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STUDIES ON THE PHYSIOLOGY OF A LAVER, *PORPHYRA TENERA* KJELLM

By

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The authors have already tried to analyse the environmental factors effecting the growth and the chemical composition of a laver, *Porphyra tenera* KJELLM. In the previous paper (1~3), it was reported that nitrogen and phosphorus contents of lavers in the cultural ground varied seasonally and locally, and were remarkably affected by the depletion of nutrients in sea water and the increase of its catalytic acivity. Furthermore, it was found that the nitrogen content of lavers increased in proportion to the water velocity when nutrient salts were very poor. The present work is an attempt to find the ecological condition suitable for the growth of lavers in connection with their physiological aspects.

Fujikawa *et al* (4, 5) reported the relation between the growth of 'Chōsen nori', *Porphyra tenera* and its physiological factors. In this paper, some experiments were made with reference to their studies and a few informations were added.

Materials and Methods

In most of the experiments the laver samples were collected from Matsushima Bay but in a few the materials were obtained from Matsukawa-ura.

Ten to 20 pieces each 5 mm square were cut off at random from the leaves of lavers obtained at the same station, and these were used for serial experiments. The average of five series were taken as representative values. The sea water used for the experiment was collected from Onagawa Bay in summer, filtered and sterilized.

The phosphorus content of the laver was colorimetrically determined after plankton analysis by Cooper's method (6), the digestion was made with sulphuric acid and perhydrole. The nitrogen content was measured by micro-Kjeldahl method of Parnas and Wagner (7).

Among the nutrient salts, ammonia was estimated by modifications of Wattenberg's method. The determination of other nutrient salts was made by the methods as described in Kaiyō-Kansoku-Hō (8).

Photosynthesis and the respiration of lavers were measured chiefly by Warburg's manometoric method and sometimes by Winkler's method.

I. On the environments for the growth of *Porphyra*

Local adundances of nitrogen contents of lavers and the indication of biological activity

It was already stated (1) that the lavers in Matsushima Bay decreased their nitrogen contents and their leaves were discoloured after January due to remarkable changes of environmental factors which occured at the cultural grounds by the inflow of oceanic water.

The results of observations in January 1954 are shown in Table 1 and Fig. 1. From Fig. 1, it is found that a remarkable decrease of nitrogen contents of the lavers arises at open sea area and extends toward the coastal area. This decrease is in accord with the course of inflow of the oceanic water(Fig. 2). Though a certain amount of variability in the nitrogen contents might depend on biological variations such as differences between mature and immature or among the different race of laver, physiological variations due to environmental factors were to be expected. Therefore, analyses of some environmental factors effecting the growth of the laver were made as follows.

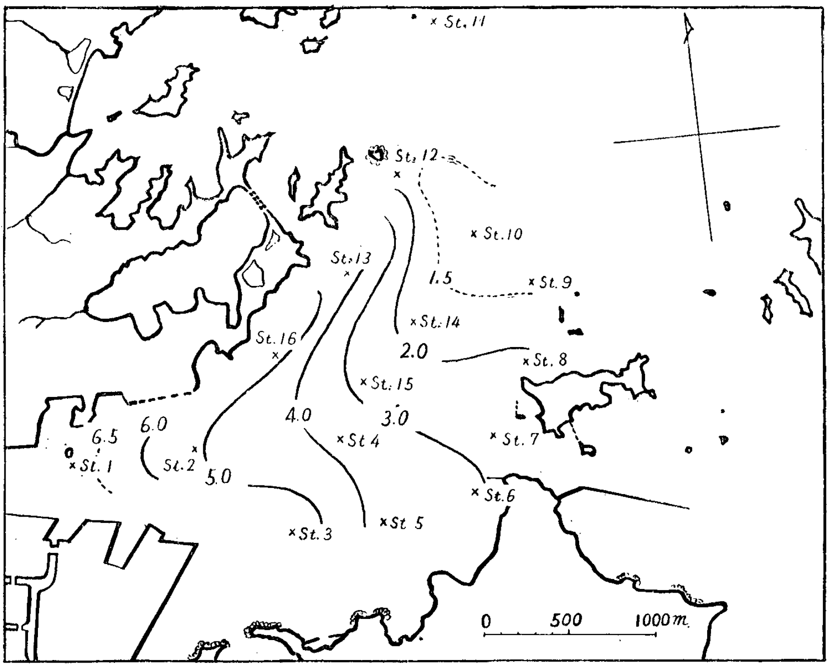


Fig. 1. Local distribution of nitrogen contents of the laver (Jan. 1954). The numerals show nitrogen contents per cent of dry weight.

Table 1. Nitrogen and phosphorus contents of the laver in Matsushima Bay in January 1954.

Sampling Date	Sampling Station	Total Nitrogen % of dry weight	Total Phosphorus % of dry weight	N : P ratio
1-27	1	6.85	.294	23.3
,,	2	5.05	.297	17.0
,,	3	5.79	.285	20.3
,,	4	4.23	.242	15.6
,,	5	3.84	.549	7.7
,,	6	2.19	.336	6.5
,,	7	2.47.	.443	5.6
,,	8	2.14	.203	10.5
,,	9	1.46	.178	8.2
,,	10	1.33	.192	6.9
,,	11	1.85	.221	8.4
,,	12	1.53	.164	9.3
,,	13	4.72	.294	16.0
,,	14	1.81	.178	10.3
,,	15	2.88	.226	12.7
,,	16	5.26	.363	14.5

