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Short communication

Two cases of fish mortality in low pH, aluminium rich water

F J Stephens[1] and M Ingram[2]

1 Research Division, Department of Fisheries, Government of Western Australia, North Beach, Australia
2 Fish Health Unit, Murdoch University, Murdoch, Western Australia, Australia

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The management of acidity in water is an environmental problem in many regions of the world. In aquaculture, acidity occurs in ponds constructed in acid sulphate soils within mangrove swamps in many tropical and some subtropical areas (Simpson & Pedini 1985; Sammut, White & Melville 1996) and in natural lakes and waterways (Baker & Schofield 1982). Disturbed soil from housing development, agriculture and mining can produce acidic run off that requires careful management (Smith & Melville 2004; Verplanck, Nordtrom, Taylor & Kimball 2004).

Precipitation of ferric iron on surfaces, including fish gills, has often been linked to acidic runoff, but there is increasing evidence that aluminium toxicity often accompanies these events. A complex series of chemical events in water precedes fish mortalities from aluminium or iron toxicity. The solubility of both metals is determined by pH and mortalities in fish can occur in poorly buffered water when large swings in pH occur because of low alkalinity (Baker & Schofield 1982). Aluminium toxicity follows polymerization of aluminium on fish gills when water pH increases from about pH 5.0–6.0 (Poléo 1995). Once the pH has increased to about pH 6.0 no further polymerization or toxicity occurs. Toxicity at a given pH is affected by factors such as fish species, water temperature and the amount of humic acid present (Baker & Schofield 1982; Poléo 1995; Peuranen, Keinanen, Tigerstedt & Vuorinen 2003). There has been considerable research into the epidemiology of aluminium toxicity in fish in the northern hemisphere but relatively little published information is available on the problem in other regions.

This study describes two mortalities where there was a significant aluminium polymerization on fish gills. One event occurred in an aquaculture pond supplied with acidic water from the void of an open cut coal mine and the other followed the addition of aluminium sulphate as a flocculant to acidic underground water.

Case 1

Silver perch, *Bidyanus bidyanus* (Mitchell), died in a pond following heavy rainfall at the start of the annual wet season. Six 1 000 000 L ponds received water from a coal mine void after treatment in a limestone-filled fluidized bed, and compost, macrophyte and settlement ponds to increase pH and remove metal ions prior to entering the aquaculture ponds (Fig. 1). The two most severely affected ponds were adjacent to the settlement pond and also received run off from the acidic soil of the settlement pond bank. Polyculture of silver perch ($n = 200$), mean 284 g, in suspended cages and freshwater crayfish, marron, *Cherax tenuimanis* ($n = 600$), mean 98 g, occurred in four of the ponds. Marron but not fish, were held in the other two ponds.

Correspondence *F J Stephens, Research Division, Department of Fisheries, Government of Western Australia, PO Box 20, North Beach, 6920 Australia (e-mail: fstephens@agric.wa.gov.au)*

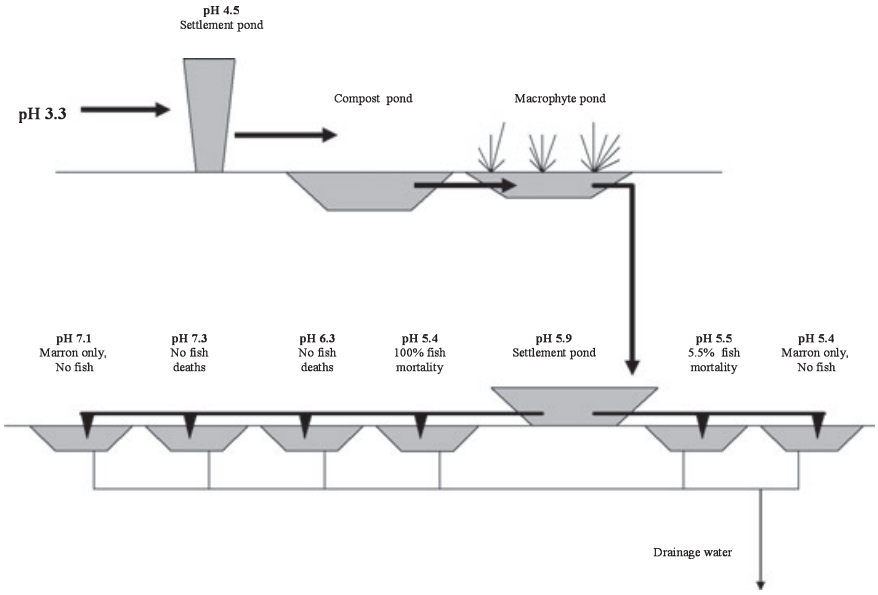

Figure 1 A schematic diagram of the layout of ponds at the facility from *Case 1*. Acidic water with negligible buffering capacity was obtained from a mine void. It passed through a bed of limestone before further conditioning in compost, macrophyte and settlement ponds and then entered a series of aquaculture ponds. Rainfall and a failure of the limestone bed to increase water alkalinity and hardness contributed to the mortality event. Adapted from a diagram supplied by Tim Storer.

