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Growth of Harmful Phytoplankton, *Heterosigma akashiwo*, in the Mixture of Surface and Deep Seawater

表層水と深層水を混合した海水中での有害植物プランクトン *Heterosigma akashiwo* の増殖

Norio YOSHIMOTO[1] and Kimio FUKAMI[2]

吉本　典生[1]・深見　公雄[2]

Abstract

Growth potential of the harmful phytoplankton taxon, *Heterosigma akashiwo*, was investigated by laboratory culture studies conducted in surface seawater (SSW), deep seawater (DSW) and the mixture (MSW) of SSW and DSW (1:1) to assess the effects of DSW drainage on natural phytoplankton community structure in coastal areas. In culture experiments using filtered seawater, the cell yields (after 14 days) of *H. akashiwo* in MSW were usually much higher than those of the mean value of SSW and DSW. However, the growth of *H. akashiwo* incubated in roughly-filtered MSW (namely, containing natural phytoplankton communities), was usually suppressed. Under relatively low phosphate concentrations and high N/P and/or Si/P ratios, *H. akashiwo* sometimes showed comparatively higher growths without natural phytoplankton (filtered MSW). These results suggested that *H. akashiwo* has the possibility to grow well in the mixture of SSW and DSW depending on the relative concentration of inorganic nutrients, and that growth of harmful phytoplankton should be considered when high amounts of DSW are drained into the coastal zone.

Key Words: Deep seawater, *Heterosigma akashiwo*, Mass drainage, Coastal ecosystems

要　旨

海洋深層水（DSW）の大量排水が沿岸海域の植物プランクトン群集に与える影響について知るために，表層水（SSW）とDSWを1：1で混合した海水（MSW）中での有害植物プランクトン *Heterosigma akashiwo* の増殖ポテンシャルについて，モデル実験系を用いて調べた．天然植物プランクトンを除去したろ過MSWで *H. akashiwo* を培養した場合の細胞収量は，DSWでの培養時とそれほど変わらず，良好に増殖することがわかった．しかし，天然植物プランクトン群集が混在しているMSWでは，ほとんどの場合 *H. akashiwo* の増殖は抑制された．一方，リン酸塩濃度が低くN/P比やSi/P比が高い場合，*H. akashiwo* は，天然植物プランクトン群集の有無にかかわらず，ほぼ同様に増殖することがわかった．以上の結果から，海洋深層水と表層海水の混合水では，無機栄養塩の組成比によっては *H. akashiwo* などの有害プランクトンの増殖が促進される可能性があり，沿岸海域へのDSWの大量排水には注意が必要であることが示唆された．

キーワード：海洋深層水，*Heterosigma akashiwo*，大量排水，沿岸生態系

[1]United Graduate School of Agricultural Science, Ehime University, 3-5-7 Tarumi, Matsuyama, Ehime 790-8566, Japan
愛媛大学大学院連合農学研究科（〒790-8566　愛媛県松山市樽味3-5-7）

[2]Graduate School of Kuroshio Science, Kochi University, 200 Monobe-otsu, Nankoku, Kochi 783-8502, Japan
高知大学大学院黒潮圏海洋科学研究科（〒783-8502　高知県南国市物部乙200）

1. Introduction

Deep seawater (DSW), which is defined as seawater below the euphotic zone, has the beneficial properties of low temperature, clean, and rich in inorganic nutrients (Nakashima, 1988; Nakashima and Toyota, 1989; Fukami, 2004). Due to these properties, DSW has been used in various industries and investigated for additional uses (Fujita and Takahashi, 2006). Among them, effective mass culture of food microalgae, based on the high concentration of inorganic nutrients, is a promising utilization of DSW (Nakashima, 1988; Fukami *et al*., 1991; Fukami *et al*., 1992; Nakashima, 1992a, 1992b; Fukami and Nishijima, 1994; Matsubayashi *et al*., 1994) and many studies have reported that DSW has the potential for effective microalgal cultivation, such as the food diatom (Fukami *et al*., 1997; Fukami *et al*., 1998). It is also pointed out that DSW can be effective for preventing or recovering from the so-called "Isoyake", the decline of seaweeds on the rocky shore (Watanabe *et al*., 2000; Fujita, 2001, 2003; Otsuka, 2006). These ideas are due to the delivery of the high inorganic nutrient concentrations to the coastal areas. The stimulation effect of DSW on the growth of microalgae, however, may be greater than on the growth of seaweeds. This means that harmful and toxic phytoplankton may grow, and red tides may break out, when mass DSW is drained into the coastal zone. Nevertheless, there are still not enough studies on the influence of DSW on the coastal ecosystem. Since the facilities for pumping and utilizing DSW have been constructed in various places in Japan, high DSW drainage may impact the coastal ecosystem beyond expected.

