Pak. J. Bot., 5 (1) : 1-9, 1973.

REPRODUCTION INDUCED BY HIGH HYDROSTATIC PRESSURE IN *CLADOPHORA GLOMERATA* (L.) KUETZ FROM BALTIC SEA.

MUSTAFA SHAMEEL*

Institut fuer Meereskunde, botanische Abteilung, Kiel (F.R. Germany)

Abstract

The after effects of hydrostatic pressure on the cellular morphology of *Cladophora glomerata* were studied. Two experimental series, one of 5 hours' and the other of 20 hours' pressure duration were conducted at 15°C; the pressure levels being: 400, 500, 800 & 1000 atm. Abnormalities in the morphology of algal cells increased with the rise of pressure. The pressure treated algae exhibited a vigorous reproduction, producing abnormally the bi-and quadriflagellated zoospores with limited power of escaping through subterminal pores. The retained zoospores metamorphosed themselves to spherical cysts. There was an initial increase in the intensity of reproduction with the increase of hydrostatic pressure as well as the time of pressure action. When both these factors reached the upper physiological limit of tolerance for the alga, the reproduction intensity decreased sharply.

Introduction

The organisms inhabiting the marine environment are exposed to different levels of hydrostatic pressure. The first laboratory research on the phyiological effects of high hydrostatic pressures was inspired by the discovery of the organisms during the voyage of the french vessel "Talisman" (1882-1883), in which the organisms were dredged from the depths of the sea as far down as 6000 m below the surface with corresponding pressure of above 600 atm. Interest on this topic has been sporadic and a number of workers have investigated different aspects of hydrostatic pressure effects on biological materials, specially the bacteria and animals (Zimmerman, 1970). The marine algae, however, were badly neglected in this connection.

Whereas hydrostatic pressure limits the extension of algae with gas filled bladders into deep waters (Damant, 1937), it generally plays no important role in the ecology of marine algae due to lack of light below euphotic zone. All metabolic processes occur in a liquid phase and since hydrostatic pressure applies only to fluid systems, it is not surprising that pressure exerts a profound influence on algal physiology. Fontaine (1929) using his very simple pressure technique was probably the first to give a preliminary information on physiological effects of pressure on a marine alga, *Ulva lactuca*. After 40 years Vidaver (1969) studied the hydrostatic pressure effects on photosynthesis of some marine algae. Shameel (1973) developed the new pressure techniques and made a detailed investigation on the hydrostatic pressure effects on the O_2 -gas exchange of a number of benthic algae. To analyse the pressure resistance, the effect of high hydrostatic pressure on the thallus weight of certain filamentous and foliacious algae has also been reported (Shameel & Ohno, 1972). In the present paper the effect of hydrostatic pressure as reproduction inducer is described.

*Present address: Institute of Marine Biology, University of Karachi, KARACHI-32 (Pakistan).

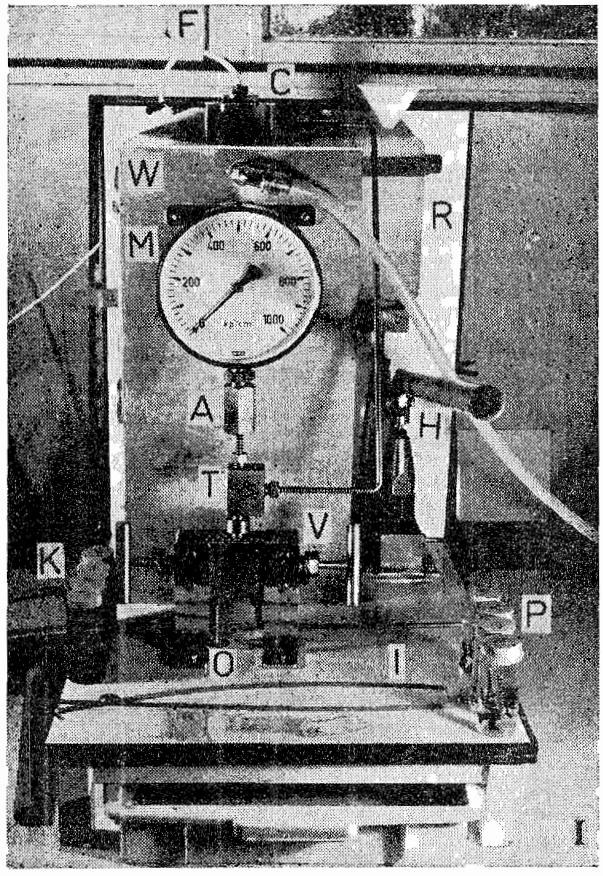

Fig. 1. Apparatus used for producing hydrostatic pressures upto 1000 atm with accessories. A=Adapter, C=upper part of the pressure chamber, F=for temperature measurement, H=Hydraulic pump, I=Iron band, K=Tubes with control algae, M=Manometer, O=Pressure outlet, P=Tubes with pressure treated algae, R=Reservoir for hydraulic fluid, T=Three-way piece, V=Double needle valve, W=temperature controlled water bath.

