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Effects of Salinity and Pre-Exposure on Acute Cadmium Toxicity to Seabass, *Lates calcarifer*

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In recent years in Malaysia, aquaculture activities have expanded into coastal areas. One of the species gaining importance is the seabass, *Lates caclarifer.* It is a marine fish tolerant to a wide range of salinities down to almost freshwater, although they are normally cultured in floating cages in estuaries with a salinity range of 10 to 25 ppt. The adults spawn in the sea , and upon hatching, the young move into mangrove areas and upstream where the salinity regime fluctuates (Ruangpanit 1986).

The seabass is commonly reared in floating cages in estuaries where the salinity may approach freshwater. However , there is increasing concern for heavy metal pollution in estuaries and coastal areas in Malaysia (Law and Singh 1986). The toxicity of heavy metals to marine organisms increases with decreasing salinity (Eisler 1971; Sunda et al. 1978), probably due to an increase in free ion concentration and, hence, metal accumulation (Engel and Fowler 1979). There is then the possibility that seabass cultured in estuaries where low salinities are frequently encountered, especially during rainy seasons, would be more susceptible to the effects of metal pollution. There is also a paucity of data on toxicity studies with the seabass.

Due to these reasons, an investigation was carried out to establish the toxicity of cadmium to the seabass at two stages of development and the influence of salinity on its toxicity.

MATERIALS AND METHODS

Seabass eggs were collected from the Fisheries Department hatchery at Tanjung Demong and hatched at 30 ppt salinity in the laboratory in 1000 L tanks. A few days after hatching, the young seabass are able to tolerate fluctuations in salinity. Young fish were fed *Artemia salina* until about 2 weeks of age, where upon the diet then consisted of finely chopped trash fish (trevally, *Carangoides* sp. and mackerel, *Scomberomorus* sp.). Toxicity tests were carried out using fish of age groups 20 days and 4 months, all from the same batch of eggs. The two size groups were chosen as these represent distinct stages of development. Fish of 20 d of age are in transition between the larval and juvenile stages and their growth is inferior at salinities less than 20 ppt. Juvenile fish however, are tolerant to freshwater (Kosutarak et al. 1984).

Seawater (30 ppt) used in the laboratory was pumped from the sea and allowed to settle before finally being filtered through 30µm cartridge filters. The required salinities were prepared by diluting seawater with dechlorinated tap water.

The static method with renewal of test solutions was employed for all toxicity tests. The test solutions were renewed every 48 hr. Stock solutions of cadmium were prepared by dissolving cadmium chloride salt in double distilled water. The procedure followed the methods reviewed by Sprague (1969). Fish were acclimated to test conditions 24 hr before exposure to metal began. Feeding was stopped 24 hr before testing and throughout the test. In tests lasting longer than 96 hr , fish were fed once in two days until satiation, in order to prevent cannibalism and to maintain the health of control and test fish (Lanno et al. 1989).

In the toxicity test with 20-d old seabass, only 20 ppt salinity was used due to the unavailablity of high salinity seawater as a result of the rainy season and poor tolerance of the fish to salinities lower than 20 ppt at this stage of development (Kosutarak et al. 1984). Twenty fish were randomly distributed into each 10-L plastic aquarium and provided with continuous aeration. The fish were exposed to six cadmium concentrations with a control receiving seawater only. Mortality was recorded every 2 hr for the first 24 hr of the test and once or twice daily thereafter for up to 16 d. The criterion for death was failure to respond to gentle stimuli. Dead fish were removed upon observation. Median lethal time was analyzed by the nomographic procedure of Litchfield (1949) and median lethal concentrations by the trimmed Spearman-Karber (computer program) method of Hamilton et al. (1977). Acclimation of the 20-d old fish to cadmium was thought to occur as very low mortalities occurred in the toxicity tests at 0.1, 0.32 and 1.0 mg Cd/L, although the exposure was prolonged up to 16 d. This phenomenon was investigated by exposing the controls (15 fish) from the toxicity test (fish previously unexposed to cadmium) and the survivors (12 fish each) at 0.1, 0.32 and 1.0 mg Cd/L to a high cadmium concentration of 32 mg/L. Their survival times were then compared statistically by the method of Litchfield and Wilcoxon (1949).

