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Effects of Formulated Ethyl Parathion on Fertilization of the Sea Urchin *Pseudechinus magellanicus* (Philippi)

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The sea urchin has been proposed as a test system for toxicity bioassays (Hangström and Lönning 1973; Jackim and Nacci 1984; Kobayashi 1971, 1973) and the effect of various pollutants on gametes and fertilization has also been studied by several authors (Mahadevan 1986; Oshida 1981; Pagano et al. 1983). It is possible to assess the effects of a certain substance on the eggs, on the sperm and on the interaction of gametes, thus determining its effect on fertilization. In particular, Castagna et al. (1981) proposed sea urchin gametes as appropriate material for toxicity bioassays.

Laboratory experiments were designed to examine the effect of a formulated ethyl parathion (F-DNTP) on the fertilization of Pseudechinus magellanicus (Philippi). Ethyl parathion was used as test substance because it is one of the most extensively employed phosphorated pesticides in Argentina. For the design of the experiments the different echinoid spawning behavior were taken into consideration. Aggregation behavior is very common in sea urchin. It is subject to feeding responses (Vadas et al. 1986) and defensive mechanisms (Pearse and Arch 1969). Echinoids have also been reported in aggregations during spawning seasons, thus showing various degrees of synchronicity. Adults of Pseudechinus magellanicus (Philippi) showed an aggregation pattern when samples were collected during the spawning season (September-January), and a few scattered individuals were also found. In both grouped and scattered individuals, gonads in varying states of maturity

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were observed.

The objectives of this investigation were: a) to determine whether sperm and eggs are equally sensitive to F-DNTP exposure and b) to assess the effect of the exposure of gametes to F-DNTP on the fertilization rate.

MATERIALS AND METHODS

Adults of Pseudechinus magellanicus (Philippi) were collected from Beagle Channel (Tierra del Fuego, Argentina). Gametes were obtained by intracoelomic injections of 0.5 M KCl. Freshly spawned eggs of five females were pooled and rinsed three times with filtered sea water. Sperm of four males was treated likewise. The oocite pool was quickly run through a series of dilutions in filtered sea water to a concentration of 50 ± 5 eggs/mL and this final pool was used for all bioassays. The egg concentration was checked in each pool dilution (Oshida et al. 1981). The sperm pool also underwent a swift dilution process, concentrations in each step assessed by Neubauer Camera counts (Castagna et al. 1981). A 50:1 sperm/egg ratio was selected because previous tests (Hernandez D, Lombardo R, Ferrari L, Tortorelli MC, Effects of Pesticides on Non-target Organisms: Toxicity of Ethyl Parathion and Carbaryl on Early Development of the Sea Urchin) showed that it was the most suitable for obtaining over 90% fertilization without polyspermy.

Spawning, handling of gametes and bioassays were carried out in an acclimatized laboratory (10 ± 1 °C). Acclimation and fertilization were performed in 250 mL glass vials; all glassware was prewashed with Extran (Merck, Germany), acid washed and then rinsed in distilled water; in all cases, filtered sea water (33‰ S, pH 7.4) was used.

Four series of experiments, differing in the pretreatment of gametes prior to fertilization, were conducted:
A) Gametes not exposed to F-DNTP prior to insemination. Fertilization took place in polluted media at different concentrations of the test substance. Gametes were suspended in filtered sea water for 1 h prior to insemination.
B) Eggs exposed to the toxicant prior to fertilization

with unexposed sperm. Eggs were incubated at different concentrations of F-DNTP for 1 h prior to insemination with sperm that had been suspended for 1 h in unpolluted sea water. Fertilization took place at the same concentrations of toxicant the eggs had been exposed to.

C) Unexposed eggs fertilized with previously exposed sperm. Eggs suspended for 1 h in unpolluted sea water were inseminated with sperm that had been incubated for 1 h at different concentrations of the test substance. Fertilization took place at the same concentrations the sperm had been exposed to.

D) Both sperm and eggs exposed to the test substance prior to insemination. Both gametes were incubated for 1 h at the same concentrations of F-DNTP, and fertilization took place at the same concentrations they had been exposed to.

