

# Understanding the Causes of Disease in European Freshwater Crayfish

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**Abstract:** *Native European freshwater crayfish (Astacida, Decapoda) are under severe pressure from habitat alteration, the introduction of nonindigenous species, and epizootic disease. Crayfish plague, an acute disease of freshwater crayfish caused by the fungus-like agent *Aphanomyces astaci*, was introduced into Europe in the mid-nineteenth century and is responsible for ongoing widespread epizootic mortality in native European populations. We reviewed recent developments and current practices in the field of crayfish pathology. The severity of crayfish plague has resulted in an overemphasis on it. Diagnostic methods for detecting fungi and fungal-like agents, and sometimes culturing them, are frequently the sole techniques used to investigate disease outbreaks in European freshwater crayfish. Consequently, the causes of a significant proportion of outbreaks are undetermined. Pathogen groups well known for causing disease in other crustaceans, such as viruses and rickettsia-like organisms, are poorly understood or unknown in European freshwater crayfish. Moreover, the pathogenic significance of some long-known pathogens of European freshwater crayfish remains obscure. For effective management of this culturally significant and threatened resource, there is an urgent need for researchers, diagnosticians, and resource managers to address the issue of disease in European freshwater crayfish from a broader perspective than has been applied previously.*

**Key Words:** *Aphanomyces astaci*, biosecurity, crayfish pathology, crayfish plague, epizootics, freshwater crayfish

Entendiendo las Causas de Enfermedad en Cangrejos Europeos de Agua Dulce

**Resumen:** *Los langostinos nativos de Europa (Astacida, Decapoda) están bajo severa presión por alteración del hábitat, la introducción de especies no nativas y una enfermedad epizootica. La peste de langostinos, una enfermedad aguda de langostinos de agua dulce producida por el agente micóide *Aphanomyces astaci*, fue introducida a Europa a mediados del siglo diecinueve y es responsable de la actual mortalidad epizootica de poblaciones Europeas nativas. Revisamos acontecimientos recientes y prácticas actuales en el campo de la patología de langostinos. La severidad de la peste de langostinos ha resultado en un excesivo énfasis en ella. Los métodos para diagnosticar, y algunas veces cultivar, hongos y agentes micoides frecuentemente son la única técnica empleada al investigar brotes de la enfermedad en langostinos de agua dulce en Europa. Consecuentemente, no están determinadas las causas de una proporción significativa de los brotes. Grupos patógenos, como virus y organismos similares a rickettsias, bien conocidos por producir enfermedades en*

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otros crustáceos son poco o nada conocidos en langostinos de agua dulce de Europa. Más aún, el significado patogénico de algunos patógenos de langostinos de agua dulce de Europa largamente conocidos es oscuro. Para el manejo efectivo de este recurso culturalmente significativo y amenazado es urgente la necesidad de investigadores, diagnosticadores y gestores de recursos para atender el asunto de la enfermedad en langostinos de agua dulce europeos desde una perspectiva más amplia que la previamente aplicada.

**Palabras Clave:** *Aphanomyces astaci*, bioseguridad, epizootia, langostinos de agua dulce, patología de langostinos, peste de langostinos

## Introduction

Freshwater crayfish (Astacida, Decapoda) are the largest mobile freshwater invertebrates, occurring in freshwater, subterranean, and semiterrestrial habitats and brackish waters, and are frequently considered keystone species (Holdich 2002a). There are three families of freshwater crayfish. The Astacidae and Cambaridae are restricted in distribution to the Northern Hemisphere (North and Central America, northeastern Asia and Europe), whereas the Parastacidae are limited to the Southern Hemisphere (Australasia, Madagascar, and South America). Although their geographic distribution in Europe is extensive, species diversity of freshwater crayfish there is much lower than in other areas. Of the 500 or more species of freshwater crayfish reported, only five are native to Europe according to the taxonomy adopted by Holdich (2002b): *Astacus astacus*, *Astacus leptodactylus*, *Astacus pachypus*, *Austropotamobius pallipes*, and *Austropotamobius torrentium*.