Survivors of the toxicity test on 20-d old seabass were analyzed for zinc and cadmium content. Zinc content was analyzed in order to investigate the effect of Cd exposure on tissue Zn content as studies by Noel-Lambot et al. (1978) on metallothionein demonstrated that zinc is displaced from binding sites in the presence of cadmium. One sample consisting of 1-3 fish from each treatment was rinsed in double distilled water and dried to constant weight at 105^{o}C . Three ml of high purity concentrated nitric acid were added to weighed aliquots of tissue in Pyrex boiling tubes. All glassware was cleaned by soaking in dilute nitric acid and rinsed with double distilled water. The mixtures were then heated to 90^{o}C for 3 hr, cooled and 1 ml of high purity hydrogen peroxide added after which heating was resumed for a further 2 hr. The solution was then made up to 10 ml with double distilled water. Blanks were similarly treated. Measurements of Cd and Zn were made by flame atomic absorption spectrophotometry with background correction. For quality control of metal analysis, three samples of a standard reference material, National Bureau of Standards Bovine Liver, were similarly digested.

Concentrations of Cd and Zn measured in the standard reference materials were within 95 percent of certified values.

In tests involving 4-month old seabass, the fish were acclimated to 5, 15 or 30 ppt salinity for 2 weeks, the salinity having been reduced gradually to the required test salinities. The toxicity test at the three salinity levels were carried out in 100-L glass tanks. A series of 4 or 5 concentrations and one control were set up for each test. Ten fish were randomly distributed among the tanks. The tests were carried out in the standard manner for a duration of 22.5 d. Toxicity data were calculated as described above for the 20-d old fish.

Fifty ml of water sample from each control and test aquarium, filtered through 0.45 μm and preserved by the addition of 50 μl concentrated nitric acid, was regularly taken for monitoring metal concentration. Cadmium concentrations, measured by flame atomic absorption spectrometry, did not differ significantly from the nominal concentration throughout the tests. Temperature (24.5 - 28.0^{o}C) and pH (7.90 -8.23) were also regularly monitored.

RESULTS AND DISCUSSION

The median lethal time (LT50) and slope function (S) values for the toxicity test on 20-d old seabass are shown in Table 1. The slope function values were similar at all concentrations.

Table 1. Median lethal time, LT50 and slope function value for 20-d old seabass, *Lates calcarifer*, at 20 ppt salinity (95 percent confidence limits).

Cadmium test concentration (mg/L)	LT 50 (hr)	Slope Function (S)
0.1	no mortality	-
0.32	10% mortality	-
1.0	566.7*	-
3.2	383.3 (304.2 - 483.3)	1.66 (1.37 - 2.02)
5.6	191.7 (166 - 221.3)	1.30 (1.19 - 1.43)
10.0	143.3 (112.8 - 182)	1.47 (1.16 - 1.57)

* LT 50 value obtained by extrapolation of toxicity curve
LT 50 and slope function (S) values were not calculated for 0.1, 0.32 and 1.0 mg Cd/L due to mortality being under 50 percent

The results of the test to determine if acclimation to Cd had occurred in the 20-d old fish, since only low mortalities were recorded, indicated that seabass pretreated at 0.32 and 1.0 mg Cd/L had significantly longer LT50 values compared to controls, whereas those pretreated at 0.1 mg Cd/L was not significantly different (Table 2). The slope function value for the 1.0 mg Cd/L pretreatment was also significantly different from controls.

Table 2. Test for acclimation to cadmium. Statistical comparison of LT50 and slope function (S) values between cadmium pretreatment or pre-exposed seabass and previously unexposed fish (controls) exposed to 32 mg Cd/L.

Pretreatment Cd conc. (mg/L)	LT50 (hr)	S	Statistical significance LT50	Statistical significance S
0 (control)	24.2(21.4 - 27.2)	1.28 (1.19 - 1.38)		
0.1	24.2(20.8 - 28.0)	1.41 (1.27 - 1.56)	n.s	n.s
0.32	31 (25.5 -37.7)	1.53 (1.32 - 1.77)	sig	n.s
1.0	38.8 (30.2 - 33.2)	1.61 (1.32 - 1.95)	sig	sig

n.s : non - significant $\alpha = 0.05$
sig : significant

The whole-body concentrations of Cd and Zn of 20-d old seabass surviving 16 d in the toxicity test are shown in Table 3. Cadmium concentration increased with exposure concentration, while Zn concentrations were undisturbed in fish exposed up to 3.2 mg Cd/L. Seabass at 5.6 mg Cd/L showed a lower but not significantly different Zn level compared with controls.

Table 3. Cadmium and zinc levels (ug/g dry wt) in whole body of seabass surviving various cadmium exposures for 16 d.

Cd exposure concentration (mg/L)	Whole body concentration (µg/g dry wt) Cd	Zn
0	1.65	236
0.1	12.5	298
0.32	21.2	256
1.0	41.4	281
3.2	101.9	229
5.6	152.4	185

Each value is from the analysis of one sample consisting of 1-3 fish each.

