



WGEUROBUS – Working Group “Towards a EUROpean OBServatory of the non-indigenous calanoid copepod *Pseudodiaptomus marinUS*”

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Abstract Since 2007, the non-indigenous calanoid copepod *Pseudodiaptomus marinus* Sato, 1913 has been increasingly recorded in numerous European sites, spreading at an unexpectedly fast pace over a short time-span. This species presents specific

biological and behavioural traits which make it of particular interest for ecological and applied research topics. On 29–30 January 2018, 29 scientists from nine European Countries established the EUROBUS (Towards a EUROpean OBServatory of the non-indigenous calanoid copepod *Pseudodiaptomus marinUS*) Working Group (WG). This WG aimed at creating a European network of institutions and researchers working on the various aspects of the

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biology and ecology of *P. marinus*, with an open forum where sharing experience and know-how among WG participants. This brought to an updated distribution map of *P. marinus* in European waters, as well as to the identification of priority research lines and potential joint initiatives under the WGEUROBUS umbrella. This contribution, stemming from the experts participating at the WG, represents the manifesto of the current and future initiatives developed within WGEUROBUS.

Keywords *Pseudodiptomus marinus* · European seas · Distribution · Ecology · Molecular approaches

Introduction

Biological invasions are recognised as a global problem driven by anthropogenic activities, and a major threat to natural ecosystems (Pooley and Queiroz 2018). The introduction of non-indigenous species (NIS) in various ecosystems may potentially lead to a homogenisation (Lodge 1993) of the biosphere, and, as a result, to biodiversity change (Katsanevakis et al. 2014). NIS establishment is not a recent phenomenon (Pooley and Queiroz 2018), but it has been experiencing a dramatically fast acceleration

over the last decades as a consequence of globalisation (Simberloff 2015).

With specific reference to marine environments, the spreading of NIS is considered a key aspect of global change, consequently its study and monitoring should be a priority for both scientists and legislators (Galil et al. 2018). Molluscs and crustaceans are the prominent contributors to NIS (e.g., Ruiz et al. 2000, 2011; Zenetos et al. 2010, 2012). Among zooplankton, free living copepods have shown great colonising capabilities both between and within continents (Lee 2016). Examples of worldwide colonizers in coastal and transitional systems include the calanoids *Acartia* (*Acanthacartia*) *tonsa* Dana, 1849 (Delpy and Pagano 2018; Marques et al. 2018b; Villate et al. 2018) and *Eurytemora affinis* Poppe, 1880 (Bollens et al. 2014; Lee 2016), and the cyclopoids *Oithona davisae* Ferrari and Orsi, 1984 (Uriarte et al. 2016; Zagami et al. 2018) and *Limnoithona tetraspina* Zhang and Li 1976 (Cordell et al. 2008).

Within the genus *Pseudodiptomus*, *P. forbesi* Poppe and Richard, 1890 and *P. inopinus* Burckhardt, 1913 are renowned global NIS (e.g., Adams et al. 2015; Bollens et al. 2012, 2014; Cordell and Morrison 1996; Cordell et al. 2007). Over the last decade, the congeneric *P. marinus* Sato, 1913 came to prominence in European basins (Sabia et al. 2015). Native to the

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Inland Sea of Japan and naturally occurring in coastal and estuarine areas of East Asia (Ohtsuka et al. 2018), *P. marinus* initiated the colonisation of several coastal and transitional areas of the Indian and Pacific Oceans in the 1950s–1960s (as reviewed in Sabia et al. 2015). Since 2007, its presence has additionally been documented in European waters (Mediterranean Sea, Black Sea, European Atlantic coasts, Southern North Sea), with a stably increasing number of records (as reviewed in Sabia et al. 2015). Wide temperature and salinity tolerance (Sabia et al. 2015; Suzuki et al. 2013; Svetlichny et al. 2019), behavioural plasticity (Sabia et al. 2014), resistance to metallic stress (Tlili et al. 2016) and possible presence of genetic divergence among populations (Sabia et al. 2017) are some of the specific biological and ecological traits of *P. marinus* which may support its capacity to become established in new environments. Each of them, either alone or in combination, may provide an adaptive advantage by which adjusting to diversified environments and so facilitating the establishment in new areas. In addition, *P. marinus* has been indicated as suitable for mass rearing providing live feed for fish larvae (Mauchline 1998), and it has been proficiently used as target species for ecotoxicological (Arias et al. 2016; Huang et al. 2006; Tlili et al. 2016, 2019) and myofibril structure (Ibrahim et al. 2015, 2016) studies.

The aspects outlined above render *P. marinus* a species of great attractiveness, not only for biological and ecological baseline studies, but also as a model organism for applied research. This fostered the establishment of the EUROBUS (Towards a

EUROpean OBServatory of the non-indigenous calanoid copepod *Pseudodiaptomus marinus*) Working Group (WG), building a network of European scientists from different institutions working on various aspects related to the biology and ecology of this recently introduced NIS. On 29–30 January 2018, a kick-off workshop was convened at the Stazione Zoologica Anton Dohrn (Naples, Italy) with the goal of evaluating the updated status of the distribution of *P. marinus* in European waters, and defining commonly agreed priority lines of research to be pursued using this species as a target organism. This contribution provides the backbone for WG activities, synthesising the results gathered during the workshop, including an updated map of distribution and ongoing spreading of *P. marinus* in European basins, and identifying the main topics that will be addressed by the WG. The main outcome from the workshop helped to identify future scientific topics to understand and advance our current knowledge on the status and trend of this species. This work is therefore considered pivotal to promote the present knowledge on *P. marinus*, and could support further establishment of other WGs focusing on different target species.

***Pseudodiaptomus marinus*: a bio-ecological sketch**

The main traits of *Pseudodiaptomus marinus* are here briefly outlined. For a more exhaustive review on the biological and ecological characters of this species, as well as an historical overview of its geographical

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spreading, the interested reader can refer to the work by Sabia et al. (2015). The centre of speciation for the genus *Pseudodiaptomus* was presumably the Indo-Malayan region (Walter 1986a, b). Members of the genus occur in tropical to temperate areas, from fresh to hypersaline waters (Sabia et al. 2015; Walter 1986b). Morphologically and behaviourally, *Pseudodiaptomus* presents intermediate adaptations to benthic and pelagic realms (Bradford-Grieve 2002): the large exopod spines, typical of benthopelagic copepods (Ohtsuka et al. 1996), are coupled with long antennules and elongated body shape, distinctive traits of pelagic species (Kiørboe 2011). Such ambivalence manifests even at behavioural level, as an epibenthic phase during daylight is replaced by pelagic dispersal at night (Bradford-Grieve 2002; Dur et al. 2010; Fancett and Kimmerer 1985).

P. marinus (Fig. 1) adheres to these general characteristics. This species tolerates wide salinity (Islam et al. 2006b; Sabia et al. 2015; Suzuki et al. 2013; Svetlichny et al. 2019) and temperature (Liang and Uye 1997; Sabia et al. 2015; Svetlichny et al. 2019) ranges, alternating epibenthic and pelagic phases (Sabia et al. 2014, 2015) while exploiting both herbivory and detritivory feeding habits (Uye and Kasahara 1983).

