KEYNOTE PRESENTATION AND ROUNDTABLE SESSION 4

CRAYFISH PATHOLOGY IN EUROPE: PAST, PRESENT AND A PROGRAMME FOR THE FUTURE

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ABSTRACT

The devastating affects of disease on European freshwater crayfish are well known as epizootics in wild populations have occurred throughout much of Europe since the mid XVIII^{e} s. After protracted and rigorous debate, the cause of the disease named crayfish plague was proved to be the fungus *Aphanomyces astaci* in 1934. In the last 70 years, much of the research conducted in the field of crayfish pathology has concentrated on fungi, especially improving diagnostic techniques for *A. astaci*. Similarly, diagnostic responses to epizootics in European crayfish have concentrated almost entirely on fungal isolation and/or identification. On the other hand, viruses have proved to be the most important pathogens in the growing global crustacean aquaculture and fishery industries. Rickettsia-like organisms (RLO) are also important. Critically, diagnostic techniques necessary to detect the full range of potential pathogens of crayfish are rarely utilized in the field of crayfish pathology in Europe. Histopathological analysis, required for the diagnosis of infections by viruses and RLOs, is absent from most European studies. Epizootics unrelated to *A. astaci* in European crayfish and epizootics in introduced American crayfish species highlight the inadequate current state of knowledge in the field. Presently, the field is ill-equipped to determine the cause(s) of these epizootics. Moreover, crayfish conservation strategies may be undermined and even detrimental to the long-term goals; eg., stocking programs may spread undetected pathogens. Therefore, critical limitations in the field of crayfish pathology have major repercussions in management of freshwater crayfish. Guiding principles and a concept for a trans-European Community research and education program were developed to address this serious issue and are presented herein.

Key-words: European freshwater crayfish, disease, pathology, research programme, education.

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PATHOLOGIE DES ÉCREVISSES EN EUROPE : PASSÉ, PRÉSENT ET UN PROGRAMME POUR LE FUTUR

RÉSUMÉ

Les effets dévastateurs des maladies des écrevisses d'eau douce européennes sont bien connus car des épizooties ont sévi dans les populations sauvages dans beaucoup de pays d'Europe depuis la moitié du XVIII^{e} siècle. Après des débats prolongés et rigoureux, il a été prouvé en 1934 que la cause de la maladie appelée peste de l'écrevisse est le champignon *Aphanomyces astaci*. Dans les 70 dernières années, une grande partie de la recherche dans le domaine de la pathologie de l'écrevisse s'est concentrée sur les mycètes, améliorant en particulier les techniques de diagnostic *d'A. astaci.* De même, les réponses diagnostiques aux épizooties des écrevisses européennes se sont concentrées presque entièrement sur l'isolement et/ou l'identification fongique. D'autre part, les virus se sont avérés être les agents pathogènes les plus sérieux vis-à-vis de l'ensemble des industries en pleine croissance de l'aquaculture et de la pêche de crustacés. Les organismes apparentés aux Rickettsia (RLO) ont également un impact important. Cependant, les techniques de diagnostic nécessaires pour détecter la gamme complète des pathogènes potentiels des écrevisses sont rarement utilisées dans le domaine de la pathologie des écrevisses en Europe. L'analyse histopathologique, exigée pour le diagnostic des infections par des virus et RLOs, est absente de la plupart des études européennes. Des épizooties non liées à *A. astaci* et affectant les écrevisses européennes*,* et des épizooties affectant les espèces introduites d'écrevisses américaines*,* mettent en évidence l'insuffisance de l'état actuel de la connaissance dans le domaine. Actuellement, ce domaine est mal équipé pour déterminer la (ou les) cause(s) des ces épizooties. De plus, des stratégies de conservation d'écrevisses peuvent être minées et même nuisibles à long terme; par exemple, les programmes de réintroduction peuvent être responsables de la dispersion de microbes pathogènes non détectés. Par conséquent, les limites critiques du domaine de la pathologie de l'écrevisse ont des répercussions majeures dans la gestion des écrevisses d'eau douce. Des principes de base et un concept de programme de recherche et d'éducation de la Communauté Européenne ont été développés pour aborder cette sérieuse question, et sont présentés ci-dessous.