Results for the toxicity test on 20-d old (Table 1) and on 4-month old fish (Table 4) show that the seabass, *Lates calcarifer* , is very tolerant to cadmium at normal salinity (30 ppt) and a long exposure time was necessary in order to get a complete response at the low test concentrations. A comparison of the tests on 4-month old seabass at 10 mg/L for example, showed a wide difference in LT 50 values between 5 and 15 ppt salinities with the value for 5 ppt being 9.8 times lower than that for 15 ppt . The LT 50 value for 15 ppt, however, was only 2.8 times lower than that for 30 ppt. The long period of exposure in this investigation on 20-d old seabass at 20 ppt salinity resulted in fish acclimating to cadmium.

Table 4. Median lethal time (LT50) and slope function (S) values and their 95 percent confidence limits for 4-month old seabass, *Lates calcarifer* .

Salinity (ppt)	Cd conc (mg/L)	LT50 (hr)	S
5	0.32	333.3	-
	1.0	61.7(50.2-75.8)	1.40(1.20-1.63)
	3.2	50.8(33.0-78.3)	2.00(0.68-2.72)
	10	16.7(7.25-38.3)	3.96(2.14-7.33)
	32	1.23(0.67-2.30)	2.79(1.77-4.41)
15	1.0	516.7	-
	3.2	466.7(350.0-625.0)	1.53(1.18-1.98)
	10	163.3(115.0-231.7)	1.78(1.37-2.30)
	32	47.5(38.3-58.7)	1.41(1.20-1.66)
30	1.0	10% mortality	-
	3.2	30% mortality	-
	10	458.3(298.3-705.0)	2.02(1.47-2.77)
	32	29.3(20.5-420.0)	1.84(1.40-2.41)

The ability for aquatic organisms to gain resistance to heavy metals after pretreatment has also been observed by other authors. Pascoe and Beattie (1979) observed resistance to cadmium in rainbow trout , *Salmo gairdneri* , alevins after pretreatment. Weis (1985) observed acclimation to cadmium by *Uca pugilator*, but only in male crabs. The ability of *Lates calcarifer* to acclimate to cadmium may be important for the survival of this species in nature, following sublethal cadmium contamination. The mechanisms for cadmium-induced tolerance may involve metallothionein. Metallothionein is possibly involved in the metabolism and detoxification of metals (Reichert et al. 1979) and its synthesis has been demonstrated in fish livers following Cd injections (Beattie and Pascoe 1979; Woodworth et al. 1983) and perfusions (Shazili and Pascoe 1987). However, in this study the metabolism of Zn was apparently unaffected by cadmium exposure (Table3), although it is known that Zn at the cellular binding sites may be replaced by Cd (Noel-Lambot et al. 1978).

Although the seabass was apparently tolerant to Cd at 15 and 30 ppt salinities, at 5 ppt salinity, the 96 hr LC50 was 1.99 mg Cd/L (Table 5). This is comparable to a 96-hr LC50 value of 1.25 mgCd/L obtained for a freshwater fish, the grass carp, *Ctenopharyngodon idella*, in studies carried out in this laboratory (unpublished data).

The fraction of total dissolved Cd present as the free ion is greater at lower salinities (Engle et al. 1981), due to a lesser degree of complexation, and as such the increased toxicity at low salinity as observed in this study. At low salinity, the pacific oyster, *Crassostrea gigas* , has been shown to accumulate greater quantities of Cd compared to oysters in high salinities (Engel and Fowler 1979). Greater toxicity at low salinity has also been observed in toxicity tests of Cd on the mud-crab, *Rhithropanopeus harrisii* (Rosenberg and Costlow 1976).

Table 5. Median lethal concentration (LC50) values at 7 and 12 days for 20-d old fish at 20 ppt salinity and at 4, 12 and 21 days for 4-mon old seabass, *Lates calcarifer* .

	Salinity (ppt)	LC50 (mg/L) 4 d	7 d	12 d	21 d
20-d fish					
	20	-	7.08 (3.90-12.8)	3.67 (2.64-5.11)	-
4-mon fish					
	5	1.99 (1.01-3.95)	-	0.75 (0.48-1.19)	-
	15	14.2 (10.6-19.0)	-	8.97 (6.28-12.8)	2.53 (0.55-11.8)
	30	19.0 (*)	-	14.2 (10.6-19.0)	7.99 (5.26-12.1)

(*) indicates unreliable confidence limits

As the first 3 years of its development is in estuaries and further upstream, where the salinity may be greatly reduced in the rainy monsoon season, the sensitivity of *Lates calcarifer* to cadmium at low salinity may be an important factor to be considered in metal pollution studies. Certainly, the early life stages of *Lates calcarifer* may be more at risk to sublethal levels of metals.

Clearly, more information is required on the response of seabass to sublethal levels of metals if relevant water quality criteria are to be established for this fish.

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