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8 Invasive Crayfish and Their Invasive Diseases in Europe with the Focus on the Virulence Evolution of the Crayfish Plague

8.1 Introduction

The high financial and cultural value of the freshwater crayfish in Europe (Lehtonen, 1975; Ackefors, 1998; Edsman, 2004; Jussila & Mannonen, 2004; Jussila *et al.*, 2014a) and the devastation of the native crayfish stocks during 20th century (Alderman, 1996; Souty-Grosset *et al.*, 2006) encouraged fisheries officers and researchers in several European countries to grasp the opportunity to introduce alien freshwater crayfish into Europe (Holdich *et al.*, 2009). The possibility was created by the eradication of the native freshwater crayfish stocks after the introduction of *Aphanomyces astaci*. This parasite was first identified in the River Po valley (Italy) in the 1850s. Afterwards, it gained access to France and Germany, and from there the disease spread rapidly to other European countries (Alderman, 1996). The recommendation that one should always take a cautious approach, stated already at the end of 19th century (e.g. Hubad, 1894), was largely ignored and the bizarre decision was made to introduce alien freshwater crayfish from the geographical region of *A. astaci*'s original distribution (Svärdson, 1965; Westman, 2000; Holdich *et al.*, 2009). Thus, the widespread introduction of different *A. astaci* strains along with their native, comparably *A. astaci*-resistant host species was initiated.

The original introduction of *A. astaci* to Europe was most probably accidental, although its vector is still not known. It took approximately 50 years before astacologists discovered the cause of the mysterious mass mortalities devastating the European crayfish stocks, but by that time, *A. astaci* had spread throughout Europe (Alderman, 1996). The resulting attempts to restore the crayfisheries resulted in an even greater catastrophe: the introduction of several novel alien pathogen strains and new host species that could function as a permanent reservoir for the pathogens. The impact was felt by both the remaining native crayfish stocks and the biodiversity of the European aquatic ecosystems (Souty-Grosset *et al.*, 2006; Nyström, 1999; Nyström *et al.*, 1999; Ruokonen, 2012).

After the turn of the millennium, strategies to deal with alien species were drafted at the EU level, as well as by individual EU member countries (e.g. MMM, 2012; EU, 2013). These strategies clearly tackled the apparent threats posed by the alien invading species. Thus, alien freshwater crayfish (defined as 'detrimental') and their diseases (defined as 'extremely detrimental') were listed as threats to the European ecosystems' biodiversity (MMM, 2012). One should note that in the case of Nordic aquatic ecosystems, a detrimental alien host species (e.g. signal crayfish, *Pacifastacus leniusculus*) is normally a chronic carrier of an extremely detrimental pathogen species (e.g. *A. astaci*). To make matters even more complicated, at least from the academic viewpoint, the alien invaders and their diseases have over time created a novel and complex environmental situation; i.e. not only is there a rapid co-evolution of the native and alien crayfish but there is also their co-adaptations against the disease agent, *A. astaci*. As a result, the resistance of both native European and alien crayfish against the crayfish plague has changed, as has the virulence of the disease agent, *A. astaci* (e.g. Jussila *et al.*, 2011, 2013a, 2014a, b; Makkonen *et al.*, 2012a, b; Makkonen, 2013).

This chapter provides an overview of the introductions of the alien species and speculates on the co-evolution of the parasite and its hosts, in addition to background about the adaptation scenarios. Recent studies on the virulence of *A. astaci* and the relationship between the disease agent and its native European and alien hosts both in the wild and laboratory conditions (Makkonen, 2013; Jussila *et al.*, 2014a,b) make it possible to speculate on the impacts of the introduction of alien species. We feel that the main issue when evaluating the introduction of an alien species should not be their potential financial benefits, but instead the focus should be on avoiding their possible extensive disastrous effects on native ecosystems. By taking a more cautious approach, it should be possible to avoid the devastating consequences presently associated with the introductions. Introducing an alien species into the natural environment is an irreversible act, how hard can it be to understand this simple fact?

8.2 European Crayfish: Indigenous Diversity and Xenodiversity

Before one can understand the spread and evolution of crayfish diseases in Europe, one must first be aware of the past and current patterns in crayfish distributions. The coexistence of crayfish species represents the main route by which a parasite can jump from one host to another. This provides favourable habitat conditions for invasive parasites such as *A. astaci*. In fact, these conditions have been changed extensively during the last 150 years after the first introduction of *A. astaci* to Europe (Alderman, 1996) and they have involved local native crayfish species extinctions, their translocations, and the introduction of new alien species (Souty-Grosset *et al.*, 2006; Holdich *et al.*, 2009; Kouba *et al.*, 2014).

The diversity of the European native crayfish can be stated to consist of five species, at least some of which most probably represent species complexes (Starobogatov, 1995; Largiader *et al.*, 2000; Trontelj *et al.*, 2005; Holdich *et al.*, 2009; Klobučar *et al.*, 2013). As estimated by Souty-Grosset *et al.* (2006), the original distribution ranges of native crayfish species in Europe were to a great extent parapatric, with the overlapping range being mainly confined to South-Eastern Europe (Figure 8.1A). The sympatric occurrence was limited to three species at most, which rarely formed syntopic populations, as indicated by the high displacement rates encountered in the case of introductions even within crayfish plague-free native crayfish species (Stucki & Romer, 2001; Gherardi, 2002). This marked segregation is emphasized by the great genetic phylogeographic divergence of crayfish populations found especially within the *Austropotamobius* genus (Fratini *et al*., 2005; Trontelj *et al*., 2005; Klobučar *et al*., 2013). However, human translocations have significantly changed species ranges and thus have led to compulsory coexistence between species. The first translocations took place in historical times, an example being the translocation of the white-clawed crayfish *(Austropotamobius pallipes)* into Western European countries (Souty-Grosset *et al*., 2006). After a devastating crayfish plague epidemic that eradicated crayfish populations across Europe, intensive crayfish translocation programs were instigated in the 20th century in many European countries in an attempt to restock the lost crayfish populations (Henttonen & Huner, 1999). These were intracontinental translocations of native species, especially of the more important economic species such as the noble crayfish *(Astacus astacus)* and narrow-clawed crayfish *(A. leptodactylus)* (Souty-Grosset *et al*., 2006; Schrimpf *et al*., 2011), but also intercontinental translocations of species that had originated from North America and Australia (Holdich *et al*., 2009; Kouba *et al*., 2014).

Unfortunately, these translocations brought together many native and alien crayfish species, which under natural conditions would not coexist. This situation promotes fierce competitive interactions between species, as well as rapid transmission of diseases. Since the crayfish plague epidemics were the main reason behind the new crayfish translocations, the species that have been introduced can generally be divided into *A. astaci*-susceptible species (native *Astacus* and *Austropotamobius* species, the introduced Australian *Cherax* species) and *A. astaci*-resistant North American species such as the signal crayfish *(P. leniusculus)* and several cambarid species, i.e. *Procambarus* and *Orconectes* (Souty-Grosset *et al*., 2006). The latter group currently encompasses nine species, which are found in natural water bodies across Europe (Kouba *et al*., 2014). The greatest xenodiversity of this group is confined to Western Europe, where most of the introductions had taken place (Figure 8.1B). In contrast, the diversity of susceptible species, currently including five native species and two *Cherax* species (Kouba *et al*., 2014), does not show such a specific regional limitation, but a core of diversity is found across continental Europe (Figure 8.1C), which was mainly a consequence of intracontinental translocations of native species. Overall, due to the recent translocations and introductions, the situation in Europe has changed significantly, and today most crayfish species currently inhabiting European waters exhibit sympatric distribution patterns, and in many cases syntopic populations, this being especially true in Western and Central Europe (Figure 8.1D). This novel biogeographic pattern in crayfish fauna in Europe has to be taken into account when dealing with disease transmissions and the evolution of the pathogen with rapid jumping from one host to another, including mixtures of different genotypes.

