EFFECT OF AMMONIUM NITRATE SOLUTIONS ON FERTILIZATION AND DEVELOPMENT OF THE SEA URCHIN, *PARECHINUS ANGULOSUS*

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ASTRACT

Eggs and sperm of *Parechinus* have been placed in sea water containing 1, 10 and 100 p.p.m. ammonium nitrate and the percentage fertilization as well as the subsequent development of the embryos compared with controls. At 10 and 100 p.p.m. there is a marked reduction in fertilization. Abnormal forms are common at 100 p.p.m. and some 95% of the population fail to reach the gastrula stage.

INTRODUCTION

This is one of a series of papers reporting the effects of ammonium nitrate pollution on the biology of animals common in Table Bay, South Africa. The work was carried out at the suggestion of Fedmis (Pty) Limited, with a view to assessing potentially harmful effects of the effluent from their Milnerton factory.

Ammonium salts and other compounds containing or giving rise to ammonia are common constituents of both industrial effluents and sewage. Such substances are toxic for a number of reasons but most particularly in that they liberate un-ionized ammonia (Wuhrmann & Woker 1948; Ball 1967). The toxicity of ammonium salts has been examined for a number of fresh-water fishes (Wuhrmann & Woker 1948; Lloyd & Herbert 1960; Hemens 1966; Merkens & Downing 1957; Ball 1967) but the effects of these salts on marine animals is less well known. Brown & Currie (1973) have examined the effect of ammonium nitrate on the marine whelk *Bullia digitalis*, while Kobayashi (1971) has studied the influence of ammonium chloride on the sea urchin *Anthocidaris crassispina*. We have been unable to find any other work involving marine invertebrates.

By far the most common method of assessing the toxicity of a pollutant, both for marine and fresh-water animals, has been the measurement of the dose necessary to kill 50% of the adult population in a stated length of time. This technique, with the incorporation of a number of more or less sophisticated modifications, is convenient and direct and is likely to be used for many years to come. However, there has been a growing awareness that the method may lead to dose assessments which are unrealistically high and that biological damage to populations may occur at far lower doses or concentrations. Sub-lethal concentrations may place the animal under stress, affect its metabolism, influence its reproductive ability, or reduce its life-span. Even if the pollutant has no observable effect on the adult in the concentration tested, it may damage the developing young or larvae, or interfere with fertilization. The effect may be lethal, preventing the attainment of adulthood, or it may result in a stunting of growth or of reproductive ability once adulthood is reached, or development may be retarded.

It is clear, therefore, that adult mortality studies, useful as they are, should at least be supplemented with more sensitive techniques, very probably involving larval development.

The use of sea urchin and other invertebrate eggs for the bioassay of toxic substances in general has been reported by Wilson & Armstrong (1952, 1954, 1958, 1961), by Okubo & Okubo (1962) and by Kobayashi (1971). The latter author reported that at a concentration of 1 p.p.m. ammonium chloride (0,33 p.p.m. NH_4) the development of the sea urchin *Anthocidaris crassispina* was normal but that at 10 p.p.m. (3,3 p.p.m. NH_4) development was retarded, though apparently leading to normal larvae. At 100 p.p.m. ammonium chloride cytolysis occurred before the 64-cell stage and at 1 000 p.p.m. unfertilized cytolysis was observed.

We decided to repeat the work of Kobayashi, using ammonium nitrate in place of ammonium chloride and applying the method to *Parechinus angulosus* (Leske) in place of *Anthocidaris*. The importance of *Parechinus* to the bionomics of the in-shore marine environment of the west coast, and particularly its relationship to the kelp-bed eco-system, has recently become apparent. We thus considered it an ideal animal for study, particularly as its pattern of reproduction and development is well known and follows the typical echinoid sequence of events.

MATERIAL AND METHODS

The breeding season of sub-littoral populations of the sea urchin may be somewhat restricted but the intertidal populations always include some individuals with mature gonads from which ripe gametes can be obtained. Thus for each series of tests about 20 animals were collected from intertidal pools at Blouberg and maintained in the laboratory in running sea water at a temperature of 15°C ($\pm$ 0,5).

The gonads of selected individuals were dissected out, male and female gonads being placed in separate dishes of sea water, still at 15°C. The gonads were then gently macerated so as to release eggs or sperm. The resulting suspensions were filtered through a 77 micron filter to remove cell debris.

Approximately 50 000 eggs were added to each of a series of dishes containing solutions of ammonium nitrate in filtered sea water, the concentrations of ammonium nitrate being 100 p.p.m., 10 p.p.m., 1 p.p.m. and 0 p.p.m. respectively (i.e., 18; 1,8; 0,18 and 0 p.p.m. NH_4). Sperm was then added in sufficient concentration to allow natural fertilization without causing effects such as polyspermy, which result from too great an excess of sperm.

Development was allowed to continue for 24 hours, samples for analysis being drawn off after 3 minutes, 10 minutes, 2,5 hours and 24 hours. A small quantity of formaldehyde was added immediately to each sample so as to halt development and preserve the cells for subsequent counting. 2 000 eggs or embryos were then counted in each sample, the state of development of each being recorded.