Mots-clés : Écrevisses européennes, maladie, pathologie, programme de recherche, éducation.

INTRODUCTION

Pathogens and the diseases that they cause are of increasing concern for conservation (LAFFERTY and GERBER, 2002). These pathogenic threats may be either exotic or endemic. The introduction of exotic pathogens may have severe consequences for native species. Prolonged periods of co-evolution often permit the establishment of relatively stable host-parasite relationships through enhanced immune functioning by the natural host and virulence attenuation by the parasite. Often this stable relationship is not replicated with a closely related naive host as it has not developed the required immune responses. The introduction of the crayfish plague fungus *Aphanomyces astaci* into Europe with native American freshwater crayfish species, and the resultant devastating consequences to native European crayfish species, is one of the most frequently quoted examples of the major threat presented to aquatic animals by exotic pathogens. However, the emphasis on this one very severe disease has resulted in a paucity of information being available to assess and manage the threats posed to European freshwater crayfish by other exotic and endemic pathogens which may also be very significant (EDGERTON, 2002a).

Freshwater crayfish are vitally significant to European society for environmental and ecological reasons. It is equally important to note the very significant cultural and economic value of freshwater crayfish throughout much of Europe, and particularly in the northern and eastern regions. Therefore, whilst the extinction of native species would be a very major loss, from the socio-economic viewpoint an even greater threat is the total loss of productive crayfish populations as this would have a very significant effect on essential parts of rural culture and incomes.

An overview of past and recent events and practices in the field highlight the lack of information on general disease, and underline the major difficulties that this situation poses to the effective management of European freshwater crayfish. Also presented is a proposal for a research and education programme which, if implemented, would have a rapid and major impact in addressing this deficiency.

HISTORY OF CRAYFISH PATHOLOGY IN EUROPE

Native European freshwater crayfish have been devastated by epizootics which have occurred throughout the continent for almost 150 years ago (ALDERMAN and POLGLASE, 1988). Such losses in an iconic and initially common-place animal precipitated a significant and widespread research effort to determine the cause of the panzootic. The first pathogen reported from European freshwater crayfish was *Psorospermium haeckeli* (HAECKEL, 1857) followed by *Thelohania contejeani* (HENNEGUY and THÉLOHAN, 1892), although the characteristic symptoms caused by the latter pathogen suggests that the disease that it causes (microsporidiosis or porcelain disease) would have been recognised much earlier.

The cause of the panzootic in European freshwater, which was referred to as the "crayfish plague", remained unknown at the turn of the twentieth century. In 1934, some 70 years subsequent to the first reported epizootics, and after rigorous debate and much earnest research, NYBELIN (1934) succeeded in performing Koch's postulates with the fungus *Aphanomyces astaci*. Thus, *A. astaci* is considered to be the causative agent for the disease termed crayfish plague (OIE, 2003a).

In the 70 years since *A. astaci* was reported, much research on European crayfish pathology has focussed on improving diagnostic techniques for *A. astaci* and on host-parasitic relationships between freshwater crayfish and particularly fungi. Definitive diagnosis for crayfish plague still relies on culturing fungi from crayfish from a population which has experienced epizootic mortality, and reinfection trials to demonstrate the susceptibility of a susceptible freshwater crayfish to the oomycete fungus (OIE, 2003a). Modern genetic-based diagnostic tests for *A. astaci*, such as those utilizing the polymerase chain reaction, have been developed and have the potential to significantly improve diagnostic capabilities for crayfish plague (CERENIUS *et al.*, 2002).

