First evidence for an established Marmorkrebs (Decapoda, Astacida, Cambaridae) population in Southwestern Germany, in syntopic occurrence with *Orconectes limosus* (Rafinesque, 1817)

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Received: 7 September 2010 / Accepted: 22 October 2010 / Published online: 29 October 2010

Abstract

Marmorkrebs are one of 12 currently known non-indigenous crayfish species (NICS) to be found in Central European waters. It is unique in the manner that there exist only females which reproduce parthenogenetically, i.e. eggs develop unfertilized and all offspring are genetically identical. Marmorkrebs have been first discovered in the German aquarium trade in the mid 1990s and became a very common pet species since then. Here, we present first evidence for a well established Marmorkrebs population in a small lake in the Upper Rhine catchment near Freiburg (Germany). The population occurs syntopically with *Orconectes limosus*, another NICS which invaded the Rhine system about 50 years ago. Morphometric and ovary weight measurements were taken from 12 Marmorkrebs specimens that were captured on July 3, 2010. The rostrum spination was pronounced and resembled the one found on a free-living individual captured in Saxony (Germany). Ovary development stages (Gonadosomatic Index) were heterogeneous and single berried females were found from early June to late July, which might indicate an asynchronous breeding habit. The relative abundance and distribution of both crayfish species were assessed by visual counts at nighttime at two occasions. Both species attained a comparable, moderate density throughout the lake margin. The Marmorkrebs was the prevalent species on shallow, swampy habitat patches, which are presumably similar to its natural prime habitats. The successful establishment of Marmorkrebs despite a pre-existing *O. limosus* population, stresses the competitive ability of Marmorkrebs. In addition to the recently suggested hypothesis that Marmorkrebs might be temperature limited in most parts of Europe, we feel that it is also necessary to consider its probable natural prime habitats and life cycle: Marmorkrebs are presumably able to colonize summer-warm, lentic habitats in most parts of Central Europe.

Key words: Marmorkrebs, marbled crayfish, non-indigenous species, invasion success, parthenogenesis, *Procambarus fallax*

Introduction

Crayfish (Astacida) are the largest mobile freshwater invertebrates and are considered as key members of littoral communities. They attain frequently a high biomass and interact with multiple trophic levels due to their omnivorous feeding habits and non-trophic activities, e.g. burrowing and bioturbation (Momot 1995; Nyström 2002). The three indigenous crayfish species (ICS) of Central Europe are largely outnumbered by 12 introduced, non-indigenous crayfish species (NICS), of which at least eight are established (Holdich et al. 2009). All ICS species are endangered throughout their Central European range and the presence of NICS is among the biggest threats to the remaining stocks (Souty-Grosset et al. 2006; Holdich et al. 2009). NICS may also have strong detrimental effects on the integrity of the receiving community, including a reduction in α-diversity and species abundance (Nyström 2002; Rodriguez et al. 2005).

The Marmorkrebs (`marbled crayfish´) is a medium sized crayfish species of unknown origin and enigmatic phylogenetic position. It was first discovered in the German pet trade in the mid 1990s and became a very popular pet species since then. It is unique in the manner that it is the only known decapod crustacean that reproduces by parthenogenesis (Scholtz et al. 2003; Martin et al. 2007; Vogt 2008): there exist only females, which lay unfertilized eggs and all offspring are genetically identical. The phylogenetic position and status of the Marmorkrebs
was long unresolved, although it has been shown to belong to the American genus *Procambarus* Ortmann, 1905 (Scholtz et al. 2003; Braband et al. 2006). Most recently, Martin et al. (2010a) suggested that the Marmorkrebs is the parthenogenetic form of *Procambarus fallax* (Hagen, 1870) and proposed the tentative scientific name *Procambarus fallax f. virginalis*. *Procambarus fallax* occurs in southern Georgia and Florida and it is therefore reasonable to assume that the Marmorkrebs also originate from the southeastern United States, although an indigenous population has never been reported.

