Status and recovery of indigenous crayfish populations after recent crayfish plague outbreaks in the Czech Republic

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The crayfish plague pathogen (Aphanomyces astaci) is one of the most important threats to indigenous European crayfish. Although it belongs among the most studied pathogens of invertebrates, only a few recent studies are available on the epidemiology of crayfish plague and its long-term effects on crayfish populations. We provide detailed data on 11 populations of European crayfish (Astacus astacus, A. leptodactylus, Austropotamobius torrentium) hit by crayfish plague in the Czech Republic between 1998 and 2011. We repeatedly surveyed the affected localities in the years following the disease outbreaks to investigate potential recovery of crayfish populations and to search for the likely sources of infection. Although the mortalities severely decimated all studied populations, European crayfish could be found in the watercourse catchments after the disease outbreaks in all but two cases. In five cases, migration barriers apparently supported crayfish survival; in two cases, the disease stopped spreading even without the presence of any barrier. Indigenous crayfish were recorded directly in the affected parts of five studied streams after some time but in most cases populations have not yet reached the original densities. Their recovery seems influenced by the population size in unaffected refuges as well as time since the outbreak. Sources of infection and transmission pathways of A. astaci apparently vary in the Czech Republic. Aphanomyces astaci of three genotype groups originating in different crayfish plague pathogen carriers were involved in the outbreaks. Direct transmission of A. astaci from invasive American crayfish present in the same stream is likely in three cases; however, these host crayfish were not recorded at the remaining localities, and long-range dispersal or other pathogen sources may be assumed. We hypothesize that chronic A. astaci infections leading to disease outbreaks under

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specific conditions may occur in some populations of indigenous crayfish in the Czech Republic.

KEY WORDS: Aphanomyces astaci, crayfish plague, European crayfish, Astacus astacus, Austropotamobius torrentium, epidemiology, field survey.

INTRODUCTION

Indigenous crayfish species have been declining in Europe during the last few decades (HOLDICH et al. 2009). Several factors resulting from human activities are responsible for this trend, e.g. habitat loss or degradation, water pollution, inappropriate fishing management and practices, and excessive water abstraction. One of the greatest dangers for European crayfish is, however, introduction and spread of non-indigenous crayfish species of North American origin and of exotic diseases, especially the crayfish plague (FÜREDER et al. 2006).

Crayfish plague is an acute stage of infection by an oomycete Aphanomyces astaci Schikora (Saprolegniales) which leads to crayfish mortality. The pathogen reproduces by motile zoospores that infect new hosts (CERENIUS et al. 1988). A. astaci seems to be of North American origin, as several crayfish species from this continent were found to have elevated resistance to the acute disease (UNESTAM 1969), being able to limit the pathogen growth only to their cuticle (CERENIUS et al. 1988). Consequently, they may act as A. astaci carriers and their populations often serve as long-term infection sources (e.g., KOZUBÍKOVÁ et al. 2009).

On the contrary, European crayfish are much more susceptible to the disease. Their immune response is usually too slow to restrain the pathogen growth, and they mostly die within days to a few weeks when infected (ALDERMAN et al. 1987). However, evidence has been growing that even these crayfish species may carry the pathogen without signs of the disease much longer than formerly expected (e.g., KOKKO et al. 2012; SVOBODA et al. 2012; KUŠAR et al. 2013; VILJAMAA-DIRKS et al. 2013). Uncovering such cases has been facilitated by a widespread use of sensitive molecular methods developed for A. astaci detection during the last decade, particularly the quantitative polymerase chain reaction (PCR) targeting the nuclear internal transcribed spacer (ITS) region (VRÅLSTAD et al. 2009). However, the phenomenon of apparent chronic infections in susceptible host species has still not been sufficiently explained. The occasionally observed higher resistance of individuals or populations of European crayfish to crayfish plague could be related to their genetic background, the A. astaci strain causing the infection, pathogen load, or local environmental conditions (MAKKONEN et al. 2012; SVOBODA et al. 2012).

The crayfish plague pathogen is considered to have been present in Europe since the second half of the 19th century when the disease gradually caused large-scale losses of European crayfish (ALDERMAN 1996). However, during the later decades of the 20th century, crayfish plague became practically forgotten in some parts of Europe where crayfish lost their economic value (e.g. KOZUBÍKOVÁ et al. 2006). This started to change again with an increasing spread of invasive North American crayfish in Europe in the last decades, and crayfish plague increasingly became an important conservation issue (FÜREDER et al. 2006).

Unlike the 19th century wave of mass mortalities, which is supposed to have been caused by one genotype group of A. astaci (HUANG et al. 1994), recent crayfish plague
outbreaks are caused also by additional pathogen genotypes originating from different host species (e.g. Filipová et al. 2013; Rezinciuc et al. 2013; Viljamaa-Dirks et al. 2013; Grandjean et al. 2014). Different A. astaci strains may show substantial variation in ecologically relevant physiological or life history traits (Rezinciuc et al. 2013), including virulence towards European crayfish (Makkonen et al. 2012; Juusila et al. 2013; Viljamaa-Dirks et al. 2013).

Only a few recent studies are available about the epidemiology of the disease and different strains of the pathogen and about long-term effects of A. astaci on the indigenous crayfish populations, although such information may be crucial for mitigation of the impact of the disease. Various reports provided some details on particular crayfish plague outbreaks, with different outcomes, ranging from apparent elimination of entire populations to the limited spread of the disease and survival of crayfish in the affected catchments (e.g. Kozubíková et al. 2008; Caprioli et al. 2013). However, data on subsequent development of crayfish populations on affected localities are usually unavailable (but see Viljamaa-Dirks et al. 2013).

In our study, we want to partly fill this gap by providing such data for several sites in the Czech Republic. This Central European country is suitable for such a follow-up study, due to relatively intensive recent research focusing on the distribution and impact of the crayfish plague pathogen. Altogether five crayfish species are found in Czech waters: indigenous Astacus astacus (Linnaeus 1758) and Austropotamobius torrentium (Schrank 1803), Astacus leptodactylus Eschscholtz 1823 (introduced from Eastern Europe in the 1890s; Horká 2006), and two North American alien species, Orconectes limosus (Rafinesque 1817) (invading apparently since the 1960s; Petrušek et al. 2006) and Pacifastacus leniusculus (Dana 1852) (introduced in 1980; Filipová et al. 2006). Despite the red list status and formal protection of all three European crayfish species, crayfish plague was not considered a relevant conservation problem until the end of the 1990s, and A. astaci presence in the country was only confirmed in 2004 (Kozubíková et al. 2006).

