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Iodate Transformation By Marine Phytoplankton

Ajcharaporn Udomkit
Old Dominion University

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by

Ajcharaporn Udomkit
B.Sc. March 1982, Chulalongkorn University, Thailand
M.Sc. April 1986, Chulalongkorn University, Thailand

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Approved by :

Dr. William M. Dunstan (Director)

Dr. George T. F. Wong

Dr. Anthony J. Provenzano, Jr.

Dr. Harold G. Marshall

ABSTRACT

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Ajcharaporn Udomkit
Old Dominion University, 1994
Director : Dr. William M. Dunstan

The speciation and distribution of iodine in the oceans are partly under biological control. Phytoplankton are suspected to mediate the transformation of iodate to iodide via reduction by the enzyme nitrate reductase. However, there has been no direct evidence to support this hypothesis.

The influence of phytoplankton on the speciation of iodine was examined with emphasis on the transformation of iodate to iodide. Six cultures of marine phytoplankton: *Skeletonema costatum, Dunaliella tertiolecta, Amphidinium carterae, Tetraselmis levis, Emiliania huxleyi,* and *Synechococcus* sp., have been examined for their ability to take up and reduce iodate under a reduced nitrate environment. In both natural and elevated iodate environments, all phytoplankton took up iodate and produced iodide. Iodate loss from the medium was not always equivalent to iodide production indicating either the accumulation of iodine in phytoplankton cells or the presence of other reduced forms of iodine besides iodide. Under an ambient iodate concentration of 359 nM, the uptake

of iodate decreased in the order of *A. carterae* > *Synechococcus* sp. > *T. levis* > *D. tertiolecta* > *E. huxleyi* > *S. costatum*. The highest rate of iodate uptake, 0.93 nM·μg chl $a^{-1}\cdot d^{-1}$, was observed in *A. carterae*, a coastal dinoflagellate. The oceanic cyanobacteria, *Synechococcus* sp., took up only 0.32 nM·μg chl $a^{-1}\cdot d^{-1}$ of iodate and released 0.31 nM·μg chl $a^{-1}\cdot d^{-1}$ as iodide. This iodide release was the highest rate among the phytoplankton tested. Because of its abundance, this cyanobacteria could act as a major producer of iodide in the ocean. On the other hand, in coastal waters the spring bloom of diatoms and dinoflagellates may be responsible for the low concentration of iodate and the presence of organic iodine. There was no evidence of inhibitory effects of high concentrations of iodate on growth and development of phytoplankton. In addition, in these experiments there was no evidence that bacterial activities were responsible for the uptake and reduction of iodate.

Studies on the transformation of iodate in the diatom *S. costatum* revealed that the changes in concentration of iodate had a significant inverse relationship with the increase of phytoplankton cell density ($R = -0.98$, P-value < 0.001, $N = 6$). The variation in iodide was best explained by the change in phaeo-pigments which are the indicator of senescent cells ($R = 0.95$, P-value $= 0.003$, $N = 6$). The ratio I:C calculated from the changes in the sum of iodate and iodide and the chlorophyll-specific photosynthetic

rate(P_{chl}) was close to those values previously reported in hydrographic data as well as in planktonic tissue by other investigators.

To examine the effect of nitrogen sources on the uptake of iodate, *S. costatum* was grown in two different media based on nitrate and ammonium as nitrogen sources. The time course variations in iodate and iodide concentration were monitored for 9 days. The decrease in iodate concentration was more intense in the culture with nitrate than in ammonium-enriched culture. The change in iodate concentration related to nitrate was highly significant (R = 0.89). The presence of ammonium ion in the media suppressed the transformation of iodate to iodide. The result implied the close relationship between iodate reduction and nitrate reduction in phytoplankton. The processes of iodate transformation may occur at the surface of or inside the phytoplankton cell. Iodate removal rate by *S. costatum* ranged from 0.10 to 0.57 nM·μg chl $a^{-1}\cdot d^{-1}$ depending on the growth stages. The removal rate was higher in the exponential phase than in the stationary phase. On the other hand, the production of iodide occurred mostly after the cell approached the stationary phase. The rate of iodide production in this species ranged from 0.01 to 0.07 nM·μg chl $a^{-1}\cdot d^{-1}$.

DEDICATION

To my beloved parents,

Manas and Absorn Udomkit

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CHAPTER ONE

GENERAL INTRODUCTION

1.1. General Introduction

The first report of the discovery of iodine was by Courtois in 1811 when he measured it in extracts of seaweed. However, it was not until 1825 that elementary iodine was detected in seawater (as reviewed by Riley 1965). Since then, studies about this element have been carried on for decades. A great deal of attention was focused on the distribution and abundance of iodine because of its important role in mammal and human physiology. Seaweeds and other marine products are an important source of iodine (as reviewed by Vinogradov, 1953). This leads to the interest in the study of iodine in the marine environment. Iodine also exists in the atmosphere and lithosphere (as reviewed by Fuge and Johnson, 1986). Recently, studies have been focused on the cycle and biogeochemistry of iodine in the oceans (Tsunogai and Henmi, 1971; Truesdale, 1978; Elderfield and Truesdale, 1980 Chapman 1983; Jickells *et al.*, 1988; Luther and Cole, 1988; Luther *et al.*,1991, Rebello *et al.*, 1990; Wong and Zhang 1992a; and Zhang 1993) as well as the exchange of this element with the atmosphere (Miyake and Tsunogai, 1963; Lovelock *et al.*, 1973; Liss and

Slater, 1974; Rahn *et al* 1976; and Chameides and Davis, 1980).

The cycling of iodine is involved in both biological and non-biological processes in the marine environment. The forms and standing crops in each reservoir are well defined, but the processes responsible for the transformation among the iodine species as well as their fluxes are partly understood. Recently, Wong (1991) has proposed the tentative cycle of dissolved iodine in the sea as given in Fig.1.1. From this figure, biological mediation will be involved in the transformation (reduction) of iodate to iodide (reaction a), the oxidation of iodide to iodate (reaction b) and the oxidation of iodide to elemental iodine (reaction c).

The major purpose of this study is to examine the interaction of iodine and biological processes in the oceans with emphasis on the role of phytoplankton on the transformation of iodate. The investigation will be concentrated on the following topics :

1. The tranformation of iodate by phytoplankton
2. Patterns of iodate transformation
3. The transformation of iodate and its products
4. The importance of phytoplankton in iodine speciation.

Fig. 1.1 A tentative cycle for of dissolved iodine species in the sea (from Wong, 1991).

(a) Reduction of iodate to iodide; Thermodynamically unfavorable; Biological mediation.

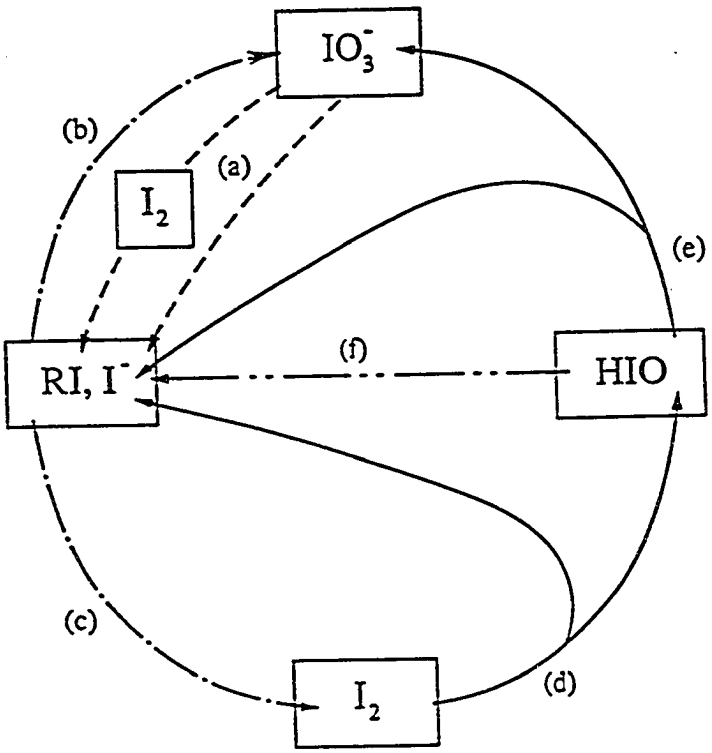
(b) Oxidation of iodide to iodate; Non-spontaneous; Biological mediation.

(c) Reduction of iodide to elemental iodine; Non-spontaneous; Biological/chemical mediation.

(d) Hydrolysis of elemental iodine to hypoiodite and iodide; Spontaneous.

(e) Disproportional of hypoiodite to iodate and iodide; Thermodynamically favorable but slow.

(f) Formation of organic iodine from hypoiodite and/or reduction of hypoiodite to iodide by reducing agents in seawater.



1.2. Literature Review

1.2.1 Speciation and Distribution of Iodine in Seawater

Seawater is the largest source of iodine in the environment (Fuge and Johnson, 1986). In seawater, iodine is known as one of the minor biophilic elements (Brewer, 1975; Elderfield and Truesdale, 1980). The concentration of total iodine in the oceans is about 60 $\mu g \cdot l^{-1}$ or 0.5 μM (Barkley and Thompson, 1960; Miyake and Tsunogai, 1963). The majority of iodine is present in the dissolved inorganic forms, iodate(IO_3^-) and iodide(I^-). Concentrations of these iodine species vary significantly with depth and geographic location. Iodate is the thermodynamically stable form. The concentration of iodate is about 0.3 to 0.4 μM in the surface water and increases with depth to approximately 0.5 μM (Wong and Brewer, 1974; Wong, 1977; Truesdale, 1978; Elderfield and Truesdale, 1980; Takayanagi and Wong, 1986; and Jickells *et al.*, 1988). The concentration of iodide, which is the metastable form of iodine, ranges from < 0.01 below the euphotic zone to 0.2 μM in the surface waters (Tsunogai and Henmi, 1971; Takayanagi and Wong, 1985; Luther and Cole, 1988; Rebello *et al.*, 1990; Luther *et al.*, 1991; Wong and Zhang, 1992a). Significant amounts of iodide are also found in bottom waters (Liss *et al.*, 1973; Herring and Liss, 1974). In contrast to oceanic waters, iodide is the predominant iodine species in anoxic waters (Wong and

Brewer, 1977; Wong *et al.*, 1985; Ullman *et al.*, 1990; Luther and Campbell, 1991). Profiles of iodine speciation in oxic and anoxic waters are shown in Fig.1.2 and Fig.1.3, respectively. In the coastal environment, the concentrations of total iodine and iodate increase with salinity but iodide concentration shows the opposite trend (Fig.1.4).

Besides these inorganic forms, small amounts of organic iodine (about 5% of total iodine) have been measured in coastal waters (Truesdale, 1975). Butler and Smith (1985) reported high concentrations of organic iodine which corresponded to 15% of total iodine in estuarine water while Luther *et al.* (1991) found that organic iodine contributed up to 70% of total iodine in some stations in the Chesapeake Bay. About 17-38% of the total iodine in the Black Sea was considered to be high molecular weight organic iodine (Luther and Campbell, 1991). Particulate iodine was found in the Atlantic Ocean (Wong *et al.*, 1976). Another form of iodine, methyl iodide, was detected in the surface eastern Pacific (Singh *et al.*, 1983). Studies for the volatile biogenic halocarbons (Loverlock *et al.*, 1973; Liss and Slater, 1974; Lovelock, 1975; Chameides and Davis, 1980; Rasmussen *et al.*, 1982; Singh *et al.*, 1983; and Moore and Tokarczyk, 1993) revealed the existence of volatile iodine in the pelagic zones. Methyl iodide was abundant in coastal waters while chloroiodomethane and its precusor di-iodomethane concentrated in surface open ocean. Moore and

Fig.1.2 Vertical profile of total iodine, iodate and iodide in the St. Lawrence Estuary at 48°58'N and 67°54'W as a representative of oxic waters (From Takayanagi and Wong 1986).

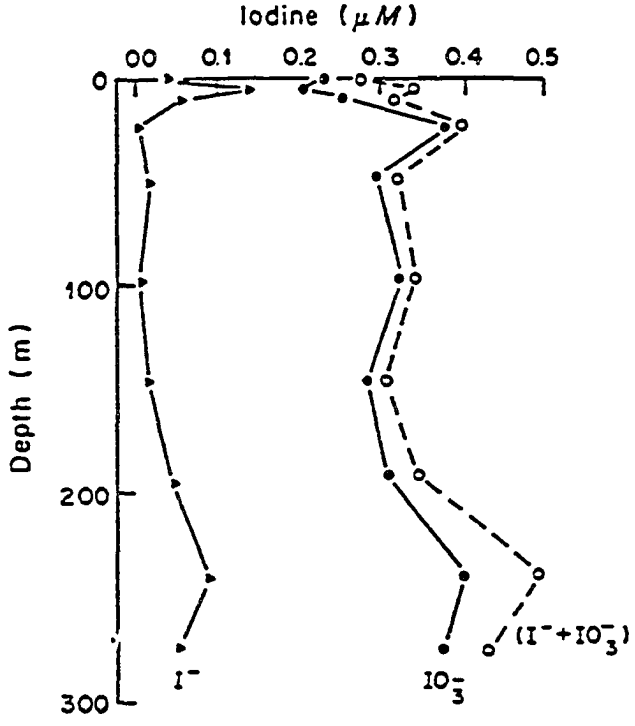


Fig.1.3 Vertical profile of iodate and iodide in the Cariaco Trench at 10°31'N and 64°45'W. (From Wong and Brewer 1977).

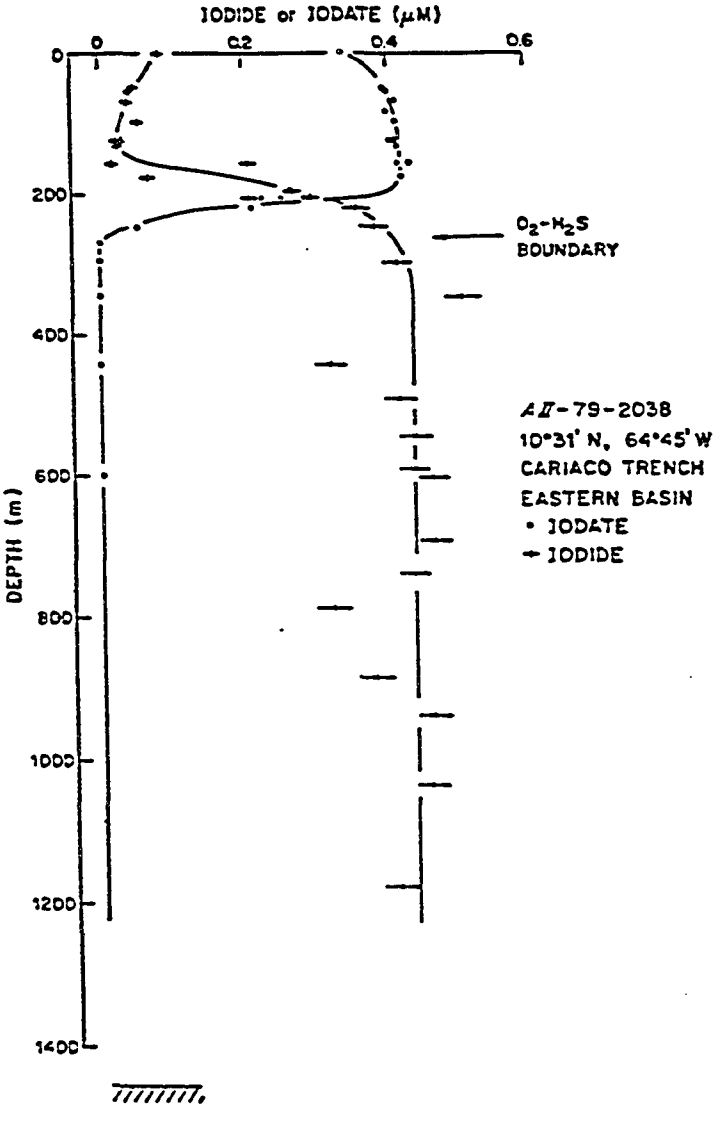
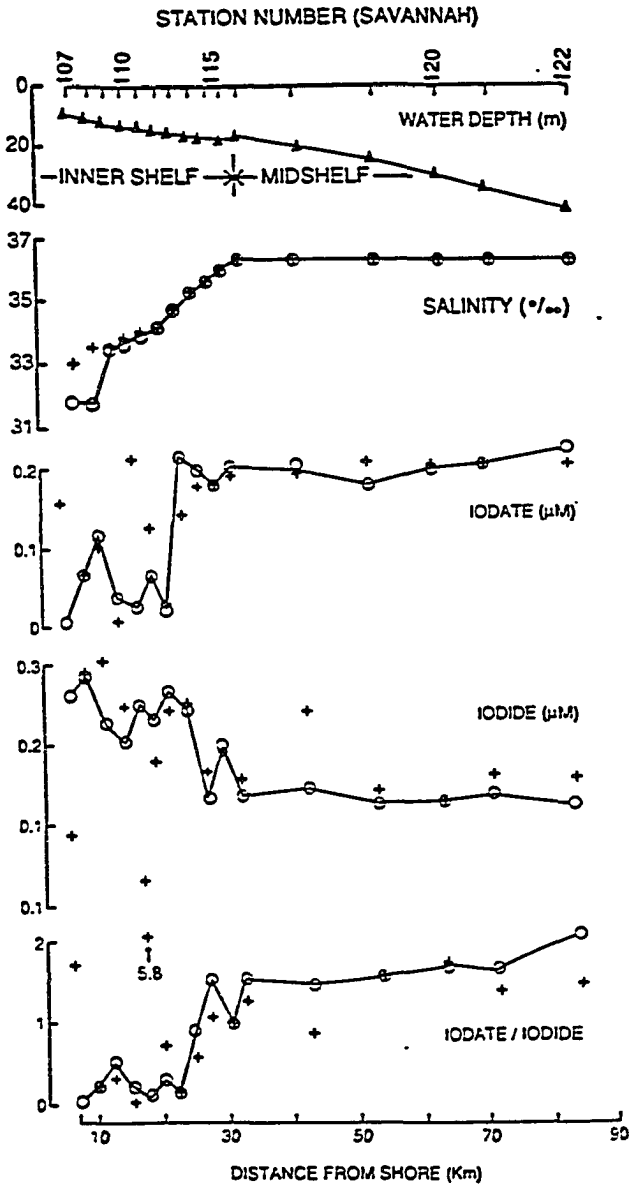


Fig.1.4 Water depth and the distribution of salinity, iodate and iodide in the surface water (O) and the bottom water (+) along the Savannah transect. (From Wong and Zhang 1992 a).



Tokarczyk (1993) suggested that both species of iodine are produced by phytoplankton. Two other species of inorganic iodine, molecular iodine(I_2) and hypoiodite(HIO), have not been directly detected in seawater but were reported to be unstable in the pH of seawater under laboratory conditions (Wong, 1980; and Wong, 1982)

1.2.2 Factors Controlling Distribution and Speciation of Iodine

1.2.2.1 Biotic Factors

Wong and Brewer (1974) demonstrated that the vertical profile of iodate was similar to that of nitrate and phosphate. Later on, Elderfield and Truesdale (1980) suggested that the coupling between total iodine and nutrients particularly phosphate represented a better relationship than that between iodate and nutrients. Several investigators have reported the close relationship between the distribution of iodine and biological activities in the surface oceans (Elderfield and Truesdale, 1980; Jickells *et al.*, 1988; Luther and Cole, 1988; Rebello *et al.*, 1990; and Luther *et al.*, 1991). High iodide concentrations were found in the surface layer of the Pacific Ocean where the productivity was also high (Tsunogai and Henmi, 1971). Truesdale (1978) suspected that the low concentrations of iodate in the near surface tropical waters of the Atlantic and Western Indian Ocean were due to biological activities, especially those of bacteria. Both

the highly significant correlations between specific total iodine and specific phosphate in the Pacific, Atlantic and Antarctic deep waters and the good agreement of the atomic ratio I:C from water samples to that of plankton composition suggest the coupling between iodine and nutrients during biological assimilation (Elderfield and Truesdale, 1980). Jickells *et al.* (1988) suspected that phytoplankton might cause the depletion of iodate in the Sargasso Sea and inshore waters around Bermuda since the declines in iodate/total iodine ratios were correlated well with the increase in chlorophyll *a* concentrations. Biological process may be responsible for the accumulation of iodide in the surface waters of the Chesapeake Bay where chemical reduction is unlikely to happen (Luther and Cole, 1988). Rebello *et al.*(1990) proposed that both iodate and iodide is taken up by phytoplankton. Iodate uptake is light dependent while the uptake of iodide occurs only at night. Wong and Zhang (1992a) suggested that the removal of dissolved iodine into particulate forms may occur in the inner shelf waters of the South Atlantic Bight.

1.2.2.2 Abiotic Factors

Besides biological control, the speciation of iodine is controlled by chemical reactions as well as by physical processes. Particle dissolution might be responsible for the specific total iodine maxima above the oxic-anoxic boundary in the Cariaco Trench while the sedimentary iodide flux might be a better explanation in the

anoxic zone of the Black Sea (Wong and Brewer, 1977). The depletion of iodate in the water column of the Yarra River estuary was partially explained by sedimentary exchange (Butler and Smith, 1985). In this case, iodate diffuses into the sediment and is reduced to iodide which diffuses back into the overlaying water. Luther and Cole (1988) suggested that the chemical reduction of iodate, by reducing agents such as sulfide and biological processes, was responsible for the presence of iodide in the anoxic bottom water of the Chesapeake Bay.

1.2.3 Bio-mediated Transformation of Iodine

The presence of iodine in seaweeds has been reported since the early 1900's. Concentration of iodine in phytoplankton and seaweeds is one thousand times greater than in seawater (Vinogradov, 1953). Kelly and Baily (1951) reported the uptake of radioactive iodide by brown algae, *Ascophyllum* sp., and concluded that iodine was dynamically assimilated and exudated by this algal species. The presence of iodo-amino acids after the uptake of radioactive iodide in four species of macroalgae indicated the incorporation of iodine in these algae (Scott, 1954). Both radioactive iodate and iodide were assimilated by green and brown algae , *Ulva rigida* and *Cystoseira crinita*, but the uptake of iodide was more intense than that of iodate (Svetasheva, 1984). The excretion product from these macroalgae, however, was only in the form of iodide. These

results suggest the bio-mediated transformation of iodine species in seawater.

Moreover, methyl iodide (iodomethane, CH_3I) was produced by many species of kelp and their associated microbes (Manley and Dastoor, 1987; and Manley and Dastoor, 1988). Other organoiodides such as iodoalkanes were released by various temperate macroalgae(Gschwend *et al.*, 1985). Iodine(I_2) was detected in an exudate from *Levringia boergensenii*(brown algae) and *Asparagopsis taxiformis*(red algae) supplied with iodide (Mairh *et al.*, 1989).

In contrast to macroalgae, the study of iodine metabolism in micro-organisms is very limited. Sugawara and Terada (1967) reported the uptake of radioactive iodate and iodide by a marine diatom *Navicula* sp. The results showed that iodide was the preferable form in comparison to iodate and the conversion of iodide to iodate occurred simultaneously with the algal growth. In 1969, Tsunogai and Sase showed that iodate in both nitrate deficient(0 mM) and nitrate limiting($\leq$ 10 mM) media can be reduced by marine bacteria which contains the enzyme nitrate reductase. The enzyme nitrate reductase extracted from the bacteria *Escherichia coli* also caused the reduction of iodate to iodide. Their results implied that organisms that can reduce nitrate are able to reduce iodate. Truesdale (1978) detected that only small amounts (less than 10 $\mu g \cdot l^{-1}$) of total iodine were taken up by six species of phytoplankton in media containing 30 $\mu g \cdot l^{-1}$ of iodate plus 50 $\mu g \cdot l^{-1}$ of

either iodate or iodide. Less than 5 $\mu g \cdot l^{-1}$ of this iodine was interconverted. Five species of phytoplankton, *Asterionella japonica, Skeletonema costatum, Dunaliella tertiolecta, Synechococcus* sp., and *Chrysochromuliina costerae,* were tested for their ability to interconvert iodate and iodide by Butler *et al.* (1981). The conversion of iodate to iodide during the senescent phase had been noticed only in two out of three cultures of *S. costatum.* Iodine content in cells of phytoplankton *Chattonella antiqua* was as high as 3.5 nM in media with 1 μM iodide addition while it was less than 0.5 μM in iodate added media (Fuse *et al.*, 1989). Most of the intracellular iodine in this phytoplankton was reported to be lipid iodine. Four other phytoplankton *Thalassiosira weissflogii, Dunaliella* sp., *Gymnodinium sunguineum* and *Heterosigma akashiwo,* accumulated more iodine from the iodate added media than from iodide enriched media when the initial iodine concentration was in the range of 0.1 to 1.0 μM. However, in higher iodine concentrations (0.01 and 1 mM), the diatom *T. weissflogii* showed a preference to accumulate more iodine in iodide media than in iodate added media. The iodine extracted from *T. weissflogii* was in a water soluble form.