Marmorkrebs have been introduced into natural ecosystems in Europe (Germany, The Netherlands and Italy; Holdich and Pöckl 2007; Marzano et al. 2009; Martin et al. 2010b) and Africa (Madagascar; Jones et al. 2008) and there is concern that its wide spread in the North American pet trade will inevitably result in releases from captivity there (Faulkes 2010). Parthenogenesis permits a high reproductive potential and there have been persistent concerns that the Marmorkrebs will become an invasive pest species (Vogt et al. 2004; Jones et al. 2008). Since the females do not need to mate in order to reproduce, one single specimen is sufficient to create a new population. Jones et al. (2008) went so far as to label the Marmorkrebs as “the perfect invader”, which is probably no understatement with regard to the situation in Madagascar. However, most records of free-living Marmorkrebs from Central Europe are based on the collection of single specimens, which led Martin et al. (2010b) to suggest that “in spite of all previous expectations, [the Marmorkrebs] does not have the potential for wide expansion within Europe”.

Here, we present the first evidence for an established Marmorkrebs population in a small lake in the Upper Rhine catchment near Freiburg (Germany). The Marmorkrebs population was found to occur syntopically with *Orconectes limosus* (Rafinesque, 1817), another NICS which invaded the Rhine system about 50 years ago. We assessed the relative abundance and distribution of both crayfish species along the shore line and examined the morphology and reproductive traits of Marmorkrebs in order to gain preliminary insights into the ecology of wild Marmorkrebs populations in temperate zones. We also discuss our findings with regard to the recent suggestion that Marmorkrebs might be temperature limited within most parts of Europe (Martin et al. 2010b).

Materials and methods

Study site and sampling

The presence of the Marmorkrebs in Lake Moosweiher (48°01’53.13"N, 7°48’18.10"E) was first confirmed by one of us (M.P.) on July 6, 2009, when an adult female of 95 mm total length was captured by snorkeling. The initial clue to the presence of Marmorkrebs in Lake Moosweiher was provided by a local pet shop owner, who told M.P. that he had repeatedly heard of Marmorkrebs in the lake. Lake Moosweiher is a small (7.6 ha; 8 m max. depth), mesotrophic lake, situated near Freiburg and is a popular swimming lake. Surface water temperature ranged between 25.7°C on July 5, 2010 to 26.2°C on July 20, 2010. The lake is fed from ground-water and features lower temperatures near the ground-water influx (22.7°C on July 20, 2010). Conductivity was low and ranged between 337 and 299 µS cm⁻¹.

In order to assess the relative crayfish abundance and distribution, the lake was visited during night time at two occasions (July 5 and July 20, 2010). At each sampling occasion, twelve sample plots, distributed along the northeastern lake margin (Figure 1), were searched for crayfish by hand lamps for one hour, beginning at 22.00 h. Plots were chosen for easy accessibility and moderate depth - deep water was avoided because it was difficult to spot crayfish in deeper areas. Plot area was estimated and varied between plots but not between sample occasions and ranged between 5 and 50 m². Each observed crayfish was identified by distinct morphological and color peculiarities (e.g. marbled colour pattern, chelae shape, spination) and noted.

On July 3, 2010, twelve Marmorkrebs were captured by hand nets and transported to the laboratory to take morphological and weight measurements. Carapace length and width as well as chelae length (*prodopus*) were measured to the nearest 0.1 mm with an electronic slide caliper. The captured crayfish were then frozen to −18°C and later dissected to calculate the gonadosomatic index (GSI = gonad weight/ fresh weight × 100). Only females with intact chelae were used for GSI calculation to avoid bias from the lower fresh weight of specimen with regenerated or missing chelae. Prior to weight measurement, specimens were put on blotting paper and subsequently gently shaken ten times to remove adherent water drops. Dissected
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gonads were also gently shaken ten times prior to weighting (Kern balance, type 822-67).

Statistical analyses

All statistical analyses were performed with SigmaPlot 10.0 (with SigmaStat 3.5 Integration). All data were tested for normality prior to analysis. Relationships between chelae length, fresh weight, carapace width and carapace length were assessed by Pearson Correlations. Weight and carapace length data were used to describe the relationship between carapace length and fresh weight by means of the power equation $y = a \times x^b$, with $x$ = carapace length. Parameters $a$ and $b$ were estimated by employing a linear regression after log transformation of the data.