Since then, numerous confirmed or suspected crayfish plague outbreaks, affecting both indigenous crayfish species, have been recorded throughout the Czech Republic (e.g. Kozubíková et al. 2008; Štambergová et al. 2009). Furthermore, recent analysis of DNA isolates from most of those outbreaks revealed that multiple genotype groups of A. astaci have been involved (Grandjean et al. 2014). In this report, we focus on the present status of crayfish populations at 11 localities in the Czech Republic affected by crayfish plague within the years 1998–2011. We searched for any crayfish at these localities repeatedly in the years after the crayfish mass mortalities and evaluated if the indigenous crayfish survived in the catchments and whether any recovery has taken place in the affected localities. Furthermore, we searched for evidence pointing to possible sources of the crayfish plague pathogen. Such data may bring better insight into the effects of crayfish plague on a natural population of indigenous crayfish in Central European conditions.

MATERIAL AND METHODS

Data collection and field search for crayfish

We included in this study all 11 confirmed crayfish plague outbreaks recorded in the Czech Republic during the last 15 years, and in addition one highly probable but unconfirmed outbreak reported in 1998–1999 (the Pšovka brook). Details on locations of the outbreaks are available in
Fig. 1. — Distribution of indigenous crayfish mass mortalities related to the crayfish plague in the Czech Republic in the period 1998–2014 (asterisks) and current distribution of American crayfish in the country (presence in ca 11 × 11 km squares of the faunistic mapping grid). Abbreviations of the localities: Be: Besének brook, Bo: Bojovský brook, C: Černý brook (the most recent crayfish plague outbreak in the Czech Republic at the time of compiling this study), K: Křivek brook, L: River Litavka, O: River Olše, Pe: tributary of Pěnenský brook, Ps: Pšovka brook, S: River Senice, U: Úpořský brook, Za: Zákolanský brook, Ze: Žebrákovský brook. Affected crayfish species: Austropotamobius torrentium (U, Za), Astacus astacus (Be, Bo, C, K, L, O, Pe, S, Ze), A. astacus and Astacus leptodactylus (Ps). Genotype groups of Aphanomyces astaci are indicated by different colours (note that the strain detected in U was related to but apparently different from those typical for the genotype group B).

Fig. 1, Table 1, and Supplementary File 1 (containing maps of catchments of all studied watercourses). We focus on 11 cases in detail: for five of them (recorded between 2008 and 2011), we describe the circumstances of the crayfish mortalities as they have not been reported before; for the rest of these outbreaks we refer to the previously published studies. The most recent crayfish plague outbreak (in the Černý brook in Western Bohemia, in January 2014) is only briefly mentioned in the discussion as only limited data were available at the time of finishing this study.

To obtain data on the development and potential recovery of crayfish populations after the mortalities, we regularly monitored the affected localities and adjacent water bodies for crayfish presence. Monitoring was performed at least once every 2 to 3 years, in the season of high crayfish activity (between April and October). We searched for crayfish manually in suitable shelters during the day or used traps at sites where manual search was not feasible. Usually, at least 50 shelters were checked at each monitored site; number of sites was chosen according to the catchment size and local conditions (we preferentially selected sites apparently most suitable for crayfish, i.e. unregulated, natural-looking parts of watercourses with enough potential shelters, and banks suitable for burrowing).

If living indigenous crayfish were found, we determined the species and returned the individual back to its habitat as soon as possible to reduce manipulation stress. If dead indigenous or living non-indigenous crayfish were found, we collected samples for molecular detection of the crayfish plague pathogen and dried or disinfected any wet equipment before its use at another site with potential occurrence of indigenous crayfish. As A. astacus and A. torrentium are protected by law in the Czech Republic, all surveys were conducted either by persons entitled to handle these species by specific permits, or under the auspices of the respective nature conservation bodies.
Table 1.

Summary of indigenous crayfish mass mortalities related to the crayfish plague recorded in the Czech Republic in 1998–2014. In all but one case (the Pšovka brook), *Aphanomyces astaci* has been confirmed in the dying crayfish.

| Year of crayfish mortality | Locality (close settlement, region) | Geographical coordinates of the mortality site | Catchment | Affected crayfish species | Who recorded crayfish mortality | *A. astaci* genotype group$^1$ | Potential *A. astaci* carriers$^2$ | Indigenous crayfish saved$^3$ | Migration barriers$^4$ | References$^5$ |
|---------------------------|-------------------------------------|-----------------------------------------------|-----------|--------------------------|-------------------------------|-----------------------------|-------------------------------|---------------------|------------------|-------------|
| 1998–1999                 | brook Pšovka (Kokořín, Central Bohemia) | From 50°23'47"N, 14°33'28"E to 50°27'44"N, 14°36'15"E | Labe      | *A. astacus, A. leptodactylus* | nature conservationists      | N/A                         | *O. hemosus*                | Yes                 | Wetlands         | B95, B99, B06, K00, K06, L07 |
| 2004                      | brook Krávec (Třinec, Silesia)       | 49°39'56"N, 18°40'09"E                         | Olš         | *A. astacus*              | local people                 | A                          | Not found                    | Yes                 | N/A              | K06         |
| 2005                      | Bojovský brook (Bojov, Central Bohemia) | 49°53'51"N, 14°21'53"E                         | Vltava     | *A. astacus*              | student excursion            | E                          | *O. hemosus*                | No                  | N/A              | K08         |
| 2005                      | Úpořský brook (Skryje, Central Bohemia) | 49°57'54"N, 13°49'12"E                          | Berounka   | *A. torrentium*          | nature conservationists      | B?                         | Not found                    | Yes                 | Pond dam         | K08         |
| 2006                      | river Olš (Jablunkov, Silesia)       | From 49°38'05"N, 18°42'26"E to 49°33'35"N, 18°49'48"E | Odra      | *A. astacus*              | fishermen                    | N/A                        | Not found                    | Yes                 | Weirs on some tributaries | K08         |
| 2007                      | tributary of Pěněnský pond (Horní Pěna, South Bohemia) | 49°05'43"N, 15°02'40"E                         | Nežárka    | *A. astacus*              | local people                 | B                          | Not found                    | Yes                 | Pond dam         | K08         |
| 2008                      | Žebrákovský brook (Světlá nad Sázavou, Vysočina region) | From 49°40'48"N, 15°23'09"E to 49°41'17"N, 15°22'52"E | Sázava    | *A. astacus*              | local people                 | B                          | Not found                    | Yes                 | Pond dam         | K13, S09   |
| 2008                      | river Senice (Lidečko, North Moravia) | 49°13'48"N, 18°02'15"E                         | Vsetínská Bečva | *A. astacus*              | nature conservationists      | A                          | Not found                    | Yes                 | N/A              | S09         |

