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INDIVIDUAL AND COMBINED EFFECTS OF TEMPERATURE AND POLYCHLORINATED BIPHENYLS ON THE GROWTH OF THREE SPECIES OF PHYTOPLANKTON*

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ABSTRACT

Two marine diatoms and a marine green alga, grown in axenic batch cultures, were exposed to polychlorinated biphenyls (PCBs) at different temperatures. PCBs did not affect the green alga's growth rate but did inhibit the growth of both diatoms. One of these, Rhizosolenia setigera, *was affected by concentrations of PCBs as low as 0·1 part per thousand million. Sensitivity of both diatoms to PCBs was highly dependent on temperature; PCB toxicity increased as the temperature tolerance limits for each species were approached. The data suggest that phytoplankton growing under suboptimal conditions may be more vulnerable to stress than when growing under optimal conditions.*

INTRODUCTION

Recent reports indicate that phytoplankton sensitivity to pollutants varies with several environmental factors. Chlorinated hydrocarbon toxicity varies with salinity (Batterton *et al.*, 1972), light intensity (MacFarlane *et al.*, 1972), geographical location (Fisher *et al.*, 1973), and interspecific competition (Mosser *et al.*, 1972*b*) and the toxicity of copper varies with pH (Kanazawa & Kanazawa, 1969) and temperature and salinity (Mandelli, 1969). These synergistic phenomena may cause the low levels of pollutants normally encountered in the environment to be as toxic as higher levels under different conditions.

We assessed the toxicity of polychlorinated biphenyls (PCBs) to three species of algae at different temperatures. The temperatures were selected on the basis of

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known growth responses of these species. We evaluated the PCB-temperature interaction for each species in an attempt to determine whether PCB toxicity increased as the temperature tolerance limits for each species were approached. PCBs were used as a stress factor because of their persistence and ubiquity in the marine environment (SCEP Task Force, 1970).

MATERIALS AND METHODS

Thalassiosira pseudonana (clone 3H), a small, estuarine, centric diatom (Guillard & Ryther, 1962), *Dunaliella tertiolecta* (clone Dun), an estuarine green alga (Eppley *et al.*, 1969), and *Rhizosolenia setigera* (clone Rhizo), a large, neritic, pennate diatom (Cupp, 1943), were grown axenically in f/2 medium (Guillard & Ryther, 1962). (Salts were added to double distilled water to yield artificial seawater with a salinity of $25^0/_{00}$ and an ionic composition equivalent to that of Instant Ocean (Aquarium Systems, Eastlake, Ohio). This medium was then enriched to form f/2, the pH was adjusted to 7·8, and the $NaHCO_3$ concentration adjusted to 200 mg/litre). *T. pseudonana* and *D. tertiolecta* were grown at 12°C, 18°C, and 25°C and *R. setigera* was cultured at 10°C and 15°C.

Exponentially-growing cells of *T. pseudonana* and *D. tertiolecta* were inoculated into sterile, tightly sealed screw-cap bottles (with Teflon lined screw-caps) containing 200 ml of medium to give initial cell densities of 10^4/ml. *R. setigera* cells were inoculated into 100 ml of medium to give an initial cell density of 48/ml. The procedure for adding the PCBs (Aroclor 1254) has been described elsewhere (Mosser *et al.*, 1972*a*). All species were tested with 0, 0·1 and 10 parts per thousand million (10^9) (ptm) of PCBs in the medium; *R. setigera* was also exposed to 1·0 ptm. Each treatment was replicated three times. All species were illuminated 14 h/day with cool white fluorescent lamps; *T. pseudonana* and *D. tertiolecta* received 3250 lumens/m^2 and *R. setigera* received 7500 lumens/m^2. Two-millilitre samples of *R. setigera* were fixed with 0·1 ml of Lugol's solution and counted with a Sedgewick-Rafter Counter. One-millilitre samples of *T. pseudonana* and *D. tertiolecta* were fixed with 0·05 ml of Lugol's solution and counted with a Speirs-Levy Eosinophil Counter. The lower inoculum size and different counting technique were used for *R. setigera* due to its relatively large cell volume ($2–3 \times 10^4$ μ^3, calculated according to Hulburt (1970)).

