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The effects of light and nitrate levels on the relationship between nitrate reductase activity and $^{15}NO_3^-$ uptake: Field observations in the East China Sea

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Abstract

Nitrate reductase activity (NRA) and $^{15}NO_3^-$ uptake (NU) were determined in the East China Sea and the adjoining Kuroshio in May 1996, at six stations covering a range of hydrographic conditions: the nutrient-rich and fresher plume of Changjiang Diluted Water along the Chinese coast, the nutrient-rich upwelling Kuroshio Subsurface Water at the shelf edge northeast of Taiwan, the oligotrophic Kuroshio Surface Water and the mixing zones among these water masses on the shelf. The values of NRA in the surface mixed layer ranged between 16 and 0.1 nM-N h^{-1}, whereas those of NU ranged between 37 and 1 nM-N h^{-1}. Higher NRA and NU were found in the frontal zone between the coastal and shelf waters and in the upwelling zone, whereas the lowest values were found in the surface Kuroshio. The NRA/Chl *a* ratio increased linearly with increasing NU/primary production ratio in the sequence: Kuroshio < coastal plume < upwelling zone and mixing zones in the shelf. This is probably a reflection of the varying nutrient condition and the relative importance of NU in sustaining the biomass in these regions.

In nitrate- and light-replete waters, the average NU/NRA was 1.0 ± 0.3. NRA was linearly related to NU so that NU = 1.08 (± 0.07)NRA (r^2 = 0.79). Thus, NRA may be used for estimating NU in these waters. In nitrate-deficient and light-replete waters, the average NU/NRA was 4 ± 4. These high and variable values of NU/NRA might have been caused by an over-estimation of NU as a result of the stimulatory effect of the added $^{15}NO_3^-$ on phytoplankton growth. Thus, NRA may be a more reliable indicator of the rate of NO_3^- uptake in oligotrophic waters. In nitrate-replete and light-deficient waters, NU did not correlate well with NRA. The average NU/NRA was 0.7 ± 0.7. These low and variable values of NU/NRA suggest a possible decoupling between NRA and NU.

By using the relationship between NU and NRA in nitrate- and light-replete waters and the depth-integrated inventory of NRA in the photic zone at each station, NU in oligotrophic waters, the coastal plume, upwelling waters and shelf waters can be estimated to be 0.45, 1.55, 3.12, and 3.59 mg-N m^{-2} h^{-1} respectively. These values fall well within the range of previously reported values in similar types of water.

The notion of subdividing primary production into new

production and recycled production was first introduced by Dugdale and Goering (1967). They defined new production as the fraction of primary production in the euphotic zone that is supported by external sources of nutrients such as the upwelling of deep water, atmospheric deposition, nitrogen fixation, and riverine input whereas recycled production is supported by the recycling of nutrients within the euphotic zone between the dissolved and the particulate phases. If the euphotic zone is at a steady state, new production will be equal to the export of organic carbon to the deep ocean (Eppley and Peterson 1979). Thus, an understanding of the governing processes and an accurate estimation of global new production have been widely recognized as crucial factors for deciphering the present imbalance in the global budget of anthropogenic carbon (Platt et al. 1992; Siegenthaler and Sarmiento 1993; Sarmiento and Le Quere 1996).

In the open oceans, away from the influence of land, new production may be supported primarily by the assimilation of nitrate. Thus, it has been estimated by measuring the uptake of ^{15}N-labeled nitrate, or NU, in incubation experiments (Eppley 1989). However, this approach has its limitations. For example, since ^{15}N is a rather abundant and naturally
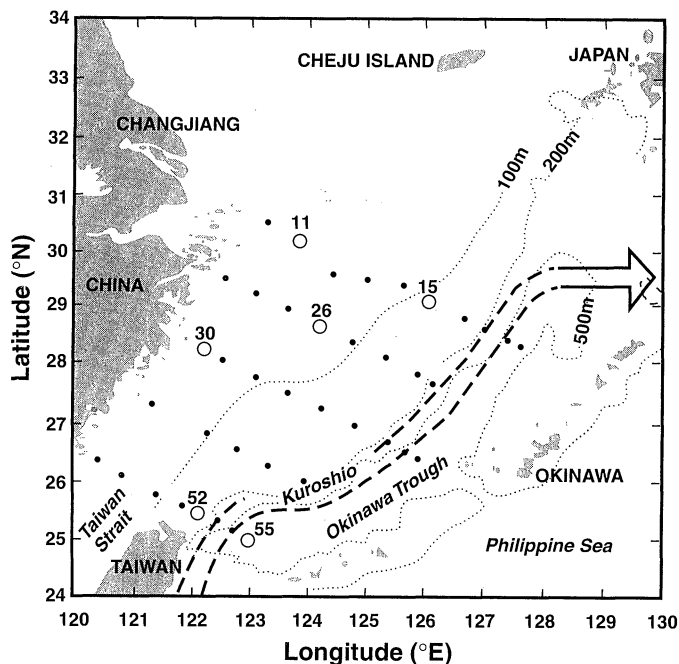
Fig. 1. The study area. Locations of stations where nitrate reductase activity (NRA) and $^{15}NO_3^-$ uptake (NU) were determined (○). Locations of other hydrographic stations (●).

occurring isotope of nitrogen, the sensitivity of the method can be affected by the relative amounts of the added and the naturally occurring $^{15}NO_3^-$. In oligotrophic waters, the nitrate concentrations can become too low to be estimated accurately by the standard method (Strickland and Parsons 1972). In such a situation, the added $^{15}NO_3^-$ may become excessive and cause artificially stimulated nitrate uptake. This can lead to high and variable results (Dugdale and Wilkerson 1986; McCarthy et al. 1992, 1996; Allen et al. 1996). Furthermore, the determination of NU requires extensive sample manipulation and the use of a mass spectrometer so that it cannot be carried out routinely on board ship. Even as a shore-based laboratory method, the associated costs and time for each analysis limit sample throughput.

Nitrate assimilation by phytoplankton involves several enzymatically mediated steps: (1) the transport of nitrate into the cell; (2) the reduction of nitrate to nitrite by nitrate reductase, (3) the reduction of nitrite to ammonia by nitrite reductase, and (4) the incorporation of ammonia-nitrogen into organic molecules (Wada and Hattori 1991). The determination of nitrate reductase activity (NRA) measures the rate of the second step under a constant set of operationally defined conditions (Eppley et al. 1969, 1970; Packard et al. 1971, 1978; Slawyk and Collos 1976; Eppley 1978; Hochman et al. 1986; Berges and Harrison 1995a). This measurement can be carried out readily onboard a ship. If nitrate reduction is the rate-limiting step in nitrate assimilation, NRA will be proportional to nitrate assimilation and thus NU so that new production may be estimated from NRA once a relationship between NRA and NU is established (Eppley et al. 1970; Packard et al. 1971; Wheeler 1983; Berges and Harrison 1993). However, although a consistent, positive, and quantitative relationship between the calculated

rates of nitrate incorporation and NRA has been found in several recent laboratory studies of phytoplankton cultures under carefully controlled conditions (Berges and Harrison 1995a,b; Berges et al. 1995), such a relationship was not observed in several earlier field studies (McCarthy and Eppley 1972; Collos and Slawyk 1977; Dortch et al. 1979; Blasco et al. 1984). The results of these earlier field studies might have been affected by the limited sensitivity of the NRA assays used (Berges and Harrison 1995a) and/or the influence of environmental factors, specifically light and nutrient conditions, on new production and NRA (Packard and Blasco 1974; Blasco and Conway 1982; Collos 1982; Blasco et al. 1984; Gao et al. 1992; Smith et al. 1992). Furthermore, most of these field studies were conducted in upwelling areas (Eppley et al. 1970; Collos and Slawyk 1977; Blasco et al. 1984; Slawyk et al. 1997). The relationships between new production and NRA in other oceanic subenvironments, such as coastal, shelf, and oligotrophic waters, have not yet been fully examined.

