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Rapid Communication

The first Marmorkrebs (Decapoda: Astacida: Cambaridae) in Scandinavia

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Abstract

Invasive crayfish have attracted much attention by scientists and policy makers in Europe, partly due to their ability to transmit diseases to native crayfish species. In December 2012, 13 specimens of a new crayfish species were found in the River Märstaån in central Sweden. Mitochondrial DNA analyses identified them as Marmorkrebs *Procambarus fallax* f. *virginalis*. It is not known if Marmorkrebs can establish reproducing populations in Sweden, and knowledge of how different stressors negatively affect the eventual reproduction of Marmorkrebs is lacking. Since the parthenogenetic Marmorkrebs has potential to become an invasive species in Sweden and Scandinavia, it may pose a serious threat to native crayfish, fish and fisheries. Swedish authorities have produced an action plan with a national strategy in order to establish a system that manages the import, movement and release of alien species and genotypes. The River Märstaån leads to Lake Mälaren, without barriers, which enhances the risk that the crayfish will also invade the lake. Due to the potential threat of further spread, it is imperative to make an action plan with a risk assessment targeted specifically towards the Marmorkrebs in River Märstaån.

Key words: *Procambarus fallax* f. *virginalis*; parthenogenetic; crayfish; disease; risk analysis; action plan

Introduction

Invasive alien species pose greater risks than previously thought for biodiversity, human health and economies. According to a report from the European Environment Agency (EEA 2012), it was calculated that these species can cause serious economic damage to agriculture, forestry and fisheries, estimated to cost at least 12 billion Euros per year in Europe alone. This damage is particularly relevant for freshwater ecosystems due to their high vulnerability to biological invasions (e.g. Rahel 2002; Riciardi et al. 2004; Scalici et al. 2009). Invasive alien species, intentionally or unintentionally introduced by humans, have been regarded as one of the greatest global threats to biological diversity (McNeely et al. 2001). In Europe, the main threat to native crayfish species has been introduced alien crayfish from North America (Holdich et al. 2009). This threat is increasing due to a facilitated trade with aquarium crayfish species, both in Europe (Chucholl 2013, Papavlasopoulou et al. 2013) and in the US (Faulkes 2010). In Sweden the North American signal crayfish (*Pacifastacus leniusculus* Dana, 1852) was legally introduced into natural waters starting in 1960, to substitute noble crayfish fisheries lost due to crayfish plague (Bohman et al. 2006). Since signal crayfish proved to be a chronic carrier of the crayfish plague agent (*Aphanomyces astaci* Schikora, 1903), strong restrictions were enforced in 1994, prohibiting new introductions. Today, illegal introductions of signal crayfish are regarded as the main threat to the native noble crayfish (*Astacus astacus* Linnaeus, 1758; Edsman and Schröder 2009; Bohman and Edsman 2011).

In November 2012, some specimens of an unknown alien cambarid crayfish, were caught in the River Märstaån in Sweden (Figure 1). The specimens were initially identified as Marmorkrebs by visual inspection. However, doubts concerning

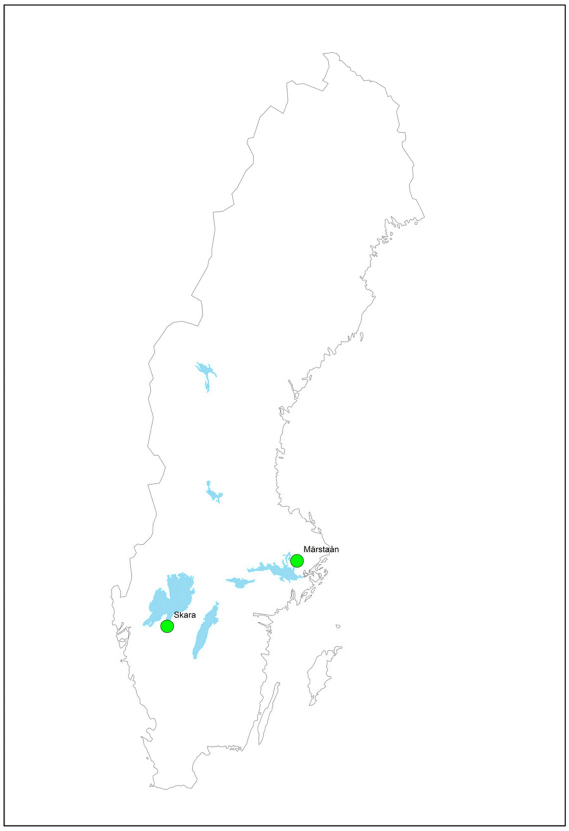

Figure 1. Map of site in Sweden. Site "Märstaån" shows the site of this study, and site "Skara" indicates the possible finding of Marmorkrebs in southern Sweden.

