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New Production Modeled in the Peru Upwelling Ecosystem from Nitrate Reductase Activity and Light

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Curso 2015/2016

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Trabajo Fin de Título para la obtención del título Máster en Oceanografía

ECOAQUA

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Grupo de Ecofisiología de Organismos Marinos

TÍTULO

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Proyecto de Investigación:

Organismo Financiador: Universidad de Las Palmas de Gran Canaria

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Grupo de Ecofisiología de Organismos Marinos

Abstract

Oceanic New Production (Dugdale and Goering, 1967) is limited by nitrate (NO_3^-), ammonium (NH_4^+), and light (Eppley et al, 1970; Packard et al, 1971). Measuring it by N-15 technique is the current gold-standard, but the data acquisition rate could be improved. Calculating it from plankton nitrate reductase activity (NR) is an inexpensive amplifier because of its high data acquisition rate (Packard et al, 1971). Here we develop a light-dependent, NO_3^- and ammonium independent model of new production based on NR activity that predicts strong new production off Peru. The model is based on measurements from the Coastal Upwelling Ecosystem Analysis (CUEA) JASON expedition from September 1976 (Packard and Jones, 1978).

Keywords: New Production, nitrate reductase, nitrate, ammonium, light, phytoplankton.

Resumen

La Producción Nueva (Dugdale and Goering, 1967) en el océano se encuentra limitada por la concentración de nitrato (NO_3^-), amonio (NH_4^+) y la cantidad de luz (Eppley et al, 1970; Packard et al, 1971). La técnica del N-15 para su determinación es el método normalmente utilizado, sin embargo, la tasa de adquisición de datos se podría mejorar. Calculando la Producción Nueva a partir de la actividad de la enzima nitrato reductasa (NR) en el plancton es una manera barata y con una alta tasa de adquisición de datos (Packard et al, 1971). Aquí desarrollamos un modelo dependiente de la luz e independiente del NO3- y amonio para calcular la Producción Nueva, basado en la actividad de la NR, que además predice grandes valores de esta nueva producción en las costas de Perú. El modelo está basado en las mediciones realizadas en la expedición JASON del programa “Coastal Upwelling Ecosystem Analysis” (CUEA) en septiembre de 1976 (Packard and Jones, 1978).

Palabras clave: Producción Nueva, nitrato reductasa, nitrato, ammono, luz, fitoplancton.

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1. Introduction

Nitrogen (N), as a major component of cells, is a key product of marine primary production. Its uptake rate is a measure of marine ecosystem productivity. However, N can be supplied to phytoplankton independently from two sources, as seen in the N cycle of Fig. 1. One uptake pattern is based on (1) oxidized N forms, primarily NO_3^-, derived from new (or allochthonous) inputs to the system and also N-fixation. Production driven by these two processes is known as "New Production". The second uptake pattern is based on (2) reduced N forms, primarily NH_4^+ and urea, supplied by bacterial nitrification or zooplankton excretion. Both processes "regenerate" protein or nucleotide bound N, in situ in the water column. This type of production is known as "Regenerated" (Dugdale and Goering, 1967).

