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The increasing distribution of *Gammarus roeselii* Gervais, 1835: first record of the non-indigenous freshwater amphipod in the sub-lacustrine Ticino River basin (Lombardy, Italy)

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Abstract

This paper reports the first record of the non-indigenous species *Gammarus roeselii* Gervais, 1835 in the sub-lacustrine Ticino River basin (Po river floodplain, Northern Italy). Up to now in Italy, this Balkanian amphipod, known as an “exotic species, well established” in Central Europe, had only been reported in the North-Eastern part of the country (Sile River basin). *Gammarus roeselii* has a low spreading potential, so its presence in other parts of Europe can only be explained by human activities. However, the available data suggests that its presence in the sub-lacustrine Ticino River basin does not currently represent a threat to the population of the native *Echinogammarus stammeri,* which is still very healthy in this area.

Key words: crustaceans, non-indigenous species, introduction, Northern Italy, Ticino River basin

Introduction

Gammarids are one of the most successful amphipod aquatic invaders in freshwater ecosystems. Their success is due to their high tolerance of environmental conditions, high reproductive rates and capacity to exploit spatial and food resources (Hesselschwerdt et al. 2008).

Nevertheless, high habitat variability may favour the coexistence between indigenous and non-indigenous species. Invasions by closely related species can lead to competition between them (Dick et al. 1993; Dick and Platvoet 2000; Van der Velde et al. 2000; Jażdżewski et al. 2004); furthermore, sharing the same resources, such as food or space, and substantial ecological overlap make such competition even more likely (Reynoldson and Bellamy 1970; Schoener 1983; Chase and Leibold 2003).

In Italy, there are currently 14 known species belonging to the genus *Echinogammarus,* and 10 belonging to the genus *Gammarus* (Ruffo and Stoch 2005). Most of these species share the same habitat and compete for the same resources. One of these gammaridean species, *Gammarus roeselii* Gervais, 1835, which originated in the Balkan area, is now considered as an “exotic species, well established” in Central Europe (Josens et al. 2005). In Italy, its distribution was restricted to the Sile River basin in the North-Eastern part of the country (Ruffo and Stoch 2005).

This paper reports the presence of the invader *G. roeselii* in another river basin in Northern Italy, thus demonstrating that this species is widening its distribution area.

Site description

The sub-lacustrine Ticino River floodplain (South-East Lombardy, Northern Italy), the largest tributary of the left bank of the Po River, is characterised by high anthropogenic pressure resulting from agriculture and industry (Garzoli et al. 2014). Nevertheless, it contains numerous

Figure 1. Actual distribution of *G. roeselii* in Northern Italy. Blue dots represent records in the Sile River basin (Ruffo and Stoch 2005); red rhombus indicates the new record in the Ticino River basin (Gaviola stream: N 45°11′27.4″; E 09°04′03.7″).

semi-natural areas such as ponds, oxbow lakes and small streams, which are often used as fish repopulation areas.

The Gaviola stream, one of the small right-bank tributaries of the Ticino River, is characterised by moderate running water and stony substrate, and is surrounded by agricultural fields (Figure 1).

During the biological survey carried out in June 2014, the water temperature was 21.6°C, with an oxygen saturation of 79% (dissolved oxygen=6.8 mg/L) and a pH of 7.2.

This small river also has a relatively good cover of macrophytes such as *Myriophyllum spicatum*, *Potamogeton pectinatus* and *P. nodosus*, *Elodea nuttalii*, *Veronica anagallis aquatica*, *Sparganium erectus* and *Callitriche* spp.

Furthermore, as is the case with all freshwater courses in the sub-lacustrine Ticino River basin, at least another two non-indigenous species are present: the crayfish *Procambarus clarkii* (Girard, 1852) (Garzoli et al. 2014) and the mollusc bivalve *Corbicula fluminea* (Müller, 1774) (Nicolini and Lodola 2011).

Materials and methods

Along a transect of 10 m, we collected three random sub-samples using a hand net (950µ mesh) with a square frame (22×23 cm, which corresponds to an area of 0.0506 m^2). Biological samples were first preserved in alcohol and subsequently counted and identified in the laboratory using a specific guide. For each species, in order to estimate the density (ind.m^{-2}), the total number of collected specimens (N) was divided by the total sampled area (0.152 m^2).

