Further studies reveal that Austropotamobius pallipes bacilliform virus (ApBV) is common in populations of native freshwater crayfish in south-eastern France

Brett F Edgerton

University of Munich, Institute of Zoology, Fish Biology and Fish Diseases, Veterinary Faculty, Kaulbachstr. 37, Munich 80539, Germany

Abstract
A small-scale histopathological survey of Austropotamobius pallipes in south-eastern France was conducted to determine the occurrence of the newly discovered Austropotamobius pallipes bacilliform virus (ApBV) and other potential pathogens. ApBV infected a high proportion of sampled crayfish from all three populations studied; in two populations ApBV infected 100% of A. pallipes examined, and in the other population ApBV was detected in 73.3%. The intensity of infection by ApBV was typically higher in the midgut and/or midgut caecum than in the hepatopancreas. All A. pallipes had low levels of lipid reserves in the hepatopancreas, and melanitic lesions in the gills were common in one population. These studies suggest that A. pallipes in this region are under significant pressure from pathogens and potentially other biotic and/or abiotic stressors. In conjunction with a previously reported small-scale histopathological survey of Astacus astacus in Finland, this study suggests that viruses commonly infect European freshwater crayfish in at least some areas. Therefore, it is critical that histopathological techniques be utilised when researching the cause of disease in European freshwater crayfish.

Introduction
Serious declines over the last 150 years in native European crayfish stocks have been well documented (Alderman, 1986). It is now widely accepted that these declines have been associated with environmental degradation, competition with introduced species, and with the presence of the crayfish plague fungus, Aphanomyces astaci.

Alderman and Polglase (1988) wrote one of the first reviews which highlighted the lack of information on viral infections in freshwater crayfish. It is noteworthy that the first report of a freshwater crayfish naturally infected by a virus was published just ten years ago (Anderson and Prior, 1992). In recent years, there has been considerable progress in identifying viruses in freshwater crayfish (Edgerton, 1999; Evans and Edgerton, 2002; Edgerton, 2002).

The first description of a natural viral infection in a European freshwater crayfish was published in the mid 1990s (Edgerton et al., 1996). Prior to this, Halder and Ahne (1988a) showed in laboratory transmission trials that Astacus astacus could act as a mechanical vector for infectious pancreatic necrosis virus which is a serious pathogen of salmonids. During these studies, the group also found an aggregate of small virus-like particles in gill
cells but showed no evidence that the virus had replicated in the crayfish (Halder and Ahne, 1988b).

A minor histopathological survey of the noble crayfish *A. astacus* in Finland, which included 15 animals from 5 populations, revealed a high occurrence of an intranuclear bacilliform virus (IBV; perhaps better known as a baculo-like virus) (Edgerton et al., 1996). In fact, *Astacus* astacus bacilliform virus (AaBV) infected 100% of the examined animals from 4 populations and over 50% from the other population. A survey in the subsequent year, which included additional populations, yielded similar results (Paasonen et al., 1999) proving that AaBV is highly prevalent in *A. astacus* in central Finland.

Recently, an IBV was associated with the near extirpation of a population of *Austropotamobius pallipes* in the Ardèche region in southern France (Edgerton et al., 2002). The results here report a small-scale survey of three other populations of *A. pallipes* from the nearby region in south-eastern France.

**Materials and Methods**

*A. pallipes* were collected by hand or in baited traps from the Azerguoise, Fermuizet and Drome Rivers in south-eastern France in late October 2001. The numbers of crayfish from each population examined in this study were 8, 15 and 11 respectively. Within several days of capture, the *A. pallipes* were killed and fixed by injection of Davidson’s solution into the cephalothorax. Immediately prior to being fixed, all crayfish from the Azerguoise and Fermuizet Rivers appeared healthy, whereas 6 animals included in the Drome River sample were moribund or apathetic. (The total number trapped from this population was approximately 70, with the remainder being used for another study.) Tissues were trimmed from the cephalothorax by cutting twice longitudinally in the dorso-ventral plane on either side of the midline to produce a full longitudinal slice of the cephalothorax of approximately three millimetres in thickness. Gill was also included in histological cassettes. Tissues were processed, and histological sections were cut at 5 µm and stained with haematoxylin and eosin routinely.