Each series of experiments was conducted by duplicate with two controls for each one. Two hours after fertilization, development was interrupted by adding 2 mL of 5% buffered formalin. Folidol-F Bayer (100 g% active principle) was used as formulated ethyl parathion. In series A, B and C the following concentrations of ethyl parathion active principle were selected: 8, 16, 32 and 64 ug/L. For series D, the concentrations were lowered to 1, 2, 4 and 8 ug/L in view of the results of previous screening tests with both eggs and sperm exposed to the test substance prior to insemination.

A sample of 1000 eggs of each concentration of each series was examined for fertilized, unfertilized, polyspermic, and abnormal embryos. The elevated fertilization membrane was used as indicator of fertilization. The number of unfertilized eggs was used to determine the effective concentration EC50. The EC50 for each series was estimated by the probit method, including Abbot's adjustment for natural responsiveness and the EC50 fiducial limits by Fieller's theorem (Finney 1971).

In an earlier series of experiments the effects of gamete age on percent fertilization was examined, in order to determine a suitable acclimation time of the gametes in the test substance solution prior to fertilization. Freshly spawned eggs were added to ageing sperm suspensions at intervals of 1, 2, 4, 8,

24 and 48 h and assays were carried out for percent fertilization. Experiments to examine egg longevity were the converse of those described above for sperm. In both cases a 50:1 sperm/egg ratio was selected.

RESULTS AND DISCUSSION

Results of experiments to examine the effect of gamete longevity on the fertilization rate are shown in Figure 1. Sperm potency remained high (over 90% fertilization) within the first 2 h, after which the fertilization of freshly spawned eggs added to the ageing sperm suspensions declined steeply. However, rates of up to 15% were detected with 24-h-old sperm. On the other hand, percent fertilization remained high (over 95%) when freshly spawned sperm was added to suspensions of 2-h-old eggs, and slowly declined to 40% fertilization after 48 h.

In accordance with these results, a 1-h period of exposure to the test substance was chosen to ensure that the process prior to fertilization (spawning, handling of gametes, rinses, dilutions, concentration controls and exposure to F-DNTP) did not exceed 2 h in all, to avoid interferences in the assessment of the toxic effects of the test substance due to gamete longevity.

In experiments to examine the effect of the exposure of sea urchin gametes to F-DNTP on the fertilization rate, abnormal embryos occurred in less than 0.003% of the total eggs examined and no polyspermic eggs were found. Percent fertilization averaged 90% in unpolluted sea water controls.

Results of the four series of experiments, shown in Figure 2, indicate that F-DNTP has a marked effect on the fertilization rate of Pseudechinus magellanicus. Percent fertilization fell rapidly with increasing concentrations of the toxicant in all four series.Even when gametes were not exposed to the test substance prior to insemination (series A), the fertilization rate was lowered by 30% when fertilization took place at 64 ug/L of F-DNTP. When gametes were exposed to the toxicant prior to insemination, the fertilization rate was drastically reduced. A F-DNTP concentration of 1.6 ug/L lowered by 90% the fertilization rate when both eggs and sperm were previously exposed to the

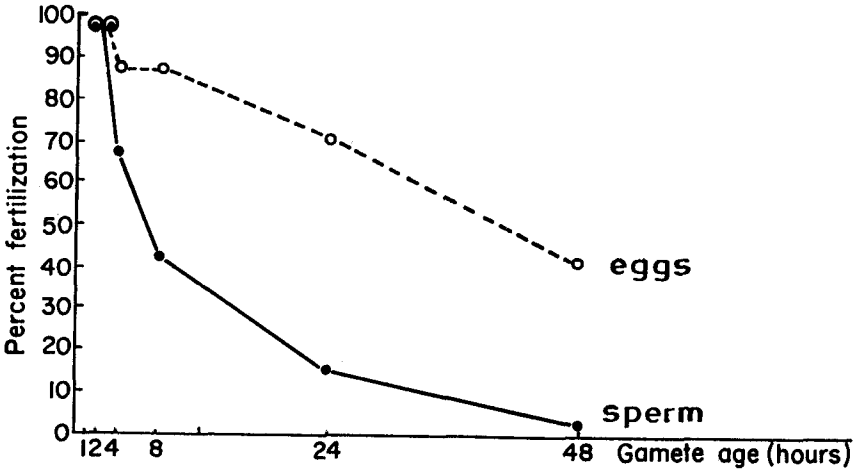

Figure 1. Percent fertilization resulting from tests of gamete longevity.

toxicant (series D).

