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COMBINED EFFECTS OF HYDROSTATIC PRESSURE AND TEMPERATURE ON THE ACTIVITY OF ALKALINE PHOSPHATASES FROM *DELESSERIA SANGUINEA* (L.) LAMOUR.

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Abstract

Activity of alkaline phosphatases from different growth forms of *D. sanguinea* from Baltic Sea and North Sea was measured under hydrostatic pressures varying from 200—800 atm at 5° and 25°C. Increasing pressure caused a decrease in the enzyme activity at 5°C but accelerated the activity at 25°C in all the growth forms. Combined effects of pressure and temperature have been explained on the basis of volume changes in the enzymatic reaction system. The pressure resistance was found to increase with the increasing morphological complexites of the thallus of different growth forms (f. *filiformis* < f. *lanceolata* < Baltic-Sea-form < Kattegat-form < North-Sea-form). It was concluded that Baltic-Sea-form, Kattegat-form and North-Sea form are merely ecophenes of the same variety i.e. *D. s.* f. *sanguinea*; and *D. s.* f. *filiformis*, *D. s.* f. *lanceolata* and *D. s.* f. *sanguinea* are the only three taxonomic varieties found in the waters of Baltic Sea and North Sea.

Introduction

Barobiology is almost 90 years old (Flügel, 1972), and the effects of hydrostatic pressure on many morphological, ecological and physiological characteristics of marine organisms are well studied (Zimmerman, 1970; Brauer, 1972; Kinne, 1972; Sleigh & Macdonald, 1972; Shameel, 1973b; Macdonald, 1976). The information regarding the combined effects of temperature and pressure on enzyme activities of marine organisms is however meagre (Shameel, 1975c), only in recent years extensive studies have been made on this aspect (Hochachka, 1976). In a previous attempt it was shown that the activity of alkaline phosphatase in some seaweeds decreased by high hydrostatic pressures at low temperatures, but at higher temperatures the activity accelerated under the same pressure conditions, and further more the changes in the enzyme activity were found to be greater in *Delesseria sanguinea* than in *Fucus vesiculosus* (Shameel, 1975b). Moreover *D. sanguinea* is very sensitive in the rates of respiration, photosynthesis and thallus growth against pressure and temperature (Shameel, 1973a, 1975a). Therefore it appeared quite interesting to study in detail the activity of alkaline phosphatases obtained from the different growth forms of *D. sanguinea* occuring in Baltic Sea and North Sea under the same conditions of hydrostatic pressure and temperature.

Delesseria sanguinea (L.) Lamouroux is a sublittoral, deep water, red alga of the order Ceramiales. It occurs in five morphologically different growth forms in Baltic Sea and North Sea: (1) North-Sea-form—which has a strongly constructed,

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brown red thallus with leaf-blades measuring upto to 10 cm in breadth and 15 cm in length; (2) Kattegat-form—which has a strong, red coloured thallus with leaf-blades of about the same dimensions as the North-Sea-form; (3) Baltic-Sea-form or *D. s.* f. *Sanguinea* (L.) Lamour.—which has a delicate, light red thallus with very small leaf-blades of 1—2 cm breadth and 5—8 cm length (Shameel, 1973a); (4) *D. s.* f. *lanceolata* C. A. Ag.—in which leaves are extremely elongated and narrow, and leaf-blade is only upto 5 mm broad; and (5) *D. s.* f. *filiformis* Levering—in which thallus is extremely reduced to thread like structures, 60—300 µm in diameter (Pankow, 1971). It is not quite clear whether these growth forms are real taxonomic varieties or merely ecotypes (Shameel 1973a). The idea of the present work was also to study this problem.

