Jap. J. Phycol. (Sôrui) 30: 279-288. December 15, 1982

Effects of physico-chemical factors and nutrients on the growth of *Heterosigma akashiwo* HADA from Osaka Bay, Japan

Makoto M. WATANABE*, Yasuo NAKAMURA*, Sakae MORI**
and Susumu YAMOCHI***

* *Water and Soil Environment Division, The National Institute for Environmental Studies, Yatabe-machi, Tsukuba, Ibaraki, 305 Japan.*
** *Faculty of Agriculture, Tohoku University, Amamiya, Sendai, Miyagi, 980 Japan.*
*** *Osaka Prefectural Fisheries Experimental Station, Misaki-cho, Sennan, Osaka, 599-03 Japan.*

WATANABE, M. M., NAKAMURA, Y., MORI, S. and YAMOCHI, S. 1982. Effects of physico-chemical factors and nutrients on the growth of *Heterosigma akashiwo* HADA from Osaka Bay, Japan. Jap. J. Phycol. 30: 279-288.

Heterosigma akashiwo HADA, Raphidophyceae, which causes heavy red tides in Osaka Bay, Japan, was obtained in axenic clonal culture by micropippette washings. The growth characteristics of the strain were examined. The optimum growth rate was obtained at temperatures of 15-25° C, salinities of 9-30‰, and light intensities above 0.034 ly·min^{-1}, and throughout the pH range examined (7.3-8.4). Nitrate and ammonium served as good nitrogen sources. Urea was not so well utilized as nitrate and ammonium, and the amino acids examined were not utilized at all. The minimum cell quota of nitrogen and the nitrate assimilation rate were 1.44 *p*mol·cell^{-1} and 0.29-1.6 *p*mol·cell^{-1}·day^{-1}, respectively. As a phosphorus source, only orthophosphate was utilized. The minimum cell quota of phosphorus and the phosphate assimilation rate were 95 *f*mol·cell^{-1} and 8-120 *f*mol·cell^{-1}·day^{-1}, respectively. Iron and vitamin B_{12} were essential for growth. These characteristics were compared with those of the other strains of *H. akashiwo*, the Fukuyama strain (IWASAKI *et al.* 1968), the Gokasho strain (IWASAKI and SASADA 1969) and the Naragansett strain (TOMAS 1978, 1979, 1980). The alga called "*H. akashiwo*" is composed of at least three physiologically and ecologically different races.

Key Index Words: *growth rate*; Heterosigma akashiwo; *minimum cell quota*; *Raphidophyceae*; *red tide*.

The marine raphidophycean flagellate, *Heterosigma akashiwo* HADA has been known as a causative organism of red tides in various areas of the coastal water of Japan. In Osaka Bay, this species occurs as a summer red tide. This red tide sometimes produces a harmful effect on fish and causes local inhabitants and fishermen to complain, because they are inclined to believe that the occurrence of the red tide is due to domestic or industrial pollution.

As mentioned by IWASAKI (1979), because a red tide is a special event in natural phytoplankton succession, more attention should be paid to the mechanisms controlling this succession. The abundance of phytoplankton in nature is regulated by a multitude of environmental factors such as nutrients, light, temperature, salinity, and grazing. Understanding these complex processes in both qualitative and quantitative terms is the ultimate objective in order to predict and control red tide outbreaks. In culture experiments, these environmental factors are reduced to a manageable number and can be investigated under defined conditions. The knowledge gained from a series of physiological studies on some red tide algal species

(IWASAKI *et al.* 1968, IWASAKI and SASADA 1969, IWASAKI 1969, 1971a, b, 1973) revealed that such studies are indispensable in explaining the most basic question about a red tide: what factors lead to mono-species patches of red tide organism.

The growth characteristics of *H. akashiwo* and its allied organisms have been studied using the strains obtained from the Fukuyama coast, Japan (IWASAKI *et al.* 1968; named *Entemosigma* sp. and thereafter transferred to *H. akashiwo*), from the Gokasho Bay, Japan (IWASAKI and SASADA 1969; named *H. inlandica*), and from the Naragansett Bay, Rhode Island, the U.S.A. (TOMAS 1978, 1979, 1980; named *Olisthodiscus luteus*). Recently, it was pointed out that these strains were morphologically conspecific and should be treated under the name of *H. akashiwo* HADA (HARA, personal communication). Some physiological differences, however, were observed between the Fukuyama strain and the Gokasho strain (IWASAKI *et al.* 1968, IWASAKI and SASADA 1969, IWASAKI 1979). It therefore seems likely that *H. akashiwo* is composed of several physiolocal races.

In the present study, as one of a series of studies aimed at defining the mechanisms of red tide outbreaks of *H. akashiwo* in Osaka Bay, culture experiments were carried out to determine the effects of physico-chemical factors, such as temperature, salinity, light intensity and pH, and of nutrients on the growth of *H. akashiwo* from Osaka bay.

