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A BioBlitz in the Viareggio Marina Area Reveals that the North American *Rhithropanopeus harrisii* (A. A. Gould, 1841) (Decapoda: Xanthoidea: Panopeidae) is Spreading Further in Italy

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Abstract: BioBlitz surveys are important events aiming to record all the living species in an area within a limited timeframe and an important tool to keep track of alien and invasive species. During a BioBlitz held in the Burlamacca Canal (Viareggio Marina Area, northern Tyrrhenian Sea, Italy), 17 taxa were found, among which an "odd" crab species. Its taxonomic identification was confirmed through an integrative taxonomic approach as the invasive Harris mud crab *Rhithropanopeus harrisii* (A. A. Gould, 1841). Subsequently, a total of 33 specimens were collected along a 5 × 1 m transect, with a density of 6.6 individuals/m^2. As the sampled material included adults and ovigerous females, we suggest that the species has already established in the area. The pathway of arrival is uncertain, although secondary spreading amidst fouling communities may have played a major role, with specimens arriving from the nearby Livorno area. Future studies may explore relationships between the alien crab and the resident biota as well as whether the species has already colonised Lake Massaciuccoli and its wetlands.

Key words: coastal monitoring, brackish waters, biological invasions, alien spreading, shipping

Introduction

Presently, biodiversity is under threat across the globe from several stressors, among which habitat loss/fragmentation, overkill, introduced species and climate change (Driscoll et al. 2012, Martínez-Megías & Rico 2021). In the last decades, significant population declines have been reported in various environments and, in general, in flagship or commercial species (Haro et al. 2000, Maynou et al. 2011, Bauer et al. 2015), although the core of biodiversity and the majority of the habitats remain understudied or even unknown worldwide (Trimble & van Aarde 2012, Sigwart & Garbett 2018). Close monitoring and periodic species inventory activities are crucial to correctly assess ecosystem changes and potential impacts on ecological and social systems (Cadotte et al. 2006, Hortal et al. 2008). However, proper scientific research on biodiversity assessment is often limited by time and budget constraints. In this view, citizen science and BioBlitz events are important tools to keep track of alien and invasive species,

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often with limited efforts; moreover, when coupled, they may provide a snapshot of the biota living in an area within a limited timeframe (Lundmark 2003, Bonney et al. 2014).

Following the reports by local anglers to one of us (G.I.) about the presence of an "odd and unknown small crab" invading the Burlamacca Canal (Viareggio Marina Area, northern Tyrrhenian Sea, Italy) since the beginning of 2021, a small BioBlitz event was organised by the Natural History Museum of Florence University, aiming to study the biota living in the area, paying particular attention to its decapod fauna. As the results obtained were potentially interesting, we hereby report about them in this article.

Materials and Methods

Study area

The Burlamacca Canal (43.8678N, 10.2622E) is a brackish waterway connecting Lake Massaciuccoli (43.8508N, 10.3103E) and the Viareggio Marina (43.8608N, 10.2389E) (northern Tyrrhenian Sea, Italy). Salinity and water movements in the canal are strongly influenced by tidal currents, although the lake generally brings through the canal inflow of nutrients to the marina (Lastrucci et al. 2017). The biota living in the canal is characterised by the presence of freshwater species living in association with brackish water ones (Lastrucci et al. 2018).

Sampling

The biota living in the Burlamacca Canal was studied on the 30th June 2021, during a small BioBlitz carried out by four of the authors of the present note (G.I., S.C., D.P. and G.S.). Invertebrate species were recorded through a visual approach or collected with the help of hand nets, whereas information about the fish species living in the canal was mostly collated through enquiring to non-professional anglers and further confirmed by showing them images of the same taxa downloaded from the web.

As one of the recorded species was presumably *Rhithropanopeus harrisii* (A. A. Gould, 1841) (Decapoda: Xanthoidea: Panopeidae) (see below), a 5 × 1 m long × wide transect (43.86756N, 10.25864E) was further explored for its presence (including specimens living on buoys and amidst floating plants) as to provide preliminary data on the abundance of the newly discovered population and on the size of the specimens living there. All recorded individuals were collected and brought to the laboratory. Measurements of the maximum carapace width (CW) were done with an electronic calliper Mitutoyo 500-197-20.