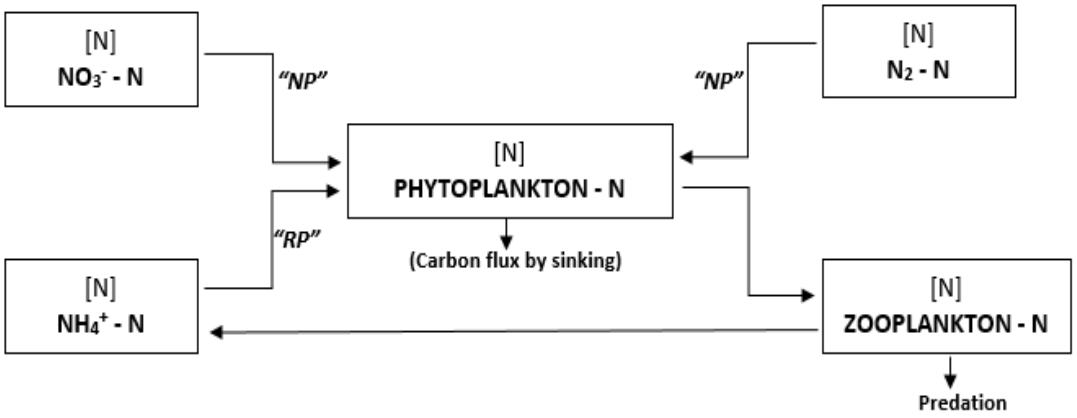


Fig. 1: Simplified model of the circulation of inorganic nitrogen (N) through the euphotic zone ecosystem. [N] represent the concentration of nitrogen in each fraction. "NP" and "RP" represent the "New production" and "Regenerated production" respectively, driven by the different sources of nitrogen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

This work focuses on the importance of nitrate-based "New Production" as a major contributor to productivity in marine ecosystems. In 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 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 by the N-14 technique (Dugdale et al., 1961; MacIsaacs and Dugdale, 1969; Dugdale and Wilkerson, 1986). However, as previous studies have shown (e.g. Packard et al., 1971), this NO_3^- uptake can be modeled from nitrate reductase activity, its dependence on nitrate and light, and its control kinetics.

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 (Fig. 2). Assimilatory NR is not found in protozoans, metazoans, or higher animals (Fig. 3).

$$NO_3^- + NADH + 2e^- + H^+ \rightarrow NO_2^- + NAD^+ + H_2O \qquad \text{(Eq. 1)}$$

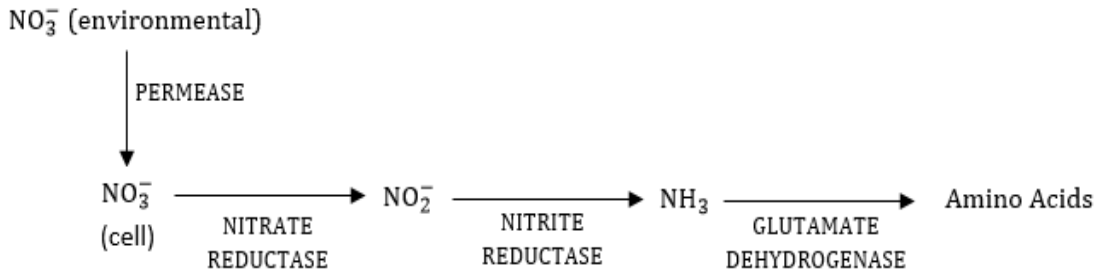


Fig. 2: Simplified model of the metabolic route for the NO_3^- conversion into protein. The different enzymes that catalyze the process are shown above. Nitrate reductase controls the biochemical reaction (modified from Packard et al., 1971).

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, 2015). NR operates in conjunction with permeases, the enzymes that are the 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 glutamate dehydrogenase (GDH), the enzyme that fixes NH_4^+ into glutamate ((COOH)-$(CH_2)_2$-$CH(NH_2)$-(COOH)), a key precursor in amino acid synthesis (Packard et al, 1971; Glibert et al, 2015).

