Progress in Oceanography 173 (2019) 78–85

Contents lists available at ScienceDirect

Progress in Oceanography

journal homepage: www.elsevier.com/locate/pocean

Calculating new production from nitrate reductase activity and light in the Peru current upwelling

S. González-Galisteo[a,*], T.T. Packard[a], M. Gómez[a], A. Herrera[a], R.C. Dugdale[b], F.P. Wilkerson[b], R.T. Barber[c], D. Blasco[d], J.P. Christensen[e], L.A. Codispoti[f]

[a] Marine Ecophysiology Group (EOMAR), IU-ECOAQUA, Universidad de Las Palmas de Gran Canaria, Canary Islands, Spain
[b] Romberg Tiburon Center, San Francisco State University, United States
[c] Nicholas School of the Environment and Earth Sciences, Duke University, United States
[d] Institut de Ciències del Mar (CSIC), Spain
[e] New England Oceanographic Laboratory, Brunswick ME, United States
[f] Horn Point Laboratory, University of Maryland Center for Environmental Science, Cambridge, MD 21613-0775, USA

ARTICLE INFO

Keywords:
Primary production
Nitrogen uptake
Nitrate
Ammonium
Light
Phytoplankton

ABSTRACT

We have calculated new production from phytoplankton nitrate reductase (NR) activity and light in the euphotic zone of the Peruvian upwelling system at 15° S. The calculation is based on unique measurements from the Coastal Upwelling Ecosystem Analysis (CUEA) JASON expedition from September 1976. The new production at the 50% light level in the euphotic zone ranged from 3.49 μM C h^{-1}, 12 km downstream from the upwelling center to 0.15 μM C h^{-1}, 46 km further downstream over the 4000 m deep Peru Trench where the upwelling was relatively weak. It compared well with ^{14}C carbon productivity measurements whose range was 0–4.2 μM C h^{-1} and 0–1.5 μM C h^{-1} for the 6 h (gross) and 24 h (net) productivity, respectively. In nitrogen units, the overall new production ranged from 4 to 510 nM of N h^{-1}. The oceanographic conditions found during September 1976 made this upwelling site an ideal one to calculate new production. Temperature in the center of the upwelling in September of 1976 reached 14.07 °C, while NO_3^- ranged from 6.65 to 7.5 μM, and NH_4^+ stayed below 0.1 μM. Chlorophyll, averaging 3.85 μg L^{-1} for the section stations in September 1976, was similar to what it was for all the stations 6 months later in March 1977 (3.23 μg L^{-1}). NR, averaging 0.20 μM N h^{-1} for the section stations in September 1976, was twice what it was for all stations, 6 month later in March 1977 (0.09 μM N h^{-1}).

1. Introduction

Nitrogen, although only 16% of the carbon in cells, is still of key importance in the geosphere-biosphere elemental transition. For this reason, the nitrogen uptake rate is an important measure of marine ecosystem productivity (Dugdale and Goering, 1967; MacIsaac and Dugdale, 1969, 1972). Nitrogen (N) can be supplied to phytoplankton independently from three sources, as seen in the N cycle of Fig. 1. One uptake pattern is based on N-fixation and uptake of N oxides, primarily NO_3^-. Production driven by these two processes is known as "New Production" or NP. The second uptake pattern is based on reduced N forms, primarily NH_4^+ and urea, supplied by bacterial remineralization or zooplankton and nekton excretion. Both processes "regenerate" simple nitrogen-rich ions or molecules from proteins or nucleotides. This type of production is known as "Regenerated Production" or RP (Dugdale and Goering, 1967). This work focuses on calculating nitrate based "New Production" because it is a major contributor to productivity in marine upwelling ecosystems. In all oceanic ecosystems, new production is the photosynthetic formation of phytoplankton biomass stimulated by nitrate fluxing from subsurface waters into the euphotic zone and by diazotroph driven nitrogen fixation (Dugdale and Goering, 1967; Dugdale et al., 1992). New production is high in all upwelling areas, in open ocean areas after major turbulent events (storms, winter mixing, etc.) and low in stratified nutrient-poor waters such as central gyres and the Arctic Ocean. Forty-five Pg yr^{-1} (10^{-15} g yr^{-1}) is a recent estimate of Global Ocean Production by the Oregon state SeaWifs program (www.science.oregonstate.edu/ocean.productivity/). With an f-ratio (Eppley and Peterson, 1979) of 0.25 (New Production /Total Production) this would mean a new production of 11 Pg yr^{-1} (3 Tg (10^{-12} g) h^{-1}), assuming the production occurred in ten daylight hours. New production is measured on bottled phytoplankton samples by the ^{15}N technique (Dugdale et al., 1961; MacIsaac

* Corresponding author.
E-mail address: sara.gonzalez115@alu.ulpgc.es (S. González-Galisteo).

https://doi.org/10.1016/j.pocean.2019.02.009
Received 23 August 2017; Received in revised form 28 June 2018; Accepted 21 February 2019
Available online 21 February 2019
0079-6611/ © 2019 Published by Elsevier Ltd.

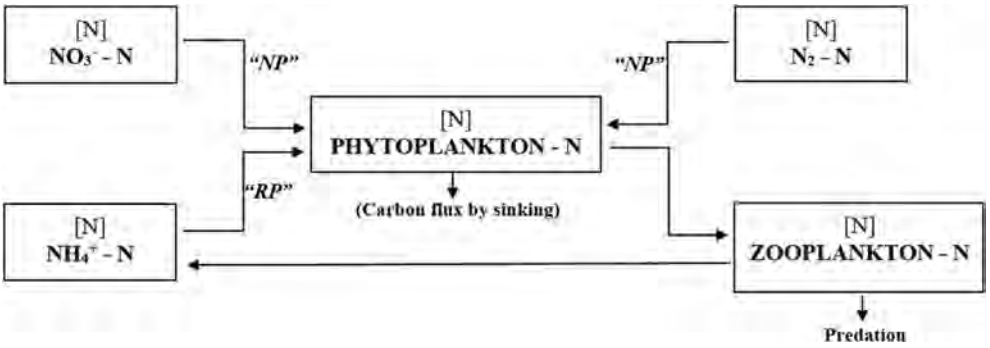

Fig. 1. Simplified model of the circulation of inorganic nitrogen (N) through the euphotic zone ecosystem. [N] represents the concentration of nitrogen in each fraction. "NP" and "RP" represent the "New Production" and "Regenerated Production" respectively, driven by the different sources of nitrogen in the system (modified from Dugdale and Goering, 1967).

