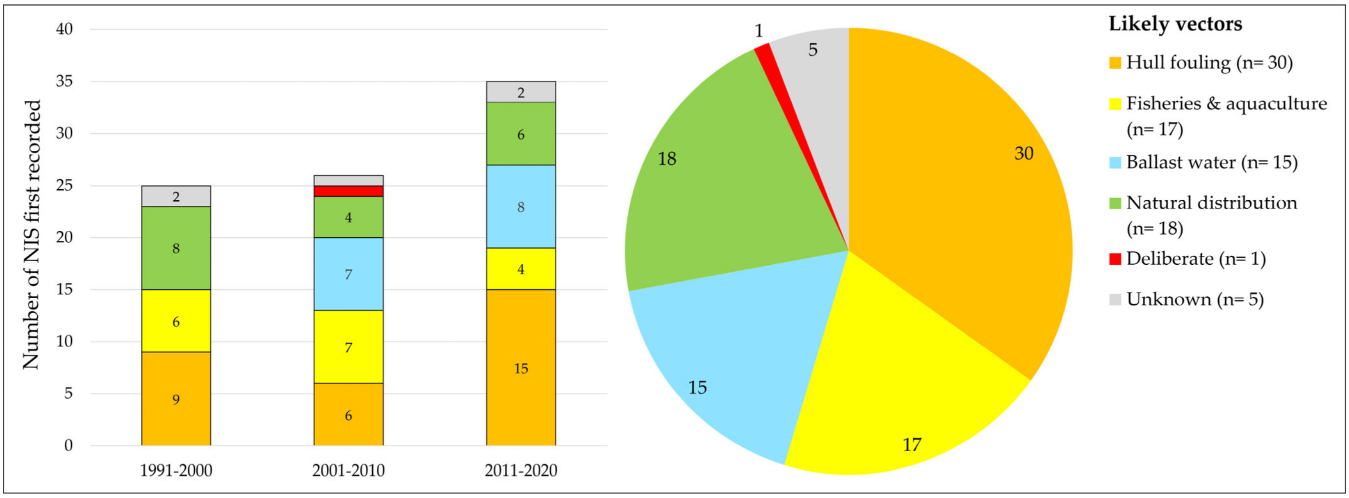


Figure 8. Likely vectors with which NIS were first introduced in the coastal waters of The Netherlands in 1991 to 2020 (after Table S1). Within these graphs, only macroflora and macrofauna species (>2 mm) are considered with a known origin outside of NW Europe, excluding endo-parasites. Bar chart: Likely vectors of first introduction in the decades 1991–2000, 2001–2010, and 2011–2020. Pie chart: Likely vectors of first introduction in the period 1991 to 2020. Vectors are ordered by the total number of NIS that are assumed to have been introduced (as specified in the pie chart) by these vectors.

As long as all of this is not considered in assessments of the most likely vectors and pathways, one should consider trends, as they are presented in Figure 8, as very rough indications at most.

3.2. *Unnoticed Establishments and Unexpected Pathways*

In June 2021, a survey focused on alien species was conducted in the Veerse Meer, a marine lake connected by a canal to the city of Middelburg in the centre of the former island of Walcheren, in the SW of The Netherlands. The survey was initiated by order of the Office for Risk Assessment and Research of The Netherlands Food and Consumer Product Safety Authority, as a baseline study [23]. The diversity of NIS in the lake, a Natura 2000 area, had never before been fully assessed. The survey, based on fieldwork expeditions in spring and summer 2020 and 2021, resulted in the discovery of 30 NIS, previously unknown to the lake, 6 of which were new records to The Netherlands [23]. Three of the latter species, i.e., a moon jellyfish and two flatworms, are discussed in more detail in the following paragraphs, based on [23] and follow-up studies, the data from which are included in Tables S2 and S3. They exemplify species that, after their discovery, turned out to actually have a much wider distribution in The Netherlands. Additional surveys provided more insight into their potential invasive pathways and vectors of spread.

3.2.1. Overlooked Establishment of the Cryptic Moon Jellyfish *Aurelia coerulea*

The Western Pacific moon jellyfish, *Aurelia coerulea*, was recently discovered to be one of the most common moon jellyfish species in the euryhaline and eurythermal coastal lagoons, marinas, and harbours of the Mediterranean Sea [33]. How long ago it established itself there remains uncertain, as moon jellyfish (*Aurelia* spp.) have often been misidentified as the supposedly cosmopolitan species *Aurelia aurita* (Linnaeus, 1758). Molecular analyses of Scorrano et al. [33] indicated, however, that *A. aurita* does not even occur in the Mediterranean. It should be considered native to Western European waters, where it is assumed to be the most common moon jellyfish species.

