AN ABSTRACT OF THE THESIS OF

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Many phytoplankton and macroalgae release dissolved organic carbon (DOC). Many macroalgae also produce and store secondary metabolites which in some case have been shown to deter their grazers. The metabolites may be released into the surrounding seawater and could inhibit primary production. On the other hand, some phytoplankton can use DOC as a supplemental nutrient. In the first part of my experimental work, I tested the toxic or stimulatory effect of released materials from macroalgae on phytoplankton growth. Two out of six macroalgal culture media showed a weak inhibitory effect on phytoplankton growth, but the effect was far less than expected. Data from these experiments provided preliminary estimates of DOC release rates of both macroalgae and phytoplankton. The release rates of phytoplankton were nearly a hundred times higher than those of macroalgae. The DOC release seemed to be positively correlated with the

surface area to volume (SA:V) ratio. This result implies that the release of DOC may be dominated by a passive diffusion.

In the second part of my experimental work, I tested whether the DOC release rates differed with growth conditions. In the macroalgal experiment, the release rates were not different between batch and continuous cultures. Extremely high DOC release in the continuous culture reported by Sieburth (1969) seemed to be a result of adverse experimental conditions. In the phytoplankton experiment, the release rates seemed to be positively correlated with growth rate. The highest release rate was observed during the exponential growth phase. This result and reports in the literature suggest that both quantitative and qualitative differences in phytoplankton DOC release are a function of growth phase. For example, phytoplankton may produce and release more nitrogen-rich low-molecular-weight organic carbon during the exponential growth phase and more carbon-rich high-molecular-weight organic carbon during the stationary growth phase.

DOC is a main food source for heterotrophic microbes. Both quantitative and qualitative variations in phytoplankton DOC release are likely to have a large influence on the microbial loop. More detailed information on the composition of the released materials and mechanism involved in the release is needed to understand these processes and the role they play in the carbon cycle in coastal ecosystems.

The Release of Total and Dissolved Organic Carbon from Macroalgae and Phytoplankton

by

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Nobuyuki Kawasaki, Author

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF TABLES (Continued)

THE RELEASE OF TOTAL AND DISSOLVED ORGANIC CARBON FROM MACROALGAE AND PHYTOPLANKTON

Chapter I

Introduction

Phytoplankton and macroalgae are the main primary producers in coastal ecosystems. Phytoplankton are usually dominant in offshore waters. In coastal environments where the euphotic zone reaches the bottom, however, macroalgae can also grow and contribute largely to the nearshore primary production. It is reported that submerged macroalgae are the second most productive ecosystem (in the range 2.9 - 7.5 kg m^{-2} y^{-1}), just below the tropical rain forest ecosystem (5 - 8 kg m^{-2} y^{-1}) (Westlake 1963, Mann 1982). At Boiler Bay, Oregon, for example, standing stock and primary production of macroalgae are a hundred times and ten times larger than those of phytoplankton, respectively (Leigh et al. 1987, Wheeler, unpublished).

Both phytoplankton and macroalgae produce organic carbon through photosynthesis. They also release substantial amounts of dissolved organic carbon (DOC) to the surrounding water. The important finding is that from 10 to 50 % of net primary production may be released by phytoplankton and macroalgae via exudation (Sieburth 1969, Khailov and Burlakova 1969, Pregnall 1983, Horner and Smith 1984, Branch and Griffiths 1988, Valiela 1988, Ramus 1992, Biddanda and Benner 1997). The DOC released can be used by heterotrophs, including bacteria, fungi and many other small organisms. Cole et al. (1988) found that the more DOC exuded by algae, the more bacteria grow in lakes and coastal waters. The release of DOC by phytoplankton and macroalgae may largely affect the microbial loop, a significant part of aquatic food webs.

Macroalgae are known to use several strategies to avoid grazing activities of herbivores including fishes, urchins, gastropods, crabs and other smaller herbivores (Lubchenco and Gaines 1981, Gaines and Lubchenco 1982, Harlin 1987, Duffy and Hay 1990). One of the strategies is a chemical defense; macroalgae produce toxic organic carbons such as polyphenols and tannins which deter herbivores (Faulkner 1984, 1986). McLachlan and Craigie (1964) collected yellow, ultraviolet-absorbing substances released from *Fucus vesiculosus* and tested their effects on seven different unicellular algae. Growth of the unicellular algae was suppressed, and most of the cells in the inhibited cultures underwent lysis. The yellow, ultraviolet-absorbing substances were identified as phenolic compounds in a later study (McLachlan and Craigie 1966). The effects of the released phenolic substances, as

well as commercially prepared phenolic substances, were similar. It has been reported that some macroalgae produce antiviral compounds (Kamet et al. 1992, Ballesteros et al. 1992, Garg et al. 1992, Ivanova et al. 1994, Jin et al. 1997). In coastal areas where water exchange is poor, these toxic substances may be accumulated and affect the entire ecosystem (Harlin 1987).

Menge et al. (1995, 1997a, 1997b) tried to determine the factors that control the ecological community structure at two sites on the Oregon coast: Boiler Bay and Strawberry Hill. At Boiler Bay, the standing stock of macroalgae estimated from Leigh et al. (1987) greatly exceeds that of phytoplankton estimated from Menge et al. (1997a) and Wheeler (unpublished). In comparison, at Strawberry Hill, the abundance of macroalgae is lower and phytoplankton are more abundant. Primary production is often controlled by inorganic nutrients that are essential for photosynthesis. At both sites, however, upwelling from May to October leads to high nutrient concentrations throughout the year (Menge et al. 1997a). Given high nutrient concentrations, both phytoplankton and macroalgae should be growing well at both sites. At Strawberry Hill, large phytoplankton blooms were observed after the upwellings. The blooms also occur at Boiler Bay, but they are much smaller than those at Strawberry Hill (Menge et al. 1995, 1997a, 1997b). What, then, causes the low abundance of phytoplankton at Boiler Bay?

There may be several factors controlling phytoplankton abundance at Boiler Bay, but one possible factor is that the abundant macroalgae release highly toxic substances and inhibit the growth of phytoplankton. The effects of these released

substances on phytoplankton may be significant due to high concentrations of macroalgae. I hypothesized that a toxic effect on phytoplankton may be observed at Boiler Bay if the rate of release of toxic substances by macroalgae exceeds the dilution of seawater by mixture as well as the decomposition of the substances by microbes. A laboratory experiment was designed and tested in order to test this hypothesis.

Several studies have examined DOC release rates of phytoplankton (Biddanda and Benner 1997) and macroalgae (Sieburth 1969, Khailov and Burlakova 1969, Fogg 1983, Horner and Smith 1984, Schramm et al. 1984). However, the experimental methods and conditions varied among these studies and therefore it is difficult to compare the DOC release rates among them. For example, different release rates were found between batch cultures and continuous cultures. Sieburth (1969) performed two different experiments to measure released substances: a closed system (batch culture) and an open system (continuous culture). In the closed system, he detected no DOC release, but found a significant increase of bacteria instead. Abundant bacteria decomposed DOC as fast as macroalgae released DOC. In the open system, with a seawater turnover rate of two - three times per hour, he detected significant DOC release and bacteria abundance remained low. Bacterial activity that decomposes organic matter into inorganic matter may be enhanced in batch cultures (Chen and Wangersky 1996b), but there have been few studies that compare batch cultures and continuous cultures in both phytoplankton and macroalgal culture experiments.

In addition to methodological differences, analytical approaches are also different among studies. ^{14}C methods have been used in many DOC release experiments for phytoplankton (e. g. Fogg et al. 1965, Derenbach and Williams 1974, Larsson and Hagstöm 1979) and macroalgae (e. g. Brylinsky 1977, Fankboner and DeBurgh 1977). The methods may be appropriate in phytoplankton experiments because small DOC storage pools inside cells which are not ^{14}C-labeled may be quickly replaced by ^{14}C-labeled DOC. However, the methods may be problematic for macroalgal experiments (Fogg 1983, Schramm et al. 1984). Because macroalgae usually have much larger internal DOC pools, some release products must originate from non-labeled photosynthates which are synthesized and stored before the experiments during short-term ^{14}C experiments (2 or 3 h). As another different analytical approach, DOC measurements have been improved by Sugimura and Suzuki (1988) who introduced the high-temperature catalytic (HTCO) method. Chen and Wangersky (1993) found that some organic compounds are resistant to oxidation, and that leads to the underestimation of organic carbon by the UV oxidation methods, but that the HTCO method recovered almost all of the compounds tested. Thus, the HTCO method appears to be a better DOC measurement than the UV and wet oxidation methods. While the HTCO method has been used for many phytoplankton experiments, no measurements have been done in macroalgal experiments. Since the ^{14}C methods may not give valid results in short- term 2macroalgal experiments, the HTCO method should be employed. I

used the HTCO method in both phytoplankton and macroalgal experiments in this study.

This study had three main objectives. The first one was to determine whether released substances from macroalgae inhibit phytoplankton growth. I chose to measure total organic carbon (TOC) or DOC in preliminary tests of toxicity because various unidentified inhibitory substances are released from macroalgae. The second objective was to determine whether TOC or DOC release rates are different between batch culture and continuous culture experiments. Sieburth (1969) found DOC release rates were significantly different between the two culture experiments and the difference was a result of bacterial activity. The last objective was to compare carbon-specific DOC release rates of macroalgae with those of phytoplankton because this result may help to understand DOC release mechanisms of macroalgae and phytoplankton.

Chapter II

TEST OF TOXIC OR STIMULATORY EFFECTS OF RELEASED SUBSTANCES FROM MACROALGAE ON PHYTOPLANKTON GROWTH

Many macroalgae use chemical defenses to deter grazers (Faulkner 1984, 1986, Harlin, 1987, Duffy and Hay 1990). Although macroalgal extracts or concentrated substances from seawater showed antialgal effects (McLachlan and Craigie 1964, 1966) and antiviral effects (Kamet et al. 1992, Ballesteros et al. 1992, Garg et al. 1992, Ivanova et al. 1994, Jin et al. 1997), no studies have been made to test the toxicity effects of release substances from live macroalgae on phytoplankton growth. If inhibitory effects on phytoplankton are observed with macroalgal culture seawater, then toxic substances may also inhibit primary productivity in coastal ecosystems. For example, at Boiler Bay where macroalgae are very abundant, the effects may be significant and lead to the low observed phytoplankton abundance (Menge et al. 1995, 1997a). On the other hand, it has been reported that some algae utilize DOC as a nutrient (Hellebust and Lewin 1977, Flynn and Butler 1986). If phytoplankton tested can use DOC as an additional nutrient, a stimulatory effect may be observed. In that case, abundant macroalgae could stimulate phytoplankton growth *in situ*.

In this chapter, I describe the experiments designed to test whether naturally released substances from macroalgae inhibit or stimulate phytoplankton growth. In addition, initial estimates of TOC and DOC release rates by both macroalgae and phytoplankton are reported. Possible causes of the differences in carbon-specific release rates are discussed.

Methods

Macroalgae Maintenance and Batch Culture DOC Release

Six species, *Hedophyllum sessile*, *Analipus japonicus*, *Odonthalia floccosa*, *Dilsea californica*, *Codium fragile* and *Ulva fenestrata* (Table II-1), were collected from Boiler Bay, Oregon, in August 1997. Dr. Gayle Hansen, an associate professor at Oregon State University, helped with the collection and identification of the macroalgae. All visible epiphytes on the macroalgae were immediately removed by hands after the macroalgae were brought back to the laboratory. The macroalgae were maintained in filtered seawater with 500 μM ammonium and 35 μM phosphate at 16 °C with photon irradiances of about 100 μE m^{-2} s^{-1} and a light: dark cycle of 12: 12 h until the time of the experiment.

For experimental incubations, macroalgae (30 - 90 g wet weight) were rinsed with filtered seawater and transferred to plastic tubs with 3 L seawater with

Table II-1 Macroalgal species used in the batch culture experiment. Dr. Gayle Hanson, an associate professor at Oregon State University, helped the collection and identification of the macroalgae.

Species	Division	Collection sites
Hedophyllum sessile	Phaeophyta (Brown Algae)	Boiler Bay
Analipus japonicus	Phaeophyta (Brown Algae)	Boiler Bay
Odonthalia floccosa	Rhodophyta (Red Algae)	Boiler Bay
Dilsea californica	Rhodophyta (Red Algae)	Boiler Bay
Codium fragile	Chlorophyta (Green Algae)	Boiler Bay
Ulva fenestrata	Chlorophyta (Green Algae)	Boiler Bay

final nutrient enrichments of 500 μM ammonium and 25 μM phosphate. About 1 - 4 g wet weight macroalgae was collected and dried in order to calculate the ratios of wet weight to dry weight (Table II-2). Seawater without macroalgae was prepared as a control. No replicate was made in this experiment. This will be discussed later.

Immediately after the macroalgae were transferred to the tubs, the seawater was stirred a few times, and 5 ml triplicate subsamples for TOC concentrations were collected as "initial" samples. TOC samples were stored in 7 ml glass vials which were acid-washed and combusted for 12 h at 500 °C prior to use. Vials were capped with Teflon-coated septa and screw caps. Triplicate subsamples were collected every 24 h for three days. After acidification with 50 μl of 85 % phosphoric acid, samples were stored at 5 °C until analyzed.

Batch Culture Phytoplankton Growth Experiment

Two cultured diatoms, *Thalassiosira pseudonana* and *Phaeodactylum tricornutum*, were used for the phytoplankton growth experiment. *Thalassiosira* is found at both high nutrient and low nutrient sites off the Oregon coast (Kokkinakis, 1987). *Phaeodactylum* is also a common coastal diatom (Halse and Syvertsen, 1997). Stock cultures were obtained from Tim Cowles, a professor at Oregon State University. Seawater collected from Hatfield Marine Science Center, Newport, Oregon, was filtered through a 0.2 micron filtration capsule for the culture medium. Nitrate and phosphate were added to the filtered seawater for the final

Table II-2 Estimation of experimental and subsample wet and dry weights of macroalgae. Subsamples were used to calculate dry/wet ratios because the experimental macroalgae could not be dried.

Species	Experimental Wet Weight (g)	Subsample Wet Weight (g)	Subsample Dry Weight (g)	Dry/Wet Ratio (%)	Estimated Experimental Dry Weight (g)
H. sessile	80.4	3.606	0.474	16.7	6.8
A. japonicus	34.1	1.236	0.239	24.5	7.9
O. floccosa	90.2	4.172	0.405	9.7	8.8
D. californica	32.9	4.292	0.260	6.1	2.0
C. fragile	40.7	1.788	0.299	13.1	10.5
U. fenestrata	32.3	1.005	0.246	19.3	6.6

concentrations of 500 μM and 25 μM, respectively. Trace metals and 0.5 ml vitamins per 1 L seawater were also added to the seawater at f/2 formulations (Guillard and Ryther 1962). Approximate 75 ml of culture medium was poured into each 125 ml glass flask and then autoclaved for 15 min. After the flask was cooled, phytoplankton were inoculated and maintained at 16 °C with photon irradiances of 100 μE m^{-2} s^{-1} and a light: dark cycles of 14: 10 h.

To determine the effects of macroalgal exuates on phytoplankton growth, 160 ml of seawater medium for the macroalgal incubations was removed from each tub on Day 1 and Day 3. No replicate was made again. Nutrients were added in the same proportions described above for maintenance of phytoplankton cultures and 150 μM silicate was also added. The "exuate" medium was then filter-sterilized by filtration through a 0.2 micron membrane filter in a sterile filtration apparatus. The filter sterilized medium was then transferred to acid-washed and baked (80 °C overnight) 125 ml culture flasks. The filtration of macroalgal seawater and the inoculation of phytoplankton were conducted in a sterile transfer chamber (Germa Free Laboratories, Inc.).

Phytoplankton growth was monitored by following changes in number of cells in control and experimental culture flasks. Cells were counted on a counting chamber (Reichert; 0.1 mm deep) through a microscope at 400 X magnification every 24 hour. Average cell abundance was determined as the mean abundance in 10 chambers when the total number of counted phytoplankton was less than 200, or 4 chambers when the total number of counted phytoplankton was more than 200.

Growth rates were calculated by using a linear regression of log transformed data, and compared statistically with two sample t-tests. Final yields were determined when the abundances of phytoplankton were relatively constant for three consecutive days. The phytoplankton abundances for last two days were averaged and used as final yield and compared statistically with two sample t-tests.

After final yields were determined, 2 ml duplicate samples were taken from each flask, and all phytoplankton were removed by glass fiber filters using a 10 ml syringe. Each filtrate was stored for later DOC analysis in 7 ml glass vials which were acid-washed and combusted for 12 h at 500 °C. Vials were capped with Teflon-coated septa and screw caps. After acidification with 50 µl of 85 % phosphoric acid, samples were stored in the refrigerator until analysis.

Total Organic Carbon Analysis

Total organic carbon was measured using a commercial high temperature combustion (HTC) instrument (Shimadzu TOC-500). Each sample was brought to room temperature, acidified (50 µl 50 % phosphoric acid) and sparged with compressed air (< 0.1 ppm CO_2) for 15 min prior to analysis to remove all inorganic carbon. Using a 100 µl syringe (Hamilton Gastight #1710), 60 µl of sample was then injected through the septum port into the vertical quartz combustion tube heated at 680 °C. The combustion tube contained two sheets of Pt gauze and quartz wool topped with Pt-Al catalyst (Shimadzu). Carbon dioxide released from the combustion was quantified by a non-dispersive infrared (NDIR)

detector after passing through an electronic dehumidifier and halogen scrubber. The carbon dioxide peaks were visually inspected to monitor and correct any drifts in baseline or peak shape and were integrated by the Shimadzu electronics.

The instrument was calibrated with a five point standard curve of potassium hydrogen phthalate dissolved in Milli-Q water, fresh standards (ranging from 30 - 430 μM) were prepared prior to each run by diluting a refrigerated stock solution (~ 8 mM) made weekly. Milli-Q water was used as the blank. Prior to running samples, I conditioned the catalyst by injecting Milli-Q water until achieving a low blank (Benner and Strom, 1993). The entire five point standard series was run before and after the analysis of samples ($r^2 > 0.998$ for all runs). Standard and sample peak area averages are based on at least three injections. I assessed instrument drift by injecting an acidified sea water sample (collected at 9°N, 140°W on February, 1992) or standards after each set of triplicates. Log sheet of standards for TOC determinations was shown on Table II-3. Sample concentrations were estimated by subtracting the entire blank (due to the instrument and Milli-Q water) from each sample and dividing by the instrument response (slope of standard curve).

Changes in the condition of the column led to some variation in the instrument response and the blank over this time period. The instrument response averaged 71.7 ± 3.3 area units per μM TOC concentration. The 9°N seawater averaged 78.3 ± 5.7 μM. Due to variations between runs, samples were normalized using the average value (n = 2 or 3) of the 9°N water samples for that day relative

Table II-3 Log sheet of slopes, intercepts, r-square of standards and 9 °N seawater samples for TOC determination.

Date	Sample	Slope	Intercept	R^2	9° N A (μM)	9° N B (μM)	9° N C (μM)	Daily Average (μM)	Ratio [1]
7/15/97	Seawater from macroalgal tubs (SM)	80.2	2893	0.9999	74.1	79.7		76.9	1.022
7/18/97	(SM)	78.4	3656	0.9996	77.1	(101.9)		77.1	1.020
7/21/97	(SM)	70.4	2410	0.9997	(94.9)	69.0		69.0	1.140
	(SM)	72.5	2685	0.9998					
7/23/97	(SM)	78.9	3199	0.9994	(104.4)	72.8		72.8	1.080
7/24/97	(SM)	72.6	1869	0.9996	(129.7)	85.5	73.6	79.6	0.988
	(SM)	74.1	2024	0.9997					
7/25/97	(SM)	67.9	2689	0.9997	87.5	80.4		84.0	0.937
7/28/97	(SM)	71.0	1998	0.9997	70.1	85.4	78.9	78.1	1.006
9/8/97	(SM)	71.2	1782	0.9998	78.2	(97.2)		78.2	1.005
	(SM)	71.9	1548	0.9999					
9/9/97	(SM)	73.0	2718	0.9985	89.1	(304.5)		89.1	0.882
	(SM)	74.2	1998	0.9994					
9/10/97	(SM)	72.5	1758	0.9997	77.4	81.4		79.4	0.990
9/11/97	(SM)	75.5	1710	0.9999	(60.3)	77.1		77.1	1.020
9/11/97	(SM)	74.2	1640	0.9998					
9/12/97	Seawater from phytoplankton flasks (SP)	67.3	2927	0.9996	78.0	(111.7)		78.0	1.008
					HMSC SW[3] (μM)	HMSC SW[3] (μM)			
9/14/97	(SP)	71.4	1764	0.9995	75.4	78.4		76.9	
9/16/97	(SP)	68.4	1431	0.9999	55.1	51.8		53.5	

Note: 1. Ratio = (Nominal Average)/(Daily Average)
2. Nominal average = 78.6 (SD±5.7)
Nominal average is the average of all 9N sea water samples during the experiment except the samples which may have been contaminated (number).
3. Seawater for standard was taken from the filtration apparatus at Hatfield Marine Science Center (HMSC), Newport, Oregon.

to the averaged 9°N sea water. Of 84 seawater samples taken from different macroalgal tubs, two (2.4%) were rejected as outliers because they had excessively high values.