(Continued)
Table 1.  
(Continued)

| Year of crayfish mortality | Locality (close settlement, region) | Geographical coordinates of the mortality site | Catchment | Affected crayfish species | Who recorded crayfish mortality | A. astaci genotype group<sup>1</sup> | Potential A. astaci carriers<sup>2</sup> | Indigenous crayfish saved<sup>3</sup> | Migration barriers<sup>4</sup> | References<sup>5</sup> |
|----------------------------|--------------------------------------|-----------------------------------------------|------------|---------------------------|---------------------------------|-----------------------------------|----------------------------------|--------------------------------|--------------------------------|--------------------------|
| 2009                       | Besének (Tišnov, South Bohemia)      | 49°21'35"N, 16°24'26"E                        | Svatka     | A. astacus                | school excursion                | A                                 | Not found                        | No                              | N/A                          | S09                     |
| 2009                       | Zákolanský brook (Okoř, Central Bohemia) | 50°10'36"N, 14°15'12"E                        | Vltava     | A. torrentium             | local sportsmen                 | E                                 | Not found                        | Yes                            | N/A                          | K13, S11                 |
| 2011                       | river Litavka (Příbram, Central Bohemia) | 49°40'09"N, 13°57'51"E                        | Berounka   | A. astacus                | local children                  | E                                 | O. limosus, E. sinensis          | Yes                            | N/A                          | K13, KB13               |
| 2014<sup>6</sup>           | Černý brook (Pec pod Čerchovem, Western Bohemia) | 49°24'06"N, 12°49'33"E                        | Radbuza    | A. astacus                | local people                    | B                                 | Not found                        | N/A                            | N/A                          |                        |

<sup>1</sup> Data obtained mostly by Grandjean et al. (2014); note that the genotype indicated as B? differs from the typical genotype group B.

<sup>2</sup> Potential A. astaci carriers detected in the same watercourse in the vicinity of the indigenous crayfish mortality site.

<sup>3</sup> Indigenous crayfish found in the catchment of the watercourse affected by crayfish plague in the year of mortality or during later surveys.

<sup>4</sup> Migration barrier(s) on the watercourse, which probably prevented spread of the disease upstream.

<sup>5</sup> B95 – Beran (1995), B99 – Beran (1999), B06 – Beran (2006), K00 – Kozák et al. (2000), K06 – Kozubíková et al. (2006), K08 – Kozubíková et al. (2008), K13 – Kozubíková & Horák (2013), KB13 – Kozubíková-Balcarová et al. (2013), L07 – Lulayová (2007), S09 – Štambregová et al. (2009), S11 – Svorová (2011).

<sup>6</sup> The most recent crayfish plague outbreak in the Czech Republic for which detailed data comparable to the rest of the described cases were not available at the time of compiling this study.
Additionally, we collected information about crayfish observations at the particular localities from literature, species occurrence database of the Nature Conservation Agency of the Czech Republic (NCA CR) and local citizens.

**Molecular detection of Aphanomyces astaci**

We confirmed *A. astaci* in the dying crayfish from all the described mass mortalities (except for the late 1990s mortality in the Pšovka brook) by multiple molecular diagnostic procedures. These results have been already partly published (see below, and Table 1 for references).

Samples of dead or dying crayfish were processed fresh or stored deep-frozen in – 80 °C or in 96% ethanol before extraction of the whole genomic DNA of each tested animal (usually from soft abdominal cuticle, or from mixed tissue samples of soft abdominal cuticle, uropods and basal limb joints). DNA extraction was performed after grinding the tissues in liquid nitrogen using DNeasy Blood & Tissue Kit (Qiagen) according to the manufacturer’s instructions.

We used PCR-based methods targeting segments of the ITS region of the nuclear ribosomal DNA, which is considered specific for *A. astaci* (Diéguez-Uribéondo et al. 2009). As the first line of evidence of *A. astaci* infection in the dead crayfish from the mass mortalities, we applied conventional PCR after Oidtmann et al. (2006) amplifying about 550-bp-long products combined with sequencing of these PCR products; all obtained sequences corresponded with the reference ones available in GenBank (see, e.g. Kozubíková et al. 2008). Consistent results suggesting *A. astaci* infection were also obtained for all the confirmed crayfish plague outbreaks (i.e. except the Pšovka brook) by the quantitative TaqMan real-time PCR after Vrálstad et al. (2009); the levels of infection for selected samples are provided in Grandjean et al. (2014).

Additionally, genotype groups of *A. astaci* strains causing the disease outbreaks were determined by analysis of nine polymorphic microsatellite markers according to Grandjean et al. (2014) for most of the described cases except for those in Pšovka brook (for which no DNA was available), and the River Olše (for which reliable genotyping failed). The results, mostly presented in Grandjean et al. (2014) and additionally including the Černý brook, are summarised in Table 1.

*Orconectes limosus* collected in the Pšovka brook, in the Vrané reservoir into which the Bojovský brook discharges, and in the River Litavka were processed in the same way as dying indigenous crayfish from mass mortalities, but only the quantitative PCR (qPCR) according to Vrálstad et al. (2009) was performed for these samples.

**RESULTS AND DISCUSSION**

**Case studies of crayfish mass mortalities and results of subsequent surveys**

Pšovka brook (crayfish mass mortalities 1998–1999). The Pšovka brook (located in the Protected Landscape Area Kokořínsko, Central Bohemia; for the map of the catchment, see Supplementary Fig. 1) is interesting due to the presence of three crayfish species, two European and one North American, allowing for a study of the dynamics of these crayfish and the crayfish plague pathogen on a long-term scale. The lowest stretch of the brook had been occupied by *O. limosus*, the central stretch by *A. astacus* together with *A. leptodactylus*, and the upper section only by *A. astacus* in the 1990s (Beran 1995). However, a mass mortality severely devastated indigenous crayfish populations in about a 10-km stretch of the brook between fishponds Štampach (50°23'47''N, 14° 33'28''E) and Stříbrník (50°27'42''N, 14°36'15''E) in 1998–1999 (Kozák et al. 2000, summarized also in Kozubíková et al. 2006). Although the crayfish plague pathogen could not be diagnosed in the dying crayfish as no suitable methods were available at that time in the Czech Republic, this disease is the most likely reason for crayfish
mortalities because of the presence of *O. limosus* in the same brook. This species had not been affected by the mortalities, and *A. astaci* was detected in its population in 2004 (60% of the tested individuals were positive for *A. astaci* DNA; KOZUBÍKOVÁ et al. 2006).

*Astacus leptodactylus* was not recorded in the Pšovka brook after the crayfish mortalities, and was considered locally extinct (BERAN 2006). Surprisingly, one adult female was found in Kokořínský důl (50°26'17"N, 14°34'50"E) in October 2013. In contrast to that species, a small population of *A. astacus* had been detected in a limited area in the most upstream section of the brook upstream from Konrádov (50°28'42"N, 14°36'23"E) already in autumn 1999 (BERAN 1999; KOZÁK et al. 2000). Survival of these crayfish could have been supported by the presence of extensive wetlands occurring discontinuously upstream of the upper range of the mass mortality. Such habitat does not seem to be suitable for *A. astacus*, and could prevent migration of infected individuals upstream, which would stop the spread of the disease.

