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EFFECT OF TEMPERATURE ON THE PRESSURE INDUCED REPRODUCTION IN *CLADOPHORA VAGABUNDA* (L.) HOEK.

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Abstract

The post-treatment influence of hydrostatic pressure on *C. vagabunda* from western Baltic Sea was studied. The cells showed an abnormality in the protoplast, which induced a vigorous reproduction. They produced quadriflagellate zoospores with limited power of escaping through subterminal pores, which later metamorphosed to spherical cysts. The rate of reproduction increased with the rise of pressure intensity upto 600 atm but decreased with further increase of pressure. The difference in behaviour of this alga from that of *C. glomerata* led to the conclusion that it is an independent species.

The pressure influence was intensified at lower and retarded at higher temperatures. The antagonistic effects of these parameters may be generalized in the barobiology of marine algae.

Introduction

Interest in the role of high hydrostatic pressure in the physiology of marine algae derives from the knowledge that pressures affect equilibria and reaction rates in shallow-water seaweeds. It is obvious that for many of them sensitivity to pressure changes could well have significance for survival, and a rise in the ambient pressure, within the biological range, can produce many different kinds of effect (Shameel, 1973c). The combined influences of temperature and pressure have not yet received the attention they deserve, and studies on pressure-induced rate of reproduction in algae still remains untapped. An efficacious reproduction depending on the pressure intensity and the time of pressure action was observed in *Cladophora glomerata* (Shameel, 1973b). During an excursion in the western Baltic Sea a morphologically similar but taxonomically disputed (Hoek, 1963; Söderström, 1965) species, *C. vagabunda* was obtained. Keeping in view the aforesaid lacuna it appeared interesting to compare the physiological behaviour of *C. vagabunda* with its taxonomic akin and to study the influence of temperature on pressure action.

Materials and Methods

Cladophora vagabunda (L.) Hoek was collected from Stein, Kiel Bight. Unialgal cultures were obtained from clean and healthy vegetative filaments. They were cultured in 50% Erdschreiber medium, which was prepared with double filtered sea water of their place of occurrence by adding 500 ml soil extract, 0.2 g K_2HPO_4 and 1.0 g KNO_3/l; the pH was adjusted to 7.5. The cultures were grown in a temperature controlled room at 15°C±0.0z and were exposed to a light intensity of 3000 lux during the 16 hours' photoperiod. After 2 weeks' growth only the healthy and non-reproductive filaments of 40—60 cells were kept in plastic tubes of 25 ml capacity containing the culture medium and were subjected to high hydrostatic pressure at varying tempera-

tures for 5 hours after which they were brought back to 15°C and were periodically examined upto 3 weeks. The methods of pressure treatment and the precautions taken during microscopic observations were the same as described earlier (Shameel, 1976).

Results

The filaments of *Cladophora vagabunda* were preliminarily subjected to 400, 500, 800 and 1000 atm for 5 hours at 15°C to test their response to higher pressures. Immediately after exposure to all the pressure levels they exhibited big vacuoles within protoplasm, varying from small (3—19 μ dia.) to larger (20—36 μ dia.) ones (Fig. 1). These changes gradually disappeared and the cells started reproducing. The effect of a pressure level of 1000 atm was exceptional, the vacuoles produced remained unchanged till the algae died within one week.

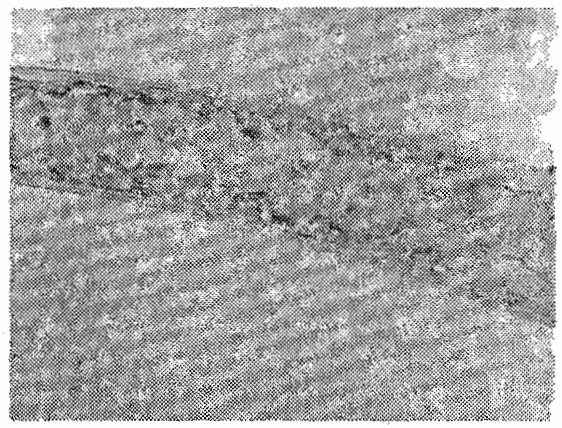

Fig. 1. Part of *Cladophora vagabunda* filament showing big vacuoles in the protoplasm immediately after subjection to 800 atm for 5 hours at 15°C.