Here, we will briefly present the status of the alien invasive crayfishes in Europe, with a special emphasis on their potential to colonise and also spread the alien disease agents that they could carry, especially *A. astaci*. The chapter should give enough background to highlight the devastating potential that the alien crayfish pose not only to their native European counterparts but also to aquatic ecosystem biodiversity.

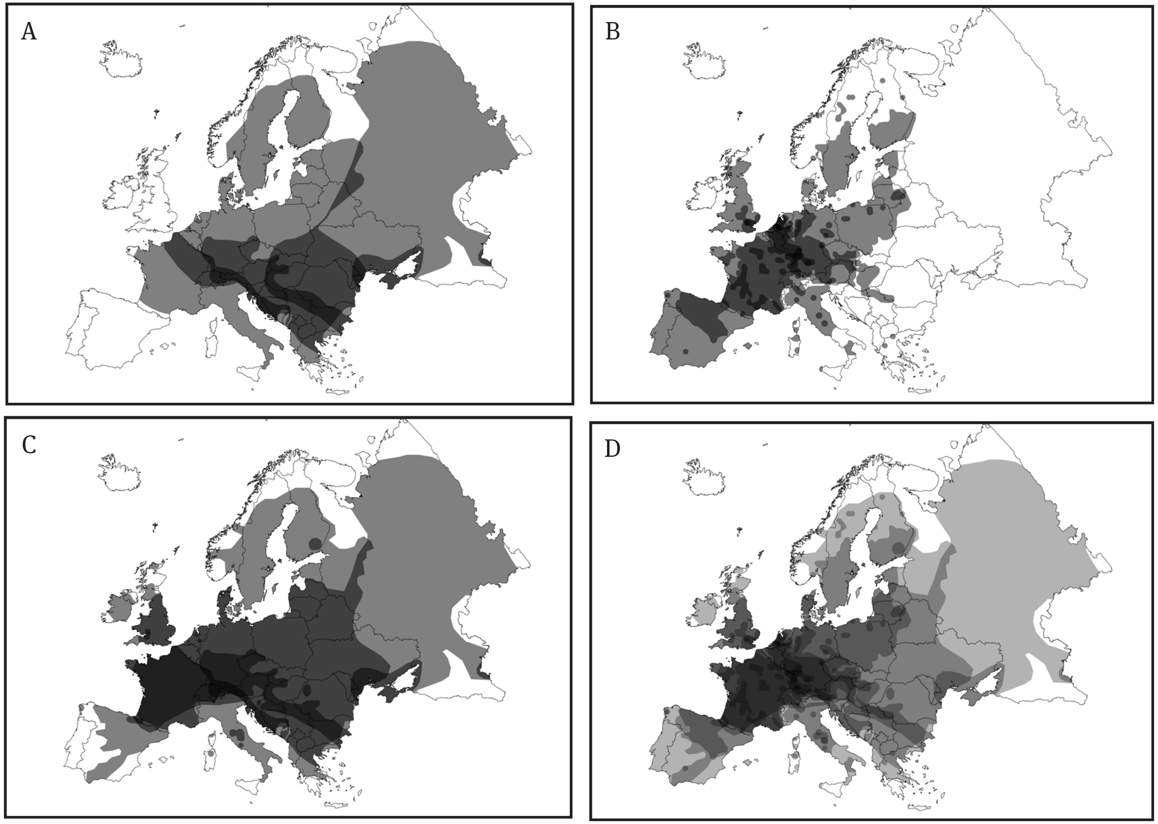

Fig. 8.1: Overlaps in the distribution ranges of crayfish species in Europe as indicated by intensity of grey shadowing (light grey indicates presence of only one crayfish species) with (A) original distribution patterns of native species (*Astacus*, *Austropotamobius*), (B) recent update of distribution patterns of introduced crayfish plague-resistant North American species (*Procambarus*, *Orconectes*, *Pacifastacus*), (C) recent update of distribution patterns of native and introduced crayfish plague-susceptible species (*Astacus*, *Austropotamobius*, *Cherax*), and (D) recent update of overall distribution patterns of all crayfish species currently present in Europe in the wild (data modified from Souty-Grosset *et al.*, 2006; Kouba *et al.*, 2014).

8.2.1 Signal Crayfish (*Pacifastacus leniusculus*)

The rationale for the introductions of the signal crayfish to Europe was the assumption that it could rejuvenate the crayfisheries in those water bodies where crayfish plague prevented the recovery of the wild native crayfish stocks (Fjälling & Fürst, 1985; Westman, 2000; Kirjavainen & Sipponen, 2004; Bohman *et al.*, 2011). The signal crayfish introductions were initiated in the late 1950s with the first experimental shipments arriving in Sweden (Svärdson, 1995; Holdich *et al.*, 2009), followed by an increasing number of introductions in Sweden and Finland. Initially, the signal crayfish were restricted to experimental farms, mostly owned by government research institutes and later to commercial farms where large numbers were transferred into natural water bodies. The introduced signal crayfish had been captured from wild stocks living in the western parts of North America, mainly from Lake Tahoe and Lake Hennessy (Abrahamsson, 1969; Westman, 1973). The introduced stocks had originated from a mixture of sites (Larson *et al.*, 2012) and it has been recently discovered that the species *Pacifastacus leniusculus* is indeed a combination of three subspecies (Agerberg & Jansson, 1995; Larson *et al.*, 2012). In addition to these introductions into Northern Europe, the signal crayfish were also transferred to continental Europe, e.g. into Austria from California and into France from Oregon (Souty-Grosset *et al.*, 2006). Currently the signal crayfish is the most widely distributed alien crayfish in Europe; in the majority of other countries it was introduced from the original Swedish stock dispersed naturally, a process which continues rapidly at the present time (Holdich *et al.*, 2009; Kouba *et al.*, 2014).

It is now generally acknowledged that the signal crayfish acts as a chronic carrier of PsI-genotype *A. astaci* (Alderman *et al.*, 1990; Bohman *et al.*, 2006; Filipová *et al.*, 2013; Viljamaa-Dirks *et al.*, 2013; Jussila *et al.*, 2014a, b) with recent evidence indicating that other *A. astaci* genotypes might also infect these crayfish (Aydin *et al.*, 2014). In addition, PsII-genotype *A. astaci* has been reported to infect the signal crayfish (Huang *et al.*, 1994). Signal crayfish also seem to be affected by opportunistic pathogens when they are living in the Nordic countries (Persson & Söderhäll, 1983; Thörnqvist & Söderhäll, 1993; Edsman *et al.*, 2015).

8.2.2 *Procambarus* Species

The most widespread and invasive *Procambarus* species in Europe is the red swamp crayfish *(Procambarus clarkii)*, which was introduced into Spain from Louisiana in 1973 (Souty-Grosset *et al.*, 2006). Due to its wide ecological adaptation to different types of water bodies, including seasonally flooded wetlands, it has spread rapidly and ultimately has become an important commercial species in Spain. This led to new illegal introductions not only throughout Spain, but also in France and Italy, and later in the Netherlands, Germany, and to many islands including Great Britain, Azores, Canary Islands, Balearic Islands, Sardinia, Sicily and even Cyprus (Holdich *et al.*, 2009). This species is still spreading rapidly across continental Europe (Kouba *et al.*, 2014), but its northern expansion to colder climates might be restricted since it is a warm water species (Souty-Grosset *et al.*, 2006).

The red swamp crayfish has long been known to be a carrier of *A. astaci* (Diéguez-Uribeondo & Söderhäll, 1993; Aquiloni *et al.*, 2011). The species is thought to be quite tolerant against this disease, but stressful conditions might cause death and extensive sporulation of *A. astaci* (Souty-Grosset *et al.*, 2006). This species has a major influence on aquatic ecosystems not only through disease transmissions to native crayfish populations, but also since it is an efficient polytrophic predator, exerting a detrimental impact on species living at several trophic levels in the aquatic animal community (Renai & Gherardi, 2004) and on macrophytes with indirect effects even at higher trophic levels, i.e. waterbirds (Souty-Grosset *et al.*, 2006).