Results

TOC release from macroalgae

TOC concentrations in the macroalgal cultures were calibrated by using the TOC standards and the 9°N seawater samples (Table II-3). The slopes of linear regressions ranged from 67.3 to 80.4 {average (±SD) = 72.9 (±3.4)}. The intercepts ranged from 1431 to 3656 {average (±SD) = 2247 (±617)}. Each linear regression showed a high R^2 value ($R^2 > 0.998$). However, TOC values of 9°N seawater samples were variable. Nine out of 26 samples showed unusually high values and were not used (See Chapter III for a more typical data set on internal standards.). The nominal average was calculated by using the rest of samples. The ratio of the nominal average to the daily average was used to correct for the daily variance of the TOC analyzer.

TOC release rates varied with species. Initial levels of TOC (Day 0) were 65 μM to 80 μM. The levels increased linearly ($R^2 > 0.98$) until Day 3 (Figure II-1). *Analipus japonicus* released the highest amounts of TOC (330 μM), while *Dilsea californica* released much less (110 μM). The control also showed a slight

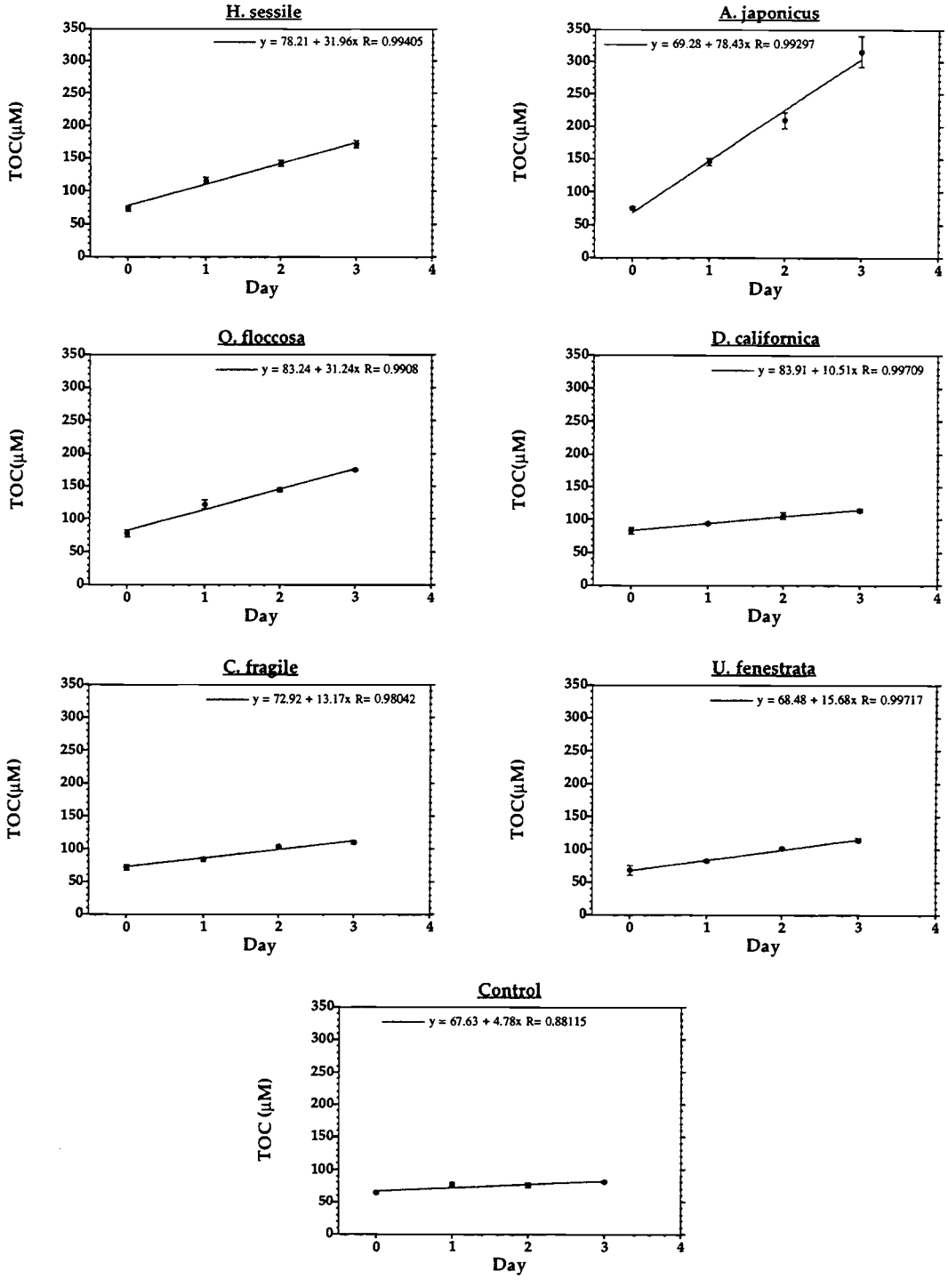

Figure II-1 TOC concentrations in the batch culture experiment. Control means seawater with no macroalgae.

increase. Even though this increase was statistically significant (two-sided p-value < 0.001), the increase may be small enough to ignore compared to the TOC increase from macroalgae.

Because dry weight varied from 2 to 10 g algal tissue per experimental chamber (Table II-2), biomass and carbon-specific release rates were calculated for each macroalgae. TOC release rates (mg C 100 g dry wt^{-1} h^{-1}) and carbon-specific TOC release rates (mg C 100 g C^{-1} h^{-1}) were shown on Table II-4. The ratio of carbon to plant tissue was estimated as 31 % by using the data of Atkinson and Smith (1983). *A. japonicus* (Brown) still showed the highest TOC release rate (1.15 mg C 100 g dry wt^{-1} h^{-1}) while *Codium fragile* (Green) and *Dilsea californica* (Red) showed low release rates (0.19 and 0.21 mg C 100 g dry wt^{-1} h^{-1}, respectively).

Phytoplankton growth rates and yields

Inhibitory or stimulatory effect of material released from the macroalgae was tested in a batch culture growth experiment with two diatoms, *Thalassiosira pseudonana* and *Phaeodactylum tricornutum*. *T. pseudonana* grew in all Day 1 culture media tested (Figure II-2), but the cells cultured on *O. floccosa* and *C. fragile* media became contaminated with flagellated cells and the abundance of *T. pseudonana* declined after day 6. Final yields from these two cultures were not included in a discussion below because of the contamination problem. All cultures showed an initial lag period of slow growth for several days and then entered an exponential phase of growth. *T. pseudonana* reached the stationary phase by day

Table II-4 Linear regression parameters for release of TOC by macroalgae and TOC release rates (mg C 100 g dry wt^{-1} h^{-1}) and carbon-specific TOC release rate (mg C 100 g C^{-1} h^{-1}) normalized to dry weight and to algal carbon.

Species	TOC release rates (± SD) (mg C 100 g dry wt^{-1} h^{-1})	Carbon-specific release rates** (± SD) (mg C 100 g C^{-1} h^{-1})	R^2
H. sessile	0.669 (±0.097)	0.207 (±0.030)	0.994
A. japonicus	1.150 (±0.106)	0.357 (±0.033)	0.993
O. floccosa	0.434 (±0.078)	0.135 (±0.024)	0.991
D. californica	0.206 (±0.011)	0.064 (±0.003)	0.997
C. fragile	0.191 (±0.030)	0.059 (±0.009)	0.980
U. fenestrata	0.958 (±0.078)	0.297 (±0.024)	0.997

Note:
**. The proportion of carbon to plant tissue was estimated as 31 % by using the data of Atkinson and Smith (1983).

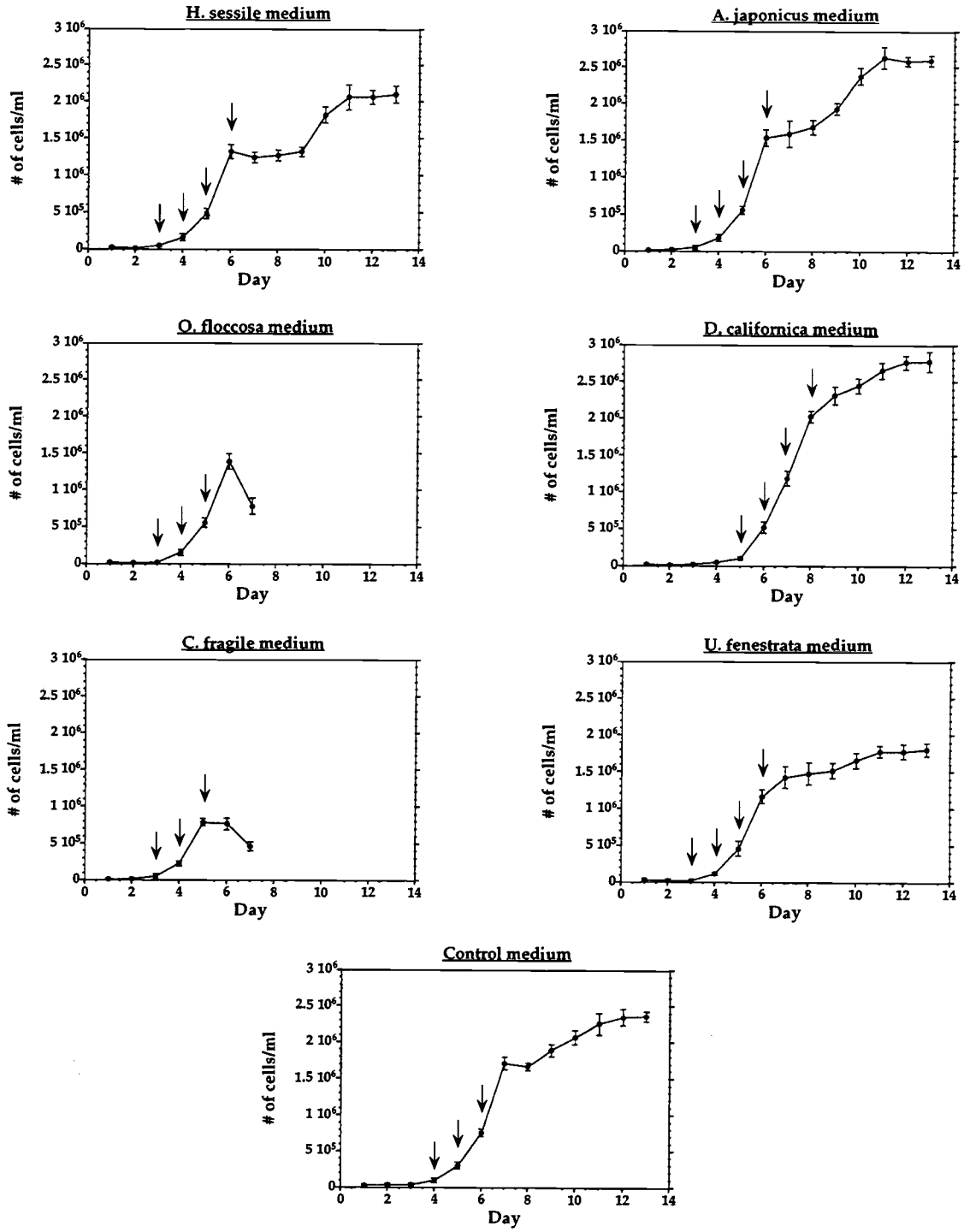

Figure II-2 Growth curves of *Thalassiosira pseudonana* in the Day 1 seawater experiment. The points indicated by arrows were points which were used for growth rate calculations.

11. Growth rates were calculated from the exponential phase cell counts as indicated by arrows in Figure II-2. The exponential phase was determined graphically from the linear increase in logarithmic number of cells (y-axis) vs day (x-axis).

P. tricornutum grew in all Day 1 culture media tested (Figure II-3). All cultures showed an initial lag period of slow growth for a few days and entered an exponential phase of growth. The time period for slow growth of *P. tricornutum* seemed to be shorter than that of *T. pseudonana*. *P. tricornutum* reached the stationary phase by day 11. Growth rates were calculated as mentioned above. The exponential growth phase was also indicated by arrows in Figure II-3.

Growth rates for *T. pseudonana* and *P. tricornutum* were all relatively high (1.49 to 2.24 div/d) on all Day 1 macroalgal culture media (Table II-5). In *T. pseudonana*, all growth rates were higher than that of the control. The ratio of the experimental growth rate to the control growth rate varied from 1.01 to 1.50. In *P. tricornutum*, on the other hand, all growth rates except *U. fenestrata* and *O. floccosa* media were lower than that of the control. The growth rates in *H. sessile* and *D. californica* media were significantly lower than that of the control (two sample t-test: both two-sided p-values < 0.01). The ratio of the experimental growth rate to the control growth rate varied from 0.76 to 1.03.

Final yields were calculated from mean phytoplankton abundance for the last two days of the culture experiment. In *T. pseudonana*, the final yields ranged from 1.80 to 2.75 (x 10^6 cells/ml) (Table II-6). The final yields from *O. floccosa*

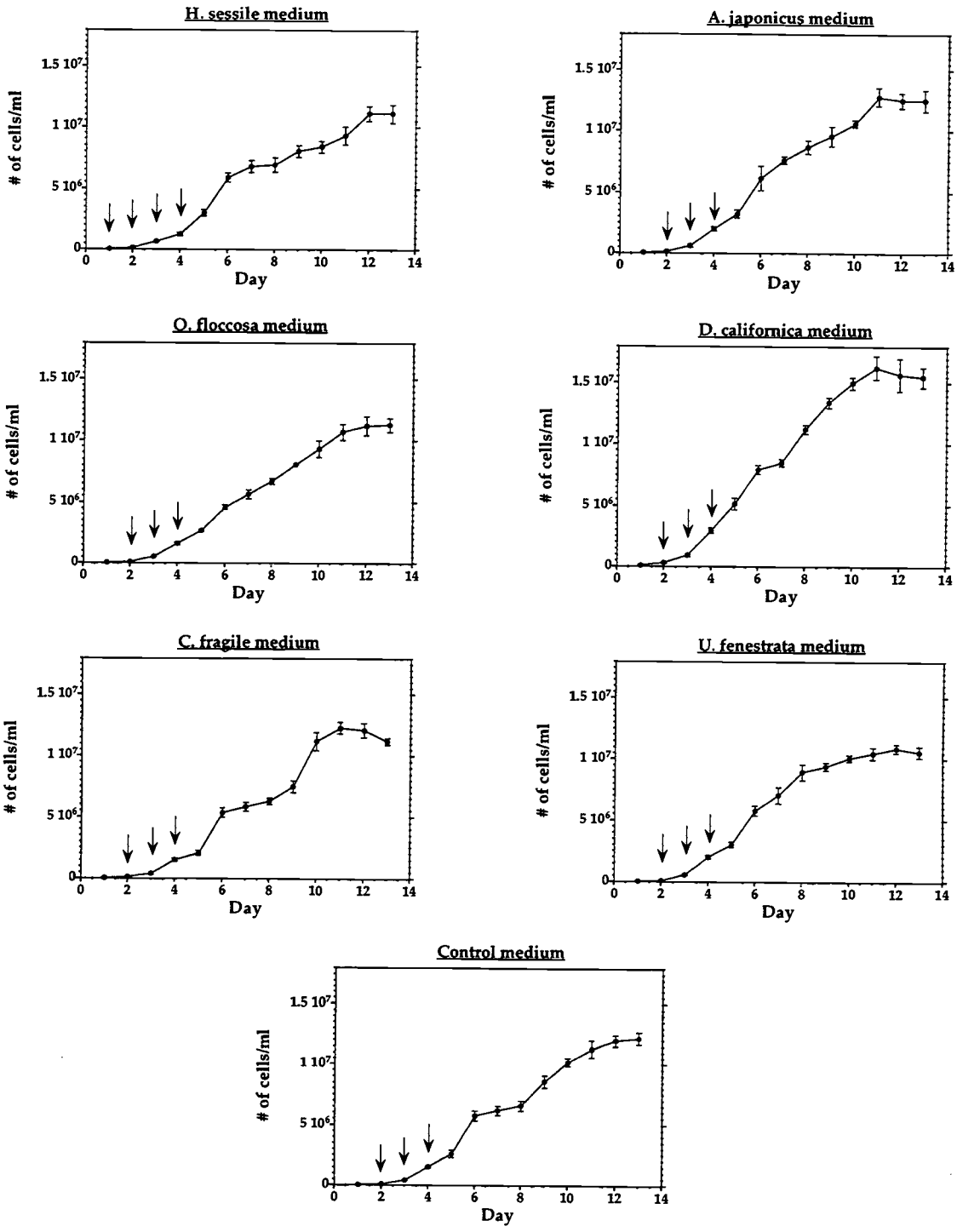

Figure II-3 Growth curves of *Phaeodactylum tricornutum* in the Day 1 seawater experiment. The points indicated by arrows were points which were used for growth rate calculations.

Table II-5 Growth rates of phytoplankton for the Day 1 experiment. For statistical difference, * shows that two-sided p-value is > 0.05, ** shows that two-sided p-value is between 0.01 and 0.05 and *** shows that two-sided p-value is < 0.01, based on two sample t-test between control and each experimental medium.

	T. pseudonana			*P. tricornutum*		
Type of seawater media	Day 1 Growth rate (±SE) (div/d)	Ratio (exp./cont.)	Statistical difference	Day 1 Growth rate (±SE) (div/d)	Ratio (exp./cont.)	Statistical difference
Control	1.49 (±0.09)	-	-	2.14 (±0.13)	-	-
H. sessile	1.63 (±0.08)	1.09	**	1.62 (±0.12)	0.76	***
A. japonicus	1.70 (±0.08)	1.14	**	1.89 (±0.13)	0.88	**
O. floccosa	2.24 (±0.13)	1.50	***	2.14 (±0.13)	1.00	*
D. californica	1.50 (±0.08)	1.01	*	1.66 (±0.09)	0.78	***
C. fragile	2.10 (±0.18)	1.41	***	1.86 (±0.07)	0.87	**
U. fenestrata	1.92 (±0.10)	1.29	***	2.20 (±0.10)	1.03	*

Table II-6 Final yields of phytoplankton for the Day 1 experiment. For statistical difference, * shows that two-sided p-value is > 0.05, ** shows that two-sided p-value is between 0.01 and 0.05 and *** shows that two-sided p-value is < 0.01, based on two sample t-test between control and each experimental medium.

Type of seawater media	*T. pseudonana* Day 1 Yield (±SD) (x 10^6 cells/ml)	Ratio (exp./cont.)	Statistical difference	*P. tricornutum* Day 1 Yield (±SD) (x 10^7 cells/ml)	Ratio (exp./cont.)	Statistical difference
Control	2.36(±0.04)	-	-	1.22(±0.05)	-	-
H. sessile	2.10(±0.02)	0.89	***	1.11(±0.02)	0.91	*
A. japonicus	2.60(±0.01)	1.10	***	1.25(±0.03)	1.02	*
O. floccosa	-	-	-	1.13(±0.02)	0.93	*
D. californica	2.75(±0.02)	1.16	***	1.55(±0.03)	1.27	***
C. fragile	-	-	-	1.11(±0.02)	0.91	*
U. fenestrata	1.80(±0.02)	0.76	***	1.08(±0.01)	0.89	**

and *C. fragile* media were not shown because of the contamination problem. The final yields from *A. japonicus* and *D. californica* media were higher than that from the control (two sample t-test: both two-sided p-values < 0.01) and the ratios of the experimental final yields to the control final yield were 1.10 to 1.16, respectively. However, those from *H. sessile* and *U. fenestrata* media were lower than that from the control (two sample t-test: both two-sided p-values < 0.01) and the ratio of the experimental final yields to the control final yield were 0.89 to 0.76, respectively.