RESULTS

Growth of *D. tertiolecta* was not affected by the PCBs at any of the temperatures tested (Fig. 1). *T. pseudonana's* growth, however, was inhibited by 10 ptm of PCBs (Fig. 1) and showed sensitivity varying with temperature. Of the three temperatures tested, *T. pseudonana's* growth rate was fastest at 25°C and slowest at

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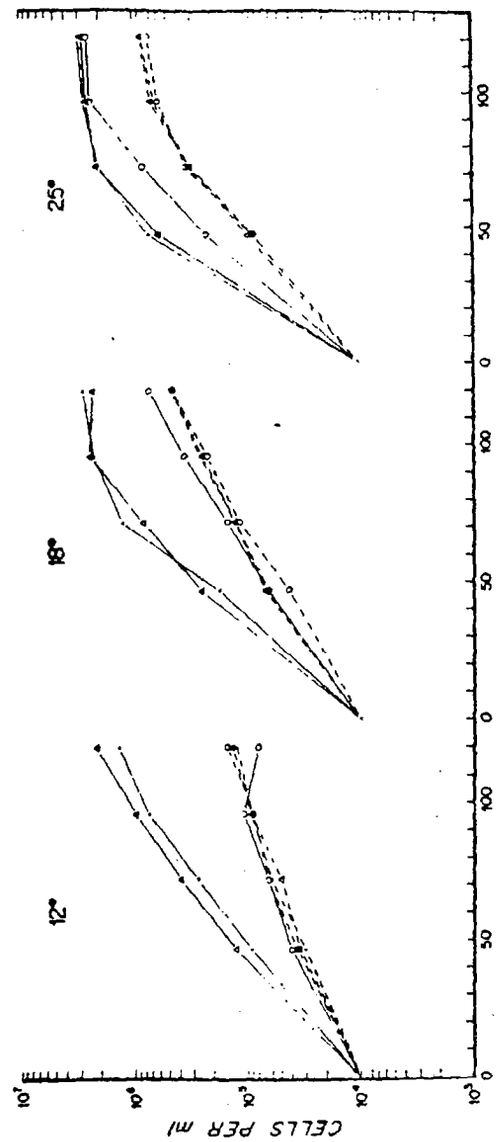

Fig. 1. Growth of *T. pseudonana* (solid lines) and *D. tertiolecta* (broken lines) at three different temperatures and with three PCB concentrations. (●——●) = 0 ptm of PCBs; (Δ——Δ) = 0·1 ptm; (○——○) = 10 ptm. Data points are the means of three replicates.

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12°C. These data are consistent with those of Guillard & Ryther (1962). Table 1 shows that 10 ptm of PCBs diminished the growth rate by 17% at 25°C, as compared with a 44% reduction at 18°C and a 58% reduction at 12°C. The lower concentration of PCBs had no significant effect on *T. pseudonana's* growth at any temperature (Fig. 1).

TABLE 1

GROWTH OF *T. pseudonana* AND *D. tertiolecta* AT THREE TEMPERATURES AND THREE CONCENTRATIONS OF PCBS. GROWTH RATES (μ) ARE EXPRESSED AS DIVISIONS PER DAY AND WERE CALCULATED ACCORDING TO THE EXPRESSION $\mu = (3.5)(\ln n_t - \ln n_{t_0})/(t - t_0)$ WHERE $t - t_0$ REPRESENTS ELAPSED TIME IN HOURS AND n_t AND n_{t_0} ARE THE POPULATION DENSITIES AT TIMES t AND t_0, RESPECTIVELY (EPPLEY & STRICKLAND, 1968). THE VALUES SHOWN (MEANS OF THREE REPLICATES) ARE GROWTH RATES FOR THE FIRST 72 H AT 18°C AND 25°C AND FOR THE FIRST 120 H AT 12°C.

Temperature	[*PCB*] *in ptm*	*Growth rate of* T. pseudonana	*% control*	*Growth rate of* D. tertiolecta	*% control*
12°C	0·0	1·43	100	0·74	100
12°C	0·1	1·57	109	0·75	101
12°C	10·0	0·60	42	0·79	107
18°C	0·0	2·34	100	1·22	100
18°C	0·1	2·15	84	1·23	101
18°C	10·0	1·31	56	1·18	97
25°C	0·0	2·57	100	1·66	100
25°C	0·1	2·59	101	1·66	100
25°C	10·0	2·13	83	1·69	102

During the first 48 hours, *R. setigera's* growth at both 10°C and 15°C was completely stopped by all concentrations of PCBs tested (Fig. 2). However, after growth resumed, the degree of inhibition was greater at 10°C than at 15°C for all PCB concentrations (Table 2).

