JOURNAL OF PLANKTON RESEARCH | VOLUME 26 | NUMBER 1 | PAGES 61–66 | 2004

Effects of temperature, salinity and irradiance on the growth of the harmful red tide dinoflagellate *Cochlodinium polykrikoides* Margalef (Dinophyceae)

DAE-IL KIM*, YUKIHIKO MATSUYAMA[1], SOU NAGASOE, MINEO YAMAGUCHI[1], YANG-HO YOON[2], YUJI OSHIMA, NOBUYOSHI IMADA AND TSUNEO HONJO

LABORATORY OF FISHERIES BIOENVIRONMENTAL SCIENCE, DIVISION OF BIORESOURCE AND BIOENVIRONMENTAL SCIENCES, KYUSHU UNIVERSITY, HAKOZAKI, FUKUOKA 812-8581, [1]HARMFUL ALGAL BLOOMS DIVISION, NATIONAL RESEARCH INSTITUTE OF FISHERIES AND ENVIRONMENT OF INLAND SEA, OHNO, SAEKI, HIROSHIMA 739-04, JAPAN AND [2]DEPARTMENT OF OCEAN ENVIRONMENT SYSTEM PROGRAM, YOSU NATIONAL UNIVERSITY, DUN-DEOK, YOSU, 550-749, KOREA

*CORRESPONDING AUTHOR: dikim@agr.kyushu-u.ac.jp

The effects of temperature, salinity and irradiance on the growth of the harmful red tide dinoflagellate Cochlodinium polykrikoides were examined in the laboratory. From 60 different combinations of temperature (10–30°C) and salinity (10–40) under saturated irradiance, C. polykrikoides exhibited its maximum specific growth rate of 0.41 day^{-1} at a combination of 25°C and salinity of 34. Optimum growth rates of >0.3 day^{-1} were observed at temperatures ranging from 21 to 26°C and at salinities from 30 to 36. The organism did not grow at temperatures ≤10°C and only grew at salinities >30 if the temperature was >15°C. It was able to grow in temperatures ranging from 15 to 30°C and at salinities from 20 to 36. These values closely resembled those observed for this species in situ. It appears as if C. polykrikoides is a stenohaline organism that prefers high salinities, indicative of offshore waters. Temperature had the greatest influence on the growth rate, followed by salinity, and then the interaction between temperature and salinity. The optimum irradiance for growth was >90 μmol m^{-2} s^{-1}. Photoinhibition did not occur at 230 μmol m^{-2} s^{-1}, which was the maximum irradiance used in this study.

INTRODUCTION

Blooms of the well-known dinoflagellate *Cochlodinium polykrikoides* Margalef have caused mass mortalities of aquacultured fish off the western coast of the island of Kyushu, Japan, and the southern coast of Korea. In Japan, the first recorded red tide of this organism occurred in the Yatsushiro Sea, Kumamoto Prefecture, in 1977 (Kumada *et al.*, 1980). Since then, blooms have been recorded frequently in the Yatsushiro Sea. Blooms also occurred occasionally in the Harima Nada of the Seto Inland Sea in the summer of 1986 (Yuki and Yoshimatsu, 1989) and in Uragami Bay, Wakayama Prefecture, in 1996 (T. Takeuchi, personal communication). Along the coast of the island of Kyushu, red tides recently occurred in the Ariake Sea off Kumamoto Prefecture, Imari Bay off Nagasaki Prefecture and Inokushi Bay off Oita Prefecture. In the summer of 2000, a massive bloom of this organism in the Yatsushiro Sea caused damage costing ~US$36.4 million.

In Korea, *C. polykrikoides* is the most prevalent dinoflagellate responsible for fish kills (Chang and Kim, 1997). The first red tide was recorded in southern Korea in 1982, and they have occurred frequently since 1989 (Kim, 1998). An extensive red tide in the summer of 1995 caused particularly heavy mortalities of aquacultured fish, which amounted to a loss of ~US$95.5 million (Kim, 1997).