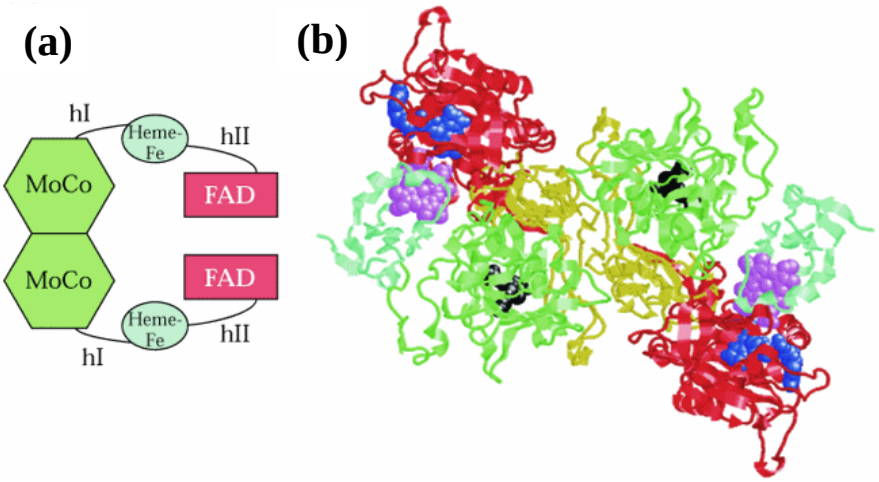


Fig. 3: (a) Functional domains of the enzyme nitrate reductase, Flavin (FAD) domain, heme (Fe) domain and molybdenum cofactor (MoCo) domain. (b) Ribbon model of the enzyme nitrate reductase. The NO_3^- reduction starts with e^- transport from NAD(P)H to the flavin domain, passing through heme domain onto NO_3^- via the molybdenum cofactor, to transform nitrate into NO_2^- (taken from Plant Biochemistry, lecture twenty-three: Nitrogen Metabolism – nitrate reduction, ammonia assimilation).

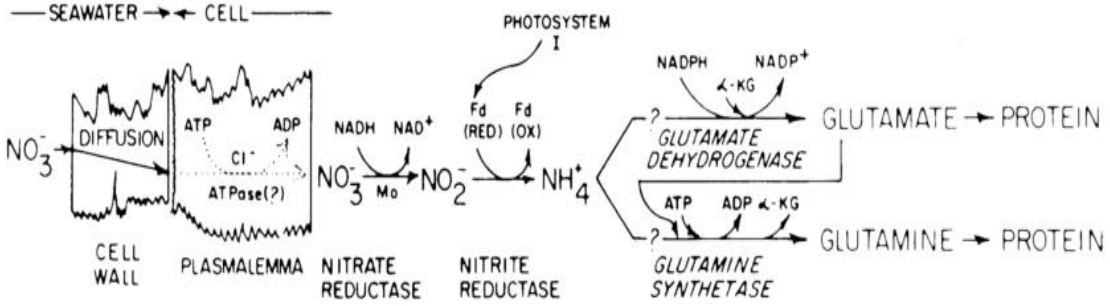


Fig. 4: Detailed scheme of the metabolic pathway for nitrate uptake and reduction in phytoplankton (taken from Packard, 1978).

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). That is why it can be used as a measure (proxy) of nitrogen uptake as well as an estimation of the NO_3^-

assimilation rate (Packard et al, 1971). 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 nitrate-rich water column by the low light. On the other hand, when NO_3^- is present and ammonium is absent, NR activity follows an endogenous diel cycle (Eppley et al, 1970; Packard et al, 1971). Because of these characteristics NR is rarely measureable 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 (Packard, Estrada and Blasco, 2004), but to date it has not been used as synoptic tool to reveal the new production in an upwelling area. Here, this is done.

We develop a light-dependent, NO_3^- -independent model of new production based on NR activity that predicts strong new production off Peru. The model is based on NR measurements from the Coastal Upwelling Ecosystem Analysis (CUEA) JASON expedition from September 1976 (Packard and Jones, 1976).

2. Material and methods

2.1. Research site:

The site of this study was 15˚ S off Pisco, Peru (Fig. 3a), 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 variability under such conditions, the JASON-76 expedition took place in the late winter and austral 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, 1976).