Research on the other long-known pathogens of European freshwater crayfish, *P. haeckeli* and *T. contejeani*, has been overshadowed, perhaps understandably, by the major emphasis on *A. astaci*. These pathogens are considered to be of much lower pathogenic significance, but actual virulence data for these species are virtually non-existent due to laboratory transmission difficulties. On a regional basis, several dedicated researchers have documented the occurrence of *P. haeckeli* and *T. contejeani*.

THE PRESENT SITUATION

Disease continues to exert a major influence on the wellbeing of native European freshwater crayfish as epizootics continue to occur throughout the continent (EDGERTON *et al.*, submitted). Furthermore, significant reductions in catches and epizootics in

American freshwater crayfish species in Europe have been observed (ROCHE, pers. comm., EDSMAN, pers. comm.).

The research emphasis on *A. astaci* has been replicated in the diagnostic sphere. Typically laboratories providing diagnostic services for European freshwater crayfish utilise predominantly, and often only, techniques applicable for the visualisation and/or identification of fungi. However, often it is the case that no fungal agent can be assigned as the cause of an epizootic in European freshwater crayfish (DIEGUEZ-URIBEONDO *et al.*, 1997; VOGT, 1999; EDGERTON *et al.*, 2002; SKURDAL and TAUGBØL, 2002). Frequently the remaining material from diseased crayfish was discarded or was unsuitable for other types of analyses because the only diagnostic analyses contemplated were for fungi. Moreover, a methodology for a broad and comprehensive diagnostic assessment for European freshwater crayfish has not been developed. Perhaps most significantly, some critical techniques typically used in diagnostic/pathological studies of other animals, such as histopathology, have been under-developed and under-utilised in the field of crayfish pathology in Europe (EDGERTON, 2000; EDGERTON *et al.*, 2002; EDGERTON, 2003).

MAJOR THREATS FROM OTHER PATHOGEN TYPES

As a consequence of the extreme focus on crayfish plague in crayfish pathology in Europe, other pathogen groups well-known for causing disease in other crustaceans are poorly known or unknown in European freshwater crayfish. Natural infections by just two viruses have been reported (EDGERTON *et al.*, 1996; EDGERTON *et al.*, 2002), the first report being published 7 years ago. Infection by a rickettsia-like or chlamydia-like organism in a European crayfish has never been reported.

Of particular concern is the lack of knowledge of viruses, as these have long been known to be serious pathogens of the other well-studied crustaceans groups of crabs (JOHNSON, 1978) and shrimp (LIGHTNER, 1996). In fact, of eight diseases of crustaceans listed by the Office International des Epizooties, seven are caused by viruses (OIE, 2003b). The remaining disease is crayfish plague.

In addition to viruses which have crayfish as their natural hosts, viruses from other hosts may threaten freshwater crayfish. White spot virus is a serious pathogen of penaeid shrimp and is listed by the OIE. White spot virus is also pathogenic to freshwater crayfish, is common and remains viable in frozen shrimp traded internationally, and is easily transmitted by susceptible hosts feeding on infected tissue (EDGERTON, 2002a). Therefore, white spot virus may represent a very significant new risk to European freshwater crayfish conservation.

These high profile pathogens and pathogen groups underline the significant threat that pathogens in addition to *A. astaci* may pose to European freshwater crayfish. However, it is important to note that, based on the current very low level of understanding of disease in freshwater crayfish, it is virtually impossible to determine the risk posed by these pathogens or pathogen groups which may be either exotic or endemic in Europe (EDGERTON, 2002a). Moreover, given the extremely low level utilization of techniques required for diagnosis of viruses and rickettsia-like organisms, there can be little confidence in even passive surveillance for the detection of potentially important pathogens in European freshwater crayfish. For example, even though it has not been detected or reported, it is entirely possible that white spot virus has already been introduced into European crayfish stocks, with or without serious consequences. Critically, biosecurity policy development is dependent on high quality data on the occurrence of pathogens. Therefore, the current poor level of knowledge of disease in European crayfish presents major difficulties in developing international biosecurity policies (EDGERTON, 2002b). Of

greater concern, current European biosecurity policies may be eminently challengeable on the grounds that freedom from certain pathogens has not been demonstrated.

