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ON ENVIRONMENTAL CHARACTERISTICS OF CULTURAL GROUNDS OF A LAVER, *PORPHYRA TENERA* KJELLMAN IN MATSUSHIMA BAY

By

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A laver, *Porphyra tenera* KJELLMAN is a red algae called 'Asakusa Nori' which Japanese are fond of. Cultural industries of laver are practised on the nutrient abundant bays or inlet along the Pacific coast of Japan. The laver is dried in the sun and supplied to markets as a dried food.

It has been recognized by a few investigators that dried lavers show seasonal and local variations in their qualities and their chemical composition, particularly nitrogen contents (Y. Okuda and S. Nakayama, 1916¹⁾; H. Matsui and Y. Fukayama 1916²⁾, K. Fujikawa, 1928, 1929³⁾ and M. Takagi, 1951⁴⁾). The quality of dried lavers produced in Matsushima Bay is far worse than that of other regions and their commercial grade falls often below the 3rd grade. We considered this cause as depending on environmental characteristics of this Bay and traced the seasonal changes of environmental factors together with nitrogen and phosphorus contents of the laver.

Materials and Methods

The observations were made from June in 1950 to June in 1951, during which period oceanographical observations were carried once or twice a month at high water interval in the neap tides. The stations of observations are illustrated in Fig. 1.

Water samples were taken at surface and bottom layers. At Station 1, 2, 13, 14, 18, 19 and 22, water samples were also collected from 2 m. and 5 m. depths besides the surface and the bottom.

The chlorinity was determined by Knudsen's method. The catalytic activity was measured by Matsudaira's method (C. Matsudaira, 1950,⁵⁾ 1952⁶⁾). Among nutrient salts, ammonia was estimated by modifications of Wattenberg's method.

Namely, the original method was slightly modified so as to prevent the formation of precipitates which had occurred often in samples with high chlorinity. The following procedure had proved highly satisfactory. Nessler's reagent was prepared as modified by L. W. Winkler (1925)⁷⁾. 25 c.c. of the sample was measured little by little into a 50 c.c. Ehrlenmyer flask in which (i) 50% Rochelle salt solution contained 0.5% NaOH was previously added, and then (ii) 1.5 c.c. of 9 N NaOH solution and (iii) 0.5 c.c. of Nessler's reagent were added slowly in the described order. After gentle mixing, the intensity of orange colour formed was colorimetrically measured according to original method. The determination of other nutrient salts was made by usual methods described in Kaiyo-Kansoku-ho (Kaiyo-Kishodai, 1940⁸⁾).

The chemical analyses of nitrogen and phosphorus contents of the laver were paralleled to the oceanographical observations. Samples were picked up from dried lavers coming into market. The origins and productive dates of these lavers had been well known. Five pieces respectively with the area of 5 cm square were cut off at random from a sample. They were balanced, dried in a thermostat at 110°C and cooled in a desiccator. And then they were balanced again and provided with the chemical analyses. The phosphorus content was colorimetrically determined after plankton analyses by Cooper's method in which the

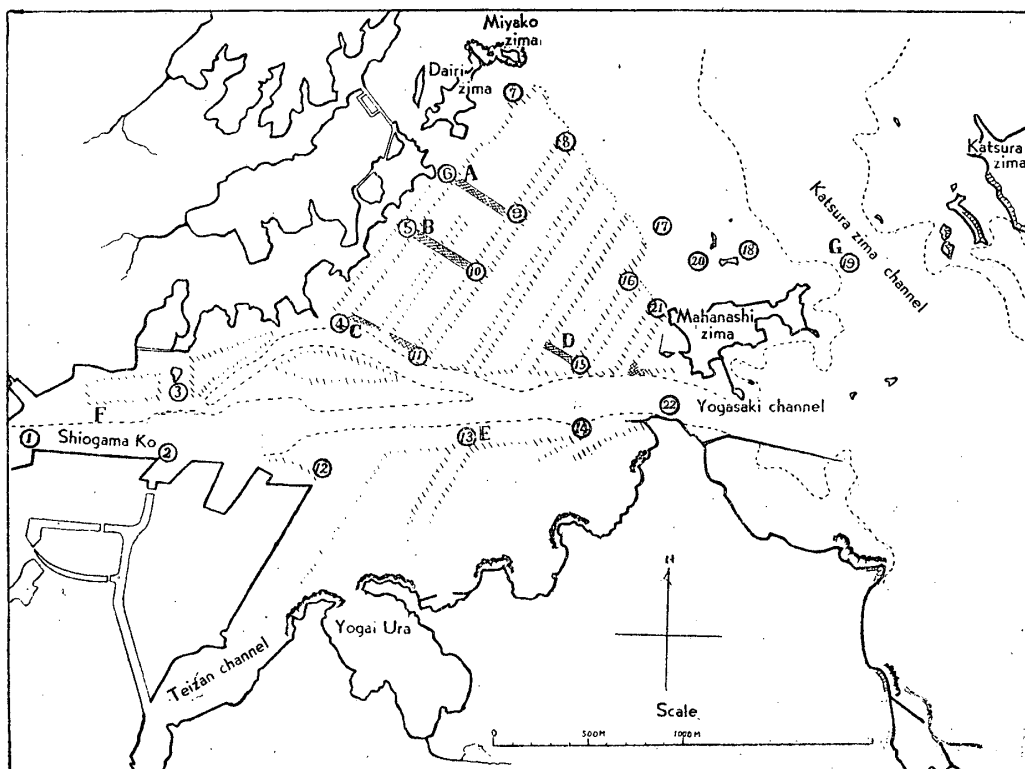


Fig. 1. Positions (A-G) of cultural grounds or laver in Matsushima Bay and survey stations (1-22).

digestion was made with sulphuric acid and perhydrol (L. H. N. Cooper, 1934⁹). The nitrogen content was measured by the micro-Kjeldahl method of Parnas and Wagner (I. K. Parnas and R. Wagner, 1921¹⁰).