the correctness of this identification relate to the close resemblance of Marmorkrebs to the North American species, *Procambarus fallax* (Hagen, 1870) and *P. alleni* (Faxon, 1884), all common in the aquarium trade in Europe (Martin et al. 2010b). However, Swedish law clearly states that import, transport and possession of all crayfish from outside Sweden is strictly forbidden (Edsman 2004). There appears to exist a "black market" for these species, mainly via the internet, hampering control by government authorities (Chucholl 2013; Faulkes 2013; Papavlasopoulou et al. 2013). It has been confirmed by traders in the aquarium market that Marmorkrebs, for example, has been sold on the Swedish black market for several years (under different names).

When an action plan for an invasive alien species is being made, it must preclude the species becoming established. Combating already established invasive species is often very difficult and costly in aquatic environments, and eradication can be almost impossible. According to the Swedish Environment Protection Agency, costs for preventative measures for a species that is expected to enter the country, vary in the range of 100,000–200,000 Euros per species and year, of which the risk and consequence analyses constitutes the largest cost. This should be compared to the costs in the order of 1.5–7 million Euros per species and year for a species that is already established (Naturvårdsverket 2011). The few cost estimates for combating established species that have been made, include a limited number of species and do not include costs such as the loss of biological diversity or cultural values. Two studies arrive at costs in the range between 1.1-9.6 billion Euros (Gren et al. 2007; Kettunen et al. 2008).

Swedish authorities have produced a national strategy and action plan including a system to manage the import, movement and release of alien species and genotypes (SEPA 2008). A three-stage hierarchal approach, proposed by the Convention on Biological Diversity, was adopted including:

1. *preventive measures* so that invasive alien species are not introduced;
2. *rapid detection and eradication*;
3. *control and containment* of established invasive alien species.

The aim of this study is to examine if Marmorkrebs has been found in Sweden, and to briefly discuss possible threats. This study will be the first step to a more comprehensive action plan, targeted specifically towards the Marmorkrebs in the River Märstaån.

Materials and methods

Study site and sampling

Between 15th November and 23rd December 2012, 13 specimens (12 live and one dead) of an unknown alien species of crayfish, was caught by a civilian in the River Märstaån, Sweden at the same site (Lat: 59.6232, Long: 17.8650). The specimens were "walking" on the substrate, and were apparently not affected by the cold water (0–2°C). Most of the crayfish were caught by landing net, but traps (Lini-traps) were also used. One crayfish was lying dead on the bottom in December and caught by a landing net. Several traps were left continuously fishing from December 2012 to March 2013, without results.

River Märstaån is a highly altered watercourse. It consists of different parts: a) meandering slow flowing areas (ca 0.4m/s), b) shallow sedimentation traps (dams) which naturally clean the regions stormwater discharge, c) underground tunnels which quickly transport the water downstream (>10m/s), or where the water is pumped to the visually restful “River Park”. The river has a constant flow, which makes the water temperature cold during winter (between 0 – 0.1°C).

Molecular analysis

For molecular genetic analysis we used partial sequences of the mitochondrial protein coding cytochrome oxidase subunit I gene (COI), also known as the “Folmer region”, the standard barcode region used for higher animal groups (Hebert et al. 2003), and the mitochondrial 12S ribosomal RNA gene, which was already applied for identifying the taxonomic position of Marmorkrebs (Martin et al. 2010a). Total DNA of eight of the 13 mentioned specimens caught in River Märstaån was extracted from alcohol-preserved muscle tissue of their walking legs by using a DNA extraction kit (DNeasy Blood and Tissue Kit, Qiagen). For amplifying the COI fragment we used the universal primer pair LCO1490/ HCO2198 designed by Folmer et al. (1994) following the slightly modified protocol described by the same authors. PCR was performed in a final volume of 25 µl with 10 to 100 ng of total DNA, 1× $(NH_4)2SO_4$ buffer, 3 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 µM of each forward and reverse primer, and 0.6 U Taq DNA polymerase. Amplification commenced at 94°C for 2 min followed by five cycles of 1 min at 96°C, 1.5 min at 45°C and 1.5 min at 72°C, afterwards succeeded by 35 cycles of 93°C for 1 min, 50°C for 1.5 min, 72°C for 1.5 min, and finished finally with a 5-min extension at 72°C. 12S rRNA fragment was amplified using the primers CF12FOR and CF12REV designed by (Braband et al. 2007) according to a standard PCR protocol with 40 cycles of 94°C for 30 s, 40°C for 30 s, and 72°C for 40 s, and with a final extension of 72°C for 5 minutes. All PCR products were purified by the MinElute® PCR Purification Kit (Qiagen) and sense and antisense strand of the fragments were sequenced by the sequencing service company LGC Genomics Berlin, Germany. The COI and the 12S datasets were aligned using the ClustalW Multiple alignment application (Thompson et al. 1994) integrated in the program BioEdit version 7.0.9.0. for Windows (Ibis Biosciences, USA; Hall 1999). Subsequently, the sequences were manually pruned to a uniform length by removing the remaining parts of the primer regions.