CHAPTER TWO

EXPERIMENTAL PROCEDURE

AND APPROACH

2. 1 Stock phytoplankton cultures and culture medium

A list of phytoplankton used in these studies is shown in Table 2.1. These phytoplankton were obtained from the Provasoli-Guillard Center for Culture of Marine Phytoplankton, Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, Maine, USA. The original cultures (about 5 ml) were then transferred into fresh media prepared from prefiltered surface seawater (salinity 30 ppt) collected from the Chesapeake Bay, Virginia. This seawater was enriched with nutrients based on the medium "f" devised by Guillard and Ryther (1962) but the concentrations of nutrients were diluted to half the strength of the original recipe ($f_{/2}$). The basic enrichment for 1 liter of $f_{/2}$ medium was :

$NaNO_3$		883	µM
$NaH_2PO_4 \cdot H_2O$		36.3	µM
$Na_2SiO_3 \cdot 9H_2O$		54	µM
Trace metals :			
$Na_2 \cdot EDTA^+$	ca	11.7	µM
$FeCl_3 \cdot 6H_2O^+$	ca	11.7	µM

Table 2.1 List of marine phytoplankton used for the experiment.

Class Scientific Name	Clone	Original Location	Ref #
Bacillariophyceae			
Skeletonema costatum (Greville) Cleve	SKEL	Milford Habor, CT	CCMP1332
Thalassiosira oceanica Hasle	13-1	Sargasso Sea	CCMP1005
Chlorophyceae			
Dunaliella tertiolecta Butcher	DUN	Unknown	CCMP1320
Dinophyceae			
Amphidinium carterae Hulburt	AMPHI	Great Pond, Falmouth,MA	CCMP1314
Prasinophyceae			
Tetraselmis levis Butcher	PLATY1	Great Pond, Falmouth,MA	CCMP896
Prymnesiophyceae			
Emiliania Huxleyi (Lohm.)	BT6	Sargasso Sea	CCMP373
Cyanophyceae			
Synechococcus sp.	DC2	33°44.9'N 67°29.8'W	CCMP1334

$CuSO_4 \cdot 5H_2O$	ca	0.04	µM
$ZnSO_4 \cdot 7H_2O$	ca	0.08	µM
$CoCl_2 \cdot 6H_2O$	ca	0.05	µM
$MnCl_2 \cdot 4H_2O$	ca	0.9	µM
$Na_2MoO_4 \cdot 2H_2O$	ca	0.03	µM
Vitamins :			
Thiamin.HCl		0.1	mg
Biotin		0.5	µg
B_{12}		0.5	µg

2.2 Stock culture maintenance

Stock cultures were maintained in incubators at 20°C under 12:12 hours light:dark cycle provided by soft-white fluorescent lights at approximately 50 $\mu E \cdot m^{-2} \cdot s^{-1}$. The cultures were transferred to a new medium every two weeks. In order to minimize bacterial activities that may interfere with the experiment, all stock cultures were previously treated with Guillard's Antibiotic Concentrated Solution which consisted of :

Penicillin G Sodium	16250 U/ml
Streptomycin Sulfate	5 mg/ml
Chloramphenicol	2 mg/ml
in 0.9% NaCl.	

This antibiotic treatment was based on the procedure described by Droop (1967).

2.3 Preparation of cultures for the experiments

Stock cultures of selected phytoplankton were first transferred to the new medium which was made from either deep Sargasso Seawater (2000 m) or artificial seawater (Morel *et al.*, 1979). The concentrations of the enriched nutrients in this medium were at $f_{/2}$ or $f_{/20}$ level as described in each experiment. After 4 to 5 days of acclimation, aliquots from this secondary stock(s) were dispersed in the experimental vessels at the desired amounts for each experiment. To study the effect of nitrogen sources on iodate uptake, stock cultures were transferred to two different media ($f_{/2}$), one with nitrate and another one with ammonium as the nitrogen source. These cultures were incubated and successively transferred into diluted media ($f_{/10}$) during the late log phase. These were the working stocks for the experiment.

2.4 Determination of phytoplankton biomass

In vivo chlorophyll fluorescence was used for routine monitoring of phytoplankton growth. Approximately 5 ml from the incubation stock was sampled and measured for chlorophyll fluorescence by a Model 100 Turner Design Fluorometer. Readings were made at the same time each day to account for diel variation. After the measurement, the

same aliquot was preserved in Lugol's solution for a microscopic cell count using a Neubauer hemocytometer. Cell density was calculated by counting cells from two hemocytometers and expressed as the number of cells per millilitre of medium. Specific growth rate (μ) during log growth phase was also calculated.

2.5 Analyses of phytoplankton pigments and iodine speciation

At least 30 ml of cell suspension from each incubation sample was concentrated onto a glass fiber filter type GFC or GF/F. Algal pigments were then extracted from these filters by mechanical grinding in 90% acetone solution. The amounts of chlorophyll *a* and phaeo-pigments were calculated as described by Strickland and Parson (1972).

The filtrates were stored and frozen for the direct determinations of iodate and iodide using an EG&G Par model 384B Polarographic analyzer with a Model 303A static mercury drop electrode in the SMDE mode. Iodate was measured by differential pulse polarographic analysis as described by Herring and Liss (1974) and Wong and Zhang (1992b). Analysis of iodide was conducted by the cathodic-stripping square wave voltammetry technique (Luther *et al.*, 1988, and Wong and Zhang, 1992b). The detection limits were about 20 nM for iodate and 2 nM in the case of iodide. The precision for both analytical methods was about 10%.

2.6 Toxicity of iodate on selected phytoplankton

To investigate the role of marine phytoplankton on the uptake of iodate, we designed bioassay experiments using the addition of iodate salt to phytoplankton cultures. However, there was a question about the amount of iodate that could be used in the experiments. Studies of the toxicity of iodate on marine phytoplankton are very limited. Fuse *et al.*(1989) reported that a flagellate *Chattonella antiqua* showed a prolong lag phase when exposed to 100 μM of iodate, while growth of a diatom *Thalassiosira weisflogii* and a flagellate *Heterosigma akashiwo* were affected at iodate concentrations of 1 mM. However, iodate concentration as high as 1 mM showed no adverse effect on the growth of the other phytoplankton, *Dunaliella* sp.(green algae) and *Gymnodinium sanguineum* (dinoflagellate). An experiment was conducted to assess the toxic level of iodate to cultures of marine phytoplankton before continuing the investigation on the transformation of iodate.

Stock cultures (50 ml each) of two diatoms, *Skeletonema costatum* and *Thalassiosira oceanica*; a chlorophyte, *Dunaliella tertiolecta*; a coccolithophorid, *Emiliania huxleyi*; and a cyanobacteria, *Synechococcus* sp.; were transferred to two liters of $f_{/2}$ media with the reduced nitrate ($f_{/20}$) concentration made from deep Sargasso Seawater (2000 m). The amount of nitrate in the media is

about 88 μM which is more realistic than 883 μM in $f_{/2}$ medium. Another reason for using this level of nitrate concentration is to shorten the phytoplankton growth period and hence incubation time. The background concentrations of iodate and iodide in this deep seawater were 359 and 12 nM, respectively. Each species was treated in duplicate with three different concentrations of iodate (up to 2000 μM) and incubated for 28 days at 20°C under 100 $\mu E \cdot m \cdot^{-2} \cdot s^{-1}$ illumination. This experimental illumination is higher than that in the stock incubation because the culture is supposed to grow at maximum conditions. *In vivo* fluorescence was periodically monitored in order to observe the abnormal in phytoplankton growth due to the toxicity of iodate.

To indicate the effect on growth of phytoplankton treated with high concentrations of iodate, two growth parameters were compared. These parameters were specific growth rate (μ) during exponential phase and the maximum yield of *in vivo* fluorescence. In each phytoplankton species there was no difference in the growth parameters between treatment with and without iodate additions (Table 2.2). *In vivo* fluorescence from each species is shown in Appendix A.

There was no evidence that high concentrations of iodates (500 to 2000 μM) cause any sublethal or lethal effect to cultures of marine phytoplankton. However, these concentration levels of iodate are unrealistic in nature,

Table 2.2 Growth rate and maximum fluorescence of phytoplankton with various iodate concentrations (SKEL = *S. costatum*, DUN = *D. tertiolecta*, BT-6 = *E. huxleyi*, 13-1 = *T. oceanica*, DC2 = *Synechococcus* sp.)

Species	Iodate addition (μM)	Growth rate (day^{-1})	Maximum fluorescence
SKEL	0	1.03	1483.9
	100	1.02	1262.3
	200	1.00	1436.1
	500	1.00	1199.5
DUN	0	1.14	2179.1
	100	1.00	1942.1
	200	1.33	1942.1
	500	1.12	1847.3
BT-6	0	0.65	3961.4
	200	0.67	3924.7
	500	0.69	3572.9
	1000	0.72	3831.9
13-1	0	0.55	1421.1
	200	0.57	1263.2
	500	0.62	1452.8
	1000	0.51	1026.2
DC2	0	0.32	74.6
	500	0.32	74.6
	1000	0.30	74.6
	2000	0.32	77.8

thus the lower iodate concentrations (5 to 25 μM) were used for the experiments.

2.7 Effect of bactetia on iodate transformation

Since Tsunogai and Sase (1969) found that nitrate reducing marine bacteria can reduce iodate to iodide, the contamination of bacteria in stock phytoplankton culture has been carefully prevented. All stock cultures are treated with an antibiotic periodically (as described before). During the experiment the control, consisting of two aliquots of filtrate from the stock culture, was inoculated into two separate sets of new media (without iodate addition and with iodate addition) and incubated in a parallel direction with the treatment samples containing algal suspension in new media. *In vivo* flourescence as well as the concentrations of iodate and iodide were monitored in the same fashion as the other treatment samples.

The results (Table 2.3 to Table 2.7) showed insignificant variations in *in vivo* fluorescence, and iodate and iodide concentrations throughout the experiments using filtrates from the cultures of *S. costatum*, *D. tertiolecta*, *A. caterae*, and *T. levis*. On the other hand, the decrease in iodate concentrations and increase in iodide concentrations were noticed in the media which contained filtrate from the cultures of *E. huxleyi* and *Synechococcus*

sp. after 6 days of incubation. However, these changes in iodate and iodide concentrations are associated with the increase in phytoplankton biomass as represented by the increase in both *in vivo* fluorescence and chlorophyll *a* content. Therefore, the variation in iodine speciation was due to the algal activities in the samples.

In conclusion, the variation in iodate and iodide concentrations in the culture media is associated only with the change in phytoplankton biomass. Bacterial activities in the culture are not responsible for this variation in iodine speciation.

Table 2.3 *In vivo* fluorescence, chlorophyll *a* content and concentrations of iodate and iodide from the samples contained the inoculum of filtrates from *S. costatum* culture in fresh media made from deep Sargasso Seawater (background iodate = 359 nM, iodide 12 nM)

Iodate Addition	Time (Day)	Fluore-scence	Algal cell (cells/ml)	Chl *a* (μg/L)	Iodate (μM)	Iodide (nM)
0 μM	0	0.2		$2.23*10^{-3}$	0.307	10.5
	3	0.2		$9.13*10^{-2}$	0.309	5.4
	7	0.2		$3.00*10^{-2}$	0.316	7.8
	14	0.1		$7.66*10^{-2}$	0.270	7.7
	28	0.1		$1.34*10^{-2}$	0.286	12.1
25 μM	0	0.2		$1.34*10^{-2}$	23.554	45.2
	3	0.4		$4.30*10^{-2}$	25.254	5.9
	7	0.3		$1.38*10^{-2}$	22.652	15.5
	14	0.5		$3.19*10^{-2}$	22.900	4.5
	28	0.2		$1.78*10^{-2}$	22.202	16.9

Table 2.4 *In vivo* fluorescence, chlorophyll *a* content and concentrations of iodate and iodide from the samples contained the inoculum of filtrates from *D. tertiolecta* culture in fresh media made from deep Sargasso Seawater (background iodate = 359 nM, iodide 12 nM)

Iodate Addition	Time (Day)	Fluore-scence	Algal cell (cells/ml)	Chl *a* (μg/L)	Iodate (μM)	Iodide (nM)
0 μM	0	0.1		$2.45*10^{-2}$	0.362	3.1
	3	0.3		$6.80*10^{-3}$	0.328	33.2
	7	0.1		$1.78*10^{-2}$	0.344	26.9
	14	0.1		$1.15*10^{-2}$	0.311	46.4
	21	0.1		$4.61*10^{-2}$	0.215	36.4
	28	0.1		$2.23*10^{-2}$	0.318	40.4
25 μM	0	0.1		$4.45*10^{-2}$	22.823	57.8
	3	0.0		$2.56*10^{-1}$	22.299	10.1
	7	0.4		$3.53*10^{-1}$	19.439	19.8
	14	0.3		$9.45*10^{-2}$	22.862	42.3
	21	0.2		$3.64*10^{-2}$	22.338	39.9
	28	0.0		$3.61*10^{-2}$	22.669	40.9

Table 2.5 *In vivo* fluorescence, chlorophyll *a* content and concentrations of iodate and iodide from the samples contained the inoculum of filtrates from *A. carterae* culture in fresh media made from deep Sargasso Seawater (background iodate = 359 nM, iodide 12 nM) [1]

Iodate Addition	Time (Day)	Fluore-scence	Algal cell (cells/ml)	Chl *a* (μg/L)	Iodate (μM)	Iodide (nM)
0 μM	0	0.0		$6.68*10^{-3}$	0.343	22.9
	3	0.3		$8.91*10^{-3}$	0.397	15.6
	7	1.2		$2.88*10^{-2}$	0.333	26.8
	14	25.6		$1.95*10^{-0}$	0.327	1.6
	20	181.9[1]	$7.1*10^{+4}$	$2.20*10^{+1}$	0.291	4.7
	28	390.0[1]	$1.3*10^{+5}$	$2.27*10^{+1}$	0.334	23.4
25 μM	0	0.0		$1.15*10^{-2}$	20.651	22.0
	3	0.7		$2.90*10^{-2}$	21.066	44.1
	7	1.3		$1.00*10^{-1}$	23.097	32.3
	14	20.4		$1.85*10^{-0}$	23.269	18.8
	20	251.4	$4.2*10^{+4}$	$2.85*10^{+1}$	17.720	78.3
	28	200.0	$1.5*10^{+5}$	$3.92*10^{-0}$	17.825	303.2

[1]The increase in *in vivo* fluorescence, algal cell density and chlorophyll *a* content in these samples indicated the presence of phytoplankton cells in the filtrates. Thus, changes in iodate and iodide in these samples were cuased by phytoplankton activities.

Table 2.6 *In vivo* fluorescence, chlorophyll *a* content and concentrations of iodate and iodide from the samples contained the inoculum of filtrates from *T. levis* culture in fresh media made from deep Sargasso Seawater (background iodate = 359 nM, iodide 12 nM) [2]

Iodate Addition	Time (Day)	Fluore-scence	Algal cell (cells/ml)	Chl *a* (μg/L)	Iodate (μM)	Iodide (nM)
0 μM	0	0.0		$5.39*10^{-2}$	0.262	10.8
	3	0.0		$2.52*10^{-2}$	0.289	0.0
	7	0.0		$4.39*10^{-2}$	0.292	23.9
	14	0.0		$1.54*10^{-1}$	0.244	19.8
	21	0.0		$3.50*10^{-2}$	0.280	30.3
	28	0.0		$5.26*10^{-2}$	0.305	17.6
25 μM	0	0.0		$2.35*10^{-2}$	16.331	1.1
	3	0.0		$3.31*10^{-1}$	20.500	10.6
	7	0.0		$2.79*10^{-2}$	19.743	0.0
	14	0.0		$1.95*10^{-1}$	19.561	12.2
	20	0.0		$4.07*10^{-2}$	20.416	20.3
	28	0.0		$5.01*10^{-2}$	19.586	18.4

[2]The increase in *in vivo* fluorescence, algal cell density and chlorophyll *a* content in these samples indicated the presence of phytoplankton cells in the filtrates. Thus, changes in iodate and iodide in these samples were cuased by phytoplankton activities.

Table 2.7 *In vivo* fluorescence, chlorophyll *a* content and concentrations of iodate and iodide from the samples contained the inoculum of filtrates from *E. huxleyi* and *Synechococcus* sp. cultures in fresh media made from deep Sargasso Seawater (background iodate 359 nM, iodide 12 nM) [3]

Iodate Addition	Time (Day)	Fluore-scence	Algal cell (cells/ml)	Chl *a* (μg/L)	Iodate (μM)	Iodide (nM)
E. huxleyi						
25 μM	0	6.6	$5.0*10^{+2}$	$2.29*10^{-1}$	24.029	60.7
	3	0.0	$1.0*10^{-0}$	$8.83*10^{-2}$	19.236	81.0
	7	12.0	$1.0*10^{-0}$	$1.49*10^{-0}$	19.722	80.3
	14	2500	$9.5*10^{+5}$	$1.34*10^{+2}$	20.735	162.4
	20	2700	$1.2*10^{+6}$	$8.93*10^{+1}$	25.147	186.3
	28	810.0	$4.4*10^{+5}$	$8.65*10^{+1}$	17.307	327.0
Synechococcus sp.						
0 μM	0	0.2		$9.49*10^{-1}$	0.358	15.6
	3	0.7		$2.73*10^{-0}$	0.303	11.1
	7	9.5		$1.74*10^{+1}$	0.256	14.8
	14	28.4		$5.71*10^{+1}$	0.162	51.6
	21	21.8		$3.68*10^{+1}$	0.152	115.8
	28	6.1		$7.72*10^{-0}$	0.080	165.8

[3]The increase in *in vivo* fluorescence, algal cell density and chlorophyll *a* content in these samples indicated the presence of phytoplankton cells in the filtrates. Thus, changes in iodate and iodide in these samples were cuased by phytoplankton activities.

CHAPTER THREE

IODATE TRANSFORMATION BY CULTURES OF MARINE PHYTOPLANKTON

3.1 Introduction

Over the past 25 years, studies of iodine in the ocean have been focused on its distribution and geochemistry (Tsunogai and Sase, 1969, Truesdale, 1978, Butler and Smith, 1985, Jickells *et al.*, 1988, Luther and Cole, 1988, Rebello *et al.*, 1990, and Wong and Zhang, 1992a). Iodine is one of the most abundant minor elements in seawater. The distribution and speciation of iodine in the oceans is controlled not only by chemical process but also biologically mediated processes (Tsunogai and Sase, 1969, Wong and Brewer, 1977, Elderfield and Truesdale, 1980, and Wong, 1991). Possible bio-transformation of iodine and its transformation rate in the ocean may have a major influence on the biogeochemical cycle of this element in the global scale. However, the importance of phytoplankton in the iodine cycle in the ocean has not yet been resolved.

Iodine exists in two major dissolved forms, iodate and iodide. Iodate is more thermodynamically stable than iodide at the pH of seawater. However, considerable amounts of iodide are measured in surface waters (Tsunogai and Henmi,

1971, Wong, 1977, Chapman, 1983; and Jickells *et al*, 1988). Thus, processes other than chemical kinetics must control the speciation of dissolved iodine in seawater. The amount of iodide in the oceans decreases with depth while iodate increases. Variations in concentrations of these two species of iodine have been associated with biological activity in the ocean (Jickells *et al*, 1988, Luther and Cole, 1988, Rebello *et al*, 1990). Results from laboratory studies on the uptake of iodine are still controversial. Sugawara and Terada (1967) reported the assimilation of iodide rather than iodate by a marine diatom. Tsunogai and Sase (1969) found marine bacteria that contain the enzyme nitrate reductase are instrumental in the reduction of iodate and production of iodide. They suggested that in surface waters, iodate can be reduced by organisms that have nitrate reductase and iodide is formed as the reduction product. According to Butler *et al.*(1981), the conversion of iodate to iodide was observed in a senescent culture of *Skeletonema costatum*. However, they concluded that the release of this iodide was caused by bacterial activity. While extensive studies on the geochemistry of iodine have been performed for decades, no conclusive studies have unequivocally identified the group of organisms responsible for the interconversion of iodate and iodide in seawater.

Based on the hypothesis that phytoplankton are the organisms mediating the conversion of iodate and iodide, we conducted experiments to demonstrate that phytoplankton

reduce iodate in carefully controlled experiments. All experimental cultures were grown in low nitrate enriched media(88 μM), which was more realistic than frequently used nitrate concentration(883 μM), with continual monitoring of bacteria activity. The rates of iodate reduction and iodide production were calculated from our experiments and interpreted in terms of rates in the natural environment.

3.2 Methods and Materials

Six species of phytoplankton were used as test organisms: a diatom, *Skeletonema costatum*(Greville) Cleve (clone SKEL); a green algae, *Dunaliella tertiolecta* Butcher(clone DUN); a dinoflagellate, *Amphidinium caterae* Hulburt(clone AMPHI); a green flagellate, *Tetraselmis levis* Butcher (clone PLATY1); a coccolithophorid, *Emiliania huxleyi*(Lohm.) clone BT6; and a cyanobacterium, *Synechococcus* sp.(clone DC2). For experiments, stock cultures of each species were transferred to fresh medium made from deep Sargasso seawater (2000 m). Nitrate in the medium was reduced to $f_{/20}$ level to simulate more realistic concentrations and algae were acclimatized for 4-5 days before each experiment.

Potassium iodate was spiked into 2 L of fresh medium containing either alga inoculum or filtrate from the same culture(as a control for possible bacterial activity). The iodate dosages were 0, 5, 10 and 25 μM for all species

except for *E. huxleyi* in which only 0 and 25 μM of iodate were used. All experimental cultures were maintained under the same environmental conditions as in the stock cultures except that the illumination was about 100 $\mu E \cdot m^{-2} \cdot s^{-1}$ to ensure the maximum algal growth. Samples for *in vivo* fluorescence, phytoplankton cell density, chlorophyll *a* content as well as iodate and iodide concentrations were cultivated on day 0, 3, 7, 14, 21, and 28. Growth rate (μ, day^{-1}) was also calculated from the relative fluorescence data during exponential-phase growth .

The determination of phytoplankton cell density, chlorophyll content and iodine speciation has been described previously in chapter two. Iodate removal rate as well as iodide production rate was calculated for both exponential and stationary phases. For the rates during exponential growth (when phytoplankton actively grew), the difference in either iodate or iodide concentrations for the first 3 days was divided by the average amount of chlorophyll *a* from the same time period. The uptake and production rates during stationary phases (day 3 to day 7 and day 7 to day 14) were calculated by dividing the difference in concentration by the maximum yield of chlorophyll *a* on day 3.

3.3 Results

3.3.1 Growth of phytoplankton in various iodate concentrations

Each species of phytoplankton grew in elevated iodate concentrations as well as in an ambient one. All cultures approached the stationary phase after three days of incubation except *Synechococcus* sp. which grew at a slow rate in comparison to other species. The variations in growth rate, maximum cell density, and maximum chlorophyll content among the samples of the same species incubated in media with and without iodate were similar (Table 3.1 and also see Appendix B). The chlorophyll content, cell density, and *in vivo* fluorescence were used to distinguish between log and stationary growth periods. For this experiment, the abundance of *S. costatum* and *D. tertiolecta* increased exponentially for the first three days, then the cultures entered stationary phase. On the other hand, the other species stayed in log phase for seven days before approaching stationary phase.