Blooms of *C. polykrikoides* have also occurred in Phosphorescent Bay in Puerto Rico (Margalef, 1961), in Puerto Quetzal in Guatemala (Rosales-Loessener *et al.*, 1996), in Quanshou Bay in China (Du *et al.*, 1993), and in Manzanillo Bay (Morales-Blake and Hernandez-Becerrill, 2001) and the Gulf of California in Mexico (Gárate-Lizárraga *et al.*, 2000). *Cochlodinium polykrikoides*

doi: 10.1093/plankt/fbh001, available online at www.plankt.oupjournals.org

blooms appear to occur in limited areas, which include the temperate region of East Asia and the subtropical region of Central America. Because these blooms obviously damage fisheries, the outbreak mechanism of the blooms needs to be clarified.

The growth features of *Karenia mikimotoi*, *Chattonella antiqua*, *Chattonella marina* and *Heterocapsa circularisquama* (which have been associated with kills of fish and shellfish) have already been investigated (Yamaguchi and Honjo, 1989; Yamaguchi *et al.*, 1991, 1997). The physiological features of *C. polykrikoides*, however, have not yet been investigated. In this study, we examined the effects of temperature, salinity and irradiance on the growth of *C. polykrikoides* under nutrient-replete laboratory conditions.

METHOD

Organism and culture conditions

A strain of *C. polykrikoides* was isolated from Furue Bay (33°23′N, 129°33′E), Nagasaki Prefecture, in January 2000. An axenic culture was obtained through repeated washing using capillary pipettes. The sterility tests were carried out using f/2pm medium (Andersen *et al.*, 1997) and fluorochrome 4′,6-diamidino-2-phenylindole staining (Porter and Feig, 1980). Silicate was eliminated from an f/2 culture medium (Guillard, 1975) and aged sea water of the Tsushima Current (salinity of 34) was used as the culture medium. The culture was maintained at 25°C under 130 μmol m^{-2} s^{-1} cool-white fluorescent illumination on a 12 h:12 h light:dark cycle.

Temperature and salinity experiments

The growth experiments were conducted in a crossed factorial design with 60 different combinations of six temperatures (10, 15, 20, 25, 27.5 and 30°C) and 10 salinities (10, 15, 20, 25, 30, 32, 34, 36, 38 and 40). Salinities <34 were prepared by diluting aged sea water with de-ionized water (Milli-Q; Millipore), and salinities >34 were prepared by evaporating sea water in a drying oven at 50°C. To decrease shock of the inocula due to a change in temperature and salinity, cultures were pre-acclimated to the desired experimental conditions by stepwise transfer over a period of 1–2 months according to the method outlined by Yamaguchi and Honjo (Yamaguchi and Honjo, 1989). If transferred cells did not grow in the experimental regime, the growth experiment was not carried out, and the growth rate at the temperature and salinity regime was regarded as zero.

Acclimated stock cultures were inoculated into three identical PP-capped test tubes (ø 18 × 160 mm; Iwaki Glass Co. Ltd), each containing 10 ml of modified f/2 medium (Guillard, 1975) without silicate for each experimental regime. All test tubes were inoculated with ~50 cells ml^{-1} and were gently shaken twice every day to prevent algal aggregation by mucus production. Every 1–2 days, 200–500 μl of the sample were fixed in a Lugol's iodine solution. Optical microscopy was used to determine the growth phase by counting the cells using a Sedgwick–Rafter counting chamber. The specific growth rates (μ; day^{-1}) of samples determined to be in the exponential growth phase were calculated according to Guillard (Guillard, 1973) by least squares fit of a straight line to the data after they had been logarithmically transformed.

Light experiments

The pre-culture was carried out at 25°C, 230 μmol m^{-2} s^{-1} under cool-white fluorescent illuminations on a 12 h: 12 h light:dark cycle. The cultures were inoculated with ~50 cells ml^{-1} in three identical PP-capped test tubes (ø 18 × 160 mm) containing 10 ml of the modified f/2 medium and were gently shaken twice every day. The 12 different irradiances (10, 30, 50, 70, 90, 110, 130, 150, 170, 190, 210 and 230 μmol m^{-2} s^{-1}) were obtained by wrapping the test tubes with UV-absorbing vinyl screens. Irradiance was measured by wrapping the receiver sensor with different UV-absorbing vinyl screens using a quantum light meter (Model LI-189; LI-COR Biosciences). Growth rates in the exponential growth phase were calculated as described above. The following formula, modified from Lederman and Tett (Lederman and Tett, 1981), was used to describe the relationship between growth rate and irradiance:

$$\mu = \mu_m \frac{I - I_0}{(K_S - I_0) + (I - I_0)} = \mu_m \frac{I - I_0}{I + (K_S - 2I_0)} \quad (1)$$

where μ is the specific growth rate (day^{-1}), μ_m is the maximum specific growth rate (day^{-1}), I is the irradiance (μmol m^{-2} s^{-1}), I_0 is the compensation irradiance (μmol m^{-2} s^{-1}) and K_S is the irradiance at $\mu_m/2$ (half-saturation light intensity).

