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Simultaneous effects of light intensity, temperature and salinity on the growth of some phytoplankton species important in aquaculture

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A 3^3 factorial experiment was used to study the simultaneous effects of light intensity, temperature and salinity on the growth oft Tetraselmis suecica, Dunaliella tertiolecta *and* Phaeodactylum tricornutum, *in mass rearing conditions. The relationship between algal growth and the simultaneous effect of studied factors was expressed by multiple regression. The coefficients of partial correlation and beta coefficients (ß), were used to quantify the relative importance of studied factors. These suggested that variation in light intensity had a more pronounced effect than a variation in temperature or medium salinity on algal growth rates. The synergistic effect between light and temperature was established for all three species.*

INTRODUCTION

Marine microalgae have been used in aquaculture to feed artemia and rotifers, they may also be used to produce chemicals, and as a food for human consumption (FABREGAS *et al.*, 1984).

The green alga *Dunaliella tertiolecta* Butcher has been cultivated for the production of glycerol and ß-carotene (BOROWITZKA, 1986) and as a source of single-cell protein (FABREGAS and HERNERO, 1985). *Tetraselmis suecica* Butcher (Prasinophyceae) and *Phaeodactilum tricornutum* Bohlin (Bacillariophyceae) may be used as food for peneaid shrimp (MOCK and NEAL, 1974), bivalves (WALNE, 1970) and rotifers for fish culture. Their growth rate in mass culture may be influenced by a range of environmental factors. The influence of these factors has been studied separately (FABREGAS *et al.*, 1984; GOLDMAN, 1977 a, b; LAING and UTTING, 1980; FALKOWSKI *et al.*, 1978).

This paper demonstrates the importance of combined effects of temperature, salinity and light intensity, and interaction between them, on the growth of *D. tertiolecta, T. suecica* and *P. tricornutum*. The aim of this study was to quantify the relationship between algal growth in mass culture and combined effects of studied environmental factors, what could be of interest in planning of rearing conditions.

MATERIAL AND METHODS

Axenic cultures of the algae were grown in Walne's medium (WALNE, 1956), 10 mg l^{-1} Na_2SiO_3 was added to the medium in which the alga *P. tricornutum* was grown. Media was sterilized by autoclaving at 121 °C for 15 minutes. Experiments were performed in 300 ml flasks with initial cell density of 3 x 10^4 - 1 x 10^5 cells ml^{-1} , 7 x 10^5 - 1 x 10^6 cells ml^{-1} and 3 x 10^4 - 1 x 10^5 cells ml^{-1} for the *T. suecica, D. tertiolecta* and *P. tricornutum*, respectively.

A 3^3 factorial experiment was used to study the combined effects of light, temperature and salinity on the growth of alga. Flasks were shaken throughout the experiments to prevent sedimentation of cells. Algal growth was observed every 24h during the exponential phase. Cells were counted on light microscope with the use of haemocytometer. Motile cells were treated with formalin before enumeration. Experiments were carried out under a 24h light regime (fluorescent lamps; 5200 K - daily light), what is practical in mass culture systems.

RESULTS AND DISCUSSION

Separate effect of light, temperature and salinity on the algal growth

Light, temperature and salinity strongly influenced growth rates of the phytoplankton species studied (Fig. 1). A proportional relationship of growth of all species to light intensity and temperature between 17 and 25 °C was established.

GOLDMAN (1977 a, b) found the optimal temperature of 20 - 30 °C for the cultivation of *D. tertiolecta*. He observed maximal growth rate at 25 °C. It was demonstrated that a lower temperature resulted in slower growth, but not in reduction of the final biomass (JÖRGENSEN, 1968; EPPLEY, 1972).

The maximum growth rate for *P. tricornutum* was in the range 15 and 25 °C (GOLDMAN *et al.*, 1976). Some authors have reported that this species also tolerated even lower temperatures. GUILLARD *et al.* (1962) and ANSELL *et al.* (1963 a, b) found the range between 10 and 15 °C as optimal.

