Relationship between the environmental factors and the dynamic state of *Skeletonema costatum*, the principal species in red tide formation in Tokyo Bay following the introduction of N and P reduction measures

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N・P 削減の導入期における東京湾の赤潮形成主要種 Skeletonema costatum の動態と環境要因の関係の変化

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要約

水質総量規制とは閉鎖性水域の水質環境基準を確保するために、環境に排出される汚濁物質の総量を一定量以下に削減する環境省告示の法制度のことである。2002 年度適用の第 5 次水質総量規制からは全リン（T-P）の削減も開始されている。富栄養な東京湾では水質総量規制による水質の改善と赤潮発生との間に関係性は見られないと言う論点があるが、規制による T-P の流入負荷と赤潮の動態（対象とする赤潮判定基準の変動）においては両者に何らかの関係性が見出せる可能性がある。本研究では重回帰分析の応用手法として「細胞数赤潮指標モデル」を作成し、モデルの説明率の推移から検証を試みた。

Key words

the total pollutant load control system, *Skeletonema costatum*, N and P reduction, red tide, Tokyo Bay

1. Introduction

The Total Pollutant Load Control System (TPLCS) was introduced in Japan in 1979 in an effort to reduce the total volume of contaminated organic substances discharged into enclosed water bodies that had significant contamination, such as Tokyo Bay, Ise Bay and the Seto Inland Sea. In order to achieve improvement in the water quality of these marine areas, specific target reductions were set in 5-year periods. Regulations related to the TPLCS were modified by revisions of the “Water Quality Pollution Control Act” and the “Law Concerning Special Measures for Conservation of the Environment of the Seto Inland Sea” in 1988. Also in 1988, the First TPLCS targeting total COD was introduced, and in 2002, the Fifth TPLCS newly designated “total nitrogen (T-N) and total phosphorus (T-P)” as a pollution monitoring parameter. The environmental impact of reducing N and P loads is thought to be noticeable from around 2002 to 2007 based on the implementation of the Fifth TPLCS along with COD reduction achieved up through the Fourth TPLCS.

In Tokyo Bay, occurrences of red tide did not show any clear trends from 2002 to 2007 of introduction of measures to reduce

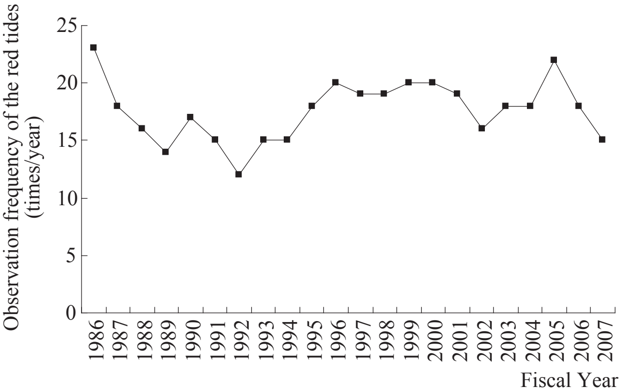

Figure 1: Observation frequency of the red tides in the inner Tokyo Bay (1986-2007 FY)

Source: Data provided by Natural Environment Division Environmental Bureau of Tokyo Metropolitan Government (2010)

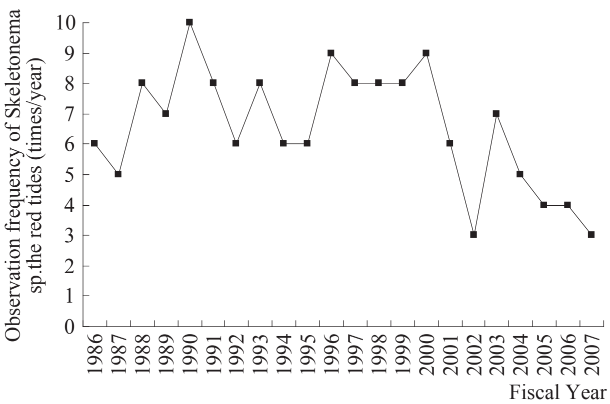

Figure 2: Observation frequency of the red tides microalgae *Skeletonema* sp. in the inner Tokyo Bay (1986-2007 FY)

Source: Data provided by ditto TMG (2010)

N and P loads (Figure 1). But the number of red tide events attributed to *S. costatum* red tide showed a decreasing trend from 2003 to 2007 (Figure 2).