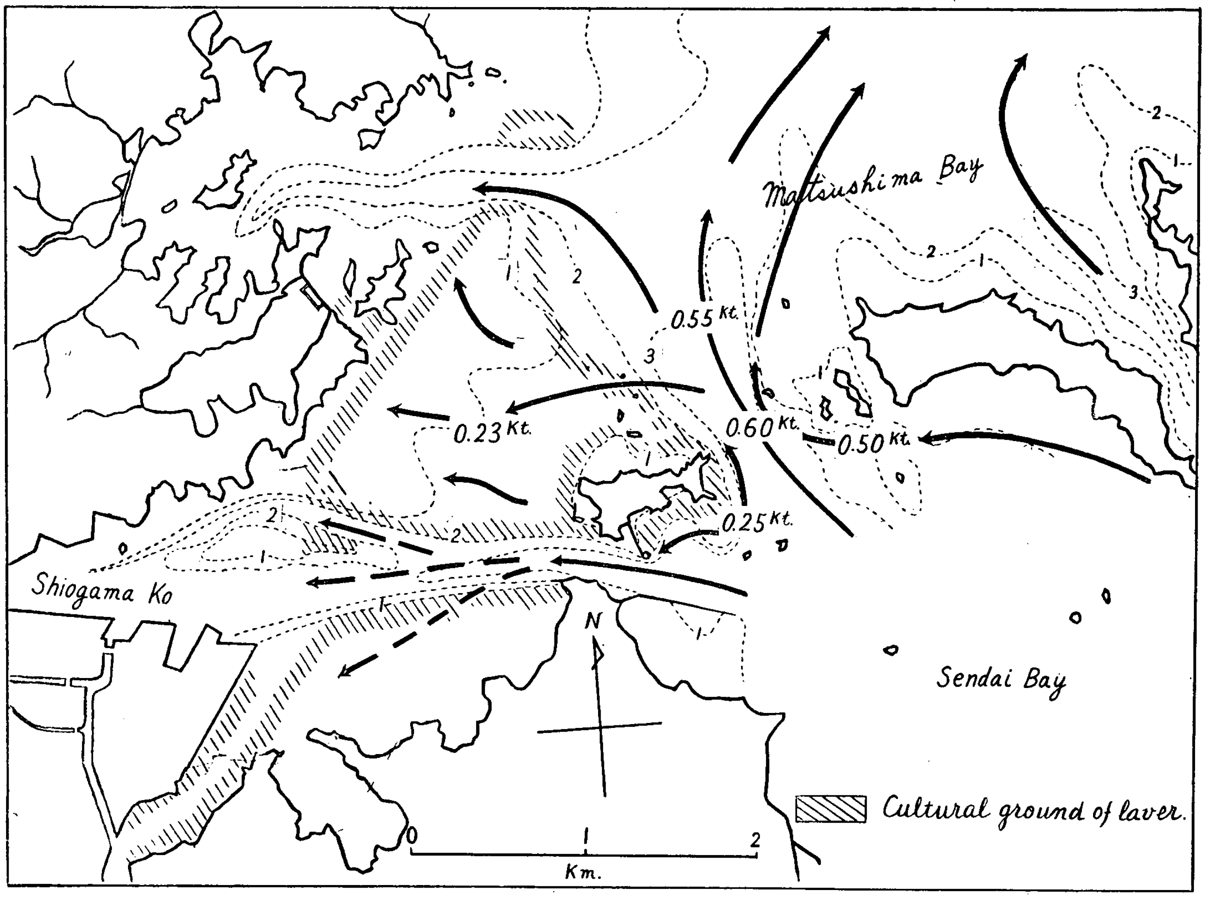


Fig. 2. Distribution of the mean currents at the moon south. (From the data of 2nd Regional Maritime Safety Head Ouarter Hydorographic Divition. Nov. 1953.).

As shown in Fig. 3 (A), (B), the photosynthesis of each samples obtained from the open sea area (St. 9), the middle area (St. 15) and the coastal area (St. 13) showed remarkable differences and had proportional relationship with the nitrogen contents. Therefore, the photosynthetic rate which has close relationship with the growth was considered to be suitable for biological activity in these experiments, though this indication may reserve some objections.

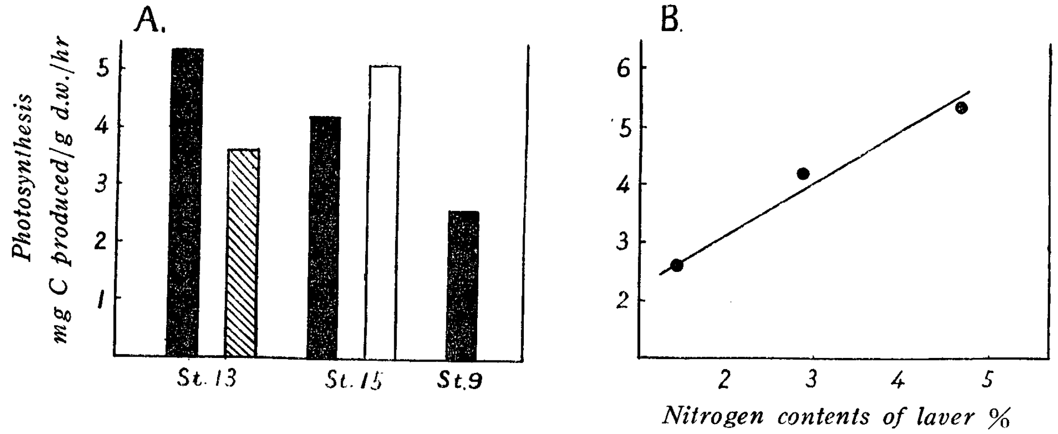


Fig. 3. Photosynthesis of the laver which grew in different areas (A), and the relation of photosynthesis to the nitrogen content of the laver (B). ■ is laver of fixed-hibi and ▨ is floating-hibi. □ cultured in miquel sea water for 2 days.

Influences of the salinity and the exposure on the growth

1. *Salinity*

This experiment was undertaken to make clear the influence of salinity of sea water on the growth of the laver.

The sea waters with different grades of chlorinity were made from distilled water and the filtered natural sea water (Cl 17.89 ‰, pH 8.3). Then the lavers which had grown in the open sea area (St. 9) and the coastal area (St. 3) were dipped respectively into these sea waters. After 20 hours the photosynthesis of each sample were measured. The results are shown in Fig. 4. The photosynthesis decreases gradually in lower chlorinity. The decrease of biological activity expressed in terms of photosynthesis appears mainly to be based on the osmotic effect. From this experiment, it is clear that *Porphyra* can adapt to the change of salinity and can live for at least 20 hours even in distilled water. Furthermore, it is concluded that the suitable salinity for the growth of the laver is in the range of chlorinity 12.00 to 18.00 per mille.