and Dugdale, 1969; Dugdale and Wilkerson, 1998). However, in theory, this NO_3^- uptake can be modeled from nitrate reductase activity, its dependence on nitrate and light, and its control kinetics (Packard et al., 1971). Assimilatory nitrate reduction in algae, plants, bacteria and archaea is catalyzed by nitrate reductase (NR), the enzyme that reduces nitrate (NO_3^-) to nitrite (NO_2^-) (Eq. (1)). It is the first step in incorporating N into protein (Glibert et al., 2016). Assimilatory NR is not found in protozoans, metazoans, or higher animals except under conditions of endosymbiosis (Packard et al., 1978; Collos and Berges, 2003). This enzyme uses as a substrate, not only NO_3^-, but the energy-rich pyridine nucleotide, reduced nicotine adenine di-nucleotide (NADH). The NR reaction oxidizes NADH to NAD^+ (oxidized nicotine adenine di-nucleotide):

$$NO_3^- + NADH + 2e^- + H^+ \rightarrow NO_2^- + NAD^+ + H_2O \quad (1)$$

In marine algae, NR can be found in the plasmalemma and other cellular membranes of diatoms, chlorophytes, and cyanobacteria (Jones and Morel, 1988; Tischner et al., 1989; Berges, 1997; Dagenais-Bellefeuille and Morse, 2013); in dinoflagellates, it can be found in chloroplasts, where most of the NO_3^- reduction takes place (Berges and Mulholland, 2008); and in chlorophytes it can be found in pyrenoids (Glibert et al., 2016). NR operates in conjunction with low affinity nitrate transporters the enzymes that are responsible for the diffusion of environmental NO_3^- into the cell; nitrite reductase (NiR), the enzyme that reduces NO_2^- to ammonium (NH_4^+); and glutamine synthetase (GS), the enzyme that fixes NH_4^+ into glutamate ((COOH)-$(CH_2)_2$-CH(NH_2)-(COOH)), a key precursor in amino acid synthesis (Glibert et al., 2016).

Nevertheless, of all the enzymes that operate in the process, the rate limiting step of the reaction is catalyzed by NR (Beevers and Hageman, 1969; Tischner, 2000; Young et al., 2007). That is why it can be used as a measure (proxy) of N-uptake as well as an estimation of the NO_3^- assimilation rate (Packard et al., 1971; Gordillo et al., 1997; Collos and Berges, 2003). It is a sensitive enzyme because it is light dependent, stimulated by NO_3^-, and inhibited by NH_4^+. In the dark, NR is normally inactivated. Also, if NO_3^- is absent or if NH_4^+ is present in seawater, NR is inactivated. In zooplankton-rich seawaters it is repressed by NH_4^+ excretion and deep in a NO_3^- -rich water column, by the low light. On the other hand, when NO_3^- is present and NH_4^+ is absent, NR activity follows an endogenous diel cycle (Eppley et al., 1970; Packard et al., 1971; Packard and Blasco, 1974; Martinez et al., 1987; Young et al., 2007). Because of these characteristics, NR is rarely measurable in the oligotrophic ocean, except during blooms, but it is easily measured in NO_3^- -rich coastal upwelling areas. Given these sensitivities, NR activity should be a useful oceanographic indicator of new production (Hung et al., 2000; Packard et al., 2004), but to date it has not been used as a synoptic tool to reveal the new production in an upwelling area. Here, this is done.

We develop a light-dependent, nitrate and ammonium independent calculation of new production based on NR activity that predicts strong new production off Peru. The calculation is based on NR measurements from the Coastal Upwelling Ecosystem Analysis (CUEA) JASON expedition from September 1976 (Packard and Jones, 1978) and the conceptual idea from Packard et al. (1971, 2004).

2. Materials and methods

2.1. Research site

The site of this study was 15°S off Pisco, Peru (Fig. 1a in Packard et al., 2015), a zone characterized by a strong and persistent upwelling (Wooster, 1961; Fernández et al., 2009). It was the focus of the Peru phase of the Coastal Upwelling Ecosystem Analysis (CUEA) program of which the JASON-76 expedition was part. To observe the upwelling at its theoretical maximum, the cruise took place in the late austral winter and spring (August, September, October and November) when the southeast trade winds intensify to the maximum (Wooster, 1961). The results presented here are from the September 10 to September 22 phase (leg IV) of JASON-76 expedition with the R/V Eastward, cruise no. E-5H-76 (Packard and Jones, 1978).

2.2. Sampling procedures

2.2.1. Seawater samples were taken with Niskin Bottles and with a Rosette

Samples were taken along a transect line (C-Line) (Fig. 1a in Packard et al., 2015) across the Peru current at 15°S every day at 13:30 h. This was done to coincide with the maximum in the NR activity. According to Fig. 2 in Eppley et al., 1970, the NR diel cycle, exhibits a maximum value, a plateau, between 10 h and 16 h. We calculated the new production per hour during this period (Eq. (2) and Table 2). The C-Line extended from the coast, at position C-1, across the Peru trench to position C-14, 200 km offshore. Two hydrosections, multiple productivity, and several deep-biology stations in addition to many ship-board experiments were made during this cruise, but only a small part of the measurements were reported (Packard et al, 2015). The productivity and deep-biology stations focused on the biological and biochemical properties as well as the nutrient chemistry in the water column. Sampling depths were established according the light. Euphotic zone samples were taken at depths where the light was 100, 50, 30, 15, 5, 1 and 0.1% of the surface incident radiation (MacIsaac and Dugdale, 1969, 1972). Samples were taken for phytoplankton productivity, inorganic nutrients (PO_4^{-3}, NO_3^-, NO_2^- and silicate), ETS activity and protein according to Packard and Jones (1978). At the same time, for NR activity, 4 L samples were taken from the rosette (Barber et al., 1978), and filtered through 4.25 cm Gelman glass fiber filters (0.7 μm pore size) and assayed for NR activity by nitrite-detection method of Hewitt and Nicholas (1964) as adapted for natural communities of marine phytoplankton by Eppley et al. (1969, 1970); Packard et al., 1971; Packard, 1973; Packard and Blasco, 1974; Martinez et al., 1987). The precision of the method was improved by quenching the reaction with Zn acetate before the ethanol, mixing the solution, and then making up to 10 ml. Berges and Harrison (1995), working with laboratory cultures of different marine phytoplankton reported a 50% improvement in this assay by adding bovine serum

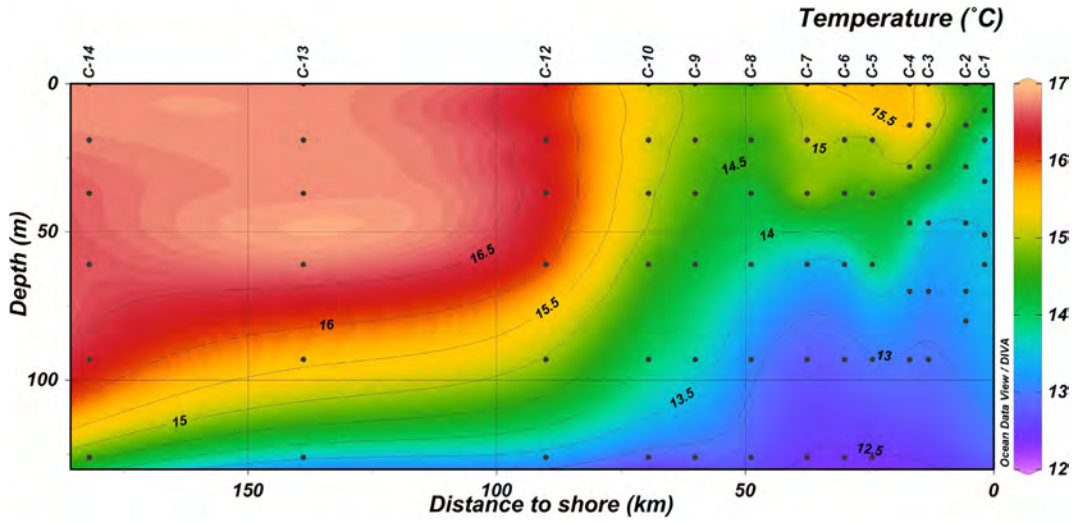

Fig. 2. Temperature (°C) of the column water along the C-line. 10th September 1976. Interpolation of the data to contour this section, as well as Fig. 3, was produced applying the DIVA gridding using the software Ocean Data View (Schlitzer, 2018).