In 2005, about 20 *A. astacus* were transferred from the upstream refuge to the brook stretch downstream of Vojtěchov (50°27'16"N, 14°35'08"E; L. BERAN unpub. data). During surveys in 2011 (MATASOVÁ 2011), 2012 and 2013, an apparently well-established *A. astacus* population was repeatedly recorded at several sites situated in about a 5 km long stretch of the Pšovka brook from the place of their transfer to the site upstream of the Harasov pond (50°24'48"N, 14°34'41"E). However, it is not clear if *A. astacus* re-colonized the central part of the brook only from the reintroduced specimens, or from the remaining population in the most upstream section of the brook, or if some individuals also survived the crayfish plague episode locally.

Between 1999 and 2012, *O. limosus* spread upstream by about 3 km and reached the area of *A. astacus* occurrence. The species must have overcome two pond dams, possibly with the aid of humans. Consequently, a zone of contact of *O. limosus* and *A. astacus* without any signs of *A. astacus* mortality was repeatedly recorded in about a 500-m long part of the brook directly upstream from the Harasov pond, in 2012 and 2013. At present, *A. astacus* significantly predominates, as only three *O. limosus* individuals were found per 66 *A. astacus* during four searches conducted in the last 2 years.

No mortality of *A. astacus* after at least 2 years of coexistence with *O. limosus* corresponds with the results of MATASOVÁ et al. (2011), who suggested that prevalence of *A. astaci* in *O. limosus* population in the Pšovka brook had a decreasing trend in 2004–2010. Additional qPCR analyses of 18 *O. limosus* individuals sampled in the Pšovka brook close to the Harasov pond in 2012 and 2013 were also consistently negative.

We assume that *A. astacus* is permanently threatened by crayfish plague in the Pšovka brook. Although recently under the detectable level, the pathogen is probably still present in the population of *O. limosus*. Thus, a new disease outbreak may be delayed but, on the long-term scale, repeated cycles of crayfish plague outbreaks and re-colonization of the brook by *A. astacus* and *A. leptodactylus* may appear or the indigenous crayfish may be entirely exterminated. To preserve at least a part of the population gene pool, *A. astacus* are occasionally transferred to alternative localities (brooks in the same area and with similar environmental conditions, previously found free of crayfish).

*Křivec brook (crayfish plague outbreak 2004)* and *River Olše* (2006). Two crayfish plague outbreaks affecting *A. astacus* were recorded in the catchment of the River Olše in Upper Silesia; one in the Křivec brook in 2004 (KOZUBÍKOVÁ et al. 2006; Table 1) and another in about a 15-km stretch of the River Olše upstream from the village Bystřice in summer 2006 (KOZUBÍKOVÁ et al. 2008; Table 1). This mass mortality probably also
reached the lower parts of at least some of the Olše tributaries (Kostkov, Hluchová-Zabínek, and the Ošetnický brook; KOZUBÍKOVÁ et al. 2008; these as well as other relevant watercourses are indicated in the map in Supplementary Fig. 2).

No crayfish were found in the Krivec brook during surveys in 2006 and 2009. The first detection of *A. astacus* in the brook since the crayfish plague outbreak was in July 2013, when 11 individuals were found in burrows at a site previously affected by the disease. It is unclear, however, whether crayfish have survived in the stream, recolonised the brook from the River Olše, or were reintroduced by local people.

Detailed mapping of crayfish distribution in the Olše catchment between the Czech-Polish border and Český Těšín (about 30 km stretch of the river) in 2004–2005, i.e. before the crayfish plague outbreak, showed that *A. astacus* had been common in that area. Crayfish had been recorded at three sites in the River Olše as well as in its 13 tributaries (altogether at 25 of 28 mapped sites; data provided by NCA CR). In contrast, in October 2006, when a detailed search for crayfish was performed at 38 sites in the Olše catchment in reaction to summer crayfish mortalities (survey ordered by NCA CR), only a few *A. astacus* were caught in two Olše tributaries, Kostkov and Lomná (see Supplementary File 1). However, these mostly negative results could have also been caused by other reasons leading to the disappearance or decline of the crayfish populations. In particular, decreased water temperature and thus lower crayfish activity in autumn, or lower experience of a person performing the survey, could have affected the results. In the Kostkov, *A. astacus* were found also in 2008 directly within the stretch affected by the 2006 outbreak. In this stream, a part of the crayfish population was separated from the lower stretch of the brook by concrete weirs, which could facilitate crayfish survival. Weirs are present also in the tributary Lomná.

In the River Olše, however, no crayfish were caught until 2011 when *A. astacus* were found at three sites; in Písek (within the area of the previous mortality), Trinec and Český Těšín (both downstream from Bystřice); see Supplementary Fig. 2. *Astacus astacus* were recorded near Bystřice also in 2013. Similarly to the situation in the river itself, crayfish had not been found in its tributaries Kopytná and Jasení (see Supplementary Fig. 2) until 2011 when they were recorded again. No crayfish at all have been caught in the Hluchová and the Ošetnický brook since the crayfish plague outbreak in Olše. However, these streams are relatively wide and stony, thus surveys of crayfish populations may be inefficient.

American crayfish have never been found in the Czech part of the River Olše watershed; the closest populations of *O. limosus* are known in Poland (KOZUBÍKOVÁ et al. 2006), and in Prudník at the Polish border about 100 km away (ĎURIŠ & HORKÁ 2007; KOZUBÍKOVÁ et al. 2008). However, the pathogen genotyping (GRANDJEAN et al. 2014) actually suggests another source of *A. astaci*. The genotype group A, previously isolated only from European indigenous crayfish (HUANG et al. 1994), was detected in *A. astaci* affected by the mortality in the Krivec brook (Table 1). The strain causing the outbreak might have thus persisted in some of the local populations of *A. astacus*. Unfortunately, genotyping of *A. astaci* from the samples from the Olše crayfish plague outbreak was not successful. Thus, although Krivec and Olše might have got infected from the same source, we cannot exclude two independent sources.

To summarize, crayfish apparently survived two disease outbreaks in the catchment (at least in tributaries of the River Olše, such as Kostkov and Lomná). Barriers on the streams or differences in population densities could play a role in preventing the disease spread upstream. Five years after the outbreak, *A. astacus* was already found in
the Olše itself. Two scenarios may explain their presence: (1) a few crayfish survived in the river but the population densities dropped under the detectable level for several years; (2) crayfish re-colonized the river from its tributaries. It seems that complex catchments with many possible refuges and discontinuous crayfish populations may reduce the overall effect of the disease, despite heavy losses during the outbreak. Moreover, the presence of *A. astaci* genotype group A, which is considered less virulent in comparison with strains from the signal crayfish (Makkonen et al. 2012; Viljamaa-Dirks et al. 2013), could have also contributed to a less harmful effects of the disease.

**Bojovský brook (crayfish plague outbreak 2005).** A crayfish plague outbreak affecting *A. astacus* was recorded in the Bojovský brook in May 2005 (for more details on this case, see Kozubíková et al. 2008 and Table 1). No *A. astacus* were found in the downstream part of the brook affected by crayfish plague during surveys repeated yearly during student excursions, nor at several sites upstream from Bojov (49°52'23''N, 14°20'58''E; Supplementary Fig. 3) and Čisovice (49°51'50''N, 14°18'46''E) investigated intensively in July 2010 and 2013.