In [36], the authors report three and one jellyfishes in Western Europe, which they identified as *Aurelia coerulea* based on DNA analyses. They do not indicate that these individuals, from Roscoff, France, and Büsum, Germany (Figure 9), respectively, prove the establishment of this alien species.

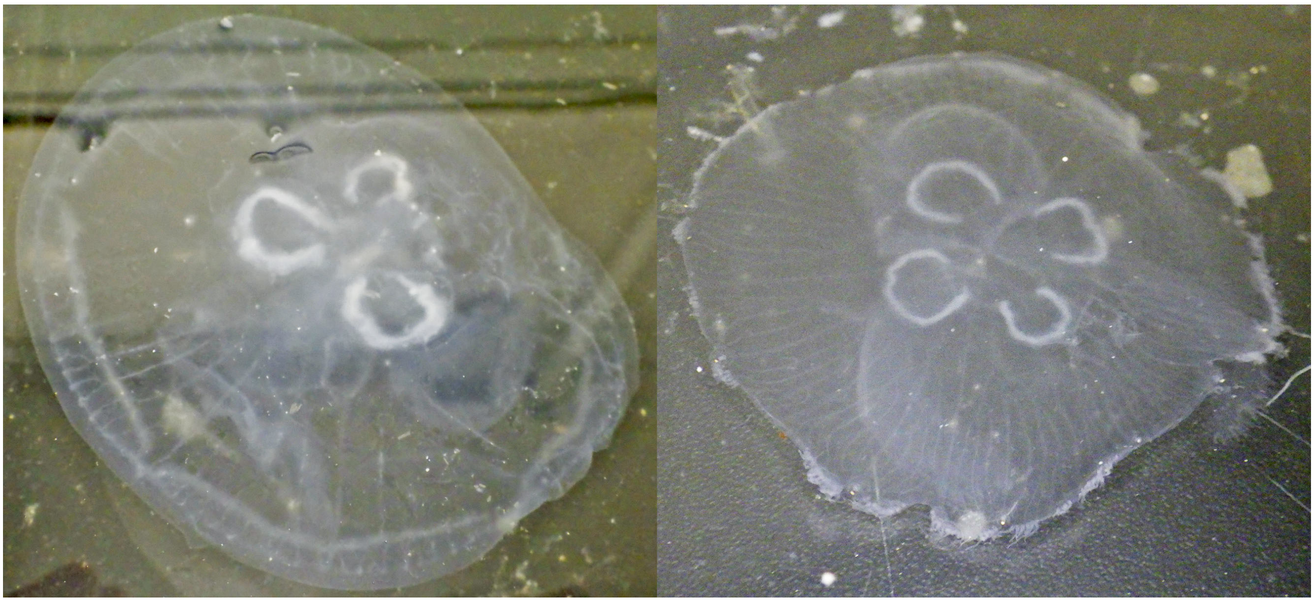
Figure 9. *Aurelia coerulea* records worldwide (red dots), according to Table S4 in [36]. Records that are indicated in this map in NW European waters concern sequences from three specimens collected off Roscoff, France, and one specimen collected near Büsum in the German part of the North Sea.

The real geographic ranges of moon jellyfish species are not well known. Various recent studies show that the *Aurelia* species can hardly be distinguished from each other based on only morphological characters [36]. The main morphological character described in the literature that may be used to differentiate between *A. aurita* and *A. coerulea* concerns the dark-orange or brownish colour of the ephyrae [33], although Lawley et al. [36] mention that this should be considered an uncertain character awaiting further in-depth studies focused on ephyrae colouration. Whereas morphological characters may not always be exclusive, molecular analyses can easily be used to differentiate between the *Aurelia* species. This approach resulted in the here-reported discovery of established populations of *Aurelia coerulea* in several inland coastal waters of The Netherlands.

On 20 July 2020, large groups of moon jellyfishes were reported in the canals of the city Middelburg (https://www.omroepzeeland.nl/nieuws/12794329/het-wemelt-van-de-kwallen-in-de-middelburgse-grachten, accessed on 9 April 2023). As was reported in the local news, moon jellyfish are usually only seen in the sea, and never that far inland in more brackish waters. A clear explanation was not given. Climate change, overfishing, eutrophication, and other anthropogenic causes are often mentioned as reasons that jellyfish populations are increasing worldwide, though often these claims are not based on robust evidence, as the long-term monitoring of jellyfish abundance is rarely performed [37]. Along the Dutch coast, the most common native jellyfish species, *Aurelia aurita* and *Rhizostoma octopus* (Gmelin, 1791), appear to have decreased in abundance [38].