In Europe at the moment, three more *Procambarus* species from North America have been identified in the wild (Kouba *et al.*, 2014): the marbled crayfish *(P. fallax* f. *virginalis)*, white river crayfish *(P.* cf. *acutus)*, and Florida crayfish *(P. alleni)*. The marbled crayfish (*Procambarus fallax*) was introduced to Europe as an aquarium pet, due to its visual appearance and possibly also for its efficient reproduction, only later discovered to be parthenogenetic (Scholtz *et al.*, 2003; Holdich *et al.*, 2009). The first documented wild population was detected in Germany in 2003 (Marten *et al.*, 2004) and since then there have been reports of small, scattered, introduced and established populations in the Netherlands, Italy and Slovakia (Soes & van Eekelen, 2006; Marzano *et al.*, 2009; Chucholl *et al.*, 2012). The species has also been found in Sweden but it does not seem to be properly established there yet (Bohman *et al.*, 2013). In fact, at least 25 independent introductions have been recorded in Europe, and the probability of expansion of this species has been shown to be very high. For this reason, it is essential that consistent trade regulations be urgently introduced in order to limit its spread and to prohibit further introductions (Chucholl, 2014). The marbled crayfish can be a carrier of *A. astaci* (Scholtz *et al.*, 2003; Holdich *et al.*, 2009) and our experiments have indicated that marbled crayfish may exhibit elevated resistance towards crayfish plague (unpublished data). In summary, it seems that the marbled crayfish is a potential colonizer and dangerous carrier of *A. astaci*. The taxonomic position of the white river crayfish is not yet clear and includes at least the white river crayfish *(P. acutus)* as well as the southern white river crayfish *(P. zonangulus)* (Kouba *et al.*, 2014). The introduction of this taxon in Europe (Spain) in the 1970s failed (Henttonen & Huner, 1999), but an established population was detected in the Netherlands in 2005 (Soes & van Eekelen, 2006) and later in Great Britain (Kouba *et al.*, 2014). At present, little is known about its possible threat to the European native crayfish. The Florida crayfish *(Procambarus alleni)* is freely available through the aquarium trade in Europe, and although individual specimens have been caught at several sites in France and Germany, it is not presently known if there is an established population (Kouba *et al.*, 2014).

8.2.3 *Orconectes* Species

The spiny-cheek crayfish *(Orconectes limosus)* is native to North America, where its original distribution area was in the Northeastern United States (Hamr, 2002). In Europe, *O. limosus* was first introduced to Poland in 1890 (Kossakowski, 1966; Aklehnovich & Razlutskij, 2013) and is thus the oldest alien crayfish species known in Europe. At present, it has been identified in at least 22 countries (Kouba *et al.*, 2014). *O. limosus* is currently spreading towards Northern Europe, having reached Lithuania (Arbačiauskas *et al.*, 2011); towards Eastern Europe in Belarus (Alkenovich & Razlutskij, 2013); and towards South Eastern Europe via the River Danube (Hudina *et al.*, 2009; Pârvulescu *et al.*, 2009). Based on genetic variability studies, there has likely been only a single incident of introduction of *O. limosus* into Europe (Filipová *et al.*, 2009; Filipová *et al.*, 2011) and thereafter the spread has occurred both naturally and by human-mediated translocations.

O. limosus has also been shown to carry *A. astaci* (Kozubíková *et al.*, 2011a; Matasová *et al.*, 2011; Pârvulescu *et al.*, 2012; Schrimpf *et al.*, 2012). However, the prevalence of the infected individuals seems to be very variable in different populations (Kozubíková *et al.*, 2011a; Matasová *et al.*, 2011) and in some cases, it seems that non-infected populations do exist. Some of these populations even coexist with native European crayfish species (Schrimpf *et al.*, 2013a). Similarly to the signal crayfish (Strand *et al.*, 2012), *O. limosus* has been shown to pose a constant threat to native European crayfish species due to the continual release of infectious *A. astaci* spores from infected individuals (Svoboda *et al.*, 2013). Although it has long been known that *O. limosus* can act as a vector for *A. astaci* (Vey *et al.*, 1983), it was only recently discovered that it also carries a novel genotype of *A. astaci* (Kozubíková *et al.*, 2011b). This Or-genotype can be differentiated from other known genotypes by the random amplification of polymorphic DNA (RAPD) technique, or with microsatellite markers (Grandjean *et al.*, 2014). Unfortunately, due to the rather limited availability of these novel strains, there is still very little information about its specific characteristics.

In addition to *O. limosus,* at least three other *Orconectes* species have been introduced into Europe since 1990, and all of them are regarded as non-susceptible *A. astaci* carriers (Schrimpf *et al.*, 2013b; Kouba *et al.*, 2014): calico crayfish *(O. immunis)*, Kentucky River crayfish (*O. juvenilis*), and virile crayfish *(O.* cf. *virilis).* The calico crayfish was first detected in the wild in 1997 in Germany (Dehus *et al.*, 1999), and since then it has rapidly colonized the Upper Rhine system both up- and downstream, and is now spreading into France (Chucholl, 2012). It is a species that exerts a major impact on the ecosystem. In the River Rhine, *O. immunis* came into contact with the previously established *O. limosus* and has ultimately displaced it (Chucholl, 2012). This represents a new perspective about recent introductions, in the sense that one may have artificially created unsustainable syntopic populations. The species has been shown to be an *A. astaci* carrier with a relatively high infection prevalence (Schrimpf *et al.*, 2013b). The Kentucky River crayfish (*O. juvenilis*) was first discovered in France in 2005 (Chucholl & Daudey, 2008), suspected to have escaped from a pond into the River Dessoubre. Though this alien population is still rather limited, it is considered to represent a threat to native European species due its potential to act as a carrier of *A. astaci*, its rapid life cycle, its high fecundity and its pollution tolerance. One attempt was made to eradicate this restricted established population but was not successful (Kouba *et al.*, 2014).

The identity of the fourth *Orconectes* species established in Europe is less clear, since it belongs to the virile crayfish *(O.* cf. *virilis)* species complex. The species introduction history in Europe is quite long, since the first imports are believed to have happened in France in 1897 and then were repeated in 1960 in Sweden, but apparently both trials failed (Souty-Grosset *et al.*, 2006). However, in 2004 a thriving population was discovered in the Netherlands (Soes & Koese, 2010), which has spread so rapidly that it has even displaced the already established *O. limosus* (Kouba *et al.*, 2014). Furthermore, their appearance in the wild, presumably after escaping from an aquarium, took place in 2004 in London (UK) from where they have started to disperse at a rate 2 km y^1 (Holdich *et al.*, 2009). Newly introduced *Orconectes* species have been demonstrated to possess a high invasive potential and dispersal capability, and one can predict that in the future they will contribute to a significant increase in overall crayfish xenodiversity in Europe.

8.2.4 *Cherax* Species

There seems to be two reasons to account for the introduction of the different *Cherax* species throughout Europe: 1) the aquarium trade (Holdich *et al.*, 2009) and 2) the possibility for farming (Souty-Grosset *et al.*, 2006). The putative aquaculture potential of these crayfish was based on experiences from Australia and also from the belief that the *Cherax* species could tolerate elevated temperatures (Morrissy, 1990; King, 1994), which were also benefits in the eyes of aquarium enthusiasts. Currently, at least two species have found their way into the wild in Europe: the yabbie (*Cherax destructor*) and the redclaw (*C. quadricarinatus*). The yabbie has been successfully introduced to Catalonia and Navarra in Spain from stock imported from California in 1983, and to Zaragosa between 1984 and 1985 (Souty-Grosset *et al.*, 2006), and Italy in 2008 (Scalici *et al.*, 2009a, b). The redclaw is extensively sold live across Europe through the aquarium trade as well as for farming (Kouba *et al.*, 2014). In 2009, this species was found to be established in a small thermal oxbow lake in Slovenia, but several single specimens have been caught in different parts of Europe as well (Jaklič & Vrezec, 2011). Both *Cherax* species are so far restricted to isolated water bodies, although their spread potential is a matter of concern.