The harmful phytoplankton taxon *Heterosigma akashiwo* is known to reach a cell density of 10^4–10^6 cells/ml and to cause red tides; such a high cell density has been observed extensively in the coastal areas around Japan (Iizuka *et al*., 2004).

In the present study, the growth potential of *H. akashiwo* in surface seawater, deep seawater and their mixture was investigated in the laboratory. We discuss the effects of mass drainage of DSW on the natural phytoplankton community structure in coastal areas in front of DSW facilities.

2. Materials and methods

2.1 Culture of *Heterosigma akashiwo* in filtered seawater

Surface seawater (SSW) and deep seawater (DSW) were collected from July 2003 to August 2004 (on 9 occasions) at the Deep Seawater Laboratory of Kochi in Muroto City, Japan, and filtered through GF/F glass fiber filters to remove phytoplankton. The mixed seawater (MSW) was prepared by mixing SSW and DSW at a ratio of 1:1. Therefore, three kinds of seawater (SSW, DSW and MSW) were used for the culture experiment in filtered seawater. *Heterosigma akashiwo* NIES-6 (Raphidophyceae) was used as the assay plankton for estimating algal growth potential (AGP) of each seawater. As cells for inoculation should have minimal intracellular concentrations of inorganic nutrients and trace metals (Nishijima and Fukami, 1994), starvation culture was carried out by preserving the *H. akashiwo* cells in aged seawater (without containing inorganic nutrients) for 5 days.

After the starvation culture, *H. akashiwo* cells were inoculated into 200 ml of 3 seawater samples (SSW, DSW and MSW) with an initial density of 1,000 cells/ml. The incubation was carried out at 20℃ under 10,000 lux with a

12:12 of L: D cycle until *H. akashiwo* reached stationary phase. Chlorophyll *a* fluorescence of *H. akashiwo* was measured in regular time intervals by Turner 10-AU Fluorometer (TURNER DESIGNS).

2.2 Culture of *H. akashiwo* in mixed seawater with natural phytoplankton communities

SSW and DSW were filtered through a 100 μm-mesh plankton net to remove larger zooplankton, and were mixed to prepare roughly-filtered MSW containing natural phytoplankton communities. MSW filtered through GF/F glass fiber filters was also prepared as a control. After the starvation culture, *H. akashiwo* was inoculated into 200ml of GF/F filtered MSW and roughly-filtered MSW with an initial density of 1,000 cells/ml. The incubation was carried out at 20℃ under 10,000 lux with a 12:12 of L:D cycle until *H. akashiwo* and other phytoplankton reached the stationary phase.

Water samples were collected in regular time intervals, and the cell density of *H. akashiwo* in the subsample was counted using epifluorescence microscopy (OLYMPUS BX60).

2.3 Nutrient analysis

Before culturing *H. akashiwo* in roughly-filtered MSW containing natural phytoplankton communities, concentrations of dissolved inorganic nitrogen (DIN: NH_4^+, NO_2 and NO_3), dissolved inorganic phosphorus (DIP), and dissolved silicate (DSi) in SSW, DSW, and MSW were determined with an automatic analyzer TRAACS-800 (BRAN + LUEBBE).

2.4 Relative Growth yield

Relative growth yield (RGY) ratios were calculated by dividing cell densities of *H. akashiwo* with natural phytoplankton communities (cultured in roughly filtered MSW) by those without other phytoplankton (cultured in GF/F filtered MSW) on the 14th day of incubation.

