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Sodium Nitrilotriacetate (NTA) Influences the Larval Development and Metamorphosis of Marine Invertebrates

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Since the early 1970s when nitrilotriacetic acid (NTA) was proposed as a substitute for polyphosphates in household laundry detergents, several studies were performed to evaluate its possible environmental impact. However, only few experiments were devoted to the study of the effects of NTA in the marine environment (Perry et al. 1984). At present there is no evidence that NTA might be toxic for adult marine animals (Eisler et al. 1972; Bott et al. 1980).

In the present work we evaluated the effects of NTA on embryonal stages of some marine animals, generally more sensitive than the adult ones.

MATERIALS AND METHODS

The test animals were chosen among common species of the littoral zone. The effects of NTA on early phases of the life cycle were studied on embryos of the mussel *Mytilus galloprovincialis* Lmk. and the sea-urchin *Paracentrotus lividus* Lmk., and on larvae of the two Ascidiacea *Botryllus schlosseri* (Pallas) and *Botrylloides leachi* (Savigny). In all experiments sea water collected at least 1 mile off-shore was used.

M. galloprovincialis is sexually ripe in winter. The spawning was stimulated by placing the animals in pre-heated (about 24° C) sea water. 30 min after the fertilization, the eggs were collected by means of a plankton net to eliminate the excess sperm, and transferred in 200-mL beakers with the experimental water. About 40 eggs per mL were present. After 40 hr at 20° C, all samples were fixed with neutralized formaldehyde (5% in sea water) and the frequencies of the 'straight-hinge' stages determined.

B. schlosseri and *B. leachi* are sessile colonial Tunicata (Ascidiacea). They are viviparous and produce non-feeding, planktonic larvae which persist for few hours before metamorphosing into a pre-adult benthic stage. Ripe colonies from the natural environ-

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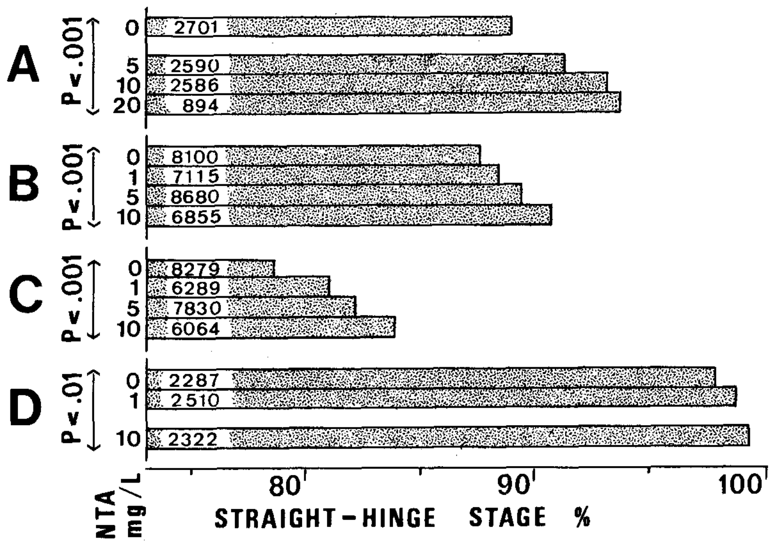

Figure 1. Frequencies of 'straight-hinge' stage in eggs of M. galloprovincialis in presence of NTA at 40 hr from fertilization. Numbers in columns indicate the total number of eggs scored. The degree of statistical significance, evaluated by G-test, is indicated. A -> D refer to separate experiments.

ment were put in vessels with filtered, 35‰ salinity, sea water. At the onset of spawning, the larvae were collected with a "Pasteur" pipette and distributed in beakers (10 larvae each) with 100 mL of treated sea water with the same salinity. For each concentration of NTA, 10 replicates (i.e, 100 larvae) were used. After 6 hr at 18°C the samples were fixed and the number of metamorphosed larvae determined.

Sperm and eggs of P. lividus were obtained by KCl-method (Ruggeri 1975) in filtered, 35 ‰. salinity, sea water. In every experiment eggs from only one female were fertilized with sperm from only one male and, after raising of the fertilization membrane, were washed to eliminate the excess sperm and distributed in 100 mL experimental water at a concentration of about 50 eggs per mL. Three replications per concentration of NTA were performed. After 40 hr at 25° C the samples were fixed with neutralized formaldehyde and filtered by a 25-mm diameter millipore filter (1.2 μ). Filters were washed with demineralized water, dessicated (60°C) and mounted on slides with oil cedar, in order to measure the somatic rods which are a good index of the initial endotrophic growth of the embryos (Marin et al.1987). Eighty 4-arm echinoplutei per replicate were measured.

