

RESEARCH ARTICLE

The low basin of the Arno River (Tuscany, Italy) as alien species hotspot: first data about *Rhithropanopeus harrisii* (Crustacea, Panopeidae)

J. Langeneck^{1*}, M. Barbieri¹, F. Maltagliati¹, A. Castelli¹

¹Dipartimento di Biologia, Università di Pisa, via Derna 1 - 56126 Pisa, Italy

*Corresponding author: Phone: +39 050 2211447; Fax: +39 050 2211410;
E-mail: klangeneck@biologia.unipi.it

Abstract

- 1 - Harbours and ports, especially if located in the nearby of brackish-water environments, can provide a significant chance to biological invasions. To date, in the Livorno port, twenty alien species have been recorded, fifteen of which are established.
- 2 - Presence, abundance, size and sex *ratio* of the mud crab *Rhithropanopeus harrisii*, a newly introduced invasive species, have been assessed in six sampling stations along the brackish-water canals between Pisa and Livorno towns. Samplings were carried out in summer and fall 2013.
- 3 - *R. harrisii* appeared fully established in the majority of the sampling stations. Reproduction occurs between May and July and sex *ratio* varied between reproductive and post-reproductive period, with females more abundant before the reproduction.
- 4 - Individuals of *R. harrisii* were more abundant in stations close to Livorno port, whereas they were scarce or sporadic in the northernmost stations, close to the main flow of the Arno River.
- 5 - Due to the high invasive potential of *R. harrisii*, a closer monitoring of brackish-water environments along the north-western Italian coast is needed, in order to assess and prevent further invasions.

Keywords: *Rhithropanopeus harrisii*, biological invasions, brackish-water habitat, alien species, ports, Ligurian Sea.

Introduction

Brackish-water environments are characterised by strong and irregular variations of the principal chemical and physical parameters, such as temperature, salinity, oxygen concentration, and often presence of pollutant agents (Cognetti, 1994; Cognetti and Maltagliati, 2000). This transitional area between freshwater, terrestrial, and marine ecosystems represents a typically stressing environment, with high risk of eutrophication, hypoxia and local

loss of large parts of the native assemblages (Cognetti, 1994; Cognetti and Maltagliati, 2000). Brackish-water environments often provide a significant chance to biological invaders (Paavola *et al.*, 2005). As already observed in several Mediterranean transitional water biotopes, native communities can be partially or largely replaced by alien species assemblages (Mizzan, 1999; Florio *et al.*, 2008). Furthermore, coastal areas, estuarine and lagoon environments have been characterised by a high rate of

human settlement and biological resource exploitation throughout history (Small and Nicholls, 2003; Lotze *et al.*, 2006). As a consequence, many anthropogenic perturbations affect brackish-water ecosystems, determining habitat alterations and changes of biotic communities structure and dynamics (Kennish, 2002). In particular, a relevant human-induced phenomenon affecting brackish-water ecosystems is represented by the introduction of a large number of alien species through ballast waters or attached to the keel of ships (Lavoie *et al.*, 1999; Sylvester, 2011). Therefore, ports can be considered hotspots

of biological invasions. The proximity to transitional environments, then, eases the settlement of stable populations of alien species (Occhipinti-Ambrogi *et al.*, 2010). The well-travelled Livorno port and the adjacent area, like other industrial ports along the Italian coasts, harbour several alien species, most of which are considered highly invasive. Documented records are available for twenty alien macrozoobenthic invertebrates overall: seven species of molluscs, three of annelids, six of crustaceans, one of bryozoans and two of tunicates (Table 1). In this paper we report the first occurrence of the dwarf mud crab

Table 1. Alien macrozoobenthic species recorded nearby the Livorno port, with year of the first sighting, establishment condition and main references.

