Bull Environ Contam Toxicol (2009) 83:474–478
DOI 10.1007/s00128-009-9799-0

Environmental and Climatic Factors Associated with Epizootic Ulcerative Syndrome (EUS) in Fish from the Zambezi Floodplains, Zambia

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Received: 7 March 2008 / Accepted: 15 June 2009 / Published online: 30 June 2009

Abstract This study was conducted to determine environmental and climatic factors associated with Epizootic Ulcerative Syndrome (EUS) in fish in the Zambezi floodplains. EUS is a fish disease that causes economic loses to the fishing industry. Streambed colour in affected water was rusty-, reddish- or yellowish- brown and pH 4.5–6.0 while pH of non affected water was 7.2. The rusty-brown precipitate on fish gills was positive for Prussian blue iron stain. Therefore, predisposing factors for EUS in the Zambezi floodplains were the acidification of ground water during drought years and eventual contamination of surface water during the floods of 2006/2007.

Keywords Water acidification · EUS outbreak · Zambezi

Fish is the most affordable source of animal protein for many people in Zambia and fishing provides income to fishermen, traders and to the local governments through fish levies. The Zambezi floodplain of the Western Province is one of the major fisheries in Zambia. From June to November 2006, a disease characterised by red spots and ulcers on the skin and very high mortality in various fish species was reported in Kazungula and Sesheke districts. The following year in June 2007, the disease reappeared in Kazungula and Sesheke and also appeared in Senanga, Mongu, Kalabo and Lukulu districts. Saprolegnia, *Aeromonas hydrophila* and *Aphanomyces invadans*, which are normally opportunistic pathogens of the seasonal Epizootic Ulcerative Syndrome (EUS), were microbiologically isolated from some samples (Hang'ombe et al. submitted). EUS has been reported in many parts of the world such as Australia (Lilley and Roberts 1997) and the USA (Blazer et al. 1999). However, this was the first time such a disease occurred in Zambia and environmental factors as well as climatic conditions associated with it, needed to be established. Most fish disease outbreaks including EUS are usually associated with environmental or climatic stress factors (Noga et al. 1998; Sanaullah et al. 2001) which include chemical changes that decrease water quality (e.g. low dissolved oxygen, improper pH and pollution) as well as physical factors such as temperature changes.

Although *A. invadans* and *A. hydrophila* have consistently been isolated from all EUS outbreaks from different countries (Lilley and Roberts 1997), none of these pathogens have been established as the primary etiological agents (Mohan and Shankar 1994) because they do not normally infect health fish in a suitable aquatic environment. EUS is generally associated with changing water quality and climatic conditions (Sanaullah et al. 2001) that may predispose the fish to the syndrome. In Australia and the Philippines outbreaks of EUS, have been associated with acidic water during periods of heavy rainfall (Sammut et al. 1996). In Bangladesh, Sanaullah et al. (2001) found that a rapid decrease in water temperature, low chloride and alkalinity with respect to hardness were significant stressors predisposing fish to EUS. Therefore, even if, the secondary infectious agents such as *A. invadans*,

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Saprolegnia and *A. hydrophila* may be the same in all EUS outbreaks world wide, the predisposing factors seem to vary from one region to the other. The objective of this study was to determine environmental and climatic factors associated with EUS outbreak in the Zambezi floodplains.

Materials and Methods

Although the reported mortality of fish peaked at the end of the flood season in June/July, it was only possible to conduct this study in September 2007. The study was conducted according to the investigation protocols in the Fish Kill Reporting and Investigation Manual (QDEH 1998). Figure 1 shows the location of the study area in Southern Africa while Fig. 2 shows sampling sites. Site 1 on Luena river in Kaoma district was the unaffected control site with no disease. The rest of the sites, 2 in Lukulu, 3 in Kalabo, 4 in Mongu, 5 on Lui river, 6 in Senanga, 7 on Kasaya river in Sesheke and 8 in Mambova, Kazungula district were affected by the disease.

At each site field tests were made on water pH using indicator strips (BDH Poole England), dissolved oxygen using a Cole-Parmer portable EW-53026-00 dissolved oxygen meter and temperature was measured using a calibrated thermometer. Observations on water colour and smell as well as aquatic plant/algal growth and surrounding human activities were also made. A digital camera was used to take photographs at the sites.