We determined sex and length of each gammarid specimen, from rostrum to telson, as suggested by Graça et al. (1994) and Van Overdijk et al. (2003). Specimens shorter than 4 mm were considered as juveniles. Furthermore, in order to describe the gammarid populations, we classified all specimens into nine size classes according to their length: I) 0–2 mm, II) 2.1–4 mm, III) 4.1–6 mm, IV) 6.1–8 mm, V) 8.1–10 mm, VI) 10.1–12 mm, VII) 12.1–14 mm, VIII) 14.1–16 mm, IX) 16.1–18 mm. As data was not normally distributed (Anderson-Darling test $p<0.05$), differences between species' lengths were tested with a Kruskall-Wallis test. Statistical analyses were all performed using the MINITAB 16 software package.

Results

In total, the estimated macrobenthos community density was 1553 ind.m^{-2}. The gammaridean specimens comprised almost 60% of the community and the rest of the assemblage was composed of Chironomidae (12.3%), *Baetis rhodani* (Pictet, 1843) (11%) and *Hydropsyche modesta* Navás 1925 (8%). We identified 108 specimens (which correspond to an estimated density of 711 ind.m^{-2}) of the indigenous species *Echinogammarus stammeri* (Karaman, 1931) and 33 specimens (which correspond to an estimate density of 217 ind.m^{-2}) of the non-indigenous species *Gammarus roeselii*, with a ratio of 3:1.

Gammarus roeselii Gervais, 1835 was easily distinguished from *E. stammeri* due to the presence of a strong mid-dorsal process on each of the three metasome segments; the number of processes varies from three (on the three metasome segments) to four, with an extra process on the last mesosome segment. Furthermore, the most characteristic features are found in the setation of the antennae and the pereiopods and 3rd uropod; both the number and the length of setae (and spines) increase with age and only become fully characteristic when the animal is sexually mature. Urosome segments 1 to 3 are always more or less elevated and laterally compressed (Karaman and Pinkster 1977) (Figure 2).

The mean length of *G. roeselii* collected in the Gaviola stream was 10.64 ± 4.47 mm. The sex

Figure 2. Specimen of *Gammarus roeselii* collected in the Gaviola stream. Photograph by Daniele Paganelli.

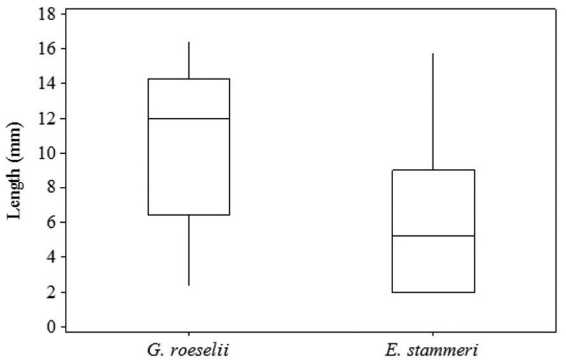

Figure 3. Box-plot of the length measurement, from rostrum to telson (mm), of *Gammarus roeselii* (N=33) and *Echinogammarus stammeri* (N=108) collected in the Gaviola stream.

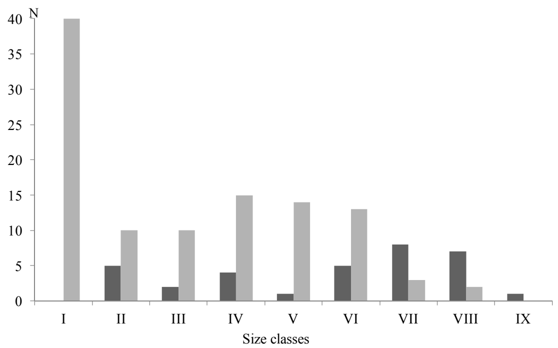

Figure 4. Body length distribution of *Gammarus roeselii* (dark grey, N=33) and *Echinogammarus stammeri* (light grey, N=108) in the Gaviola stream. Size classes: I) 0-2 mm, II) 2.1-4 mm, III) 4.1-6 mm, IV) 6.1-8 mm, V) 8.1-10 mm, VI) 10.1-12 mm, VII) 12.1-14 mm, VIII) 14.1-16 mm, IX) 16.1-18 mm.

ratio found for this species is almost 1:2 (10 males and 18 females). The body length of female specimens varied from 5.6 to 13.6 mm, and male specimens from 12.4 to 16.4 mm; furthermore, we collected three mature brooding females and five juvenile specimens (Figure 3).

