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INVASION NOTE

First records of the non-indigenous signal crayfish (*Pacifastacus leniusculus*) and its threat to noble crayfish (*Astacus astacus*) populations in Estonia

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Abstract This study gives an overview of status and distribution of signal crayfish (*Pacifastacus leniusculus*), the first NICS in Estonia and its influence on native noble crayfish (*Astacus astacus*) populations. The first specimen of signal crayfish was caught during the monitoring of noble crayfish in North Estonia in 2008. The signal crayfish has since been found in three additional sites. Test fishing has indicated that the abundance of signal crayfish has been fluctuating between years and among localities. It has had strong negative impact on abundance of one noble crayfish population. The disconnected distribution of signal crayfish strongly suggests that these populations are the result of human-assisted introductions. Real-time PCR analyses proved that signal crayfish carry the causative agent of the crayfish plague, an oomycete *Aphanomyces astaci*, thus contributing to its spread. Mortalities in noble crayfish populations had been caused by *A. astaci* strains from A, B and E genotype group.

Keywords Invasive crayfish · Native crayfish · *Aphanomyces astaci* · Disease outbreak · Genotyping

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Introduction

The introduction of non-indigenous crayfish species (NICS) is one of the major causes of extinction of indigenous crayfish species (ICS) in European freshwaters (Holdich et al. 2009). Progressively spreading NICS often exhibit devastating effects on ICS stocks as well as on entire ecosystems across European countries (Rodríguez et al. 2005).

The North American (NA) signal crayfish (*Pacifastacus leniusculus*) is the most widespread NICS in Europe, being first introduced to Sweden in 1959. By 2014, it occured in at least 29 European regions (Kouba et al. 2014). Crayfish species of NA origin are latent carriers of the crayfish plague (Alderman et al. 1990) caused by the oomycete *Aphanomyces astaci* (Unestam 1972). Crayfish plague is lethal in most cases to all crayfish species not originating from NA (OIE 2012).

The noble crayfish (*Astacus astacus*) is the only ICS in Estonia and occurs in more than 255 sites (lakes and river stretches), but most sites present low densities, except for some populations in South-Eastern Estonia and on the island of Saaremaa (Paaver and Hurt 2009). The main factors causing the decline of Estonian crayfish populations since early 1900s are crayfish plague, habitat deterioration, mink (*Mustela vison*) and eel (*Anguilla anguilla*) predation and fishing (Tuusti et al. 1993).

Until 2008 Estonia was one of the last countries in Europe where NICS were not recorded. To protect native noble crayfish, the Estonian Nature Conservation Act prohibits introduction of non-native species into the wild, bringing live specimens of the signal crayfish, narrow-clawed crayfish (*Astacus leptodactylus*) and spiny-cheek crayfish (*Orconectes limosus*) into Estonia, or conducting transactions with live specimens of these species.

In 2016, there were 23 noble crayfish farms having operating licences from the Veterinary and Food Board in Estonia. However, crayfish plague outbreaks, together with information about efficient and cheap exploitation of signal crayfish populations in Finland, increased the interest of crayfish farmers and owners of water bodies in introducing crayfish plague resistant crayfish species. Illegal introductions of crayfish plague-carrying NICS create potential dangers to native noble crayfish populations and crayfish farms, because they are already spread in water bodies of neighbouring countries such as Sweden, Finland and Latvia (Kouba et al. 2014).

The aim of this study was to get an overview of the status and distribution of signal crayfish and its involvement in a series of plague outbreaks taken place recently in noble crayfish populations in Estonia.

Detection and distribution of signal crayfish in Estonia

Signal crayfish have now been found in four sites in Estonia (Supplementary Table 1; Fig. 1). The first three signal crayfish populations were found during monitoring of noble crayfish carried out by the Department of Aquaculture of Estonian University of Life Sciences (EULS) by means of cylindrical Mjärde Lini traps. The fourth signal crayfish population was found by local fishermen. After the discovery of signal crayfish, monitoring was conducted at all these sites yearly (Supplementary Table 1). For every trapping session, catch per unit effort (CPUE; the number of caught crayfish per trap night) was recorded at each site (Supplementary Table 1). Information about the occurrence of noble crayfish in these rivers before the detection of signal crayfish was obtained from a database of standardized test fishings and crayfish stockings of the Department of Aquaculture of EULS which was held since 2003 and includes data back to 1989.