The East China Sea is one of the larger and more productive marginal seas of the world (Fig. 1). It extends from the Cheju Island, at about 33°20'N, in the north to the northern coast of the island of Taiwan, at about 25°N, in the south. It is bounded to the east by the Kuroshio and to the west by continental China. Extensive exchanges occur between the East China Sea and the Kuroshio. The warm, saline, and nutrient poor Kuroshio Surface Water finds its way to the shelf through frontal processes. The cold and nutrient-rich Kuroshio Subsurface Water can reach the shelf through topographically induced upwelling (Chern and Wang 1990; Chern et al. 1990; Su et al. 1990; Wong et al. 1991; Hsueh et al. 1992; Liu et al. 1992a,b; Chen et al. 1995; Gong and Liu 1995; Chen 1996; Gong et al. 1996). A year-round permanent upwelling center is present at the shelf edge northeast of Taiwan (Liu et al. 1992b). At this location, the upwelling is strong enough at times that its influence can become clearly evident even in the surface waters. Presently, it is unclear whether there may be other upwelling centers along the remainder of the shelf edge. The East China Sea is also the receiving water of the outflow from Changjiang, one of the larger rivers of the world with a flow rate of 979 km^3 yr^{-1} (Milliman and Jin 1985). This results in the presence of the fresh, cold, and nutrient-rich Changjiang Diluted Water along the Chinese coast (Wong et al. 1998). Another source of surface water to the Sea is the Taiwan Current Warm Water which enters the Sea through the Taiwan Strait from the south. This water is nutrient poor, saline, and warm but its temperature and salinity are slightly lower than those of the Kuroshio Surface Water. Thus, the major surface water masses in the East China Sea are the Changjiang Diluted Water, the Taiwan Current Warm Water, the Kuroshio Surface Water, and the upwelling Kuroshio Subsurface Water (Liu et al. 1992a; Chen et al. 1995; Gong and Liu 1995). These water masses exhibit a wide range of nutrient, light, and/or hydrographic conditions. In this study, the relationships between NU and NRA in the East China Sea under different light and nitrate concentrations were examined.

Materials and methods

Sampling—Forty stations were occupied in five transects across the East China Sea between 2 and 15 May 1996 aboard the R/V *Ocean Research I* during Cruise ORI-449 of the Kuroshio Edge Exchange Processes (KEEP) Study. The locations of the stations are shown in Fig. 1.

Hydrographic and nutrient measurements—At each station, the distributions of temperature, salinity, and fluorescence were recorded with a SeaBird model SBE9/11 conductivity–temperature–depth (CTD) recorder. Photosynthetically active radiance (PAR) was recorded with a Biospherical Instruments Model QSP200L radiometer. Discrete water samples were collected with GO-FLO bottles mounted onto a Rosette sampling assembly (General Oceanic). Subsamples were then obtained for the determination of salinity, nitrite, and (nitrate + nitrite).

Subsamples were returned to the shore-based laboratory for the determination of salinity with an Autosal salinometer. The precision for this measurement was ±0.003. Nitrite and (nitrate + nitrite) were determined on board ship by the standard pink azo dye method which has been adapted for use with a flow injection analyzer (Morris and Riley 1963; Strickland and Parsons 1972; Gardner et al. 1976; Pai et al. 1990; Gong 1992; Liu et al. 1992a,b). The precision for the determination of nitrate and nitrite were ±0.3 and ±0.03 μM respectively.

Chl a, primary production, $^{15}NO_3^-$ uptake and NRA assay—Subsamples were obtained at six stations: Sta. 11, 15, 26, 30, 52, and 55 (Fig. 1) for the determination of chlorophyll *a*, primary production, $^{15}NO_3^-$ uptake and NRA. Sta. 52 and 55 were occupied twice within a 6-hour period (Sta. 52-1 and 52-3, and Sta. 55-3 and 55-5) to study the temporal changes in these parameters. Chlorophyll *a* (Chl *a*) was determined by the method of Strickland and Parsons (1972) as modified by Gong et al. (1993) and Chen et al. (1999). Seawater (100 ml) was filtered through 47-mm Whatman GF/F glass fiber filters on board ship, then stored immediately at −20°C and returned to the laboratory for further processing. In the laboratory, the filters were ground in and extracted with 10 ml of 90% acetone at 4°C for 2 h. Then, the mixture was centrifuged for 10 min at 3,000 rpm. The concentration of Chl *a* in the supernatant liquid was measured fluorimetrically with a Turner model 10-AU-005 fluorometer. The fluorometer was calibrated against a standard prepared from pure Chl *a* (Sigma Chemical) The precision in the determination of Chl *a* was ±8% at 0.1 mg m^{-3}.

Primary production was determined by the uptake of ^{14}C-labeled bicarbonate by the method of Parsons et al. (1984) as modified by Shiah et al. (1995). Seawater was filtered on board ship through a 200 μm mesh-size net to remove the larger zooplankton (Strickland and Parsons 1972). It was then transferred, together with 10 μCi of NaH$^{14}CO_3$, into a 250-ml acid-cleaned polycarbonate bottle (Nalgene). The sample was incubated under in situ conditions for 6 h. After the incubation, the sample was filtered through a 25-mm Whatman GF/F glass fiber filter under low vacuum (<100 mm Hg). The filter was stored in the dark at −20°C and

returned to the shore-based laboratory where it was analyzed for its ^{14}C content by liquid scintillation counting using a Packard Model 2700TR liquid scintillation counter. The precision in the counting statistics was about ±1%.

The uptake of nitrate was determined by the method of Dugdale and Wilkerson (1986) by measuring the uptake of added $^{15}NO_3^-$. On board ship, seawater was filtered through a 200-μm mesh-size net. The concentration of nitrate in the sample was determined on board ship. When there was a detectable concentration of nitrate in the sample, an amount of $^{15}NO_3^-$ equivalent to 10% of that concentration was added to the filtrate in a 1-liter polycarbonate bottle. (If the concentration of nitrate was below detection limit, 100 nM of $^{15}NO_3^-$ was added to the sample.) The mixture was incubated under in situ conditions for 6 h. Upon the termination of the incubation, the sample was filtered through 25-mm Whatman GF/F glass fiber filters. The filters were stored in a freezer at −20°C and were returned to a shore-based laboratory for the determination of their ^{15}N content by mass spectrometry using a Carlo-Erba NA1400 elemental analyzer which fed into a VG622 Micromass mass spectrometer. The mean standard deviation in the determination of the ^{15}N to ^{14}N atomic ratio was about ±0.006%. The rate of nitrate uptake was calculated by using the protocols for the Joint Global Ocean Flux Study (UNESCO 1994). Thus,

$$\text{Nitrate uptake (nM h}^{-1}) = (^{15}Nxs \cdot PNt)/(^{15}Nenr \cdot t) \quad \text{and}$$

$$^{15}Nenr = [(100 \cdot {}^{15}N)/({}^{15}N + {}^{14}N)] - {}^{15}Nn$$

where *t* is the incubation time in hours, ^{15}Nxs is the excess ^{15}N (measured ^{15}N minus ^{15}N natural abundance, 0.366 atom%) in the postincubation particulate sample, PNt is the particulate nitrogen content of the sample after incubation in units of nM, $^{15}Nenr$ is the ^{15}N enrichment in the dissolved fraction, ^{15}N is the concentration of labeled N in nM, ^{14}N is the concentration of unlabeled N in nM and ^{15}Nn is the natural abundance of ^{15}N. In this scheme, the effect of nitrate regeneration was not accounted for.