The Tokyo Metropolitan Government defines a red tide as having the following characteristics: (1) sea water is brownish-red, yellowish-brown, green, etc.; (2) transparency is lowered to less than 1.5 m; (3) microscope observation shows a large number of red tide phytoplankton, and (4) the chlorophyll concentration (total volume of chlorophyll-a and pheo-pigments by the Lorenzen method) is ≥ 50 mg/m^3 (excluding zooplankton without chlorophyll). This study uses the same standard to designate red tide events.

A previous report examining the significance of the relationship between the dynamic state of red tide and nutrients found a "significant correspondence between the number of red tide occurrences and water quality control (Honjo, 1991)" in Seto Inland Sea. However, the red tide phenomenon is complex, and even detailed studies of nutrient loading and red tide occurrences have turned up no direct relation between red tide and nutrient salt (Okaichi, 1997)".

Here, we examine the trends in the abundance of *S. costatum*, a diatom which appears throughout all seasons in the inner bays (Kokubo, 1960). *S. costatum* was the most dominant species in the red tide surveys by the Tokyo Metropolitan Government and was found to often cause red tides. Multiple regression analysis was used to analyze the variation in *S. costatum* by volume when it "is not in an active proliferation phase (*Chl-a*/pheo-pigment ratio of 0-0.9.)." Typically, red tide is a difficult phenomenon to characterize biologically or statistically, as it is an abnormal proliferation of plankton However, in the non-bloom phase, a normal distribution can be obtained and used in statistical analysis. This is an important attempt to clarify the factors related to a reduction of *S. costatum*, and it may also permit elucidation of the relationship between the reduction in N and P and red tide (Suzuki, 2007; 2008).

2. Materials and methods

2.1 Data sets

Observation data obtained from monthly water quality surveys from 1986 to 2005 (Table 1) at the 8 fixed points in Tokyo Bay (Figure 3) were conducted by the Environmental Bureau of Tokyo Metropolitan Government Weather data were obtained from the Tokyo District Meteorological Observatory (http://www.data.jma.go.jp/obd/stats/etrn/index.php).

A total of 1248 observations from 1986 to 1998 and 672 observations from 1999 to 2005 were categorized by conditions and used in analysis. The data was split into two groups at 1999 to reflect the reduction in the volume of inflow load from 1999 to 2004 (inflow load, or total effluent amount, data is announced every five years, corresponding to the duration of the TPLCS.

For the candidate objective variables (hereafter, "Red Tide Index"), the number of cells of *Skeletonema* sp. was selected because it is a dominant species in the "red tide judgment standard" and it has a tendency to be reduced. Variance inflation factors were considered as candidate explanatory variables and were selected from the survey items that do not exhibit multicollinearity.

Since the number of cells is a pure biotic indicator, a mul-

Table 1: The list of observation items in "The water quality investigation result in fiscal year 2005"