In *P. tricornutum*, the final yields ranged from 1.08 to 1.55 (x 10^7 cells/ml). The final yields in *A. japonicus* and *D. californica* media were higher than that of the control and the ratios of the experimental final yields to the control final yield were 1.02 to 1.27, respectively. The rest of four media showed lower final yields than the control, even though the values were not different statistically (F-test, $F_{5, 10}$ = 0.42, two sided p-value > 0.5). The ratios ranged from 0.89 to 0.93 (Table II-6). No consistent inhibitory or stimulatory effect on the final yields was observed.

Compared to Day 1 macroalgal media, Day 3 media contained more TOC released from macroalgae (Figure II-1). If there is an inhibitory or stimulatory effect of TOC released from the macroalgae on phytoplankton, the effect would have been shown more significantly on the Day 3 experiment than on the Day 1 experiment.

T. pseudonana grew in all Day 3 culture media tested (Figure II-4). Growth curves of *T. pseudonana* on the Day 3 experiment were similar to those on the Day 1 experiment. All the growth rates except from *A. japonicus* and *U. fenestrata*

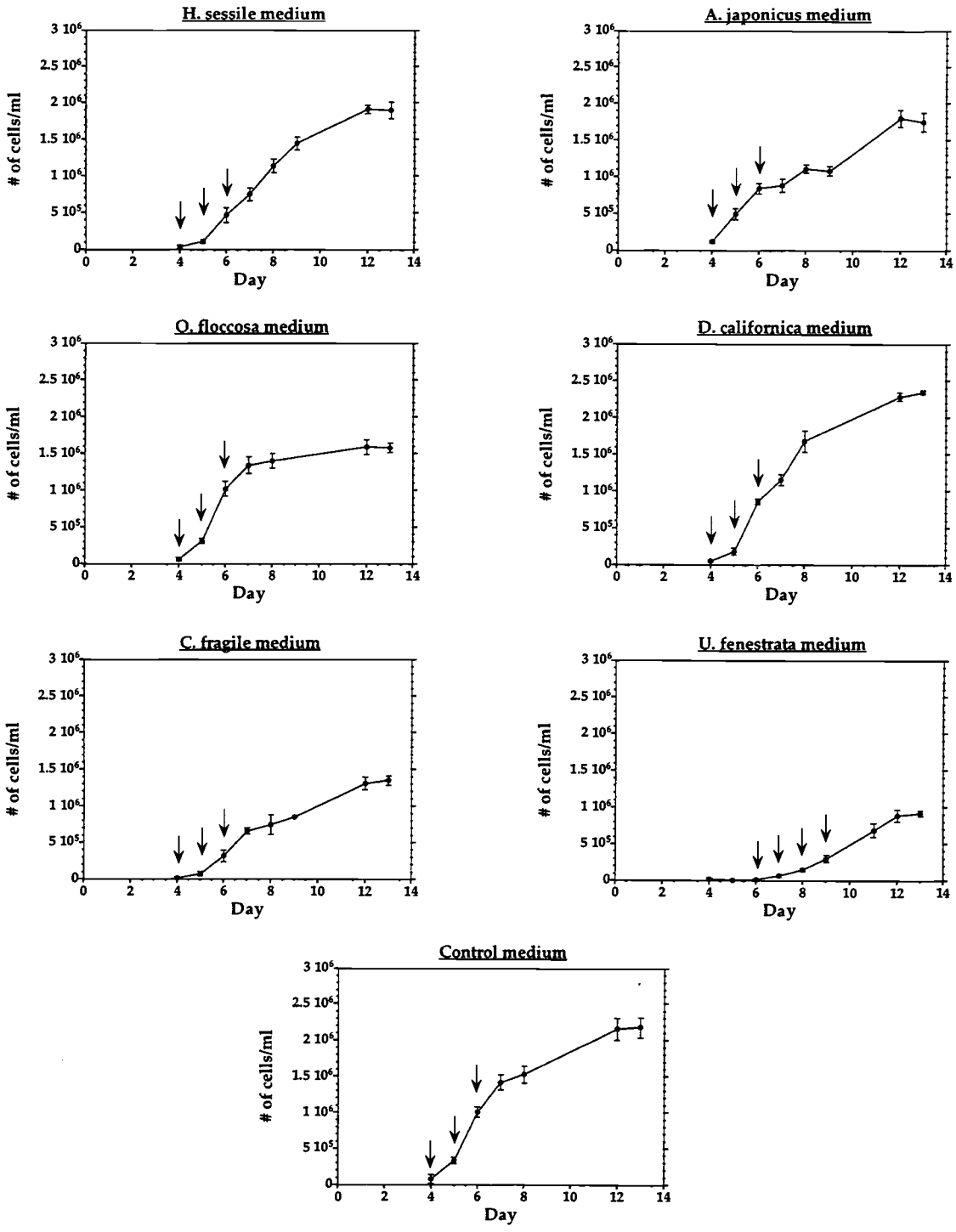

Figure II-4 Growth curves of *Thalassiosira pseudonana* in the Day 3 seawater experiment. The points indicated by arrows were points which were used for growth rate calculations.

media were slightly higher than that from the control, although the differences were not statistically significant (two sample t-test: two-sided p-values from *H. sessile*, *D. californica* and *C. fragile* > 0.05) except in *O. floccosa* medium (two sample t-test: two-sided p-value = 0.03, moderately significant) (Table II-7). The ratios of the experimental growth rate to the control growth rate varied from 1.04 to 1.13. However, *A. japonicus* and *U. fenestrata* media showed significantly lower growth rates than the control (two sample t-test: both two-sided p-value < 0.01) and the ratios of the experimental growth rate to the control growth rate were 0.82 and 0.60, respectively. In *P. tricornutum*, on the other hand, all growth rates except in *D. californica* medium were lower than that of the control (Figure II-5, Table II-7). The growth rates in *U. fenestrata and A. japonicus* media also showed the lowest and the second lowest (1.46 and 1.51 div/d, respectively). However, the differences were only moderately significant (two sided p-values were between 0.01 and 0.05). The ratios of the experimental growth rate to the control growth rate varied from 0.81 to 1.02.

Final yields were calculated as described above. In *T. pseudonana*, the final yields ranged from 0.92 to 2.35 (x 10^6 cells/ml) (Table II-8). All final yields except with *D. californica* medium were significantly lower than that of the control (two sample t-test: all two-sided p-values < 0.01). *U. fenestrata* medium showed the lowest final yield, and the value reached only less than half of that observed in control medium. The ratios of the experimental final yields to the control final yield were from 0.42 to 1.08. In *P. tricornutum*, the final yields ranged from 0.82 to 1.24

Table II-7 Growth rates of phytoplankton for the Day 3 experiment. For statistical difference, * shows that two-sided p-value is > 0.05, ** shows that two-sided p-value is between 0.01 and 0.05 and *** shows that two-sided p-value is < 0.01, based on two sample t-test between control and each experimental medium.

	T. pseudonana			*P. tricornutum*		
Type of seawater media	Day 3 Growth rate(±SE)(div./day)	Ratio (exp./cont.)	Statistical difference	Day 3 Growth rate(±SE)(div./day)	Ratio (exp./cont.)	Statistical difference
Control	1.87 (±0.10)	-	-	1.80 (±0.11)	-	-
H. sessile	1.94 (±0.19)	1.04	*	1.75 (±0.12)	0.95	*
A. japonicus	1.53 (±0.11)	0.82	***	1.51 (±0.12)	0.83	**
O. floccosa	2.12 (±0.13)	1.13	**	1.78 (±0.17)	0.97	*
D. californica	2.01 (±0.10)	1.07	*	1.84 (±0.10)	1.02	*
C. fragile	1.96 (±0.14)	1.05	*	1.66 (±0.13)	0.92	*
U. fenestrata	1.13 (±0.09)	0.60	***	1.46 (±0.15)	0.81	**

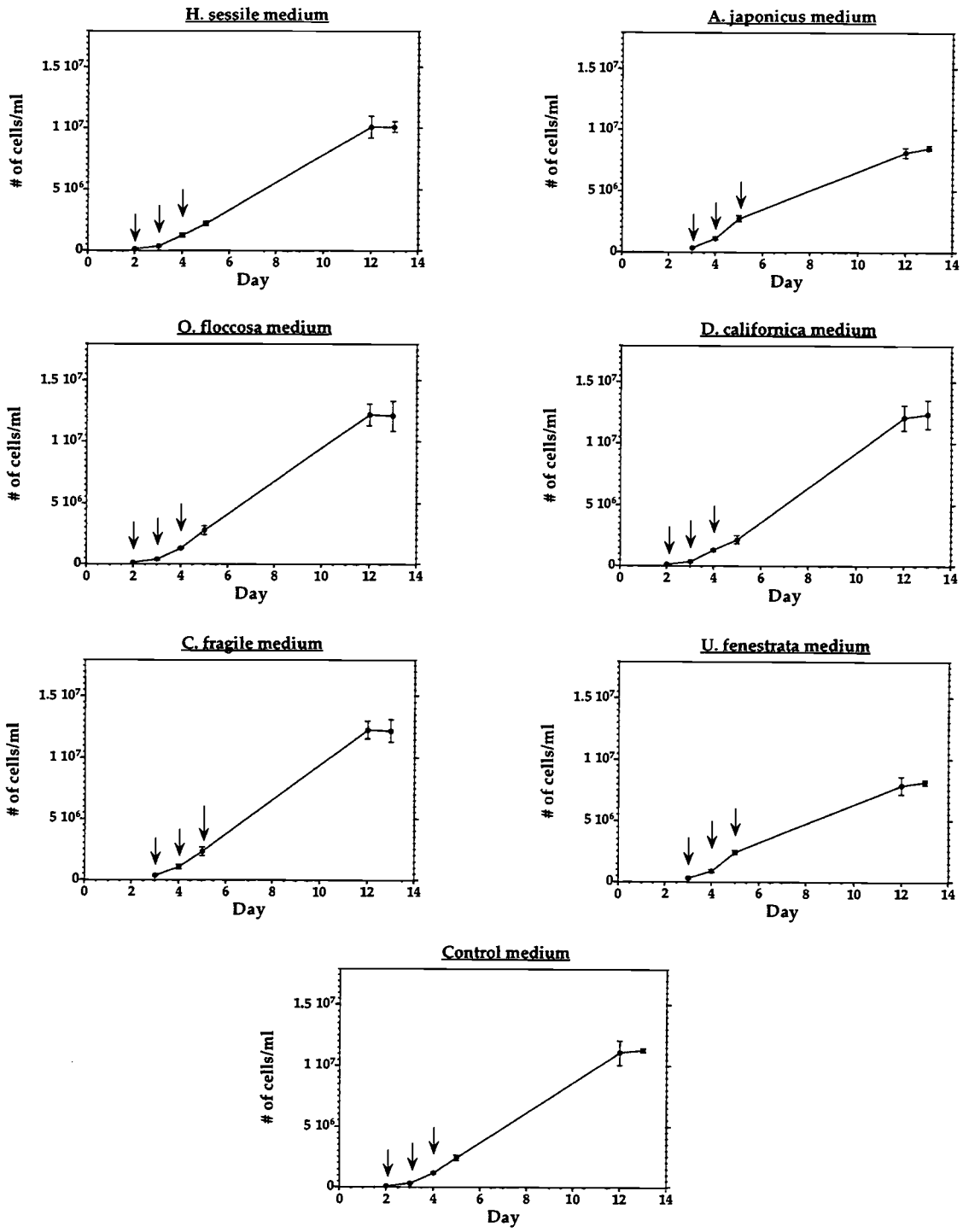

Figure II-5 Growth curves of *Phaeodactylum tricornutum* in the Day 3 seawater experiment. The points indicated by arrows were points which were used for growth rate calculations.

Table II-8 Final yields of phytoplankton for the Day 3 experiment. For statistical difference, * shows that two-sided p-value is > 0.05, ** shows that two-sided p-value is between 0.01 and 0.05 and *** shows that two-sided p-value is < 0.01, based on two sample t-test between control and each experimental medium.

Type of seawater media	*T. pseudonana* Day 3 Yield(±SD) (x 10^6 cells/ml)	Ratio (exp./cont.)	Statistical difference	*P. tricornutum* Day 3 Yield(±SD) (x 10^7 cells/ml)	Ratio (exp./cont.)	Statistical difference
Control	2.18(±0.02)	-	-	1.13(±0.02)	-	-
H. sessile	1.90(±0.01)	0.87	***	1.01(±0.04)	0.90	**
A. japonicus	1.75(±0.02)	0.80	***	0.85(±0.01)	0.75	***
O. floccosa	1.58(±0.04)	0.67	***	1.21(±0.01)	1.07	**
D. californica	2.35(±0.03)	1.08	***	1.24(±0.02)	1.10	**
C. fragile	1.34(±0.01)	0.61	***	1.22(±0.01)	1.08	**
U. fenestrata	0.92(±0.02)	0.42	***	0.82(±0.02)	0.73	***

(x 10^7 cells/ml) (Table II-8). The values in *A. japonicus* and *U. fenestrata* media were significantly lower than that in the control (two sample t-test: both two-sided p-values < 0.01). The ratios of the experimental final yields to the control final yield were 0.75 in *A. japonicus* medium and 0.72 in *U. fenestrata* medium, respectively. The final yields in the rest of the media did not show significant differences from that in the control (two sample t-test: all two-sided p-values > 0.1). The ratios of the experimental final yields to the control final yield ranged from 0.90 to 1.10.

These results were then compared between the Day 1 and Day 3 experiments in order to examine if the inhibitory or stimulatory effect would be more significant on Day 3 media than on Day 1 media. In *T. pseudonana*, the growth rates in control, *H. sessile* and *D. californica* media were significantly lower in Day 1 media than in Day 3 media (two sample t-test, all two-sided p-values < 0.01) (Table II-9). The values in the rest of media were higher for Day 1 than for Day 3 media. The value in *U. fenestrata* medium was significantly lower on Day 3 than on Day 1 medium (two sample t-test, two-sided p-value < 0.01). The ratio of the growth rate for Day 3 to Day 1 medium was only 0.59. For the final yields, *O. floccosa* and *C. fragile* media could not be compared the results between the Day 1 and Day 3 experiments because of the contamination problem on the Day 1 experiments. However, all of the other media showed significantly lower final yields in the Day 3 experiment compared with the Day 1 experiment (two sample t-test, all two-sided p-values < 0.01) (Table II-10). *A. japonicus* and *U. fenestrata*

Table II-9 Comparison of growth rates of phytoplankton between the Day 1 and the Day 3 experiments. For statistical difference, * shows that two-sided p-value is > 0.05, ** shows that two-sided p-value is between 0.01 and 0.05 and *** shows that two-sided p-value is < 0.01, based on two sample t-test between Day 1 and Day 3 media.

Type of seawater media	*T. pseudonana*				*P. tricornutum*			
	Growth rates (div./day)		Ratio(Day3/Day1)	Statistical difference	Growth rates (div./day)		Ratio(Day3/Day1)	Statistical difference
	Day 1	Day 3			Day1	Day3		
Control	1.49	1.87	1.26	***	2.14	1.80	0.84	***
H. sessile	1.63	1.94	1.19	***	1.62	1.75	1.08	**
A. japonicus	1.70	1.53	0.90	**	1.89	1.51	0.80	***
O. floccosa	2.24	2.12	0.95	*	2.14	1.78	0.83	***
D. californica	1.50	2.01	1.34	***	1.66	1.84	1.11	**
C. fragile	2.10	1.96	0.93	**	1.86	1.66	0.89	**
U. fenestrata	1.92	1.13	0.59	***	2.20	1.46	0.66	***

Table II-10 Comparison of final yields of phytoplankton between the Day 1 and the Day 3 experiments. For statistical difference, * shows that two-sided p-value is > 0.05, ** shows that two-sided p-value is between 0.01 and 0.05 and *** shows that two-sided p-value is < 0.01, based on two sample t-test between Day 1 and Day 3 media.

Type of seawater media	*T. pseudonana* Final Yields (x 10^6 cells/ml)		Ratio(Day3/Day1)	Statistical difference	*P. tricornutum* Final Yields (x 10^7 cells/ml)		Ratio(Day3/Day1)	Statistical difference
	Day 1	Day 3			Day1	Day3		
Control	2.36	2.18	0.92	***	1.22	1.13	0.93	**
H. sessile	2.10	1.90	0.90	***	1.11	1.01	0.92	**
A. japonicus	2.60	1.75	0.67	***	1.25	0.85	0.68	***
O. floccosa	-	1.58	-	-	1.13	1.21	1.07	**
D. californica	2.75	2.35	0.85	***	1.55	1.24	0.80	***
C. fragile	-	1.34	-	-	1.11	1.22	1.10	***
U. fenestrata	1.80	0.92	0.51	***	1.08	0.82	0.76	***

media especially showed much lower final yields in Day 3 media and the ratios of the final yields for Day 3 to Day 1 media were 0.67 and 0.51, respectively.

For *P. tricornutum*, *H. sessile* and *D. californica* media showed higher growth rates in Day 3 media than those in Day 1 media even though the differences were moderately significant (two sample t-test, both of two-sided p-values were between 0.01 and 0.05) (Table II-9). The other media showed lower growth rates in the Day 3 experiment compared with the Day 1 experiment. *U. fenestrata* medium showed the largest difference in the final yield between the two experiments. The ratio of the final yield for Day 3 to Day 1 was only 0.66. *O. floccosa* and *C. fragile* media showed higher final yields in the Day 3 experiment than in the Day 1 experiment (Table II-10). The rest of the media showed lower final yields in Day 3 media. *A. japonicus* and *U. fenestrata* media especially showed much lower final yields in the Day 3 experiment. The ratios of the final yields for Day 3 to Day 1 media were 0.68 and 0.76, respectively.

TOC release from phytoplankton

During the 14 days of phytoplankton growth experiment, TOC levels in all flasks increased in *T. pseudonana* (Table II-11). For the Day 1 experiment, the initial levels of TOC were between 65 and 84 μM and the final DOC levels increased and ranged from 514 to 1518 μM. Assuming that the initial levels of TOC were equal to those of DOC because the seawater was filtered by a 0.2 micron filtration capsule prior to use, the differences between the initial and final DOC

Table II-11 DOC release rates and carbon-specific release rates of *Thalassiosira pseudonana*. The release rates from *O. floccosa* and *C. fragile* on the Day 1 experiment were not calculated because of the contamination problem.

	Type of seawater media	Initial TOC (μM)	Final DOC (μM)	Difference (μM) (Final - Initial)	Final yield (x 10^6 cells/ml)	Release rate (pmol DOC $cell^{-1}$ d^{-1})	Carbon-specific release rate (pmol DOC pmol C^{-1} d^{-1})
Day1	Control	64.8	1035	970	2.36	0.029	0.0070
	H. sessile	78.4	516	438	2.10	0.015	0.0035
	A. japonicus	83.5	724	641	2.60	0.018	0.0042
	O. floccosa	74.0	514	440	-	-	-
	D. californica	75.6	1518	1443	2.75	0.038	0.0089
	C. fragile	72.4	594	521	-	-	-
	U. fenestrata	68.7	749	680	1.80	0.027	0.0064
Day3	Control	81.1	957	876	2.18	0.029	0.0068
	H. sessile	175	1207	1032	1.90	0.039	0.0092
	A. japonicus	316	986	666	1.75	0.038	0.0092
	O. floccosa	172	743	571	1.58	0.026	0.0062
	D. californica	115	1040	926	2.35	0.020	0.0049
	C. fragile	110	1106	996	1.34	0.053	0.0126
	U. fenestrata	115	309	195	0.92	0.015	0.0036

levels were estimated and variable (from 438 to 1443 μM). For the Day 3 experiment, the initial levels of TOC were between 81 and 316 μM, and the final DOC levels were from 310 to 1207 μM. The differences between the initial and final levels were also variable (from 195 to 1032 μM).

TOC levels in all flasks also increased for *P. tricornutum* (Table II-12). On the Day 1 experiment, the initial levels of TOC were between 65 and 84 μM, and the final DOC levels were from 320 to 498 μM. The differences between the initial and final DOC levels varied from 247 to 433 μM. For the Day 3 experiment, the initial levels of TOC were between 81 and 316 μM, and the final DOC levels were from 344 to 528 μM. The differences between the initial and final levels varied from 190 to 413 μM. The final DOC levels for *P. tricornutum* was less variable than those for *T. pseudonana*.

