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RESEARCH NOTE

TOXICITY OF AMMONIA TO EARLY LIFE STAGES OF RAINBOW TROUT (*SALMO GAIRDNERI*)

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Abstract—The toxicity of ammonia to early life stages of rainbow trout (*Salmo gairdneri*) was examined in hard fresh water. When exposure began within 24 h of fertilisation and proceeded for 73 days, severe mortality (> 70%) occurred, particularly among the eggs, at concentrations of unionised ammonia as low as 0.027 mg l^{-1} as NH_3. When exposure did not start until the eyed-egg stage (*c.* 24 days) only 40% of the eggs, yolk-sac fry and fry (but especially the fry) died at 0.27 mg l^{-1} as NH_3. Both the standards proposed for protecting fish communities and the protocols drawn up for assessing the effects of chemicals should take into account the sensitivity of early life stages of freshwater fish. In particular, in evaluating chemicals, exposure should begin as soon as possible after fertilisation.

Key words—ammonia, toxicity, rainbow trout eggs and fry, fresh water

INTRODUCTION

The derivation of Environmental Quality Standards for chemicals requires relevant toxicity test methods to assess the chemical's potential for causing harm in the aquatic environment. To this end there has been a tendency towards longer studies and tests on the most sensitive parts of the life cycle. In fish these two elements come together as "early life stage" (ELS) tests. International standards do not yet exist for such tests but most discussions in this area seem to agree that there is a need to provide data on:

(i) mortality (observed daily) at egg, larval and juvenile (fry) stages;
(ii) the length of time from fertilisation to hatching;
(iii) possibly the length of time from hatching to full resorption of yolk; and
(iv) abnormalities in development.

This note briefly describes the results of such a test of the effects of ammonia on rainbow trout (*Salmo gairdneri*).

Ammonia is generally considered to be typical of poisons which act in such a way that median asymptotic lethal concentrations ("threshold LC_{50}s") are expressed within a few days of exposure rather than weeks, at least with post-fry stages [see, for example, Ball (1967) and Solbé *et al.* (1985)]. This does not exclude the possibility of longer term effects occurring at other stages of the life cycle. Indeed Calamari *et al.* (1981) demonstrated a 72-day LC_{50} of 0.056 mg l^{-1} as undissociated ammonia to rainbow trout eggs and fry. In addition, however, they showed that in terms of 96 h LC_{50} values sensitivity increased throughout the 71 days following fertilisation. The study reported here was similar to the former approach of Calamari *et al.*, but was also designed to indicate the effects on the result of beginning the test either as soon after fertilisation as possible, or when the eggs had already reached the "eyed" stage.

METHODS

Flow-through techniques were used. Six tanks each of 15 l. capacity were fitted with egg-holding trays and downstream weirs so that a gentle flow of water or test solutions was maintained around the eggs. Fry, once hatched, could fall between the glass rods supporting the eggs but were prevented from leaving the tanks by mesh screens.

The tanks were opaque and fitted with opaque lids, the latter being partially removed before the fry began to feed independently (on a proprietary fry food).

Analytical grade ammonium chloride was delivered in aqueous stock solution to free-piston burettes whose action was controlled by concentric-siphon dosers (Shurben, 1978). The dosers mixed the stock solution with dilution water (a natural, hard, unchlorinated, borehole water) and passed it to the test vessels. One vessel acted as a control and received no test solution. Details of the conditions in each vessel are given in the Results section.

Two studies were carried out. In Test A the eggs (591–737 in each vessel) were exposed from within one day of fertilisation. (The unusually large number of eggs permitted withdrawal of eggs, larvae and fry for wet and dry weight determinations.) Test B began 24 days later when the eggs, fertilised on the same day as those used in Test A, had reached the eyed stage. From 136 to 174 were used per vessel.

The observations indicated in the Introduction were made every day for 73 days (Test A) or 49 days (Test B).

