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A preliminary analysis of the pigments from ice algae in the adjacent waters of Great Wall Station, Antarctica

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Abstract This paper reports the results of separation and identification of the pigments from ice algae in the adjacent waters (62°12′30″S～62°14′30″S, 58°53′W～58°57′W) of Great Wall Station, Antarctica during the icing period (from June 1988～December 1988) and the discussion is also made on the composition and seasonal variations of the pigments of ice algae in that area as well as their roles in marine ecosystems. The results indicate that 15 kinds of pigments have been separated from ice algae, of which 13 kinds can be identified. They are respectively: carotene, pheophytin-a, chlorophyll-a, -b, -c, xanthophyll, fucoxanthin, chlorophyllide-a, violaxanthin, pheophorbide-a, chlorophyllin-a, derivative of chlorophyll-c (diadinoxanthin), but two kinds of pigments can not be identified. There are distinct seasonal variations in the pigments of ice algae at that sea area.

Key words Antarctica, Great Wall Station, adjacent waters, pigments of ice algae

1 Introduction

Antarctic ice algae were first reported by Bunt and Wood (1963). In 1956 Bunt first observed in the adjacent waters of Mawson Station a colored layer of very low transparency at the bottom of 1.4 m sea ice, and he also saved this phenomenon later at McMurdo Station. Afterwards, a large amount of ice algae from the sea ice in the adjacent waters of the Ross Island or nearby McMurdo Station, Antarctica were identified and their kinds, quantity and distribution were also reported (Bunt and Wood, 1963; Bunt, 1963).

The ice algae grow principally at the bottom of sea ice, where a brown-colored layer can be seen distinctly. So the bottom of sea ice is sometimes called sea ice layer or colored layer. The colored layer of antarctic sea ice is the result of the growth of the micro-algae, mainly diatoms. The micro-algae in sea ice are usually called ice algae (Bunt and Wood, 1963; Bunt, 1968). What interests people is that large icicles as long as a few meters sometimes can be observed in the lower layer of sea ice, to which loose and soft ice crystals are attached. It is still not clear how the formation of these icicles are formed and whether they are related to the layer of ice algae, but there are scholars concerned who have really observed fishes and zooplankton feeding around the icicles. Hence, it has been thought that these icicles are probably the places for the inhabitation, feeding and shelter of fishes and zooplankton.

The survival, growth and reproduction of marine algae depend upon chlorophyll capable of carrying out photosynthesis, so the studies on plant pigments have increasingly received great attention of the scholars concerned. Quite a number of scholars have made successive studies on the kind, quantity distribution, seasonal variation, biomass and productivity of ice algae and their ecological environment in other sea areas of Antarctica (Bunt and Wood, 1963; Hoshiai, 1969). But owing to limitation of the conditions, only few reports have so far been made of researches on antarctic ice algae pigments.

The authors have conducted the separation and identification of the ice algae pigment samples from the adjacent waters of the Great Wall Bay, Antarctica, discussed the composition and seasonal variations of sea ice pigments and studied the role of ice algae in the marine ecosystem of that sea area.

2 Materials and methods

The materials used in this paper were icicles and ice blocks (lumps) collected monthly during the period of the formation of sea ice (June ~ December 1988) in the adjacent waters (62°12′30″S ~ 62°14′30″S, 58°53′W ~ 58°57′W). During June ~ July mixed ice blocks were used for separation, whereas during August ~ December mixed icicles were used. The collected ice samples melted naturally indoors, a part of which was used exclusively for the classification of phytoplankton (reported in another paper), a definite quantity of the other part was filtered with suction and concentrated on 0.8 μm micropore filter membranes and freeze-stored at −30°C and brought homeward for analysis. The ice samples were first extracted with 90% acetone solution, then separated by thin-layer chromatography, and the pigments of ice algae were identified according to the R_f values of various marine algal pigments reported by Jeffrey (1961).

3 Result and discussion

The relationship between the growth of ice algae and the thickness of ice-snow layer is rather complicated. The color and compactness of the ice-snow layer have rather marked influences on the growth of the ice algae (Zhang and Lu, 1986) and also on the quantity and kind of the pigments of ice algae.