Nitrate reductase activity was determined on board ship by a modified version of the method of Hochman et al. (1986). Seawater (20 liters at Sta. 55 and 4 to 8 liters at the other stations) was collected just after sunrise and filtered through a 47-mm Gelman type A/E glass fiber filter. The filter was transferred to a beaker together with 1 ml of a phosphate buffer (150 mM of K_2HPO_4 adjusted to a pH of 7.6), 50 μl of toluene, 0.2 ml of 6.5 mM NADH (Sigma Chemical) and 0.2 ml of 0.1 M potassium nitrate. The beaker was placed on a vortex mixer and mixed for 5 min at room temperature, ≈20°C. Then, the reaction was terminated by pipetting 1 ml of the slurry from the beaker into a centrifuge tube containing 1.7 ml of 0.13 M ZnSO$_4$ at 97°C. After the solution was allowed to cool, 0.2 ml of 1 N NaOH was added and the mixture was centrifuged for 20 min at 4,000 rpm. Two milliliters of the supernatant liquid was removed for the determination of nitrite by adding 0.1 ml of a 2% (w/v) sulfanilamide solution in 15% hydrochloric acid and 0.1 ml of a 0.3% (w/v) *N*-1-naphthylethylenediamine hydrochloride solution to the supernatant liquid and measuring the absorbance of the azo dye formed at 545 nm (Strickland and Parsons 1972) with a Brinkman PC-800 probe colorimeter

(a) Temperature (°C)

(b) Salinity

(c) Nitrate (μM)

(d) Chlorophyll-a (mg m⁻³)
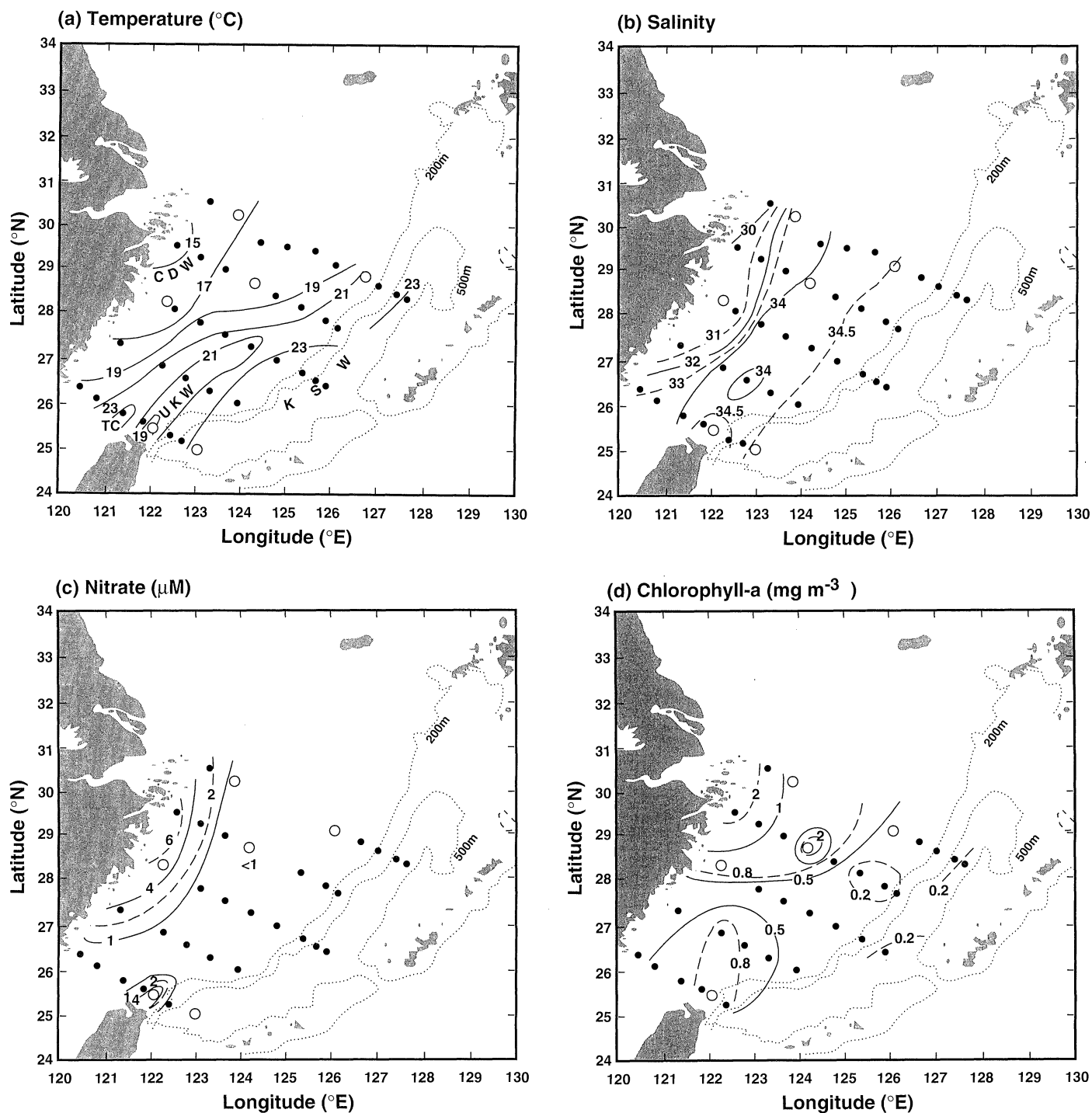
Fig. 2.   The surface distribution of (A) temperature, (B) salinity, (C) nitrate, and (D) Chl *a* in the study area. CDW, Changjiang diluted water; KSW, Kuroshio surface water; UKW, upwelling Kuroshio subsurface water; TC, Taiwan current warm water. Station positions refer to Fig. 1.

equipped with a probe tip with a 2-cm light path. The precision in repeated determinations of NRA in a sample was ±5%. Since nitrite could be detected down to about 0.1 μM, the corresponding detection limits for the determination of NRA would be 0.4 and 0.1 nM-N h⁻¹ when 5 and 20 liters of sample were processed respectively. The procedural blank of the method included the reagent blank and the blank due

to the presence of intracellular nitrite. The reagent blank corresponded to 0.03 and <0.01 nM-N h⁻¹ when sample volumes of 5 and 20 liters were used. The presence of intracellular nitrite gave rise to a blank that corresponded to NRA of 0.2 nM-N h⁻¹ mg Chl *a*⁻¹ m³. The results reported here had been corrected for these blanks. The use of Gelman A/ E filters (nominal pore size 1.0 μm) for the determination

Fig. 3. The vertical distribution of (A) temperature, salinity and σ_Θ, (B) nitrate, Chl a, and %PAR, and (C) NRA and NU at Sta. 11. Horizontal dashed line denotes PZD_{10}. Horizontal thin solid line denotes MLD.

Fig. 4. Same as Fig. 3 at Sta. 15.

of NRA and Whatman GF/F filters (nominal pore size 0.7 μm) for the determination of primary production, $^{15}NO_3^-$ uptake, and Chl a may lead to an undersampling of the picoplankton. However, the diff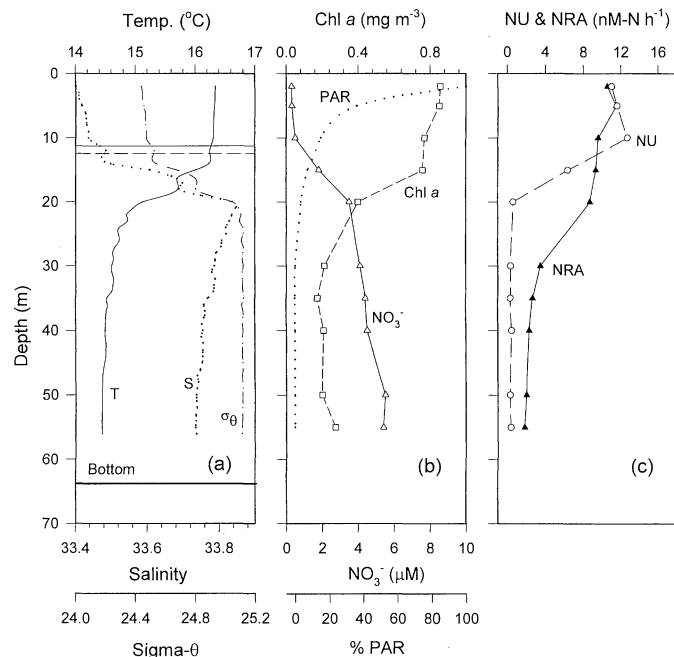erence in the retention efficiency between these two types of filters should be minimal since others have shown that filters with even larger differences in nominal pore size retain similar fractions of the phytoplankton (Venrick et al. 1987). Furthermore, the dominant types of phytoplankton in the study area are larger phytoplankton, such as Skeletonema costatum, Thalassiosira spp., Thalassionema nitzschiodies, and Trichodesmium spp. (Chen 1995), which should be retained effectively by both kinds of filters.