As well as complicating international biosecurity policy, this lack of information on freshwater crayfish pathogens has major implications for regional biosecurity issues. Significantly, in many European countries there are policies for restocking of native crayfish populations to restore fisheries and to enhance conservation efforts. In only a few countries are there mandatory health checks prior to stocking, and in such cases the diagnostic tests applied are for visible signs of disease and the detection of readily identifiable parasites such as *Psorospermium*. Therefore, there is no chance of detecting infections by a whole range of other pathogens including viruses, rickettsia-like organisms, and so on. In other words, the majority of restockings are performed without any knowledge of the disease status of the stock, and in the remaining minority of cases the knowledge is very limited. It is entirely possible, even likely, that restocking programs for freshwater crayfish are resulting in the dispersal of pathogens, some of which may be seriously detrimental to freshwater crayfish populations.

BACKGROUND DISCUSSIONS IN THE ROUNDTABLE

A number of issues were highlighted in the disease roundtable. Firstly, disease was considered to be one of the most significant limitations on freshwater crayfish production and conservation in Europe. Epizootics in European crayfish are very frequent, and contribute to significant pessimism that native European crayfish may become extinct in some countries in the foreseeable future. Crayfish plague was considered to be an extremely important disease. It was also noted that there are many instances where *A. astaci* can not be associated with epizootics in native freshwater crayfish as attempts to isolate the fungus were negative when material supplied was of high quality. Moreover, there are concerns that diagnostic processes are prolonged, and managers are unable to make timely decisions. Often epizootics in native freshwater crayfish do not result in complete mortality, or the mortality in the lake is patchy, suggesting that multiple factors are involved (eg. genetics of host or *A. astaci*, water currents, or other pathogens). In some of these cases the diagnosis given by authorities was crayfish plague. However, OIE (2003a) states that for crayfish plague “100% mortality is the norm” in susceptible species. Moreover, there have been cases where different laboratories within the same country have provided different diagnoses from the same samples. Taken together, these observations have resulted in a reduced level of confidence in diagnostic services.

Epizootics in American crayfish species have been observed in a number of countries. In Sweden, Finland and Spain there have been epizootics in signal crayfish, *Pacifastacus leniusculus*, with losses of up to 90% observed across all size classes. In Sweden the epizootics have typically followed very severe winters. Hypotheses for this relationship involved stress-related factors, or even biophysical relationships between the crayfish, *A. astaci* and ice. However, the involvement of *A. astaci* with these epizootics is unknown. In Sweden, often the best producing signal crayfish lakes have the highest incidence of *A. astaci*-associated melanised lesions. Farmed populations of signal crayfish in Sweden are tested every two years (50 animals), and on only a few occasions has *P. haeckeli* been found. Therefore, it was considered unlikely that an increase in the occurrence of *P. haeckeli* has resulted in an increased incidence of crayfish plague disease in signal crayfish. In Finland there have been at least three epizootics in signal crayfish populations; two were wild populations (from lake Puujärvi and lake Katuma) and Olkiluoto crayfish farm has also been affected at least twice. It was generally agreed that there was virtually no data available to make any assessment on the likely cause of epizootics in signal crayfish.

In Scandinavia there have been a number of lakes in which the native noble crayfish, *A. astacus*, and signal crayfish have co-existed for prolonged periods, several decades in some cases. Generally in such instances the native population declined gradually. These observations question two very widely held views, that: 1) all signal crayfish populations are carriers of *A. astaci*, and 2) all noble crayfish are highly susceptible to *A. astaci*.

Additional constraints in researching and managing disease in freshwater crayfish were discussed. In some countries fisheries legislation hampers our understanding of disease; eg. the closed season on trapping crayfish in some countries, such as Finland, means that fisherman are unaware of an epizootic until after it has occurred. There is a general difficulty in resourcing (i.e. funding) research and field studies on disease in European freshwater crayfish. This flows through to an inability to retain young and enthusiastic scientists after they have been trained with valuable and rare skills. Significantly, there is a shortage of trained expertise in overall crayfish pathology in research and diagnostic disciplines in Europe.