3.1 *Kind of ice algal pigments*

By using thin-layer chromatography, a preliminary study was made on the composition of the algal pigments from the adjacent waters of Great Wall Station, Antarctica. The result of the separation of the pigments is shown in Fig. 1. The kinds, R_f values and colors of the ice algae pigments are listed in Table 1.

From Fig. 1 and Table 1, it can be seen that in the sea area of Great Wall Station, Antarctica, there are many pigment spots (about 10, each $>$5 mm in diameter), showing that the ice algae pigments in that sea area are many in kind and their contents are high. In the same volume of water sample the content of pigments from ice alage is 10

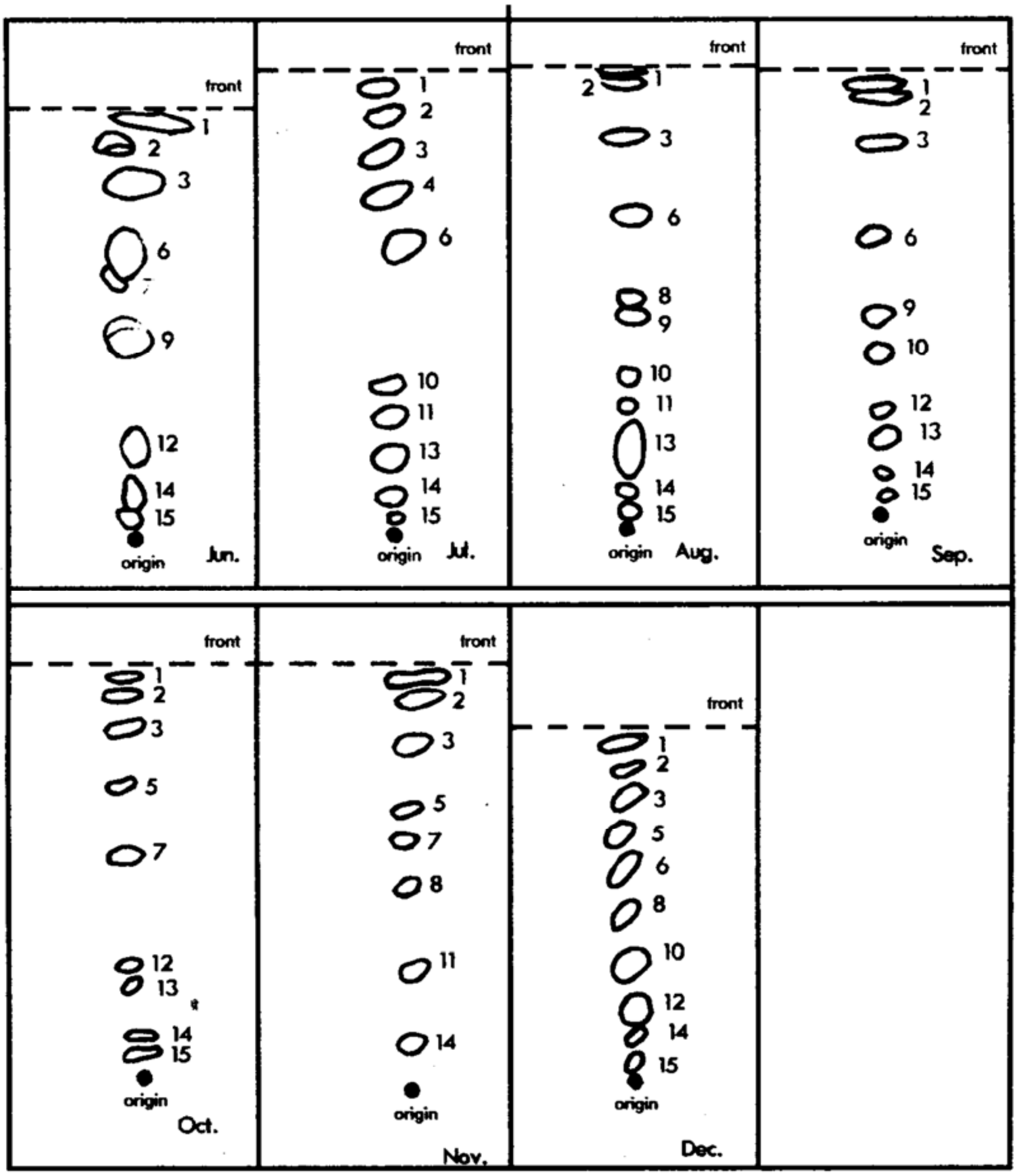

Fig. 1. Thin-layer chromatograms of the ice algae pigments. Developer: acetic acid : ether : petroleum ether (30～60℃) = 1 : 25 : 25 (v/v/v); Development temperature: 19℃; Development time: 7～10 min.

～100 times that from sea water. For example, the content of antarctic ice algae chlorophyll-a was determined to be 81.69 mg/m^3 (Zhang and Lu, 1986) in November 1982, whereas in the same month, that in the sea water was 8.24 mg/m^3. Sometimes the chlorophyll-a concentration in the ice algae communities in the bottom of the sea ice is even 2000 times that in the seawater below the sea ice (Chen and Xiao, 1986).

Fifteen kinds of ice algae pigments in that sea area have been separated and 13 identified. The sequence of spots as arranged in order of the magnitudes of their R_f values are listed in Table 1, from which it can be seen that the kinds of pigments separated from the ice algae in that sea area are consistent with those from phytoplankton in sea water (Yu *et al.