The Distribution of Pseudodiaptomus marinus in European and Neighbouring Waters—A Rolling Review

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Abstract: Among non-native copepods, the calanoid Pseudodiaptomus marinus Sato, 1913 is the species probably spreading at the fastest pace in European and neighbouring waters since its first record in the Adriatic Sea in 2007. In this contribution, we provide an update on the distribution of P. marinus in the Mediterranean and Black Seas, along the Atlantic coasts of Europe, in the English Channel and in the southern North Sea. Starting from a previous distribution overview, we include here original and recently (2019–2023) published data to show the novel introduction of this species in different geographical areas, and its secondary spreading in already colonised regions. The picture drawn in this work confirms the strong ability of P. marinus to settle in environments characterised by extremely diverse abiotic conditions, and to take advantage of different vectors of introduction. The data presented allow speculations on realistic future introductions of P. marinus and on the potential extension of its distribution range.

Keywords: Pseudodiaptomus marinus; copepod; non-indigenous species; European waters
1. Introduction

One of the effects of globalisation and anthropic pressure on aquatic natural systems is the huge amount of species (~10,000) transported daily worldwide [1], with a concomitant increase in the rate of introduction of non-indigenous species (NIS) (also known as alien, allochthonous, introduced, non-native species) in new areas [2]. Zooplanktonic organisms are among the most efficient at colonising regions outside their native range [3]. In most cases (90%), these include holoplanktonic species, principally in freshwater (62%) rather than haline (estuarine, brackish and marine; 38%) systems [3].

Among marine zooplankters, copepods are known to be efficient invaders both within and between continents (as discussed in [4]). These millimetre-sized crustaceans can be efficiently introduced through trans-oceanic ships, as they dominate the ballast waters zooplankton community (e.g., [5–7]). Additionally, documented evidence also reports the introduction of alien copepods through natural and/or human-made canals (e.g., [8]), as well as through aquaculture/mariculture (e.g., [9,10]). In the pelagic marine environment, examples of globally successful invaders include the cyclopoids Oithona davisae Ferrari & Orsi, 1984 [8,9,11–13] and Limnoithona tetraspina Zhang & Li, 1976 [14,15] and the calanoids Acartia (Acanthacartia) tonsa Dana, 1849 [16,17], Pseudodiaptomus forbesi Poppe & Richard, 1890 and Pseudodiaptomus inopinus Burckhardt, 1913 [14,18,19].

Over the last few years, another representative of the genus Pseudodiaptomus has made its appearance in European and neighbouring waters (ENW), namely, Pseudodiaptomus marinus Sato, 1913. Following its first record in the Adriatic Sea (northernmost part of the Mediterranean Sea) in 2007 [20], this species has rapidly spread not only across the entire Mediterranean Sea but also in the Black Sea, along the Atlantic coasts of Europe, in the English Channel and in the southern North Sea (as reviewed in [21,22]), with a >450% increase over a four-year time window (2015–2019) [22]. In some European countries, such as Croatia and Italy, P. marinus has been included in the NIS list monitored under the Marine Strategy Framework Directive (MSFD, 2008/56/EC) (Descriptor 1: Biodiversity and Descriptor 2: Non-indigenous species). The supposed primary vector of introduction is ballast waters, while secondary spread through coastal circulation and local ship traffic may favour its further dispersal [22]. The ability of this species to establish in diverse environments is likely supported by specific traits, including a wide salinity tolerance [23], behavioural plasticity (including a day–night alternation of epibenthic and pelagic phases) [24] and genetic diversity [25].

This contribution is intended as a periodic revision of P. marinus occurrence in the study area, following previous reviews ([21,22,26]; as of fall 2019). Such rolling reviews can provide an almost real-time view of the spread of this NIS and of its successful establishment in introduced areas. This work stems from the activities of the ICES WGEUROBUS (Towards a EURopean OBservatory of the non-indigenous calanoid copepod Pseudodiaptomus marinus) [22]. The emerging scenario manifests the ongoing arrival of P. marinus in new regions and its further spreading from already colonised areas via secondary introduction. The results presented provide elements to make hypotheses on the occurrence of P. marinus at a global scale and are contextualised in the general framework of global-scale marine invasions.

2. Materials and Methods

Starting from the scenario depicted in fall 2019 [22], a survey of the published literature reporting new records of P. marinus in ENW was carried out. For details on materials and methods employed, the interested reader is invited to refer to the cited works. In addition, new records are presented here as original data. In this case, as the materials and methods may differ from site to site, specific details are provided in the description of each record.

The study area has been regionalised according to the description given by the European Environment Agency [27], in line with scientific usage [28,29]. For each region, a west–east direction is followed in the listing of the records.
Figure 1 provides a map of the distribution of *P. marinus* in ENW including past (up to fall 2019), new published (fall 2019 to date) and original (this work) records. Table 1 summarises the temperature and salinity values (or ranges, depending on the site) associated with the *P. marinus* records in the survey literature and in the original data presented here.
Figure 1. Cont.
Figure 1. Cont.
Figure 1. *Pseudodiaptomus marinus* distribution in European and neighbouring waters. The data include published reports (orange symbols) and original records (green symbols). To improve the readability of the map in more densely crowded areas, insets are provided for the following sectors: (a) English Channel and southeastern North Sea; (b) southern North Sea; (c) Gulf of Naples; (d) western and northern Adriatic Sea; (e) eastern Adriatic Sea; (f) Gulf of Trieste. The full bibliographic references of the literature cited are provided in Appendix A. Details on the geographic coordinates of each site are provided in the Supplementary Material.
Table 1. Temperature (°C) and salinity values (or ranges, depending on data availability) associated with the occurrence of *Pseudodiaptomus marinus* in European and neighbouring waters, as derived from the literature survey and the original data presented in this work.