Current estimates designate up to one-half of all freshwater crayfish as threatened with population decline or extinction (Taylor 2002). Factors associated with these declines are habitat loss and degradation, over-harvesting, and introduction of nonindigenous species. In Europe the most important factor in freshwater crayfish population declines has been introduction of nonindigenous species and the resultant competition and, more important, cotranslocation of the fungus-like agent *Aphanomyces astaci*. *A. astaci* is carried by North American freshwater crayfish species as a subclinical infection, but it is acutely pathogenic to native European freshwater crayfish (Alderman & Polglase 1988). Crayfish plague, the disease caused by *A. astaci*, is perhaps the most commonly cited case of incursion of an exotic disease in aquatic animals, as a result of the ecological and socioeconomic significance of freshwater crayfish, and the severity and longevity of the crayfish plague panzootic. Populations of all species of native European freshwater crayfish from throughout Europe have been affected over the last 150 years. The crayfish plague panzootic was one of 29 cases that Laferty and Gerber (2002) considered in concluding that pathogens are of increasing concern for conservation.

Baillie and Groombridge (1996) list *A. astacus*, *A. pallipes*, and *A. torrentium* as vulnerable as a result of range

reductions and population declines. These same three species are listed as protected in Appendix III of the Bern Convention, meaning that their exploitation and harvesting are subject to controls. Additionally, *A. pallipes* is listed in Appendix V of the European Habitats Directive, requiring the designation of special areas of conservation for its protection. These documents, and the initiatives they represent, attest to the conservation value of these species and of native European freshwater crayfish in general.

## History of Epizootics in European Freshwater Crayfish

According to Alderman and Polglase (1988), there is general agreement that the first epizootic of crayfish plague occurred in the Po Valley in Italy in the 1860s. By the early 1900s, the epizootics had spread and affected freshwater crayfish populations in most of central, northern, and eastern Europe. Only isolated populations of freshwater crayfish in these regions were unaffected (Alderman & Polglase 1988). Continued spread in the latter half of the 1900s resulted in epizootics in freshwater crayfish populations in Norway (Taugbøl et al. 1993), England (Alderman et al. 1984), Ireland (Reynolds 1988), Spain (Dieguez-Uribeondo et al. 1997), and Turkey (Baran & Soylu 1989). These were officially recorded as being due to crayfish plague, with *A. astaci* being isolated in the latter four cases.

In early investigations into the epizootics, many pathogens, including bacteria, fungi, protozoa, and metazoans found in association with dying crayfish, were proposed as the aetiological agent (Alderman & Polglase 1988). Moreover, the extensive range of gross signs exhibited by diseased animals suggested to some investigators that a number of different diseases were acting in different regions (Alderman & Polglase 1988). The confusion among astacologists of the early twentieth century is illustrated by the following statement: animals affected by the epizootics presented “a clinical picture with such an unbelievably variable symptom complex, unlike anything produced for any other known epizootic disease” (Schikora 1922). Although a detailed case for a fungal etiology for the epizootics was presented in the

very early 1900s (Schikora 1903; Schikora 1906), it was not until the latter 1920s that this view gained general acceptance (Alderman & Polglase 1988). Shortly afterwards, Nybelin (1934) demonstrated that *A. astaci*, isolated from diseased crayfish, was highly pathogenic to European freshwater crayfish. Thus, *A. astaci* was accepted as the causative agent of crayfish plague.

The origin of *A. astaci* was unknown until Unestam (1972) provided indirect evidence that North American crayfish are the natural hosts for the fungus by proving that these species are resistant to disease. North American crayfish are subclinical carriers. Unestam (1976) reported that freshwater crayfish species belonging to the Parastacidae and native to Australia and New Guinea are as highly susceptible to crayfish plague as the European crayfish. The latter report, however, was based on a small number of animals and provided no survival data for control crayfish kept under the same experimental conditions, which were far from optimal and included a water temperature near the lower lethal limit for these tropical and subtropical species. Molecular tools have since provided direct evidence of the role of North American crayfish as vectors of *A. astaci* (Huang et al. 1994). North American freshwater crayfish have been widely translocated in Europe, both intentionally, to replace lost native populations, and unintentionally. The American signal crayfish (*Pacifastacus leniusculus*) in particular has been widely stocked in northern Europe. Other American freshwater crayfish, including the red swamp crayfish (*Procambarus clarkii*) and *Orconectes* species, have been introduced and have spread in more southern regions of Europe. Fish transfers are a potential mechanism for dispersal of crayfish plague because *A. astaci* remains viable on fish scales and in cuticle in fish guts (Alderman & Polglase 1988; Oidtmann et al. 2002).

