Ecological Research Series

EFFECTS OF SELECTED WASTEWATER CHLORINATION PRODUCTS AND CAPTAN ON MARINE ALGAE

Environmental Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Gulf Breeze, Florida 32561

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EFFECTS OF SELECTED WASTEWATER CHLORINATION PRODUCTS AND CAPTAN ON MARINE ALGAE

by

Harish C. Sikka and Gary L. Butler
Syracuse Research Corporation
Syracuse, New York 13210

Grant No. R803943010

Project Officer

Gerald E. Walsh
Environmental Research Laboratory
Gulf Breeze, Florida 32561

ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
GULF BREEZE, FLORIDA 32561

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FORWARD

Organochlorine compounds such as pesticides have been known to be pollutants in water for many years. Biological effects of similar compounds that are formed during chlorination of waste water are poorly known. The Environmental Research Laboratory, Gulf Breeze, has long supported research on effects of organochlorine compounds on living things. Data from that research has been used for setting water quality standards in estuaries.

The research reported here demonstrates effects of organochlorine compounds formed during the waste water treatment process, and the fungicide captan, on growth of marine unicellular algae. Synergistic effects of some of the compounds demonstrates, in a simple way, the complex nature of pollutant interactions and their effects on algal productivity.

Gerald E. Walsh
Project Officer
Environmental Research Laboratory
Gulf Breeze, Florida

ABSTRACT

The investigation was undertaken with the overall objective of determining for marine phytoplankton the effects of both certain stable chloro-organic compounds produced during chlorination of sewage effluents and the pesticide captan.

3-Chlorobenzoic acid (1 or 10 ppm) had either slight or no effect on growth of Dunaliella or Porphyridium. It inhibited growth of Skeletonema at 10 ppm, but had no marked effect at 1 ppm.

5-Chlorouracil at 1 or 10 ppm did not affect Skeletonema, but stimulated growth of Dunaliella initially.

4-Chlororesorcinol had no effect on Dunaliella at 1 ppm, but 10 ppm of the chemical caused a small decrease in growth. The chemical produced an initial stimulation in growth of Porphyridium, followed by an inhibition. Growth of Skeletonema was inhibited by 4-chlororesorcinol at concentrations ranging from 1 to 10 ppm.

Treatment with 3-chlorophenol stimulated growth of Dunaliella. Skeletonema growth was inhibited at concentrations higher than 2.5 ppm, but showed some stimulation at 1 ppm. At 1 ppm it stimulated growth of Porphyridium, but was slightly inhibitory at 5 ppm.

A combination of 3-chlorophenol and 4-chlororesorcinol interacted synergistically to reduce Skeletonema growth.

Captan suppressed growth of Dunaliella and Porphyridium at a concentration of 5 ppm. Slight stimulation in growth of the two organisms was noticed in the presence of 0.1 and 1 ppm of the fungicide. Captan was inhibitory to Skeletonema at concentrations ranging from 0.25 to 5 ppm. Treatment of Skeletonema with 0.5 ppm of captan for 30 minutes caused a substantial reduction in photosynthetic $^{14}CO_2$ fixation.

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EFFECT OF SELECTED WASTEWATER CHLORINATION PRODUCTS ON MARINE ALGAE

SECTION 1

INTRODUCTION

Chlorination is a widely used practice for disinfecting municipal waste-waters or combined municipal-industrial wastes before they are discharged into receiving waters. The quantity of chlorine used for sewage treatment is expected to increase as municipalities not presently treating their sewage are required to comply with the treatment standards mandated by the Federal Water Pollution Control Act by July 1, 1977 (U.S. Government Public Law, 92-500; 1972). Additionally, an increasing number of industries have been required to provide waste-water treatment facilities which often include effluent chlorination.

Some organic compounds may escape secondary treatment or be only partially degraded and as a result may react with chlorine, yielding persistent, potentially toxic organic compounds. Glaze et al. (1973) and Jolley (1974, 1975) have shown that chlorine-containing organic compounds are produced when sewage effluents are chlorinated. Jolley (1975) identified seventeen stable, chlorine-containing organic compounds in domestic waste-water effluent which had been chlorinated to a 1 to 2 mg/ℓ chlorine residual. Some of these compounds included chlorinated phenols, aromatic acids, purines, and pyrimidines. Barnhart and Campbell (1972) studied the reaction of chlorine with organic chemicals which are known to be present in industrial waste-water effluents. They observed that chlorine reacted readily with phenol, m-cresol, and aniline under conventional effluent treatment conditions. Based on the assumption that 90,700 metric tons of chlorine are used annually in the U.S. for disinfecting waste-water, Jolley (1975) estimated that approximately 4,535 metric tons of stable chlorine-containing organic compounds would be released to the receiving water.

The presence of chloro groups is known to render benzenoid compounds more resistant to microbiological degradation (Alexander and Lustigman, 1966), which suggests a potential for accumulation of these compounds in receiving waters. The introduction of chlorinated organics into the aquatic environment is of great environmental concern because of their potential toxicity to various organisms. In order to fully evaluate the impact of waste-water chlorination on the aquatic environment, it is necessary that we know effects and fate of chlorination products in the biota. The toxicity of residual chlorine on aquatic life has been extensively investigated and was recently reviewed by Brungs (1973). However, very little information is available on effects of stable chlorine-containing organic compounds that may have been produced during the chlorination process. Gehrs et al. (1974) reported

that 5-chlorouracil and 4-chlororesorcinol, which are among the constituents of chlorinated effluents, decreased the hatchability of carp eggs at concentrations as low as 1 ppb.

Phytoplankton constitute a vital part of the aquatic biota. They contribute to the oxygen supply in the aquatic environment through their photosynthetic activity. An adverse effect on growth of phytoplankton, which represent the first link in the aquatic food chain, may have adverse effects on the entire ecosystem. Algae may also play an important role in determining the fate of chloro-organics in the aquatic ecosystem. They may remove the chemicals from the environment by adsorption and/or absorption and may subsequently metabolize them. The metabolites may be more toxic and/or more persistent than the parent chemical and so may present additional pollution problems. Chemicals which are accumulated by algae may be transferred to higher trophic levels with potentially deleterious effects on man.

The present study was undertaken to investigate in marine phytoplankton the effects of selected stable organic compounds produced during chlorination of sewage effluents. The results of this study will provide information needed for establishing guidelines for the discharge of chlorine-treated waste-waters into the aquatic ecosystems.

SECTION 2

CONCLUSIONS

Our studies have shown that certain chloro-organic compounds produced during chlorination of sewage effluents such as 3-chlorophenol, 3-chlorobenzoic acid, 4-chlororesorcinol and 5-chlorouracil affect the growth of *Dunaliella tertiolecta*, *Skeletonema costatum*, and *Porphyridium sp.* at concentrations considerably higher than those expected to be found in the environment. A combination of 3-chlorophenol and 4-chlororesorcinol interacted synergistically to inhibit the growth of *Skeletonema*. As our results are based on the effects on a limited number of species, it is not possible to make a general statement concerning the toxicity of these chemicals to phytoplankton.

SECTION 3

RECOMMENDATIONS

1. To fully assess the impact of chloro-organic compounds on phytoplankton, we suggest that the effect of these chemicals on additional algal species be examined.

2. It is likely that algae in the bodies of water receiving chlorinated waste-waters will be exposed to several chloro-organic chemicals simultaneously. As a result there may be synergistic or antagonistic effects produced by the interaction of these chemicals. Therefore, it is recommended that the effects of chloro-organic compounds be studied in the presence of other similar compounds.

3. It is quite likely that the chloro-organic compounds may be accumulated by phytoplankton. An accumulation of these chemicals by phytoplankton is of ecological significance because the chemicals can be transferred to higher trophic levels. We suggest that the uptake and metabolism of these chemicals by phytoplankton be examined.

SECTION 4

MATERIALS AND METHODS

CHEMICALS

The chemicals tested for toxicity to phytoplankton included 3-chlorophenol, 3-chlorobenzoic acid, 4-chlororesorcinol and 5-chlorouracil. They are among the several stable chlorine-containing organic compounds which Jolley (1975) identified in chlorinated sewage effluents. These chemicals were selected because their concentrations in chlorinated sewage effluents were relatively high (1-5 μg/ℓ) compared to those of other chlorine-containing compounds and because nothing was known about their toxicity to phytoplankton. 3-Chlorobenzoic acid (99+%), 3-chlorophenol and 4-chlororesorcinol (99%) were purchased from Aldrich Chemical Company, and 5-chlorouracil (A-grade) from Calbiochem.

CULTURING OF ORGANISMS

The three species representing different algal divisions used in this study were Skeletonema costatum (Bacillariophyta), Dunaliella tertiolecta (Chlorophyta), and Porphyridium sp. (Rhodophyta). They were obtained from the University of Rhode Island, Narragansett Marine Laboratory. The cultures were bacteria-free and grown axenically on Modified Burkholder's artificial seawater medium recommended by the Environmental Research Laboratory, EPA, Corvallis, Oregon (1974). The medium was supplemented with the following: 16.48 mg N/l as $NaNO_3$, 4.45 mg P/l as K_2HPO_4, 4.94 mg Si/l as $Na_2SiO_3.9H_2O$, 1 μg/l vitamin B_{12}, 1 μg/l biotin, 0.2 mg/l thiamin-HCl and 1 ml/l NAAM trace metal mix (0.1856 g H_3BO_3; 0.416 g $MnCl_2.4H_2O$; 0.032 g $ZnCl_2$; 1.428 mg $COCl_2.6H_2O$; 0.0214 mg $CuCl_2.2H_2O$; 7.26 mg $Na_2MoO_4.2H_2O$ per liter). The salinity was adjusted to 30 ppt and the pH to 7.2 After filtration through a 0.45 μm membrane filter, 1 ml/l sterilized Fe-EDTA solution (33.05 μg Fe/ml as $FeCl_3$ + 300 μg/ml Na_2 EDTA) was added aseptically The cultures were grown on a reciprocating shaker under controlled environmental conditions, 16 hours of light per day at 4304 lux and a temperature of 22° ± 1°C.

GROWTH STUDIES

Exponentially growing cells were inoculated into 100 ml of sterile medium to give an initial density of 10^4 cells per ml. Stock solutions of the chemicals were prepared in acetone. The chemicals in acetone solution

were added to the medium so that the final concentration of carrier solvent was 0.01%. The control consisted of growth medium containing 0.01% acetone. Each treatment was replicated 5-6 times. Aliquots of cell suspension were removed at various intervals following treatment and growth of algae was determined by measuring absorbance at 650 nm in a Cary spectrophotometer. The results were analyzed statistically by using the paired difference test (Mendenhall, 1975).

SECTION 5

RESULTS

In our initial studies on chloro-organic chemicals and phytoplankton, the organisms were cultured in aged, synthetic seawater medium (Rila Products) supplemented with nutrients recommended by Guillard and Ryther (1962). Growth of untreated algae varied considerably in these experiments. In an attempt to achieve reproducibility in growth rates, we cultured the algae in a medium of better-defined composition than that prepared from the Rila Marine Mix. For this purpose, the artificial seawater-medium recommended by the Environmental Research Laboratory, EP, Corvallis, Oregon, was used in subsequent studies.

EFFECT OF THE TEST CHEMICALS ON PHYTOPLANKTON GROWTH

The results concerning effects of 3-chlorobenzoic acid, 5-chlorouracil, 4-chlororesorcinol and 3-chlorophenol on growth of Dunaliella, Skeletonema, and Porphyridium are reported below. Inhibition or stimulation of growth, when reported, is statistically significant at the 95% confidence level.