The release rates per cell (pmol DOC $cell^{-1}$ d^{-1}) were calculated, assuming that DOC release by the phytoplankton was constant. Then, the carbon-specific release rates (pmol DOC pmol C^{-1} d^{-1}), were calculated assuming that each cell of *T. pseudonana* and *P. tricornutum* contain 4.2 and 0.8 pmol carbon per cell, respectively (Brzezinski 1985). For *T. pseudonana*, the release rates from *O. floccosa* and *C. fragile* media could not be calculated because of the contamination problem described above. The release rates and carbon-specific release rates of the other cultures varied from 0.015 to 0.053 {average (±SD) = 0.025 (±0.015)} (pmol DOC $cell^{-1}$ d^{-1}) and from 0.0035 to 0.0126{average (±SD) = 0.0059 (±0.0035)} (pmol DOC pmol C^{-1} d^{-1}), respectively (Table II-11). For *P. tricornutum*, the release

Table II-12 DOC release rates and carbon-specific release rates of *Phaeodactylum tricornutum.*

	Type of seawater media	Initial TOC (μM)	Final DOC (μM)	Difference (μM) (Final - Initial)	Final yield (x 10^7 cells/ml)	Release rate (pmol DOC $cell^{-1}$ d^{-1})	Carbon-specific release rate (pmol DOC pmol C^{-1} d^{-1})
Day1	Control	64.8	498	433	1.22	0.0025	0.0032
	H. sessile	78.4	458	391	1.11	0.0024	0.0031
	A. japonicus	83.5	462	378	1.25	0.0022	0.0027
	O. floccosa	74.0	365	291	1.13	0.0018	0.0023
	D. californica	75.6	447	371	1.55	0.0017	0.0021
	C. fragile	72.4	320	247	1.11	0.0016	0.0020
	U. fenestrata	68.7	445	376	1.08	0.0025	0.0031
Day3	Control	81.1	393	312	1.13	0.0020	0.0025
	H. sessile	175	514	339	1.01	0.0024	0.0030
	A. japonicus	316	505	190	0.85	0.0035	0.0043
	O. floccosa	172	344	172	1.12	0.0011	0.0014
	D. californica	115	528	413	1.24	0.0011	0.0014
	C. fragile	110	446	336	1.22	0.0020	0.0025
	U. fenestrata	115	528	413	0.92	0.0036	0.0045

rates and carbon-specific release rates varied from 0.0011 to 0.0036 {average (±SD) = 0.0022 (±0.0007)} (pmol DOC $cell^{-1}$ d^{-1}) and from 0.0014 to 0.0045 {average(±SD) = 0.0027 (±0.0009)} (pmol DOC pmol C^{-1} d^{-1}), respectively (Table II-12).

Comparison of TOC Release Rates between Macroalgae and Phytoplankton

The carbon-specific release rates were used to compare the release rates between macroalgae and phytoplankton. The release rates of macroalgae ranged from 1.42 $x10^{-5}$ to 8.57 $x10^{-5}$ (pmol TOC pmol C^{-1} d^{-1}) whereas the release rates of phytoplankton ranged from 271 $x10^{-5}$ to 588 x 10^{-5} (pmol DOC pmol C^{-1} d^{-1}). Thus, phytoplankton release rates were about 50 - 200 times higher than those of macroalgae (Table II-13).

Discussion

TOC Release from Macroalgae

In this experiment, all macroalgae tested released TOC. The release of each macroalga was almost linear ($R^2 > 0.98$) through the experiment. Thus, the release rates were calculated by using all plots from day 0 to 3. *A. japonicus* (Phaeophyta) had the highest release rate. Sieburth (1969) reported that Phaeophyta released

Table II-13 Comparison of carbon-specific release rates between macroalgae and phytoplankton.

Species name	Carbon-specific release rate (mg C 100 g C^{-1} h^{-1})	Carbon-specific release rate (x 10^{-5} pmol TOC (or DOC) pmol C^{-1} d^{-1})
Macroalgae		
H. sessile	0.207	4.97
A. japonicus	0.357	8.57
O. floccosa	0.135	3.24
D. californica	0.064	1.54
C. fragile	0.059	1.42
U. fenestrata	0.297	4.58
Phytoplankton		
T. pseudonana		588
P. tricornutum		271

much more TOC than the other two macroalgal groups. However, in my experiment, *U. fenestrata* (Chlorophyta), had the second highest release rate. A significant difference of TOC release rates among the three algal divisions was not observed in my experiments.

Compared to results reported by Sieburth (1969), Khailov and Burlakova (1969) and Pregnall (1983), TOC release rates in my experiment were far lower (up to 100 times lower) (Table II-14). The rates, however, were as low as those of Horner and Smith (1984). Laboratory and field studies of DOC release rates showed a large range of values (Horner and Smith 1984). Causes leading to the wide range of DOC release rates among macroalgae will be discussed later in Chapter III because the experiments in Chapter III focused on examining the DOC release of both macroalgae and phytoplankton in different conditions.

The TOC values also increased in the control tub. Because the top of the tub was open during the experiment, the additional organic carbon may have been provided into seawater from the air. However, even though the increase was statistically significant (p-value < 0.01), the change was still small enough to neglect compared to the increase of TOC by macroalgae.

Phytoplankton Growth Experiment

Both phytoplankton species grew well in all culture media except two flasks of *T. pseudonana* which became contaminated with flagellated organisms. By day 6 of the experiment, the flagellated organisms had consumed most of the *T.*

Table II-14 Comparison of TOC or DOC release rates by macroalgae.

	Species	TOC/DOC release rates (mgC 100g dry wt^{-1} h^{-1})	Data sources
(Phaeophyta)	*Hedophyllum sessile*	0.67	This experiment
	Analipus japonicus	1.15	This experiment
	Laminaria digitata	44.2	Sieburth (1969)
	Laminaria agardhii	17.4	Sieburth (1969)
	Fucus vesiculosus	39.2	Sieburth (1969)
	Fucus vesiculosus	37.2- 130	Khalihov and Burkova (1969)
	Padina japonica	14.0	Schramm et al. (1984)
(Rhodophyta)	*Odontholia floccoca*	0.43	This experiment
	Dilsea californica	0.21	This experiment
	Chondrus crispus	4.4	Sieburth (1969)
	Polysiponia harveyi	0.4	Sieburth (1969)
	Rhodimenia palmata	89.9- 304	Khalihov and Burkova (1969)
	Gracilaria acerosa	4.6	Schramm et al. (1984)
(Chlorophyta)	*Codium fragile*	0.19	This experiment
	Ulva fenestrata	0.96	This experiment
	Ulva lactuca	20.8	Sieburth (1969)
	Ulva lactuca	0.14	Horner and Smith (1984)
	Enteromorpha prolifera	26.0	Pregnall (1983)

pseudonana. All of the flasks had been acid-washed for a day and then baked at 80 °C in the oven for another day before the experiment. The temperature may not be high enough to kill these organisms in the two flasks and may have led to the contamination.

For the Day 1 macroalgal culture media, some media may have stimulated or inhibited phytoplankton growth compared to the control media, but the effect was neither strong nor consistent. For example, even though *H. sessile* medium had lower final yields in both phytoplankton species than the control (Table II-6), it had higher growth rate for *T. pseudonana*, but lower for *P. tricornutum* than the control (Table II-5). For the Day 3 macroalgal culture media, the results did show consistent inhibitory effects on both phytoplankton species for two macroalgal media, *A. japonicus* and *U. fenestrata*. The ratios of the growth rates of *T. pseudonana* and *P. tricornutum* in *A. japonicus* medium to those in the control were 0.82 and 0.83, respectively and in *U. fenestrata* medium, the ratios were 0.60 and 0.81, respectively (Table II-7). The ratios of the final yields of *T. pseudonana* and *P. tricornutum* in *A. japonicus* medium to those in the control were 0.80 and 0.75, respectively and in *U. fenestrata* medium, the ratios were 0.42 and 0.73, respectively (Table II-8). The rest of macroalgal media did not show any consistent stimulatory or inhibitory effects on phytoplankton growth.

Although some weak inhibitory effects on phytoplankton growth were observed for two out of six macroalgal media tested in this experiment, the results are suggestive but inconclusive because of the lack of replication. The original

intent of this experiment was to screen as many macroalgae as possible for inhibitory effects on phytoplankton. If any of the macroalgal media had shown a greater inhibitory effect, then the experiment would have been repeated for those species. Since no strong effects were observed, no replication was attempted.

Nonetheless, the results do not support the hypothesis that release of inhibitory stubstances by macroalgae is the main cause of low phytoplankton abundance at Boiler Bay. Since phytoplankton abundance is ten times lower at Boiler Bay than at Strawberry Hill, any inhibitory release products would have to have a very large effect on either growth rates or final yields. Four of six macroalgal media showed no inhibitory effect. Two species, *U. fenestrata* and *A. japonicus* inhibited growth by 40 % and 16 % respectively, and reduced final yields by 60 % and 25 % respectively. Even if all the macroalgae at Boiler Bay were as inhibitory as *Ulva*, the effect is too small to explain the very low abundance of phytoplankton at that site.

There are a few other possibilities to explain low phytoplankton abundance at Boiler Bay. One is that herbivores, filter feeders and grazers may control phytoplankton abundance. In the tidal zones, the main consumers of phytoplankton are mussels and barnacles, and zooplankton such as copepods play only a minor role as grazers. Menge et al. (1997b) found that both barnacles and mussels were always more abundant at Strawberry Hill than at Boiler Bay. Thus, grazing does not appear to control phytoplankton abundance at Boiler Bay. Another possibility is that rates of nearshore water exchange are different as a result of differences in

rates of coastal upwelling and nearshore circulation patterns. These differences may provide more favorable conditions for phytoplankton retention and growth at Strawberry Hill (Menge et al. 1997a, 1997b). However, a better understanding of the nearshore circulation patterns is needed to test this hypothesis.

In conclusion, I found suggestive results that some macroalgae release substances which can inhibit phytoplankton growth, but the results are not sufficient to explain the low phytoplankton abundance at Boiler Bay.

DOC Release from Phytoplankton

Both phytoplankton species released DOC during the two-week experiment. The DOC release rates were calculated assuming that the DOC release was constant during the experiment because samples were taken only the first and last day of the experiment. The rates ranged from 0.0027 (pmol DOC pmol C^{-1} d^{-1}) in *P. tricornutum* to 0.0059 (pmol DOC pmol C^{-1} d^{-1}) in *T. pseudonana*. These values are one order magnitude lower than those reported by Biddanda and Benner (1997) (Table II-15). For some phytoplankton species, the DOC release rates seem to be maximum during the exponential growth phase (Biddanda and Benner 1997). Thus, the values calculated in my experiment could be underestimated. It would be necessary to sample more frequently (e.g. at least every three days) to solve this problem.

Table II-15 Comparison of carbon-specific release rates of phytoplankton with the data of Biddanda and Benner (1997).

Phytoplankton Species	DOC Release Rates (pmol DOC pmol C^{-1} d^{-1})	Data Sources
T. pseudonana	0.0059	This experiment
P. tricornutum	0.0027	This experiment
Skeletonema costatum	0.067	Biddanda and Benner (1997)
Synechococcus bacillaris	0.012	Biddanda and Benner (1997)
Pheocystis sp.	0.038	Biddanda and Benner (1997)
Emiliania huxleyi	0.025	Biddanda and Benner (1997)

Comparison of TOC/DOC Release Rates between Macroalgae and Phytoplankton

First of all, to compare the release rates between two groups, the rates were converted into the same unit. Carbon-specific release rates by phytoplankton were about two orders of magnitude higher than those of macroalgae (Table II-13). Because the DOC release rates for macroalgae in this experiment were nearly two orders of magnitude lower than those reported by Sieburth (1969), Khailov and Burlakova (1969) and Pregnall (1983) (Table II-14), a comparison between macroalgae is still difficult. However, since the release rates of phytoplankton appear to have been significantly underestimated in the present experiment, the DOC release by phytoplankton in fact may be high compared with that of macroalgae.

There may be several explanations for phytoplankton having higher DOC release rates than macroalgae. One possibility is that phytoplankton have much higher surface area to volume (SA:V) ratios than macroalgae. Since both groups acquire nutrients and excrete metabolites across the entire surface of area of cells or thallus, the rate of uptake and release should be a function of SA:V ratios. There are two transport mechanisms in phytoplankton and macroalgae. One is an active transport which is defined as transport in a direction opposite to that predicted from chemical and electrical driving forces acting on solute. Large molecules such as polysaccharides have very low diffusion coefficients and may require the active transport mechanisms for significant release across the cell membrane.

The other transport mechanism is a passive transport in which the membrane merely acts as a barrier permitting migration of solute on solvent to take place at a reduced rate. It has been suggested that release of low-molecular-weight organic carbon (LMWOC), such as free amino acids (FAA) and free sugars, may not be an active transport, but simply a result of unavoidable passive permeation through the cell membrane (Hellebust 1974, Mague et al. 1980, Bjørnsen 1988). The SA:V ratios of phytoplankton usually range from 10^4 to 10^5 (cm^2/cm^3) (Lewis 1976, Malone 1980), while those of macroalgae range from 10^1 to 10^2 (cm^2/cm^3) (Rosenberg and Ramus 1984). The relationship between nutrient uptake and SA:V ratio has been well examined, with rates of uptake being positively correlated with SA:V ratio in macroalgae (Rosenberg and Ramus 1984, Hein et al. 1995, Taylor et al. 1998) and phytoplankton (Lewis 1976, Chisholm 1992). Rosenberg and Ramus (1984) examined the relationship of nutrient uptake and SA:V ratio of four macroalgae including *Ulva curvata* and *Codium decorticatum*. They reported that the SA:V ratio of *U. curvata* was about 20 times higher than that of *C. decorticatum* and the uptake of ammonium was about 12 times higher. In my experiment, the TOC release rate of *U. fenestrata* was about 5 times higher than that of *C. fragile*. The TOC release rates also seem to be positively correlated with SA:V ratio in macroalgae. This may imply that phytoplankton should have higher DOC release rates due to higher SA:V ratios compared to macroalgae.

In passive diffusion, the release rate through the cell or tissue membrane depends on the concentration gradient across the membrane, its diffusion

coefficient, and the permeability of the membrane for each substance. The flux of a substance out of a cell or tissue is given by Yeagle (1991):

$$\frac{\partial C}{dt} = A\frac{DmK}{l}(Ci - Co),$$

Where A is the surface area of the cell, D_m is the diffusion coefficient of the substance in the membrane, K is the partition coefficient between the membrane and the lipid phases, l is the thickness of the membrane and Ci and Co are the concentrations of the substance inside and outside the membrane, respectively. The term D_mK/l is defined as the permeability coefficient (P) and has the dimensions of velocity.

If the concentrations of the substance inside the membrane, the permeability coefficient, or both are significantly different between phytoplankton and macroalgae, the difference(s) may also affect the DOC release rates. Internal pools of LMWOC are mainly FAA and sugars. Average FAA pool is about 10 % of total cellular nitrogen in phytoplankton (Wheeler 1983) while 10 - 15 % in macroalgae (Naldi and Wheeler 1999). Carbohydrates, a main carbon source in both phytoplankton and macroalgae, include free sugars. Average carbohydrate is about 10 – 15 % of the cell dry weight in phytoplankton (Parson et al. 1961, Darley 1977, Brown and Jeffrey 1992, Chu et al. 1996) and 40 – 60 % of the tissue dry weight in macroalgae (Montgomery and Gerking 1980, Bird et al. 1982, Lawrence and McClintock 1988) Macroalgae allocate substantial amounts of carbon to their cell walls. When soluble carbohydrates such as mono-, di-, oligosaccharides are compared, they are interestingly only 1 – 2 % of the cell or tissue dry weight in

both groups (Lawrence and McClintock 1988, Brown and Jeffrey 1992), although the ratios of total carbohydrates to dry weight are very different. However, both carbohydrate and FAA pools significantly vary with nutrient conditions (Bird et al. 1982, Miyazaki and Miyashita 1993, Chu et al. 1996). Thus, internal pools of LMWOC may not be significantly different between two groups, but rather may be more strongly influenced by nutrient conditions.

Stadelmann (1962) reported permeability of many substances from various algal cells. A high permeability to glucose and sucrose was found in some diatoms, but this is a not typical characteristic of diatoms, but species-specific (Stadelmann 1962). Moreover, the permeability measurement of organic carbon especially for macroalgae has been scarce. Therefore, I could not find similarities or differences of permeability between phytoplankton and macroalgae.

In conclusion, DOC may be released mainly by a passive transport, and the DOC release rate may be positively correlated with SA:V ratio. Phytoplankton usually have such high SA:V ratio (at least 100 times higher than macroalgae) and the release rates of DOC are also high. The composition of internal DOC pools seems to be similar between macroalgae and phytoplankton even though it may change significantly with different nutrient conditions. More data on permeability coefficients is needed for a thorough analysis of the underlying cause(s) of different DOC release rates between two groups. In addition, the effects of nutrients and other culture conditions need to be examined in more detail.

Chapter III

COMPARISON OF TOC/DOC RELEASE BETWEEN MACROALGAE AND PHYTOPLANTKON FOR DIFFERENT CULTURE CONDITIONS

In Chapter II, it was confirmed that both macroalgae and phytoplankton release organic carbon. However, I found that DOC release rates as well as ratios of DOC released to total fixed carbon reported in literature were substantially different in macroalgal experiments. The ratio of DOC released to total fixed carbon ranged from 0.0002 % (Fankboner and DeBurgh 1977) to 40 % (Sieburth 1969 and Khailov and Burlakova 1969). Sieburth (1969) reported that enormous amounts of DOC were released from macroalgae tested in his continuous culture experiment, but none in his batch culture experiment.

In this chapter, I describe experiments designed to test whether DOC release rates as well as the ratio of DOC released to total fixed carbon were different between batch culture and continuous culture experiments for both phytoplankton and macroalgae. In phytoplankton batch culture experiments, samplings were made more frequently (every two or three days) to eliminate the underestimation of release rates pointed out in Chapter II.

Methods

Macroalgal Experiment (Continuous Culture)

Continuous cultures of *Palmaria mollis* (Rhodophyceae) were provided by Carl Demetropoulos (OSU Hatfield Marine Science Center). The macroalga was kept in 18 L tanks in which seawater was exchanged approximately once a day. There were six different nutrient conditions controlling the macroalgal growth, but in this study three conditions were chosen for sampling TOC, DOC, POC, PON and bacterial samples: (1) NO_3^- media with a high C: N ratio (21.2), (2) NO_3^- + NH_4^+ media with a high C: N ratio, (3) NO_3^- + NH_4^+ media with a low C: N ratio (5.5) (Table III-1). Samples were taken from two tanks of each nutrient condition and three media tanks to measure initial seawater conditions. Triplicate 5 ml TOC samples were taken directly from each tank into 7 ml glass vials as described in Chapter II. Triplicate 50 ml seawater was taken by acid-washed 60 ml syringes and then filtered through 25 mm GF/F filters to estimate POC and PON. Triplicate 5 ml samples of the filtered seawater were stored in the glass vials for DOC samples. The filters were stored in vacutainers for POC/PON samples. The vacutainers were then stored in a freezer to prevent POC/PON from being decomposed by bacteria. Additional 10 ml samples of seawater were also taken from each tank for bacterial samples. Formaldehyde was added to the seawater for preservation at the final concentration of 5 %.

TOC and DOC samples were measured as described in Chapter II except with the use of new standard seawater. The deep seawater which was taken from 1000 m deep at NH-85 (44° 39' N, 126° 03' W) was used for standard seawater sample because some contamination was found with the 9°N seawater which was previously used as standard seawater sample.

Table III-1 Three nutrient conditions in the continuous culture experiment of *P. mollis*. Each different nutrient condition is referred to NO_3^-, NO_3^- + NH_4^+ (high C) and NO_3^- + NH_4^+ (low C).

Media	Nitrogen sources	C: N
NO_3^-	100 % NO_3^-	21.2
NO_3^- + NH_4^+ (high C)	NO_3^-: NH_4^+ = 1: 2	21.2
NO_3^- + NH_4^+ (low C)	NO_3^-: NH_4^+ = 1: 2	5.5

After return to the lab at OSU, POC and PON samples were measured using a CNS analyzer (Carlo Erba NA-1500) (Verardo et al. 1990). The GF/F filters were placed into a glass chamber with 12 N HCl for acid-fuming for a day and dried completely in a 60 °C oven for another day. Then, each filter was packed into a tin capsule. The instrument was calibrated with a five point standard curve of L-cystine (Macroanalysis). The standards (ranging from 0.1 – 1 mg) were prepared prior to each run by using a microbalance (Cahn C-31). The five point standard series was run before the analysis of samples to test the instrument condition. Additional standards were run between every six samples. Triplicate of blank tin capsules and blank GF/F filters were also measured to estimate carbon and nitrogen

blanks. Sample concentrations were then estimated by subtracting both of the blanks.