Species	First sighting	Established	Main reference(s)
<i>Rapana venosa</i>	1987	no	Paolini, 1987
<i>Bursatella leachii</i>	2006	no	Crocetta <i>et al.</i> , 2009
<i>Xenostrobus securis</i>	2006	yes	Giusti <i>et al.</i> , 2008; Barbieri <i>et al.</i> , 2011
<i>Arcuatula senhousia</i>	2001	yes	Campani <i>et al.</i> , 2004
<i>Fulvia fragilis</i>	2003	yes	Crocetta, 2005; Bartolini <i>et al.</i> , 2010
<i>Venerupis philippinarum</i>	unknown	yes	Crocetta, 2012
<i>Theora lubrica</i>	2001	yes	Campani <i>et al.</i> , 2004
<i>Hydroides elegans</i>	unknown	yes	Lupi, 2012
<i>Hydroides dianthus</i>	unknown	yes	Lupi, 2012
<i>Ficopomatus enigmaticus</i>	unknown	yes	-
<i>Procambarus clarkii</i>	unknown	yes	Barbaresi and Gherardi, 2000
<i>Panulirus regius</i>	2010	no	Frogliata <i>et al.</i> , 2012
<i>Callinectes sapidus</i>	2005	no	Bisconti and Silvi, 2005
<i>Portunus segnis</i>	2004	no	Crocetta, 2006
<i>Rhithropanopeus harrisi</i>	2013	yes	present study
<i>Caprella scaura</i>	2008	yes	Occhipinti-Ambrogi in Galil, 2008
<i>Electra tenella</i>	2012	yes	Rosso in Thessalou-Legaki <i>et al.</i> , 2012
<i>Microcosmus squamiger</i>	2008	yes	Ramos in Galil, 2008
<i>Styela plicata</i>	unknown	yes	Lupi, 2012

Rhithropanopeus harrisii (Gould, 1841) (Fig. 1), with settled abundant populations in the canals north of Livorno port. Although along the Northern European coasts the invasion of *R. harrisii* dates back to the second half of the XIX century (Maitland, 1874), this species was firstly reported from the Mediterranean Sea only twenty years ago, in the Po River delta (Mizzan and Zanella, 1996). Further records are referred still to the Northern Adriatic Sea, south of the Po delta (Fabbri and Landi, 1999), and farther north in the Venice Lagoon (Mizzan, 2005). The species has been also reported from the Étang de Berre, near Marseille in France (Noël, 2001) and from the Gulf of Tunis in Tunisia (Ben Souissi *et al.*, 2004). This species shows a high degree of adaptability to a wide range of environmental conditions, being able to tolerate wide variations of salinity and temperature (Christiansen and Costlow, 1975; Boyle *et al.*, 2010). Typical bio-ecological characteristics favouring species' invasive potential are its high reproductive rate, wide spectrum of food items, and scarcity of predators and competitors (Turoboyski, 1973; Hegele-Drywa and Normant, 2009).

Materials and methods

Study area

The low basin of the Arno River is connected to the Livorno port by the 11 km long and

approximately 3.5 m deep Navicelli canal, which connects the floodway Scolmatore dell'Arno about 1 km from the mouth, shortly north of the port. The floodway branches off the Arno just downstream of Pontedera town, 40 km from the river mouth and 28 km off the coast, and receives also the water of some lesser tributaries, whereas the Navicelli canal branches at the level of Pisa town, about 6 km from the river mouth (Fig. 2). The area is affected by strong organic pollution, due to the sewers of the whole basin, and by a less pronounced heavy metal pollution, related mainly to the industrial activities in the neighbourhood of Firenze and to the numerous shipyards located along the canals (Leoni and Sartori, 1997). The biocoenoses present in the study area are typical of brackish-water, stressed environments where the salinity varies both seasonally and geographically from nearly freshwater (4 psu) to typical marine values.