Morphological and molecular identifications

Identification of the resident biota was carried out in the field with magnifying glasses or in the laboratory with a Nikon SMZ645 stereomicroscope. However, to further verify the morphological identification of *R. harrisii*, total genomic DNA was also extracted from the fifth pereiopod of two specimens as described in Crocetta et al. (2020). Partial sequences of the *cytochrome c oxidase subunit I* (COI) gene were amplified using the primers COL6b (5′-ACAAATCATAAAGATATYGG-3′) and COH6 (5′-ADACTTCDGGRTGDCCAAARAAYCA-3′) (Schubart & Huber 2006) in 25 µl volume reaction (Tanduo et al. 2021a). Polymerase chain reactions (PCRs) were performed as in Tanduo et al. (2021b). The PCRs products were purified and Sanger sequenced through an automated DNA sequencer (ABI PRISM 3730). The chromatograms for each sequence obtained were checked, assembled and edited using BioEdit version 7.0.0 (Hall 2011) and and finally compared with reference sequences from the NCBI nucleotide (NT) database using BLASTn (Morgulis et al. 2008).

Results

The BioBlitz revealed the presence of 17 taxa (Table 1). The studied area was dominated by floating plants, among which the alien *Ceratophyllum demersum* L., 1753 and *Myriophyllum spicatum* L., 1753, as well as by the alien serpulid tubeworm *Ficopomatus enigmaticus* Fauvel, 1923, that forms aggregate fouling cables, buoys and floating devices and provides shelter to many organisms, including native and alien arthropods. Among them, several specimens potentially ascribable to the alien crab *R. harrisii* were found (Fig. 1). We recorded a rather diverse fish fauna, accounting for seven taxa and including the native protected species *Anguilla anguilla* (Linnaeus, 1758) as well as four alien freshwater species. Finally, single individuals of a non-indigenous freshwater turtle (*Trachemys* sp.) and of the coypu *Myocastor coypus* (Molina, 1782) were observed.

The morphology of the "odd" crab specimens agreed with the diagnostic characters of *R. harrisii* reported by Christiansen (1969) and Roche & Torchin (2007). A 593 base pairs partial sequence of the COI gene was obtained from both specimens, with sequences resulting identical one to each other. They showed a 98.10–100 % similarity with ~100 COI sequences of *R. harrisii*, including those from two complete mitochondrial genomes

Table 1. Taxa recorded during the BioBlitz carried out in the Burlamacca Canal (Italy), preceded by their status in Italy. Abbreviations used: A – alien; N – native.

Floating plants	**Chordata (Fishes)**
(A) *Ceratophyllum demersum* Linnaeus, 1753	(N) *Anguilla anguilla* (Linnaeus, 1758)
(A) *Myriophyllum spicatum* Linnaeus, 1753	(A) *Cyprinus carpio* Linnaeus, 1758
Annelida	(A) *Gambusia* sp.
(A) *Ficopomatus enigmaticus* Fauvel, 1923	(A) *Lepomis gibbosus* (Linnaeus, 1758)
Arthropoda (Amphipoda)	(A) *Micropterus salmoides* (Lacepède, 1802)
(A) *Grandidierella japonica* Stephensen, 1938	(N) *Mugil cephalus* Linnaeus, 1758
Arthropoda (Isopoda)	(N) *Squalius* cf. *cephalus* (Linnaeus, 1758)
(N) *Lekanesphaera hookeri* (Leach, 1814)	**Chordata (Reptilia)**
Arthropoda (Decapoda)	(A) *Trachemys* sp.
(N) *Palaemon* cf. *elegans* Rathke, 1837	**Chordata (Mammalia)**
(N) *Carcinus aestuarii* Nardo, 1847	(A) *Myocastor coypus* (Molina, 1782)
(A) *Rhithropanopeus harrisii* (A.A. Gould, 1841)	