3. Results

3.1 Growth of *H. akashiwo* in filtered seawater

Growth curves of *H. akashiwo* cultured in filtered SSW, DSW and MSW collected on 30 April 2002 and 13 May 2004 are shown in Fig. 1, as the representative of several series of experiments. *H. akashiwo* hardly grew in SSW, but grew well in DSW and MSW after a lag period of a few days. The maximum chlorophyll *a* fluorescence in DSW was obtained after 2 weeks. In MSW, chlorophyll *a* fluorescence was approximately half of that in DSW (Fig. 1).

3.2 Growth of *H. akashiwo* in mixed seawater with natural phytoplankton communities

Growth curves of *H. akashiwo* in MSW collected in several different sampling occasions with natural phytoplankton are shown in Fig. 2, as the representative of several series of experiments. Growth of *H. akashiwo* in the roughly-filtered MSW with natural phytoplankton communities was usually lower than in GF/F filtered MSW without natural phytoplankton communities. *H. akashiwo* decreased from the 3rd or the 4th day and disappeared on the 12th to the 14th day (Fig. 2A and 2B). However, in some occasions like 25 March and 13 May 2004, *H. akashiwo* grew well even with natural phytoplankton communities (Fig. 2C and 2D).

3.3 Change in nutrient concentrations

Seasonal changes in DIN, DIP and DSi concentrations are shown in Figs. 3A–3C, respectively. Concentration of DIN in SSW, MSW and DSW varied in the range of 0.53–2.0 μM, 12–17 μM

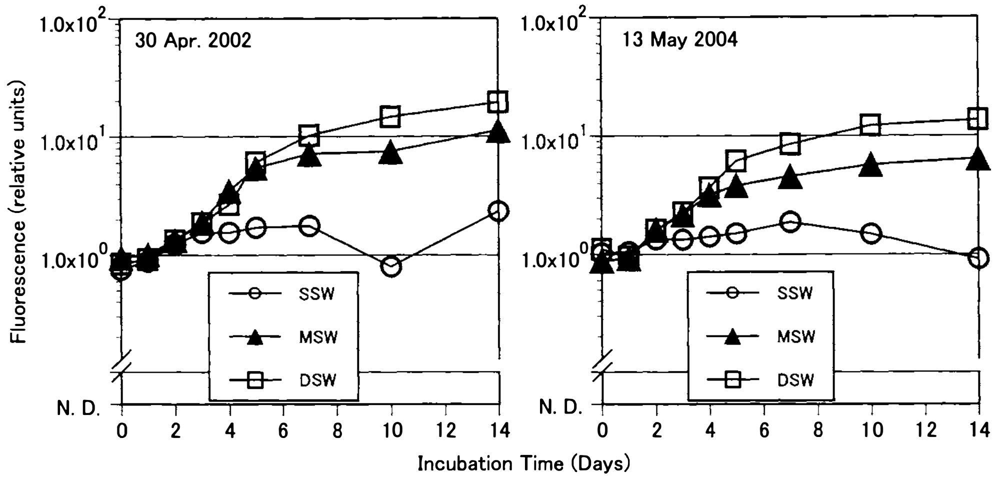

Fig. 1. Growth curves of *Heterosigma akashiwo* in GF/F-filtered surface seawater (SSW: ○), deep seawater (DSW: □) and mixed seawater (MSW; SSW:DSW = 1:1: ▲) collected on 30 April 2002 and 13 May 2004. N. D.: not detected.