Statistical analysis

Analysis of variance (ANOVA) was used to determine the effects of temperature and salinity on the growth rate. Cubic polynomial equations were determined based on the ANOVA results (Yamaguchi and Honjo, 1989; Yamaguchi *et al.*, 1991, 1997; Ellegaard *et al.*,

1993). The computer application SPSS 11.0J for Windows (SPSS, Chicago, IL, USA) was used for the statistical analysis.

RESULTS

Effect of temperature and salinity on growth

Growth of the organism was not observed at temperatures ≤10°C, but was observed at salinities >30 at 15°C (Figure 1A). Dependent on the salinity, *C. polykrikoides* grew at temperatures >20°C. The maximum growth rate (0.41 day^{-1}) was obtained at 25°C and a salinity of 34. In addition, specific growth rates >0.3 day^{-1} occurred when the combinations of temperature and salinity were from 21 to 26°C and between 30 and 36, respectively.

A two-factor ANOVA indicated significant effects of temperature and salinity on the growth rates of *C. polykrikodes* at the 0.1% level. Sixty-five and 21% of total sum of squares were accounted for by the sum of squares for temperature and salinity, respectively (Table I).

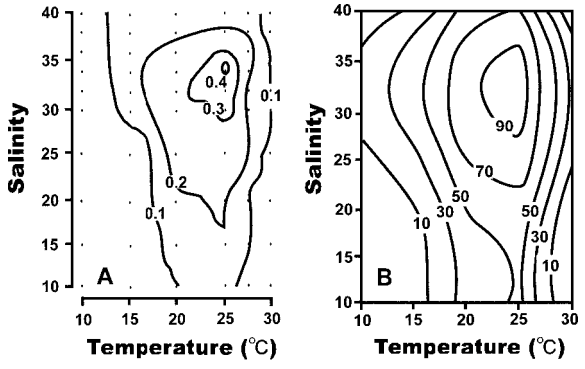

Fig. 1. Response surface contour plots of (**A**) specific growth rate (day^{-1}) and (**B**) percent of *C. polykrikoides* as a function of temperature and salinity.

On the basis of the ANOVA results, cubic equations of the form:

$$\mu = b_{00} + b_{10}T + b_{01}S + b_{11}TS + \ldots + b_{30}T^3 + b_{03}S^3 \quad (2)$$

where μ is the specific growth rate, T is temperature, S is salinity and b_{nn} are regression coefficients, were fitted by the stepwise forward regression method. The multiple regressions of the specific growth rate of *C. polykrikoides* on temperature and salinity obtained were as follows:

$$\mu = 0.946 - 0.177T - 0.0474S + 0.01218T^2 + 0.0026745S^2 - 0.00023757T^3 - 0.00004003S^3$$

This regression equation provided a good fit to the observed data (as shown in Table II); the adjusted R^2 value is 0.82. A response surface contour plot is shown in Figure 1B. The range of temperature and salinity comprising 90–100% of optimum is found at 21.3–26.2°C and 27.6–36.7, respectively.

Effect of irradiance on growth

Cochlodinium polykrikoides did not grow at 10 μmol m^{-2} s^{-1}, and the growth rate at 30 μmol m^{-2} s^{-1} was 0.12 day^{-1} (Figure 2). Growth of the organism saturated at 90 μmol m^{-2} s^{-1}. The optimum irradiance for growth was >90 μmol m^{-2} s^{-1}. Photoinhibition did not occur at 230 μmol m^{-2} s^{-1}, which was the maximum irradiance used in this study. The following hyperbolic equation described the exponential growth phase:

$$\mu = 0.35\ \frac{I - 10.38}{I + 24.31}$$

The compensation irradiance (I_0) was 10.38 μmol m^{-2} s^{-1}. The maximum growth rate (μ_m) and half-saturation irradiance (K_S) were 0.35 ± 0.06 day^{-1} and 45.06 ± 0.22 μmol m^{-2} s^{-1}, respectively.