PROGRAMME TO RESPOND TO DISEASE THREATS TO EUROPEAN FRESHWATER CRAYFISH

The roundtable concluded that it was extremely difficult to develop management strategies for disease in European freshwater crayfish with such a low level of confidence in the current level of knowledge. Therefore, a program to address this major deficiency was developed.

A conceptual framework (Figure 1) and a set of guiding principles were developed. It was recognised that crayfish plague was an extremely important disease. However, it was recognised that other pathogens must also be considered, such as viruses and rickettsia-like organisms. The role of the environment in the expression of disease was also noted. The need for improved diagnostic techniques and widespread adoption of these techniques was seen as critical. As far as practicable, these diagnostic techniques should be quick, cheap and standardised. These diagnostic techniques should then be applied to diagnostic response to disease outbreaks and in pre-stocking health checks in national and regional laboratories on farmed or wild stocks. Data from disease investigations should be incorporated into an EU disease register for freshwater crayfish to assist in the management of crayfish stocks, to prevent spread of disease. The development of the program should involve a network of laboratories throughout Europe, including research and fish disease diagnostic laboratories, EU and national funding, and contain a significant education component to transfer information to regional laboratories, field officers, farmers and the general public.

The proposed program involves the establishment of a key centre for research which would be responsible for administering the programme and for the primary research role, as well as a strong education role (Figure 2). The key centre would initially develop the standardised diagnostic techniques. Once developed, the key centre would play an education roll by conducting a workshop with all of the partner research centres and a representative from each of the European community national fish disease reference laboratories to extend the techniques to all countries in the EU. To ensure that these techniques are embedded in the field in Europe, it would be extremely beneficial if the participants immediately applied those skills on returning by each conducting a small scale health survey of crayfish in their countries. The information gained on the occurrence of pathogens would be extremely valuable, as would the archival material obtained in such surveys. Individual partner centres could then focus their research on the most pressing issues in crayfish health, with one partner centre each concentrating on crayfish plague, white spot virus (and other viruses), health of signal crayfish, and other important pathogens discovered. Finally, a robust education effort will be