albumen (BSA) to the crude enzyme homogenate. However, their data on the diatom, *Thalassiosira pseudonana* was ambiguously reported (Fig. 3 in Berges and Harrison, 1995). Consequently, we hesitate, without stronger data, to apply their 50% correction to our natural phytoplankton community samples from the Peru current. Accordingly, we base our new production calculation on our original data (Packard and Jones, 1978).

3. Theory and calculations

As said above, new production is the photosynthetic formation of phytoplankton biomass driven by NO_3^- input into the euphotic zone and by N fixation produced by diazotrophic organisms. However, in most coastal upwelling systems, the diazotrophic contribution is practically null compared with the production driven by NO_3^- assimilation. This can be seen in the N-productivity measurements found in the upwelled and the peripheral water masses off Vietnam in July 2003 and July 2004 (Loick-Wilde et al., 2016). Their study showed, in Table 1, that the contribution of N fixation to total primary production (PP) was less than 10% of the PP driven by NO_3^- assimilation, hence, we neglect, in our study, any diazotroph contribution and consider only the NO_3^- assimilation. A similar conclusion was made from the N-fixation measurements of Fernandez et al. (2011). They calculated a N-fixation of 60 pmol L^{-1} h^{-1} for the Peru-Chile upwelling, where pmol signifies 10^{-12} mol of N. The N-regenerated productivity for this region at the same time was 6.3 nmol L^{-1} h^{-1}, a hundred-fold larger than the N-fixation (Fernández et al., 2009). In their case, the new production at that time was lower than the regenerated production because the NH_4^+ regeneration rates were high (2.8 nmol NH_4^+ L^{-1} h^{-1}). This finding simplifies the calculation presented below.

3.1. Calculating new production

The new production calculation, on an hourly basis, was founded on: (1) the knowledge that NR regulates the first step in the phytoplankton NO_3^- assimilation processes; (2) the assumption that nitrogen fixation, here, by the diazotrophs is negligible; (3) that a measurement of NR activity yields the V_{max}, the potential or the capacity of the enzyme reaction to reduce NO_3^- to NO_2^- (Eq. (2)); (4) the dependence of NO_3^- uptake and NR activity on light and [NO_3^-] (MacIsaac and Dugdale, 1969, 1972; Packard, 1973; Packard and Blasco, 1974; Packard et al., 1971, 1978), (5) the inverse dependence of NR on [NH_4^+] (Packard et al., 1971; Packard and Blasco, 1974; Blasco and Packard, 1974; Glibert et al., 2016) and (6) that the [NH_4^+] in the euphotic zone off 15°S was too low to inhibit NR (Kogelshatz et al., 1978). In ocean waters charged with ample NO_3^-, and relatively low NH_4^+ the calculation can be expressed as a light-dependent, single substrate Michaelis-Menten equation (Eq. (2)):

$$New\ \ production = \frac{NR \cdot [light]}{K_m + [light]} \tag{2}$$

Here, NR represents the V_{max} of the NR-catalysed NO_3^- reduction reaction, expressed in μM h^{-1}. [Light] is the light level as a % of surface radiation (%I_0). The K_m which we call the "light-K" (K_{LT}), is the NR Michaelis-Menten constant for light (Packard, 1973; Martinez et al., 1987). The calculated new production is expressed in μM h^{-1}, the same units as the NR. The measurements show that, in the euphotic zone, NO_3^- along the C-Line was always above 6 μM (Table 1), and that the NH_4^+ was always below 0.1 μM (Kogelshatz et al., 1978). These conditions support the simplification of the enzyme kinetics to a single substrate case based on light. Accordingly, we can write Eq. (2) for the New Production Rate (NPR) as a differential equation (Eq. (3)):

$$NPR = \frac{[\partial NO_3^-]}{\partial t} = \frac{[NR] \cdot [h_\nu]}{K_{LT} + [h_\nu]} \tag{3}$$

where [NR] is, again, the V_{max} of the NO_3^- reduction reaction, expressed in μM h^{-1}. [h_ν] is [light] expressed in %I_0, and K_{LT} is 2.4% I_0, taken as the average of the light-Ks from two previous upwelling studies off Northwest Africa (Table 1 in Packard, 1973 and Table 2 in Martínez et al., 1987). Here, we assume that the light-K in the Northwest Africa upwelling describes, to a first approximation, the affinity of light in the phytoplankton community of the Peru upwelling. Resulting NPR is expressed in μM h^{-1}.

3.2. Oceanographic conditions

Off Peru, low sea surface temperatures, as in Fig. 2, indicate strong upwelling intensity with water originating at 120 m, 200 km offshore. This is consistent with the descriptions of this upwelling system (Smith 1968; Dugdale, 1972; MacIsaac et al., 1985; Dugdale et al., 1995). In September 1976, classified as an ENSO transition year (Rykaczewski and Checkley, 2008; Santoso et al., 2014), the upwelling was more intense than in previous months (Kogelshatz et al., 1978; Packard et al., 2015). Sea surface temperatures at C-3, the center of the upwelling, were known to be low (MacIsaac et al., 1985). However, in September 1976, at the surface of station C3, temperatures dropped to as low as

Table 1
Biochemical characteristics of Peruvian upwelling along the C-line during JASON-76 R/V Eastward cruise no. E-5H-67 in September 1976. In column 1 are the station position and number, time of sampling and day in September 1976. All data have been converted to L^{-1} basis from the original data. Coordinates and oceanographic characteristic for these stations are shown in Table 1 of Packard et al., 2015. Original data are from CUEA data reports (Packard and Jones, 1978; Kogelshatz et al., 1978).