Materials and Methods

The material used in the present study was determined after Pankow (1971) as *Cladophora glomerata* (L.) KUETZ. The algae were collected near Moeltenort (Kiel Bight, western Baltic Sea) during July to October 1971 and were brought in thermos flasks within a few hours to the constant temperature rooms. They were kept in small plastic boxes (10 x 10 x 4 cm) in double filtered sea water (always adjusted to 15 ‰ S), in which 420 mg $NaHCO_3$/l was added. Air bubbling kept the water circulating and the illumination of about 5000 lux was obtained from cool white lamps (type: OSRAM universal white, 40 W), which alternated with a 12 hours' rhythm of light and darkness.

After allowing for two weeks' gradual adaptation from 5°, 10° to 15°C experimental temperature the healthy, uninjured, free from epiphytes and non-reproductive algae were selected and subjected to different hydrostatic pressures; pressure levels used were 400, 500, 800 and 1000 atm. For this purpose they were kept in PVC-tubes (P, Fig. 1) of 6.5 ml capacity and four such tubes were placed in the pressure chamber. The pressure apparatus (Fig. 1) consists of a hydraulic pump (H), a double needle valve (V) of AMINCO (U.S.A.), a manometer (M) and a pressure chamber (C) hanging in a temperature constant water bath (W). Reference may be made to Shameel (1973) for detailed description of this apparatus and the technique employed for exerting high hydrostatic pressure. The controls were kept in similar but black painted PVC-tubes (K). During pressure application the control tubes were kept in an aluminium chamber under similar experimental conditions except atmospheric pressure.

After the removal of pressure the algae were observed in culture medium under Zeiss Winkel microscope employing phase contrast assembly. The microscopy was done in aquarium, where the temperature was 15°C ± 3, the time of observation and the light intensity used were kept to a minimum to avoid sudden temperature changes. For the study of nuclear division the algal filaments were kept in picro-aniline blue solution for 10 minutes in covered Petri dish (Gerlach, 1969, p.204). Later on they were washed thoroughly with tap water and were finally mounted in glycerine as a temporary preparation.

Results

The pressure effects on *Cladophora glomerata* kept under 400, 500, 800 and 1000 atm for 5 hours at 15°C are shown in Table 1. The algal thalli exhibited no morphological changes after treatment to 400 atm, but after subjection to 500 atm big vacuoles measuring 2-15 μ in diameter were visible within protoplasm. These vacuoles disappeared slowly. After 24 hours 95% of the changed cells showed this abnormality whereas after 2 days 80%, after 3 days 60%, after 4 days 30% and after 5 days no morphologically changed cells were left. The control algae showed no such vacuole formation.

When the pressure was increased to 800 atm, the size of the vacuoles also increased. The smaller vacuoles were 3-20 μ in diameter and the bigger ones measured 20-36 μ (Fig. 2A). The vacuoles disappeared quickly as compared to those of 500 atm. After 24

TABLE 1. Percent of morphologically changed cells in *Cladophora glomerata* after subjection to hydrostatic pressure for 5 hours at 15°C.

Pressure applied (atm)	Days after subjection to pressure					
	0	1	2	3	4	5
400	0	0	0	0	0	0
500	100	95	80	60	30	0
800	100	20	5	0	0	0
1000	100	100	100	100	100	100

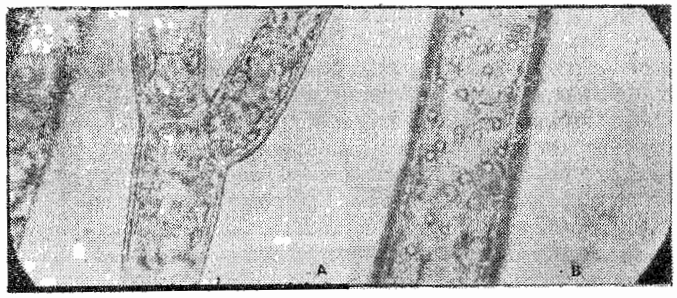

Fig. 2. Part of *Cladophora glomerata* filaments showing cellular morphology, A. immediately after subjection to 800 atm for 5 hours at 15°C. B. control alga kept under similar conditions except atmospheric pressure.