Materials and Methods

The North-Sea-form was dredged from Heligoland, the Kattegat—form from Fornäs, the Baltic-Sea-form from Kieler Aussenförde, and f. *filiformis* and f. *lanceolata* from Boknis Eck (Fig. 1). The algal thalli were kept in double filtered

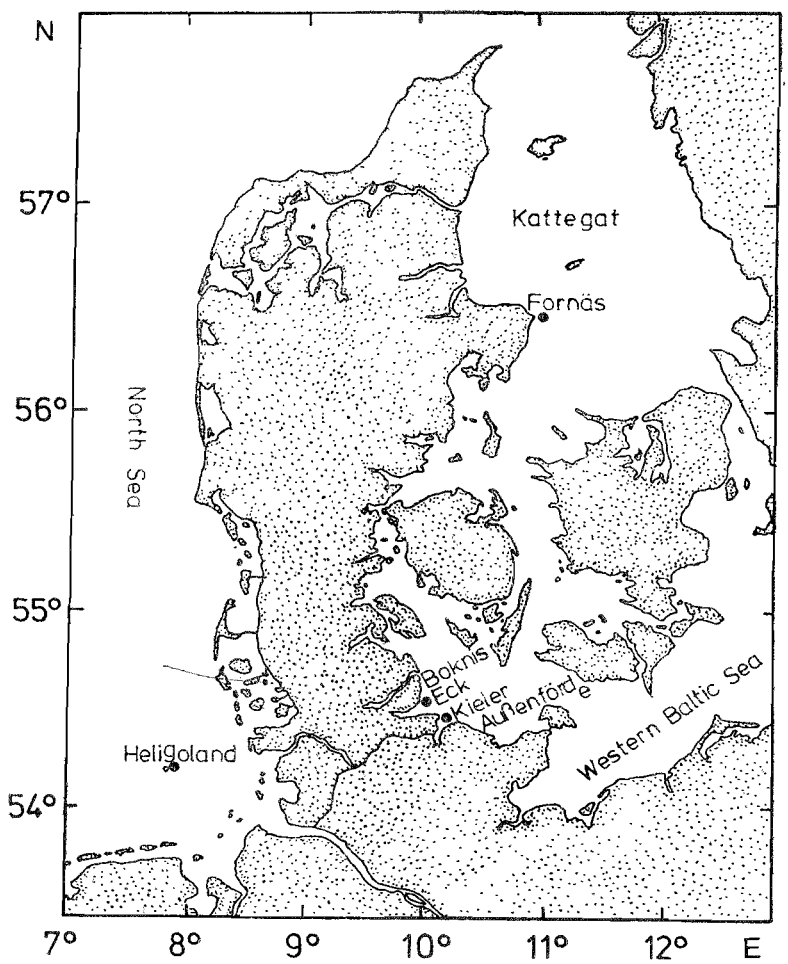

Fig. 1. Map showing the places (●) in Baltic Sea and North Sea, where from the different growth forms of *D. sanguinea* were dredged

sea water collected from their respective place of occurrence, to which 420 mg $NaHCO_3$/l was added, in quadrangular plastic boxes of 10 X 10 X 4 cm at 5°C for a few days. Eight such boxes were placed in 2 rows and aerated as shown in Fig. 2. Air bubbling kept the water circulating and the illumination (5000 lux) was obtained from cool white lamps of 40 W, which alternated with a 12 hours' rythm of light and darkness.

Two experimental series for 5°C and 25°C were set up under pressure levels of 200, 400, 600 and 800 atm. For the details of pressure apparatus and techniques employed reference may be made to Shameel (1973a). The alkaline phosphatase activity was measured as mentioned previously (Shameel, 1975b). For every tempe-erature — pressure combination 8 independent experiments were conducted. mean value of enzyme activity thus obtained at one tempera and ture hydrostatic pressure was compared with the values measured at the same temperature and atmospheric pressure set at 100. The relative enzyme activity was thus expressed as "percent of control".