Mean pH of water in the void was 3.4 ± 0.1 with a total aluminium content of 16 ± 2 mg L^{-1} (Table 1). Normally the pH of water leaving the limestone-filled fluidized bed was 6.2 ± 0.1 and 7.6 ± 0.4 when it entered the ponds, but on the day of peak mortality the pH of water in the worst affected pond was 5.4 and water leaving the fluidized bed was pH 4.5 (Fig. 1). The low pH was attributed to 'armouring' of limestone in the limestone-filled fluidized bed, which prevented adequate treatment to increase alkalinity, hardness and pH. Total aluminium in the pond water was 0.22 mg L^{-1}. A total of 94 mm of rain had fallen in the 24 days preceding fish mortality (Fig. 2), breaking a long dry period, and water temperature had decreased from approximately 21–13 °C during this period. In the two ponds in which the fish died, the pond water had cleared and became a pale grey/green in the days prior to the onset of fish mortality.

The fish in the two affected ponds were lethargic and inappetant in the days prior to the mortality event, with the fish in the worst affected pond having the lowest feed intake. Fifty per cent of the

Table 1 Composition of unfiltered water from the coal mine void in *Case 1*. Treatment prior to its use in aquaculture ponds was designed to increase its pH and buffering capacity and remove dissolved and suspended metal ions

Element	mg L^{-1}
Al	15
Ca	19
Cd	0.001
Cu	0.01
Fe	3.5
K	6.7
Mg	32
Mn	1.5
Na	95
Pb	0.02

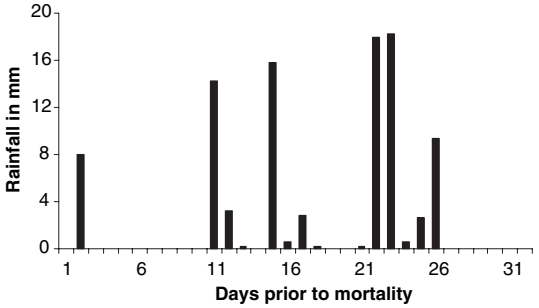

Figure 2 Rainfall in the month prior to the start of the fish mortality event in *Case 1*. There had been several days of rain after a long dry period, including 8 mm on the day prior to the day dead fish were first noticed. The two affected ponds were adjacent to the banks of the settlement pond that contained some acidic soil. Acidic runoff from these banks and a service road contributed to the low pH in some ponds.

fish in this pond died on day 1 and by the fourth day all of the fish were dead. On the day mortalities started, 11 fish also died in the second worst affected pond but no further mortalities occurred in this pond. No marron mortalities were reported and the number and condition of marron at harvest several months later were within the accepted industry standards and similar in all six ponds.

Two live fish were submitted for diagnosis on day 2 of mortality. The fish were in good body condition and had large deposits of fat in the coelomic cavity. There were no external skin lesions but the gills were pale and coated with a fine white/yellowish material. No parasites were seen in skin scrapings and gill biopsies. The livers were pale and friable. Haematocrit and leucocrit were 37.5% and 1.3%, and 39% and 1.4%, respectively. A heavy growth of *Saprolegnia* sp. was obtained from the gills.

Histologically, moderate to severe, diffuse hypertrophy and hyperplasia of gill epithelium and fusion of secondary lamellae were evident (Fig. 3). Aggregations of fungal hyphae and rod-shaped bacteria were visible between secondary lamellae. Aluminium was detected within lamellar epithelial cells, on gill epithelium and within organic matter between gill lamellae using the staining technique of Havas (1986). Iron was also seen between gill lamellae using Perl's stain. There was severe, diffuse fatty degeneration of hepatocytes and some hepatocyte nuclei were enlarged with marginated chromatin (Fig. 4). The lumen of kidney tubules contained protein and sloughed cells. There were elevated numbers of melanomacrophages in the spleen and thymus. Transmission electron microscopy of the liver showed widespread degeneration of hepatocytes with fatty change and chromatolysis (Fig. 5). Gill epithelial cells were necrotic and the epithelium was infiltrated with macrophages (Fig. 6). No bacteria were cultured from the liver, blood or gill. It was concluded that death had resulted from respiratory and osmoregulatory dysfunction caused by the large amounts of aluminium and iron-containing compounds on the gills and infection with *Saprolegnia* sp.

