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Effect of chronic ammonia exposure on growth of European seabass (Dicentrarchus labrax) juveniles

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Abstract: Chronic effects of ammonia were studied in juvenile seabass, Dicentrarchus labrax (mean WEIGHT=11 g), exposed for 63 days to eight stable ammonia concentrations, ranging from 0.24 to 0.90 mg l−1 unionised ammonia nitrogen (UIA-N), respectively, from 6.1 to 22.3 mg l−1 total ammonia nitrogen (TA-N). Temperature (21.8 °C), pH (8.0), salinity (37.0 ppt), and oxygen concentration (over 80% saturation at the outlet) were maintained constant. Fish were fed using a self-feeder device, and they were starved during the last 8 days. Mortality of 28.9 and 42.6% occurred within the first 8 days at the two highest UIA-N concentrations, respectively, 0.90 and 0.88 mg l−1. From days 0 to 55, a 1.8-fold increase in weight gain was observed under the 0.90-mg l−1 UIA-N condition, compared to a 3.4-fold increase in the control. Weight gains were negatively correlated to ambient ammonia concentrations. Weight loss, or a transient period of growth stagnation, was observed from the onset of ammonia exposure to day 13 in seabass exposed to concentrations above 0.43 mg l−1 UIA-N. After day 13, weight gains were observed in all groups, indicating that the fish were able to adapt to increased ambient ammonia concentrations over time. By the end of the experiment, plasma ammonia levels were positively related to ambient ammonia concentrations, and oxygen consumption recorded in fasting fish was significantly dependent on ammonia concentrations. In seabass juveniles, the 0.26-mg l−1 UIA-N concentration, under an average pH of 8.0, can be considered as a safe long-term limit conditions in seawater.

Keywords: Ammonia; Chronic toxicity; Seabass; Growth; Plasma ammonia

Introduction

Ammonia and urea are the two main nitrogenous products excreted by teleost fish (Forster and Goldstein, 1969), with ammonia usually representing 75 to 90 % of nitrogenous excretion (Handy and Poxton, 1993). Ammonia is mainly excreted as the un-ionised form NH_3 (UIA). In seawater, NH_3 ionises to form NH_4^+. The relative proportion of the two forms depends upon pH, temperature and, to a lesser extent, salinity (Whitfield, 1974; Bower and Bidwell, 1978). In seawater, ammonia is measured as total ammonia nitrogen (TAN), which represents the sum of UIA-N and NH_4^+-N. The NH_3 molecule is non-polar and readily soluble in lipids. It is 300 to 400 times more toxic than NH_4^+ (Thurston *et al*., 1981; Haywood, 1983). Under intensive rearing conditions, and particularly when effluent water is re-used, ambient ammonia concentrations may reach levels that limit fish survival and growth (Haywood, 1983).

The acute and chronic toxicities of ammonia have been extensively reviewed for freshwater species (Ruffier *et al*., 1981; Haywood, 1983; Russo and Thurston, 1991; Handy and Poxton, 1993 ; Tomasso, 1994) but ammonia toxicity data for marine fish species or salmonids in sea water are relatively scarce (Alderson, 1979; Wajsbrot *et al*.,1993; Tudor *et al*., 1994; Person-Le Ruyet *et al*., 1995, 1997a, b; Fivelstad *et al*., 1995). Further, the chronic effects of exposure to ammonia in seawater have rarely been examined (Person-Le Ruyet and Bœuf, 1998). However, « safe levels » for growth, usually extrapolated from LC50 data, are reported to range from 0.05 to 0.2 mg l^{-1} UIA-N (Ruffier *et al*., 1981, Handy and Poxton, 1993), depending on species, age and environment (oxygen concentration, pH). Lethal concentration for 50% of the population

(96-h LC50) have been reported to be 1.7 mg l^{-1} UIA-N (40.0 mg l^{-1} TAN) in seabass juvenile (Person-Le Ruyet *et al.*, 1995).

Seabass farming in sea cages and land-based systems is developing rapidly in the Mediterranean region, with production increasing from 4700 mt in 1991 to 23000 mt in 1997, to some 50000 mt in 2000 (Paquotte P., comm. pers.). For economic and environmental reasons, culture systems using recirculating water are being developed, and as a result, there is a need to define the environmental quality standards required for fish growth. The purpose of this study is to provide information about the chronic effects of ammonia exposure on survival and growth of seabass juvenile.