3.3.2 Changes in iodate and iodide concentrations

The concentrations of iodate and iodide in the culture media were monitored during the course of the experiment (see Appendix B). For all species, algal growth was accompanied by a decrease in iodate and an increase in iodide in the medium. Fig.3.1 and Fig.3.2 represented the fluorescence and chlorophyll as well as the amounts of iodate and iodide in the cultures of *S. costatum.* Samples for iodate and iodide analyses were taken on a routine basis on days 0, 3, 7, 14, 21, and 28 day. The rates of iodate

uptake as well as iodide production per unit chlorophyll for each species were calculated for log (3 days for *S. costatum* and *D.tertiolecta*, and seven days for the rest) and stationary phase(3-14 days for the first two species and 7-14 days for the others). At ambient iodate concentration of 359 nM (no iodate addition), the highest uptake rate of iodate is observed in *A. cartarea* (0.93 nM·μg $Chla^{-1}$· day^{-1}) followed by *Synechococcus* sp., *D. tertiolecta*, *T. levis*, and *S. costatum*. The lowest rate was in *E. huxleyi*, 0.03 nM·μg $Chla^{-1}$ ·day^{-1}, (Fig.3.3). The production rate of iodide range from 0.31 nM·μg $Chla^{-1}$· day^{-1} in *Synechococcus* sp. to 0.03 nM·μg $Chla^{-1}$· day^{-1} in *S. costatum* culture (Fig.3.4). Both iodate removal rate and iodide production rate in phytoplankton cultures tended to decrease from log phase to stationary phase. The rate of iodate removal and iodide production generally increased with increasing iodate concentrations (Fig.3.5 and Fig.3.6). The ratio of iodate to iodide as shown in Table 3.2 revealed two different patterns in the removal of iodate. The first group which included *S. costatum*, *D. tertiolecta*, and *A. carterae* had high iodate removal rate in comparison to iodide production (ratio IO_3:I > 1) Other species, *T. levis*, *E. huxleyi*, and *Synechococcus* sp., produced iodide at the same rate as removed iodate (ratio IO_3:I ≤ 1).

Table 3.1 Growth rate, maximum cell density and maximum chlorophyll *a* content in cultures of six phytoplankton treated with various iodate concentrations.

Clone	Iodate addition (μM)	Growth rate (day^{-1})	Maximum cell density ($cells \cdot ml^{-1}$)	Maximum chlorophyll *a* ($\mu g \cdot L^{-1}$)
SKEL	0	0.92	$8.29*10^5$	$9.81*10^1$
	5	0.97	$7.39*10^5$	$1.04*10^2$
	10	0.91	$6.74*10^5$	$2.16*10^2$
	25	0.95	$7.21*10^5$	$1.04*10^2$
DUN	0	0.97	$5.44*10^5$	$1.14*10^2$
	5	1.00	$5.44*10^5$	$1.31*10^2$
	10	0.99	$5.92*10^5$	$1.37*10^2$
	25	1.01	$6.75*10^5$	$1.31*10^2$
AMPHI	0	0.49	$1.22*10^5$	$3.10*10^1$
	5	0.52	$1.49*10^5$	$2.67*10^1$
	10	0.51	$1.43*10^5$	$2.67*10^1$
	25	0.54	$1.57*10^5$	$3.59*10^1$
PLATY1	0	0.80	$3.00*10^5$	$4.43*10^1$
	5	0.82	$2.91*10^5$	$3.76*10^1$
	10	0.82	$3.08*10^5$	$1.93*10^1$
	25	0.81	$2.93*10^5$	$2.95*10^1$
BT-6	0	0.60	$8.77*10^5$	$1.04*10^2$
	25	0.55	$9.15*10^5$	$8.64*10^1$
DC2	0	0.37		$7.32*10^1$
	5	0.33		$7.11*10^1$
	10	0.32		$6.84*10^1$
	25	0.30		$8.03*10^1$

Fig. 3.1 Relative fluorescence and chlorophyll contents in cultures of *S. costatum* in deep seawater enriched media with various levels of iodate addition.

A. Relative fluorescence

B. Chlorophyll *a* concentration

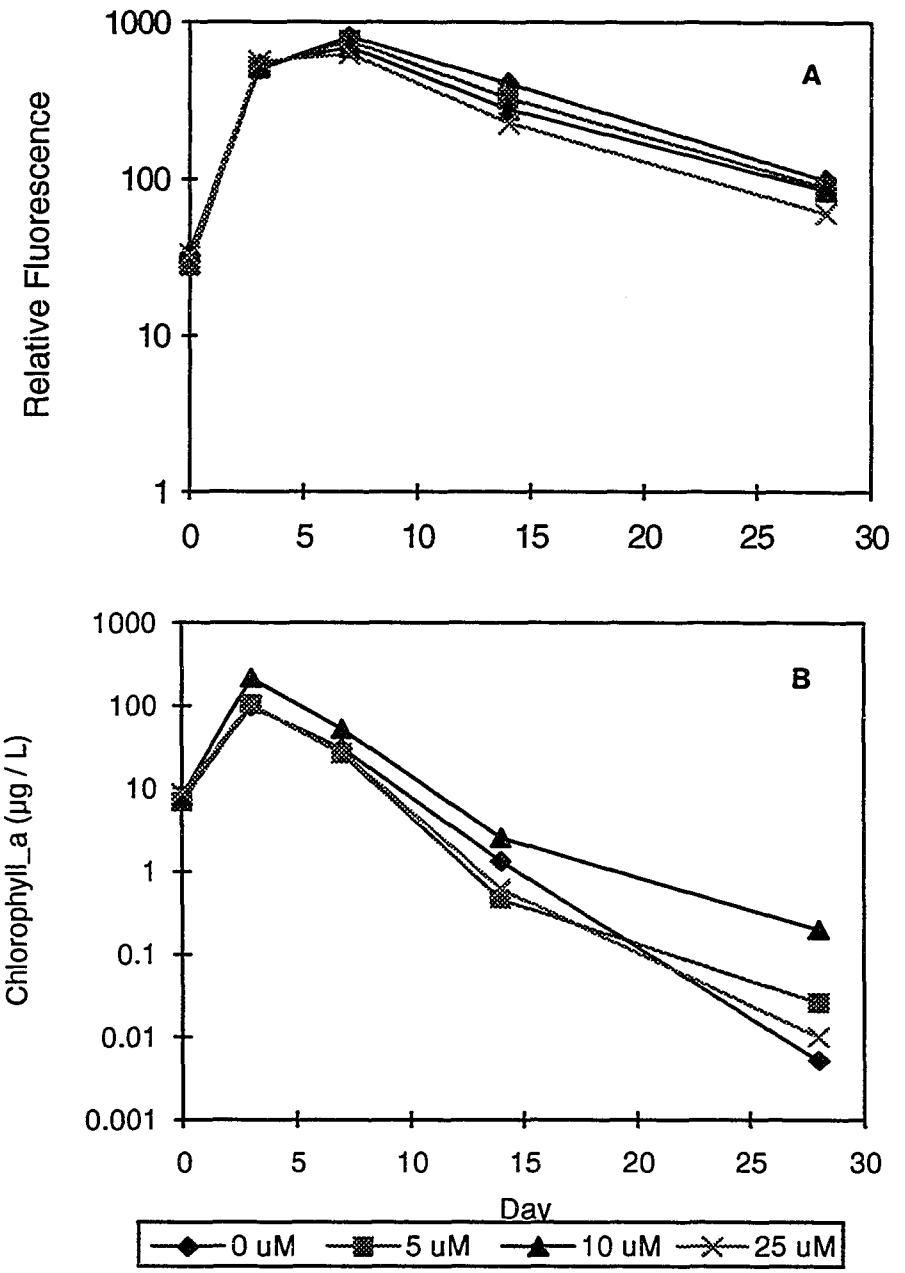
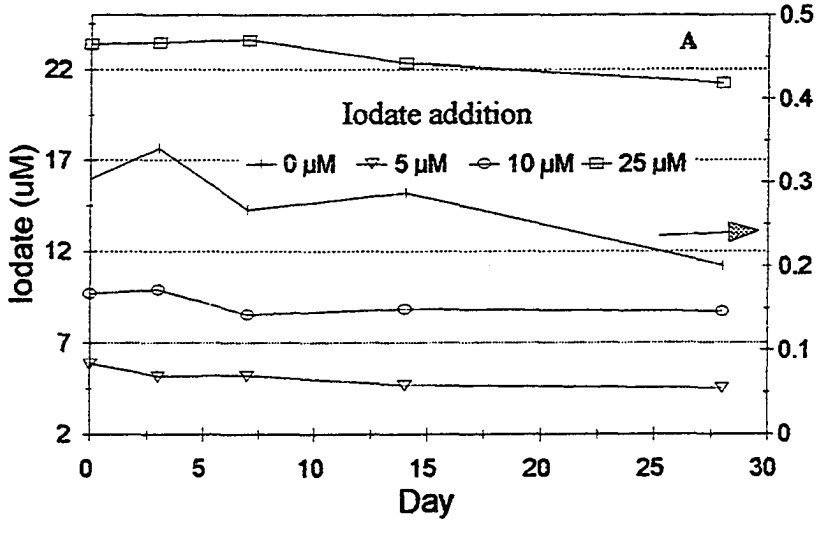


Fig. 3.2 Iodine speciation in the culture of *S. costatum* over 28 days incubation.

A. Iodate concentration.

B. Iodide concentration.



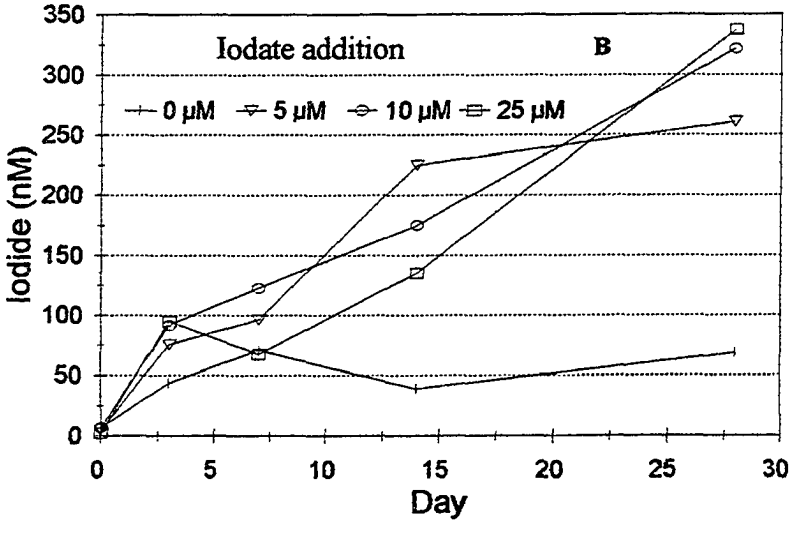


Fig. 3.3 Rates of iodate removal in an ambient iodate environment during the first 14 days of experiment. (AMPHI = *A. carterae*, BT6 = *E. huxleyi*, DC2 = *Synechococcus* sp., DUN = *D. tertiolecta* , PLATY1 = *T. levis*, and SKEL = *S. costatum*).

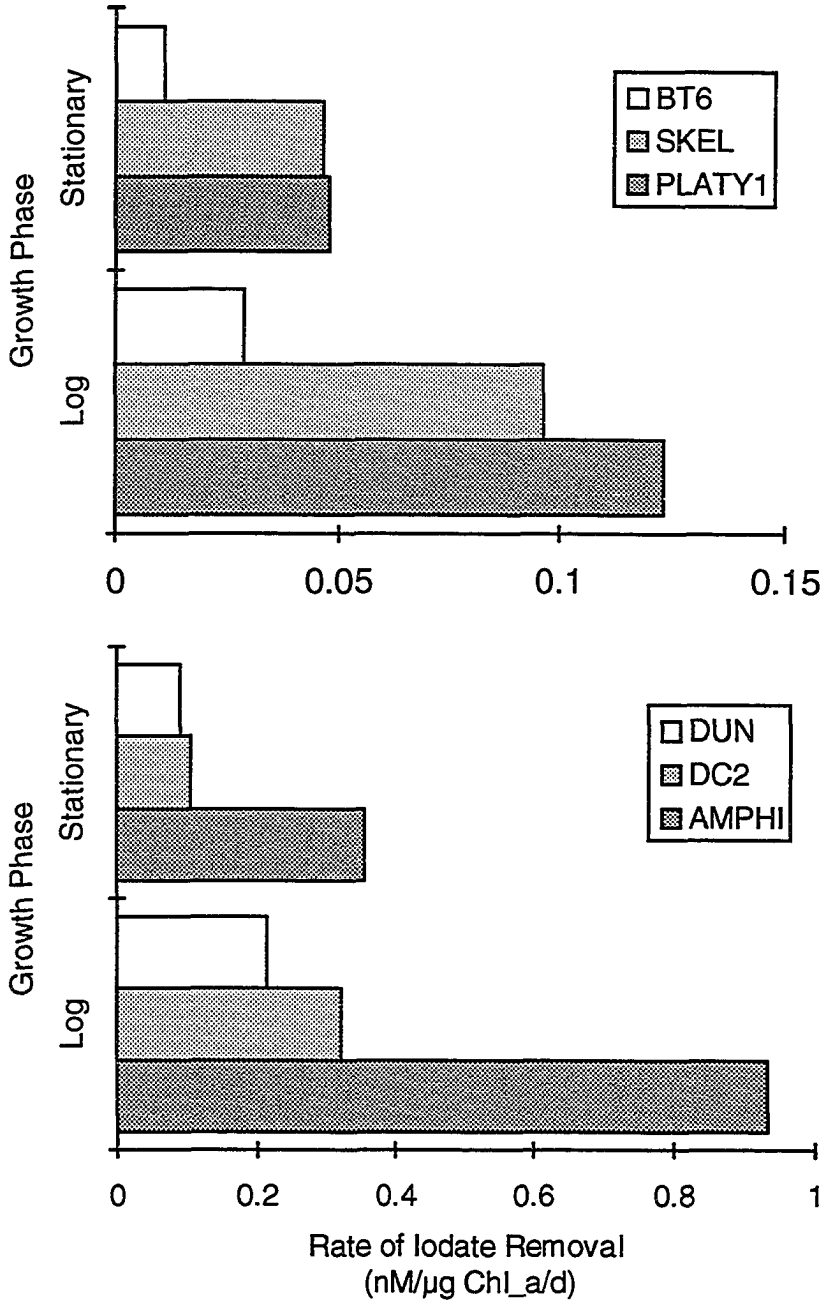


Fig. 3.4 Rates of iodide production in an ambient iodate environment during the first 14 days of experiment. (AMPHI = *A. carterae*, BT6 = *E. huxleyi*, DC2 = *Synechococcus* sp., DUN = *D. tertiolecta* , PLATY1 = *T. levis*, and SKEL = *S. costatum*).

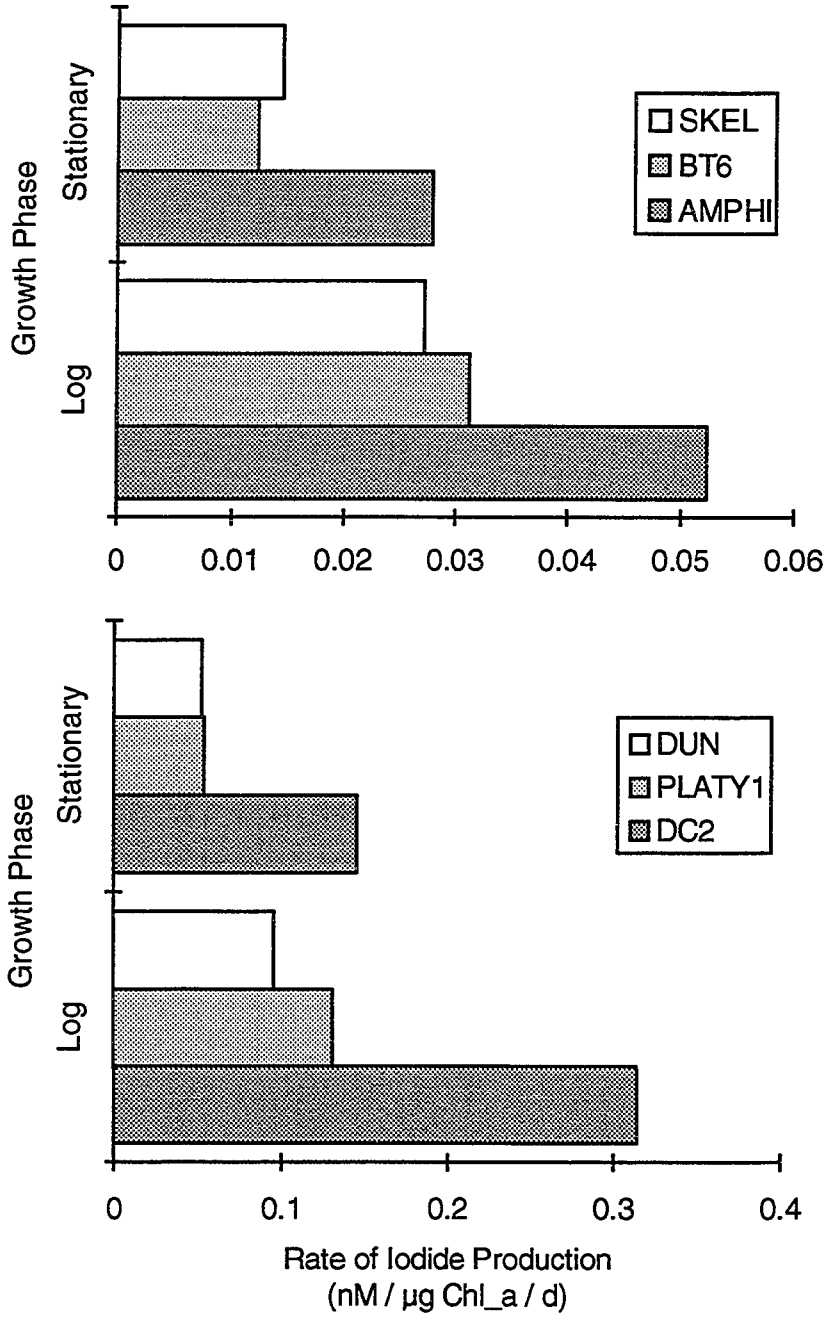


Fig. 3.5 Rates of iodate removal by cultures of phytoplankton in media containing various concentrations of iodate. (AMPHI = *A. carterae*, BT6 = *E. huxleyi*, DC2 = *Synechococcus* sp., DUN = *D. tertiolecta* , PLATY1 = *T. levis*, and SKEL = *S. costatum*).

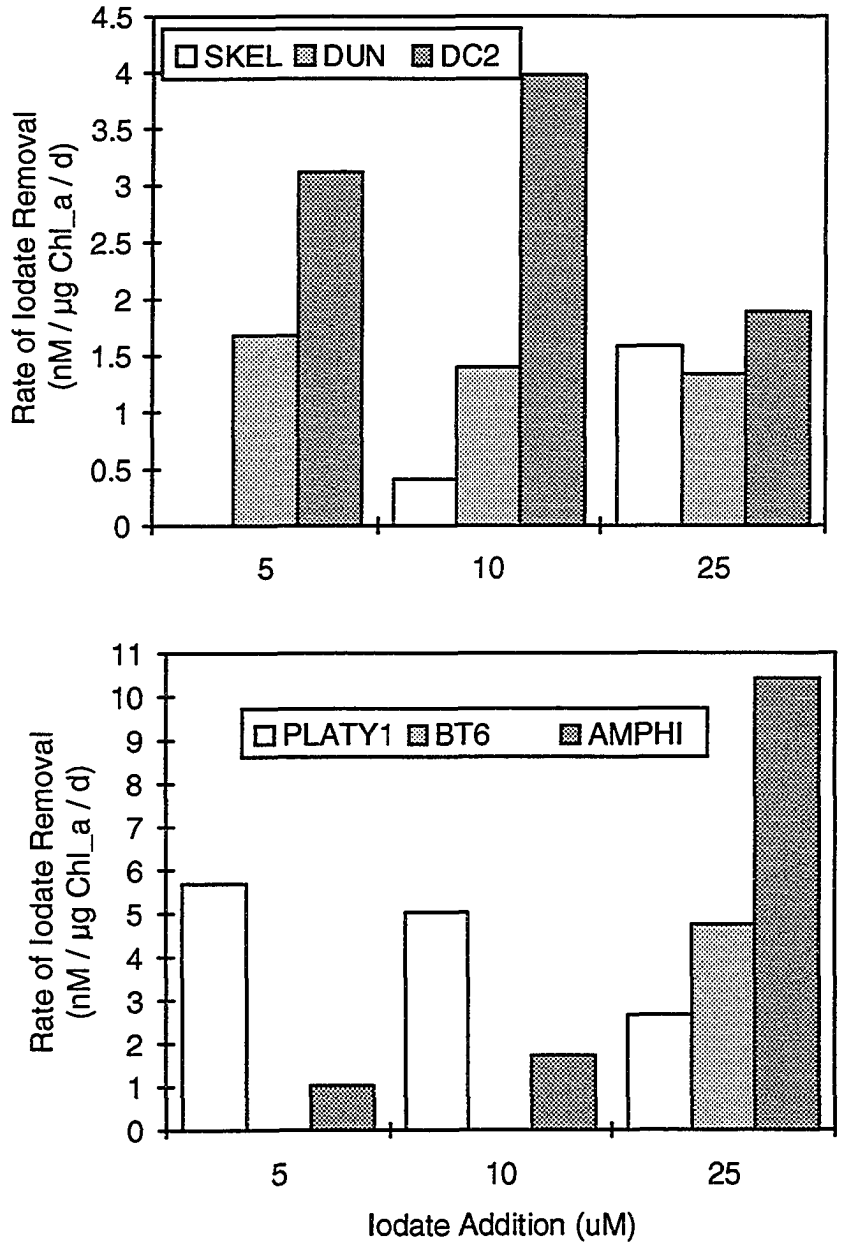
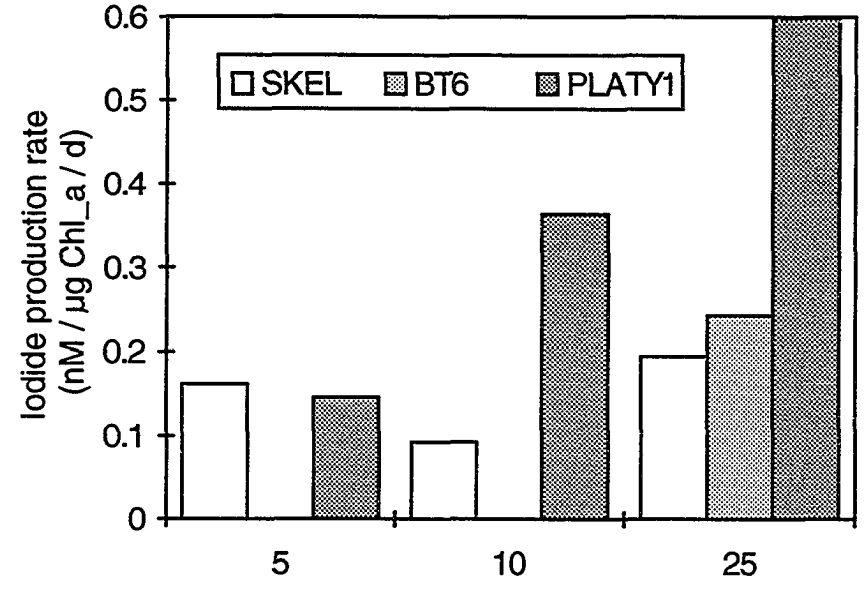


Fig. 3.6 Rates of iodide production by cultures of phytoplankton in media containing various concentrations of iodate. (AMPHI = *A. carterae*, BT6 = *E. huxleyi*, DC2 = *Synechococcus* sp., DUN = *D. tertiolecta* , PLATY1 = *T. levis*, and SKEL = *S. costatum*).



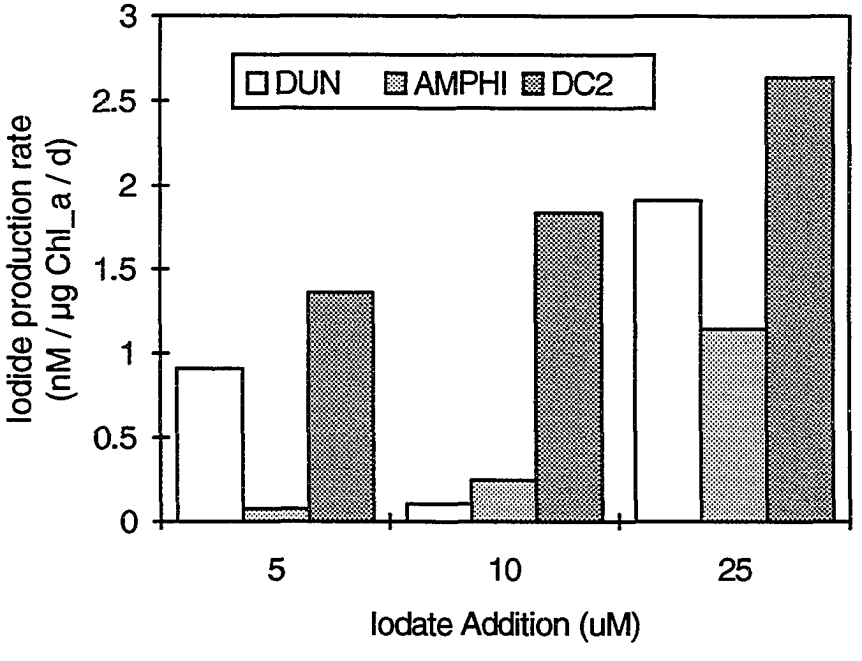


Table 3.2 Ratio of iodate removal to iodide production by six species of phytoplankton grown in deep seawater enriched media without iodate addition (background iodate concentration 359 nM).