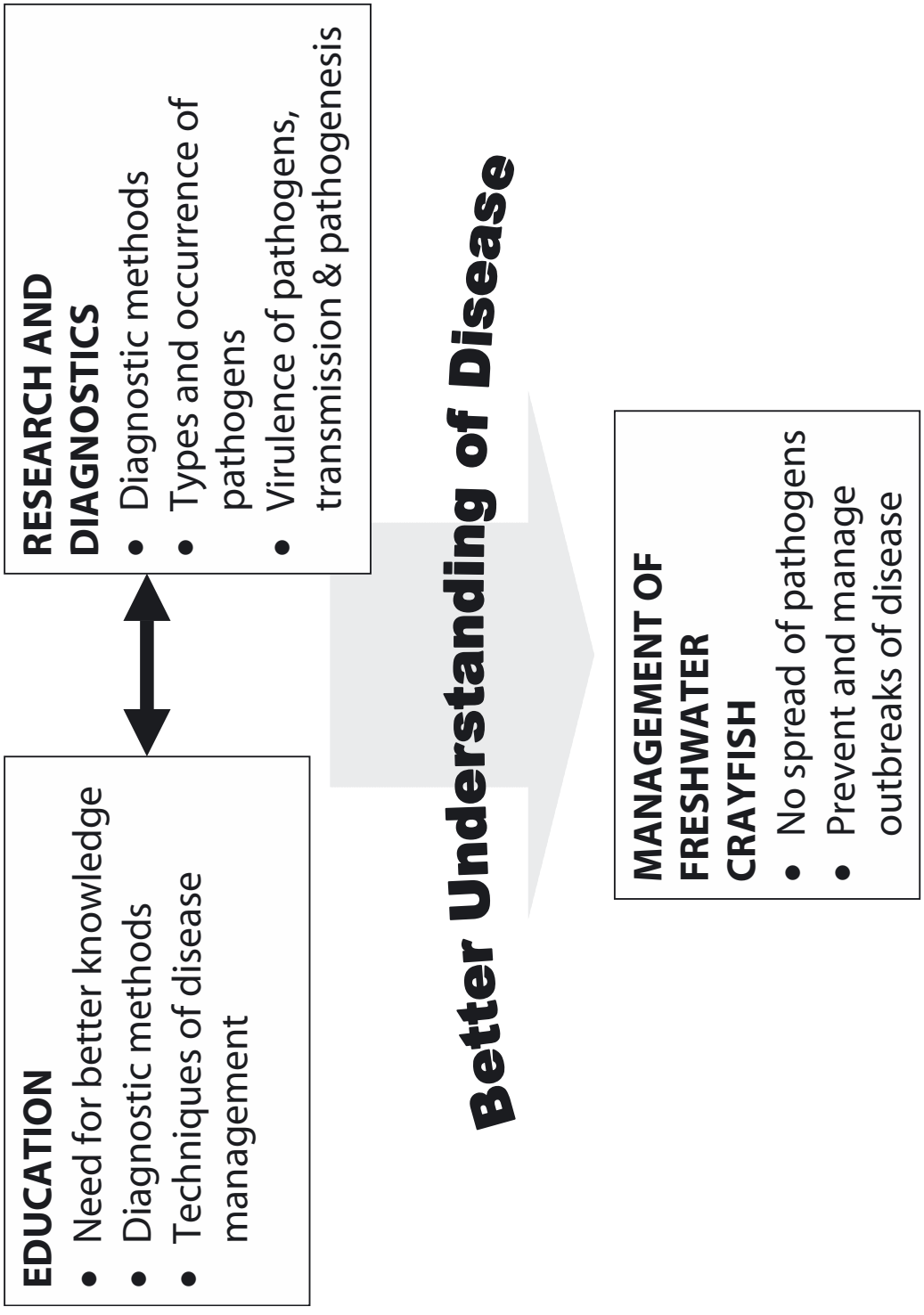

Figure 1
Conceptual framework for a programme to respond to the disease threats to European freshwater crayfish.

Figure 1
Cadre conceptuel d'un programme pour répondre aux menaces engendrées par les maladies des écrevisses européennes.