Materials and methods

Fish and rearing conditions

Seabass were reared at Ifremer Station in Palavas from eggs to day 132 in semi-closed systems (Covès *et al.*, 1991). Fish were graded and those within the weight range (4.0 ± 1.4 g) were randomly distributed (230 fish per tank) amongst 9 circular tanks (effective volume $1m^3$) in a sound-proof facility. The fish were acclimated to the rearing conditions for 41 days prior the experiment. The experiment then lasted 63 consecutive days. Fish were fed from day 1 to 55 using expanded pellets (54.3% proteins, 15.3% crude fat), using a self-demand system described by Boujard *et al.* (1992). When the fish activated a rod positioned below the water surface, an electric pulse was generated to stimulate an electric feeder that delivers a predetermined amount of feed (1.5 to 2.0 g). Every day, the feeding system was turn off between 9:00 h and 10:00 h in

order to replenish the feeders and to check for the presence of uneaten pellets collected in a sedimentation trap located at the outlet of each tank. From day 56 to day 63, fish were starved.

Tanks were supplied with running sea water, sand-filtered at 15 μm, UV sterilised, heated, degassed in a packed column and dispatched to the tanks by gravity. Flow rate was fixed at 1 m^3 h^{-1} (accuracy of the flow-meter : ± 0.05 m^3 h^{-1}) in order to maintain oxygen saturation systematically above 80% and to provide self-cleaning of the tank. Temperature was maintained at 21.80 ± 0.15 °C (mean ± standard deviation (SD)) using a heat-exchanger and checked hourly. Salinity (37.0 ± 2.5 ppt) was checked once a day, and pH (7.99 ± 0.14) once a day. Light intensity at the water surface was 250 lux, and the photoperiod was maintained at 16h light - 8h dark including a 30 min artificial dawn and dusk, using incandescent lamp (OSRAM Decor Silver E27).

Experimental design

Eight different ammonia solutions of eight different TAN concentrations, ranging 14.4-57.7 g l^{-1} TAN, were delivered in the inlet of eight tanks from day 1 to day 63, using a peristaltic pump (8 channels, flow rate around 5 ml min^{-1}). These concentrated solutions were obtained by dissolving 61 to 245 g l^{-1} ammonium chloride powder (NH_4Cl, BASF®, 99.5 % purity) in tap water. Inputs of ammonia were calculated to obtain a range of eight concentrations, from 10 to 40% of the 96-h LC50s reported for seabass juvenile by Person-Le Ruyet *et al*. (1995), in the rearing tanks. Due to the preciseness of the peristaltic pump, the range of the concentrations failed to demonstrate equal steps, but every flow rate was constant with time. A ninth tank (control) did not receive ammonia solution. The ambient ammonia concentration within each

tank was checked daily in a 24h-pooled sample of water, obtained from the effluent water and poured in a 2 l bottle where 5 ml of 99.5% chloroform were added for stabilisation (Dosdat *et al*., 1992). The TAN concentration was then determined by the indophenol method (Bower and Holm-Hansen, 1980), using a Technicon Analyser®. For each tank, the mean TAN concentrations ± SD were calculated from daily data. The pH was measured at the outlet using a Tacussel PHN81® pH-meter, coupled with a Tacussel TC100® probe containing a saturated solution of KCl/AgCl (Tacussel KS120 ®) and an Ag/AgCl reference electrode (Johansson and Wedborg, 1980). UIA-N concentrations were calculated from TAN according to pH, temperature and salinity, using the equation of Johansson and Wedborg (1980). Therefore, the percentage of UIA-N to TAN was given by the following equation :

$$\%NH3 = 100/[1+10^{(\log K_1 - pH)}]$$

with $$\log K_1 = -0.467 + 0.00113 \times S + 2.887.9 \times T^{-1}$$

where K_1 is the dissociation constant, S (in g l^{-1}) the salinity and T the temperature (°K)

In every tanks, at day 0, 13, 27, 41 and 55, the fish, fasted for 24 h, were anaesthetised (using a solution containing 150 µl l^{-1}, ethylene-glycol-monophenyl-ether), counted and 50 of them were randomly collected and individually weighed to the nearest 0.01 g. In order to avoid a lowering of the oxygen concentration due to increasing biomass, stocking density was reduced on day 27 by removing at random 130 fish per tank. Fish were weighed again to establish an initial weight. Mortality was counted daily.

The mean wet weights ± standard deviation were calculated as the arithmetic mean from the samples of 50 fishes. The average individual weight gain at day 55 was calculated as Wf – Wi,

where Wf is the final mean wet weight (n=50) at day 55 and Wi is the initial mean wet weight (n=50).