Analysis of variance, G-test and comparison intervals of means were calulated and applied as suggested by Sokal & Rohlf (1981).

RESULTS AND DISCUSSION

Figure 1 shows the results of four experiments on developing eggs

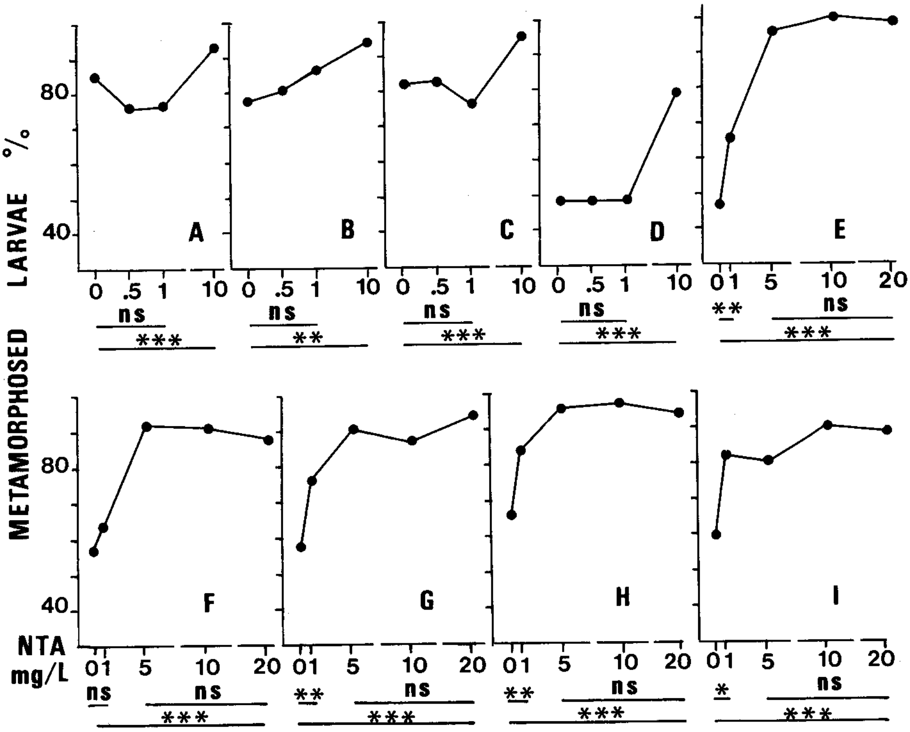

Figure 2. Frequencies of metamorphosed larvae of *B. schlosseri* in presence of NTA at 6 hr after spawning. The degree of statistical significance, evaluated by G-test, is indicated: ns = not significant; * = P<0.05; ** = P<0.01; *** = P<0.001.

of *M. galloprovincialis*. An apparently small, but statistically highly significant, rise in frequencies of the 'straight-hinge' stage with the increasing NTA concentrations is present in all tests.

The effect of NTA on the metamorphosis rate of the larvae of *B. schlosseri* was tested in nine experiments whose results are illustrated in Figure 2. NTA induces an increased frequency of metamorphosed larvae at concentrations between 1-5 mg/L. With increased concentrations of NTA no further increase in the response is noticed.

Similar results were obtained in experiments on larvae of *B. leachi* (Figure 3), but the latter species seems to be less sensitive to NTA, since only 4 of 6 experiments gave positive responses.

The greatest sensitivity to NTA was shown by developing eggs of *P. lividus* (Figure 4). A highly significant increase in somatic rod lenght is already evident at concentrations as low as 0.05 - 0.1 mg/L. Above 1 mg/L, no further significant increase with increasing concentrations is noticed. Four more experiments were performed using both natural and artificial sea water prepared in the laboratory (Marin et al. 1987), with and without 10 mg/L of NTA.