Investigations into the cause of mortality in European crayfish led to the reporting of a number of potential pathogens other than *A. astaci*, including protozoans, bacteria, fungi and other fungus-like agents, and *Psorospermium haeckeli*. Many of these organisms were originally described in freshwater crayfish over 100 years ago (Fig. 1). Even so, the pathogenic significance to European freshwater crayfish of many of these potential pathogens remains obscure.

## Recent Advances in Crayfish Pathology

A significant development for the study of crayfish plague has been the recent development of genetic-based research and diagnostic tools. The research tools have been used effectively to study the relatedness of various strains of *A. astaci* to establish an epidemiological picture of the crayfish plague panzootic in Europe (Huang et al. 1994; Dieguez-Uribeondo et al. 1995). The diagnostic tools, such as polymerase chain reaction, permit rapid

and significantly less laborious confirmation of infection by *A. astaci* (Cerenius et al. 2002).

Since the 1980s, the increasing occurrence of semi-intensive and intensive farming of freshwater crayfish, predominantly of species native to Australia, has allowed the closer monitoring of the health status of those crayfish populations. As a result, many pathogens and potential pathogens of native Australian freshwater crayfish have been reported in the intervening period, including viruses, bacteria, rickettsia-like organisms, and endoparasitic ciliates (Edgerton 2002a; Evans & Edgerton 2002).

Viruses are common, serious pathogens of aquatic animals. Of 35 diseases of aquatic animals listed by the Office International des Epizooties (OIE), 16 are caused by viruses (OIE 2003). Seven of 8 diseases of crustaceans listed by the OIE are caused by viruses. The remaining OIE-listed disease of crustaceans is crayfish plague. The first viral infection of a crustacean was reported by Vago (1966). However, the first viral infection in a natural population of freshwater crayfish was not reported until over 25 years later (Anderson & Prior 1992).

Edgerton et al. (1996) were the first to report a viral infection in a natural population of native European freshwater crayfish. These authors found that an intranuclear bacilliform virus infected 100% of examined *A. astacus* from four populations and 53.3% from another population in central Finland. The high prevalence of *Astacus astacus* bacilliform virus (AaBV) in *A. astacus* in Finland was confirmed by similar surveys in subsequent years (Paasonen et al. 1999). A second intranuclear bacilliform virus was recently found in association with an epizootic that resulted in the near extirpation of a population of *A. pallipes* in southern France (Edgerton et al. 2002). A small-scale survey has since shown that the virus was common in another three populations of *A. pallipes* located near the first population. The virus infected 100% of crayfish from two populations and infected 73.3% of crayfish from another population (Edgerton 2003).

White spot syndrome virus (WSSV) has been panzootic in shrimp farms throughout Asia since the early 1990s and in South America since the late 1990s. White spot syndrome virus is an OIE-listed disease. In a farm pond, WSSV typically causes 100% mortality in penaeid shrimp within 3–5 days of the first observation of animals showing clinical signs of white spot disease. Infectious WSSV is known to be common in commercial shrimp products because of its high prevalence in the major prawn-producing areas, both in farms and in the wild (Lo & Kou 1998). Moreover WSSV can remain viable for extended periods in frozen tissues (Nunan et al. 1998), as can most viruses. The first occurrence of infection by WSSV in freshwater crayfish was reported by Richman et al. (1997), who discovered that the virus was the cause of epizootics, with up to 90% mortality, in *Procambarus* sp. and *Orconectes punctimanus* sent to the National Zoo in Baltimore, Maryland (U.S.A.). The source of the virus was frozen prawns,



Figure 1. Timeline of significant events in the field of crayfish pathology (not to scale). Key: #, relates to Europe; \*, relates to United States; +, relates to Australia.

which were fed to the crayfish prior to shipment to Baltimore (Lightner et al. 1997). Subsequent experimental transmission trials have proven the potential for WSSV to cause serious mortality in freshwater crayfish from all three families (Wang et al. 1998; Shi et al. 2000; Corbel et al. 2001; Jiravanichpaisal et al. 2001; Edgerton 2004).

