Mediterranean Marine Science

Vol 15, No 3 (2014)

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doi: 10.12681/mms.712

Research Article

Mediterranean Marine Science
Indexed in WoS (Web of Science, ISI Thomson) and SCOPUS
The journal is available on line at http://www.medit-mar-sc.net
DOI: http://dx.doi.org/10.12681/mms.712

Record of an established population of *Palaemon macrodactylus* Rathbun, 1902 (Decapoda, Palaemonidae) in the Mediterranean Sea: confirming a prediction

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Handling Editor: Argyro Zenetos

Received: 29 November 2013; Accepted: 23 April 2014; Published on line: 18 September 2014

Abstract

The capture of larvae of *Palaemon macrodactylus* off Mallorca (Balearic Islands) has been recently reported as evidence of a potential presence of a population of this species in the Mediterranean Sea. Photos of this species, taken during dives in the Sacca di Goro (northern Adriatic) were published in the same year, but no specimen could be collected at that time. Herein, we report the capture in 2013 of numerous individuals of the oriental shrimp, including ovigerous females, both in the Sacca di Goro and in the Lagoon of Grado and Marano (northern Adriatic Sea, Italy), confirming the existence of a well-established population of this species in the Mediterranean Sea.

Keywords: *Palaemon macrodactylus*, introduced species, genetic analysis, new record, Mediterranean Sea, Adriatic Sea.

Introduction

Palaemon macrodactylus Rathbun, 1902 is an estuarine shrimp native to the north-western Pacific, reported in waters around Japan, China and Korea (Newman, 1963). After its first record outside the native area by Newman (1963), who reported its presence in San Francisco Bay, USA since 1957, and a second one in the 1970s from southern Australia by Buckworth (1979), the species has undergone a dramatic expansion since the 1990s. It has been reported from the Atlantic coast of Europe (Ashelby *et al*., 2004; Cuesta *et al*., 2004; Lavesque *et al*., 2010), the Black Sea (Raykov *et al*., 2010) and the Atlantic coast of South and North America (Spivak *et al*., 2006; Warkentine & Rachlin, 2010). A detailed chronology of the introductions of this species, probably by accidental transport in ship ballast water, followed by local spread of larvae and adults by natural dispersion processes, is given by Ashelby *et al*. (2013).

In all cases, *Palaemon macrodactylus* was found in estuaries, bays, lagoons, and brackish waters, which are its common habitat, although there is one exception: Mar del Plata Harbour, Argentina (Spivak *et al*., 2006) where this species lives in a marine habitat but with several physiological problems, possibly linked to the stress of the high salinity conditions (Vázquez *et al*., 2012). However, laboratory tests have proven that *P. macrodactylus* can tolerate a very wide range of salinities (Born, 1968).

The presence of *P. macrodactylus* in the western Mediterranean Sea has been inferred from the presence of a few larvae (one zoea VI in 2005, and seven zoeae III in 2010) in plankton samples collected in marine waters off Mallorca, Balearic Islands (Torres *et al*., 2012). Ashelby *et al*. (2013) suggested the possibility that these larvae might have been released by a passing vessel during offshore ballast water exchange, instead of originating from a nearby still undetected population as hypothesized by Torres *et al*. (2012). Photographic evidence (no specimens could be collected at that time) of the presence of *P. macrodactylus* in the lagoon of Sacca di Goro (northern Adriatic Sea) was provided by Rinaldi (2012).

In this study, the presence in the Mediterranean Sea of a well-established population of *P. macrodactylus* is confirmed from adults collected in 2013 in brackish habitat in the Lagoon of Grado and Marano and in the Sacca di Goro, and in marine habitat off the Po Delta. New photographic evidence of its presence, already in spring 2011, in the