The growth rate of *D. tertiolecta* and *P. tricornutum* increased proportionally with the salinity values used in the experiment. *T. suecica* showed maximal growth rate at salinity of 25 ‰. Above and below this value growth rate decreased (Fig. 1).

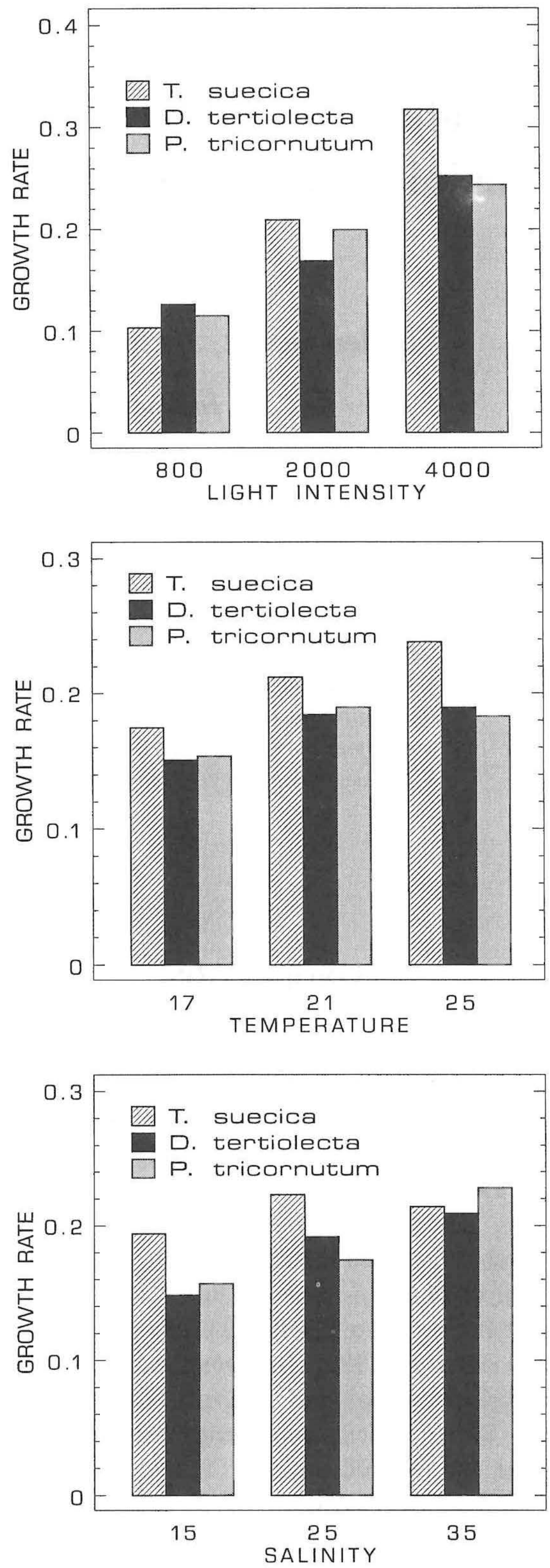

Fig. 1. Effect of light intensity (Lux), temperature (°C) and salinity (‰) on the growth rate (day^{-1}) of *T. suecica*, *D. tertiolecta* and *P. tricornutum*

D. tertiolecta is able to tolerate a wide range of salt concentration. It adjusts the osmotic potential by changing the internal concentrations of glycerol (BEN-AMOTZ and ARRON, 1973; 1978). Optimal condition for obtaining a maximum growth rate in the stationary phase of *T. suecica* culture was between 25 - 30 ‰. Ranges of optimal salinity for growth in media prepared from artificial seawater were found to be the same (LAING and UTTING, 1980). Below 25 ‰ cellular density decreased proportionally to the salinity.

Simultaneous effect of light, temperature and salinity on algal growth

Simultaneous effects of light, temperature and salinity on algal growth were examined using different combinations of these factors according to a 3^3 factorial design (all combination of 3 factors were done at 3 levels) (Table 1). The relationship between algal growth rates and the simultaneous effects of the factors studied was expressed by multiple regression (Table 2).