3-Chlorobenzoic Acid

The chemical at a concentration of 1 ppm produced no effect on growth of Dunaliella or Porphyridium (Figures 1 and 2). It did not affect Skeletonema within the first week of treatment, but inhibited its growth by 10-12% during the second week (Figure 3). At a concentration of 10 ppm, Porphyridium was not affected; however, the growth of Dunaliella was inhibited 4-12% during the first five days following treatment. Subsequently, the Dunaliella cells treated with 10 ppm of 3-chlorobenzoic acid showed the same growth as the control.

5-Chlorouracil

Figure 4 shows the effect of 1 and 10 ppm of 5-chlorouracil on the growth of Skeletonema and Dunaliella. The chemical, at concentrations of 1 and 10 ppm, did not significantly affect the growth of Skeletonema until 7 days following treatment. However, at both concentrations a significant stimulation of growth (3-9%) was noticed on days 10 through 14. 5-Chlorouracil, at concentrations of 1 and 10 ppm, stimulated the growth of Dunaliella through day 10; subsequently, the stimulatory effect was eliminated and after 14 days the treated cultures showed the same growth as the control.

4-Chlororesorcinol

This chemical, at the concentration of 1 ppm, did not inhibit growth of Dunaliella (Figure 5). In the cultures treated with 10 ppm of 4-chlororesorcinol, a 13% reduction in growth was observed on day 3; however, after day 6, the treated cultures showed the same growth as the controls. Chlororesorcinol was more toxic to Skeletonema. Figures 5 and 6 show the effect of 4-chlororesorcinol on Skeletonema at concentrations ranging from 1 to 10 ppm. Growth decreased with an increase in concentration of the chemical. 4-Chlororesorcinol at concentrations of 1.0, 2.5, 5.0, 7.5, and 10 ppm reduced growth of Skeletonema by 22, 39, 62, 72, and 100%, respectively after 3 days of treatment. Subsequently, the cells in all treatments except 10 ppm recovered from the effect of the chemical so that growth proceeded at almost the same rate as those in the controls when the study was terminated. The growth of Skeletonema was completely suppressed in the presence of 10 ppm of 4-chlororesorcinol.

Figure 7 shows the effect of chlororesorcinol on growth of Porphyridium. A stimulation (38-33%) in the growth of the organism was noticed on days 3 and 4 after treatment with 1 ppm of the chemical, followed by a small inhibition (5-14%) on days 6 through 14. In the cultures treated with 10 ppm of chlororesorcinol, an increase (18-34%) in growth was noticed on days 3 and 4. This initial stimulation was followed by an inhibition in growth (6-21%) after day 6.

3-Chlorophenol

A significant stimulation (8-18%) in the growth of Dunaliella was noticed on day 4 through day 14 after treatment with 1 ppm of 3-chlorophenol (Figure 8). Treatment with 10 ppm of the chemical also stimulated growth, but the maximum increase in growth was only 12%.

Growth of Skeletonema was significantly increased (9-13%) on day 4 through day 6 following treatment with 1 ppm of 3-chlorophenol (Figure 9); however, after day 6, growth in the treated cultures was the same as in the controls. The concentration of 10 ppm completely suppressed growth of Skeletonema. At concentrations ranging from 2.5 to 7.5 ppm, growth of Skeletonema decreased with increase in concentration (Figure 10). At concentrations of 2.5, 5.0, and 7.5 ppm the chemical caused decrease in growth of the organism by 20, 82, and 97%, respectively, following 7 days of treatment.

Figure 11 shows the effect of 3-chorophenol on growth of Porphyridium. It did not significantly affect growth of the alga on the concentration of 0.1 ppm. The cultures treated with 1 ppm of the chemical showed a 12% increase in growth after one week as compared to the control. However, a slight inhibition in growth (5%) was observed in the cells exposed to 5 ppm of chlorophenol.

INTERACTION OF 3-CHLOROPHENOL AND 4-CHLORORESORCINOL

It is quite likely that the bodies of water receiving chlorinated waste-waters, phytoplankton may be exposed to several chloro-organic chemicals simultaneously. As a result, there may be synergistic or antagonistic effects produced by their interaction. We observed that 3-chlorophenol and 4-chlororesorcinol are toxic to Skeletonema individually. In order to ascertain if the two chemicals interacted, we examined their effect in combination with one another on growth of alga.

At a concentration of 2.5 ppm, 3-chlorophenol and 4-chlororesorcinol reduced growth of Skeletonema by 26 and 17%, respectively, on day 3 after treatment; by day 10, inhibition had decreased to 5 and 8%, respectively. When algae were exposed to a combination of 2.5 ppm each of 3-chlorophenol and 4-chlororesorcinol, the cells showed a time lag in growth until the 7th day after treatment, but growth subsequently proceeded at almost the same rate as those in the control (Figure 12). In cultures treated with 5 ppm of either of the chemicals alone, a lag in cell growth was noticed until day 7 following treatment, but afterwards the growth proceeded at a rate close to that of the control. However, treatment with a combination of 5 ppm of each of the two chemicals completely inhibited growth, and the cells failed to recover for the duration of the experiment. These findings indicate an interaction between 3-chlorophenol and 4-chlororesorcinol in inhibiting the growth of Skeletonema.

The following formula described by Colby (1967) was used to ascertain whether the effect produced by a combination of the two chemicals was synergistic or additive: $E = XY/100$; where

E = expected growth as a percentage of control with the test chemicals

X = growth as a percentage of control with chemical A

Y = growth as a percentage of control with chemical B

When the observed value is less than the expected value, the combination is synergistic. When the observed and expected values are equal, the effect is additive. If the observed value is greater than the expected value, antagonism is indicated. When Skeletonema was exposed to a combination of 3-chlorophenol and 4-chlororesorcinol, the growth was reduced below the expected value indicating that the two chemicals interacted synergistically (Table 1).

COMPARATIVE EFFECTS OF CHLORINATED AND NON-CHLORINATED ORGANIC COMPOUNDS ON THE GROWTH OF PHYTOPLANKTON

A wide variety of organic chemicals including phenolic compounds are present in municipal water supplies and waste-water treatment plant effluents. In order to determine if the chlorination of organic compounds present in

waste waters alters their toxicity to phytoplankton, we compared the effects of 3-chlorophenol and 4-chlororesorcinol, which were found to be toxic in our studies, with their non-chlorinated analogs, i.e., phenol and resorcinol. Treatment of Skeletonema with 5 ppm of phenol or resorcinol caused a small inhibition (less than 5%) over a two-week period. In contrast, the growth of the organism was reduced by 83 and 77% after 3 days of treatment with 3-chlorophenol and 4-chlororesorcinol, respectively, which suggests that chlorine-containing compounds present in sewage effluents may be more toxic to phytoplankton than parent compounds that would be present in the effluent prior to chlorination.

TABLE 1. EFFECT OF 3-CHLOROPHENOL AND 4-CHLORORESORCINOL AND THE COMBINATION OF 3-CHLOROPHENOL AND 4-CHLORORESORCINOL ON GROWTH OF SKELETONEMA

Chemical		Days after Treatment	Growth Response % of Control[a]	
3-chlorophenol	2.5 ppm	3	74	
"	2.5 ppm	7	85	
"	2.5 ppm	14	96	
3-chlorophenol	5.0 ppm	3	14	
"	5.0 ppm	7	9	
"	5.0 ppm	14	58	
4-chlororesorcinol	2.5 ppm	3	83	
"	2.5 ppm	7	81	
"	2.5 ppm	14	94	
4-chlororesorcinol	5.0 ppm	3	22	
"	5.0 ppm	7	10	
"	5.0 ppm	14	51	
3-chlorophenol +	2.5 ppm	3	0	(61)
4-chlororesorcinol	2.5 ppm	7	0	(69)
		14	75	(90)
3-chlorophenol +	5.0 ppm			
4-chlororesorcinol	5.0 ppm			
		14	0	(29)

[a] Expected responses for combinations are shown in parentheses following each observed response.

SECTION 6

DISCUSSION

Our findings have shown that certain chloro-organic compounds present in effluents of sewage treatment plants affect the growth of phytoplankton at concentrations considerably higher than those expected to be found in the environment. The toxicities of chlorophenol, chlororesorcinol, chlorobenzoic acid, and chlorouracil to phytoplankton varied among the species. The chemicals, up to 10 ppm, either had no effect or caused stimulation in growth of Dunaliella and Porphyridium. However, Skeletonema was found to be more sensitive to the effect of the chloro-organic chemicals than the other two organisms. At 1 ppm, none of the chemicals except 4-chlororesorcinol appeared to inhibit growth of Skeletonema. However, at a higher concentration (10 ppm), 3-chlorobenzoic acid, and 3-chlorophenol were found to be toxic.

These findings suggest that phytoplankton are less sensitive to the chloro-organic compounds than other aquatic organisms. Gehrs et al. (1974) observed that both 5-chlorouracil and 4-chlororesorcinol decreased the hatchability of carp eggs at concentrations as low as 1 ppb. A lack of effect of the test chemicals on phytoplankton growth does not rule out the possibility that the chemicals may have ecologically significant effects. It is quite likely that these chemicals may be accumulated by phytoplankton. An accumulation of the chemicals by phytoplankton is of ecological significance because these chemicals may be transferred to higher trophic levels. Therefore, the uptake and accumulation of the chloro-organic chemicals by phytoplankton need to be investigated in order to fully access their impact.

It was observed that 3-chlorophenol and 5-chlorouracil caused an initial stimulation in growth of the organisms, followed by a decline in the growth rate. The reason for this stimulation is not known; however, the subsequent decrease may be explained by assuming that biological or non-biological transformations of the chemicals resulted in formation of products which caused inhibition of growth. In the case of the other chemicals, an initial lag in the growth of the organisms was observed, but subsequently the cells recovered and growth resumed at the same rate as in the untreated cells. In nature, these brief periods of growth stimulation or inhibition may have a profound effect on species diversity in phytoplankton communities. During these periods, the faster-growing, less susceptible organisms may gain a competitive advantage over the more sensitive species and may eventually become the dominant species in the community. A change in species composition of phytoplankton communities may produce an impact on organisms at higher trophic levels since many marine animals, particularly zooplankton and oyster and clam larvae, graze selectively.

3-Chlorophenol and 4-chlororesorcinol acted synergistically in inhibiting growth of Skeletonema, which suggests that interaction among chloro-organic chemicals should be taken into account in evaluating their effects. In nature, where several chloro-organic chemicals may be present simultaneously in bodies of water receiving chlorinated waste-waters, the combined effect of these chemicals will be expected to be different from that of the individual chemicals.