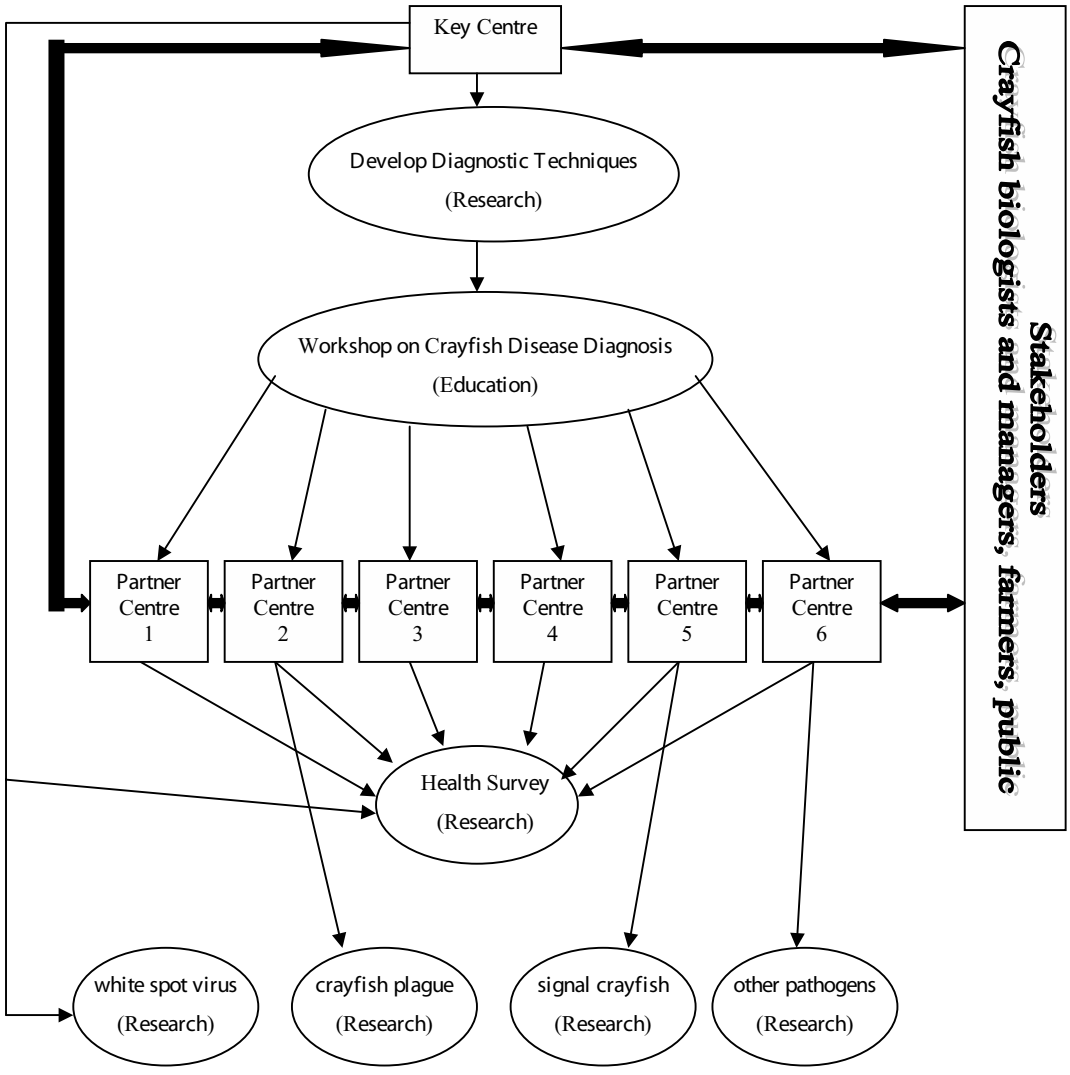

Figure 2

Proposed programme to respond to the disease threats to European freshwater crayfish. Note that partner centres may be national fish disease reference laboratories or independent laboratories (e.g. at universities). It is envisaged that there would be at least one partner centre from each EU member country. After developing skills in crayfish disease diagnosis, some partner centres would continue to research the most pressing issues which relate mostly to native crayfish, though one stream would concentrate on disease in signal crayfish.

Figure 2

Programme proposé pour répondre aux menaces engendrées par les maladies des écrevisses européennes. Notez que les centres partenaires peuvent être des laboratoires nationaux de références sur les maladies des poissons ou des laboratoires indépendants (c.-à.-d. au sein des universités). Il est envisagé qu'il y ait au moins un partenaire par pays membre de la communauté européenne. Après avoir développé des compétences dans le diagnostic des maladies chez les écrevisses, quelques centres partenaires continueraient d'obtenir les connaissances les plus urgentes en ce qui concerne préférentiellement les écrevisses natives, bien qu'une équipe pourrait se concentrer sur les maladies de l'écrevisse signal.

required to ensure that the information gathered from the program is presented to the stakeholders (crayfish managers, farmers and general public) in a useful form. This could be in the form of leaflets to raise their awareness to the threat of disease to crayfish populations, and providing information on signs of disease in freshwater crayfish, what to do when sick or dying crayfish are observed, and how to play a role in reducing the impact of disease in freshwater crayfish. The Internet should also be a major tool in the education effort.

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