2. *Exposure*

In the experiment on the influence of exposure on the biological activity of the laver, the following two samples were used. One of them was exposed directly to the air after being taken out from the sea water and the other was exposed to the air after the moisture was absorbed by filter papers. After

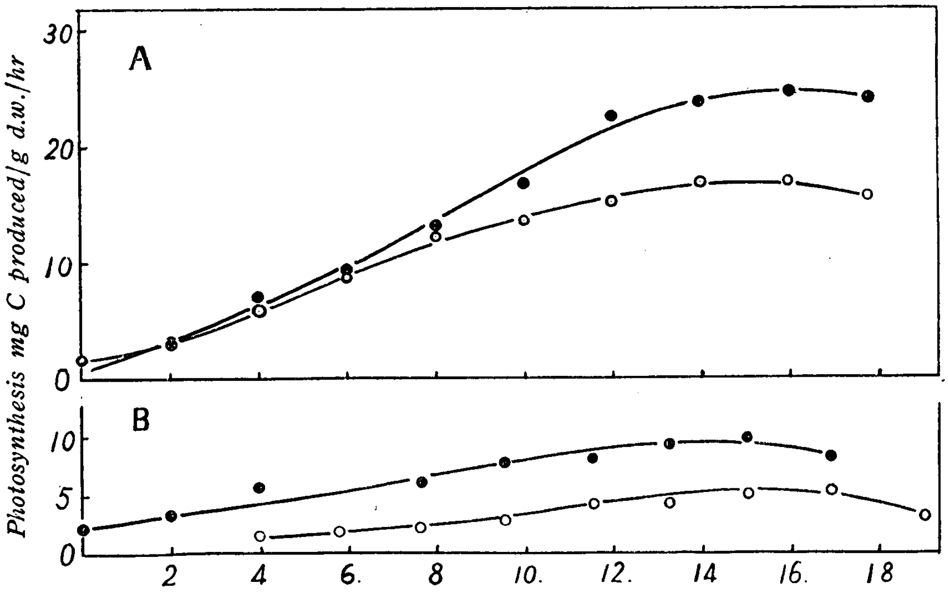


Fig. 4. Relation between photosynthesis and chlorinity. A. Radiation 1.43 cal per cm² per min. B. Radiation 0.4 cal. The dots indicate the laver collected at St, 3 ; the circles are those from St. 9.

exposure of each samples indoors for a few days, their photosynthetic rates were measured. The relationship between the time of exposure and the photosynthetic rate is shown in Fig. 5. The exposure had no significant effect on the biological activity of the laver during a period of less than nine days. However, whether these results will agree with the exposure under natural environments is uncertain, because the exposure may be influenced by meteorological factors such as solar radiation, humidity of air, wind velocity and etc. On the other hand, it had been observed that the biological activity of the laver which grew on a *fixed-hibi showed higher value than that of *floating-hibi

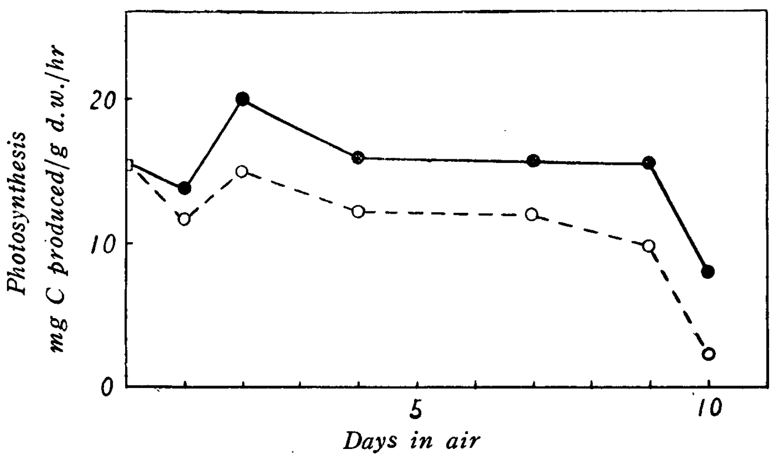


Fig. 5. Effect of the exposure on photosythesis.

* Fixed-hibi is exposed to air at low tide, and floating-hibi is floating near the surface of the water continually by the buoys.

(Fig. 3). The cause of this difference in biological activity is not yet clear, though it is considered that it may be due to the accumlative effect of the exposure under the natural environments.

Light

The influence of light quality on the growth of the laver had been reported by Fujikawa (4).

In the present study, the relationship between the growth of the laver and light intensity has been investigated. Light intensity was measured as total radiation cal per cm^2 per minute in the experiment. Analyses were made under the conditions of direct sunlight and electric light. The relationship of photosynthesis and solar radiation is not yet solved, because there is a qualitative difference between sunlight and electric light. However, the highest photosynthetic rate, showing the value of 45.6 ml oxygen (24.5 mg carbon) per gram of dry weight per hour, has been observed at 1.43 cal per cm^2 per minute in total radiation. In the present stage, it is considered that the value shows roughly the upper limit. The lower limit has not been determined accurately due to the insensitivity of methods of measurement. With regard to this relationship, more precise experiments are necessary.

Temperature

1. *Photosynthesis.*

In this experiment, two laver samples which had grown in different places were used, and their photosynthesis were measured at a constant illumination

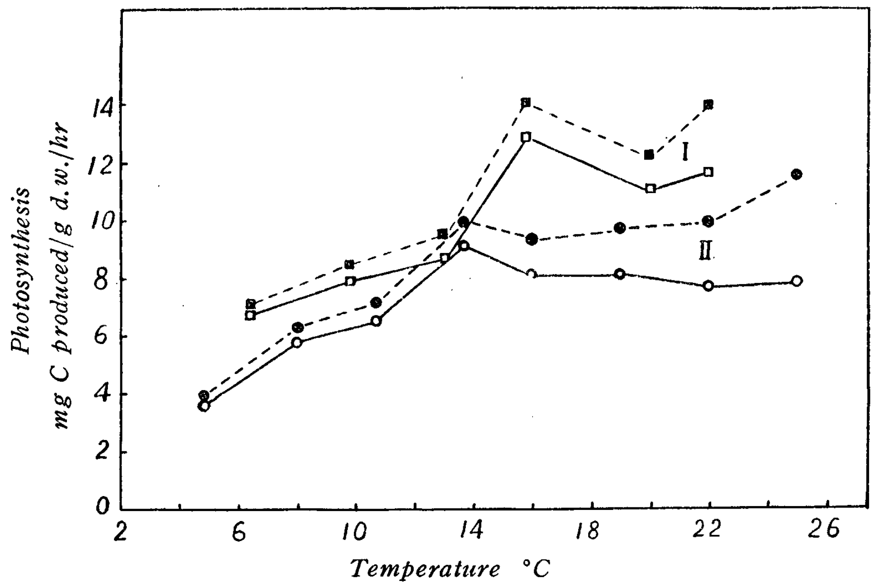


Fig. 6. Relation between photosynthesis and temperature. The solid line shows the net assimilation. The broken line shows the photosynthetic rate. I is laver from st. 13 and II is laver from st. 3.

of two 150 w electric lamps under different water temperatures for 40 minutes after being exposed to the light for 20 minutes. The time factor as pointed out by Blackman (9) was not taken into consideration. The sample I was collected from St. 13 and sample II from St. 3. The relation between the photosynthetic rate of the lavers and the water temperature is shown by the broken line in Fig. 6. As shown in the figure, the photosynthetic rate and the critical point of temperature in which a further increase of tempearture does not increase the photosynthetic rate differed between the two samples. The critical temperature upon the photosynthetic rate was 13.8°C and 15.8°C respectively. This range of temperature corresponds to the water temperature at the middle of Novemver in Matsushima Bay and Matsukawa-ura inlet.