The next year, in June 2021, numerous moon jellyfish with a seemingly atypical red colour were noticed within a baseline study of the non-indigenous species in the Veerse Meer [23]. This marine lake is connected to Middelburg by a canal, which can explain how the jellyfishes got there. Because of their atypical colour, twenty individuals were collected with a large zooplankton net at different sites. They varied in life stages from large medusae to small ephyra and were conditionally identified as native common moon jellyfishes (*Aurelia aurita*) that had fed on a reddish-coloured phytoplankton species. To increase the chances of detecting non-indigenous species, DNA analyses were carried out as standard during this survey in case of any doubt regarding species identifications.

To analyse the collected *Aurelia* specimens, the DNA was extracted using a CTAB-based protocol based on [39], with the adjustment of using the premade CTAB-Lysis buffer from BioChemica (A4150), and dissolving the DNA pellet in Tris-EDTA buffer (pH 8.0) instead of MilliQ. The Qiagen Rotor-Gene Q standard PCR mix was used with the specific primers LCOjf and HCOcato from [40] for the Rotor-Gene Q apparatus optimised PCR-program with the following steps: 95 °C for 5 min, 2× (95 °C for 4′; 51 °C for 2′, 72 °C for 2′), 43× (95 °C for 45″; 50 °C for 45″, 72 °C for 1′), an extension at 72 °C for 5′, and an HRM step from 75 °C to 90 °C at a rate of 0.1 °C every 2 s. The HRM analyses clearly show two groups of PCR products, suggesting the presence of two species of *Aurelia*. Subsequent sequencing of the PCR products at Macrogen Europe confirmed this. Both the common native moon jellyfish *Aurelia aurita* and the NW Pacific cryptic moon jellyfish *Aurelia coerulea* were present among the individuals collected. The HRM analyses indicated that COI PCR products of *A. aurita* melt around 81.2–81.6 °C, and those of *A. coerulea* melt around 81.8–82.7 °C. This difference was consistently found, enabling the identification of specimens based on only the HRM analyses at the end of a PCR. Concerning the medusae, no obvious morphological differences could be seen between the two species (Figure 10). To validate the HRM-based identification method, a selection of PCR products was sequenced, all confirming the validity of the method: Genbank Accession numbers OQ940539-OQ940549.



(**a**) (**b**)

Figure 10. Moon jellyfish medusae of the two species that live in a panmictic population in The Netherlands. (**a**) Collected in the Veerse Meer: *Aurelia coerulea* as confirmed by DNA analyses (Genbank acc. no. OQ940549). Size: about 10 cm in diameter. (**b**) Collected in the Oosterschelde: *Aurelia aurita* as confirmed by DNA analyses (Genbank acc. no. OQ940541). Size: about 9 cm in diameter.

Following the above confirmation of the presence of *Aurelia coerulea* in Dutch waters, moon jellyfish were collected with a large zooplankton net within the Marine Alien Species Detection Network along the whole Dutch coastline in the late summer of 2021 to assess the spread and establishment of *A. coerulea* [24] (Table S2). Additionally, specifically aimed at finding the polyps of *A. coerulea*, some sampling was carried out in the fall of 2022.

Based on subsequent DNA analyses, several conclusions are possible.

1. *Aurelia coerulea* is established in a panmictic population together with *A. aurita* in the inland marine lakes Veerse Meer and Grevelingen (Figure 11). Moon jellyfish collected at more exposed sites along the North Sea and within the Oosterschelde and southern Westerschelde represented *A. aurita*. The establishment of *A. coerulea* within the sheltered inland marine lakes of The Netherlands agrees with the fact that this species has a lagoonal- or harbour-limited distribution within the Mediterranean [33]. The presence of *A. coerulea* in the northern Westerschelde, in the port of Vlissingen (Sloehaven), also supports this and may be linked to the canal through Walcheren, which connects the Veerse Meer with the Westerschelde, near the Sloehaven. The high numbers of moon jellyfish sighted in the canals in Middelburg in 2020, mentioned earlier, may have concerned this jellyfish species, as well.