TABLE 2

GROWTH OF *R. setigera* AT TWO TEMPERATURES AND FOUR CONCENTRATIONS OF PCBS. GROWTH RATES ARE EXPRESSED AS DIVISIONS PER DAY AND WERE CALCULATED AS SHOWN IN TABLE 1. THE VALUES SHOWN (MEANS OF THREE REPLICATES) ARE GROWTH RATES FOR THE FIRST 192 H AT 10°C AND FOR THE FIRST 144 H AT 15°C.

Temperature	[*PCB*] *in ptm*	*Growth rate of* R. setigera	*% control*
10°C	0·0	0·60	100
10°C	0·1	0·49	82
10°C	1·0	0·46	77
10°C	10·0	0·49	82
15°C	0·0	0·86	100
15°C	0·1	0·81	95
15°C	1·0	0·75	87
15°C	10·0	0·73	85

A two-way analysis of variance (Sokal & Rohlf, 1969) of *T. pseudonana's* growth rates showed that temperature, 10 ptm of PCBs, and the interaction term between temperature and PCB treatment were all significant ($P < 0.001$), indicating that its organochlorine sensitivity was highly dependent on the temperature (Table 3).

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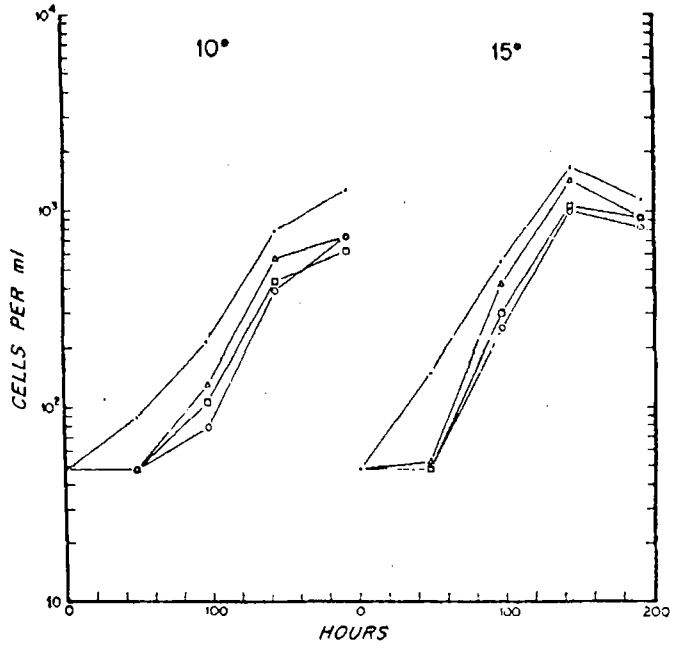

Fig. 2. Growth of *R. setigera* at two different temperatures and with four PCB concentrations. (●——●) = 0 ptm of PCBs; (Δ——Δ) = 0·1 ptm; (□——□) = 1·0 ptm; (○——○) = 10 ptm. Data points are the means of three replicates.

TABLE 3

TWO-WAY ANALYSES OF VARIANCE OF THE GROWTH RATES OF *T. pseudonana* (*T.p.*) AND *D. tertiolecta* (*D.t.*), SHOWING THE EFFECTS OF PCB TREATMENT (10 PTM), TEMPERATURE, AND PCB-TEMPERATURE INTERACTIONS. THESE ANALYSES WERE BASED ON THE GROWTH RATES GIVEN IN TABLE 1.

Source of variation	*df*		*SS*		*Fs*	
	T.p.	D.t.	T.p.	D.t.	T.p.	D.t.
PCB treatment	1	1	2·66	0·001	806·1 ($P < 0·001$)	0·125 n.s.
Temperature	2	2	5·4	2·49	818·2 ($P < 0·001$)	155·3 ($P < 0·001$)
PCB × Temperature interaction	2	2	0·28	0·008	42·4 ($P < 0·001$)	0·50 n.s.
Error	12	12	0·04	0·10		

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A two-way analysis of variance of *R. setigera's* growth rates (Table 4) showed that 0·1 ptm of PCBs inhibited growth ($P < 0·025$), that temperature affected growth ($P < 0·001$), and that the interaction term between temperature and 0·1 ptm of PCBs was also significant ($P < 0·001$).