Wind direction velocity (m/s)	Sal	PCB (mg/L)	Iprobenfos (mg/L)
Total depth (m)	Cl- (mg/L)	CCl_4 (mg/L)	Cd (mg/L)
Collection depth (m)	Conductivity	Trichlomethylene (mg/L)	MBAS (mg/L)
Weather	PON (mg/L)	Tetrachloroethylene (mg/L)	NH_4-N (mg/L)
Low water time (t, m)	TOC (mg/L)	1, 3-Dichloropropylene (mg/L)	NO_2-N (mg/L)
High water time(t, m)	D-TOC (mg/L)	Thiuram (mg/L)	NO_3-N (mg/L)
Temperature (°C)	DOC (mg/L)	Simazine (mg/L)	PO_4-P (mg/L)
Water temperature (°C)	Chl-a (mg/m^3)	Thiobencarb (mg/L)	1, 2-dichloroethane (mg/L)
Color	Quantity of plankton Deposition (mL/m^3)	Benzene (mg/L)	1, 1-dichloroethylene (mg/L)
Odor		Se (mg/L)	Cis-1, 2-dichlomethylene (mg/L)
Transparency (m)	Chl-a + phaeopigment (mg/m^3)	F (mg/L)	1, 1, 1-trichlomethane (mg/L)
pH	DCOD (mg/L)	B (mg/L)	1, 1, 2-trichlomethane (mg/L)
DO (mg/L)	Cyanide (mg/L)	Phenols (mg/L)	Dichloromethane (mg/L)
COD (mg/L)	Org-P (mg/L)	Cu (mg/L)	Normalhexane extracts (mg/L)
Coliform group bacteria (MPN/100 mL)	Pb (mg/L)	Zn (mg/L)	Trihalomethane (mg/L)
	Cr (VI) (mg/L)	D-Fe (mg/L)	Ignition loss (mg/L)
T-N (mg/L)	As (mg/L)	D-Mn (mg/L)	Dry loss (mg/L)
SS (mg/L)	Total Hg (mg/L)	Cr (mg/L)	Total sulfide (mg/L)
T-P (mg/L)	R-Hg (mg/L)	EPN (mg/L)	Oxidation reduction potential (mV)

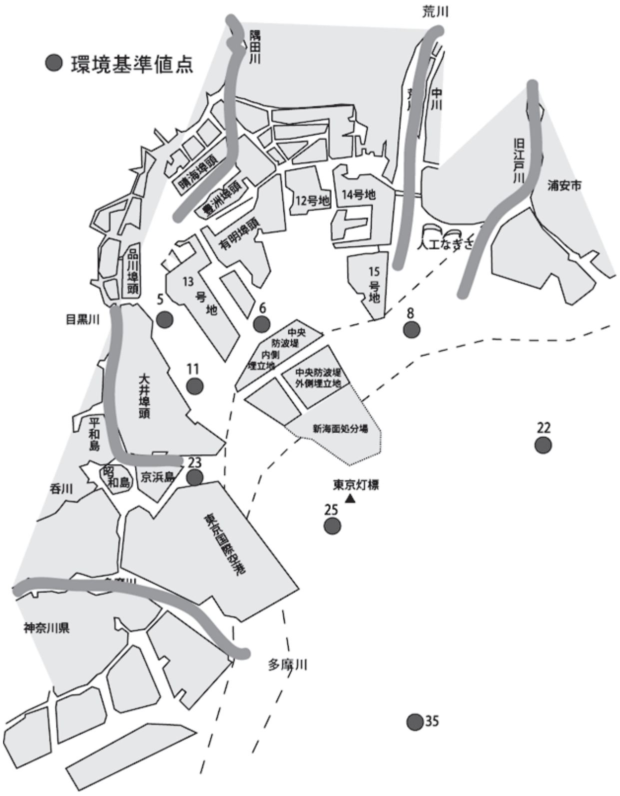

Figure 3: The location of sampling stations in the inner Tokyo Bay
Source: Redrawn from TokyoMetropolitan government. http://www2.kankyo.metro.tokyo.jp/kansi/mizu/image/kaiiki.pdf