Table I: Summary of ANOVA of growth rate of C. polykrikoides as a function of temperature and salinity

Source of variation	d.f.	Sum of squares	Mean square	*F*
Temperature	5	0.495	0.099	42.758***
Salinity	9	0.158	0.017	7.507***
Error	45	0.105	0.002	
Total	59	0.758		

***$P < 0.001$.

Table II: Statistical testing and standard errors for the parameters of the model for the influence of temperature (T) and salinity (S) on growth rate

Parameter	Estimate	SE	*t*-statistic
Intercept	0.94597622	0.28922	3.271**
T	–0.17701511	0.04225	–4.189***
S	–0.04740523	0.02129	–2.227*
TT	0.01218113	0.00224	5.449***
SS	0.00267454	0.00090	2.971**
TTT	–0.00023757	0.00004	–6.390***
SSS	–0.00004003	0.00001	–3.394***

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

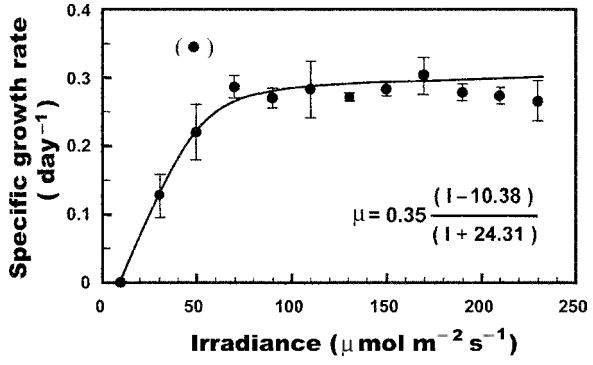

Fig. 2. Specific growth rate (day^{-1}) of *C. polykrikoides* as a function of light intensities at constant temperature and salinity. Bars represent the standard deviation ($n = 3$). The point in parentheses was omitted to calculate the rectangular hyperbola equation.

DISCUSSION

Ecologically, there are three aspects common to red tides. First, there is an increase in population size, called initiation. Secondly, there is support, e.g. suitable environmental conditions such as temperature, salinity, nutrients and growth factors, and finally, the maintenance and transport of blooms by hydrologic and meteorologic forces (Steidinger, 1975). An understanding of the life cycle and population growth of the causative organism is important to elucidate the outbreak mechanisms of red tides.

The results of growth experiments of *C. polykrikoides* at various temperature and salinity combinations indicated that the growth of the organism was dependent on the temperature and salinity. The maximum growth rate (0.41 day^{-1}) was obtained at 25°C and a salinity of 34 (Figure 1). These results on optimum temperature and salinity for *C. polykrikoides* were much higher than for the representative red tide species in western Japan, except for *H. circuarisquma* at 30°C and salinity of 30 (Yamaguchi *et al.*, 1997). The optimum values of temperatures and salinities were obtained for *Ch. antiqua* at 25°C and 25, respectively (Yamaguchi *et al.*, 1991), for *Ch. marina* at 25°C and a salinity of 20 (Yamaguchi *et al.*, 1991), for *Ch. verruculosa* at 15°C and a salinity of 25 (Yamaguchi *et al.*, 1997), for *K. mikimotoi* at 25°C and a salinity of 25 (Yamaguchi and Honjo, 1989), and for *Gymnodinium catenatum* at 25°C and a salinity of 30 (Yamamoto *et al.*, 2002). Therefore, these results indicate that *C. polykrikoides* grows best in the higher temperatures and salinities present in summer, predominating over other species in such conditions. In addition, it can be inferred that this species originates from habitats with high temperature and salinity, such as subtropical or tropical waters.

Ranges of temperatures and salinities during the red tide period of *C. polykrikoides* are summarized in Table III. Many blooms occurred within the optimal temperature and salinity ranges of 21–26°C and 30–36. Blooms in Inokushi Bay (18.9–20.3°C) and Uragami Bay (16–22°C) occurred in lower than optimal temperature ranges. However, Inokushi Bay and Uragami Bay are both isolated and may have different strains of *C. polykrikoides* having different physiological features.