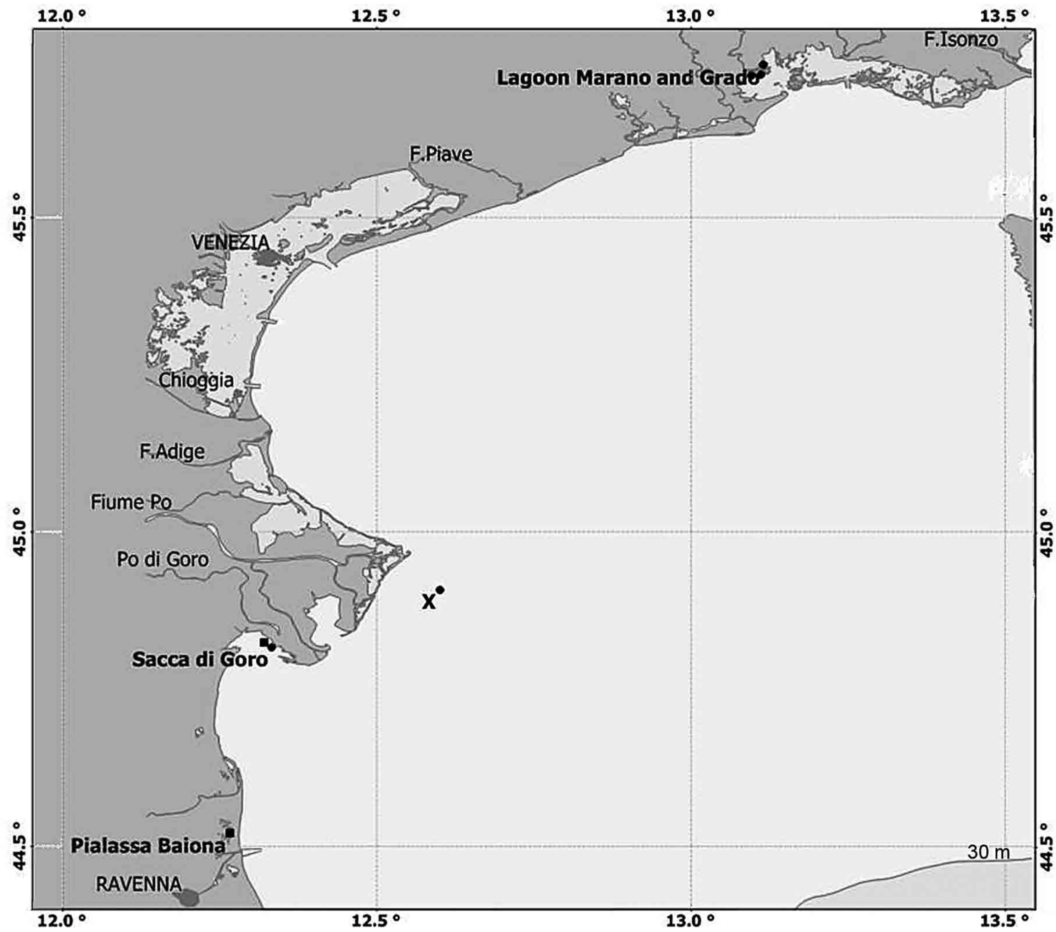

Fig.1: Northern Adriatic Sea, presence of *Palaemon macrodactylus*: ● collected, ■ photographed

Pialassa Baiona, a system of brackish shallow ponds and canals connected to the Ravenna harbour, the second commercial port in the Adriatic, is also added (Fig. 1).

Materials and Methods

Sampling methods

In the Sacca di Goro, the sample of *Palaemon macrodactylus*, associated with *P. adspersus*, was collected in July 2013 with a hand-net. In the Lagoon of Grado and Marano, six stations were sampled on 8th May 2013, after the finding of P. macrodactylus in the landings of local fishermen. Four stations (TME 1 – 4) were located in mesohaline waters in the inner area of the lagoon, where specimens had been previously caught by fishermen, and two stations (TEU 3 – 4) were placed in euhaline waters on eelgrass bed (Tab. 1). The sampling was performed by means of fyke nets with a mesh size of 0.7 cm, positioned with the aid of local fishermen. Samples were collected 24 hours after the setting of the nets and the main physico-chemical parameters of surface water were recorded (temperature, salinity) in addition to the habitat type. Samples were frozen (-20°C) on site and later, in the laboratory, all shrimps were sorted for identification and fixed in ethanol (90%).

The specimen collected at sea was caught with a beam-trawl, cod-end mesh size 40 mm stretch, during the 2013 survey of Adriatic flatfish stocks.