Materials and Methods

A crude culture strain of *H. akashiwo* was isolated in August, 1979 from Tanigawa Fishing Port, Osaka Bay, Japan. A clonal axenic culture strain (OHE-1) was obtained using the sterile micropipette washing method. Modified ASP-7 medium (Table 1) was used as the basal medium. This was sterilized by autoclaving (120°C, 20 min.). The culture vessels were 500 m*l* Erlenmeyer flasks containing 200 m*l* of the medium. All cultures were inoculated with living cells to a concentration of 500-1,000 cells·ml^{-1} and incubated in growth chambers having a photoperiod of 12: 12 LD (lights on at 0800 and off at 2000).

The effects of temperature, light intensity, salinity and pH on the growth of this strain were examied. Growth was measured from changes in cell number by counting living cells using a 1 m*l* counting chamber of the Sedwick-Raftor type (Fujimoto Co.). As cell division occurs between 0500 and 1100 (WATANABE *et al.* in prep.), counts were made at 1300 or 1400 on consecutive days for up to 8 days. After day 8, counts were made at about 3 day intervals until the growth reached a stationary phase. The relative growth constant (k) in the exponential phase was calculated by the least squares fit of:

$$\ln N = \ln No + kt \quad (1)$$

where No=initial cell concentration; N= cell concentration after t days from inoculation.

Nutrient utilization in this strain was studied. The growth yield was measured by counting cell numbers using an Improved Neubauer Haemocytometer 3 weeks after inoculation. The organism was precultured once in medium lacking the compound to be tested. The experiments determining the minimum cell quota and assimilation rate of nitrogen and phosphorus were conducted using 500 m*l* Erlenmeyer flasks with 200 m*l* of nitrate limited medium (about 50 μM nitrate) or phosphate limited medium (about 10 μM orthophosphate). Diel changes of ambient nitrate and phosphate concentrations were determined from glass fiber filtered samples using a Technicon Autoanalyzer A-II. Cell counts were made by the same method followed in measuring growth rate. The cell quota of nitrogen or phosphorus at time t was obtained by the following equation:

$$q = \frac{S_T - S}{N} \quad (2)$$

where q=cell quota at time t; S=ambient nitrate or phosphate concentration at time

t; S_T=total nitrogen or phosphorus concentration, which was calculated from the equation, $S_T=So+No\cdot q_m$ (So=initial nutrient concentration; q_m=minimum cell quota); N =cell concentration at time t. The minimum cell quota (q_m) was obtained by the equation (2) when the ambient nitrate or phosphate concentration became undetectable and the terminal cell concentration was obtained. The nitrate or phosphate assimilation rate was measured from:

$$v=q\cdot\frac{d\ln N}{dt}+\frac{dq}{dt} \qquad (3)$$

where v=assimilation rate of nitrate or phosphate. All of these nutritional studies were done at temperature of 20±1°C and a light intensity of 0.04 ly·min^{-1}.

In all experiments, a bacteria-free check was done using STP medium (PROVASOLI *et al.* 1957).

Results

Effect of temperature: Growth at different temperatures was examined at a light intensity of 0.04 ly·min^{-1}. The results are shown in Fig. 1. Growth occurred at all temperatures examined. At temperatures from 10 to 30°C, a 3-fold difference in growth rates (k=0.2—0.64 day^{-1}) were observed as well as a 12-fold difference in cell concentration (0.25—3×10^5 cells·ml^{-1}). The optimal growth rate was observed at temperatures of 15—25°C (k=0.64day^{-1}). At temperatures of 30 or 10°C, the growth rate was drastically reduced. The terminal cell concentration had a maximum value of 3×10^5 cells·ml^{-1} at 15—20°C and declined thereafter with decreasing or increasing temperatures.

Effect of salinity: Growth at different salinities was tested at 20±1°C and 0.04 ly·min^{-1} by varying the total amount of the major salts (NaCl, $MgSO_4\cdot 7H_2O$, KCl and $CaCl_2\cdot 2H_2O$) of the basal medium without changing the ratios of these salts. The results are shown in Fig. 2. Growth was observed throughout the salinity range tested. The growth rate and cell concentration were maximum (0.64 day^{-1} and 3×10^5 cells·ml^{-1}, respectively) at salinities of 9-30‰, and declined thereafter with increasing salinity.

Effect of light intensity: Growth at different light intensities was examined at 20±1°C. In this experiment, the upper portion of culture flasks were covered with black paper and light was supplied from the bottom of the flasks. Different intensities of light were obtained by neutral density screening. The light intensity at the inside of culture flasks was measured by an underwater spherical (4π collector) quantum sensor (Bio-spherical Instruments, Inc., San Diego, U. S. A. model QSL-100) with a non selective response to quantum flux between 400-700 nm. The results are shown in Fig. 3. Growth was recognized at light intensities

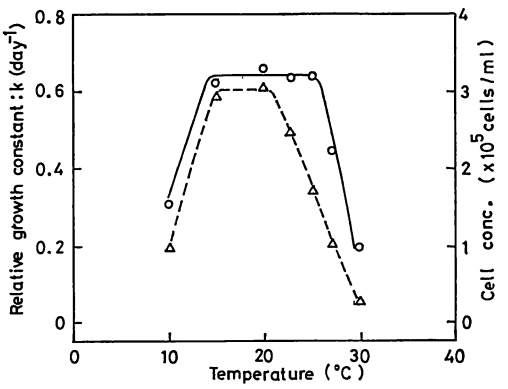

Fig. 1. Growth of *H. akashiwo* at different temperatures. ○: Growth rate. △: Cell concentration.