<table>
<thead>
<tr>
<th>Site</th>
<th>Temperature</th>
<th>Salinity</th>
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<tbody>
<tr>
<td>Bay of Biscay and the Iberian Coast</td>
<td></td>
<td></td>
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<tr>
<td>Peniche (PT)</td>
<td>15.5–17.1 °C</td>
<td>35.0–35.1</td>
</tr>
<tr>
<td>Celtic Seas</td>
<td></td>
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<tr>
<td>Bristol Channel (UK)</td>
<td>n.a.</td>
<td>n.a.</td>
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<tr>
<td>Greater North Sea</td>
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<tr>
<td>East Anglia (UK)</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>East Kent (UK)</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>Isle of Wight (UK)</td>
<td>n.a.</td>
<td>n.a.</td>
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<tr>
<td>River Blackwater (UK)</td>
<td>n.a.</td>
<td>n.a.</td>
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<tr>
<td>River Humber (UK)</td>
<td>n.a.</td>
<td>n.a.</td>
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<td>River Thames (UK)</td>
<td>n.a.</td>
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<tr>
<td>Southampton (UK)</td>
<td>n.a.</td>
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<td>South Kent (UK)</td>
<td>n.a.</td>
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<td>Southeast Yorkshire (UK)</td>
<td>n.a.</td>
<td>n.a.</td>
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<tr>
<td>The Eastern Scheldt estuary (NL) [25]</td>
<td>~21 °C</td>
<td>~31</td>
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<tr>
<td>Limfjord (DK)</td>
<td>18 °C</td>
<td>29</td>
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<tr>
<td>Hirtshals (DK)</td>
<td>12.2–12.8 °C</td>
<td>31.0–32.5</td>
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<tr>
<td>Western Mediterranean Sea</td>
<td></td>
<td></td>
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<tr>
<td>Leghorn (IT)—Nov 2020 §1</td>
<td>17.7 °C</td>
<td>38.3</td>
</tr>
<tr>
<td>— Nov 2021 §2</td>
<td>17.3 °C</td>
<td>38.1</td>
</tr>
<tr>
<td>Gulf of Naples—Sarno River (IT) §3</td>
<td>15.3 °C</td>
<td>37.6</td>
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<tr>
<td>Gulf of Naples—Torre del Greco (IT) §4</td>
<td>14.9 °C</td>
<td>37.9</td>
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<tr>
<td>Gulf of Naples—Between Torre del Greco and Torre Annunziata (IT)</td>
<td>17.4–28.1 °C</td>
<td>36.9–37.9</td>
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<tr>
<td>Gulf of Pozzuoli (IT)</td>
<td>15.2–27.0 °C</td>
<td>37.6–38.0</td>
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<tr>
<td>Gulf of Pozzuoli (IT) [26]</td>
<td>17.4–27.3 °C</td>
<td>37.5–37.7</td>
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<tr>
<td>Gulf of Salerno (IT) §5</td>
<td>16.6 °C</td>
<td>38.5</td>
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<tr>
<td>Ionian Sea and the Central Mediterranean Sea</td>
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<tr>
<td>Marina di Ragusa (IT) §6</td>
<td>17.3 °C</td>
<td>38.7</td>
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<tr>
<td>Adriatic Sea</td>
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<tr>
<td>Emilia Romagna coasts (IT) [27]</td>
<td>6.8–15.4 °C</td>
<td>33.3–39.9</td>
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<tr>
<td>Gulf of Trieste (IT)</td>
<td>9.6–25.9 °C</td>
<td>34.3–38.5</td>
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<tr>
<td>Marano and Grado Lagoon (IT)</td>
<td>5.0–30.0 °C</td>
<td>4.7–35.4</td>
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<tr>
<td>Croatian ports (HR) [28,29]</td>
<td>n.a.</td>
<td>n.a.</td>
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<tr>
<td>Neretva River (HR) [30]</td>
<td>10.6–25.5 °C</td>
<td>0.0–38.4</td>
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<td>Aegean-Levantine Sea</td>
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<tr>
<td>Thessaloniki Bay (GR) [31]</td>
<td>17.2–31.0 °C</td>
<td>34.8–38.5</td>
</tr>
<tr>
<td>Thermoikos Gulf (GR)</td>
<td>14.7–27.7 °C</td>
<td>34.9–38.5</td>
</tr>
<tr>
<td>Iskenderun Bay (TR) [32,33]</td>
<td>15.2–16.1 °C</td>
<td>38.5–38.8</td>
</tr>
<tr>
<td>Izmir Bay (TR) §4</td>
<td>19 °C</td>
<td>38.9</td>
</tr>
<tr>
<td>Yenifoca Bay (TR) [35,36]</td>
<td>14.6–15.1 °C</td>
<td>39.2</td>
</tr>
<tr>
<td>Hadera monitoring station (IL) [37]</td>
<td>16.2–32.4 °C</td>
<td>38.3–40.4</td>
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<tr>
<td>Black Sea</td>
<td></td>
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<tr>
<td>Izmit Bay (TR) [38]</td>
<td>15.6–23.2 °C</td>
<td>29.5–38.7</td>
</tr>
</tbody>
</table>

Vertically integrated values: §1 0–5 m; §2 0–50 m; §3 0–10 m; §4 0–90 m; §5 0–50 m; §6 0–4.5 m. § surface values.