Identification methods

Photographed specimens were identified by rostrum shape and presence of a distinctive white dorsal stripe running along the carapace and abdomen. Moreover, considering that some previous records of *P. macrodactylus* were misidentified as recently demonstrated by Ashelby *et al.* (2013), DNA barcode identification was also carried out to confirm taxonomic identification.

DNA extraction, amplification and sequencing

The DNA barcode identification of the shrimps was based on partial sequence of the 16S rDNA gene. Total genomic DNA was extracted from the muscle tissue of one pereiopod of one ovigerous female and incubated for 20 hours in 300 µl lysis buffer at 65ºC. Protein was precipitated by the addition of 100 µl of 7.5 M ammonium acetate and subsequent centrifugation, and DNA precipitation was obtained by the addition of 300 µl of isopropanol and consecutive centrifugation. The resulting pellet was washed with ethanol (70%), dried, and finally re-suspended in Milli-Q distilled water.

Target mitochondrial DNA from the 16S rDNA gene was amplified with polymerase chain reaction (PCR) using the following cycling conditions: 2 min at 95ºC, 40 cycles of 20s at 95ºC, 20s at 45-48ºC, 45s at 72ºC, and 5 min 72ºC. Primers 1472 (5´- AGA TAG AAA CCA ACC TGG -3´) (Crandall & Fitzpatrick, 1996) and 16L2 (5´-TGC CTG TTT ATC AAA AAC AT-3´) (Schubart *et al.*, 2002) were used to amplify 533bp. PCR product was sent to Biomedal´s Laboratories to be purified and then bidirectionally sequenced.

Sequences were edited using the Chromas version 2.0 software, and then a BLAST search was executed at NCBI webpage to get the sequence that best matches our sequence.

Results

Palaemon macrodactylus Rathbun, 1902 (Fig. 2 a, b)

Material examined: a) Lagoon of Grado and Marano (see Table 1 for coordinates and depth), 8 May 2013: 24 specimens (3 ovigerous females); the DNA voucher specimen has been deposited in the Invertebrate Collection of the Museo Friulano di Storia Naturale (Udine), under accession number zi-02149. b) Sacca di Goro (Po River Delta), depth 1 m, July 2013: 6 females (3 ovigerous), carapace length 11.0 – 12.2 mm. c) Four miles SE of Punta Maestra (44°54.2'N 12°34.5'E, 44°54.7'N 12°38.3'E), depth 18 – 27 m, beam trawl, 11 December 2013: 1 female, carapace length 13.6 mm.

The specimen from the Po region, at present in the CF collection will be deposited in the near future in the Museo Civico di Storia Naturale in Verona.

One specimen, later identified as *P. macrodactylus*, was first photographed on 13 April 2011 in the Piallassa Baiona among *Gracilaria* sp. (Fig. 2a) and another one was photographed on 22 June 2011 in the Sacca di Goro on a mussel bed (Fig. 2b; see also Rinaldi 2012, pg. 331). Its presence in Sacca di Goro was definitely confirmed by the collection of 6 specimens in July 2013.

A total of 24 individuals of *P. macrodactylus* were found in three out of the six stations sampled in the Lagoon of Grado and Marano (TME2, TME3 and TME4) (Table 1). Ovigerous females were only found in TME4 (3 specimens), where the abundance of *P. macrodactylus* was higher. Sampling also yielded the following autochthonous species: *Palaemon adspersus, P. elegans*, and *Crangon crangon*. *Palaemon adspersus* was the most abundant species, especially in the euhaline area of the lagoon, where eelgrass bed is the prevalent habitat, and the salinity ranged between 28.0 and 33.6 PSU (Table 1).

The BLAST search resulted in a 100% match of the 533 bp of the 16S sequence obtained in the present study with the 2 sequences of *P. macrodactylus* deposited at Genbank. One sequence (511 bp) belongs to a specimen from Tokyo