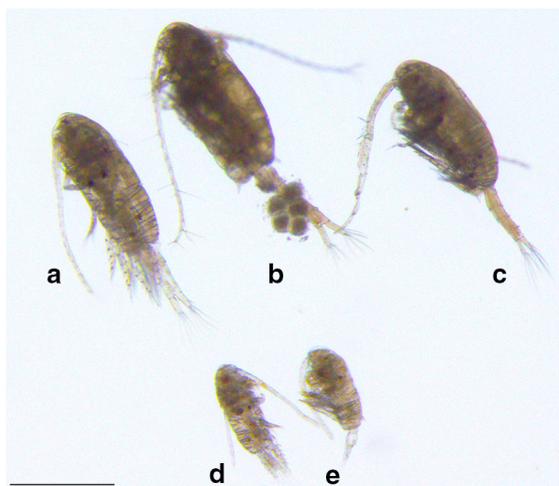


Fig. 1 *Pseudodiaptomus marinus* individuals from the Gulf of Trieste (Northern Adriatic Sea, Italy); **a** late copepodite stage (CV); **b** ovigerous female; **c** adult male; **d**, **e** early copepodite stages; scale bar: 500 μ m

Non-indigenous or invasive? A semantic clarification

The lack of a uniform and agreed terminology in bioinvasion science has often engendered debate among scientists (e.g., Colautti and MacIsaac 2004; Falk-Petersen et al. 2006; Galil et al. 2014; Occhipinti-Ambrogi and Galil 2004), resulting in a surfeit of synonyms or in the inhomogeneous usage of the same terms among authors. Most of the controversy revolves around the usage of the adjective “invasive”, often adopted as equivalent to non-indigenous (Colautti and MacIsaac 2004).

Pseudodiaptomus marinus has been frequently labelled as invasive in its common acceptance of non-indigenous (e.g., Deschutter et al. 2018; Rajakaruna and Lewis 2017; Rajakaruna et al. 2012; Sabia et al. 2014, 2015, 2017; Tlili et al. 2016, 2019). *P. marinus* is typically included among the rare species, but even when it ranks among the first components of the copepod assemblage its percentage contribution is nonetheless low (8%: Deschutter et al. 2018; < 3%: Islam et al. 2006b; 13%: Lučić et al. 2015; < 1%: Pansera et al. 2014). With the only exception of the Agua Hedionda lagoon (California, USA) (Fleminger and Hendrix Kramer 1988), where it has become the most dominant late-spring species, to date the introduction of *P. marinus* has not been associated with any documented impact neither on the pelagic plankton communities nor on the health of the receiving basin (Sabia et al. 2015). On these grounds, this species should more appropriately be defined as non-indigenous (or appropriate literature-confirmed synonyms). Equivalently, following the approach conceptualised by Colautti and MacIsaac (2004), *P. marinus* could be categorised as a stage IVa NIS, i.e. an alien widespread but rare. Consistently, *P. marinus* can be considered widespread sensu Galil et al. (2014), as this species has already expanded in more than ten Countries.

The expanding distribution of *Pseudodiaptomus marinus* in European waters

Over the last decade, *Pseudodiaptomus marinus* has been increasingly recorded in European waters: Mediterranean Sea (de Olazabal and Tirelli 2011; Delpy et al. 2012; Erdoğan and Ertan 2014; Karuza

et al. 2016; Lučić et al. 2015; Pansera et al. 2014; Sabia et al. 2015; Stefanni et al. 2018; Vidjak et al. 2019; Zagami and Brugnano 2013), Black Sea (Garbazej et al. 2016), European Atlantic coasts (Brylinski et al. 2012; Reyes-Martínez and González-Gordillo 2019; Richirt et al. 2019; Uriarte et al. 2016), and Southern North Sea (Deschutter et al. 2018; Günther et al. 2018; Jha et al. 2013; Wootton et al. 2018). In November 2017, *P. marinus* was also found in three coastal sites in the Gulf of Gabés (Tunisia) (33.90° N, 10.20° E) (N. Makhlouf, M. Nejb Daly Yahia and M. Pagano, pers. comm.). This latter constitutes the first record of this NIS in North-African waters, as confirmed by its absence in previous surveys carried out in the same area (Jenhani et al. 2019).

Out of the several possible means of NIS introduction (Minchin et al. 2009; Ojaveer et al. 2018), transoceanic ships are considered the primary vector for *P. marinus*, as this species has often been reported in ballast water samples (Cabrini et al. 2019; Choi et al. 2005; Cordell et al. 2008; Lawrence and Cordell 2010). Aquaculture also seems to be a plausible way of introduction, specifically for those environments close to transoceanic ship traffic (de Olazabal and Tirelli 2011; Fleminger and Hendrix Kramer 1988; Sabia et al. 2015). Secondary spread introduction through coastal circulation and intra-coastal ship traffic has also been deemed realistically applicable (Cabrini et al. 2019; Deschutter et al. 2018; Jha et al. 2013; Lučić et al. 2015), as well as attachment to hull fouling (Lučić et al. 2015). In this view, *P. marinus* configures as a polyvectic species sensu Carlton and Ruiz (2005).

Most of the research of WG members focuses on the spatio-temporal distribution of *P. marinus* in European waters (Fig. 2, top panel). The majority of the occurrences of *P. marinus* was recorded in coastal/neritic waters, followed by transitional systems (estuaries and lagoons) (Fig. 2, central panel). The taxonomic identification is mostly based on morphological keys (body shape, segmentation of P3–P4, structural details of P5), while genetic analyses have been performed only in a very small number of cases (Fig. 2, bottom panel). This document provides an overview of the occurrence, distribution and settlement of *P. marinus* in different European basins (Fig. 3). Compared to a previous snapshot (Sabia et al. 2015), an increase in > 450% of records has been scored over the 2015–2019 period (from 23 to 129; see

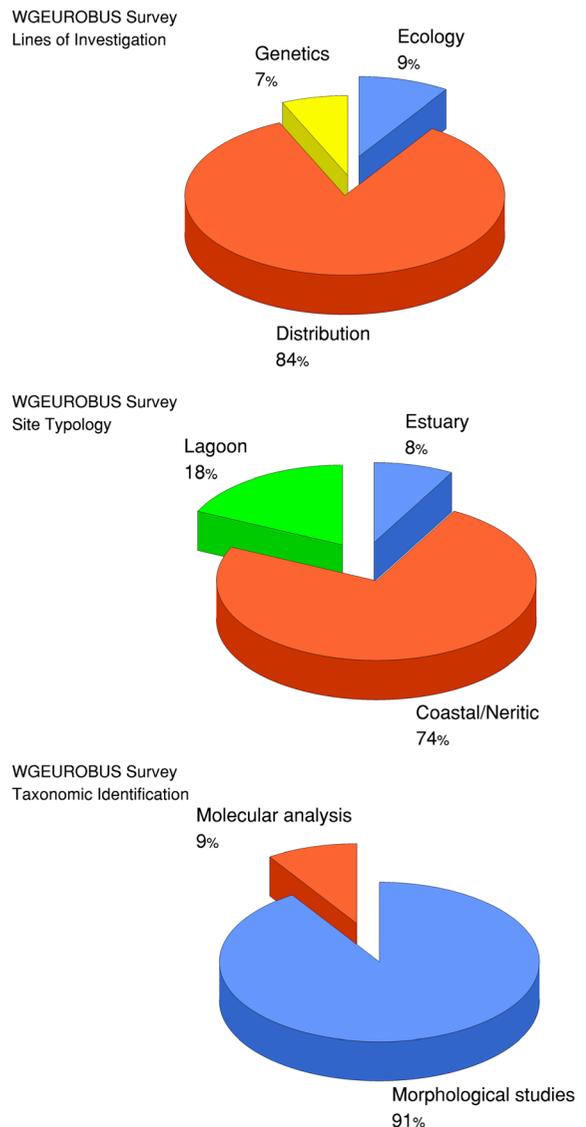


Fig. 2 WGEUROBUS survey piecharts reporting on: the percentage contribution to major lines of research among WG participants (distribution, ecology and genetics) (top panel); the site typologies covered by WGEUROBUS (coastal/neritic, lagoon, estuary) (central panel); the method of taxonomic identification used (morphological, molecular) (bottom panel)

Supplementary Material for details), > 55% presented here as original data. For each geographical area, an overview of the literature-based (labelled as LD in the reference to Fig. 3) and WGEUROBUS-updated (labelled as ED, with geographic coordinates) distributions will be given, highlighting the spreading dynamics (new introductions or establishments) of