Bacterial subsamples (2 ml) of each duplicate water sample were stained with DAPI (4'-6-diamidino-2-phenylidole), according to a procedure modified from Suzuki et al. (1993). DAPI samples were stained with 20 µl of 1 mg ml^{-1} DAPI stock solution (25 µg ml^{-1} final concentration) for 7 min, then filtered through 0.2 µm black Nuclepore polycarbonate filters mounted on prewetted 0.45 µm Nuclepore Membrafil cellulosic filters. Sample filters were removed from the filtration tower under vacuum to prevent cells from floating off the filter. The filters were then mounted on glass slides with a drop of Cargille type A immersion oil on the slide beneath the filter and a drop of immersion oil on top of the filter beneath the cover slip.

For each prepared slide, the number of bacterial cells were counted within an ocular grid in seven randomly selected fields (magnification, 1250 X). Microscopy was performed with a Zeiss Universal epifluorescence microscope equipped with a 75-W xenon lamp. The counts were made with Zeiss UV filter set 47, 77, 02 (G 365 excitation filter, FT 395 beam splitter, and LP 420 barrier filter).

Macroalgal Experiment (Batch Culture)

After the continuous culture experiment, additional samples of *P. mollis* were brought back to the lab for a batch culture experiment. For experimental incubations, the macroalga (25 – 40 g wet weight) was transferred to eight plastic

tubs with 3 L seawater. Three different nutrient conditions as described in the continuous culture experiment were prepared in each of two tubs (Table III-1). Two tubs with NO_3^- medium but no macroalga were prepared for the control. The macroalga was maintained at 14 °C with photon irradiances of about 100 $\mu E\ m^{-2}\ s^{-1}$ and a light: dark cycle of 14: 10 h during the experiment. Duplicate subsamples of about 5 g wet weight macroalga were collected and dried in order to calculate the ratio of dry weight to wet weight (Table III-2).

Triplicate samples of TOC, POC and PON were taken every 24 h with the same procedure as described in the continuous culture experiment in this Chapter. Triplicate DOC samples were also taken for Day 0 and Day 3. After the final sampling, the wet weight of all experimental macroalga was measured to estimate their growth.

Phytoplankton Experiment (Continuous Culture)

A motile, unicellular green alga, *Dunaliella tertiolecta*, was used for both continuous and batch culture experiments. The continuous culture was provided by Ricardo Letelier (OSU). *D. tertiolecta* was kept in a 2 L tank and 200 ml seawater medium in the tank was exchanged per day. The light condition was similar to the 5 m deep water column where *D. tertiolecta* was sampled. Triplicate samples for DOC, POC, PON and cell counts were taken from incoming medium and inside tank. All of the measurements were the same as in the previous experiments.

Table III-2 Estimation of experimental and subsample wet and dry weights of *P. mollis*.

Nutrient conditions	Initial wet weight (g)	Final wet weight (g)	Initial estimated dry weight (g)	Final estimated dry weight (g)
NO_3^- – 1	27.7	35.9	3.6	4.7
NO_3^- – 2	40.2	47.8	5.2	6.2
NO_3^- + NH_4^+ (high C) – 1	28.1	39.1	3.8	5.1
NO_3^- + NH_4^+ (high C) – 2	37.1	48.9	4.8	6.4
NO_3^- + NH_4^+ (low C) – 1	28.2	36.0	3.7	4.7
NO_3^- + NH_4^+ (low C) – 2	31.6	38.9	4.1	5.1

Note) * Two subsamples were taken in order to calculate a dry/wet ratio. Mean = 13 %.

Subsample	Wet weight (g)	Dry weight (g)	Dry/Wet ratio (%)
1	3.566	0.466	13.1
2	3.425	0.441	12.9

Phytoplankton Experiment (Batch Culture)

The stock culture of *D. tertiolecta* was obtained from Tim Cowles (OSU). The seawater collected from HMSC was filtered with a 0.2 micron filtration apparatus for the culture medium. The nutrients (nitrate, phosphate, vitamins and trace metals) were added to the seawater as described in the previous phytoplankton experiment.

Approximately 100 ml of culture medium was poured into twenty-eight 125 ml flasks, and *D. tertiolecta* was inoculated into each flask and maintained at 14 °C with photon irradiances of about 100 $\mu E\ m^{-2}\ s^{-1}$ and a light: dark cycle of 14: 10 h. Triplicate flasks were randomly sampled for POC, PON, DOC and cell counts on day 0, 2, 4, 6, 9, 12 and 15. TOC samples were also taken, but measured only on day 0 and 2 because POC increased so much that TOC concentration was far beyond the instrument capacity after day 4. Subsamples of 20 ml seawater were filtered through GF/F filters. The filters were stored into vacutainers and frozen at – 5 °C until analysis. Triplicate 5 ml samples of DOC were taken from the filtered seawater. Because *D. tertiolecta* was motile, one droplet of Lugol's was added to the samples prior to cell counts in order to kill the cells.

Results

Macroalgal Experiment (Continuous Culture)

TOC and DOC measurements were calibrated by using the TOC standards and deep seawater (Table III-3). The slopes of linear regressions ranged from 61.5 to 83.6 {average (±SD) = 71.3 (±6.1)}. The intercepts ranged from 829 to 1876 {average (±SD) = 1255 (±297)}. Each linear regression showed a high R^2 value ($R^2 > 0.998$). TOC values of the deep seawater samples were also stable. 48 samples were run for the experiments, and none of them showed any evidence of contamination. Nominal average and corrections for internal standards were calculated as described in Chapter II (Table III-3).

Initial TOC concentrations in the media ranged from 292 to 358 (μM) (Figure III-1). The values were much higher than those in the previous experiment (70 – 80 μM in the initial condition), even though both were collected from HMSC. Excess carbon contents in this experiment may have come from added nutrient solutions. The trace metal solution chelated with EDTA (Ethylene Diamine Tetra Acetic acid) which contains 6 carbon atoms per molecule was used to prevent iron from precipitating in this experiment. This effect will be discussed later.

TOC concentrations in all culture tanks were higher than those in media tanks (two sided t-test, NO_3^- medium: two sided p-value < 0.001, $NO_3^- + NH_4^+$ (high C) medium: two sided p-value = 0.006, and $NO_3^- + NH_4^+$ (low C) medium: two sided p-value = 0.001) (Figure III-1), which implied that *P. mollis* released

Table III-3 Log sheet of slopes, intercepts, r-square of standards and deep seawater samples for TOC/DOC determination. Daily average was the average of three daily deep seawater samples. Internal Standard Correction = (Nominal Average)/(Daily Average). Nominal average was the average of all 48 deep seawater samples.

Date	Sample	Slope	Intercept	R^2	Deep seawater 1 (μM)	Deep seawater 2 (μM)	Deep seawater 3 (μM)	Daily Average	Internal Standard Correction
7/10/98	*D. tertiolecta* (Batch 1)	83.6	1025	1.0000	42.5	43.1	41.4	42.3	1.063
7/11/98	*D. tertiolecta* (Batch 2)	71.5	1294	0.9996	38.8	36.7	37.3	37.6	0.944
7/16/98	*D. tertiolecta* (Batch 3)	74.7	1523	0.9998	40.1	41.3	39.4	40.3	1.011
7/26/98	*D. tertiolecta* (Cont.)	75.5	1131	0.9986	43.2	38.6	39.7	40.5	1.017
10/3/98	*P. mollis* (Cont. 1)	72.0	1374	0.9999	38.2	36.2	37.1	37.2	0.934
10/7/98	*P. mollis* (Cont. 2)	76.1	829	0.9993	41.6	40.0	42.5	41.4	1.039
10/13/98	*P. mollis* (Cont. 3)	69.0	1291	0.9997	40.5	39.1	39.0	39.5	0.993
10/16/98	*P. mollis* (Cont. 4)	63.9	816	0.9985	39.1	39.3	41.6	40.4	1.005
10/24/98	*P. mollis* (Batch 1)	65.0	973	1.0000	40.8	36.6	41.3	39.6	0.994
10/24/98	*P. mollis* (Batch 2)	61.8	927	0.9997	40.9	38.7	40.6	40.1	1.006
10/30/98	*P. mollis* (Batch 3)	69.7	1355	0.9998	41.7	40.5	39.8	40.7	1.021
11./7/98	*P. mollis* (Batch 4)	75.8	1356	0.9990	37.0	41.8	38.9	39.2	0.985
11/11/98	*P. mollis* (Batch 5)	73.6	1641	0.9994	40.0	39.7	39.0	39.6	0.994
11/13/98	*P. mollis* (Batch 6)	61.5	1476	0.9996	38.7	37.3	38.9	38.3	0.962
11/25/98	*P. mollis* (Batch 7)	77.7	1198	0.9999	43.7	39.7	39.6	41.0	1.030
12/3/98	Nutrients	68.6	1876	0.9998	38.6	37.0	37.4	37.7	0.947
	Average	71.3	1255				Nominal	39.8	
	SD	6.1	297				SD	1.8	

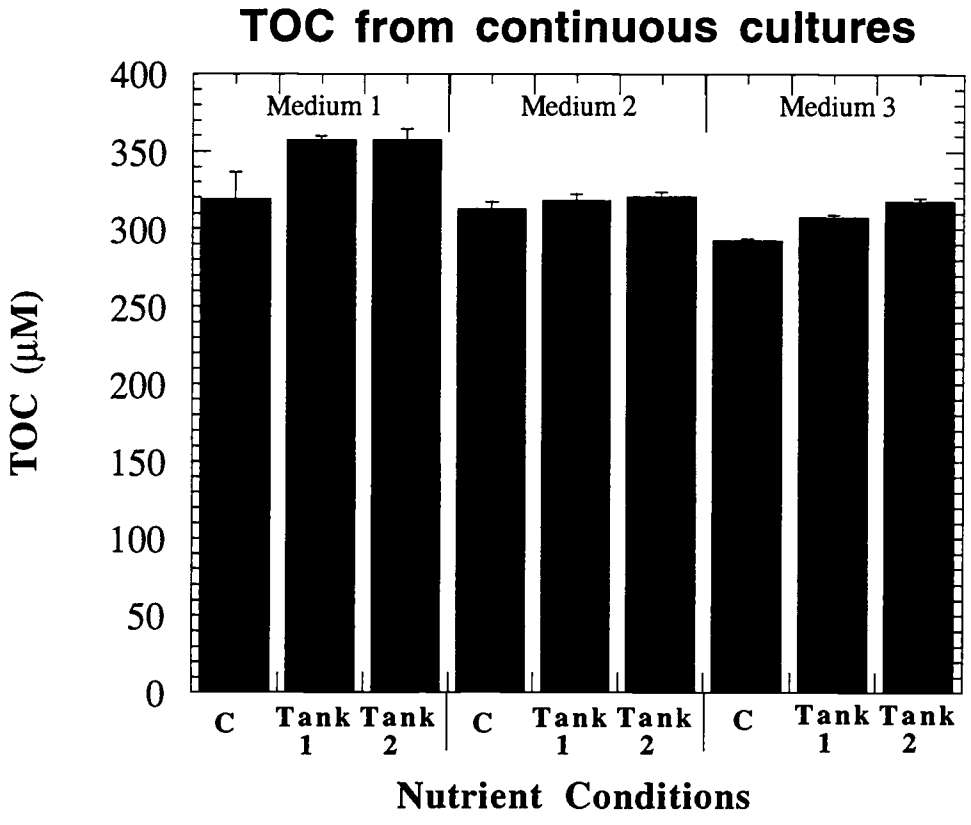

Figure III-1 TOC concentrations in the continuous cultures of *P. mollis*. Control (= C) seawater was sampled before *P. mollis* was introduced.

Medium 1 = NO_3^-

Medium 2 = NO_3^- + NH_4^+ (high C)

Medium 3 = NO_3^- + NH_4^+ (low C)

TOC into water during the experiment. TOC release rates (mg C 100 g dry wt^{-1} h^{-1}) and carbon-specific TOC release rates (mg C 100 g C^{-1} h^{-1}) were calculated by using the estimated experimental dry weight (Table III-4). Macroalga had the highest release rate (0.940 mg C 100 g dry wt^{-1} h^{-1}) in NO_3^- medium, the lowest release rate (0.158 mg C 100 g dry wt^{-1} h^{-1}) in NO_3^- + NH_4^+ (high C) medium.

Bacterial numbers ranged from 1.2 to 2.0 (x 10^6/ml) (Figure III-2). The numbers were not statistically different between each medium tank and experimental tank (two sided t-test, NO_3^- medium: two sided p-value = 0.24, NO_3^- + NH_4^+ (high C) medium: two sided p-value = 0.12, and NO_3^- + NH_4^+ (low C) medium: two sided p-value = 0.65). The dilution of media (once per day) may have kept bacteria from accumulating in the experimental tanks. However, the numbers of bacteria in this culture were much higher than the 300 cells/ml reported by Sieburth (1969).

Initial POC concentrations ranged from 5.5 to 23.3 (μM) in the media tanks. POC increased in the experimental tanks to concentrations of 64.5 to 84.5 (μM). POC release rates were calculated and ranged from 0.12 to 0.21 (mg C 100 g dry wt^{-1} h^{-1}). PON values ranged from 6.3 to 9.8 (μM) in the media, but the values increased only a little in the experimental tanks (Table III-5). Ratios of released organic carbon to total fixed carbon ranged from 1.5 % to 2.7 % (Table III-5).

Table III-4 Calculation of TOC release rate (mg C 100 g dry wt^{-1} h^{-1}) and carbon-specific TOC release rate (mg C 100 g C^{-1} h^{-1}). TOC samples were taken from media tanks with no *P. mollis* and *P. mollis* kept tanks. Carbon content of tissue was estimated as 31 % by using the data of Atkinson and Smith (1983). Dry weight was estimated as 13 % of wet weight by using the data of batch culture experiment (Table III-2).

Tank	TOC (μM) (±SD)	Wet weight of *P. mollis* (g) (±SD)	Estimated dry weight of *P. mollis* (g) (±SD)	TOC release rate (mg C 100g dry wt^{-1} h^{-1}) (±SD)	Carbon-specific TOC release rate (mg C 100g C^{-1} h^{-1}) (±SD)
NO_3^- medium	319 (±17.1)	-	-	-	-
NO_3^- tank	358 (±4.6)	282 (±4.5)	36.7 (±0.6)	0.940 (±0.014)	0.291 (±0.004)
NO_3^-+ NH_4^+ (high C) medium	313 (±6.2)	-	-	-	-
NO_3^-+ NH_4^+ (high C) tank	320 (±3.2)	286 (±7.0)	37.2 (±0.9)	0.158 (±0.026)	0.049 (±0.008)
NO_3^-+ NH_4^+ (low C) medium	292 (±1.5)	-	-	-	-
NO_3^-+ NH_4^+ (low C) tank	314 (±6.5)	285 (±5.0)	37.1 (±0.7)	0.491 (±0.118)	0.152 (±0.037)

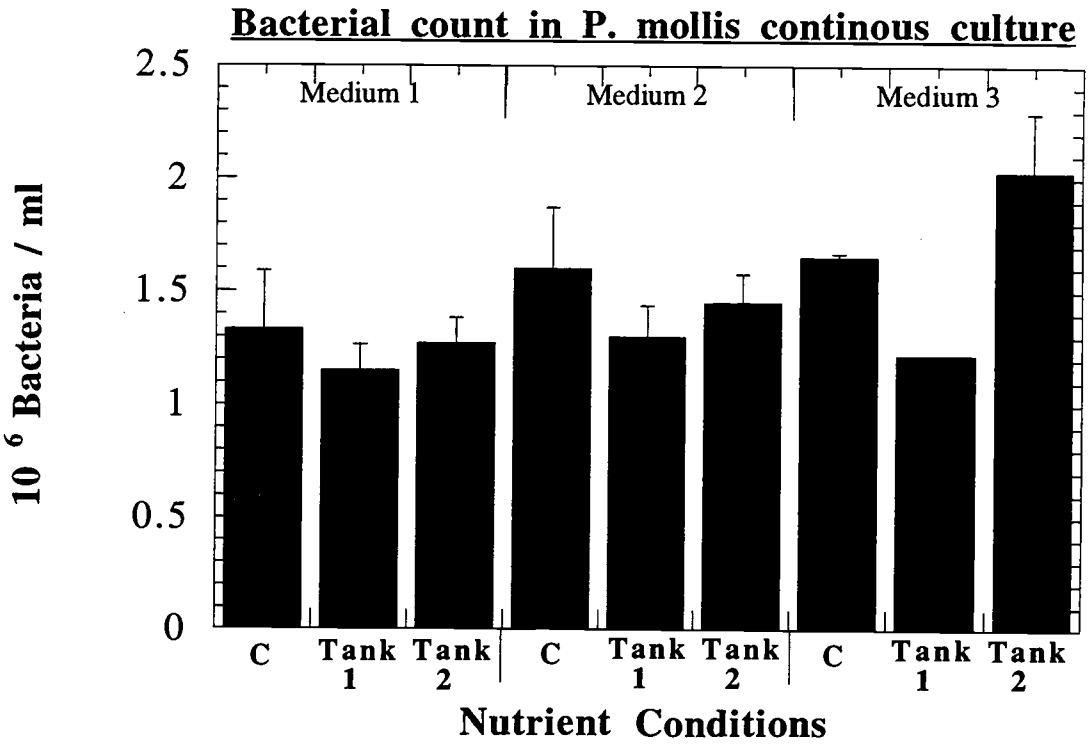

Figure III-2 Bacterial numbers in the continuous cultures of *P. mollis*.
Control (= C) seawater was sampled before *P. mollis* was introduced.
Medium 1 = NO_3^-

Medium 2 = NO_3^- + NH_4^+ (high C)

Medium 3 = NO_3^- + NH_4^+ (low C)

Table III-5 POC and PON concentrations and the ratio of released organic carbon to total fixed carbon in the continuous culture experiment of *P. mollis*. Samples were taken from media tanks with no *P. mollis* and *P. mollis* kept tanks. POC release rates (mg C 100 g dry wt^{-1} h^{-1}) were estimated as described for TOC release rates.

Nutrient Conditions	POC (μM) (±SD)	PON (μM) (±SD)	POC: PON	POC release rate (mg C 100 g dry wt^{-1} h^{-1})	Ratio of released organic carbon to total fixed carbon (%)
NO_3^- medium	13.0 (±8.6)	6.3 (±1.0)	2.1	-	-
NO_3^- tank - 1	75.3 (±23.1)	11.8 (±0.5)	6.4	0.18	2.4
NO_3^- tank - 2	84.5(±4.5)	7.6 (±0.7)	11.2	0.21	2.7
NO_3^- + NH_4^+ (high C) medium	5.5 (±0.5)	5.9 (±1.8)	0.9	-	-
NO_3^- + NH_4^+ (high C) tank - 1	64.5 (±22.7)	7.8 (±1.1)	8.3	0.17	2.1
NO_3^- + NH_4^+ (high C) tank - 2	65.5 (±4.8)	6.8 (±0.9)	9.7	0.17	2.1
NO_3^- + NH_4^+ (low C) medium	23.3 (±7.9)	8.1 (±3.2)	2.9	-	-
NO_3^- + NH_4^+ (low C) tank - 1	75.2 (±10.3)	13.2 (±2.2)	5.7	0.15	1.9
NO_3^- + NH_4^+ (low C) tank - 2	64.5 (±18.8)	10.1 (±1.4)	6.4	0.12	1.5

Macroalgal Experiment (Batch Culture)

The wet weight of *P. mollis* was measured on the first and last day of the experiment. All of the macroalga in the experimental tubs grew with time, and the growth rates ranged from 0.056 (±0.018) (d^{-1}) in NO_3^- + NH_4^+ (low C) medium to 0.101 (±0.013) (d^{-1}) in NO_3^- + NH_4^+ (high C) medium (Table III-2). However, even though the growth rate in NO_3^- + NH_4^+ (high C) medium was nearly twice as high as that in NO_3^- + NH_4^+ (low C) medium, it was not different statistically ($F_{3,6}$ = 2.333, p-value > 0.1) due to small number of samples (n = 6). This result still corresponded to that by Demetropoulos (personal comm.) who reported *P. mollis* in NO_3^- medium and NO_3^- + NH_4^+ (high C) medium showed the higher growth rates than that NO_3^- + NH_4^+ (low C) medium in his continuous culture experiment.