According to Fujikawa *et al* (4), *Porphyra* called 'Chōsen nori' showed the highest photosynthetic rate at 26°C when the samples were dipped at once into the sea water of different water temperature. In the present experiments, *Porphyra* showed no such high photosynthetic rate at 26°C.

In general, it has been said that optimum temperature for photosynthesis did not exist. It seems worth mentioning that the suitable temperature for the photosynthesis of the laver was about 14°C in the present experiment.

2. *Respiration*

In the experiment of respiration of the laver, the oxygen consumption per unit weight was measured by the manometric method. The respiratory coefficient was expressed as milli-grams of carbon consumed per hour per gram of dry weight. The amount of carbon consumed was calculated from the oxygen consumption by Fleming's table (10). Fig. 7 shows the relation between

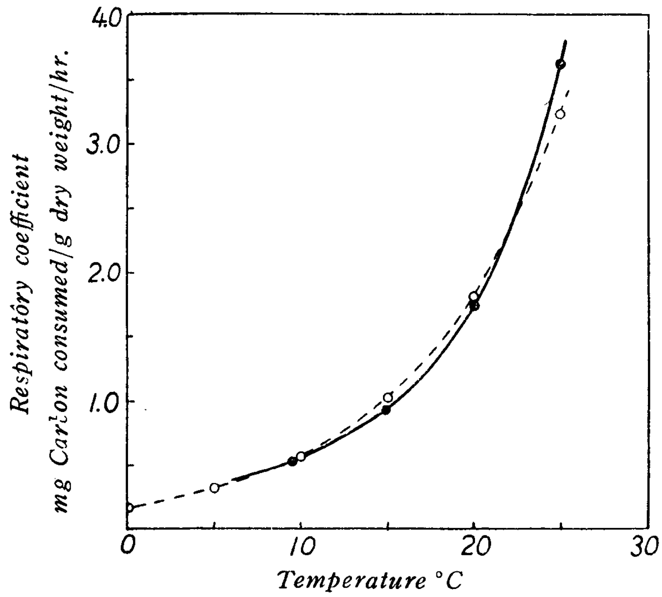


Fig. 7. Relation between respiration and temperature. The broken line is the curve obtained from equation $R_T = R_0 e^{ST}$.

respiration and temperature. From this result, it is apparent that there is an exponential increase in the respiratory coefficient of the laver.

Riley (11) showed that the temperature effecting the respiratory coefficient of phytoplankton can be expressed as

$$R_T = R_0 e^{ST}$$

in which R_T is the respiratory coefficient in grams of carbon per day per gram of phytoplankton carbon at the temperature T; R_0, the respiratory coefficient at 0°C, is 0.0I75; and the constant s is 0.069 from the Long Island Sound data.

In the present study, if the equation $R_T = R_0 e^{ST}$ is used for the respiration of the laver, the values chosen for R_0 and s will be respectively 0.178 and 0.116. The respiratory coefficient calculated in such manner is illustrated in Fig. 7 (broken line). This agrees fairly well with the result of the experiment (solid line). Therefore, it seems possible to use the equation for the estimation of respiration coefficient of laver. Ketchum (I2) reported that the normal carbon content of various algae varied from 51 to 56 per cent of the ash free dry weight. If Ketchum's result can be extended to the carbon content of laver, and the respiratory coefficient is defined as shown by Riley, the values of R_0 and s will be respectively 0.0078 and 0.116.

3. *Net assimiration*

The production of carbon will be obtained by subtracting the respiratory rate from the photosynthetic rate. Thus, the carbon production of the laver was estimated from the data. This is shown by the solid line in Fig. 7. Generally, the respiratory rate determines what proportion of the products of photosynthesis will be available for the production of new plant tissue. However, the respiratory rate was small comparatively in low temperature in this experiment. It will be expected that variations in the photosynthesis induced by environmental changes are rather more remarkable than respiratory rate. Therefore, more precise investigations on the environmental factors, especially the chemical factors such as unidentified growth factor, heavy metals, catalytic activity, chelating agent and etc. are required.

II. On mineral nutrition of *Porphyra*

Carbon

It will be probably rare that the production of plant material in natural environments is limited by the supply of inorganic carbon since the total carbonate is generally present in great excess. The carbon dioxide concentration, however, may be a limiting factor on the growth of the laver in the cultural ground where there are no currents, because cultural industries of lavers are apparently practised intensively.

The experiment was carried out to ascertain the influence of the CO_2 partial pressure on the growth of the laver. The precise method of controlling the CO_2 partial pressure of culture media will be described in an other report. Young lavers which were about 1.0 mm in length were cultured at 10.0±2.0°C together with their substrata (a piece of slender bamboo) in miquel sea water (Cl 18.88 ‰, total alkalinity 2.48 milli-equivalents) in equilibrium with different CO_2 partial pressures of atmosphere as shown in Table 2. After 40 days, the body length of laver were measured.

Table 2. Relation between CO_2 partial pressure and laver growth.

Percent of volume or pressure		0.03	0.5	1.0	1.5	2.0
Partial pressure, Torr		0.228	3.80	7.60	11.40	15.20
pH		8.30	7.46	7.00	6.73	6.55
Body length mm	Initial	1.0	1.0	1.0	1.0	1.0
	After 40 days	9.0	25.0	12.0	6.0	3.0

As seen in Table 2, the highest growth was observed at 0.5 per cent CO_2 in air. And a partial pressure of CO_2 higher than 1.0 per cent inhibited the growth. It was found that the inhibitory effect was also due to the decrease of pH other than the direct action of CO_2 gas.

Osterlind (13) (14) showed that *Scenedesmus quadiricauda* can also utillize bicarbonate ions although *Chlorella pyrenoidosa* can not. Steemann Nielsen (15) (16) showed that bicarbonate ions can be utillized by other aquatic plants. It seems reasonable, therfore, to suppose that the laver can also utilize bicarbonate ions. Furthermore, as already shown by Osterlind(17) and Ketchum(18), it seems possible to presume that carbonate ions can not serve directly as a source of carbon in photosynthesis.