The first specimen of signal crayfish was caught in Mustjõgi River, Harju County in 2008 (Fig. 1). This river is 38 km long with a catchment area of 98.8 km^2. The noble crayfish population had become extinct there but recovered after restockings in 1997–1999. Test fishings in 2005 showed the presence of noble crayfish (CPUE 1.2), but after the detection of signal crayfish in 2008, noble crayfish were not found except in 2014 (a single specimen). No signal or noble crayfish were found in extensive test fishings in Mustjõgi River and its tributaries in 2009 (Supplementary Table 1). In 2010–2012 few signal crayfish were found at the same site, in a 25–30 m long section of the river (Supplementary Table 1). During the years 2013–2016 no signal crayfish were caught in test fishings.

The second signal crayfish population was found in 2010 on the island of Saaremaa in Riksu Stream, which is 19.6 km long with a catchment area of 49.4 km^2. Additional test fishing with an increased number of trap nights was carried out and 61 (CPUE 0.12) signal crayfish were caught from a 500 m section downstream of the previous site in Riksu Stream (Supplementary Table 1). Noble crayfish were not found at that site although in the early 2000s there was a noble crayfish population. About 50 noble crayfish were caught per 10 m in 2002 (unpublished data of EULS). In 2011 and 2012 a new site was found just upstream but the number of signal crayfish in the total catch was lower despite an increase in the number of trap nights (Supplementary Table 1). In 2013, new signal crayfish sites were found further upstream, and up to 2014 signal crayfish occurred in about three km section of Riksu Stream. The total catch in 2013–2016 had increased (CPUE 0.19 up to 3.31) compared to the first 3 years (Supplementary Table 1). The estimated migration rate of signal crayfish in Riksu Stream during the last 2 years has been one km upstream.

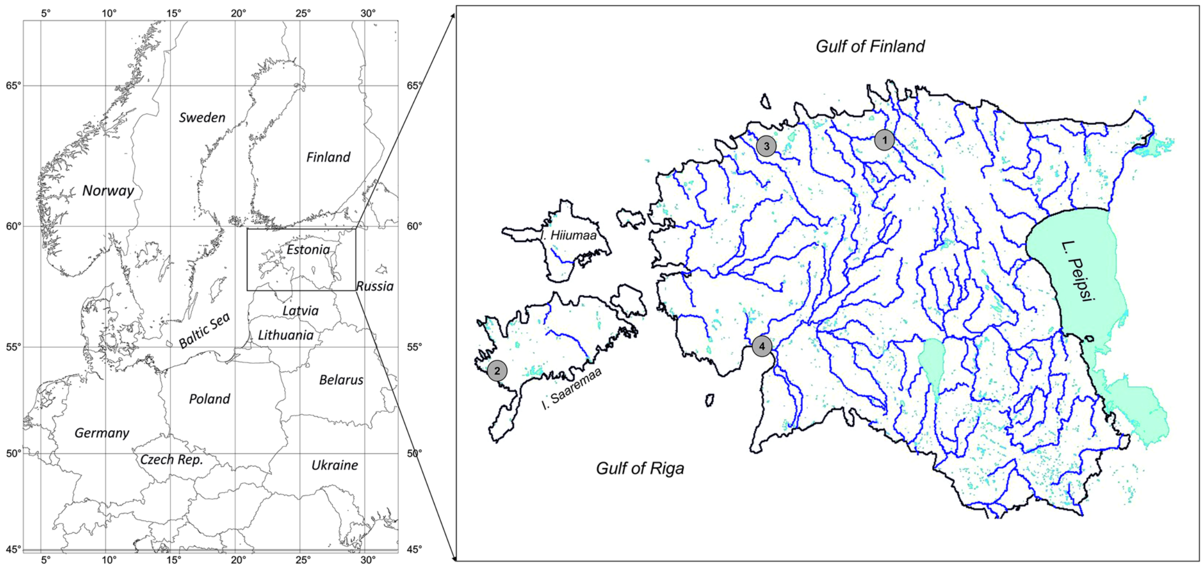

Fig. 1 The signal crayfish locations in Estonia and the year of first reporting: *1* Mustjõgi River, Harju County (2008); *2* Riksu Stream, island of Saaremaa (2010); *3* Vääna River, Harju County (2012); *4* Pärnu River, Pärnu County (2016)

