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SHORT COMMUNICATION

Additional record of the non-indigenous copepod *Pseudodiaptomus marinus* (Sato, 1913) in the Adriatic Sea

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The Indo-Pacific egg-carrying copepod Pseudodiaptomus marinus Sato, 1913 *was recorded for the first time in the Port of Koper, Slovenia (Gulf of Trieste) in February 2015. This is the fourth finding of this species in the Mediterranean Sea, and the third for the Adriatic Sea. A rather high abundance of 73 ind. m^{-3} was recorded, which is considerably higher than previous findings in the Adriatic. Maritime transport is presumed to be the main cause of its introduction, primarily through ballast water release. Repeat sampling in May confirmed the presence of this copepod, indicating the possibility that the species has established a stable population in the Port of Koper.*

Key words: Copepods, *Pseudodiaptomus marinus*, non-indigenous species, biological invasion, ballast water, Mediterranean

INTRODUCTION

Planktonic copepods are among the most numerous organisms in all marine ecosystems. They represent the most diverse and abundant taxonomic group in ballast tanks; however, their density decreases with the age of the ballast water (CHU *et al.*, 1997). There are a relatively limited number of records of non-indigenous planktonic copepods in the Mediterranean, although this sea is considered to be one of the world areas most affected by biological invasion (ZENETOS *et al.*, 2010, 2012).

Pseudodiaptomus marinus Sato, 1913 is a free-living pelagic copepod, indigenous to the Northwestern Pacific Ocean (WALTER 1987). It was accurately described by SATO (1913) from samples collected off the west coast of Japan. The first invasion was recorded in the 1960s, when the species was transferred *via* ballast waters to the Hawaiian archipelago (JONES, 1966). Now *P. marinus* occurs worldwide in tropical and temperate areas, in fresh to hyper-saline waters (WALTER, 1986). The first record of *P. marinus* in European Atlantic waters was reported in October 2010 along the coast of France (BRYLINSKI *et al.*, 2012). Later, JHA *et al.* (2013) found specimens of this species in a sample taken in the German Bight (German exclusive economic zone) and later between the Netherlands and the British coast.

The introduction of *P. marinus* into the Mediterranean also seems to be due to maritime transport. The first observation of this copepod in the Adriatic Sea was in November 2007 near Rimini on the western coast (DE OLAZABAL &

TIRELLI, 2011). In October 2008 some specimens were recorded in Lake Faro, a small coastal area along the northeastern Sicilian coast (southern Tyrrhenian Sea), where nowadays a stable population of this species is established (SABIA *et al.*, 2012, 2014). In May 2009, this species was found in an artificial channel located near the Port of Monfalcone in the Gulf of Trieste (northern Adriatic Sea) (DE OLAZABAL & TIRELLI, 2011). The aim of this paper is to report another record of the non-indigenous calanoid copepod *P. marinus* on the northern Adriatic coast, specifically in the Port of Koper.

MATERIAL AND METHODS

Description of sampling location

Sampling was conducted in the Port of Koper, Slovenia, following the sampling protocol of the Port Baseline Survey, which is one of the activities envisaged by the BALMAS project (IPA Adriatic Cross-Border Cooperation Programme). The Port of Koper is the only Slovenian international seaport, located in the southeastern part of the Gulf of Trieste (Adriatic Sea) (Fig. 1). The port is divided into three basins (basins I, II, III) with two piers, which provide specialized terminals to handle a variety of shipments and goods. Basins I and II have a maximum depth of approx. 14 m, while basin III has a depth of about 18 m. The anchorage area for waiting ships is located 2 to 5 km outside the port with a depth varying from 17 to 19 m. The river Rižana, which is the principal freshwater supply in the area, discharges mainly into basin II carrying urban and industrial waste waters.