Results

Distribution and relative abundance

In total, 56 Marmorkrebs and 48 Orconectes limosus were recorded from Lake Moosweiher. Both species occurred syntopically at nine sample plots, whereas two plots had only Marmorkrebs (Figure 1). The latter two plots (one and six) were adjacent to each other and featured a shallow, soft-bottomed backwater area, with an extensive detritus layer and dead wood. At one plot (eleven) we found no crayfish. Total crayfish density was estimated at 0.07 to 1.50 individuals m$^{-2}$ and was highest on plots seven (1.50) and five (1.33). On both plots, Marmorkrebs was more abundant than O. limosus (0.83 vs. 0.67 and 1.00 vs. 0.33 individuals m$^{-2}$, respectively). Mean total crayfish abundance was estimated at 0.27 individuals m$^{-2}$, with both species being equally abundant.

Morphology and reproductive traits

Chelae length, carapace width and fresh weight correlated significantly positive with carapace length (Pearson Correlation: correlation coefficient $= 0.92$, $p < 0.001$, correlation coefficient $= 0.98$, $p < 0.001$; correlation coefficient $= 0.96$, $p < 0.001$, respectively; Figure 2). The linear regression coefficients are summarized in Table 1. The rostrum spination of all twelve examined Marmorkrebs resembled the one found on a free-living individual captured in Saxony, i.e. the tip of the acumen, the lateral spines on the rostrum and the anterior angle of the postorbital ridges were pronounced and acute (Martin et al. 2010b; Figure 3).

The GSI values of the examined females ranged between 0.24 and 4.9 (mean $= 0.9 +/- 1.4$ SD, $N = 11$) and did not correlate with carapace length (Pearson Correlation: $p > 0.05$; Figure 4). Egg bearing females were captured on June 10, July 9 and July 21, 2010 (Figure 3). Fecundity was determined from three females and was seven, 160 and 724 eggs.

Discussion

The considerable number of specimens observed, the lake-wide distribution and the presence of different size classes, as well as reproducing individuals, strongly suggest that Marmorkrebs has established a stable population in Lake Moosweiher. This conclusion is further supported by a Marmorkrebs record made in summer 2009 and reported observations that date back even further. The presented findings are the first evidence for an established, free-living
Marmorkrebs population in Germany and possibly even Europe. There exist some uncertainties about the status of Marmorkrebs populations in the Netherlands, where the species was reported from the wild in Dordrecht in 2004 (Soes and van Eekelen 2006) and was still present there in 2008 (according to www.marmorkrebs.org). With the possible Dutch exception, all previously published records of Marmorkrebs in Europe were based on single specimens (summarized in Martin et al. 2010b). Martin et al. (2010b) attributed the apparent lack of records of established wild Marmorkrebs populations from Europe to the fact that Marmorkrebs might fail to establish sustaining populations. The species is very common in the pet trade and the records of single Marmorkrebs specimens across Europe stress the inability of the pet trade to keep animals captive (unpubl. data; Duggan 2010). The first stage of the invasion process, i.e. the introduction into the wild is certainly accomplished; however, subsequent establishment success seems to be poor.

In order to understand and predict the invasive success of an introduced species, the authors feel that it is important to consider its natural life history and habitat preferences. Since there are no known natural Marmorkrebs populations it is reasonable to consider the life history and habitat requirements of its closest relative Procambarus fallax (Scholtz et al. 2003; Vogt 2008; Martin et al. 2010a): Although P. fallax may occur in streams and rivers, it seems to prefer lentic or slow flowing habitats and is typically found in marshes, wet prairies and sloughs (Hendrix and Loftus 2000). This preference is also reflected by the common name of P. fallax: slough crayfish (Dorn et al. 2005). Procambarus fallax inhabits also temporary wetlands, which feature seasonal dry-downs during which crayfish retreat into refugia or simple burrows (Hendrix and Loftus 2000; Martin et al. 2010a). Although crayfish might reproduce within the refugia or burrows, growth is most likely inhibited or slowed down. In this respect, they are adapted to a boom and bust scenario, i.e. growth and recruitment during inundation and reduced activity during dry periods. Granted that Marmorkrebs has a similar natural life history, this boom and bust strategy might facilitate its success at moderate European temperatures: while growth and recruitment is most likely inhibited during winter months, Marmorkrebs might be able to thrive during the