In comparison, *E. stammeri* specimens were significantly shorter than *G. roeselii* (K-Wallis test: H=28.41; DF=1; p=0.000) with a mean length of 5.80 ± 3.83 mm and a sex ratio of 1:4 in favour of females (11 males and 47 females, two of them with eggs); the rest of the population was composed of 50 juvenile specimens. The length of *E. stammeri* female specimens varied from 4.2 to 11.2 mm, while the length of male specimens varied from 9.8 to 15.8 mm (Figure 3).

Using the body length distribution, we observed a greater number of *E. stammeri* specimens in the first (0–2 mm) to the sixth size-classes (10–12 mm), whereas in the seventh to the ninth size-classes (>12.1mm) there was a greater number of *G. roeselii* specimens (Figure 4).

Discussion and Conclusion

Gammarus roeselii is a species of Balkan origin, but it is usually defined as an old alien coloniser, well established but not invasive (Karaman and Pinkster 1977; Jażdżewski and Roux 1988).

Despite the fact that *G. roeselii* has a low spread potential (Grabowski et al. 2007), it is expanding its distribution area in Italy; its presence in the sub-lacustrine Ticino area represents a new record for this species. Up to now, its distribution in Italy was limited to the Sile basin, in the North-Eastern part of the country where it was accidentally introduced (Ruffo and Stoch 2005). However, it is difficult to identify the vectors of *G. roeselii* introduction to this area. The Ticino river basin and the Sile basin are not directly connected, thus the spread of this freshwater amphipod could be due to human activities such as aquaculture, as reported for example for another amphipod invasive species, *Dikerogammarus villosus* (Sowinski, 1894) in Lake Garda (Casellato et al. 2006). Another possible introduction vector could be fish repopulation, or alternatively it may have been accidentally introduced as a productive food source for commercial fish as is the case in Eastern European artificial reservoirs (Grabowski et al. 2007). Furthermore, we cannot exclude overland transport as another possible introduction vector for *G. roeselii*. Even though we did not find any data on the ability of *G. roeselii* to survive in dry conditions, it is known that gammarids can survive out of the water for a few days. For example, according to Martens and Grabow (2008), *D. villosus* can survive for up to 6 days out of the water. Indeed, Bacela-Spychalska et al. (2013) stated that *G. roeselii* has a greater ability to stay attached to different materials (e.g. ropes and neoprene diving suites) than *Gammarus pulex* (although less than *D. villous*) which could favour the colonisation of new environments.

Gammarus roeselii reaches a higher density in small rivers with moderate water currents and abundance of plants by using such biotopes as refuge (Meijerinmg 1991; Starry et al. 1998; Pöckl et al. 2003; Mayer et al. 2012). Moreover, it can survive in lower oxygen concentrations and higher temperatures than *E. stammeri*, which prefers fresh running water (Karaman 1993; Kley et al. 2009). In the Gaviola stream, where aquatic flora is abundant and water flow is low, the environmental conditions are suitable for the growth of *G. roeselii,* as confirmed by the presence of brooding females and juvenile specimens in our samples. This finding also suggests that the record of *G. roeselii* is not accidental, but that there is a well-established population.

In our study area, the density ratio between *E. stammeri* and *G. roeselii* is still in favour of the former indigenous species, even though the specimens of the latter species reach larger size classes. Its larger size could be considered an advantage for food competition and reproduction; furthermore, its efficient antipredator behaviours and the presence of the robust spines on its metasomes may offer *G. roeselii* an advantage during the colonisation process of new habitats, as suggested by Bollache et al. (2006) and Lagrue et al. (2011).

However, these two amphipod species can survive in sympatry: they live in the same area, use similar resources and, so far, the presence of the Balkan species is not a threat to the indigenous species in the Ticino River area.

Indeed, our results suggest that the population of *E. stammeri* is still very healthy: it has a high density, numerous juvenile specimens and brooding females.

In the future, it would be useful to check the presence of the non-indigenous species *G. roeselii* in other small rivers in the area around the Ticino river basin and define its distribution in the main course of the Ticino River. In addition, further research should be undertaken on the competition between the native and alien species, their food preferences, and population genetics of *G. roeselii* in order to infer important aspects of the invasion process of this species.

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