Nitrogen

1. *Nitrogen reqirments*

As already shown in the previous paper, nitrogen contents of lavers varied considerablly according to their environments, showing the values fluctuating from 1.93 to 10.05 per cent of dry weight. It seems difficult, therefore, that the normal nitrogen requirement is induced from these nitrogen contents of laver. On the other hand, the disappearence of gloss and coloration (turning brackish purple to yellowish) were usually observed when nitrogen contents decreased below about 5 per cent of dry weight, and the biological activity of laver also was decreased. (See Fig. 3, B.) From these results, it is worth mentioning that normal nitrogen requirement of laver is from 5.0 to 6.5 per cent of dry weight.

On the other hand, the absolute nitrogen requirement of laver is assumed as about 1.40 per cent of dry weight, because the biological activity of the laver which contained the nitrogen of 1.43 per cent showed a very low value.

2. *Availability of different forms of nitrogen*

Many investigations have shown that both ammonia and nitrate are readily available sources of nitrogen in algal cultures (19~22). It had been found that ammonium nitrogen was more rapidly assimilated than nitrate nitrogen from the same solution in cultures of the marine diatom *Nitzschea closterium* by ZoBell (23) and in mixed phytoplankton population by Harvey (24).

An experiment was therefore made in which equal quantities (N. 1 mg/L) of ammonium chloride, potassium nitrate, ammonium nitrate and urea respectively were added to sea waters as the nitrogen source. Each sea water further was enriched with 0.2 mg phosphate P per liter. The results are shown in Fig. 8, from which it is clear that ammonium was utilized more rapidly than nitrate nitrogen, and that urea was also utilized rapidly.

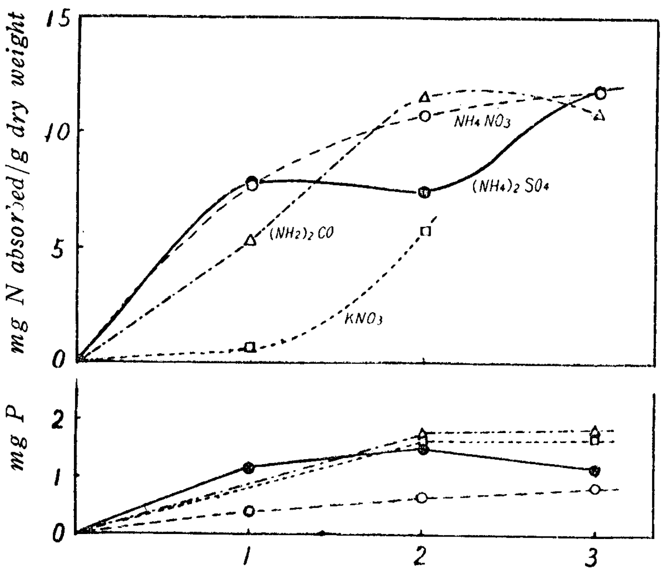


Fig. 8. Absorption of nutrient salts. Upper figure shows the utilization of various nitrogen sources: dots $(NH_4)_2SO_4$; circles NH_4NO_3; squares, KNO_3; triangles, $(NH_2)_2CO$.
Lower figure shows the utilization of phosphate P. (Signs are the same for both figures).

Harvey (24) showed that practically all of the ammonium nitrogen was assimilated before there was any appreciable utilization of nitrate nitrogen, even though the latter was 60 times more concentrated in the enriched sea water. It is apparent, therefore, that the more rapid utilization of ammonium was not merely due to the more rapid diffusion of its ions than that of the nitrate ions. It is important that Harvey's results with phytoplankton can be applied also to the laver.

3. *Optimum concentration of nitrogen*

Ketchum (18) described that the optimim concentration of the nitrogen supply is apparently very different for different species. Therefore the following experiment was made to investigate the optimum concentration of nitrogen for the growth of the laver. Table 3 shows the photosynthesis in different concentrations of nitrogen. The samples were cultured for 20 hours in the respective sea waters before photosynthesis was measured. From Table 3, it is clear that the concentration of nitrogen had no remarkable effect on the growth or photosynthetic behavior in a short time when the concentrations were less than 50 mg N per liter. Furthermore, it may be concluded that the deficiency of nitrogen does not inhibit a complete carbohydrate metabolism in a short time.

Table 3. Relation between photosynthesis and concentration of nitrogen.

(A)

NH_4NO_3 ($N\mu g/l$)	KH_2PO_4 ($P\mu g/l$)	Photosynthesis ($O_2\mu l$/hour/mg)
trace	trace	22.4
500	77	27.4
2000	310	24.6
10000	1550	24.9
25000	3860	23.1
50000	7730	27.9

(B)

NH_4NO_3 ($N\mu g/l$)	KH_2PO_4 ($P\mu g/l$)	Photosynthesis ($O_2\mu l$/hour/mg)
trace	trace	23.6
15	30	25.0
30	30	25.0
50	30	24.2
70	30	27.8
100	30	26.2
140	30	27.4
210	30	27.3

In the higher concentrations of nitrogen, however, it is considered that the inhibitory effect on the growth of the laver may appear after a longer time and may differ according to the nitrogen source. The following experiment was made to understand these effects. The laver samples which were collected from a sterile area (St. 9) were cultured in various sea waters enriched with phosphorus (2.8 mg/L) and contained respectively ammonium nitrate, potassium nitrate, urea and ammonium chloride 20 mg N per liter. After an hour and a week the biological activity in each sea water was measured respectively. In a short time, there was no significant difference among them. After a week, however, the result as shown in Fig. 9 was observed. It was remarkable that the biological activity of the laver decreased when the nitrogen was supplied as ammonium chloride and did not increase when the nitrogen was supplied as urea, though they were utilized rapidly by the laver in initial culture.

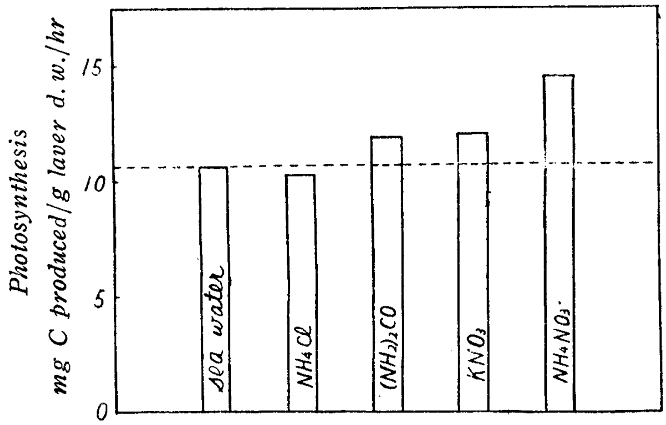


Fig. 9. Photosythesis of the laver in the sea water containing various nitrogen suorce.