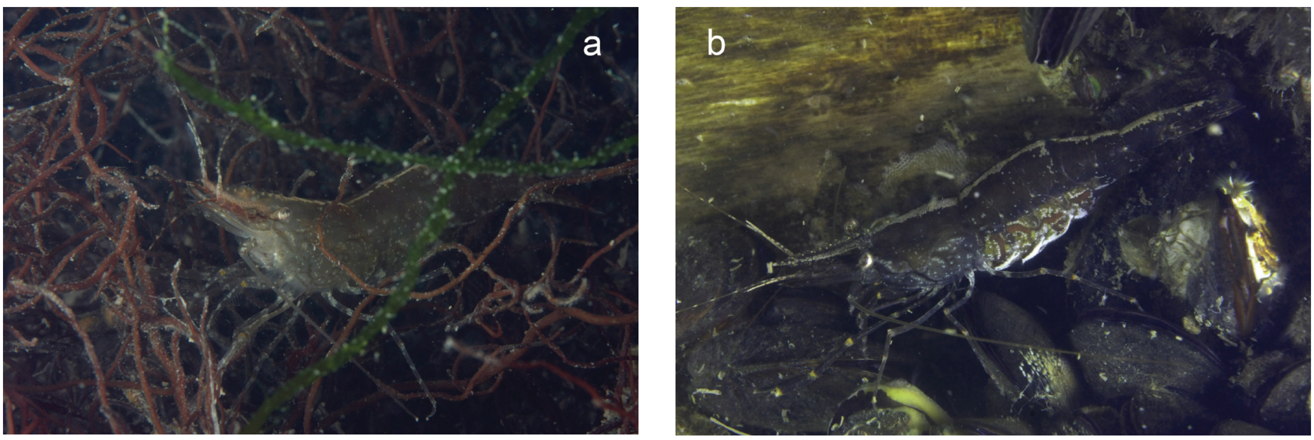

Fig. 2: *Palaemon macrodactylus*, northern Adriatic Sea: a) Pialassa Baiona, 13 April 2011; b) Sacca di Goro, 22 June 2011 (photo A. Rinaldi).

Table 1. Stations sampled in the Lagoon of Grado and Marano: geographical coordinates, depth and habitat type (UMF: unvegetated muddy flat; EGB: eelgrass bed), physico-chemical parameters, and catch of shrimps.

Station	TME1	TME2	TME3	TME4	TEU3	TEU4
Coordinates	45°45'57''N 13°11'38''E	45°44'31''N 13°06'58''E	45°43'40''N 13°06'43''E	45°43'29''N 13°05'48''E	45°43'41''N 13°15'47''E	45°44'13''N 13°13'31''E
Depth (m)	0.8	1.0	0.8	0.8	0.5	1.0
Habitat	UMF	UMF	UMF	UMF	EGB	EGB
Temperature (°C)	20.6	15.9	19.9	18.4	20.3	19.9
Salinity (psu)	19.6	0.36	20.5	13.5	33.6	28.0
Palaemon adspersus	18	0	170	68	136	154
Palaemon elegans	47	2	7	34	0	0
Palaemon macrodactylus	0	4	3	17	0	0
Crangon crangon	0	0	1	0	0	0

Bay (Japan) (accession number: DQ642875), and the other one (539 bp) to a specimen from the Guadalquivir River (Spain) (accession number: JQ042297). These data fully confirm the correct identification of our specimens based on morphological characteristics.

Discussion

The Mediterranean Sea is an ecosystem that is highly vulnerable to the introduction of alien species due to connections with the Atlantic Ocean through the Strait of Gibraltar and with the Red Sea and Indian Ocean through the Suez Canal. Their exponential increase in recent years is a matter of concern (Zenetos *et al*., 2012). Moreover, the busy maritime traffic between Mediterranean harbours accelerates the movements of species beyond their natural dispersion processes, due to the accidental transport of adults and larvae associated to ship fouling and ballast water (Zibrowius, 1992). The ultimate settlement of these species in the Mediterranean Sea depends primarily on their habitat requirements in addition to the propagule pressure induced by maritime traffic and globalization. Therefore, the Levantine Sea, the warmest sector of the Mediterranean, has been invaded by a large number of species native of the tropical Red Sea and Indian Ocean, whereas in the Northern Adriatic Sea, the cooler sector, the settled alien species are mainly native from the temperate regions of the West Atlantic and West Pacific Oceans (Galil, 2009).