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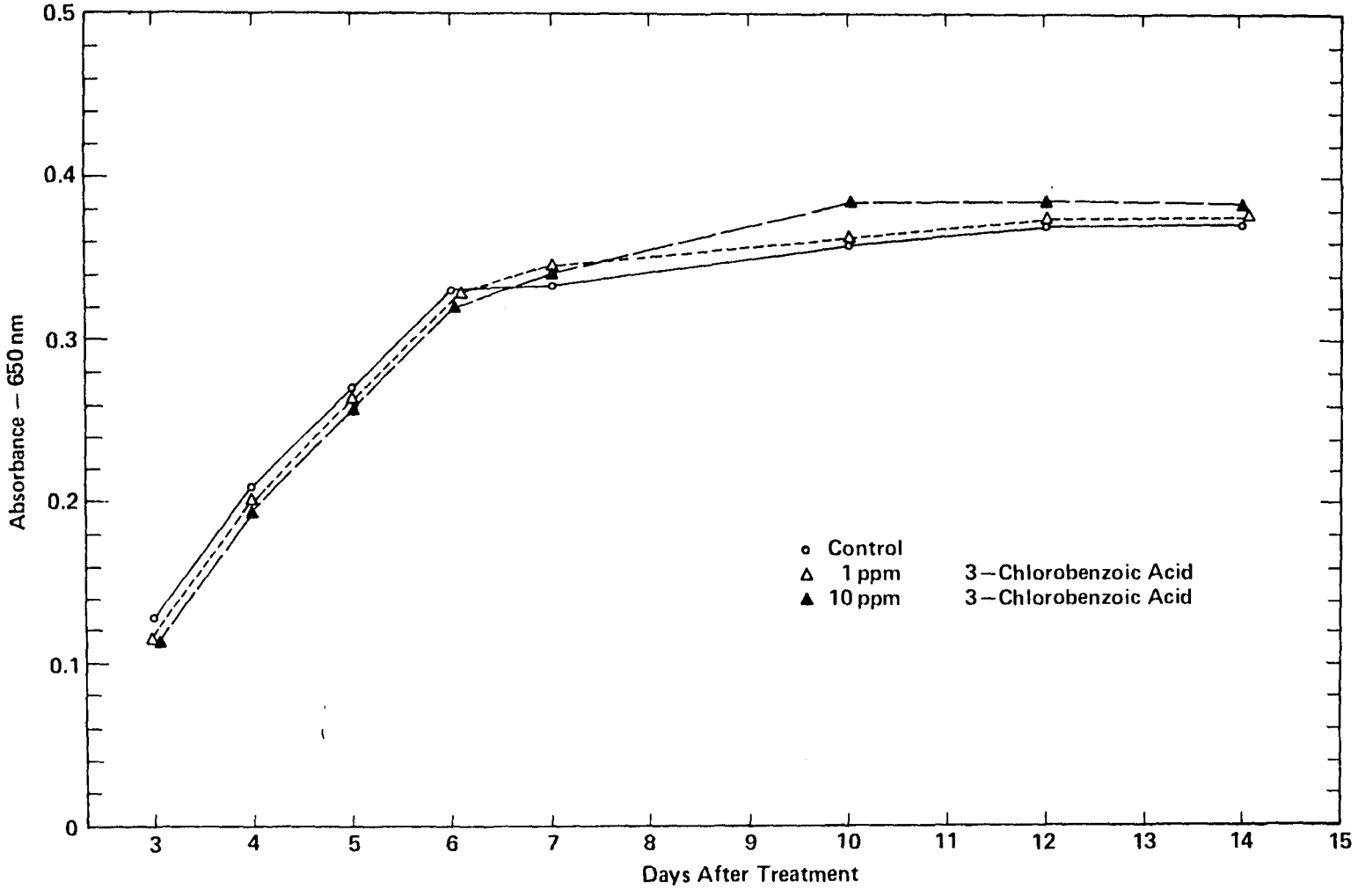

Figure 1. The effect of 3-chlorobenzoic acid on the growth of Dunaliella.

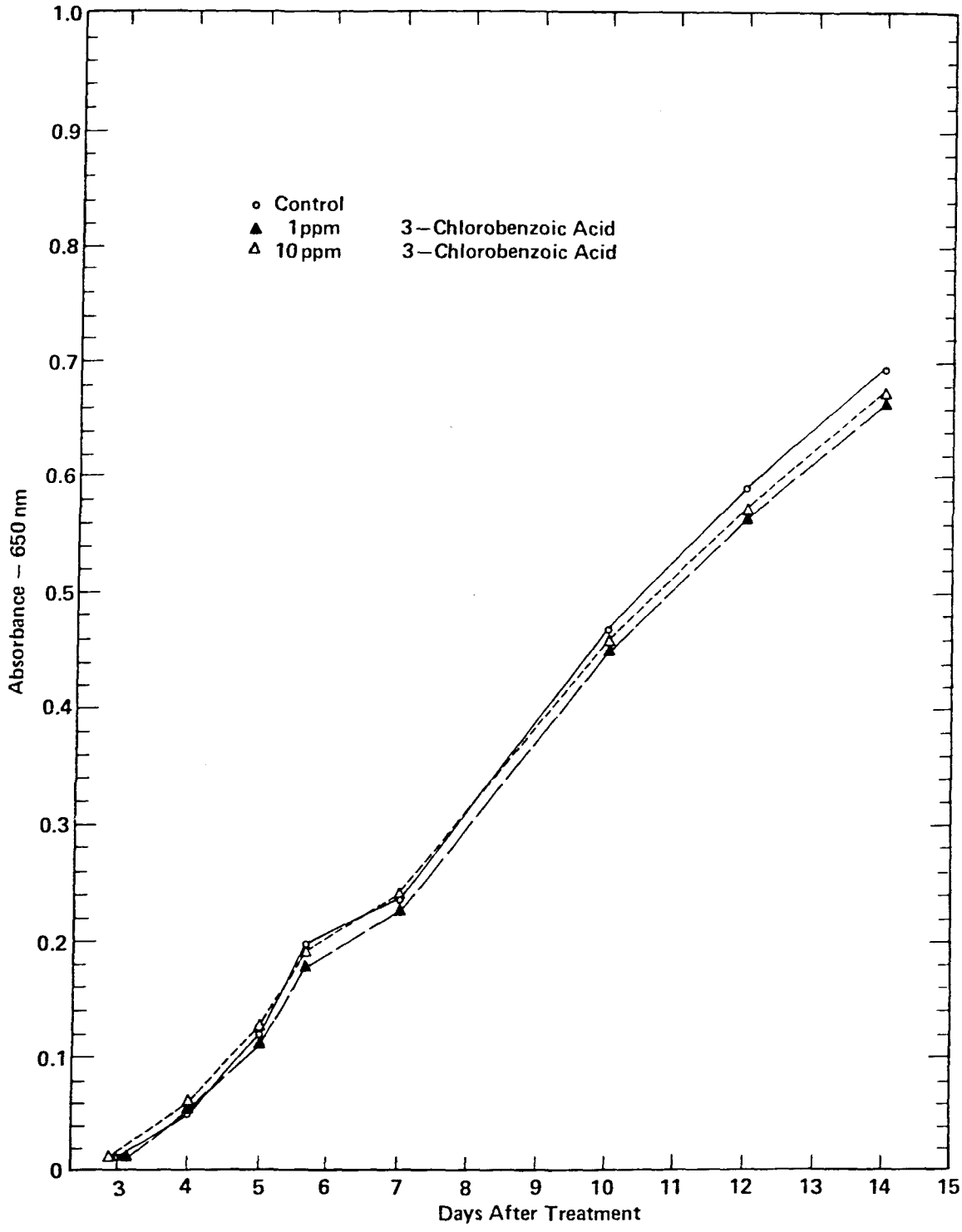

Figure 2. The effect of 3-chlorobenzoic acid on the growth of Porphyridium.

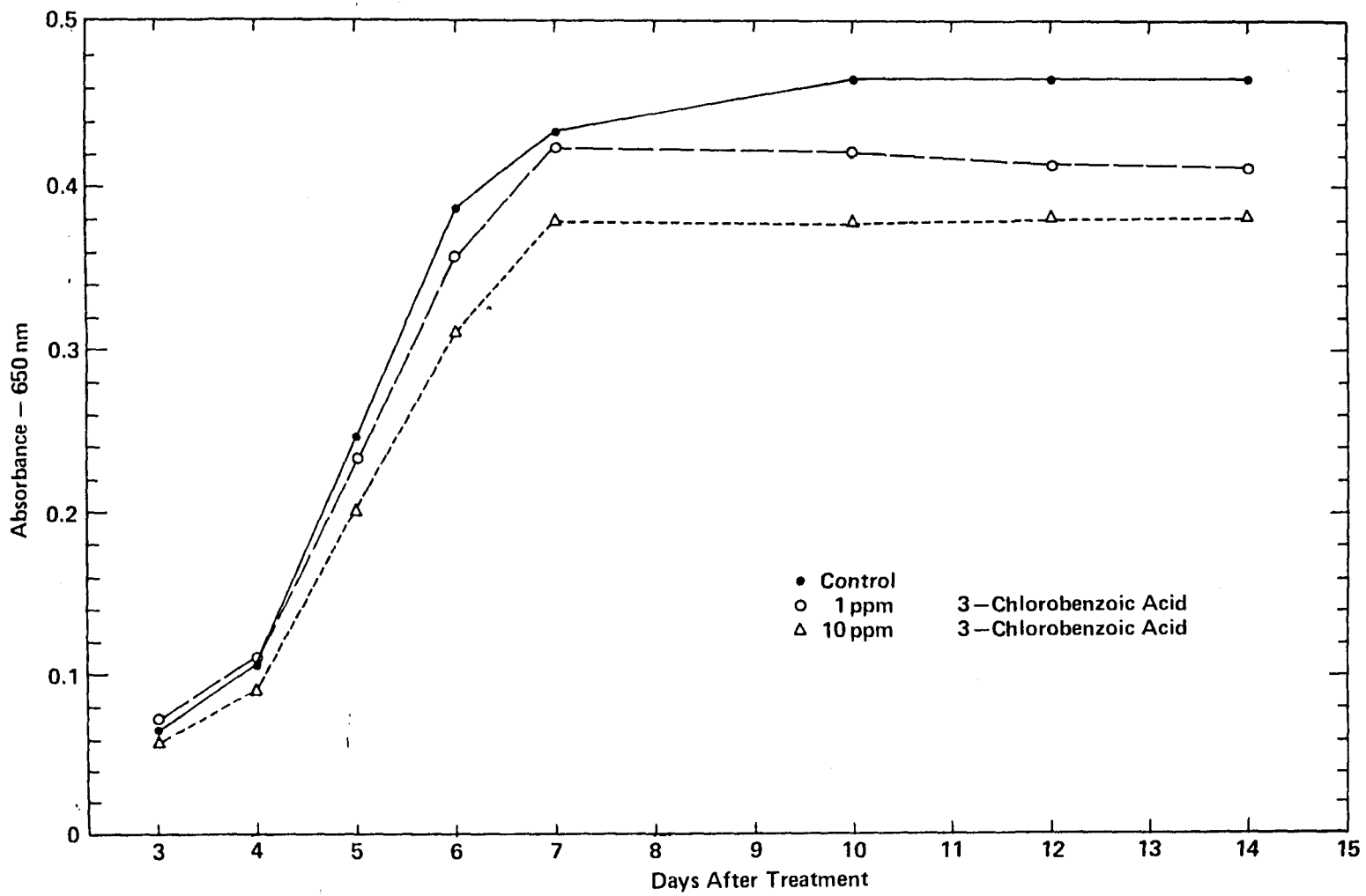

Figure 3. The effect of 3-chlorobenzoic acid on the growth of Skeletonema.