*, 1986). However, the content of the chlorophyll-a in sea water is only 1/10 ～1/100 of that in the same volume of sea ice algae. Four to five liters of seawater sample were needed for filtration so that the distinct spots and color of the phytoplankton pigments in seawater can be seen after separation, but only 0.5～1.5 L were needed after the melting of the mixed icicles. As to the number of pigment spots in a chromatogram, 7～8 kinds of pigments, occupying 50% of the kinds of the whole year can be separated in 6 months (January, February, April, June, November and December) of a year; 5 or less than 5 kinds of pigment can be separated in September and October, amounting to 33.3% of the kinds in the whole year. But in that sea area, over 8 kinds of ice algae pigments could be separated in all the months from June to December.

Table 1. Kind, R_f value and color of the ice algae pigments

Spot	Name of pigment	Color	R_f value						
			Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
1	Carotene	Pale yellow	0.98	0.98	0.98	0.98	0.98	0.98	0.98
2	Pheophytin-a	Gray*	0.92	0.91	0.96	0.92	0.96	0.92	0.91
3	Chlorophyll-a	Yellow green	0.82	0.85	0.84	0.83	0.94	0.82	0.81
4	Xanthophyll	Yellow	-	0.79	-	-	-	-	-
5	Diadinoxanthin	Pale yellow	-	-	-	-	0.70	0.69	0.72
6	Chlorophyll-b	Pale yellow	0.67	0.67	0.68	0.65	-	-	-
7	---	Yellow	0.58	-	-	-	0.54	0.59	-
8	Fucoxanthin	Yellow	-	-	0.50	-	-	0.49	0.49
9	Chlorophyllin	Yellow	0.44	-	0.44	0.46	-	-	-
10	---	Pale brown	-	0.37	0.34	0.37	-	-	-
11	Chlorophyllide-a	Yellow green	-	0.29	0.27	-	-	-	-
*12	Pheophorbide-a	Gray	0.23	-	-	0.24	0.26	-	0.23
13	Violaxanthin	Pale yellow	-	0.19	0.18	0.19	0.20	-	-
14	Chlorophyll-c	Pale yellow	0.12	0.10	0.09	0.11	0.08	0.12	0.14
15	Der	Pale yellow	0.06	0.05	0.04	0.06	0.02	-	-

* The colorless light spot is gray.

