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Short-Term Effects on Sea Trumpeter (*Therapon humeralis*) of Oxidizing Iron(II) in Seawater

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The toxicity of acid-iron wastes from titanium dioxide plants has been tested on a number of fish species (Kinne and Rosenthal 1967; Wilson *et al.* 1974; Lehtinen 1980; Lehtinen *et al.* 1984). Such effluents are typically high in sulfuric acid and iron(II), and also contain lower levels of several heavy metals and suspended solids (Edmonds 1981; Lehtinen *et al.* 1984). Preliminary acute toxicity experiments on marine and estuarine animals, including fish, suggested that the hydrogen ion concentration of the acid-iron waste was the active toxic component (Wilson *et al.* 1974; Lehtonen and Tuunainen 1974).

The precipitate of hydrated iron(III) oxide resulting from acid-iron waste entering a water body caused physical blockage of the gill epithelium in fish and was responsible for their impaired performance in a rotary-flow test (Lehtinen 1980). Lehtinen and Klingstedt (1983) examined the precipitate formed on the gills of fish subjected to acid-iron wastes, and found, in addition to iron, high levels of titanium, potassium and phosphorus. Larsson *et al.* (1980) studied the sublethal effects of titanium dioxide industrial effluent on the flounder (*Platichthys flesus*) and concluded that physiological disturbances resulted from mechanical action of the hydrated iron(III) oxide precipitate on their gill tissue and/or from a disturbed gill function caused by biochemical action of heavy metals co-precipitated from the acid-iron waste. A direct effect of the heavy metals in solution was considered improbable.

An earlier study (Edmonds 1981) showed that oxidizing solutions of iron(II) were more toxic to fish than a suspension of oxidized iron(III) containing an equivalent quantity of iron. Apparently iron exerts its toxicity as soluble iron(II) oxidizes to insoluble flocculent iron(III). The experiments described here were undertaken to test this hypothesis with the sea trumpeter, *Therapon humeralis*. This fish species is common in marine embayments of Western Australia, and is readily maintained in experimental aquaria.

MATERIALS AND METHODS

Sea trumpeter, *Therapon humeralis* (total length 80-105 mm), were collected by beach seine from Whitfords beach near Perth, Western Australia, and maintained in filtered flowing seawater (18-22°C, salinity 34.5-36$^{o}/_{oo}$, pH 8.1) at the Western Australian Marine Research Laboratories. They were held at the test temperature

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(20° or 25°C) for seven days prior to experimentation. Water temperature was maintained at within 0.2°C of the nominal value by use of a thermostat. Experiments were carried out in a rectangular glass tank (25 x 40 x 30 cm deep) containing seawater (20 L) aerated with filtered air (575 mL/min). The vigorous air flow maintained oxygen levels at or near saturation during the experiments, and ensured that the iron remained in suspension following its precipitation.

Acute toxicity tests were carried out in seawater (salinity $35^{o}/_{oo}$, pH 8.1) at 20° and 25°C under static conditions. For each of 14 individual tests, ten fish were transferred to the experimental glass tank and given two hours to adjust to the tank environment. Crystals of $FeSO_4.7H_2O$ were added to the water and rapidly dissolved by mixing to produce nominal iron(II) concentrations of 8, 10, 12, 14, 16, 18 or 20 mg/L. The tank was checked hourly during the first 8 hr, and thereafter at 8 hr intervals; dead fish were removed. Tests were usually terminated after 24 hr (although some tests lasted 72 hr) because it was clear that toxic conditions were short-lived (<8 hr). Survivors were transferred to holding tanks and observed for further mortality for several weeks before their release to the sea. During this period, fish swam and fed normally and showed no signs of distress. 24 hr LC_{50} values were calculated by the probit method as described by Reish and Oshida (1987).

In a separate experiment, the rate of iron oxidation and precipitation was determined at 20° and 25°C by measuring the amount of iron left in solution at various times for up to 60 minutes following the addition of $FeSO_4.7H_2O$ (equivalent to a nominal value of 20 mg Fe/L, actual value 19.7 mg Fe/L). These experiments were carried out, without fish, under the same conditions employed in the acute toxicity tests. Water samples (10 mL) were removed by syringe and filtered through a 0.1 µm membrane filter into a tube containing 1 drop of 50% HCl. Filtrates, containing unreacted iron(II) now stabilised at the low pH, were analysed for iron by atomic absorption spectroscopy using Varian instrumentation. The oxygen levels and pH of the seawater were monitored using a Titron RD-25 oxygen meter and a Philips PW 9409 digital pH meter.