Field and laboratory methods

Six sampling stations were selected along the Navicelli and Scolmatore dell'Arno canals: four were located in the Navicelli canal (S1-S4), one at the confluence between the two canals (S5) and the sixth in the Scolmatore dell'Arno canal, upstream of the confluence (S6), approximately 3-4 km far from each other (Fig. 2). In each station sampling

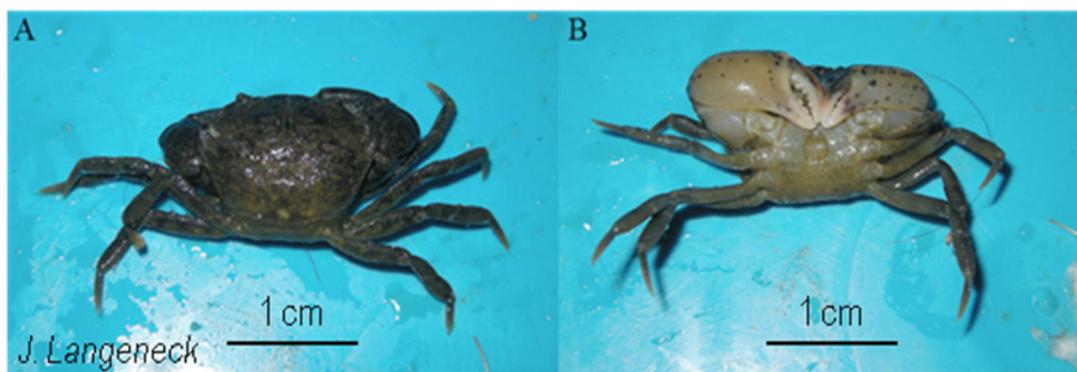


Figure 1. Male individual of *Rhithropanopeus harrisii*. A: dorsal view; B: ventral view.

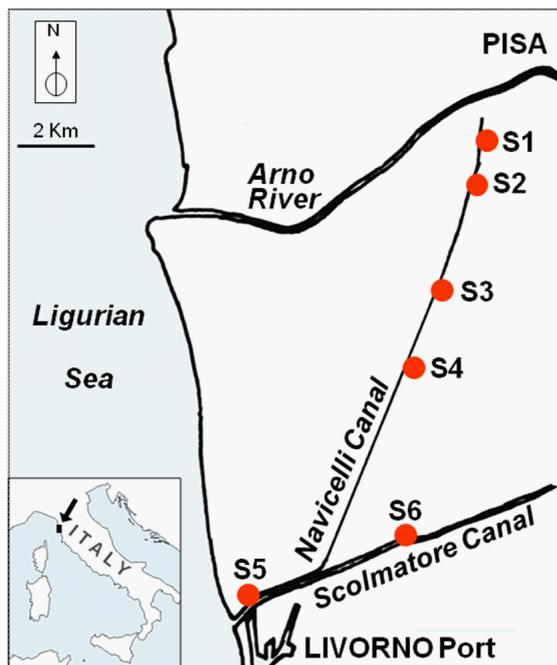


Figure 2. Location of *Rhithropanopeus harrisii* sampling sites.

design provided four randomly allocated samples, in order to gather information on local variability. The sampling was carried out by means of a steel sampling net measuring 25 x 20 x 20 cm with 1 mm mesh following Castelli *et al.* (1996). Samples were sorted in laboratory and the specimens of *Rhithropanopeus harrisii* were measured with a Vernier calliper and sexed where possible. The significance of size difference between the sexes has been checked by means of the *t*-test. The dominant concretion-building species and other macrofaunal organisms were also annotated. Samplings were carried out in all stations on summer (June-July) 2013 and fall (October) 2013. Moreover, additional samples were collected only in S4 and S6 on May 2013 and in S4 on September 2013. About fifty individuals of *R. harrisii* were stored in 96% ethanol at -20 °C as vouchers in the collection and for possible future molecular studies.

Results

Assemblage composition

The walls of the canals were colonized by two different assemblages, the most common is characterized by reefs of the serpulid polychaete *Ficopomatus enigmaticus* (21 of 24 samples in summer, 19 of 24 samples in fall), the less common being represented by concretions of the barnacle *Balanus eburneus*, the alien pigmy mussel *Xenostrobus securis* and undetermined filamentous green algae. Generally, *B. eburneus* and *F. enigmaticus* are mutually exclusive, whereas a high number of *X. securis* specimens can be found also in *F. enigmaticus* reefs, as observed by Barbieri *et al.* (2011). *X. securis* was found in all sampling stations both in summer and fall, whereas *R. harrisii* was reported from four stations in summer, and five in fall. As regards other species, the most widespread was the polychaete *Hediste diversicolor* (five stations in summer, four in fall), followed by the common eel *Anguilla anguilla* (three stations in both seasons), *Alitta succinea* (two stations in both seasons), and the brackish-water shrimps *Palaemon adspersus* (three stations in fall) and *Palaemonetes antennarius* (one station in summer, two in fall).