The third signal crayfish population was found in 2012 in Vääna River, Harju County. It is 64.3 km long with a catchment area of 315 km^2. In the end of the 1990s there was a dense noble crayfish population (CPUE 4.8). The situation in Vääna River was different from other rivers because both noble crayfish and signal crayfish are living in sympatry. In 2012 one noble crayfish and one signal crayfish were found in 135 trap nights (Supplementary Table 1). In 2013, test fishing included more traps (300 trap nights) and one signal crayfish was found. At the same time, noble crayfish dominated in catch. In 2014, abundance of both species had increased. In 2015 and 2016 fewer signal and noble crayfish were caught using the same number of traps (Supplementary Table 1).

The fourth signal crayfish population was found in 2016 by local fishermen in Pärnu Bay, 2 km from the coast and in the mouth of Pärnu River in the middle of Pärnu City (Pärnu County). Pärnu River is 144.5 km long with a catchment area of 6836.5 km^2. In a following test fishing 16 signal crayfish and one noble crayfish were caught from the river during 100 trap nights (Supplementary Table 1; Fig. 1).

Detection of crayfish plague

To get an overview of crayfish plague occurrence in Estonia samples from 16 populations where mortalities had occurred or from cage experiments were analysed for *A. astaci* (Supplementary Table 2). Molecular tests for crayfish plague detection were conducted in four different laboratories based on Oidtmann et al. (2004) and Vrålstad et al. (2009) methodology (Supplementary Table 2). *Aphanomyces astaci* multilocus genotype (SSR) was determined according to Grandjean et al. (2014) methodology (Supplementary Table 2).

Multilocus genotype group was determined in case of four crayfish mass mortalities. A sample from Laugi Stream (2007) on the island of Saaremaa showed *A. astaci* multilocus genotype SSR-E and a sample from Pärlijõgi River (2010) in the Võru County showed *A. astaci* multilocus genotype SSR-B. Other two cases in Härjanurme fish and crayfish farm (2010) in Jõgeva County and Avijõgi River (2015) in the East-Viru County exhibited *A. astaci* multilocus genotype SSR-A (Supplementary Table 2). Crayfish plague analyses in signal crayfish watercourses—Mustjõgi River, Riksu Stream and Vääna River indicated presence of *A. astaci* (Supplementary Table 2) but genotype could not be determined.

Discussion

The spread of NICS is a combination of natural expansion and human-assisted introductions which may be both deliberate and accidental (Peay and Füreder 2011). In our case, natural migration is not an issue since the discovered locations are not connected to the waters inhabited by the species in neighbouring countries. Also, there is no direct connection between the signal crayfish-inhabited rivers in Estonia. The main vector of introduction of NICS in Estonia may be the international trade with alive crayfish and the interest of increasing number of crayfish farmers (Paaver and Hurt 2009) in the introduction of alien species. The threat to the noble crayfish from illegal introduction, catching, trade and farming of NICS (Paaver and Hurt 2009) set a need for the improved legislation restricting the spread of NICS. These changes were included into the Nature Conservation Act and adopted in 2004.

Our study showed that the crayfish plague was detected at first time on the island of Saaremaa in 2006, which was 4 years earlier than found signal crayfish. Crayfish plague caused collapse of the stocks in the three crayfish farms during 2006–2007 and in two wild populations, in rivers that were connected to the Pähkla fish and crayfish farm on the island of Saaremaa (Supplementary Table 2). Analysis of one sample from 2007 Laugi Stream outbreak showed *A. astaci* multilocus genotype SSR-E which is originally isolated from spiny-cheek crayfish (*Orconectes limosus*) (Kozubíková et al. 2011). Based on these data we might assume that at least the occurrence of crayfish plague genotype group E on the island of Saaremaa could be linked to the trade with alive crayfish with Lithuania. No live *O. limosus* specimens have been found in Estonia so far but its occurrence is confirmed in Latvia (Briede 2011) and Lithuania (Arbăciauskas et al. 2011). *Aphanomyces astaci* could spread to the island of Saaremaa also by the careless transfer of contaminated crayfish traps or alive fish transport. The presence of genotype group A involved in Härjanurme fish and crayfish farm (2010) and Avijõgi River (2015) mass mortalities and genotype group B (strain originating from *P. leniusculus*) in Pärlijõgi River (2010) was not really surprising. Recently, Vrålstad et al. (2014) and Maguire et al. (2016) reported that both genotype groups were responsible of a large series of outbreak in *A. astacus* stock in Norway and Croatia, respectively. The same explanation can be given to the outbreak in Pärlijõgi River where was moderate density of noble crayfish population (CPUE 3.0) in the middle of 2000s. In 2010 detected crayfish plague outbreak and *A. astaci* genotype group analysis revealed B strain (Supplementary Table 2). Signal crayfish has never been detected in this river. Pärlijõgi River belongs to Gauja River watershed in Estonia and Latvia, but by data of Briede (2011), signal crayfish does not habit there. However, we cannot exclude other animals (e.g. semiaquatic mammals or birds), which can also spread the spores (Makkonen et al. 2013).