Significantly, of the 29 cases considered by Lafferty and Gerber (2002) in which infectious diseases have influenced host species of conservation concern, the highest number of cases (12) involved viruses.

Rickettsia-like organisms also cause disease in aquatic animals, including invertebrates. Two rickettsia-like organisms have been associated with mortality in the Australian freshwater crayfish *Cherax quadricarinatus* (Owens et al. 1992; Ketterer et al. 1992; Edgerton & Prior 1999). Similar agents have been reported in two European crustaceans in the marine crab *Carcinus mediterraneus* in the Mediterranean Sea (Bonami & Pappalardo 1980) and the freshwater amphipod *Rivulogammarus pulex* in northern Sweden (Larsson 1982). However, no

rickettsia-like organisms have been reported from freshwater crayfish in Europe.

### Why Some Pathogen Groups Are Understudied

Given that there has been significant interest in diseases of European freshwater crayfish for almost 150 years and that viruses have a proven ability to cause serious disease in aquatic animals, especially crustaceans, it is curious that viruses were not detected in the native European freshwater crayfish fauna until recently. The possibility that viruses were only recently introduced to European crayfish populations cannot be discounted on the current evidence. Nonetheless, assessment of the literature and current practices in the field reveal that disease diagnosticians and researchers working with freshwater crayfish typically concentrate on techniques appropriate only for the detection of fungi and fungus-like organisms. Occasionally, general techniques for the identification of bacteria, epibionts, and some readily recognized parasites, such as *Psorospermium* and microsporidians, are also used. Conversely, histopathology is rarely utilized and there is a general lack of expertise in this discipline in Europe.

Almost without exception, all of the 50+ viruses reported from crustaceans were discovered by electron microscopy conducted on tissue preparations after lesions were detected with a light microscope. There are no cell lines from crustaceans that could be used for laboratory isolations of viruses, as is routine for other animals such as fish and insects. Many other pathogens of crustaceans, including rickettsia-like organisms, were similarly discovered during histopathological analyses. Subsequent to their discovery, researchers have developed additional diagnostic tests, including genetically based tests, for detecting important crustacean pathogens such as WSSV. Histopathology remains the most important diagnostic tool for studying diseases in crustaceans because it allows the detection of a broad spectrum of potentially important pathogens. It is most likely that the low level of expertise in and utilization of histopathology in the study of disease in European crayfish is the primary reason for the lack of discovery and poor knowledge of these pathogen groups (Edgerton 2000; Edgerton et al. 2002).

### Case Histories of Recent Epizootics in Freshwater Crayfish

There are now numerous reports (Dieguez-Uribeondo et al. 1997; Vogt 1999; Edgerton et al. 2002; Skurdal & Taugbøl 2002), and many more anecdotes of epizootics in native European freshwater crayfish which could not be associated with crayfish plague even though techniques and samples were appropriate for detection of that pathogen. Moreover, there have recently been re-

ports (Dieguez-Uribeondo et al. 1997) and anecdotes of epizootics in introduced American crayfish species. Following are several country-specific case histories that underscore these facts.

#### Norway

It is generally accepted that four watersheds in Norway have been struck by the crayfish plague, but *A. astaci* has never been isolated and used for infection experiments to provide a reliable diagnosis. The diagnosis of crayfish plague has thus been based on the disease's history—100% mortality of crayfish, no other organisms affected—and on direct microscopy showing fungal hyphae characteristic of *Aphanomyces* (Taugbøl et al. 1993).

Since 1995, three (among several) cases of total mortality of *A. astacus* have been thoroughly investigated. None could be associated with crayfish plague even though techniques and samples were appropriate. A range of fungi were isolated and considered secondary invaders in all cases (Taugbøl 1996, 1999; and diagnoses from the National Veterinary Institute). In the first two cases, diagnostic tests were restricted to analyses for *A. astaci* and other fungus-like organisms and observations of fresh tissues. In the last case, histological examination of one *A. astacus* showed that intranuclear inclusions consistent with infection by *Astacus astacus* bacilliform virus were present in the midgut but at a very low level (B.E., unpublished data).