### Results

Eosinophilic inclusions in hypertrophic nuclei of epithelial cells of the mesenteron (Figures 1-3), consistent with infection by *Austropotamobius pallipes* bacilliform virus (ApBV), were detected in *A. pallipes* from all three populations (Table 1). These lesions were detected in one hundred percent of *A. pallipes* examined from the Azerguoise and Drome Rivers, and in 73.3% of *A. pallipes* from the Fermuizet River.

A total of 30 *A. pallipes* infected by ApBV were examined in this study. All three tissues known to be susceptible to infection by ApBV, hepatopancreas, midgut and midgut caecum, were present in sections of 17 of those animals. In eight of them, lesions were detected only

<table>
<thead>
<tr>
<th>Population</th>
<th>No. animals</th>
<th>No. infected (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azerguoise R</td>
<td>8</td>
<td>8 (100)</td>
</tr>
<tr>
<td>Fermuizet R</td>
<td>15</td>
<td>11 (73.3)</td>
</tr>
<tr>
<td>Drome R</td>
<td>11</td>
<td>11 (100)</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td>30 (88.2)</td>
</tr>
</tbody>
</table>

Table 1 – Data on the occurrence of *Austropotamobius pallipes* bacilliform virus in *Austropotamobius pallipes* populations in south-eastern France.
Figures 1-3: *Austropotamobius pallipes* infected by *Austropotamobius pallipes* bacilliform virus (ApBV). H&E. 1) Infected cells (arrows) in the hepatopancreatic tubule epithelium; note that infected cells have hypertrophic nuclei which contain eosinophilic inclusions. Scale Bar = 10 \( \mu m \). 2) Infected cells (arrows) in the midgut caecum. Scale Bar = 32 \( \mu m \). 3) Infected cells (arrows) in the midgut. Scale Bar = 32 \( \mu m \). FG = foregut. Figure 4: Hepatopancreatocytes of *Austropotamobius pallipes* infected by *Austropotamobius pallipes* bacilliform virus in the process of sloughing from the epithelium (arrows). Scale Bar = 32 \( \mu m \). H&E. Figures 5-6: Gill filaments of *Austropotamobius pallipes* from the Drome River displaying melanitic lesions. H&E. 5) In the majority of cases, the lesions (arrows) were limited to melanisation in the cuticle. Scale Bar = 64 \( \mu m \). 6) In some cases the lesions were more invasive and resulted in truncation of filaments with significant melanisation at the margin. Scale Bar = 32 \( \mu m \).
in the hepatopancreas. Conversely, in only one animal infected cells were observed in the midgut and/or midgut caecum and not in the hepatopancreas. However, in most cases the intensity of infection (i.e. the proportion of cells displaying the lesions) in the midgut and/or midgut caecum epithelia was considerably higher than in the hepatopancreatic tubule epithelium. In the hepatopancreatic epithelia, infected cells were most commonly observed in the antechamber and main collecting ducts, and were occasionally observed medially, but never in the apical tips, of tubules. On the other hand, infected cells were occasionally observed between cells undergoing mitosis in the midgut caecum epithelia. Cells infected by ApBV were occasionally observed in the process of sloughing from the hepatopancreatic tubule epithelium or in the lumen of hepatopancreatic tubules (Figure 4).

Melanisation of gill filaments was observed in eight *A. pallipes* from the Drome River. In most cases, melanin was deposited in the cuticle with a small number of cells adhered to the inner surface of it (Figure 5). On some occasions, the lesions were more invasive and were marked by truncation of gill filaments (Figure 6). One of the moribund *A. pallipes* from the Drome River, which did not display melanitic lesions in the gills, was infected by a microsporidian. All *A. pallipes* from all populations had low to very low levels of lipid vacuolation in the hepatopancreas.

**Discussion**

The low to very low level of lipid stores in the hepatopancreas of *A. pallipes* from all populations is surprising given that the crayfish were collected in mid Autumn. This may be an indication of significant pressure on these populations, either from a deteriorating environment, chronic infections, and/or other stressors. The melanitic lesions in the gills of the *A. pallipes* from the Drome River, which may be due to fungal or bacterial infections, is further evidence of such pressures on this population.