3. Results

3.1. *Pseudodiaptomus marinus* Records from Published Literature

- Greater North Sea

  The presence of *P. marinus* was recently recorded in the Eastern Scheldt estuary (the Netherlands; southern North Sea) in September 2018, at a temperature of ~21 °C and a salinity of ~31 [30]. The occurrence of copepodites suggests active reproduction in the estuary. The arrival of this NIS may have been possible through a connection with the adjacent Western Scheldt, where *P. marinus* has been occasionally found since 2011, although other vectors of introduction could have worked as the estuary is subject to intense human activities including shellfish culture ([25] and references therein).

- Western Mediterranean Sea

  In the Gulf of Naples (Italy; central Tyrrhenian Sea), *P. marinus* was collected for the first time in offshore net samples (0–50 m depth layer) in December 2013 and April 2014 [22].
At the Long-Term Ecological Research site LTER_EU_IT_061 (also labelled as LTER-MC), this NIS was first found only in July 2014 [21], but eDNA metabarcoding (V4-18S rRNA) samples revealed the presence of this species since July 2011 [31]. Afterwards, this NIS was only seldom found in this area, mostly as copepodes.

The Gulf of Pozzuoli, in the northern part of the Gulf of Naples, is characterised by depths between 60 and 110 m and includes an industrial district affecting the surrounding environment [32]. Individuals of *P. marinus* were found in May and July 2019 [32]. Specimens were found with vertical tows in the 0–15 m layer with abundances of 5.6 (males) and 18.7 (copepodes) ind. m\(^{-3}\) and in the 0–50 m layer with a copepodite abundance of 3.4 ind. m\(^{-3}\). The supposed vector of introduction was mariculture, as samples were collected close to mussel farms.

**Adriatic Sea**

*P. marinus* was found during autumn and winter (November to January) in the period 2015–2017 along the coasts of Emilia Romagna (Italy; northern Adriatic Sea) [33]. This NIS was found at low abundance (maximum abundance: 17.1 ind. m\(^{-3}\)), at temperatures between 6.8 and 15.4 °C and salinities between 33.3 and 39.9, over a depth range of 8–25 m [33].

In the Croatian part of the eastern Adriatic, *P. marinus* was first detected in 2015 in the Šibenik Bay located in the Krka River estuary. Specimens of *P. marinus* were found in vertical net hauls collected after sunset in the central part of the bay (depth 36 m), which is consistent with the observed vertical distribution pattern of this species in its native environment [34]. Subsequent monitoring (2016–2022) confirmed the establishment of the *P. marinus* population at anchoring sites in the Šibenik port (depth 6 m) regardless of season, with all life stages present. In quick succession, additional occurrences of *P. marinus* were recorded in other estuarine areas of the eastern Adriatic closely associated with shipping activities: in 2018 at a fixed station in the port of Ploče (delta of the Neretva River, depth ca. 10 m) [22] and in 2020 in the northern (cargo) port of Split located in the eastern part of Kaštela Bay (18 m) [35]. In 2021, evidence of the dispersal of *P. marinus* from the original site in the north port of Split to the wider Kaštela Bay area was found. Apparently, all cases of *P. marinus* presence in the eastern Adriatic share some common features: locations in or near harbours, nocturnal presence in the water column, evidence of established populations and no signs of changes in the local zooplankton community. Considering the detection sites, ballast water is likely to be the main vector of introduction, followed by secondary dispersal to nearby areas.

Recently, genetic sequences irrevocably assigned to *P. marinus* were determined in four other Croatian ports using DNA metabarcoding (V4-18S rRNA) [36]. The results of this study confirmed previous detections in the ports of Šibenik, Ploče, and the northern port of Split and revealed new distributions of *P. marinus* spanning from the northern to the southern Croatian Adriatic coast (ports of Pula, Zadar and Dubrovnik and the city port of Split).

The Neretva River is the largest watercourse on the eastern coast of the Adriatic Sea, and the Neretva Delta is one of the most important and fertile agricultural areas in Croatia. Following the first record of *P. marinus* in the delta [22], a further one was tallied in August 2021 during a one-time check of the zooplankton composition in the upper reaches of the Neretva River at the station Opuzen, with two separate vertical hauls from the bottom to the surface using a modified Nansen net with a 125 μm mesh size [37]. Three individuals (two males and one female) were recorded at this time. Additional detections of this copepod were noted during the monitoring of zooplankton at the same station from May 2022 to January 2023: two males and one copepodite on July 28th; two copepodes, one male and one female on August 17th; one male in October; one male on November 3rd. The entire area is subject to constant stress from humans and fluctuations in water balance, as well as the recent introduction of several NIS [38,39].
Aegean-Levantine Sea

_P. marinus_ was reported in Thessaloniki Bay (Greece) in the North Aegean Sea in August, September and October 2021 [40] during monitoring samplings at a shore-based fixed station on the urban sea front. This shallow bay has restricted water circulation. Salinity and nutrient inputs are variable due to the inflows of rivers around the bay and of the Black Sea [41,42]. Additionally, this bay is undergoing anthropogenic eutrophication pressure due to urban and industrial activities. The sampling station is next to the port of Thessaloniki, one of the main Mediterranean ports with a high traffic load and commercial maritime transport [43]; thus, ship ballast waters can be considered the main vector for the introduction of _P. marinus_ into the bay [40].