The strain used for the laboratory study was isolated from seawater at 14.7°C from Furue Bay in January 2000. Furthermore, this species did not grow at temperatures ≤10°C, and only little growth occurred at 15°C. Kim *et al.* reported hyaline cysts of *C. polykrikoides* (Kim *et al.*, 2002). However, these results and the results from Table III, which show that *C. polykrikoides* red tides were observed in water temperatures of 16–29°C in the field, suggest that vegetative cells of *C. polykrikoides* can overwinter where water temperatures >14–15°C are maintained during the winter season.

The salinity range for optimal growth (>0.3 day^{-1}) was 30–36. When the cells cultivated at a salinity of 25

Table III: The ranges of water temperature and salinity during the occurrence of C. polykrikoides red tide in the field

Location and year	Temperature (°C)	Salinity	Reference
Phosphorescent Bay, Puerto Rico, 1958	29.8–30.1	35.3–35.8	Margalef (1961)
Yatsushiro Sea, Japan, 1978	23.0–29.9	31.7–34.2	Honda *et al.* (1980)
Quanshou Bay, China, 1990	22.4–26.7	31.3–33.8	Du *et al.* (1993)
Korea southern coast, Republic of Korea, 1995–1998	22.5–27.0	30.0–33.4	NFRDI[a] (1998); Suh *et al.* (2000)
Uragami Bay, Japan, 1996	16.0–22.0	•	T. Takeuchi, unpublished
Manzanillo Bay, Mexico, 1999–2000	25.5	34.5–34.7	Morales-Blake and Hernandez-Becerrill (2001)
Yatsushiro Sea, Japan, 2000	24.5–26.6	32.0–33.0	Kim *et al.*, unpublished
Gulf of California, Mexico, 2000	29–31	•	Garate-Lizarraga and Bustillos-Guzman (2000)
Inokushi Bay, Japan, 2002	18.9–20.3	32.6–34.6	K. Miyamura, personal communication

[a]National Fisheries Research and Development Institute.

were inoculated into salinities of 20 and 15 without acclimation, the chains of cells became solitary. In addition, small cells with light or pale pigment and abnormal cells with many projections were also observed. Accordingly, these results indicated that *C. polykrikoides* were not able to survive in salinities of 20–15. Similar morphological changes were observed when the cells were inoculated from a salinity of 34 into one of 40. Therefore, the upper limit for survival was salinities of 36–40. Table III shows that *C. polykrikoides* was found in the Yatsushiro Sea in 2000–2002 in salinities that varied from 30.0 to 34.5. Moreover, the blooms of this organism occurred when the salinity varied from 31.7 to 35.8. These results indicated that the organism might be a stenohaline species that prefers high salinities indicative of offshore waters.

As described above, multiple regression analysis was used to predict the growth rate of this organism under favorable conditions of light and nutrients (Table II). Based on this equation, *in situ* water temperature and salinity data were used to estimate and predict the occurrence of *C. polykrikoides* red tides using the method described by Toda *et al.* (Toda *et al.*, 1994).

The compensation irradiance (I_0) for *C. polykrikoides* was 10.38 μmol m^{-2} s^{-1}. The value was similar to 10.31 μmol m^{-2} s^{-1} found for *Ch. antiqua* (Yamaguchi *et al.*, 1991) and much higher than the 0.7 μmol m^{-2} s^{-1} for *K. mikimotoi* (Yamaguchi and Honjo, 1989), both of which occurred coincidentally in the summer at Yatsushiro Sea. *Cochlodinium polykrikoides* and *Ch. antiqua* have a higher irradiance requirement than *K. mikimotoi*. *Cochlodinium polykrikoides* did not show photoinhibition at the maximum irradiance used in this study—230 μmol m^{-2} s^{-1}. Kirk (Kirk, 1983) reported that the maximum irradiance was an average of 2000 μmol m^{-2} s^{-1} at the surface layer in the field, which is 10-fold higher than that in this study. To examine photoinhibition in this species, further study is necessary under higher irradiance than that used in the present study.