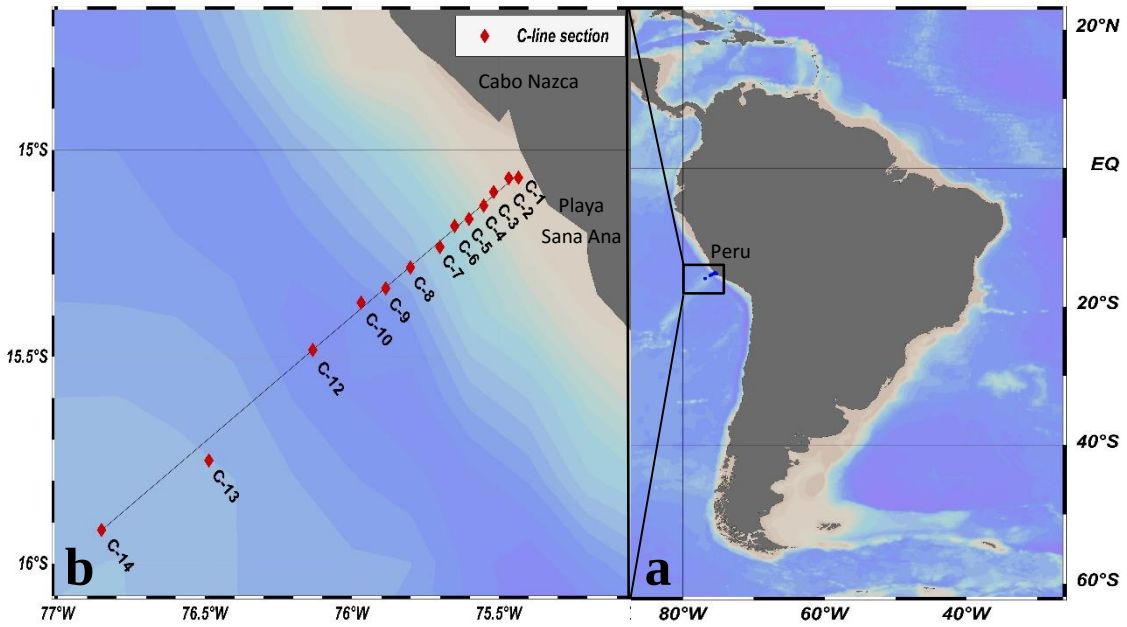


Fig. 5: (a) Location of the site of study, in the Peruvian coast at 15˚ S. (b) C-line section orthogonal to the Peruvian coast at 15˚ S.

2.2. Sampling procedures:

Seawater samples were taken with Niskin Bottles and with a Rosette Sampler along a transect line ("C"-Line) (Fig.3b) across the Peru current at 15˚S. The C-line extended from the coast, at position C-1, across the Peru trench (Fig. 3) to position C-14, 182 km offshore (Fig. 4). Two hydrosections, multiple productivity, and several deep-biology stations were taken during this cruise. 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. Four L samples were taken from the morning productivity rosette (Barber et al, 1978), and filtered through 4.25cm Gelman glass fiber filters (0.7µm pore size). These samples were assayed for NR activity by nitrite-detection method of Hewett and Nicholas, (1964) as modified by Eppley et al. (1969) and Packard et al, (1971). 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. Subsamples 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 (1976).