_P. marinus_ was recorded for the first time in İzmir Bay, Aegean Sea, in November 2015, at a station 29 m deep [44]. İzmir Bay is located on the Turkish coast in the eastern part of the Aegean Sea. Several monitoring studies showed that this NIS easily inhabited the İzmir Bay ecosystem and distributed throughout the bay, with preference for the more productive inner and middle parts of İzmir Bay [44]. The trophic structure of the bay is gradually changing from hypertrophic in the inner to oligotrophic in the outer region, with chlorophyll-α values of 1.0–25.4 and 0.1–2.6 µg L⁻¹ in the middle-inner and outer bay regions, respectively [45]. The inner bay is shallow and heavily influenced by anthropogenic pressures and river inputs. The TCDD İzmir, Alsancak international port, in the inner bay plays an important role in the transport of several alien species, likely including _P. marinus_ [44].

In March 2022, _P. marinus_ was also found in Yenifoça Bay (Turkey), adjacent to İzmir Bay, where it was collected at only one station 46 m deep [46,47].

A recent finding of _P. marinus_ in the southeastern Levantine Sea was reported at the Hadera meteo-marine station (Israel) 26 m deep [48]. Monthly samplings were performed in the framework of the Israeli National Monitoring Programme between September 2019 and December 2021 using vertical hauls of WP2 net (mesh size: 200 µm; diameter: 57 cm), in which _P. marinus_ was identified since February 2020. The initial identification was made using DNA metabarcoding (COI mtRNA and 18S V9 rRNA). Following the initial indication, _P. marinus_ specimens were found in the corresponding preserved samples. During the sampling period, the annual mean of the water column integrated values of temperature, salinity and chlorophyll-α fluctuated between 16.2 and 32.4 °C, 38.3 and 40.4 and 0.05 and 0.92 µg L⁻¹, respectively [48].

In the coastal waters of İskenderun Bay (Turkey; northeastern Levantine Sea), only one female individual of _P. marinus_ was found for the first time in March 2022, in the framework of the Integrated Marine Pollution Monitoring Program [49,50]. The Levantine Sea is ultra-oligotrophic, but in İskenderun Bay, productivity is two to four times higher and chlorophyll-α is 0.11–2.86 µg L⁻¹ [51]. _P. marinus_ could have been transported by the longshore current from the Israeli coast into İskenderun Bay or through ship ballast waters. İskenderun Bay has many national and international harbours and ports that facilitate NIS introductions [52,53].

Black Sea

The first occurrence of _P. marinus_ in the Marmara Sea was recorded in August 2020 [54]. Only two males and three copepodite specimens were found near the offshore waters of Hereke city in İzmit Bay (Turkey), over a water column depth of 106 m. İzmit Bay is an extension of the Marmara Sea at its northeastern-most part. It has a two-layered water system: the upper layer is less saline and originates from the Black Sea, whereas the lower layer originates from the highly saline Mediterranean Sea. The highest chlorophyll-α values in the Marmara Sea are generally recorded in the east, including in İzmit Bay. The average seasonal chlorophyll-α values range from 1.5 to 9.6 mg L⁻¹, with the highest value of 18 mg L⁻¹ measured in the middle of the bay [55]. İzmit Bay is highly eutrophic and suffers from intense anthropogenic, industrial and maritime transport pressures. Its ecological status is classified as “bad” in the inner part and “poor” in its outer region [56]. This bay
has an important geographic position, and several large and small ports are located in İzmit bay [54]. *P. marinus* could have been introduced to İzmit Bay by shipping or currents originating in the Aegean Sea. The Marmara Sea ecosystem has favourable conditions for the numerical growth of *P. marinus* populations. This species was found just before the massive mucilage event in April 2021 [57]. Indeed, mucilage events lead to covering the bottom and the destruction of benthic habitats [58]. Since *P. marinus* is a hyperbenthic species [21,22], this phenomenon could affect its persistence in the Marmara Sea. Future monitoring programs can test this hypothesis.

3.2. New Records of *Pseudodiaptomus marinus* in ENW

- **Bay of Biscay and the Iberian Coast**

  During the zooplankton monitoring campaigns carried out near the Peniche peninsula (western coast of Portugal), at a sheltered coastal area (ca. 15 m depth), a few *P. marinus* specimens (two ovigerous females, one non-ovigerous female and four copepodites) were observed in November and December 2020. Samples were collected during the day by vertical tow from just above the seabed, approximately 10 m deep using a WP2 net (mesh size: 200 µm; mouth diameter: 57 cm) and preserved in a 4% buffered formaldehyde seawater solution.

  This region is characterised by a strongly seasonal upwelling regime, especially during the spring–summer months [59]. Since 1981, it was designated as a natural reserve, along with the Berlengas archipelago, and in 1998, it was declared a Marine Protected Area and in 2011, it was declared a Biosphere Reserve World Heritage by UNESCO. The region is also characterised by two important geomorphological structures, Cape Carvoeiro and the Nazare Canyon, with a significant influence on the physical environment and the ecological features of the region. These structures interact with the circulation associated with coastal upwelling to intensify primary production in the ecosystem. The western coast of Portugal is an important traffic maritime route, so it can be inferred that shipping played a role in the introduction of *P. marinus* into the area.