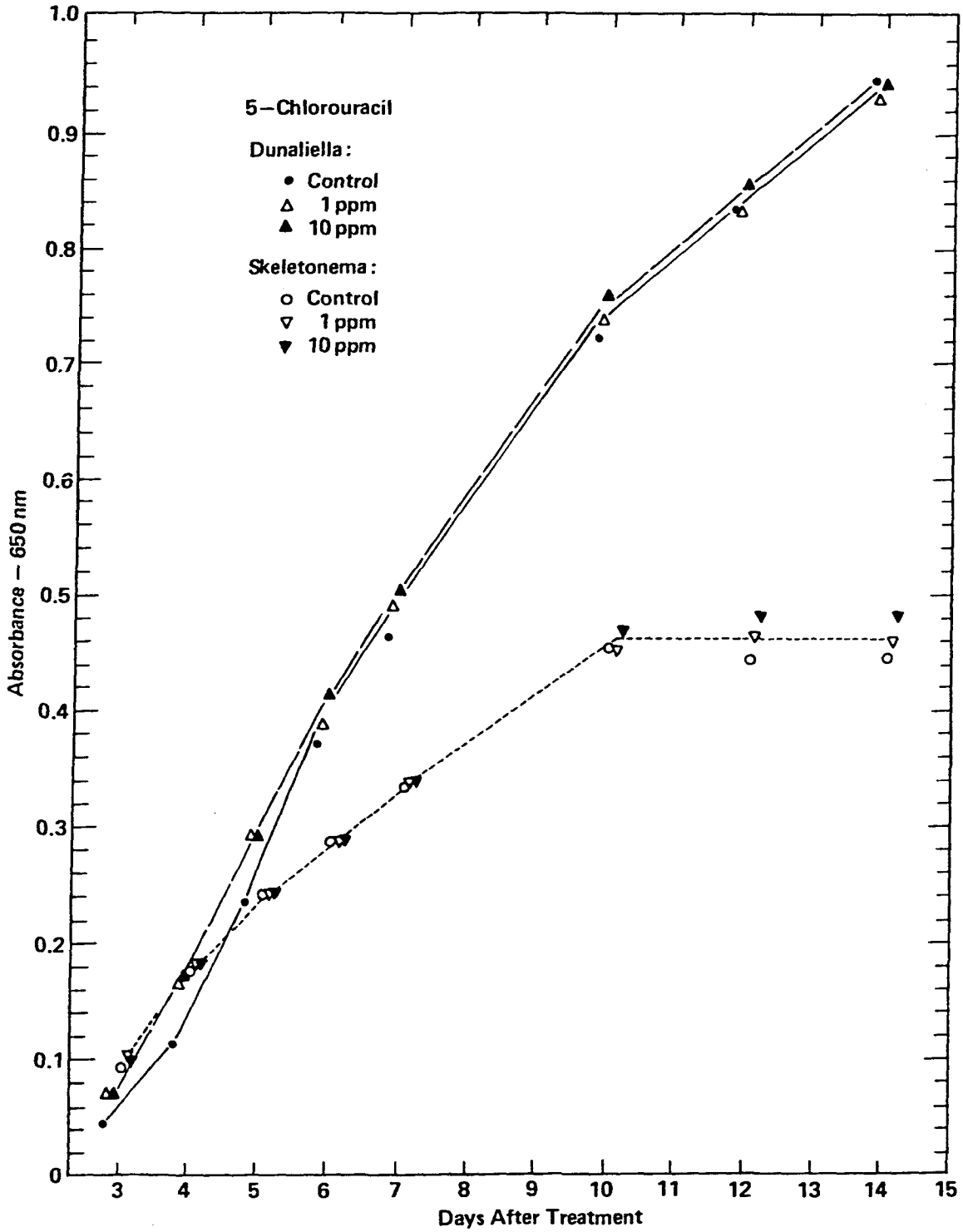

Figure 4. The effect of 5-chlorouracil on the growth of Dunaliella and Skeletonema.

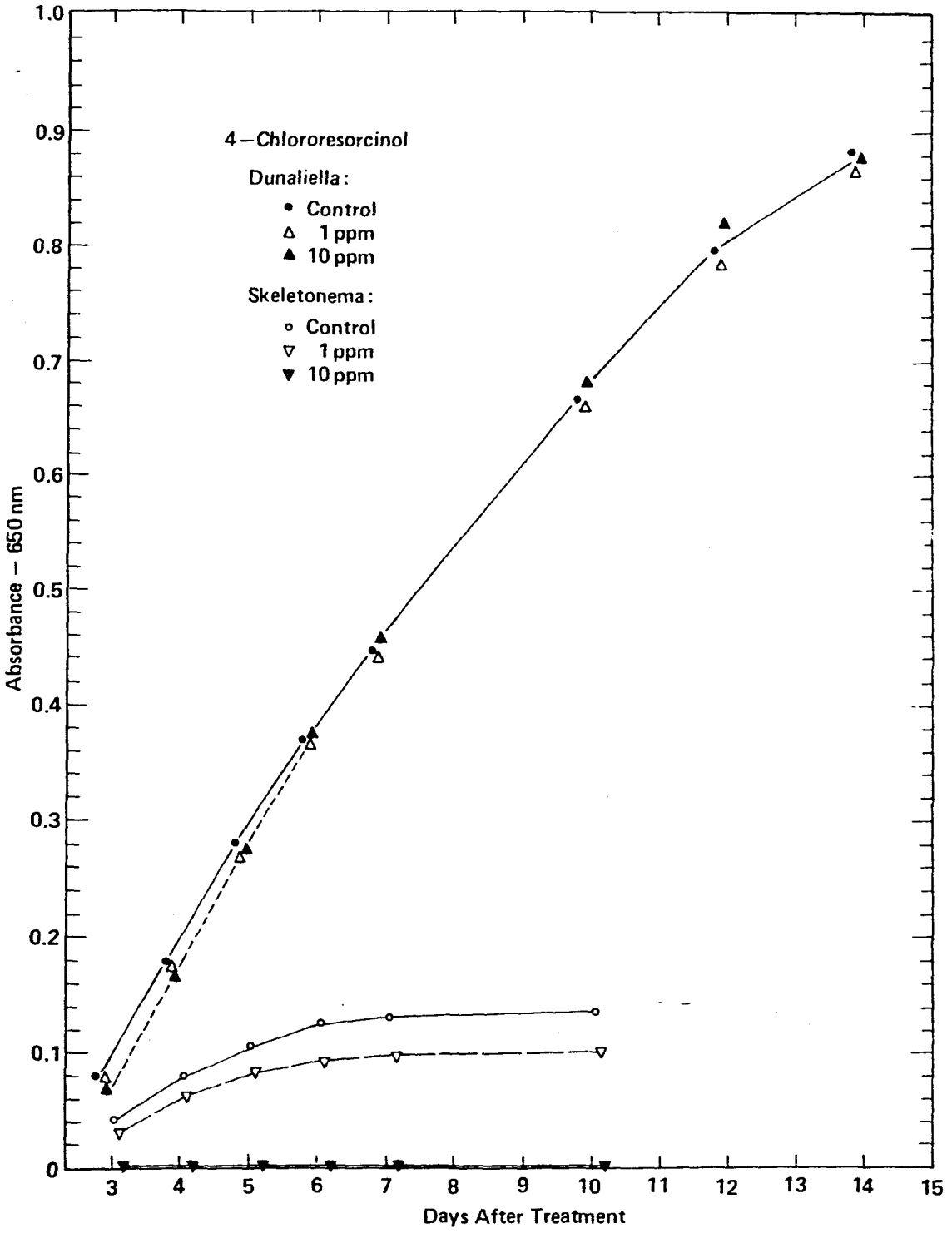

Figure 5. The effect of 4-chlororesorcinol on the growth of Dunaliella and Skeletonema.

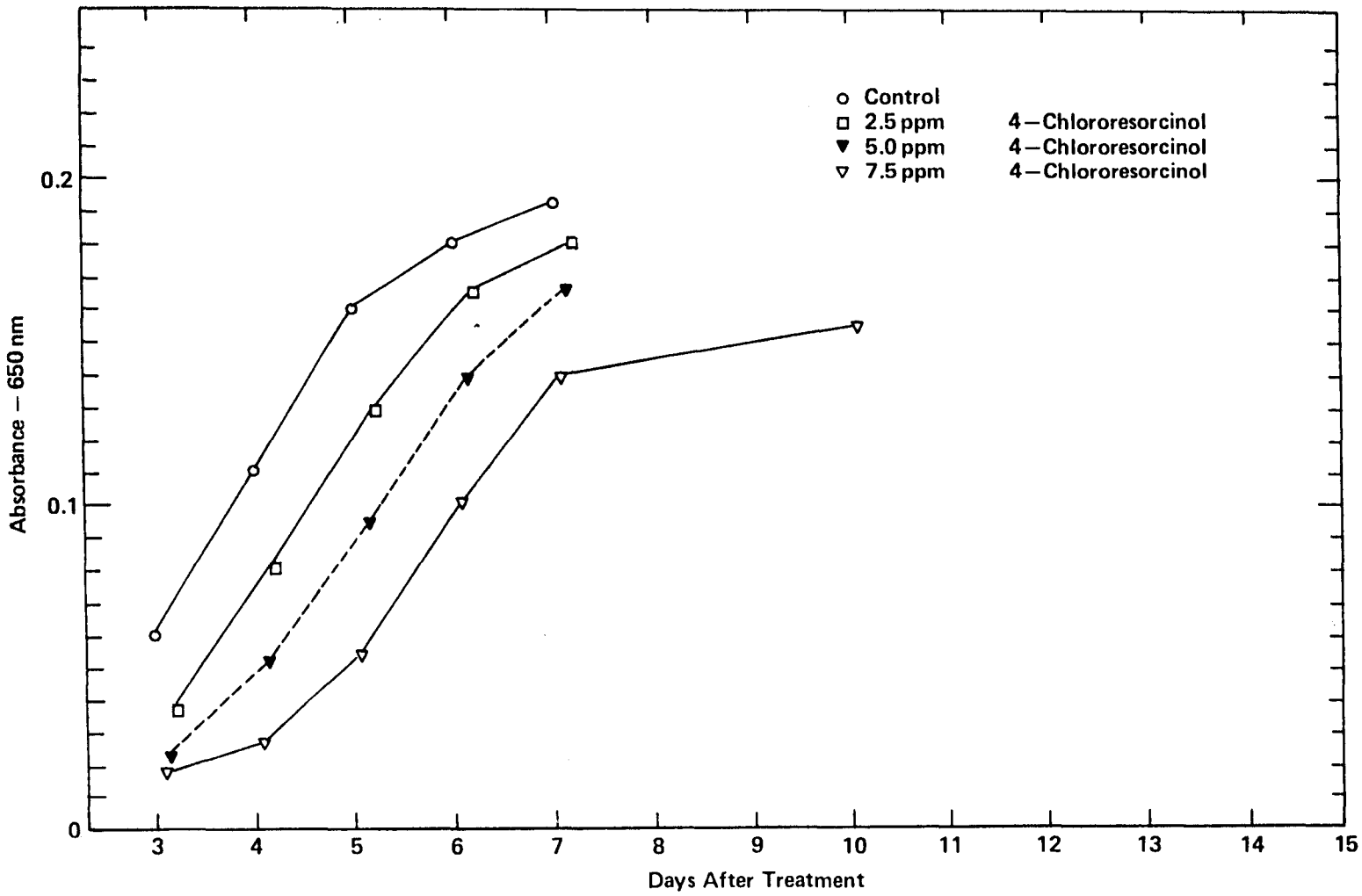

Figure 6. The effect of 4-chlororesorcinol on the growth of Skeletonema.