tiple regression equation to predict the trajectory of *S. costatum* red tides based cell stages and abundances in each stage was attempted. That is, the resource limiting growth differs by the stages of the cells. As the method of condition analysis, a database of observational data (analysis, N = 1089; assumption of regression equation: N = 1089) with no active red tide events and few *Skeletonema* sp. cells was created from the survey result database for the years 1986 to 2005. Then, in consideration of auxotrophy of each stage of the cells, the data (N = 1089) were categorized by conditions for the logarithmic, non-proliferation and non-active proliferation phases. The ratio of *Chl-a* to pheopigment was considered to reflect the non-active and active phases of the primary dominant species, *Skeletonema* sp., in the ranges 0 to 0.9 (N = 73) and ≥ 1 (N = 1016). However, since an equation with statistically significant was not obtained for the active proliferation phase, and therefore, an equation which predicts the number of log phase *Skeletonema* sp. cells in the non-active proliferation phase as the objective variable (Red Tide Index model) was selected. Objective variables were selected from the factors having high data adequacy and showing a normal distribution (for *Mesodinium rubrum* was converted by Box-Cox conversion prior to analysis) and calculated by the sequential selection method with the variable censoring standard as $\alpha = 0.15$. Finally, the equation (Red Tide Index model) was applied to the database values from 1999 to 2005, and the number of log phase *Skeletonema* sp. cells in the non-active proliferation phase was assumed in order to estimate the contribution ratio of the explanatory variables selected before and after the Total Pollutant Load Controls drastically changed in 1999. Minitab14 was used for analysis and optimization of the equation.

3. Results

Multiple regression equation in the assumed non-active proliferation phase with 73 data points

log (*Skeletonema* sp.) = –21.522 + 0.5387DO + 0.8895log (*Mesodinium rubrum*) + 0.8063 average wind speed on the previous day –0.14477Sal. + 2.831pH + 4.646T-P

Here, the contribution ratio for the revised degrees of freedom was $R^2_{adj.} = 0.618$. Therefore, approximately 60 % of the variation in the measured values could be explained by the abundance of log phase *Skeletonema* sp.in this multiple regression equation. Variance inflation factors (VIFs) are as follows: dissolved oxygen (DO), 2.8; *Mesodinium*, 1.1; average wind speed on the previous day, 1.2; salinity, 1.6; pH, 3.1; and T-P, 1.2. Thus, multicollinearity is not suspected.

The p-value of the multiple regression equation for the non-active proliferation phase was statistically significant ($p < 0.05$). The correlation (R^2) between the estimated log phase *Skeletonema* sp. and the measured value from 1999 to 2003 show a breakdown by period as follows: 0.618 for 1986 to 1998, 0.648 for 1999 to 2001, and 0.279 for 2002 to 2005. By year R^2 for 1999-2001 showed a contribution ratio of approximately 60-70 %, while the R^2 for 2002-2005 rapidly dropped.

These results demonstrate that the equation (Red Tide Index model) is a good representation the data set before application of the Fifth TPLCS, but due to significant environmental changes, the multiple regression equation could no longer be used to explain "number of log phase (*Skeletonema* sp.) cells in the non-active proliferation phase". A "standardized multiple regression equation" was also developed in order to understand the correlation in the changes of environmental factors due to the implementation of the Fifth TPLCS. The letter "Sta" indicates standardized data. The multiple regression equation developed for six variables using 73 data points from 1986 to 1998 had almost identical $R^2_{adj.}$ values and only standardized variables was as follows:

Sta log (*Skeletonema* sp.) = 0.560 Sta DO + 0.246 Sta log (*Mesodinium*) + 0.348 Sta average wind speed on the previous day – 0.357 Sta Sal. + 0.349 Sta pH + 0.175 Sta T-P
$R^2_{adj.} = 0.618$

However, using the same six variables in a multiple regression equation for data from 2002 (right after the implementation of the Fifth TPLCS) produced an equation with a P value that was significant for mean wind speed on the previous day and

T-P but not for DO, log phase *Mesodinium* abundance, sality, pH, as the VIFs were over 10 for parameters DO, pH, and T-P. Therefore, the best multiple regression equation by step-wise of 6 variables in the following was selected.

Sta log (*Skeletonema* sp.) = 1.07 Sta DO + 0.995 Sta T-P
$R^2_{adj.}$ =0.669

VIFs were 1.4 for both DO and T-P, and multicollinearity is not suspected. P value between the equation and explanatory variables is smaller than 0.05, which is significant. The correlations among explanatory variables for 1986 to 1998, 1999 to 2001, and 2002 are shown in Tables 2-4, respectively.