- **Celtic Seas**

  In 2022, the UK government commissioned, for the first time, a routine monitoring programme of zooplankton sampling at inshore sites around England, using morning–early evening standard vertical hauls through a WP2 net (mesh size: 200 µm; mouth diameter: 57 cm). The programme started in August 2022 and covered shallow (depth range 10–30 m) inshore sites. In the Celtic Seas region, *P. marinus* was found for the first time along the western coast of England in the Bristol Channel as a rare species in September 2022. Considering the strong anthropic pressure of the system, ballast waters can be assumed as the primary vector of introduction.

- **Greater North Sea**

  In the framework of the same UK government monitoring programme discussed above, *P. marinus* was recorded in numerous sites along English east and south coasts. In August 2022, this species was reported for the first time in Southampton, Isle of Wight, mouth of River Blackwater and South Kent. In September 2022, the NIS was recorded in East Kent and East Anglia, while in October, it also appeared in the mouth of the Thames River and in Southeast Yorkshire. In November 2022, *P. marinus* was also spotted in the mouth of the Humber River. Samples contained males, gravid females and juvenile copepodite stages. Most often, the NIS occurred as a rare species, but on occasions it was the dominant or second component of the samples, scoring a maximum abundance of 730 ind. m$^{-3}$. Samples with the highest abundance were located near significant sources of freshwater input and large ports. Ballast waters can be assumed as the primary vector of introduction, with coastal circulation acting as a possible principal secondary spreading mechanism.

*P. marinus* was also found as part of a multispecies culture in an artificial lagoon used for the production of flatfish for restocking in the western part of the Limfjord (Denmark) on 15 September 2021. The temperature was 18 °C and the salinity was 29 at the time of
the sampling. *P. marinus* was probably introduced to the lagoons by the inlet of water with a North Sea origin. The lagoon was kept for the next year’s production, but *P. marinus* apparently did not survive during winter.

Two specimens of *P. marinus* (a female and a copepodite) were found offshore Hirtshals (Denmark), at an 18 m deep site, on 11 November 2022, in the frame of a national NOVANA programme with monthly samplings. The sampling location, on the mid-eastern North Sea coast, is characterized by a permanently thermally mixed water column. *P. marinus* occurred at salinities from 31.0 to 32.5, at temperatures from 12.2 to 12.8 °C, in fully oxygenated waters. The coastline has great importance as a nursery and spawning ground for fish, shrimp and other larger crustaceans.

- **Western Mediterranean Sea**

*P. marinus* was recorded for the first time in the pelagic waters off the Tuscany coast, 12 nautical miles from Leghorn harbour (Italy; southern Ligurian Sea), in November 2020, at a station 100 m deep. Only one female and two copepodite specimens were found, collected with night-time horizontal surface sampling (0–5 m). Subsequent monitoring in the same area, conducted seasonally, resulted in the collection of three ovigerous female specimens the following autumn, in November 2021, by night-time vertical sampling from 50 m depth to the surface. The zooplankton samples were taken with a modified WP2 net (mesh size: 300 µm; mouth diameter: 60 cm). The investigated sector, located off the Italian coast, on the border between the northern Tyrrhenian and the Ligurian Seas, is characterized by the large extension of the continental shelf and the limited depth (100 m), even at a considerable distance from the coast (18 miles) [60]. The coastal area of the sampling sites is strongly influenced by anthropogenic pressure, with both tourist and commercial activities. The Leghorn harbour is one of the main commercial ports in the western Mediterranean Sea; consequently, the introduction of *P. marinus* into the area was likely mediated by ballast waters.

In the Gulf of Pozzuoli (Italy; central Tyrrenhian Sea), several *P. marinus* individuals (females, males and copepodites) were found on 19 July 2018 in the framework of the ABBaCo Project. The population abundance was 22.2 ind. m\(^{-3}\) at a coastal station situated near Nisida island mussel farms (at the boundary between the Gulf of Pozzuoli and the Gulf of Naples), while the NIS ranked as a rare species at a site inside the Gulf of Pozzuoli; its first record in this area was dated to 2018 rather than 2019 [32].

In the innermost southeasternmost part of the Gulf of Naples (Italy; central Tyrrenhian Sea), *P. marinus* was found as a rare species (one female) in July 2020 in the framework of the NEREA project. The sampling area is affected by the presence of the Sarno River, which carries high concentrations of inorganic and organic pollutants and is considered one of the most polluted rivers in Europe [61]. It is to be noted that in previous samplings in the same area (2002–2005 and 2007–2009), this species was not recorded. At present, it is difficult to formulate hypotheses on the introduction pathway of *P. marinus* in this area, but a realistic possibility is the arrival through secondary spread (water currents, attachment to hull fouling) or from neighbouring introduced areas (e.g., LITER_EU_IT_061).

In April 2021, ten adult females and one male were also found off Torre del Greco, a locality positioned south-east from LITER_EU_IT_061 station in the Gulf of Naples. Subsequently, in June 2021, during the sampling activities of the PO FEAMP project, one female was found as a rare species (0.14 ind. m\(^{-3}\)) at a station between Torre del Greco and Torre Annunziata (further south-east from Torre del Greco) in the 0–25 m layer. As for the Gulf of Pozzuoli, in these two cases, the samples were also collected near mussel farms, supporting the introduction through shellfish culture.