Results and Discussion

The relative alkaline phosphatase activity obtained from five different growth forms of *Delesseria sanguinea* show one thing in common that temperature and hydrstatic pressure are antagonistic to each other in their effects (Fig. 3). At 5°C the enzyme activity was decelerated by step-wise increase of pressure from 200 to 800 atm, while at 2°C the increasing pressure accelerated the activity progressively in all of the 5 growth forms. The lower temperature, therefore appears to be more severe in effect than the higher one. It has been observed that the activity of lactate dehydrogenase (LDH) of *Asterias rubens* was increasingly inhibited with augmenting

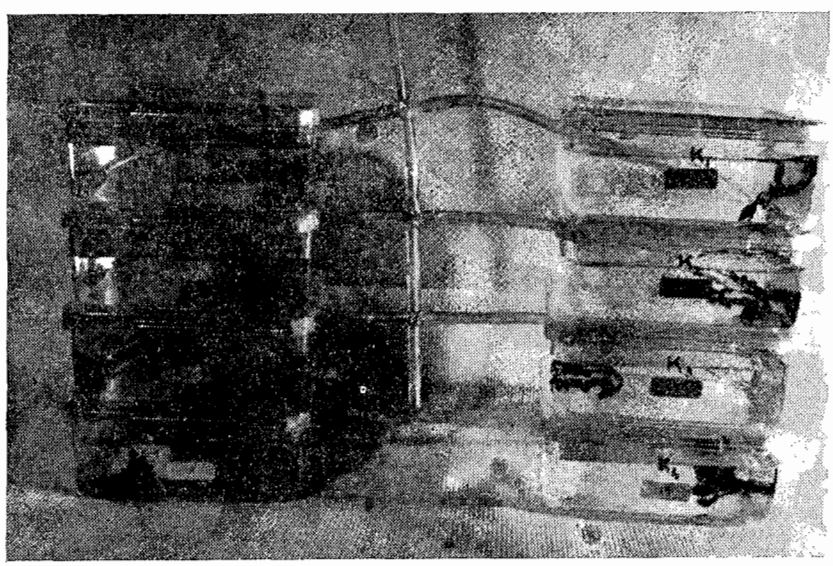

Fig. 2. Arrangement for culturing 8 simultaneous samples of algal specimens with the centrally running aeration system

pressure and decreasing temperature (Neuhoff & Theede, 1975). Alterantive non-covalent contributions to binding substrate and substrate analogue by LDH were found to be stabilized at low temperatures and high pressures in *Antimora rostrata* (Hochachka, 1976). High hydrostatic pressure and low temperatures are the two environmental parameters which directly affect on the function of enzyme in deep -sea organisms, therefore their mode of action must be clearly understood, and for that purpose one has to determine first the thermodynamic parameters.

The effect of hydrostatic pressure on enzyme reaction rate depends on the volume change occuring during activation of enzyme—substrate (ES)—complex. Pressure will affect the reaction rate if the volume of the system containing the ground state reactants differs from the volume of system containing the activated reactants.