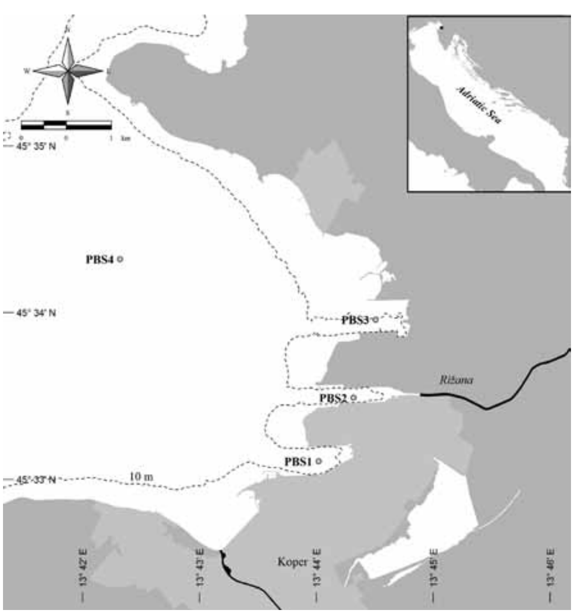

Fig. 1. Study area with sampling locations

Fieldwork

Sampling was carried out at four fixed stations: three of them (PBS1, PBS2, PBS3) located in the three port basins (I, II and III, respectively) with the fourth station (PBS4) located at the anchorage area outside the port (Fig. 1). Zooplankton and phytoplankton were sampled and environmental parameters recorded during four campaigns in order to encompass seasonal characteristics: 9th May 2014, 28th July 2014, 17th November 2014 and 9th February 2015. Samplings were always carried out in the morning between 9:00 and 12:00. In order to ascertain whether findings of *Pseudodiaptomus marinus* were only coincidental or if a stable population had been established, we performed an additional sampling on May 11, 2015, between 10 and 11 p.m. only on station PBS1. Four samples were collected.

Supporting environmental parameters (temperature, salinity, oxygen, pH and light) and transparency were also recorded using a CTD probe (equipped with the LI-192SA Underwater Quantum sensor) and a Secchi disc. Samples for determination of chlorophyll *a* as a proxy of phytoplankton biomass were collected at the sea surface of each station using a 5 l Niskin bottle.

Mesozooplankton samples were collected by vertical hauls with a WP2 net (200 µm mesh size, 55 cm in diameter and 150 cm in length) from the bottom to the surface, depending on the station depth. Samples were preserved in a 4% formaldehyde-seawater buffered solution.

Analysis

Chlorophyll *a* concentrations, corrected for phaeopigments, were determined fluorometrically (HOLM-HANSEN *et al.*, 1965) on a Trilogy Fluorometer (Turner Designs). Subsamples of 400 ml were filtered onto GF/F glass-fibre filters, extracted in 90 % acetone and measured for fluorescence.

Zooplankton taxonomic identification and copepod counts were performed using the Olympus SZH stereomicroscope at 40x and 80x magnifications.

RESULTS

Pseudodiaptomus marinus specimens (Fig. 2) were recorded during the February 2015 cruise at stations PBS1 and PBS2. Species density and physical-chemical characteristics of the sea water are presented in Table 1. The light conditions of the sea water at that time were, particularly on stations PBS1 and PBS2, quite limited as we measured less than 100 µmol photon m^{-2} s^{-1} at a depth of only 2 m. Irradiance decreased rapidly thereafter throughout the water column (< 10 µmol photon m^{-2} s^{-1} at the bottom). Light intensities in February 2015 were on average the lowest among all sampling campaigns. The extremely low Secchi depth measured on station PBS1 (0.2 m) indicated highly disturbed water due to sediment resuspension, whereas on other stations this was not observed (Secchi depth 2-3 m). At station PBS1 a high concentration of phaeopigments, i.e. degradation products of chlorophyll *a,* was also measured, equaling the concentration of actively growing phytoplankton biomass (Table 1).

Considerably higher abundances of *P. marinus* were noted at PBS1 station with 219 individuals in total or 73 ind. m^{-3}. Out of that number, there were 116 females (39 ind. m^{-3}), 99 males (33 ind. m^{-3}), and fourth copepodites stage (CV4) (2 ind. m^{-3}). Seven females were ovigerous, with 14 eggs in sack. Female lengths ranged from 1.54 to 1.69 mm, and male from 1.38 to 1.40 mm. *Pseudodiaptomus marinus* represented 13 % of the total copepod abundance at PBS1 station and, with *Paracalanus parvus,* shared the third highest percentage. The dominant copepods were *Acartia clausi* (28 %) and *Oithona similis* (26 %).

Table 1. Station information, average values of environmental and phytoplankton parameters, and Pseudodiaptomus marinus density in February 2015 (surface values only)*

Station	Location	Depth (m)	Secchi depth (m)	Temperature (°C)	Salinity	Oxygen (%)	Chl *a* (µg L^{-1})*	Phaeopigments (µg L^{-1})*	*P. marinus* (ind. m^{-3})
PBS1	13°44.021’ E; 45°33.108’ N	13.1	0.3	8.86±0.01	37.29±<0.01	98.52±0.03	0.70	0.70	73
PBS2	13°44.338’ E; 45°33.506’ N	13.7	2	9.11±0.23	37.32±0.15	98.28±0.23	0.45	0.29	< 1