Table 1. Growth rates (day^{-1}) of *T. suecica* (T. s), *D. tertiolecta* (D. t) and *P. tricornutum* (P. t) in the different combinations of light intensity (Lux), temperature (°C) and salinity (‰).

Light intensity	Temperature	Salinity	k (day^{-1})		
			T. s	D. t	P. t
800	17	15	0.05	0.09	0.05
800	17	25	0.08	0.10	0.06
800	17	35	0.07	0.11	0.12
800	21	15	0.09	0.10	0.10
800	21	25	0.12	0.14	0.12
800	21	35	0.11	0.15	0.15
800	25	15	0.12	0.11	0.11
800	25	25	0.15	0.16	0.14
800	25	35	0.14	0.18	0.18
2000	17	15	0.16	0.12	0.15
2000	17	25	0.19	0.13	0.17
2000	17	35	0.18	0.15	0.20
2000	21	15	0.20	0.13	0.18
2000	21	25	0.22	0.18	0.19
2000	21	35	0.21	0.20	0.24
2000	25	15	0.22	0.15	0.19
2000	25	25	0.25	0.22	0.20
2000	25	35	0.24	0.23	0.26
4000	17	15	0.26	0.20	0.18
4000	17	25	0.29	0.21	0.20
4000	17	35	0.28	0.23	0.25
4000	21	15	0.30	0.21	0.20
4000	21	25	0.33	0.26	0.23
4000	21	35	0.32	0.28	0.28
4000	25	15	0.34	0.22	0.24
4000	25	25	0.37	0.32	0.26
4000	25	35	0.36	0.34	0.35

Table 2. Simultaneous effect of light (L), temperature (T) and salinity (SAL) on the growth rates of *T. suecica* (T. s), *D. tertiolecta* (D. t) and *P. tricornutum* (P. t).

Alga	Variable	R	R_p	a	b	β - coeff.	R^2
T. s	L	0.93	0.99		0.00007	0.93	
	T	0.31	0.88	- 0.146	0.00875	0.31	0.95
	SAL	0.08	0.45		0.00094	0.08	
D. t	L	0.80	0.95		0.00004	0.80	
	T	0.41	0.85	- 0.154	0.00820	0.41	0.91
	SAL	0.37	0.83		0.00300	0.37	
P. t	L	0.76	0.92		0.00004	0.75	
	T	0.37	0.76	- 0.150	0.00760	0.37	0.93
	SAL	0.43	0.80		0.00350	0.43	

R - coefficient of correlation
R_p - coefficient of partial correlation
a, b - coefficients of multiple regression
β - coeff. - regression coefficients stated in terms of their standard deviations
R^2 - coefficient of multiple determination

The coefficients of multiple determination (R^2), which measures the overall degree of association between the algal growth rate and independent variables was over 0.90 for all three species. That is, light, temperature and salinity account for more than 90% of the variance in the growth rates.

The relative importance of the factors studied was demonstrated by the coefficients of partial correlation. The correlation between growth rate and light intensity, the individual effects of temperature and salinity being excluded was very similar to that when the effects of these factor were included. The coefficient of correlation between growth rate and temperature, and between growth rate and salinity was considerably greater when the effect of light was removed. This suggested that variations in light intensity affected algal growth much more than the variation in temperature and salinity. Beta coefficients (β, regression coefficients stated in terms of their standard deviation) were also useful in determining the relative importance of these three independent variables. Each increase of one standard deviation in the values of light intensity will be accompanied (if temperature and salinity stay constant) by a decrease of 0.93, 0.80 and 0.75 standard deviation in the values of growth rate of *T. suecica, D. tertiolecta* and *P. tricornutum*, respectively (Table 2.). β-coefficient of light intensity was lower for *P. tricornutum* than for the other two species. It could be explained by the results published by NELSON *et al.* (1979). They obtained relatively high growth rate of *P. tricornutum* at low light intensity. The increase of light intensity had less effect on the growth of *P. tricornutum* than on any other species (FALKOWSKI *et al.*, 1978).