The invasive crayfish *Procambarus clarkii*, more related to freshwater environments, was collected only in the low salinity S6 in summer, and the estuarine crab *Carcinus aestuarii*, a native species possibly affected by the competition with *R. harrisii*, was reported only in S4 in fall (Table 2). Moreover, empty shells of *Arcuatula senhousia* were found amongst alive individuals of *X. securis* in S6.

Distribution and abundance of Rhithropanopeus harrisii

Rhithropanopeus harrisii was at first recorded in S4 and S6 (May 2013), successively in all stations except S1 and S2 (summer); finally,

Table 2. Distribution of the macrobenthic species across sampled sites in summer (SU) and fall (FA) 2013.

	S1		S2		S3		S4		S5		S6	
	SU	FA										
<i>Xenostrobus securis</i>	x	x	x	x	x	x	x	x	x	x	x	x
<i>Rhithropanopeus harrisii</i>	-	-	-	x	x	x	x	x	x	x	x	x
<i>Hediste diversicolor</i>	x	x	x	x	x	-	x	x	-	-	x	x
<i>Alitta succinea</i>	-	-	-	-	x	x	x	x	-	-	-	-
<i>Palaemonetes antennarius</i>	-	-	-	-	-	-	-	-	x	x	x	-
<i>Palaemon adspersus</i>	-	-	-	-	-	-	x	-	x	-	x	-
<i>Procambarus clarkii</i>	-	-	-	-	-	-	-	-	-	-	x	-
<i>Carcinus aestuarii</i>	-	-	-	-	-	-	-	x	-	-	-	-
<i>Anguilla anguilla</i>	-	x	-	-	x	-	x	x	-	-	x	x

a single large male has been found also in S2 in fall. Recruits (< 4 mm carapace width, CW) were abundant in summer both in S5 and S6. The species appeared well-spread in the majority of the stations considered. Ovigerous females of *R. harrisii* were observed only between May and July and only in S3, S4 and S5 sampling stations,

although recruits have been reported also from S6, where the species appeared to be quite common. Before the reproduction the sex ratio appeared strongly skewed towards females, whereas it tended to 1:1, or was skewed towards males after the reproduction, in fall samples (Fig. 3). Average size of the crabs strongly decreased in fall, after the

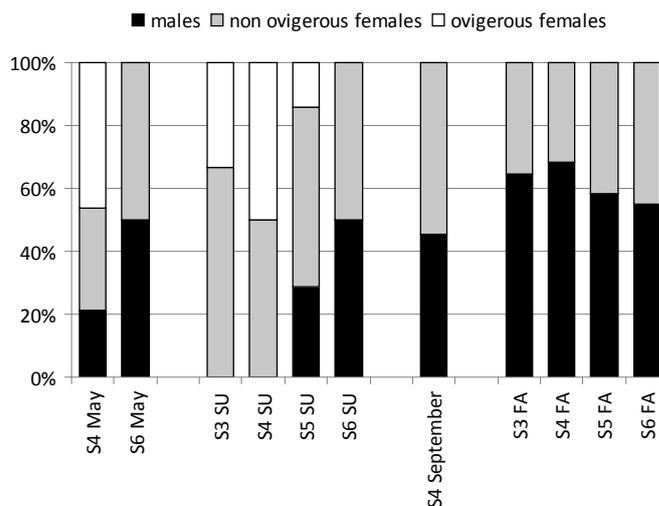


Figure 3. *Rhithropanopeus harrisii*. Variation of sex ratio and proportions of ovigerous and non-ovigerous females across sampling stations. Station codes are as in Fig. 2, SU: summer; FA: fall.

recruitment (Tab. 3), due to the contribution of a large amount of juveniles, as well as the reduced number and size of adults. In fact, specimens over 10 mm of CW constituted the 75-100% of spring and summer samples, whereas in fall samples only 12-35% of the specimens exceeded this size. Sexes did not usually differ in size: before spawning and immediately after, in some samples males were significantly larger, whereas in fall samples sexes do not differ, with the exception of S5, where females were larger.