In conclusion, the results of the present study indicate that *C. polykrikoides* prefers high salinity, temperature and irradiance in summer. These results provide important information for understanding the mechanism of *C. polykrikoides* blooms and in developing the technology to predict blooms of this organism in the field. Further study is necessary to examine the physiological characteristics of each strain isolated from various seawaters, overwintering aspects of the life cycle and nutrient uptake to further understand and characterize red tide outbreaks of this organism.

ACKNOWLEDGEMENTS

We are pleased to acknowledge Mr M. Iwasaki of the Fisheries Research Center of Goshoura Cho, Kumamoto Prefecture, who kindly helped in the field sampling and data collection. We also thank Mr K. Miyamura of the Marine Fisheries Research Center, Oita Prefecture, for providing useful information.

REFERENCES

Andersen, R. A., Morton, S. L. and Sexton J. P. (1997) Provasoli–Guillard National Center for Culture of Marine Phytoplankton 1997 list of strains. *J. Phycol.*, **33(Suppl.)**, 1–75.

Chang, M. and Kim, W. S. (1997) Prologue and epilogue to the first international symposium on plankton blooms. *Ocean Res. (Seoul)*, **19**, 135–137.

Du, Q., Huang, Y. and Wang, X. (1993) Toxic dinoflagellate red tide by a *Cochlodinium* sp. along the coast of Fujian, China. In Smayda, T. J. and Shimizu, Y. (eds), *Toxic Phytoplankton Blooms in the Sea*. Elsevier, New York, pp. 235–238.

Ellegaard, M., Christensen, N. F. and Moestrup, Ø. (1993) Temperature and salinity effects on growth of a non-chain-forming strain of

Gymnodinium catenatum (Dinophyceae) established from a cyst from recent sediments in the Sound (Øresund), Denmark. *J. Phycol.*, **29**, 418–426.

Gárate-Lizárraga, L., Bustillos-Guzmán, J. J., Morquecho, L. and Lechuga-Déveze, C. (2000) First outbreak of *Cochlodinium polykrikoides* in the Gulf of California. *Harmful Algae News*, Intergovernmental Oceanographic Commission of UNESCO, **21**, 7 pp.

Guillard, R. R. L. (1973) Division rates. In Stein, J. R. (ed.), *Handbook of Phycological Methods. Culture Methods and Growth Measurements*. Cambridge University Press, Cambridge, pp. 289–311.

Guillard, R. R. L. (1975) Culture of phytoplankton for feeding marine invertebrates. In Smith, W. L. and Chanley, M. H. (eds), *Cultures of Marine Invertebrate Animals*. Plenum Press, New York, pp. 29–60.

Honda, A., Ishida, K. and Miyamura, S. (1980) III. Yatsushiro Kaiiki, Yatsushiro Kai-1. In Fisheries Agency, Fukuokaken Suisan Shikenjyou, Sagaken Suisan Shikenjyou, Nagasaki Suisan Shikenjyou, Kumamoto Suisan Shikenjyou and Kagoshima Suisan Shikenjyou (eds), *Kyushu Seiganiki Akashiwo Yosatsu Chosa Houkokusho*, pp. 108–123 (in Japanese).

Kim, C. H., Cho, H. J., Shin, J. B., Moon, C. H. and Matsuoka, K. (2002) Regeneration from hyaline cysts of *Cochlodinium polykrikoides* (Gymnodiniales, Dinophyceae), a red tide organism along the Korean coast. *Phycologia*, **41**, 667–669.

Kim, H. G. (1997) Recent harmful algal blooms and mitigation strategies in Korea. *Ocean Res. (Seoul)*, **19**, 185–192.

Kim, H. G. (1998) Harmful algal blooms in Korean coastal waters focused on three fish-killing dinoflagellates. In Kim, H. G., Lee, S. G. and Lee, C. K. (eds), *Harmful Algal Blooms in Korea and China*. National Fisheries Research and Development Institute, Pusan, Republic of Korea, pp. 1–20.

Kirk, J. T. O. (1983) *Light and Photosynthesis in Aquatic Ecosystems*. Cambridge University Press, Cambridge, pp. 219–253.