The rate of accumulation/depuration of hydrated iron(III) oxide on the gills of fish was studied by subjecting groups of ten (20°C) or nine (25°C) fish to sublethal levels of iron (8 mg/L). An individual live fish was removed from the tank at intervals following the addition of iron (see Fig. 2), and immediately sacrificed. Four fish that had not been subjected to iron served as controls. Gills were excised and placed into pre-weighed 50-mL beakers. The gills were dried (105°C, 16 hr), reweighed and the dried gills digested with a mixture of nitric/perchloric acids (5:1) before analysis for iron by atomic absorption spectroscopy.

RESULTS AND DISCUSSION

The chemistry of the oxidation of iron(II) in seawater is complex (Roekens and Van Grieken 1983), and has been the subject of some dispute (Davison 1984; Roekens and Van Grieken 1984). In regard to the toxicity tests described in this paper, the chemistry can be simplified to two stages. First, oxidation of iron(II) to iron(III) by the dissolved oxygen in seawater, and second, the hydrolysis of iron(III) liberating hydrogen ions and resulting in the precipitation of iron as hydrated iron(III) oxide floc. The rate of iron(II) oxidation is pH dependent at pH <8.45, and Roekens and Van Grieken (1983) demonstrated a tenfold decrease in this oxidation rate when the pH of seawater was reduced from 7.96 to 7.32.

Addition of solid iron(II) sulfate to the seawater resulted in virtually instantaneous dissolution of the iron followed by its oxidation, hydrolysis and precipitation as hydrated iron(III) oxide. The observed changes in water chemistry (Table 1) were as expected from the work of Roekens and Van Grieken (1983). The rate of formation of the hydrated iron(III) oxide was fast initially but then slowed markedly because of the decrease in pH resulting from the hydrolysis.

Table 1. Changes in water chemistry following addition of iron(II) at 20 mg/L (nr: not recorded)

Time (min)	pH		$[O_2]$ mg/L		Iron(III) as % of total iron	
	20°C	25°C	20°C	25°C	20°C	25°C
0	8.08	8.12	7.0	6.3	0	0
2	7.27	7.18	6.3	5.4	nr	nr
3	7.18	nr	6.2	nr	51	58
6	7.03	6.93	6.2	5.4	59.5	69.5
10	6.95	6.86	6.4	5.6	67.5	76.5
20	6.91	6.88	6.7	6.0	77.0	86.0
40	6.99	7.05	6.8	6.1	89.0	96.5
60	7.12	7.3	7.0	6.1	97.5	99.5

The plots of percent survival against time (Fig. 1) provided information about the mode of toxic action of iron(II). Most mortality occurred within four hours of addition of iron(II). During this early period, fish constantly broke the surface of the water to gulp air - behaviour consistent with extreme oxygen deprivation. Fish that survived this critical early period returned to their normal swimming pattern after about 6 hr. No further mortality was observed for these fish nor did they show any obvious signs of distress up to the end of the test (t=24 hr or 72 hr). At all iron concentrations, death occurred more quickly at the higher temperature (Fig. 1). The 24 hr LC_{50} values (with 95% confidence limits) were 16.7 mg/L (15.6-17.9 mg/L) at 20°C and 11.8 mg/L (10.4-13.5 mg/L) at 25°C. It should be noted that, because of the nature of the toxicant and its mode of toxic action, these LC_{50} values are highly dependent on the experimental conditions (e.g. pH, oxygen concentration).

Possible contributing factors to the acute toxicity observed in these tests include reduced pH, iron(II) in solution, and mechanical blockage of the gills resulting from precipitation of iron(III) onto the gill filaments of the fish. Previous studies