Annual Change of Environmental Factors in the Cultural Grounds

Matsushima Bay is a shallow inlet with an average depth of less than 2 meters, connecting itself with Sendai Bay in the south through five narrow straits. The tidal range is a little over 1 meter on an average. The cultural grounds of laver are situated at south-western area of the inlet. Positions of main cultural grounds are shown in Fig. 1 (A-F). The available data in the oceanographical observations on the annual changes of environmental factors—transparency, temperature, chlorinity, catalytic activity and nutrient salts were respectively arranged and summarized in each cultural ground. In the following statements, general features on the annual change of each environmental factor will be described in detail.

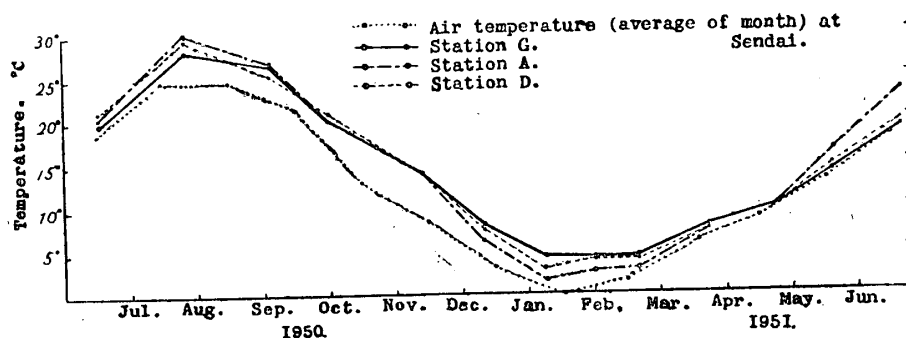


Fig. 2. Temperature cycle in Matsushima-Bay.

1) Water Temperature

The annual change of temperature in the surface and bottom waters in cultural grounds is shown in Fig. 2 for Station A, D and G by which the temperature of inlet water has been characterized. Generally, water temperatures changed with air temperatures and the differences between the temperatures in the surface and the bottom were insignificantly small as the cultural grounds were shallow enough to be affected by winds and tides. From March on, the temperature rose gradually and reached to 10°C in May and 20°C at the end of June or in the early days of July. The maximum temperature of over 31°C was read at Station A in early August. From middle of August the temperature began to fall and it was 21°C at the end of September, 10°C in December and it fell as low as 2-3°C at the early of January. Such tendency was quite in accord with the result

observed during two years from April in 1942 to April in 1944 by T. Imai and his co-workers¹¹⁾ at Mangoku-ura Inlet situated in the east of this inlet.

2) Chlorinity

The distribution of chlorinity was chiefly influenced by the water inflow through Katsurazima Strait by tidal current though significant effects of rain