Water and sediment samples were collected in duplicate at all sites. Water sample for nitrate and phosphorus analysis was filtered on site and collected in 500 mL plastic bottles rinsed with distilled water. Water for heavy metal and pesticide analysis was collected in 500 mL glass bottles rinsed with hydrochloric acid and methanol respectively. Sediment samples for heavy metal analysis were collected in 500 mL glass jars rinsed with hydrochloric acid while those for pesticides were collected in glass jars rinsed with methanol. Fish freshly caught by fishermen using gill nets were collected from both affected and non affected sites. Out of the total number of fish collected from affected site half had red skin spots or ulcers and the other half without any obvious signs of illness. Although many species were affected, only 6 *Barbus poechii* were collected per site and used for Prussian blue stain for iron because of their small gill size and suitability for direct examination without the standard histological processing which causes the loss of the unfixed precipitates clogged in the gills. Ten mixed fish species of Serranochromis, Oreochromis and Sargochromis per site were collected for pesticide and heavy metal analysis. Fish were individually wrapped in aluminium foil and all samples were transported to the laboratory in cooler boxes containing ice packs. Water samples for nitrate and phosphorus analysis were stored in a freezer at −20°C while the other samples were refrigerated at 4°C and analyzed within 7 days from collection.

Water, sediment and fish samples were analysed in duplicate in the laboratory for heavy metals using an atomic absorption spectrophotometer (AAS) ANA 182, Tokyo Photo Electric Co Ltd. A Hitachi 263-50 gas chromatography with an electron capture detector (GC-ECD) was used for the detection of pesticides from water, sediment and fish extracts. Phosphates and nitrates in water were determined by the ammonium molybdate and chromotropic acid

Fig. 1 Location of study area in Zambia and Southern Africa. Map adapted from www.africamap.com

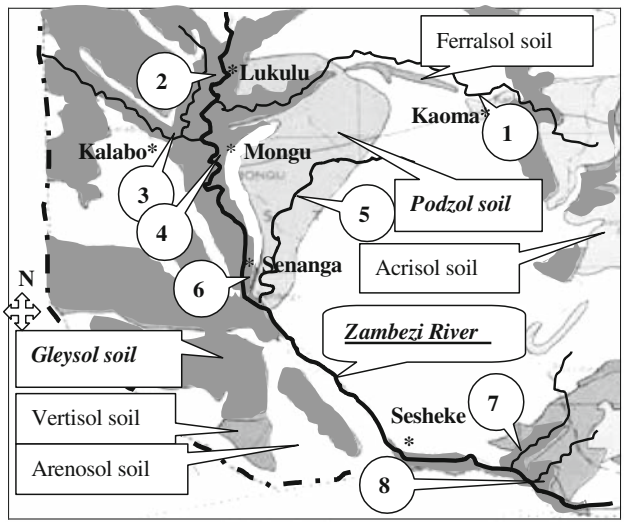

Fig. 2 Soil types at different sampling sites. Site *1* was not affected while sites *2–8* were in affected areas. Map adapted from GRZ (1986)

spectrophotometric methods respectively. Pieces of gill filaments from small *B. poechii* fish were directly laid flat on microscopic slides, stained with Perls Prussian blue without any tissue processing and examined under a microscope at 40× magnification for greenish-blue to blue coloured precipitates. Photographs of stained gills were taken through the eye piece of the microscope using a digital camera. Both field and laboratory results were subjected to the interpretation guide in appendix 5 of the Fish Kill Reporting and Investigation Manual (QDEH 1998) in order to make a conclusion on the major factors involved.

Flood water level records on little Zambezi river for the year 2002–2007 were obtained from the Water Affairs Department in Mongu. Little Zambezi is a branch of the main Zambezi river that latter re-joins it, forming a large island in the flood plain. Water level records were a compilation of daily readings from meters permanently fixed on the river bank.