The source of infection which caused the disease outbreak remained unknown for 6 years. In 2011, however, a dense population of *O. limosus* was confirmed in the reservoir Vrané (49°54'37''N, 14°23'16''E) on the River Vltava into which Bojovský brook discharges, and a few individuals were found also in the mouth of the brook. This finding, facilitated by temporarily lowered water level in the reservoir, is in accordance with the detection of *A. astaci* of the genotype group E in the dying *A. astacus* (Grandjean et al. 2014). This genotype group has been isolated previously from *O. limosus* (Kozubíková et al. 2011). Moreover, all 23 *O. limosus* caught in the reservoir tested positive for *A. astaci* (Kozubíková-Balcarová unpub. data).

**Úpořský brook (crayfish plague outbreak 2005).** An extensive mass mortality of *A. torrentium* caused by the crayfish plague was recorded in the central part of the Úpořský brook in the nature reserve Týřov located in the Protected Landscape Area Křivoklátsko in June 2005 (for further details, see Kozubíková et al. 2008, Table 1, and Supplementary Fig. 4). The source of infection for this crayfish plague outbreak could have been another crayfish mass mortality in a nearby brook, which occurred in spring 2005, but *A. astaci* could not be confirmed there due to unavailability of samples (Kozubíková et al. 2008). Microsatellite genotyping detected an *A. astaci* strain closely related but distinct from typical strains of the genotype group B (indicated as B? in Fig. 1 and Table 1; Grandjean et al. 2014) in dying crayfish from the Úpořský brook. This suggests *P. leniusculus* might have been the primary source of infection. However, as these American crayfish are not known to occur in the whole of Central Bohemia (Fig. 1), long-distance transmission of the disease, probably related to human activities, is suspected.

A strong population of *A. torrentium* has been saved from the disease in the upper part of the brook called the Míza. The 2-m-high dam of Kučerův pond (49°57'41''N, 13°50'40''E) apparently prevented migration of infected crayfish upstream (Kozubíková et al. 2008). Tens to hundreds of crayfish were repeatedly found in this upper part of the brook during regular surveys in the following years (NCA CR).

*Austropotamobius torrentium* might also have survived the disease episode in the catchment of the Hořejší brook, which discharges into the Úpořský brook about 150 m downstream from the above-mentioned dam. Crayfish could be found in the Hořejší brook at least since 2008 (A. Hoffmannová, NCA CR). However, we are not aware of any
surveys in this stream between 2005 and 2007; thus it is not clear whether the crayfish plague outbreak affected this brook or not.

Until 2009, when one *A. torrentium* was found about 0.9 km under the dam of Kučerův pond (A. Hoffmannová, NCA CR), crayfish were considered extinct in the whole part of the Úpořský brook affected by crayfish plague (as no report is available on their detection there despite repeated surveys). In the following years, individual crayfish were repeatedly found in the Úpořský brook but no further than about 1.3 km downstream from the Kučerův pond dam (NCA CR). We consider this a gradual colonisation of the downstream parts by crayfish from the upstream refuges in Míza and Hořejší brook. In 2013, however, we obtained unofficial information (L. Vápeník pers. comm.) that individual *A. torrentium* used to be regularly observed in the stretch of the Úpořský brook affected by crayfish plague 1.5–3.5 km downstream from the Kučerův pond dam between 2002 and 2012. If this information is correct, it would mean that some crayfish survived the crayfish plague outbreak directly in the stream or in its small forest tributaries without any migration barriers.

In any case, it is apparent that the *A. torrentium* population in the Úpořský brook downstream from Kučerův pond is still very rare and that it has been severely devastated by the crayfish plague outbreak. Recovery of the population seems to be very slow despite the fact that rich crayfish populations survived in the brook catchment.

*Tributary of Pěnenský pond (crayfish plague outbreak 2007).* A crayfish plague outbreak affecting *A. astacus* occurred in the stretch of the brook and its small side tributaries between Pěnenský pond and a small forest pond, Sígl (49°05′39″N, 15°03′00″E), in July 2007 (for more details, see Kožubíková et al. 2008, Table 1, and Supplementary Fig. 5).

After the mass mortality, crayfish could be repeatedly found only upstream from the Sígl pond. However, this part of the brook seems a low-quality habitat for crayfish. It is very narrow (usually 30–50 cm) and shallow, partly straightened and runs through predominantly spruce forest. Altogether, this leads to low supply of shelters and food. Indeed, the crayfish population seems to be very sparse there, as only one to two individuals were recorded each time during surveys in 2008, 2010 and 2012. Survival of crayfish upstream of Sígl pond could have been supported by a 1-m-high dam and by the fact that the pond is substantially filled with muddy sediment and densely overgrown with aquatic vegetation and may not be inhabited by crayfish. Thus, the pond could play the role of a migration barrier preventing spread of the disease upstream.

In the part of the stream previously affected by crayfish plague, no crayfish were found despite yearly surveys until 2012. One large male *A. astacus* was captured in this section in 2012, but no crayfish were found in the subsequent survey in 2013. It is not clear if this single record is due to spontaneous re-colonization from some upstream part of the brook’s watershed, local survival of some individuals during the disease outbreak, or re-colonization from the downstream pond (in which the status of the crayfish population is unknown).

The origin of infection is unclear as well. *Aphanomyces astaci* strain of group B was detected in dying crayfish (Table 1, Grandjean et al. 2014), suggesting *P. leniusculus* as the original source (Huang et al. 1994). Indeed, *P. leniusculus* used to be observed in a fishpond near Lomy u Kunžaku (about 9 km from the affected brook but in a different catchment) during the period 1990–2002 (Filipova et al. 2006). However, *P. leniusculus* were not recorded at that site later (A. Petrusek unpub. data). Although not known from anywhere else in the vicinity of the affected brook, we cannot exclude that *P. leniusculus* have been spread from the pond to other sites (such as Pěnenský...
pond) by people and came into contact with *A. astacus*, or that the pathogen spores were transmitted from such undetected populations due to human activities. Alternatively, one may speculate about long-distance dispersal from neighbouring regions of Austria where *P. leniusculus* is more widespread (Pöckl 1999; Kouba et al. 2014).

**Žebrákovský brook (crayfish plague outbreak 2008).** This case has been mentioned in Štambergová et al. (2009) without further details. A mass mortality of *A. astacus* was detected in the lower part of the Žebrákovský brook between the village Horní Březinka (49°40′48″N, 15°23′09″E) and the dam of Lánský pond (49°41′19″N, 15°22′58″E) in August 2008 (Table 1 and Supplementary Fig. 6). Tens of dead, dying and daytime-active crayfish were found outside their shelters. Some of the still-living individuals were scratching their bodies or showed spasmatic movements of the limbs and whole bodies. Other invertebrates and fish remained unaffected.