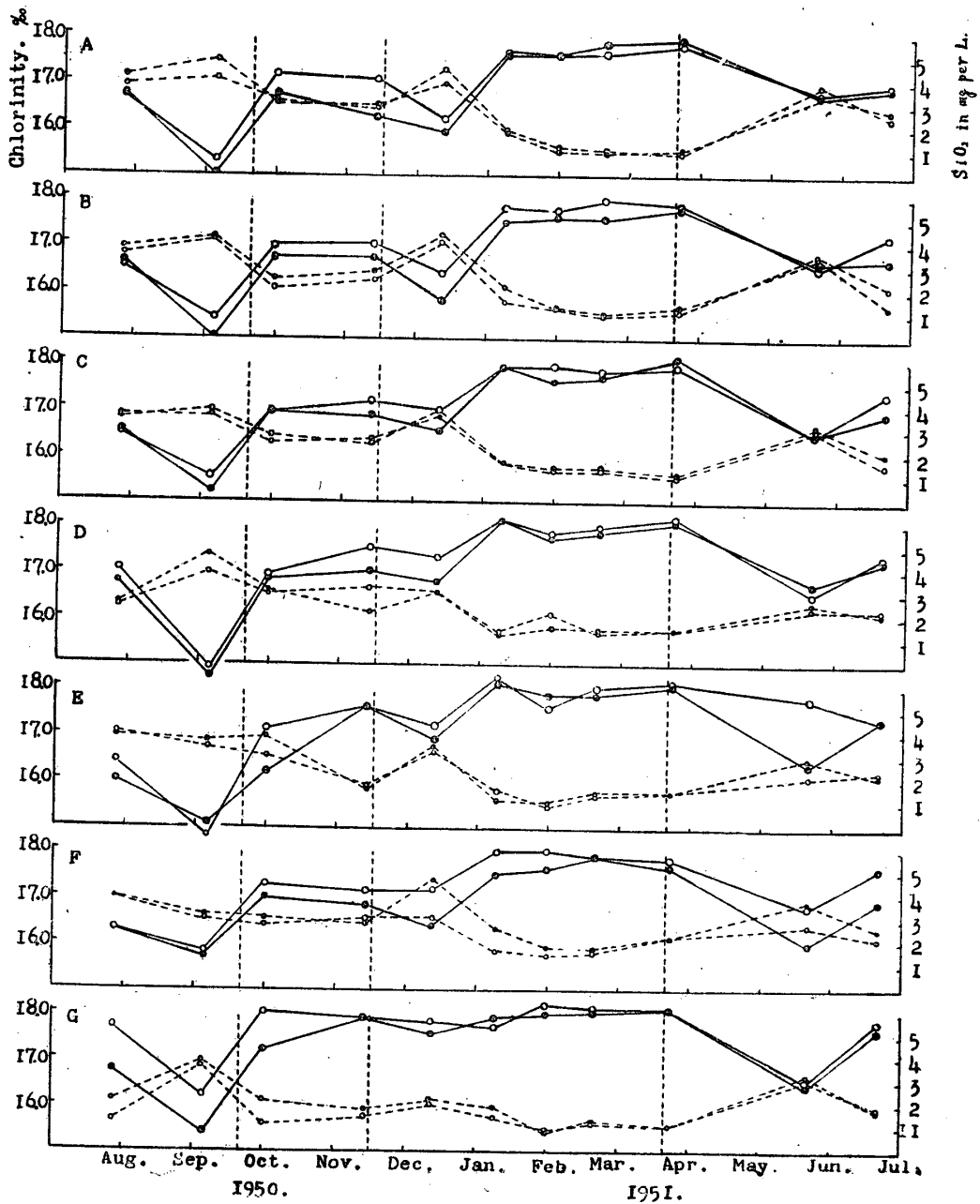


Fig. 3. Annual changes of chlorinity and silicate in cultural grounds. Solid lines show chlorinity and dotted lines silicate. Dots are the surface; circles are the bottom.

fall were observed occasionally in a part of cultural grounds (Fig. 3). The chlorinity changed in the range of 15‰ to 18‰ during a year. During the period of the cultivation, the fluctuation was remarkable in the north-western cultural ground (Station A and B). An outstanding increase of chlorinity occurred in January all over the grounds recording the maximum value 18‰. This high chlorinity remained for two months until late in April. Such marked change was considered doubtlessly as a result of the inflow of Sendai Bay water affected by cold current Oyashio and would cause the decline of nitrogen content in the laver accompanying the change of other environmental factors as will be stated later.

3) *Silicate*

The annual cycle of silicate is shown in Fig. 3. The amount of silicate had a strong negative relationship to the chlorinity. The rain fall was generally supposed to have a positive effect on the amount of silicate because increased river-water would bring a vast amount of silicate from the land. The predominant negative relationship and the local variation were therefore readily explainable. The amount of silicate had fluctuated from about 1 mg to 4mg of SiO_2 per liter. The remarkable decrease occurred in January and a scanty amount as low as 1.5 mg per liter observed from January to March was indicative of an effect by the inflow of oceanic water

4) *Catalytic Activity*

As already reported by H. W. Harvey (1925)¹²⁾ and C. Matsudaira (1950),⁵⁾ the catalytic activity of oceanic waters was higher than coastal or inshore waters in many cases. Therefore, we could trace the inflow of oceanic water by characteristic changes of catalytic activity. On the other hand, it had been ascertained in subsequent experiments, that the catalytic activity was affected by copper content and negative catalysts in sea-water and that the intensity of the activity had controlled the growth of diatom as an environmental factor (Matsudaira, 1952).⁶⁾ In this stage, we considered it worth while to know cyclic changes of catalytic activity in this inlet from an ecological standpoint of the laver.

The annual changes of catalytic activity is shown in Fig. 4 for Stations A~G. The surface water was taken directly with glass bottles without using water-sampling instrument from which the copper would dissolve out in the sample. Therefore, the data of catalytic activity in the surface water were considered as representing accurate values of the activity.

Generally speaking, the catalytic activity had the highest value in August. Once it began to fall suddenly in September, till it had reached to the minimum value in December. An outstanding increase had occurred at the middle of January and it continued to March. This marked fluctuation of the activity