In the simple correlation charts in Tables 2-4, the differences in the average values were verified at 95 % significant level based on the calculated values. Comparing data in Tables 2 and 3, which is from before and after the Fifth TPLCS, respectively, reveals significant differences in log phase *Skeletonema* sp. abundance, pH, sality, T-P, log phase *Mesodinium* abundance and mean wind speed on the previous day. In comparisons of both average values, from 4.75 to 5.94 in log phase *Skeletonema* sp. values; from 8.00 to 7.86 in pH; from 24.27 to 28.24 in sality; from 0.22 to 0.13 in T-P, from 0.45 to -0.37 in log phase *Mesodinium* abundance and from 3.46 to 3.07 in the mean wind speed on the previous day.

Similarly, in comparisons of the simple correlations, it is remarkable that the correlations for pH/DO (from 0.82 to 0.95) and T-N/T-P (from 0.84 to 0.93) in Tables 4 after the Fifth

Table 2: Correlation of selected target valuable (FY1986 to 1998)

No. items	1	2	3	4	5	6	7	8	9	10	11
M	16.15	16.75	7.93	7.60	23.64	2.75	0.19	2.32	0.22	3.71	5.68
SD	5.88	4.73	0.34	2.83	6.71	0.99	0.10	0.77	0.75	1.18	2.72
1 temp.		0.90**	0.08	–0.06	–0.50**	0.16**	0.32**	0.32**	0.40**	–0.42**	0.18
2 W.temp.			–0.05	–0.26*	–0.56**	0.22†	0.37**	0.20†	0.37**	–0.38**	0.02
3 pH				0.78**	0.54**	–0.46**	–0.27*	0.28*	–0.03	0.14*	0.59**
4 DO					0.43**	–0.34**	–0.29*	0.27*	–0.07	–0.03	0.60**
5 Sal.						–0.47**	–0.29*	–0.07	–0.22†	0.27*	0.06
6 T-N							0.79**	0.42**	0.03	–0.08	–0.06
7 T-P								0.58**	–0.03	–0.17	–0.043
8 DOC									–0.06	0.13	0.35**
9 logMeso										–0.11	0.24*
10 average wind speed on the previous day											0.23†
11 log (*Skeletonema* sp.)											

† < 0.10, * < 0.05, ** < 0.01

Table 3: Correlation of selected target valuable (FY1999 to 2001)

No. items	1	2	3	4	5	6	7	8	9	10	11
M	17.24	17.97	8.00	8.03	24.27	2.67	0.22	2.16	0.45	3.46	4.75
SD	6.32	4.29	0.41	3.22	5.88	1.31	0.23	0.63	0.90	0.85	3.02
1 temp.		0.94**	0.25	0.03	–0.59**	0.08	–0.14	0.10	0.32	0.13	0.46*
2 W.temp.			0.17	–0.09	–0.55**	0.05	–0.22	–0.04	0.31	–0.01	0.26
3 pH				0.82**	0.35	–0.50	–0.49*	0.04	0.56**	0.03	0.73**
4 DO					0.17	–0.10	–0.11	0.45*	0.52*	0.04	0.72**
5 Sal.						–0.65**	–0.39	–0.45*	–0.11	–0.22	–0.18
6 T-N							0.84**	0.80**	–0.16	–0.01	–0.05
7 T-P								0.75**	–0.25	–0.013	–0.12
8 DOC									0.14	0.10	0.39†
9 logMeso										–0.15	0.37†
10 average wind speed on the previous day											0.40†
11 log (*Skeletonema* sp.)											

† < 0.10, * < 0.05, ** < 0.01

TPLCS became higher among the air temperature/water temperature (from 0.90 to 0.94), pH/DO (from 0.78 to 0.82) and T-N/T-P (from 0.79 to 0.84) than those shown in Tables 2 and 3.