The Gulf of Salerno (Italy; central-southern Tyrrenhian Sea) presents an average depth similar to that of the Gulf of Naples (260 and 170 m, respectively), but it is more open to oligotrophic waters from the Tyrrenhian Sea while the human impact is less pronounced [62]. One *P. marinus* female was found in September 2019 in the framework of a monitoring program in the 0–50 m layer at an offshore station during the discharge of sediments dredged from the nearby port of Salerno, which may be assumed as the origin
point. Samples collected in the port by vertical hauls did not reveal any presence of *P. marinus*. It is thus reasonable to assume that the species was collected from the bottom, where it stays during the morning, in a layer not sampled by the plankton net.

- **Ionian Sea and the Central Mediterranean Sea**

  Several specimens (ovigerous female, male and copepodites) of *P. marinus* were collected in front of Marina di Ragusa (Sicily, Italy), a coastal area facing the Malta Channel. Specimens were collected in November 2022 by daytime vertical and horizontal hauls carried out near the coast, over a water column that ranged from 3 to 9.5 m in depth. In this area, *P. marinus* reaches a maximum abundance of 3.5 ind. m$^{-3}$ at a depth of 4.5 m. The Malta–Sicily Channel is part of the Sicily Channel system, where waters and thermohaline properties between the eastern and western Mediterranean basins mix together. Topographically, the extensive continental shelf between the Sicilian coast and the island of Malta is characterized by a plateau with an average depth of 150 m [63]. The monitored stretch of coastline is characterized by a high intensity of industrial settlements, mainly in the field of hydrocarbon supply and processing. For this reason, it can be hypothesized that the introduction of *P. marinus* was mediated by commercial maritime traffic, particularly through ballast water from ships.

- **Adriatic Sea**

  In the Gulf of Trieste (Italy; northern Adriatic Sea), *P. marinus* has been recorded since 2009, when it was found for the first time in the harbour of Monfalcone [20], probably introduced by ballast waters or as a consequence of aquaculture activities. Since then, it was observed in several samples collected at sampling stations in the central part of the gulf, near the harbour of Trieste and at the coastal LTER_EU_IT_056 station [22]. As in samples collected from January 2006 to December 2017 with WP2 vertical nets (mesh size: 200 µm; mouth diameter: 57 cm) [22], *P. marinus* was also present year-round during January 2018–December 2022, mainly as copepodites that were frequently found in summer and autumn. The abundance of *P. marinus* in this last period never reached the maximal values previously observed (172.6 ind. m$^{-3}$), with the highest values of 15.4 and 15.1 ind. m$^{-3}$ in September 2020 and July 2021, respectively.

  Recently, *P. marinus* has also been spreading in the neighbouring Marano and Grado Lagoon, which, together with the Venice Lagoon, is one of the two most important coastal systems in northeastern Italy, located between the Friuli Venezia Giulia lowlands and the northern Adriatic Sea. The Marano and Grado Lagoon is a shallow basin (average depth 1 m) bounded by the Tagliamento River to the west and by the Isonzo River to the east. It extends parallel to the northermmost coast of the Adriatic Sea for a length of about 32 km and covers an area of 160 km$^2$. This basin plays an important role for fishery, fish and shellfish farming, and it has been designated as a site of the Natura 2000 network. Moreover, it is a Special Area of Conservation (SAC—IT3320037) and a Special Protection Area (SPA—IT3320037). The northernmost part of the basin hosts an industrial area along with the harbour of Porto Nogaro, an international commercial port with a total cargo handled from 2012 to 2022 ranging from around 900,000 to 1,500,000 t y$^{-1}$ [64] accessible through a navigable canal crossing the lagoon.

  Zooplankton surveys in the Marano and Grado Lagoon began in March 2019 and were conducted monthly from January 2020 to November 2021, interrupted only in March and April 2020 due to the COVID-19 pandemic. Samplings were performed during the daytime with a Bongo net equipped with a 200 µm net, and floats were attached to support the net collectors. The net was towed at low speed (<1 m s$^{-1}$) for about five minutes, allowing an average of 6,000 L of water to be filtered per tow. *P. marinus* was found in 49 of the 164 samples analysed, in a wide range of temperature (5.0–30.0 °C) and salinity (4.7–35.4) values. Although *P. marinus* was not always observed at all sites, it was present in almost every sampling, with abundances ranging from 0.1 to 19.8 ind. m$^{-3}$ in February 2021 and July 2019, respectively. In addition to net zooplankton surveys, in spring and autumn 2021, water samples (5 L at each station) for eDNA analyses were collected at
16 stations and filtered through 1.2 µm PES membrane filters (PALL Laboratory, Port Washington, NY, USA). eDNA metabarcoding targeting the mitochondrial cytochrome-c-oxidase I gene (COI) using mlCOIintF and jgHCO2198 primers [65,66] allowed for the first time the detection of *P. marinus* in the lagoon (one site on 27 September 2021) with this approach. The introduction of *P. marinus* in the lagoon was probably associated with the extensive aquaculture activities in the area (as hypothesised also to explain its presence in the artificial channel near the harbour of Monfalcone [20]), but arrival by ballast waters cannot be ruled out.