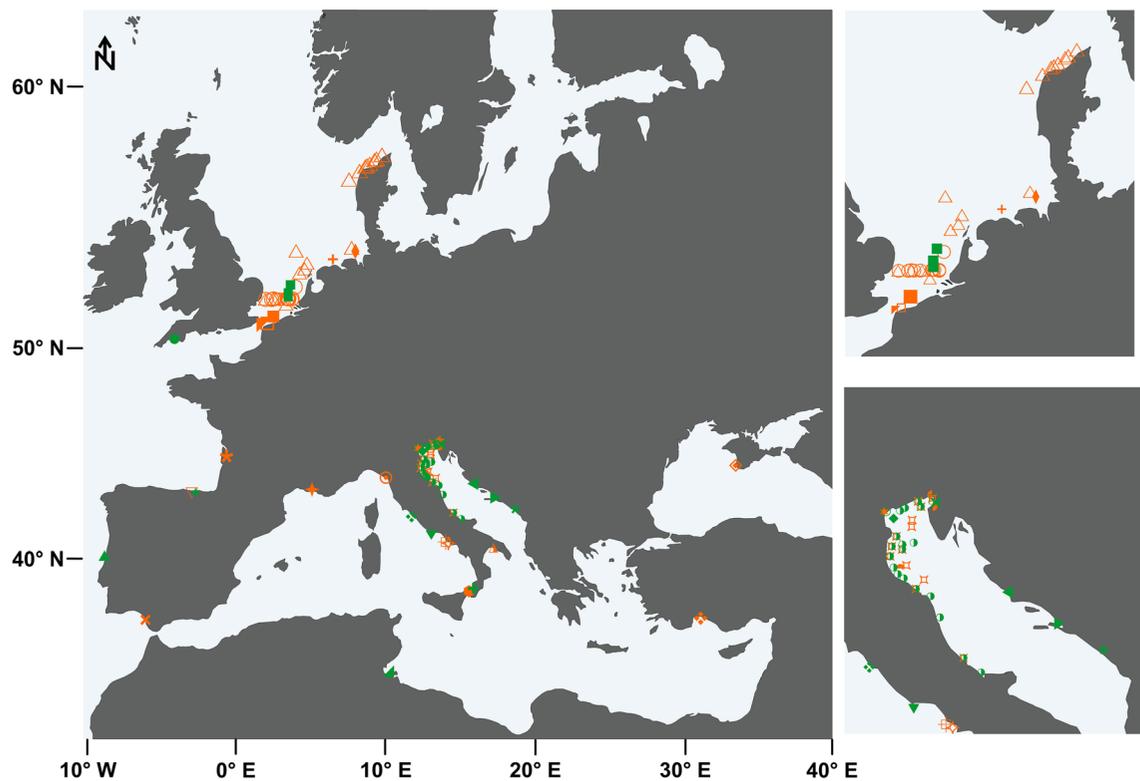


Fig. 3 Distribution of the Asian alien copepod *Pseudodiaptomus marinus* in European waters. Orange symbols for literature data (labelled as LD in the text): Δ : Southeastern North Sea (CPR Survey) (Wootton et al. 2018); \blacklozenge : Southern North Sea (Günther et al. 2018); \oplus : Southern North Sea (German EEZ) (Jha et al. 2013); \circ : Southern North Sea (CPR Survey) (Jha et al. 2013); \blacksquare : Southern North Sea (Belgian waters) (Deschutter et al. 2018); \square : Gravelines (Brylinski et al. 2012); \blacktriangledown : Calais (Brylinski et al. 2012); \star : Gironde Estuary (Brylinski et al. 2012; Richirt et al. 2019); ∇ : Estuary of Bilbao (Albaina et al. 2016; Uriarte et al. 2016); \times : Guadalquivir Estuary (Reyes-Martínez and González-Gordillo 2019); \blacklozenge : Berre Lagoon (Delpy et al. 2012); \odot : Marina di Carrara (Sabia et al. 2015); \oplus : Lake Fusaro (Sabia et al. 2015); \diamond : Gulf of Naples (multiple sites) (Sabia et al. 2015); \bullet : Lake Faro and Lake Ganzirri (Pansera et al. 2014; Sabia et al. 2015; Zagami and Brugnano 2013); \blacktriangle : Mar Piccolo of Taranto (Karuza et al. 2016); \boxplus : Adriatic Sea (Sept. 2014 surveys; multiple sites) (Stefanni et al. 2018); \blacktriangle : Rimini (de Olazabal and Tirelli 2011); \star : Port of Venice (Vidjak et al. 2019); \blacklozenge : Monfalcone (de Olazabal and

Tirelli 2011) and Port of Trieste (Vidjak et al. 2019); \blacklozenge : Port of Koper (Lučić et al. 2015); \blacklozenge : Köprüçay (Erdoğan and Ertan 2014); \blacklozenge : Sevastopol Bay (Garbazej et al. 2016). Green symbols for WGEUROBUS original data (labelled as ED in the text) presented in this study: \blacksquare : Southern North Sea (CPR surveys); \bullet : Plymouth Sound; \star : Estuary of Urdaibai; \blacktriangle : Mondego Estuary; \blacklozenge : Civitavecchia harbour; \blacktriangledown : San Felice Circeo; \oplus : Gioia Tauro and Strait of Messina; \circ : Adriatic Sea (2011–2014 surveys; multiple sites); \circ : Venice Lagoon (multiple sites); \blacklozenge : Gulf of Venice; \square : Gulf of Trieste (LTER-C1); \times : Gulf of Trieste (multiple sites); \blacktriangleleft : Šibenik Bay; \blacktriangleright : Neretva River; \star : Boka Kotorska; \blacktriangleleft : Gulf of Gabès. Insets in the right panel provide a more detailed view of the North Sea (upper panel) and Adriatic Sea (lower panel) basins. Coastline data: NOAA National Geophysical Data Center, Coastline extracted: WLC (World Coast Line), Date Retrieved: 08 January, 2013, <http://www.ngdc.noaa.gov/mgg/shorelines/shorelines.html>. Details on geographic coordinates of each site are provided in the Supplementary Material

P. marinus in different European basins. Ancillary information is provided as Supplementary Material.

Southern North Sea and English Channel

The North Sea is one of the busiest shipping areas in the world, second only to the South China Sea, with

over seven thousand ships per day passing through its waters. The majority of main ports (Rotterdam, Antwerp, Amsterdam, Bremerhaven, London, and Immingham) are located in the Southern sector of the North Sea and are typically accessed via a route through the English Channel (Nilsson et al. 2018). The first observations of *P. marinus* in the region were

made from samples taken in Calais harbour and in Gravelines (France) in 2010 (Brylinski et al. 2012), followed by further records in Southern North Sea (Deschutter et al. 2018; Günther et al. 2018; Jha et al. 2013; Wootton et al. 2018) (Fig. 3, LD).

The CPR (Continuous Plankton Recorder) Survey is the longest running, most geographically extensive, marine biological survey in the world (Wootton et al. 2018) (Fig. 3, LD). The CPR, usually towed behind ships of opportunity (SOOPS), uses a 270 µm mesh, catching both phytoplankton and zooplankton, and typically samples at a depth of 7–10 m (Reid et al. 2003). Upon the first ever sighting in the CPR Survey in October 2011 (Jha et al. 2013), *P. marinus* remained in samples along the same transect through winter 2011 until January 2012. This species is typically associated with shallow coastal waters, however the depth along this transect reaches up to 30 m, with specimens found up to 50 km from the shore. In subsequent years, *P. marinus* has persisted in this region and has spread northwards, with specimens appearing on samples off the north coast of Denmark (Wootton et al. 2018). So far, in CPR samples, *P. marinus* has only occurred in the Southern North Sea and in North Sea coastal waters of the Netherlands, Germany and Denmark, and only during autumn/winter months (from September through to March) (CPR data 2011–January 2018; <https://doi.org/10.7487/2019.66.1.1173>). Specimens tend to occur in low numbers (1.0 up to 11.0 individuals per sample), with the majority of records represented by adult females, and in samples taken around dusk or in other low light conditions. The presence of *P. marinus* in this area is confirmed by original data here presented, validating the occurrence of this species in the Southern North Sea (Fig. 3, ED). Interestingly, outside of routine CPR analysis, the presence of *P. marinus* has also been regularly observed in net caught material (used for educational outreach purposes) collected around marinas near the CPR laboratory in Plymouth (England) (50.36° N, 4.14° W), and the surrounding area (M. Wootton, pers. obs.) (Figure 3, ED).