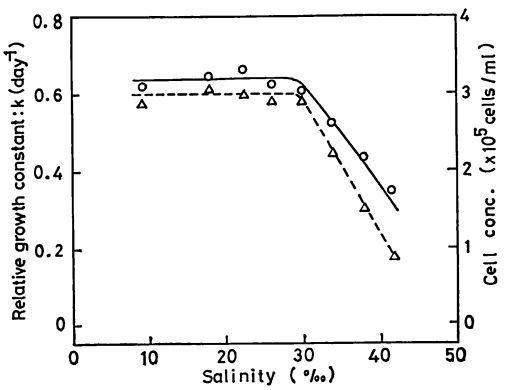

Fig. 2. Growth of *H. akashiwo* at different salinities. ○: Growth rate. △: Cell concentration.

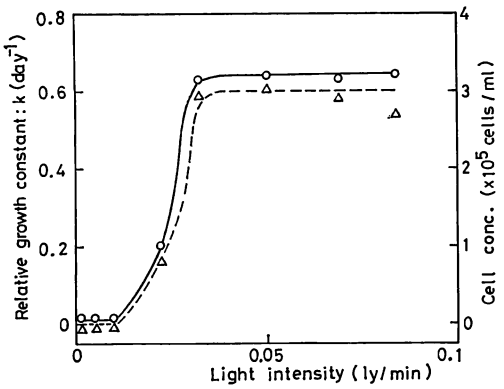

Fig. 3. Growth of *H. akashiwo* at different light intensities. ○: Growth rate. △: Cell concentration.

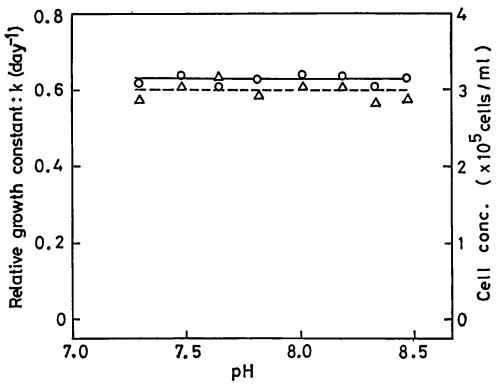

Fig. 4. Growth of *H. akashiwo* at different pH. ○: Growth rate. △: Cell concentration.

above 0.022 ly·min^{-1} and growth rate and cell concentration were maximum (0.65 day^{-1} and 3×10^5 cells·ml^{-1}, respectively) above 0.034 ly·min^{-1}.

Effect of pH: Growth at different pHs was examined at 20±1°C and 0.04 ly·min^{-1}. Within the range of pH 7.3—8.4, the final pH of cultured media changed very slightly from the initial pH (within 0.03 pH units). At pHs higher than 8.5, however, cultured media contained precipitation and showed considerably different pH values from the initial ones. Only the results of experiments carried out at pH 7.3—8.4 are shown in Fig. 4. The growth rate and cell concentration were maximum (0.63 day^{-1} and 3×10^5 cells·ml^{-1}, respectively) at these pH values.

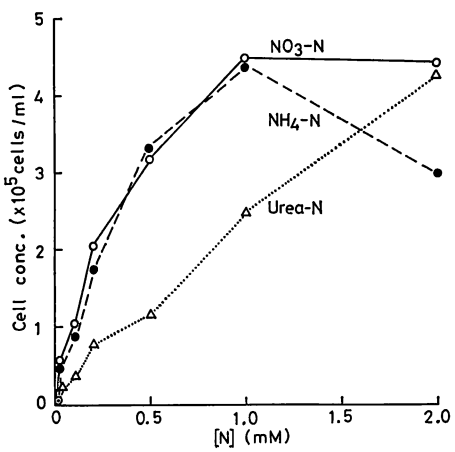

Fig. 5. The effect of nitrogen sources on the growth yield of *H. akashiwo*.

Utilization of nitrogen sources: As inorganic nitrogen sources, both nitrate (as $NaNO_3$) and ammonium (as NH_4Cl) were examined. Both compounds served as good nitrogen sources (Fig. 5). A maximum cell concentration of 4.5×10^5 cells·ml^{-1} was obtained using above 1 mM nitrate and at 1 mM of ammonium. Growth was slightly inhibited at 2 mM of ammonium, but not with the same concentration of nitrate. As organic nitrogen sources, urea and 3 kinds of amino acids (glycine, D.L.- alanine and L-glutamate) were examined. The medium containing urea was sterilized by membrane filtration (Milipore, 0.45μm HA). Urea was not utilized as effectively as nitrate and ammonium (cf. Fig. 5), and the amino acids were not utilized at all.