Oxygen concentrations of inlet and outlet water from each tank was monitored using the methodology described by Lemarié *et al*. (1992). A part of inflowing and outflowing water was diverted through solenoid valves to a measure chamber where oxygen concentration was measured using a Ysi 58® oxymeter. Oxygen data were recorded on a data logger (Grant-Squirrel® SQ16-4V-1D). Recording, opening and closing of each solenoid valve were managed by an automatic controller device. Oxygen concentrations were measured every 40 min. during 5 consecutive days, (from day 58 to 63) on starved fish in the control, 0.26, 0.43, 0.64, and 0.88 mg l^{-1} UIA-N conditions. Oxygen uptake (MO_2) in each tank was calculated as :

$$MO_2 \text{ (mg kg}^{-1} \text{ h}^{-1}) = ([O_2] \text{ outlet} - [O_2] \text{ intlet}) \times \text{flow rate (l h}^{-1}) \times \text{fish biomass}^{-1} \text{ (kg)}$$

In the same tanks, on day 57 blood samples were collected, on 14 individuals per tanks, from vessels at the caudal peduncle using free-ammonium salt heparinized syringe, pooled in pairs to get enough blood and immediately centrifuged. Plasma TAN contents were determined within one hour using the enzymatic kit Sigma Diagnostic UV-170®.

Experimental data on growth at the end of each period were processed by one-way analysis of variance (ANOVA). Concerning data on water quality and triggering activity, possible differences among treatments were tested by repeated measures ANOVA (Zar, 1984). Pairwise comparisons between the means were made using the Student-Newman-Keuls (SNK) test. The accepted level of significance was $P<0.05$.

Results

Environmental conditions :

The environmental conditions within the rearing tanks (temperature, salinity, oxygen concentration, pH) were stable. The variation coefficients (CV%, Table 1) of the eight ambient TAN concentrations tested were low, ranging from 11 to 19 %. The pH was stable in all the treatments, ranging from 7.89 to 8.13 during the whole experiment. This meant a good correlation between the UIA-N fraction and TAN. Under the experimental conditions, the average ambient UIA-N to TAN ratio was 4.0%. Repeated measures one way ANOVA on oxygen levels in the 9 tanks during the 55 first days demonstrated that there were no significative difference among the treatments ($F(432; 8) = 0.543$, $n = 55$).

Table 1 – Ammonia concentrations (mean ± SD, mg l^{-1}) in seawater during the 63 days experiment. Values bearing common superscript in the same column are not significantly different (P<0.05).

Tank	Number of days	Measured TAN (mg l^{-1})		Calculated UIA-N (mg l^{-1})
		mean ± SD	C.V (%)	mean ± SD
Control	63	0.4 ± 0.1^{a}	18.9	0.01 ± 0.0^{a}
C1	63	6.1 ± 0.8^{b}	13.5	0.24 ± 0.04^{b}
C2	63	6.6 ± 0.7^{c}	10.7	0.26 ± 0.04^{c}
C3	63	10.6 ± 1.2^{d}	11.3	0.43 ± 0.05^{d}
C4	63	13.3 ± 2.6^{e}	19.3	0.53 ± 0.10^{e}
C5	63	15.9 ± 1.8^{f}	11.8	0.64 ± 0.08^{f}
C6	63	17.7 ± 3.0^{g}	17.0	0.71 ± 0.12^{g}
C7	63	21.7 ± 2.3^{h}	11.1	0.88 ± 0.10^{h}
C8	63	22.3 ± 3.1^{h}	14.0	0.90 ± 0.13^{h}