Case 2

Goldfish, *Carassius auratus* (L.), and koi, *Cyprinus carpio* L., died in a pond supplied with groundwater from a lined borehole. Water was pumped into a fibreglass settling pond, held for several days to oxygenate and to expel hydrogen sulphide and then treated with aluminium sulphate to reduce turbidity before being pumped into a polyethylene lined pond. All fish ($n = 6$) died within 48 h of stocking in the pond. They had increased respiratory effort, pale gills with a pale yellow/brown granular coating and pale, eroded areas of skin. Water analysis is shown in Table 2. Previously 82 of 100 barramundi, *Lates calcarifer* (Bloch), fingerlings had died within 72 h of introduction to a pond under similar circumstances. There were no mortalities in other ponds on the property that were stocked with goldfish and koi in water from the same source and supposedly treated in the same way, but water in

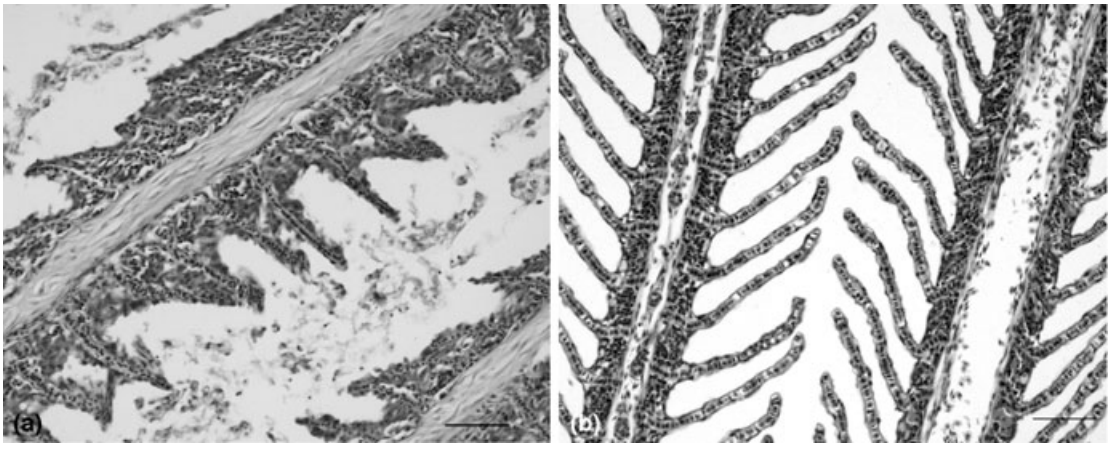

Figure 3 Histology of the gill. (a) Gill from an affected silver perch from *Case 1* showing severe, diffuse hyperplasia of the epithelium of secondary gill lamellae, necrotic epithelial cells and organic and inorganic debris in the interlamellar space. Aluminium and iron were identified in the debris using histological staining techniques. Aluminium was also seen within cells in the hyperplastic tissue. (b) Gill from unaffected silver perch in ponds from a different aquaculture facility (bars = 50 μm).

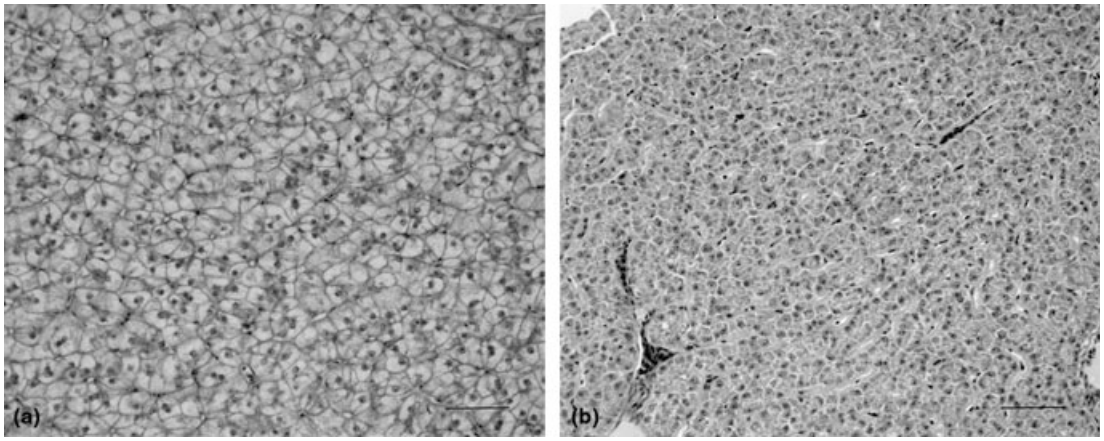

Figure 4 Histology of the liver. (a) Liver of an affected silver perch from *Case 1* showing severe, diffuse fatty change and swelling of hepatocytes. (b) Liver from an unaffected pond-reared silver perch that was feeding on pond fauna at a different aquaculture facility (bars = 50 μm).

these ponds had much higher pH and alkalinity (Table 2), presumably from contact with added crushed limestone.