CUEA C-line section	Depth (m)	NR (μM h^{-1})	Chl. α (μg l^{-1})	Prot. (μM)	NO_3^- (μM)	NO_2^- (μM)	SiO_4 (μM)	PO_4 (μM)	^{14}C(24 h) (μM C h^{-1})	^{14}C(6 h) (μM C h^{-1})
C10 (18)	0	0.02892	1.06	3.61	11.92	0.52	9.7	0.94	1.64	3.41
Day 16	5	0.02313	0.9	3.05	11.93	0.51	9.6	0.93	1.73	4.63
13:43	8	0.03709	1	2.7	11.82	0.58	9.6	0.91	1.73	4.3
	15	0.01552	0.96	2.47	11.82	0.58	9.7	0.91	1.51	3.64
	24	0.03229	1.13	4.81	12.1	0.58	10.1	0.94	0.37	1.51
	36	0.01453	0.67	3.82	12.54	0.54	11	0.98	0.16	0.59
C8 (19)	0	0.13923	4.11	8.41	12.42	0.82	11.3	1.17	9.65	17.2
Day 17	4	0.22473	3.8	8.68	12.56	0.64	11	1.17	10.15	18
13:30	8	0.2378	3.75	8.27	13.2	0.72	11.5	1.19	6.47	21.18
	12	0.1926	3.55	8.47	13.24	0.68	11.6	1.18	4.5	11.2
	19	0.15081	3.59	6.42	12.93	0.67	11.3	1.16	1.2	3.36
	29	0.06557	2.62	6.84	14.15	1.21	12.5	1.25	0.07	1.09
C5 (20)	0	0.42414	6.96	17.91	9.21	0.79	5.6	0.93	18.24	26.81
Day 18	3	0.53399	8.34	18.99	9.24	0.76	5.6	0.93	14.91	32.45
13:25	6	0.4779	8.08	21.62	9.76	0.8	5.7	0.94	12.18	30.64
	9	0.56735	6.93	20.38	9.79	0.81	6	0.97	9.64	31.99
	14	0.21918	7.1	–	11.59	0.77	7.3	1.03	2.22	8.77
	21	0.07376	3.99	5.18	14.15	0.93	11.2	1.16	0.53	2.14
C3 (21)	0	0.4042	3.67	6.43	15.24	0.76	22.2	1.44	8.08	12.97
Day 19	5	0.40927	3.14	5.66	15.19	0.73	21.8	1.42	5.54	13.57
13:27	9	0.33345	3.15	5.39	16.62	0.58	21.7	1.47	3.92	10.66
	14	0.25155	2.36	4.91	16.45	0.63	22.9	1.51	2.4	6.88
	21	0.05584	1.64	2.98	16.28	0.72	24.8	1.51	0.56	1.3
	33	0.01605	0.86	1.01	17.5	0.58	26.9	1.63	0.11	0.05
C1 (22)	0	0.19273	3.06	7.07	12.93	1	25	1.51	6.84	8.46
Day 20	4	0.18613	3.37	8.73	13.03	1.01	25.2	1.51	5.44	11.66
13:28	6	0.12456	3.01	9.36	12.76	1.03	25.3	1.5	5.2	13.28
	10	0.13893	3.01	6.66	13.37	1.05	25.3	1.53	3.62	9.28
	16	0.12085	2.47	6.21	13.4	0.92	24.9	1.53	0.98	3.88
	24	0.03896	2.07	–	14.73	1.13	27.2	1.64	0.37	0.47
C12 (35)	0	0.39047	7.47	–	7.36	0.68	4	0.88	16.64	25.91
Day 22	3	0.40368	7.09	–	6.99	0.65	3.7	0.84	13.51	50.34
13:28	5	0.29856	6.72	–	7.42	0.62	3.9	0.86	10.12	25.06
	9	0.28159	6.51	–	7.29	0.79	4.3	0.84	7.74	23.66
	13	0.27529	2.87	–	6.65	1.03	3.5	0.85	2.47	8.88
	21	0.04239	7.8	–	9.56	1.6	8.2	0.97	0.08	1.51

14.10 °C (Table 1, Packard et al., 2015). This was more than 2 degrees lower than the temperatures were from 28 March to 8 April 1976 when the average temperature at station C3 was 16.65 °C (Packard et al., 1978) and more than 2 degrees lower than they were the following spring (1977), when temperatures of 17.17 °C were recorded. This was also more than 1 degree lower than temperatures were 10 years later in austral spring (August-September) during the French Paciprod cruise of the R/V Jean Charcot (Minas et al., 1990; Dugdale et al., 1995). From a study of the same C-Line section made during the 1986 Paciprod cruise in austral spring, the upwelled waters at C-3, as in 1976, originated at 120 m far offshore, in the vicinity of C-14. As for the phytoplankton at C-3, on JASON-76, large and dense populations of diatoms, most belonging to the group Chaetoceros, were found in the first meters of the water column (Rojas de Mendiola, 1976).

3.3. Productivity stations

Following the description in the methodology section, productivity and biochemical measurements are presented in (Table 1). In September 1976, NO_3^- ranged from 6.65 to 17.5 μM throughout the euphotic zone from C-1 to C-12. It never fell below 6.65 μM. High NO_3^- levels (15.24 μM) occurred at the sea surface (0 m) at the upwelling center, C-3. The maximum (17.5 μM) was reached at the bottom of the euphotic zone at C-3 (Sta 21, Tables 1 and 2). The lower values of NO_3^- at C-5 and C-12 reflected biological uptake (Table 1). A NO_2^- section (not shown) had two subsurface maxima (1.1 μM) at the bottom of the euphotic zone. The first (1.13 μM) occurred at C-1, near the coast, and extended vertically all the way to the surface. The second was a NO_2^- patch spreading horizontally from C-Line positions C-5 to C-8, between 21 and 29 m (Table 1). A third subsurface maximum of 1.6 μM occurred at 20 m at C-Line position C-12; this one coincided with a NO_3^- minimum zone in the waters above. Overall, the NO_2^- concentrations in the euphotic zone ranged from 0.51 to 1.6 μM, with a water column minimum at C-10 (Table 1) A chlorophyll section (not shown) revealed two maxima, the first one at C-5 and the second one at C-12 (Table 1). Both reached 8 mg m^{-3}. The first was displaced offshore by about 10 km from the silicate and NO_3^- maxima at the upwelling center; the second coincided with a silicate and NO_3^- minimum (Table 1) and extended down to 40 m in the water column. Ten years later (in 1986), on the Paciprod cruise (Minas et al, 1990), chlorophyll concentrations were much lower, between 20% and 50% of the JASON-76 chlorophyll. In addition, the chlorophyll maximum in 1986 was displaced about 30 km offshore from its position at C-5 in 1976 (Table 1). As for the silicate distribution, it was high over the shelf in the upwelling area, ranging from 21.7 to 27.2 μM (from C-1 to C-3) and low seaward of the Peru Trench, averaging around 7.3 μM (from C-10 to C-12) (Table 1). The high levels of silicate in the upwelling center were about twice what Dugdale et al. (1995) reports from the Paciprod cruise at the same place in spring 1986. However, offshore, the nitrate and silicate returned to their normal 1:1 ratio (Dugdale, 1972; Dugdale et al., 1995).

Table 2
Calculations of New Production (NP) per liter of seawater in terms of nitrogen and carbon. They are based on Eqs. (2) and (3), using light and nitrate reductase (NR) activity along the C-line. NP in terms of carbon was obtained by multiplying NP, in terms of nitrogen, by a factor of 6.6, the Redfield ratio for the conversion of N into C (Geider and La Roche, 2002).