	Iodate Removal : Iodide Production		
Phytoplankton	Log Phase	Stationary Phase	Average
SKEL	3.6	3.2	3.4
DUN	2.2	1.8	2.0
AMPHI	17.8	12.8	15.3
PLATY1	0.9	0.9	0.9
BT6	0.9	0.9	0.9
DC2	1.0	0.7	0.9

3.4 Discussion

3.4.1 Removal of Iodate and production of iodide

The removal of iodate from culture media accompanied by the production of iodide in our experiments demonstrates the reduction of iodate to iodide by marine phytoplankton. Based on the fact that iodate can be reduced by the enzyme nitrate reductase (Tsunogai and Sase, 1969), then the reduction of iodate may occur at the same site as nitrate reduction. Butz and Jackson (1977), proposed the uptake and reduction of nitrate by the membrane bound nitrate reductase. Serra (1978) proposed the regulation model for nitrate utilization in a diatom *S. costatum*. In this model, nitrate is transported intracellularly where the reduction takes place. Therefore, the reduction of iodate may occur either at the cell surface or inside the phytoplankton cell.

The rate and magnitude of iodate removal and iodide production varied with the initial concentration of iodate in the culture. They were also a function of growth phase and were species specific. In all cases, the rate of iodate removal tended to increase in elevated iodate concentrations. For example, the dinoflagellate *A. carterae* took up 0.93 $nM \cdot \mu g\ Chla^{-1} \cdot day^{-1}$ of iodate at an ambient iodate concentration of 359 nM, the rate increased to 10.4 $nM \cdot \mu g\ Chla^{-1} \cdot day^{-1}$ in 25 μM of added iodate. Other coastal species, *T. levis*, *D. tertiolecta*, and *S. costatum*, showed the ability to remove iodate at rates slower than *A.*

cartarea. The oceanic phytoplankton *Synechococcus* sp. took up 0.32 nM·μg $Chla^{-1}$·day^{-1} in the ambient concentration of iodate. Iodate removal by *E. huxleyi* was smaller than the cyanobacteria .

The increase in iodide during the course of the experiments indicates that iodate in phytoplankton culture media was reduced to iodide. In all phytoplankton cultures, the rate of iodide production appears to be higher in log phase than in stationary phase. The major iodide producer from this study is *Synechococcus* sp. which is an oceanic phytoplankton. This species, together with *E.huxleyi* and the estuarine phytoplankton, *T. levis*, produced iodide approximately at the same rate as iodate removal in ambient iodate concentration (359 nM). The ratio of iodate to iodide in these species was close to one and indicated that most of iodate removed from phytoplankton media was reduced to iodide and released into the media. Other species, *S. costatum*, *D. tertiolecta*, and *A. carterae*, had higher ratio of iodate to iodide which indicated either the intracellular accumulation of iodine or the possibility of iodide-based reactions in the cultures. *S. costatum*, a widely distributed coastal phytoplankton, released about 28% of the iodate taken up as iodide, while *A. carterae* released only 6% of influx iodate as iodide.

At present, there have been no reports on either iodide accumulation or organic iodine formation by marine phytoplankton. However, there are extensive studies about

the accumulation of iodine by macroalgae especially in the brown algae and red algae. According to Vinogradov (1953), iodine in algae can occur in different compounds such as iodide salts, organic compounds, and free iodine. Meguro et al. (1967) suggested that approximately 85% of reserved iodine in kelp appears in the form of iodide. In the case of *A. carterae*, the majority of iodide produced by the reduction of iodate may accumulate intracellularly. On the other hand, the discrepancy of iodate influx and iodide efflux in this dinoflagellate may suggest the formation of other form(s) of iodine. This iodine species may occur as the result of metabolic incorporation of iodide, since Scott (1954) as well as Tong and Chaikoff (1955) reported that radiolabelled iodide was incorporated into iodotyrosine by macroalgae. Another possibility is the intracellular production of volatile organic iodine such as methyl iodide that had been reported in phytoplankton by Harvey (Chameides and Davis, 1980) and Manley and Milligen (1991).

Because the production of iodide is species specific, spatial and temporal distributions of phytoplankton may affect the speciation of iodine in the ocean. For example, blooms of dinoflagellates, like *A. carterae*, may be responsible for the low concentration of iodate in coastal environments. The reduction products from these blooms may exist in the form of either iodide or organic iodine in coastal waters. In this case, seasonal variations in iodine speciation should be observed. Jickells *et al.*(1988)

reported the seasonal changes in the speciation of dissolved iodine in waters around Bermuda and in the Sargasso Sea and suggested these variations were partly due to a biological mechanism. The presence of organic iodine in the surface water of Chesapeake Bay as observed by Luther *et al.*(1991) is suspected to be controlled by the biological as well as photochemical processes.

3.4.2 Comparison with previous studies

The role of phytoplankton in iodate reduction is confirmed by our study. However, this result is in contrast to previously published studies. Using radiolabelled iodine, Sugawara and Terada (1967) found that iodate uptake by the marine diatom *Navicula* was relatively small in comparison to iodide uptake. They also reported that high concentrations of iodate have an adverse effect on this organism. We did not observe this in our experiments. Because of the differences in experimental organisms as well as experimental conditions, it is difficult to compare their result with ours. In our experiment, medium was made from deep seawater that contained approximately 22 nM of iodide and 359 nM of iodate while the medium in their experiments used surface water with ambient concentrations of 130 nM and 250 nM of iodide and iodate, respectively. In this case, the uptake of iodine may depend on the ratio concentrations of available iodine species. Also the amount of nitrate added in our medium (88 μM) was less than the amount used in

their experiment (124 μM). High nitrate concentration may affect the transformation of iodine since Tsunogai and Sase (1969) found that the uptake of radioactive iodate by the diatom *Navicula* was suppressed by nitrate.

In the studies by Truesdale (1978), phytoplankton belonging to three different taxa: four chlorophytes, a diatom, and a chrysophyte, showed insignificant interconversion between iodate and iodide. Experimental detail is lacking in this paper and it is impractical to compare his results to my results.

Butler et al. (1981) reported the decrease in iodate concentration from two of the three cultures of *S. costatum* after 6 days of incubation. They suggested that this iodate loss was caused by bacterial activity. However, no information on bacteria monitoring was reported in that work. In comparison to our study, their culture medium contained a higher nitrate concentration(about 883 μM) than the natural level. This high nitrate may have competed with iodate (Tsunogai and Sase, 1969) for the transport as well as reduction site and suppressed the transformation of iodate. After the exhaustion of nitrate (day 6), the reduction of iodate may resume. Also, there is the possibility of bacterial removal of iodate since phytoplankton should approach stationary phase by that time. Unfortunately, the authors did not provide data about the biomass or growth of phytoplankton to support this possibility.

3.4.3 Role of phytoplankton in iodine speciation

I propose here that phytoplankton may act as a biological mediator of iodine speciation in the ocean. In seawater, dissolved inorganic iodine in form of iodate may be transformed by phytoplankton and released as iodide. This iodide may accumulate in plankton cells or undergo further biochemical processes. Most iodide in the cell may be released extracellularly during cell lysis. If we consider *Synechococcus* sp. as representative of phytoplankton in the photic zone of the ocean in an area such as Sargasso Sea, the rate of iodate removal, 0.32 $nM \cdot \mu g\ Chla^{-1} \cdot\ day^{-1}$, is almost equal to the iodide production rate of 0.31 $nM \cdot \mu g\ Chla^{-1} \cdot\ day^{-1}$ from our experiment. The mean chlorophyll *a* in this region is 0.23 $\mu g\ Chla \cdot L^{-1}$ (Jickells *et al.*,1988). Then, the iodate transformation rate would be 0.07 $nM \cdot day^{-1}$ which is equal to the production of iodide. Taking into account the pool of iodate of approximately 0.45 μM in the deep oceanic waters (Wong and Brewer, 1974; Elderfield and Truesdale, 1980) and 0.33 μM in the Sargasso Sea (Jickells *et al.*, 1988), the time for 0.45 μM of iodate to be transformed until the concentration reaches 0.33 μM in surface waters would be $\{(0.45 - 0.33)\mu M\}/(0.07*10^{-3}\ \mu M/d)$ which is equal to 4.7 years. In the same way, the time required for the accumulation of iodide in seawater from 0 μM in deep seawater (Wong *et al.*, 1985) to 0.1 μM in surface waters (Wong, 1977; and Luther *et al.*, 1988) would be about 4 years. These time periods are very short in comparison to

an average residence time of surface water which is about 241 years in the North Atlantic Ocean (Pickard and Emery, 1988). Thus, the result implies the important role of phytoplankton in iodine speciation by the transformation of iodate to iodide.

Although the rates of iodate removal and iodide production by the diatom, *S. costatum*, are low among the phytoplankton tested, the high abundance of this species during the bloom can cause the dramatic depletion of iodate in the coastal waters. For a bloom with an average chlorophyll content of 11.4 μg Chl*a*·L^{-1} (Eppley *et al.*, 1977) and iodate removal rate of 0.10 nM·μg Chl*a*$^{-1}$ ·day^{-1}, the diatom bloom can take up iodate at the rate of 1.10 nM·day^{-1}. In the same fashion, the calculated iodide production by the bloom would be 0.31 nM·day^{-1}. In the case of a dinoflagellate bloom (assuming that the bloom species take up iodate at the same rate as *A. carterae*), the calculated iodate removal rate will be 10.64 nM·day^{-1}. Therefore, during a spring bloom of diatoms or dinoflagellates an average iodate removal rate would be 5.87 nM·day^{-1}. The average amount of iodate is 0.06 μM for the inner shelf as reported by Wong and Zhang(1992a) and the average concentration of iodate in oceanic waters is 0.33 μM (Jickells *et al.*, 1988). If we assume that iodate in the coastal areas are transported from the oceans, the time required to remove iodate to the concentration existing in the coastal waters would be about 46 days which is in good

agreement with the residence time of shelf water in the middle Atlantic Bight which ranges from 50 to 350 days (Mountain, 1991). The result also indicates the importance of iodate transformation by phytoplankton in the coastal region especially during the spring phytoplankton blooms.

3.5 Conclusion

In summary, all six species of phytoplankton that representing different taxa have the ability to take up and reduce iodate. The reduction product, iodide, is released to the surrounding medium. The effect of bacteria in the culture on the iodate transformation is negligible. The removal rate of iodate as well as the production rate of iodide by phytoplankton is species specific. Phytoplankton may act as an important mediator in the speciation of iodine through the transformation of iodate to iodide in both coastal and oceanic environments. The calculated turn over time of iodate in the coastal waters is in the order of months while in the open ocean it is a matter of years. There is also the possibility that iodate transformed by phytoplankton may act as a substrate for the formation of both volatile and non-volatile organic iodine in the marine environment.

CHAPTER FOUR

PATTERN OF IODATE UPTAKE IN THE DIATOM *Skeletonema costatum*

4.1 Introduction

Studies on the distribution and speciation of iodine in seawater have been conducted since the first detection of iodine in Baltic Water in 1825 (Wong, 1991). The distribution of iodate, the most thermodynamically stable and the most abundant form of iodine, in South Atlantic waters showed nutrient-like behavior and might be under biological mediation (Wong and Brewer, 1974). Later, many investigations on the distribution of iodate, iodide and total iodine also revealed the possibility of biological influence on iodine speciation in marine environments (Elderfield and Truesdale, 1980, Jickells *et al.*, 1988, and Luther and Cole, 1988).

Besides the chemical control of the speciation of iodine, the biological control by marine flora and micro-organisms was expected since iodine was concentrated in marine organisms especially macroalgae (Virnogradov, 1953). Also, micro-organisms such as diatom and nitrate-reducing bacteria were reported to be able to mediate the interconversion of dissolved inorganic iodine species in laboratories (Sugawara and Terada, 1967, Tsunogai and Sase, 1969, and Butler *et al.*, 1981). Rebello *et al.* (1990)

hypothesized that phytoplankton can assimilate both iodate and iodide at different times of the day.

Our previous studies showed that cultures of marine phytoplankton removed iodate from the media and released iodide (Udomkit et al., in preparation). Using cultures of the marine diatom *Skeletonema costatum,* we demonstrated here that the uptake of iodate takes place during the exponential phase while the release of iodide occurs in the senescent phase.

4.2 Methods and Materials

Skeletonema costatum Cleve (clone SKEL) was acclimated in $f_{/20}$ enriched media made from artificial seawater (Morel *et al.*, 1979). The culture was maintained at 20°C with 12:12 light:dark cycle provided by soft white fluorescent light at 70 $\mu E \cdot m^{-2} \cdot s^{-1}$ for four days. Inoculums from this culture was transferred to fresh media with 300 nM iodate addition. The experiment was carried out under the same environment as mentioned above.

Routine sampling was conducted on day 0, 1, 2, 3, 4, and 9. *In vivo* fluorescence was measured from replicate samples and the same aliquots were preserved for further cell counting. The analyses of chlorophyll *a*, iodate and iodide were performed as described in chapter two. Changes in the concentration of iodate and iodide were calculated

from the difference in concentration divided by an average amount of chlorophyll over the same time period.

Photosynthetic rate was measured by the uptake of radioactive carbonate ,$^{14}CO_3^{2-}$, (Parsons *et al.*, 1984). The amount of 2.5 μCi of $^{14}CO_3^{2-}$ was added to a certain amount of culture and incubated for 3 hours. Phytoplankton cells were filtered onto a 0.45 μ Millipore membrane filter. The radioactivity from fixed carbon in the cells was then measured with a Packard Liquid Scintillation Counter model 460C with 90% efficiency. The photosynthetic rate (mg C·m^{-3}·hr^{-1}) was calculated after the subtraction of the dark carbon fixation. The chlorophyll-specific photosynthetic rate (P_{chl}, mg C·mg chl*a*$^{-1}$·d^{-1}) was also determined for each sampling day.

4.3 Results

The abundance of *S. costatum* increased exponentially during the first four days of the experiment with the maximum yield of $43.5*10^5$ cells·ml^{-1} on day 4 (Fig.4.1). Growth rate during log phase was 0.64±0.06 d^{-1}. Chlorophyll *a* (Fig.4.2) showed the same pattern but the maximum chlorophyll content of 41.84±5.578 μg chl*a*·L^{-1} (or mg chl*a*·m^{-3}) occurred on day 3. The amounts of phaeo-pigments which are chlorophyll degradation products were also presented in the same figure. Phaeo-pigments increased

Fig. 4.1 Cell density of *S. costatum* in media with 300 nM iodate addition during 9 days of incubation. (Data presented here is an average value from replicate samples with standard error)

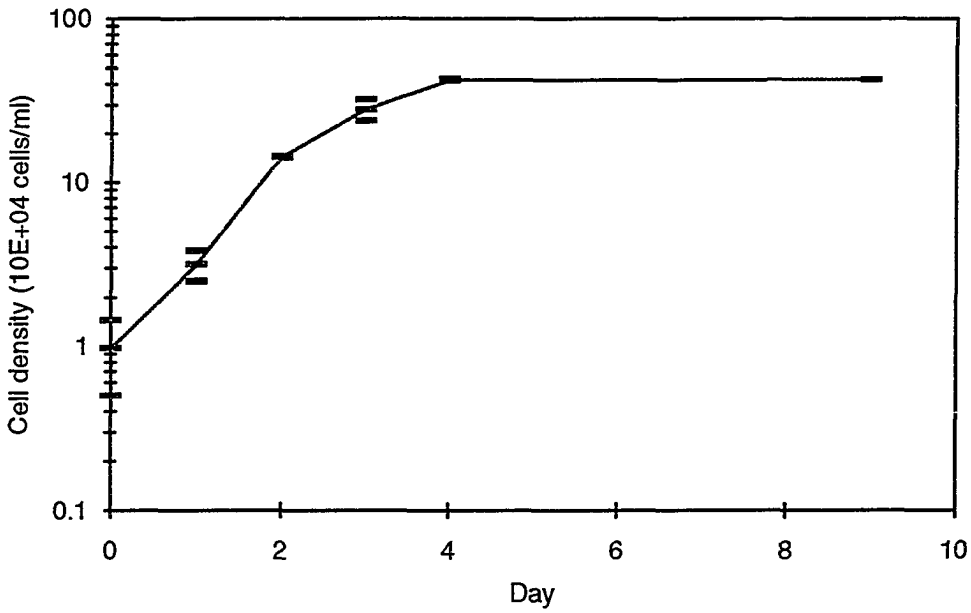
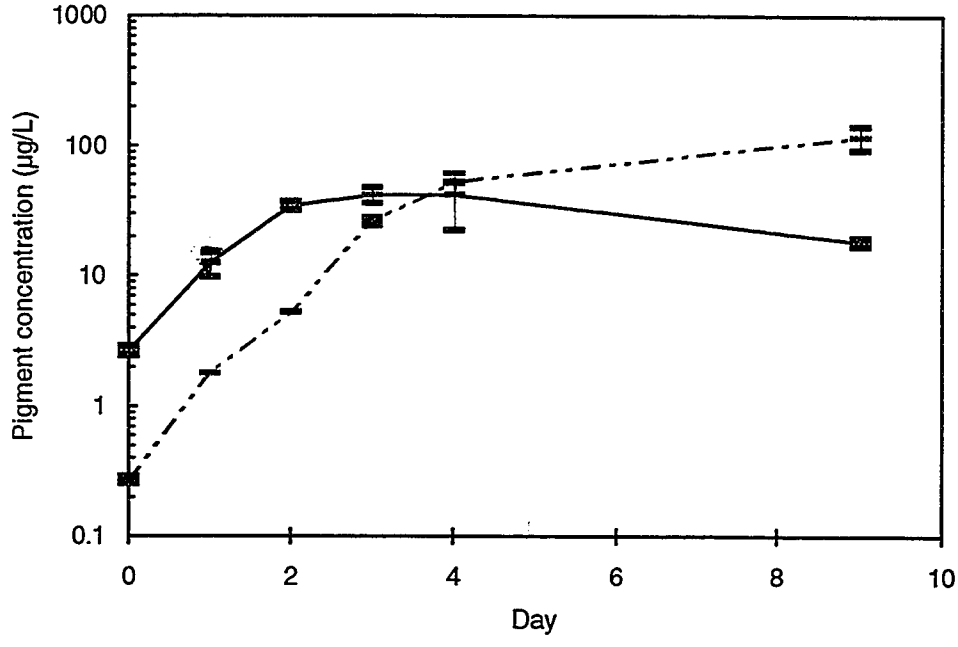


Fig. 4.2 Concentrations of chlorophyll *a* and phaeo-pigments from cultures of *S. costatum* with 300 nM iodate addition during 9 days of incubation. (—— concentration of chlorophyll *a* and -·-· concentration of phaeo-pigments. Data were presented as an average value with standard error).



progressively during the experiment and the highest concentration was found in the stationary phase which indicated the lysis of phytoplankton cells.

Average photosynthetic rates increased from 151 ± 7.20 mg $C \cdot m^{-3} \cdot d^{-1}$ at the beginning, to 3110 ± 220 mg $C \cdot m^{-3} \cdot d^{-1}$ toward the end of the experiment (Fig.4.3). The chlorophyll-specific photosynthetic rate (P_{chl}) by *S. costatum* is shown in Fig.4.4. The results indicate the gradual increase in carbon fixation rate throughout the experiment.

Changes in iodate and iodide concentrations were displayed in Fig.4.5. Iodate concentration decreased rapidly during exponential phase while iodide accumulation started at day 3 of incubation. The variation in iodide concentration during the first three days was insignificant.

Rates of iodate uptake and iodide production were calculated for each day by dividing the change in iodine concentrations with an average value of chlorophyll during the same time period. However, the rates on day 9 were calculated using the maximum value of chlorophyll *a* on day 4, which represented the biomass of phytoplankton at the beginning of stationary phase, instead of the average value between day 4 and day 9. Iodate uptake rates ranged from 0.08 to 1.09 $nM \cdot mg\ chla^{-1} \cdot d^{-1}$ (Fig.4.6). Production of iodide was negligible for the first two days. The production rates started from 0.12 $nM \cdot mg\ chla^{-1} \cdot d^{-1}$ on day 3 to 0.33 $nM \cdot mg\ chla^{-1} \cdot d^{-1}$ on the next day then dropped to

Fig. 4.3 Rate of photosynethesis (as measured by radioactive carbon fixation) by *S. costatum* in artificial $f_{/20}$ media with 300 nM iodate addition (Data was presented as an average value with standard error).

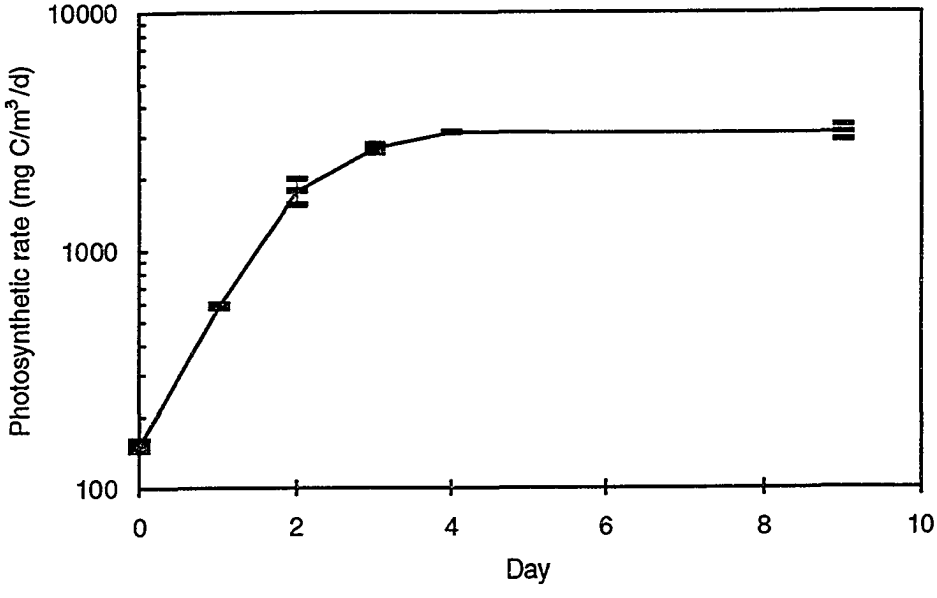


Fig. 4.4 Chlorophyll-specific photosynthetic rate (P_{chl}) in iodate added culture of *S. costatum* (Data presented was an average value with standard error).