As an alien species, *Cherax* pose a potential threat to the native European crayfish even though they are regarded as susceptible to *A. astaci* infection. The population of yabbie in Navarra has actually been successfully eradicated by introducing *A. astaci*, and there have been reports of devastating outbreaks of crayfish plague in farmed redclaws at Sicily (Kouba *et al.*, 2014). Furthermore, all *Cherax* species introduced to Europe are fast growing and reproduce efficiently (Lawrence & Jones, 2002), and thus they possess the potential for colonization and to spread further if there is not an immediate instigation of an eradication campaign of existing established populations (Tricarico *et al.*, 2010).

8.3 Signal Crayfish in the Nordic Context

The widespread introduction of the signal crayfish into Europe was initiated in the Nordic Countries in order to revitalise the crayfisheries; a void had been created by the eradication of the majority of the wild noble crayfish stocks by crayfish plague epidemics and changes in aquatic ecosystems. The driving force behind these rather hasty actions was the tradition of crayfish trapping and related cultural festivities (Jussila *et al.*, 2014a). The signal crayfish has now achieved an established position in the Nordic countries and currently accounts for the majority of the annual crayfish catch (Jussila & Mannonen, 2004; Fiskeriverket, 2005). Its commercial value and the general relevance of crayfish as the centrepiece of crayfish parties—an important occasion in the late summer in the Nordic countries—has allowed these alien signal crayfish to effectively take the place of the native noble crayfish both in Nordic aquatic ecosystems and as the crayfish being celebrated and consumed in crayfish parties (Ackefors, 1998). Unfortunately, the negative aspects of the signal crayfish on the aquatic ecosystem (Nyström, 1999; Ruokonen, 2012), and especially on the native crayfish (Bohman *et al.*, 2006), have been largely ignored and the continuous spreading of this invasive alien crayfish has even been encouraged (Jussila *et al.*, 2014a).

In Finland and Sweden, the first signal crayfish introductions were restricted to the southern parts of the countries (Jussila & Mannonen, 2004; Kirjavainen & Sipponen, 2004). There were several reasons for limiting the introductions to the southern parts, e.g. the environmental threats of the signal crayfish and the protection of the existing productive noble crayfish stocks. However, once the introductions of the signal crayfish had started, there were also illegal introductions. Now the signal crayfish has spread very efficiently, paying no heed to various national and regional crayfisheries strategies, legal proceedings against illegal stockings, and intensive and innovative information campaigns (Jussila *et al.*, 2014a). The strong cultural ties between the crayfish and the Nordic peoples have only assisted the spread of the alien signal crayfish, similar to the situation with the native noble crayfish some 150 years earlier (Kilpinen, 2003). This has created a situation where alien crayfish stocks are growing in numbers, crayfish plague is commonplace, and the very existence of the native noble crayfish is threatened.

Contrary to earlier belief, the signal crayfish has been proven to be susceptible to *A. astaci* (Persson & Söderhäll, 1983; Thörnqvist & Söderhäll, 1993; Aydin *et al.*, 2014) to such an extent that several collapses of wild stocks have been reported (Jussila *et al.*, 2014a, b; Sandström *et al.*, 2014). The signal crayfish has been shown to act as a chronic *A. astaci* carrier (Alderman *et al.*, 1990; Bohman *et al.*, 2006; Diéguez-Uribeondo, 2006; Jussila *et al.*, 2014a, b), thus spreading the disease presumed to be the virulent PsI-genotype. This chronic crayfish plague infection has even been noted to decrease their commercial value (e.g. Jussila *et al.*, 2013b). There have been collapses of the signal crayfish populations, but other effects have also been reported (Smith & Söderhäll, 1986; Pakkasmaa, 2006; Jussila *et al.*, 2013b; Edsman *et al.*, 2015; Jussila *et al.*, 2014a, b). The present rate of stock collapses in the natural environment has been estimated to be at least 10% of all known stocks in Finland and Sweden (Sahlin *et al.*, 2010; Jussila *et al.*, 2014a; L. Edsman, personal communication, November 13, 2013). Another stressful factor on the stocks of signal crayfish are the increasingly unpredictable micro-climatic changes, like very rapid water cooling in autumn or the lakes becoming ice-covered but then thawing soon afterwards, with the latter process possibly repeating itself several times during one winter. As the climate becomes warmer, the habitat suitable for the signal crayfish is spreading northwards (Capinha *et al.*, 2013) and the belief that the native noble crayfish stocks would be protected by the long cold winters may be naïve. It is also worth noting that crayfish immune defences seem to have an innate seasonal clock (Gruber *et al.*, 2014b), and the related patterns may be distorted by predicted climatic change. So far, the 65th northern parallel has been taken as the upper limit for the signal crayfish because it cannot reproduce in colder climatic conditions (Heinimaa & Pursiainen, 2010), but milder winters will tend to push that boundary further north.

The signal crayfish is having an adverse effect on the biodiversity of aquatic ecosystems (Nyström, 1999; Ruokonen, 2012; Ercoli, 2014) since it is exerting an impact on macro-invertebrate richness and community composition. The signal crayfish acts as a link between littoral and profundal areas and may even interfere with the abundance of fish parasites. In addition to acting on the ecosystem level, huge signal crayfish populations can interfere with traditional ways of fishing by becoming tangled in fishermen's nets, as some of the best sites for catching whitefish (*Coregonus* sp.) are the stony hard-bottom shallows of the water bodies, which signal crayfish also inhabit.

8.4 The Diseases of the Invasive Crayfish

8.4.1 Crayfish Plague (*Aphanomyces astaci*)

The first mass mortalities of native crayfish were detected in Lombardy (Italy) in 1859 (Cornalia, 1860). During the next three decades, the crayfish plague spread rapidly from France and Germany all over Continental Europe, both east and west, mainly along large river watersheds (Alderman, 1996), reaching Finland via Russia in 1893 (Järvi, 1910). From Finland, the disease then spread to Sweden in 1907 and from Sweden to Norway in 1971, but only in the late 1970s and 1980s did it extend into Spain, Greece and further to Turkey, and to Great Britain and Ireland (Alderman, 1996). During this first wave of epidemics, human activities associated with the crayfish trade were the main reason for the fast spread of the disease (Alderman, 1996). The vector for the first disease wave was most likely an infected crayfish of North American origin (Unestam, 1972; Unestam, 1975a, b). However, the species of this first vector still remains unidentified (Makkonen, 2013). In Europe, there is now a large area where the alien pathogen *A. astaci* has probably been coexisting with crayfish populations for 100 years or more. This area is bounded by France in the west, Italy and the northern Balkans in the south, Russia in the east and Finland and Sweden in the north (Figure 8.2). This represents almost the entire continent of Europe and encompasses the distribution ranges of more or less all native crayfish species, potentially allowing gradual adaptation of the pathogen to native crayfish species populations (e.g. Gruber *et al.*, 2014a).

The first wave of crayfish plague spread was mainly reported as mass mortalities and population eradications in the native crayfish stock. However, there was a second wave of crayfish plague epidemics during the 1990s due to introductions and therapid spread of alien North American crayfish species, which act as *A. astaci* carriers (Persson & Söderhäll, 1983; Huang *et al.*, 1994; Vennerström *et al.*, 1998; Oidtmann *et al.*, 1999; Vogt, 1999; Oidtmann *et al.*, 2006; Kozubíková *et al.*, 2008). Both crayfish plague epidemic waves probably overlapped in time at least in the 1980s and 1990s, which is well documented in many regions, for example in the Czech Republic (Kozubíková *et al.*, 2008).