hours there were only 20% changed cells to be seen, after 2 days 5% and after 3 days practically no changed cells were left. After subjection to 1000 atm the size of the vacuoles remained the same as above, but the change was irreversible and the algae died after one week. The controls (Fig. 2B) on the other hand remained healthy and unchanged.

During the course of the investigation it was observed that the pressed algae exhibited a vigorous reproduction as compared to the controls. Therefore two experimental series were set up: in one series the pressure duration was 5 hours and in the other 20 hours. The pressure levels used were 400, 500, 800 and 1000 atm, and the microscopic observations followed periodically up to 3 weeks after pressure treatment as shown in Table 2.

TABLE 2. Percentage of reproducing cells in *Cladophora glomerata* after subjection to hydrostatic pressure at 15°C.

Pressure applied (atm)	Duration of Pressure (hours)	Days after subjection to pressure						
		0	1	3	5	7	14	21
400	5	0	0	0	0	0	0	0
400	20	0	0	0	5	10	20	20
500	5	0	0	5	20	40	50	50
500	20	20	30	40	60	90	90	90
800	5	5	5	10	15	25	30	30
800	20	0	0	0	0	0	0	0
1000	5	0	0	0	0	0	0	0

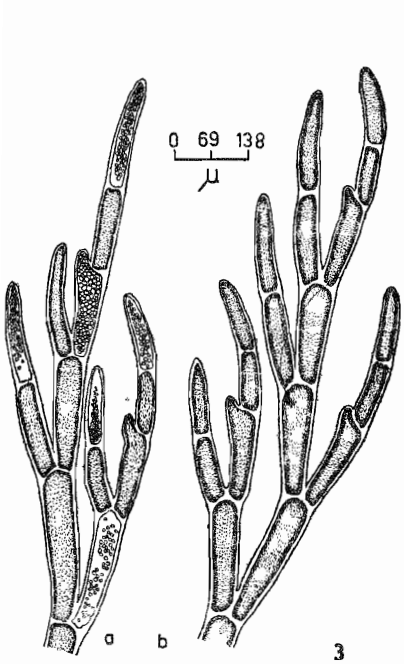

Fig. 3. Filaments of *Cladophora glomerata* a. zoospore formation immediately after subjection to 500 atm for 20 hours at 15°C. b. control thallus at atmospheric pressure.

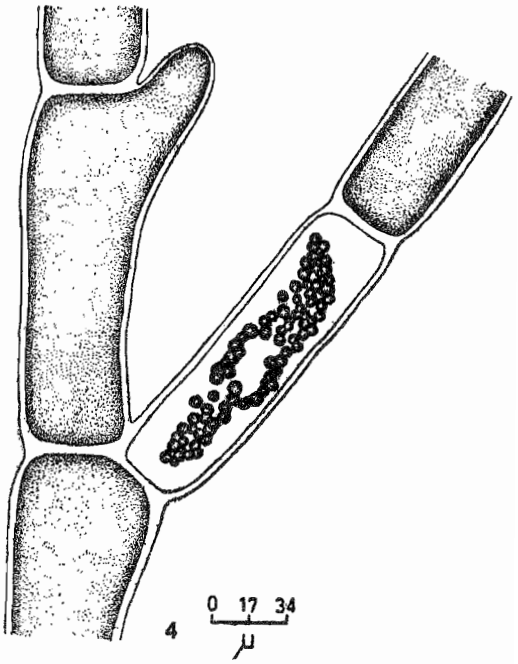

Fig. 4. A part of *Cladophora glomerata* filament showing cyst formation 2 days after subjection to 500 atm for 20 hours at 15°C.

The algae showed practically no change as compared to the control after 5 hours' subjection to 400 atm. As the pressure duration was increased to 20 hours, the algal cells remained morphologically unchanged but about 5% of them exhibited a noticeable reproduction on the 5th day. After one week about 10% of the pressed cells showed reproduction. The percentage of the reproducing cells increased to 20% after 2 weeks with no further increase at subsequent observations of 3 week's experimental period. The control algae exhibited no reproduction.