Results

Hydrography, light, nutrient, and Chl a—The surface distributions of temperature, salinity, nitrate and Chl a are shown in Fig. 2. (Station positions refer to Fig. 1.) The four major surface water masses were distinctly identifiable. The fresh, cold, and nitrate-rich Changjiang Diluted Water formed a band along the Chinese coast extending southward from the mouth of the Changjiang in the northwestern corner of the study area. Sta. 30 (Fig. 1) was located within this plume of fresher water. Sta. 11 and Sta. 26 were located at the seaward side of the frontal region of this plume. The highest concentration of Chl a in the study area, 2.44 mg m^{-3}, was found at Sta. 26. The warm and saline Kuroshio Surface Water was found along the shelf edge. Sta. 55 was located in the Kuroshio, whereas Sta. 15 was located on the shelf side of the frontal zone of the Kuroshio. The upwelling
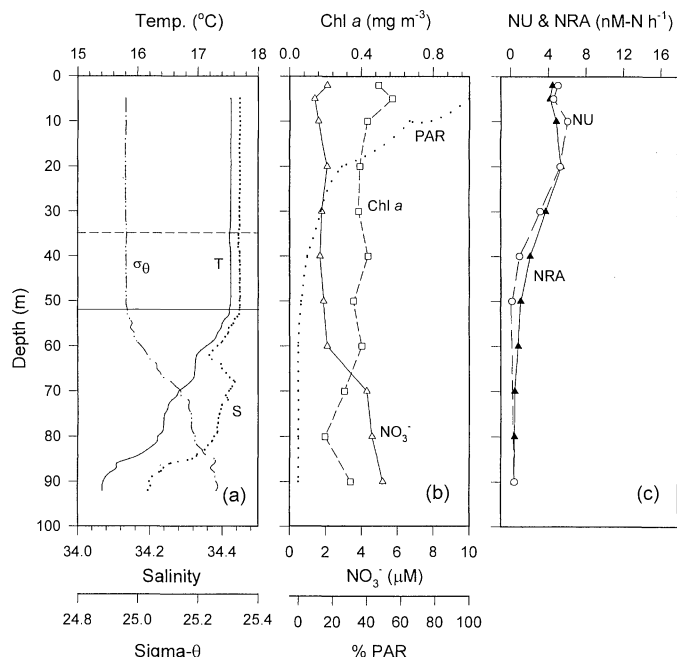
Kuroshio Subsurface Water manifested itself as a patch of colder and nutrient-rich water with moderately high concentrations of Chl a (>0.8 mg m^{-3}) northeast of Taiwan. Sta. 52 was located near the center of this upwelling dome. A small patch of warm (>23°C) water was found northwest of Taiwan in the Taiwan Strait, and was probably the Taiwan Current Warm Water.

The vertical distributions of temperature, salinity, sigma-Θ, nitrate, Chl a, and %PAR (% of the PAR at the sea surface) at Sta. 11, 15, 26, 30, 52, and 55 are shown in Figs. 3–8. The mixed layer depth (MLD, the depth at which the density gradient increased abruptly by a factor of more than 2) and the photic zone depth (PZD_{10}, the depth at which %PAR dropped to 10%) are also indicated in the figures. The averages of some of the characteristics of the water at these stations over the depths of the mixed layers are summarized in Table 1. Stations with shallow MLD and PZD_{10} (Table 2), high Chl a, and strong pycnoclines were found in the coastal fresh water plume (Sta. 30) and at the frontal region of the plume (Sta. 11 and 26). However, high concentrations of nitrate (6 μM) were found only at Sta. 30. The concentration of nitrate at the other two stations was <0.5 μM. At Sta. 26, the mixed layer was particularly shallow (3 m) and the surface concentration of Chl a was exceptionally high (2.4 mg m^{-3}). At Sta. 30, the fresher surface mixed layer was underlain by a layer of colder water between 13 and 20 m. Below 20 m, both temperature and salinity increased with depth. In the upwelling region at Sta. 52, as in the coastal plume, there was a shallow MLD and PZD_{10}, and high Chl a and high nitrate were again found. In contrast, in the oligotrophic Kuroshio at Sta. 55, the MLD and the PZD_{10} were much deeper, whereas the concentrations of nitrate and Chl a were low. At the frontal region between the Kuroshio and the shelf water, at Sta. 15, the MLD was the
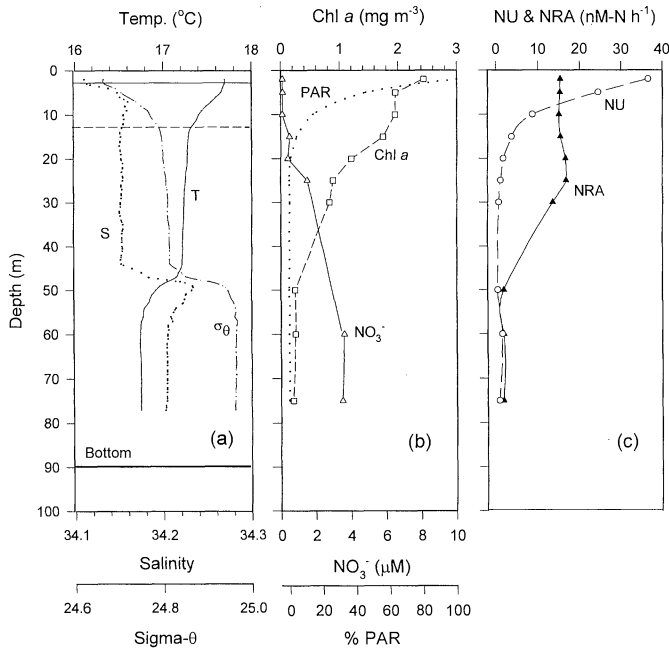
Fig. 5.   Same as Fig. 3 at Sta. 26.
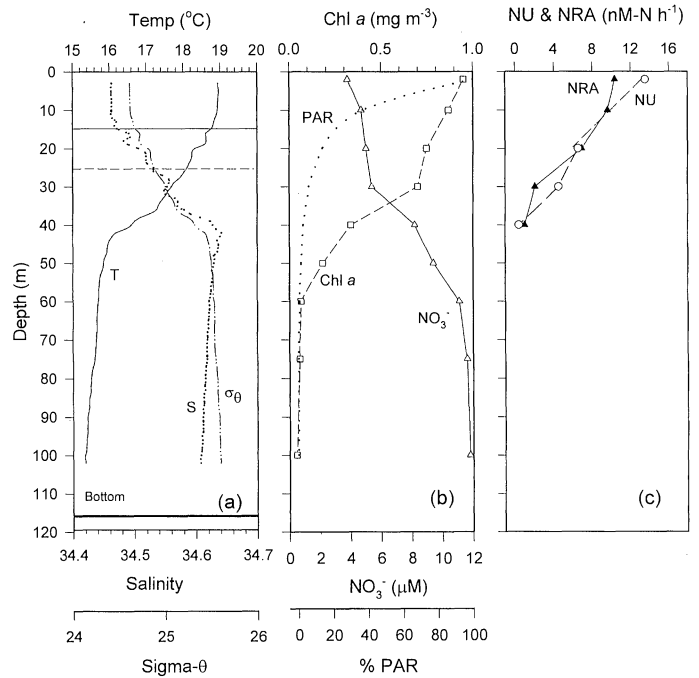
Fig. 7.   Same as Fig. 3 at Sta. 52-3.

deepest among these six stations (52 m) and exceeded the PZD (34 m), and this was accompanied by intermediate levels of nitrate and Chl *a*.

*NRA and NU–*As a first approximation, the vertical distributions of NRA and NU were usually similar to each other and to Chl *a* (Figs. 3–8). Significant levels of NRA and NU were confined primarily to within the MLD, the PZD_{10} or both. At greater depths, they dropped abruptly with depth to

a background value. In the mixed layer, higher NRA and NU were found at the frontal zone of the coastal plume at Sta. 11 and at Sta. 26 (Table 1). The values at Sta. 26 (NRA = 15 nM-N h^{-1}; NU = 37 nM-N h^{-1}) were exceptionally high. Within the plume, at Sta. 30, NRA and NU were considerably lower. These values were similar to those observed at the frontal zone between the Kuroshio and the shelf water at Sta. 15. High NRA and NU were also found in the up-

Fig. 6.   Same as Fig. 3 at Sta. 30.