Kumada, K., Takeda, K. and Aramaki, T. (1980) III. Yatsushiro Kaiiki, Yatsushiro Kai-2. In Fisheries Agency, Fukuokaken Suisan Shikenjyou, Sagaken Suisan Shikenjyou, Nagasaki Suisan Shikenjyou, Kumamoto Suisan Shikenjyou and Kagoshima Suisan Shikenjyou (eds), *Kyushu Seiganiki Akashiwo Yosatsu Chosa Houkokusho*, pp. 125–136 (in Japanese).

Lederman, T. C. and Tett, P. (1981) Problems in modeling the photosynthesis–light relationship for phytoplankton. *Bot. Mar.*, **24**, 125–134.

Margalef, R. (1961) Hidrografía y fitoplancton de un área marina de la costa meridional de Puerto Rico. *Invest. Pesq. Tomo*, **XVIII**, 33–96 (in Spanish).

Morales-Blake, A. and Hernández-Becerrill, D. (2001) Unusual HABs Manzanillo Bay, Colima, Mexico. *Harmful Algae News*, Intergovernmental Oceanographic Commission of UNESCO, **22**, 6 pp.

National Fisheries Research and Development Institute (1998) *Recent Red Tides in Korean Coastal Waters*, 2nd edn. Pusan, Republic of Korea, 292 pp. (in Korean).

Porter, K. G. and Feig, Y. S. (1980) The use of DAPI for identifying and counting aquatic microflora. *Limnol. Oceanogr.*, **25**, 943–948.

Rosales-Loessener, F., Matsuoka, K., Fukuyo, Y. and Sanchez, E. H. (1996) Cysts of harmful dinoflagellates found from Pacific coastal waters of Guatemala. In Yasumoto, T., Oshima, Y. and Fukuyo, Y. (eds), *Harmful and Toxic Algal Blooms*. Intergovernmental Oceanographic Commission of UNESCO, Sendai, pp. 193–195.

Steidinger, K. A. (1975) Basic factors influencing red tides. In LoCicero, V. R. (ed.), *Proceedings of the First International Conference on Toxic Dinoflagellates*. Massachusetts Science and Technology Foundation, Massachusetts, pp. 153–162.

Suh, Y. S., Kim, J. H. and Kim, H. G. (2000) Relationship between sea surface temperature derived from NOAA satellites and *Cochlodinium polykrikoides* red tide occurrence in Korea coastal waters. *J. Korean Environ. Sci. Soc.*, **9**, 215–221.

Toda, S., Abo, K., Honjo, T., Yamaguchi, M. and Matsuyama, Y. (1994) Effect of water exchange on the growth of the red-tide dinoflagellate *Gymnodinium nagasakiense* in an inlet of Gokasho Bay, Japan. *Bull. Plankton Soc. Jpn*, **34**, 201–203.

Yamaguchi, M. and Honjo, T. (1989) Effect of temperature, salinity and irradiance on the growth of the noxious red tide flagellate *Gymnodinium nagasakiense* (Dinophyceae). *Nippon Suisan Gakkaishi*, **55**, 2029–2036.

Yamaguchi, M., Imai, I. and Honjo, T. (1991) Effect of temperature, salinity and irradiance on the growth of the noxious red tide flagellate *Chattonella antiqua* and *C. marina* (Rhaphidophyceae). *Nippon Suisan Gakkaishi*, **57**, 1277–1284.

Yamaguchi, M., Itakura, S., Nagasaki, K., Matsuyama, Y., Uchida, T. and Imai, I. (1997) Effect of temperature and salinity on the growth of the red tide flagellates *Heterocapsa circularisquama* (Dinophyceae) and *Chattonella verruculosa* (Rhaphidophyceae). *J. Plankton Res.*, **19**, 1167–1174.

Yamamoto, T., Oh, S. J. and Kataoka, Y. (2002) Effects of temperature, salinity and irradiance on the growth of the toxic dinoflagellate *Gymnodinium catenatum* (Dinophyceae) isolated from Hiroshima Bay, Japan. *Fish. Sci.*, **68**, 356–363.

Yuki, K. and Yoshimatsu, S. (1989) Two fish-killing species of *Cochlodinium* from Harima Nada, Seto Inland Sea, Japan. In Okaichi, T., Anderson, D. M. and Nemoto, T. (eds), *Red Tides: Biology, Environmental Science, and Toxicology*. Elsevier, Amsterdam, pp. 451–454.

Received on November 18, 2002; accepted on September 23, 2003