The main groups of ice algae in that sea area can also be known preliminarily from the kinds of the ice algae pigments. From Fig. 1 and Table 1, it can be seen that during the freezing period carotene, chlorophyll-a, pheophytin-a and chlorophyll-c can all be separated from the algae in the adjacent waters of Great Wall Station. All these pigments are indispensable to marine diatoms. Though dinoflagellates and chrysophytes also contain these pigments, yet their contents are all lower than those of diatoms. In marine diatoms, the chlorophyll-a content amounts to 85% of the total weight of chlorophyll, whereas in dinoflagellates, it amounts to 63～77% of the total (Zheng *et al.*, 1984). Hence it is inferred that the above pigments are mostly provided by the diatoms. This conclusion is quite consistent with that obtained in the identification of diatoms as the dominant species of the phytoplankton from the sea ice in that sea area by the traditional taxonomic method (Yu *et al.*, 1986).

3.2 The seasonal changes of ice algae pigments

Usually, people use chlorophyll-a content in waters as an index for the standing stock of phytoplankton. Hence, the determination of chlorophyll-a content in water bodies has become one of the important parameters in the marine ecological research. In view of investigations, obvious geographical and seasonal changes can be seen in the distribution of the standing stock of phytoplankton in the Southern Ocean and waters around Antarctica. Theoretically, the standing stock of the phytoplankton in the offshore waters of Antarctica and the subantarctic islands should be far greater than that in the open seas. For instance, the Scott Sea, the Ross Sea and the southwestern waters of the Weddell Sea which lie on the west of the Antarctic Peninsula are very rich in plankton (El-sayed, 1970). Hence we use the seasonal changes of chlorophyll-a to study those in ice algae pigments. Fig. 2 shows the kinds of ice algae pigments in different months (the number of spots are used temporarily to represent the various kinds of pigments).

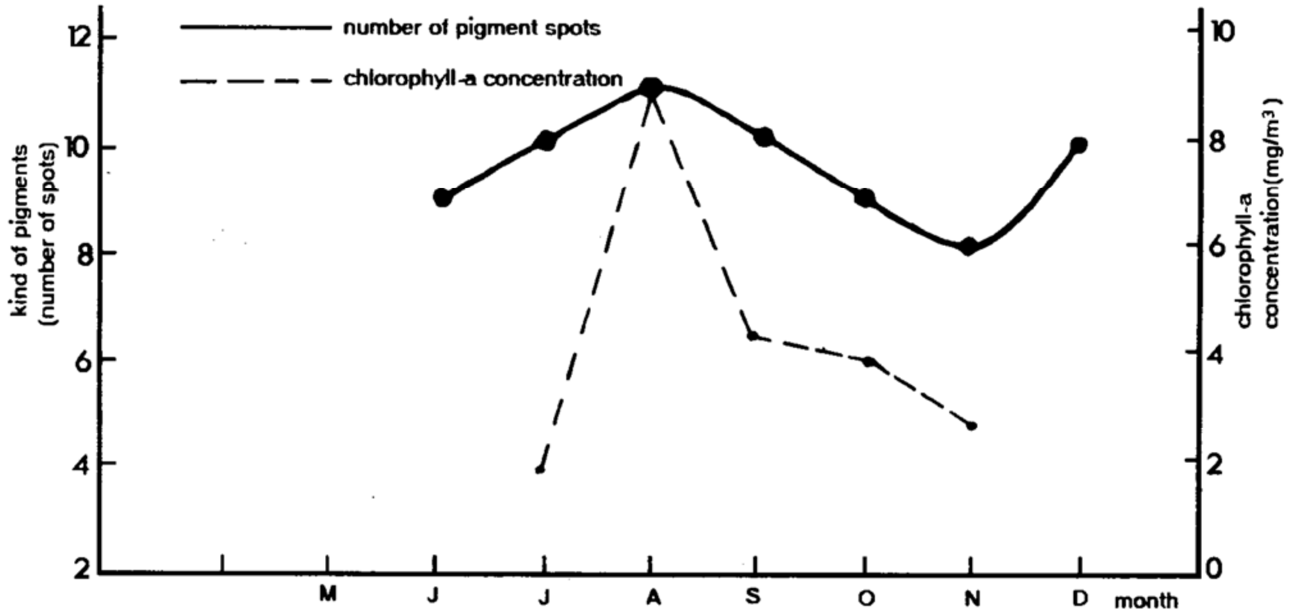

Fig. 2. Number of spots of the ice algae pigments and chlorophyll-a concentration in different months.