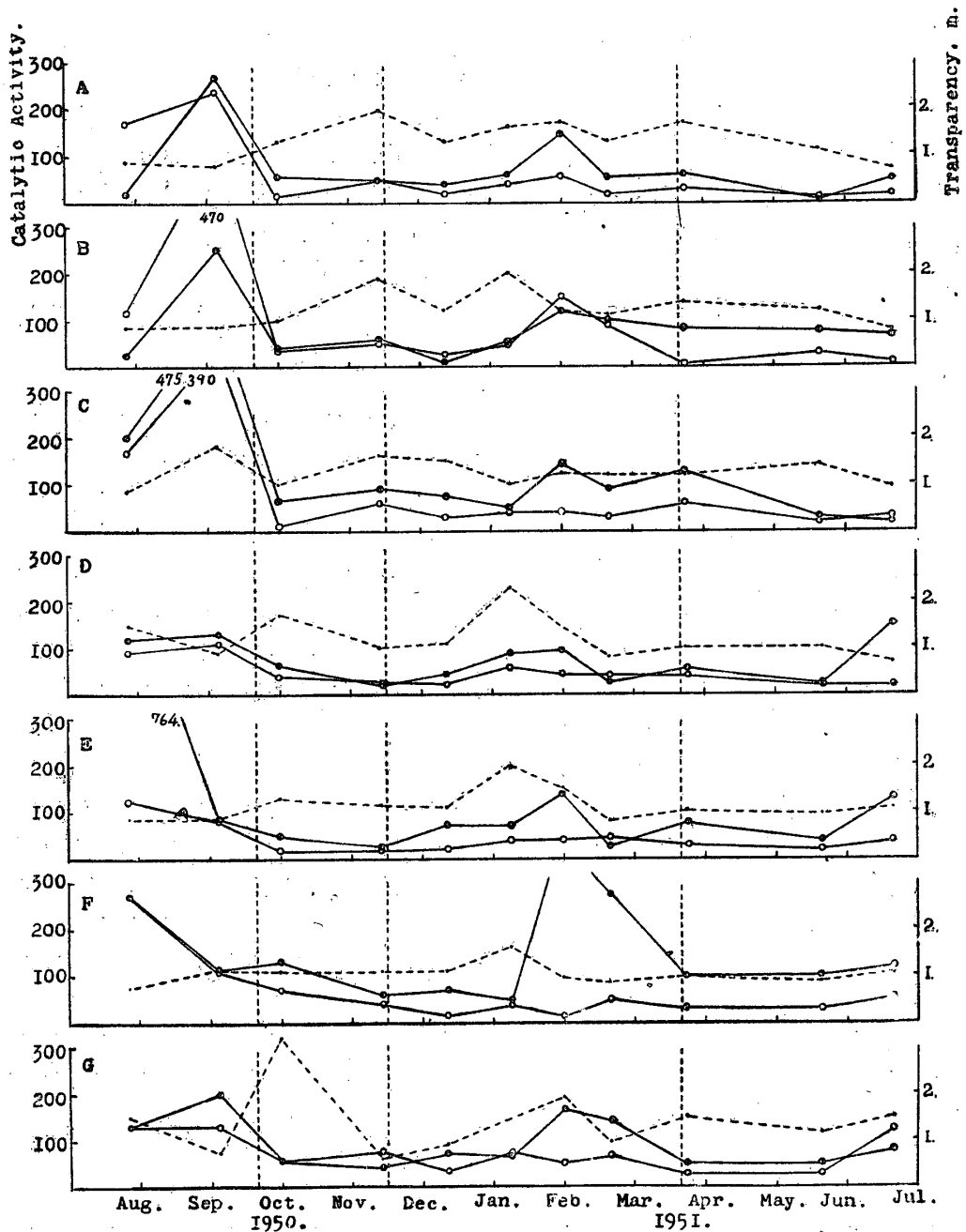


Fig. 4. Annual changes of catalytic activity and transparency in cultural grounds. Solid lines indicate catalytic activity. ($K_{30} \times 10^3$) Dots are the surface and circles are the bottom. Dotted lines show transparency.

had fairly coincided with that of temperature and chlorinity though it was more obscure as compared to the latter. This suggested, therefore, that the inflow of oceanic water masses possesses high activity through Sendai Bay.

During the period of observations, the activity of bottom waters was generally lower than those of the surface and showed a characteristic of fluctuation similar to that of transparency. (dotted line in Fig. 4). As the decrease of transparency in this inlet had been occasionally observed when fine muds and detrituses were

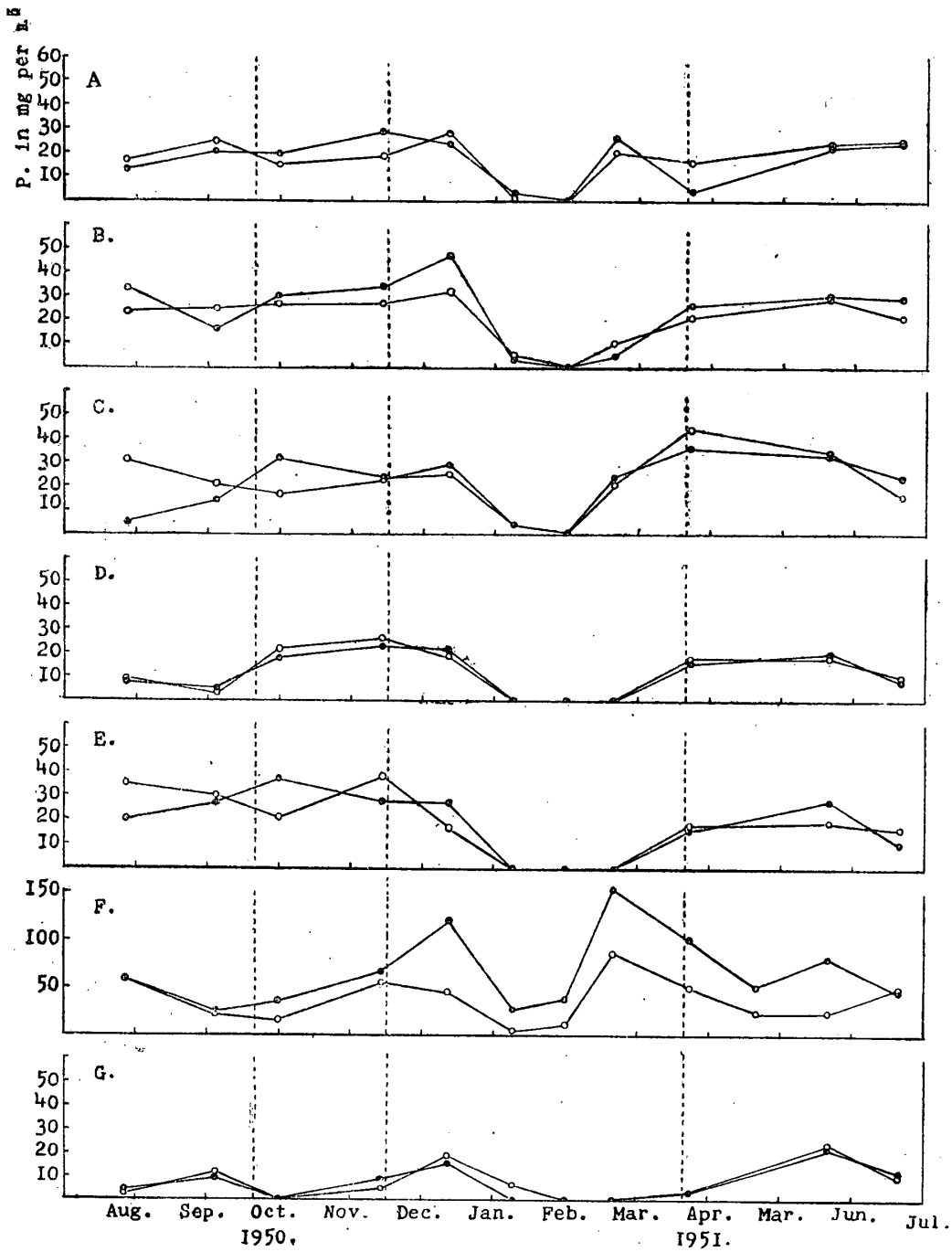


Fig. 5. Annual change of phosphate in cultural grounds.
(Signs are the same as Fig. 3.)

floated from the bottom by the turbulence of water, the change of activity in bottom layer was considered as representing partially the influence of negative catalysts liberated from bottom muds.