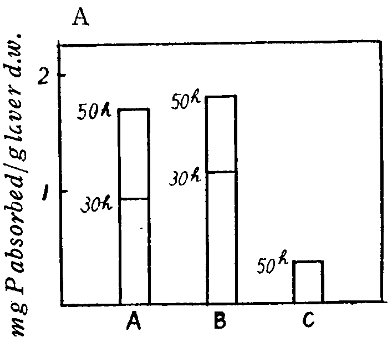
Rodhe (25) found that the initial growth rate of *Ankistrodesmus falcatus* was equal for nitrogen concentrations from 0.15 to 10.0 mg N per liter whether the nitrogen was supplied as ammonium chloride or calcium nitrate. After a longer period, the growth rate decreased in higher ammonical nitrogen concentrations, being less in the 10 mg than in the 5 mg N per liter after six or more days. He attributed this inhibitory effect to the rapid assimilation of CO_2 by highly productive culture with resultant pH values of 11 or higher. The result was similar to Rodhe's results when the nitrogen was supplied as ammonium chloride. The increase of pH, however, was slight, and the variation of pH to be considered as an inhibitory effect could not be observed. Therefore, it could not be considered that this inhibitory effect merely resulted from the increase of pH. In so far as this experiment is concerned, this inhibitory effect seems to be due to the high ammonium ion concentrations rather than pH. However, it can be said in general that the laver may be relatively insensitive to the change of nitrogen concentration in sea water (provided it is present in high concentration).

Phosphorus

1. *The absorption of phosphorus*

Various experiments on phosphorus uptake by the laver were carried out. The amount of phosphorus in the form of phosphate absorbed by the laver was estimated from the phosphorus remaining in the solution. The first experiment was made on the phosphorus uptake by lavers which grew under different environments. The result is shown in Fig. 10, A.

The relation between the absorption and the concentration of phosphorus is shown in Fig. 10, B from which it is found that the absorption of phosphorus was increased with the phosphorus concentration in the sea water, but the increase rate began to decrease gradually when phosphorus concentration



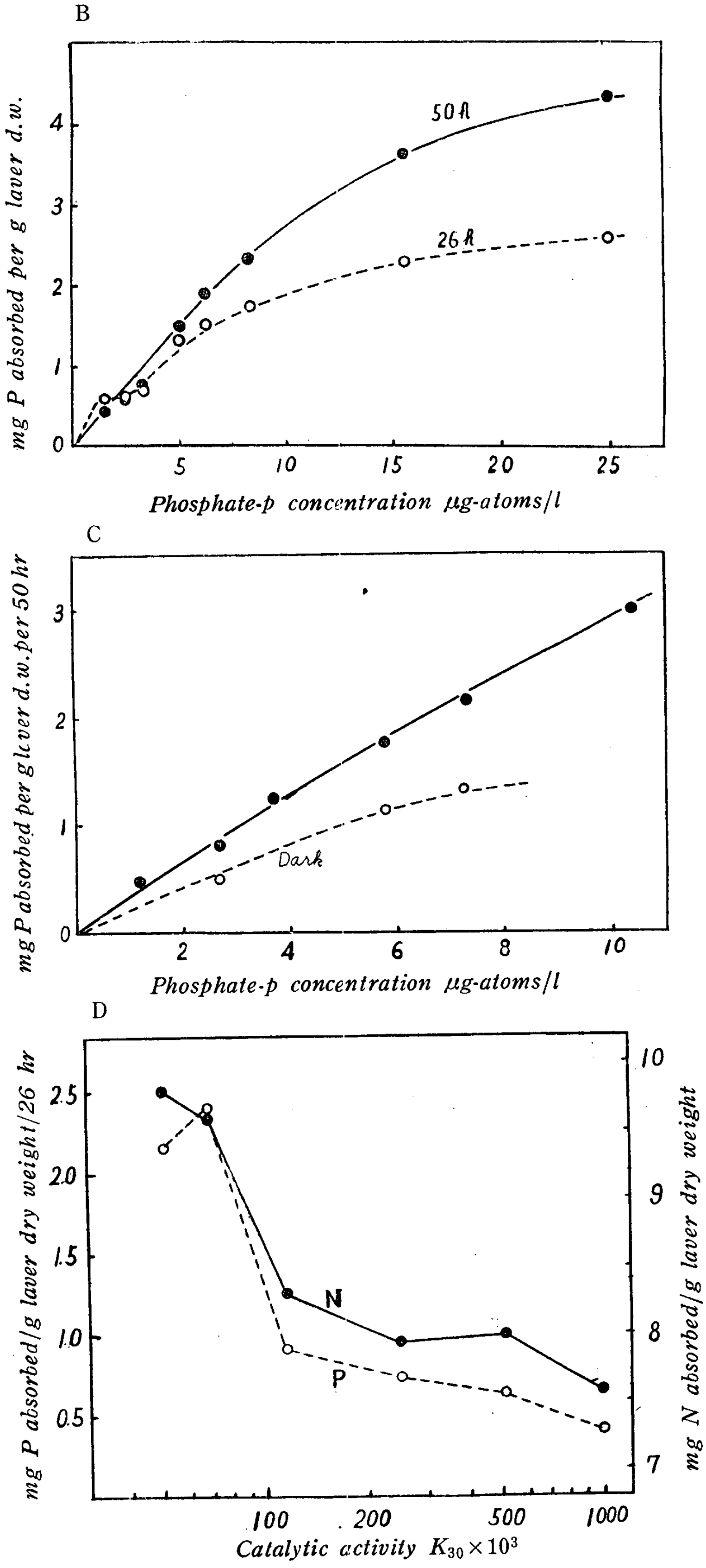


Fig. 10. The absorption of phosphorus. A. shows the absorption of phosphorus by the laver which grew in different areas. B. Relation between absorption of P and its concentration in the surrounding water. C. Effect of illumination on the uptake of P. D. Effect of catalytic activity on the uptake.

reached to about 10 μg-atoms per liter. Furthermore, as can be seen from Fig. 10, C, phosphorus was absorbed by the laver in the dark.