CUEA C-line section	Depth (m)	hν (Ly min^{-1})	hν (%)	NR (μM h^{-1})	NO_3^- (μM)	New production (μM N h^{-1})	New production (μM C h^{-1})
C10 (18)	0	35.1	100	0.03	11.92	0.03	0.19
Day 16	5	17.55	50	0.02	11.93	0.02	0.15
13:43	8	10.53	30	0.04	11.82	0.04	0.24
	15	5.265	15	0.02	11.82	0.02	0.10
	24	1.755	5	0.03	12.10	0.03	0.21
	36	0.351	1	0.01	12.54	0.01	0.09
C8 (19)	0	54.8	100	0.14	12.42	0.14	0.92
Day 17	4	27.4	50	0.22	12.56	0.22	1.48
13:30	8	16.44	30	0.24	13.20	0.24	1.56
	12	8.22	15	0.19	13.24	0.19	1.26
	19	2.74	5	0.15	12.93	0.15	0.97
	29	0.548	1	0.07	14.15	0.06	0.41
C5 (20)	0	73.8	100	0.42	9.21	0.42	2.79
Day 18	3	36.9	50	0.53	9.24	0.53	3.49
13:25	6	22.14	30	0.48	9.76	0.47	3.10
	9	11.07	15	0.57	9.79	0.55	3.61
	14	3.69	5	0.22	11.59	0.21	1.39
	21	0.738	1	0.07	14.15	0.07	0.45
C3 (21)	0	49.6	100	0.40	15.24	0.40	2.66
Day 19	5	24.8	50	0.41	15.19	0.41	2.68
13:27	9	14.88	30	0.33	16.62	0.33	2.18
	14	7.44	15	0.25	16.45	0.25	1.63
	21	2.48	5	0.06	16.28	0.06	0.36
	33	0.496	1	0.02	17.50	0.02	0.10
C1 (22)	0	72	100	0.19	12.93	0.19	1.27
Day 20	4	36	50	0.19	13.03	0.19	1.22
13:28	6	21.6	30	0.12	12.76	0.12	0.82
	10	10.8	15	0.14	13.37	0.14	0.91
	16	3.6	5	0.12	13.40	0.12	0.78
	24	0.72	1	0.04	14.73	0.04	0.25
C12 (35)	0	42.1	100	0.39	7.36	0.39	2.57
Day 22	3	21.5	50	0.40	6.99	0.40	2.64
13:28	5	12.63	30	0.30	7.42	0.30	1.95
	9	6.32	15	0.28	7.29	0.28	1.82
	13	2.11	5	0.28	6.65	0.26	1.72
	21	0.42	1	0.04	9.56	0.04	0.27

The decrease was an indication of diatom uptake (Dugdale and Wilkerson, 1998), but with the silicate nearly twice the nitrate in the upwelling center it was unlikely to be controlling new production as Dugdale et al. (1995) found from modeling this ecosystem 10 years later.

The carbon productivity (^{14}C) distributions are shown in Table 1 and Fig. 3 as gross productivity. Measurements reveal highest values in the near-surface water for both the 6 and 24 h incubation bottles. Overall, the two productivity measurements ranged from 0 to 4.2 and 0 to 1.5 μM of carbon per hour, respectively. Here, we interpret the 6 h C-productivity as gross or total productivity and the 24 h C-productivity as net productivity. Using the Redfield C:N ratio of 6.6 (Geider and La Roche, 2002) these ranges become 0–0.64 and 0–0.23 μM N h^{-1}. Both correlate with the NR as expected if new production is related to carbon productivity (Fig. 3).

The new production calculation, the NR measurements, and the light intensity measurements in both ly min^{-1} and $\%I_0$ are presented in Table 2. Nitrate reductase (NR) was measured in the euphotic zone throughout the water column at all stations (Tables 1 and 2). It ranged from 0 to 0.56 μM h^{-1} in September 1976, lower than NR measured in March 1977 which ranged from 0 to 0.92 μM h^{-1}. Here, in contrast to the mesopelagic (subsurface) bacterial NR measurements made along the C-Line in fall, 1977 (Packard et al., 1978), special attention was paid to the phytoplankton in the light-rich surface waters where nitrate was plentiful and NR activity was extraordinary high. The NR vertical distribution revealed high values near the surface, where light is unlimited. Two maxima appear, the first and most intense one was at C-5; the second occurred at C-12. Both coincided with new production maxima calculated according Eq. (3) and represented in Table 2 and Fig. 3. From Table 2 the new production ranged from 4 to 550 nM N h^{-1} (0.03–3.61 μM C h^{-1}) along the C-Line in September 1976. During the same season 10 years later (1986) the new production, modeled from nutrient and hydrographic measurements and a new concept of silicate limitation of diatom growth (Dugdale et al., 1992), ranged from 5 to 10 nM N h^{-1}. This model also found that, at that time, ammonium-based regenerated production ranged from 7.5 to 2.5 nM N h^{-1}. In our study in 1976, the ammonium levels were low and from the levels shown in Table 1 and 2, the total productivity was composed entirely of new production. Otherwise the slope we found in the correlation of NR and Chl-α would have been much lower than 0.96. In any case, the NR-based calculation predicted a two-order of magnitude range of new production for 1976.

4. Discussion

From our previous research (MacIsaac and Dugdale, 1969; Eppley et al., 1970; Packard et al., 1971; Blasco and Packard, 1974; Dugdale, 1985; MacIsaac et al., 1985; Martinez et al., 1987), we knew that NR activity was enhanced by light, followed a diel cycle, was stimulated by nitrate and was inhibited by NH_4^+. Glibert et al. (2016) confirms these properties of NR. We also knew that the production off Peru is driven by the vertical flux of inorganic nutrients, especially NO_3^-. Since