#### Finland

Diagnosis of crayfish plague in Finland by the National Veterinary and Food Research Institute (EELA) requires exposure of susceptible crayfish to the fungus that has been isolated from diseased crayfish. Modern molecular tools may also be used. Histopathology has occasionally been used in Finland as a diagnostic tool for freshwater crayfish since *Astacus astacus* bacilliform virus was discovered in 1995.

From 1999 to 2002, 137 epizootics in *A. astacus* were analysed at EELA (S. Viljamaa-Dirks, personal communication). Of these, 35 epizootics were verified as crayfish plague through isolation and identification of *A. astaci*. Ten epizootics were verified as crayfish plague based on pathological, anatomical, or histological analyses without the isolation of *A. astaci*. Furthermore, other pathological changes were evident in 11 epizootics (e.g., unidentified trauma, hepatopancreatic infection). In 81 epizootics the etiologies could not be verified. For 38 of these epizootics, however, case-history features suggested that crayfish plague may have been involved (S. Viljamaa-Dirks, personal communication), but there was no diagnostic evidence.

During the past decade there have also been suspected crayfish plague outbreaks in established, productive populations of wild signal crayfish (*P. leniusculus*) in Finland.

In such cases, noble crayfish (*A. astacus*) were typically present initially and were eradicated. The signal crayfish stock usually recovered in a few years, with crayfish commonly showing visible signs of chronic infection by *A. astaci*.

### Sweden

Currently, only the OIE recommendations for diagnosis of crayfish plague are routinely carried out in Sweden, with additional assessments for *Psorospermium baeckeli* and microsporidiosis. In 2003 five cases of suspected epizootic mortality in lake populations of American signal crayfish (*P. leniusculus*) were reported to the National Board of Fisheries (L.E., unpublished data). Dead or moribund crayfish were observed at the lake bottom in two cases. In these five lakes, the catch of *P. leniusculus* had declined by 60% to over 90% from the previous year. *A. astaci* was known to be endemic in two populations.

### France

*A. astaci* was not reported from the wild in France from 1912 to the 1990s. Throughout the 1990s, *A. astaci* was associated with epizootics in native European crayfish in western and northwestern France (Machino & Dieguez-Uribeondo 1998; Neveu 1998, 2000; Neveu & Bachelier 2003). In the late 1990s and early 2000s, epizootics were also observed in populations of native (*A. pallipes*) and introduced (*Orconectes limosus*) freshwater crayfish in southeastern France (H. Watt & P. Roche, personal communication).

One such epizootic in *A. pallipes* was associated with a high prevalence of infection by an intranuclear bacilliform virus (Edgerton et al. 2002). However, although *Austropotamobius pallipes* bacilliform virus showed a high level of infection in some tissues, there was insufficient evidence to conclude that it was the sole cause of mortality in the population.

### Spain

Epizootics in native freshwater crayfish in Spain were observed in 1958, 1965, and 1975 (Cuellar & Coll 1983). However, *A. astaci* was not isolated from crayfish collected during an epizootic until 1978, after significant stockings of native American crayfish species had occurred throughout Spain in the early 1970s (Dieguez-Uribeondo et al. 1997). In the late 1970s and early 1980s, epizootic mortality in native crayfish populations occurred throughout Spain, drastically reducing the distribution of *A. pallipes*.

Dieguez-Uribeondo et al. (1997) studied 21 crayfish epizootics in Spain between 1994 and 1997. Again, diagnostic analyses concentrated on visualizing and isolating fungi and fungus-like organisms. Crayfish plague was diagnosed as the cause of 10 out of 16 epizootics in *A.*

*pallipes* from the wild and farms; one epizootic each was diagnosed as being due to saprolegniasis, pollution, or water quality; and for three epizootics the cause could not be determined. Five of the epizootics were found in the native American species *P. leniusculus*. Two of these episodes were diagnosed as being due to crayfish plague, two were diagnosed as being due to pollution, and the cause of one epizootic could not be determined.