Fig. 8.   Same as Fig. 3 at Sta. 55-3.

Table 1. Characteristics of the mixed layer in the different hydrographic regimes of the East China Sea.

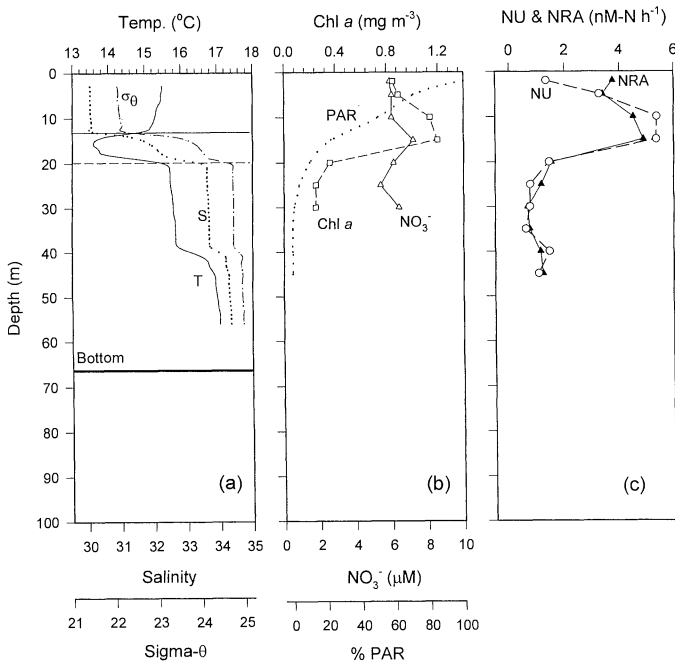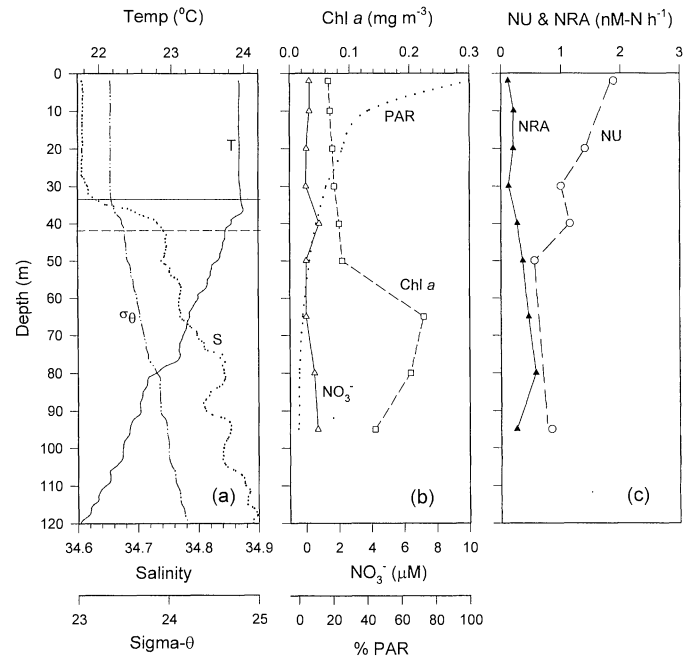| Hydrographic regime	Sta.	Mixed-layer depth (m)	S	T °C	NO_3^- (μM)	Chl a (mg m^{-3})	NU (nM-N h^{-1})	NRA (nM-N h^{-1})	NU/NRA
Coastal plume-shelf frontal region	11	12	33.4	16.3	0.4	0.83	11.8	10.6	1.11
Shelf-Kuroshio frontal region	15	52	34.4	17.5	1.8	0.43	3.6	3.7	0.98
Coastal plume-shelf frontal region	26	3	34.1	17.7	0.1	2.44	36.5	15.4	2.38
Coastal plume	30	13	30.1	15.4	6.0	0.96	3.3	4.7	0.71
Upwelling center	52-1	15	34.5	18.5	5.0	0.77	ND*	5.3	ND
Upwelling center	52-3	15	34.5	18.9	4.3	0.90	13.5	10.0	1.35
Kuroshio	55-3	33	34.6	23.9	<0.1	0.07	1.4	0.17	8.65
Kuroshio	55-5	35	34.6	24.0	<0.1	0.06	ND	0.13	ND

* ND, no data.

welling zone at Sta. 52. The lowest NRA and NU were found in the oligotrophic Kuroshio at Sta. 55. Below the MLD, on occasions, NRA did not decrease with depth in step with NU as it seemed to drop less abruptly with depth. This phenomenon was clearly evident at Sta. 26 where high NRA persisted down to about 30 m, whereas NU had decreased to a residual level below 15 m.

Temporal variations—Sta. 52 and 55 were sampled a second time within six hours from the time when the data in Figs. 7, 8 were collected. The two sets of results are compared in Fig. 9. At Sta. 55, the hydrography did not change significantly between the casts as indicated by their similar temperature profiles. The corresponding distributions of NRA in these two casts were also approximately the same. The high degree of reproducibility in these profiles gives credence to the reliability of our modified method of Hochman et al. (1986) for the determination of NRA. At Sta. 52, between the two casts, there was a warming of the surface water by about 0.5°C and a shoaling of the thermocline. The corresponding distribution of NRA also showed higher surface NRA and a shoaling of the depth at which the NRA dropped noticeably. The depth-integrated inventories of NRA through PZD_{01}, the photic zone depth down to 1% of %PAR, with averages of 2.88 ± 18% and 0.42 ± 18% mg-N m^{-2} h^{-1} at Sta. 52 and 55 respectively, did not vary greatly between casts (Table 3).

Discussion

Light, nitrate concentrations, and NU/NRA—Light is one of the primary environmental factors that can affect nitrate assimilation (Packard and Blasco 1974; Gao et al. 1992; Smith et al. 1992; Berges et al. 1995). PZD_{01} has been used traditionally as the base of the euphotic zone below which light becomes a limiting factor of photosynthetic activities (Parsons and Takahashi 1977). The relationship between NU/NRA and nitrate at depths where %PAR exceeded 10% is shown in Fig. 10A. At these depths, the availability of light should no longer be a limiting factor for nitrate assimilation. At nitrate concentrations above 1 μM, all the data points coalesced around a ratio of about 1. The average was 1.0 ± 0.3 (Table 4). Thus, there was an almost 1:1 relationship between NRA and NU under light- and nutrient-replete conditions. Below a nitrate concentration of 1 μM, there were much larger scatter. Some exceedingly high values of NU/NRA, up to >15, were found and the average was 4 ± 4 (Table 4). These excess NU relative to NRA in light-replete but nutrient-deficient waters could have been an artifact which was caused by the $^{15}NO_3^-$ added for the determination of NU (Eppley et al. 1977; Dugdale and Wilkerson 1986; McCarthy et al. 1996). In nitrate-deficient waters, the availability of nitrate may become the determining factor that limits nitrate assimilation. The amount of $^{15}NO_3^-$ added in the determination of NU was intended to be 10%

Table 2. Characteristics of the photic zone to PZD_{10} in the different hydrographic regimes of the East China Sea.*

Sta.	PZD_{10} (m)	NRA/Chl a (nM-N h^{-1} mg^{-1} m^3)	NU_{10} (mg-N m^{-2} h^{-1})	NU_{10}' (mg-N m^{-2} h^{-1})	NU_{10}'/NU_{10}	NU_{10} (mg-C m^{-2} d^{-1})	PP_{10} (mg-C m^{-2} d^{-1})	NU_{10}/PP_{10}
11	13	12.8	2.09	2.06	0.99	142	344	0.41
15	34	10.2	2.25	2.35	1.04	153	625	0.25
26	12	7.3	3.67	2.77	0.75	250	505	0.50
30	20	4.8	1.05	1.36	1.30	72	467	0.15
52-1	24	7.2	ND†	1.85	ND	ND	ND	ND
52-3	25	10.4	3.32	3.21	0.97	226	1,007	0.22
55-3	41	2.6	0.80	0.11	0.14	8‡	153	0.05
55-5	63	4.1	ND	0.23	ND	ND	ND	ND