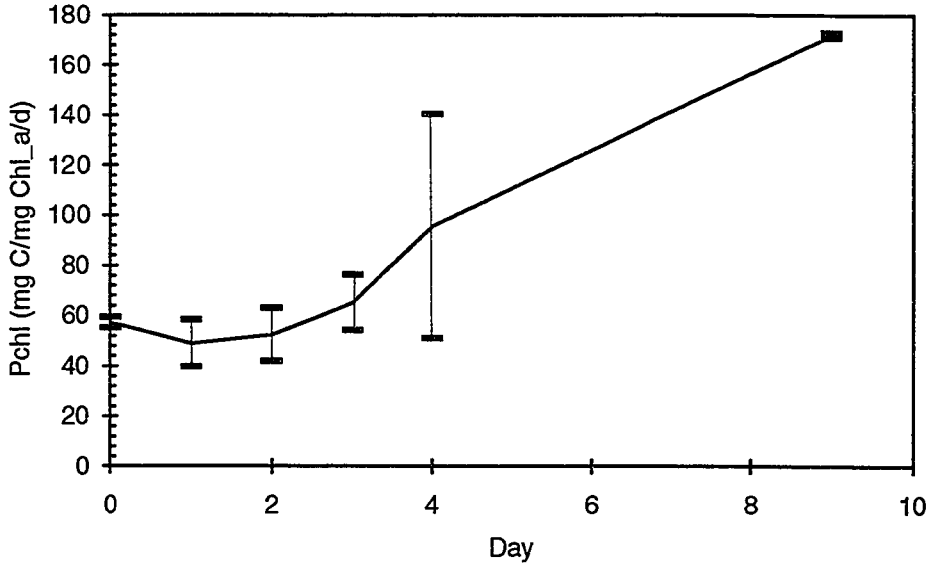
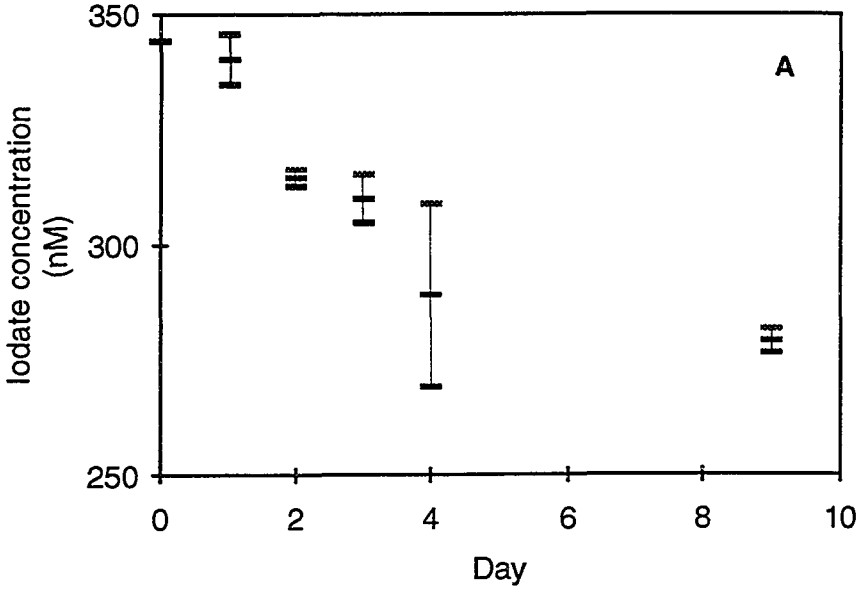


Fig. 4.5 Changes in concentrations of iodate and iodide in iodate added cultures of *S. costatum* during 9 days incubation (Data presented was an average value with standard error).

A. Concentration of iodate

B. Concentration of iodide



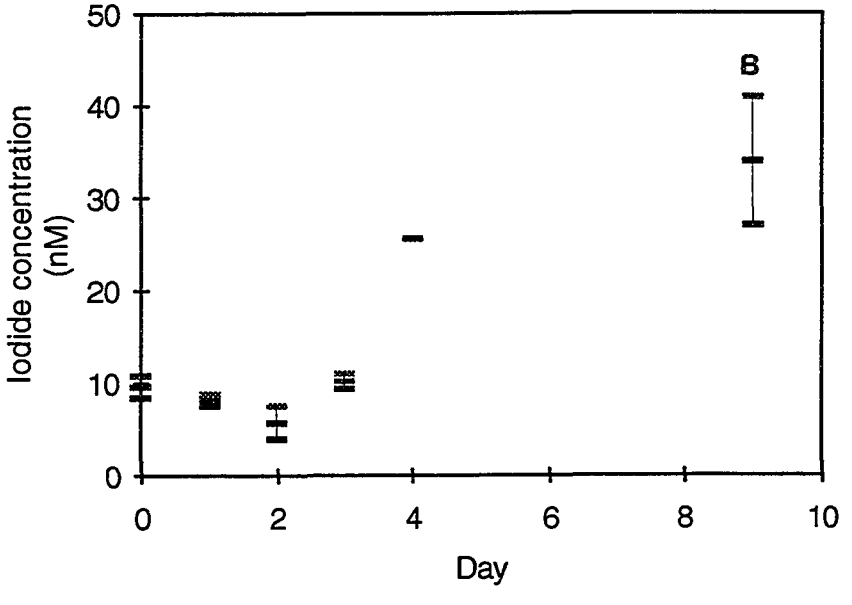
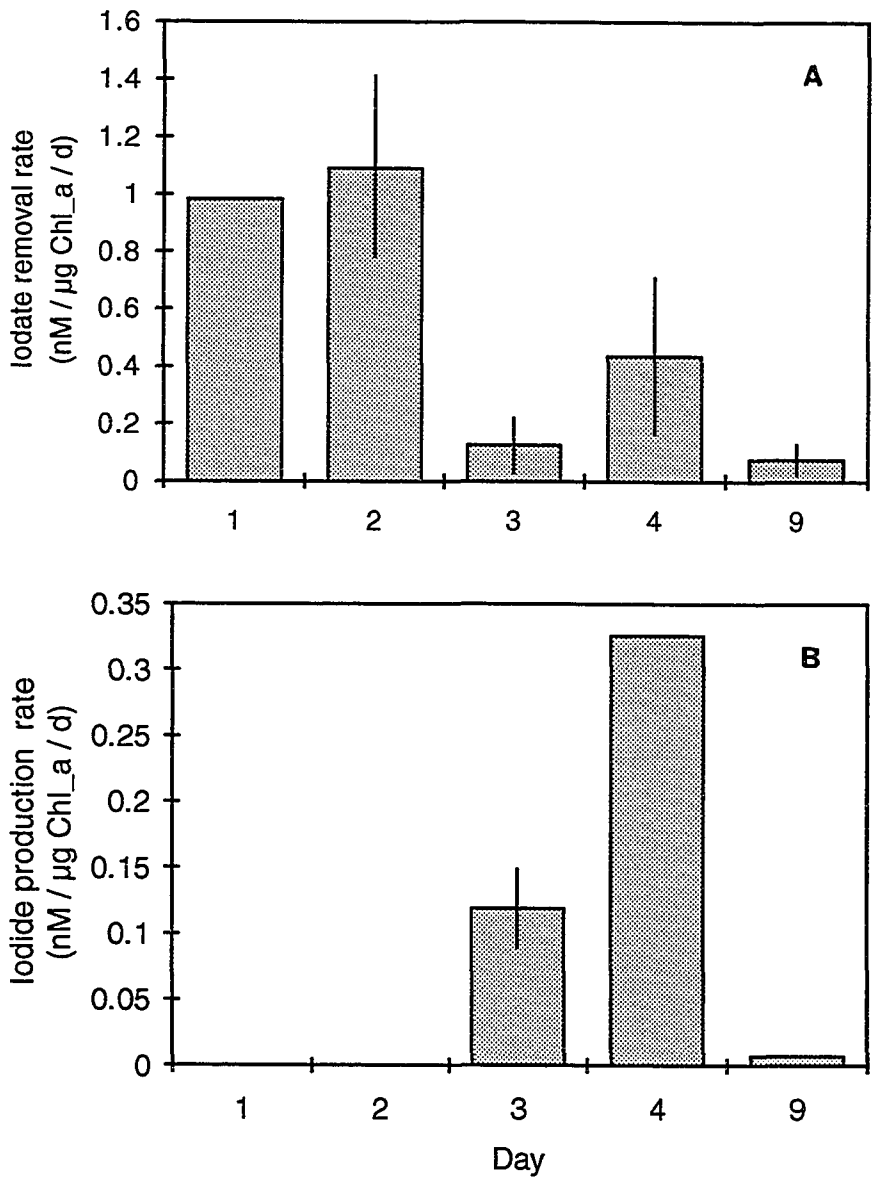


Fig. 4.6 Rate of changes in iodate and iodide concentrations in iodate-added cultures of *S. costatum* .

A. Iodate uptake rate.

B. Iodide production rate.



less than 0.01 nM·mg $chla^{-1}\cdot d^{-1}$ toward the end of the experiment. The results (Fig.4.6) indicated that during exponential phase *S. costatum* took up iodate at a rate greater than in the later stage. On the contrary, iodide production was higher when the culture approached stationary phase than it was in log phase.

4.4 Discussion

The Pearson product moment correlation coefficients among iodine species and the parameters representing phytoplankton growth were determined (Table 4.1). Iodate concentrations decreased significantly ($P < 0.050$) with the increase in cell density, photosynthetic rate, and the amount of phaeo-pigments. The highest significant figure was found between the amount of iodate and the abundance of phytoplankton and the relationship was expressed as :

$$\text{Iodate(nM)} = 342.899 - 1.366E\text{-}4 * \text{cell density(cells}\cdot\text{ml}^{-1})$$
where $R^2 = 0.976$, P value < 0.001, and $N = 6$.

On the other hand, iodide concentration showed positive correlation with the amount of phaeo-pigments and cell density. The high significant correlation between iodide and phaeo-pigments was represented by :

$$\text{Iodide(nM)} = 7.438 + 0.239 * \text{Phaeo-pigments}(\mu g\cdot L^{-1})$$
where $R^2 = 0.908$, P value $= 0.003$, and $N = 6$.

Table 4.1. Relationship between the concentrations of dissolved inorganic iodine (iodate and iodide) and the growth parameters from culture of *S. costatum*. (* indicates statistically significant values).

Iodine species	Growth parameter	Correlation coefficient	P value
Iodate (nM)	Cell density ($cell \cdot l^{-1}$)	-0.976	< 0.001 *
	Chlorophyll *a* ($mg \cdot m^{-3}$)	-0.575	0.233
	Phaeo-pigments ($mg \cdot m^{-3}$)	-0.873	0.023 *
	Photosynthetic rate ($mg\ C \cdot m^{-3} \cdot hr^{-1}$)	-0.962	0.002 *
Iodide (nM)	Cell density ($cell \cdot l^{-1}$)	0.838	0.037 *
	Chlorophyll *a* ($mg \cdot m^{-3}$)	0.092	0.862
	Phaeo-pigments ($mg \cdot m^{-3}$)	0.953	0.003 *
	Photosynthetic rate ($mg\ C \cdot m^{-3} \cdot hr^{-1}$)	0.701	0.120

The correlation coefficient(R) and P value between iodate and iodide were -0.832 and 0.040, respectively. This indicated that as iodate in the culture media decreased, iodide in the same sample tended to increase significantly.

A highly significant inverse relationship between iodate and the growth parameters, cell density and photosynthetic rate, suggested that iodate was removed from the media during the growth of phytoplankton. The positive relationship between concentration of iodide and cell number as well as photosynthetic rate indicated the accumulation of iodide in the culture media. The high significant correlation between iodide concentrations and phaeo-pigments, which are the degradation products from phytoplankton, implied that iodide was released from degraded phytoplankton cells. The removal rate of iodate by *S. costatum* on day 3 in this experiment was 0.13 nM·μg chl $a^{-1}\cdot d^{-1}$ which was in the same order as the rate reported from the previous experiment, 0.10 nM·μg chl $a^{-1}\cdot d^{-1}$, in the same species. However, the iodide production rate from this experiment was higher than that from the previous experiment. The difference in the production rate may be due to the growth status of *S. costatum*. The culture media in the previous experiment was more enriched with nutrients, except nitrate, than the media in this experiment. This high concentration of nutrients especially of silicate, might prolong phytoplankton growth, thus the production of

iodide occurred at the slower rate than when this diatom was grown in media with less nutrients.

Assuming that the iodate depleted from the media entered phytoplankton cells and was reduced to iodide which was later released extracellularly, the ratio I:C was calculated from the rate of total iodine (iodate plus iodide) depleted from the media divided by the average P_{chl} on the same day. An average I:C ratio in exponential phase and stationary phase was $6.5*10^{-3}$ and $1.3*10^{-4}$, respectively. The I:C ratio in the stationary phase is comparable to the ratio in natural phytoplankton. This stationary phase I:C ratio was in the same range of those ratios reported from the hydrographic data ($1.0*10^{-4}$) and plankton composition ($1.4*10^{-4}$) by Elderfield and Truesdale (1980). Thus, iodate may possibly enter phytoplankton cells via the uptake from the culture media. The high I:C ratio during exponential phase indicated the accumulation of iodine by phytoplankton during active growth phase where *S. costatum* exhibited high vegetative growth and reproduction. This may be comparable to high iodine requirement during spore formation and vegetative growth in brown algae, *Ectocarpus siliculous* observed by Wooley and Lewin (1973).

4.5 Conclusion

In summary, we demonstrated that a marine phytoplankton *S. costatum* took up iodate from the culture media during exponential growth phase. This iodate was reduced to iodide intracellularly. Phytoplankton may accumulate iodine during the active growth phase. In senescent phase, storage iodine is released extracellularly as iodide. The rate of iodate removal from this experiment ranged from 0.08 to 1.09 $nM \cdot mg\ chla^{-1} \cdot d^{-1}$, while the production rate of iodide ranged from 0.12 to 0.33 $nM \cdot mg\ chla^{-1} \cdot d^{-1}$. This result indicates that phytoplankton can mediate the transformation of iodine species, hence, implies the importance of phytoplankton on the speciation of iodine in the marine environment.

CHAPTER FIVE

INFLUENCES OF NITROGEN SOURCES ON THE TRANSFORMATION OF IODATE BY *Skeletonema costatum*

5.1 Introduction

Iodine in marine environments exists predominantly in dissolved forms, iodate and iodide (Winkler, 1916). Studies on the distribution of iodine in the world's oceans reveal that high concentrations of iodine can be expected in areas of high biological productivity (Tsunogai and Sase, 1969, and Tsunogai and Henmi, 1971). Elderfield and Truesdale (1980) reported the depletion of iodine from surface waters and suggested a relationship between the distribution of iodine and nutrients. They concluded that the iodine cycle in the marine environment was under biological control. The uptake of radioactive iodide and iodate was observed in a cultured diatom *Navicula* sp. (Sugawara and Terada, 1967). Later, Tsunaogai and Sase (1969) demonstrated that nitrate reducing bacteria can reduce iodate and suggested the mechanism of iodate reduction by the enzyme nitrate reductase. However, there has been no direct evidence of the uptake of iodate by marine phytoplankton.

In order to determine the influence of nitrogen sources on the transformation of iodate by phytoplankton, batch cultures of the diatom *Skeletonema costatum* were grown in both nitrate and ammonium-enriched artificial media with the addition of 300 nM iodate. In the media prepared with nitrate nitrogen, the diatom was capable of taking up significant amounts of iodate and reducing this iodate to iodide which was later released. The presence of ammonium ions inhibited the removal of iodate by this phytoplankton.

5.2 Methods and Materials

In order to acclimatize the algae for an experiment, stock cultures of *Skeletonema costatum* Greville (clone SKEL) were inoculated into two sets of $f_{/10}$ media made from surface seawater enriched with either nitrate nitrogen or ammonia nitrogen. These media contained approximately 177 μM of ammonium-nitrogen or nitrate-nitrogen. After 4 days of incubation, the cultures were transferred into media made from artificial seawater (Morel et al., 1979) with the enriched nutrient level of $f_{/20}$. The nitrogen sources (about 88 μM) were the same as in the previous stocks. These cultures were incubated for another 4 days. Then, an aliquot of 5 ml. from each working stock culture was transferred to 100 ml of fresh $f_{/20}$ media with 0.300 μM iodate addition in 125 ml sterile Erlenmeyer flasks. These media were also prepared from artificial seawater enriched

with either nitrate or ammonia. All the treatments were prepared in triplicate.

In vivo fluorescence was read daily to monitor growth in order to determine the best sampling periods which turned out to be at the beginning of the experiment as well as on day 2, 3, 4, 7, 8, and 9. An aliquot of 5 ml was sampled for *in vivo* fluorescence, then preserved in Lugol's solution for the estimation of cell density. Samples for the determination of chlorophyll *a* as well as iodate and iodide were collected after the fluorescent measurement. The sampling procedures, preservation of the samples, and the chemical analyses were mentioned previously in chapter two. One sample of filtrates was used for the determination of nitrate or ammonium nitrogen (Parson *et al.*,1984). Growth of phytoplankton was monitored by the growth rate (μ) during log phase growth,by chlorophyll *a* concentration, by *in vivo* fluorescence and by cell density.

The rates of iodate uptake, iodide production, and nutrient uptake during exponential growth phase were calculated by dividing the difference in concentration during the desired period with either an average chlorophyll content or cell density. The rates in stationary phase were retrieved by dividing the change in concentration by the maximum chlorophyll or the average cell density during stationary phase. These rates were reported with 99% confidence interval.

5.3 Results

5.3.1 Growth of *S. costatum* in NO_3^- vs. NH_4^+ - enriched media

S. costatum grew in NO_3^- as well as in NH_4^+ enriched media. *In vivo* fluorescence and cell density in both cultures were similar (Fig.5.1 and Fig.5.2). *S. costatum* demonstrated exponential growth for 3 days after the inoculation before approaching stationary phase. Maximum cell density was approximately 4.5 $*10^5$ cells$\cdot ml^{-1}$ in both cases. Growth rate (μ) calculated from *in vivo* fluorescence during exponential growth(3 days) was 0.69 per day in media enriched with nitrate while the one in ammonium-enriched media was 0.63 per day. The amounts of extracted chlorophyll *a* as well as phaeo-pigments are also shown in Fig.5.1 and Fig.5.2. Maximum chlorophyll *a* found on the third day of the experiment was 34.4±1.3 and 46.4±9.1 μ g chla$\cdot L^{-1}$ in cultures with nitrate and ammonium respectively. The concentration of phaeo-pigments increased exponentially during the first four days of incubation and reached a maximum of 62 μg phaeo-pigment$\cdot L^{-1}$.

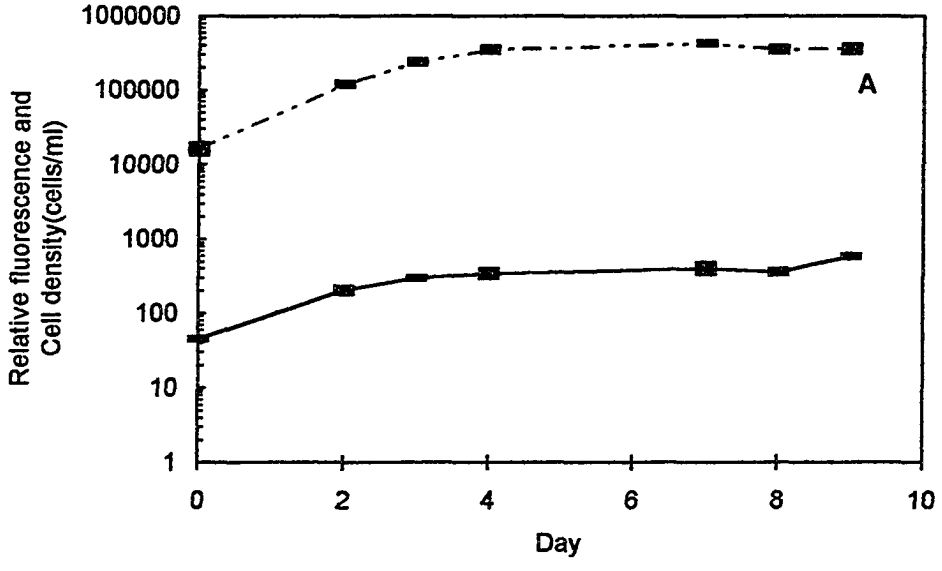
5.3.2 Nutrient and iodine concentrations in nitrate-enriched cultures of *S. costatum*

Concentrations of NO_3^- in the cultures decreased with time (Fig.5.3). The depletion of nitrate was rapid during the first four days of the experiment. At the end of the

Fig. 5.1 Growth and biomass of *S. costatum* in nitrate-enriched media with 300 nM iodate addition.

A. Average cell density (-···-) and *in vivo* fluorescence (———)

B. Chlorophyll *a* concentration (———) and phaeo-pigment concentration (-···-)



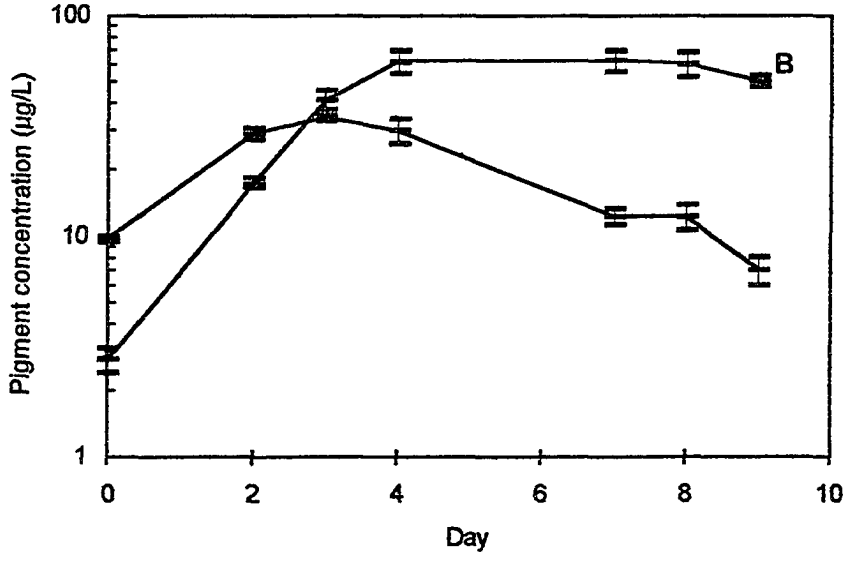
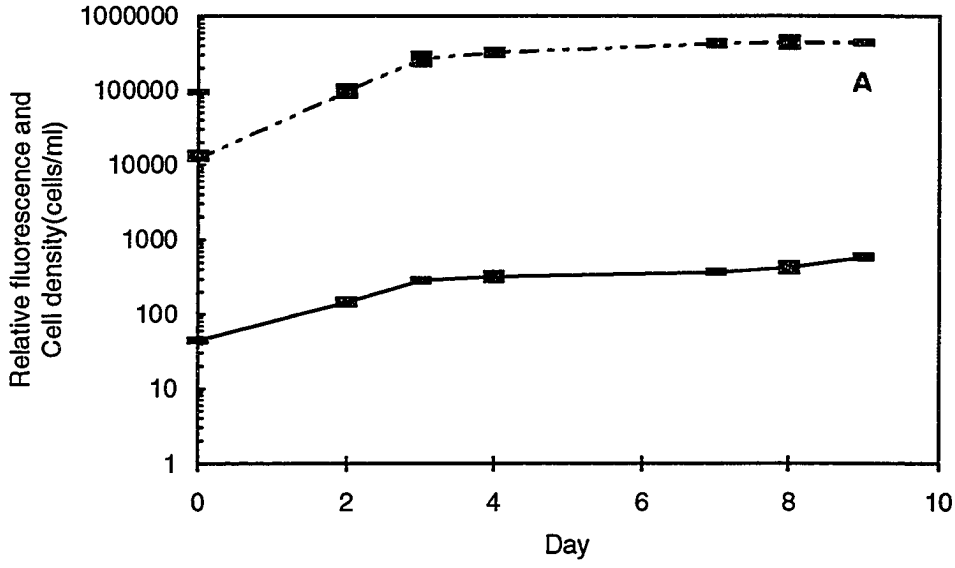


Fig. 5.2 Growth and biomass of *S. costatum* in ammonium-enriched media with 300 nM iodate addition.