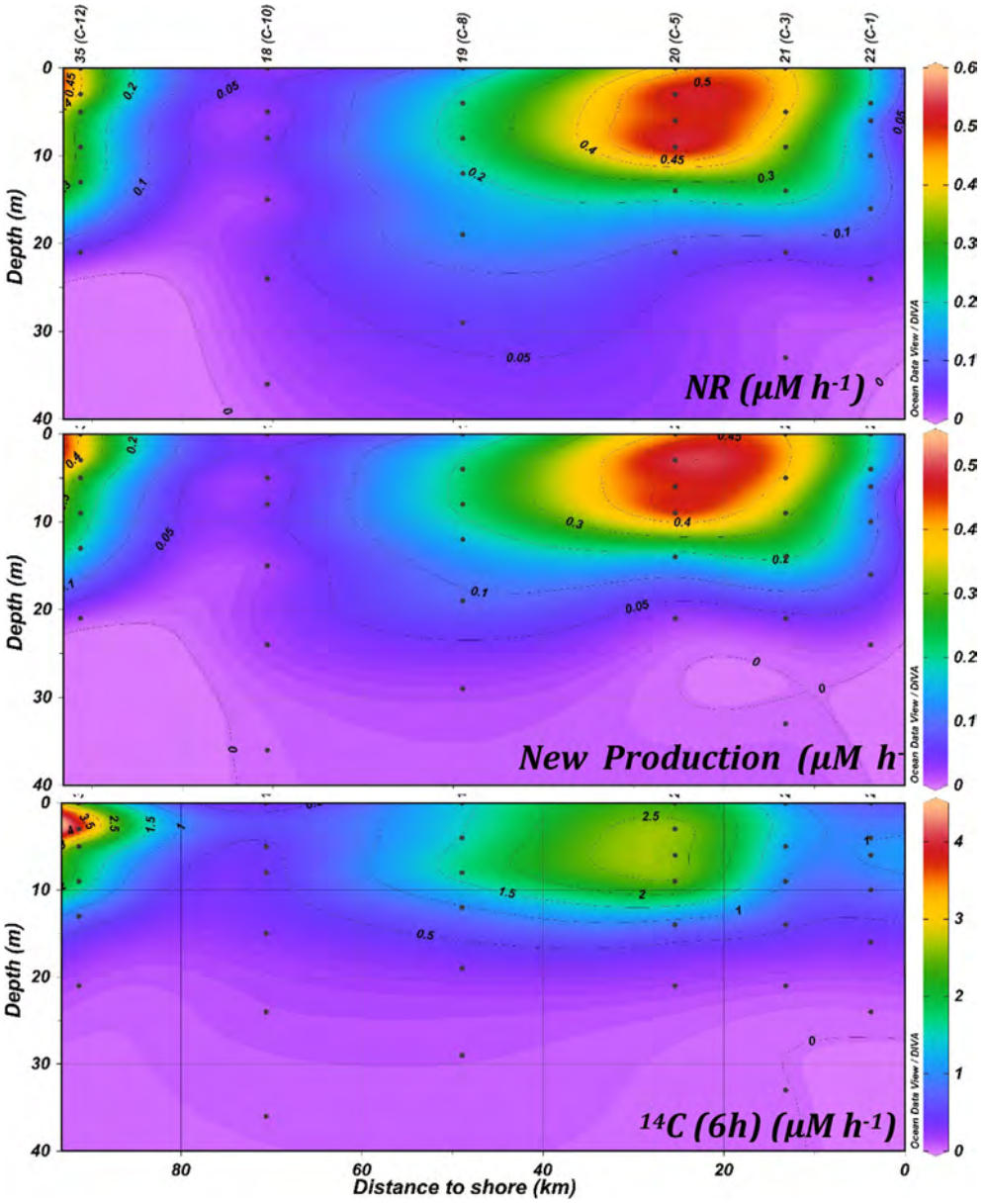

Fig. 3. Distribution of the Nitrate Reductase activity expressed in mM of N, New production, calculated in terms of Nitrogen and ^{14}C productivity calculated from 6 h deck-incubated bottled phytoplankton, along the C-Line from C-1 to C-12 in the upwelling system of Peru coast, from 16th to 22th September 1976.

Table 3
New production, per sea-surface area, with biological and chemical water column properties integrated over the euphotic zone (Ez). All the integrated values are expressed per m^2. The cross-shelf section was made on 16th–22th September 1976 during the JASON-76 R/V Eastward cruise no. E-5H-67. Original data are from CUEA data reports (Kogelshatz et al., 1978; Packard and Jones, 1978). New production in carbon can be calculated using the C:N Redfield Ratio of 6.6 (Geider and La Roche, 2002). Average values per m^3 for the Ez properties (above) can be calculated, dividing by Ez.

CUEA C-line section	Ez (m)	NR (mmol h^{-1})	Chl. a (mg)	Protein (mmol)	NO_3^- (mmol)	NO_2^- (mmol)	SiO_4 (mmol)	PO_4 (mmol)	^{14}C (24 h) (mmol C h^{-1})	^{14}C (6 h) (mmol C h^{-1})	New Prod. (mmol N h^{-1})
C10 (18) Day 16	36	0.9	34.82	127.91	433.47	20.21	360.3	33.65	3.05	8.1	0.69
C8 (19) Day 17	29	4.8	101.56	219.98	429.98	22.7	334.95	34.38	12.42	23.9	3.88
C5 (20) Day 18	21	7.51	143.99	179.27	229.04	16.98	149.3	21.13	13.53	34.8	6.39
C3 (21) Day 19	33	6.49	72.38	129.63	539.61	21.9	785.65	49.79	6.92	16.28	5.81
C1 (22) Day 20	24	3	65.88	120.34	322.8	24.33	611.1	36.97	6	13.93	2.47
C12 (35) Day 22	21	5.44	123.55	–	158.1	20.25	97.95	18.34	11.27	32.82	4.49

phytoplankton NO_3^- assimilation is the main component of new production (Dugdale and Goering, 1967; Dugdale, 1985) and since NR controls it, NR should serve as a measure, a proxy, for potential new production. Packard et al. (1971) hypothesized that nitrate uptake (RNO_3^-) would be a linear function of NR, related to NR by a constant (C) so that RNO_3^- would equal C·[NR]. Blasco et al. (1984) showed that, although true, the linear relation was clouded by high variability to the point where it was not too useful, by itself. Here, we explore the use of Michaelis-Menten kinetics (Eq. (2) and (3)) to reduce this variability and to improve the prediction of RNO_3^- from NR. New production by the ^{15}N method was not made on this cruise, so the new production prediction from NR can only be verified by its relationship with the 6 h ^{14}C productivity measurements. This was done here and the results argues that the calculated new production and gross production are effectively equal and that regenerated production played an insignificant role in this part of the Peru upwelling in September 1976. Table 3 and Fig. 3 show that new production ranged from 0.69 to 6.39 mmol N $m^{-2}h^{-1}$. About 10 km downstream between C-4 and C-5, just over the shelf edge occurred the new production maximum at 6.39 mmol N $m^{-2}h^{-1}$. Further offshore over 2000 m of ocean, in the middle of the eastern wall of the Peru trench, the new production was at its minimum, 0.69 mmol N $m^{-2}h^{-1}$. Further offshore (90 km), perhaps in response to Ekman pumping in response to wind stress curl (Pickett and Paduan, 2003), the new production rose to 4.49 mmol N $m^{-2}h^{-1}$ at C-12 (Table 3, Fig. 3). It is interesting that the global total new production (Duce et al., 2008), normalized by the ocean's surface-area ($357{\cdot}10^6$ km^2) was 0.044 mmol N $m^{-2}h^{-1}$, about two orders of magnitude lower than our calculations for the Peru upwelling. This is to be expected because most of the world's oceans are oligotrophic with negligible new production.

Since phytoplankton nitrogen metabolism is responsible for NR activity in the euphotic zone, NR should be correlated with chlorophyll, but except for Eppley et al. (1969) and Eppley et al. (1970) the literature is not strong on this topic. We checked this relationship with the integrated data in Table 3 and found a relationship that is described by the equation NR = 0.049 + 0.273 (r^2 = 0.67, n = 6). This means that the NR/Chl ratio is 49 μmol NO_3^- reduced per μg Chl a. Eppley et al. (1970) report 35 μmol NO_3^- reduced per μg Chl a. for the same location in the Peruvian upwelling in 1969.