Behaviour and mortality

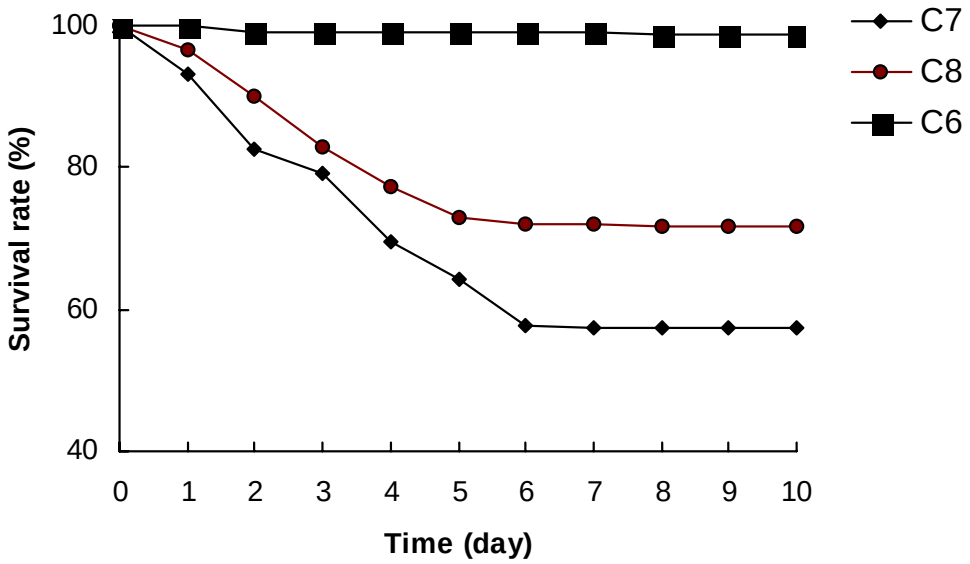
Changes in the swimming behaviour (disorientation and erratic swimming), in gill ventilation, and in the colour of fish (darkened skin) were observed within two hours after addition of ammonia in the rearing tanks. They were noticed until 15 days of ammonia exposure for the two highest concentrations. This observation was correlated with the feeding triggering activity (*i.e.* the number of actuations on the rod), which was significantly different (repeated measures one way ANOVA; $F(72,8) = 14.25$; $n = 12$) among the treatments from day 1 to 12. The number of actuations was the highest in the 0.43, 0.53 and 0.64 mg l^{-1} UIA-N conditions (Table 2). It decreased for the two highest concentrations. This typical observation was still perceptible at the end of the feeding period (day 41-55; Table 2).

No mortality was observed from control up to 0.71 mg l^{-1} UIA-N (17.7 mg l^{-1} TAN) concentrations. In the 0.90 and 0.88 mg l^{-1} UIA-N groups, respectively 29 and 43 % of the fish died between day 0 and 8. No mortality was observed after (Figure 1) .

Table 2 - Average daily number of actuations (mean ± SD) by fish under various ambient ammonia conditions. Values bearing common superscript in the same row are not significantly different (P<0.05).

	Control	C1	C2	C3	C4	C5	C6	C7	C8
Day 1-12	72.1 ± 18.6 [a]	51.5 ± 8.7 [a]	59.9 15.5 [a]	123.1 ± 21.9 [b]	129.2 ± 48.0 [b]	158.6 ± 41.6 [b]	120.2 ± 19.3 [b]	114.2 ± 25.5 [b]	68.7 ± 16.4 [a]
Day 41-55	46.9 ± 14.6 [a]	47.2 ± 19.1 [a]	55.3 31.8 [a]	110.6 ± 24.5 [c]	179.1 ± 34.8 [e]	124.8 ± 18.7 [c]	91.6 ± 12.8 [b]	144.7 ± 14.3 [d]	73.7 ± 25.6 [b]

Figure 1 – Cumulative survival rate during the first 10 days in the three tanks where mortality occurred.



Fish growth

In all groups of fish exposed to ammonia, except for the two lower concentrations, marked effects on growth were observed (Table 3). At day 13, weight increase was significantly lower in fish exposed to 0.24 and 0.26 mg l^{-1} UIA-N than in the control. Fish growth was stopped in the 0.43, 0.53 and 0.64 mg l^{-1} UIA-N groups, while fish lost weight when ammonia concentration was over 0.71 mg l^{-1} UIA-N. From day 27, individual weight gains were observed in all fish groups. From day 41, fish submitted to the two lowest concentrations where not significantly different from the control. Over the 55-day feeding period, mean weights increased by 3.4 fold in the controls and only by 1.8 in the highest ammonia concentrations tested. Figure 2 shows the weight gain, expressed as a percentage of the weight gain by the control fish, in relation with ammonia concentration in seawater. This relative growth performance index was linearly and negatively correlated to the ammonia concentrations, and can be described by the equation:

$$y = -83.03x + 102.1 \qquad r^2 = 0.97$$

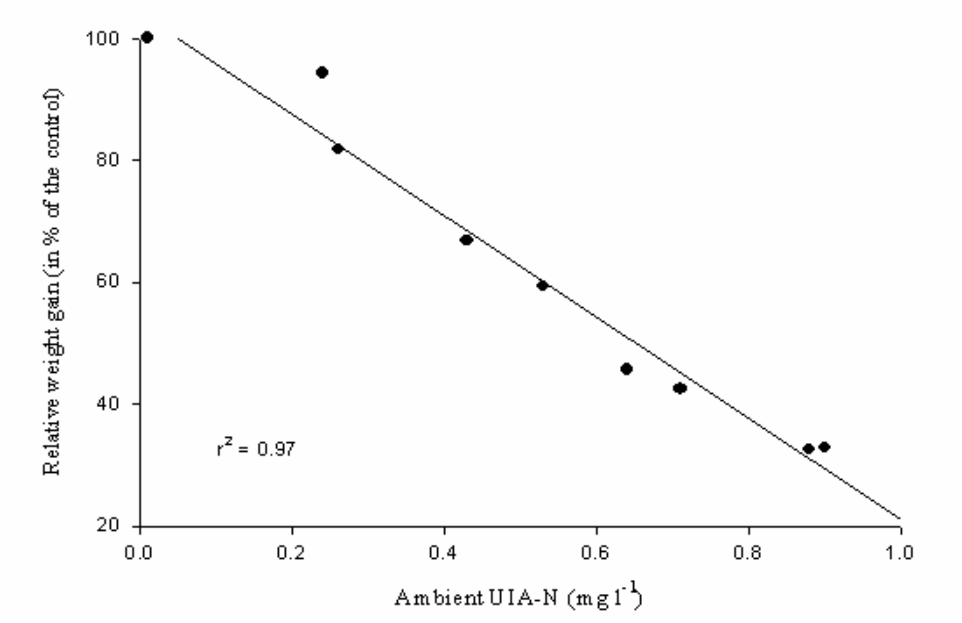
where y is the % of growth compared to the control and x is the UIA-N concentration in mg l^{-1}. The weight increase at day 55 was 90% of the control fish at 0.14 mg l^{-1} UIA-N (3.4 mg l^{-1} TAN) and 50 % at 0.62 mg l^{-1} UIA-N (15.3 mg.l^{-1} TAN).

During the first 55 days, the average daily food intake for the two lower concentrations (respectively 2.2 and 2.6 % of the standing biomass) was similar to the control (2.5%). Due to high food spillage, estimation of uneaten pellets was not possible in fish submitted to higher TAN concentrations. Apparent food delivery for the higher concentration conditions did not represent the actual intake by the fish.

Table 3 – Fish weights in grammes (mean ± SD, n = 50) of seabass juveniles exposed to ammonia in seawater at different sampling days. Values in the same column marked with a common superscript are not significantly different (P<0.05).

	UIA-N (mg l^{-1})	day 0	day 13	day 27	day 41	day 55
Control	0.01	12.1 ± 2.8	16.6 ± 4.3[a]	24.5 ± 6.3 [a]	31.6 ± 8.3[a]	40.1 ± 9.3[a]
C1	0.24	10.6 ± 2.4	15.4 ± 4.1[b]	22.1 ± 6.1[b]	28.1 ± 7.6[a]	37.0 ± 9.2[a]
C2	0.26	11.2 ± 2.5	14.8 ± 3.7[b]	20.9 ± 5.6[b]	29.8 ± 8.0[a]	34.1 ± 8.8[a]
C3	0.43	11.2 ± 3.0	11.4 ± 2.9[c]	16.3 ± 4.5[c]	23.5 ± 7.3[b]	29.9 ± 8.3[b]
C4	0.53	10.9 ± 2.6	11.2 ± 3.0[c]	15.6 ± 3.7[c]	20.9 ± 5.4[b]	27.5 ± 7.5[b]
C5	0.64	12.0 ± 2.4	11.2 ± 2.3[c]	14.9 ± 4.2[c]	17.6 ± 5.5[d]	24.8 ± 5.7[c]
C6	0.71	12.1 ± 3.1	10.4 ± 2.9[c]	13.8 ± 3.5[d]	19.3 ± 4.9[c]	24.0 ± 5.8[c]
C7	0.88	11.6 ± 3.3	9.9 ± 2.8[c]	12.3 ± 3.2[d]	17.3 ± 3.8[d]	20.7 ± 5.3[d]
C8	0.90	11.4 ± 2.7	9.9 ± 2.3[c]	12.7 ± 3.0[d]	17.5 ± 3.5[d]	20.6 ± 4.7[d]
F(441, 8)		1.98	32.04	45.47	40.55	41.17

Fig. 2. Weight gain expressed as a % of the weight gain of the control fish in relation with ambient ammonia concentration in seawater on the 55-day trial.