Disease control

The National Veterinary Institute in Sweden (SVA) analyzed nine sample specimens for crayfish plague, eight specimens for white-spot virus syndrome, and two specimens for bacteria.

Crayfish plague was examined using real-time PCR (Vrålstad et al. 2009), and consisted of a body sample per individual (skin/shell and internal organs), in addition to an extra gill sample from one individual. Cultivation on agar (PG-1) was performed on one specimen.

A virus study was performed using real-time PCR for White Spot Syndrome Virus (WSSV) causing White Spot Disease (WSD), eight crayfish were studied (one organ samples per specimen).

Bacterial examination was carried out by plating organ material from two specimens on Tyes agar and blood agar, two standard culture media.

Results and discussion

The molecular genetic analysis revealed that all eight examined specimen from River Märstaån were absolutely identical for both the 658 base pairs COI sequence (GenBank® accession number (acc. no. KF033123) and the 370 base pairs 12S sequence (acc. no. KF033122). They showed also a 100 percent identity to the Marmorkrebs from the laboratory culture of the Humboldt-Universität zu Berlin (acc. no. COI/12S HM358010/ HM358014) and to the Marmorkrebs found in Saxony, Germany (acc.no. COI/12S HM358011/ HM358015) respectively (Martin et al. 2010b). In addition, the COI sequences of the Swedish specimen corresponded completely with that of the Marmorkrebs detected in Baden-Württemberg, Germany (acc. no. JF438007) (Filipová et al. 2011). This result suggests that the cambarid crayfishes caught in River Märstaån are doubtless Marmorkrebs and thus, this finding in Sweden is the world’s northernmost discovery of Marmorkrebs in natural waters. It is also the first finding in the European “subcontinent” Scandinavia.

The caught specimens fell into two size classes, with total length of 35 mm (9 individuals) and 80mm (3 individuals) respectively. This may be due to one of two causes: 1) someone emptied

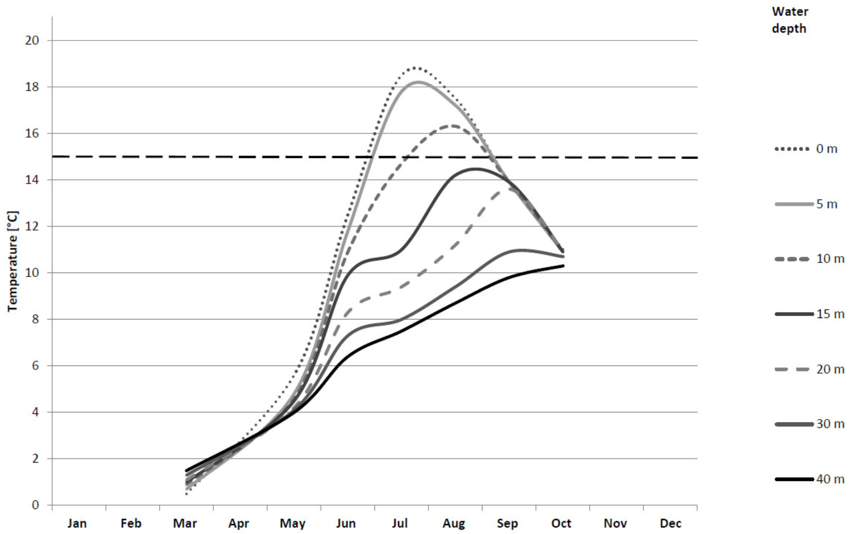

Figure 2. Main water temperature in different depths of lake Mälaren (Prästfjärden) recorded from 1981 to 1985 (ILEC 1999). Dashed horizontal line indicates the temperature of 15°C at which Marmorkrebs cease their reproduction (Seitz et al. 2005).