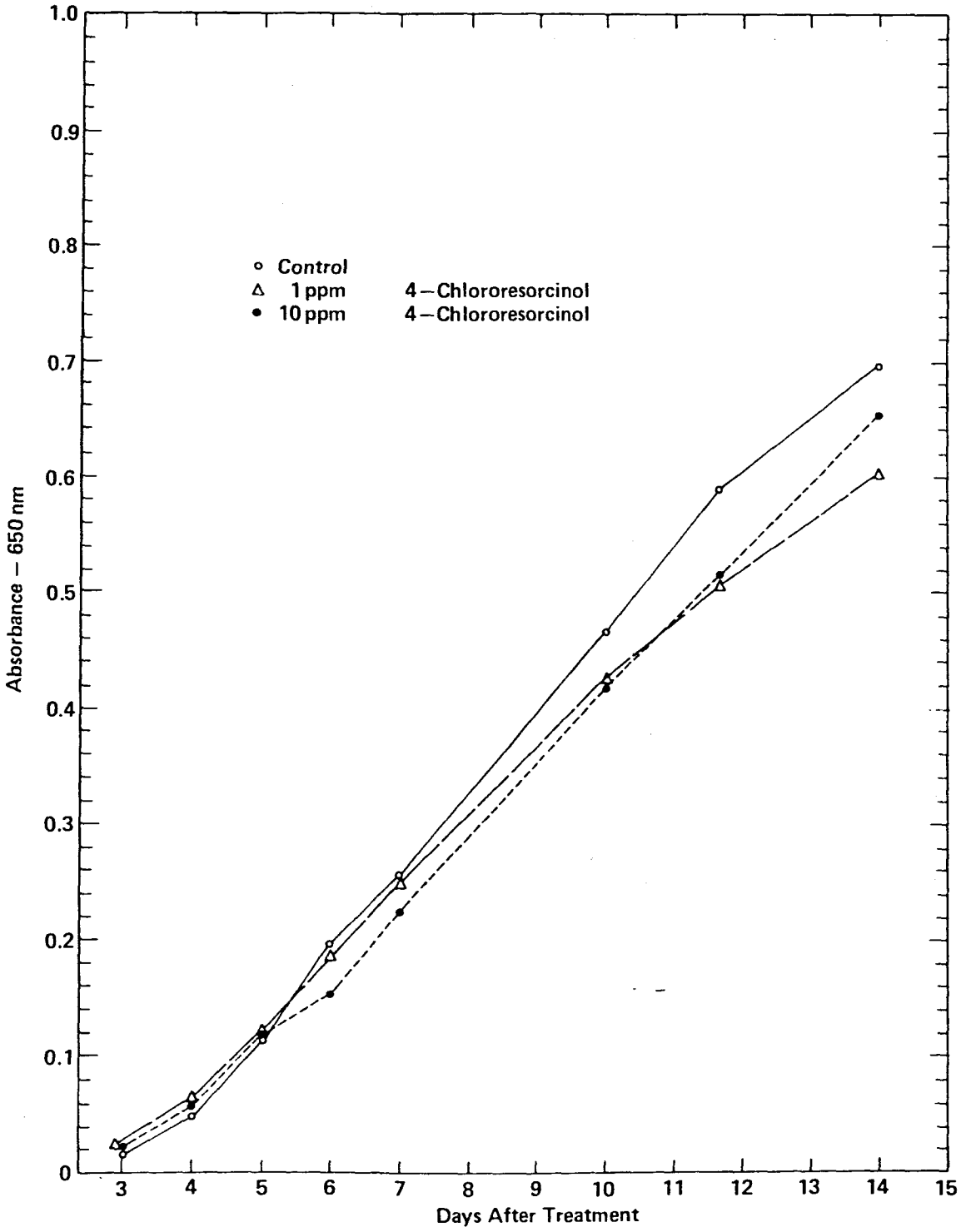

Figure 7. The effect of 4-chlororesorcinol on the growth of Porphyridium.

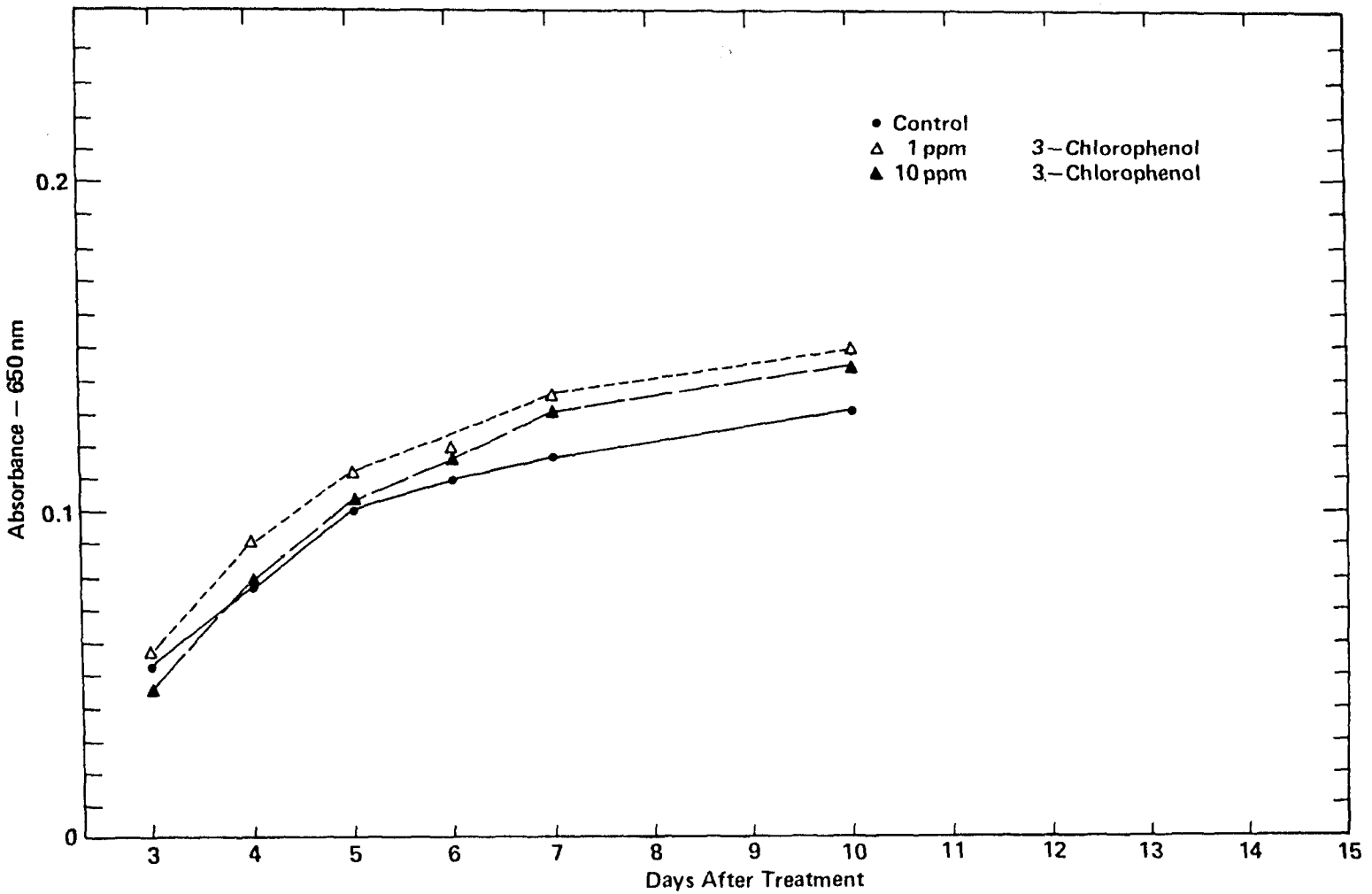

Figure 8. The effect of 3-chlorophenol on the growth of Dunaliella.

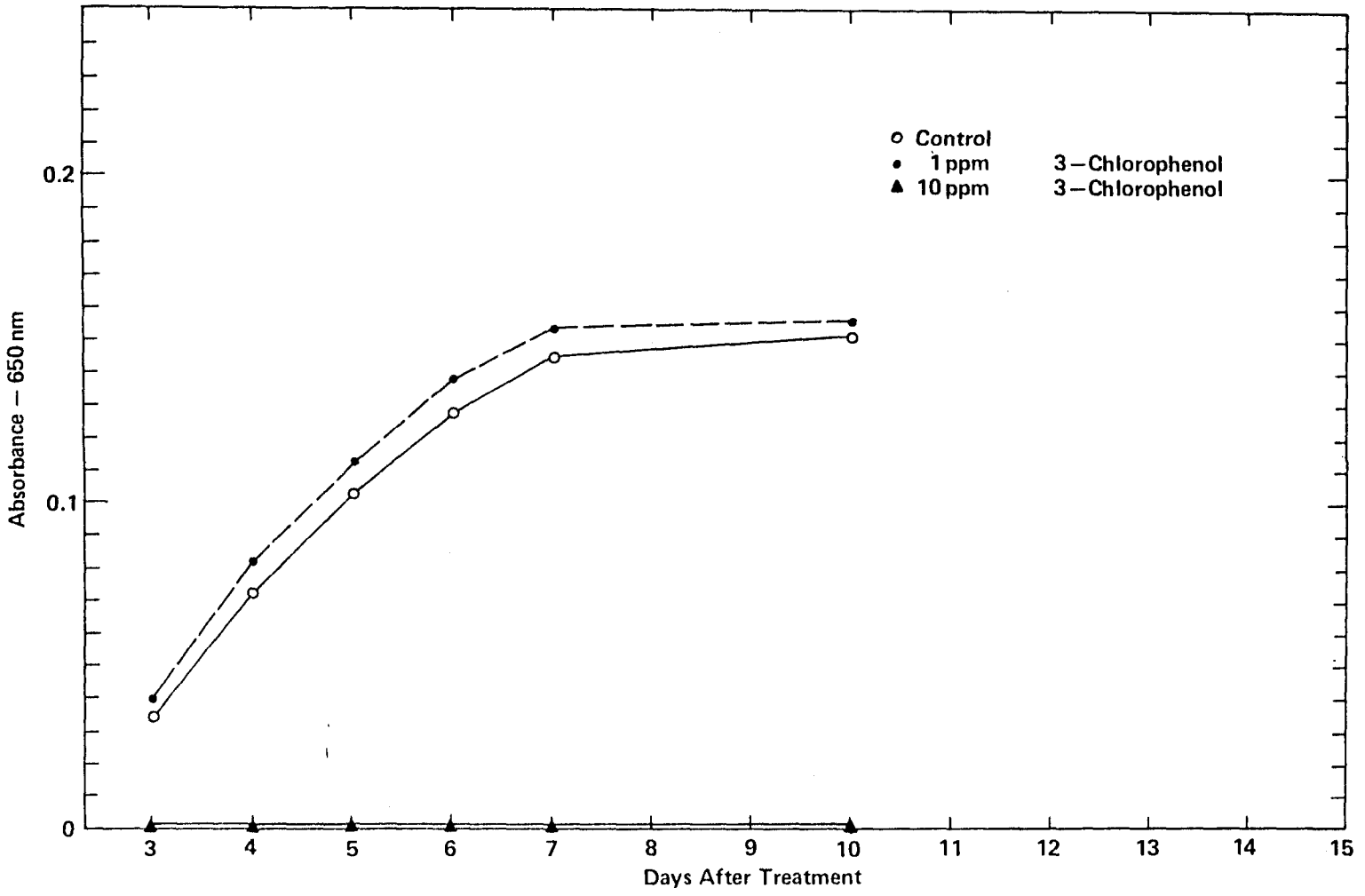

Figure 9. The effect of 3-chlorophenol on the growth of Skeletonema.