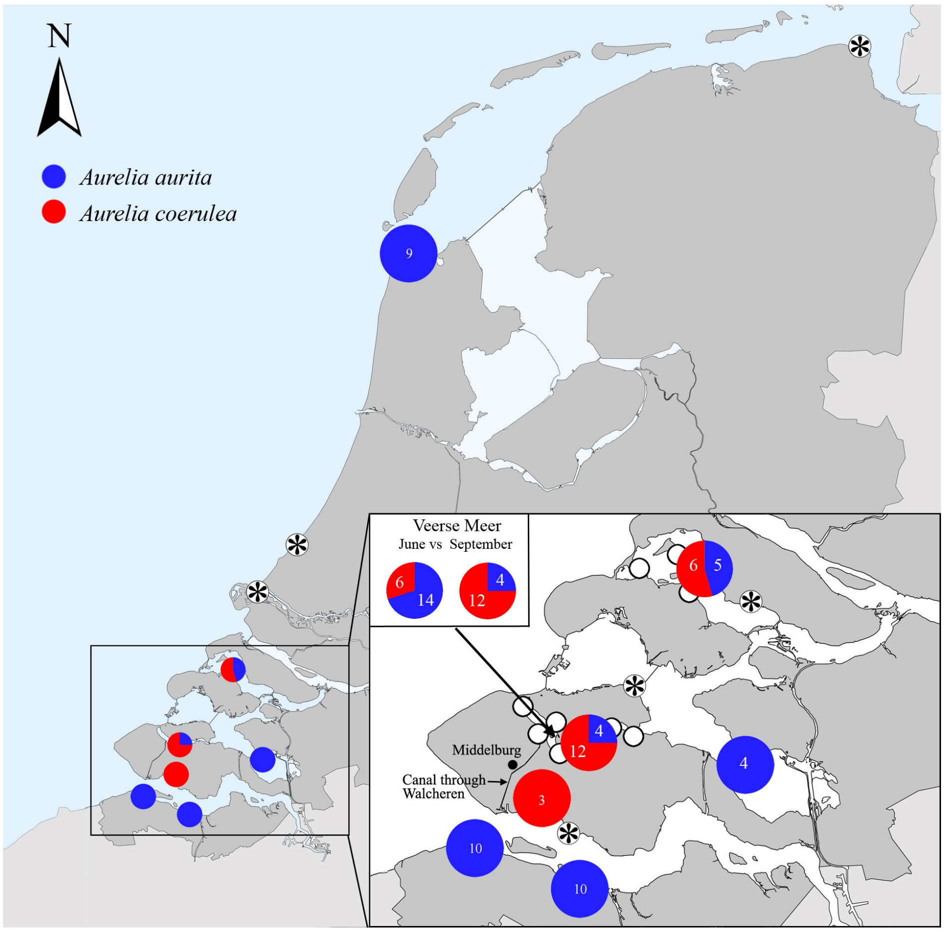


Figure 11. *Aurelia* spp. distribution in the late summer of 2021. Exact dates and sampling locations are given in Table S2. Sites are shown where moon jellyfish were caught with a large zooplankton net after they were spotted within 15 min by looking over the water surface. Pie charts indicate the numbers of *Aurelia aurita* and *A. coerulea* specimens caught at these sites. Several sites within the lakes Veerse Meer and Grevelingen were sampled. Their exact locations are indicated in the map with the symbol ○ and specified in Table S2. Within the pie charts made for these lakes, these sites were combined. Locations that are marked with a ✱ were visited in September/October 2021, but no moon jellyfish were sighted within 15 min of looking (Table S2). As is illustrated in a pie chart based on samples taken in the early summer of 2021 in the Veerse Meer, *A. aurita* was the most dominant moon jellyfish, while *A. coerulea* was the dominant moon jellyfish there in late summer. Still unpublished results of the monitoring in 2022 indicate the same seasonal pattern.

2. At least within the Veerse Meer, *Aurelia aurita* is the dominant species in early summer, while *A. coerulea* is dominant in late summer. Thus, the two species peak after each other in early to late summer. As a result, high numbers of moon jellyfish (*Aurelia* spp.) can be encountered in the lake throughout the summer.
3. Collecting and sequencing *Aurelia* polyps from settlement plates in the summer of 2021 deployed at a depth of 1 m in the Veerse Meer resulted in the detection of only *A. aurita*. Specifically aimed at detecting the whereabouts of *A. coerulea* polyps in the lake, on 29 October 2022, several medusae were collected from a beach off Geersdijk, where polyps were collected from oyster shells the same day by scuba diving between 8 and 10 m in depth. Subsequent DNA analyses of four medusae and six polyps taken from different shells indicated that they all were *A. coerulea* (Genbank nrs: OQ940545-OQ940549). Detecting the polyps was not assumed to be simple, taking into account that only the polyps of *A. aurita* were detected in the Veerse Meer in 2021, whereas a recent study by van Walraven et al. [38], specifically focused on finding the polyps of all jellyfish species occurring in the southern North Sea area, also resulted in only the detection of *A. aurita*.

Concerning the introduction vector of *A. coerulea* in The Netherlands, there are several possibilities. The species may have been introduced in the port of Vlissingen (Sloehaven) by ballast water arriving with a ship from the Mediterranean. Subsequently, the medusae may have used the canal through Walcheren, via Middelburg, towards the Veerse Meer.

An alternative possibility to consider concerns the glass eels, which are caught in southern European waters and released in the Veerse Meer in support of the endangered European eel, i.e., *Anguilla anguilla* (Linnaeus, 1758) population, for restocking purposes, following Council Regulation (EC) No 1100/2007 [41]. It remains to be studied whether the ephyra stages of jellyfish can travel along with these glass eels.