The algae pressed under 500 atm for 5 hours exibited morphologically changed cells with big vaculoes within the protoplasm. This change decreased in due course of time and was replaced by increasing intensity of reproduction. On the 3rd day after pressure treatment 5% of the cells showed reproduction, on 5th day it was seen in 20% of the cells. On 7th day the reproduction increased to 40%, in 2 weeks it became 50% with no further increase thereafter. However as the pressure duration was increased to 20 hours, about 20% cells exhibited reproduction (Fig. 3a) and 80% cells showed cell changes immediately after pressure removal. With increasing duration the reproduction increased to a maximum of 90% in a week with corresponding decrease in the cellular changes. There was no further change. The controls (Fig. 3b) showed practically no reproduction.

The algae kept under 800 atm for 5 hours showed 95% changed cells (Fig. 2A) and 5% reproducing cells immediately after pressure removal. In 3 days the reproducing cells increased to 10% and there were about 30% dead cells. In 5 days there were

no changed cells to be seen, 50% cells were dead and 15% of the living cells showed reproduction. In a week 60% cells were already dead and 25% of the living cells exhibited reproduction. In 2 weeks 30% of the surviving cells were showing reproduction with no further increase thereafter (Table 2). As the pressure duration was increased to 20 hours, 20 % cells were already dead and 80% changed very strongly, as seen immediately after pressure release. In 24 hours after pressure removal 50% cells were dead and in 3 days the mortality increased to 100%. There was no reproduction. The algae subjected to 1000 atm for 5 hours also showed no reproduction, but irreversible changes in cell morphology.

The mode of reproduction in the pressure treated algae was very peculiar. Karyokinesis was followed by accumulation of cytoplasm around each nucleus. The cells thus appeared being filled with rounded small bodies, which later on became pear-shaped and developed 2-4 flagella of equal size. These zooids were 10. 5 - 12.0μ long and 7. 5- 10.0μ broad and had a small parietal chloroplast lying at the posterior end of each zooid. After the development of cilia they showed very active movement. Meanwhile a small circular pore was produced always at the uppermost end of the lateral wall, in the terminal cells it being next to the extremity. It is surprising that in only a few cells the zooids were able to come out, although the opening was present. In only 2% of the reproducing cells 3/4th of the zooids succeeded in coming out, in 5% cells half of the produced zooids emerged and in 10% cases 1/4 th zooids managed to escape. The remaining ones slowly lost their movement, discarded the cilia and finally modified themselves into some what spherical cysts or thick walled aplanospores encrusted with $CaCO_3$ (Fig. 4). These cysts were $1\frac{1}{2}$—2 times bigger than the zooids. In no case a pairing or fusion between two escaped zooids was observed. Attempts made to study the type of nuclear division preceding zooid formation were not successful. In no nucleus the chromosomes were rendered distinctly visible.

When similar experiments were conducted with *Acrosiphonia centralis*, *Bryopsis plumosa*, *Chaetomorpha linum*, *Ectocarpus siliculosus* and *Spongomorpha lanosa*, no reproduction was induced by hydrostatic pressures even upto 800 atm.

Discussion

This study elucidates the after effects of hydrostatic pressure on cellular morphology. The effects of this parameter on biological systems become extremely important since upto 400 atm there was no observable change in the cells of *Cladophora glomerata*, but beyond that the cells exhibited abnormalities. This is probably the lowest physiological limit of tolerance for the alga. The abnormalities in the morphology of algal cells increased with the increasing pressure. Upto 800 atm these changes were reversible, thus showing the highest physiological limit of tolerance. Beyond this limit the hydrostatic pressure behaved to be lethal, and the algae died after one week. It may be mentioned that *Ulva lactuca* exposed to 800 atm for an hour usually died within a few days (Fontaine, 1929). This upper physiological limit of tolerance thus seems to be variable in different marine algae.