A. Average cell density (-···-) and *in vivo* fluorescence (———)

B. Chlorophyll *a* concentration (———) and phaeo-pigment concentration (-···-)



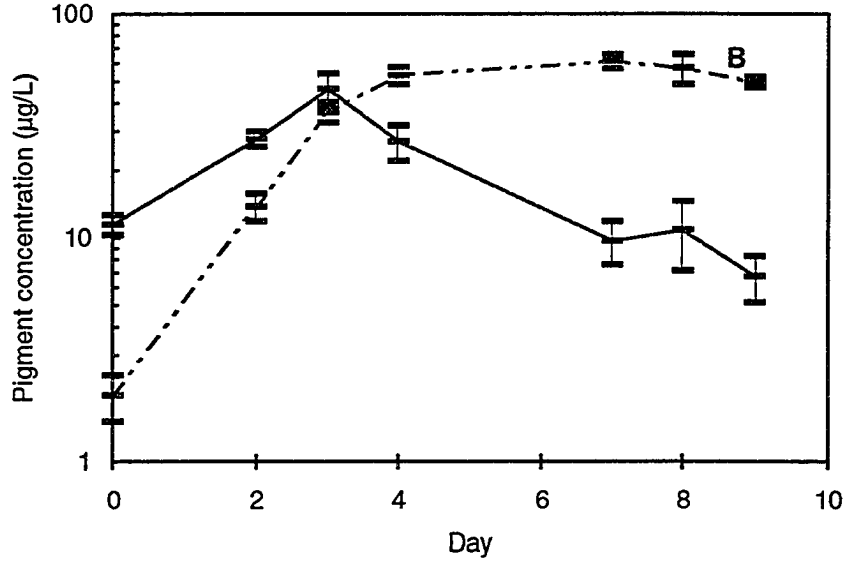
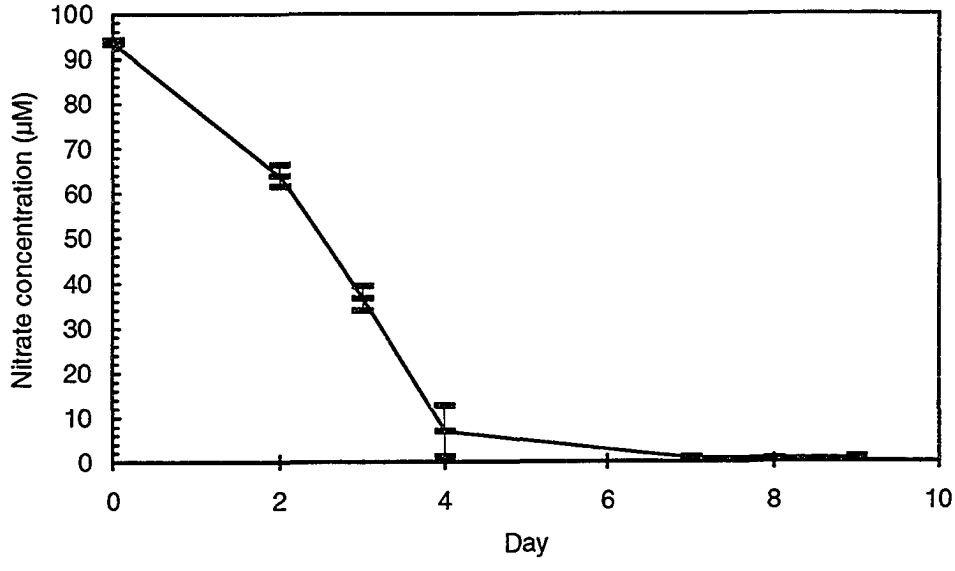


Fig. 5.3 Changes in nitrate concentration in culture media of *S. costatum* during the experiment (Data shown are average concentrations with standard error bars).



experiment(day 9), nitrate concentrations in triplicate samples were less than 1 μM. Since the chlorophyll concentration indicated that the log growth phase lasted only three days, thus the rate of nitrate uptake was calculated from the difference in nitrate concentrations for these three days. From the initial nitrate addition of 88.3 μM, nitrate concentrations diminished at an average rate of 0.78±0.08 $\mu M \cdot \mu g\ chla^{-1} \cdot d^{-1}$ during the exponential phase (3 days). After three days, the uptake was 0.17±0.03 $\mu M \cdot \mu g\ chla^{-1} \cdot d^{-1}$. The concentrations of nitrite in the same samples were less than 0.20 μM throughout the experiment.

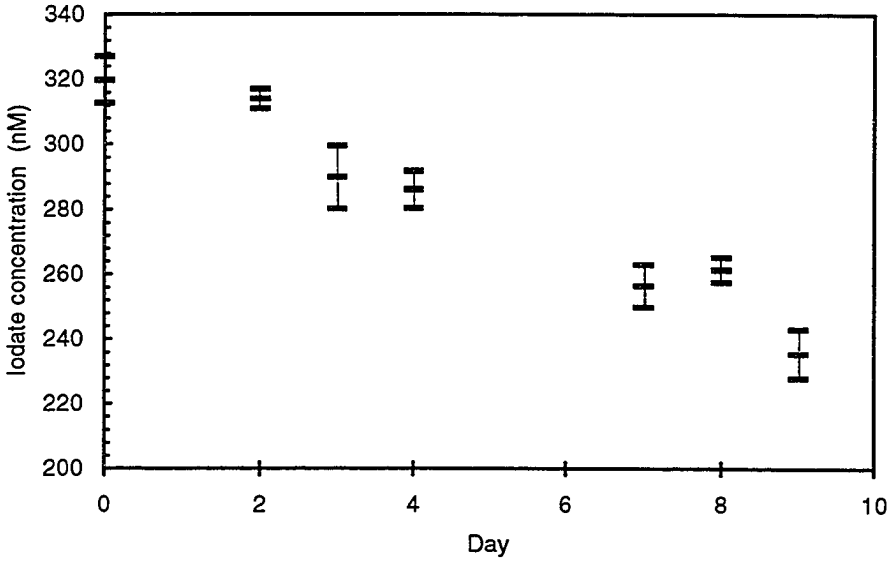
Changes in iodate and iodide concentrations in nitrate-grown cultures are shown in Fig.5.4. The background concentration of 50 nM iodate and 30 nM iodide were from the impure salts used in media preparation. An additional 300 nM of iodate was added at the beginning of the experiment. In nitrate-grown cultures, the depletion of iodate concentration was uniform throughout the experiment and was followed by increased iodide concentrations (Fig.5.4). The amount of iodate diminished from 320±72 nM to 236±75 nM by the end of the experiment. Iodide increased from 244±10 nM at the beginning to 79±6 M at the end of the experiment. Total inorganic iodine (iodate plus iodide) changed from 344 ±7 nM to 315±8 nM during the incubation period.

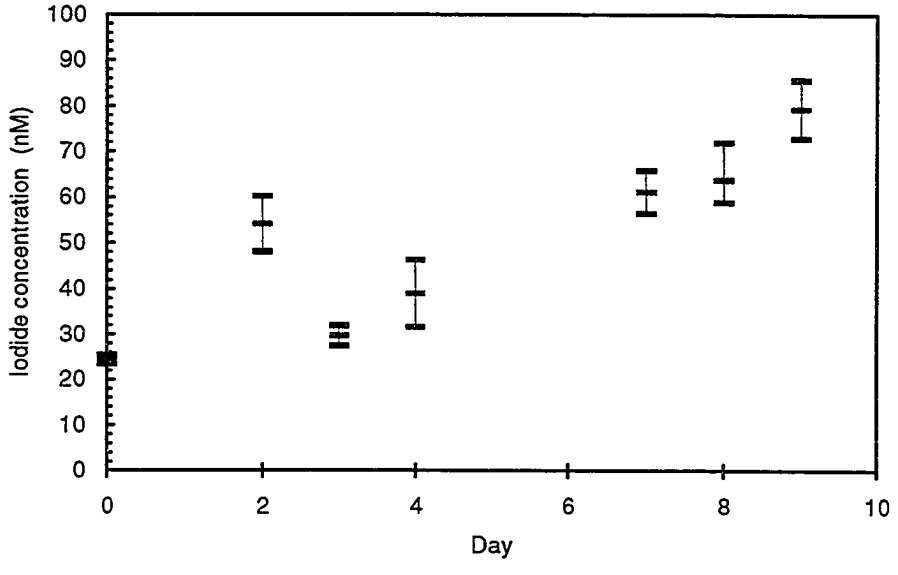
Rate of iodate disappearance was 0.57±0.27 and 0.26± 0.16 $nM \cdot \mu g\ chl\ a^{-1} \cdot d^{-1}$ during log (0-3 day) and stationary

Fig. 5.4 Variation in iodate and iodide concentrations in nitrate-enriched cultures of *S. costatum* with 300 nM iodate addition (Data shown represent average concentrations with standard error bars).

A. Iodate concentration

B. Iodide concentration





(3-9 day) phase, respectively. Iodide was produced at the rate of 0.07±0.04 nM·μg chl $a^{-1}\cdot d^{-1}$ during log phase and 0.24±0.05 nM·μg chl $a^{-1}\cdot d^{-1}$ in stationary phase.

5.3.3 Nutrient and iodine concentrations in ammonium-enriched cultures of *S. costatum*

Concentrations of ammonium-nitrogen decreased from 88.3 μM at the beginning to approximately 4 μM at the end of the experiment (Fig.5.5). An average ammonium uptake rate was 1.00±0.02 μM·μg chl$a^{-1}\cdot d^{-1}$ during the exponential phase (the first three days of incubation) and 0.03±0.03 μM·μg chl a^{-1} $\cdot d^{-1}$ in the stationary phase.

The variations in iodate and iodide concentrations shown in Fig.5.6 indicated two patterns of the concentration changes. During the first three days of the incubation, the changes in both iodate and iodide concentrations were insignificant. After day three, when the ammonium concentration was exhausted from the media, the concentration of iodate decreased while iodide concentration increased. Iodate depleted from 360±9 nM on day 3 to 316±8 nM at the end of the experiment. Concentration of iodide increased from 214±3 nM to 361±7 nM. Iodate was removed from the media at the rate of 0.15±0.12 nM·μg chl a^{-1} $\cdot d^{-1}$ while iodide was produced at 0.05±0.03 nM·μg chl a^{-1} $\cdot d^{-1}$.

Fig. 5.5 Changes in ammonium concentrations in culture media of *S. costatum* during the experiment (Data represent average concentrations with standard error bars).

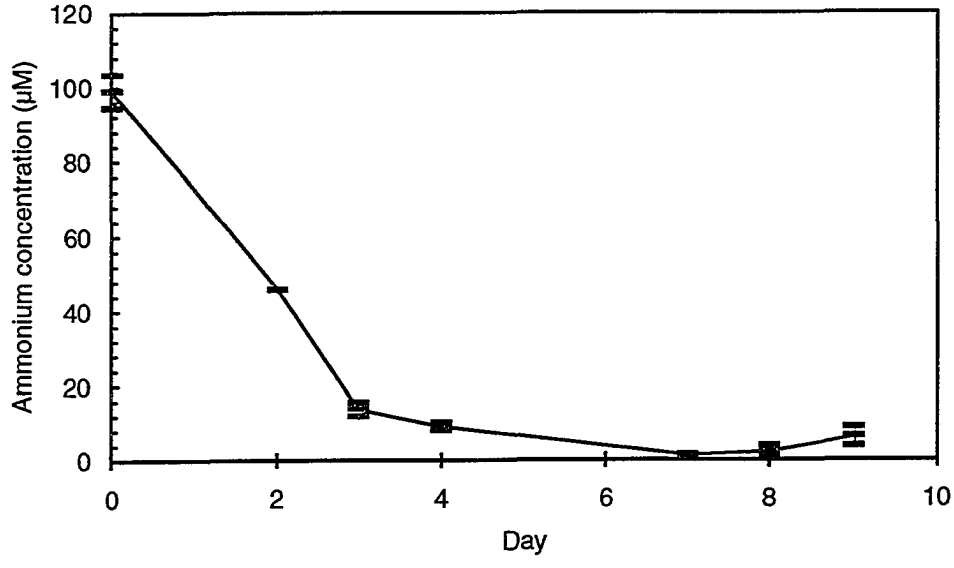
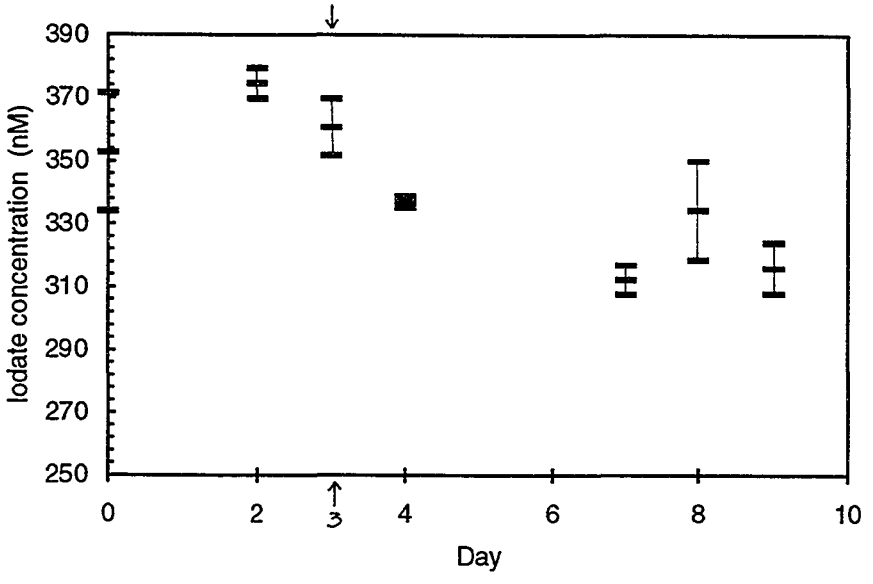
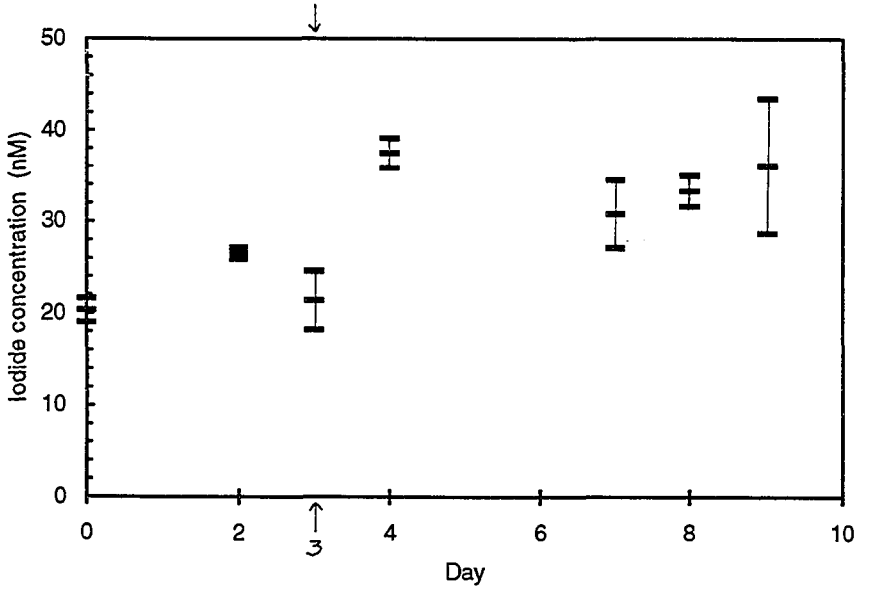


Fig. 5.6 Variation in iodate and iodide concentrations in ammonium-enriched culture of *S. costatum* with 300 nM iodate addition (Data represent average concentrations with standard error bars).

A. Iodate concentration

B. Iodide concentration





5.4 Discussion

5.4.1 Growth of *S. costatum* in NO_3^- and NH_4^+ enriched media

S. costatum showed no significant difference in growth in NO_3^- enriched media in comparison to NH_4^+ enriched media as shown by *in vivo* fluorescence, cell density and the amount of photosynthetic pigments. The amount of chlorophyll in both culture media declined earlier than cell density which is not unusual in phytoplankton culture. Growth rate of *S. costatum* in nitrate-based media is higher than that in ammonium-enriched media. This situation has also been reported in other groups of phytoplankton (Dortch, 1990).

5.4.2 Changes in nitrate and ammonium concentrations

Balch (1985) reported the K_m and V_{max} for nitrate uptake by *S. costatum* of 1.24 $\mu M \cdot cell^{-1} \cdot hr^{-1}$ and 8.5 $fM \cdot cell^{-1} \cdot hr^{-1}$. At a nitrate level of 88.3 μM, as in this experiment, the calculated nitrate uptake rate would be 0.20 $pM \cdot cell^{-1} \cdot d^{-1}$. In this experiment, the rate of nitrate uptake by the same phytoplankton was 0.78 $\mu M \cdot \mu g$ $chla^{-1} \cdot d^{-1}$ or 0.15 $nM \cdot cell^{-1} \cdot d^{-1}$ which is higher than the one calculated by Balch's uptake parameters. The concentration of nitrate in this experiment is 88.3 μM which is higher than 33 μM used in Balch's. Since the uptake rate is substrate dependent, then the difference in nitrate concentration may

affect its uptake rate. The uptake rate of ammonium is greater than that of nitrate, 1.00 $\mu M \cdot \mu g$ chl $a^{-1} \cdot d^{-1}$ or 0.23 $nM \cdot cell^{-1} \cdot d^{-1}$. This result, however, indicated that *S. costatum* in this study took up ammonium at rate faster than nitrate.

5.4.3 Variations in iodate and iodide concentration in nitrate-enriched cultures of *S. costatum*

Changes in iodate concentrations in nitrate-grown cultures show a high inverse relationship with the density of phytoplankton as well as with the *in vivo* fluorescence but not with the amount of extracted chlorophyll (Table.5.1). The variation in iodate concentration also followed the change in nitrate concentration. On the other hand, iodide exhibited positive correlation with both cell fluorescence and cell density. The concentration of iodide tended to increase with the decrease in either nitrate or iodate concentration. The decrease of iodate and subsequent increase in iodide in NO_3^- grown cultures with iodate addition indicated the conversion of iodate to iodide by *S. costatum*. Iodate was transformed to iodide by phytoplankton and iodide was subsequently released into the media. Rates of iodate disappearance of 0.57±0.28 and 0.26 ±0.16 $nM \cdot \mu g$ $chla^{-1} \cdot d^{-1}$ were in the same order of those reported in chapter four which were 0.51±0.20 and 0.12±0.01 $nM \cdot \mu g$ $chla^{-1} \cdot d^{-1}$ in log and stationary phase, respectively. Rate of iodide production during log phase of 0.07±0.04 nM·

Table 5.1 Correlation matrix of growth parameters and concentrations of nutrients and dissolved iodine in the nitrate-enriched cultures of *Skeletonema costatum* .

	Fluorescence	Cell density	Chlorophyll *a*	Phaeo-pigments	Nitrate	Nitrite	Iodate	Iodide
Fluorescence	1	0.865	-0.241	0.787	-0.895	-0.746	-0.945	0.663
Cell density		1	-0.146	0.978	-0.986	-0.924	-0.875	0.700
Chlorophyll *a*			1	-0.026	0.141	0.297	0.478	-0.494
Phaeo-pigments				1	-0.975	-0.931	-0.791	0.556
Nitrate					1	0.912	0.890	-0.640
Nitrite						1	0.833	-0.534
Iodate							1	-0.736
Iodide								1

μg $chla^{-1} \cdot d^{-1}$ was higher than that in the previous chapter (0.01±0.01 nM·μg $chla^{-1} \cdot d^{-1}$). Iodide production rates during stationary phase, 0.24±0.05 vs. 0.10±0.08 nM·μg $chla^{-1} \cdot d^{-1}$,were quite comparable in both experiments.

The ratio of nitrate uptake to iodate disappearance from this experiment was approximately 1377:1 and 670:1 for log and stationary growth phase, respectively. This result indicated the possible competitive uptake between iodate and nitrate. These ratios are higher than the nitrate:iodate molar ratio in seawater of 357:1 as reported by Wong and Brewer (1974)and the concentration ratio of nitrate:iodate in the culture media of 294:1 at the beginning of the experiment. However, the stationary phase ratio is in the same order of the molar ratio in seawater and in the media. This discrepancy may be due to the excess amount of nitrate-nitrogen in this experiment in comparison to the nitrate concentration in their report.

5.4.4 Variations in iodate and iodide concentration in ammonium-enriched cultures of *S. costatum*

The variations in both iodate and iodide concentration in the culture enriched with ammonium were insignificant during the first three days of incubation. During this period, phytoplankton took up about 86% of available ammonium and the cell number increased exponentially but the concentrations of iodate and iodide were quite uniform. From day three to the end of the experiment, the amount of

ammonium ions in the media depleted slowly and the concentration was less than 10 μM on the last day of the experiment. During this stationary phase, iodate in the media decreased about 45 nM and iodide increased about 15 nM. This result indicated that iodate removal and transformation were suppressed in the presence of ammonium ions.

Serra *et al.*(1978) and Collos *et al.*(1992) found that nitrate transport into the phytoplankton *S. costatum* involved not only the carrier-mediated transport but also the diffusion process. The presence of ammonium ions in the environment inhibits the assimilation of nitrate at the level of nitrate transport into the cell rather than the reduction process inside the cell. Ammonium ions affect the permease that is responsible for nitrate transport, thus inactivates nitrate transfer by this carrier. However, a certain amount of nitrate can diffuse into the cell. In this case, if iodate mimics nitrate behavior, iodate uptake by *S. costatum* can be inhibited by high concentration of ammonium during the first three days of incubation. This may cause a change in iodate concentration in the media containing ammonium.

5.4.5 Iodate transformation by *S. costatum*

The uptake of iodine was first reported in studies of macroalgae (Kelly and Baily 1951, Scott 1954, Tong and Chaikoff 1955, Klemperer 1957, and Svetasheva 1984).

However, the studies in marine phytoplankton were quite limited (Kelly and Baily, 1951; Sugawara and Terada 1967; Truesdale 1978; Butler *et al.*, 1981). My results revealed that maximum iodate disappearance from the culture media occurred during exponential growth. This period coincided with high vegetative growth and reproduction in *S. costatum* and was supported by Wooley and Lewin's 1978 report of high iodine requirement of the brown algae *Ectocarpus siliculous* during spore formation and vegetative growth. The fact that *S. costatum* took up iodate but excreted only iodide was similar to brown algae *Cystoseira crinita* (Svetasheva, 1984).

The total amount of iodine (iodate + iodide) in both nitrate and ammonium-enriched cultures decreased throughout the experiment. In both cases, the amount of total iodine lost was about 30 nM. There are many possible explanations for this discrepancy. First, phytoplankton may be able to store iodine intracellularly as had been reported in various red and brown seaweeds (Klemperer, 1957; Westlund *et al.*,1981; and Mairh *et al.*,1989). Iodide may be the major storage form of iodine in phytoplankton as it is in macroalgae(Meguro *et al.*,1967). Secondly, iodide once produced may act as the substrate for further biochemical processes inside the phytoplankton cell, i.e. incorporation into organic molecules in the cells, or it may react with other chemical reagents in the culture media. Organic iodine such as iodo-tyrosine has been discovered in

macroalgae (Scott, 1954; Tong and Chaikoff, 1955; and Meguro *et al.*, 1967). Another possible iodine species resulting from iodide metabolism may be the volatile halogenated organic compound, methyl iodide(CH_3I), as reported in macroalgae by Gschwind *et al.*, 1985; Manley and Dastoor, 1987; and Manley and Dastoor 1988, and in phytoplankton by Manley and Milligen (1991). Finally, iodide excreted into the medium may react with other active molecules in the ambient environment.

5.4.6 Relationship between iodate and nitrate uptake

The experimental results showed a high positive correlation between iodate and nitrate and the significant decrease in iodate concentration in nitrate-grown cultures. In addition, the presence of ammonium ions also suppresses the removal of iodate and the transformation of iodate to iodide in the same way as it affects nitrate uptake. Thus, iodate may behave in the same fashion as nitrate. The uptake of iodate by phytoplankton may mimic that of nitrate. In this case, iodate in the media may enter phytoplankton cells by two different processes. The first one is probable the diffusion process. The latter relies on the existence of nitrate and can be inhibited in the presence of ammonium. It may involve an active carrier-mediated transport as observed in the uptake of nitrate by *S. costatum* (Serra *et al.*,1978).

Once inside the cell, iodate can be reduced to iodide. The reduction of iodate may occur by the activity of the enzyme nitrate reductase(NR) since the extract of this enzyme shows the ability to reduce iodate to iodide (Tsunogai and Sase, 1969). Since the uptake of nitrate does not coincide with nitrate reductase and ammonium can repress only the nitrate carrier (Butz and Jackson, 1977; and Serra *et al.*,1978), ammonium in the media can influence the uptake process but not the activity of NR. In this case, it explains the increase of iodide produced by the reduction of iodate in the ammonium-enriched media after ammonium ions were exhausted.

5.5 Conclusion

In summary, our results revealed that under laboratory conditions, the diatom *S. costatum* transformed iodate to iodide. Iodate in the media showed a close relationship with nitrate and may mimic the behavior of nitrate. Thus, phytoplankton takes up iodate from the media and reduces it to iodide. The transport of iodate may occur by either the diffusion process or the carrier-mediated pathway. In the presence of ammonium, the active transport may be repressed and iodate can enter phytoplankton cells only when ammonium ions are depleted.

CHAPTER SIX

GENERAL DISCUSSION AND CONCLUSION

6.1 General Discussion

6.1.1 Iodate removal

These experiments show that phytoplankton of different taxonomic groups are capable of taking up iodate from the culture media. This result confirms the hypothesis that phytoplankton, the primary producer in the oceans, plays a significant role in the biogeochemistry cycle of iodine. The uptake of iodate is species specific but there is not sufficient data to demonstrate a pattern among different phytoplankton taxa. The study of the diatom *S. costatum* suggested the coupling of iodate removal and nitrate uptake by phytoplankton, and also, the inhibition of the iodate uptake in the presence of ammonium ion.