The experiment on nitrogen utilization was followed over an 8 day period in a nitrate limited medium with 57.4 μM of $NaNO_3$. The results are shown in Fig. 6. Ambient nitrate was depleted after day 5, but cells increased up to day 7. The terminal cell concentration was 4.0×10^4 cells·ml^{-1}. From the equation (2), the minimum cell quota of nitrogen was obtained as 1.44 *p*mol·cell^{-1} (=20.16 *p*g·N·cell^{-1}). The cell quota ranged from 1.44 to 2.12 *p*mol·cell^{-1} and the nitrate assimilation rate from 0.29 to 1.6 *p*mol·cell^{-1}·day^{-1}. The highest values were obtained at day 4, when cells were

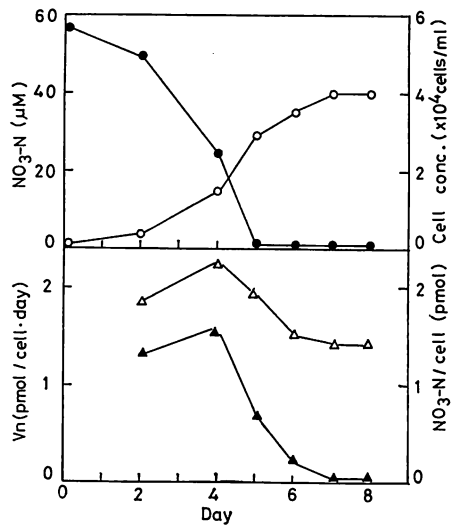

Fig. 6. Changes of ambient nitrate concentration in medium (●), cell concentration (○), V_n: nitrate assimilation rate (▲) and cell quota of nitrogen (△).

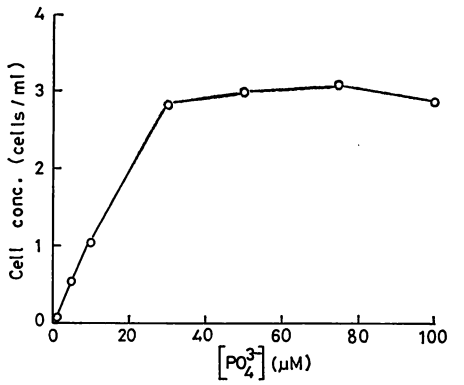

Fig. 7. The effect of phosphate on the growth yield of *H. akashiwo*.

actively growing.

Utilization of phosphorus sources: As phosphorus sources, glycerophosphate (β-$Na_2C_3H_5(OH)_2PO_4 \cdot 5H_2O$) and orthophosphate ($NaH_2PO_4 \cdot 2H_2O$) were tested. Growth was observed only in orthophosphate. A maximum cell concentration of 3×10^6 cells·ml^{-1} was obtained using above 30 μM of phosphate (Fig. 7).

The experiment on phosphorus utilization was followed over a 10 day period in a phosphate limited medium with 5.1 μM of $NaH_2PO_4 \cdot 2H_2O$. The results are shown in Fig. 8. Ambient phosphate was depleted after day 6, but cells increased up to day 8. The terminal cell concentration in this culture was 5.8×10^4 cells·ml^{-1}. The minimum cell quota of phosphorus was obtained as 95 $f$mol·cell^{-1}. The cell quota ranged from 95 to 220 $f$mol·cell^{-1} and the phosphate assimilation rate from 8 to 115 $f$mol·cell^{-1}·day^{-1}. The highest value weres obtained at days

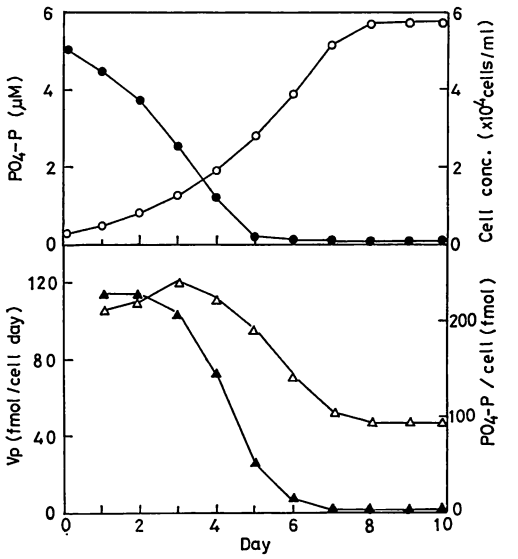

Fig. 8. Changes of ambient phosphate concentration in medium (●), cell concentration (○), V_p: phosphate assimilation rate (▲), and cell quota (△) of phosphorus.

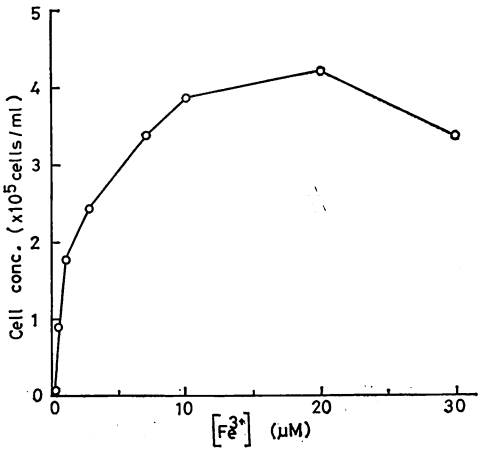

Fig. 9. The effect of iron on the growth yield of *H. akashiwo*.