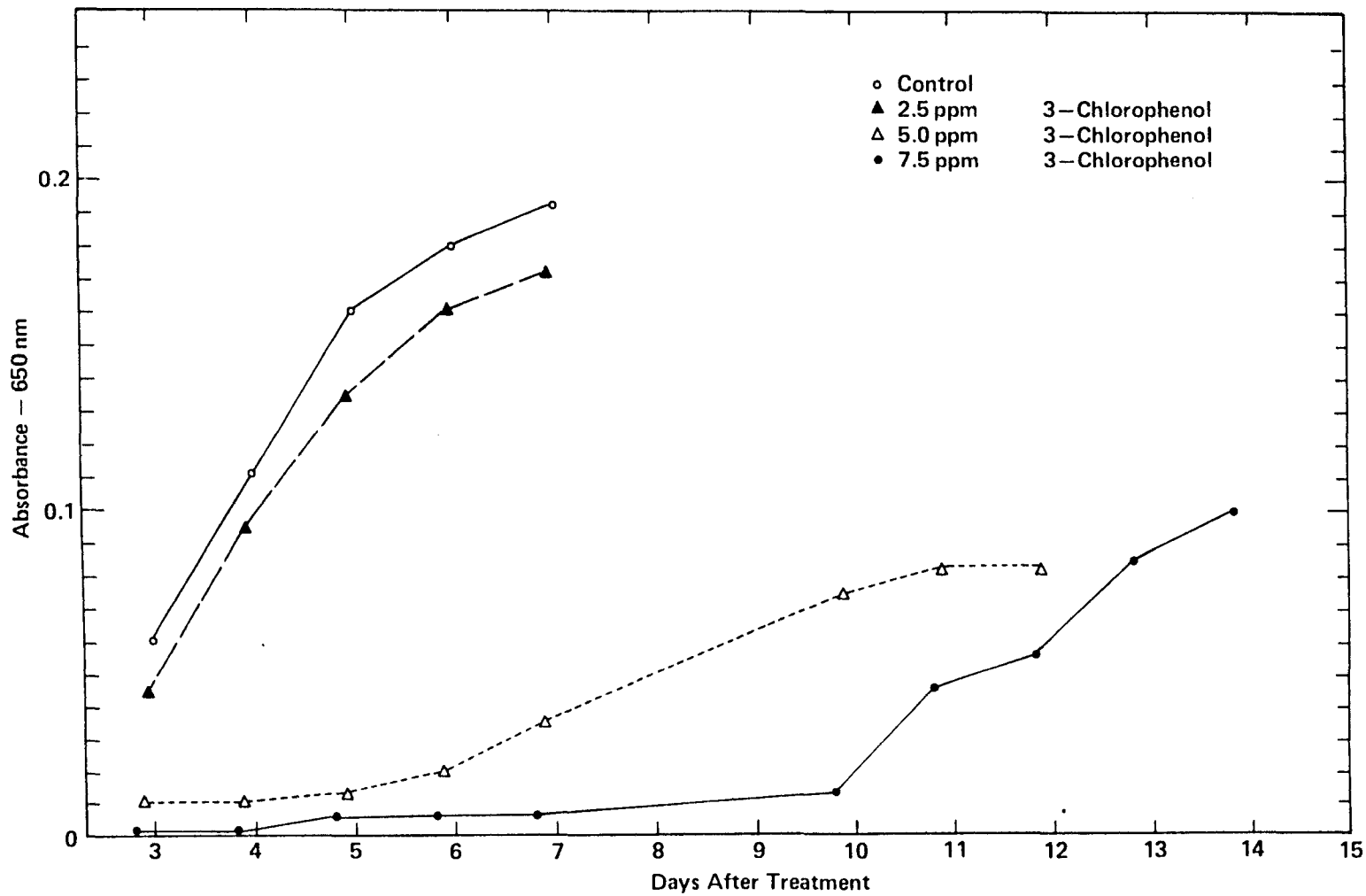

Figure 10. The effect of 3-chlorophenol on the growth of Skeletonema.

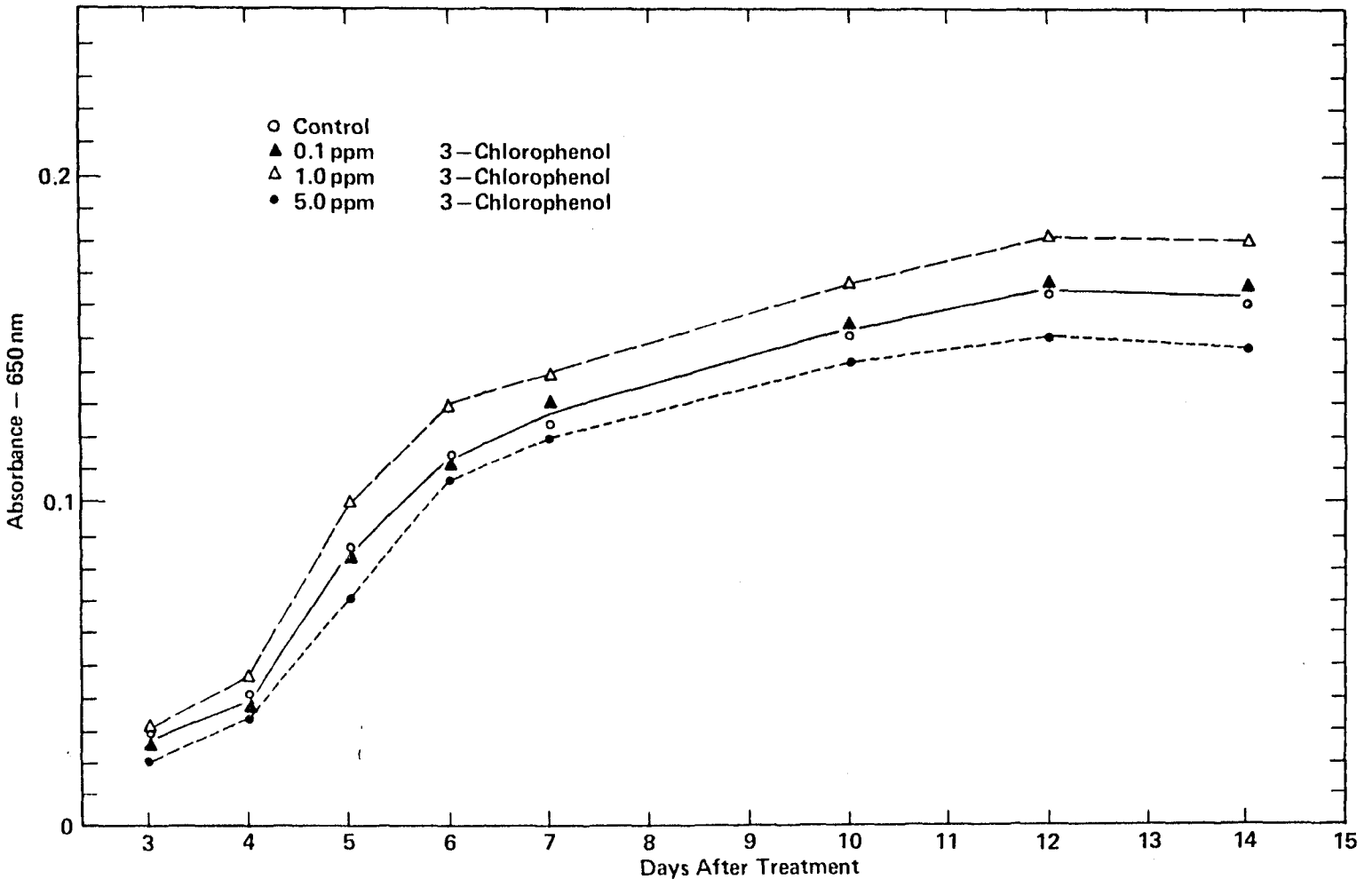

Figure 11. The effect of 3-chlorophenol on the growth of Porphyridium.

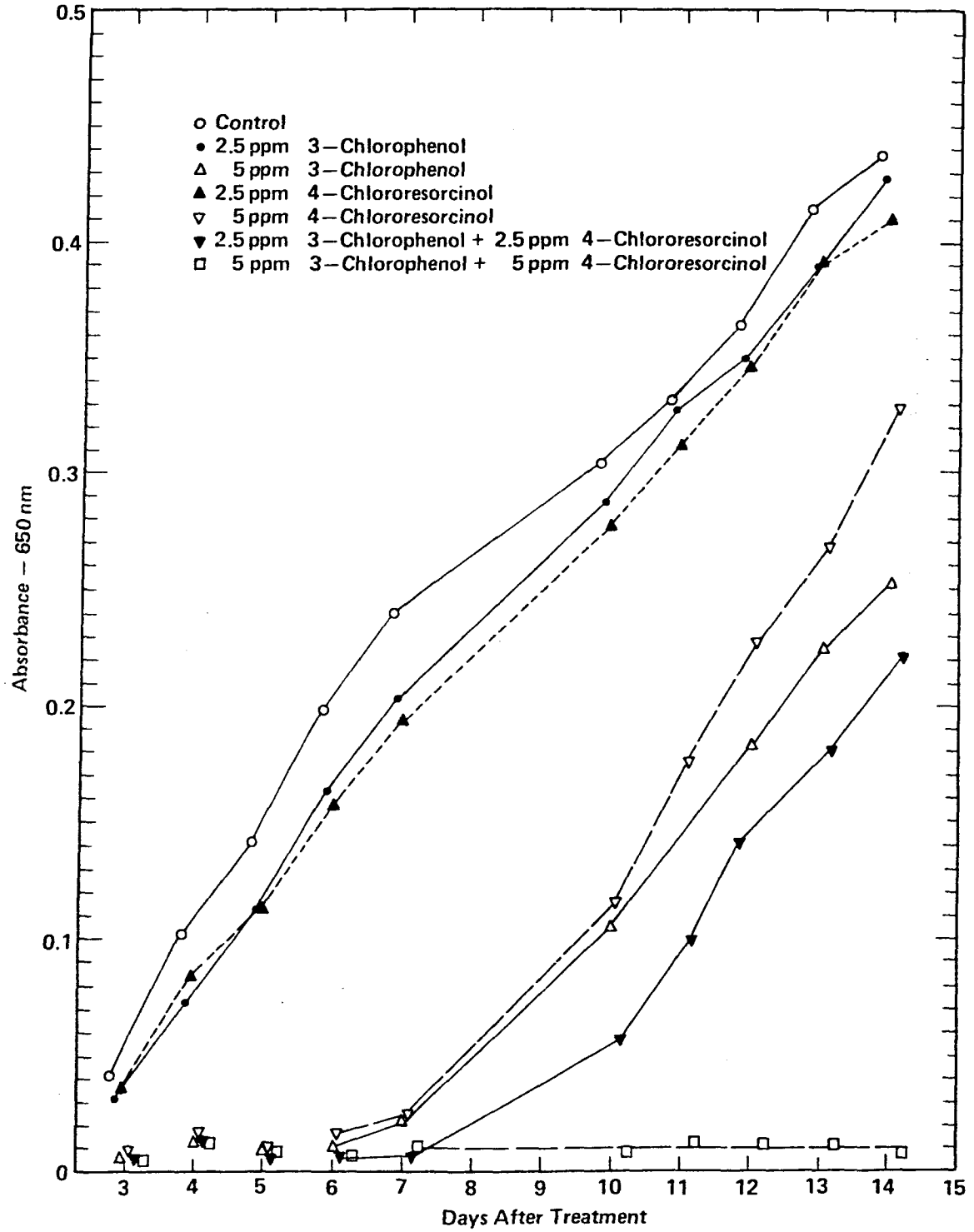

Figure 12. The effect of 3-chlorophenol and 4-chlororesorcinol singly and in combination on the growth of Skeletonema.