The filaments were next exposed to 300, 400, 500, 600, 700, 800, 900 and 1000 atm for 5 hours at 15°C to find out the pressure level which induces the highest rate of reproduction. Subjection of algae to 300 atm, even upto 3 weeks induced no reproduction; the pressure level of 400 atm induced a mild reproduction 3 days after pressure treatment which gradually increased further (Table 1). As the pressure was increased from 400 atm the rate of reproduction gradually increased attaining a maximum rate at 600 atm. With a further increase of pressure, however, the rate of reproduction declined, and at 1000 atm no reproduction was induced.

During this investigation about 20% mortality was observed in the filaments exposed to 800 atm and 50% mortality in those subjected to 900 atm. All the filaments treated to 1000 atm died within one week. Furthermore it was observed that after exposure to 400—900 atm the rate of reproduction increased rapidly at the beginning and reached quite close to the maximum value in each case 1 week after subjection to pressure (Table 1). There was a slight increase in the rate of reproduction in

TABLE 1. Percentage of reproducing cells in *Cladophora vagabunda* after subjection to hydrostatic pressure for 5 hours at 15°C.

Pressure (atm)	Days after subjection to pressure						
	0	1	3	5	7	14	21
300	0	0	0	0	0	0	0
400	0	0	5	10	20	30	30
500	0	5	10	25	40	50	50
600	0	5	15	30	50	60	60
700	0	5	10	30	45	55	55
800	0	5	10	20	35	40	40
900	0	0	5	10	20	25	25
1000	0	0	0	0	0	0	0

the second week which remained unaltered during the third week. The control algae remained healthy and manifested no reproduction, throughout the period of investigation.

After the determination of optimum level of hydrostatic pressure for reproduction, *C. vagabunda* thalli were treated with 600 atm for 5 hours at 5°, 10°, 15°, 20°, and 25°C. During this time the control algae were also kept at the same temperature but at atmospheric pressure. Immediately after the pressure-temperature treatment they were brought back to 15°C and the intensity of induced rate of reproduction was determined. The algal cells showed an outburst of reproduction when treated with pressure at 5°C (Table 2), the only temperature which induced reproduction imme-

TABLE 2. Percentage of reproducing cells in *Cladophora vagabunda* after subjection to 600 atm for 5 hours at different temperatures.

Temperature (°C)	Days after subjection to pressure						
	0	1	3	5	7	14	21
5	5	20	50	60	70	80	80
10	0	10	20	35	55	65	65
15	0	5	15	30	50	60	60
20	0	5	10	25	40	45	45
25	0	0	5	20	35	40	40

diately after pressure release. After 2 weeks' time about 80% of the pressure treated cells reproduced while the control algae exhibited practically no reproduction. The reproduction-inducing effect of hydrostatic pressure gradually decreased with increasing temperature. Two weeks after pressure treatment the percentage of reproducing cells fell from 80 to 60% at 15°C, and further to 40% at 25°C. The control algae kept initially at 20°C for 5 hours also showed about 10% reproduction and those at 25°C exhibited 20% reproduction after 3 weeks.

The karyokinesis was followed by the accumulation of cytoplasm around each nucleus, which later became pear shaped and developed 4 equal sized flagella of terminal orientation. The swarmers were 14—16 μ long and 9—10 μ broad with a parietal chloroplast lying at one side of the posterior end. Although in most of the reproducing cells a small circular pore was produced next to the extremity, only 10% cells could escape. About 90% zooids discarded their cilia after an active phase of movement and were modified to spherical cysts, which were 1½—2 times bigger than the swarmers (Fig. 2). No pairing or fusion was noticed between the emerged zooids. Almost all the swarmers produced in the temperature-affected control algae emerged from the reproducing cells.

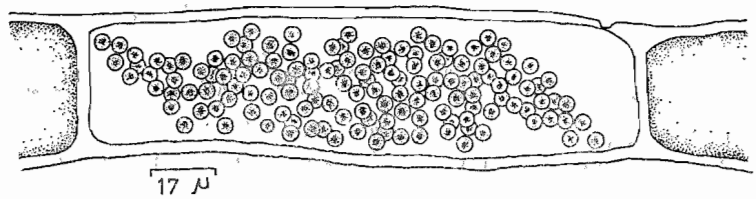

Fig. 2. Part of *Cladophora vagabunda* filament showing cyst formation 5 days after subjection to 600 atm for 5 hours at 15°C.