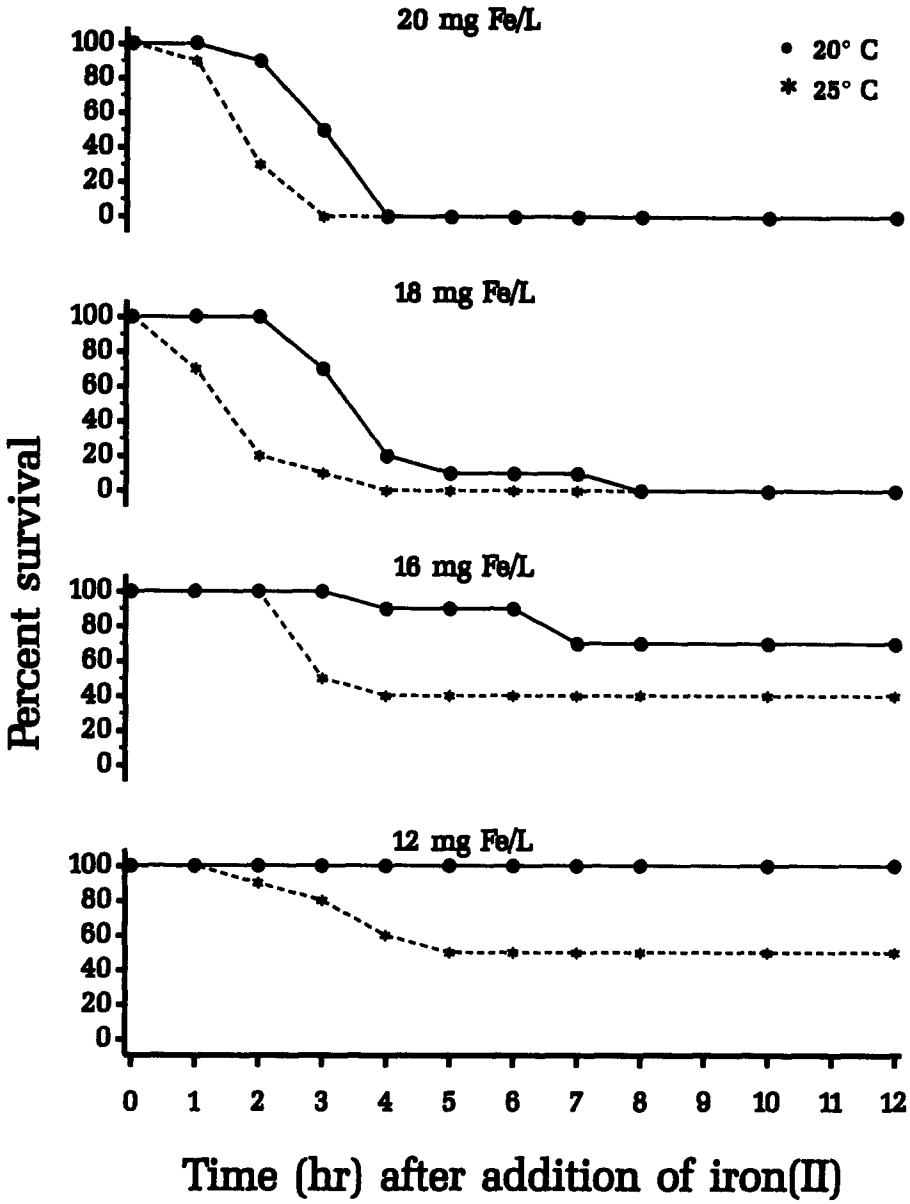

Figure 1. Percent survival of 10 *Therapon humeralis* versus time at 20° and 25°C at four iron(II) concentrations. No mortality occurred at 8 mg/L at either temperature. Data for the 10 and 14 mg/L experiments were consistent with the results plotted here but have been omitted to enhance clarity.