1-3, when cells were actively growing.

Trace matal requirement: Trace metal requirements were examined for iron, manganese, cobalt and zinc. Among them, only an iron requirement was detected. A maximum cell concentration of about 4×10^5 cells·ml^{-1} was obtained using 10 and 20 μM of iron (Fig. 9).

Vitamin requirement: Vitamin requirements were tested for vitamin B_{12} and vitamin mix S_3 of Provasoli (cf. Table 1), which contains thiamine, biotin and 7 other vitamins. It was found (Fig. 10) that only vitamin B_{12} was essential for growth, while the other vitamins were not. No stimulative effect was recognized when vitamin mix S_3 was added to the medium containing 1 $\mu g \cdot l^{-1}$ of vitamin B_{12}.

Table 1. Composition of ASP-7 modified medium.

NaCl	25.0 g	NTA	70.0 mg
$MgSO_4\cdot7H_2O$	9.0 g	$Na_2EDTA\cdot2H_2O$	30.0 mg
KCl	0.7 g	$FeCl_3\cdot6H_2O$	1.9 mg
$Cacl_2\cdot2H_2O$	0.3 g	$CoSO_4\cdot7H_2O$	28.0 μg
$NaNO_3$	50.0 mg	$ZnSO_4\cdot7H_2O$	1.4 mg
$NaH_2PO_4\cdot2H_2O$	20.0 mg	$MnCl_2\cdot4H_2O$	1.0 mg
$Na_2SiO_3\cdot9H_2O$	10.0 mg	H_3BO_3	34.0 mg
Vitamin B_{12}	1.0 μg	H_2O	1000.0 m*l*
Vitamin mix S_3*	10.0 m*l*	pH	8.0
TRIS	1.0 g		

* One m*l* of vitamin mix S_3 contains biotin: 0.1 μg, thiamine: 50 μg, nicotic acid: 10 μg, Ca panthothenate: 10 μg, *p*-amino benzoic acid: 1 μg, inositol: 0.5 mg, folic acid: 0.2 μg, thimine: 0.3 mg (PROVASOLI 1963).

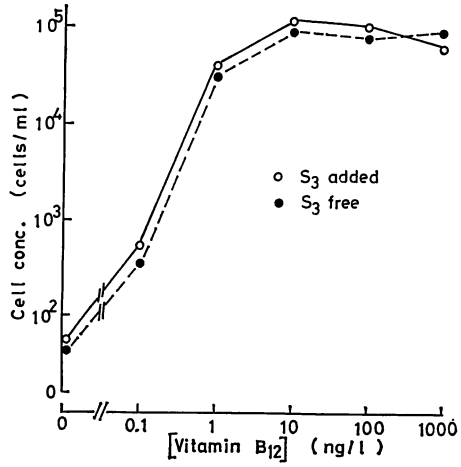

Fig. 10. The effect of vitamins on growth yield of *H. akashiwo*.

Discussion

As shown in Results, the Osaka strain used in the present study was eurythermal and euryhaline. This feature has been observed in most red tide flagellates (IWASAKI 1979). The light intensity above which the maximum growth could be obtained was 0.034 ly·min^{-1}. This value is lower than the values for *Dunaliella tertiolecta* (0.15 ly·min^{-1}), *Amphidinium carteri* (0.1 ly·min^{-1}) and *Skeletonema costatum* (0.075 ly·min^{-1}) reported by JITTS *et al.* (1964). This suggests that *H. akashiwo* has the ability to form dense populations even in turbid coastal waters of low light intensity. Maximum growth was observed throughout the pH range tested (7.3-8.4). Either nitrate or ammonium served as a good nitrogen source for the Osaka strain, as reported for most algal species (IWASAKI 1979). On the contrary, urea was not utilized as effectively as nitrate and ammonium, and amino acids did not seem to serve as nitrogen sources. As to the phosphorus source, only orthophosphate was utilized. Although it has been reported that glycerophosphate was utilized for almost all red tide flagellates (MAHONEY and MCLAUGHLIN 1977, IWASAKI 1979), glycerophosphates was not utilized by this strain. It is probable that this strain does not have alkaline phosphatase activity. As shown in the nitrate and phosphate utilization experiments, the minimum cell quota of nitrogen and phosphorus of this strain were 1.44 *p*mol·$cell^{-1}$ and 95 *f*mol·$cell^{-1}$, respectively. From this result, a N: P ratio of cell subsistence is calculated as 15.2 and this value is similar to the N: P ratios reported for several phytoplankters (STRICKLAND *et al.* 1969, RHEE and GOTHAM 1981). The cell quota and assimilation rate of these two nutrients showed daily changes. The values

were high when cells were actively growing and became low with a decreasing growth rate. From this finding, it is suggested that the cell quota and assimilation rate have a close relationship to growth. This problem will be analyzed in a future report on growth kinetics under nitrate or phosphate limited continuous culture. Both iron and vitamin B_{12} were needed for the growth of this strain, as reported for most red tide flagellates (IWASAKI 1979). Although it has been said that manganese and zinc were essential elements for growth of alage (O'KELLEY 1974), this strain showed maximum growth even when these elements were not added. However, trace amounts of manganese and zinc usually appear as contaminants in commercial reagents of NaCl and $MgSO_4 \cdot 7H_2O$, which are the major salts of the modified ASP-7 medium. It is probable that these trace amounts suffice for the growth of this strain.