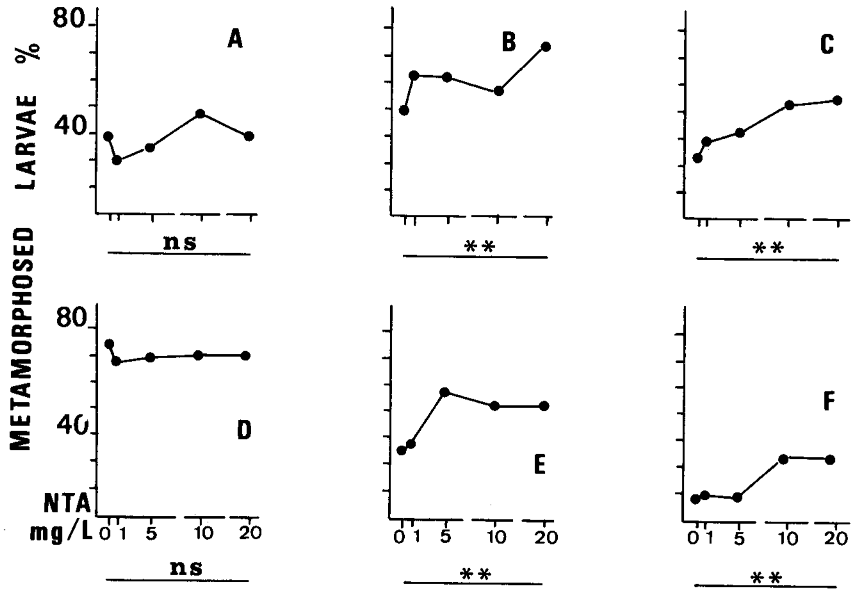

Figure 3. Frequencies of metamorphosed larvae of B. leachi in presence of NTA at 6 hr from spawning. Symbols as in Fig.2.

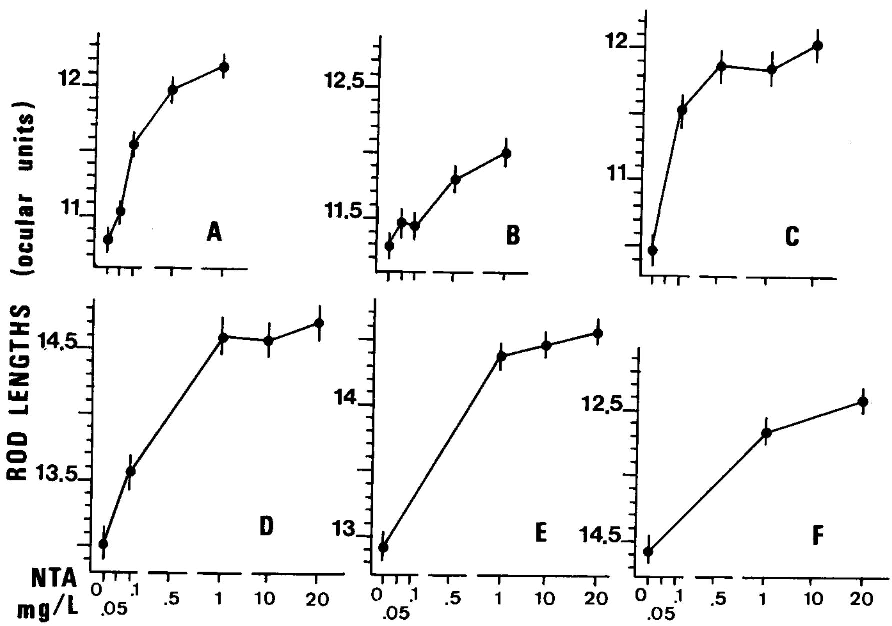

Figure 4. Mean rod lengths of young plutei of P. lividus at 40 hr from fertilization in presence of NTA. Vertical bars represent 95% comparison intervals of means.

The results (Figure 5) confirm the larger echinopluteus size in the presence of NTA, but indicate that bigger mean differences between treated and untreated embryos are present when natural sea

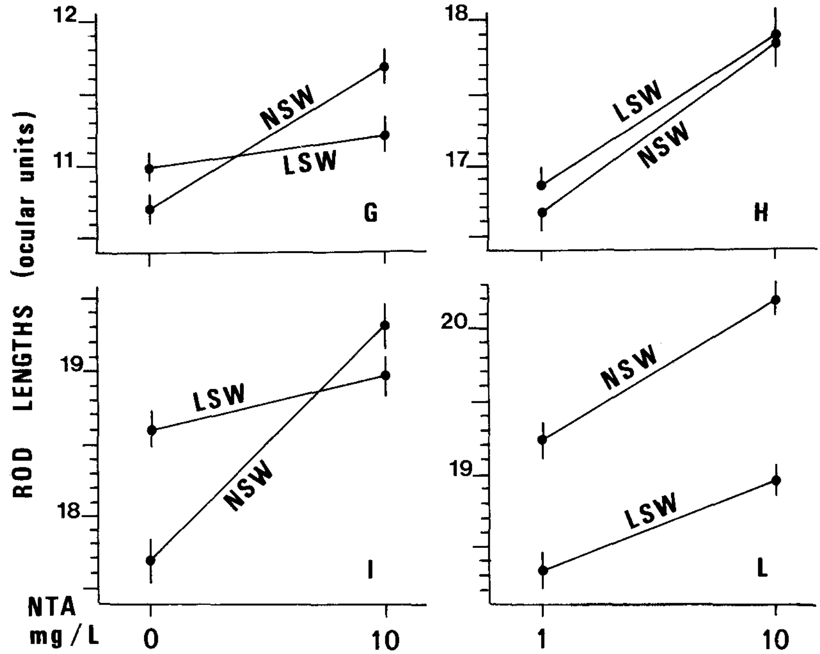

Figure 5. Mean rod lengths of young plutei of P. lividus at 40 hr from fertilization in presence of NTA. Experiments in natural and artificial sea water. Vertical bars represent 95% confidence intervals of mean.

