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Jumalon, Nepheronia A.

Aquaculture Department, Southeast Asian Fisheries Development Center

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## Acute toxicity of unionized ammonia to milkfish (*Chanos chanos* Forsskal) fry

Nepheronia A. Jumalon

The toxicity of ammonia to fish is well-studied (Smith and Piper, 1975; Robinette, 1976). This toxicity depends on the amount of unionized or free ammonia in solution rather than the dissociated form, as demonstrated by Wuhrmann and Woker (1948 in Hazel *et al.*, 1971) and Downing and Merkens (1955).

Mortality of milkfish fry under culture condition is sometimes attributed to toxic levels of ammonia, but no evidence has been given to prove this. A study on the effects of varying concentrations of ammonia to milkfish fry, would, therefore, contribute important information which can be used in the maintenance of good water quality to insure maximum survival.

Two runs of static 96h bioassays (refer to APHA, 1971) were conducted at the Sabalo Laboratory, SEAFDEC, Iloilo to determine the median lethal concentration (LC 50) of unionized ammonia (NH<sub>3</sub>) to milkfish fry. Test concentrations were based on exploratory 24h and 48h bioassays and were made in three replicates. Reagent grade ammonium chloride (NH<sub>4</sub>Cl) was used to adjust the level of unionized ammonia.

One liter beakers were randomly distributed and each was filled with 800 ml seawater of varying NH<sub>3</sub> concentrations and provided with gentle aeration before the fry were stocked at a rate of 100 per beaker. Fry were obtained from the coasts of Nanga, Guimbal and were acclimated without feeding in 20-liter aquarium filled 3/4 with conditioned seawater two days prior to the experiment. Length and weight of fry averaged (n=100) 12.58 mm and 3.92 mg, respectively, with ranges of 11.00-14.55 mm and 1.60-6.86 mg. In all runs, mortality observed during the acclimation period was either zero or less than 1%.

Mortality in the different test concentrations was recorded every hour and fry were removed immediately when they die. Dead fry were preserved in 10% buffered formalin and submitted to the Micro-technique Laboratory for histological sections of the gills. Sections were cut at 6-8μ and stained with haematoxylin and eosin.

The following physico-chemical parameters were monitored every morning: temperature (using a lab. thermometer) salinity (using an AO refractometer) and ammonia concentration using the Indophenol Blue Method (FAO, 1975). The following, on the other hand, were analyzed before and at the end of the experiment: dissolved oxygen using the micro-winkler titration method (based from APH, 1971), pH (using a TOA ph meter) and total alkalinity using the titration method (APHA, 1971).

Surviving fry from 26.08 to 31.28 ppm were taken at the end of the 96h bioassay, and kept in recovery aquaria filled with untreated seawater and fed sufficiently with *Chlorella/Brachionus*. After 10 days, samples were taken for histological sections to check gill condition.

Physico-chemical condition did not vary markedly among the treatments. From the averages calculated for each test concentration, the following values were obtained.

	<u>Ranges</u>	<u>Averages (n=7)</u>
Temperature	28.00–29.64°C	28.84±0.7472
Salinity	33.73–35.63‰	34.75±0.8826
Dissolved oxygen	4.04–4.88 ppm	4.58±0.3099
pH	7.60–7.93	7.76
Total alkalinity	136.00–145.66 ppm	140.36±3.4361

The 96h median lethal concentration, determined by the Reed-Muench method (Woelf, 1968), was calculated at 28.09 ppm  $\text{NH}_3$ , 29.69 ppm. Table 1 gives the results obtained. Even at high concentrations of unionized ammonia, most of the fry mortality occurred after 48 to 96 hours exposure.

Examination of the gill condition of fry exposed to different  $\text{NH}_3$  concentrations shows that severe gill damage occurs only at concentrations above 20 ppm, especially above the LC 50. Gills of fish exposed to 11.25 ppm are comparable to those of control fish, though slight changes may take place. Figures 1 to 4 show some of the gill sections made.

The high LC 50 value obtained shows that milkfish fry has a great tolerance to ammonia. In fact, fry with severely-damaged gills can still recover days after it is returned to favorable culture condition, as indicated by Figures 1-4. Considering that ammonia concentration is usually below 1 ppm even in a pond where there is accumulation of "lab-lab" (Jumalon, 1978) or in feeding trials (Mangalik, 1979), it can be deduced that observed mortalities of milkfish fry under culture conditions are not due to ammonia toxicity.