As mentioned before, physiological studies on *H. akashiwo* have already been carried out using Fukuyama strain (IWASAKI *et al.* 1968), Gokasho strain (IWASAKI and SASADA 1969) and Naragansett strain (TOMAS 1978, 1979, 1980). Although the known characteristics for the Osaka strain have not all been examined in these strains, the existing characteristics for these strains are compared with those of the Osaka strain in Table 2. As shown in the table, the Fukuyama strain is characterized by a preference for low salinity at a narrow range and low pH and utilization of organic phosphrus. From this, it was suggested that the Fukuyama strain had the ability to form dense populations in seawater with an enhanced nutrient supply, low salinities and a low pH, resulting from water washed from the land after a heavy rainfall or from the discharge of domestic waste or sewage (IWASAKI *et al.* 1968). The Gokasho strain is characterized by a preference for a low salinity and a high pH, a utilization of organic nitrogen and phosphorus and a great growth enhancement by purine and pyrimidine. From this, the Gokasho strain seemed to be well adapted to organically polluted waters having high COD values, a low salinity and a high pH (IWASAKI and SASADA 1969). The

Table 2. Physiological characteristics of 4 strains

Strains / Characteristics	Fukuyama strain (IWASAKI *et al.* 1968)	Gokasho strain (IWASAKI and SASADA 1969)	Naragansett strain (TOMAS 1978, 1979, 1980)	Osaka strain
Temperature (Optimum)	not examined	not examined	15-25° C	15-25° C
Salinity (Optimum)	27‰	10-14‰	10-40‰	9-31‰
Light intensity (Optimum)	not examined	not examined	0.028 ly·min^{-1}(a)	0.034 ly·min^{-1}(b)
pH (Optimum)	7.5	8.5-9.0	not examined	7.3-8.4
Utilization of N-sources	NO_3-N and NH_4-N. (Urea was utilized.)	NO_3-N and NH_4-N. (Urea, uric acid, asparagine and arginine were utilized.)	NO_3-N and NH_4-N. (Minimum cell quota of N: 24.00 pg·N·cell^{-1})	NO_3-N and NH_4-N. (Urea was utilized.) (Minimum cell quota of N: 20.16 pg·N·cell^{-1})
Utilization of P-sources	PO_4-P and glycerophosphate.	PO_4-P and glycerophosphate. (Adenylic acid and guanylic acid were utilized.)	PO_4-P only. (Alkaline phosphatase activity was not detected.)	PO_4-P only.
Utilization of vitamins	B_{12} (A little growth enhancement by vitamin mix S_3)	B_{12} (A great growth enhancement by purine and pyrimidine.)	not examined	B_{12}

a: optimum as photosynthetic activity. b: optimum as growth rate.

Naragansett and Osaka strains seem to have similar physiological natures. They are characterized by a wide tolerance of different temperatures and salinities and no ability of organic phosphorus utilization. From this, it can be said that the Osaka and Naragansett strains are ecologically less favoured only in seawater containing insufficient inorganic phosphorus when compared with the Fukuyama and Gokasho strains, because of the inability of organic phophorus utilization.

Thus it appears that the alga called "*H. akashiwo*" is composed of at least three physiologically different races and that these differences are ecologically meaningful. Most current phycologists seem to regard the physiological characteristics of paticular strains as minor differences which have no relation with the species problem, relying on the classical morphological species concept. However, some reproductively isolated populations (biological species) and ecologically differentiated populations (ecospecies) have been recognized in a single morphological species of algae and higher plants (TURRESON 1922, 1925, WATANABE 1977, WATANABE and ICHIMURA 1978a, b, 1982, ICHIMURA 1981). It will be worthy to analyze the differences observed among 4 strains of *H. akashiwo* from the view-point of biological species concept (MAYR 1963).

In 1978, a five-modal pattern red tide outbreak of *H. akashiwo* in Osaka Bay was observed (YAMOCHI unpub. data). First a red tide of *H. akashiwo* occurred in early June. Thereafter, the red tide occurred 4 times with intervals of 30-40 days until early October. During the outbreak periods, the temperature ranged from 20 to 27.5°C, the salinity ranged from 13 to 33‰, the pH ranged from 8.0 to 8.4 and the light intensity at the surface, where *H. akashiwo* concentrates during the daytime by vertical migration (WATANABE *et al.* in prep.), ranged from 0.04 to 1.0 ly·min^{-1} during the daytime. The experimental results for the growth of Osaka strain of *H. akashiwo* under these conditions (k=0.64-0.45 day^{-1} and 1-3×10^5 cells·ml^{-1}) clearly indicate the potentiality for the establishment of the *H. akashiwo* bloom. Although these physico-chemical factors appear to be major factors governing an *H. akashiwo* bloom, the five-modal pattern of the red tide outbreak cannot be explained by these factors alone. According to Yamochi (unpub. data), a red tide bloom of *H. akashiwo* occurred when the salinity showed a tendency to decline. In nature, changes of salinity imply not only those of osmotic pressure, but also those of nutrient concentration. The abundance cycle appears to be affected by nutrient dynamics, because osmotic pressure is considered to be optimal for the growth of *H. akashiwo*. Unfortunately, the detailed nutrient dynamics in Osaka Bay have not yet been elucidated. Based on the present work, detailed field observations of this species should be made to assess the causative factors regulating the red tide outbreak of *H. akashiwo*.