- **Aegean-Levantine Sea**

  Thessaloniki Bay (Greece) is the upper part of the Thermaikos Gulf, which is a marine ecosystem with high complexity that can be divided into three parts: Thessaloniki Bay, the inner Thermaikos Gulf and the outer Thermaikos Gulf. The latter is connected to the Aegean Sea with water exchange taking place and is also influenced by the inflow of the Black Sea waters [67]. Following the first record in Thessaloniki Bay in 2021 [40], an analysis of earlier collected samples from the outer Thermaikos Gulf in 2016 recorded the presence of *P. marinus*. One adult female, one adult male and two copepodites were identified. The adult female was found in a surface sample collected at 3 m depth, while the male and copepodite specimens were found in samples collected from deeper layers (45.5 and 64.5 m, respectively) with temperature ranging from 14.7 to 27.7 °C. These specimens collected in 2016 could have arrived in the Thermaikos Gulf either through ballast waters or through the coastal circulation of waters from the Aegean and the Black Sea, where *P. marinus* was recorded for the first time in 2016 [68]. These new findings bring forward five years the arrival of *P. marinus* in the basin, from 2021 [40] to 2016.

4. **Discussion**

  According to a recent census [69], 874 NIS have been recorded in strictly European marine waters as of 2020, at a rate of 21 new introductions per year over the 2012–2017 period. Their distribution is uneven, with the highest number of aliens found in Italy, France, Spain and Greece, probably due to several factors including increased monitoring efforts and the density of gateways and pathways [29]. Among the species more capable of establishing in a highly diversified range of environments is the calanoid copepod *Pseudodiaptomus marinus*. In a few years after its first spotting in the Adriatic Sea in 2007 [20], this species has recorded a fast spreading in European and neighbouring waters, with a process that is still ongoing. As reviewed in the present contribution, compared to a previous snapshot [22], the distribution of *P. marinus* has further expanded in different sectors of ENW, likely due to either new introductions or secondary spreading from already introduced environments. The presence of *P. marinus* is now verified in eight out of the ten MSFD subregions, now including the Celtic Seas compared to [22]. This spread is particularly impressive considering that in [29] the occurrence of *P. marinus* was verified only in four subregions (Western Mediterranean, Ionian Sea and the Central Mediterranean Sea, Adriatic Sea, Greater North Sea). The continuous monitoring of NIS occurrence is fundamental to assess the real-time evolution of its spreading [70]. Such activity would also crucially benefit from an increase in the use of molecular tools [3,29]. Indeed, metabarcoding studies on plankton communities are contributing to the early detection of non-indigenous and even rare species [71]. With specific reference to *P. marinus* from ENW, over the time window investigated in the present work, new sequences have been made available from the area of interest [31,48,54,72], adding to previous molecular studies [25,73–77]. The increase in new validated reference sequences for this species (e.g., [48,72]) is fundamental for a proper molecular identification of *P. marinus* using metabarcoding approaches (eDNA and/or organismal DNA) (e.g., [31,48]), as well as in the investigation of the genetic connections of geographically distant populations (e.g., [76]).

  As discussed in [22], *P. marinus* can exploit different vectors of introduction in new environments, e.g., ballast waters and aquaculture/mariculture, configuring as a polyvectic species [78]. At a regional scale, the secondary arrival of this NIS can be favoured by the
local current regimes [22]. For example, the spread of *P. marinus* in the North Sea might have been supported by the mostly cyclonic circulation of the basin [79]. The first observations of this NIS in Calais harbour (France) in 2010 [26] were followed by the first occurrences of *P. marinus* in the Continuous Plankton Recorder samples in 2011 [80] and then further east in subsequent years ([22,75,81,82], present study). Recently, it was suggested that species inhabiting the Adriatic Sea could be transported to the Turkish Levantine coast by the Bimodal Oscillation System (BiOS) [83]. This process leads to switching the circulation patterns of the North Ionian Gyre (NIG) between cyclonic and anticyclonic on decadal intervals [84]. BiOS can affect the thermohaline properties in the southern Adriatic Sea and also affect the Levantine Surface Water (LSW) and Levantine Intermediate Water (LIW) via the Ionian Jet current [85]. Therefore, it is hypothesised that *P. marinus* could have arrived in the Körprüçay estuary by the BiOS mechanism or even more likely by ship ballast waters. More research on this topic is needed, likely including the use of molecular tools to reconstruct the phylogeographic connections of the two populations. The original data presented here also identify another possible introduction pathway, i.e., the discharge of sediments dredged in areas where the copepod has already established, as proposed for the Gulf of Salerno (see Section 3.2).

The data presented in this work (both from the literature and original) confirm the ability of *P. marinus* to establish in a variety of environments, as discussed in previous reviews [21,22,26]. This species can establish in coastal areas as well as in estuaries and coastal lagoons, in a wide range of temperatures and salinities. Species of the genus *Pseudodiaptomus* are considered rare, if not completely absent, below the 10 m isobath [86]. The data included here verify the ability of *P. marinus* to establish at sites where the bottom depth is deeper than this threshold, as shown also in [22]. Currently available information also suggests no latitudinal preference in this NIS.