With the limited data available, it is unclear if polyps could easily have been transported within hull fouling communities on pleasure crafts, as all polyps found on settlement plates deployed at 1 m depth from floating docks belonged to the common native moon jellyfish *Aurelia aurita*. The polyps of *A. coerulea* were only found at one site, at much greater depths, on the bottom of the Veerse Meer.

Natural distribution from the Mediterranean cannot entirely be excluded as a possibility. In that case, *A. coerulea* medusae would have drifted along with the south to north residual currents along the Western European coast up to The Netherlands, using several stepping stones along the way. Anyway, knowing that this species has established itself in the coastal waters of The Netherlands indicates that it may have also established itself elsewhere in Western Europe, being overlooked because of its morphological resemblance to *Aurelia aurita*.

The introduction of *A. coerulea* can have several ecological and economic impacts. In the canonical metagenetic life cycle of many scyphozoan jellyfish, the adult medusae produce planula larvae that settle on a substrate, developing into polyps that bud off juvenile jellyfish called ephyrae, growing into adults again [42]. *A. coerulea* is known to be able to bypass the polyp stage, producing ephyrae directly from planula larvae. This direct development allows the species to form higher densities in the short term [43]. The addition of high densities of *A. coerulea* in late summer and autumn could increase grazing pressure on zooplankton, next to the already high grazing pressure exerted by another invasive species, the ctenophore *Mnemiopsis leidyi* A. Agassiz, 1865, which is also abundant in late summer and autumn [38].

The direct impacts of jellyfish on humans include a decrease in the appeal of areas for coastal tourism, the clogging of fishing gear, and the clogging of power plant intakes (as reviewed in [44,45]). In Korean waters, *A. coerulea* blooms have a severe economic impact through interfering with fisheries and clogging power plant intakes, which has prompted efforts to remove polyps and medusae [46]. In the Dutch port of Sloehaven, where *A. coerulea* is reported in this study, *Aurelia* sp. medusae clogged a power plant intake [47], suggesting that similar problems in Dutch waters are possible.

The establishment of the cryptic moon jellyfish *Aurelia coerulea* in the coastal waters of The Netherlands highlights various aspects to be considered regarding potential new introductions and establishments of non-indigenous marine species. Because cryptic invasive species may go unnoticed until they have established themselves and begun to cause harm to the environment [48], awareness of the existence of cryptic species and tools and knowledge to detect them is important for management and monitoring. There are several signs that can suggest the presence of a new cryptic species:

- It suddenly occurs outside of its "traditional" habitat;
- It suddenly occurs in different environmental conditions, such as temperature or salinity range;
- Its seasonal patterns suddenly change.

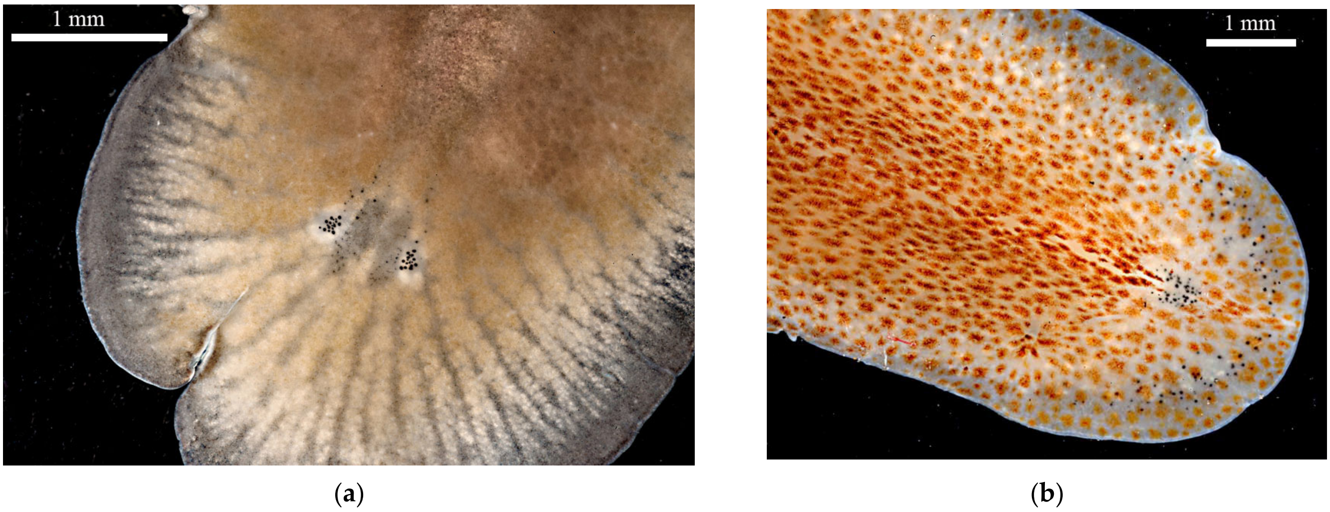
Changes in these parameters are often attributed to climate change, but might also be indicative of the presence of a recently introduced cryptic NIS, such as *A. coerulea*. DNA analyses can often be used to confirm this. Similar studies could be performed on other invasive species that are known or suspected to be cryptic species complexes, such as the hydroid *Cordylophora caspia* (Pallas, 1771) [18,49] and the tubeworm *Ficopomatus enigmaticus* (Fauvel, 1923) [50].