Acknowledgements

The authors wish to express our sincere thanks to Prof. H. IWASAKI, Mie University, for his critical reading of the manuscript; to Dr. M. WATANABE, The National Institute for Environmental Studies, for his helpful encouragement; to Dr. HARA, Tsukuba University, for his advice and help regarding the taxonomical problems of *H. akashiwo*.

References

ICHIMURA, T. 1981. Mating types and reproductive isolation in *Closterium ehrenbergii* Meneghini. Bot. Mag. Tokyo **94**: 325-334.

IWASASAKI, H. 1969. Studies on the red tide flagellates-III. On *Peridinium hagoei* Schiller appeared in Gokasho Bay, Shima Peninsula. Bull. Plankt. Soc. Japan **16**: 132-139.

IWASAKI, H. 1971a. Studies of the red tide flagellates-V. On *Polykrikos shwarzi* Butchili. Bull. Jap. Soc. Sci. Fish. **37**: 606-609.

Iwasaki, H. 1971b. Studies on the red tide flagellates-VI. On *Eutreptiella* sp. and *Exuviaella* sp. appeared in Bingo-Nada, The Seto Inland Sea, in 1970. J. Oceanogr. Soc. Japan **27**: 152-157.

IWASAKI, H. 1973. The physiological characteristics of neritic red tide flagellates. Bull. Plankt. Soc. Japan **19**: 104-114.

IWASAKI, H. 1979. Physiological ecology of red tide flagellates, p. 357-393. *In* M. LEVANDOWSKY and S.H. HUTNER (ed.), Biochemistry and physiology of protozoa. vol. 1. Academic Press.

IWASAKI, H., T. FUJIYAMA and YAMASHITA, E. 1968. Studies on the red tide dinoflagellates-I. On *Entemosigma* sp. appeared in coastal area of Fukuyama. J. Fac. Fish. Anim. Husb. Hiroshima Univ. **7**: 259-267.

IWASAKI, H. and SASADA, K. 1969. Studies on the red tide flagellates-II. On *Heterosigma inlandica* appeared in Gokasho Bay, Shima Peninsula. Bull. Jap. Soc. Sci. Fish. **35**: 943-947.

JITTS, H.R., C.D. McALLISTER, K. STEPHENS and STRICKLAND, J.D.H. 1964. The cell division rate of some marine phytoplankters as a function of light and temperature. J. Fish. Res. Bd. Canada, **21**: 139-157.

MAHONEY, J.B. and McLAUGHLIN, J.J.A. 1977. The association of phytoflagellate blooms in lower New York Bay with hypertrophication. J. exp. mar. Biol. Ecol. **28**: 53-65.

MAYR, E. 1963. Animal species and evolution. The Belknap Press of Harvard Univ. Press, New York.

O'KELLEY, J.C. 1974. Inorganic nutrients, p. 610-635. *In* W.D.P. STEWART (ed.), Algal physiology and biochemistry. Univ. California Press., Berkeley and Los Angeles.

PROVASOLI, L. 1963. Growine marine seaweeds, p. 9-17. *In* D. DeVIRVILLE and J. FELDMANN (ed.), Proc. Int. Seaweed Symp. Pergamon Press, Oxford.

PROVASOLI, L., J.J. MacLAUGHLIN and DROOP, M.R. 1957. The development of artificial media for marine algae. Arch. Microbiol. **25**: 392-428.

RHEE, G.Y. and GOTHAM, I.J. 1981. Optimum N: P ratios and coexistence of planktonic algae. J. Phycol. **16**: 486-489.

STRICKLAND, J.D.H., O. HOLM-HANSEN, R.W. EPPLEY and LINN, R.J. 1969. The use of a deep tank in plankton ecology. I. Studies of the growth and composition of phytoplankton crops at low nutrient levels. Limnol. Oceanogr. **14**: 23-34.

TOMAS, C.R. 1978. *Olisthodiscus luteus* (Cryptophyceae) I. Effects of salinity and temperature on growth, motility and survival. J. Phycol. **14**: 309-313.

TOMAS, C.R. 1979. *Olisthodiscus luteus* (Cryptophyceae) III. Uptake and utilization of nitrogen and phosphorus. J. Phycol. **15**: 5-12.

TOMAS, C.R. 1980. *Olisthodiscus luteus* (Cryptophyceae) IV. Effects of light intensity and temperature on photosynthesis, and cellular composition. J. Phycol. **16**: 149-156.

TURRESON, G. 1922. The genotype response of the plant species to the habitat. Hereditas **3**: 211-350.