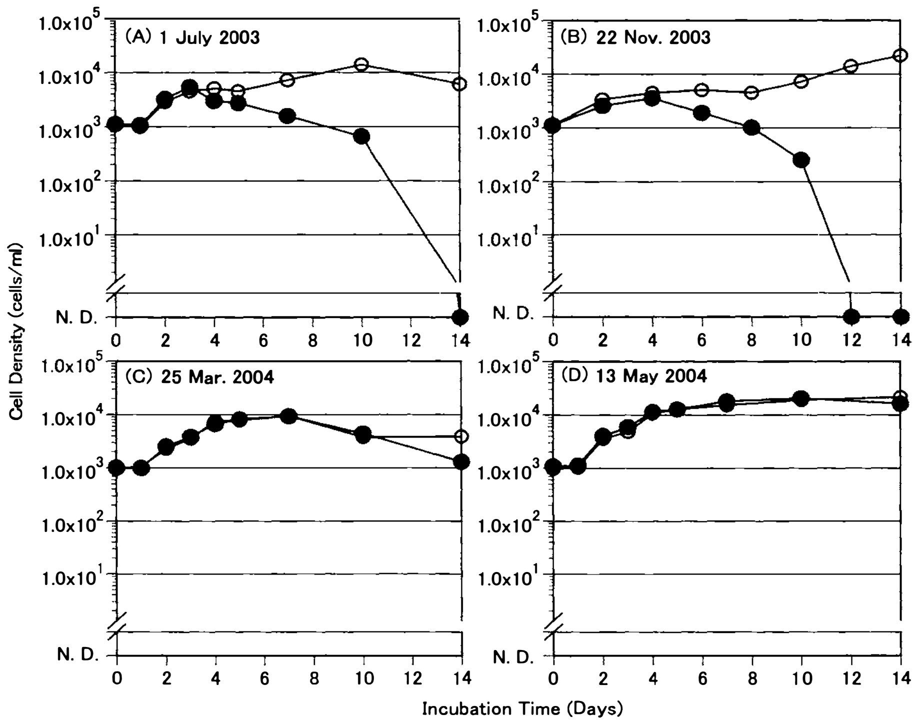

Fig. 2. Growth curves of H. *akashiwo* in mixed seawater (MSW; SSW:DSW = 1:1) without (○) and with (●) natural phytoplankton. Date of commencement of experiments are 1 July in 2003 (A), 22 November in 2003 (B), 25 March in 2004 (C), and 13 May in 2004 (D). N. D.: not detected.

and 23-30 μM, respectively (Fig. 3A). Concentrations of DIP in SSW, MSW and DSW varied in the range of 0-0.22 μM, 0.28-1.1 μM and 1.3-1.9 μM, respectively (Fig. 3B). Concentrations of DSi in SSW, MSW and DSW varied in the range of 0.73 - 24 μM, 18 - 36 μM and 35 - 50 μM, respectively (Fig. 3C). Concentrations of each nutrient in MSW were near the mean values of DSW and SSW.

N/P ratios in MSW varied in the range of 13-43 and increased from May to June, 2004 (Fig. 4A). N/Si ratios in MSW varied in the range of