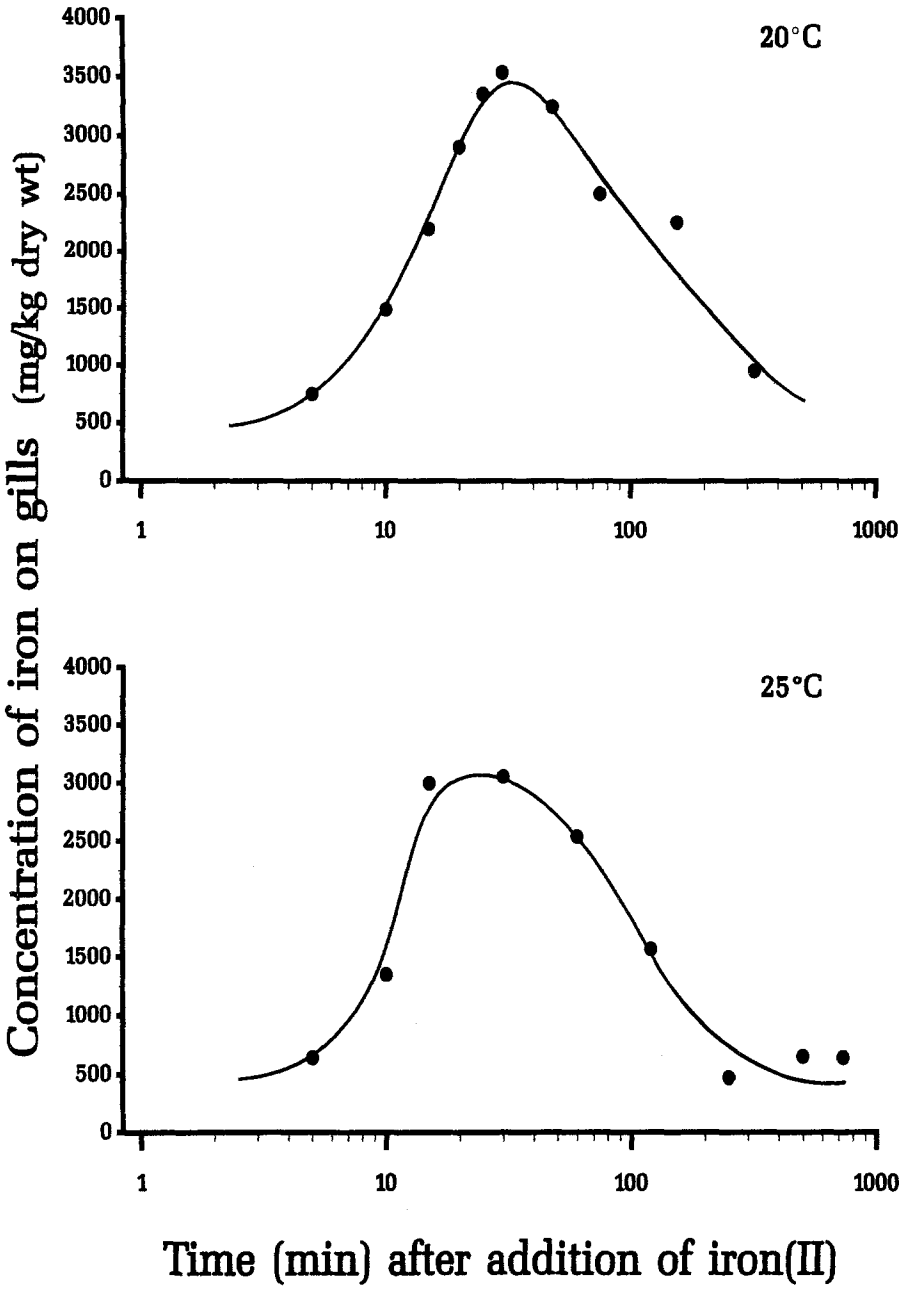

Figure 2. Accumulation/depuration of iron(III) oxide on gills of *Therapon humeralis* at 20° and 25°C. Lines have been fitted by eye. The mean background level of iron in gills of *T. humeralis* was 510 mg/kg dry weight (n=4, SD=54).

on the effects of low pH on marine fish suggest that the slight reduction in pH recorded in the present study (Table 1) was unlikely to affect the test fish (Carter 1964; Edmonds 1981). Soluble iron(II) was not expected to be a significant contributor to the overall toxicity of the system because of the short half-life (*ca* 3 min, see Table 1) of this chemical species in solution.

Visual examination of the gills of dead sea trumpeter following the toxicity tests revealed large accumulations of iron(III) oxide suggesting that the major cause of death was mechanical blockage of the gills resulting from precipitation of the iron onto the gill filaments. The observation that fish died sooner at the higher temperature is consistent with this hypothesis. Metabolic rate and oxygen demand are increased at the higher temperature and gill blockage would be more debilitating for fish at 25°C compared with those at 20°C. The results reported here also support the mechanism of toxic effects for acid-iron wastes proposed by others (Lehtinen 1980; Larsson *et al.* 1980; Lehtinen and Klingstedt 1983). In the present study, however, the mode of toxic action was free from the possible contributing effects of other constituents in the acid-iron waste water.

The accumulation with time of iron on sea trumpeter gills is shown in Fig. 2. The levels rise sharply from the natural or background level (510 mg Fe/kg dry weight) during the first 30 minutes of a sublethal exposure (8 mg Fe/L). After 30 min exposure the gills contained over 3,000 mg Fe/kg dry weight) and the fish showed obvious signs of hypoxia. From this point, however, the fish were able to cleanse their gills of the floc and virtually all of the contaminating iron was removed within five hours. The pattern of accumulation/depuration of iron was similar at 20° and 25°C. Possibly, the accumulation rate was faster at 25°C (consistent with a faster rate of Fe(III) production, see Table 1), and this may have contributed to the apparent increased toxicity at the higher temperature (Fig. 1).

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