Discussion

The present study reports the first occurrence of established breeding populations of *R. harrisii* for the western Italian coasts. Reproduction occurs between May and July, earlier than that observed in higher-latitude populations, where eggs are laid in late summer or early fall (Turoboyski, 1973; Fowler *et al.*, 2013). The variation of the

sex ratio is quite interesting, as in summer samples it is skewed towards females, in fall samples towards males or 1:1. A 1:1 sex ratio was reported by most authors (Turoboyski, 1973; Roche and Torchin, 2007), although in some introduced populations it can be strongly skewed towards males (Vortsman, 1939; Mizzan and Zanella, 1996). Interestingly, a female-skewed sex ratio has never been reported for this species. Sex *ratio* in *R. harrisii* generally shows no seasonal variation (Turoboyski, 1973), therefore, variation between the reproductive and post-reproductive seasons is somewhat puzzling. We can argue that the settlement process is currently ongoing, and the lesser abundance of the species in the most distant stations (S2 and S3) is consistent with that. This hypothesis, however, does not account for the seasonal variation of sex *ratio*; the variation, moreover, has been observed only where ovigerous females were reported, also in S3,

Table 3. *Rhithropanopaeus harrisii*. Mean values (\pm standard deviation) of individual carapace width (CW) in the total samples and both sexes in the stations where the species has been found. Differences between sexes were assessed by t-test.

Sample	N	CW (mm)	CW ♂ ♂ (mm)	CW ♀ ♀ (mm)	t-test
S4 May	35	12.91 \pm 1.68	14.93 \pm 2,24	12.36 \pm 0.84	ns
S6 May	8	11.48 \pm 1.96	12.53 \pm 2.23	10.43 \pm 0.68	*
S3 summer	3	10.13 \pm 1.76	-	10.13 \pm 1.76	-
S4 summer	4	12.43 \pm 1.72	-	12.43 \pm 1.72	-
S5 summer	7	13.01 \pm 1.38	12.33 \pm 2.72	13.29 \pm 0.82	ns
S6 summer	4	10.88 \pm 1.04	11.95 \pm 0.78	9.80 \pm 0.28	*
S4 September	108	9.06 \pm 2.67	9.70 \pm 2.89	8.23 \pm 2.13	*
S3 fall	18	8.84 \pm 1.62	8.68 \pm 1.55	9.15 \pm 1.84	ns
S4 fall	41	9.18 \pm 2.22	9.34 \pm 2.18	8.84 \pm 2.35	ns
S5 fall	61	6.23 \pm 1.82	5.75 \pm 1.61	6.81 \pm 1.91	*
S6 fall	58	7.66 \pm 2.46	7.79 \pm 2.89	7.48 \pm 1.79	ns

S4 and S5. However, differences in sampling procedures between the present investigation and previous studies could account for the above mentioned discrepancies.

After reproduction, crab average size (measured as CW) decreased substantially (Tab. 3). This outcome is certainly due to both the dominance of juveniles in the samples and a strong reduction in number of large adults. In fact, for samples with comparable sizes, before reproduction, large adults (over 10 mm CW) constituted the 75-100% of the sample; whereas, after reproduction, large adults were only the 12-35%. A possible explanation, also suitable for the seasonal variation of sex *ratio*, is an increase in the mortality rate of large adults after reproduction. However, being the life cycle typically iteroparous (Turoboyski, 1973), it is more likely that the observed variations of sex *ratio* are the result of a behavioural post-reproductive response. A possible explanation could take into account the displacement of large adults towards the bottom of the canal, letting to juveniles the upper part of the habitat characterised by *F. enigmaticus* concretions. Males were slightly larger than females and the maximum size was higher in males (18.55 mm versus 14.50 mm CW), consistently with Turoboyski's (1973) and Roche and Torchin's (2007) findings, although there was often no significant difference between sexes and in one case females were significantly larger. *R. harrisii* appears to be fully established in the study area, at least in most of the investigated stations; whereas it is sporadic in S2 and absent in S1, where *Xenostrobus securis* is relatively abundant. *R. harrisii* can be considered omnivorous, feeding mainly on detritus (Czerniejewsky and Rybczyk, 2008), but it can also prey on sessile or slow moving invertebrates (Czerniejewsky and Rybczyk, 2008; Hegele-Driwa and Normant, 2009). In the present work, individuals of *R. harrisii* have been observed feeding on native nereidid polychaetes such as *Hediste*