Finally, it seems worth mentioning that the high catalytic activity in summer season decreased sharply to a level as low as $K_{30} \times 10^3 \cdot 100$ at the end of September during which month the collection of laver-spores had been accustomed to be practised, and it remained at this level during the favorable season of the cultivation which stretches from October to the end of December.

5) *Phosphate*

As shown in Fig. 5, the annual changes of phosphate at Stations A~E showed the intermediate features between two extremes at Station F and G, each in its degree depending on relative amount of interchanging water by tidal currents. At Station G which showed the characteristic change of Sendai Bay water, the amount of phosphate was very scanty all over the year showing only less than 10 mg of $PO_4\text{-P}$ per cubic meter, except two peaks as low as 20 mg of $PO_4\text{-P}$ per cubic meter observed in the middle of December and May. The null amount of phosphate observed during two months from January to February and which would suppress the normal growth of laver was of a special outstanding significance.

The amount of phosphate at Station F near the harbour was far more abundant comparing with Station G and showed the typical cyclic change of phosphate in this inlet. The phosphate in the surface water began to increase in early October and reached to the value of 120 mg of $PO_4\text{-P}$ per cubic meter in the middle of December. Then it rapidly reduced to the minimum value as low as 15 mg per cubic meter in January, probably resulting from the inflow of Sendai Bay water with a scanty amount of phosphate. But recovery was very rapid and in the end of February, the highest value of 153 mg per cubic meter was observed again. Then it gradually decreased to as much as 60 mg per cubic meter at the end of April, and remained at this level with slight fluctuation through the summer season until it decreased to below 30 mg per cubic meter in September. The change of phosphate in the bottom water was fairly paralleled to the surface water but the amount of it was far less than the latter. It was concluded, therefore that the observed high values in the surface was not due to localized phosphorus regeneration in the bottom mud but depending on the influence of the phosphate-rich polluted water in the inside of the harbour.

The changes of phosphate at other stations corresponded generally to the mixing proportions of the harbour water with Sendai Bay water. This indicated that the phosphate in the inlet had been supplied by the nutrient-rich harbour water. The amount of phosphate at A~E which was main cultural grounds of laver in this inlet was fairly uniform all over the year except the two months of January and February and had kept the value of 20-30 mg of $PO_4\text{-P}$ per