The above procedure was carried out on three separate occasions. The results of the first two were very similar and are combined in Tables 1 to 3. While the third test run was being undertaken, a malfunction in the cooling system led to higher and more variable temperatures being experienced; the results of this series of experiments are thus excluded from the tabular summaries, though it may be stated that the trends observed did not differ from those encountered in the first two series.

RESULTS

The first clear result of the experiments is that concentrations of ammonium nitrate as low as 10 p.p.m. have a markedly adverse effect on fertilization. X^2 tests on the figures on which the percentages are based show that this effect is significant at the 0,01 % level. The influence of 100 p.p.m. ammonium nitrate is still more marked. It is not just that fertilization is retarded, for even after 24 hours some 62 to 66 % of the eggs remain unfertilized as compared with less than 10 % of the controls.

However, of the eggs which achieve fertilization, no significant difference can be demonstrated between the controls and those subjected to 10 p.p.m. of the pollutant as far as subsequent development is concerned, the likelihood of attaining the gastrula stage within 24 hours being virtually the same. In contrast to this finding, the eggs which are successfully fertilized in 100 p.p.m. ammonium nitrate have little chance of attaining the gastrula stage, severe abnormalities developing at about the 4-cell stage.

DISCUSSION

The work so far carried out on the effects of ammonium nitrate on South African marine invertebrates (Brown & Currie 1973; Brown in press; Currie & Brown in press) shows that there is a very wide range of tolerance among these forms. At one end of the tolerance range the sandy-beach whelk, *Bullia*, is not killed within 7 days by initial concentrations of ammonium nitrate as high as 1 500 p.p.m., though stress occurs above 100 p.p.m. and there is evidence that the animals can detect a concentration of about 50 p.p.m. (Brown & Currie 1973). In contrast, the present paper demonstrates that a concentration of 10 p.p.m. has a marked effect on fertilization in *Parechinus*. It may be suspected that concentrations lower than 10 p.p.m. may have some effect, though this was not measurable at 1 p.p.m.

These results are similar to those obtained by Kobayashi (1971), testing the effect of ammonium chloride on *Anthocidaris*. One might not, in fact, expect the toxicity of ammonium nitrate and ammonium chloride to differ widely (Hemens 1966), though Currie & Brown (in preparation) have produced evidence that certain abnormalities induced in black mussel embryos subjected to ammonium nitrate are due either to the nitrate component or to derived nitrite, the fraction of un-ionized ammonia having quite different effects.

In any pollution study it is useful to know the tolerance of different stages in the life history of the same species. An investigation of the tolerance of adults of *Parechinus* to ammonium nitrate is a project for future consideration, though it may be stated here that in a short series of trial experiments performed by one of us (Brown unpublished) and using the rate of dislodgement from a vertical surface as the criterion, it was not possible to demonstrate any effect of ammonium nitrate solutions up to 75 p.p.m. It may well prove to be a general rule that developing embryos or the gametes themselves are more susceptible to pollution than are adults of the same species, but this principle remains to be tested in specific cases.

TABLE 1

EFFECT OF AMMONIUM NITRATE CONCENTRATION ON FERTILIZATION IN *Parechinus*

Concentration of NH_4NO_3	*After* 3 *minutes*		*After* 10 *minutes*	
	% *Fertilized*	% *Unfertilized*	% *Fertilized*	% *Unfertilized*
0 (control)	20,57	79,43	60,68	39,32
1 p.p.m.	22,05	77,95	44,61	55,39
10 p.p.m.	8,88	91,12	37,20	62,80
100 p.p.m.	3.09	96,91	11,20	88,80

TABLE 2

STATE OF DEVELOPMENT OF *Parechinus* AFTER TWO AND A HALF HOURS IN VARIOUS CONCENTRATIONS OF AMMONIUM NITRATE

Concentration of NH_4NO_3	% *Unfertilized*	% 1-*cell*	% 2-*cell*	% 4-*cell*	% 8-*cell*
0 (control)	11,08	8,91	10,27	67,03	2,70
1 p.p.m.	14,70	6,58	14,07	62,87	2,39
10 p.p.m.	55,58	2,23	3,72	37,20	1,19
100 p.p.m.	56,44	2,45	6,13	32,51*	2,15

* High percentage of abnormality

TABLE 3

STATE OF DEVELOPMENT OF *Parechinus* AFTER TWENTY-FOUR HOURS IN VARIOUS CONCENTRATIONS OF AMMONIUM NITRATE

Concentration of NH_4NO_3	% *Unfertilized*	% 1-*cell*	% 2-*cell*	% 4-*cell*	% *Gastrula*
0 (control)	9,85	0	0,56	0	89,57
1 p.p.m.	15,47	0	0	1,017	83,30
10 p.p.m.	65,99	0	0	1,57	33,42
100 p.p.m.	61,95	0	0	31,22*	4,89

* High percentage of abnormality

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