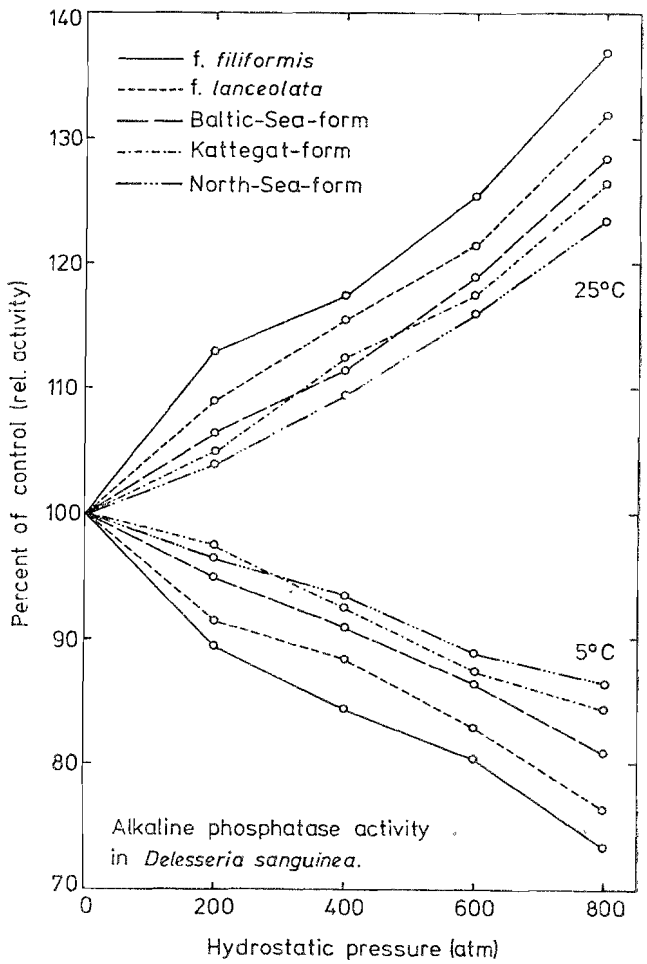

Fig. 3. Relative enzyme activity expressed in terms of control percentage in different growth forms of *D. sanguinea* (L.) Lamour. as affected by high hydrostatic pressures at 5° and 25°C.

and pressure will affect the equilibrium state of the reaction if the volume of the system containing reaction products differs from the volume of the system containing reactants. At constant temperature and pressure the enthalpy change during reaction may be obtained by the equation:

$$\triangle H = \triangle E + P\triangle V$$

where E is internal energy, P is hydrostatic pressure and V is volume indicating pressure-volume work performed (Low & Somero, 1975). The standard free energy change can thus be found out as:

$$\triangle G\ddagger = \triangle E + P\triangle V - T\triangle S$$

where T is absolute temperature and S entropy. The free energy of activation of the reaction may similarly be determined as:

$$\triangle G\ddagger = \triangle E\ddagger + P\triangle V\ddagger - T\triangle S\ddagger \text{ , therefore}$$
$$= \triangle H\ddagger - T\triangle S\ddagger \text{ , whereas}$$
$$\triangle H\ddagger = Ea - RT$$
$$\triangle S\ddagger = 4.576\ (\log k - 10.753 - \log T + \frac{Ea}{4.576})$$

where Ea is the external energy of activation, R is gas constant and k is the rate constant (modified after Lehrer & Barker, 1970).

Under elevated pressure the positive $\triangle V\ddagger$ will lead to an increase in $\triangle G\ddagger$, therefore increased pressure will favour the conversion of A to B, if in the equilibrium A=B the volume of the system containing A is greater than the volume of the system containing B. In a similar way hydrostatic pressure will accelerate the reaction velocity, if the volume of the system containing the ground state ES-complex is greater than the volume of the system containing the activated complex (ES). The volume change of activation is obtained by the equation:

$$\triangle V\ddagger = 2.3\ RT\frac{\log kp_1 - \log kp_2}{p_2 - p_1}$$

where kp_1 and kp_2 are velocity constants at pressures p_1 and p_2 (modified after Johnson & Eyring, 1970).

The influence of temperature on enzyme reaction rates can be described by Arrhenius equation. where rate constant is found out as:

$$k = Ae^{-ER/T}$$

where A is a constant and E is the activation energy (Baldwin *et al.*, 1975). Temperature effects are based on the formation of a high energy activated complex, which later on decomposes to give free enzyme and products. Probability of forming the activated complex governs the rate of overall reaction and in turn depends on the free energy of activation and temperature. Therefore at a given temperature the reaction rate is determined by the magnitude of $\triangle G\ddagger$ Temperature and pressure interact, so that enzyme reaction rates depend on the relative contributions of these

parameters. While increasing temperature always tends to increase the kinetic energy of the reactants and therefore to increase enzyme reaction rate, increasing hydrostatic pressure may increase, decrease or have no influence on the reaction rate which depends on the relative volumes of activated ES-complex and the reactants.

The five growth forms of *D. sanguinea* differ in their pressure resistance. As far as the pressure influence on alkaline phosphatase activity is concerned *D. s.* f. *filiformis* appears to be the least resistant and North-Sea-form to be the most resistant (Fig. 3). It is probably due to the reason that North-Sea-form lives in the normal conditions of salinity (30‰ S), which is typical of the world oceans, and *D. s.* f. *filiformis* inhabits the already stressed condition of low salinity (15‰ S). It has already been observed that the pressure resistance of *D. sanguinea* increases with increasing salinity in the external medium (Shameel, 1973a). According to their pressure resistance the five growth forms of this species may be arranged as follows:

North-Sea-form > Kattegat-form > Baltic-Sea-form > f. *lanceolata* > f. *filiformis*.