**Table 1. Analysis by the Reed-Muench Method**

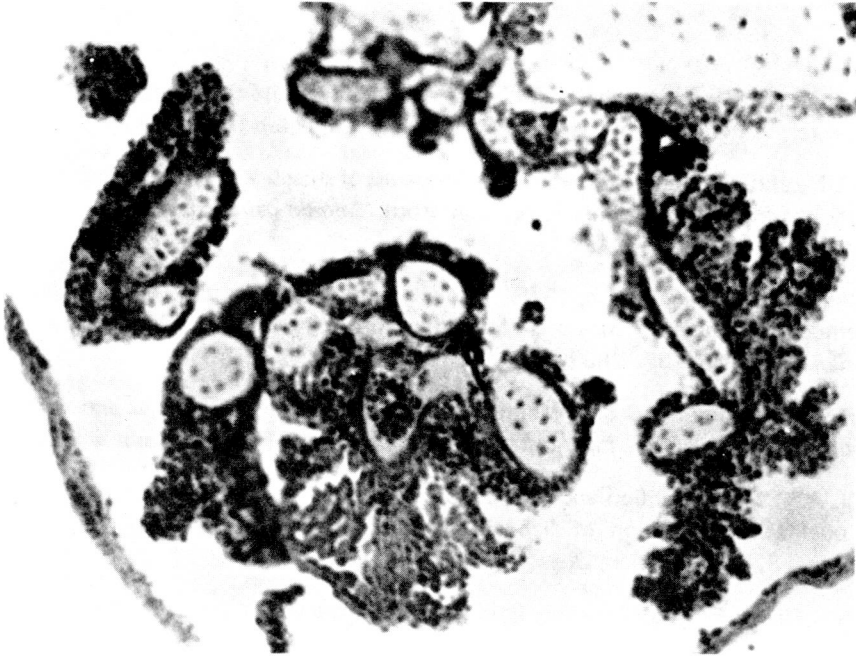
$\text{NH}_3$ Concentration (ppm)	Dose (ppm $\text{NH}_3$ ) Log scale	N	Number		Accumulated		Cumulative	
			dead	alive	No. dead	No. alive	Total	% mortality
0.69 (control)	-0.1612	100	0	100	0	494	494	0.00
11.25	1.0512	100	3	97	3	394	397	0.76
20.81	1.3183	100	10	90	13	297	310	4.19
26.08	1.4163	100	28	72	41	207	248	16.53
26.91	1.4299	100	44	56	85	135	220	38.64
29.66	1.4722	100	58	42	143	79	222	64.81
31.28	1.4953	100	63	37	206	37	243	84.77



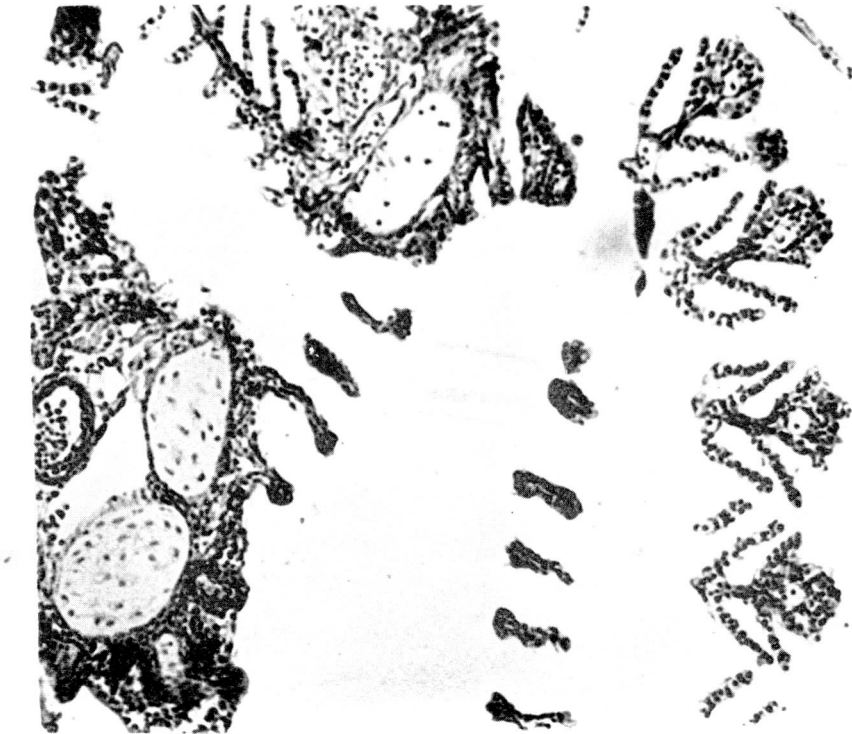
**Fig. 1. Gill section from control fish. 0.69 ppm  $\text{NH}_3$  x 67.**



**Fig. 2. Gill section from fry exposed to 26.08 ppm  $\text{NH}_3$ . Hyperplasia of gill epithelium has resulted to fusion of some lamellae. x67.**



**Fig. 3** Gill section from fry exposed to 29.66 ppm  $\text{NH}_3$ . Severe hyperplasia of gill epithelium has resulted to fusion of lamellae. x67.



**Fig. 4.** Gill section from surviving fry after 96 hours exposure to 29.66 ppm  $\text{NH}_3$  followed by a 10-day recovery period. Note that gills are starting to become normal again. x67.

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