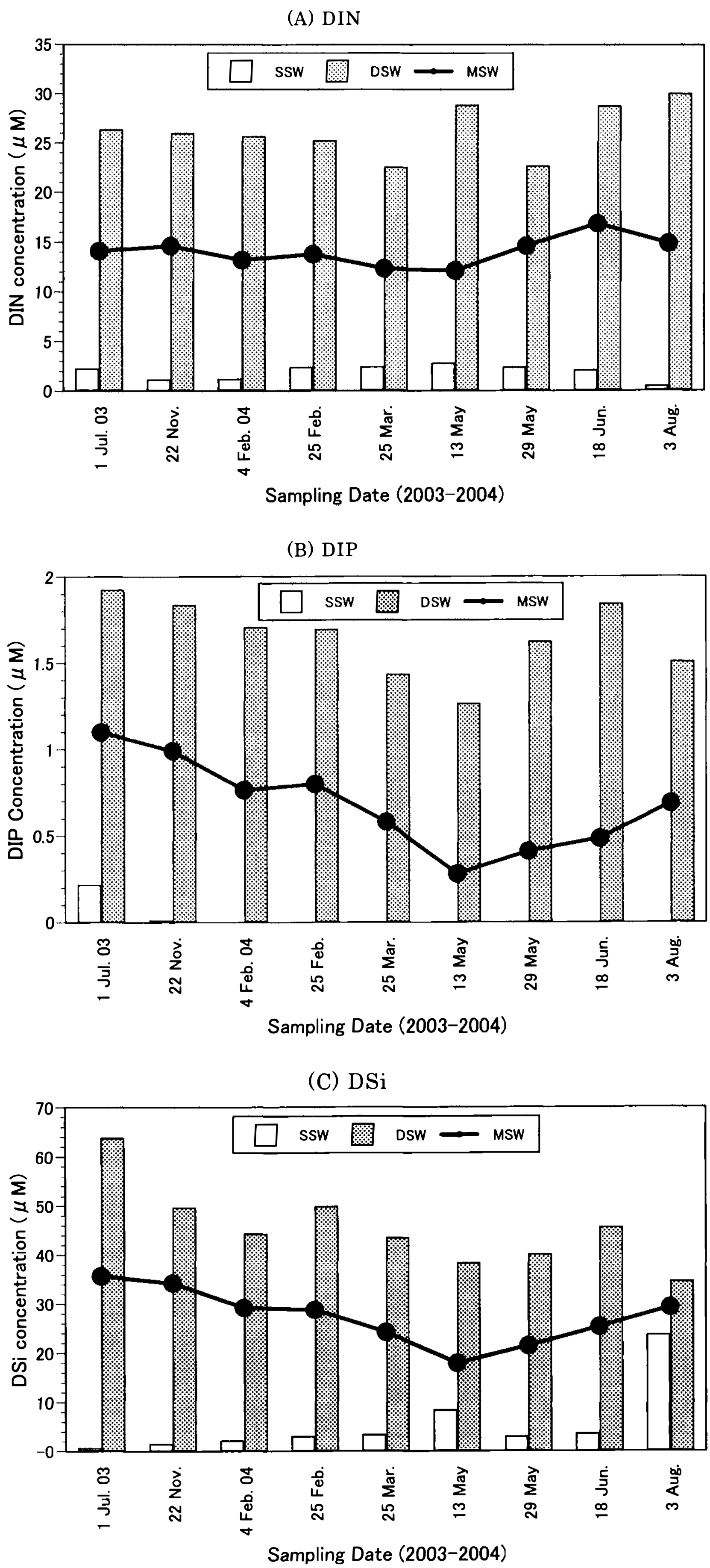

Fig. 3. Changes in DIN (A), DIP (B), and DSi (C) concentrations in surface seawater (SSW: □), deep seawater (DSW: □) and mixed seawater (MSW; SSW:DSW=1:1:●) from July, 2003 to August, 2004.