* PZD_{10}, Photic zone depth to 10% of surface photosynthetically active radiance (PAR). NU_{10}, NU_{10}', PP_{10}, Depth integrated NU, NU estimated from NRA and primary production to PZD_{10}. NU_{10}/PP_{10}, Both in mg-C m^{-2} d^{-1}.
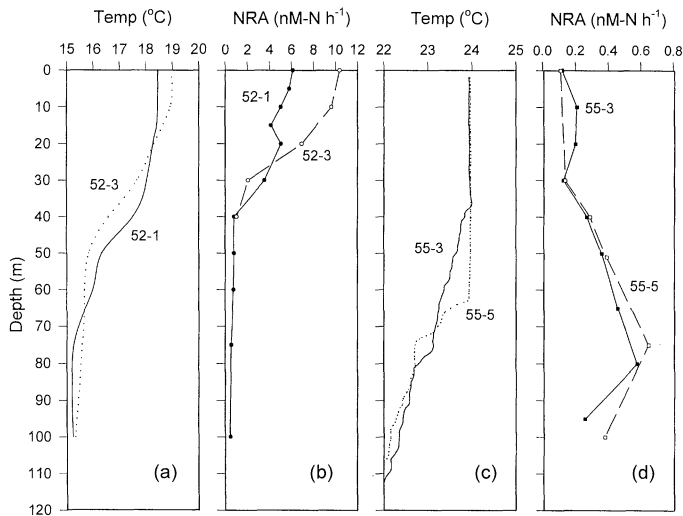† ND, No data.
‡ Estimated from NU_{10}'.

Fig. 9. The vertical distribution of temperature and NRA in two casts at Sta. 52 (A and B) and Sta. 55 (C and D).

of the ambient concentration. However, in these oligotrophic waters, the ambient concentrations of nitrate were frequently below the detection limit of the analytical method used. In these cases, 100 nM of $^{15}NO_3^-$ was added to the sample. Thus, the exact molar ratio between the added amount of $^{15}NO_3^-$ and the ambient concentration was not known. It was probably variable from sample to sample and it could have been significantly larger than 10%. If the added $^{15}NO_3^-$ had become excessive and had stimulated additional phytoplankton growth, nitrate assimilation would have been over-estimated by NU. On the other hand, NRA does not suffer from this source of error. Thus, in oligotrophic waters, NRA may be a more reliable indicator of nitrate assimilation.

The relationship between NU/NRA and %PAR at %PAR >1% in waters with nitrate exceeding 1 μM is shown in Fig. 10B. In these waters, the availability of nitrate should no longer be a limiting factor for nitrate assimilation. At %PAR above 10%, NU/NRA coalesced around a constant value close to 1. Between %PAR of 1% and 10%, there was much greater scatter. NU/NRA tended to be lower than those in the light-replete waters. The average NU/NRA was 0.7 ±

0.7 (Table 4). Previous investigators (Blasco et al. 1984; McCarthy et al. 1996; Slawyk et al. 1997) have suggested that NRA represents a retrospective integration of the potential in nitrate reduction, whereas NU is a prospective integration of nitrate assimilation by the phytoplankton cells. Although NRA is inhibited in the dark and is activated by light, the adjustment is not instantaneous. The adjustment time from a dark condition to a light condition may take a fraction of an hour to hours before a steady-state level of NRA is reached (Eppley et al. 1970; Packard and Blasco 1974; Gao et al. 1992; Smith et al. 1992; Berges et al. 1995). Thus, when nitrate reductase is extracted from a collection of phytoplankton cells for the determination of NRA, the NRA found includes the influence of a sum of the histories of the light conditions experienced by the cells prior to sampling. On the other hand, NU represents nitrate assimilation during the incubation period under the light condition following sample collection. NRA and NU are equivalent if the environmental conditions have remained the same through out the time periods over which these two measurements integrate. However, under light-deficient and possibly light-limiting conditions, the light histories of the cells may be variable and may include more light-abundant conditions as a result of vertical mixing, vertical migration or both. The cells that have experienced higher light condition will contribute enzyme activities that are higher than what would be expected relative to the light condition at which the sample is collected. Moreover, these light-deficient and nitrate-replete conditions were found in or close to the nitracline. Ward et al. (1989) and Eppley and Koeve (1990) suggested that nitrate may be formed in these waters by nitrification. The nitrate formed may dilute the $^{15}NO_3^-$ added and cause an underestimation of NU. The low and highly variable values of NU/NRA observed suggest a possible decoupling between NU and NRA which may be explained by the overestimation of nitrate assimilation by NRA, the underestimation of nitrate assimilation by NU or both.

Relationships between NRA and NU, and NRA and Chl-a—The relationships between NRA and NU (Fig. 10) suggest that the decoupling between NRA and NU occurs at concentrations of nitrate below 1 μM, %PAR below 10% or

Table 3. Characteristics of the photic zone to PZD_{01} in the different hydrographic regimes of the East China Sea.*

Sta.	PZD_{01} (m)	NU_{01}	NRA_{01}	NU_{01}'	NU_{01}'/NU_{01}	NU_{01}	PP_{01}	NU_{01}/PP_{01}
		(mg-N m^{-2} h^{-1})				(mg-C m^{-2} d^{-1})		
11	29	2.61	3.59	3.90	1.50	178	556	0.32
15	36	2.31	2.25	2.44	1.06	157	645	0.24
26	19	4.06	4.09	4.43	1.09	276	604	0.46
30	29	1.17	1.43	1.55	1.32	80	489	0.16
52-1	40	ND†	2.35	2.55	ND	ND	ND	ND
52-3	45	4.02	3.41	3.70	0.92	274	1,155	0.24
55-3	78	1.16	0.34	0.36	0.32	25‡	294	0.08
55-5	100	ND	0.49	0.53	ND	ND	ND	ND

* PZD_{01}, Photic zone depth to 1% of surface photosynthetically active radiance (PAR). NU_{01}, NU_{01}', NRA_{01}, PP_{01}, Depth integrated NU, NU estimated from NRA, NRA and primary production to PZD_{01}. NU_{01}/PP_{01}, Both in mg-C m^{-2} d^{-1}.
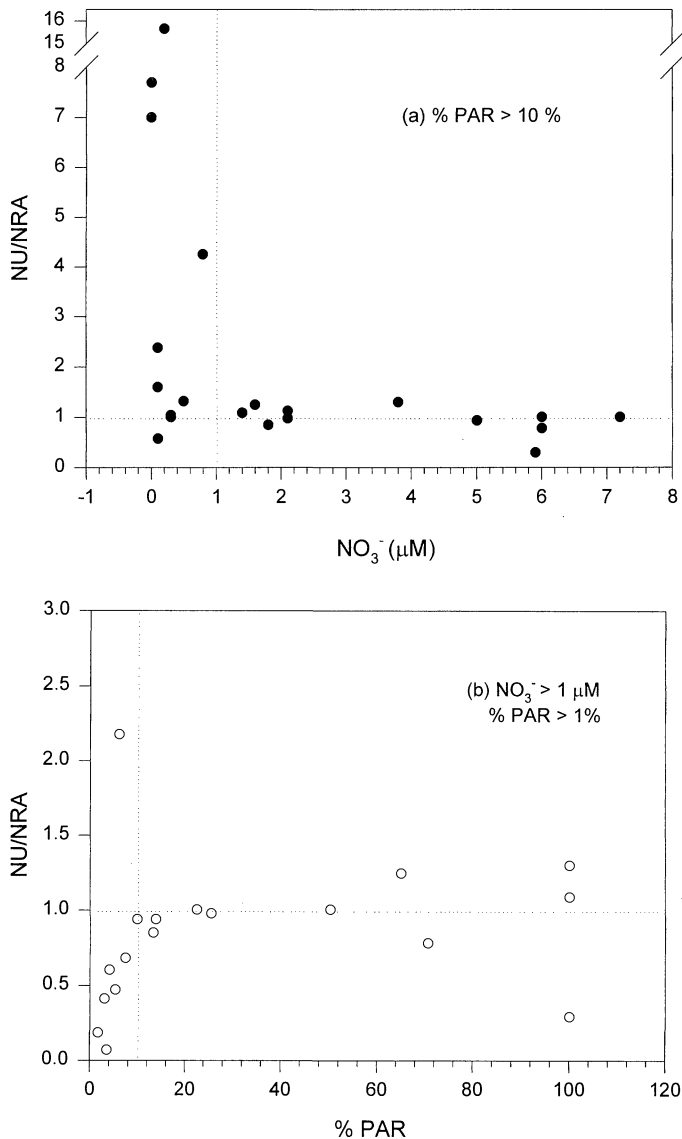† ND, No data.
‡ Estimated from NRA_{01}.