There is no information on how signal crayfish spread to Estonia, but this may already have happened before 2008. No evidence about legal introductions of NICS to Estonia has been found. Strict regulation of import of live crayfish to certain European countries (Peay 2009) and intensive proactive conservation measures to protect the ICS (Holdich et al. 2009) have not been sufficient to reduce the trade and spread of NICS (Peay 2009). Trade in ornamental freshwater crayfish has grown rapidly in the last decade and many aquarium shops in Estonia have sold marbled crayfish that are able to survive in North European countries and transmit the crayfish plague (Keller et al. 2014; Kaldre et al. 2015; Mrugala et al. 2015).

Our data indicate different patterns of development of introduced NICS populations. So far, signal crayfish have been responsible for disappearance of the noble crayfish population in Estonia at least in two sites—Mustjõgi River and Riksu Stream. In the Mustjõgi River noble crayfish have not been found after the discovery of signal crayfish, but today, the signal crayfish population seems to have been lost as well. The last signal crayfish in Mustjõgi River was seen in 2012. During 2015 and 2016, 1 000 noble crayfish in total have been restocked to the Mustjõgi River and have been survived so far. Five noble crayfish (CPUE 0.1) were caught in 2016 from the same place where the signal crayfish was detected. Signal crayfish were probably brought to the island of Saaremaa in 2004–2005 and have been responsible for the disappearance the noble crayfish population in the Riksu Stream in the beginning of the 2000s. First detected in 2010, during the last 6 years the signal crayfish population has been growing and there are no obstacles to spread in both directions—either downstream (via Riksu Lake towards the sea) or upstream. Until 2013, there were attempts to dry part of the river inhabited by signal crayfish by constructing a new side channel. However, new sites with signal crayfish occurred upstream, making this activity senseless and turning the possible eradication almost impossible. The case of Pärnu bay also shows that signal crayfish can tolerate brackish (salinity 2–3 ppt) conditions and spread via coastal areas.

Noble crayfish populations did not disappear from all the signal crayfish locations. Although signal crayfish are chronic carriers of *A. astaci* and are also capable of occupying habitats of European ICS, having wider environmental tolerance (Peay and Füreder 2011), in Estonia noble crayfish still persist in sympatry with signal crayfish in two localities—in Vääna and Pärnu Rivers. Signal crayfish were found together with noble crayfish in a small (200 m) site in Vääna River in 2012 and test fishing in each year showed the presence of both species at the same site (Supplementary Table 1). Crayfish plague analyses of signal crayfish showed a low level of *A. astaci*, but noble crayfish were not infected (Supplementary Table 2). The number of analysed specimens was small and period of our study short, thus monitoring including more individuals and longer time should be carried out. Still our study confirmed that permanent coexistence between ICS and NICS is possible as also described in other studies (Schrimpf et al. 2013; James et al. 2017).

Conclusions

Despite a ban on introduction, signal crayfish have been recorded at four Estonian sites since 2008 and have caused the disappearance of noble crayfish populations at least in two sites so far. Abundance of signal crayfish has been fluctuating between years, increased in one population, is low in two localities and disappeared from one place. The pattern of distribution and the fact that the water bodies with signal crayfish localities are not connected, strongly suggest that these populations are the result of illegal human-assisted introductions. It is confirmed by the fact that many outbreaks of crayfish plague in native *A. astacus* populations are caused by different *A. astaci* genotype groups—A, B and E.

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