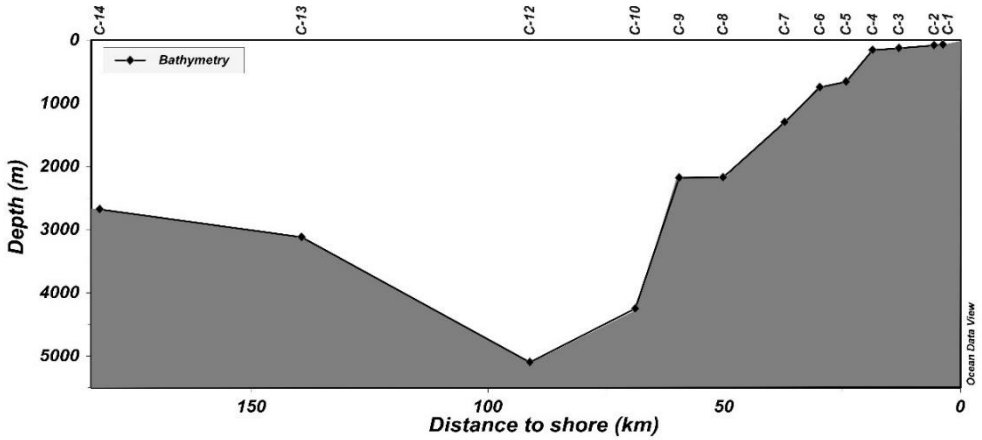


Fig. 6: CUEA c-line through the Peru upwelling and Trench at 15˚ S. Peru coast on the right. The closest C-line position, C-1, was 2.7 km from the coast. C-14 was 185.2 km from the coast, located west of the Peru-Chile trench.

2.3. Modeling:

The new production model was based 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, 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; Packard, 1973; 1978), (5) its inverse dependence on NH_4^+ (Packard et at, 1971; Packard and Blasco, 1974; Glibert et al, 2015, and (6) that the NH_4^+ in the euphotic zone off 15° S would not inhibit NR.

$$v = \frac{V_{max}\,[S]}{K_m + [S]} \qquad \text{(Eq. 2)}$$

$$New\ Production = \frac{NR\ \cdot\ (light)}{K_m\ +\ (light)} \qquad \text{(Eq. 3)}$$

In ocean waters charged with ample NO_3^-, and relatively low NH_4^+ the model can be expressed as a single substrate Michaelis-Menten equation (Eq.3) where K_m = the NR Michaelis constant for light (Packard, 1973; Martinez et al., 1988), NR is the V_{max} of the NO_3^- reduction reaction, and [light] is the light level as a % of surface radiation, sometimes expressed in Langleys (Martinez et al., 1987). Fig. 8a shows that the euphotic zone NO_3^- along the C-Line was always above 4 μM, and that the NH_4^+ was always below

0.1 μM, and supports the simplification of the enzyme kinetics to a single substrate case. Accordingly, we can write Eq. 3 for the New Production Rate (NPR) as a differential equation (Eq. 4):

$$NPR = - (\partial[NO_3^-]/\partial t) = [NR][h\nu]/(K_{Lt} + [h\nu]) \qquad \text{(Eq. 4)}$$

Where NR is the V_{max} of NR, K_{Lt} = the NR Michaelis constant for Light. Light [hν] was measured and reported in both % I_0 and Langleys. A K_{lt} of 21.4 ly min^{-1} (2.5% I_0), from a previous upwelling study (Martinez et al, 1987) was used here.

3. Results:

Off Peru, low sea surface temperatures usually indicate upwelling intensity (Dugdale, 1972). In September 1976, classified as an El Niño year, it was observed that the upwelling was more intense than in previous months. Sea surface temperatures at C-3, the center of the upwelling, in September 1976 averaged 14.07 ˚C (Fig. 7, table 1) while from 28 March to 8 April 1976 the average temperature was 16.65˚C (Packard et al, 1978). Large and dense populations of diatoms, most belonging to the group, Chaetoceros, were found in the first meters of the water column, in the euphotic zone (Rojas de Mendiola, 1976), especially at C-3.