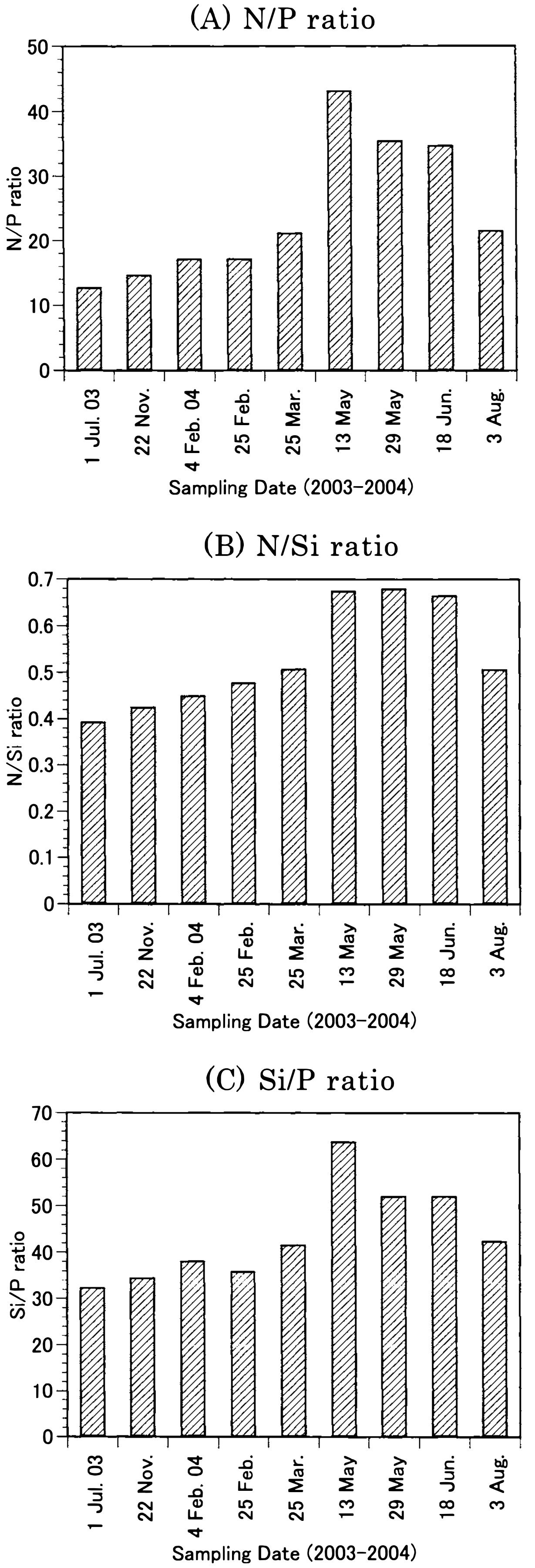

Fig. 4. Seasonal changes in N/P ratio (A), N/Si ratio (B), and Si/P ratio (C) (in mol) in mixed seawater (MSW) from July, 2003 to August, 2004.

0.39-0.68 and also increased from May to June, 2004 (Fig. 4B). Si/P ratios in MSW varied in the range of 29-64, and they increased from May to June, 2004 (Fig. 4C). These results were due to the low concentration of DIP from May to June, 2004 (Fig. 3B).

Relative growth yield (RGY) ratios were plotted against either N/P (Fig. 5A), N/Si (Fig. 5B) or Si/P (Fig. 5C). RGY values were significantly correlated with N/P ($p < 0.05$; $r = 0.55$) (Fig. 5A), N/Si ($p < 0.05$; $r = 0.51$) (Fig. 5B) and Si/P ($p < 0.05$; $r = 0.54$) (Fig. 5C).

4. Discussion

In comparison with the growth of *H. akashiwo* in GF/F-filtered MSW, the cell yields of *H. akashiwo* were not so much less than those in DSW but much better than SSW (Fig. 1), though the concentrations of inorganic nutrient in MSW were approximately half of DSW. These results suggest that *H. akashiwo* has the possibility to grow well in the vicinity of a DSW outlet where the mass amount of DSW is drained.

However, the growth of *H. akashiwo* in MSW was usually suppressed when natural phytoplankton communities were present (Fig. 2A and 2B). The growth of *H. akashiwo* with natural phytoplankton communities was correlated to changes in the nutrient concentration ratios, such as N/P, N/Si and Si/P (Fig. 4). The results suggest that low concentrations of phosphorus and/or silicate in MSW resulted in relatively good growth of *H. akashiwo* (Figs. 5A, 5B), in particular at low phosphorus concentrations (Fig. 5C).

The N/P ratio of *H. akashiwo* cell material was reported as 15.2 (Watanabe *et al.*, 1982), 11.1-25.0 (Miyata *et al.*, 1986) and 29.0 (Hosaka, 1992). The optimum N/P ratio of *H. akashiwo* cells, on