To illustrate the spread and effects of *A. astaci* in crayfish stocks, we will use two countries as case studies, one from the south and the other from the north of Europe. In Slovenia in southern Europe, the first wave of crayfish plague was detected quite early in 1880, and lasted until 1935 (Franke, 1889; Šulgaj, 1937). The disease spread from the River Danube drainage basin and affected crayfish populations in all the main rivers draining into the River Danube (Figure 8.3). There was no record of any outbreaks of crayfish plague in western Slovenia in the Adriatic drainage or in adjacent parts of northern Italy despite their close vicinity to the first European reported occurrence of crayfish plague in the River Po. As already indicated by Alderman (1996), this may not necessarily reflect some kind of limitation of disease spread to Italy, but it may be a result of poor documentation due to national boundaries in this region, i.e. between Italy and the Austro-Hungarian Empire. However, the Museum of Natural History in Vienna has preserved specimens of the white-clawed crayfish with clear signs of crayfish plague infection; these specimens were collected in 1892 near Gorica (Gorizia) by the River Soča (Isonzo) from the Adriatic drainage region (A. Vrezec & M. Jaklič, unpublished). This indicates that the spread from the River Po was actually more extensive than that described by Alderman (1996). After the outbreaks of crayfish plague, there were collapses in almost all crayfish populations in the large rivers in Slovenia, especially of the noble crayfish, which had previously been an important and heavily trapped species (Šulgaj, 1937) but were never to be revitalized. Numerous attempts at restocking noble crayfish populations were either completely unsuccessful or limited to only smaller streams (Budihna, 1996). In 2003 and 2007, the first alien signal crayfish invasions took place in the River Mura and River Drava from populations introduced into Austria (Bertok *et al.*, 2003; Vrezec *et al.*, 2013), and these animals were known to be *A. astaci* carriers (Kušar *et al.*, 2013). This is the first incident of a crayfish plague disease agent occurring in Slovenia after 1935, although no mass mortality outbreaks have been described so far.

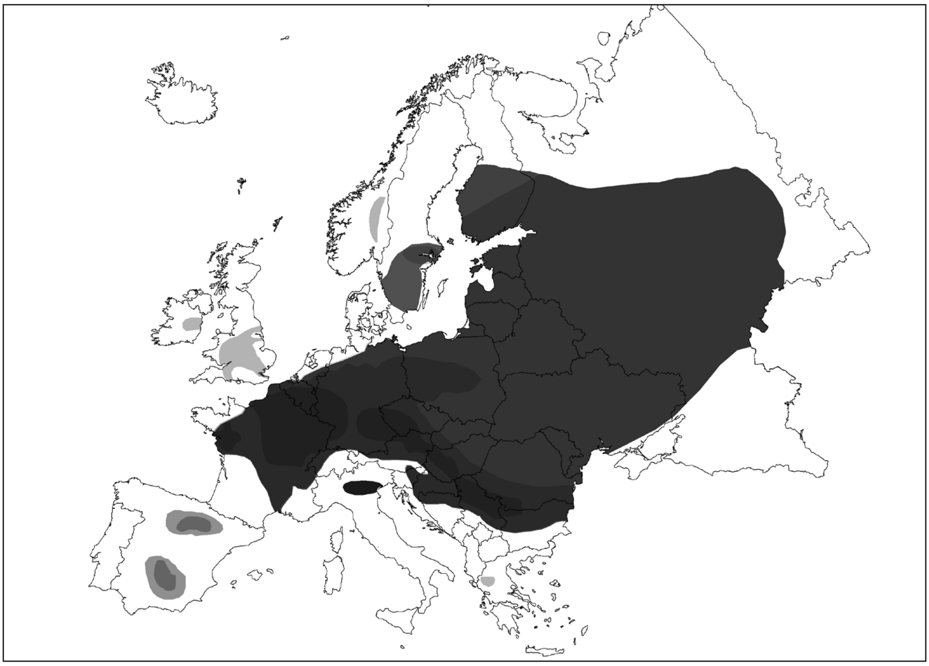

Fig. 8.2: Dynamics of the first wave of crayfish plague expansion across Europe between 1860 and 1995 (modified after Alderman, 1996). The expansion is shown according to the following time periods: 1860–1869 (the darkest), 1870–1879, 1880–1889, 1890–1899, 1900–1909, 1910–1929, 1950–1969, 1970–1979, and 1980–1995 (the brightest).

Our second case is Finland in northern Europe, where the first wave of crayfish plague epidemics represented an enormous setback to crayfish fishermen and the associated trade (Järvi, 1910). Noble crayfish were intensively restocked into the affected lakes, but quite often after the recovery of the population to an exploitable level within 10 to 20 years, the disease struck again, causing a new collapse (Fürst, 1995; Erkamo *et al.*, 2010). As a solution to the chronic crayfish plague infection problem, re-stockings with the supposedly "crayfish plague resistant" signal crayfish were initiated during the 1960s (Westman, 1973). Subsequently it was discovered that the signal crayfish often carried the *A. astaci* infection. Thus, the second and still ongoing wave of outbreaks of crayfish plague in Finland can be traced to the introduction of these alien

North American crayfish species. These species were able to spread the novel strains of *A. astaci* permanently to new areas, when new habitats became colonized. Surprisingly, the distribution and prevalence of *A. astaci* in North America is unclear (Huang *et al.*, 1994; Makkonen *et al.*, 2012a), since the disease agent does not trigger dramatic population collapses there.

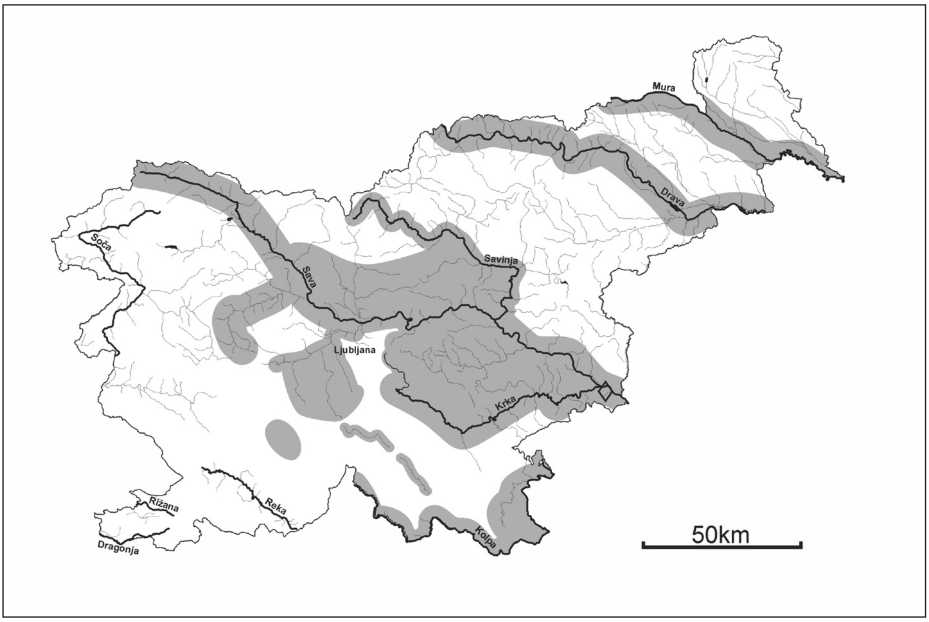

Fig. 8.3: The estimated distribution (light grey) of *Aphanomyces astaci* in the first crayfish plague wave in Slovenia, which took place between 1880 and 1935 (after Kušar *et al.*, 2013).