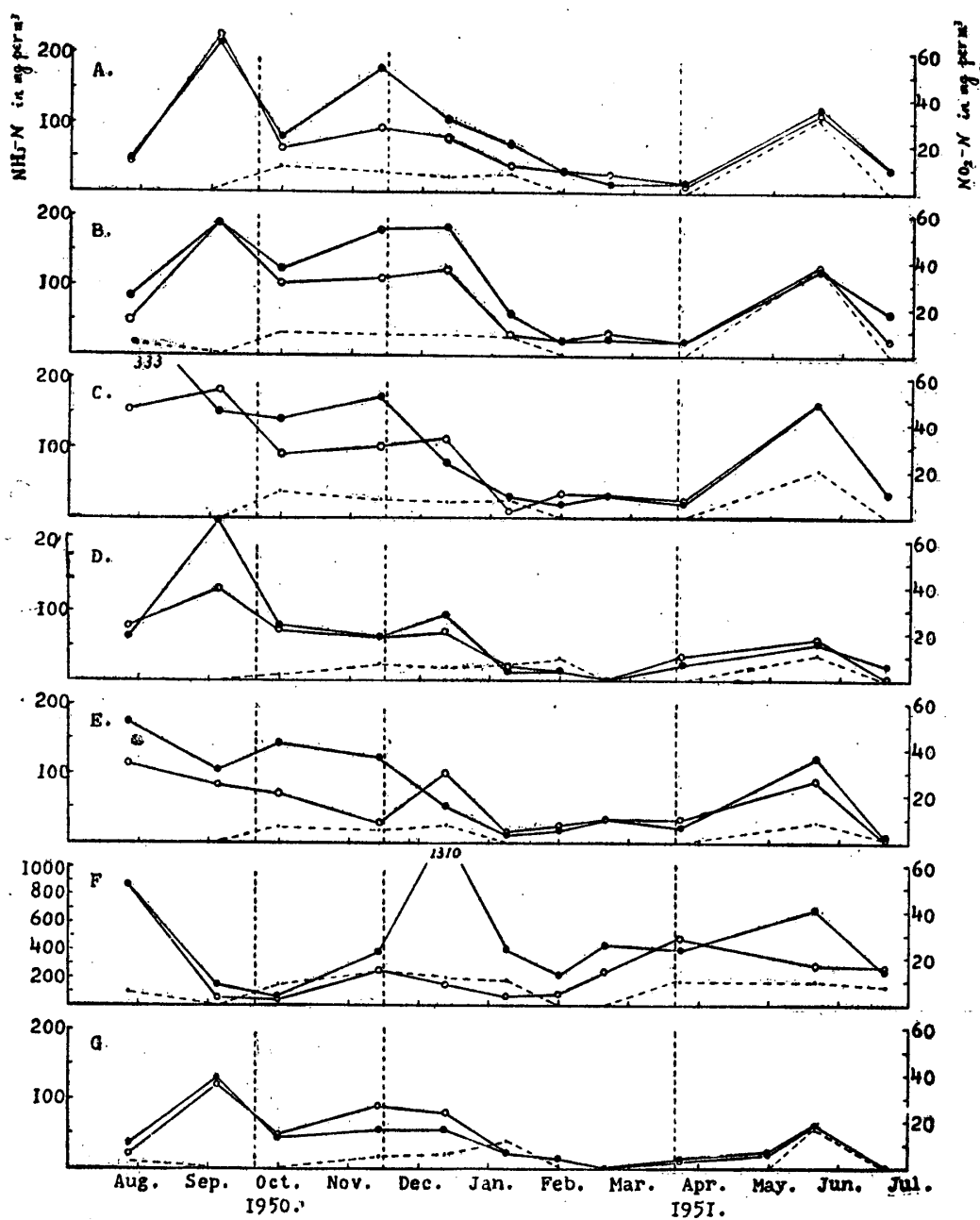


Fig. 6. Annual change of Ammonia and nitrite in cultural grounds. Solid lines indicate ammonia and dotted lines nitrite. (Signs are the same as Fig. 3.)

cubic meter.

6) Ammonia and Nitrite

Seasonal changes of ammonia showed a trend of fluctuation similar to that of phosphate. Namely, the fluctuation of ammonia in the main cultural grounds (St. A~E) depended upon the characteristic changes of two water masses at

Stations F and G shown in Fig. 6.

The surface water at Station G was very poor in the amount of ammonia and did not go over 150 mg of $\text{NH}_3\text{-N}$ per cubic meter in its maximum. The values below 25 mg per cubic meter during the period from January to March gave an indication of complete control of the laver crop by this nutrient factor. Comparing with Station G, the amount of ammonia in the surface water at Station F was far more abundant and had exceeded usually 100 mg of $\text{NH}_3\text{-N}$ per cubic meter. The high concentration in summer with the value of about 900 mg per cubic meter followed by low one of about 100–200 mg per cubic meter in September and then it was rapidly recovered until it reached to the highest value of 1320 mg per cubic meter in December. In January, it dropped suddenly to the level of 200 mg per cubic meter and remained at this level during the rest of year with slight fluctuations.

The amount of ammonia at other stations was fairly uniform during the year showing the values fluctuated from 100 mg to 800 mg of $\text{NH}_3\text{-N}$ per cubic meter but during three months from January to March when the interchange of the inlet water with Sendai Bay water was dominant it had decreased to a very scanty amount of less than 25 mg per cubic meter. Therefore, the most part of ammonia in the cultural grounds was supposed to be supplied by the nutrient-rich harbour water in the same manner as in the case of phosphate.

The annual change of nitrite in the surface water is shown by dotted line in

Table 1.

Sampling Date.	Sampling Station.	Moisture. % of dry weight.	Total Nitrogen. % of dry weight.	Phosphorus. % of dry weight.	N : P
12- 8	A	17.0	7.22±0.32	0.694±0.031	10.4±0.60
12-16	A	15.2	6.46±1.11	0.619±0.086	10.4±3.02
1- 5	A	12.4	6.49±0.20	0.574±0.035	11.3±0.95
1- 5	A(9)	8.7	5.25±0.63	0.495±0.029	10.6±1.75
12-16	B(5)	7.9	9.37±0.95	0.673±0.029	13.9±1.57
12-16	C	13.4	6.19±0.40	0.446±0.095	13.9±2.78
12-26	C	13.7	5.26±0.51	0.490±0.013	10.8±1.14
1-26	C	13.5	3.94±0.31	0.311±0.013	12.7±0.59
2-10	C	14.3	3.44±0.61	0.333±0.022	10.3±1.47
12.	D(21)	7.8	8.79±1.35	0.606±0.102	14.5±3.68
1.	D(21)	8.3	6.92±0.66	0.531±0.024	13.0±1.51
2- 8	D	5.3	7.24±0.44	0.607±0.018	11.9±0.37
2-24	D	6.2	7.24±0.83	0.422±0.102	17.1±3.68
3.	D	11.5	4.27±0.65	0.295±0.013	14.5±1.84
12-12	F	7.2	10.05±0.94	0.729±0.203	13.8±3.95
12-25	F	7.1	7.49±0.26	0.661±0.034	11.3±0.76
12- 5	F(12)	4.6	6.31±0.17	0.589±0.173	10.7±3.00
12-17	F(3)	7.7	9.12±1.69	0.814±0.142	11.2±0.84

Fig. 6. The nitrite began to appear in September and remained at the level of 5–10 mg of $\text{NO}_2\text{-N}$ per cubic meter to December. Then, it disappeared during the period from January to March and was followed by the maximum as high as 30 mg per cubic meter in May, and then disappeared again in the summer season. The amount of nitrite in the cultural grounds during the period of laver cultivation from October to March did not go over 10 mg of $\text{NO}_2\text{-N}$ per cubic meter. This value was far less than that of off Shinagawa in Tokyo Bay observed by Y. Matsue (1936).¹³⁾

Though the nitrate was considered as a significant nutrient factor of laver, unfortunately we failed to have accurate analyses because the nitrate in this inlet was too poor. But we obtained the rough value of 30 mg of $\text{NO}_3\text{-N}$ per cubic meter in the maximum using Harvey's method. According to the results obtained by Y. Matsue (1936)¹³⁾ and L. H. N. Cooper (1937),¹⁴⁾ respectively in Tokyo Bay and English Channel, the amount of nitrate was about eight times the amount of the nitrite. When we assume this relation the nitrate in this inlet should be in the quantity not exceeding 80 mg of $\text{NO}_3\text{-N}$ per cubic meter.