Transitional systems on the European Atlantic coasts

The first appearance of *P. marinus* was in the southern sector of the Bay of Biscay and dates back to 2010 in

the estuary of Bilbao (Albaina et al. 2016; Uriarte et al. 2016), followed by occurrence in the Gironde Estuary (Brylinski et al. 2012; Richirt et al. 2019), and since 2016 in the Guadalquivir Estuary (Reyes-Martínez and González-Gordillo 2019) (Fig. 3, LD).

The estuaries of Bilbao and Urdaibai (Spain) are small (ca. 12–22 km long), mesotidal systems that are seawater dominated at high tide. They are located very near each other (ca. 25 km apart) and share a temperate oceanic climate. In these two estuaries, monthly samplings of zooplankton by horizontal tows of 200 µm mesh size nets were carried out at high tide during neap tides since 1998. *P. marinus* was first observed in the estuary of Bilbao in 2010 (Uriarte et al. 2016) (Fig. 3, LD) and it reached a maximum abundance of 1102.0 ind m⁻³ in 2015. In the estuary of Urdaibai (43.35° N, 2.67° W), *P. marinus* was recorded for the first time in 2011, but up until 2015 it has only been observed in two samples (1.0–2.0 ind. m⁻³) (Fig. 3, ED). In both estuaries (Bilbao and Urdaibai) *P. marinus* occurrences have been registered at salinities between 28.8 and 34.5, over a wide range of water temperatures (11.9–22.7 °C), but with maximum abundances in summer.

The Mondego estuary (Portugal) (40.13° N, 8.83° W) is a small (8.6 km²), shallow (2–10 m), mesotidal system with two arms with different hydrological characteristics, the south arm (7 km long) being shallower (2–4 m), more stratified and with a longer residence time than the north arm (21 km long) (Marques et al. 2006). Ongoing monthly zooplankton samplings at high tide by horizontal subsurface hauls, using a 335 µm mesh plankton net (Marques et al. 2006), started in 2003 and *P. marinus* was first recorded in March 2011 (Fig. 3, ED). Its presence in the estuary is patchy with several outbursts (from < 0.1 to 39.7 ind. m⁻³), with peaks in September, and high interannual variations in abundance. The occurrence of *P. marinus* has been observed over a wide range of surface water temperatures (11.8–27.0 °C) and salinities (11.3–34.8), but its presence in the estuary seems to be related mainly to the intrusion of marine waters, occurring during events of low freshwater input, as noted for marine species due to drought events (e.g., Marques et al. 2018a and references therein).

Mediterranean Sea

Berre Lagoon

Located in the Northwestern Mediterranean Sea, Berre Lagoon (France) is one of the largest European lagoons with an area of 155 km². This shallow coastal basin (mean depth ~ 6 m) has undergone significant hydrological and morphological changes over the decades (Delpy and Pagano 2018; Delpy et al. 2012). Over time, the introduction of several alien species was noticed, and among them the copepod *P. marinus* (Fig. 3, LD), detected for the first time in January 2010 (Delpy et al. 2012, 2016).

The vertical hauls performed during the day with a modified WP2 plankton net (1.2 m long, 50 cm diameter of opening area and 80 µm mesh size) did not allow the sampling of adults. Therefore, divers scratched the sediments and managed to collect a few adults identified as *P. marinus*. In 2010, *P. marinus* was occasional in Berre Lagoon with 1.0–3.0 ind. m⁻³ in winter and late summer, whereas it was particularly abundant at Port de Bouc, the exchange zone between the lagoon and the Mediterranean Sea, with up to 728.0 ind. m⁻³ in July. This spatial distribution highlights a potential introduction via ballast water. In 2011, *P. marinus* presented the same range of values at Port de Bouc (10.0–113.0 ind. m⁻³) and in Berre Lagoon (7.0–107.0 ind. m⁻³), but with different temporal variations. Highest abundances were first observed in late spring and summer in the lagoon, and then in autumn at Port de Bouc. Even if *P. marinus* occurred over wide ranges of temperature (5.8–25.6 °C) and salinity (16.5–38.0), the increase in its abundance seemed to respond to an increase in temperature (> 17 °C) and in chlorophyll *a* concentration (> 1 µg L⁻¹) in both sites.

Tyrrhenian and Ionian Seas

P. marinus was found in the Northern Tyrrhenian Sea in December 2008 in Marina di Carrara (Italy) (Sabia et al. 2015), in samples collected in a coastal area (10 m depth) near the harbour (Fig. 3, LD). In the Southern Tyrrhenian Sea, the first record of *P. marinus* dates back to 2011 when a few adult individuals were occasionally found in March and July in the brackish Lake Fusaro (Italy) (Fig. 3, LD) (Sabia et al. 2015). This NIS was also found in the Mar Piccolo of Taranto

(Ionian Sea, Italy) (Fig. 3, LD) over a 1-year sampling program together with the non-indigenous cyclopoid *Oithona brevicornis* Giesbrecht, 1891 (Karuza et al. 2016). This basin is site of the largest Italian mussel farming, and has been subject to intense anthropic stress for decades (Karuza et al. 2016).

In the Gulf of Naples (Italy), the presence of *P. marinus* was recorded for the first time offshore in samples collected in the upper 50 m over the 100 m isobath in December 2013 and April 2014 with abundance of 2.5 ind. m⁻³ and 0.1 ind. m⁻³, respectively (Sabia et al. 2015) (Fig. 3, LD). *P. marinus* was noticed for the first time at the LTER-MC station on 1st July 2014 (one adult male). The site is located 2 nautical miles off the city of Naples, at the border between the littoral and the open-water systems, and is characterized by a strong seasonal signal (Mazzocchi et al. 2012; Ribera d'Alcalà et al. 2004). *P. marinus* occurred seldom at the station, more frequently as copepodites, with generally very low abundances and a maximum of 16.0 ind. m⁻³ in July 2017 (Fig. 3, LD). Very recently, the analysis of high-throughput sequencing of the V4-18S rDNA of metazoan assemblages in surface waters has revealed the presence of *P. marinus* at LTER-MC in samples from October to December 2013, therefore moving backwards its appearance at this monitoring site (I. Di Capua and M. G. Mazzocchi, unpubl. data).

Monitoring activities carried out in the framework of the Marine Strategy Framework Directive (MSFD) (Descriptor 1: Biodiversity) revealed the presence of *P. marinus* in offshore samples collected in July 2017 in the area of the San Felice Circeo promontory (south of Rome, Italy) (Fig. 3, ED). The samples were collected at two stations at 3 and 6 nautical miles from the coast (41.17° N, 13.10° E; 41.13° N, 13.11° E) (Fig. 3, ED), with an abundance of 3.2 ind. m⁻³ and 4.1 ind. m⁻³, respectively. In relation to the “Descriptor 2: Non-indigenous species” of MSFD, samples showed the occurrence of *P. marinus* over the period 2015–2017 in Civitavecchia harbour (north of Rome, Italy) in two monitoring points: one in the loading and unloading area (42.10° N, 11.78° E) and one at the harbour entrance (42.10° N, 11.77° E) (Fig. 3, ED). This species was recorded for the first time in both stations in November 2015, with a maximum abundance of 7.7 ind. m⁻³. Since the first occurrence, *P. marinus* was constantly found in the samples, more frequently during the autumn period.