an entire aquarium of crayfish into the river (a common phenomenon according to Souty-Grosset et al 2006) or 2) the crayfish had been established for a longer time period and reproduced in the river. A third possibility (however unlikely) is that someone may have sent specimens from his own aquarium, signifying that the sample specimens were never in the River Märstaån at all. The Marmorkrebs (English: marbled crayfish) *Procambarus fallax* (Hagen, 1870) f. *virginalis* (Martin et al. 2010a) is the only decapod with obligate parthenogenesis known to date (Scholtz et al. 2003). This means, that a single individual can establish a whole population of female clones (Martin et al. 2007). The Marmorkrebs possesses pronounced r-selected characteristics; it matures early, is fast growing, has high fecundity and can reproduce several times a year (Jones et al. 2008; Seitz et al. 2005). In addition, it may act as a vector for crayfish plague and other diseases (Culas 2003; Vogt et al. 2004), Marmorkrebs thus has potential to become an invasive species in Sweden and Scandinavia, posing a serious threat to native crayfish, fish and fisheries.

No infection by *Aphanomyces astaci* or WSSV could be detected by preliminary analyses made by the National Veterinary Institute in Sweden (SVA). No bacteria of interest were found by SVA. However, disease cannot be ruled out in the population since so few individuals were tested. The crayfish may also carry other parasites and diseases apart from the ones so far examined (e.g. Vogt et al. 2004).

Several recent studies and reviews deal with the potential ecological threat in Europe from Marmorkrebs introductions. Chucholl et al. (2012) warned of an increase of established population in natural waters. The findings include both specimens in running waters, and established populations in more lentic areas. In Lake Moosweiher, where Marmorkrebs has been established for a few years, they coexist with other invasive crayfish, possibly enhancing their ability as vectors to transmit diseases to native species. Feria and Faulkes (2011) created several distribution models to forecast the potential dispersal of Marmorkrebs in Southern and Central Europe. Most models suggest that relatively small areas of Europe are suitable habitat for Marmorkrebs, although one model predicts that most of Southern and Central Europe could be suitable. Northern Europe (i.e. Scandinavia) was not included in the models by Feria and Faulkes. The crucial factor is whether water temperatures are within optimal limits for the Marmorkrebs to establish reproducing populations. It has been argued that colder temperatures in Northern Europe may decrease the spreading of Marmorkrebs (Martin et al. 2010b).

Due to the climate in Sweden, it is entirely possible that reproduction will not occur since waters here are cold, the winter is long and warm periods are short. Yet, an adult crayfish survives cold water by lowering its activity and slowing down its body functions. For example, the Marmorkrebs is known to have survived in water under ice cover during winter (Pfeiffer 2005). In River Märstaån's many shallow ponds (0.5–1.5m deep) heat up quickly during summer, and consequently reach optimal reproduction temperatures (20–25°C), even though the summer lasts for only a few months (June – August). Thus there may be potential for Marmorkrebs reproduction at this site. However, as Seitz et al. (2005) have shown, reproduction of Marmorkrebs stops below a water temperature of 15 °C, and 20 °C marks the lower border of optimal conditions for this crayfish species with respect to growth and mortality. As Figure 2 shows there is only a short time window for reproduction during the summer months in the area where the Marmorkrebs specimens have been found. Furthermore, most of the time temperatures do not support propagation of Marmorkrebs in Swedish waters. It is fair to state that the Swedish climate will have negative effects on the establishment potential of Marmorkrebs, but we do not yet know to what extent. Nevertheless, since invasive species sometimes behave unpredictably (IUCN 2000; Phillips et al 2008; Zanden and Olden 2008), conservation action is required.

Close to the site where the Marmorkrebs were found, the water flows down a tunnel, which quickly carries the water about 1km downstream. From this site there is no barrier before the river meets Lake Mälaren, which enhances the risk for the crayfish to invade the lake. Lake Mälaren is Sweden's third biggest lake and a drinking water reservoir for 2 million people. The lake is also important for recreation, fisheries and tourism. Due to its importance, an action plan for Lake Mälaren, which states that "the water should not contain organisms that could threaten human health or the environment", has been adopted (Naturvårdsverket 2011).

Due to the potential threat for an invasion of natural waters, our first objective is to assess whether the Marmorkrebs has established a reproducing population. This includes monitoring its distribution and density within the river system, which also includes making an action plan and performing risk assessment for the Marmorkrebs in River Märstaån.

While writing this manuscript, a possible Marmorkrebs (after inspection from photography) was found 30 June 2013 on a new site, a pond in southern Sweden (Lat 58.3900, Long 13.1823; figure 1/Skara). A more precise verification is in progress.

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