From Fig. 2, it can be seen that during June～December the seasonal changes in the kinds of the ice algae pigments in each month is quite obvious. From June the number of pigment spots begins to rise and reaches the highest peak (11 spots), then it drops and reaches the minimum in November and only 8 pigment spots are separated, and up to December the number tends to rise again, which is because the thicknesses of the ice-snow in different months, as well as the environmental conditions are different. Hence the kinds of ice algae pigments also show differences in varying degrees.

Chlorophyll-a showed the same tendency of variation. In July, the chlorophyll-a content was low (1.759 mg/m^3), the change ranging from 1.080～2.383 mg/m^3. In taking icicle samples, the snow covering the ice should be removed first. In July the snow was 7～15 cm thick and the thickness of sea ice 42 cm; in August the chlorophyll-a content increased sharply to 8.977 mg/m^3. In September it dropped to 4.913 mg/m^3 and in October and November it was 4.0595 mg/m^3 and 2.347 mg/m^3 repectively. With the ice layer gradually becoming thinner, the chlorophyll-a content rapidly dropped. The phytoplankton in sea water swims up to the ice layer for photosynthesis. According to the photophilous habit of organisms, it is considered that when the ice becomes thicker more phytoplankters will swim up to ice layer, the kind and number of ice algae will sharply increase, and the chlorophyll-a content becomes higher. In August the maximum thickness of the icicles was already 77 cm; at this time the chlorophyll-a content of the icicles was 40 times that in the same volume of sea water. From the beginning of freezing to the melting of ice, the chlorophyll-a content of sea ice is generally higher than that in the same volume of sea water. This result coincides with the counting of the phytoplankton (Yu *et al.*, 1986).

3.3 The effect of ice algae pigments on ice algae

One of the important undertakers of marine primary productivity in Antarctica is the ice algae. The reason why ice algae can exist extensively in both the fixed and floating ice regions is that they have immediate relation to the quantity of pigments that can cause them to carry out photosynthesis. The pigment that can cause marine algae to carry out photosynthesis is mainly chlorophyll, which, therefore, is also called by some the photosynthetic pigment. There are still some other accessary pigments as carotene, etc. That is to say, in a certain sea area if the phytoplankton pigments are various and their contents are high the algae certainly are more in kind, and in quantity, and the marine productivity is higher.

Different populations of ice algae are composed of different kinds of pigments. Even the pigments are identical in kind, their quantities are different, that is to say, the same kind but different quantity of pigments will form different kind of phytoplankton (algae). The growth and reproduction of ice algae depend upon the photosynthesis of ice algae pigments. Ice algae bear the biological phototaxis and photophilous characteristics through which the ice algae pigments have an effect on the ice algae.

3.4 The effect of ice algae on marine ecosystems

As the structures, physical and chemical characteristics of sea ice are different from distributions of the algae there are also differences in their primary productivity. According to the report by Hashiai (1981a, 1981b) on the biomass of the bottom layer of the sea ice in the adjacent waters of Syowa (Szoux) station: the chlorophyll-a concentration for April was 829 mg/m^3, 0.10 mg/m^3 for August, and greater than 100 mg/m^3 for December (Chen and Xiao, 1986).

Palmisano and Sullivan (1983) did field simulated experiments on photosynthesis of microalgae in sea ice in McMurdo Sound and obtained the data that each mg of chlorophyll-a can fix 0.6～7.5 mg carbon each day. Meconville *et al.* melted the bottom of the sea ice samples from the waters nearby Casey Station, Australia, to simulate the field conditions and obtained the data that the primary productivity for November was as high as 81 μg • C per hour per liter of water sample and in the middle of January dropped to 2.8 μg • C. Obviously, viewed either from standing stock or from biomass, both the main significance and value of sea ice microalgae in the antarctic marine ecosystem admit of no negligence.