A population of *A. astacus* evidently survived upstream from Lánský pond, as up to 20 living individuals were repeatedly recorded between this pond and the dam of the reservoir Kristiánka (49°41′53″N, 15°22′56″E) during surveys in 2008 (October), 2009, 2010 and 2013. According to the owner of Lánský pond, crayfish were found in the pond when it was drained for fish harvesting in autumn 2008. Thus, the disease spread upstream was apparently stopped by about a 50-cm step at the outflow of the pond dam supplemented by a concrete area below it, which could have prevented crayfish migration upstream. Additionally, when the crayfish mortality was recorded, two wooden boards about 30 cm high were temporarily installed transversally to the brook under the dam to further limit the migration of infected individuals. Although barriers of this height are unlikely to prevent crayfish spread in the long term (Řimalová-Kadlecová & Bíly 2013), apparently they have been sufficient to stop disease transmission during the relatively short period of the acute crayfish plague outbreak.

No crayfish were recorded downstream from Lánský pond as well as in the stretch of the brook near its discharge to the River Sázava (49°39′41″N, 15°23′20″E) during repeated later surveys, although spontaneous re-colonization of the downstream parts by crayfish from Lánský pond seems to be possible. If this is already taking place, the population of *A. astacus* under the pond remains under the detectable level.

Although no crayfish species other than *A. astacus* are known in the catchment of Žebrákovský brook, detection of *A. astaci* from the genotype group B (Table 1, Grandjean et al. 2014) suggests *P. leniusculus* as the original source of infection (Huang et al. 1994). We cannot exclude presence of this species in the brook or its small tributaries and ponds on them, or in the River Sázava. In fact, a well-established dense population of *P. leniusculus* has been recorded recently in the brook Staviště discharging into the Sázava in Žďár nad Sázavou (49°34′12″N, 15°56′16″E), about 40 km away from the Žebrákovský brook (60 km upstream along the river course). Such a downstream distance in a large river may be covered by a quickly expanding *P. leniusculus* population in less than 3 years (Hudina et al. 2009), thus its undetected presence in the river is possible (but see Imhoff et al. 2011 and references therein for examples of much slower downstream dispersal rate of this crayfish in the United Kingdom).

**Senice (crayfish plague outbreak 2008).** A crayfish plague outbreak affecting *A. astacus* occurred in the River Senice downstream from the village Lidečko (Table 1, Supplementary Fig. 7) in August 2008 (this case was mentioned without details in
S̆T̆AMBERGOVÁ et al. 2009). About 20 dead crayfish in different stages of decomposition and one living individual were found during two surveys while other invertebrates and fish remained unaffected (M. BOJDÁ pers. comm.).

No crayfish were caught during a survey in the affected part of the river or at two additional sites 1 km and 8 km upstream from it in July 2010, but two alive A. astacus and crayfish burrows were recorded at the site about 8 km upstream from the site of mass mortality (upstream from Horní Lideč, 49°11'8''N, 18°4'24''E) during fish monitoring in October 2012 (NCA CR). Although no crayfish were found during the following extensive survey at the site of the mass mortality and in four additional sites upstream in 2013, it seems that a scarce population of A. astacus still survives in the catchment of the River Senice. This is supported by information from a local hydrobiologist who has observed a few A. astacus in the upper stretch of the river (near the villages Valašská Senice and Francova Lhota) in recent years (M. HOLZER pers. comm.).

The source of infection remains unknown and it does not seem that the pathogen was transmitted directly from American crayfish, as these are not known from the catchment of the River Bečva, into which the River Senice discharges. Moreover, A. astaci from genotype group A, originally isolated from infected European crayfish (HUANG et al. 1994) and presumably responsible for the 19th century mass mortalities, was detected in the dead crayfish from the River Senice (Table 1, GRANDJEAN et al. 2014). This could point to either chronic pathogen survival in some local indigenous crayfish populations (see VILJAMAA-DIRKS et al. 2011, 2013), or its long-distance spread.

Besének (crayfish plague outbreak 2009). A mass mortality of A. astacus in the Besének brook (case mentioned without details in S̆T̆AMBERGOVÁ et al. 2009) near its confluence with the River Svratka and in the vicinity of the nature reserve Květnice was reported in May 2009 (Table 1, Supplementary Fig. 8). About 3 weeks later, 10 dead or dying crayfish together with two living individuals with no signs of disease hidden in shelters were found in the stretch of the brook about 300 m upstream from the site of the first recorded mortality, suggesting spread of the mortality upstream. Other invertebrates and fish were unaffected.

It seems at present that A. astacus has been completely lost in the Besének brook and no population survived even in the upstream parts of the brook. Neither crayfish nor signs of their presence were found in the affected part of the brook in August 2009 (NCA CR) and during regular spring pupil excursions focusing on hydrobiology (A. JANKOVSKÁ pers. comm.). The same negative results were obtained in the upper parts of the brook during detailed surveys in 2010 and 2013.

Similar to the above-mentioned case, A. astaci from genotype group A was detected in the dead crayfish from Besének brook (Table 1, GRANDJEAN et al. 2014). The source of the pathogen and way of its spread thus remain unknown.

Zákolský brook (crayfish plague outbreak 2009). The history of crayfish populations in the Zákolský brook is partly described in Czech by SVOBODOVÁ (2011). An A. torrentium mortality caused by the crayfish plague was recorded in August 2009 in the Zákolský brook in Podholí (50°10'36''N, 14°15'12''E, see Supplementary Fig. 9). Approximately 10 dead and two living crayfish were recorded at the same site about 3 weeks later; surprisingly, most of them were found in shelters. Other invertebrates and fish were not affected. Already a few weeks earlier (in July 2009), only two small A. torrentium were caught in the Zákolský brook near its confluence with the Dřetovický brook (50°11'00''N, 14°14'53''E) about 1.5 km downstream from Podholí during a
regular survey, although crayfish used to occur there in high population densities in the previous years (VLACH et al. 2009). No A. torrentium were found at this site in September 2009. If the local disappearance of crayfish at this site was related to a crayfish plague outbreak, this might suggest spread of the mortality upstream.

However, tens of living A. torrentium with no signs of disease were recorded in the Zákolanský brook about 1 km upstream from the mortality site near Nový Mlyn (50°10'15''N, 14°16'01''E) in autumn 2009, although no migration barriers are present on the brook between Podholí and Nový Mlyn. The same results were obtained during a detailed survey in the whole Zákolanský brook in 2010; no crayfish were detected in Podholí and downstream from it, while apparently untouched A. torrentium and A. astacus populations were found in the brook near Nový Mlyn and further upstream.