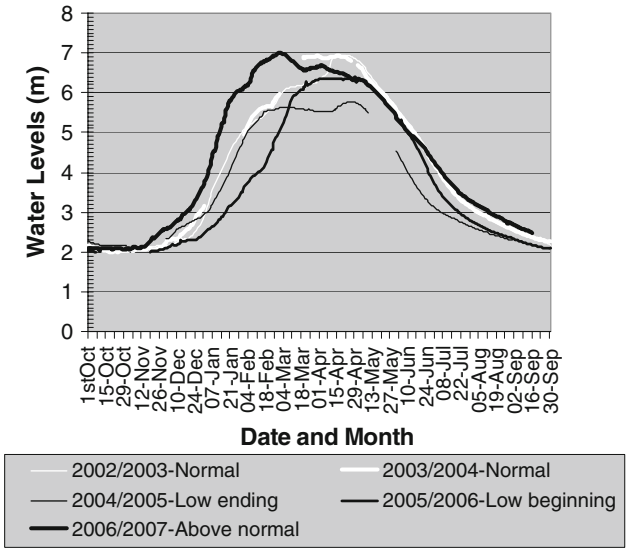

Fig. 3 Water levels at Matongo, Mongu, on Little Zambezi river within the floodplain for the year 2002/2003 to 2006/2007 rain seasons

Results and Discussion

The mortality of fish due to the EUS outbreak in the study area peaked at the end of the flood season in June/July, but the study was conducted in September 2007 when fish were no longer dying but simply showing red skin spots or ulcer and sometimes scars of healing. Figure 3 shows that the rainfall and flooding patterns from 2002 to 2004 were normal. The drought year of 2004/2005 was the most critical since it recorded the lowest flood levels because the rain that had started normally ended earlier than normal. The rain season of 2005/2006 caused very severe floods only in Kazungula and Sesheke districts that were followed by the first EUS outbreak of 2006 only in the two districts, while there was a partial drought in other parts of the province including Mongu. The consecutive drought and partial drought years were followed by the massive floods of 2006/2007 that had the longest duration and highest water levels and the subsequent EUS outbreak of 2007 in all but one district of Western Province.

Water tests showed that, in affected areas the pH was 4.5–6.0 and the colour of the streambed varied from rusty-brown to reddish-brown or yellowish-brown compared to the greenish water of non affected sites (Table 1; Fig. 4a–d). Acidic water usually dissolves minerals such as iron and aluminium, but when the pH increases, the dissolved iron precipitates as iron hydroxide that forms deposits on the streambed, submerged plants and gills of fish. Levels of iron and aluminium in water were very low due to the precipitation that had already taken place at the time of sampling. Perls Prussian blue stain of gills gave a positive greenish-blue colour of the rusty-brown precipitates indicating the presence of iron in the precipitates (Fig. 4e, f). The water hardness as well as calcium and magnesium ion concentrations were very low at all sites, implying that the buffering capacity was equally low and was even lowered further in acidic areas (Table 1).

Causes of water acidification can be anthropological or natural. In this study area there were no nearby industries that could have contaminated the water with acid.

Table 1 Surface water pH, colour and other parameters for different water bodies

Type of water body	Parameter measured in water							
	n	Streambed colour	pH	Hardness mg/L	Ca mg/L	Mg mg/L	Fe mg/L	Al mg/L
A	11	Rusty, reddish or yellowish-brown	5.25 ± 0.75	3.1 ± 0.4	1.6 ± 0.3	0.10 ± 0.01	0.053 ± 0.004	<0.03 ± 0.00
B	8	Greenish	6.50 ± 0.30	7.8 ± 0.9	2.5 ± 0.3	0.20 ± 0.01	<0.003 ± 0.000	<0.03 ± 0.00
C	10	Greenish	7.28 ± 0.28	21.7 ± 1.3	7.0 ± 0.6	1. 0 ± 0.03	<0.003 ± 0.000	<0.03 ± 0.00

A Affected lagoons, lakes and small tributaries with no or slow water current

B Non-affected small tributaries with fast water current and Zambezi river backwaters

C Non-affected Zambezi river at any site, Kabompo river and Luena river

Fig. 4 Cloudy streambed in some affected areas due to (**a**) *rusty-brown* (**b**) *yellowish-brown* and (**c**) *reddish-brown* precipitates at sites 5, 2, and 4 respectively: (**d**) Clear appearance of *greenish* streambed of non-affected site 1: (**e**), (**f**) *Greenish-blue* iron hydroxide precipitates (*dark dots*) on gills of *Barbus poechii* fish (x40 magnification) following Perl's Prussiun blue stain. (Color figure online)