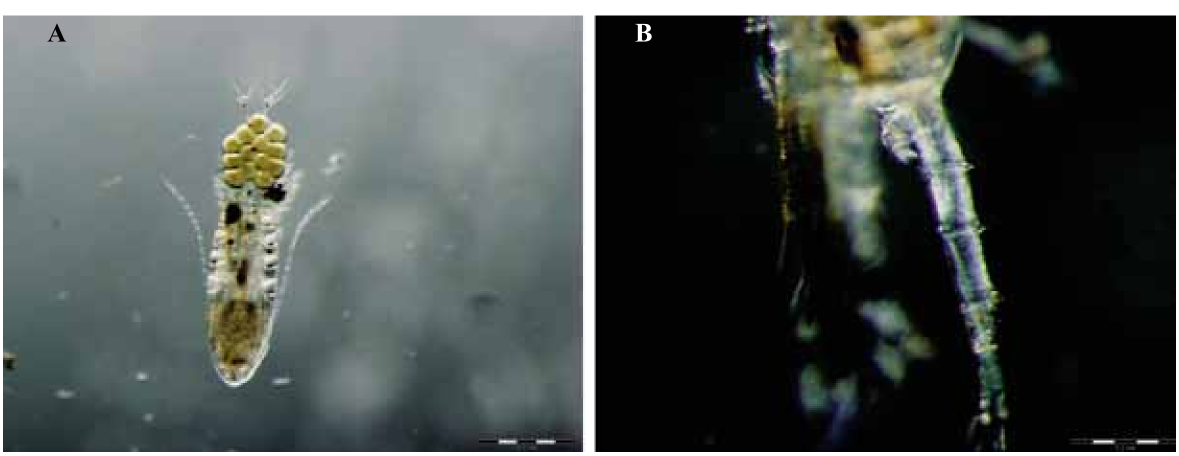

Fig. 2. Pseudodiaptomus marinus Sato, 1913. Egg-carrying female, dorsal view (A); Female genital segment, lateral view (B)

At PBS2 station, *P. marinus* was scarce and only 8 specimens were found. Five were males and three were females. One female was ovigerous.

During the additional sampling on May 11, considerably lower abundances compared to the February sampling were recorded since only 3 to 5 individuals (or 1.39±0.41 ind. m^{-3}) were found in all samples collected at station PBS1. Among them, two females were ovigerous. The dinoflagellate *Noctiluca scintillans* and the copepod *A. clausi* were at the time dominant since they represented 50 % and 42 % of total zooplankton density (6987±174 ind. m^{-3}), respectively.

DISCUSSION

Pseudodiaptomus marinus is one of the most well-known planktonic species considered to be introduced to new environments by human activities (JONES, 1966; GRINDLEY & GRICE, 1969; DE OLAZABAL & TIRELLI, 2011; BRYLINSKI *et* al., 2012). It is generally introduced into coastal areas, primarily into ports by trans-oceanic ships. After initial adaptation this species often becomes numerous and frequently constitutes the main component in the recipient zooplankton communities (SABIA *et al*., 2014).

Egg-carrying copepods have lower fecundity and feeding rates, and longer egg hatching times compared to free-spawning species (KIØRBOE & SABATINI, 1994). These characteristics reduce the mortality of eggs, which was also directly confirmed for *P. marinus* by LIANG & UYE (1997b).

Pseudodiaptomus marinus has recently colonized European coastal marine environments. In the Mediterranean Sea, this copepod species was found in only two regions before now (DE OLAZABAL & TIRELLI, 2011; SABIA et al., 2012, 2014). The Mediterranean basin is the shipping route of vessels transiting from Asia to Europe via the Suez Canal. The fact that *P. marinus* is not yet recorded in the Eastern Mediterranean (ZENETOS *et al*., 2010, 2012) indicates that the species is not a Lessepsian migrant and that it was introduced to new areas primarily by shipping, mainly from ballast water release. Furthermore, species of the genus *Pseudodiaptomus* are known to attach to ships' fouling and in that way transfer to other areas (JACOBS, 1961; GRINDLEY & GRICE, 1969).

In February 2015, we found a considerable high abundance of *P. marinus* at only one sampling station in the Port of Koper. We assume that the tugboat maneuver that occurred just prior to sampling time in the first basin caused extensive sediment resuspension and mixing of the water column (personal observation). This was indicated by a low Secchi depth and very limited light conditions, as well as by a relatively high concentration of phaeopigments. High concentrations of chlorophyll *a* degradation products are usually observed in the bottom layer, while in the water column phaeopigments are generally much lower in concentration than that of actively growing phytoplankton biomass. It could be that the resuspension and mixing lifted copepods that are concentrated near the bottom during the day, which resulted in higher densities of *P. marinus* in the water column as compared to the relatively undisturbed station PBS2 and the repeat sampling in May.

Compared to other European seas where this copepod has been found, our densities and percentages to total copepod numbers are considerably higher. BRYLINSKI *et al.* (2012) collected a very low number of specimens along the northern coast of France, i.e. two ovigerous females and a few copepodites CV stages (0.2 to 4.0 ind. m^{-3}). YHA *et al.* (2013) reported 67 specimens for the North Sea, of which 15 were males, 10 were females and 42 were copepodites, representing densities of 0.05, 0.03 and 0.13 ind. m^{-3}, respectively. DE OLAZABAL & TIRELLI (2011) collected 11 specimens of *P. marinus* near Rimini on the Adriatic coast and 4 specimens in an artificial channel at Port of Monfalcone. A particularly high *P. marinus* abundance was observed in Lake Faro (Sicily, Italy), with an annual maximum of 3900 ind. m^{-3}(SABIA *et al.,* 2014). In our area *P. marinus* became the fourth most abundant copepod species, exceeded only by species from the families Acartiidae and Oithonidae.