3.2.2. Two Pacific Flatworms Introduced to the NE Atlantic

Introduced marine species belong to a variety of taxonomic groups, with certain taxonomic groups being particularly well-represented, as is illustrated. Accounts of human-mediated transoceanic introductions of polyclad flatworms are relatively few [51], however. We hypothesise that the number of recorded polyclad introductions is underestimated, since in many routine monitoring programmes, flatworms are identified only to phylum, class, or order and not any further.

Only five non-indigenous flatworm species were recorded until 2020 along the Dutch coast (Figure 3). Two of these are *Stylochus flevensis* Hofker, 1930, which was originally thought to be endemic to a small inland sea in The Netherlands, the Zuiderzee [52], and *Stylochus necopinata* (Sluys, Faubel, Rajagopal & van der Velde, 2005). They are extremely similar in external appearance and are both recorded for the North Sea Canal (Figure 1). As the type material of *S. flevensis* could not be traced, Sluys et al. [53] could not completely rule out the possibility that these taxa are actually conspecific. Although they almost certainly originate from a different part of the world, their origin remains cryptogenic. A third introduced polyclad flatworm, also recorded in the North Sea Canal, is *Euplana gracilis* (Girard, 1850) [54]. The fourth and fifth introduced flatworm species recorded, i.e., *Notocomplana koreana* (Kato, 1937) (Figure 12a) and *Prosthiostomum wagurensis* Kato, 1944 (Figure 12b), both originate from the Pacific. They were first discovered (or at least identified) in 2020 during an NIS-focused survey in the Veerse Meer, which also resulted in the discovery of the cryptic moon jellyfish *Aurelia coerulea*. Molecular analyses enabled the identification of these species, as based on external morphology alone, closely related flatworm species are often difficult or even impossible to distinguish from each other. Both species could be identified after flatworm photos, and advice on their morphology and molecular analyses was given by Dr. Tsuyuki (pers. comm.), who has been involved in various studies describing Pacific flatworm species, based on both morphology and molecular analyses (e.g., [55]).

To analyse the flatworm specimens, the DNA was extracted in a similar manner as described above for the moon jellyfish. The Qiagen Rotor-Gene Q standard PCR mix was used, with the flatworm primers HRNT-F2 and HRNT-R2 [55] aimed at the marker 28S, and the primers HRpra2 and HRprb2-2 [55] aimed at the marker COI. For these two markers, the PCR programs that were used in a Rotor-Gene Q apparatus consisted of the following steps: for the marker 28S: 95 °C for 5′, 55× (95 °C for 2″; 55 °C for 10″, 72 °C for 60″), extension at 72 °C for 5′, and an HRM step going from 75 °C to 90 °C at a rate of 0.1 °C every 2 s; for the marker COI: 95 °C for 4′, 55× (95 °C for 60″; 48 °C for 60″, 72 °C for 60″), extension at 72 °C for 8′, and an HRM step going from 75 °C to 90 °C at a rate of 0.1 °C every 2 s.



(**a**) (**b**)

Figure 12. Pacific flatworms first recorded in The Netherlands. (**a**) *Notocomplana koreana*; (**b**) *Prosthiostomum wagurensis*.

To assess the spread and establishment of the two flatworm species, specimens were collected from various substrates and sites within the Marine Alien Species Detection Network (Figure 3) along the whole Dutch coastline in mainly the late summer of 2021. When processing samples, special attention was given to collecting flatworms, for example, from scrape samples, to be photographed alive in detail on both sides in a Petri dish with seawater, before fixing them in ethanol 96% and cutting off a small piece of tissue for DNA analyses. Based on subsequent DNA analyses, a distribution map was made illustrating the spread of the Pacific species *Notocomplana koreana* and *Prosthiostomum wagurensis* along the Dutch coastline (Figure 13). For these flatworms, the following conclusions can be drawn:

1. *Prosthiostomum wagurensis* and *Notocomplana koreana* both originate from the Pacific and were both first identified based on samples collected in the Veerse Meer. Although one may expect a similar introduction pathway, their distribution outside of this inland seawater lake does not support this view.
2. The presence of *P. wagurensis* could only be validated based on DNA analyses in sheltered inland seawater systems, such as the Veerse Meer and the Grevelingen (Figure 1). Taking into account that flatworms not distinguishable from *P. wagurensis* in terms of external features had already been noticed during earlier unpublished work in and near the ports of Rotterdam (Netherlands) on 22 May 2019, Vlissingen (Netherlands) on 27 May 2019, and Zeebrugge (Belgium) on 2 October 2019 (Faasse, unpublished), it appears most likely that the species was introduced into these ports and inland waters by ship hull fouling, possibly using the more sheltered parts of ports in Europe as stepping stones. Where in Europe the species was first introduced remains uncertain.
3. The presence of *N. koreana* was validated based on DNA analyses in the same sheltered inland seawater systems as *P. wagurensis*, but various additional specimens were collected by scrape sampling from 5 out of the 13 navigational buoys on the open North Sea that were searched for NIS in 2021. This indicates that this species can establish itself well in highly exposed habitats, and that it may be using the navigational buoys on the open sea off the west coast of Europe, in addition to ports, as stepping stones. Its potential presence in ports was already indicated by a study based on samples taken in 2017 in the port of Vlissingen (Sloehaven), in the Westerschelde (Figure 1). Although no flatworms were noticed at that time, metabarcoding indicated the presence of the DNA (COI) of *N. koreana* in a bulk sample taken from a settlement plate [56].

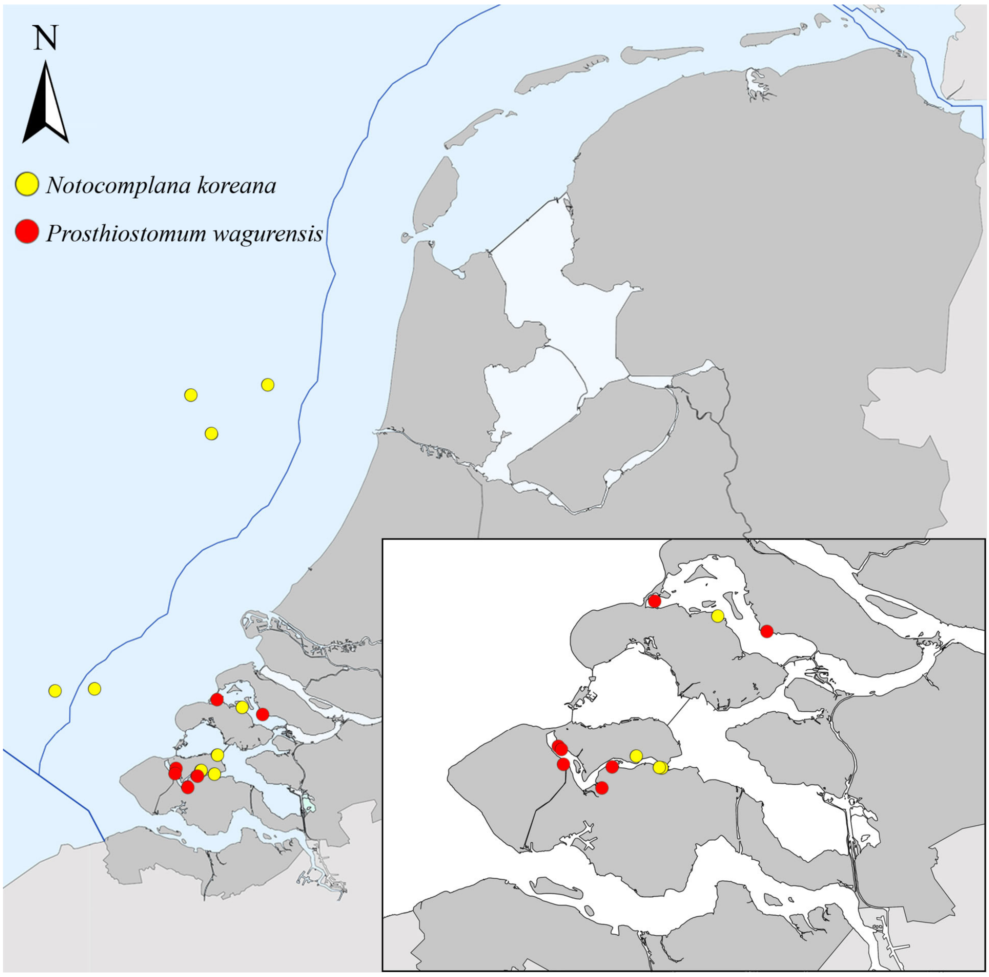


Figure 13. *Notocomplana koreana* and *Prosthiostomum wagurensis* distribution as confirmed by DNA analyses of specimens collected in 2020–2021. Exact dates and sampling locations are given in Table S3.

The establishments of the Pacific flatworms *Prosthiostomum wagurensis* and *Notocomplana koreana* highlight various aspects to be considered regarding potential new introductions and establishments of non-indigenous marine species.