TURRESON, G. 1925. The plant species in relation to habitat and climate. Hereditas **6**: 147-236.

WATANABE, M.M. 1977. Biosystematics in *Closterium* of sexual unicellular green algae and *Calothrix* and *Spirulina* of asexual filamentous blue-green algae, with special reference to the analyses of natural populations. Dr. thesis of Hokkaido University.

WATANABE, M.M. and ICHIMURA, T. 1978a. Biosystematic studies of the *Closterium peracerosum-strigosum-littorale* complex. II. Reproductive isolation and morphological variation among the several populations from the Northern Kanto Area in Japan. Bot. Mag. Tokyo **91**: 1-10.

WATANABE, M.M. and ICHIMURA, T. 1978b. Biosystematic studies of the *Closterium peracerosum-strigosum-littorale* complex. III. Degrees of sexual isolation among the three population groups from the Northern Kanto Area. Bot. Mag. Tokyo **91**: 11-24.

WATANABE, M.M. and ICHIMURA, T. 1982. Biosystematic studies of the *Closterium peracerosum-strigosum-littorale* complex. IV. Hybrid breakdown between two closely related groups, Group II-A and Group II-B. Bot. Mag. Tokyo **95**: 241-247.

渡辺 信*・中村泰男*・森 栄・矢持 進***：大阪湾に出現した *Heterosigma akashiwo* の増殖特性**

大阪湾に赤潮として出現した *Heterosigma akashiwo* をミクロピペット法で分離して無菌培養株を得，その増殖特性を解析した。本株は水温 15-25° C，塩分 9～30‰，照度 0.034 ly•min^{-1} 以上で，また調べられた pH 範囲 (7.3～8.4) すべてで最大の増殖を示した。増殖の為の窒素源としては硝酸態窒素，アンモニア態窒素双方がよく利用されたが，低濃度の尿素は硝酸態窒素，アンモニア態窒素ほどには利用されず，また，アミノ酸は全く利用されなかった。本株の硝酸態窒素同化速度は 0.29—1.6 *p*mol•cell^{-1}•day^{-1} であり，又細胞内窒素最小含有量は 1.44 *p*mol•cell^{-1} であった。燐源としてはオルト燐酸のみが利用された。燐酸の同化速度は 8-120 *f*mol•cell^{-1}•day^{-1} であり，又細胞内燐最小含有量は 95 *f*mol•cell^{-1} であった。鉄，ビタミン B_{12} は本株の増殖に必須であった。本研究で明らかとなった本株の増殖特性と *H. akashiwo* の他の株，福山株 (IWASAKI *et al.*, 1968)，五ケ所株 (IWASAKI and SASADA 1969)，Naragansett 株 (TOMAS 1978, 1979, 1980) の増殖特性を比較・検討したところ，*H. akashiwo* は少なくとも3種の生理生態的に異った群よりなることが判明した。(*305 茨城県筑波郡谷田部町小野川，国立公害研究所水質土壌環境部 **980 仙台市提通雨宮町1の1，東北大・農 **599-03 大阪府泉南郡岬町多奈川谷川2926—1，大阪府水産試験場)

案 内

——緑藻類の分類大系に関するシンポジウム——

Symposium on:

The Systematics of the Green Algae

上記のシンポジウムが分類学協会 (The Systematics Association) 後援のもとに，1983年3月29日～31日に英国ロンドンの The Polytechnic of North London において開かれます。シンポジウムは主に紹待講演によって構成され，英語が使われます。第1次サーキュラーによると，プログラムの概略や参加申し込み手続きなどは下記の通りである。

プログラム

第1日 緑藻分類の検討，緑藻分類学への超微構造と生化学の寄与及び環境がもたらす変異によって引き起される問題等を含むジネラル・トピックス

第2日 Desmids, Ulotrichales, Cladophorales など，特定現生藻類に関する検討

第3日 現生・化石藻を含むその他の特定分類群の検討

予定紹待講演者

A. J. BROOK (U. K.), R. L. CHAPMAN (U. S. A.), G. ELLIOT (U. K.), E. KESSLER (F. R. G.), G. M. LOKHORST (Netherlands), K. R. MATTOX (U. S. A.), M. MELKONIAN (F. R. G). J. D. PICKETT-HEAPS (U. S. A.), F. E. ROUND (U. K.), C. VAN DEN HOEK (Netherlands), P. C. SILVA (U. S. A.), C. J. O'KELLY (U. S. A.)

ポスターセッション

シンポジュウム期間中，ポスターセッションが計画されているので，希望者は申し込むこと。

宿泊その他

会場のポリテクニック・ホステルにおける宿泊料（朝・夜食込み）は約12ポンド/日，昼食4ポンド。参加費（お茶代含まず）15ポンド。学生10ポンド

第2次案内は1983年1月に郵送される予定で，全プログラムとポストシンポジュウムワークショップ（4月1日～2日）に関する詳細が示されることになっている。参加登録と詳細の問合わせは

DR D. E. G. IRVINE
Department of Food and Biological Sciences
The Polytechnic of North London
Holloway Road
London N7 8DB
ENGLAND