Some combinations of variables with high correlations were assumed to be caused by the change in the oceanic condition after the Fifth TPLCS among variables which are not selected in the "standardized partial regression equation" after the Fifth TPLCS. Multicollinearity sometimes occurs when the correlation between independent variables with high standard deviation co-occur in the equation; nonetheless, it is not observed between the variables which were used as the equation as observed in establishment of equation because the applicable factors were suppressor variables. Furthermore, although salinity and T-P are significant when formulated as multiple regression in the simple correlation chart, they are observed as not showing significant differences before and after log phase *Skeletonema* sp. abundance and the Fifth TPLCS. Among the equations which have been selected by step-wise are DO, log phase *Mesodinium* abundance, mean wind speed on the previous day, salinity, pH and T-P, which are explanatory variables of the multiple regression equation. When the equation without salinity and T-P is chosen, it becomes impossible to create an equation with a high contribution ratio, which is the purpose of this study. Thus, salinity and T-P were not deleted from the equation because multicollinearity was not observed and these environmental factors are important as explanatory variables.

Moreover, although the influence of pH (2.831), DO (0.5387), and similar variables could be observed in the partial regression coefficients in the "multiple regression equation", it could be confirmed that the level of correlation did not have an abnormal influence on the values of each coefficient compared to the values of the standard partial regression coefficients in the "standardized partial regression equation". In the equation before the Fifth TPLCS, the environmental factors by the standard partial regression coefficients before the Fifth TPLCS were DO, Sal, pH, average wind speed of the day before, log (*Mesodinium*) and T-P, and in the equation after the Control, the environmental factors by the standard partial regression coefficients before the Control became DO and T-P.

4. Discussion

Skeletonema sp. is the most dominant phytoplankton species in Tokyo Bay. Of the environmental variables selected for inclusions in this study through evaluation of the multiple regression equation and the standardized multiple regression equation, the following six explanatory variables with a close relationship to red tide were selected (Iwasaki,1980; Okaichi, 1997; The Japanese Society of Fisheries Science, 1980): DO, salinity, pH, average wind speed on the previous day, log phase *Mesodinium* and T-P. For selection of the equation variables and the contribution ratio, data from "A Report on the Survey Result of the Secondary Pollution in Tokyo Bay" (Water Quality Conservation Division the Environmental Bureau of Tokyo Metropolitan Government,1984) and "Red Tide Simulation Using Fourier Analysis and Multiple Regression Equation"(Ouchi, 1982: 1986) were used. Salinity and P (T-P) were commonly used in the comparison of the explanatory variables of this equation. No multicollinearity was observed for DO, pH, mean wind speed on the previous day and log phase *Mesodinium* abundance. The reason why the objective variables were determined as log phase *Skeletonema* sp. was to provide normality and also to create the equation with high contribution ratio, but the contribution rate was higher than them, which allowed to accomplish it.

Significant differences were observed in log phase *Skeletonema* sp. abundance, pH, salinity, T-P, log phase *Mesodinium* abundance and average wind speed of the previous day based on

Table 4: Correlation of selected target valuable (2002)

No. items	1	2	3	4	5	6	7	8	9	10	11
M	15.84	17.08	7.86	7.60	28.24	2.18	0.13	2.38	–0.37	3.07	5.94
SD	6.02	3.46	0.16	1.33	2.70	1.26	0.08	0.69	0.41	0.71	1.53
1 temp.		0.96**	0.21	0.19	–0.70**	0.26	0.42†	0.38†	0.38†	0.02	0.67*
2 W.temp.			0.15	0.18	–0.72**	0.34	0.53†	0.55*	0.55*	0.01	0.78**
3 pH				0.95**	0.44†	–0.73**	–0.67**	0.09	–0.09	0.05	0.38
4 DO					0.39†	–0.61*	–0.55*	0.04	–0.04	0.20	0.45†
5 Sal.						–0.76**	–0.83**	–0.32	–0.32	0.09	–0.34†
6 T-N							0.93**	0.35	0.35	0.21	0.09
7 T-P								0.48†	0.48†	0.20	0.34
8 DOC									0.64*	0.18	0.76**
9 logMeso										–0.003	0.55†
10 average wind speed on the previous day											0.024
11 log (*Skeletonema* sp.)											