Adult individuals were found in particular in the loading and unloading area, being less abundant at the harbour entrance. The largest abundance over the 2015–2017 sampling time window was recorded in the loading zone in September 2017, with a score of 12.3 ind. m⁻³.

In July 2014, *P. marinus* was collected in the Strait of Messina (Sicily, Italy) (38.32° N, 15.49° E) (Fig. 3, ED), in a surface Manta-net sample during the TARA-Mediterranean survey (M. G. Mazzocchi, unpubl. data), in a marine area close to Lake Faro which had already been invaded by *P. marinus* since 2008 (Sabia et al. 2015). In autumn 2018, *P. marinus* was also found in the port of Gioia Tauro (Calabria, Italy) (38.44° N, 15.90° E) in samples collected in the framework of the MSFD (F. Cicero, pers. comm.) (Fig. 3, ED).

Lake Faro and Lake Ganzirri

In Lake Faro (Sicily, Italy), *P. marinus* has recurred annually since 2008 (Pansera et al. 2014; Sabia et al. 2015; Zagami and Brugnano 2013), and recently it has started spreading also in the neighbouring Lake Ganzirri (Sabia et al. 2015) (Fig. 3, LD). In Lake Faro, *P. marinus* samples were collected monthly from January to December 2014 using a WP2 standard zooplankton net (mouth diameter 57 cm, mesh size 200 µm), vertically towed from 15 m depth to the surface in the central part of the lake. *P. marinus* was found throughout the entire sampling period. It occurred at temperatures ranging between 12.8 and 29.0 °C and salinities varying from 26.5 to 37.0. Higher seasonal abundances were recorded from June to October, with highest density of adults and nauplii in June (temperature: 24.5 °C; salinity: 31.4) and October (temperature: 23.4 °C; salinity: 34.8). Even if it is considered a demersal copepod species, it has become one of the most common planktonic species (but with limited percentage contribution) owing to the presence of an anoxic bottom layer.

The occurrence of this species in Faro and Ganzirri lakes is incompatible with ships' ballast water transfers, because of shallow channels that connect them with the Strait of Messina. Nonetheless, Lake Faro is an importation centre of living molluscs (*Crassostrea gigas*, *Ostrea edulis* and *Mytilus galloprovincialis*) from European aquaculture sites. The significant number of newly described copepod species (Baviera

et al. 2007; Brugnano et al. 2010; Zagami et al. 2008), as well as first records of species typical of remote biogeographic regions (Cosentino and Giacobbe 2011; Cosentino et al. 2009; Zagami et al. 2005, 2018), are most probably related to the importation of molluscs for aquaculture activities.

Adriatic Sea

The first records of *P. marinus* in the Mediterranean Sea date back to more than 10 years ago, when it was observed in the Northern Adriatic near Rimini (Italy) in 2007 and in the harbour of Monfalcone (Italy) in 2009 (de Olazabal and Tirelli 2011) (Fig. 3, LD). The occurrence of this NIS in the Adriatic basin was then further observed in the ports of Koper (Slovenia) (Lučić et al. 2015; Vidjak et al. 2019), Trieste and Venice (Italy) (Vidjak et al. 2019), as well as at several sites in the Northern Adriatic basin and at one coastal station in the Southern Adriatic (Stefanni et al. 2018) (Fig. 3, LD). *P. marinus* has also been found in the ballast water from ships docked in Italian (Trieste, Venice, Ancona, Bari) and Slovenian (Koper) ports (Cabrini et al. 2019).

Several new records were reported in the framework of WGEUROBUS survey. In the Gulf of Trieste (Italy), zooplankton was collected during daytime by vertical hauls of WP2 net, from near-bottom to surface. The sampling sites comprised the LTER-C1 station (45.70° N, 13.70° E) and several stations located in the central area of the Gulf of Trieste, as well as in the harbours of Trieste and Monfalcone (see Supplementary Material) (Fig. 3, ED). Overall, *P. marinus* was found in 62 of the 349 samples analysed from January 2006 to December 2017 (temperature: 8.5–23.0 °C; salinity: 30.0–38.0); juveniles were dominant throughout the year while males were observed only in autumn. The presence of *P. marinus* increased during the last 4 years (maximum abundance: 172.6 ind. m⁻³), and occasionally it arrived to represent the second most abundant zooplanktonic taxon (e.g., November 2016).

Zooplankton were collected during day-time by horizontal and vertical hauls of WP2 and Apstein nets (200 µm mesh size) at eight stations located in the Venice Lagoon (Italy) (see Supplementary Material) from May 2014 to February 2017, and at one station at sea (Gulf of Venice; 45.30° N, 12.50° E) from May 2014 to November 2017 (Fig. 3, ED). *P. marinus* was

present throughout the year in the lagoon (temperature: 6.3–31.5 °C; salinity: 19.7–35.5), with highest abundance of 54.4 ind. m⁻³. It was less frequent and less abundant (maximum abundance: 2.8 ind. m⁻³) at the sea station (temperature: 10.6–26.0 °C; salinity: 32.5–38.3).

In the Šibenik Bay (43.74° N, 15.88° E) located in the Krka River estuary (Croatia), *P. marinus* was first identified in November 2015 in the central bay area (Fig. 3, ED). After that, samplings were further carried out in the nearby shallow port of Šibenik (6–9 m depth), presumably the point of origin, and in the deeper central part of the bay (35 m), using vertically hauled plankton nets equipped with either 125 or 53 µm meshes. Overall, from November 2015 to April 2018, *P. marinus* was identified in six samples, collected either at sunset or at night, and in one sample collected during the day. Abundances were highly variable, extending over 5.0 ind. m⁻³ (June 2016) to 59.0 ind. m⁻³ (February 2017) in the port area, and from < 1.0 ind. m⁻³ (December 2016) to 557.0 ind. m⁻³ (November 2015) in the central part of the bay. *P. marinus* was observed in conditions of surface salinity ranging 5.0–32.2 and surface temperature between 9.4 and 22.8 °C.

A recent find (December 2018) of *P. marinus* is in the delta of Neretva River (43.06° N, 17.42° E), at the eastern coast of South Adriatic Sea (Croatia) (Fig. 3, ED). The population was recorded in the shallow part (12 m) of a semi-enclosed bay near the town of Ploče. The sampling station was located in the vicinity of the second largest Croatian cargo port. Sampling was performed with a vertical bottom-surface haul of a 125 µm Nansen net in December 2018, in the morning hours (around 8 a.m.). The total abundance of *P. marinus* was relatively small (8.4 ind. m⁻³). The temperature conditions varied between 16.9 °C at the surface and 18.6 °C at bottom, while salinity ranged between 31.4 and 38.4 at surface and bottom, respectively.

P. marinus has also been recorded for the first time in the Boka Kotorska Bay (Montenegro) (42.48° N, 18.72° E) (Fig. 3, ED). Samples were collected in October 2018 with vertical hauls using a 125 µm net, from the bottom (61 m) to the surface, and over the deepest layer (61–32 m). Abundances were 14.4 ind. m⁻³ and 3.7 ind. m⁻³, respectively (M. Hure, pers. comm.).

Four surveys were carried out along the Northeastern coast of the Adriatic Sea, from Trieste (NE Italy) to the Gargano Peninsula (SE Italy) in September 2011–2014 (see Supplementary Materials). Zooplankton samples were collected during the entire day cycle by vertical hauls of WP2 net within the first 100 m of the water, and *P. marinus* was found in 26 out of the 76 analysed samples. The highest abundance of 4729.0 ind. m⁻³ was observed in 2012 at a coastal station near Rimini, sampled at night (Fig. 3, ED). All samples collected in 2014 survey (46 samples) were analysed by a multi-marker metabarcoding approach which pointed out that *P. marinus* enlarged its distribution in the whole Northern Adriatic Sea (Stefanni et al. 2018).