Initial TOC concentrations in all of 8 tubs ranged from 293 to 306 (μM) which was as high as those in the continuous culture experiments. TOC values increased with time through the experiment, but the increase was not linear (Figure III-3). Because additional nutrients were provided to prevent nutrient depletion in the tubs between day 1 and 2, the TOC values also increased dramatically. After all measurements, triplicate samples of nutrient stocks were taken and measured in order to know how much carbon each nutrient contained. Each nutrient stock was diluted to the concentration equal to that in the experiment with double distilled water. Only the trace metal solution contained high amounts of carbon (193 μM) which led to excess carbon in the experimental media.

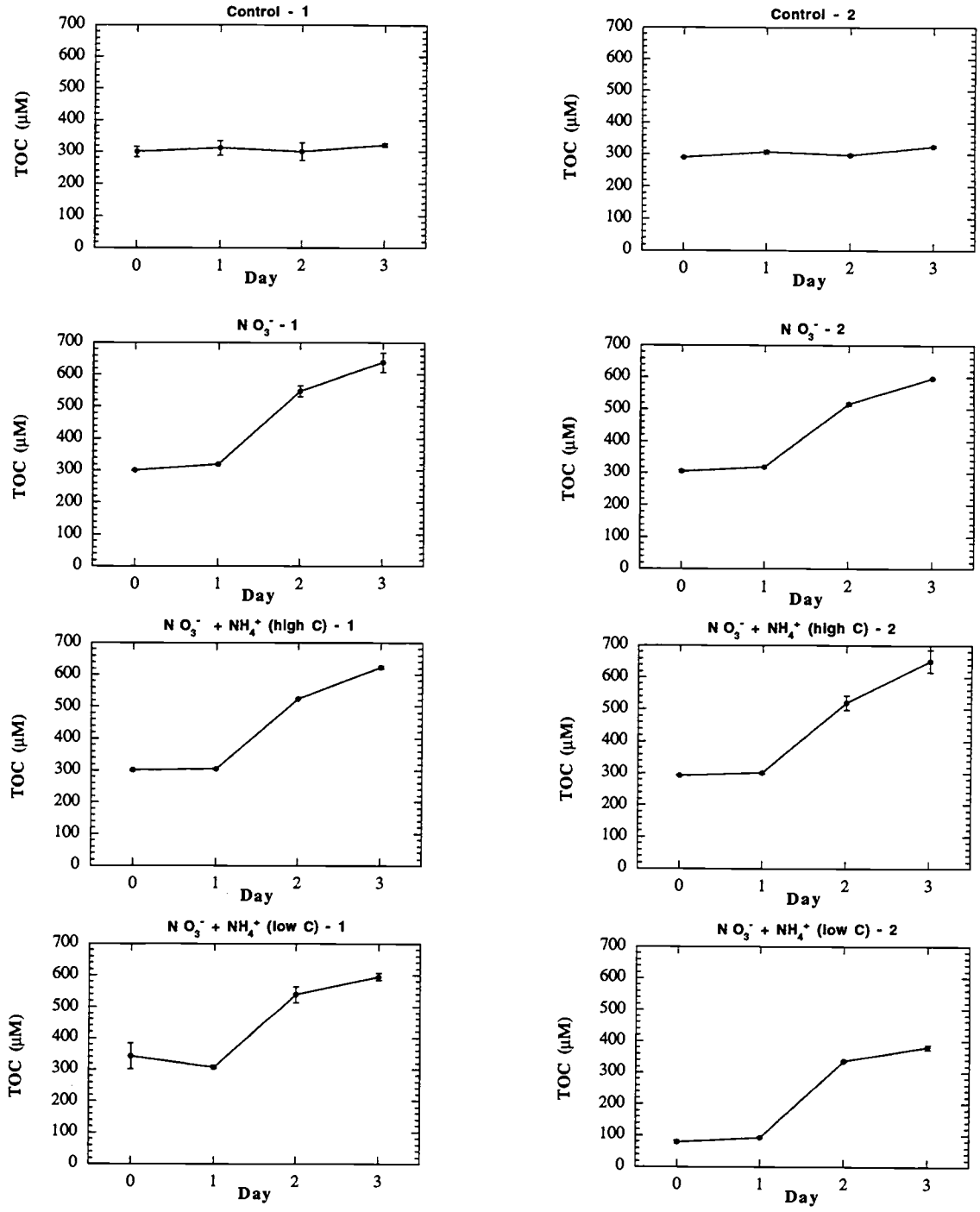

Figure III-3 TOC concentrations for 3-day batch culture experiments of *P. mollis*. More nutrients were added to all tubs except control tubs between day 1 and 2.

Thus, TOC release rates and carbon-specific TOC release rates were calculated separately between days 0 - 1 and days 2 - 3 (Table III-6). Between days 0 - 1, the TOC release rates and carbon-specific TOC release rates ranged from 0.13 to 0.78 (mg C 100 g dry wt^{-1} h^{-1}) and from 0.04 to 0.24 (mg C 100 g C^{-1} h^{-1}), respectively (Table III-6). NO_3^- medium and NO_3^- + NH_4^+ (high C) medium had higher release rates than NO_3^- + NH_4^+ (low C) medium even though the difference was not statistically significant ($F_{3,6}$ = 1.962, p-value > 0.1) due to small number of samples (n = 6). The values were as low as those of the continuous culture experiments and previous experiments. Ratios of released organic carbon to total fixed carbon ranged from 0.5 % to 1 %. Significant different ratios among the different nutrient conditions were not observed ($F_{3,6}$ = 2.55, p-value > 0.1).

Between days 2 - 3, both rates ranged from 1.32 to 2.80 (mg C 100 g dry wt^{-1} h^{-1}) and from 0.41 to 0.87 (mg C 100 g C^{-1} h^{-1}), respectively (Table III-7). The values were about three times higher than those between days 0 - 1. NO_3^- medium and NO_3^- + NH_4^+ (high C) medium also had higher release rates than NO_3^- + NH_4^+ (low C) medium even though the difference was not statistically significant ($F_{3,6}$ = 2.14, p-value > 0.1) due to small number of samples (n = 6). Ratios of released organic carbon to total fixed carbon ranged from 0.6 % to 2.2 %. NO_3^- + NH_4^+ (low C) medium had higher ratio than the others ($F_{3,6}$ = 11.37, p-value < 0.001).

Bacterial numbers in the initial condition were very low (less than 1.5 x 10^5 ml^{-1}), which was an order magnitude lower than observed in the continuous culture media. Because the media in the continuous culture had been made for seven days

Table III-6 TOC release rates and ratios of released TOC to total fixed carbon between days 0 - 1 in the batch culture experiment of *P. mollis*. Both rates and ratios were calculated separately between days 0 - 1 and days 2 - 3 because of the addition of nutrients which contained significant amounts of organic carbon between day 1 and 2. The calculations were shown below.

Nutrient Condition	TOC release rate* (mg C 100 g dry wt^{-1} h^{-1}) between days 0 - 1 (±SD)	Ratio of released TOC to total fixed carbon between days 0 - 1 (%)	Growth rate (d^{-1})** (±SD)
NO_3^-	0.57 (±0.30)	0.81 (±0.14)	0.072 (±0.020)
NO_3^- + NH_4^+ (high C)	0.38 (±0.01)	0.53 (±0.12)	0.101 (±0.013)
NO_3^- + NH_4^+ (low C)	0.20 (±0.09)	1.00 (±0.35)	0.056 (±0.018)

Note)
* TOC release rates (mg C/100 g dry wt/h) were calculated from the formula below.

$$\text{TOC release rate} = \frac{(\text{Final TOC} - \text{Initial TOC})\ (\text{mmol/L}) \times 12\ (\text{C mg/mmol}) \times \text{seawater (L)} \times 100}{\text{Plant dry weight (g dry wt)} \times \text{Time (h)}}$$

Example) TOC release rate in NO_3^- between day 0 and 1

$$\text{TOC release rate} = \frac{(319.5 - 299.3) \times 10^{-3}\ (\text{mmol/L}) \times 12\ (\text{C mg/mmol}) \times 3\ (\text{L}) \times 100}{3.87\ (\text{g dry wt}) \times 24\ (\text{h})} = 0.78\ (\text{mg C/100 g dry wt/h})$$

** Growth rate was calculated by using a following formula:
Growth rate = ln(final experimental wet weight/initial experimental wet weight)/3 days (in Table III-2)

Table III-7 TOC release rates and ratios of released TOC to total fixed carbon between days 2 - 3 in the batch culture experiment of *P. mollis*. Both rates and ratios were calculated separately between days 0 - 1 and days 2 - 3 because of the addition of nutrients which contained significant amounts of organic carbon between day 1 and 2. The calculations were shown below.

Nutrient Conditions	TOC release rate* (mg C 100 g dry wt^{-1} h^{-1}) between days 2 - 3 (±SD)	Ratio of released TOC to total fixed carbon (%) between days 2 - 3	Growth rate (d^{-1})** (±SD)
NO_3^-	2.14 (±0.44)	1.22 (±0.03)	0.072 (±0.020)
NO_3^- + NH_4^+ (high C)	2.68 (±0.17)	0.64 (±0.01)	0.101 (±0.013)
NO_3^- + NH_4^+ (low C)	1.34 (±0.03)	2.15 (±0.49)	0.056 (±0.018)

Note)
* TOC release rates (mg C/100 g dry wt/h) were calculated from the formula below.

$$\text{TOC release rate} = \frac{(\text{Final TOC} - \text{Initial TOC})\ (\text{mmol/L}) \times 12\ (\text{C mg/mmol}) \times \text{seawater (L)} \times 100}{\text{Plant dry weight (g dry wt)} \times \text{Time (h)}}$$

Example) TOC release rate in NO_3^- between day 2 and 3

$$\text{TOC release rate} = \frac{(619.7 - 547.9) \times 10^{-3}\ (\text{mmol/L}) \times 12\ (\text{C mg/mmol}) \times 3\ (\text{L}) \times 100}{4.54\ (\text{g dry wt}) \times 24\ (\text{h})} = 0.78\ (\text{mg C/100 g dry wt/h})$$

** Growth rate was calculated by using a following formula:
Growth rate = ln(final experimental wet weight/initial experimental wet weight)/3 days (in Table III-2)

prior to sampling, bacteria may have accumulated in these media for seven days. The bacterial numbers increased with time except in NO_3^- - 2 tub (Figure III-4). The values specially increased between days 2 - 3 in most of the cultures. The final concentration of bacteria was about ten times higher than the initial concentration and as high as those in the continuous culture experiment.

Both POC and PON values varied (Figure III-5). In most cases, POC and PON decreased initially, then POC increased by a factor of about 3, and remained high. PON increased by a factor of about 2, and stayed high except in control. In the control, PON stayed low until day 1, then increased slowly.

Phytoplankton Experiment (Continuous Culture)

The DOC concentration in incoming media was 347 μM (±9.0). The initial values were much higher than observed in the previous phytoplankton experiment as well as in the phytoplankton batch culture experiment described below. The value increased to 551 μM (±5.4) in the experimental tank with *D. tertiolecta*. This result indicates that *D. tertiolecta* releases DOC. I compare release rates and cellular parameters to data reported by Falkowski and Owens (1980) and Biddanda and Benner (1997) below.

The DOC release rate for *D. tertiolecta* in the continuous culture was 0.012 (pmol DOC pmol cell C^{-1} d^{-1}). The value was compared to those of different phytoplankton (Biddanda and Benner 1997). It was as low as *Synechococcus bacillaris*, but lower than *Emiliania huxleyi*, *Phaeocystis* sp. and *Skeletonema*

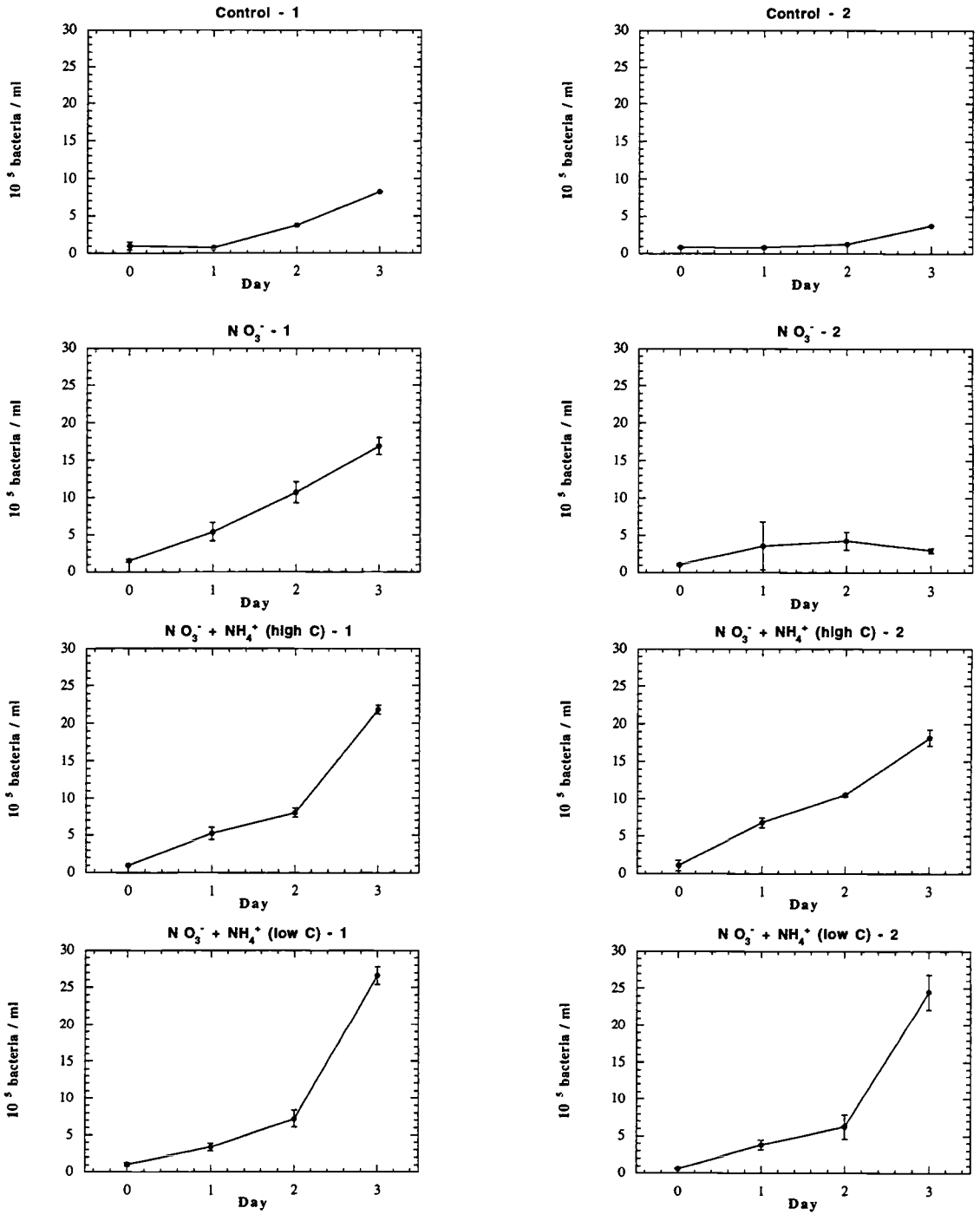

Figure III-4 Bacterial numbers for 3-day batch culture experiments of *P. mollis*. Bacteria were counted every 24 hours.

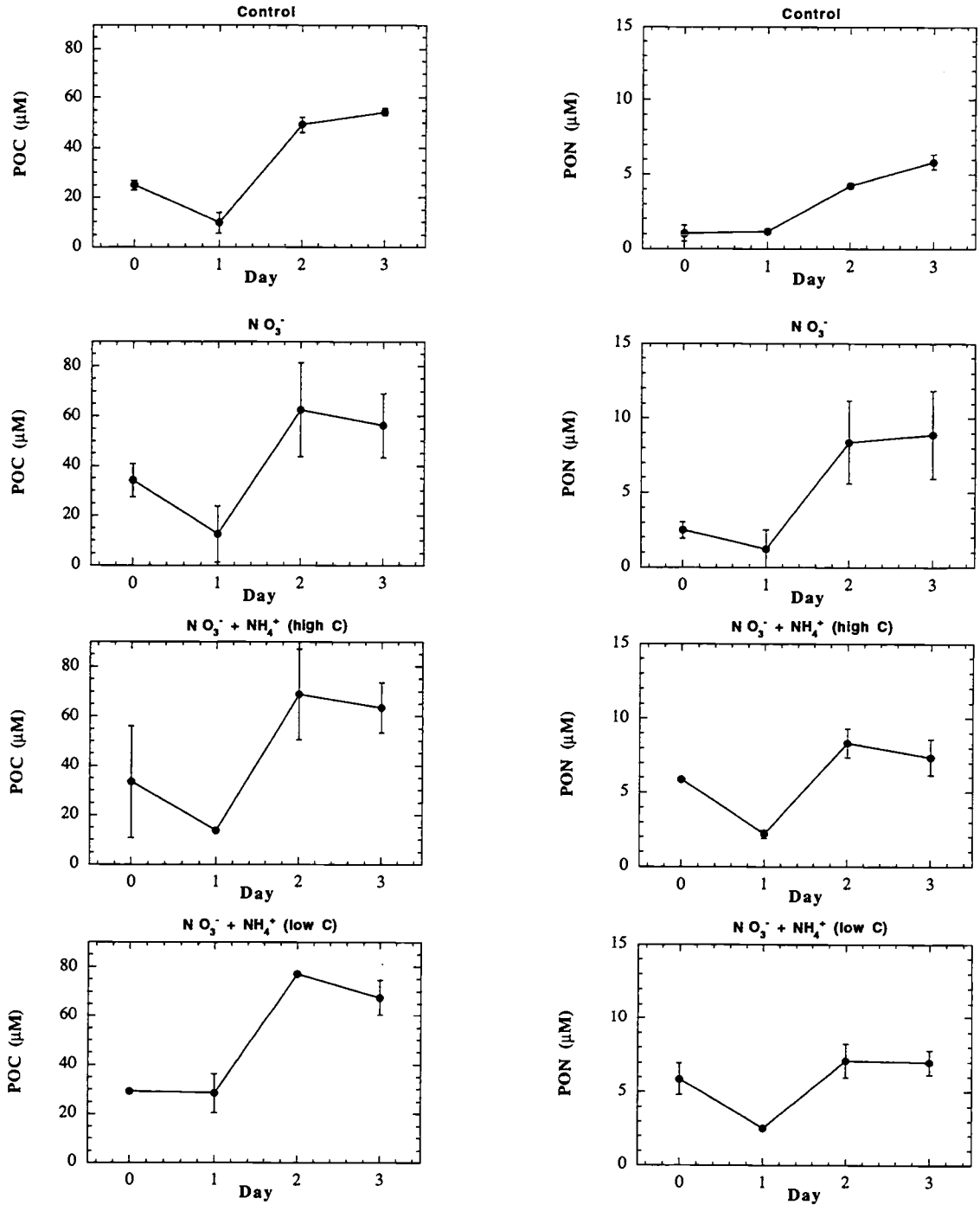

Figure III-5 POM concentrations for 3-day batch culture experiments of *P. mollis*. More nutrients were added to all tubs except control tubs between day 1 and 2.

costatum (Table III-8). POC and PON were measured to estimate how much carbon and nitrogen each cell contained. Compared to cellular parameters of *D. tertiolecta* reported by Falkowski and Owens (1980), carbon per cell was twice as high, but nitrogen was similar. The ratio of POC to PON was 7.3, which was close to Redfield ratio. The ratio of POC to DOC was 8.1, which implied that *D. tertiolecta* released about 11 % of total fixed carbon as DOC into the surrounding seawater.

Phytoplankton Experiment (Batch Culture)

D. tertiolecta growth showed an initial lag period for two days and then entered an exponential growth phase. *D. tertiolecta* reached the stationary phase by day 12 (Figure III-6). Maximum growth rate was calculated from the exponential growth phase determined as described in Chapter II. The exponential growth phase was indicated by arrows in Figure III-6. The maximum growth rate for *D. tertiolecta* was 0.89 d^{-1}. *D. tertiolecta* grew slowly after the exponential growth phase. The average growth rate was 0.17 d^{-1}, which was similar to that observed in the continuous culture experiment (0.10 d^{-1}) (Table III-8).

The initial DOC concentration in experimental medium was 110 μM, which was much lower than that observed in the continuous culture experiment, because EDTA was not used in the trace metal solution. The DOC value increased with time, and the final value reached 710 μM (Figure III-7A). *D. tertiolecta* kept releasing DOC during the experiment, but not constantly. The DOC release rate was high [0.052 – 0.097 (pmol DOC pmol cell C^{-1} d^{-1})] during the exponential

Table III-8 Comparison of DOC release rates by phytoplankton with the data of Biddanda and Benner (1997).