† < 0.10, * < 0.05, ** < 0.01

simple correlation charts (Tables 4). Despite the reduction in red tide occurrence, the number of log phase *Skeletonema* sp. cells increased, which is consistent with the observation of highly variable cell concentration numbers in red tide occurrences and a large standard deviation. Salinity increased, but according to Suzumura et al. (2000), P undergoes adsorption in low salinity conditions, which is opposite of elution, which is observed in conditions of low salinity. The zooplankton, *Mesodinium*, is a predator of *Skeletonema* sp. (example presented by Okaichi) (1997). Mean wind speed on the previous day is not a significant explanatory variable after the implementation of the Fifth TPLCS, but as the coefficient of average wind speed of the previous day is approaching 0, the relationship between wind and log phase *Skeletonema* sp. can be considered as one of the factors. Another study has shown that disturbance of the upper and lower water layers by strong wind is disadvantageous for phytoplanktons, such as *Skeletonema*, which lack the ability to actively move within the water column (Yoshida et al., 1998).

On the other hand, the relation between T-P and DOC, DO and pH, which can be assumed when using suppressor variables from the above. There is naturally a high correlation between air temperature and water temperature. For the relationship between pH and DO, as photosynthesis proceeds, both DO and pH increase. The level of correlation between T-N and T-P can be considered to be caused by Redfield ratio, which is phytoplankton's nutrition intake ratio if most of them are from phytoplanktons although intake concentration and outward concentration differ. Another factor in determining the ratio of N:P is the chemical composition of agricultural fertilizer, industrial effluent water and sewage that enters enclosed bodies of water. In the data categorized by growth condition, if the determined N:P ratio is larger than 16, P is considered to be limiting. Historical accumulation of T-N and T-P in Tokyo Bay and Ise Bay cannot be easily removed, and the P budget must specifically be investigated in this equation.

According to Okaichi (1997), the major limiting factor of *Skeletonema* proliferation is P, and volume of proliferation increases by lack of P, and sometimes can be induced by adding P alone. A report in Okaichi's book shows that the nutrient salt content of *Skeletonema* sp. differs from the Redfield ratio and that P content is remarkably large (Manabe, 1979; 1980). This explains why *Skeletonema* sp. is one of the species that is sensitive to P remediation. Auxotrophy differs by type of plankton, and while inorganic N and P are the main nutrients in supporting red tide events, these are rarely related to the occurrence of dinoflagellate red tides (Iwasaki, 1980).

Taken together, both T-P and T-N load were changed by the implementation of the TPLCS and, consequently, *Skeletonema* sp. and the Red Tide Index changed at the same time. It is very likely the link between T-P load in sea water and the Red Tide Index was not clear because both T-N and T-P loads were significantly reduced by the Fifth TPLCS, which changed the environmental factor linkage.

On the other hand, in a comparison of coefficients of simple correlation and standardized multiple regression, T-P was not a significant explanatory variable related to the Red Tide Index before and after the Control. However, the changes in the equation before and after the implementation of the Fifth TPLCS are relevant, and therefore, it is highly possible that Red Tide Index log phase *Skeletonema* sp. and such environmental linkage also changed before and after the implementation of the Fifth TPLCS. This suggests that N and P load reduction has the possibility to change the course of *S. costatum* red tide occurrences.

The impact of the Fifth TPLCS years its implementation is most evident in the reduction of N and P loading. However, many aspects related to the impact still remain to be elucidated, such as the effect of N and P residence time and P elution from the sea sediments.

Acknowledgments

I would like to express my sincere gratitude to Professor Tsuneo Takeuchi, Graduate School of Environmental Studies, Nagoya University, who provided advice for this thesis from the aspect of the policy of Total Pollutant Load Control. And I also thank the Natural Environment Division of the Environmental Bureau of the Tokyo Metropolitan Government for providing documents that were invaluable for conducting this study. Funding was provided by the scientific research fund (special researcher grant to Chika Suzuki) and the "Women Researcher Promotion Program" research skill-up grant (Chika Suzuki) .

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(Received March 21, 2013; accepted March 26, 2013)