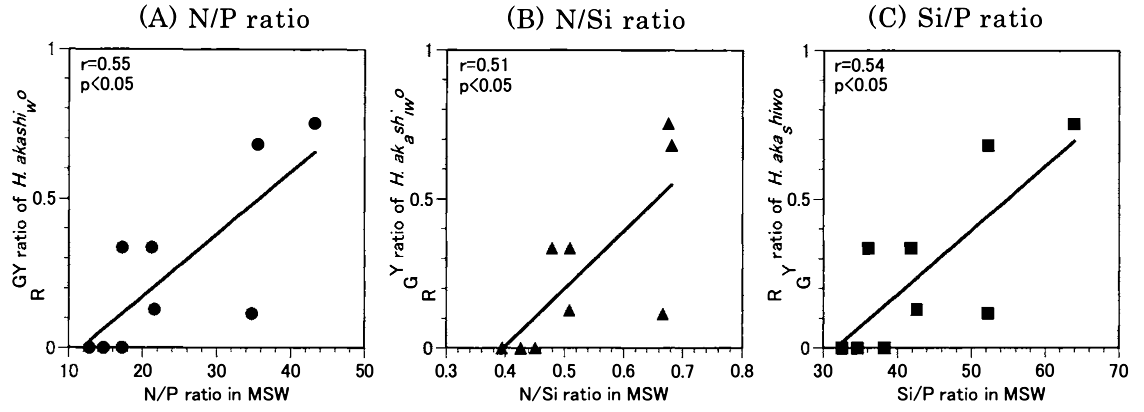

Fig. 5. Correlation between relative growth yield (RGY) ratios of *H. akashiwo* and N/P (A), N/Si (B), and Si/P (C) ratios in mixed seawater (MSW).

the other hand, was 64.8 (Nishijima and Fukami, 1993), which means *H. akashiwo* prefers relatively high N/P ratios. In this study, it was found that *H. akashiwo* grew better when the concentration of phosphorus was relatively low (Figs. 5A, 5C). When *H. akashiwo* was cultured under phosphorus-limited or even phosphorus-depleted conditions, constant cell abundances were maintained for some period (Kohata *et al.*, 1982). Another study showed that *H. akashiwo* took up phosphorus and rapidly accumulated it in the cell (Watanabe *et al.*, 1987). These results suggest that *H. akashiwo* utilizes low concentrations of phosphorus more effectively than other phytoplankton communities and that *H. akashiwo* has the possibility to grow when natural phytoplankton communities can not grow well due to the phosphorus limitation. In addition, it was found that *H. akashiwo* did not grow when the concentration of silicate was high (Figs. 5B, 5C). In such occasions, diatoms grew predominantly in natural phytoplankton communities in the present study (data not shown), and subsequently, the growth of *H. akashiwo* was suppressed.

Our data showed that the growth of *H. akashiwo* could be suppressed when natural phytoplankton communities are present, even though high nutrients are supplied by mass drainage of DSW. However, *H. akashiwo* has the possibility to grow well depending on the relative concentration of phosphorus and/or silicate (Fig. 5). Therefore, we must consider that we may have some risk of the growth of harmful phytoplankton when huge amounts of DSW are drained in the coastal zone.

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References

Fujita, D. (2001) Algal recovery on coralline-covered cobbles collected from an urchin-dominated barren ground in flowing deep-sea water. Deep Ocean Wat. Res., 2, 57-64 (in Japanese with English abstract).

Fujita, D. (2003) Algal recovery on coralline-covered cobbles collected from an urchin-dominated barren ground in flowing deep-sea water II. Deep Ocean Wat. Res., 4, 1-9 (in Japanese with English abstract).

Fujita, D. and M. Takahashi (2006) Utilization of deep seawater resources -from basic to practical applications-. Seizando-Shoten, Tokyo, 209 pp. (in Japanese).

Fukami, K. (2004) Kuroshio and deep seawater. Farming Japan, 38, 10-16.

Fukami, K., A. Kawai, M. Asada, M. Okabe, T. Hotta, T. Moriyama, S. Doi, T. Nishijima, M. Yamaguchi and M. Taniguchi (1998) Continuous and simultaneous cultivation of benthic food diatom *Nitzschia* sp. and abalone *Haliotis sieboldii* by using deep seawater. J. Mar. Biotechnol., 6, 237-240.

Fukami, K., T. Nishijima (1994) Culturing of food diatom with deep seawater and effect of adding some bacteria isolated from deep seawater. Kaiyo Monthly, 26, 139-145 (in Japanese).

Fukami, K., T. Nishijima and Y. Hata (1991) Isolation of bacteria promoting the growth of microalgae from deep seawater and its effects. Kuroshio (Rep. Inst. Kuroshio Sphere., Kochi Univ.), 5, 17-21 (in Japanese).

Fukami, K., T. Nishijima and Y. Hata (1992) Availability of deep seawater and effects of bacteria isolated from deep seawater on the mass culture of food microalga *Chaetoceros ceratosporum*. Nippon Suisan Gakkaishi, 58, 931-936.