Direct field observations have shown different spawning behaviors in echinoids: scattered individuals spawning synchronously or asynchronously; no active aggregation, scattered groups spawning; aggregated individuals spawning synchronously or asynchronously with a short time lag. Even in slow moving waters, percent fertilization is noticeably reduced (20%) if the animals do not spawn synchronously in close proximity (Pennington 1985). Results of series D suggest that fertilization will be much impaired in scattered individuals spawning synchronously or asynchronously even in waters with low concentrations of the toxicant.

Table 1. Parameters of toxicity curves for series A, B, C and D.

Experiment	EC50 (ug/L)	EC50 95% Fiducial limits	Slope	Correlation Coefficient
A	92.2	81.5 - 106.6	1.51	0.96
B	26.9	24.1 - 30.5	1.16	0.96
C	13.5	12.9 - 14.1	1.95	0.95
D	0.9	0.8 - 1.0	2.04	0.91

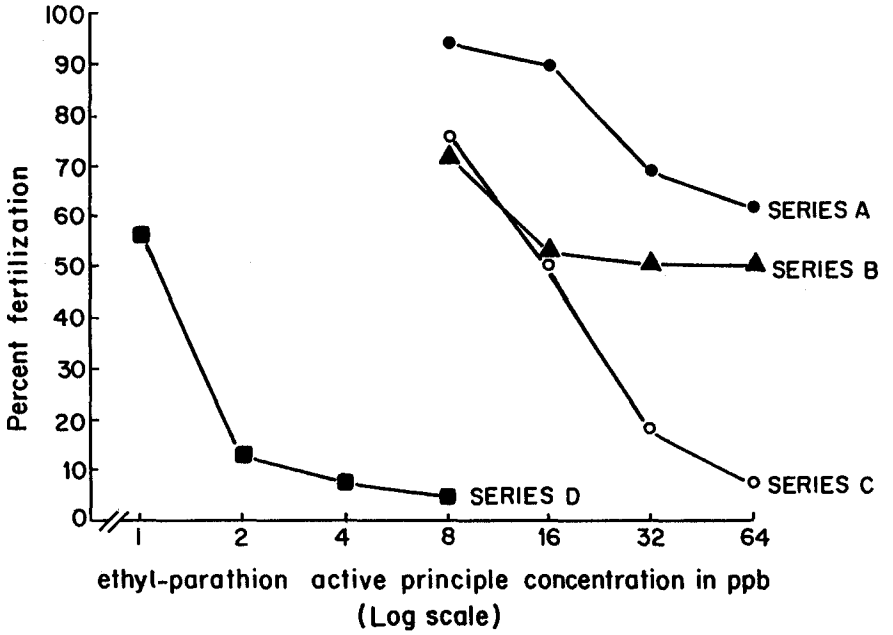

Figure 2. Response of sea urchin gametes exposed to F-DNTP

Sperm were found to be more susceptible to the toxic effects of F-DNTP than eggs. This can be clearly seen in Table 1. The EC50 estimated for series C (exposed sperm) was significantly lower (50%) than the EC50 for series B (exposed eggs).

Although pretreatment of both gametes yields the highest percentages of unfertilized eggs, the exposure of sperm only to high concentrations of F-DNTP may produce similar results to those obtained pretreating both gametes in lower concentrations. In this respect, the effect of F-DNTP on the fertilization rate was more dose-dependent in sperm than in eggs (the slope of series C probit line is twice that of series B, Table 1).

Accordingly, if individuals are scattered, or grouped but spawn asynchronously with a certain time lag, the effect of F-DNTP on fertilization would be more pronounced, especially if sperm were released before the eggs and remained in polluted water for some time prior to fertilization, as shown by the results of series B and C.

In our experiments, pretreatment of both gametes

(series D) with F-DNTP resulted in an EC50 90 times lower than that of series A, in which gametes were not pretreated. Nevertheless, a 50% decrease in the fertilization rate occurred in only 90 ug/L F-DNTP, when gametes were not pretreated (series A). It therefore seems that low concentrations of F-DNTP may produce a harmful effect on the fertilization even when spawning occurs synchronously in aggregated individuals, in which case gametes would only be exposed to the pollutant for a short period of time prior to fertilization.

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