TABLE 4

TWO-WAY ANALYSIS OF VARIANCE OF THE GROWTH RATES OF *R. setigera* SHOWING THE EFFECTS OF PCB TREATMENT (0·1 PTM), TEMPERATURE, AND PCB-TEMPERATURE INTERACTIONS. THIS ANALYSIS WAS BASED ON THE GROWTH RATES GIVEN IN TABLE 2.

Source of variation	*df*	*SS*	*Fs*
PCB treatment	1	0·02	8·0 ($P < 0·025$)
Temperature	1	0·11	44·0 ($P < 0·001$)
PCB × temperature interaction	1	0·15	60·0 ($P < 0·001$)
Error	8	0·02	

DISCUSSION

Polychlorinated biphenyls are extremely persistent and widespread in nature (Peakall & Lincer, 1970; SCEP Task Force, 1970). Due to their low solubility in water and high solubility in lipids (Peakall & Lincer, 1970), organisms, including phytoplankton, concentrate them by many orders of magnitude from surrounding waters (Södergren, 1971; Harvey *et al.*, 1973). Thus, organisms containing substantial amounts of PCBs may occur in waters with low or non-detectable concentrations (Holden, 1970). Nevertheless, levels in open ocean waters exceeding amounts necessary to inhibit *R. setigera's* growth in pure culture do occur (Harvey *et al.*, 1973).

Concentrations of chlorinated hydrocarbons as low as 0·1 ptm have not previously been reported to affect the growth rate of an algal species in pure culture, as occurred with *R. setigera*. Since phytoplankton are opportunistic organisms (Hutchinson, 1967), competing species might well take advantage of *R. setigera's* abnormally long lag phase and exploit the resources of an area during this lag. Such occurrences have been demonstrated in the laboratory with mixed algal cultures (Mosser *et al.*, 1972*b*). Alteration of the species composition of natural phytoplankton communities could also affect other trophic levels, because many zooplankters graze and digest selectively (Marshall & Orr, 1952; Bainbridge, 1958; Provasoli *et al.*, 1959, Curl & McLeod, 1961; Lee *et al.*, 1966; Richman & Rogers, 1969), as do oyster and clam larvae (Davis & Guillard, 1958) and other herbivores (Wood, 1965).

It is noteworthy that *T. pseudonana's* growth was inhibited more at temperatures at which its cell division rate was lower; that is, it grew slowest at 12°C and was inhibited most at this temperature. At 25°C, inhibition by PCBs was minimal.

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Similarly, *R. setigera's* growth was inhibited more at the temperature at which it grew more slowly. Thus, the sensitivity of phytoplankters to chemical stress is dependent on the conditions in which they are living; toxicity tests should be interpreted accordingly.

As conditions change to produce an environment that is not optimal for growth, resident organisms become more susceptible to stress. Experiments with animals support this conclusion. For example, clam larvae (Calabrese, 1969) and mussel larvae (Brenko & Calabrese, 1969) had narrower temperature tolerance ranges when the salinity was unfavourable. Lobster survival (McLeese, 1956) and fish development (Kinne & Kinne, 1962) were similarly affected by interactions of oxygen, salinity and temperature. Environmental stress (including pollutants) would therefore tend to narrow the niche of a sensitive organism. Thus, Vernberg & Vernberg (1972) found that mercury reduced temperature and salinity tolerance of fiddler crabs and Jitts *et al.* (1964) found that the light and temperature niches of two phytoplankton species were substantially narrowed by a chemical poisoning of an unknown nature.

The decrease of a species' resistance to stress as the tolerance limits of one or a set of environmental conditions are approached may have important implications on successional patterns in nature. As environmental conditions change so that they are no longer optimal for a particular algal species, that species would then be more vulnerable to stress than it would under its optimal conditions and therefore less capable of competing successfully with other species for resources. Its competitor(s) would then be able to replace it rapidly. Such phenomena may accelerate the succession of phytoplankton species and may partially account for the rapidity with which it normally occurs in nature (Raymont, 1963).

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