Eastern Mediterranean Sea

Studies on the occurrence and distribution of *P. marinus* in the Eastern Mediterranean are limited. The use of methodology likely not optimised for the sampling of *P. marinus* (i.e., vertical tows during the morning), as well as the oligotrophic high saline environment make the investigation of the importance of this species quite demanding. *P. marinus* has been so far recorded only in Köprüçay (Fig. 3, LD), an estuarine zone located south of Turkey (Erdoğan and Ertan 2014). The Köprüçay estuary is classified as a medium sized estuary, near the town of Serik. Zooplankton community structure and biomass were investigated monthly at five stations in the Köprüçay estuary during 2009, using a standard plankton net of 55 µm mesh size. The net was towed horizontally just beneath the surface. Throughout the year, salinity ranged between 0.1 and 36.0, whereas chlorophyll *a* values varied from 1.22 to 4.12 µg L⁻¹. Among the zooplankton, *P. marinus* was recorded in the collected samples, however no information is available on the abundance and seasonal distribution of this species in Köprüçay estuary.

Black Sea

In Sevastopol Bay (Northern Black Sea), *P. marinus* was initially discovered in 2016 in a sample taken during a routine plankton survey conducted since 2002 (Garbazy et al. 2016) (Fig. 3, LD). Samples were collected twice a month at two stations in the central part of the bay and in its mouth by vertical hauls through the whole water column using a Juday

plankton net (mouth area 0.1 m² and 150 µm mesh size). Additionally, zooplankton samples were collected in 2016 from a pier not far from the bay mouth. A few females and copepodite stages of *P. marinus* were collected for the first time in the centre of the bay in late September 2016. About a month later, females and copepodite stages of *P. marinus* were found at all the stations in significant abundance in the bay mouth (103.0 ind. m⁻³) and at the centre of the bay (1373.0 ind. m⁻³). This was the highest abundance of the species to date. In 2017, the species was found as copepodite stages and nauplii in autumn with a maximum in November (234.0 ind. m⁻³). The occurrence of *P. marinus* in Sevastopol Bay indicates that the species has successfully adapted to the relatively low salinities (17.0–17.8) and low winter temperatures (down to 5.2 °C) of Sevastopol Bay (Kuftarkova et al. 2006).

Insight into physiological traits supporting the spreading of *Pseudodiaptomus marinus*

Species traits are fundamental descriptors of the organisms' response to a disturbance, including climate change and biological invasions, with cascading effects over several hierarchical levels from the individual to the population (Kissling et al. 2018). As mentioned in the Introduction, the successful spreading of *Pseudodiaptomus marinus* could be sustained by specific physiological traits, providing metabolic and biological plasticity sensu lato facilitating the establishment in new sites. To shed light on these aspects, research lines will be developed within WGEUROBUS to assess the physiological performances of *P. marinus* under different experimental conditions, thus providing new elements to appreciate the specific adaptations of this NIS to a large variety of environmental parameters.

The documented euryhalinity and eurythermicity of *P. marinus* (Sabia et al. 2015; Suzuki et al. 2013) can provide an additional advantage in the spreading into new environments. A recent investigation within WGEUROBUS framework (Svetlichny et al. 2019) on a laboratory reared population showed that the salinity tolerance range of *P. marinus* adults is comprised between 3.0 and 45.0. Such a wide salinity window can represent a potentially advantageous trait, allowing this species to establish in extremely diverse

environments. From a behavioural perspective, an increase in ambient temperature from 12.0 to 27.0 °C led to an increase in locomotor activity, while a reduction in temperature to 8.0 °C seemingly induced a torpid state in *P. marinus* females. This latter response might be considered an overwintering strategy to endure harsh wintertime conditions, such as those experienced in the Black Sea, providing this species with another element of adaptability.

The respiration rate of *P. marinus* was preliminarily measured on individuals collected in Sevastopol Bay (Black Sea) and reared in the laboratory. In active *P. marinus* adult females, the specific respiration rate was 4.5 µL O₂ mg DW⁻¹ h⁻¹, while in males it was measured as 3.6 µL O₂ mg DW⁻¹ h⁻¹. It is interesting to note that, although males swim more actively and extensively than females (Sabia et al. 2014), their respiration rate is lower likely owing to the dependence of this parameter on body weight (Ikeda et al. 2001). Ikeda et al. (2001) reported a respiration rate of 0.06 µL O₂ ind⁻¹ h⁻¹ at 14 °C in *P. marinus* females, which is equal to a specific respiration rate of 4.0 µL O₂ mg DW⁻¹ h⁻¹, while Lehette et al. (2016) calculated a value of about 7.0 µL O₂ mg DW⁻¹ h⁻¹ at 24 °C in females of the congeneric *Pseudodiaptomus annandalei* Sewell, 1919. Considering a Q₁₀ = 2, these measurements translate into 6.8 and 6.1 µL O₂ mg DW⁻¹ h⁻¹ for *P. marinus* and *P. annandalei*, respectively. The respiration rate of *P. marinus* from the Black Sea was thus in line with literature data, while being almost half that recorded for the Black Sea holoplanktonic calanoids *Pseudocalanus elongatus* Brady, 1865 and *Acartia clausi* Giesbrecht, 1889 (Svetlichny et al. 2017). Based on these preliminary evidences, it is possible to speculate that the reduced oxygen demand in *P. marinus* may favour its settlement in particularly hostile environments such as the hypoxic waters of Lake Faro (Italy) (Sabia et al. 2015).

Pseudodiaptomus marinus: threat or opportunity?

As a consequence of the anthropocentrically biased view of negative consequences related to the introduction of alien species (Katsanevakis et al. 2014), the majority of bioinvasion literature focuses on alien species whose introduction has impacted the receiving environment (Briggs 2012). This contrasts with the evidence that NISs can leave unaltered (if not

increase) biodiversity (Briggs 2012; Katsanevakis et al. 2014), and in some cases their introduction can even be beneficial for the conservation of the environment (Schlaepfer et al. 2011), but the debate in the scientific community is still vibrant (Russell and Blackburn 2017).

The open discussion forum held during the WGEUROBUS workshop was centred on a driving question: should *Pseudodiaptomus marinus* be considered a threat, or should it be seen as an opportunity for scientific and economic development? This theme arose from the evidence that, despite being included in an Australian early warning list (Hayes et al. 2005), the arrival of *P. marinus* has not been associated (at least so far) with negative impacts on any of the receiving basins. On the other hand, the spreading of this species is proceeding at a very fast pace, likely sustained by increasing global ship traffic (Tournadre 2014), and the real impact may be yet to come. In addition, as mentioned in the Introduction, this species is particularly prone to use as a model organism for applied research topics as well as live feed for fish larvae. The WG thus identified some priority research lines to be developed in the framework of the group activities focusing on target topics, defining future challenges and opportunities of research development.

Monitoring *Pseudodiaptomus marinus* distribution

As recently pointed out by Chiba et al. (2018), zooplankton monitoring programmes have deepened the understanding of the mechanisms by which marine ecosystems react to local and global stressors. The constant monitoring of aquatic systems represents the first-assessment tool to record the arrival of a new NIS (Belmonte 2018; Boxshall 2007; Zenetos et al. 2010), and can contribute to the development of the global Aichi Targets indicators (Chiba et al. 2018). In this field, WGEUROBUS research can benefit from already established and ongoing programmes (e.g., LTER series, CPR surveys) to constantly survey the spreading and settlement of *P. marinus*, but it may at the same time prompt specific sampling efforts to integrate present knowledge on both the distributional and seasonal patterns of this species.