Fukami, K., S. Nishimura, M. Ogusa, M. Asada and T. Nishijima (1997) Continuous culture with deep seawater of a benthic food diatom *Nitzschia* sp.. Hydrobiologia, 358, 245-249.

Hosaka, M. (1992) Growth characteristics of strain of *Heterosigma akashiwo* (HADA) HADA isolated from Tokyo Bay, Japan. Bull. Plankton Soc. Jpn., 39, 49-58.

Iizuka, S., S. Uye, A. Mitsutani and Y. Ishida (2004) Ecolagical problems of red tide. In "Red Tides." (ed. by T. Okaichi), TERRAPUB/Kluwer, Tokyo, pp. 179-257.

Kohata, K., M. M. Watanabe, Y. Nakamura and M. Watanabe (1982) Growth and phosphate uptake kinetics in *Olisthodiscus luteus* grown on synchronized batch cultures. Res. Rep. Natl. Inst. Environ. Stud., 30, 95-111 (in Japanese with English abstract).

Matsubayashi, T., I. Maruyama, S. Kido, Y. Ando, T. Nakashima and T. Toyota (1994) Effects of deep seawater on the growth of several species of marine micro-algae. J. Appl. Phycol., 6, 75-77.

Miyata, K., A. Hattori and A. Ohtsuki (1986) Variation of cellular phosphorus composition of *Skeletonema costatum* and *Heterosigma akashiwo* grown in chemostats. Mar. Biol., 93, 291-297.

Nakashima, T. (1988) Effects of deep sea water on the growth of a marine diatom species *Skeletonema costatum*. Bull. Plankton Soc. Jpn., 35, 45-55 (in Japanese with English abstract).

Nakashima, T. (1992a) Factors liberating growth lag of a diatom, *Skeletonema costatum*, in deep sea water I. Liberation by adding various organic matter. Bull. Plankton Soc. Jpn., 38, 93-104 (in Japanese with English abstract).

Nakashima, T. (1992b) Factors liberating growth lag of a diatom, *Skeletonema costatum*, in deep sea water II. Liberation by mixing with surface sea water. Bull. Plankton Soc. Jpn., 38, 105-111 (in Japanese with English abstract).

Nakashima, T. and T. Toyota (1989) Artificial upwelling of deep seawater. Kaiyo Monthly, 21, 618-625 (in Japanese).

Nishijima, T. and K. Fukami (1993) Effect of N:P ratio on the growth of phytoplankton, 2. Raphidophyceae and Bacillariophyceae. In "Effect of N:P Ratio in Water on Aquatic Organisms." (ed. by Y. Yoshida), Koseisyakoseikaku, Tokyo, pp. 20-28 (in Japanese).

Nishijima, T. and K. Fukami (1994) Ecology of algicidal microorganisms, 2. Evaluation of algal growth potential by red tide microalgae. In "Prevention and Control of Red Tide Microalgae by Microorganisms." (ed. by Y. Ishida and I. Sugahara), Koseisyakoseikaku, Tokyo, pp. 22-32. (in Japanese).

Otsuka, K. (2006) Estimation of effects of deep ocean water discharge on seaweed bed ecosystem at Muroto coastal area. Fisheries Engineering, 43, 21-33 (in Japanese with English abstract).

Watanabe, M., K. Kohata and M. Kunugi (1987) ^{31}P nuclear magnetic resonance study of intracellular phosphate pools and polyphosphate metabolism in *Heterosigma akashiwo* (HADA) HADA (Raphidophyceae). J. Phycol., 23, 54-62.

Watanabe, M., Y. Nakamura, S. Mori and S. Yamochi (1982) Effects of physico-chemical factors and nutrients on the growth of *Heterosigma akashiwo* HADA from Osaka Bay, Japan. Jpn. J. Phycol., 30, 279-288.

Watanabe, M., M. Taniguchi, T. Ikeda, M. Komatsu, K. Takatsuki and S. Kanamaki (2000) Fertilization of coastal area by deep seawater. Kaiyo Monthly extra, 22, 160-169 (in Japanese).

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