Because of the extremely cryptic habits of flatworms, they are prone to being transported in ship hull fouling, and due to the presence of a pelagic larval stage, there is a possibility of transportation in ballast water. One has to be aware, however, that not all alien flatworms will follow the same pathways into and throughout Europe. Even though the species described here both originate from the Pacific and were both first detected in the same water system, it seems that one of them prefers more sheltered habitats, while the other appears to additionally establish itself in highly exposed open sea habitats. This makes navigational buoys and possibly other manmade constructions (e.g., wind turbines) on open seas, such as the North Sea, potential stepping stones for species such as *N. koreana*.

Most likely, both species have been introduced by hull fouling, although it cannot be excluded completely that they arrived in The Netherlands by natural spread, using ports and/or buoys as stepping stones. How and where these species were first introduced in Europe from the Pacific will probably remain uncertain, as the monitoring of ports and buoys for the presence of NIS has not always been common practice. In more recent years, for example, within the alien species monitoring network of The Netherlands, monitoring such potential hotspots and stepping stones has become more common. As a result, introduction pathways and vectors of NIS may become easier to trace. Tracing back where and when species were first introduced can further be aided by eDNA studies, as was conducted in the Port of Vlissingen in 2017 [56], during which the genetic material of *N. koreana* was detected three years before the first individuals were found and identified in the coastal waters of The Netherlands.

4. Conclusions

Information on temporal and spatial trends regarding NIS introductions can provide insights into pathways, vectors, and hotspots, while facilitating horizon scanning. This information is often sparsely available, however. Zenetos et al. [9] have, therefore, presented a baseline at European and subregional levels, whereby countries such as France and Denmark have published reviews on marine NIS on a national level, evaluating and analysing origins, pathways, and establishment [10,11]. Additionally, we here provide an overview of all marine and brackish water NIS that were recorded from The Netherlands, for which it is assumed that they dispersed outside of their native region with human aid. Based on macrofauna and macroflora species that have their origin outside the NE Atlantic, temporal trends over the last three decades were studied for the taxonomic groups, species origin, introduction vectors, and water systems in which the NIS were first recorded. These analyses showed, for example, 1: that bryozoan NIS were not recorded before the 1990s and are becoming an increasingly important group; 2: that NIS monitoring effort has been more equally divided over the water systems of The Netherlands since around 2010, probably resulting in new NIS records being more evenly divided over these water systems; and 3: that trends in likely vectors and pathways should be considered very rough indications at most.

Some NIS may have been introduced to The Netherlands straight away from their native range (primary introduction), while others may have first been introduced to other countries in the NE Atlantic area, with subsequent spread to The Netherlands by different vectors or natural dispersal (secondary introduction). An additional difficulty in analysing trends in NIS data is the fact that the establishment of new NIS depends on statistics, including the chance of uptake of a species by a vector, the chance of survival during transport, and the chances of release, survival, and reproduction in the receptor region. Especially if the numbers of records are low, relatively high differences between years or decades may for some part be explained “just by chance”.

To be able to trace back pathways and vectors, species have to be detected at a relatively early stage, before they have the opportunity to spread further. Based on three NIS that were recently recorded for the first time in The Netherlands, we learned to be aware of cryptic species. Such species may remain unnoticed because of a morphological resemblance to native species. In some cases, climate change may be misleadingly used to explain why certain allegedly native species suddenly start to be noticed outside of their “traditional habitats”, in a different environment, or in a different season of the year. As the establishment of the cryptic Pacific moon jellyfish *Aurelia coerulea* in The Netherlands shows, a newly introduced cryptic species should routinely be considered as a potential explanation of such “abnormalities”, possibly in addition to climate change. It is important to realise that ongoing monitoring programs were originally not focused on NIS, and thus were not designed to detect those NIS and may therefore have missed them. This may happen when a certain habitat is not sampled, or because the organisms in question are fixed in a way that hampers their identification. Within the present study, the discovery of two Pacific flatworm species is described, both identified in a NIS-focused Veerse Meer survey, based on morphology and DNA barcoding in 2020–2021. Subsequent sampling within the Marine Alien Species Detection Network of The Netherlands and unpublished naturalists’ observations of flatworms with similar morphologies revealed a much wider occurrence in The Netherlands. It is also illustrated how one of these flatworm species may use the navigational buoys in the North Sea as stepping stones, while the other species appears to prefer more sheltered habitats in inland waters and ports. Such data on NIS are essential for assessing pathways, vectors, and associated hotspots, both at present and in the future.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/d15060719/s1: Table S1: An overview of the NIS that have been recorded in the coastal waters of The Netherlands (Excel file). Table S2: *Aurelia* spp. sampling locations (Excel file). Table S3: *Notocomplana koreana* and *Prosthiostomum wagurensis* sampling locations (Excel file).

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