6.1.2 Reduction of iodate and production of iodide by phytoplankton

Iodate taken up by phytoplankton is reduced to iodide. The reduction or transformation of iodate may occur either inside phytoplankton cell or on the cell surface. The mechanism of this process is still unclear. Because of its chemical similarities to nitrate (Wong 1991), iodate may behave in the same fashion as nitrate. Tsunogai and Sase

(1969) showed that iodate can be reduced by the enzyme nitrate reductase extracted from nitrate reducing bacteria . Therefore, this enzyme may be responsible for the reduction of iodate in phytoplankton. The results demonstrate that the amount of iodate lost from culture media during the experiment is in close association with nitrate and the concentration of iodide shows an inverse relationship with nitrate. This implies that iodate reduction occurs in the same fashion as nitrate reduction. Then, all or some of the reducing product, iodide, is excreted extracellularly.

The production of iodide by marine phytoplankton is also species specific. Small phytoplankton, i.e. *E. huxleyi* and *Synechococcus* sp., produced more iodide than large phytoplankton. However, the amount of iodide detected in the culture media may not be equal to the gross production since phytoplankton may accumulate iodide intracellularly or iodide may be an intermediate for further biochemical processes in the cells.

6.1.3 Possible metabolism of iodide

There is a distinctive pattern of iodate uptake and iodide production among different phytoplankton species used in this study. Rates of iodide production in *Synechococcus* sp. and *E. huxleyi* are close to their iodate uptake rates. On the other hand, there are large differences between iodate uptake rates and iodide production rates in *A. carterae* and *S. costatum*. This disagreement suggests that

iodine can be stored intracellularly in some phytoplankton. The storage form of iodine is most likely to be iodide since it is the dominant form of iodine that accumulates in macroalgae (Meguro et al. 1967). Another possibility is that iodide produced from the reduction of iodate acts as a substrate for other reactions. Iodide can be incorporated to organic molecules such as iodo-tyrosine as reported in macroalgae (Scott 1954; and Tong and Chaikoff, 1955). Moreover, iodide may react with the algal metabolite, dimethyl-β-propiothetin (DMPT), to produce methyl iodide(CH_3I) as proposed by White (1982) and Brickman *et al.* (1985). This reaction is confirmed by the production of methyl iodide by phytoplankton (Manley and Milligen 1991).

The time lag between iodate uptake and iodide production in the culture of *S. costatum* (see Chapter five) also supports the possibility of both intracellular iodide accumulation and metabolism. Besides, the significant positive correlation between iodide and algal degradation pigments suggests that iodide is released when the culture approaches senescent phase.

6.1.4 Hypothetical model of iodate uptake by marine phytoplankton

Fig.6.1 is a proposed scheme of iodate transformation by phytoplankton. In this model, iodate is taken up by phytoplankton. The transformation, in this case applied to the reduction of iodate, may occur either intracellularly or

at the cell surface. Because of the close relationship between the concentrations of iodate and nitrate, the suppress of iodate removal by ammonium ions, and the ability of the enzyme nitrate reductase to reduce iodate to iodide is reasonable. It is most likely that the transformation of iodate to iodide occurs in the same fashion as nitrate reduction. In this case, iodate may be transported into phytoplankton cell. The transport process [a] consists of two pathways, diffusion and carrier-mediated processes. The uptake may be light independent as in nitrate transport (Balch, 1985). Intracellular iodate is reduced to iodide (process [b]) possibly by the enzyme nitrate reductase. There is also a possibility that the reduction of iodate may occur at the cell surface and iodide is released into the culture media. A certain amount of iodide may be accumulated intracellularly and excreted extracellularly, especially during cell lysis. I also propose in this model other products of iodate reduction. There is a possibility that extracellular iodide may react with the algal metabolites, dimethyl-β-propiothetin (dimethylsulfonium compound), to form volatile methyl iodide(iodomethane) as suggested by White (1982), Brinckman et al.(1985), and Manley and Dastoor (1987) as represent by a reaction [d]. However, this has not been quantitatively confirmed. On the other hand, intracellular iodide may be incorporated to organic compounds (as in reaction [c]) such as iodotyrosine (Scott 1954; and Tong and Chaikoff 1955; Klemperer 1957; and

Meguro et al., 1967) or lipid halogens (Hewson and Hager 1980) which has been reported in macroalgae . This organic iodine can be stored inside the cell or exuded during cell lysis. This explanation seems to fit well with the presence of organic iodine in the surface estuarine waters as reported by Truesdale(1975), Butler and Smith(1985), Jickells et al.(1988), and Luther et al.(1988).

Even though we demonstrated the uptake and reduction of iodate by marine phytoplankton, the question about the importance of iodine in phytoplankton is still unknown. Tong and Chaikoff (1955) and Klemperer (1957) assumed that iodine was necessary for growth of red and brown algae. Fries (1966); Pedens•n (1969); and Woolery and Lewin (1973) reported the demand for iodine in the growth and development of selected brown and red algae. On the contrary, our results did not show the enhanced growth of phytoplankton with iodine additions. Further investigations are necessary to understand the bio-mediated role of phytoplankton in iodine cycle as well as the importance of iodate to phytoplankton. Suggested future work on this topic should concern the verification of this laboratory discovery with the natural population of phytoplnkton in field studies. The processes that may be responsible for the transformation of iodate, and the relationship between substrates and environmental conditions on these processes need further investigation.

Fig. 6.1 A hypothetical model of iodate uptake by a phytoplankter

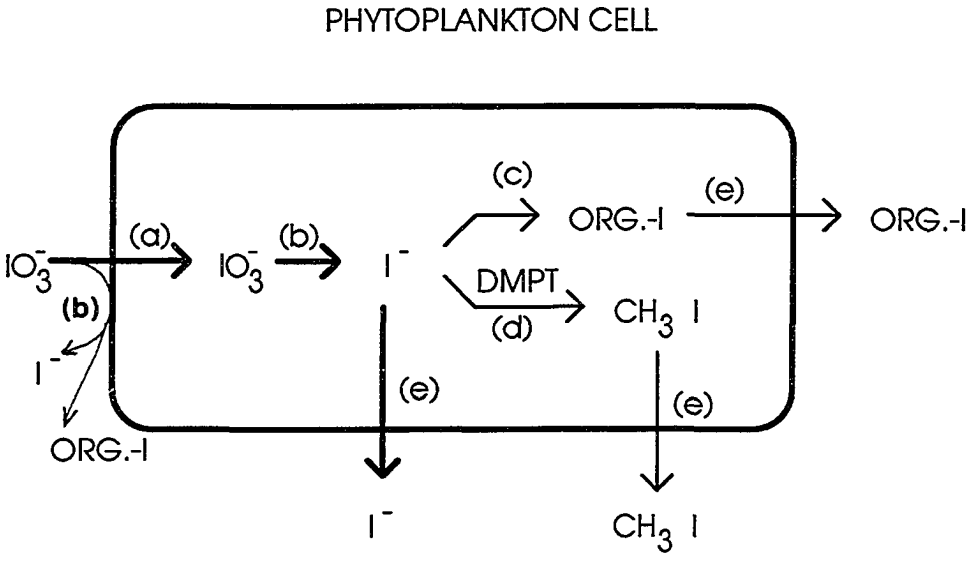
(a). Transport processes

(b). Reduction process

(c). Incorporation to macromolecules

(d). Reaction with algal metabolite

(e). Excretion during phytoplankton growth or when cell lysis.



6.2 Conclusion

In summary, my results demonstrate that marine phytoplankton are able to take up iodine in the form of iodate. This iodate is reduced to iodide which may act as an important substrate for further iodine metabolism. The transformation of iodate is species specific and occurs simultaneously with phytoplankton growth. Iodate uptake is intense during exponential growth and has a close relationship with nitrate uptake.

The uptake and reduction of iodate by phytoplankton suggests the significant role of phytoplankton in the biogeochemical cycle of iodine. Since the reduction of iodate to iodide is thermodynamically unfavorable in seawater, the bio-mediated reduction by phytoplankton will be one of the significant process that produces iodide besides the reduction by chemical agents. Iodide produced from the reduction of iodate is not only a precursor of many other chemical reactions in marine environments but also a significant species of iodine exchanges between the oceans and the atmosphere. Iodide, if being accumulated in phytoplankton, and possibly its assimilated products may be transferred through the higher trophic levels in the food chain. Thus, the transformation of iodate to iodide by phytoplankton may not only cause the existence of iodide but also affect the cycle of iodine in the environment. However, the speciation and distribution of iodide and

iodate will depend on the spatial and temporal distribution of phytoplankton in the oceans. Thus, phytoplankton play an important role in the cycle of iodine.

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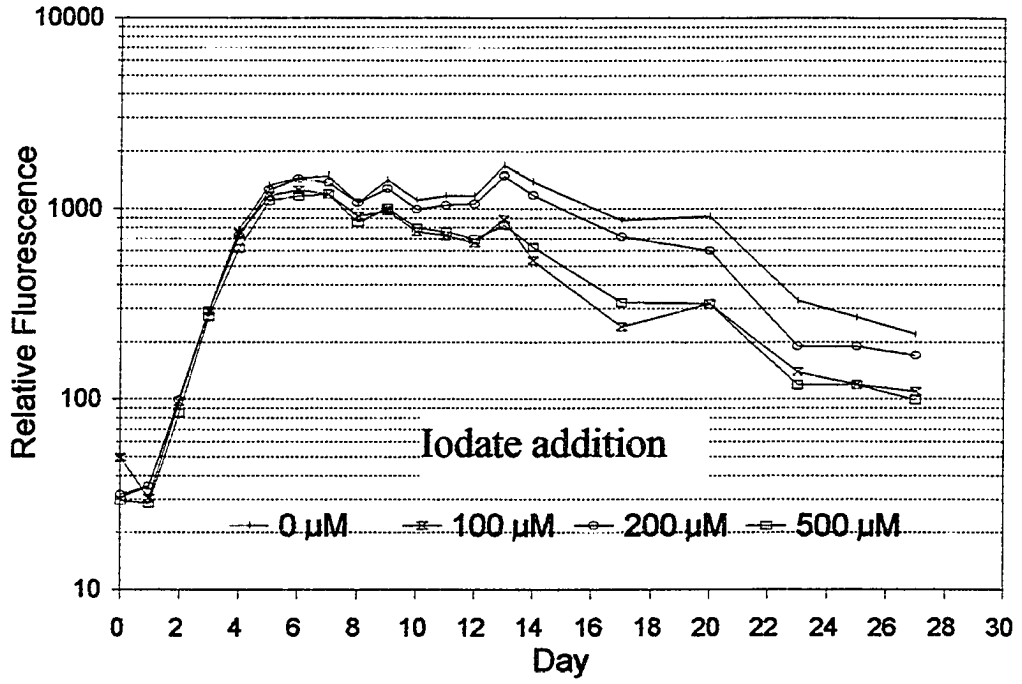
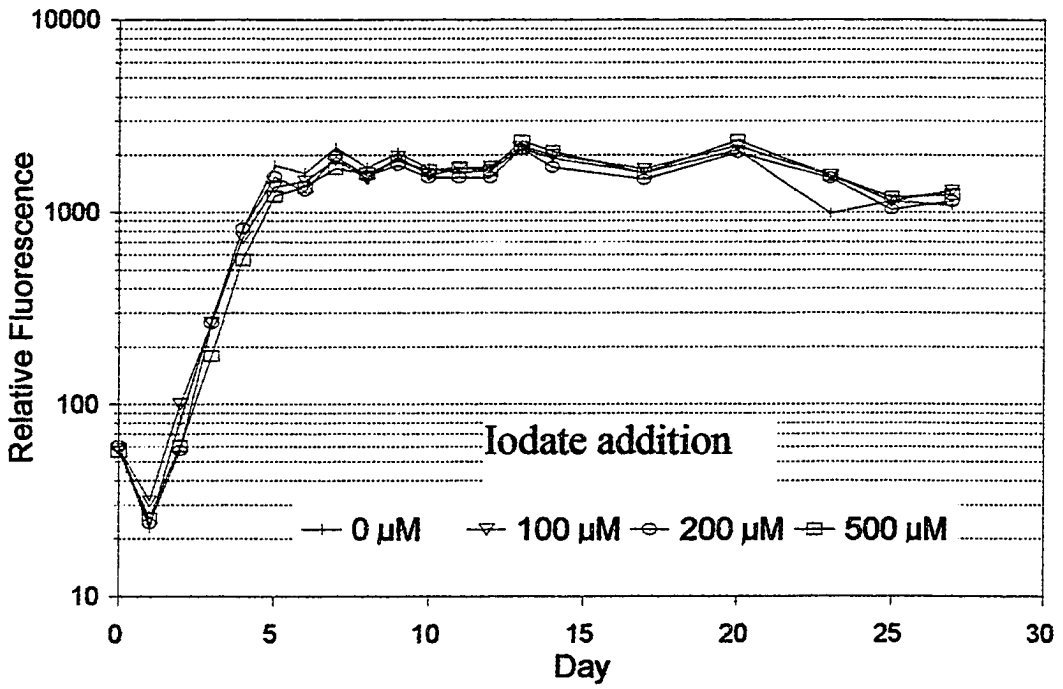
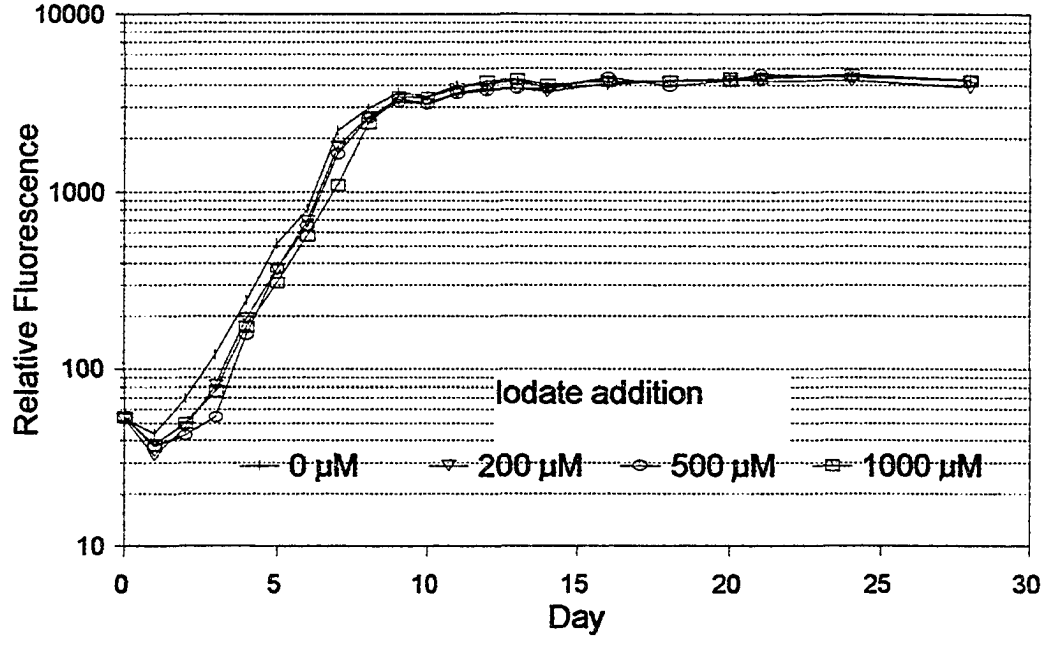
Appendix A.1: *In vivo* fluorescence from culture of *S. costatum* in deep seawater-enriched media with iodate addition



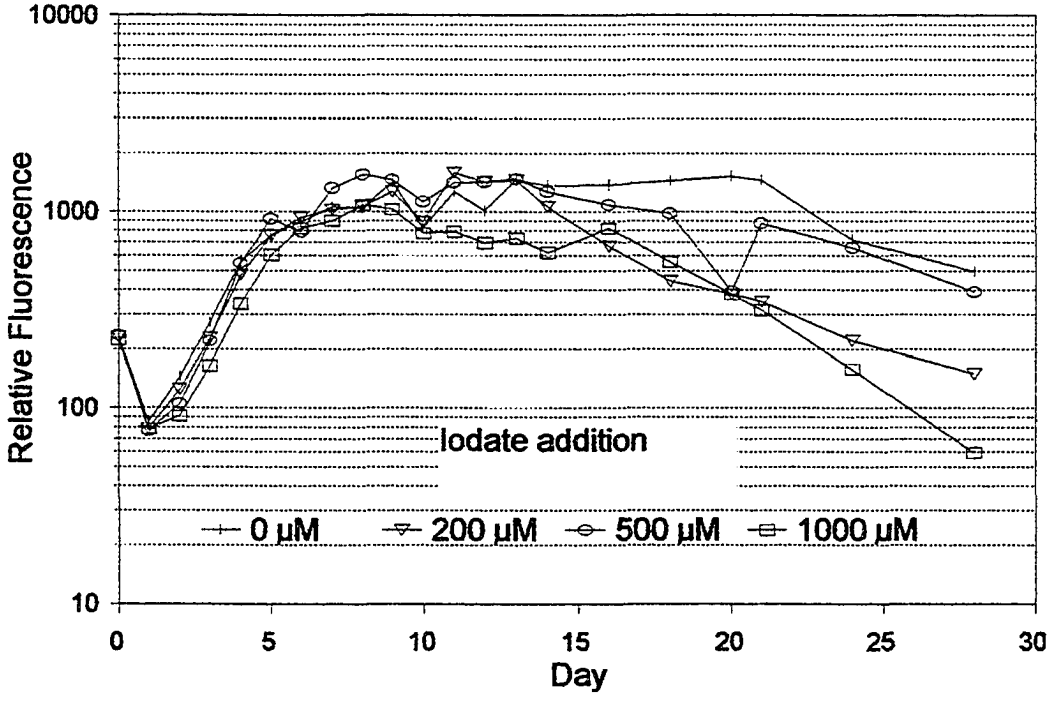
Appendix A.2: *In vivo* fluorescence from culture of *D. tertiolecta* in deep seawater-enriched media with iodate addition



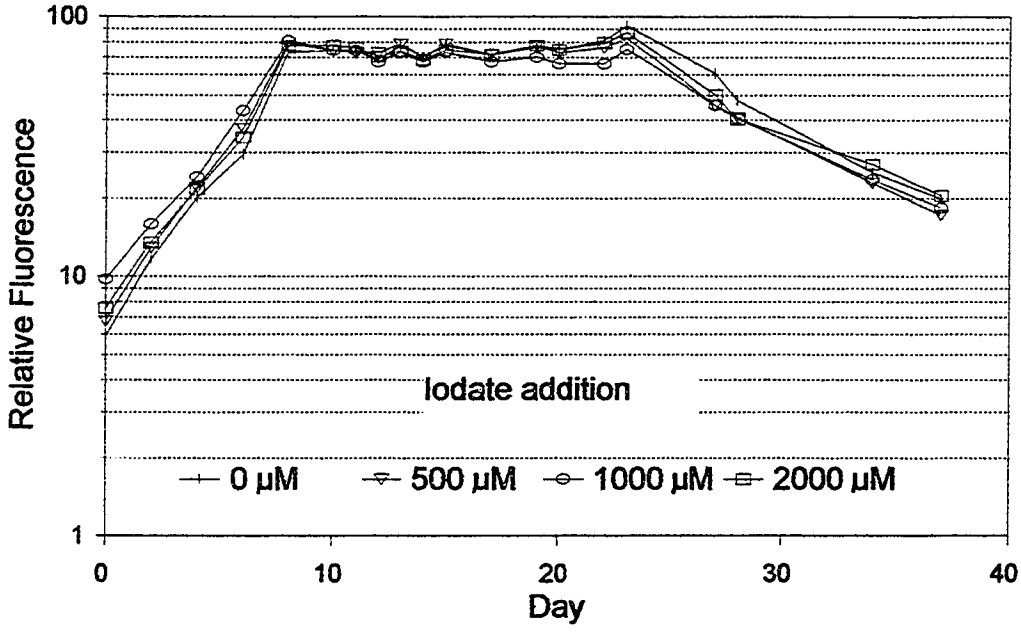
Appendix A.3: *In vivo* fluorescence from culture of *E. huxleyi* in deep seawater-enriched media with iodate addition



Appendix A.4: *In vivo* fluorescence from culture of *T. oceanica* in deep seawater-enriched media with iodate addition



Appendix A.5: *In vivo* fluorescence from culture of *Synechococcus* sp. in deep seawater-enriched media with iodate addition



Appendix A.6: In vivo fluorescence from cultures of phytoplankton in deep seawater enriched media with iodate additions

Species	Time (Day)	Iodate addition (μM) 0	100	200	500
SKEL	0	30.48	49.76	31.55	29.4
	1	35.08	30.23	35.08	28.62
	2	100.12	96.96	98.54	84.32
	3	290	290	290	270
	4	778.8	748.8	723.8	618.8
	5	1326	1168	1262.8	1104.8
	6	1451.9	1262.3	1436.1	1167.5
	7	1483.9	1199.5	1373.3	1199.5
	8	1073.4	915.4	1073.4	852.2
	9	1420.7	978.3	1278.5	1009.9
	10	1105	757.4	994.4	789
	11	1168.2	725.8	1041.8	757.4
	12	1168	662.4	1057.4	694
	13	1673.5	883.5	1483.9	820.3
	14	1389.4	536.2	1184	631
	17	870	240	710	320
	20	915.1	314.7	599.1	314.7
	23	328.4	138.4	188.4	118.4
	25	268.9	118.9	188.9	118.9
	27	219	109	169	99
DUN	0	57.94	58.48	60.64	56.86
	1	22.69	31.31	23.77	25.39
	2	82.74	100.12	57.46	60.62
	3	270	265	265	180
	4	813.8	733.8	823.8	563.8
	5	1752.6	1357.6	1531.4	1215.4
	6	1594.1	1451.9	1293.9	1357.1
	7	2179.1	1878.9	1942.1	1689.3
	8	1705.4	1500	1547.4	1594.8
	9	2036.9	1942.1	1768.3	1847.3
	10	1689.6	1563.2	1515.8	1658
	11	1610.6	1689.6	1515.8	1705.4
	12	1657.8	1721	1515.6	1657.8
	13	2149.9	2194.9	2131.7	2352.9
	14	1926.6	1989.8	1721.2	2084.6
	17	1610.4	1673.6	1484	1594.6
	20	2115.9	2226.5	2052.7	2368.7
	23	982.6	1552.4	1500.6	1552.4
	25	1138.5	1138.5	1034.9	1190.3
	27	1086.8	1294	1138.6	1242.2

Appendix A.6: (cont'd)

Species	Time (Day)	Iodate addition (μM) 0	200	500	1000
BT6	0	52.61	53.15	53.69	54.23
	1	43.7	32.93	36.7	37.77
	2	69.11	47.57	42.72	50.26
	3	120.66	81.16	54.3	75.63
	4	245.49	194.92	157	174.38
	5	511.5	366.5	369	309
	6	801.6	689.1	649.1	571.6
	7	2210.8	1800	1642	1104.8
	8	2951.5	2640.7	2588.9	2433.5
	9	3625.2	3314.4	3210.8	3418
	10	3443.7	3132.9	3107	3392.9
	11	3961.4	3624.7	3572.9	3831.9
	12	4091	3832	3728.4	4220.5
	13	4194.7	3832.1	3832.1	4298.3
	14	3858.8	3677.5	3832.9	3988.3
	16	3987.4	4091	4401.8	4220.5
	18	4246	4194.2	3935.2	4194.2
	20	4298.1	4349.9	4246.3	4246.3
	21	4479.8	4220.8	4557.5	4350.3
	24	4428.1	4246.8	4454	4583.5
	28	4272.4	3883.9	4246.5	4246.5
13-1	0	229.98	228.4	233.14	220.5
	1	84.32	78	75.84	78
	2	144.46	123.92	104.96	90.74
	3	274	226.5	219	164
	4	554	474	544	339
	5	754.4	757.4	915.4	599.4
	6	883.9	931.3	789.1	820.7
	7	1057.4	1025.8	1318.1	899.4
	8	1041.7	1057.5	1547.3	1081.2
	9	1421.1	1263.2	1452.8	1026.2
	10	820.6	883.8	1120.8	774.2
	11	1262.7	1587.7	1412.8	788.7
	12	1010	1420.8	1420.8	694
	13	1452.5	1452.5	1468.3	725.7
	14	1358.5	1058.6	1264	615.9
	16	1373.4	662.4	1081.1	820.4
	18	1439.36	440.8	978	551.4
	20	1515.5	377.9	390.9	377.9
	21	1452.7	346.7	868.1	315.1
	24	718.1	220.4	654.9	157.2
	28	494	149	389	59

Appendix A.6 : (cont'd)