EFFECT OF CAPTAN ON MARINE ALGAE

SECTION 1

INTRODUCTION

Captan (N-trichloromethylthio-4-cyclohexane-1,2-dicarboximide) is one of the fungicides widely used in coastal areas. Usage of this chemical may increase in the future as it is a registered substitute for certain fungicides which have been reported to be ecologically hazardous. Captan may be introduced into the estuarine environment through run-off from treated coastal lands or drift from fungicide application. The effects of captan on terrestrial organisms have been investigated, but relatively little research has been done concerning effects of the fungicide on phytoplankton which represent the first link in the aquatic food chain. Knowledge of the effects of pesticides on primary producers is important in predicting their impact on the marine environment. We have investigated the effects of captan on growth and photosynthesis in selected species of marine phytoplankton.

SECTION 2

CONCLUSIONS AND RECOMMENDATIONS

The results indicate that marine phytoplankton vary in their sensitivity to the fungicide captan. *Skeletonema* is considerably more sensitive to the fungicide than *Dunaliella* and *Porphyridium*. Treatment with 0.5 ppm of captan caused a substantial inhibition of photosynthesis in *Skeletonema*.

Our results indicate the following areas of interest: (1) the effect of captan on additional algal species should be examined, (2) the effect of captan in phytoplankton should be studied in the presence of other pesticides and environmental contaminants, and (3) the uptake and metabolism of captan by phytoplankton should be investigated.

SECTION 3

MATERIALS AND METHODS

GROWTH STUDIES

The effect of captan was examined in Skeletonema costatum (Bacillariophyta), Dunaliella tertiolecta (Chlorophyta), and Porphyridium sp. (Rhodophyta). The procedures for culturing the organisms and measuring their growth were similar to those used in determining the effects of chloro-organic compounds. Desired volumes of captan solution in acetone were added to the cell suspension so that the final concentration of acetone was 0.01%. The control cell suspension contained a similar amount of acetone.

PHOTOSYNTHESIS

The photosynthetic activity of Skeletonema was determined by measuring $^{14}CO_2$ fixation by the cells. The cell suspension (10^4 cells/ml) was preincubated with captan for 30 minutes on a reciprocating shaker at 22°C under a light intensity of approximately 10,750 lux. Following preincubation with the fungicide, $NaH^{14}CO_3$ was added to the cell suspension and the cells were allowed to fix $^{14}CO_2$ for 60 minutes. Aliquots of cell suspension were removed at selected times after addition of $NaH^{14}CO_3$, filtered through a 0.45 mμ Millipore filter and the cells were washed with the growth medium. To determine the amount of $^{14}CO_2$ fixed by the cells, the filter discs with the cells were transferred to a scintillation fluid and counted for ^{14}C in a Nuclear Chicago liquid scintillation counter.

SECTION 4

RESULTS

EFFECT ON GROWTH

The effect of captan on the growth of Dunaliella is shown in Figure 13. The fungicide, at 0.1 and 1 ppm, significantly stimulated growth by 6 and 20%, respectively, after 7 days of treatment. However, 5 ppm of captan completely inhibited growth of the alga.

Figure 14 shows the effect of captan on growth of Porphyridium. The fungicide, at 0.1 ppm, stimulated growth by 23% through day 4, but subsequently growth proceeded at almost the same rate as that in the controls. Treatment with 1 ppm of the chemical did not significantly affect growth. Growth of cultures exposed to 5 ppm captan was completely suppressed during the first 3 days following treatment; however, the cells recovered subsequently and the inhibition decreased to 18% by day 12.

Skeletonema was more sensitive to captan than either Dunaliella or Porphyridium (Figures 15 and 16). The fungicide inhibited growth of the organism at concentrations ranging from 0.25 to 5 ppm, inhibition increasing with an increase in captan concentration. Cells exposed to 0.25-1 ppm of captan showed a time lag of growth with the duration of the lag period increasing with an increase in concentration of the fungicide. Following the lag period, the growth of the treated cells proceeded at almost the same rate as that in the controls. However, at 5 ppm, captan damaged the cells so severely that they never resumed growth.

EFFECT ON PHOTOSYNTHESIS

To ascertain the mechanism by which captan causes toxicity in Skeletonema, the effect of the fungicide on photosynthesis was examined. Photosynthetic CO_2 fixation by Skeletonema was used as a measure of photosynthetic activity. Since CO_2 fixation is the result of both light and dark reactions of photosynthesis, an adverse effect on any of the photosynthetic reactions will be reflected in reduced CO_2 fixation. Exposure of Skeletonema to 0.5 and 1 ppm of captan for 30 minutes reduced $^{14}CO_2$ fixation by 83 and 93%, respectively (Table 1).

TABLE 1. EFFECT OF CAPTAN ON PHOTOSYNTHETIC $^{14}CO_2$ FIXATION BY SKELETONEMA

Treatment	Total ^{14}C fixed (dpm x 10^{-3}) after	
	30 min.	60 min.
Control	16.7	37.4
Captan 0.5 ppm	2.8	2.9
Captan 1.0 ppm	1.0	1.2

SECTION 5

DISCUSSION

Our findings indicate that different species of marine phytoplankton vary in their response to captan. Skeletonema is considerably more sensitive to the fungicide than Dunaliella or Porphyridium. Captan was toxic to Skeletonema at a concentration as low as 0.25 ppm. The toxic action of captan has been suggested to be due to its ability to react readily with cellular sulfhydryl groups (Lukens, 1971; Lukens and Sisler, 1958; Owens and Blaak, 1960; Richmond and Somers, 1966). The trichloromethylthio moiety produced as a result of the reaction of thiols with captan is also capable of reacting with sulfhydryl and amino groups. Because captan may inhibit enzymes and coenzymes with functional sulfhydryl and amino groups known to operate in many areas of cell metabolism, the toxicity of the fungicide to phytoplankton may result from its interference with any number of biochemical processes. Our findings indicate that inhibition of photosynthesis by captan is an important mechanism by which the fungicide causes growth inhibition in Skeletonema. The present studies do not identify the mechanism(s) by which the fungicide inhibits photosynthesis. However, on the basis of the chemical reactivity of captan, it may be postulated that the fungicide inhibits both the light-mediated reactions and the reactions in the carbon-reduction cycle of photosynthesis.

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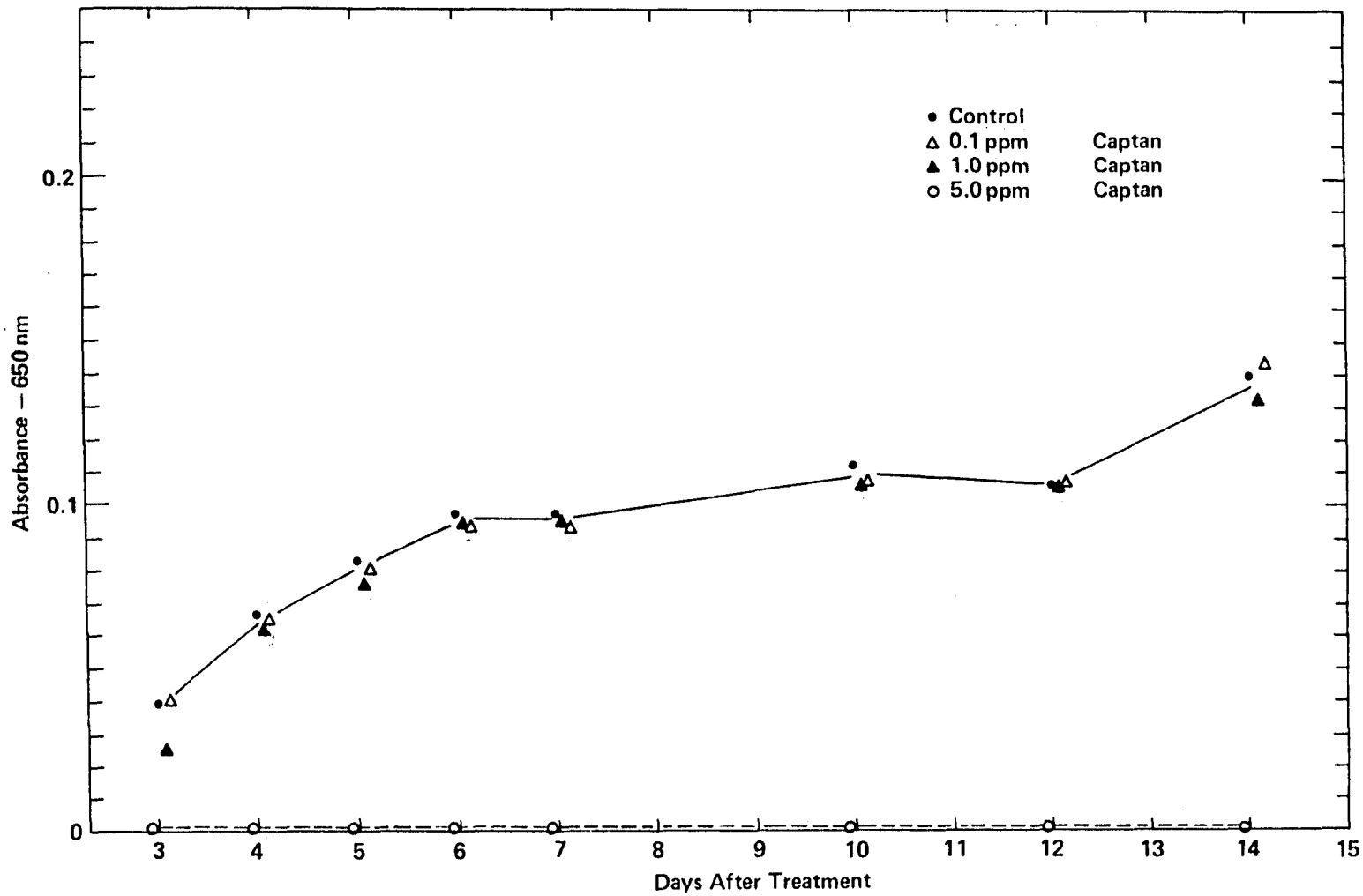

Figure 13. The effect of captan on the growth of Dunaliella.