Influences of catalytic activity (26) upon the absorption of phosphorus is shown in Fig. 10. D. It was obvious that the absorption had been inhibited strongly by the activity above $K_{30} \times 10^3$ 80. From these experiments, it can be concluded that the absorption of nutrients is mainly due to the diffusion based on the concentration gradient between the cell and the sea water and controlled also by the physiological state of the cells.

3. *Forms of phosphorus in the cells*

From the above experiments on the phosphorus uptake by the laver, it was suggested that the laver would have a phosphate content corresponding to that of the environment. Firstly the laver which grew in an area rich in nutrients were washed respectively by distilled water and artificial sea water for 50 hours. Then, the amount of phosphate liberated from the laver were measured. In the artificial sea water, the liberation of phosphate could not be observed. In the distilled water, however, the liberation of phosphate P from the cells were observed. This result shows that inorganic phosphate exists in the cell and was not adsorbed.

Next, the following three samples; 1) cultured in miquel's sea water for a day, 2) cultured in miquel's sea water for two days and 3) cultured in miquel's sea wated for two days and then washed by artificial sea water for a day, were heated to 45°C in distilled water, and left for 20 hours. Then they were again heated to 100°C. The amount of phosphorus liberated from the laver cells by such treatments was measured. As shown in Table 4, different amounts of phsphorus were liberated by the breakdown of laver cells by these heatings. It is suggested, therefore, that laver are able to store phosphorus in excess of their requirements, as found by Matsue(27) in *Skeletonema costatum* culture. This phosphate is liberated by heating and not removed by the washing of the laver with the water.

Table 4. Liberation of phosphorus by heating. (μg/10cm² laver)

Sample	Times in miquel sea-water	Washing with artificial sea-water	In 45°C			In 100°C	Total average
				After 20 hr	Total		
1	24 hr	— — —	1.1 1.5 1.8	1.25 0.00 0.56	2.34 1.50 2.36	8.1 7.1 7.9	9.80
2	48 hr	— — —	3.6 2.0 1.8	6.54 10.08 0.75	10.14 12.08 2.50	18.5 21.3 19.2	27.94
3	48 hr	0.5 0.5 0.5	1.6 1.0 0.7	0.00 0.57 0.30	1.6 1.57 1.05	8.1 7.1 7.1	9.21

3. *Optimum concentration of phosphorus*

As already reported in a previous paper, the phosphorus contents of lavers reduced remarkably in the concentration below 10 μg (0.32 μg-atoms) P per liter, but the phosphorus above 20 μg (0.65 μg-atoms) P per liter had no significant effect upon them.

An experiment was made to decide the lower phosphorus concentration which limits the growth. Preliminary observations indicated that the photosynthesis had taken place normally in sterile sea water for a certain period of time. Therefore, two different samples, (1) collected in a coastal area rich in nutrients (St. 3) and (2) collected at a comparatively sterile area (St. 9) and then cultured in phosphorus deficient sea water for two days, were prepared. These lavers were cultured in each sea water which differed in phosphorus concentrations as shown in Fig. 11. After 20 hours, the photosynthetic rate of each cultures were measured under the same conditions.

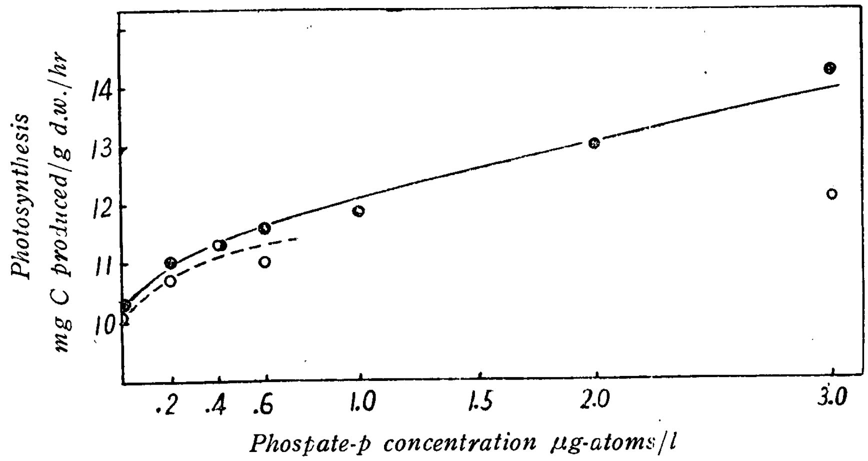


Fig. 11. Relation between photosynthesis and phosphorus concentration of the surrounding water. (sample 2)

In the former samples (I), phosphorus concentration had no effect on photosynthesis. In the phosphorus deficient laver the results as shown in Fig. 11 were obtained. From Fig. 11, it is found that the photosynthesis of the laver was affected by phosphorus concentrations in sea water, particularly at the concentration less than 0.4 to 0.6 μg-atoms P per liter. Therefore, it must be concluded that phosphorus concentrations below 0.4 to 0.6 μg-atoms P per liter affect the normal growth of the laver in much the same way as it affects the phosphorus contents of laver. It seems impossible to presume the upper limit of concentration for optimum growth of the laver, because it may change by various environmental factors.

Chu (28) showed, however, that the upper limit which permited the maximum growth rate for various algae varied from 8.9 to 17.8 mg P per liter, higher concentrations being inhibitory. It will be expected that Chu's results can be extended to the laver.

The data on the amount of phosphate contained in the laver, as already reported by authors (1, 2) show considerable variation, the values fluctuating from 0.077 to 0.81 per cent of dry weight. The phosphorus content of the laver can be expected to vary, according to the phosphorus concentration in the sea water and to the physiological state of the laver. It is difficult and uncertain, therefore, to estimate the phosphorus requirement.

The ratio of nitrogen to phosphorus

In the previous papers (1, 2), it was shown that the ratio of nitrogen to phosphorus in the laver varied in wide range from 5.6 to 23.3 and was about 11 in average at Matsushima Bay, but the ratio varied more remarkably in Matsukawa-ura. In Matsukawa-ura, it had been observed that the phosphorus content of the laver grown at the area located near the mouth of the inlet took approximately a constant value independent of the nitrogen content while at the inner part of the inlet where water was stagnant, it was proportional to the nitrogen content. In the experiment on absorption of both nitrogen and phosphorus, it was not observed that a constant ratio existed between nitrogen and phosphorus in their uptake, though the amount of phosphorus uptake increased slightly with the increase of nitrogen uptake. Furthermore, as can be seen in Fig. 8, it is clear that the absorption of phosphorus was not affected by the forms of nitrogen. To ascertain the relation between photosynthesis and absorption of nutrient salts, an experiment was tried. In the experiment, the lavers were cultured in nutrient rich sea water, and then the amount of oxygen produced by the photosynthesis and the amount of nitrogen and phosphorus absorbed by the laver were measured. Analyses were made in the interval as shown in Fig. 12. Table 5 and Fig. 12 show the expeiremental results. From the results, it seems clear that the absorption of nutrient salts have nothing to do with photosynthesis. Therefore, it is impossible to introduce the ratio of $O_2 : N : P$ from this experiment.