Discussion

Immediately after exposure to different pressure intensitities *Cladophora vagabunda* displayed large vacuoles, which gradualy disappeared. This vacuolization was probably a step towards the division of protoplast for the purpose of reproduction, which might be due to a gradual encroachment of the protoplasm on the central vacuole as has already been sugges ed (Shameel, 1973b). The mode of reproduction in the pressure-treated specimens of this species was similar to that observed in the case of *C. glomerata*. The swarmers produced by *C. vagabunda* were undoubtedly zoospores, because the zooids were quadriflagellate and agreed in their measurement with those given by Hoek (1963, p. 159) for zoospores produced by the specimens collected from the same locality, while gametes are always biflagellate and comparatively smaller in dimensions (Hoek, 1963; Pankow, 1971; Gams, 1974). Moreover no coupling or fusion was noticed in them, which confirms their asexual nature.

The present study provided an opportunity to compare the physiological characteristics and to elucidate the interspecific delimitations between morphologically overlapping *C. vagabunda* and *C. glomerata*. Though both the species resembled in their responses against hydrostatic pressure in their cell morphology as well as rate of reproduction, the former appeared to be more sensitive to pressure than the latter. *C. glomerata* did not show any changes in the morphology or reproduction after an exposure to 400 atm for 5 hours (Shameel 1973b), but its taxonomic akin. *C. vaga-*

bunda showed vacuolization of protoplast as well as a feeble reproduction after the same treatment. At all the comparable levels of pressure treatment the induced rate of reproduction in *C. vagabunda* was higher than *C. glomerata*. They also differ in their lowest and highest physiological limits of pressure tolerance, which lie at 300 and 700 atm respectively in the case of former alga as compared to 400 and 800 atm of the latter (Shameel, *loc. cit.*). As the subspecies or ecotypes of the same species behave similarly in their responses against pressure (Shameel, 1977). it becomes evident that *C. glomerata* and *C. vagabunda* are two independent species, and hydrostatic pressure induces specific responses in them.

C. glomerata reproduces only by asexual biflagellate zoospores (Hoek 1963; Söderström 1963; Sakai, 1964; Pankow, 1971; Wartenberg, 1972), but in the previous study we observed the swarmers with 2—4 flagella of equal size (Shameel, 1973b). Hoek (1963) found a casual aggregation and fusion of 2 biflagellate zoospores quite regularly in material collected from Kiel Canal. Our material was collected from a place very close to this locality, therefore the quadriflagellate swarmers observed by us might have resulted from the fusion, which probably escaped our observation (Shameel *loc. cit.*). It is true that bi-and quadriflagellate zooids were observed within the same zooidangium. Our attempts to study the type of nuclear division preceding zooidogenesis were unfortunately not successful.

Cladophora is not the only algal genus showing the pressure-induced reproduction. The discharge of gametes and zoospores has been noticed in other marine as well as fresh water algae after release of high hydrostatic pressure surrounding them (Table 3). Although a detailed investigation has not been carried out in any alga other than *C. glomerata*, their remains no doubt that the response of these green algae to high pressure is due to a metabolic shock which induces a sudden reproduction. How the higher algae, especially brown and red seaweeds respond to hydrostatic pressure would be an interesting problem of barobiology.

TABLE 3. Reproduction-inducing effect of high hydrostatic pressure observed on different green algae.

Green algae	Pressure (atm)	Duration (hour)	Observer
Chlorella ellipsoidea.	135 — 700	10 — 48	Lue-Kim, 1971
Chlorela pyrenoidosa	340	—	Vidaver, 1972
Cladophora glomerata	400 — 800	5 — 20	Shameel, 1973b
Cryptomonad (undet.)	300 — 500	5	Sturm. 1957
Ulva lobata	1000	—	Vidaver. 1972

The parameters of hydrostatic pressure and temperature manifest an interrelationship in their influence on the rate of reproduction. The low temperatures intensify the pressure effect and the higher ones prevent the pressure influence, that is why the pressure induced rate of reproduction is maximum at 5°C and minimum at 25°C. The latter temperature made the pressure very mild in its effect and reproduction was induced in only half of the cells as compared to those at former.

TABLE 4. Antagonistic effects of hydrostatic pressure and temperature observed on different biological reactions of seaweeds.