Histologically the fish had severe, diffuse hyperplasia of the epithelium of gill lamellae. Organic and inorganic matter between the primary lamellae

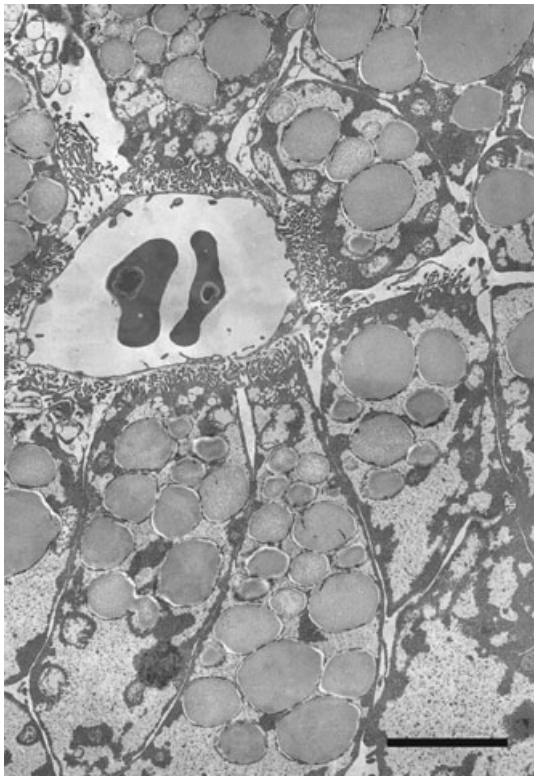

Figure 5 Electron micrograph of the liver of a silver perch from *Case 1*. There is swelling and lipid accumulation. Cytoplasmic organelles are ballooned and protein has precipitated around lipid droplets and on the cell membrane. The integrity of microvilli bordering the sinusoid suggests that the hepatocytes remain viable (bar = 15 μm).

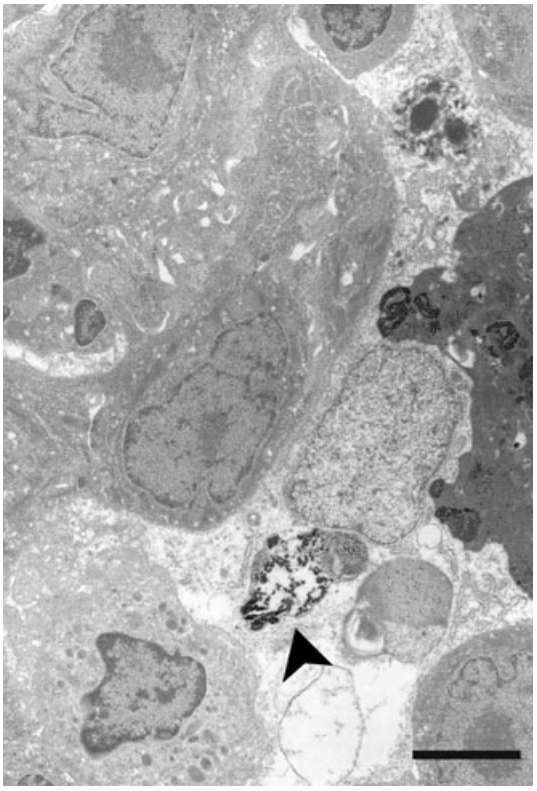

Figure 6 Electron micrograph of hyperplastic gill epithelium of a silver perch from *Case 1* showing necrosis and degenerative changes. There is vacuolar degeneration of cytoplasmic organelles. Macrophages are present and some electron dense material (arrow) may be aluminium (bar = 7.5 μm).