Compared to Sabia et al. (2015), the original data presented in this document together with the latest (2015–2019) literature records provide a bird's eye view of the present state European distribution of

P. marinus. The current picture thus depicted demonstrates that the spreading of this NIS is a still developing process, and that introduced environments may be used as stepping stones for further secondary spreading. Model simulations considering the net reproductive rate R_0 as a function of ambient water temperature, assuming both annual means (Rajakaruna et al. 2012) and monthly variations (Rajakaruna and Lewis 2017), set a theoretical boundary to potentially invisable habitats in correspondence of the English Channel-North Sea edge. At present, the northernmost recordings of *P. marinus* in the North Sea (Deschutter et al. 2018; Günther et al. 2018; Wootton et al. 2018; original data presented here) are actually set outside such limit, suggesting a degree of adaptability to temperature fluctuations stronger than that predicted numerically. In light of this, the occurrence of *P. marinus* in the Skagerrak (Wootton et al. 2018) may be a precursor of a further spreading in the Kattegat and, on a longer term, in the Baltic Sea.

To date, *P. marinus* has never been recorded in the multi-annual zooplankton time series collected in the neritic station in the south of the Mallorca Island (Balearic Islands, Spain) (Fernandez de Puelles et al. 2003, 2004, 2007). Whether this absence is due to unsuitable environmental conditions favouring its settlement or to undersampling is presently impossible to assess, and specific investigations should be carried out. In the former case, the comprehension of the mechanisms hampering the establishment of *P. marinus* would provide precious information to understand the interactions of this copepod with its environment.

Being present in 7 out of the 10 European marine subregions identified in the framework of the MSFD, *P. marinus* ranks as one of the most widespread copepod NIS in Europe, following *A. tonsa* which has established in 8 subregions (Tsiamis et al. 2019). The continuous update of the distribution map of *P. marinus* will monitor the spreading process and the establishment of this species in already introduced regions. Such information could be uploaded in information systems, e.g. AquaNIS (www.corpi.ku.lt/databases/aquanis) and EASIN (<https://easin.jrc.ec.europa.eu/>), thus providing the scientific community a real-time scenario of the evolving distribution of *P. marinus*.

As reviewed in the Introduction, the successful establishment is likely sustained by great plasticity (e.g., physiological, behavioural, genetic), providing

an adaptive advantage by which adjusting to the environmental characteristics of the recipient basin. The assessment of the invasive potential of *P. marinus* can be better appreciated considering its physiological responses. Preliminary reports on respiration rates (present work), together with trials investigating the salinity and temperature resistance (Svetlichny et al. 2019), can help to elucidate the biological traits which can support the establishment success of this species.

Impact on receiving environment

Data from in situ samplings presented in previous sections show a general stable settlement of *P. marinus* upon its first recording in the newly colonised environments. As of present, no impact on resident pelagic copepod community has been documented upon the arrival of this NIS, with the only exception of Agua Hedionda lagoon (Fleminger and Hendrix Kramer 1988). Introduced species, however, can interact with one or more native species at different spatial and temporal scales (Katsanevakis et al. 2014; Ojaveer et al. 2015), thus their potential impact on the receiving environment should be investigated accordingly. The presumed non-invasiveness of this species, based on the absence of documented detrimental effects on the pelagic community of the receiving basin, should not reduce the attention towards it. The traditional separation between studies on planktonic and benthic copepods and their ecology has prevented the definition of an *unicum* among these compartments (Belmonte 2018). With specific reference to *P. marinus*, owing to its epibenthic behaviour it would be fundamental to analyse any potential effect on the benthic community in terms of competitive exclusion. Moreover, this species can represent a food source for higher predators, such as carnivore copepods (Uye and Kayano 1994a, b), chaetognaths (Nagasawa and Marumo 1984; Nomura et al. 2007) as well as larval and juvenile fish (Bryant and Arnold 2007; Islam et al. 2006a). Its introduction may therefore provide higher trophic level predators an optional alimentary choice, with repercussions on the flow of energy and matter among the components of the food web that should be carefully addressed in ecological studies.

Sampling protocols and vectors of introduction

NIS studies are intrinsically characterised by a degree of uncertainty needing quantification (Katsanevakis and Moustakas 2018). With specific reference to *P. marinus*, such uncertainties are related to potentially inappropriate sampling protocols, to the possible presence of multiple vectors of introduction, and to a poor knowledge of its impact on receiving systems. All these topics play a key role in understanding the introduction and establishment of *P. marinus* in new areas, and as such they all deserve priority in future research lines.

As already discussed in previous works (Brylinski et al. 2012; de Olazabal and Tirelli 2011; Deschutter et al. 2018; Lučić et al. 2015; Sabia et al. 2015), the sampling protocols for *P. marinus* should be optimised to account for its nycthemeral changes in vertical position. Such issue is particularly critical for all the species alternating benthic and pelagic behaviour, as discussed also for *P. annandalei* (Blanda et al. 2015). Failure to address this topic, for example monitoring the presence of *P. marinus* only in the mesozooplankton samples collected along the water column, would ineluctably lead to underestimations of the actual abundance of the species in the sampled sites. A practical example is discussed in Lučić et al. (2015), who reported that the resuspension of sediment owing to tugboat manoeuvre in the Port of Koper (Slovenia) led to a higher than the usual abundance of *P. marinus* in the morning time.

Within WGEUROBUS discussion, some possible solutions have been identified in the sampling of the epibenthic layer during daylight and/or performing vertical tows after dusk. This could imply performing close-to-the-bottom horizontal hauls or using bottom samplers (e.g., sledges or pumps), in order to collect samples as close as possible to the bottom. These solutions, however, may be impracticable when collecting samples in the framework of well-established programs (e.g., LTER monitorings, CPR surveys), which use standardised protocols to ensure comparability of the data collected over the years. Nocturnal vertical tows, although highly efficient, demand dedicated activities considerably increasing field-time and cost. As such, tailored sampling strategies could be employed in sampling campaigns specifically conceived for the monitoring of *P. marinus*. Among other reported successful sampling strategies for

various Pseudodiaptomidae are emergence traps, diver-towed nets near the bottom and sledge tows (Rios-Jara and González 2000; Saint-Jean and Pagano 1990; Youngbluth 1982), all feasible in field campaigns targeting marine benthic or demersal NIS in general.

As discussed in a previous section, documented proofs demonstrate the arrival of *P. marinus* in new environments through different vectors. For each newly invaded region, it is thus fundamental to point out all the possible means of arrival, in order to properly monitor and manage possible re-introductions. As multiple introduction events may occur in a given area, it would be important to corroborate this information with molecular analyses to unveil the origin area of the NIS.

Pseudodiaptomus marinus for aquaculture and nutraceutical applications

Globally, aquaculture is the fastest growing food sector in the world, providing about 39% of fish for human consumption (FAO 2016). The promotion of research on new species is one of the central actions proposed by the EU to provide the industry with sustainable resources (European Commission 2002). In the last decade, copepods have received great attention as live prey for commercial and ornamental fish aquaculture (Hansen 2017). This is mainly due to their optimal size spectrum, high quality biochemical composition and possibility to control their nutritional value via dedicated algal food formulation. Among calanoid copepods, which constitute the natural food source for most marine fish larvae and adults at sea, *A. tonsa*, *Temora stylifera* Dana, 1849 and *Centropages typicus* Krøyer, 1849 have been proposed for commercial and ornamental tropical fish larviculture (Buttino et al. 2012; Drillet et al. 2011; Olivotto et al. 2008), whose polyunsaturated fatty acids (PUFA) content is a reflection of their diets. An exception might be represented by *P. annandalei*, commonly used as live feed in Indo-Pacific aquaculture (Blanda et al. 2015; Celino et al. 2012; Lee et al. 2010; Rayner et al. 2017a). This species seems to be able to elongate and desaturate FAs assimilated via the algal food (Rayner et al. 2017b), suggesting that calanoids spending much of the daytime associated with detritus-dominated sediments may have strong plasticity in exploiting low FA diets. It is thus reasonable to assume

that also *P. marinus*, by alternating epibenthic and pelagic behaviour, may have such potential. The ease of cultivating this species (Mauchline 1998; Uye 2005), the optimisation of developmental times using targeted temperature and diet (Huang et al. 2006; Liang and Uye 1997; Uye and Onbé 1975), together with a detailed characterisation of its lipid spectrum, would open up the way to the potential use of *P. marinus* as staple feed in first feeding fish trial as a more sustainable and environmentally friendly resource.