Phytoplankton Species	Experimental Release Rates (pmol DOC pmol cell C^{-1} d^{-1})	Data Sources
D. tertiolecta (Continuous culture)	0.012	This experiment
D. tertiolecta (Batch culture)	0.010- 0.097	This experiment
Skeletonema costatum	0.067	Biddanda and Benner (1997)
Synechococcus bacillaris	0.012	Biddanda and Benner (1997)
Phaeocystis sp.	0.038	Biddanda and Benner (1997)
Emiliania huxleyi	0.025	Biddanda and Benner (1997)

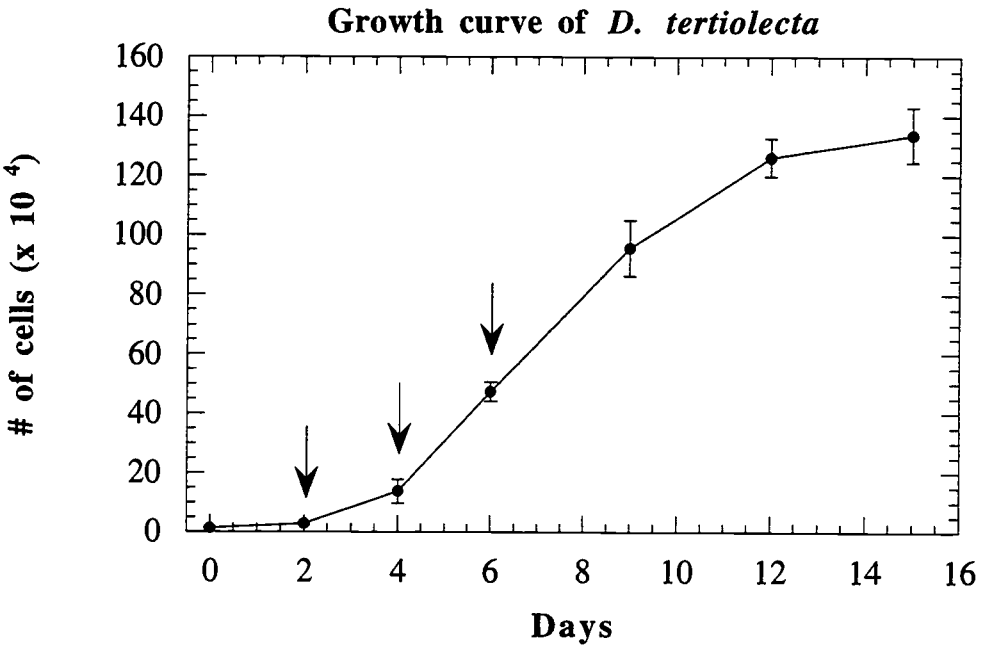

Figure III-6 Growth curve of *D. tertiolecta* in the batch culture experiment. Maxium growth rate (= 0.89 d^{-1}) was calculated by using points which were indicated by arrows.

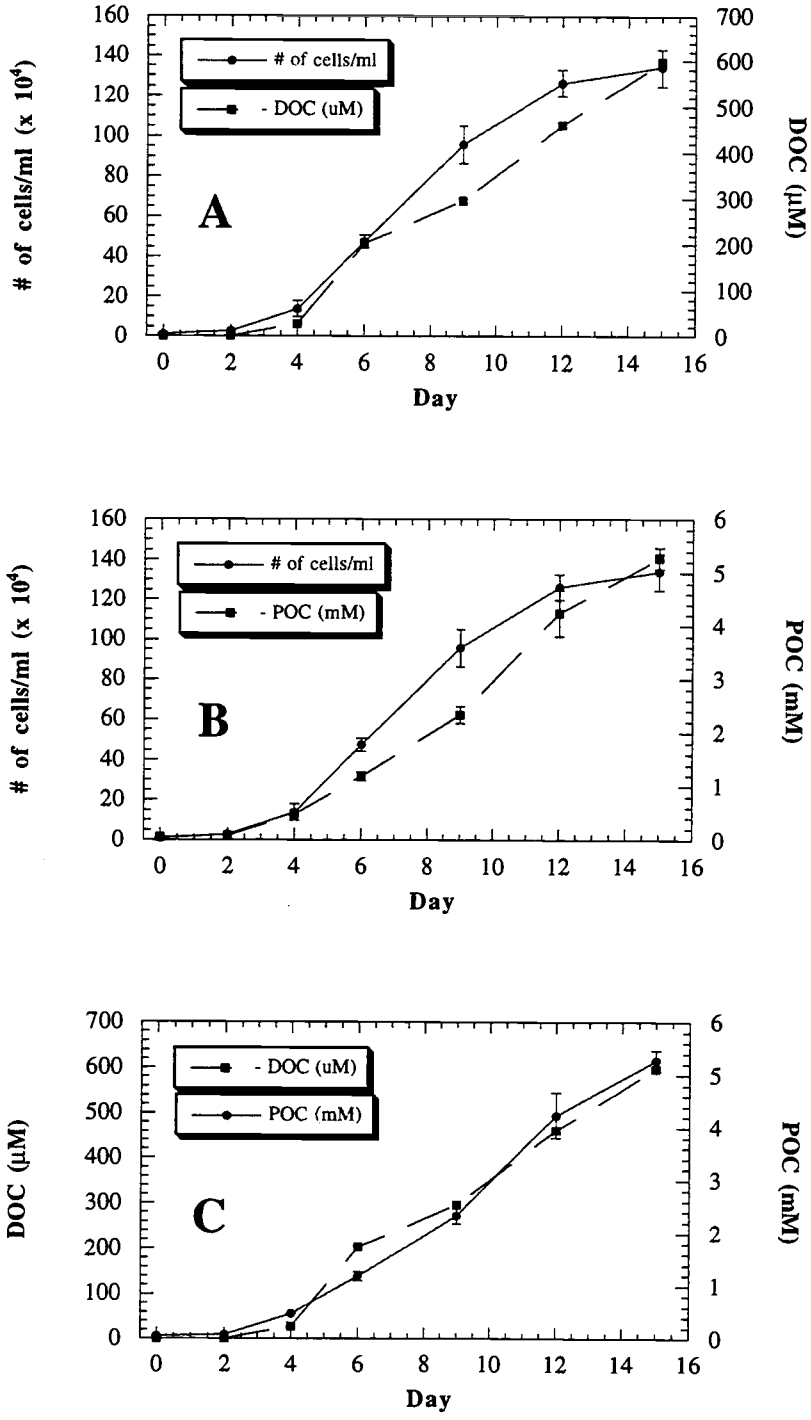

Figure III-7 Relationships between :
A. Cell numbers vs. DOC release
B. Cell numbers vs. POC
C. POC vs. PON
in *D. tertiolecta* batch culture experiment.

growth phase, and lower during the stationary growth phase [0.010 – 0.018 (pmol DOC pmol cell C^{-1} d^{-1})] (Table III-8 and 9).

POC also increased with time (Table III-10, Figure III-7A and B). POC corresponded to *D. tertiolecta* cell numbers, but cell carbon varied with the culture growth phase (Table III-10). When inoculated, *D. tertiolecta* contained the highest amounts of carbon per cell. During the exponential growth phase, the carbon content per cell decreased, and then increased gradually with time and was close to that of the initial condition at the end of the experiment. PON values showed a different pattern. PON increased with time until day 6, and remained constant after that (Figure III-7C). *D. tertiolecta* may have consumed all nitrogenous nutrient by day 6. Because the number of *D. tertiolecta* cells still increased after day 6, the nitrogen content per cell decreased with time. At the final sampling, the nitrogen contents per cell (0.17 pmol N/cell) decreased to about 25 % of that observed during the exponential growth phase (0.68 pmol N/cell) (Table III-10).

Thus, the ratio of POC to PON changed dramatically. During the exponential growth phase, the ratio was about 8, which was similar to that observed in the continuous experiment, but reached ~ 20 in the end of the experiment. The ratio of POC to DOC during the exponential growth phase was 5.8, which meant that about 15 % of total fixed carbon was released as DOC. The ratio during the stationary growth phase increased to about 8.5, which was similar to that in the continuous experiment. The result meant that about 11 % of total fixed carbon was released as DOC (Table III-10).

Table III-9 Comparison of DOC release rates in the batch culture experiment of *D. tertiolecta.*

Days	DOC release rate (pmol DOC pmol cell C^{-1} day^{-1})	Growth phase	Average DOC release rate on each growth phase (pmol DOC pmol cell C^{-1} day^{-1}) (±SD)
0 - 2	0.0	Lag period	0
2 - 4	0.052	Exponential	0.075 (±0.032)
4 - 6	0.097	Exponential	
6 - 9	0.018	Stationary	0.015 (±0.004)
9 - 12	0.017	Stationary	
12 - 15	0.010	Stationary	

Table III-10 Comparison of POC, PON, and DOC in the batch culture experiment of *D. tertiolecta*.

Day	DOC (μM)*	POC (μM) (±SD)	PON (μM) (±SD)	POC : PON	POC : DOC	# of cells (x10^4)/ml	pmol C/cell (±SD)	pmol N/cell (±SD)
0	0	53 ±7.3	3.4 ±0.2	15.7	-	1.3	4.20 ±0.70	0.27 ±0.02
2	0	79 ±10.5	11.4 ±2.3	6.9	-	2.8	2.85 ±0.38	0.41 ±0.10
4	27.3	477 ±1.4	85.4 ±0.3	5.6	17.5	13.8	3.46 ±0.01	0.62 ±0.01
6	204	1188 ±82.5	189 ±23.5	6.3	5.8	47.4	2.51 ±0.17	0.43 ±0.01
9	297	2334 ±155	215 ±0.5	10.9	7.9	95.6	2.44 ±0.20	0.22 ±0.01
12	461	4242 ±431	224 ±10.4	19.0	9.2	126	3.36 ±0.42	0.17 ±0.02
15	597	5274 ±198	229 ±4.0	23.0	8.8	134	3.94 ±0.18	0.16 ±0.02
						Mean (±SD)	3.25 ±0.68	0.33 ±0.17
					Falkowski and Owens (1980)	(±SD)	2.81 ±0.46	0.80 ±0.26

* DOC values were measured values minus the initial DOC values (116 μM) of the seawater.

Growth rate = 0.89 (d^{-1})

Discussion

Comparison of Macroalgal Experiment between Batch and Continuous Cultures

In both culture experiments, initial TOC concentrations were high compared with those in previous experiment (Chapter II). EDTA, which contains 6 carbon atoms per molecule, was added in the trace metal solution. The diluted metal solution contained about 200 μM TOC. Because TOC concentration in initial seawater collected at HMSC was about 110 μM, high TOC values were mainly a result of the addition of carbon from the trace metal solution.

TOC release rates and carbon-specific TOC release rates were compared between continuous culture and batch culture experiments and with data reported in the literature (Table III-11). The values in the continuous culture were as low as those of the batch culture between days 0 - 1, and lower (by a factor of 5) than those between days 2 - 3. These results are different from those of Sieburth (1969), where no DOC was released in his batch cultures, but enormous amounts of DOC were released in his continuous cultures. Sieburth concluded that the increase of bacteria in the batch culture decomposed all released DOC. In my experiment, bacteria did not increase in the incubation tanks (Figure III-2 and 4), but the initial number of bacteria in the continuous culture was about 10 times higher than that in the batch culture experiment. Because the culture media was held in 180 L tanks for a week, bacteria must have grown in the tanks before the sampling. High initial bacteria in the continuous culture may have caused low TOC release rates.

Table III-11 Comparison of TOC or DOC release rates of *P. mollis* with literature and the previous experiment.

	Species	TOC/DOC release rates (mg C 100 g dry wt^{-1} h^{-1})	Data sources
(Phaeophyta)	*Hedophyllum sessile*	0.67	Present work
	Analipus japonicus	1.15	Present work
	Fucus vesiculosus	39.2	Sieburth (1969)
	Fucus vesiculosus	37.2- 130	Khalihov and Burlakova (1969)
	Padina japonica	14.0	Schramm et al. (1984)
(Rhodophyta)	*Palmaria mollis* (Continuous Culture)	0.16- 0.94	Present work
	Palmaria mollis (Batch Culture)	0.20- 2.7	Present work
	Odontholia floccoca	0.43	Present work
	Dilsea californica	0.21	Present work
	Rhodimenia palmata	89.9- 304	Khalihov and Burlakova (1969)
	Gracilaria acerosa	4.6	Schramm et al. (1984)
(Chlorophyta)	*Codium fragile*	0.19	Present work
	Ulva fenestrata	0.96	Present work
	Ulva lactuca	20.8	Sieburth (1969)
	Ulva lactuca	0.14	Horner and Smith (1984)
	Enteromorpha prolifera	26.0	Pregnall (1983)

However, the rates between day 2 and 3 in the batch culture were higher than those between day 0 and 1 although the number of bacteria increased and, at the end of the experiment, reached as high as that in the continuous culture. Thus, bacterial activity may not be as significant as reported by Sieburth (1969). TOC release of *P. mollis* may not be different between continuous and batch culture. The results reported by Sieburth (1969) may have been a result of experimental conditions such as wounding and other stress effects as pointed out by Moebus and Johnson (1974) and Schramm et al. (1984). In the continuous experiment designed by Sieburth (1969), 10 - 20 g wet weight of algae were packed into only a 125 ml incubation chamber. In my batch culture experiment, 30 - 40 g wet weight of *P. mollis* was incubated into 3 L seawater and in my continuous culture experiment, about 180 g wet weight of *P. mollis* was incubated into 18 L seawater. Sieburth also wired the algae to a screen which rotated at 50 to 60 rpm and may have caused wounding. Wounding and other stress effects may have caused extra release of organic carbon in Sieburth's continuous culture.

TOC release by *P. mollis* ranges from 0.5 % to 2.7 % of total fixed carbon in this experiment (Table III-6, 7 and 8). The values were not significantly different between batch and continuous culture experiments. These values were far lower than found by Khailov and Burlakova (1969) and Sieburth (1969), who found that up to 40 % of net photosynthetic production was released as dissolved organic matter by the brown algae tested. As mentioned above, the high value of organic carbon release may be a result of adverse experimental conditions. On the other

hand, my values were much higher than found by Fankboner and DeBurgh (1977). They estimated that only 0.0002 % of total fixed carbon was released as DOC. Schramm et al. (1984) reported that determination of ^{14}C-labeled exudates in short-term labeling experiments performed by Brylinski (1977) and by Fankboner and DeBurgh (1977) were seriously underestimated for several reasons. In particular, some release products may originate from non-labeled photosynthetic products which were produced before the experiment and released during the experiment. This would certainly underestimate total exudation, especially during short-term incubations (2 - 3 h), if a significant time lag exists between the incorporation of inorganic ^{14}C and the subsequent release of labeled organic compounds.

The DOC or TOC release rates in my experiments, however, were close to those estimated by Fogg (1983) and Schramm et al. (1984). Schramm et al. (1984) used a chemical measurement, comparable to the HTCO method employed in my experiment. Because chemical analyses measure bulk DOC directly, the methodological problems encountered with the ^{14}C method are eliminated. Fogg (1983) and Schramm et al. (1984) concluded that 1 – 5 % of net photosynthetic production may be released from marine macroalgae when methodological and analytical errors (e.g. consumption by heterotrophs and detrimental experimental effects) are carefully avoided.

In the macroalgal continuous culture, POC concentrations were always higher in the macroalgal tanks than in the media tanks. POC release rates were calculated and ranged from 0.12 to 0.21 (mg C 100 g dry wt^{-1} h^{-1}) (Table III-5). The

rates were about 20 % of TOC release rates in the NO_3^- nutrient condition, 40 % of those in the $NO_3^- + NH_4^+$ (low C) nutrient condition, and nearly 100 % of those in the $NO_3^- + NH_4^+$ (high C) nutrient condition. *P. mollis* may release significant amounts of carbon as POC. However, I observed that some diatoms also grew in all of the continuous culture tanks. Diatoms attach to the surface of *P. mollis* and some cells may have been released to the seawater medium in the tanks. It is difficult to estimate how much TOC is derived from diatoms, and how much is from *P. mollis*. In the batch culture experiment, POC concentrations did increase, but not linearly (Figure III-5). The overall POC increase, however, was much smaller than observed in the continuous culture experiment because the accumulation of diatoms was not observed in the batch culture experiment.

In conclusion, DOC or TOC release rates of *P. mollis* in continuous and batch cultures were similar, i.e. within a factor of two. The extremely high values of organic carbon release in the continuous culture experiment reported by Sieburth (1969) may be a result of adverse experimental conditions. The decomposition of DOC by heterotrophic microbes is still important, but this activity does not appear to be as fast as the release of TOC by macroalgae.

Comparison of Phytoplankton Experiments between Batch and Continuous Cultures

In both types of culture experiments, it was confirmed that *D. tertiolecta* released DOC in all growth phases. However, DOC release rates seemed to vary with growth phases, and they may be positively correlated with growth rates. In the

batch culture experiment, the rates were high (0.052 – 0.097 pmol DOC pmol cell C^{-1} d^{-1}) during the exponential growth phase while low (0.010 – 0.018 pmol DOC pmol cell C^{-1} d^{-1}) during the stationary phase. In the continuous culture, the rate was low (0.012 pmol DOC pmol cell C^{-1} d^{-1}), but the growth rate was also low.

Chen and Wangersky (1993, 1996a) and Myklestand et al. (1989) reported that a diatom, *Chaetoceros* sp. had the highest DOC release rates during the exponential phase. Other workers found that phytoplankton release the most DOC after their blooms (Goldman et al. 1992). Rates of DOC release during phytoplankton growth appear to vary among taxa (Biddanda and Benner, 1997). Chen and Wangersky (1996a) also found that while exudation of DOC can accompany an exponential growth phase, the major release of DOC during stationary phase is probably a result of cell lysis. They also reported that most DOC (> 80 %) released by phytoplankton were low-molecular-weight (LMW) DOC (< 10000 Da) during their growth experiment, but as cells reached at the stationary phase, a ratio of carbon-rich high-molecular-weight (HMW) DOC (> 10000 Da) to LMW DOC increased. They concluded that LMW DOC was more rapidly decomposed by bacteria than HMW DOC, and the ratio of HMW DOC to LMW DOC increased.

In addition to variations in bacterial activity, phytoplankton cells may release more HMW DOC during their stationary growth phase than during their exponential growth phase. In my experiment, the C: N ratio of cells increased with time and reached 20 in the end of the experiment. This value was close to the ratio

of HMW DOC released by 4 different phytoplankton tested by Biddanda and Benner (1997). It may be anticipated that more HMW DOC remains as storage material in the cells during the stationary growth phase. If the HMW DOC release is dominated by a passive diffusion, more HMW DOC would be released from the cells during the stationary growth phase. In addition, it takes bacteria much longer time to decompose HMW DOC than LMW DOC (Chen and Wangersky 1996b). Although average HMW DOC pools are about 30 % of total released DOC pools in phytoplankton (Biddanda and Benner 1997), HMW DOC may be accumulated in water column during the cell growth whereas LMW DOC may be decomposed quickly by heterotrophs. Thus, the combination of cell lysis and relatively high HMW DOC release during the stationary growth phase may cause significant organic carbon release after the bloom as predicted by Goldman et al. (1992) and Williams (1995).

Comparison of Macroalgal and Phytoplankton Experiments

As described in Chapter II, TOC/DOC release rates of *P. mollis* was converted to carbon-specific release rates for comparison with *D. tertiolecta*. This time, because the DOC release rate of *D. tertiolecta* was measured more frequently, the underestimation of the value pointed out in Chapter II appears to be eliminated. Release rates for *D. tertiolecta* ranged from 1000- 9700 (x 10^{-5} pmol DOC pmol C^{-1} d^{-1}) while in *P. mollis*, the rates ranged from 1.2 to 20 (x 10^{-5} pmol DOC pmol C^{-1} d^{-}

[1]). This result suggests that the surface area to volume (SA:V) ratio may be positively correlated with DOC release.

In the *D. tertiolecta* experiment, DOC release rates appear to be positively correlated with their growth rates. In the *P. mollis* experiment, the rates also seemed to be positively correlated with their growth rates but only in the batch culture experiment. The NO_3^- + NH_4^+ (low C) medium had both the lowest growth rates and TOC release rates of all media. However, in the continuous cultures, the NO_3^- + NH_4^+ (low C) medium had higher TOC release rate than the NO_3^- + NH_4^+ (low C) medium (Table III-4). Because the differences of growth rates in the macroalgal experiment were not significant statistically due to small number of samples (n=6), it is hard to discuss the relationship between growth rate and TOC release rate. Moreover, in the macroalgal experiment, although nutrient conditions were different, macroalgae were never nutrient-limited or light-limited. Therefore, significant differences were not expected among different nutrient conditions.