Although carbon fixation is composed of both new and regenerated production, if the NH_4^+ levels are low, as during JASON-76, then new production is the dominant process and should correlate with carbon productivity (Eppley and Peterson, 1979). Plotting integrated values of new production against integrated values of both 6 h and 24 h ^{14}C productivity (Table 3) should reveal a correlation with the regression equations,

$$\text{New Production} = 0.96\ ^{14}C\ (6\,h) + 5.34\ (r^2 = 0.54,\ n = 6)$$

and

$$\text{New Production} = 3.27\ ^{14}C\ (24\,h) - 0.24\ (r^2 = 0.63,\ n = 6),$$

respectively. The 6 h based ^{14}C productivity data should be close to gross production. The fact that a ^{14}C determined-gross productivity versus an NR-derived new production calculation has nearly a 1:1 relationship (slope = 0.9597), supports the assumption that new production dominates, and that regenerated productivity is negligible. Accordingly, the f-ratio should be close to 1.

5. Conclusions

The heuristic, enzyme-kinetic based calculation developed here predicts realistic levels of new production but will need future comparison with new production measured by the ^{15}N technique. The main conclusions of this work are set forth below.

New production, here, ranging between 0.004 and 0.55 μM of N h^{-1}, (0.03–3.61 μM C h^{-1}), has the same distribution pattern as the ^{14}C-based primary production (Fig. 3). The ^{14}C (24 h) and ^{14}C (6 h), representing net and gross productivity respectively, ranged between 0–1.5 and 0–4.2 μM C h^{-1}.

New production, here, was highest 12 km downstream of the upwelling center and 25 km upstream of where there co-occured a maximum in the water column respiration, carbon flux and heterotrophic energy production, and a minimum in the nutrient retention efficiency and benthic respiration (Packard et al., 2015). This was at the CUEA C-Line positions C-5, a position over the upper part of the continental slope.

Temperature across the Peru shelf at 15°S, in September 1976, clearly evidenced strong upwelling. Temperatures as low as 14.07 °C were found in the upwelling center at C-3. This temperature was more than 2 °C lower than it was at the same location in March 1977 (16.65 °C).

At this time, NO_3^- and NO_2^- over the Peruvian shelf and trench ranged between 6.65–17.5 μM and 0.51–1.6 μM, respectively, while the NH_4^+ was less than 0.1 μM. These conditions made this section an ideal research site to study and calculate new production.

Chlorophyll averaged 3.85 μg L^{-1}. This was slightly higher than it was in March 1977 (3.23 μg L^{-1}). NR averaged 0.21 μM N h^{-1} for the cross-shelf section on 16–22 September 1976. This was twice what it was for all stations in March 1977 (0.09 μM N h^{-1}).

Acknowledgements

We thank J. Ammerman, P. Coble and J. Kogelschatz for help with the cruise. SGG. was self-funded. NSF (USA) grant, OCE 75-23718A01, to TTP, funded JASON-76. TTP was supported by TIAA-CREF, Social Security (USA), and al by the Canary Islands CEI: Tricontinental Atlantic Campus. This work was completed while SGG was a student in the ULPGC Graduate Program in Oceanography.

References

Barber, R.T., Huntsman, S., Kogelschatz, J., Smith, W., Jones, B., 1978. Coastal Upwelling Ecosystems Analysis. Data Report 49. Carbon, Chlorophyll and Light Extinction from JOINT II. CUEA Data Report. Technical Report. Bigelow Laboratory for Ocean Sciences.

Beevers, L., Hageman, R.H., 1969. Nitrate reduction in higher plants. Annu. Rev. Plant Physiol. 20, 495–522.

Berges, J.A., 1997. Miniview: Algal nitrate reductases. Eur. J. Phycol. 32, 3–8.

Berges, J.A., Harrison, P.J., 1995. Nitrate reductase activity quantitatively predicts the rate of nitrate incorporation under steady state light limitation: A revised assay and characterization of the enzyme in three species of marine phytoplankton. Limnol. Oceanogr. 40, 82–93.

Berges, J.A., Mulholland, M.R., 2008. Enzymes and Nitrogen Cycling.

Blasco, D., MacIsaac, J.J., Packard, T.T., Dugdale, R.C., Hancock, A., Angeles, L., 1984. Relationship between nitrate reductase and nitrate uptake in phytoplankton in the Peru upwelling region. Limnol. Oceanogr. 29, 275–286.

Blasco, T., Packard, T., 1974. Nitrate reductase measurements in upwelling regions: Baja California and Northwest Africa. I. Significance of the distribution. Tethys 6, 239–246.

Collos, Y., Berges, J.A., 2003. Nitrogen metabolism in phytoplankton. In: Marine Ecology: Encyclopedia of Life Support Systems (EOLSS). Eolss Publishers, Oxford, UK, pp. 262–280.

Dagenais-Bellefeuille, S., Morse, D., 2013. Putting the N in dinoflagellates. Front. Microbiol. 4, 369.

Duce, R.A., LaRoche, J., Altieri, K., Arrigo, K., Baker, A.R., Capone, D.G., Cornell, S., Dentener, F., Galloway, J., Ganeshram, R.S., Geider, R.J., Jickells, T., Kuypers, M.M., Langlois, R., Liss, P.S., Liu, S.M., Middelburg, J.J., Moore, C.M., Nickovic, S., Oschlies, A., Pedersen, T., Prospero, J., Schlitzer, R., Seitzinger, S., Sorensen, L.L., Uematsu, M., Ulloa, O., Voss, M., Ward, B., Zamora, L., 2008. Impacts of atmospheric anthropogenic nitrogen on the open ocean. Science 320, 893–897.

Dugdale, R., Wilkerson, F., Minas, H., 1995. The role of a silicate pump in driving new production. Deep Sea Res. Part I: Oceanogr. Res. Papers 42, 697–719.

Dugdale, R.C., 1972. Chemical oceanography and primary productivity. Geoforum 3, 47–61.

Dugdale, R.C., 1985. The effects of varying nutrient concentration on biological production in upwelling regions. California Cooper. Ocean. Fish. Investig. 26, 93–96.

Dugdale, R.C., Goering, J.J., 1967. Uptake of new and regenerated forms of nitrogen in primary productivity. Limnol. Oceanogr. 12, 196–206.