water is employed. The significance of this interaction was tested by means of a two-way analysis of variance: it was significant in experiments G ($P<0.001$), I ($P<0.001$) and L ($P<0.01$). The occurrence of larger echinopluteus size in artificial sea water in comparison with that obtained in natural sea water, is a frequent phenomenon due to the unavoidable pollution of coastal sea water. In two successive experiments the effect of NTA on the developmental rate of the latter organisms was studied by sampling the experimental lots every 2 hr, from 18 to 24 hr, after fertilization. NTA seems to speed up the progression from the 'prism' to the 'young pluteus' stage (Figure 6).

The results of our experiments confirm the lack of in vivo toxicity of NTA on aquatic organisms already shown by other authors (Eisler et al. 1972; Barica et al. 1973; Bott et al. 1980). Our results generally agree with several reports on the effects of NTA on microorganisms, insects, mammals, as well as on several types of cultured cells (see for references: Venier et al. 1987).

Present data clearly indicate that NTA is able to influence the early development of marine invertebrates already at concentrations as low as those that may be present in natural environments (Bartholomew and Pfaender 1983).

The strong capacity to sequester metal ions as water soluble complexes is considered as the real hazard posed by the intro-

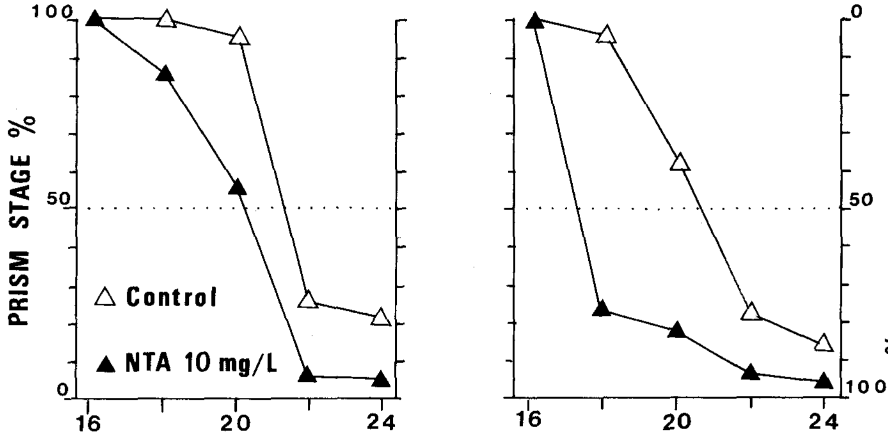

Figure 6. Pattern of prism stage frequencies in eggs of *P. lividus* developing in presence of NTA in two successive experiments. In abscissa, hours from fertilization.

duction of NTA in natural environment (Perry et al. 1984). In fact several insoluble metals increase their toxicity after their solubilization by NTA (Montaldi et al. 1987). NTA also interacts with soluble metals, in some cases lowering or removing their toxicity (Allen et al. 1980; Muramoto 1980). In fact the toxicity of trace metals seems to be dependent on the concentration of the free metal ions (Allen et al. 1980), as strongly chelated metals are usually not readily taken-up by the organisms (Mottola 1974).

This property of the chelating agent may partially explain the results of our experiments. Trace toxic metals are normally present in sea water (in particular in the coastal zones). In general they are complexed in a labile form with anionic salinity components (Nurnberg & Raspor 1981), which presumably are easily lost at the membrane level, allowing the penetration of the metal. In the presence of NTA much stronger links are formed between NTA and metals, resulting in the detoxification of the sea water. However the results obtained in experiments performed with artificial laboratory sea water (G-L, Figure 5) show that other mechanisms must be involved. In fact in the artificial medium, in which heavy metals are absent, the effect of NTA is still present, though not as strongly as in natural sea water. As shown by Raspor et al. (1977) and Nurnberg & Raspor (1981), in sea water some relevant salinity components, in particular the alkaline earth ions Ca(II) and Mg(II), compete for the ligand NTA. The chelation of such ions might alter the ion ratio, and, considering the high biological significance of the involved cations, this could explain the observed effect on the sensitive developmental stages. In conclusion, in evaluating the possible effects of the presence of NTA in natural sea water, the possibility of an alteration of the ion ratio should not be neglected.

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