

Investigation into mortalities of wels catfish (*Silurus glanis*) in a lake in southern England

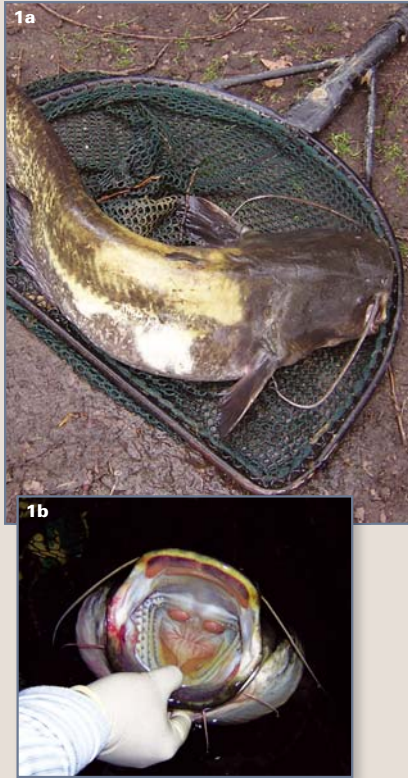


Figure 1: Catfish in rigor showing spinal curvature (Figure 1a). Unusual gaping behaviour seen in fish 1 (Figure 1b).

Introduction

Fish mortalities occurred in a lake in southern England between May and September 2005. The lake, a gravel pit, contained a mixture of specimen-sized coarse fish and wels catfish (*Silurus glanis*). Due to excessive weed growth the lake was treated with a Terbutryn based herbicide in April 2005. After the herbicide treatment the fishery suffered from a bacterial bloom and subsequent water quality issues including low oxygen levels. Fish deaths occurred throughout May 2005, 90% of which were carp (*Cyprinus carpio*). Water quality parameters had stabilised by the end of the month and deaths ceased. The fishery was re-opened for angling with fish feeding well and no apparent health problems.

During June, wels catfish were failing to recover after capture. The fishery was closed and monitored for the next few weeks and fourteen dead catfish were removed from the lake. The fishery was test fished in early July resulting in the capture of 8 carp, 4 grass carp (*Ctenopharyngodon idella*), 5 bream (*Abramis brama*) and 3 catfish. Two of the catfish died within seconds of being landed, the remaining catfish had died before reaching the net. The test was suspended and monitoring took place with no apparent deaths amongst the other species caught and returned to the lake. A further test was carried out in mid July during which time another catfish died on capture. Other fish species appeared unaffected.

The Cefas Fish Health Inspectorate were contacted to investigate these mortalities and attended the lake in late September.

Methods

Diagnostic tests

- Virology – brain, kidney and spleen samples were taken and placed into transport media and inoculated onto BF, EP and CCO cell lines.
- Bacteriology – kidney swabs were plated onto TSA medium and incubated at 22°C for 48 hours.
- Histology – brain, gill, heart, kidney, liver and spleen samples were taken and placed in neutral buffered formalin (NBF). Samples were processed using standard histological techniques. Slides were stained using haematoxylin and eosin (H&E). In addition, liver sections were stained using Perls' Prussian blue for the presence of ferric iron.
- Electron microscopy – samples of heart, spleen, kidney, liver and gill were fixed in 2.5% glutaraldehyde in sodium cacodylate buffer, post fixed in osmium tetroxide and processed for transmission electron microscopy (TEM) using routine methods.
- Blood – PCR was carried out using Ictalurid Herpesvirus (IcHV) primer sets

Results

Clinical signs and pathology

Almost immediately on landing the body went into rigor (Figure 1). This was also observed in catfish mortalities prior to this investigation. Externally, both fish appeared normal. Internally, gross pathology of the visceral organs was absent. Additionally, fish 1 displayed flaring of the opercula and gaping (not seen in previous mortalities).

Microorganisms and parasites were not detected in the samples examined and PCR for IcHV was negative. However, histopathological changes were observed in the kidney and liver of both fish sampled, with limited pathology also present in the gill. Specific renal pathology included tubular nephrosis appearing as increased eosinophilia of the tubule cells and detachment from the basal lamina (Figure 2). Haematopoietic tissue appeared unremarkable. The principle changes in the liver were the presence of yellowish/brown pigment within hepatocytes (Figures 3 & 4) which were positive for ferric iron (Figures 4 & 5) indicative of hemosiderin. Ultrastructural examination of the liver provided evidence of limited cytopathic effect associated with the electron dense pigment granules (Figures 8 to 10). Limited gill pathology was observed in fish 2, consisting of mild epithelial hyperplasia and congestion of the branchial vessels (Figure 6). Other tissues appeared within normal limits.