Table 5. Relation between photosynthesis and absorption of nitrogen and phosphorus. ($10 cm^2$ laver)

Time (hr)	O_2 produced (μl)	P absorbed (μg)	N absorbed (μg)	$O_2 : P$	$O_2 : N$	N : P
2	25	1.3	116.7	19.2	0.215	90
4	51	0.9	153.7	56.7	0.331	170
6	74	2.5	187	27.6	0.396	75
8	97	3.2	213	30.3	0.456	66.6
9.5	117	4.0	210	29.2	0.556	52.5
12	158	4.6	220	34.4	0.719	48.0
16	210	4.8	228	43.8	0.920	47.5
22	273		257		1.06	

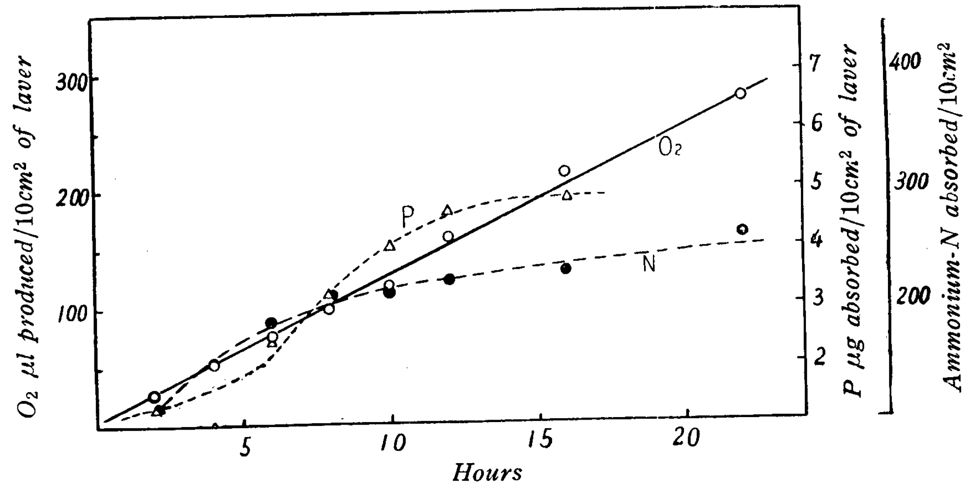


Fig. 12. Relation between oxygen produced by photosynthesis and nitrogen, phosphorus utilized by laver: dots, nitrogen; triangles, phosphorus; circles, oxygen.

Already, the ratio of nitrogen to phosphorus in marine phytoplankton had been shown as given in the following table by Redfield (29), Cooper (30) and Harvey (24).

			Ratio N/P by weight.
Redfield	Bay of Fundy, *Thalassiosira Nordenskioldii*		13.4
	Off Nova Scotea coast, mixed diatom community		6.9
Cooper	English Channel, mixed diatom community,	3, IV, '34	9.6
	,, ,, mainly *Rhizosolenia*,	15, V, '34	7.6
	,, ,, mainly *Rhizosolenia*,	24, V, '34	7.0
Harvey	,, ,, mixed diatom community		10.4

As is obvious from these results, the ratio differs by investigators.

Matsue(27) showed that the ratio of N : P was 12, in *Skeletonema costatum*, and found that the diatom absorbed and stored the phosphorus exceeding its requirements. He concluded that the ratio of N : P in cultured *Skeletonema* varies in a wide range from 2.1 to 12.2 corresponding to the excess of phosphorus stored in the cells. In the laver, the values from 5.6 to 41.2 on the ratio of N : P had been observed to date. Though the laver contained phosphorus which was liberated by the heating of cells, whether the phosphorus was the type of storage is not yet evident.

In the previous observations at Matsukawa-ura and in Matsushima Bay (2) (1), the amount of phosphorus necessary for normal phosphorus metabolism of the laver was assumed as about 0.2 per cent of the dry weight of the laver. Therefore, the variation of the ratio of N : P in the laver was considered to be based on the absorption in excess of the requirement. As the result, it is concluded that the amount of phosphorus contained in *Porphyra* shows considerable variation by environmental conditions and its physiological state and biological activity also varies not only by nutrient salts in the sea wates but by other complex chemical factors.

Summary

Studies were made to find the ecological condition suitable for the growth of lavers, using the photosynthetic rate which has close relationship with the growth as an index of biological activity. The obtained results are summarized as follows;

(1) Laver can adapt in wide range to the change of salinity and endures at least for 20 hours even in distilled water. The suitable chlorinity for the growth of laver are from 12.00 to 18.00 per mille.

(2) The exposure has no signifiant effect on the biological activity of the laver in less than nine days and the laver which grew on a fixed-hibi showed higher value than that of the floating-hibi in the biological activity.

(3) The highest photosynthetic rate was observed at 1.43 gcal per cm^2 per min. in total radiation.

(4) The critical point of temperature which a further increase of temperature does not increase the photosynthetic rate differed by the condition under which the growth took place, and the photosynthetic rate does not always show a regular variation.

(5) The respiratory rate of the laver can be experssed as

$$R_T = R_0 e^{ST}$$

in which R_T is the respiratory coefficient in mg of carbon per hour per gram of dry weight at the temperature T; R_0, the respiratory coefficient at 0°C., is 0.178; and the constant s is 0.116.

(6) The laver showed the highest growth rate at the CO_2 partial pressure of ~~0.228~~ 3.80 Torr.

(7) The concentration of nitrogen has no effect on the growth or photosynthetic behavior in short periods when the concentrations are less than 50 mg N per liter.

(8) The diffusion based on concentration gradient is the main cause of nutrient absorption, but it is controlled also by the physiological state of the cells.

(9) The photosynthesis of the phosphorus deficient laver is affected by phosphorus concentrations in sea water, particularly, in the concentration less than 0.4 to 0.6 μg-atoms phosphate P per liter.

(10) The ratio of N : P in the laver showed no constant value.

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