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Opinion paper

Current techniques, approaches and knowledge in diagnosis of crayfish plague and other crayfish diseases

Round table 3 (chairman: Javier Diéguez-Uribeondo)

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Crayfish diseases are one of the main causes of crayfish decline in Europe and specifically, crayfish plague (Edgerton *et al.*, 2004). Knowledge of its causative agent, *Aphanomyces astaci* (Oomycetes), is still one of the crucial key aspects in crayfish management, and designing and implementing action plans for restoring the European indigenous crayfish species (ICS). In the Regional European Crayfish Workshop dealing with the Future of Native Crayfish in Europe held in Pisek, Czech Republic, a number of researchers joined a round table focused on crayfish diseases, and discussed the main problems and needs in crayfish disease studies. The data discussed only refer to the countries represented in this group discussion (Czech Republic, Estonia, Finland, France, Italy, Spain and Sweden) and the main topics are discussed next.

CRAYFISH PLAGUE: CURRENT SITUATION AND ACTIONS IN EUROPE

The situation and actions among European countries regarding the impact, monitoring, control and diagnosis of this disease are diverse. This is mainly due to: (1) the distinct presence, distribution and spread of non-indigenous crayfish species (NICS), and (2) the existence or not of crayfish plague specialists and ongoing research on this matter.

In each country, the number of detected and diagnosed mortalities due to crayfish plague varies from a couple of cases to a dozen cases per year. Thus, in Finland, Spain, Sweden and Estonia the number of crayfish plague cases diagnosed is around 10 per year, in the Czech Republic from 5 to 8 per year, in France around 2 per year, and a recently reported case in Italy. The number of cases does not appear to be related to actual status but rather to the intensity of research carried out.

In general, crayfish mass mortalities are detected by chance and the correct diagnosis depends on whether they are reported, and whether there is an available laboratory with skills in *A. astaci* identification. In some countries, there are laboratories with specialists where samples can be routinely sent and where established protocols for collecting and submitting samples exist, *i.e.*, Czech Republic, Finland, Sweden and Spain.

In this round table, researchers pointed out the active presence of crayfish plague and its continuous threat to indigenous populations of European freshwater crayfish. Researchers, in addition, emphasized the need to have in each country: a center of reference for diagnosing the disease, and a protocol for collecting and submitting samples. These aspects are crucial in order to efficiently detect, monitor and prevent the presence and effect of crayfish plague and distinguish it from other mass mortality events in freshwater crayfish.

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APPLICATION OF MOLECULAR TECHNIQUES

Aphanomyces astaci is a difficult organism to study and problems are associated with submission of crayfish samples, availability of expertise in laboratories, difficulty in isolating and maintaining strains of *A. astaci*, and interpreting the results of samples. The advent of molecular techniques, however, has allowed the development of new methods for rapid and reliable identification of *A. astaci* (Oidtmann *et al.*, 2006; Ballesteros *et al.*, 2007; Hochwimmer *et al.*, 2009; Kozubíková *et al.*, 2009; Vrålstad *et al.*, 2009). More research, however, is needed on *Aphanomyces* biodiversity and molecular markers, since in recent years, a number of new *Aphanomyces* species have been described, and it has been found that some species are present in crayfish and provide false positives (Ballesteros *et al.*, 2006, 2007; Oidtmann *et al.*, 2006; Diéguez-Uribeondo *et al.*, 2009). Improvement of the currently developed molecular techniques for diagnosing crayfish plague is also allowing us to improve the specificity of molecular tests. The combined knowledge of disease history, culture-based or histological studies and molecular studies are key aspects for providing accurate diagnosis.

CRAYFISH PLAGUE SPREAD AND EPIDEMIOLOGY

Crayfish plague is the most studied invertebrate disease, and its causative agent *A. astaci* is one of the best-known pathogens (see reviews by Söderhäll and Cerenius, 1999; Diéguez-Uribeondo *et al.*, 2006; Cerenius *et al.*, 2009). The biology and phylogeny of *A. astaci* has been extensively studied and this knowledge has allowed us to achieve important goals in crayfish management. However, there are still some gaps in the knowledge that need further studies such as the existence of low-virulence strains. This aspect has been noticed in Finland and Sweden (Viljamaa-Dirks and Heinikainen, 2006; Diéguez-Uribeondo and Söderhäll, unpublished, respectively). The presence of low-virulence strains could mean that some strains can stay in native crayfish for long periods similar to chronic interaction of *A. astaci* in their natural carriers, *i.e.*, North American crayfish, and eventually manifest as acute plague. This is an important issue in management since it emphasizes the need for carrying out health controls in native crayfish for restocking. On the other hand, the presence of such strains would also mean that it could be native crayfish with an enhanced resistance. Further studies need to be done in this exciting area of research.

OTHER CRAYFISH DISEASES

Other important crayfish diseases are: virosis, porcelain disease caused by *Thelohania* spp., psorospermiasis caused by *Psorospermium* spp., and others are related to high density of ectoparasites or ectocommensals such as branchiobdelidan species (Diéguez-Uribeondo *et al.*, 2006). These diseases are only of particular importance on scarce occasions and economic impact is more relevant in crayfish farming than in natural populations.

FINAL REMARKS

The European ICS are endangered species and in some regions are at risk of extinction. The habitats where ICS can be restored are restricted by the distribution of NICS: in many countries, the ICS have been forced to live in limited habitats continuously threatened by crayfish plague and also other diseases. Thus, the future situation is linked to crayfish plague and NICS distribution and therefore, studies on biodiversity, biology, diagnosis and epidemiology of crayfish plague as well as spread of NICS are crucial for development of action plans and strategies for ICS conservation. Moreover, research on other crayfish diseases should also not be neglected.

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