The infective units of *A. astaci*, zoospores, are viable in temperatures between 2 and 25°C (Unestam, 1969). The Pc-genotype of *A. astaci* is an exception, since it has been shown to have an even higher temperature optimum as an adaptation to warmer environmental conditions (Diéquez-Uribeondo *et al.*, 1995). American crayfish species, which are carriers of *A. astaci,* have been demonstrated to constantly release minor but nonetheless lethal amounts of spores into the ambient water (Diéquez-Uribeondo & Söderhäll, 1993; Strand *et al.*, 2012; Svoboda *et al.*, 2013). There is a continual production of the spores, although the largest numbers of spores are released during molting and at the death of the crayfish (Strand *et al.*, 2012; Svoboda *et al.*, 2013), as had been previously postulated (Oidtmann *et al.*, 2002). Therefore, the presence of introduced species carrying *A. astaci* do pose a serious and ever-present risk to the surrounding native populations (Strand *et al.*, 2012; Svoboda *et al.*, 2013; Strand *et al.*, 2014).

Currently, five genotypes of *A. astaci* are known (Table 8.1) to infect native and alien crayfish in Europe (Huang *et al.*, 1994; Diéguez-Uribeondo *et al.*, 1995; Kozubíková *et al.*, 2011b). The As-genotype has been traced back to the first invasion of *A. astaci*, before the importation of signal crayfish. The PsI-genotype and PsII-genotype have been found not only in the signal crayfish, but also in the native European species after signal crayfish introductions. The PsI-genotype has been attributed to the signal crayfish originating from Lake Tahoe and Lake Hennessey (USA), which were the main sources of the imported animals (Abrahamsson, 1969; Westman, 1973). A single isolate of the PsII-genotype has been linked to the introduction of signal crayfish from Lake Pitt (Canada) to Sweden (Huang *et al.*, 1994) but, based on current knowledge, the PsII-genotype is not widely present in Europe (Söderhäll & Cerenius, 1999). The Pc-genotype was brought here along with the red swamp crayfish (Diéguez-Uribeondo & Söderhäll, 1993; Diéguez-Uribeondo *et al.*, 1995) and the Or-genotype accompanied the spiny-cheek crayfish (Kozubíková *et al.*, 2011b).

Tab. 8.1: Genotypes of *Aphanomyces astaci*.

Genotype	Original host	Reference
As	unknown	Huang *et al.* (1994)
PsI	Signal crayfish (*P. leniusculus,* Lake Tahoe, USA)	Huang *et al.* (1994)
PsII	Signal crayfish (*P. leniusculus,* Lake Pitt, Canada)	Huang *et al.* (1994)
Pc	Red-swamp crayfish (*P. clarkii,* Louisiana, USA)	Diéguez-Uribeondo *et al.* (1995)
Or	Spiny-cheek crayfish (*O. limosus,* USA)	Kozubíková *et al.* (2011a)

Laboratory infection trials have shown extensive variation in the virulence of different *A. astaci* strains (Makkonen *et al.*, 2012b; Makkonen *et al.*, 2014). In general, strains of PsI-genotype seemed to possess considerably higher virulence (Makkonen *et al.*, 2012b; Jussila *et al.*, 2013a; Makkonen *et al.*, 2014; Gruber *et al.*, 2014a) in comparison with the strains of As-genotype which, on the other hand, appear to be more variable in their virulence (Makkonen *et al.*, 2012b; Makkonen *et al.*, 2014). Furthermore, latent crayfish plague infections without mass mortalities have recently been reported in the native European noble crayfish (Jussila *et al.*, 2011; Viljamaa-Dirks *et al.*, 2011), narrow-clawed crayfish (Kokko *et al.*, 2012; Svoboda *et al.*, 2012), and stone crayfish (Kušar *et al.*, 2013) populations, and most recently in the white-clawed crayfish (Manfrin & Pretto, 2014).

8.5 The Adaptation of *Aphanomyces astaci* in Europe

8.5.1 The Virulence Evolution of the *Aphanomyces astaci*

A. astaci has been under high selective pressure to adapt to the European crayfish hosts and its new environmental conditions since its arrival in the 1860s in the River Po valley (Alderman, 1996). The highly susceptible European crayfish species did not allow *A. astaci* to establish a long-term habitat, as these animals were mostly eradicated in crayfish plague epidemics. In this situation, the parasite itself was facing an evolutionary dead-end, as it was destroying the wild native European crayfish stocks, i.e. its habitat. Of course, the European crayfish were also encountering a major challenge to their existence, and they had to find some way to adapt to this novel disease, which was being efficiently spread by human activity (Kilpinen, 2003). Time was quickly running out for both the parasite and its host populations.

Within 100 years, there is indirect evidence to indicate that some of the European crayfish populations may have survived repeated crayfish plague epidemics (Fürst, 1995; Kilpinen, 2003), and there is now new evidence from Turkey that the narrow clawed crayfish stocks under certain circumstances could survive, at least to some extent, a crayfish plague epidemic (Harlioğlu, 2004; Kokko *et al.*, 2012; Svoboda *et al.*, 2012) and even recover afterwards. In this section, we will present some potential scenarios that could explain the newly established balance between the parasite and its hosts, i.e. the *A. astaci* – native European crayfish co-evolutive adaptation process. From an evolutionary point of view, the parasite-host interaction usually results in strong selection pressure for the parasite, and not the host, to favour a low virulent parasite which infects but does not kill the host (Tokeshi, 1999).

8.5.2 The Adaptation of *Aphanomyces astaci* to its Native and Alien Hosts

It has been long presumed that *A. astaci* may have adapted to its European hosts and that its virulence may have declined, but it is only in the last decade that it has been possible to demonstrate this experimentally (Jussila *et al.*, 2011; Makkonen, 2013; Jussila *et al.*, 2014a). Wild native European crayfish stocks, which are viable and producing commercial catch, have been shown to be latent carriers of *A. astaci* (Jussila *et al.*, 2011, Viljamaa-Dirks *et al.*, 2011). Laboratory-scale infection studies have revealed significant virulence differences among *A. astaci* isolates, both among and within the As-genotype and PsI-genotype, and even the existence of very low virulent isolates (Makkonen *et al.*, 2012; Jussila *et al.*, 2013a; Makkonen, 2013).

The alien crayfish, especially the signal crayfish, have been shown to be susceptible to *A. astaci*, which points to the high virulence of the PsI-genotype and possibly a lowered resistance of the signal crayfish towards *A. astaci* (Jussila *et al.*, 2014a; Aydin *et al.*, 2014). Laboratory experiments have demonstrated that the PsI-genotype of

A. astaci, although highly virulent, is also capable of exhibiting significant virulence variation (Jussila *et al.*, 2013a). This indicates that even European based PsI-genotypes could be adapting, while the presence of a permanent host habitat for the PsI-genotype *A. astaci* allows for the maintenance of high virulence without the immediate threat of the parasite's evolutionary suicide due to the outbreak of a devastating crayfish plague epidemic. Thus, the adaptation pressure of *A. astaci* strains capable of infecting North American crayfish species in Europe is significantly different than that of the *A. astaci* strains infecting only native European crayfish species.

8.5.2.1 The Effect of the Host Jumps on the Adaptation Process

The As-genotype *A. astaci*, after its presumed arrival in Europe in the 1850s (Alderman, 1996), had access to a variety of host habitats among the European native crayfish spectrum, a total of five different species (Souty-Grosset *et al.*, 2006). The European crayfish species were all susceptible to *A. astaci*, and the outcome of the crayfish plague epidemic during the first decades was a complete elimination of host populations. If it had not had assistance from humans, the disease might have had a short history in Europe, but the disease agent was unintentionally aided by transferring it to new water bodies and populations. The rapid and efficient spreading allowed for both the constant presence of epidemics and chance to host jump from one European crayfish species to the next and, apparently, to jump back to the species that it had already infected.