However, further mortalities of A. torrentium and A. astacus were observed in the Zákolanský brook between Nový Mlyn and the village of Číčovice (50°09'22''N, 14°15'02''E) in April 2011. Unfortunately, no suitable samples for detection of A. astaci were available from this mortality; we could only find part of a claw and one whole dried A. astacus on the bank of the brook upstream from Okořský pond (50°09'44''N, 14°15'40''E). Although the test for A. astaci was negative, and water analyses indicated organic pollution (SVOBODOVÁ 2011), we cannot exclude crayfish plague as the reason for these mortalities as well. However, spread of the disease from the downstream parts of the brook does not seem to be the case due to about a 6-m-high weir upstream from Nový Mlyn and about a 4-m-high dam at Okořský pond. Moreover, although indigenous crayfish populations were severely devastated, individual crayfish could be found in this area in 2012 (J. SVOBODOVÁ unpub. data). Austropotamobius torrentium and A. astacus also survive in the Zákolanský brook and some of its tributaries upstream from Číčovice (SVOBODOVÁ 2011, D. FISCHER unpub. data).

More factors might have been involved in mortalities of indigenous crayfish in the Zákolanský brook, as the local water quality has been repeatedly negatively affected (SVOBODOVÁ et al. 2012), and the crayfish plague pathogen was confirmed in the brook as well. Although American crayfish have not been detected in the brook itself until now, O. limosus occur in the River Vltava into which the Zákolanský brook discharges, and may inhabit the mouth of the brook (which is only 10 km downstream from the confluence of Zákolanský and Dřetovický brooks). A population of O. limosus heavily infected with A. astaci is known also from a town pond in Smečno (MATASOVÁ et al. 2011) in the catchment of the Zákolanský brook. Microsatellite genotyping revealed the presence of the A. astaci genotype group E, thus also supporting the assumption that O. limosus was the original source of infection for this mass mortality (Table 1, GRANDJEAN et al. 2014). The reason why the upstream spread of the disease stopped in 2009 despite the absence of apparent migration barriers is unclear, but a similar case has been already described in Italy (CAPIROLI et al. 2013). A feasible option may be a heterogeneous distribution of the crayfish populations in the brook, but data directly supporting this hypothesis are lacking.

Litavka (crayfish plague outbreak 2011). A mass mortality of A. astacus was recorded in the upper part of the River Litavka in a town park in Příbram, downstream from Vysokopecský pond between the end of August and beginning of October 2011 (Table 1, Supplementary Fig. 10). Hundreds of dead and dying as well as living crayfish could be found in the stream (ca 2 m wide in this part) outside shelters, and the mortality did not affect other aquatic animals. The numbers of dying crayfish were surprisingly high, as only one individual of A. astacus had been found (in 2010) during
multiple regular surveys for crayfish in the previous years. In this case, crayfish plague was not only confirmed with molecular analyses, but axenic laboratory cultures of A. astaci were obtained from dying crayfish as well (more details on the isolation procedure and results are available in KOZUBÍKOVÁ-BALCAROVÁ et al. 2013).

Further surveys in 2011, 2012 and 2013 showed that the crayfish plague outbreak remained localized in the upper part of the River Litavka. Important populations of indigenous crayfish in the river catchment (A. astacus in Obecnický, Ohrazenický, Jalový, Červený, Stroupinský, and Suchomastský brooks and A. torrentium in Stroupinský, Kubovský and Bzovský brooks; see Supplementary Fig. 10) were not affected by the disease (NCA CR).

Moreover, A. astacus survived even in the River Litavka itself approximately 25 km downstream and further from the mortality site. This might have been facilitated by industrial pollution (e.g. high concentrations of heavy metals) decreasing water quality downstream from the town of Příbram, which could have two effects: (1) no crayfish probably occurred in that part of the river, or the population was very sparse; thus, discontinuity in the host presence and subsequent limited transmission of the pathogen among the hosts could play a role; (2) A. astaci zoospores could have been affected by the pollutants as well, and their viability and spread downstream might have been reduced.

Although genetic analysis showed that the pathogen detected in dying A. astacus from the River Litavka, as well as A. astaci cultures obtained from them, belonged to the genotype group E (Table 1, GRANDJEAN et al. 2014) originally isolated from O. limosus (KOZUBÍKOVÁ et al. 2011), the source of infection remained unknown until 2013. In September 2013, however, two potential A. astaci carrier species were found in the River Litavka. Several individuals of O. limosus were caught directly at the site of the crayfish plague outbreak, and body parts of O. limosus were found by local children in Vysokopecký pond just upstream of it. Moreover, one individual of the Chinese mitten crab, Eriocheir sinensis H. Milne Edwards 1853, which also may be infected by the disease (BENISCH 1940; SVOBODA et al. 2014), was caught in a hydroelectric power plant connected to the Litavka near the farmstead Janov (49°50'15"N, 13°58'32"E), already in the zone of A. astacus occurrence. Two more crabs had been observed there already in 2012 (J. HAVRLÍK pers. comm.) and, according to children from a school in Příbram, crabs had been released to Litavka by some locals already in 2011.

Six O. limosus and one E. sinensis individual available for DNA analysis tested negative for presence of the pathogen. However, given the relatively low number of samples, we suppose that O. limosus is most likely the original carrier of the infection transmitted to A. astacus. Even if the pathogen prevalence in its populations in the River Litavka is low at present, the species may expand further downstream, and indigenous crayfish surviving in the River Litavka in its tributaries thus remain under severe threat from the crayfish plague. As O. limosus must have been introduced actively by humans to the Vysokopecký pond, serious risk exists that such introductions will be repeated locally.

GENERAL DISCUSSION

Our study confirms that the crayfish plague remains one of the most important threats to indigenous crayfish populations in the Czech Republic. Between 2008 and 2011, five crayfish plague outbreaks were detected in the country, a frequency similar to that in the period 2004–2007 reported previously by KOZUBÍKOVÁ et al. (2006, 2008). Additional crayfish plague outbreaks still occur in the country. While finishing this study (in January 2014), a mass mortality of several hundreds A. astacus was recorded in the Černý brook and in the
adjacent pond U Mlýnečku near Pec pod Čerchovem (Table 1, Supplementary Fig. 11), and *A. astaci* of the genotype group B was confirmed in collected moribund crayfish. The source of infection remains unclear and there is a high risk that the disease will spread downstream and hit populations of *A. torrentium* occurring in the same catchment.

Similar frequency of confirmed crayfish plague outbreaks as in the Czech Republic has been reported also in Germany (C. CHUCHOLL pers. comm.) and France (DIÉGUEZ-URIBEONDO 2009). This is much less than in Fennoscandia (Sweden and Finland) where tens to hundreds of sites affected by the crayfish plague are confirmed yearly (DIÉGUEZ-URIBEONDO 2009; HOLDICH et al. 2009; VILJAMAA-DIRKS et al. 2013). Such differences may simply result from varying quality of the reporting systems, and the level of public awareness on crayfish and crayfish plague. We presume that additional outbreaks probably remain undetected or unreported in the Czech Republic (see discussion in KOZUBIKOVÁ et al. 2008). However, the different attitude to crayfish (widespread exploitation in the Fennoscandian countries vs low commercial interest in the central European ones; HOLDICH et al. 2009), possibly related to the frequency of illegal introductions of American crayfish (BOHMAN et al. 2011), could play a role. Unfortunately, limited data on the frequency of crayfish plague outbreaks are available from other Central European countries where crayfish are not significantly exploited (such as Poland, Slovakia, Hungary and Austria). Therefore, a more detailed comparison of the situation in different European regions is difficult.