diversicolor and *Alitta succinea*, as well as on dead shrimps and fishes. Because of its high tolerance to wide variations of salinity, *R. harrisii* will be probably able to invade also S1 and the main flow of the Arno River, as already observed for *X. securis* (Barbieri *et al.*, 2011), which has a similar tolerance and adaptability to low salinity values (Boyle *et al.*, 2010; Adarraga and Martínez, 2012). Currently, twenty species of alien macrobenthic invertebrates have been reported from the study area. Nine of them are species typical of brackish-water environments, of which seven are established with breeding populations; whereas eleven are strictly marine, of which eight are established. *Ficopomatus enigmaticus*, *Hydroides elegans*, *H. dianthus* and *Styela plicata* are by now historical introductions, dating back to the second half of the XIX century or the beginning of the XX century (Harant and Vernieres, 1933; Bianchi, 1981). Despite the current agreement about their status of alien in the Mediterranean Sea, their arrival in this basin cannot be precisely traced, even with modern molecular methods (Lupi, 2012). Since the massive use of ballast waters was not common at the time, these species have been probably introduced as fouling components. *Venerupis philippinarum* and *Procambarus clarkii* have been introduced in the second half of the XX century for aquaculture purposes, although for these species introduction dates and pathways are largely unknown (Barbaresi and Gherardi, 2000; Breber, 2002). Secondary introduction mechanisms are probably related to farm escapees. Although *P. clarkii* is generally considered a freshwater invader, this species is capable to live, moult and mate with salinity up to 25 psu (Casellato and Masiero, 2011). Due to these bio-ecological characteristics, some authors argued that it could also represent a threat for brackish-water ecosystems (Scalici *et al.*, 2010; Casellato and Masiero, 2011) and

its presence in some of our samples supports this hypothesis. *Rapana venosa*, *Bursatella leachii*, *Panulirus regius*, *Portunus segnis* and *Callinectes sapidus* have not been reported after the first record (see references in Tab.1); hence, they shall be considered as not established in the study area. In this work we considered *P. regius* as alien species despite the striking hypothesis of the human-unrelated arrival of early life stages through Gibraltar (Frogliola *et al.*, 2012) because its introduction via ballast water is anyway more likely. Ballast waters are probably also responsible for the introduction of the remaining species, that result established in the study area (Crocetta, 2012).

Conclusions

Large ports offer important chances to the introduction of alien species. The proximity of brackish-water environments, characterised by low resistance to biological invasions, favours the establishment of the invaders (Paavola *et al.*, 2005). Successively to the establishment of breeding populations, brackish-water environments may function as source of recruits towards proximate favourable habitats, which can be also invaded. The system constituted by Livorno port and the canalisation of the Arno River is a typical example of such a situation. The large majority of the alien species recorded in the brackish-water environment in the proximity of Livorno port appears fully established. The recent building of a small harbour on the mouth of the Arno River will probably enhance the possibility of invasive brackish-water species spreading in the north Tuscany coast.

The dwarf mud crab *Rhithropanopeus harrisi*, recorded for the first time in coastal biotopes of the Ligurian Sea, appears to be completely established in the canals of the low basin of the Arno River. Given the species' bio-ecological features, we expect its invasion

in other brackish-water environments of the Ligurian Sea. Therefore we recommend a closer monitoring of such biotopes, in order to trace and possibly prevent the spread of *R. harrisi* and the consequent alterations of native biological communities it may produce.

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