Oxygen uptake and plasma ammonia concentration

Oxygen consumption by fish starved for 5 to 10 days ranged from 205 mg O_2 kg^{-1} h^{-1} for the control group to 368 mg O_2 kg^{-1} h^{-1} for the 0.88 mg l^{-1} UIA-N group. It was positively correlated to ammonia concentrations as shown in Figure 3. The relation can be described by the following equation :

$$y = 199.7x + 191.1 \qquad r^2 = 0.93$$

where y is the oxygen consumption (mg kg^{-1} h^{-1}) and x the UIA-N concentration (mg l^{-1}).

In seabass, acclimated to ammonia for 57 days and starved for 2 days, plasma TAN was also dependant on ambient ammonia concentration as shown in Figure 4 according to the following equation :

$$y = 0.48x + 3.32 \qquad r^2 = 0.97$$

where y is the plasma TAN concentration (mg l^{-1}) and x the TAN concentration in seawater(mg l^{-1}).

Fig. 3. Oxygen uptake in relation with ambient ammonia concentration in seawater. Vertical bars represent the standard deviation of the mean.

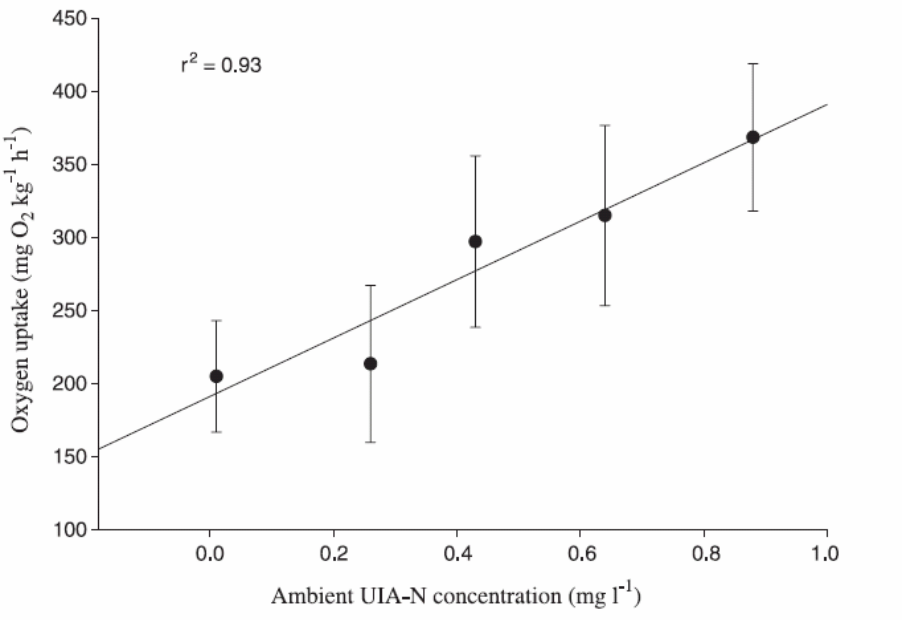
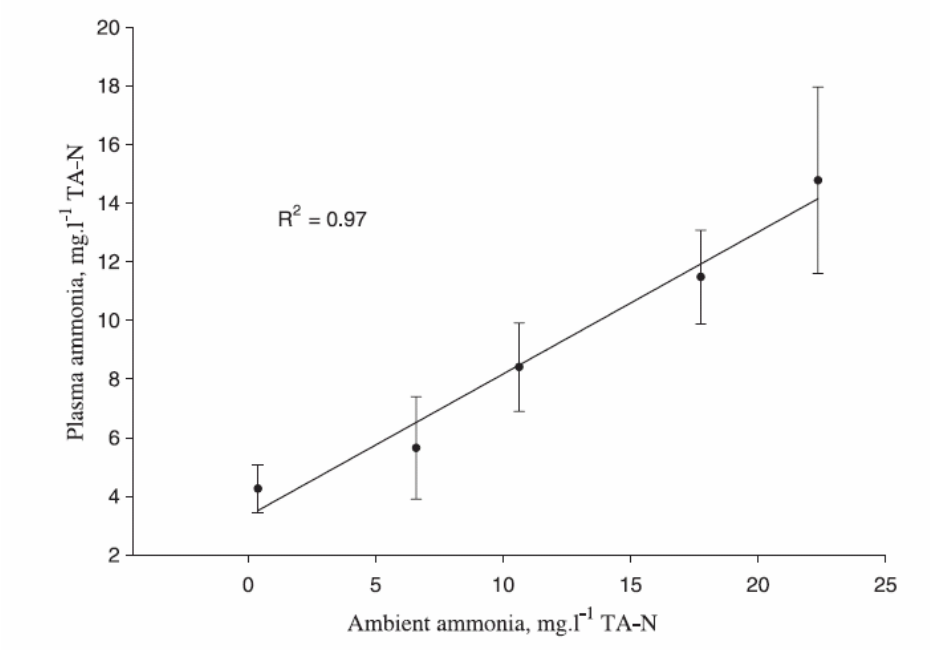


Fig. 4. Plasma TA-N in relation with ambient ammonia concentration in seawater. Vertical bars represents standard deviation of the mean.