Species	Time (Day)	Iodate addition (μM) 0	500	1000	2000
SYN	0	5.85	6.75	9.76	7.55
	2	11.66	12.92	15.92	13.55
	4	20.07	21.97	24.18	21.65
	6	29.49	37.03	43.56	34.34
	8	73.06	79.38	80.96	77.8
	10	74.64	74.64	74.64	77.8
	11	74.54	72.96	74.54	76.12
	12	68.62	72.57	67.04	70.2
	13	78	78.79	73.26	74.05
	14	70.1	70.1	66.94	68.52
	15	78	78.79	73.26	74.84
	17	71.68	71.68	66.94	71.68
	19	76.42	78	70.1	78
	20	74.84	72.68	66.15	74.84
	22	81.06	76.32	66.05	79.48
	23	92.42	82.94	75.04	86.89
	27	60.83	45.75	45.75	50.06
	28	47.8	40.9	40.63	40.36
	34	25.19	23.03	23.57	27.07
	37	20	17.31	18.39	20.54

Appendix B.1 : In vivo fluorescence, cell density, pigment concentrations,and concentrations of iodate and iodide in cultures of S. costatum in deep seawater enriched media with iodate additions. (Data for Chapter 3)

Iodate Addition (µM)	Time (Day)	In vivo Fluorescence	Cell density (Cells/ml)	Chl_a (µg/L)	Phaeo-pigment (µg/L)	Iodate (µM)	Iodide (nM)
0	0	32.2	8.50E+03	7.20E+00	8.34E-01	0.304	7.2
	3	502.8	3.83E+05	9.81E+01	2.66E+01	0.341	44.0
	7	803.7	8.29E+05	3.04E+01	4.26E+01	0.267	70.9
	14	407.8	4.76E+05	1.32E+00	5.73E+00	0.287	39.2
	28	99.02		5.21E-03	5.49E-01	0.201	68.7
5	0	28.2	1.55E+04	6.93E+00	2.85E+00	5.871	4.9
	3	512.8	4.22E+05	1.04E+02	2.46E+01	5.154	75.7
	7	756.3	7.39E+05	2.60E+01	4.58E+01	5.191	97.0
	14	327.8	5.84E+05	4.63E-01	7.96E+00	4.683	225.1
	28	89.54		2.61E-02	6.67E-01	4.505	260.7
10	0	34.2	2.13E+04	8.00E+00	-6.64E-01	9.731	6.8
	3	527.8	3.91E+05	2.16E+02	4.95E+01	9.937	91.8
	7	693.1	6.74E+05	5.26E+01	8.26E+01	8.570	122.9
	14	277.8	5.64E+05	2.54E+00	1.36E+01	8.864	174.9
	28	86.06		2.02E-01	1.22E+00	8.721	321.7
25	0	32.2	1.70E+04	8.80E+00	-7.65E-01	23.412	2.5
	3	557.8	3.74E+05	1.04E+02	2.91E+01	23.466	95.1
	7	629.9	7.21E+05	2.74E+01	3.64E+01	23.627	67.6
	14	227.8	5.77E+05	6.17E-01	8.61E+00	22.354	135.3
	28	61.1		1.00E-02	4.36E-01	21.283	337.9

Appendix B.2 : In vivo fluorescence, cell density, pigment concentrations,and concentrations of iodate and iodide in cultures of D. tertiolecta in deep seawater enriched media with iodate additions. (Data for Chapter 3)

Iodate Addition (µM)	Time (Day)	In vivo Fluorescence	Cell density (Cells/ml)	Chl_a (µg/L)	Phaeo-pigment (µg/L)	Iodate (µM)	Iodide (nM)
0	0	25.2	1.35E+03	9.60E+00	5.42E+00	0.382	60.4
	3	462.9	2.19E+05	1.14E+02	5.01E+01	0.311	44.7
	7	851.1	5.23E+05	8.94E+01	6.55E+01	0.326	67.3
	14	857.9	4.47E+05	3.86E+01	3.34E+01	0.289	109.4
	21	598.0	5.44E+05	3.47E+01	2.72E+01	0.216	113.0
	28	592.9	6.18E+05	1.90E+01	2.25E+01	0.123	239.7
5	0	25.7	1.95E+04	8.80E+00	5.52E+00	6.405	27.6
	3	507.9	2.86E+05	1.31E+02	6.99E+01	5.191	53.4
	7	851.1	4.56E+05	9.76E+01	7.78E+01	4.842	91.8
	14	897.9	5.44E+05	4.29E+01	3.94E+01	4.293	673.6
	21	628.0	5.21E+05	2.99E+01	2.64E+01	4.091	798.6
	28	547.9	5.28E+05	2.65E+01	2.68E+01	3.225	1931.2
10	0	25.9	1.80E+04	1.01E+01	6.07E+00	9.991	14.4
	3	497.9	2.93E+05	1.37E+02	7.16E+01	8.297	29.9
	7	819.5	4.42E+05	9.76E+01	8.14E+01	7.643	105.2
	14	945.9	5.92E+05	4.24E+01	3.46E+01	7.287	337.7
	21	648.0	4.16E+05	2.99E+01	2.69E+01	7.009	1194.7
	28	547.9	6.71E+05	2.60E+01	2.45E+01	6.709	2257.5
25	0	23.7	1.80E+04	9.06E+00	5.60E+00	18.863	33.4
	3	492.9	3.03E+05	1.31E+02	6.99E+01	18.543	79.2
	7	819.5	4.44E+05	9.48E+01	7.69E+01	17.937	214.9
	14	882.7	6.75E+05	4.69E+01	4.46E+01	17.816	561.9
	21	688.0	4.33E+05	2.80E+01	2.64E+01	17.755	1437.5
	28	587.9	6.63E+05	1.40E+01	1.70E+01	15.877	4265.9

Appendix B.3 : In vivo fluorescence, cell density, pigment concentrations, and concentrations of iodate and iodide in cultures of A. carterae in deep seawater enriched media with iodate additions. (Data for Chapter 3)

Iodate Addition (µM)	Time (Day)	In vivo Fluorescence	Cell density (Cells/ml)	Chl_a (µg/L)	Phaeo-pigment (µg/L)	Iodate (µM)	Iodide (nM)
0	0	22.4	5.50E+03	5.854E+00	6.532E-01	0.363	38.5
	3	47.4	1.55E+04	1.253E+01	1.788E+00	0.367	39.4
	7	333.6	7.70E+04	3.105E+01	9.119E+00	0.320	52.1
	14	208.6	1.22E+05	1.439E+01	6.906E+00	0.150	55.5
	20	148.6	1.52E+05	7.334E+00	4.585E+00	0.121	62.6
	28	145.36	1.27E+05	6.787E-01	2.630E+00	0.122	60.2
5	0	21.4	5.00E+03	7.381E+00	6.702E-01	4.703	43.7
	3	43.4	1.45E+04	1.013E+01	1.394E+00	4.654	63.5
	7	348.6	6.80E+04	2.670E+01	8.277E+00	4.921	62.5
	14	168.6	1.49E+05	7.635E-01	3.538E+00	4.224	94.8
	20	118.6	1.32E+05	7.974E-01	2.688E+00	4.523	95.5
	28	116.92	1.38E+05	5.938E-01	1.832E+00	4.350	69.0
10	0	21.4	3.00E+03	7.041E+00	6.787E-01	9.237	43.6
	3	43.4	9.50E+03	8.529E+00	1.946E+00	9.038	20.8
	7	328.6	8.00E+04	2.670E+01	7.111E+00	8.748	90.5
	14	158.6	1.43E+05	2.087E+00	3.317E+00	8.338	149.8
	20	118.6	1.42E+05	1.086E+00	2.333E+00	8.060	110.1
	28	110.6	1.26E+05	8.210E-01	1.740E+00	8.841	125.8
25	0	23.4	3.50E+03	6.363E+00	8.059E-01	21.429	23.6
	3	45.4	1.75E+04	1.002E+01	1.850E+00	23.258	41.9
	7	388.6	9.55E+04	3.594E+01	6.548E+00	21.017	118.9
	14	168.6	1.57E+05	1.866E+00	2.986E+00	21.697	233.7
	20	123.6	1.39E+05	1.866E+00	2.876E+00	18.195	487.2
	28	126.4	1.37E+05	5.747E-01	1.560E+00	17.972	524.1

Appendix B.4 : In vivo fluorescence, cell density, pigment concentrations, and concentrations of iodate and iodide in cultures of T. levis in deep seawater enriched media with iodate additions. (Data for Chapter 3)

Iodate Addition (µM)	Time (Day)	In vivo Fluorescence	Cell density (Cells/ml)	Chl_a (µg/L)	Phaeo-pigment (µg/L)	Iodate (µM)	Iodide (nM)
0	0	10.74	1.00E+03	1.913E-01	2.832E-01	0.404	0.0
	3	116.90	4.55E+04	9.595E+00	6.466E+00	0.271	0.0
	7	900.60	3.00E+05	4.435E+01	3.901E+01	0.297	21.7
	14	1185.00	2.90E+05	1.759E+01	2.096E+01	0.294	37.2
	21	1042.80	2.79E+05	2.106E+00	3.028E+00	0.290	63.0
	28	537.20	1.74E+05	3.349E-01	1.023E+00	0.280	43.0
5	0	11.38	3.00E+03	2.453E-01	3.309E-01	4.591	1.2
	3	132.70	5.15E+04	7.729E+00	5.888E+00	4.293	7.8
	7	916.40	2.65E+05	3.760E+01	3.818E+01	2.279	25.3
	14	1232.40	2.69E+05	5.997E+00	8.406E+00	2.300	56.6
	21	1074.40	2.91E+05	4.465E-01	8.796E-01	2.271	71.2
	28	632.00	1.89E+05	2.456E-01	7.859E-01	2.259	50.2
10	0	11.06	3.50E+03	2.633E-01	3.247E-01	8.626	0.0
	3	129.60	4.15E+04	1.058E+01	9.022E+00	8.189	10.1
	7	1042.80	3.08E+05	1.928E+01	2.320E+01	7.859	42.4
	14	1169.20	2.51E+05	1.222E+00	1.955E+00	8.181	84.3
	21	979.60	2.60E+05	4.577E-01	6.695E-01	7.112	81.8
	28	600.40	1.88E+05	2.203E-01	6.521E-01	7.248	96.0
25	0	11.69	2.00E+03	3.273E-01	4.651E-01	18.442	14.1
	3	132.70	4.80E+04	8.995E+00	8.026E+00	18.871	8.2
	7	1011.20	2.93E+05	2.950E+01	3.744E+01	20.109	62.0
	14	1200.80	2.47E+05	9.595E+00	1.240E+01	20.460	192.9
	21	948.00	2.52E+05	8.093E-01	1.364E+00	19.308	199.3
	28	505.60	1.88E+05	5.581E-01	1.284E+00	18.437	191.5

Appendix B.5 : In vivo fluorescence, cell density, pigment concentrations, and concentrations of iodate and iodide in cultures of E. huxleyi in deep seawater enriched media with iodate additions. (Data for Chapter 3)

Iodate Addition (µM)	Time (Day)	In vivo Fluorescence	Cell density (Cells/ml)	Chl_a (µg/L)	Phaeo-pigment (µg/L)	Iodate (µM)	Iodide (nM)
0	0	66.36	3.10E+04	3.702E+00	8.699E-01	0.256	14.7
	3	167.48	1.09E+05	1.546E+01	3.396E+00	0.230	32.0
	7	1832.80	8.37E+05	1.039E+02	2.464E+01	0.269	31.8
	14	2050.00	8.77E+05	9.762E+01	2.295E+01	0.276	54.7
	21	1600.00	7.09E+05	7.809E+01	3.152E+01	0.194	54.8
	28	680.00	8.40E+05	2.885E+01	1.650E+01	0.238	51.0
25	0	66.36	3.10E+04	4.072E+00	5.599E-01	18.964	45.2
	3	173.80	8.95E+04	1.493E+01	3.230E+00	21.718	62.6
	7	1564.20	6.57E+05	8.509E+01	1.895E+01	18.638	131.2
	14	1700.00	8.46E+05	8.646E+01	1.950E+01	17.514	164.5
	21	1400.00	9.15E+05	8.088E+01	1.411E+01	18.528	345.2
	28	660.00	7.08E+05	3.905E+01	4.797E+00	15.277	216.9

Appendix B.6 : In vivo fluorescence, cell density, pigment concentrations and concentrations of iodate and iodide in cultures of Synechococcus sp. in deep seawater enriched media with iodate additions. (Data for Chapter 3)

Iodate Addition (µM)	Time (Day)	In vivo Fluorescence	Chl_a (µg/L)	Pheao-pigment (µg/L)	Iodate (µM)	Iodide (nM)
0	0	3.00	4.836E+00	-3.818E-02	0.272	23.9
	3	11.38	2.314E+01	1.490E+00	0.271	154.4
	7	41.08	7.321E+01	1.174E+01	0.198	181.1
	14	31.60	5.872E+01	2.667E+00	0.133	322.4
	21	11.20	1.928E+01	9.256E-01	0.062	311.1
	28	3.90	4.118E+00	7.004E-01	0.105	358.5
5	0	3.80	5.319E+00	-2.545E-02	5.001	35.2
	3	12.96	2.401E+01	2.327E+00	4.954	70.0
	7	37.92	7.112E+01	5.606E+00	4.273	319.1
	14	25.28	4.715E+01	2.869E+00	3.547	888.3
	21	9.52	1.612E+01	1.271E+00	3.090	930.8
	28	3.20	3.398E+00	6.345E-01	2.794	1252.6
10	0	4.00	4.836E+00	-3.818E-02	9.651	54.5
	3	13.59	2.545E+01	1.828E+00	6.813	14.7
	7	37.92	6.485E+01	6.401E+00	7.717	465.1
	14	25.28	4.228E+01	2.418E+00	7.050	1042.4
	21	8.96	1.609E+01	9.731E-01	6.733	1199.0
	28	3.70	3.838E+00	4.566E-01	5.196	1582.8
25	0	5.70	5.663E+00	-3.818E-02	17.934	14.4
	3	13.59	2.545E+01	1.828E+00	19.431	87.5
	7	45.82	8.033E+01	6.267E+00	17.748	624.9
	14	31.60	5.073E+01	3.290E+00	16.727	1808.8
	21	12.88	1.774E+01	1.963E+00	16.808	2575.6
	28	4.30	4.198E+00	5.157E-01	16.936	2283.0

Appendix C: Parameters associated with growth and concentrations of iodate and iodide in artificial enriched cultures of *S. costatum* with 300 nM iodate additions. (Data for Chapter 4)

Parameter	Sample	Time (Day) 0	1	2	3	4	9
Cell density (cells /ml)	1	14500	38000	143500	239000	435000	427500
	2	5000	25000	142500	322000	420000	428000
	mean	9750	31500	143000	280500	427500	427750
	sd	4750	6500	500	41500	7500	250
Fluorescence	1	25.28	48.47	210.00	320.00	360.00	884.80
	2	25.60	48.47	180.00	360.00	350.00	948.00
	mean	25.44	48.47	195.00	340.00	355.00	916.40
	sd	0.16	0.00	15.00	20.00	5.00	31.60
Chl_a (µg / L)	1	2.43	15.08	31.67	47.41	22.31	16.73
	2	2.87	9.80	36.95	36.26	61.36	19.52
	mean	2.65	12.44	34.31	41.84	41.84	18.13
	sd	0.22	2.64	2.64	5.58	19.52	1.39
Phaeo-pigments (µg/ L)	1	0.29	1.78	5.25	24.25	53.13	92.67
	2	0.25	1.78	5.25	27.86	51.82	142.71
	mean	0.27	1.78	5.25	26.06	52.47	117.69
	sd	0.02	0.00	0.00	1.80	0.66	25.02
Iodate (nM)	1	344.1	334.7	316.4	315.2	269.1	286.4
	2	344.1	345.6	267.1	295.1	250.3	275.3
	mean	344.1	340.2	291.7	305.2	259.7	280.9
	sd	0.0	5.5	24.7	10.1	9.4	5.6
Iodide (nM)	1	10.8	8.8	7.5	11.0	25.6	27.0
	2	8.4	7.5	4.0	9.2	9.0	40.8
	mean	9.6	8.2	5.7	10.1	17.3	33.9
	sd	1.2	0.6	1.8	0.9	8.3	6.9

Appendix D: Parameters associated with growth of Skeletonema costatum in nitrate enriched media with 300 nM iodate addition. (Data for Chapter 5)

Parameter	Sample	Time (Day) 0	2	3	4	7	8	9
In vivo	1	44.8	230.7	310.0	380.0	389.3	379.7	600.0
fluorescence	2	44.8	192.8	290.0	360.0	339.3	329.7	590.0
(relative	3	45.8	192.8	290.0	290.0	489.3	369.7	570.0
unit)	mean	45.1	205.4	296.7	343.3	406.0	359.7	586.7
	sd	0.6	21.9	11.5	47.3	76.4	26.5	15.3
Cell density	1	16500	114000	257000	349000	408500	388000	328000
(cells/ml)	2	13000	118000	229000	375500	424500	337000	411000
	3	19000	127000	230500	325714	449500	335000	344000
	mean	16167	119667	238833	350071	427500	353333	361000
	sd	3014	6658	15751	24910	20664	30039	44034
Chlorophyll_a	1	9.43	26.92	35.90	24.68	12.34	14.58	5.61
(µg/L)	2	10.05	31.41	33.65	31.41	11.22	11.22	7.85
	3	10.05	29.17	33.65	33.65	13.46	11.22	7.85
	mean	9.8	29.2	34.4	29.9	12.3	12.3	7.1
	sd	0.4	2.2	1.3	4.7	1.1	1.9	1.3
Phaeo-pigments	1	2.88	18.60	43.01	66.37	54.43	49.15	45.98
(µg/L)	2	3.13	17.15	45.26	68.75	61.62	67.69	52.84
	3	2.25	16.36	36.15	51.33	71.52	64.66	52.84
	mean	2.8	17.4	41.5	62.1	62.5	60.5	50.6
	sd	0.5	1.1	4.7	9.4	8.6	9.9	4.0
Chlorophyll	1	4.753	8.569	8.636	15.398	31.550	26.038	106.977
specific	2	4.456	6.137	8.618	11.462	30.248	29.392	75.138
fluorescence	3	4.555	6.609	8.618	8.618	36.350	32.958	72.591
(relative	mean	4.588	7.105	8.624	11.826	32.716	29.462	84.902
unit)	sd	0.151	1.289	0.011	3.405	3.214	3.460	19.160

Appendix D: Parameters associated with growth of Skeletonema costatum in ammonium enriched media with 300 nM iodate addition. (Data for Chapter 5)

Parameter	Sample	Day 0	2	3	4	7	8	9
In vivo	1	45.8	158.0	300.0	360.0	379.3	469.7	560.0
fluorescence	2	43.8	132.7	280.0	330.0	359.3	349.7	600.0
(relative	3	42.8	148.5	280.0	280.0	369.3	459.7	600.0
unit)	mean	44.1	146.4	286.7	323.3	369.3	426.4	586.7
	sd	1.5	12.8	11.5	40.4	10.0	66.6	23.1
Cell density	1	11000	78000	251500	300000	413000	496000	419000
(cells/ml)	2	15500	109000	226000	359000	461500	353000	447000
	3	9500	102000	323000	307000	409500	474000	436000
	mean	12000	96333	266833	322000	428000	441000	434000
	sd	3122	16258	50285	32234	29065	77000	14107
Chlorophyll a	1	11.55	29.17	44.87	20.19	11.22	15.70	5.61
(µg/L)	2	12.88	24.68	38.14	31.41	6.73	6.73	5.61
	3	10.05	29.17	56.09	29.17	11.22	10.10	8.97
	mean	11.5	27.7	46.4	26.9	9.7	10.8	6.7
	sd	1.4	2.6	9.1	5.9	2.6	4.5	1.9
Phaeo-pigments	1	2.06	13.32	37.07	55.68	55.55	63.20	50.54
(µg/L)	2	2.51	11.74	31.66	56.61	66.11	44.86	45.98
	3	1.37	16.36	41.03	46.71	61.62	62.74	51.72
	mean	2.0	13.8	36.6	53.0	61.1	56.9	49.4
	sd	0.6	2.3	4.7	5.5	5.3	10.5	3.0
Chlorophyll	1	3.964	5.417	6.686	17.829	33.814	29.909	99.845
specific	2	3.400	5.378	7.342	10.507	53.384	51.958	106.977
fluorescence	3	4.257	5.092	4.992	9.600	32.922	45.534	66.860
(relative	mean	3.874	5.296	6.340	12.646	40.040	42.467	91.227
unit)	sd	0.435	0.177	1.212	4.512	11.565	11.340	21.402

Appendix D: Concentrations of nitrate, nitrite, iodate, and iodide in nitrate enriched cultures of Skeletonema costatum with 300 nM iodate addition. (Data for Chapter 5)

Parameter	Sample	Day 0	2	3	4	7	8	9
Nitrate	1	93.446	60.658	34.206	5.804	1.035	0.809	0.725
(µg/L)	2	93.370	64.510	40.450	0.666	0.761	0.798	0.702
	3	94.397	66.455	35.300	14.571		0.809	1.160
	mean	93.738	63.874	36.652	7.014	0.898	0.805	0.862
	sd	0.572	2.950	3.334	7.031	0.193	0.006	0.258
Nitrite	1	0.028	0.119	0.158	0.063	0.032	0.008	0.016
(µg/L)	2	0.012	0.115	0.139	0.024	0.012	0.008	0.020
	3	0.020	0.103	0.135	0.119	0.008	0.008	0.016
	mean	0.020	0.112	0.144	0.069	0.017	0.008	0.017
	sd	0.008	0.008	0.013	0.048	0.013	0.000	0.002
Iodate	1	315	308	309	287	258	256	221
(nM)	2	334	319	280	276	244	259	244
	3	310	314	280	296	267	269	242
	mean	320	314	290	286	256	261	236
	sd	12	5	17	10	11	7	13
Iodide	1	26	63	34	46	70	60	86
(nM)	2	24	42	28	46	58	73	85
	3	23	57	30	25	55	58	67
	mean	24	54	31	39	61	64	79
	sd	2	11	3	12	8	8	11

Appendix D: Concentrations of ammonium-nitrogen, iodate and iodide in ammonium-enriched cultures of Skeletonema costatum with 300 nM iodate addition. (Data for Chapter 5)

Parameter	Sample	Day 0	2	3	4	7	8	9
Ammonium	1	95.464	46.086	12.509	8.559	1.129	4.515	3.950
(µg/L)	2	105.340	46.086	16.459	8.559	1.129	1.552	5.135
	3	96.452	46.086	12.509	10.534	1.552	0.705	9.876
	mean	99.085	46.086	13.826	9.217	1.270	2.257	6.320
	sd	5.439	ERR	2.281	1.140	0.244	2.000	3.135
Iodate	1	365	381	371	333	313	364	308
(nM)	2	377	377	343	338	320	328	332
	3	316	365	368	339	304	311	308
	mean	353	374	361	337	312	334	316
	sd	32	9	16	3	8	27	14
Iodide	1	18	25	27	36	36	37	46
(nM)	2	21	27	16	36	24	32	22
	3	22	27	21	41	33	31	40
	mean	20	26	21	37	31	33	36
	sd	2	1	6	3	6	3	13

Ajcharaporn Udomkit

Place of Birth : Bangkok, Thailand
Date of Birth : October 25, 1959

Education

1986 Master of Science in Marine Biology, Chulalongkorn University, Bangkok, Thailand.

1982 Bachelor of Science in Marine Sciences, Chulalongkorn University, Bangkok, Thailand.

Publication

1991 Frolich, P. N., Blanc, V., Mortlock, R. A., Chillrud, S. N., Dunstan, W. M., Udomkit, A., and Peng, T.-H. River fluxes of dissolved silica to the ocean were higher during glacials: Ge/Si in diatoms, rivers and oceans. *Paleoceanography* 7(6): 739-767.

1986 Sudara, S.; Udomkit, A.; and Manthachitry, V. Demersal zooplankton associated with coral heads at Sichang Islands, Thailand. *Galaxea* 5: 195-202.

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Presentations

1993 Reduction of iodate by cultures of marine phytoplankton. with W. M. Dunstan and G. T. F. Wong. ASLO&SWS 1993 Annual Meetings. University of Alberta, Edmonton, Canada.

1991 Experiments on phytoplankton influence on iodine speciation. with W. M. Dunstan and G. T. F. Wong. Fourth International Phycological Congress, Duke University, North Carolina.

Positions

1987 Instructor, Department of Marine Sciences, Chulalongkorn University, Bangkok, Thailand.

1984 Marine Biologist, Department of Fisheries, Bangkok, Thailand.

Memberships

American Society of Limnology and Oceanography
Phycological Society of America
The Oceanography Society