Table 2 Chemical analysis of water in *Case 2*. The treated underground water had $NaHCO_3$ added to increase pH prior to the addition of aluminium sulphate (alum) to flocculate suspended solids such as tannins. Fish toxicity occurred when pH was not adequately controlled during treatment

	Pond with dead fish	Underground water	Treated underground water 48 h after alum addition	Unaffected pond
pH	4.2	5.4	6.5	7.4
Alkalinity (mg L^{-1} $CaCO_3$)	0	0	100	222
Hardness (mg L^{-1} $CaCO_3$)	26	0	36	36
K (mg L^{-1})	4.9	6.1	4.6	7.0
S (mg L^{-1})	29.5	2.9	26.6	60.0
Na (mg L^{-1})	42.9	32	106	220
Ca (mg L^{-1})	10.6	0.8	2.3	5.5
Mg (mg L^{-1})	6.5	2.8	5.2	5.8
Cu (mg L^{-1})	< 0.05	< 0.05	< 0.05	< 0.05
Zn (mg L^{-1})	0.36	< 0.05	< 0.05	< 0.05
Fe (mg L^{-1})	< 0.05	< 0.05	< 0.05	< 0.05
Cl (mg L^{-1})	62.4	64	66	115
Al (mg L^{-1})	0.7	0.7	0.4	< 0.05

was positive for aluminium, but negative for iron. No parasites were found on the biopsies of gill and skin and no bacteria were isolated.

The history and lesions in both cases were consistent with aluminium toxicity. In *Case 1*, the reduction in turbidity prior to fish deaths and the low pH and poor buffering capacity of the water, together with high total levels of aluminium are typical of other reports of aluminium toxicity (Brown, Morley, Sanderson & Tait 1983; Sammut *et al.* 1996). In the second case, the addition of aluminium sulphate to poorly buffered acidic water to flocculate suspended solids, possibly tannins, resulted in the mortality.

Although fish mortalities associated with aluminium precipitation onto gills in low pH water have been reported for over 20 years (Baker & Schofield 1982), the chemistry and epidemiology of these events continue to be studied. Recent research suggests that a rise in pH through the pH range 5.0–6.5, rather than low pH *per se*, is the major factor responsible for fish mortality (Poléo 1995; Poléo, Østbye, Øxnevad, Andersen, Heibo & Vøllestad 1997). These authors attribute mortality to instantaneous polymerization of soluble aluminium when the pH of aluminium-rich water rises above approximately pH 5.0. Once polymerization has occurred the thickening of the water-plasma diffusion barrier is the most likely cause of the fish mortality due to the respiratory and acid base disturbances (Poléo 1995; Peuranen *et al.* 2003). This may have resulted in the severe necrosis noted in electron micrographs from *Case 1*. Larger fish in warmer water with a low content of some solids such as humic acid are more likely to die from the effects of aluminium in acidic water (Peuranen *et al.* 2003). The fish that are more tolerant of environmental hypoxia seem more likely to survive (Poléo *et al.* 1997).

Verplanck *et al.* (2004) investigated the chemistry of metal-rich water from mine sites and their findings substantiate the pathogenesis of aluminium toxicity that was postulated by Poléo (1995). Verplanck *et al.* (2004) showed that when water pH was 3.83, all aluminium and iron were dissolved and in ionic form. As pH increased from 3.83 to 6.62, the total aluminium and iron concentration in unfiltered water remained unchanged, but dissolved aluminium and iron were replaced by particulate suspended and colloidal aluminium and iron. This change was the most marked between pH 3.83 and 5.13.

There have been occasional reports of aluminium and/or iron toxicity in aquaculture ventures (Langdon 1987) often when bore or groundwater is used without adequate prior aeration. Reduced turbidity often precedes mortalities when aluminium is released from soil and clay at low pH and flocculates suspended solids (Brown *et al.* 1983; Sammut *et al.* 1996).

Infection with *Saprolegnia* sp. undoubtedly contributed to fish mortality in *Case 1* and probably resulted from stress, including suboptimal water chemistry and the recent decline in water temperature (Landos, Callinan, Stuart, Raed, Boyd, Nixon, Mifsud & Beakes 2002), but there was none of the skin lesions typically seen in saprolegniasis (Puckridge, Walker, Langdon, Daley & Beakes 1989). No fish were submitted from the other ponds, so it was impossible to ascertain the relative effects of *Saprolegnia* infection and water chemistry in the worst affected pond. Marron in the same ponds did not die and might

have been less exposed to the polymerization process when water pH increased. Alternatively, marron may be more resistant to the effects of aluminium polymerization on the gills because the exoskeleton may render it less attractive as a nucleus for aluminium polymerization.

These mortality events highlight some aspects of water chemistry that require careful management before use for aquaculture. Both facilities have successfully managed their water treatment by increasing their monitoring of pH and improved management of water chemistry and have had no further associated mortalities.

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