The ratio of released TOC to total fixed carbon was compared between the two types of algae. In *D. tertiolecta*, DOC released ranged from 10 % to 15 % of total fixed carbon while, in *P. mollis*, the ratio of DOC released to total fixed carbon ranged from 0.5 % to 2.7 %. Higher ratios in phytoplankton may also be a result of high SA:V ratio. Alternatively, macroalgae use significant amounts of carbon for structural components and this may lead to a low ratio of DOC released to total fixed carbon.

In the phytoplankton experiment, the ratio of released DOC of total fixed carbon increased with their growth rate. When the growth rate was low (0.1 - 0.2 d^{-1}), about 10 % of total fixed carbon was released as DOC. When the growth rate was high (0.9 d^{-1}), about 15 % of total fixed carbon was released as DOC. Thus, for phytoplankton, the ratio of DOC released to total fixed carbon appears to be positively correlated with the growth rate. In the *P. mollis* batch cultures, the lowest growth rate condition (NO_3^- + NH_4^+ (low C) medium) showed the highest ratio of released organic carbon to total fixed carbon while the highest growth rate condition (NO_3^- + NH_4^+ (high C) medium) showed the lowest ratio. In the continuous cultures, on the other hand, the NO_3^- + NH_4^+ (low C) medium showed the lowest ratio (Table III-5). I could not find any consistent relationship between growth rate and a ratio of released organic carbon to total fixed carbon in *P. mollis*.

In conclusion, TOC/DOC release rates may be positively correlated with SA:V ratio. Because phytoplankton have a high SA:V ratio compared to macroalgae, the DOC release rates in *D. tertiolecta* were at least 50 times higher than those of macroalga, *P. mollis*. For *D. tertiolecta*, the ratio of released DOC to total fixed carbon appears be correlated with growth rate. For *P. mollis*, no relationship between growth rate and release rates could be discerned.

Chapter IV

Summary

There were three main goals in this thesis. The first goal was to test the toxic or stimulatory effect of released substances from macroalgae on phytoplankton growth. No consistent stimulatory effect was observed, but a weak inhibitory effect on phytoplankton growth was observed from two out of six species tested. *U. fenestrata* inhibited growth rates and final yields of tested phytoplankton by 25 % and 40 %, respectively, but the effect was not strong enough to support my hypothesis.

The second goal was to determine whether DOC release rates are different between batch and continuous culture experiments. The release rates of *P. mollis* were similar between the two culture conditions and much lower than those reported by Sieburth (1969). My results suggest that the extreme high release rates of macroalgae tested in Sieburth's continuous culture may be a result of adverse experimental conditions.

In the *D. tertiolecta* experiment, the release rates were also similar for the two types of cultures. The rates, however, appear to be positively correlated with growth rate. Based on the dramatic increase of cell C: N ratios during the stationary phase in the batch culture experiment, different types of DOC may be released

between the exponential and stationary phases. Low-molecular-weight DOC (LMW DOC) (C: N ratio = ~ 7) is usually released during fast growth, but substantial amounts of carbon-rich high-molecular-weight DOC (HMW DOC) (C: N ratio = ~20) may be released during the stationary phase. LMW DOC may be decomposed more quickly by microbial heterotrophs than HMW DOC. Thus, the apparently higher HMW DOC release during the stationary growth phase may be in part due to slower bacterial utilization. These processes may all contribute to the accumulation of organic carbon after the bloom or in mid-to late summer as predicted by Goldman et al. (1992) and Williams (1995).

The ratios of released organic carbon to total fixed carbon ranged from 0.5 % to 2.7 % in this macroalgal experiment. The ratios were also much lower than those observed Sieburth (1969) who found up to 40 % of total fixed carbon was released as DOC. This difference may be a result of adverse experimental conditions used by Sieburth (1969). On the other hand, my results were much higher than those found by Fankboner and DeBurgh (1977) who estimated only 0.0002 % of total fixed carbon is released as DOC. The ^{14}C method in macroalgal experiments employed by Fankboner and DeBurgh (1977) may severely underestimate DOC release because some release products may originate from non-^{14}C-labeled photosynthetic products that were produced before the experiment and released during the experiment. The ratios in my experiments were similar to those reported by Fogg (1983) and Schramm et al. (1984) who concluded that 1 - 5 % of total fixed carbon may be released from healthy macroalgae. In the phytoplankton

experiment, the ratio of released organic carbon to total fixed carbon ranged from 10 % to 15 %. The ratio may be also positively correlated with phytoplankton growth rates.

The last goal was to determine factor(s) which influence the release of DOC. When the carbon-specific release rates were compared between phytoplankton and macroalgae, phytoplankton had at least 50 times higher DOC release rates than macroalgae. This indicates that DOC release may be dominated by a passive diffusion because the release by the passive diffusion is positively correlated with a surface area to volume (SA:V) ratio. Phytoplankton have higher SA:V ratios (10^4 - 10^5 in terms of cm^2/cm^3) than macroalgae (10^1 - 10^2 in terms of cm^2/cm^3) and this may cause higher DOC release rates in phytoplankton than those in macroalgae.

To deepen the understanding of DOC release mechanisms of phytoplankton and macroalgae, the following experiments are suggested for future study. Samples for DOC release rates should be taken for several different growth conditions and different species to test whether the relationship that the rates are positively correlated with growth rates is a general characteristic or a species-specific characteristic. At the same time, samples should also be taken for identification of the major products that are released throughout growth. The analyses may indicate what kind of substances phytoplankton and macroalgae produce and release for different growth conditions and growth phases.

DOC is a main food source for microbial heterotrophs. Different compositions of DOC may influence the structure of microbial loops. The information obtained in future studies will provide useful predictions of the structure and activity of the microbial loops as well as carbon and nitrogen cycles in aquatic ecosystems.

Bibliography

Atkinson, M. J. and S. V. Smith, 1983. C:N:P ratios of benthic marine plants. *Limnol. Oceanogr.*, 28: 568-574.

Ballesteros, E., D. Martin and M. J. Uriz, 1992. Biological activity of extracts from some Mediterranean macrophytes. *Bot. Mar.*, 35: 481-485.

Benner, R. and M. Strom, 1993. A critical evaluation of the analytical blank associated with DOC measurement by high-temperature catalytic oxidation. *Mar. Chem.*, 41: 153-160.

Biddanda, B. and R. Benner, 1997. Carbon, nitrogen, and carbohydrate fluxes during the production of particulate and dissolved organic matter by marine phytoplankton. *Limnol. Oceanogr.*, 42: 506-518.

Bird, K. T., C. Habig and T. DeBusk, 1982. Nitrogen allocation and storage patterns in *Gracilaria tikvahiae* (Rhodophyta). *J. Phycol.*, 18: 344-348.

Bjørnsen, P. K., 1988. Phytoplankton exudation of organic matter: why do healthy cells do it? *Limnol. Oceanogr.*, 33: 151-154.

Branch, G. H. and C. L. Griffiths, 1988. The Benguela ecosystem Part V. the coastal zone. *Oceanogr. Mar. Biol. Annu. Rev.*, 26: 395-486.

Brown, M. R. and S. W. Jeffrey, 1992. Biochemical composition of microalgae from the green algal classes Chlorophyceae and Prasinophyceae. 1. Amino acids, sugars and pigments. *J. Exp. Mar. Biol. Ecol.*, 161: 91-113.

Brylinsky, M., 1977. Release of dissolved organic matter by some marine macrophytes. *Mar. Biol.*, 39: 213-220.

Brzezinski, M. A., 1985. The Si:C:N ratio of marine diatoms: interspecific variability and the effect of some environmental variables. *J. Phycol.*, 21: 347-357.

Chen, W. and P. J. Wangersky, 1993. High-temperature combustion analysis of dissolved organic carbon produced in phytoplankton cultures. *Mar. Chem.*, 41: 167-171.

Chen, W. and P. J. Wangersky, 1996a. Production of dissolved organic carbon in phytoplankton cultures as measured by high-temperature combustion

catalytic oxidation and ultraviolet photo-oxidation methods. *J. Plankton Res.*, 18: 1201-1211.

Chen, W. and P. J. Wangersky, 1996b. Rates of microbial degradation of dissolved organic carbon from phytoplankton cultures. *J. Plankton Res.*, 18: 1521-1533.

Chisholm, S. W., 1992. Phytoplankton size. In: P. G. Falkowski and A. D. Woodhead (Editors), *Primary productivity and biogeochemical cycles in the sea*. Plenum Press, New York, pp. 213-237.

Chu, W. L., S. M. Phang and S. H. Goh, 1996. Environmental effects on growth and biochemical composition of *Nitzscia inconspicua* Grunow. *J. Appl. Phycol.*, 8: 389-396.

Cole, J. J., S. Findley and M. L. Pace, 1988. Bacterial production in fresh and salt water ecosystems: a cross-system overview. *Mar. Ecol. Prog. Ser.*, 4: 1-10.

Darley, W. M., 1977. Biochemical composition. In: D. Werner (Editor), The biology of diatoms. University of California Press, Berkley and Los Angels, pp. 198-223.

Derenbach, J. B. and P. J. LeB. Williams, 1974. Autotrophic and bacterial production: Fractionations by differential filtration. *Mar. Biol.*, 25: 263-267.

Duffy, J. E. and M. E. Hay, 1990. Seaweed adaptations to herbivory. *Biosci.*, 40:368-375

Falkowski, P. G. and T. G. Owens, 1980. Light-shade adaptation: two strategies in marine phytoplankton. *Plant Physiol.*, 66: 592-595.

Fankboner, P. V. and M. E. DeBurgh, 1977. Diurnal exudation of ^{14}C-labelled compounds by the large kelp *Macrocystis integrifolia* Bory. *J. Exp. Mar. Biol. Ecol.*, 26: 151-162.

Faulkner, D. J., 1984. Marine natural products: metabolites of marine algae and herbivorous marine mollusks. *Nat. Prod. Rep.*, 1: 251-280.

Faulkner, D. J., 1986. Marine natural products. *Nat. Prod. Rep.*, 3: 2-33.

Flynn, K. J. and I. Butler, 1986. Nitrogen sources for the growth of marine microalgae: role of dissolved free amino acids. *Mar. Ecol. Prog. Ser.*, 34: 281-304.

Fogg G. E., 1983. The ecological significance of extracellular products of phytoplankton photosynthesis. *Bot. Mar.*, 26: 3-14.

Fogg G. E., C. Nalewajko and W. D. Watt, 1965. Extracellular products of phytoplankton photosynthesis. *Proc. R. Soc. Lond. Ser. B*, 162: 517-534.

Gaines, S. D. and J. Lubchenco, 1982. A unified approach to marine plant-herbivore interactions. II. Biography. *Ann. Rev. Ecol. Syst.*, 13: 111-138.

Garg, H. S., M. Sharma and D. S. Bhakuni, 1992. An antiviral sphingosine derivative from the green alga *Ulva fasciata. Tetraheadron Letters*, 33: 1641-1644.

Goldman, J. C., D. A. Hansell and M. R. Dennett, 1992. Chemical characterization of three large oceanic diatoms: potential impact on water column chemistry. *Mar. Ecol. Prog. Ser.*, 88: 257-270.

Guillard, R. R. L. and J. H. Ryther, 1962. Studies on marine planktonic diatoms. I. *Cyclotella nana* Hustedt, and *Detonula conforvacea* (Cleve) Gran. *Can. J. Microbiol.*, 8: 229-239.

Harlin, M. M., 1987. Allelochemistry in marine macroalgae. *Cri. Rev. Plant Sci.*, 5: 237-249.

Hasle, G. R. and E. E. Syvertson, 1997. Marine diatoms. In: C. R. Tomas (Editor), *Identifying marine phytoplankton.* Academic Press, New York, pp. 5-386.

Hein, M., M. F. Pedersen and K. Sand-Jensen, 1995. Size-dependent nitrogen uptake in micro- and macroalgae. *Mar. Ecol. Prog. Ser.*, 118: 247-253.

Hellebust, J. A., 1974. Extracellular products. In: W. D. P. Stewart (Editor), *Algal physiology and biochemistry.* Blackwell Scientific Publications, 989 pp.

Hellebust, J. A. and J. Lewin, 1977. Heterotrophic nutrition. In: D. Werner, (Editor), *Botany Monographs.* Blackwell Scientific Publication, Oxford, pp. 169-197.

Horner, S. M. J. and D. F. Smith, 1984. Distinguishing modes of dissolved organic carbon production in marine macrophytes by tracer kinetic analysis. *Mar. Biol.*, 81: 231-236.

Ivanova, V., R. Rouseva, M. Kolarova, J. Serkedjieva, R. Racher and N. Menolova, 1994. Isolation of a polysaccharide with antiviral effect from *Ulva lactuca. Prepar. Biochem.*, 24: 83-97.

Jin, H. J., J. H. Kim, C. H. Sohn, R. E. DeWreede, T. J. Choi, G. H. N. Towers J. B. Hudson and Y. K. Hong, 1997. Inhibition of *Taq* DNA polymerase by seaweed extracts from British Columbia, Canada and Korea. *J. Appl. Phycol.*, 9: 383-388.

Kamet, S. Y., S. Wahidulla, L. D'Souza, C. G. Naik, V. Ambiye, D. S. Bhakuni, A. K. Goel, H. S. Garg and R. C. Srimal, 1992. Bioactivity of marine organisms. *Bot. Mar.*, 35: 161-164.

Khailov, K. M. and Z. P. Burlakova, 1969. Release of dissolved organic matter by marine seaweeds and distribution of their total organic production to inshore communities. *Limnol. Oceanogr.*, 14: 521-527.

Kokkinnakis, S. A., 1987. Utilization of inorganic and organic nitrogen by phytoplankton off the Washington and Oregon coasts. A thesis presented to the degree of Master of Science, Oregon State University, Oregon, USA, 103p.

Larsson, U. and Å. Hagström, 1979. Phytoplankton exudate release as an energy source for the growth of pelagic bacteria. *Mar. Biol.*, 52:199-206.

Lawrence, J. M. and J. B. McClintock, 1988. Allocation of organic material and energy to the holdfast, stipe and fronds in *Postelsia palmaeformis* (Phaeophyta: Laminariales) on the California coast. *Mar. Biol.*, 99: 151-155.

Leigh, E. G., R. T. Paine, J. F. Quinn and T. H. Suchanek, 1987. Wave energy and intertidal productivity. *Proc. Natl. Acad. Sci. USA*, 84: 1314-1318.

Lewis, W. M., 1976. Surface/volume ratio: implications for phytoplankton morphology. *Science*, 192: 885-887.

Lubchenco, J. and S. D. Gaines, 1981. A unified approach to marine plant-herbivore interactions. I. Population and communities. *Ann. Rev. Ecol. Syst.*, 12: 405-437.

Mague, T. H., E. Friberg, D. J. Hules, 1980. Extracellular release of carbon by marine phytoplankton: a physiological approach. *Limnol. Oceanogr.*, 25: 262-279.

Malone, T. C., 1980. Algal size. In: I. Morris (Editor), *The physiological ecology of phytoplankton*. University of California Press, Berkley and Los Angeles, pp. 433-463.

Mann, K. H., 1982. *Ecology of coastal waters: a system approach.* University of California Press, 322 pp.

McLachlan, J. and J. S. Craigie, 1964. Algal inhibition by yellow ultraviolet-absorbing substances from Fucus vesiculosms. *Can. J. Bot.*, 42: 287-292.

McLachlan, J. and J. S. Craigie, 1966. Antialgal activity of some simple phenols. *J. Phycol.*, 2: 133-135.

Menge B. A., B. A. Daley and P. A. Wheeler, 1995. Control of interaction strength in marine benthic communities. In: G. A. Polis and R. Winelliner (Editors), *Food webs: integration of pattern and dynamics.* Chapman and Hall, New York, pp. 258-274.

Menge, B. A., B. A. Daley, P. A. Wheeler and P. T. Strub, 1997a. Rocky intertidal oceanography: an association between community structure and nearshore phytoplankton concentration. *Limnol. Oceanogr.*, 42: 57-66.

Menge, B. A., B. A. Daley, P. A. Wheeler, E. Dahlhoff, E. Sanford and P. T. Strub, 1997b. Benthic-pelagic links and rocky intertidal communities: evidence for bottom-up effects on top-down control. *Proc. Natl. Acad. Sci. USA*, 94: 14530-14535.

Miyashita, S. and T. Miyazaki, 1993. Effects of addition on phytoplankton in Lake Nakamura, Japan. I. Changes in biochemical components. *Hydrobiologia*, 254: 43-51.

Moebus, K. and K. M. Johnson, 1974. Exudation of dissolved organic carbon by brown algae. *Mar. Biol.*, 26: 117-125.

Montgomery, W. L. and S. D. Gerking, 1980. Marine macroalgae as foods for fishes: an evaluation of potential food quality. *Environ. Biol. Fish.*, 5: 143-153.

Myklestad, S. M., O. Holm-Hansen, K. M. Varum and B. E. Volcani, 1989. Rate of release of extracellular amino acids and carbohydrates from the marine diatom *Chaetoceros affinis. J. Plankton Res.*, 11: 763-773.

Naldi, M. and P. A. Wheeler, 1999. Changes in nitrogen pools in *Ulva fenestrata* (Chlorophyta) and *Gracilaria pacifica* (Rhodophyta) under nitrate and ammonium enrichment. *J. Phycol.*, 35: 70-77.

Parson, T. R., K. Stephens and J. D. H. Strickland, 1961. On the chemical composition of eleven species of marine phytoplankters. *J. Fish. Res. Bd. Can.*, 18: 1001-1016.

Pregnall, A. M., 1983. Release of dissolved organic carbon from the estuarine intertidal macroalga *Enteromorpha prolifera. Mar. Biol.*, 73: 37-42.

Ramus, J., 1992. Productivity of seaweeds. In: P. G. Faulkowski and A. D. Woodhead (Editors), *Primary productivity and biochemical cycles in the sea*. Plenum Press, New York, pp. 239-255.

Rosenberg, G. and J. Ramus, 1984. Uptake of inorganic nitrogen and seaweed surface area: volume ratio. *Aquat. Bot.*, 19: 65-72.

Schramm, W., E. Gualberto and C. Orosco, 1984. Release of dissolved organic matter from marine tropical reef plants: temperature and desiccation effect *Botanica Marina*, 27: 71-77.

Sieburth, J. M., 1969. Studies on algal substances in the sea. III. The production of extracellular organic matter by littoral marine algae. *J. Exp. Mar. Biol. Ecol.*, 3: 290-309.

Stadelmann, E. J., 1962. Permeability. In: R. A. Lewin (Editor), *Physiology and biochemistry of algae*. Academic press, New York, pp. 493-528.

Sugimura, Y. and Y. Suzuki, 1988. A high-temperature catalytic oxidation method for the determination of non-volatile dissolved organic carbon in seawater by direct injection of a liquid sample. *Mar. Chem.*, 24: 105-131.

Suzuki, M. T., E. B. Sherr and B. F. Sherr, 1993. DAPI direct counting underestimates bacterial abundances and average cell size compared to AO direct counting. *Limnol. Oceanogr.*, 38: 1566-1570.

Taylor, R. B., J. T. A. Peck and T. A. V. Rees, 1998. Scaling of ammonium uptake by seaweeds to surface area: volume ratio: geographical variation and the role of uptake by passive diffusion. *Mar. Ecol. Prog. Ser.*, 169: 143-148.

Valiela, I., 1991. Ecology of water columns. In: R. S. K. Barnes and K. H. Mann (Editors), *Fundamentals of aquatic ecology*. Blackwell Scientific Publications, pp. 29-56.

Verardo, D. J., P. N. Froelich and A. McLutyre, 1990. Determination of organic carbon and nitrogen in marine sediments using the Carlo Erba NA-1500 analyses. *Deep-Sea Res.* 37: 157-165.

Westlake, D. F., 1963. Comparisons of plant productivity. *Biol. Rev.*, 38: 385-429.

Wheeler, P. A., 1983. Phytoplankton nitrogen metabolism. In: E. J. Carpenter and D. C. Capone (Editors), *Nitrogen in the Marine Environment.* Academic Press, pp. 309-346.

Yeagle, P., 1991. The structure of biological membranes. CRC Press. 1227 pp.