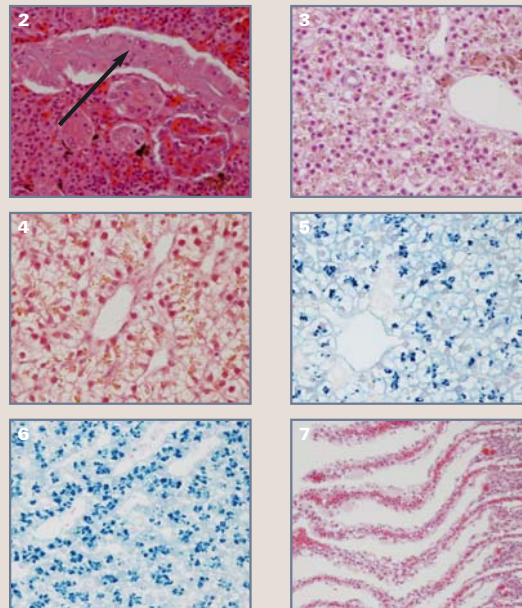


Figure 2: Section through the kidney of fish 1 showing acute nephrosis of the tubular epithelium. Note tubule epithelial cell eosinophilia (arrow). H&E stain.

Figures 3 & 4: Livers from fish 1 and 2 respectively showing the presence of yellowish/brown pigment granules within the hepatocytes. H&E stain.

Figures 5 & 6: Liver sections from fish 1 and 2 respectively stained with Perls method for ferric iron. Note the positive staining of the pigment granules (dark blue), indicative of hemosiderin. Figure 7: Section through the gill of fish 2 showing mild epithelial hyperplasia. H&E stain.

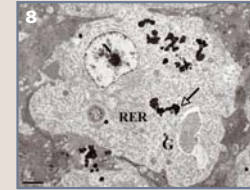


Figure 8: Liver cell from fish 2 containing electron dense pigment within the cytoplasm (arrows). Nucleus (N) and rough endoplasmic reticulum (RER) can be seen clearly and cytoplasm contains abundant glycogen (G). Scale bar = 2µm. TEM micrograph.



Figure 9: Liver from fish 2 showing nucleus (N), rough endoplasmic reticulum (RER), glycogen (G) and electron dense deposit (arrow). The affected cell is surrounded by degenerate cells (*) which also contain electron dense pigment. Scale bar = 2µm. TEM micrograph.

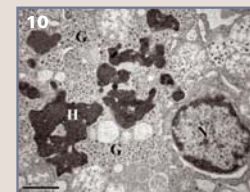


Figure 10: Higher power view of cytoplasm of a liver cell from fish 2. The nucleus (N) is clearly seen as are deposits of electron dense granules of hemosiderin (H). Glycogen (G) is abundant throughout the cytoplasm. Scale bar = 1µm. TEM micrograph.

Discussion

The finding of hepatic hemosiderosis is significant even though only two fish were sampled. The deposition of this pigment is most frequently associated with the breakdown of red blood cells resulting in anaemia. This possibility is supported by the behaviour of affected fish, including fish 1 which showed signs of acute respiratory stress on capture. During the period of catfish mortalities no other species were involved, although carp species died initially during the spring of 2005. It is interesting to speculate that physiologically, the catfish were able to survive the prevalent conditions following the application of the herbicide but were chronically affected resulting in the pathology observed. Later, when further stressed during capture they rapidly succumbed.

The testing for infectious agents provided negative results and the apparent absence of parasites was unexpected. The importance of gathering information on the history of the site, including stocking and management practices is clear. In this case we were not able to identify the cause of the observed mortalities but the pathological features observed in the catfish suggest that exposure to toxicants is a possibility. The release of algal toxins during the period of excessive weed growth may have contributed to the pathology seen but signs of hepatotoxicity, which might be expected if algal toxins were present, were not evident in the two fish examined. Herbicide toxicity appears unlikely since reported effects have only been recorded at significantly higher concentrations than the dosing applied to the lake. A common problem affecting freshwater aquaria, so called "brown blood disease" is associated with nitrite toxicity, which causes methemoglobinemia resulting in hypoxia. Affected fish may appear unaffected but succumb rapidly on exertion, in a similar fashion to the symptoms observed in the catfish during this investigation. The nitrite levels within the lake were not measured and this possibility cannot be ruled out. This investigation has highlighted the need for an integrated approach when investigating unexpected mortalities, including samples for water chemistry and potential toxins.

Acknowledgement

The authors wish to acknowledge Mr Mike Barras for his valuable contribution to this investigation.