The Mediterranean Sea is a hotspot of biodiversity (Coll *et al*., 2010), with a significant number of endemisms related to its richness in habitats. The estuarine habitats, located mainly in the northern sector, are more prone to be colonized by non-indigenous temperate species well-adapted to brackish waters. A number of alien species from different phyla has been reported in the recent past from the North Adriatic lagoons (Froglia & Speranza, 1993; Mizzan, 1999; Occhipinti Ambrogi, 2000). According to Occhipinti Ambrogi *et al*. (2011) the Lagoon of Venice is the "main hotspot of introduction" in Italy. The findings of a certain number of them were also first records for the Mediterranean Sea. The present record of a well-established population of the oriental shrimp adds a new case to the list.

Palaemon macrodactylus has sustained a fast and wider expansion worldwide since the 1990s (reviewed in Ashelby *et al*., 2013). Due to the presence of important international harbours in the vicinity of the places where it was found, there is a general agreement that accidental ship transportation is the main vector of its introduction (Ashelby *et al*., 2013). This is surely the case for the North Adriatic, even if the precise date of introduction is not determinable. The existence of a well-established population, including ovigerous females, was proven only in 2013, but, on photographic evidence, the presence of the species in the brackish water system near the port of Ravenna can be dated back to at least two years earlier. If Ravenna harbour was the first Adriatic stepping stone for *P. macrodactylus*, the settlement in the Lagoon of Grado and Marano could originate from a secondary spread of larvae in ballast waters by cargo vessels that operate between Ravenna and Porto Nogaro. A natural dispersal of larvae seems unlikely because the general current pattern is anti-clockwise in the North Adriatic Sea. But the possibility of active along-shore movements of adults should also be considered in the light of the capture in December 2013 of one specimen offshore of the Po Delta in euhaline waters (36.85 PSU, 12.7°C). The origin is also difficult to determine because ships arrive both from areas of native distribution (Asia) as well as from areas of secondary introduction.

It is difficult to predict the impact of this alien species on the native fauna but competition with the congeneric species *P. adspersus* and *P. elegans* can be easily hypothesized since the species were found at the same sites. In the few cases where these interactions have been studied, non significant displacement or decline of native species have been observed, due to the capabilities of the oriental shrimp to live in a wide range of salinities, thus avoiding direct competition with similar species (González-Ortegón *et al*., 2010), and to exploit under-used niches (Béguer *et al*., 2011). However, *P. macrodactylus* shows tolerance to a broad range of temperatures, salinities, oxygen variations (Born, 1968; González-Ortegón *et al*., 2010), and pollution (Siegfried, 1980). Hence, in case of deterioration of environmental conditions (contamination, hypoxia, etc.), it might be favoured against the native species. Another way to impact on native species is to transmit pathogens such as fungi, bacteria and viruses. Several of these pathogens have been detected in *P. macrodactylus* (Ashelby *et al*., 2013), where the most dangerous seems to be the white spot syndrome virus (WSSV) detected in the Argentinean populations of P. macrodactylus (Martorelli *et al*., 2012). As penaeids are quite sensitive to the WSSV, some concern may arise for the farming of *Marsupenaeus japonicus*, carried on since 1980's in some North Adriatic lagoons, and for the fishery of the autochthonous stock of *Melicertus kerathurus*.

Ashelby *et al*. (2013) suggested that the oriental shrimp will likely diffuse in the near future in north-eastern Europe (Baltic Sea), southern Norway, north-western Africa, and pointed out that invasion of the Mediterranean region might already have begun (based on the larval evidence reported by Torres *et al*., 2012). The present data fully confirm this prediction and we can expect to see this shrimp in other Mediterranean brackish habitats in the near future.

To limit the introduction of aquatic species through shipping is a difficult task, despite existing international regulations (Katsanevakis *et al*., 2013). These alien species, once established, cannot be eradicated. In the best cases (marketable species) they can only be controlled. Therefore, the protection and conservation of the native biota is of particular importance to prevent the deterioration of habitats that facilitate the settlement of opportunistic euryoecious alien species.

Acknowledgements

We wish to thank fishermen Tiziano Ghenda and Angelo Filippo from Marano for their reporting of a "strange shrimp" and Edoardo Turolla for the collection of shrimps in the Sacca di Goro. We also thank Elvira Pensa, Giovanni Casolino, Paolo Glerean, Gianna Innocenti and Sammy De Grave for their help and Alessandro D'Aietti of ARPA FVG for providing the physico-chemical data. Thanks are also due to three anonymous referees and Argyro Zenetos for her editorial work.

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