Compared to distributions obtained by model simulations accounting for the species net reproductive rate as a function of water temperature [87,88], *P. marinus* has clearly established in theoretically non-invadable regions, confirming its settlement in areas potentially unsuitable (southern North Sea, Levantine Basin, Black Sea) ([22,75,81,82], present study). It is worth underlining that in [21], the absence (as of 2015) of *P. marinus* in the eastern Mediterranean Sea was supposed to be related to a hypothetical haline restriction of this copepod to salinities < 38.5. Subsequent salinity tolerance experiments [23], however, reported an upper limit to values up to 44, and the records of *P. marinus* in the Levantine Basin [48,49] validate its ability to spread outside its theoretical ecological and geographical boundaries, pointing to species-specific physiological traits supporting a plastic adaptation to a wide range of abiotic conditions [22,23].

The present distribution of *P. marinus* may actually be underestimated. Owing to its epibenthic behaviour, the chances of collecting enough specimens during day-time samplings are reduced, especially considering that vertical tows never allow sampling near the bottom. To date, the introduction of *P. marinus* has not had negative impacts on the pelagic communities of the receiving environments, with the only exception of the Agua Hedionda Lagoon (Agua Hedionda Lagoon, CA, USA) [86], although no information is presently available for the benthic ecosystems where this NIS lives during the morning [22]. In light of this, night-time samplings could be of great help to more efficiently reveal the real occurrence of this species.

As pointed out by [3], the majority of zooplankton invasions are reported for species with documented severe ecological impacts in urbanised and commercially important areas, likely more intensively monitored by different research institutions. Efforts should be devoted to the collection of samples from regions with limited coverage, also resorting to the possibility of using metabarcoding approaches to detect NIS, including *P. marinus* that typically occurs in few numbers and is therefore often rare in zooplankton samples.

The reports available allow us to make tentative hypotheses on the future distribution of *P. marinus*. It may be very likely that this NIS will appear along the North African coasts (where it has been reported so far only in the Gulf of Gabès [22]) and at more sites in
the Black Sea, in the Aegean Sea, along the Atlantic coastline and in the English Channel, where this species is already introduced. The occurrence of *P. marinus* along the Danish coast in the southern North Sea suggests a potential future introduction, as well as in the Baltic Sea, a basin where biological invasions have already occurred [89], also including zooplanktonic organisms [90]. The temperature and salinity conditions of the Baltic Sea may be compliant with the tolerance limits of *P. marinus* [23], although to date no record of this species has occurred.

Over the last twenty years, an increase in non-indigenous zooplankton organisms has been evidenced, owing to an increase in awareness and in the number of invaders [3]. Based on its great spreading capacities, *P. marinus* is a target species to improve our knowledge of the mechanisms favouring the introduction of NIS in receiving environments worldwide.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jmse11061238/s1, File S1: published and original *P. marinus* distribution data in ENW.

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Appendix A
This appendix lists the bibliographic references used in the preparation of Figure 1. The references [A1–A10] are ranked in alphabetical order.


References
2. Simberloff, D. Non-native invasive species and novel ecosystems. F1000Prime Rep. 2015, 7, 47. [CrossRef]

19. Adams, J.B.; Bollens, S.M.; Bishop, J.G. Predation on the invasive copepod, 

12. Pansera, M.; Camatti, E.; Schroeder, A.; Zagami, G.; Bergamasco, A. The non-indigenous 


31. Di Capua, I.; Piredda, R.; Mazzocchi, M.G.; Zingone, A. Metazoan diversity and seasonality through eDNA metabarcoding at a 

25. Sabia, L.; Di Capua, I.; Percopo, I.; Uttieri, M.; Amato, A. ITS2 in calanoid copepods: Reconstructing phylogenetic relationships 


46. Terbıyık Kurt, T.; Başıktepe, S.; Velasquez, X.; Guy-Haim, T. New Record of Pseudodiaptomus marinus in the Yenifoça Bay (Aegean Sea); Department of Marine Biology, Faculty of Fisheries, Çukurova University: Adana, Turkey, in prep.

47. TÜBİTAK-MRC and İzmir Metropolitan Municipality. Monitoring of İzmir Bay Water Quality and Terrestrial Inputs and Developing Recommendations for the Pollution Prevention (İZİZ); TÜBİTAK-MAM ve İzmir Metropolitan Municipality: Kocaeli, Turkey, 2023; p. 522T203.


49. Terbıyık Kurt, T.; Başıktepe, Ş.; Velasquez, X.; Guy-Haim, T. Recent Introduction of Non-Indigeneous Copepods Species Pseudodiaptomus marinus in the İskenderun Bay (Northeastern Mediterranean); Department of Marine Biology, Faculty of Fisheries, Çukurova University: Adana, Turkey, in prep.


59. Leitão, F.; Baptista, V.; Vieira, V.; Silva, P.L.; Relvas, P.; Teodósio, M.A. A 60-year time series analyses of the upwelling along the Portuguese coast. Water 2019, 11, 1285. [CrossRef]
63. Reyes Suarez, N.C.; Cook, M.S.; Gací, M.; Paduan, J.D.; Drago, A.; Cardin, V. Sea surface circulation structures in the Malta-Sicily Channel from remote sensing data. Water 2019, 11, 1589. [CrossRef]
83. Mutlu, E.; Özvarol, Y. Recent record of *Oceania armata* and near-past records of other gelatinous organisms in the Turkish waters presumably derived by basin-scale current. *COMU J. Mar. Sci. Fish.* 2022, 5, 48–55. [CrossRef]


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