Royo et al. (2002) examined dead and ill *A. pallipes* that were collected regularly from the Spanish national park Picos de Europa over a 2-year period. The mortality episode was chronic, and there was no obvious effect on population number. Diagnostic techniques included gross examination, microscopic examination of soft cuticle and gills from fresh samples and samples fixed in 70% ethanol, and fungal cultures from fresh samples. The diagnoses included aggression, association with moulting, association with storms, fungal diseases, epibionts, bacteria, and unknown. The authors recognized the need to include other diagnostic techniques, such as histopathology and bacteriology, to determine the actual causes of mortality.

The salient features of these case histories are as follows: (1) *A. astaci* has been associated with a large proportion of epizootics in native crayfish in Europe; (2) a significant proportion of epizootics and a chronic mortality episode in native European crayfish were unrelated to *A. astaci*; (3) there have been epizootics in native American freshwater crayfish in Europe; and (4) in almost all cases, diagnostic techniques suitable only for identifying fungi and fungus-like organisms, and less frequently *Psorospermium* and microsporidians, were used by researchers and diagnosticians.

It is likely that there were some false-negative results for *A. astaci* in the above case histories. Also important to consider, however, is the possible coinvolvement of pathogens in disease outbreaks. For example, *P. leniusculus*, which is considered highly resistant to crayfish plague, will succumb to the disease if coinfecting by *P. baeckeli* (Thörnquist & Söderhäll 1993). This is particularly interesting because *P. baeckeli* is often considered to have low virulence. *P. baeckeli* is common in native European crayfish, for which extensive surveys have been performed (Taugbøl & Skurdal 1995; Henttonen et al. 1995; Vogt 1999). Intranuclear bacilliform viruses are also common in the native European crayfish populations that have been surveyed. Significantly, an epizootic in *A. astacus* in the Finnish lake Suurlaus in 1996 (H. Saksman, personal communication) was preceded by a histopathological survey in 1995 in which all examined crayfish were infected by *Astacus astacus* bacilliform virus (Edgerton et al. 1996). The cause of the epizootic was not determined because of a lack of samples. During a roundtable discussion on crayfish plague at a meeting of European crayfish biologists and resource managers held in 2001, there was general agreement that the possible involvement of

these common pathogens with outbreaks of disease associated with other pathogens, including *A. astaci*, had not been studied (Cerenius et al. 2002). Moreover, researchers working with crayfish plague have found that crayfish from certain populations and individual crayfish appear to have increased resistance to the fungus (Cerenius et al. 2002). However, studies have not been conducted that draw any correlation to the underlying health status of crayfish, including the presence of coinfecting pathogens.

The overriding conclusions from these case histories are that diseases other than crayfish plague cause epizootics in native and introduced crayfish in Europe. Currently there is a poor level of general understanding of the causes of disease in European freshwater crayfish, and the field is poorly equipped to diagnose and respond to infections by nonfungal pathogens.

### Implications for Management of European Freshwater Crayfish

The implications of this poor level of understanding are serious. An important aspect of managing crayfish populations in Europe, for species conservation and for enhancement of fisheries, has been restocking of waterbodies with adults or juvenile crayfish that have been produced in hatcheries. In some European countries it is general practice for crayfish to be subjected to health checks prior to stocking, though in many countries there are no mandated health checks. In those countries where there are health checks, these typically include only examinations for clinical signs of disease and parasites. To our knowledge, based on networking in the field and an email circulated to all European Community national reference laboratories for fish disease, histopathological examination of freshwater crayfish is not required by any European regulations. Therefore, there is virtually zero probability that viral infections, and many other potentially serious pathogens, will be detected during health examinations prior to stocking of native crayfish. Moreover, given the poor level of understanding of long-known and common pathogens such as *P. haeckeli*, there is confusion over how to treat such positive results in terms of whether or not to permit stocking of infected crayfish.