Table 1. Linear regression statistics. Regression equations are \( y = a \times x + b \) for carapace width and chelae length and \( y = 10^b \times x^a \) for fresh weight, respectively. \( x = \) carapace length [mm].

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<tr>
<td>carapace width [mm]</td>
<td>0.510</td>
<td>-2.034</td>
<td>0.98</td>
<td>&lt; 0.001</td>
<td>11</td>
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<tr>
<td>chelae length [mm]</td>
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<td>-10.742</td>
<td>0.98</td>
<td>&lt; 0.001</td>
<td>11</td>
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<tr>
<td>fresh weight [g]</td>
<td>3.211</td>
<td>-3.978</td>
<td>0.99</td>
<td>&lt; 0.001</td>
<td>12</td>
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Figure 3. Left: Rostrum spination of Marmorkrebs from Lake Moosweiher. Arrows indicate acute spines that are usually less pronounced or absent in laboratory specimens (Martin et al. 2010b; pers. obs.). Right: Egg bearing Marmorkrebs, captured from Lake Moosweiher on July 9, 2010. The female carried 724 eggs. Photographs by CC (left) and MP (right).

Figure 4. Gonadosomatic Index (GSI) of Marmorkrebs captured from Lake Moosweiher on July 3, 2010.

warm summer months. It was shown that Marmorkrebs can survive ice cover (Pfeiffer 2005) and reproduces at temperatures above 15°C (Seitz et al. 2005). The inactivity forced by low temperatures during winter might not be that different from its natural life history as one would expect, because Marmorkrebs might also exhibit periods of inactivity within its natural habitat. Similarly, the red swamp crayfish, *Procambarus clarkii* (Girard, 1852), was often considered as ‘warm-water’ species (Henttonen and Huner 1999), but proved recently to be able to thrive also at cooler climates at higher latitudes and altitudes (Frutiger et al. 1999; Mueller 2007; Ellis and England 2008; Dümpelmann et al. 2009; unpubl. data). The red swamp crayfish has a comparable natural prime habitat to *P. fallax* and shows considerable life cycle plasticity, including reduced activity during winter in Central Europe (Frutiger et al. 1999; unpubl. data). The case of *P. clarkii*
illustrates well that *Procambarus* species can cope with seasonally low temperatures and may exceed expectations of their invasive potential that are based on assumed temperature constraints (cf. Bohl et al. 1989). Although this is speculative with regard to Marmorkrebs, it gives rise to the idea that temperature constraints are not the single factor that determines the invasive success (cf. Martin et al. 2010b).

Based on the habitat preferences of its closest relatives, it is reasonable to expect that introduced Marmorkrebs will be especially successful in lentic habitats, such as ponds, shallow lakes, backwaters and wetlands. This assumption is supported by reports from Madagascar, where Marmorkrebs thrives in brick pits, drainage ditches, rice fields and fish ponds (Jones et al. 2008). Martin et al. (2010b) on the other hand, found a single specimen in a lowland brook in Saxony. They were unsuccessful in detecting further specimens, which indicates that the Marmorkrebs was not able to propagate. They concluded that the temperatures were probably too low. However, the failure of the Saxony specimen to establish a population might also be related to the unsuitable habitat. Marmorkrebs need temperatures between 20 and 25°C for optimal development and reproduction (Seitz et al. 2005) and Martin et al. (2010b) argue that these temperatures are significantly higher than that found in most European waters. While this holds certainly true for many rivers and streams, small or shallow lentic habitats may offer more favorable temperatures for the Marmorkrebs, because those habitats heat up faster and reach higher summer temperatures than most lotic habitats. Interestingly, shallow lentic habitats are presumably also close to the natural prime habitat of the Marmorkrebs as pointed out above. In the present study, surface water temperatures clearly exceeded 20°C, thus permitting optimal temperature conditions for growth and recruitment. We found both freshly moulted and breeding individuals. Lake Moosweiher features also shallow, swampy areas (plots one and six), at which Marmorkrebs occurred exclusively.