RESULTS

Table 1 sets out the conditions in each test vessel and the response, in terms of mortality, of each life

Table 1. Test conditions and responses of early life stages of rainbow trout to ammonia—mean values, with SD in parentheses (for other conditions, see Results section)

Concentrations of ammonia as N (mg^{-1})		Mortality % individuals entering stage			Mortality (total) %*
Total	Undissociated	Egg	Yolk-sac fry	Fry	
Test A (started within 24 h of fertilisation)					
0.14 (0.17)	—	8.8	1.6	2.6	12.6
2.55 (0.41)	0.022 (0.004)	65.2	12.6	4.9	71.1
4.52 (0.43)	0.039 (0.009)	78.5	33.6	2.7	86.1
8.00 (0.49)	0.069 (0.013)	87.3	44.1	0	92.9
15.50 (0.57)	0.128 (0.012)	72.1	26.8	4.7	80.6
25.77 (1.65)	0.219 (0.040)	97.9	61.5	20.0	99.4
Test B (started 24 days after fertilisation)					
0.19 (0.20)	—	2.2	4.0	3.6	9.5
2.59 (0.39)	0.023 (0.004)	3.3	9.6	11.6	22.7
4.74 (0.19)	0.041 (0.10)	3.2	5.7	2.5	11.0
8.15 (0.49)	0.072 (0.015)	3.4	3.8	3.5	10.3
15.72 (0.53)	0.130 (0.012)	3.4	11.2	8.3	21.3
25.92 (2.28)	0.226 (0.046)	3.3	14.0	27.5	39.7

*The "total mortality" was calculated separately to take account of the eggs and fry removed from each vessel for the measurement of dry matter content.

stage. In addition the hardness and alkalinity were 263 ± 2.3 and $243 \pm 3.2\ mg\ l^{-1}$ as $CaCO_3$, respectively, while the concentration of dissolved oxygen (DO) equalled or exceeded 95% ASV during the pre-hatching stages and 92 and 76% ASV in the larval and post-larval periods. (In the latter the mean DO was 88% ASV.) The temperature of the water was not controlled but ranged from 11.5 to 17.0°C: the overall mean was 14.9 ± 1.1°C. The pH was controlled using small additions of carbon dioxide to the aeration flow as necessary. The control pH values (7.52 ± 0.05) were never more than 0.07 pH units higher than the lowest value from the test vessels. Individual daily pH and temperature values were used in calculating the concentrations of undissociated ammonia given in Table 1 from the levels for total ammonia found on analysis and also presented in the table.

The results indicate that concentrations of undissociated ammonia as low as $0.022\ mg\ l^{-1}$ as N (0.027 as NH_3) could have a marked effect on the survival of rainbow trout eggs and fry if exposure began within 24 h of fertilisation. Mortality was heaviest in the egg but yolk-sac fry were still vulnerable. When exposed only from the eyed stage onwards rainbow trout seemed less susceptible. Seven weeks of exposure at a concentration of $0.23\ mg\ l^{-1}$ as N did not produce a 50% kill. The data for periods to eyeing, hatching or resorption of yolk and for dry-matter content varied little between treatments and no concentration-related sub-lethal responses could be detected.

DISCUSSION

The tentative water quality standards proposed by the European Inland Fisheries Advisory Commission (EIFAC) (Alabaster and Lloyd, 1982), although reporting the work of Calamari *et al.* (1981), cited above, suggested that the lowest lethal concentration of unionised ammonia found for salmonid fish was $0.2\ mg\ l^{-1}$ as NH_3. This present note supports the findings of Calamari *et al.* that lower concentrations may be toxic. In contrast to the Italian study, however, severe effects on rainbow trout eggs were demonstrated in waters containing concentrations of unionised ammonia at least as low as $0.022\ mg\ l^{-1}$ as N, or $0.027\ mg\ l^{-1}$ as NH_3 whereas Calamari *et al.* recorded 96 h LC_{50}s declining from $>0.49\ mg\ l^{-1}$ as NH_3 in eggs exposed from days 1 to 5 after fertilisation to $0.16\ mg\ l^{-1}$ as NH_3 for fry exposed from days 71 to 75. In both studies, however, there was a clear indication that longer periods of exposure led to much lower LC_{50} values than those obtained in, say, 96 h.

This present study's result, at least as far as rainbow trout is concerned, considerably erodes the safety factor incorporated by EIFAC in proposing a tentative standard of $0.025\,mg\,l^{-1}$ as NH_3 for all species of European freshwater fish. However, for salmonids, the standard is based on a 95 percentile while the lethal levels reported here were average values. While this suggests a wider margin of safety, the situation may still be a cause for concern where lower temperatures exist. (Such conditions may occur at spawning time and when, in any case, sewage works effluents, a source of ammonia, may not be as fully nitrified as in warmer weather.)

On the other hand, if early life stage tests are to become part of the routine pre-marketing testing of chemicals the results given here suggest that exposure should begin as soon as possible after fertilisation. By delaying the start of the test until the eggs are "eyed" sensitive stages may be missed in the assessment of a chemical's capacity to harm freshwater fish.

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