The As-genotype *A. astaci* was thus allowed a longer time to adapt to novel conditions and access to crayfish that were highly susceptible to the disease. One could postulate that this scenario was fundamental for the lately discovered putative better balance between *A. astaci* and European crayfish (Jussila *et al.*, 2014a). The theory that there was a chronic crayfish plague infection was proposed in the last century (e.g. Fürst, 1995), but only verified during the past decade (Jussila *et al.*, 2011; Viljamaa-Dirks *et al.*, 2011). Since then, there have been several reports of native European crayfish being apparent latent carriers of *A. astaci* (Kokko *et al.*, 2012; Svoboda *et al.*, 2012; Kušar *et al.*, 2013; Viljamaa-Dirks *et al.*, 2013). In addition to the host jumps as such, the different *A. astaci* strains have been transferred between different aquatic habitats, i.e. different ambient aquatic conditions, which include, for example, differences in water quality and temperature regime. These habitat jumps would have added to the adaptation pressure on *A. astaci*, as perhaps reflected in the differing environmental preferences of the individual *A. astaci* genotypes (e.g. Dieguez-Uribeondo *et al.*, 1995).

The mass introduction of the alien North American crayfish into Europe changed the host species range and habitat for *A. astaci*, i.e. several relatively resistant crayfish species were intentionally introduced all over Europe, creating rather complicated and overlapping alien species distributions (see Fig. 8.1d). Since it seemed that they were often carrying *A. astaci*, some discovered to be novel genotypes (Holdich *et al.*, 2009; Viljamaa-Dirks *et al.*, 2013), the parasites may have felt themselves fortunate now to have been conveniently provided with a permanent host reservoir. There are also theoretical reasons to believe that the As-genotype *A. astaci*, which had probably already adapted to co-existing with the European crayfish, were no longer capable of infecting the newly introduced alien crayfish, despite the fact that they had originated from the same geographical region (i.e. North America) where they could well have had an overlapping distribution. On the other hand, the novel genotypes were able to infect both the native European crayfish and the newly introduced alien counterparts. The former case was responsible for population collapses (e.g. Kirjavainen & Sipponen, 2004; Bohman *et al.*, 2006) but in the latter case, only minor gross symptoms and very rarely increased mortality were originally reported. However, during recent decades there have been several reports of population collapses in the signal crayfish (Sahlin *et al.*, 2010; Jussila *et al.*, 2014a, b; Sandström *et al*, 2014) and it is now obvious that the alien North American crayfish are susceptible to *A. astaci* when they are living under European conditions (Persson & Söderhäll, 1983; Thörnqvist & Söderhäll, 1993; Aydin *et al.*, 2014), i.e. this is evidence of the evolution of a balance, albeit a labile balance, between the disease and its North American hosts.

To complicate matters further, it has been shown that there is considerable genetic variation even within different *A. astaci* genotypes (Makkonen *et al.*, 2012a) and that As-genotype *A. astaci* may be capable of infecting signal crayfish (Aydin *et al.*, 2014). One could hypothesize that the crayfish populations during different crayfish plague epidemics could be expressing different *A. astaci* strains and the *A. astaci* genotype itself would be largely host species-specific. Thus, these host jumps would contribute to the spectrum of differences within and among the *A. astaci* genotypes, thus encouraging the adaptation of the various different *A. astaci* strains to European host habitats.

8.5.3 Selection Pressure by Native Crayfish Hosts in Europe

Currently, the infected host crayfish seem to be expressing certain *A. astaci* genotypes and quite often the analyses indicate that only one genotype is infecting an individual host. As there are several *A. astaci* genotypes and species present in Europe, this could be evidence of some kind of superinfection-type infection (May & Nowak, 1994) occurring during crayfish plague epidemics, with normally only one genotype been detected in the infected crayfish.

There are a few possible scenarios to explain the adaptation pressures on *A. astaci* in its native hosts during an infection (Figure 8.4), and we will present some simplified models here. At present, the most common scenario has been one with a high host mortality, which means that there is no special need for *A. astaci* to adapt, since this situation guarantees optimum conditions for sporulation and thus maximizes the probability of further infections. However, this scenario demands that some host habitat is preserved, since otherwise the maintenance of high virulence could cause complete eradication of the *A. astaci* host habitat. Subpopulation(s) of the host crayfish might survive in specific cases; there may be individuals with elevated resistance or certain environmental conditions may promote survival. This scenario ensures high sporulation, but might accidentally kill all of the hosts and could thus be the type I suicide situation.

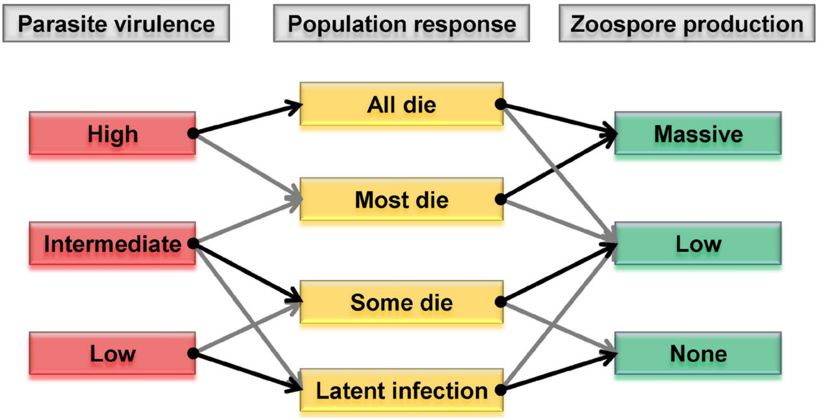

Fig. 8.4: A schematic representation of possible *A. astaci* adaptative scenarios and outcomes when the parasite infects native European crayfish species. Black arrows indicate the most probable outcomes and grey arrows indicate alternative options.

The second scenario would be an *A. astaci* infection with lowered host mortality (Figure 8.4), which would result in the survival of the majority of native host crayfishes and a low overall spore density in the water. This represents the other end of the infection continuum in comparison with the scenario described in the previous paragraph. This would make possible a latent crayfish plague infection in a native host crayfish population. Alternatively, a chronic crayfish plague infection with more severe gross symptoms would also be possible with a slightly higher host crayfish mortality rate. This would require a rather delicately balanced relationship between *A. astaci* and its native host crayfish. There might also be occasional acute *A. astaci* infection phases, as a result of changes in the ambient conditions or different levels of stress in host crayfish leading to a window of opportunity for mass sporulation of *A. astaci*. In this case, *A. astaci* might need to adopt a different tactic for reproduction other than asexual sporulation or, alternatively, it would need to rely on the possibility that a low level sporulation would nonetheless ensure further infections. This scenario raises the risk level of a cessation of *A. astaci* infection and lowered sporulation, and could thus be the type II suicide situation.

The third scenario would be that an *A. astaci* infection would be capable of inducing an intermediate level mortality (Fig. 8.4), where part of the population would survive and mortalities would also be delayed, allowing for a longer time span for sporulation and a lower density of infective spores released at any given time. This would result in lower infective pressure and a situation where perhaps those hosts in better physiological condition or those that were more resistant towards *A. astaci* might survive. This scenario could lead to an acute epidemic followed by a longer latent infection phase. Occasional acute phases might occur and they should ensure sufficient sporulation and thus the avoidance of both type I and type II suicide situations.

8.5.4 Selection Pressure in North American Hosts in Europe

The novel environmental pressures in conjunction with opportunistic European parasites have modified the resistance of the alien signal crayfish to plague disease in Europe and this has greatly altered the relationship between *A. astaci* and its alien crayfish hosts. The outcome has been very different from the unrealistically optimistic predictions. The epidemics of crayfish plague have proved to be more detrimental to alien crayfish than expected and the reasons behind the observed changes have yet to be clarified. The *A. astaci* RAPD-genotypes that have been detected in the alien crayfish species are normally highly virulent even though the PsI-genotype strains do exhibit some variations in their virulence (Jussila *et al.*, 2013a). The adaptation scenarios in this situation are far more limited (Figure 8.5) than those in the *A. astaci* genotypes responsible for epidemics in native European crayfish. There is one key difference compared to *A. astaci* infecting native European crayfish, i.e. there is only a negligible possibility for these *A. astaci* genotypes to adopt the suicide route option.