Nitrogen and Phosphorus Contents of Laver

The result of analyses on nitrogen and phosphorus contents of lavers is shown in Table I.

Nitrogen contents varied seasonally and locally showing values fluctuating from 3.44 to 10.05 in per cent of dry weight. Generally speaking, the amount of nitrogen in lavers produced early in December showed high values with about 7 per cent of dry weight and it began to decrease rapidly after the middle of January. However, this trend differed considerably in its degree among cultural grounds. According to the result of Y. Okuda and S. Nakayama,¹⁾ the commercial grade of the laver could be estimated by the amount of total nitrogen. Namely, the total nitrogen in the 1st grade was above 6 per cent of dry weight and the others below 5 per cent. Therefore, the commercial grade of lavers produced in this inlet after January was assumed to be below 2nd grade so far as nitrogen analyses are concerned. Furthermore, in such samples the disappearances of gloss and colouration (turning from blackish purple to yellowish) were usually observed accompanying the decrease of total nitrogen.

Phosphorus contents fluctuated between 0.81 and 0.3 per cent of dry weight. They were approximately proportional to the nitrogen contents. The N:P ratio was about 11:1 except for the existence of certain variations. The phosphorus content of the samples at Station D was slightly less than the others, in spite of the abundant nitrogen contents. Therefore, the N:P ratio was extraordinarily high showing the value of 12~17:1. This appeared to be the effects of nutrient factors as will be discussed in the following chapter.

Relationship of Nitrogen and Phosphorus Contents of Lavers to Environmental Factors

As already noted above, it had been observed that the lavers in Matsushima Bay decreased not only their nitrogen contents but their leaves were discoloured and disintegrated after January when remarkable changes of environmental factors had occurred at cultural grounds by the inflow of oceanic water. On the other hand, it is well known that the amount of total nitrogen in lavers tended to decrease especially in late seasons of cultivation and also varied locally among cultural grounds.¹⁾²⁾³⁾⁴⁾ Though a certain amount of variability in nitrogen contents might depend on biological variations such as differences between mature and immature or among different races of lavers, the physiological variations due to environmental factors were to be expected. Then, in order to analyse the effect of environmental factors on the disorder of lavers, nitrogen and phosphorus contents of lavers, as shown in Table I, were plotted against each environmental factors after the lavers had been collected. Among environmental factors, the ammonia, the phosphate concentrations and the catalytic activity of sea-water gave significant affects.

The relationship between nitrogen contents of lavers and the concentrations of ammonia in sea-water are shown in Fig. 7A. At Stations A, B, C and F, the nitrogen contents of lavers were fairly proportional to the concentration of ammonia at the range of 20 mg to 150 mg of $\text{NH}_3\text{-N}$ per cubic meter (dots and solid line). At Station D, the effect of ammonia concentration upon the nitrogen contents was far smaller than that of other stations (circles and dotted line).

Fig. 7B shows the relation of phosphorus contents of lavers to phosphate concentrations. Dots in the figure are the values obtained at Stations A, B, C and F and circles at Station D. As can be seen in the figure, the phosphorus contents of lavers reduce remarkably at the concentration below 10 mg of $\text{PO}_4\text{-P}$ per cubic meter but the phosphate, above 20 mg of $\text{PO}_4\text{-P}$ per cubic meter had no significant effect upon them.

H. W. Harvey (1933)¹⁵⁾ reported that the photosynthetic rates of diatom was affected by the depletion of nutrients such as phosphate, ammonia and nitrate. G. A. Riley (1946)¹⁶⁾ observed the correlation between photosynthetic rate of phytoplankton and phosphate concentration in sea-water during the six cruises to Georges Bank between September 1939 and June 1940. When the rates had been averaged for different ranges of phosphate concentration, there was a pronounced reduction in the average rate when the phosphate fell below about 0.5 to 0.6 mg-atom of P per cubic meter. B. H. Ketchum (1939)¹⁷⁾ reported a decrease in the growth rate of experimental cultures of *Nitzschia closterium* when the phosphate concentration was less than 50 gamma of PO_4 per liter (0.55 mg-