Fig. 10. The relationship between (A) NU/NRA and nitrate at depths where %PAR>10%, and, (B) NU/NRA and %PAR at locations where [NO$_3^-$] > 1 μM and %PAR > 1%.

Fig. 11. The relationship between NU and NRA in waters with (A) [NO$_3^-$] > 1 μM and %PAR > 10%, (B) [NO$_3^-$] < 1 μM and %PAR > 10%, and (C) [NO$_3^-$] > 1 μM and 1%<%PAR < 10%. The model II regression line is shown as a solid line in (A) and as a dashed line in (B) and (C). The 1:1 line is shown as a dotted line in (A). The solid lines in (B) and (C) denote the regression relationship in (A).

both. By taking these two criteria into consideration, four types of water may be identified: (A) nitrate- and light-replete waters, (B) nitrate-deficient and light-replete water, (C) nitrate-replete and light-deficient water, and (D) nitrate- and light-deficient water. The relationships between NU and NRA in the first three types of water were estimated by the reduced major axis method or model II regression analysis (Laws and Archie 1981) through the origin and the results are shown in Fig. 11 and summarized in Table 4. As expected from the previous discussions, NU was strongly correlated with NRA under nitrate- and light-replete conditions. The uncertainty of the slope, which represented NU/NRA, was about ±6%. Thus, in this type of water, both NU and NRA may be used for estimating the rate of nitrate assimilation. Given that NRA may be determined more readily, NU may be estimated from NRA once a relationship be-

tween the two can be established empirically by measuring both parameters simultaneously in a small number of samples. In type (B) water, the slope and its standard deviation were larger and the correlation was poorer than those of type (A) water. If the poorer correlation is indeed due to the difficulties in estimating nitrate assimilation accurately with NU, NRA may be a useful supplementary tool for this purpose. Nitrate assimilation again may be estimated from NRA and the relationship between NRA and NU. The relationship may be established from a small number of samples of this kind by applying strict controls on the experimental conditions used in the determination of NU (Allen et al. 1996). Data from the anomalous Sta. 26 contribute significantly to the overall variability in this group of data points. In the relationship between NU and NRA in type (C) water, the slope was about half of that in type (A) water, and the cor-

Table 4. Relationship between NU and NRA under different combinations of light and nitrate conditions.

Type	Light (%PAR)	NO$_3^-$ (μM)	Average NU/NRA	NU vs NRA* Slope	r^2	N	Characteristic
A	>10	>1	1.0 ± 0.3	1.08 ± 0.07	0.79	12	Both NRA and NU are indicative of NO$_3^-$ assimilation
B	>10	<1	4 ± 4	1.5 ± 0.2	0.65	10	NRA may be more indicative of NO$_3^-$ assimilation
C	<10	>1	0.7 ± 0.7	0.6 ± 0.2	0.19	7	NU may be more indicative of NO$_3^-$ assimilation
D	<10	<1	0.9 ± 0.7	Insufficient data		2	

* Model II linear regression through the origin. NU and NRA in nM-N h^{-1}. N, number of data points; r, correction coefficient.

relation was even poorer than in type (B) water. The slope of the linear relationship had an uncertainty of ±30%. Thus, NRA does not represent NU well and cannot be used readily for estimating the rate of nitrate assimilation in this type of water. A relationship between NU and NRA cannot be estimated for type (D) water since this type of water was represented by only two data points.

The average ratio of NRA/Chl a in the photic zone where %PAR exceeded 10% at each station is listed in Table 2. Among the three major surface water masses: the oligotrophic Kuroshio Surface Water at Sta. 55, the upwelling Kuroshio Subsurface Water at Sta. 52 and the Changjiang Diluted Water at Sta. 30, the highest NRA/Chl a, 8.8 nM-N h^{-1} mg^{-1} m^3 (the average at Sta. 52-1 and 52-3), was found in the upwelling water. In the other two water masses, the values of NRA/Chl a were about one half or less of this value. The average ratios of NRA/Chl a at the other three stations in the frontal zones between the coastal plume and the shelf water or between the Kuroshio and the shelf water were also high, being either similar to (Sta. 26) or even higher than (Sta. 11 and 15) that in the upwelling zone.
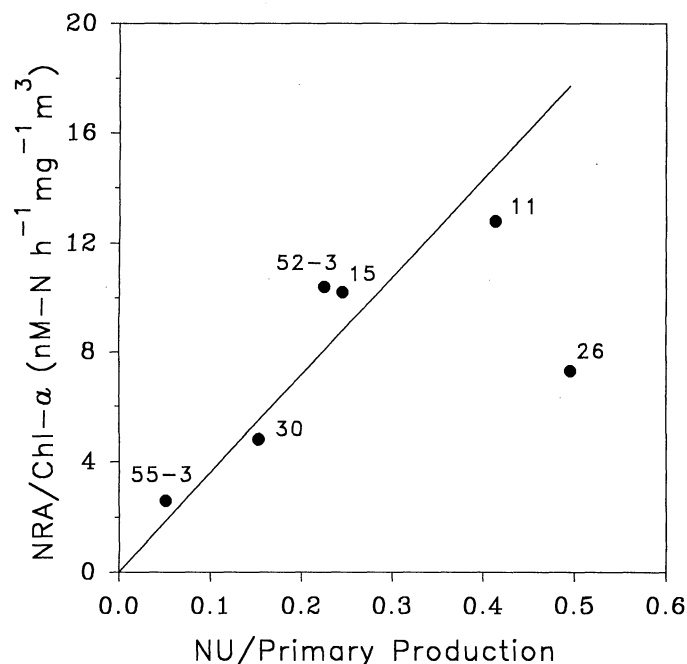

Fig. 12. The relationship between average NRA/Chl a and the NU/primary production within PZD$_{10}$. The station number is indicated next to each data point. The solid line represents the model II regression line when the data point from Sta. 26 is excluded.

There are two possible explanations for these differences. First, different assemblages of phytoplankton have been found in the different zones in the East China Sea (Chen 1995). The differences in NRA/Chl a might have reflected their varying abilities to utilize nitrate. Secondly, the nutrient conditions in the different zones might have been different and nutrient conditions can affect NRA/Chl a (Rhee 1979). An observed phytoplankton biomass may be sustained by a variety of combined nitrogen: nitrate, nitrite, ammonia and dissolved organic nitrogen (DON), whereas NRA measures the utilization of only nitrate. Thus, in an area where new production is supported by nitrate uptake and where new production relative to primary production, or the f-ratio, is high, the NRA/Chl a will be high. On the other hand, when other forms of combined nitrogen are used to maintain the biomass, the NRA/Chl a and f-ratio will be low (Harrison et al. 1987). The f-ratio is expected to be higher in upwelling waters than in the oligotrophic ocean where new production is limited by the availability of nitrate (Eppley 1989). In coastal waters, the presence of ammonia and/or DON may suppress the assimilation of nitrate and lead to lower f-ratios and low NRA/Chl a (Eppley et al. 1969; Packard and Blasco 1974; Dortch et al. 1979; Eppley 1989; Berges et al. 1995). Whereas NU/primary production is not exactly equivalent to the f-ratio unless new production is supported predominantly by nitrate uptake since new production can include other processes such as nitrogen fixation, its geographical variations still seem to follow this pattern (Table 3). NU/primary production increased from 0.08 in the oligotrophic Kuroshio water to 0.16 in the coastal Changjiang Diluted Water to 0.24 in the upwelling water to 0.24 to 0.46 in the frontal zones in the shelf. (In view of the possible artifact in the direct determination of NU in oligotrophic waters, the NU at Sta. 55 was estimated from the NRA inventory and its relationship to NU derived in this study (Table 4) so that NU = 1.08 NRA.) These values of NU/primary production are similar to those of the f-ratios found in similar types of marine sub-environments (Eppley 1989). The suppression of nitrate uptake which would result in lower NU/primary production in the coastal Changjiang Diluted Water relative to the upwelling water is a distinct possibility. Although neither dissolved organic nitrogen (DON) nor ammonia was measured in this study, it is highly likely that at least ammonia may be present in the coastal waters since Changjiang, the major source of nutrients to these waters, is also an ammonia-rich river (Edmond et al. 1985). The relationship between NU/ primary production and the average NRA/Chl a within PZD$_{10}$ is shown in Fig. 12. (The data are listed in Table 2.) There is a general trend of increasing NRA/Chl a with in-

creasing NU/primary production. If the data point from Sta. 26, where peculiar hydrographic properties were found, is excluded, they are linearly related and strongly correlated with each other so that

NRA/Chl *a* (nM-N h^{-1} mg^{-1} m^3)

$\quad$ = 36($\pm$4)(NU/primary production);$\quad$ $r^2 = 0.83$.