Biological reaction	Seaweed	Pressure (atm)	Temperature (°C)	Duration (hour)	Observer
Activity of alkaline phosphatase	*Delesseria sanguinea*	200 — 800	5 — 25	1	Shameel, 1975b
	Ecotypes of *D. sanguinea*	200 — 800	5 — 25	1	Shameel, 1977
	Fucus vesiculosus	200 — 800	5 — 25	1	Shameel, 1975b
Cell viability	*Delesseria sanguinea*	100 — 800	5 — 30	6	Shameel, 1973a
	Prophyra perforata	680 — 1360	15 — 30	1/6	Vidaver, 1969
Changes in cell morphology	*Bryopsis plumosa*	200 — 800	5 — 15	5	Shameel, 1976
Exudation of organic substances	*Fucus vesiculosus*	800	5 — 40	4	Shameel, 1978b
Growth:					
mature thalli	*Porphyra perforata*	680 — 1360	15 — 30	1/6	Vidaver, 1969
young thalli	*Delesseria sanguinea*	200	5 — 22	2	Shameel, 1975a
Photosynthesis:					
a-spike	*Porphyra perforata*	70 — 1430	10 — 30	—	Vidaver, 1969
	Ulva lobata	70 — 1430	10 — 30	—	Vidaver, 1969
O_2 gush	*Porphyra perforata*	70 — 1430	10 — 30	—	Vidaver, 1969
	Ulva lobata	70 — 1430	10 — 30	—	Vidaver, 1969
photosynthetic enhancement	*Porphyra perforata*	340 — 1160	5 — 25	—	Vidaver, 1969
rate of photosynthesis	*Delesseria sanguinea*	100 — 800	5 — 30	6	Shameel, 1973a
	Fucus vesiculosus	100 — 800	5 — 30	6	Shameel, 1973a
	Porphyra umbilicalis	600	5 — 30	5	Shameel, 1978a
steady O_2 evolution rate	*Porphyra perforata*	170 — 1230	10 — 30	—	Vidaver, 1969
	Ulva lobata	170 — 1230	10 — 30	—	Vidaver, 1969
Rate of respiration	*Delesseria sanguinea*	100 — 800	5 — 30	6	Shameel, 1973a
	Fucus vesiculosus	100 — 800	5 — 30	6	Shameel, 1973a
	Porphyra umbilicalis	600	5 — 30	5	Shameel, 1978a

Similar observations regarding the antagonistic effects of temperture and hydrostatic pressure on different biological reactions have been made in different seaweeds in our as well as other laboratories (Table 4). Therefore the pressure effects in relation to temperature may be generalized in the case of marine algae. Hydrostatic pressure is of complementary importance with temperature in influencing the rate of biological activities of marine algae. As a matter of fact it is clear that while hydrostatic pressure and temperature generally affect the biological reactions in opposite directions, it holds true over a narrow range only. There is an optimal pressure for each temperature, at which the biological response is greatest, and a change occurring in any parameter decreases either the rate of reaction or the biological response.

The control algae kept at 20°—25°C showed a slight reproduction. This was purely a temperature effect, therefore at 25°C the pressure effect was negligible. The formation of gametangia in *Halicystis parvula* (*Derbesia tenuissima*) and sporangia in *Undaria pinnatifida* increase with increasing temperature (Gessner, 1970). In many cases, seaweeds inhabiting sublittoral areas fructify during summer. In *Ulva lobata* abrupt changes in temperature, illumination or salinity also induce reproduction (Vidaver, 1972).

Generally the pressure-temperature effects are interpreted in terms of the theory of absolute reaction rate (Vidaver, 1969). Although the kinetics of biological reactions can, in general, be predicted by this theory, its application has certain drawbacks. Firstly, some heat-induced protein denaturations may occur with a decrease rather than an increase in the final volume. Secondly, irrespective of the recent investigations of Hochachka (1976) on deep-sea biochemistry it is not possible to directly measure activation volume changes. Apart from these obstacles this theory has been widely applied, due to the fact that reactions involving proteins are extremely sensitive to relatively small changes in pressure or temperature in comparison to the ordinary chemical reactions (Vidaver, *loc. cit.*). In analysing the responses of an alga to hydrostatic pressure and temperature, one is likely to find several mechanisms involved in the reactions.

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