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The Effects of Chlorination of Wastewater on Fertilization in Some Marine Invertebrates

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Abstract

Unchlorinated domestic sewage was found to be a relatively weak inhibitor of external fertilization in 3 marine invertebrates. Chlorinated sewage was a potent spermicide, active in inhibiting fertilization in concentrations of available chlorine as low as 0.05 ppm. Sodium hypochlorite in seawater duplicated the effect, and excess sodium thiosulfate terminated it. The possibility of chlorine disinfection affecting reproductive success in the vicinity of outfalls is discussed.

Introduction

Chlorination is commonly used as a disinfectant for sewage water and an antifouling agent for power-plant cooling water systems. The effects of chlorination of cooling water systems on phytoplankton growth rate, photosynthesis, and respiration have recently been examined in marine, estuarine, and fresh-water situations (Hamilton *et al.*, 1970; Hirayama and Hirano, 1970; Brook and Baker, 1972). These studies have demonstrated reduced primary productivity following passage of water through a generating plant during periods of chlorination. The projected consequences of this depression in the marine and estuarine studies were minimal; the impact on river water may be greater.

The present report, which arose from a study of the effects of sewage on embryonic development, concerns the effects of chlorination on the external fertilization of 3 marine invertebrates: an echinoid, an echiuroid, and a polychaetous annelid.

Materials and Methods

Shedding of gametes of the sea urchin *Strongylocentrotus purpuratus* was induced by intracoelomic injection of 0.5 M KCl. Eggs were collected in seawater and washed at least 2 times; sperm was collected as the undiluted seminal fluid, and stored at 5 °C. Shedding of gametes of the echiuroid *Urechis caupo* was induced by insertion of a glass pipet into the gonopores (Gould, 1967); eggs were washed into seawater, and the sperm stored as undiluted semen. Release of gametes of the annelid *Phragmatopoma californica* was induced by removing individuals from their tubes. Eggs and sperm were stored in seawater.

The bulk of experiments utilized *Strongylocentrotus purpuratus* gametes. Fertilization success was determined 20 min after insemination by scoring presence or absence of the hyaline layer, using 200 eggs per sample. Fertilization in the controls always exceeded 98%. Sperm concentrations of 0.033, 0.010 and 0.0033% v/v were used, which correspond to approximately 1.6×10^7, 4.8×10^6 and 1.6×10^6 sperm/ml.

Fresh chlorinated and unchlorinated sewage was obtained from the Pacific Grove, California sewage treatment facility each morning at 9.00 hrs. Sodium hypochlorite was used to prepare solutions of chlorinated seawater, and excess sodium thiosulfate was used to neutralize the chlorine in sewage or seawater. Thiosulfate had no effect on fertilization at the concentrations used (up to 1 mM). Available chlorine was assayed by the standard iodometric method (Farber, 1960).

Results

Strongylocentrotus purpuratus

Unchlorinated sewage is a relatively weak inhibitor of fertilization; a 5 min exposure of a 0.0033% sperm suspension to 10% sewage, followed by addition of eggs, results in 80% fertilization. The daily composition of the sewage is sufficiently variable to make this effect typical rather than absolute; sewage potency may vary from $\frac{1}{2}$ to 2 times this amount.

As seen in Table 1, chlorinated sewage is drastically more detrimental to fertilization, and a 0.5% dilution of moderately chlorinated sewage (11 ppm undiluted) significantly reduces fertilization success. The potency of the chlorinated sewage as a fertilization inhibitor decreases with time. In another experiment, a 1% solution of sewage, diluted in sea water, was aged at room temperature for 72 h. At 0.033% sperm concentration, the percent fertilization increased from 5 to 71% over this time period.

Table 1. *Strongylocentrotus purpuratus. Fertilization success following 5 min exposure of sperm to dilutions of chlorinated sewage, hypochlorite solutions, and chlorinated sewage treated with excess thiosulfate*

Sperm concentration (%)	Chlorinated sewage concentration			Hypochlorite concentration			Chlorinated sewage treated with $Na_2S_2O_3$		
	0.20% (0.022 ppm)	0.50% (0.055 ppm)	1.0% (0.11 ppm)	0.025 ppm	0.064 ppm	0.125 ppm	2.0%	5.0%	10%
	Percent fertilization								
0.033	100	100	14	100	100	6	100	100	100
0.010	100	56	9	100	23	1	99	99	98
0.0033	100	1	0	100	2	1	90	65	5

This inhibition of fertilization results from the chlorine. To demonstrate this, hypochlorite was added to seawater to a level comparable to that found in sewage; as seen in Table 1, a sodium hypochlorite solution of 0.125 ppm available chlorine was a slightly better inhibitor than sewage with 0.110 ppm chlorine.

Further evidence, also summarized in Table 1, comes from experiments in which the chlorine in sewage was chemically reduced with thiosulfate. The strength of the sewage as an inhibitor falls to that of unchlorinated sewage following thiosulfate treatment. Correlative evidence that chlorine is the active agent comes from the observation that the inhibition varied directly with assayed chlorine content. Chlorine levels in the sewage varied from 10 to 45 ppm, and the observed inhibition varied accordingly.