Given that the combination of NRA and Chl *a* can be measured more readily than the combination of NU and primary production and, in fact, Chl *a* may be estimated from fluorescence recorded by a sensor (Gong et al. 1993), this relationship suggests that NRA/Chl *a* may potentially be used as a supplement for mapping the distribution of NU/primary production, and even the *f*-ratio if new production is dominated by nitrate uptake, in a given area.

Depth-integrated NRA and NU—The commonly accepted depth of the euphotic zone is PZD$_{01}$. The depth-integrated NRA and NU down to PZD$_{01}$, NRA$_{01}$, and NU$_{01}$, at each station are listed in Table 3. In general, the spatial variations in integrated NRA and NU were similar. Higher values were found in the frontal zones at Sta. 26, 11, and 15 and in the upwelling Kuroshio Subsurface Water at Sta. 52-1 and 52-3. In contrast, even though the Changjiang Diluted Water is nitrate-rich, the depth-integrated NRA and NU in these waters at Sta. 30 were significantly lower. These suppressed levels of NRA and NU are consistent with the possibility that nitrate assimilation in coastal waters may be suppressed by the presence of other reduced forms of combined nitrogen, such as ammonia, DON, or both (Harrison et al. 1987). The lowest depth-integrated NRA and NU were found in the oligotrophic Kuroshio at Sta. 55–3 and 55–5. The lower NU and NRA in the Kuroshio relative to those in the upwelling region are consistent with the expected decrease in the f-ratio from the upwelling region to the open oceans (Eppley 1989).

NU$_{01}$ may also be estimated from NRA$_{01}$ by using the relationship between NU and NRA in light- and nitrate-replete waters shown in Table 4 and these estimated values, NU$_{01}'$, are listed in Table 3. NU$_{01}'$ in the oligotrophic waters (the average of Sta. 55–3 and 55–5), the coastal plume (Sta. 30), the upwelling water (the average of Sta. 52-1 and 52-3) and the shelf waters (the average of Sta. 11, 15, and 26) were 0.45, 1.55, 3.12, and 3.59 mg-N m^{-2} h^{-1} respectively. With the exception of the oligotrophic water at Sta. 55, the estimated values are similar, although frequently slightly higher than, those determined by ^{15}NO$_3^-$ uptake. The ratio of the estimated values to the measured values at these five stations ranged from 0.92 to 1.32 and the average was 1.18 $\pm$ 0.23. The slightly higher estimated values were probably caused by the decoupling between NRA and NU in waters with %PAR between 1% and 10%. When the estimated NU and the measured NU were integrated only down to PZD$_{10}$, NU$_{10}'$, and NU$_{10}$ (Table 2), NU$_{10}'$/NU$_{10}$ ranged from 0.75 to 1.30 and the average ratio dropped to 1.01 $\pm$ 0.19. However, since the contribution of this type of water, with %PAR between 1% and 10%, to the total estimated depth-integrated NU down to PZD$_{01}$ was usually small (an average of 12% at Sta. 11, 15, 26, and 30 where the estimated to measured

NU exceeded 1), the effect was minimal in view of the other uncertainties that are inherent in the determination of NRA and NU.

Eppley (1989) has compiled the recent estimates of NU. In five upwelling areas, with the exception of one extraordinarily high value, he reported a range of 1.6–8.1 mg-N m^{-2} h^{-1} which yields an average of 3.9 $\pm$ 3.1 mg-N m^{-2} h^{-1}. Allen et al. (1996) found NU at 3.7 mg-N m^{-2} h^{-1} in the nutrient-rich water in the center of a cyclonic mesoscale eddy. Our value of 4.0 mg-N m^{-2} h^{-1} (Sta. 52-3) falls well within this general range of reported values. For coastal regions, Eppley (1989) reported a range of 0.8 to 8 mg-N m^{-2} h^{-1}. Our values of 2.3–4.1 mg-N m^{-2} h^{-1} (Sta. 11, 15, and 26) and an average value of 3.0 mg-N m^{-2} h^{-1} in the shelf waters outside of the plume of Diluted Changjiang Water also fall well within these reported values.

In the oligotrophic Kuroshio at Sta. 55–3, the value of NU estimated from NRA, NU$_{01}'$, (0.36 mg-N m^{-2} h^{-1}) was only about a third of that determined directly (Table 3). Again, the discrepancy might have been caused by the stimulatory effect of the ^{15}NO$_3^-$ added. By using a method for the determination of nitrate at nM level (Garside 1982), Eppley and Koeve (1990) determined the uptake of nitrate by directly measuring the disappearance of ambient nitrate and they reported NU of 0.55 $\pm$ 0.5 mg-N m^{-2} h^{-1} during the day and 0.43 $\pm$ 0.28 mg-N m^{-2} h^{-1} at night in the eastern subtropical Atlantic Ocean where low concentrations (sub-μM) of nitrate were found in the euphotic zone. With due precautions taken to avoid the artifact that may be introduced by the added ^{15}NO$_3^-$, Allen et al. (1996) found that the NU in the oligotrophic North Pacific was 0.42 mg-N m^{-2} h^{-1}. Our values of NU estimated from NRA in the oligotrophic Kuroshio (0.36 and 0.53 mg-N m^{-2} h^{-1} at Sta. 55–3 and 55–5) are quite comparable to these values. On the other hand, our measured value of NU (1.16 mg-N m^{-2} h^{-1}) is significantly higher than these reported values. Thus, in oligotrophic waters, NRA may well be a more reliable tool for estimating NU than the traditional procedure of measuring ^{15}NO$_3^-$ uptake without a simultaneous determination of the ambient concentration of nitrate by using an analytical method with a detection limit at the nanomolar level.

Conclusions

Although the measurements of NRA and NU in a field sampling program may be affected differently under different light and nitrate conditions, NRA and NU are strongly, quantitatively, and linearly related to each other under light- and nitrate-replete conditions. The relationship between NRA and NU may be established with a relatively small number of simultaneous determinations of NRA and NU. Once the relationship is found for a study area, it may be used for estimating NU from the determination of NRA alone. Since NRA may be determined readily on board ship with relative ease, it is potentially a powerful, time-saving, and economical supplementary tool for expanding the present data base on NU in the marine environment. This experimental approach can be especially helpful in estimating NU in the oligotrophic oceans where a precise determination of nitrate assimilation by measuring NU is difficult.

The distributions of NU and NRA in the East China Sea are related to the nitrate- and light- conditions in the different surface water masses. The NRA, NU, and NRA/Chl *a* in the upwelling water exceeded those in the oligotrophic Kuroshio Surface Water and in the fresher coastal plume by more than a factor of two. Primary production in the upwelling water was probably supported to a large extent by the utilization of the plentiful nitrate and its NU/primary production, 0.24, was high. Although the coastal plume was also nitrate replete, the possible presence of other reduced combined nitrogen could have suppressed the uptake of nitrate and this resulted in a relatively low NU/primary production of 0.16. In the oligotrophic water, nitrate uptake was limited by the availability of nitrate so that recycled production was made necessary by this nitrate-deficient condition and the NU/primary production, 0.08, was lower still.

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