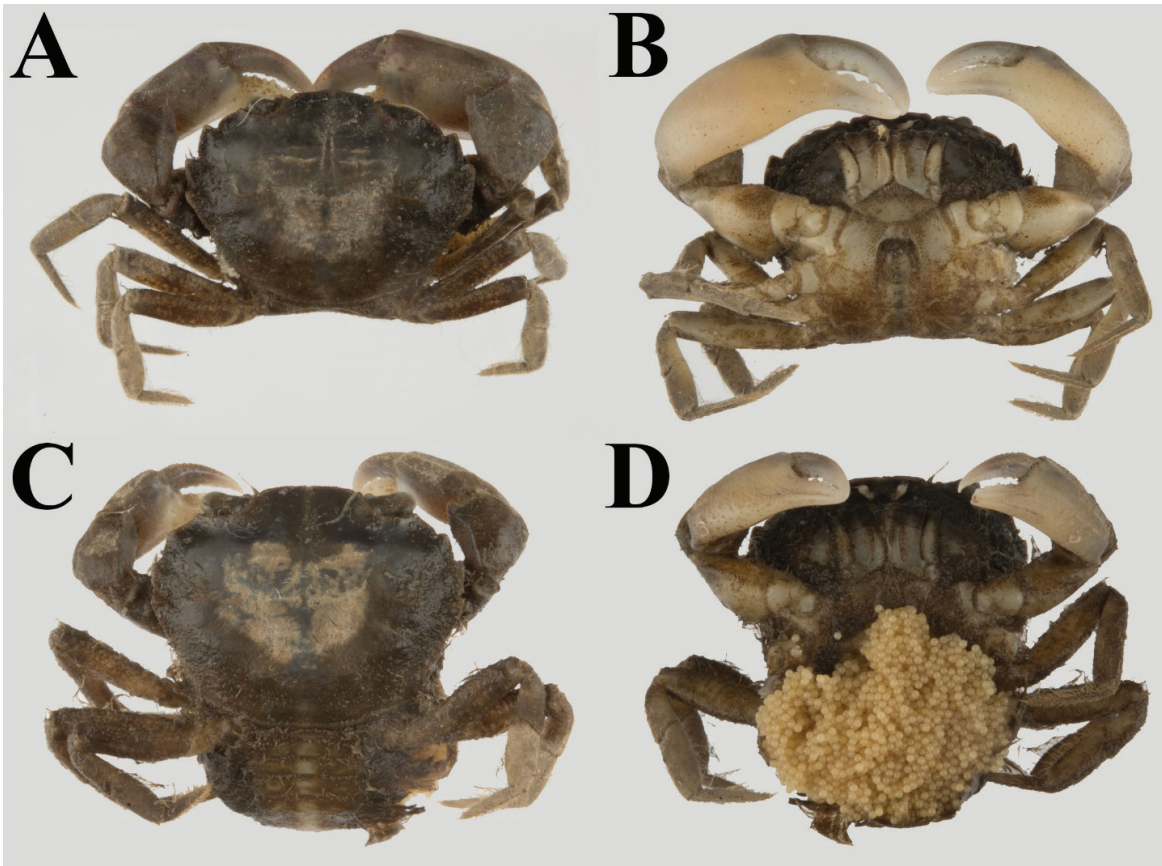

Fig. 1. The Harris mud crab *Rhithropanopeus harrisii* from the Burlamacca Canal (Italy). Specimens not to scale. A–B. Dorsal and ventral view of an adult male, CW 17.4 mm. C–D. Dorsal and ventral view of an ovigerous female, CW 12 mm.

(MW446896–7), whereas a lower similarity (≤91.23 %) was retrieved with all the other taxa deposited in GenBank. Thus, molecular results confirmed the morphological identification of the species. The single haplotype was deposited in GenBank, under the code ON461369.

A total of 33 specimens of *R. harrisii* were collected along the transect, corresponding to an average of 6.6 individuals/m^2. Males were 15 (CW: 9.8–17.4 mm), all showing dimorphic claws with the right being larger, whereas females were 18 (CW: 8.1–12.6 mm), all with claws of similar size. Among them, five were ovigerous (CW: 8.1–12 mm). Males resulted bigger in sizes (t test: 2,086;