The lethality is most probably due to a disruption in the outer plasma membrane of the cell. Pressure application can affect the regulatory function of plasma membranes (Murakami, 1963). *Chaetoceros decipiens* collected from a depth of 40m showed chromatophores aggregated in the centre, while the surface phytoplankton exibited scattered chromatophores (Saifullah & Steven, 1973). High hydrostatic pressure could cause a solation of the chromatophore membrane, which would then assume a spheroid

shape, due to structural alteration of the constituent proteins. Animal cells tend to become spherical under pressure, presumably because of loss of protein structure (Marsland & Brown, 1936). Vidaver (1969) observed a pressure induced disruption of the chloroplast in *Ulva lobata* cells. Shameel & Ohno (1972) found out that after subjection to hydrostatic pressures between 750 and 1000 atm on *Delesseria sanguinea* and *Phycodrys sinuosa* the plastid membranes were ruptured and the pigment molecules were dissolved in cytoplasm. In these circumstances the cell viability becomes doubtful. Sustained application of high hydrostatic pressure thus appears to be lethal to algal tissues. Although pressures of a few hundred atmospheres do not bring any drastic change in the molecular volume of the cell constituents (Shameel, 1973), yet any change, influencing the different macromolecules within the cell, may be effective enough to disturb the equilibria of the synchronous biochemical pathways, which in turn might cause a disorder in the metabolism leading finally to the death of the cell.

The decreasing cell morphological changes with simultaneous increase in the rate of reproduction suggest that these changes are actually a step towards division of protoplast for the purpose of reproduction, which might be due to a gradual encroachment of the protoplasm on the central vacuole. After completion of the nuclear divisions there is a progressive cleavage into uninucleate protoplasts by means of a progressive vacuolization (Czempyrek, 1930). This pressure induced intensity of reproduction depends on the amount of hydrostatic pressure applied as well as the duration of pressure action. With the increase of both these factors the reproduction intensity also increases, but when these factors have reached the upper physiological limit of tolerance for the alga, it decreases sharply. Beyond this limit both the factors act harmfully. This confirms the previous observations made on the O_2-gas exchange of non-filamentous benthic algae (Shameel, 1973).

Sturm (1957) has suggested that the pressure action is composed of two components: one of them depends on the quantity of stimulus, which is a product of pressure level and the duration of pressure application. At the beginning it is stimulatory and then becomes injurious to the algal growth. The other one is determined mainly by the quantity of pressure applied and causes a reversible injury to the growth of algae upto a definite pressure level. Observations made on the cellular morphology and the reproduction intensity of *C. glomerata* can thus be explained on a similar basis, thus indirectly confirming the above mentioned hypothesis.

The zooids produced by the reproducing cells are most probably zoospores, because no coupling or fusion between them was observed. Moreover the pear shaped quadriflagellate nature of the zooids confirms this view. Apart from this the biflagellate zooids were also present, which might be confused with gametes, as gametes are always biflagellate in different species of *Cladophora* (Czempyrek, 1930). These biflagellate zooids are also zoospores, because their measurement does not coincide with that of gametes as given by List (1930). Although in *Cladophora* the zoospores, are generally quadriflagellate, but in *C. glomerata* they are known to be biflagellate (List, 1930; Schussnig, 1928b), The period, in which algae were collected, is also indicative of zoospore formation. In *C. glomerata* zoospore-formation takes place at intervals throughout the year, while gametes are only formed in spring at the end of a longer period of zoospore-formation (List, 1930).

C. glomerata individuals are diploid and unlike other species of this genus this is atypical in that nuclear divisions immediately preceding formation of zoospores are mitotic, meiosis occuring just before gametogenesis (List, 1930; Schussnig, 1928a).

Certain peculiarities in the formation of chromosomes and in their mode of separation were, however, noted in the first nuclear divisions leading to the formation of zoospores (List, 1930). Attempts made to study the type of nuclear division preceding zoospore formation in the pressure treated algae failed partly due to methodological difficulties and partly due to small size of chromosomes.

Coincident with the cytoplasmic cleavage there is a development of a small lens-shaped area in the lateral wall. The gelatinization and bursting of this area produce a lateral circular pore through which the zoospores escape (List, 1930). The formation of a pore at the uppermost end of the lateral wall and the retention of zoospores by pressure treated algae are probably the results of a disturbed metabolism. Formation of thick walled aplanospores, which are dormant stages in the life cycle, is probably induced due to unfavourable condition, here being high hydrostatic pressure. In *C. glomerata* the cells of a number of short branched flaments become filled with food reserves and are sometimes encrusted with carbonate of lime. They are produced during unfavourable conditions of the environment. These structures persist after the remaining parts of the thalli have disappeared and have given rise to new threads in the next season (Cholnoky, 1930).