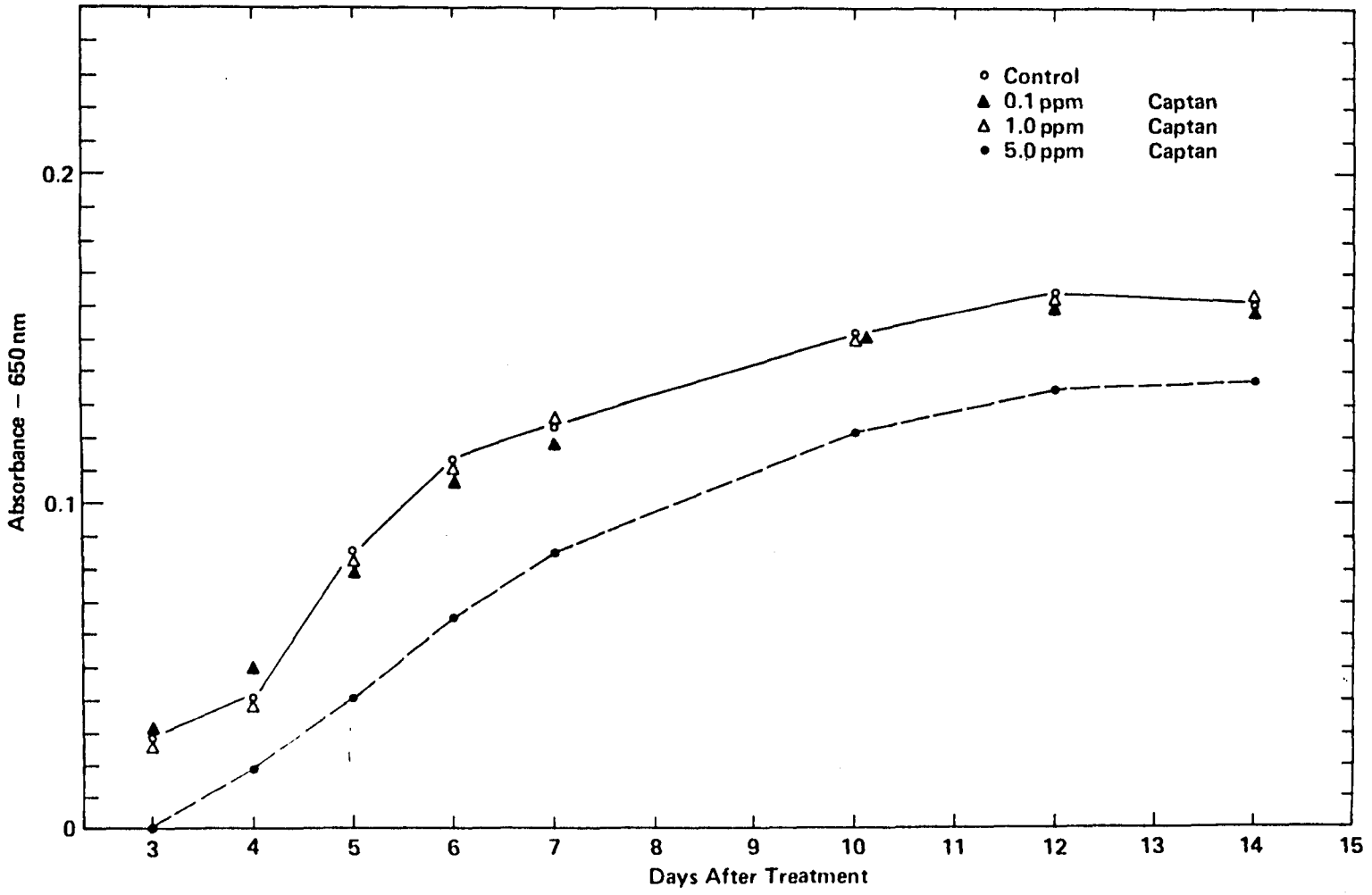

Figure 14. The effect of captan on the growth of Porphyridium.

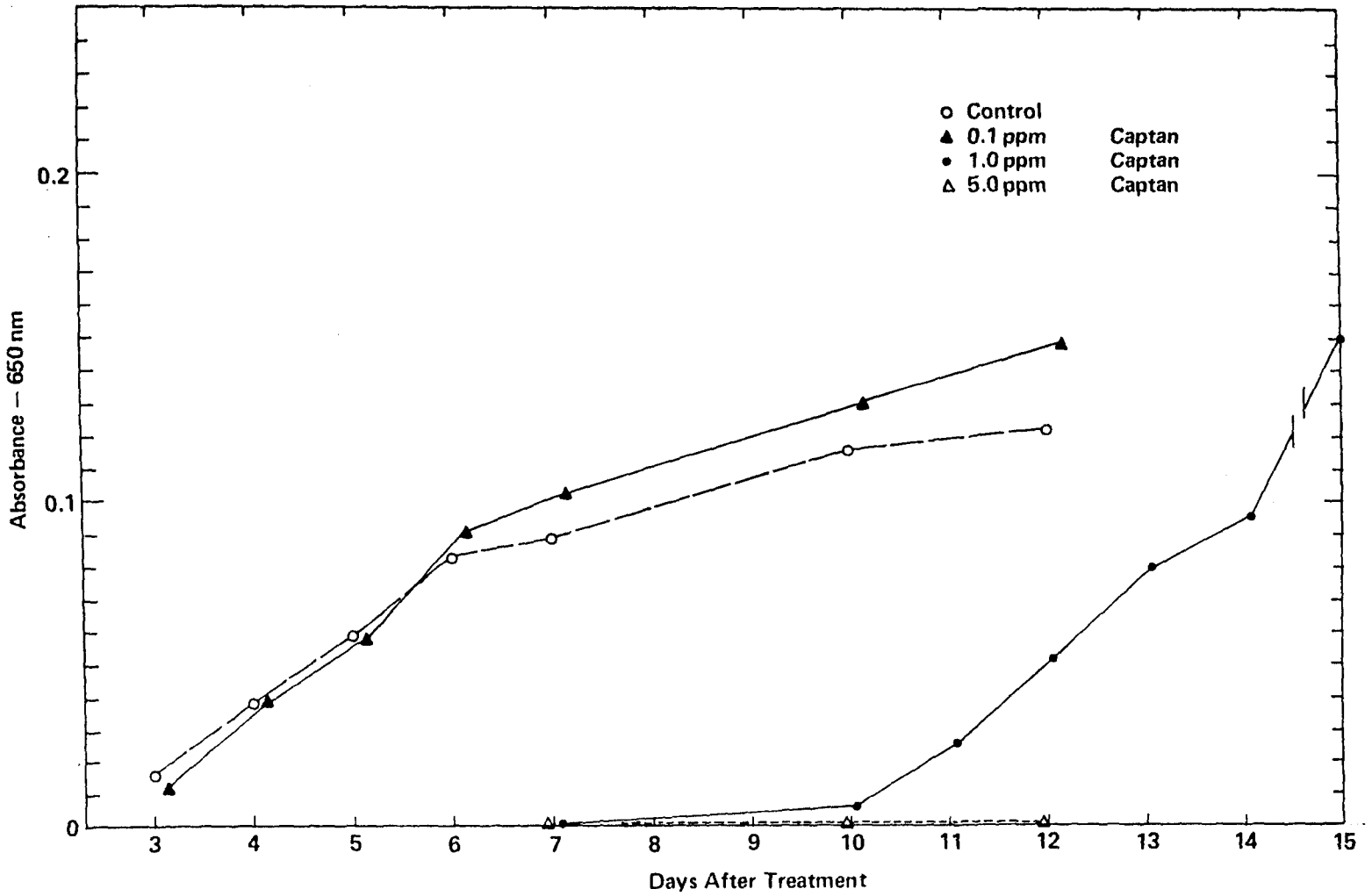

Figure 15. The effect of captan on the growth of Skeletonema.

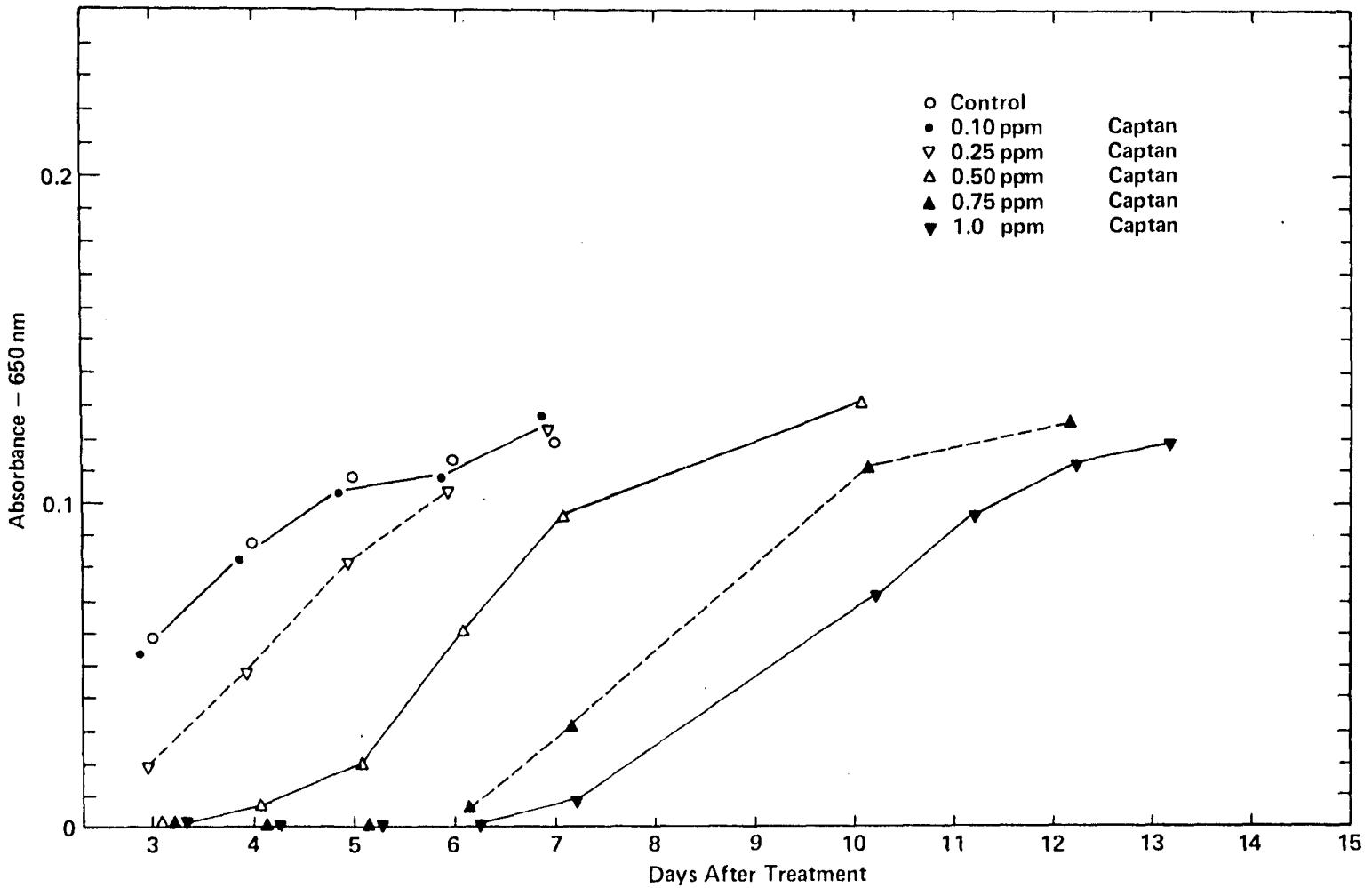

Figure 16. The effect of captan on the growth of Skeletonema.

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15. SUPPLEMENTARY NOTES

16. ABSTRACT

Effects of stable chloro-organic compounds formed during chlorination of sewage effluents on growth of marine unicellular algae were determined. Captan suppressed growth of Dunaliella tertiolecta and Porphyridium cruentum at 5 ppm. Growth of Skeletonema costatum was inhibited by 0.25 ppm captan.

3-Chlorobenzoic acid inhibited growth of S. costatum at 10 ppm but had no effect on D. tertiolecta or P. cruentum. There was no effect of 1-10 ppm 5-chlorouracil on S. costatum, but growth of D. tertiolecta was stimulated initially. Growth of S. costatum was inhibited by 1 ppm 4-chlororesorcinol, and 10 ppm inhibited growth of P. cruentum. At 1 ppm, 3-chlorophenol stimulated growth of all three species, but growth of S. costatum was inhibited by 2.5 ppm.

A combination of 3-chlorophenol and 4-chlororesorcinol interacted synergistically to reduce growth of S. costatum.

It is concluded that chloro-organic compounds formed during chlorination of sewage effluent are not an immediate threat to marine unicellular algae.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Algae, Marine, Phytoplankton, Growth, Sewage Treatment, Water Pollution	Dunaliella, Skeletonema, Porphyridium, Chlorobenzoic acid, chlorophenol, chlororesorcinol, captan	

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