The pressure resistant forms avoid the danger of metabolic disturbances, if they are able to maintain the pressure induced changes in the enzyme activity within a limit. It is of great interest to observe that the pressure resistance of the different growth forms of *D. sanguinea* increases with the increasing morphological complexities of the thallus. The pressure resistance may be considered as a genetically fixed, physiological characteristic of different varieties of the same species.

It is quite interesting to note that *D. sanguinea* f. *filiformis* and *D. s.* f. *lanceolata* are distinct in the combined effects of pressure and temperature on their enzyme activity as compared to the other forms, but the other three growth forms do not differentiate among themselves so clearly (Fig. 3). This confirms the previous assumption that the morphological differences between Baltic-Sea-form, Kattegat-form and the North-Sea-form are not due to genetically fixed characters but they are mainly due to the differences in the hydrographic conditions of their places of occurence (Shameel, 1973a). It would therefore appear that these three forms have no taxonomic significance and are merely three ecophes of the same variety i.e. *D. s.* f. *sanguinea*. Therefore from the taxonomic point of view there are only three varieties of *D. sanguinea* found in the waters of Baltic Sea and North Sea: *D. s.* f. *filiformis*, *D. s.* f. *lanceolata* and *D. s.* f. *sanguinea*.

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References

Baldwin, J., K.B. Storey, and P.W. Hochachka, 1975. Lactate dehydrogenase M_4 of an abyssal fish: strategies for function at low temperature and high pressure. Comp. Biochem. Physiol., **52B:** 19-23.

Brauer, R.W. 1972. Barobiology and the experimental biology of the deep sea. N. Carolina Sea Grant Prog., Chapel Hill, 428 pp.

Flügel, H. 1972. Ninety years high-pressure research. Proc. R.S. (B), **73:** 279-285.

Hochachka, P.W. 1976. Biochemistry at depth. Pergamon Press, N.Y., 202 pp.

Johnson, F.H. and H. Eyring, 1970. The kinetic basis of pressure effects in biology and chemistry. *In:* Zimmerman, A.M. (ed.) 1970, pp. 1-44.

Kinne, O. 1972. Marine ecology. Vol. I, Part 3, Wiley Inter-Science, N.Y., pp. 1323-1450.

Lehrer, G.M. and R. Barker, 1970. Conformational changes in rabbit muscle aldolase. Kinetic Studies. Biochem. J., **9:** 1533-1539.

Low, P.S. and G.N. Somero. 1975. Pressure effects on enzyme structure and function *in vitro* and under simulated *in vivo* conditions. Comp. Biochem. Physiol., **52B:** 67-74.

Macdonald, A.G. 1976. Physiological aspects of deep sea biology. Monog. Physiol. Soc., Cambridge, **31:** 1-450.

Neuhoff, H.G. and H. Theede, 1975. Kombinationswirkungen von Temperaturund hydrostatischem Druck auf *Asterias rubens*. Kieler Meeresforsch., **31:** 32-43.

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

Shameel, M. 1973a. Untersuchungen über den Einfluss des hydrostatischen Druckes auf den O_2-Gaswechsel mariner benthischer Algen. Int. Revue ges. Hydrobiol., **58:** 741-782.

Shameel, M. 1973b. Barobiology of marine algae. Nabatia, **1:** 15-22.

Shameel, M. 1975a. Untersuchungen über die Wirkung des hydrostatischen Druckes auf das Wachstum von *Delesseria sanguinea* (L.) Lamour. aus der westlichen Ostsee. Hydrobiologia, **47:** 209-230.

Shameel, M. 1975b. Activity of alkaline phosphatase in some seaweeds under the influence of hydrostatic pressure and temperature. Pak. J. Bot., **7:** 169-173.

Shameel, M. 1975c. Influence of hydrostatic pressure on the enzyme systems of marine organisms. Pak. J. Sci., **27:** (in press).

Sleigh, M.A. and A.G. Macdonald, 1972. The effects of pressure on organisms. Symp. Soc. Exper. Biol., **26:** 1-516.

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