Dugdale, R.C., Menzel, D.W., Ryther, J.H., 1961. Nitrogen fixation in the Sargasso Sea. Deep Sea Res. 7, 297–300.
Dugdale, R.C., Wilkerson, F.P., 1998. Silicate regulation of new production in the equatorial Pacific upwelling. Nature 391, 270–273.
Dugdale, R.C., Wilkerson, F.P., Barber, R.T., Chavez, F.P., 1992. Estimating new production in the equatorial pacific ocean at 150°W. J. Geophys. Res. 97, 681–686.
Eppley, R.W., Packard, T.T., MacIsaac, J.J., 1970. Nitrate reductase in Peru current phytoplankton. Mar. Biol. 6, 195–199.
Eppley, R.W., Peterson, B.J., 1979. Particulate organic matter flux and planktonic new production in the deep ocean. Nature 282, 677–680.
Eppley, R.W., Rogers, J.N., McCarthy, J.J., 1969. Half-saturation constants for uptake of nitrate and ammonium by marine phytoplankton. Limnol. Oceanogr. 14, 912–920.
Fernández, C., Farías, L., Alcaman, M.E., 2009. Primary production and nitrogen regeneration processes in surface waters of the Peruvian upwelling system. Prog. Oceanogr. 83, 159–168.
Fernández, C., Farías, L., Ulloa, O., 2011. Nitrogen fixation in denitrified marine waters. PLoS ONE 6, 1–9.
Geider, R., La Roche, J., 2002. Redfield revisited: variability of C:N: P in marine microalgae and its biochemical basis. Eur. J. Phycol. 37, 1–17.
Glibert, P.M., Wilkerson, F.P., Dugdale, R.C., Raven, J.A., Dupont, C.L., Leavitt, P.R., Parker, A.E., Burkholder, J.M., Kana, T.M., 2016. Pluses and minuses of ammonium and nitrate uptake and assimilation by phytoplankton and implications for productivity and community composition, with emphasis on nitrogen-enriched conditions. Limnol. Oceanogr. 61, 165–197.
Gordillo, F.J.L., Jiménez, C., Corzo, A., Niell, F.X., 1997. Optimized nitrate reductase assay predicts the rate of nitrate utilization in the halotolerant microalga Dunaliella viridis. J. Appl. Phycol. 9, 99–106.
Hewitt, E.J., Nicholas, D.J., 1964. Enzymes of inorganic nitrogen metabolism. Modern Methods Plants Anal. 7, 67–162.
Hung, C.C., Wong, G., Liu, K.K., Shiah, F.K., Gong, G.C., 2000. The effects of light and nitrate levels on the relationship between nitrate reductase activity and 15NO3 - uptake: Field observations in the East China Sea. Limnol. Oceanogr. 45, 836–848.
Jones, G.J., Morel, F.M., 1988. Plasmalemma redox activity in the diatom Thalassiosira: a possible role for nitrate reductase. Plant Physiol. 87, 143–147.
Kogelshatz, J., Shepherd, R., Whitledge, T., Codispoti, L., Huyer, A., 1978. JOINT-II JASON 76 hydro data, R/V EASTWARD Cruises E-5F-76 through E-5L-76, International Decade of Ocean Exploration, CUEA Data Rep., vol. 38.
Loick-Wilde, N., Weber, S.C., Conroy, B.J., Capone, D.G., Coles, V.J., Medeiros, P.M., Steinberg, D.K., Montoya, J.P., 2016. Nitrogen sources and net growth efficiency of zooplankton in three Amazon River plume food webs. Limnol. Oceanogr. 61, 460–481.
MacIsaac, J.J., Dugdale, R.C., 1969. The kinetics of nitrate and ammonia uptake by natural populations of marine phytoplankton. Deep Sea Res. 16, 45–57.
MacIsaac, J.J., Dugdale, R.C., 1972. Interactions of light and inorganic nitrogen in controlling nitrogen uptake in the sea. Deep-Sea Res. Oceanogr. Abstr. 19, 209–232.
MacIsaac, J.J., Dugdale, R.C., Barber, R.T., Blasco, D., Packard, T.T., 1985. Primary production cycle in an upwelling center. Deep-Sea Res. 32, 503–529.
Martínez, R., Packard, T.T., Blasco, D., 1987. Light effects and diel variations of nitrate reductase activity in phytoplankton from the northwest Africa upwelling region. Deep Sea Res. 34, 741–753.
Minas, H.J., Coste, J.B., Minas, M., Raimbault, P., 1990. Conditions hydrologiques, chimiques et production primaire dans les upwellings du Perou et des iles Galapagos, en regime d'hiver austral (campagne Paciprod). Oceanol. Acta 10, 383–390.
Packard, T., Blasco, D., MacIsaac, J., Dugdale, R., 1971. Variations of nitrate reductase in marine phytoplankton. Investigaciones Pesqueras 35, 209–219.
Packard, T.T., 1973. The light dependence of nitrate reductase in marine phytoplankton. Limnol. Oceanogr. 18, 466–469.
Packard, T.T., Blasco, D., 1974. Nitrate reductase activity in upwelling regions. 2. Ammonia and Light dependence. Tethys 269–280.
Packard, T.T., Blasco, D., Estrada, M., 2004. Modeling physiological processes in plankton on enzyme kinetic principles. Scientia Marina 68, 49–56.
Packard, T.T., Dugdale, R.C., Goering, J.J., Barber, R.T., 1978. Nitrate reductase activity in the subsurface waters of the Peru Current. J. Mar. Res. 36, 59–76.
Packard, T.T., Jones, V., 1978. Biochemistry and ecology of the Peru Current: The JASON expedition to the Peru upwelling system, September 1976, CUEA. Technical Report, 46. Technical Report. Bigelow Laboratory for Ocean Sciences.
Packard, T.T., Osma, N., Fernández-Urruzola, I., Codispoti, L.A., Christensen, J.P., Gómez, M., 2015. Peruvian upwelling plankton respiration: Calculations of carbon flux, nutrient retention efficiency, and heterotrophic energy production. Biogeosciences 12, 2641–2654.
Pickett, M.H., Paduan, J.D., 2003. Ekman transport and pumping in the California Current based on the U.S. Navy's high-resolution atmospheric model (COAMPS). J. Geophys. Res. 108, 1–10.
Rykaczewski, R., Checkley, D., 2008. Influence of ocean winds on the pelagic ecosystem in upwelling regions. PNAS 105, 1065–1970.
Rojas de Mendiola, B., 1976. Phytoplankton analysis of the station samples: The JASON expedition to the Peru upwelling system, September 1976, CUEA. Technical Report, 46. Technical Report. Bigelow Laboratory for Ocean Sciences.
Santoso, A., McGregor, S., Jin, F.F., Cai, W., England, M.H., An, S.I., McPhaden, M.J., Guilyardi, E., 2014. Late-twentieth-century emergence of the El Niño propagation asymmetry and future projections. Nature 504, 126–130.
Schlitzer, R., 2018. Ocean Data View, https://odv.awi.de.
Smith, R., 1968. Upwelling. Oceanogr. Mar. Biol. Ann. Rev. 6, 11–46.
Tischner, R., 2000. Nitrate uptake and reduction in plants. Plant, Cell Environ. 23, 1005–1024.
Tischner, R., Ward, M.R., Huffaker, R.C., 1989. Evidence for a plasmamembrane-bound nitrate reductase involved in nitrate uptake of Chlorella sorokiniana. Planta 1, 19–24.
Wooster, W.S., 1961. Yearly changes in the Peru current. Limnol. Oceanogr. 222–226.
Young, E.B., Dring, M.J., Savidge, G., Birkett, D.A., Berges, J.A., 2007. Seasonal variations in nitrate reductase activity and internal N pools in intertidal brown algae are correlated with ambient nitrate concentrations. Plant, Cell Environ. 30, 764–774.