ApBV was discovered during an investigation of the cause of epizootic mortality in a wild population of *A. pallipes* (Edgerton et al., 2002). In that study, hepatopancreatic tissues for histology were either dissected prior to fixation, or remained in the cephalothorax which was cut in half longitudinally prior to being placed in fixative. As a consequence of using these techniques, midgut was observed in only two animals in that study. The level of infection by ApBV in the midgut in both of those cases was higher than was observed in the hepatopancreas of each animal. In the present study, cephalothoraxes were fixed whole and were processed in a manner which allowed for the preparation of longitudinal sections in the dorso-ventral plane along the midline or parallel and near to it. This technique regularly produces sections which contain the open-ended alimentary tract, from the oesophagus to the hindgut, severed at the posterior margin of the section, and including the midgut and midgut caecum. This study adds to the initial observations by Edgerton et al. (2002) that the level of infection by ApBV in the midgut and/or midgut caecum may be considerably higher than in the hepatopancreas. On the other hand, in the present study it was more common for ApBV to be detected in the hepatopancreas and not in the midgut and/or midgut caecum, than vice versa. When investigating mortality in
A. pallipes it is important to ensure that sections contain midgut and midgut caecum to determine the involvement of ApBV. However, this study suggests that the prevalence of ApBV in a population will not be significantly understated if few sections contain midgut and midgut caecum.

The histopathological survey of A. astacus in Finland reported by Paasonen et al. (1999) was a continuation of the study reported in Edgerton et al. (1996). Therefore, the study reported herein is only the second reported histopathological survey of European freshwater crayfish. Both histopathological surveys were on a limited scale, involving small numbers of crayfish from small numbers of populations in a restricted geographic region. However, both studies showed that viruses were common in the sampled populations, and suggest that viral infections may be common in freshwater crayfish throughout Europe. It should be noted that the data from Drome River are affected by a slightly positive bias as the sample contained six animals which were apathetic or moribund and, therefore, more likely to carry infections. Moreover, the number of A. pallipes sampled in this study from each population was limited by difficulties in catching this relatively reclusive species and by concern for the species’ well-being since it is vulnerable to range reductions and population declines (Baille and Groobridge, 1996). Though the number of analysed A. pallipes was too low to establish the prevalence of ApBV, it is clear that the virus is common in these populations.

These findings present two interesting questions: 1) if viruses do commonly infect European freshwater crayfish, why were they undetected until very recently even though there has been significant research interest in crayfish diseases in Europe for 150 years? and 2) what is the significance of viral infections in European freshwater crayfish?

There are several possible answers for the first question; that the viruses were recently introduced, were previously present at levels so low as to make their detection improbable, or that researchers and diagnosticians were not frequently using diagnostic tools applicable for their detection. Though the first two possibilities can not be excluded, it does appear, both from the literature and the author’s own understanding of the field, that the latter factor has been very significant. Histopathology, which is required for detection of virus infections in freshwater crayfish in the absence of established cell lines, is significantly underutilised in the diagnosis and study of disease in European freshwater crayfish. The great majority of freshwater crayfish disease diagnosticians and researchers focus mostly on mycological, and to a lesser extent bacteriological and general parasitological techniques. Though this emphasis is understandable, it is clear that it has resulted in a limited overall knowledge of pathogens of European freshwater crayfish. It therefore follows that the significance of viral infections, and of many other disease conditions, in European freshwater crayfish will remain unknown until the fundamental diagnostic tool of histopathology is much more widely utilised.

Acknowledgments
This research was initiated whilst the author was in receipt of a Research Fellowship from...
the Centre National de la Recherche Scientifique in France, and concluded whilst in receipt of an Alexander von Humboldt Research Fellowship in Germany. Drs. Philippe Roch and JR Bonami (France), and Prof. Rudolf Hoffmann and Dr. Birgit Oidtmann (Germany) are thanked for hosting the author during the tenure of these fellowships. Pascal Roche, Benoit Froment and Jean-Yves Patingre of the Conseil Supérieur de la Pêche in France are thanked for their enthusiastic support of this project and for organising and carrying out the collection of crayfish.

References