Discussion

This study presents results concerning the long-term effects of ambient ammonia on juvenile seabass and supplements acute toxicity tests performed on the same species (Tudor *et al*., 1994, Person-Le Ruyet *et al*., 1995). Environmental parameters (pH, dissolved oxygen, temperature and salinity) were stable and were similar among treatments and were not limiting for growth (Lemarié *et al.,* 2000). Thus, the effects we observed on growth were attributed to ammonia, which varied among treatments. The growth of the fish in the control tank was in the range of values reported recently in the literature (Quemener *et al*., 1999; Peres and Oliva-Teles, 1999; Pichavant *et al.,* 2001).

The experiment confirmed that ammonia is stressful for the fish even at low concentrations. The effect of ammonia is immediate, linear and dose dependant. Person-Le Ruyet *et al*. (1998) have shown that ammonia entered fish within 15 minutes of exposure. The observed effects on the changes in behaviour were similar to those reviewed by Haywood (1983). They were associated with erratic actuations on self-feeders and with an increase in the oxygen consumption. These feeder actuations were not a consequence of feeding activity, but of abnormal swimming at the surface. They were the result of the build up of ammonia in the blood and other tissues (brain, liver, muscle) which has negative effects on synaptic connections of the central nervous system and the NMDA (N-Methyl-D-aspartic Acid) receptor activity (Russo and Thurston, 1991; Tomasso, 1994; Montfort *et al.,* 2000).

Survival was 100% from the control to the 0.71 mg l^{-1} UIA-N level, which represents 42% of the 96-h LC50 (1.7 mg l^{-1} UIA-N) in the species (Person-Le Ruyet *et al*., 1995). This maximum

ammonia level for no mortality was higher than in turbot juveniles (Person-Le Ruyet *et al*., 1997b). Mortality was recorded for the highest ammonia concentrations : 43% of the fish died after 8 day exposure at 0.88 mg l^{-1} UIA-N, which can be considered as a rough estimate of the 8 day LC50. Comparison of various LC50 estimates on seabass, seabream, turbot and some freshwater species is given in Table 4. From our study, juvenile seabass appeared more sensitive than turbot, and comparable to seabream, as it was predicted by Person-Le Ruyet *et al*. (1995).

Table 4 – Comparison of various ammonia toxicity levels in some fish species.

	Species	TAN (mg l^{-1})	UIA-N (mg l^{-1})	Authors
4-day LC50	Seabass	40	1.7	Person-Le Ruyet *et al*., 1995
4-day LC50	Seabream	57	2.5	Person-Le Ruyet *et al*., 1995
4-day LC50	Turbot	59	2.6	Person-Le Ruyet *et al*., 1995
4-day LC50	Catfish	45	1.6	Colt and Tchobanoglous, 1978
4-day LC50	Rainbow trout	22	0.3-0.6	Haywood, 1983
8-day LC50	Seabass	>22.3	>0.9	This study
20-day LC50	Seabream	15.7	0.89	Wajsbrot *et al.*, 1993
28-day LC50	Turbot	38	1	Person-Le Ruyet and Bœuf, 1998
20-day EC50	Seabream	15.7	0.89	Wajsbrot *et al.*, 1993
28-day EC50	Turbot	17-19	0.50-0.65	Person-Le Ruyet *et al*., 1998
55-day EC50	Turbot	17-21	0.60-0.75	Person-Le Ruyet *et al*.,1998
55-day EC50	Seabass	22	0.9	This study

LC50: lethal concentration for 50% of the population.
EC50 : concentration reducing growth by 50%.

At day 27, a weight gain is recorded in all the treatments, indicating that the fish globally adapted to their environment, and that the physiological disturbances were partly reversed after day 13 (Person-Le Ruyet *et al*.,1998). The physiological processes were then sufficiently re-

organised to enable growth. Physiological effects include mostly higher activities of glutamine synthetase, which transforms glutamate (a highly potent excitatory neurotransmitter) into glutamine by adding a NH_3 molecule, in the brain in non-ureotelic fish (Wang and Walsh, 2000). Detoxification of ammonia to urea is the other pathway that can be utilised by many fish species (Wood 1993). After 27 days of ammonia exposure, acclimated seabass expressed a lower growth than control fish at all ammonia levels. At that time, in all the treatments, the fish could not compensate for the initial growth delay recorded at day 13.