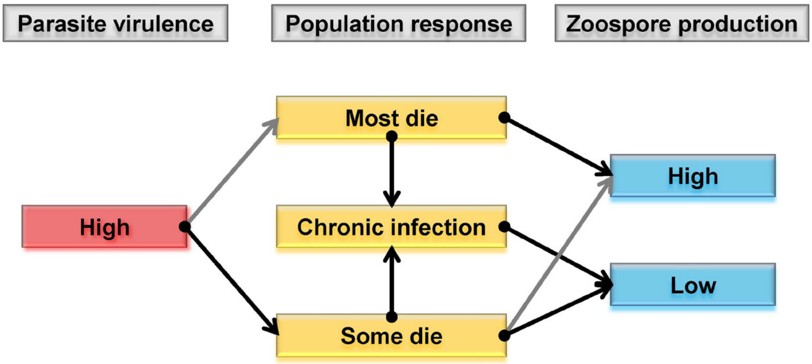

Fig. 8.5: A schematic representation of *A. astaci* adaptative scenarios and outcomes for infecting alien crayfish species in Europe. Black arrows indicate the most probable outcomes and grey arrows indicate alternative options.

The incidents of collapsed signal crayfish populations reported from the Nordic countries (Edgerton & Jussila, 2004; Sahlin *et al.*, 2010; Sandström *et al*, 2014; Jussila *et al.*, 2014b) indicate that the alien crayfish might be losing their advantage in terms of disease resistance as they adapt to European conditions. This could provide *A. astaci* with a novel platform for mass sporulation (Figure 8.4), as it has been shown that the alien crayfish release only small numbers of spores during chronic crayfish plague infections, but there is a peak in sporulation when the condition of the host crayfish starts to deteriorate (Strand *et al.*, 2012). This, together with the well reported role of alien crayfish as a permanent host habitat for *A. astaci* (Holdich *et al.*, 2009), would further increase the risk of the even wider spreading of *A. astaci*. The host jumps, which can occur as a consequence of the close proximity of different populations of crayfish species, are also a factor triggering evolution of *A. astaci*, although the host jumps would only be back and forth between the noble crayfish and the signal crayfish populations, as is happening in the Nordic countries. The network for host jumps, bearing in mind that *A. astaci* has permanent populations of alien crayfish as host habitats, would be even more complex in continental Europe (Figure 8.1).

It is clear that the signal crayfish can act as a platform for *A. astaci* sporulation, quite possibly promoting interactions between different *A. astaci* strains. The sporulation from chronically infected signal crayfish is continual but limited (Figure 8.4; Strand *et al.*, 2012), and there are indications that the signal crayfish may be infected with several different *A. astaci* strains (Aydin *et al.*, 2014). The possibility of multiple infections is extremely interesting, and although this scenario has not been reported, were it to occur it would permit *A. astaci* to exchange genetic information.

The co-infections with different parasites have been reported to cause different syndromes in the signal crayfish (Edsman *et al.*, 2015) and even increased mortality (Thörnqvist & Söderhäll, 1993; Aydin *et al.*, 2014). The crayfish plague epidemics could thus be either superinfections causing elevated mortality or co-infections (May & Nowak, 1995) where the gross symptoms are variable and seem to allow opportunistic parasites to avoid destruction by the weakened immune system in the host (Persson & Söderhäll, 1993). This would raise the possibility of interactions between the parasites during an acute infection and possibility for genetic recombination between closely related species.

There are reports that signal crayfish are only infected with the PsI-genotype *A. astaci* (Jussila *et al.*, 2014b) or, at least, are expressing only that genotype. It has been shown that the PsI-genotype *A. astaci* is highly virulent (Jussila *et al.*, 2013a; Makkonen, 2013; Gruber *et al.*, 2014a) and this species appears to require high virulence in order to effectively complete its life cycle in the signal crayfish via successful mass sporulation. The change in the host-parasite balance between the PsI-genotype *A. astaci* and its signal crayfish hosts favouring *A. astaci* sporulation would create an alternative adaptation platform for the *A. astaci* in Europe. In this case, the less resistant signal crayfish populations would ensure an even more successful spreading of the highly virulent *A. astaci* throughout European aquatic ecosystems and then the native crayfish would face an even greater threat from alien crayfish and their lethal diseases.

8.6 Acknowledgements

We are grateful to have received the strategic funding of the University of Eastern Finland, which allowed us to do much of the work discussed here. LIFE+ CrayMate (LIFE12 INF/FI/233) has provided essential support. We are grateful to Andrej Kapla (Ljubljana, Slovenia) who prepared the maps presented within this chapter. We are thankful to Dr. Ewen MacDonald (University of Eastern Finland) for revising the language. In a bizarre way, we wish to acknowledge the work of those misguided individuals who are still attempting to spread these alien crayfish species — it means that those of us trying to frustrate their efforts will never be out of work or run out of topics about which we can argue. And finally, please consider carefully and always exercise caution before you start to play around with Mother Nature.

In a nutshell

- *Aphanomyces astaci* belongs to the class of Oomycetes, a diverse group of fungus-like organisms, including not only a wide variety of plant and animal pathogens, but also saprophytic species. *A. astaci* itself is a very specific parasite infecting the freshwater crayfish of North America. However, in the European crayfish, it causes a lethal disease known as crayfish plague. The pathogen spreads from host to host by producing swimming zoospores; should a suitable host be found, these then encyst, germinate, and start to grow hyphae into the host tissues. A massive sporulation occurs with the death of the host. In contrast, in the case of the disease-resistant North American crayfish species, there is a continual low level sporulation.
- The introduction in Europe of *A. astaci* and the repeated introductions of its North American host species are a classic example of a man-made ecological disaster, stemming from the naïve belief that the manipulation of an ecosystem would be straightforward. Currently, the native European crayfish is on the brink of extinction and the alien crayfish species which were supposed to replace the eradicated native stocks display many signs and symptoms of maladaptation. *A. astaci* itself has apparently adapted rather well to European conditions, and seems to be currently co-evolving at multiple levels, while maintaining contact with its relatively resistant hosts as new crayfish stocks of North American origin are imported into Europe. One can presume that this multiple-host-species pattern is likely to guarantee *A. astaci*'s survival even in the most severe crayfish plague epidemics. One could also predict that this will inevitably lead to the total eradication of the remaining native European crayfish stocks.
- The introduction of the *A. astaci* to Europe, though it was purely accidental, has not only seriously devastated native crayfish populations throughout Europe, but also resulted in further damage due to misguided management attempts to rectify the situation.

- According to data about wild crayfish populations, it seems that the adaptation of *A. astaci* allowing it to sometimes coexist with the native European crayfish has taken less than 150 years. The indirect evidence for this proposal, originally obtained from the records of chronic crayfish plague infections of native European crayfish populations, especially the noble crayfish *(Astacus astacus)*, has recently been verified using qPCR techniques.
- In the Nordic countries, there is much anecdotal evidence describing possible population collapses of the signal crayfish *(Pacifastacus leniusculus)* due to crayfish plague epidemics. Previously, the signal crayfish had been thought to be highly resistant to crayfish plague infection. Recent observations from the wild populations indicate that crayfish plague and subsequent infections by opportunistic parasites, such as the *Fusarium* sp. complex, may lower the reproduction potential of the wild signal crayfish stocks. Alarmingly, it has also been shown that the signal crayfish could be susceptible to *A. astaci* of both the PsI-genotype and As-genotype, which means that associated population collapses could well be possible in these crayfish. These recent findings seem to confirm the long-established theories about the reduced *A. astaci* resistance in signal crayfish.
- These conclusions, which are no longer merely theoretical but are now based on research findings, clearly indicate that further introduction of alien crayfish species, and especially their diseases, are predicted to pose severe problems to the receiving ecosystems, and the consequences would not be limited to certain target species, but would often spread through the whole ecosystem. The fundamental concept of always erring on the side of caution is unfortunately all too often ignored. Although this creates circumstances that are very interesting from an academic view point, they are very detrimental to the organisms and biodiversity in these ecosystems.

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