All crayfish plague outbreaks described in this study were recorded in small- or middle-sized streams. Such habitats are typical for indigenous crayfish in the Czech Republic (ŠTAMBERGOVÁ et al. 2009) and, moreover, dying crayfish are more noticeable in smaller streams. Although a few cases of crayfish mass mortalities or sudden disappearances of *A. astacus* have recently been detected in Czech standing waters (fish ponds, flooded quarries), either samples were unavailable for *A. astaci* detection in dying crayfish, or the pathogen was not confirmed (KOZUBIKOVÁ et al. 2008; KOZUBIKOVÁ-BALCAROVÁ unpub. data). Thus, our experience is based specifically on crayfish plague outbreaks in running waters.

In such environments, spread of the crayfish mortality is expected downstream as well as upstream from the initial point of the disease outbreak. In both directions, migration of infected crayfish is the main mechanism of spread; downstream transmission of infection is additionally supported by the water current carrying *A. astaci* zoospores. However, as the indigenous crayfish population seems to have been entirely exterminated from the whole brook catchment in only two cases (in Besének and Bojovský brooks) out of 11 described in this study, mechanisms limiting disease spread and thus supporting crayfish survival must have been frequently involved.

Migration barriers on the streams have been already recognized as an effective measure preventing upstream spread of diseases (see RAHEL 2013). Indeed, in three crayfish plague outbreaks mentioned in this study, vertical pond dams apparently saved parts of indigenous crayfish populations. In one case, wetlands with suboptimal conditions for crayfish, and thus low population density, could play such a role. Finally, weirs in the River Olše might have stopped spread of the disease to some of its tributaries. However, no such apparent barriers are present between the sites of crayfish plague outbreaks and crayfish survival in the Zákolanský brook and in the River Litavka. Crayfish survival there might rather have been facilitated by fragmentation of crayfish populations and the limited life span of zoospores (see above).

Most interesting and promising with regards to population survival and recovery are findings of living indigenous crayfish directly at the sites of previous mass
mortalities (in Pšovka, Křivec, Úpořský brook, River Olše, and a tributary of Pěnenský pond). However, we usually cannot distinguish if crayfish presence resulted from recolonization of the particular sites by crayfish from refuges, unreported reintroductions or local survival of some individuals. Such survivors might have escaped infection by \textit{A. astaci}, but the possibility that individuals got infected during crayfish plague outbreaks but did not die cannot be excluded. Studies on the infection presence in the living indigenous crayfish could elucidate whether chronically infected indigenous crayfish occur in the wild also in the Czech Republic. It is possible, as all three European crayfish species occurring in the country have already been confirmed to carry sub-lethal infections elsewhere (\textit{A. astacus} in Finland: \textsc{Viljamaa-Dirks} et al. 2013; \textit{A. leptodactylus} in Romania and Turkey: \textsc{Kokko} et al. 2012; \textsc{Schrmpf} et al. 2012; \textit{A. torrentium} in Slovenia: \textsc{Kušar} et al. 2013).

Apparent recovery took place in the Pšovka brook and the River Olše catchment, leading to the establishment of prosperous populations in the areas affected previously by crayfish plague. Pšovka brook, however, is the stream where the mass mortality had occurred earliest of all studied sites, already in the late 1990s, and population recovery was probably reinforced by translocation of a certain number of \textit{A. astacus} individuals. In the rest of the brooks mentioned in the previous paragraph, only individual crayfish or very sparse populations have been detected in the plague-affected sites up to now. Thus, reintroductions of crayfish from refuges may be helpful, as barriers on the watercourses such as ponds may prevent not only spread of diseases upstream but also slow down the spread of crayfish downstream. However, such activities are only suitable in absence of American crayfish.

The crayfish plague pathogen is apparently transmitted by different pathways and from various sources in the Czech Republic, as supported by detection of three genotype groups of \textit{A. astaci} involved in the described outbreaks (\textsc{Grandjean} et al. 2014). American crayfish (\textit{O. limosus}) were found only in three of the affected streams close to the sites of indigenous crayfish mortalities (Table 1). In such cases, these crayfish are the most probable sources of infection, which is in concordance with detection of \textit{A. astaci} originally obtained from this species (genotype group E; \textsc{Kozubíková} et al. 2011) in the Bojovský brook and the River Litavka. However, it took 6 and 2 years, respectively, before \textit{O. limosus} presence was confirmed at these particular sites. This suggests that also in some other cases (in particular Zákolanský and Žebrákovský brooks, and the Pěnenský pond tributary), American crayfish may live in the vicinity of the crayfish plague outbreaks sites but stay so far undetected.

However, in the remaining cases, direct transmission of the pathogen from American crayfish seems unlikely. In the Úpořský brook, transmission of \textit{A. astaci} zoospores with wet fishing equipment has been suggested (\textsc{Kozubíková} et al. 2008). In the Křivec, Besének and Senice, long-term persistence of infection in populations of indigenous crayfish cannot be excluded, as \textit{A. astaci} of the genotype group A (\textsc{Huang} et al. 1994) has been detected. This pathogen group could have spread relatively recently to the country. However, given the observations of chronic infections in indigenous European crayfish populations (see above), an intriguing scenario cannot be excluded that \textit{A. astaci} had survived in the country since the mass mortalities of indigenous crayfish in the nineteenth century. A hypothesis that such a pathogen survives over long periods in some partially resistant host populations and occasionally spreads to naïve ones should be tested. Our results confirm that this pathogen genotype group is still able to decimate indigenous populations severely, as also reported by \textsc{Viljamaa-Dirks} et al. (2013).
In our study, we have not observed any apparent differences among crayfish plague outbreaks caused by different genotype groups of the pathogen, nor could we see any differences in subsequent recovery of the affected crayfish populations. The development of crayfish populations after the disease outbreaks probably depends on many additional factors, such as genetic variation, local environmental conditions and time passed since the outbreak. Further research is necessary to reveal if co-evolution among the hosts and various pathogen strains takes place in Central Europe, and which conditions may reduce negative effects of crayfish plague on the indigenous crayfish populations.

Despite survival of some indigenous crayfish after most of the crayfish plague outbreaks described in this study, crayfish populations were seriously decimated in all cases we are aware of. Such bottlenecks could make the populations more vulnerable to other negative environmental factors. Although recovery of the populations seems to take place at some sites, it is slow and in all but two cases the crayfish populations have not yet reached the density before the disease outbreaks. Monitoring of the populations should continue to improve understanding of the long-term effect of crayfish plague on the indigenous crayfish in the Czech Republic.

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