

**Pathology of *Mytilus galloprovincialis* L. reproductive organs
produced by Nitrogen-compounds**

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Many of mineral and organic compounds or breakdown products may affect the natural environment; LC50, ET50 (LITCHFIELD & WILCOXON, 1949) as well as several methods of growth potential estimation or marine bioassay (BERLAND et al. 1972; MAESTRINI et al. 1984 a, b) were applied to get knowledge concerning their effects on marine living beings. There are only few data on their behaviour and pathways through the ecosystems. SOLBE (1988) defined "the study of the pathways, fate and effects of chemicals to and in environment: ECOTOXICOLOGY". And we emphasize: any physical or chemical parameter must be included as potential harmful factors, when it reaches a critical size or concentration level. This is a new tool that helps us to understand biological effects of pollutant and learn how to protect our natural resources. Heavy eutrophicated environments present high levels of N-NH₄ and N-urea. N-NH₄ and N-urea reached into Romanian inshore area a significant increase as the process of eutrophication grown up in the last years. Ammonia reached in 1989 maximum values of 240-1,417 µg l⁻¹ and urea 201-2,200 µg l⁻¹ (PECHEANU, personal communication). Both N-compounds were found to be toxic - on *Mytilus galloprovincialis* in long term exposure - when they reach a high concentration level.

MATERIAL AND METHODS

Mussels were collected from an area less influenced by the outfalls, cleaned out of periphyton and acclimated at laboratory conditions for 30 days. They were experimented in static renewal, continuous air supply, 20 - 22°C, light/darks : 16/8 hrs., each vassel (10 l) containing 32 individuals (height 6-7 cm). There were used 8 experimental vassels representing duplicates for : control ; 1‰, 5‰, and 10‰ (in mg) N-NH₄ and N-urea. The sea water was changed every day and then contaminated with N-compounds up to experimental level. Mussels were fed with *Chloromonas* : 1.25 x 10⁶ cel/mussel/8 times a day. Algae were separated from culture medium by centrifugation and rinsed with clean sea water. Mussels were sacrificed after 72 days of exposure. Gonad cycles was determined and numerical ranking of a sample was valued : O - resting ; 1 - immature ; 2 - developing ; 3A - ripe ; 3B - spawning ; 3C - redeveloping ; 3D - spent. Both gonad squash observations and histological preparations were performed.

RESULTS

Ovula and sperm release was observed only in control, during August. The follicles size did not show great differences between controls and variants, except NO₃NH₄ exposures (Table 1).

Table 1 :
The size class frequency of follicles after 72 days of experimentation (N-compounds as mg ‰ and size in µ)

Analyzed sample	no. of samples	X	Follicles size			Follicles size class (%)			
			SD	min.	max.	1-100	100-200	200-400	400-600
Control	32	225	127	71	550	9.37	46.87	28.12	15.63
Urea 1‰	32	204	107	57	471	15.15	45.45	33.33	6.06
Urea 5‰	32	152	152	71	385	21.87	62.50	15.62	0
Urea 10‰	32	304	128	143	607	0	25.00	59.37	15.63
NO ₃ NH ₄ 1‰	32	223	89	64	407	2.63	47.37	47.37	2.6
NO ₃ NH ₄ 10‰	32	265	79	164	499	0	25.00	71.87	3.13

The gonad stages (Table 2) proved that urea and NO₃NH₄ produced an interrupting of reproductive cycle, but what stage, it depended on the nitrogen species and their concentrations.

Table 2 :
The gonads stages

Analyzed sample	no. of samples	Frequency of each stage (%)							
		0	1	2	3A	3B	3C	3D	3E
Starting point of experiment	100	0	0	0	2	4	20	74	0
Control	32	0	100	0	0	0	0	0	0
Urea 1‰	32	0	0	0	0	0	28.89	71.11	0
Urea 5‰	32	0	0	0	0	0	16.67	83.33	0
Urea 10‰	32	0	0	0	0	0	3.12	90.62	0
NO ₃ NH ₄ 1‰	32	0	0	0	0	0	31.25	68.75	0
NO ₃ NH ₄ 10‰	32	0	0	0	0	0	5.77	94.28	0

At the end of experiment, 100% of the control individuals resumed the reproductive cycle as they were found to be immature : gonads presented islands of rudimentary reproductive tissue in the matrix. All variants showed in different percentages either 3C : developing (new oocytes being found at the margin of the follicle ; male follicles show a reformation of the lamellae of sperm), or 3D : spent (in females residual oocytes reabsorbed ; follicles in the males decrease in size and the remaining sperm are broken down by amoebocytes) stages.

When 10 mg‰ urea was used, a stage (3E) beyond the 3D was observed ; 90.62% of individuals presented follicles completely spent, and they looked like big and irregular holes with no tendency of recovering.

In conclusion, the increase of nitrogen compounds in an eutrophicated environment could produce a pathology of gonads.

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