Vidaver (1972) has observed a discharge of gametes and zoospores in *Ulva lactuca* thalli immediately after release of pressures upto 1000 atm. Sturm (1957) also observed in a colourless and not clearly determined Cryptomonad a rapidly increasing reproduction after subjection to hydrostatic pressures of 300—500 atm for 5 hours. The response of algae to hydrostatic pressure in all such observations seems to be due to a metabolic shock, which induces a sudden reproduction.

Occurrence of no reproduction in the pressure treated *Acrosiphonia centralis*, *Bryopsis plumosa*, *Chaetomorpha lin m*, *Ectocarpus siliculosus* and *Spongomorpha lanosa* suggests that different algae of similar morphological constitution respond to hydrostatic pressure quite differently. Although some of them are very sensititive to pressures (Shameel & Ohno, 1972), the differences in response are probably due to different enzyme systems of these algae whose proteinacious constituents undergo change in their molecular volume differently under the same level of hydrostatic pressure. Many of the observed pressure effects on intact cells may be directly related to the effects of pressure on the protein components of the membrane systems. There is no evidence to indicate what effect pressure might have on lipids in general and on lipid component of the membrane systems.

Acknowledgements

I am greatly indebted to Late Prof. Fritz Gessner and the members of "botanische Abteilung, Institut fuer Meereskunde, Kiel" for their cooperation and help in conducting this research. I wish to record my sincere thanks to D.A.A.D., Bonn (F.R. Germany) for providing me the financial support for this investigation. I am thankful to Dr. S.A. Qadir for going through this manuscript critically.

References

Cholnoky, B. 1930. Die Dauerorgane von *Cladophora glomerata*. Zeitschr. Bot., **22**: 545-585.

Czempyrek, H. 1930. Beitrag zur Kenntnis der Schwaermerbildung bei der Gattung *Cladophora*. Arch. Protistenk., **72**: 433-452.

Damant, G.C. 1937. Storage of oxygen in the bladders of the seaweed *Ascophyllum nodosum* and their adaptation to hydrostatic pressure. J. Exper. Biol., **1**: 198-209.

Fontaine, M. 1929. De l'influence comparee de la pression sur la respiration et la photosynthese des algues. Compt. Ren. Soc. Biol., **53**: 912-91.

Gerlach, D. 1969. Botanische Mikrotechnik. Georg—Thieme Verlag, Stuttgart., 298 pp.

List, H. 1930. Die Entwicklungsgeschichte von *Cladophora glomerata* KUETZ. Arch. Protistenk., **72**: 453-481.

Marsland, D.A. and D.E.S. Brown. 1936. Amoeboid movement at high hydrostatic pressure. J. Cell. Comp. Physiol., **8**: 167-178.

Murakami, T.H. 1963. Effect of high hydrostatic pressure on the permeability of plasma membrane under the various temperatures. Symp. Cell. Chem., **13**: 147-156 (in japanese).

Pankow, H. 1971. Algenflora der Ostsee. I. Benthos. Gustav Fischer Verlag, Stuttgart., 419 pp.

Saifullah S.M. and D.M. Steven. 1973. Seasonal succession of certain species of phytoplankton from St. Margaret's Bay, N.S. Pak. J. Bot., **5**: 11-17

Schussnig, B. 1928a. Die Reduktionsteilung bei *Cladophora glomerata*. Oesterr. Bot. Zeitschr., **77**: 62-67.

Schussning, B. 1928b. Zur Entwicklungsgeschichte der Siphoneen I. Ber. Deutsch. Bot. Ges., **46**: 481-490.

Shameel, M. 1973. Untersuchungen ueber den Einfluess des hydrostatischen Druckes auf den O_2-Gaswechsel mariner benthischer Algen. Int. Revue ges. Hydrobiol., **58** (5): (in press).

Shameel, M. and M. Ohno. 1972. Some observations on the effect of high hydrostatic pressure on the thallus weight of certain marine algae. Rep. Usa Mar. Biol. Stat., **19** (2): 1-6 (in japanese).

Sturm, G. 1957. Die Wirkung hoher hydrostatischer Druecke auf Suesswasseralgen. Archiv f. Mikrobiol, **28**: 109-125.

Vidaver, W. 1969. Hydrostatic pressure effects on photosynthesis. Int. Revue ges. Hydrobiol., **54**: 697-747.

Vidaver, W. 1972. Pressure—plants. In O. Kinne (ed.): Marine Ecology, Vol. I, Part 3, Wiley Inter—Science, New York., pp. 1389—1405.

Zimmerman, A.M. 1970. High pressure effects on cellular processes. Acad. Press, New York., 324 pp.