In its native environment of the Inland Sea of Japan, where this copepod was first described, *P. marinus* reaches densities of more than 1000 ind. m^{-3} (UYE *et al*., 1982; LIANG & UYE, 1997b). *Pseudodiaptomus marinus* populations, which are now distributed from tropical to northern Japanese and Russian waters (see BRYLINSKI, 2012), indicate that this species has a high survival potential, posing little limitation to its potential spread to new areas. In the Inland Sea of Japan, this copepod reproduces throughout the year (8.9-28.2 °C) in a salinity range from 28.6 to 32.2 (LIANG & UYE, 1997a). In the Sacramento-San Joaquin Estuary, California, it inhabits the environment within a salinity range of 6.1-9.5, and reaches a maximum density of 390 ind. m^{-3} (ORSI & WALTER, 1991). Our salinity values at station PBS1 were higher than those reported above (on average 37.3), which indicates the wide ecological adaptation of this species.

In our study females (53 %) slightly outnumbered males (47 %). UYE *et al.* (1982) noted that the ratios were nearly equal in both sexes during the warmer season when *P. marinus* reached its abundance peak, whereas adult females were more numerous than males during the winter. We noted a very small number of eggs in female sacks and a high ratio of body size for both sexes. These results are in accordance with the biometry and reproductive characteristics for this species in its native environment during the winter period (UYE *et al*., 1982), confirming dependence on sea water temperature. Inverse relationship patterns between adult body sizes and temperature were essentially similar to those of many neritic copepods (ATKINSON, 1994).

There are three possibilities regarding *P. marinus* introduction to the area of the Port of Koper: 1) it could be spread to Koper by currents from the nearby Port of Monfalcone, since both sites are located in the Gulf of Trieste; 2) it could be attached as a fouling organism and transferred with local maritime traffic from the Port of Monfalcone; 3) it could be dispersed by ballast waters from trans-oceanic ships. We suppose that ballast water release was the main cause of this introduction, since this copepod was numerous only in one port's basin (PBS1). In the neighboring basin (PBS2) only a few individuals were recorded, while at the other two stations (PBS3 and PBS4) no specimens were found. The Port of Koper is open to international maritime traffic. Studies of cargo flows and shipping patterns show that this port is connected directly or through Mediterranean hubs with various ports of the world (DAVID *et al*., 2006). However, the ballast water discharge assessment showed that Koper is mainly a recipient port of ballast waters which originate almost exclusively from areas inside the Mediterranean region (PERKOVIĆ *et al*., 2004). In this case, one can assume that this species could be numerous in some other areas of the Mediterranean, as well.

During the May sampling we found several individuals of *P. marinus*, among them one ovigerous female. Although this abundance was much lower compared to the February values, this nevertheless suggests that the presence of *P. marinus* in the southeastern part of the Gulf of Trieste is not coincidental and that a stable population might have established itself.

High densities of *P. marinus* in plankton samples were recorded only when the shallow water column was highly mixed. While on other stations with a relatively undisturbed water column, densities of *P. marinus* were much lower or nonexistent. This could be in line with the biological traits of this species, which remains on or near the bottom during the day, then migrates to the water column at dusk (WALTER, 1986). Thus, for future monitoring of this copepod population in our area, it is necessary to adjust the sampling to times of reduced daylight or to night sampling.

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Dodatni nalaz alohtone vrste veslonošca *Pseudodiaptomus marinus* (Sato, 1913) u Jadranskom moru

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SAŽETAK

Indo-pacifička vrsta veslonošca *Pseudodiaptomus marinus* je po prvi put zabilježena u veljači 2015. godine u luci Kopar, Slovenija. Ovo je četvrti nalaz ove vrste u Sredozemnom moru, od kojih su tri u Jadranskom moru. Utvrđene su visoke vrijednosti od 73 ind. m^{-3}, znatno više od prije zabilježenih za Jadran. Smatramo da je morski transport glavni uzrok unosa ovog veslonošca, prvenstveno putem balastnih voda. Ponovljenim uzorkovanjem u svibnju na istoj postaju potvrđeno je njegova stalna prisutnost što ukazuje na mogućnost uspostavljanja stabilne populacije u luci Kopar.

Ključne riječi: Veslonošci, *Pseudodiaptomus marinus*, unošene vrste, biološka invazija, balastne vode, Sredozemno more