The β-coefficient values for temperature and salinity were much lower, suggesting that light intensity probably accounts for much more of the actual change in growth rate than do temperature and salinity. It was also evident that temperature have a greater effect than salinity on the growth rate of *T. suecica* and *D. tertiolecta*, what could be explained by reported tolerance of these species to changes in salinity (BEN-AMOTZ *et al.*, 1978; FABREGAS *et al.*, 1984). Salinity found to be of greater significance for the growth of *P. tricornutum.*

Effect of interaction between studied factors on the algal growth

Multifactor analysis of variance confirmed significant ($P < 0.001$) separate effects of light, temperature and salinity on algal growth rates.

A significant interaction (dependence of the effect of one factor on the level of another) between light intensity and temperature was found for all three phytoplankton species (Table 3). It indicated that the effects of light and tem-

Table 3. Multifactor analysis of variance. Effects of interaction between light (L), temperature (T) and salinity on the growth rates of *T. suecica* (T. s), *D. tertiolecta* (D. t) and *P. tricornutum* (P. t).

Spec.	Source of variation	SS	df	MS	F	P
T. s	MAIN EFFECTS	0.231	6	0.038	1000.0	< 0.0001
	INTERACTIONS	3.55E-4	12	2.96E-5	8.0	< 0.005
	L x T	3.26E-4	4	8.15E-5	22.0	< 0.001
	L x SAL	1.48E-5	4	3.70E-6	1.0	n.s.
	T x SAL	1.48E-5	4	3.70E-6	1.0	n.s.
D. t	MAIN EFFECTS	0.110	6	0.018	287.9	< 0.0001
	INTERACTIONS	5.38E-3	12	4.48E-4	7.0	< 0.01
	L x T	7.56E-4	4	1.89E-4	4.0	< 0.05
	L x SAL	5.78E-4	4	1.44E-4	2.3	n.s.
	T x SAL	4.04E-3	4	1.01E-3	15.8	< 0.0005
P. t	MAIN EFFECTS	0.117	6	0.020	217.9	< 0.0001
	INTERACTIONS	2.56E-3	12	2.13E-4	2.4	n.s.
	L x T	1.35E-3	4	3.37E-4	3.8	< 0.05
	L x SAL	7.04E-4	4	1.76E-4	2.0	n.s.
	T x SAL	5.03E-4	4	1.26E-4	1.4	n.s.

n.s. - not significant (P > 0.1)

perature were not simply additive but synergistic. That is, the simultaneous effect of light and temperature on the algal growth was found to be significantly greater than when each of these factors was acting independently. A synergistic effect between temperature and salinity was only found for *D. tertiolecta*.

In comparison to a number of studies dealing with separate effects of different environmental factors on the growth of algae, there are very few studies reporting combined effect of these factors. FABREGAS *et al.* (1984) studied growth of *T. suecica* in cultures with different salinities and nutrient concentration, and found salinity to be more important factor when it was related to the nutrient concentration.

This study demonstrated the importance of the combined effects of light intensity, temperature and salinity and their interaction on the growth of phytoplankton species important in aquaculture. This relationship was described by the equation which may approximate conditions relevant for a desirable growth rate of produced algal populations within studied ranges. From a practical point of view this knowledge could be useful in planning of rearing conditions in mass production. However, this area calls for further investigation.

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Istovremeno djelovanje intenziteta svjetlosti, temperature i saliniteta na rast nekih fitoplanktonskih vrsta važnih u akvakulturi

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KRATKI SADRŽAJ

Istodobno djelovanje intenziteta svjetlosti, temperature i saliniteta na rast fitoplanktonskih vrsta *Tetraselmis suecica, Dunaliella tertiolecta* i *Phaeodactylum tricornutum* u uvjetima masovnog uzgoja, istraživan je pomoću 3^3 faktorskog eksperimenta. Odnos rasta alga i istodobnog utjecaja ispitivanih faktora opisan je jednadžbama multiple regresije. Na osnovi koeficijenata parcijalne korelacije, te beta koeficijenata (β) utvrđeno je da su varijacije u intenzitetu svjetlosti imale znatno veći utjecaj na rast alga nego što su to imale varijacije temperature i saliniteta. Sinergistički efekt između intenziteta svjetlosti i temperature utvrđen je kod sve tri vrste alga.