Given that the habitat type has probably an influence on the invasion success, it is not surprising that there exists a lack of records of established wild Marmorkrebs populations from Europe: Small, lentic systems are often not monitored by routine sampling (e.g. within the scope of the EU Habitats Directive and Water Framework Directive). In fact, all recent Marmorkrebs records from Europe were made coincidentally (Marzano et al. 2009; Martin et al. 2010b). This is also well illustrated by the present study, where the initial clue to the presence of Marmorkrebs was obtained by mere chance. The estimated number of unreported Marmorkrebs populations is therefore presumably high, because Marmorkrebs may establish stable populations especially in under-sampled habitat types. The currently published records represent most likely only the ‘tip of the iceberg’.

We also obtained preliminary morphological and reproductive data. The length-weight relationship of the individuals examined shows that growth is not isometric, which coincides with results of laboratory studies on Marmorkrebs (Seitz et al. 2005). The length-weight regression coefficients are similar to values reported for female *Procambarus clarkii* (Huner and Barr 1991; unpubl. data). Interestingly, the rostrum spination of Marmorkrebs from Lake Moosweiher resembles that of one found on a free-living individual captured in Saxony (Figure 3), which might support the tentative interpretation that the spines are a sign of predator-induced plasticity (Martin et al. 2010b). However, this needs further investigation. Ovary development stages (i.e. GSI values) were heterogeneous and single berried females were found from early June to late July (cf. Figure 4), which might indicate an asynchronous breeding habit. In Madagascar, approximately 12 % of Marmorkrebs sampled were breeding, both in summer and winter (Jones et al. 2008). Both findings suggest that individuals breed independently of each other whenever conditions are favorable (e.g. at water temperatures above 15°C). However, it is not clear whether individuals are able to propagate more than once per year in Lake Moosweiher. Marmorkrebs reproduce approximately every three months at temperatures between 20 and 25°C (Seitz et al. 2005). These temperatures can be attained in Lake Moosweiher for three months and individuals breeding in early June might be able to reproduce again by late summer.

The presented findings demonstrate that Marmorkrebs are able to establish wild populations in Central Europe. Its invasion success is presumably affected by both temperature and habitat type: summer-warm, lentic habitats may be colonized readily. Based on a comparison of climate data of its probable native range and its established range (assessed
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in CLIMATCH v.1.0; Invasive Animals CRC, Bureau of Rural Sciences 2008), we expect that Marmorkrebs are also able to develop reproductively active populations on the Iberian and the Balkan Peninsula, in Italy, France and possibly in parts of England and Eastern Europe. The pre-existence of another crayfish species does not seem to mitigate the potential of Marmorkrebs to establish a stable population (cf. Jimenez and Faulkes 2010). The Marmorkrebs population in Lake Moosweiher poses a latent risk to a population of the highly endangered white-clawed crayfish (Austropotamobius pallipes, Lereboullet, 1858), which is situated within 3.6 km linear distance. Future research should therefore address the potential of Marmorkrebs to spread via smaller brooks or over land. Moreover, its population ecology and interactions with other crayfish species (here Orconectes limosus) are only poorly understood and need more attention (Jimenez and Faulkes 2010). Unlike other NICS, Marmorkrebs don’t need to maintain a minimum viable population size, which makes eradication difficult, or even impossible. We stress that it is therefore urgent to minimize the risk of further Marmorkrebs introductions.

Acknowledgements

We are grateful to Z. Faulkes, C. Lukhaup, G. Mayer and the local fishing association (ASV Freiburg, H.P. Beil and G. Nuss) for valuable information and support. The helpful comments of three referees are gratefully acknowledged.

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