Garrison *et al.* (1982) pointed out that ice algae have a function as seeding cells. In antarctic winter ice algae are stored as seeding cells in sea ice layer, the formation of seeding cells is as follows: in autumn sea water begins to freeze and the phytoplanktons still float in sea water; at the beginning of winter, the sea ice becomes thick and the falling snow covers the ice, but the phytoplankton in water bodies are still in a state of hiding. When lightening time and temperature markedly drop, the algae stop growing. When the upper part of ice is covered by snow, new ice grows at its bottom. In this way, ice algae are stored as seeding cells in the sea ice layer. In spring, as temperature rises and lightening time prolongs the phytoplankton in ice begin to grow and reproduce. Moreover, the algae blooms accelerated the speed of the absorption of sun light (Chen and Xiao, 1986), which, as a result, caused the accumulated ice to melt more rapidly. In summer, the melting of ice released the phytoplankton stored in the ice layer. After entering the water columns, these algae get sufficient sunlight and suitable temperature and grow and multiply rapidly to become the important undertakers of primary productivity in water columns (Garrison *et al.*, 1982).

Buinitsky obtained the result in his experiment that the maximum quantity of sea ice algae, amounted to 3700×10^4 cells per liter of sea ice water. Ice algae are also very nutritious. According to calculation, the organic matter of 100 g of diatom cells can produce 2196 Joules of heat (Zhang and Lu, 1986). Therefore, viewed from the content of ice algae pigments and biomass, the effect of ice algae on the antarctic marine primary productivity admits of no negligence and it is also probably the main source of nutrition for the phytoplankton in antarctic fixed and floating ice areas.

References

Bunt, J. S. (1963): Diatoms of antarctic sea-ice as agents of primary production. *Nature*, 199, 155—157.

Bunt, J. S. and Wood, E. J. (1963): Microalgae and antarctic sea-ice. *Nature*, 199, 1245—1255.

Bunt, J. S. (1968): Microalgae of antarctic pack ice zone. Symposium on antarctic oceanography, Ed. by Currie, R. I., Scott Polar Research Institute, Cambridge, 198—219.

Chen Xingqun and Xiao Yichang (1986): General status of microscopic organisms in antarctic sea ice. *Marine Science*, 10(4), 40—50 (in chinese).

El-Sayed, S. Z. (1970): On the productivity of the Southern Ocean (Atlantic and Pacific Sectors). In: Antarctic Ecology, Ed. by Holdyate, M. W., Academic Press, New York, 1, 119—135.

Garrison, D. L., Buck, K. R. and Silven, M. W. (1982): Ice algae communities in the Weddell Sea, Antarctic. *J. U. S.*, 17, 157—159.

Hoshiai, T. (1969): Ecological observations of the colored layer of the sea-ice at Syowa Station. *Antarctic Rec.*, 34, 60—72.

Hoshiai, T. (1981a): Solar radiation and sibility of the under surface of sea-ice algae proliferation, *Antarctic Rec.*, 73, 23—29.

Hoshiai, T. (1981b): Proliferation of ice algae in the Syowa Station area. *Mem. Natl. Inst. Polar, Ser. E. (Biol. Med. Sci.)*, 14, 1—12.

Jeffrey, S. W. (1961) : Paper chromatographic separation of chlorophylls and carotenoids from marine algae, *Biochem. J.*, 80, 336—342.

Palmisano, A. C. and Sullivan, C. W. (1983): Physiology of sea ice diatoms, I. Dark survival of three polar diatoms. *Can. J. Microbiol.*, 29, 157—160.

Yu Jianluan, Li Ruixiang and Huang Fengpeng (1986): A preliminary study on the ecology of the phytoplankton in Great Wall Bay. *Antarctic Research* (Chinese Edition), 4(4), 34—39.

Zhang Kuncheng and Lu Peiding (1986) : Some ecological observations on antarctic ice algae. A collection of Antarctic Scientific Exploration. No. 3, Ocean Press, Beijing, 49—59(in Chinese).

Zheng Zhong, Li Xiaoqing and Xu Zhenzu (1984): Marine planktology. Ocean Press, 17 (in Chinese).