In addition to aquaculture applications, copepods in general, and *P. marinus* in particular, may also have biotechnological potential as a possible source of nutraceuticals, defined as products isolated from food providing health benefits to its basic nutritional value. To date, only one copepod-derived nutraceutical is available on the market. The Norwegian bio-marine company Calanus AS is currently exploiting the sustainable large-scale commercial harvesting of freshly collected *Calanus finmarchicus* Gunnerus, 1770 from the North Sea. Although the Norwegian Fisheries Management Authorities have recently developed a management plan allowing the commercial harvesting of 254,000 tonnes of *C. finmarchicus* per year, it still remains to be evaluated the long-term impact of this large-scale biomass exploitation on the northern hemisphere. For the same reasons expressed in relation to aquaculture applications, *P. marinus* may represent a sustainable source of new molecules for nutraceutical use: combining ecological, chemical and molecular information could speed up the possibility of using this species as a model organism for aquaculture and biotechnology applications.

Molecular perspectives in the study of *Pseudodiaptomus marinus*

Molecular tools may provide critical information not only in the detection of species integrating taxonomic identifications (Ojaveer et al. 2018), but also in understanding the genetic relationships among populations inhabiting different areas (Ohtsuka et al. 2018). As pointed out by some authors (e.g., Bron et al. 2011; Tarrant et al. 2019), copepod studies can benefit from molecular approaches to study different aspects of biology, ecology (e.g., distribution, occurrence and co-occurrence with other taxa, food web interactions, etc.), (eco)physiology and taxonomy. Although the so

called ‘-omics’ studies are growing for marine copepods as well (Amato and Carotenuto 2018), relatively little information is available for *P. marinus*. Despite the numerous records in different localities, scant are the reports of molecular identification of this NIS. The first ones in European coastal waters date to 2013 in the Iberian Peninsula (Abad et al. 2016; Albaina et al. 2016), followed by identifications in Southern Italy (Sabia et al. 2017) and in the Adriatic Sea (Stefanni et al. 2018). In the estuary of Bilbao, a metabarcoding (mBC) approach compared to visual enumeration of the samples by microscopic inspections was applied (Abad et al. 2016; Albaina et al. 2016). The hyper-variable domain V9 of the small subunit of the nuclear ribosomal DNA (18S rDNA) was amplified and sequenced from environmental DNA (eDNA). Before assigning sequences to operational taxonomic units (OTUs) by BLAST, a custom reference database was produced. The comparison of mBC with visual counts identified a rather pronounced difference in the occurrence of *P. marinus* (Abad et al. 2016). A different approach was implemented for the samples from Lake Faro (Sabia et al. 2017). *P. marinus* was morphologically identified, then the second internal transcribed spacer of the rDNA (ITS2 rDNA) was sequenced and compared to the sequences present in the GenBank (NCBI) from Korean samples (Sabia et al. 2017), revealing a significant divergence though very closely related on the Bayesian phylogenetic tree. A network analysis showed only private haplotypes, suggesting high levels of divergence at this *locus* (Sabia et al. 2017). The evidence of different adult female body lengths (Table I in Sabia et al. 2015), coupled with a 4.4% divergence in ITS2 sequences between Italian and Korean specimens (Sabia et al. 2017), point to the possible presence of cryptic species or different morphs. Stefanni et al. (2018) integrated molecular (COI and 18S) and morphological analyses to examine the mesozooplankton assemblage collected from the Northern and Western Adriatic Sea. Their results showed the spreading of *P. marinus* in the basin since its first record in 2007 (de Olazabal and Tirelli 2011), with higher abundances in the vicinity of the ports of the Northern Adriatic Sea. Recently, a combination of a new COI minibarcode primer (124 bp) and the use of an appropriate reference library (the DNA barcode COI for North Sea metazoans at the Senckenberg Institute in Wilhelmshaven) allowed the detection of *P. marinus* in extracellular

eDNA samples collected at different sampling sites and during different seasons in the German Bight (North Sea) (Günther et al. 2018). These results suggest that such tools could be used to detect and monitor the spreading of non-indigenous or invasive species.

A recent study was carried out on different *P. marinus* populations from Asia and San Francisco Estuary in order to track the origin of invasion on the Pacific coast of USA (Ohtsuka et al. 2018). The mitochondrial cytochrome b (cyt *b* mtDNA) was sequenced, haplotypes analysed and FST calculated. The authors concluded that *P. marinus* from Japan invaded San Francisco multiple times. The few reports published to date on molecular identification of this NIS reveal a lack of consistency about the molecular marker used. The 18S rDNA and ITS2 rDNA (Abad et al. 2016; Sabia et al. 2017) and the cyt *b* mtDNA and COI mtDNA (Albaina et al. 2016; Ohtsuka et al. 2018) were used almost similarly. Unfortunately, these results are not truly comparable nor a more extensive analysis can be performed as different markers were used. As a consequence, a homogenisation of protocols and approaches is urged to understand whether multiple introductions have occurred in the European seas, thus casting light on the arrival and spreading process.

Further advanced molecular studies can be envisaged in order to disentangle genomic features which might confer *P. marinus* its ability to survive in very different environments. Whole genome sequencing, assembly and annotation can be beneficial to develop a molecular tool set for this species as well as for calanoids in general. This line of investigation might take advantage of the techniques and technologies available today in order to rapidly increase our knowledge on the biology, ecology, evolution and development of this NIS, as well as on its biotechnological potential.

Concluding remarks

To date, most of the bioinvasion literature is centred upon macroorganisms (Soares et al. 2018), although some authors (Marques 2011; Ojaveer et al. 2015, 2018) warned about the limited knowledge on the invasion process by microorganisms and their associated consequences. Copepods do fall into this

category (Soares et al. 2018), notwithstanding their contribution to zooplankton biomass and diversity, and their pivotal role in the functioning of aquatic ecosystems (Uttieri 2018). The invasive potential of some copepod species, and in a number of instances the impactful consequences of their introduction—such as competitive exclusion or spatial segregation of endemic species—are well documented in the literature (e.g., Delpy and Pagano 2018; Marques et al. 2018b; Uriarte et al. 2016; Villate et al. 2018; Zagami et al. 2018). Amid copepod NIS is the calanoid *Pseudodiaptomus marinus*, whose spread in European waters has been reviewed in Brylinski et al. (2012) and Sabia et al. (2015). The fast rate of dissemination of this NIS, together with its potential uses in both baseline and applied research, provided the seed for the establishment of the WGEUROBUS, aimed at facilitating knowledge sharing and joint collaborations within the scientific community. WGEUROBUS activities have been formalised under the ICES Expert Group umbrella in Fall 2018, providing an institutional endorsement to the WG activities at international level.

The discussion held during the workshop in 2018 demonstrated the diversified expertise of WG participants, which covers a great range of disciplines providing a strong scientific support over several research topics. This opportunity will help to further enhance the present understanding of the ecological impacts of NIS introductions, and will continue to support the scientific direction of ecological studies.

In its current conceptualization, the WG gathers experts from European countries only. As obvious, however, the topics covered by the WG have no geographical limits, and contributors are welcome to participate over discussion and data sharing initiatives. In particular, the distribution of *P. marinus* in the Mediterranean basin needs to be integrated with reports from Levantine area and North-African Countries, as also demonstrated by the recent findings of this species in Tunisian waters. Future implementations of WGEUROBUS activities will account for this issue, fostering the integration with other groups (e.g., MEDZOO: Working Group on Mediterranean Zooplankton Ecology; <http://medzoo.bio/>) as well as other ICES WGs to get a more robust description of *P. marinus* spreading.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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