Contemporary government policy development emphasizes robust assessment of scientific data and opinion. When deciding on whether to permit new importations of living animals or even animal-based products, governments are required to substantiate decisions by adherence to internationally accepted standards, such as those developed by the OIE, or by completion of scientific import risk analyses. One of the greatest challenges to conducting these assessments for freshwater crayfish is the lack of information on their pathogenic faunas (Edgerton 2002b,

2002c). First, given the underutilization of important diagnostic tools, the risk analyst must be aware that many potentially significant pathogens will not have been reported and thus cannot be included in the assessment. This may apply equally to importing and exporting countries. Second, when similar pathogens have been reported from both countries, rarely have thorough studies been done to determine whether there are species or strain-related differences. Clearly, the lack of information on crayfish pathogens is a severe impediment to the development of effective biosecurity policy.

### Conclusions

In crustaceans there are often limited specific signs of a disease. Most dying crayfish exhibit nonspecific signs of disease, such as anorexia, lethargy, and possibly autotomy, all of which have been associated with infection by a wide range of pathogens of crustaceans. Therefore, clinical symptoms are of less significance in crayfish pathology than in that of vertebrates. Moreover, diagnostic techniques for crayfish plague remain complicated, though the widespread adoption of PCR techniques has the potential to simplify the process. Without PCR proper diagnosis of crayfish plague requires the isolation of an oomycete belonging to the genus *Aphanomyces*, followed by a transmission trial to confirm that the *Aphanomyces* is pathogenic to a susceptible freshwater crayfish species. As a consequence of the prolonged difficulties at the turn of the twentieth century in determining that the cause of crayfish plague was *A. astaci*, the remaining difficulties in diagnosing this agent, and the rather nonspecific and descriptive name given to the disease, there has been an overwhelming tendency in the field to conclude that all epizootics in European crayfish were caused by this one disease. Currently, CRAYNET, a thematic network funded by the European Union, is gathering data to produce an atlas of crayfish plague distribution, which will include diagnostic data. This will provide additional information on the level of incomplete diagnosis and misdiagnosis of crayfish plague.

Our goal here is not to create confusion as to the cause of crayfish plague disease. Many research groups on many occasions have clearly demonstrated that *A. astaci* is a serious pathogen of freshwater crayfish. Moreover, the OIE accepts that *A. astaci* is the aetiological agent for crayfish plague. Neither do we suggest that any of the viruses recently reported in European freshwater crayfish are serious pathogens; too few data exist for these potential pathogens to base even a preliminary assessment of their pathological significance. Nor do we suggest that the OIE-listed and significant viral pathogen of crustaceans, WSSV, has been introduced to Europe. However, detection of WSSV, if it were present and having a serious impact on

European freshwater crayfish populations, is impossible with the current practices in the field.

That there are outbreaks of disease, at times associated with either chronic or epizootic mortality, in native European crayfish that are not associated with *A. astaci* is clear. Moreover, because the few regional studies show that some potential pathogens, such as *P. baeckeli* and intranuclear bacilliform viruses, are common in freshwater crayfish populations, it seems certain that these potential pathogens concomitantly infect freshwater crayfish infected by *A. astaci*. The significance of these coinfections on the pathogenesis of crayfish plague is undetermined. Finally, recent observations of epizootics in native American crayfish species in Europe show that there are factors—possibly including a reduced resistance to crayfish plague due to stress, infection and coinfection by other pathogens, or increased virulence of *A. astaci*—that have a serious impact on production of this introduced species. Therefore, it is clear that there is a general and significant lack of understanding of the causes of disease in freshwater crayfish in Europe.

The extreme emphasis on *A. astaci* has created inertia in European astacology, which has curtailed researchers, state fish-disease diagnosticians, and resource managers from fully assessing and considering the existence of other serious pathogens of freshwater crayfish and the ensuing consequences. As a result, basic skills in crayfish pathology have been lost or underdeveloped. Moreover, some management schemes aimed at conserving native European freshwater crayfish are less likely to be effective, and might actually be harmful, in achieving their goal because of a lack of appreciation of the presence or significance of certain pathogens. Therefore, it is imperative that this inertia be arrested and that concentrated effort be given to studying the full range of pathogens affecting European freshwater crayfish for the protection of these culturally significant and threatened species.

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