The effect of chlorination is primarily on the sperm and not the egg. A 5 min incubation of eggs in 0.77 ppm hypochlorite, followed by washing or thiosulfate reduction to remove hypochlorite, resulted in no effect on fertilizability. Incubation of sperm in a 10-fold lower hypochlorite concentration resulted in a loss of fertilizing ability which could not be restored by washing or thiosulfate treatment. Microscopic examination shows that this loss of fertilizing ability is correlated with loss of sperm motility. There was also a tendency for flagellar detachment.

The spermicidal action of chlorine is rapid. Excess thiosulfate was added 1 sec after introduction of sperm into a 1.9 ppm chlorine solution. No fertilization with 0.0033% sperm was observed.

Other Phyla

Less extensive work was carried out with species from 2 other phyla to determine if the chlorine sensitivity of fertilization was general. The effects of hypochlorite on *Urechis caupo* sperm were determined by incubating a 0.0020% suspension of sperm with varying concentrations of hypochlorite for 5 min. Two control sperm suspensions were used, one containing only seawater and one containing seawater to which hypochlorite and excess thiosulfate had been added prior to introduction of sperm. As seen in Table 2, complete inhibition of fertilization was only observed after exposure to 0.40 ppm chlorine.

Table 2. *Other phyla. Fertilization success with Urechis caupo gametes following 5 min exposure to a 0.0020 % suspension of sperm to hypochlorite solutions. Percent of Phragmatopoma californica sperm exhibiting motility after similar treatment is also given*

Chlorine concentration (ppm)	*Urechis caupo* (Fertilization success) (%)	*Phragmatopoma californica* (Motility observed) (%)
Controls	100	95
0.20	78	78
0.40	0	25
1.0	0	14

A suspension of sperm from *Phragmatopoma californica* was treated with varying concentrations of hypochlorite for 5 min. Samples were then checked microscopically for evidence of motility, scoring as motile any observable thrashing movements. Quantitation was possible because the motile sperm stick to the glass microscope slide and can be directly counted. The results (Table 2) show that motility is affected by all concentrations tested. Concentrations of 0.40 ppm and greater resulted in major structural aberrations, including apparent flagellar bifurcation. Sperm exhibiting motility above 0.40 ppm had slow and small movements as compared to normal sperm.

Discussion and Conclusions

The present study and that of Kobayashi (1971) indicate that fertilization and early development provide sensitive bioassays for some pollutants in the marine environment. Unchlorinated domestic sewage weakly affected fertilization, but chlorinated sewage was a powerful inhibitor. The effects of hypochlorite on fertilization are more pronounced than the reported effects on phytoplankton photosynthesis (Hamilton *et al.*, 1970; Hirayama and Hirano, 1970; Brook and Baker, 1972). Thus, a significant inhibition of fertilization was observed at chlorine concentrations below

0.05 ppm when dilute suspensions of sperm were treated.

The potential effects of chlorine disinfection on the marine environment are of great interest. Important factors are (1) frequency of disinfection treatments; (2) peak concentration of chlorine delivered to the outfall/diffuser system; (3) effective ratio of wastewater to initial diluting water; (4) patterns and extent of water mass movements through the oceanographic seasons; (5) chlorine demand of the receiving waters; (6) stability of the disinfectant in the environment. A key factor with respect to fertilization is the peak concentration of chlorine reached during breeding periods.

The outfall studied, a small (6×10^6 l/day) primitive system emptying directly into the intertidal zone, employed very high concentrations of chlorine (> 40 ppm) routinely in disinfection procedures. At these chlorine levels, even a 1000-fold initial dilution of the raw sewage would be insufficient to protect fertilization.

More modern outfalls employ extensive diffuser systems at significant depths and distances from shore. For example, the large (1.5×10^9 l/day) White Point installation for Los Angeles County, California, delivers effluent between depths of 50 and 60 m at distances of 1.4 to 2.8 km from shore (Grigg and Kiwala, 1970). Engineering design parameters ensure a minimum ratio of 100:1 of initial diluting water to wastewater (N. Brooks, personal communication). Chlorine disinfection is only used sporadically, and chlorine levels at the outfall are kept below 1.0 ppm (N. Brooks, personal communication). Thus, the projected consequences of such outfalls on fertilization are minimal. In fact, proliferation of sea urchins, attributable to other aspects of the pollution, has been observed in the White Point area (North, 1964).

The results presented here suggest the importance of controlled levels of chlorine disinfection in sewage and power-plant effluents. Fertilization is a sensitive indicator of chlorine levels, and used in concert with studies of further embryonic development and measurement of phytoplankton productivity, may be of value in assessing the impact of wastewaters on their receiving waters.

Summary

1. Unchlorinated sewage is a relatively mild inhibitor of external marine fertilization.

2. Chlorinated sewage is a very potent fertilization inhibitor, active in concentrations as low as 0.05 ppm available chlorine.

3. Primary effects of both chlorinated and unchlorinated sewage are on sperm.

4. Use of chlorine disinfection in sewage outfalls could contribute to reproductive failure in externally fertilizing marine invertebrates in the vicinity of the initial diluting water of such outfalls.

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