Statistically, after 55 days, the growth performances of seabass juveniles were not affected by the two lowest ammonia concentrations, i.e. 0.24 and 0.26 mg l^{-1} UIA-N, (respectively 6.1 and 6.6 mg.l^{-1} TAN), even if the fish expressed respectively 94 and 82% of the growth of the control fish. In that sense, some compensatory growth might have occurred between day 27 and day 55. Behavioral and physiological disturbances were still perceptible by the end of the experiment as shown by triggering activity, increased oxygen uptake and plasma ammonia accumulation. The concentration of no-observable effect have been reported to be 0.11-0.18 mg l^{-1} UIA-N in turbot juveniles (Person-Le Ruyet and Bœuf, 1998) and 0.27 mg l^{-1} UIA-N in seabream juveniles (Wajsbrot *et al*., 1993). These values are closed to the 0.24-0.26 mg l^{-1} UIA-N we observed in seabass, which represents 16% of the 96-h LC50. These data suggest that there are no major differences in the long-term sensitivity to ammonia among marine species, and that their long-term sensitivity is lower than in most salmonids (Arillo *et al*., 1981, Russo and Thurston, 1991).

Seabass fish farms in flow-through system usually operate at fish loading density of 50 kg m^{-3} (Lemarié *et al*., 1998). In that condition, ammonia concentrations never exceed 1.2 mg l^{-1} TAN

(0.05 mg l^{-1} UIA-N) (Dosdat *et al*., 1996; Lemarié *et al*., 1998). Therefore, in those rearing conditions, the effects of ammonia on fish growth would remain low and would be economically acceptable. When using recirculating systems, ammonia levels can easily reach 10 to 15 mg l^{-1} TAN, which are usually considered as the upper allowable limits by the industry (Blancheton, personal communication). Nevertheless, in those systems the pH continuously decreases due to the build up of CO_2 from fish and of H^+ from the nitrifying bacteria (Covès and Gasset, 1994). Optimal pH in seabass appeared to be 7.0 (Lemarié *et al*., 2000). In these conditions, UIA-N would amount at the most 0.1 mg l^{-1}, which would result in a very slight growth reduction (Figure 2) and would be below the concentration of no-observable effect. Nevertheless, considering that UIA-N and $NH4^+$ are more toxic at low pH and that ammonia toxicity is mainly due to UIA, safe ammonia levels in those systems have to be re-assessed.

Blood plasma TAN concentrations were positively correlated to ambient ammonia levels, as previously described in freshwater and seawater species (Arillo *et al*., 1981; Person-Le Ruyet *et al*., 1995, 1997a,b; Knoph and Thorud, 1996). In all ammonia exposed groups, they remain lower than the external TAN concentration in water (iso concentration was 6.4 mg l^{-1} TAN). The coefficient of the linear regression between plasma and ambient TAN (0.48) was close to the ones ((0.49-0.63)) reported by Person-Le Ruyet *et al*. (1997) in turbot in shorter term experiments (28-42 days), indicating that seabass can support ammonia in the long-term in the same way. The seabass appeared to adapt to a 3-fold increase in plasma TAN, relative to controls, without any mortality, but growth seemed to be significantly affected by plasma TAN concentrations over 8 mg l^{-1} TAN. This threshold level seems to be higher in turbot when it has

been reported to be 10 mg l^{-1} plasma TAN (Person-Le Ruyet and Bœuf, 1998). This is consistent with a higher sensitivity of seabass to ammonia.

Oxygen consumption in fasted fish was correlated to ammonia level in ambient seawater. Higher O_2 consumption in ammonia-exposed groups is related to the observed hyper-ventilation. These results are in agreement with observations by Knoph (1996). MO_2 increased with ammonia level and was 1.5 times higher than the control under the highest ammonia concentration. The oxygen consumption by the control fish was similar to the value given by Lemarié *et al*. (1992).

To conclude, more information are required to determine precisely safe levels for ammonia in sea bass and particularly their interactions with other environmental factors, specially when pH and CO_2 conditions vary. The reduced growth of seabass observed when ammonia concentration increases, specially in recirculating systems (Blancheton *et al.*, 2001), can be attributed not only to UIA-N concentration but to other parameters as pH, CO_2 or NO_2^- which could act in synergy and affect growth potential. Further, investigations on the physiological mechanisms involved in long-term toxicity and adaptation to ammonia are still required.

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