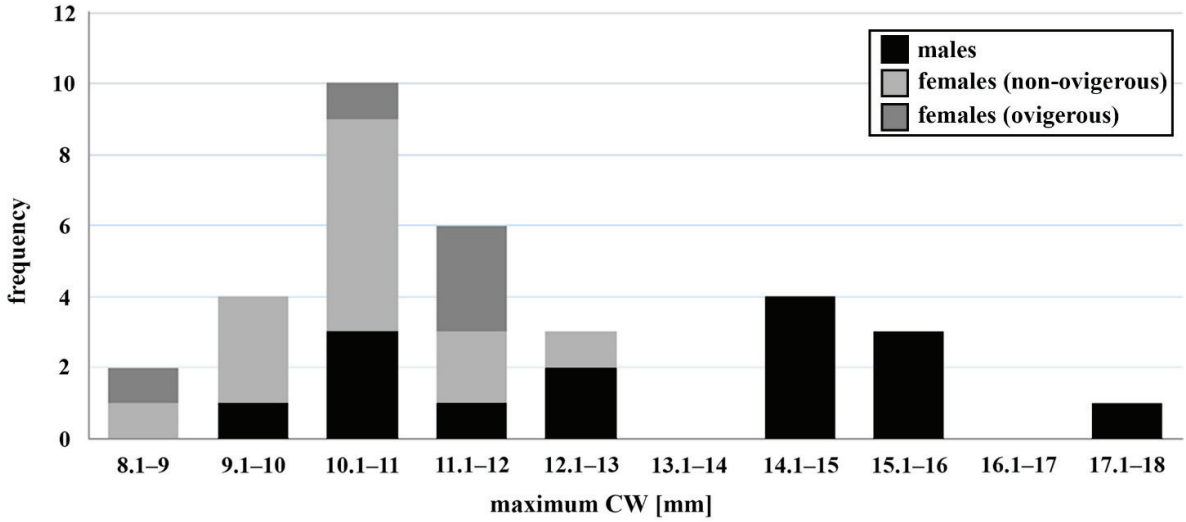

Fig. 2. Histogram of the carapace width [mm] – frequency distribution of *Rhithropanopeus harrisii* in the investigated transect (N = 33), with specimens divided in males and ovigerous/non-ovigerous females.

df=20; P<0.00055), and ovigerous females were found in the lower size-classes, as evidenced by the histogram of the width-frequency distribution (Fig. 2). All crab samples were preserved and stored in the Zoological collection of the Natural History Museum, Florence University, under the code MZUF 5140.

Discussion

Our results confirm the role of BioBlitz as an important tool to keep track of the biodiversity of an area in a limited timeframe (Karns et al. 2010, McMullin et al. 2018). In fact, not only we hereby report an account of the biota living in an understudied environment but also highlight the presence of *R. harrisii* in the Burlamacca Canal for the first time. Thus, we widen the known distribution of this species in Italy (Tyrrhenian Sea coast) after nearly ten years since the last record.

The Harris mud crab *R. harrisii* is a species native to the Atlantic coast of North America, introduced unintentionally in over 20 countries and several continents worldwide (Roche & Torchin 2007, Roche et al. 2009). It also accounts for one of the earliest crustaceans invading Europe, with specimens found in Holland (Zuiderzee) as early as in 1874, when they were originally thought to be a newly discovered species and described as *Pilumnus tridentatus* Maitland, 1874 (see Maitland 1874). In the Mediterranean Sea, the species was first found in 1994 in the north Adriatic Sea, where it was then recorded from the Laguna di Grado and Marano to Cesenatico area (Mizzan & Zanella 1996, Fabbri & Landi 1999, Museo di Storia Naturale di Venezia 2022). Later, it was found off the coast of Marseille (Noel 2001), in the Gulf of Tunis (Ben Souissi et al. 2004) and, in 2013, in Livorno harbour and in the brackish canals behind the Port (Langeneck et al. 2015). The latest record was from Olbia Port (Sardinia, Italy) in 2014 (Ferrario et al. 2017). Indeed, taking into account the successful invasion history of the species, its further spreading in the Mediterranean Sea was highly expected. However, if not for the combined efforts highlighted here, it would have presumably passed unnoticed for additional time. Unfortunately, there are no certainties regarding the possible pathway of arrival of the Harris mud crab in the area. Nevertheless, possible vectors of introduction for this species are the accidental transport through vessel fouling, ballast water and oyster shipments, as well as fish stocking (Cohen & Carlton 1995, Roche & Torchin 2007); among them, secondary spreading amidst fouling communities may have played a major role also here, with specimens presumably arriving from the nearby Livorno harbour area due to recreational boating.

Finally, no studies ever quantified the impact of this species on native communities but inferred evidence suggests that it may alter interactions among species and cause economic damage through competition with native species, alteration of food webs and fouling of water suction pipes (Roche & Torchin 2007). Moreover, the species is known to tolerate anoxia and pollutants (Normant et al. 2004) and a wide range of environmental conditions, including salinity and temperature (Petersen 2006), all characteristics supposed to have facilitat-

ed its success as a global opportunistic invader. Also in the present case, no evidence of impacts was collected. However, the Burlamacca Canal is characterised by poor environmental conditions and biota with a high percentage (~65 %) of alien species, and the presence of *R. harrisii* in high densities, including adults and ovigerous females, confirms previous statements and suggests its establishment in the area. Future studies may thus explore relationships between the alien crab and the resident biota, as well as whether the species has already colonised Lake Massaciuccoli and its wetlands.

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