Therefore, the acid was draining from natural sources in the soil. Natural sources of acids could come from decomposing organic matter releasing humic and other organic acids or from the oxidation of iron pyrite in the soil. Low rainfall from 2004 to 2006 followed by severe floods of 2007 were favourable conditions for iron pyrite oxidation and sulphuric acid production followed by surface water contamination with acid. The rusty-brown to reddish-brown or yellowish-brown appearance of the streambed in affected areas and gill analysis showing the presence of iron hydroxide provide further evidence of iron pyrite oxidation in the soil. The disease was more prevalent in portions of rivers or streams with slow water current and lagoons that were found in areas with either gleysol or podzol type of soil (Fig. 2). Gleysol units are also found in podzol soils. Gleysol is a type of soil that exhibits a greyish colour due to anaerobic wetland conditions. Soils maps of Zambia do not sub classify gleysol distribution according to sulphur content. Thionic gleysols, latter called thionic fluvisols includes soils that have a horizon containing an amount of sulphides and/or elementary sulphur which is high enough to cause acidification of the soil upon oxidation (FAO/UNESCO 1968, 1970).

This study shows that when the necessary environmental conditions were met, acid was produced in the soil in quantities large enough to contaminate the water in the floodplains. This means that some of the waterlogged gleysols in the study area (Fig. 2) contain iron pyrite. The high water table has all along prevented oxygen in the air from reacting with the iron sulphides. When the water table was lowered due to the drought of 2004/2005 and reduced rainfall of 2005/2006, iron pyrite was exposed to air and oxidised to produce sulphuric acid. This resulted in acidification of ground water and, eventually, surface waters during the floods of 2006/2007. Acidic water with pH below 3.5 dissolves metals such as iron, aluminium and manganese, but as the pH gradually rises to above 3.5, iron

hydroxide precipitates as a reddish-brown or rusty-brown pigment that settles at the bottom of the river, coating submerged vegetation and also clogging gills of fish (QDEH 1998; Hoehn and Sizemore 1977). Most fish species are killed by pH less than 4.0, while pH of 4.0–6.0 is stressful and predisposes then to disease. Therefore, the low pH and the clogging of gills with iron hydroxide precipitates caused severe stress on the fish making them susceptible to infection by opportunistic infections that caused the syndrome in the fish. Contamination of natural water ways with acid runoff from the soil have been reported in other parts of the world such as Australia (Hart et al. 1987). Furthermore, massive fish kills following heavy rain seasons have been associated with acidic water and high aluminium (Witter et al. 1990) and precipitates of iron salts. Experimental exposure of fish to low pH has been shown to cause changes in epidermal structures and in severe cases has resulted in epidermal necrosis (Daye and Garside 1976; Calliman et al. 2005). Result of this study, also supports the mounting evidence that EUS outbreaks are triggered by non-infectious factors associated with changes in the aquatic environment. For example EUS outbreaks in Australia and the Philippines, have been associated with acidic water draining from acid sulphate soils during periods of heavy rainfall (Sammut et al. 1996), with tributaries draining acid sulphate areas recording the highest prevalence. In Bangladesh, rapid decrease in water temperature, low chloride and alkalinity with respect to hardness were found to be the major predisposing factors to EUS outbreak in fish (Sanaullah et al. 2001).

In this study, the occurrence of the disease outbreak at the end of the flood season, when there was slow water current in tributaries of the Zambezi river and separation of lakes and lagoons from the main floodwater lead to a reduction in the dilution effect of the water. The reduced dilution effect of the water, coupled with the low buffering capacity due to its low hardness resulted in increased acidity. These pH changes also caused dissolution and precipitation of iron hydroxide, therefore, contributing to the fish syndrome.

In conclusion, this study has demonstrated for the first time in Zambia that the main predisposing environmental factors associated with EUS outbreak of fish reported in the year 2006 and 2007 in the Zambezi floodplains, was the acidification of ground water during years of drought and eventual, contamination of surface waters during the massive floods of 2006/2007. The occurrence of each EUS outbreak was at the end of the flood season, when there was reduced dilution effect due to the slow, soft water current in affected tributaries of the Zambezi river and separation of lakes and lagoons from the main floodwater. The disease was more prevalent in lagoons, lakes and tributaries of the Zambezi river with slow water current, where the pH was less than 6.0, therefore, affected fish that were found in the Zambezi river which had a normal pH of 7.2, were originating from its affected tributaries.

Acknowledgments We wish to thank the Ministry of Agriculture and Cooperatives through the Department of Fisheries for funding this investigation. The authors are grateful to the Water Affairs Department for providing flood records and to all fishermen and other members of the general public for providing information.

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