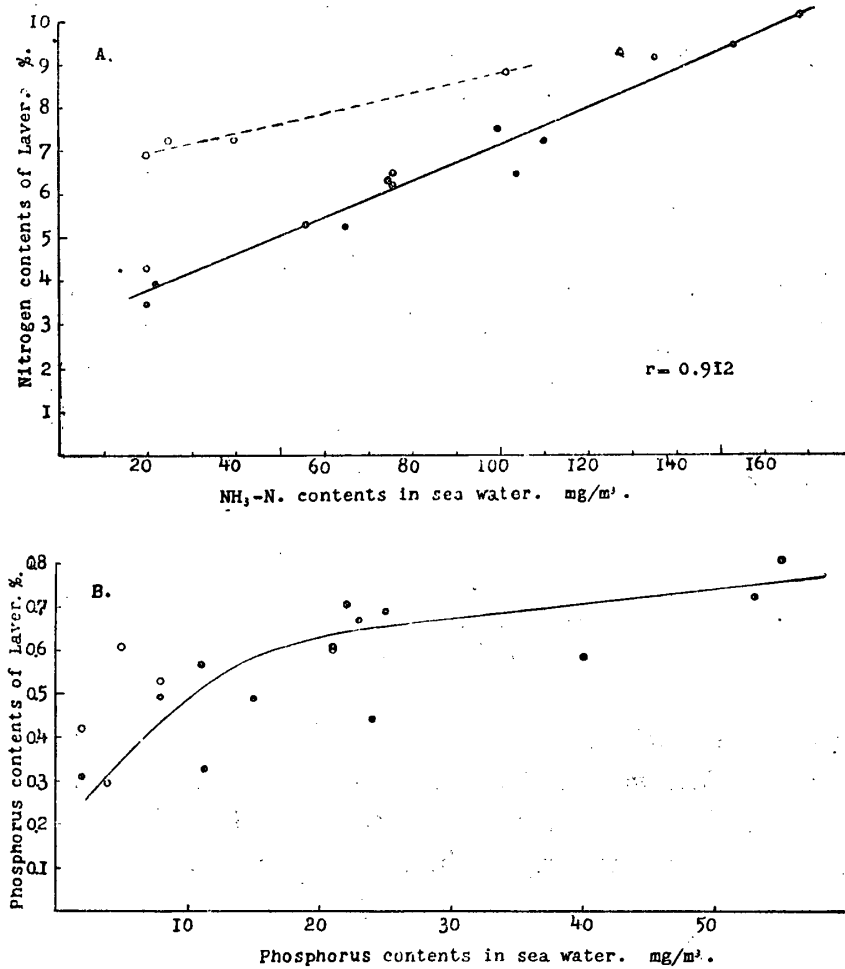


Fig. 7. Correlation between nitrogen and phosphorus contents in laver and nutrients factors. A. shows nitrogen contents of laver plotted against ammonium concentration in the sea water.

B. shows relationship between the phosphorus and phosphate concentration in the sea water. Circles show that of St. D.

atom per M³). Therefore, as far as the diatom physiology can be extended to the laver, it seems reasonable to suppose that nutrient depletion affects the normal growth of lavers in much the same way as it affects nitrogen and phosphorus contents of lavers. In such cases, normal growth of lavers would be limited when the ammonia fell below 150 mg of NH₃-N per cubic meter and the phosphate concentration would be less than 10 mg of PO₄-P per cubic meter. Practically, the discolouration or the integration of lavers was observed at Stations A, B and C when the ammonia and phosphate concentrations became free in January. However, there was a case in which the growth of lavers had been hardly affected by the nutrient depletion as at Station D. This would be easily explainable

by the amount of interchanging water because Station D had more stronger tidal currents through Yogasaki Strait than the other stations.

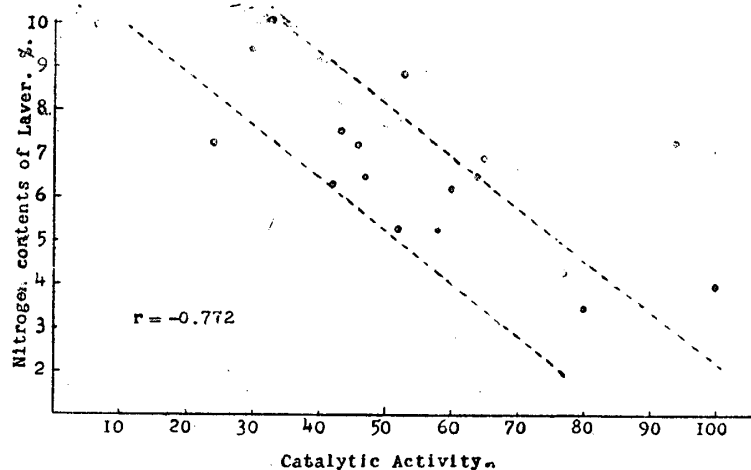


Fig. 8. Correlation between nitrogen contents of laver and catalytic activity. ($K_{30} \times 10^3$).

Fig. 8 shows the correlation between nitrogen contents of lavers and the catalytic activity of surface waters. The notation is the same as Fig. 7. The plots were slightly more scattered than in the case of nutrient factors. The better results might be obtained if complete data on the catalytic activity were available since this factor was locally and seasonally very variable. However, it was obvious that the nitrogen contents had been strongly inhibited by the activity above $K_{30} \times 10^3 \cdot 80$. The result was similar to that of a diatom, *Skeletonema costatum* as already reported by an author (1952).⁶⁾ Finally, it was worth while noting that the value of activity to limit the normal growth of lavers was lower than $K_{30} \times 10^3 \cdot 100$ of *Skeletonema*. Furthermore, to attain more precise analyses of the effect of activity the experimental approaches would be required.

Summary

The paper presented the environmental characteristics of cultural grounds of a laver, *Porphyra tenera* KJELLMAN in Matsushima Bay and the seasonal changes of its nitrogen and phosphorus contents. Data were provided with oceanographical observations stretched from June in 1950 to June in 1951 and paralleled chemical analyses of the laver.

The obtained results are summarized as follows ;

(1) Remarkable changes of a few basic environmental factors—water, temperature, chlorinity, catalytic activity and nutrient salts, occurred with all over the cultural grounds in January when the growth of laver was impeded and they

were indicative of an effect by the inflow of oceanic water.

(2) Analytical results of nutrient salts indicated that nutrients of lavers in this inlet were supplied chiefly by nutrient-rich harbour waters.

(3) Chemical analyses of the laver showed that nitrogen contents varied seasonally and locally, showing the values fluctuating from 3.33 to 10.05 in per cent of dry weights. The phosphorus contents were between 0.81 and 0.30 per cent of dry weights. The N : P ratio was about 11 : 1 except for the existence of certain variations.

(4) Nitrogen and phosphorus contents were remarkably affected by the depletion of nutrients and the increase of catalytic activity. When the ammonia concentration fell below about 150 mg of $\text{NH}_3\text{-N}$ per cubic meter and the phosphate was less than 10 mg of $\text{PO}_4\text{-P}$ per cubic meter, nitrogen contents of lavers decreased approximately proportional to the depletion of their concentrations in sea-water. The increase of catalytic activity behaved in the same manner as nutrient factors.

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