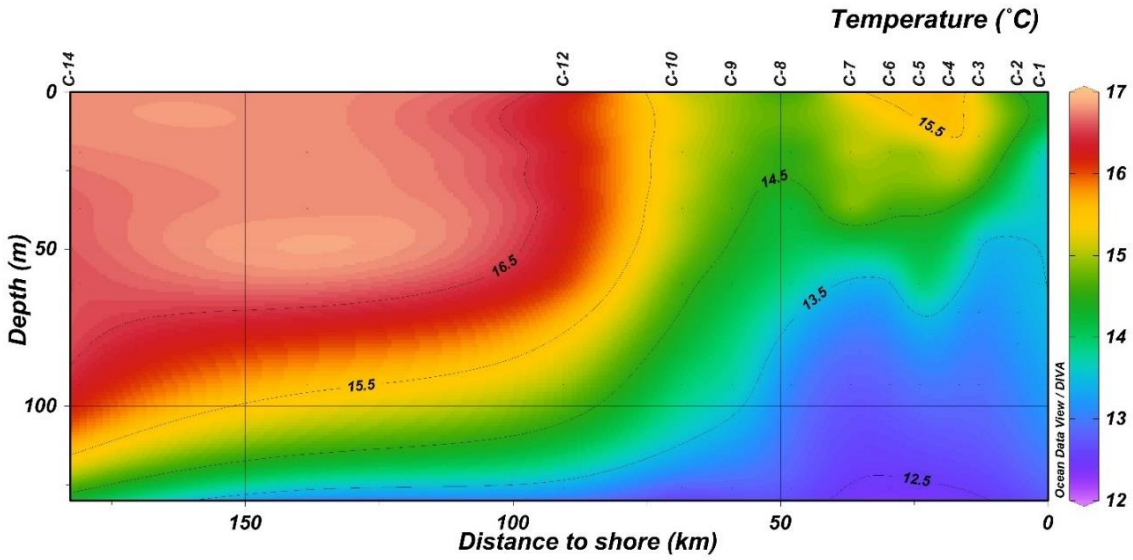


Fig. 7: Temperature (˚C) of the column water along the C-line. 10th September, 1976.

Table 1: Oceanographic characteristics of Peruvian upwelling along the C-line during JASON-76 R/V Eastward cruise no. E-5H-67 in 10th September 1976. Original data are present in CUEA data reports (Packard and Jones, 1976). **Σ** is sigma-t [i.e., (density -1)*1000].

CUEA C-line section	Coordinates	Day	Total depth (m)	Temperature (˚C)	Salinity (PSU)	Σ
C-1	15˚04.0' S 75˚26.2' W	10	74	13.86	34.888	26.148
C-2	15˚04.5' S 75˚28.0' W	10	86	13.82	34.887	26.157
C-3	15˚06.6' S 75˚31.5' W	10	130	14.08	34.888	26.101
C-4	15˚08.0' S 75˚33.8' W	10	160	14.45	34.9	26.038
C-5	15˚10.0' S 75˚36.6' W	10	660	14.10	34.91	26.107
C-6	15˚11.2' S 75˚39.2' W	10	750	13.94	34.895	26.122
C-7	15˚14.3' S 75˚42.2' W	10	1300	14.06	34.897	26.108
C-8	15˚17.2' S 75˚48.5' W	10	2175	13.77	34.893	26.155
C-9	15˚20.4' S 75˚53.7' W	10	2180	14.29	34.917	26.078
C-10	15˚22.9' S 75˚58.9'W	10	4250	14.38	34.92	26.063
C-12	15˚29.0' S 76˚08.0' W	10	5100	15.61	35.04	25.885
C-13	15˚45.3' S 76˚29.8' W	10	3120	16.04	35.019	25.833
C-14	15˚55.8' S 76˚51.5' W	10	2680	16.3	35.018	25.78

Table 2: Integrated values of the key biological and chemical parameters measured at the productivity stations along the C-line in the euphotic zone during JASON-76 R/V Eastward cruise no. E-5H-67 from 16th to 22th of September 1976. Original data are present in CUEA data reports (Packard and Jones, 1976). Integrated units represented in cursive.

CUEA C-line section (JASON station)	Coordinates	Day	Total depth (m)	Chl. α *mg/m²*	Prot. *mmol/m²*	NO_3^- *mmol/m²*	NO_2^- *mmol/m²*	Silicate *mmol/m²*	^{14}C (6h) *mmol/m²/h*	^{14}C (24h) *mmol/m²/h*
C-1 (22)	15˚03.2' S 75˚26.0' W	20	74	65.88	120.34	322.8	24.33	611.1	13.93	6
C-3 (21)	15˚05.9' S 75˚31.4' W	19	130	72.38	129.63	539.61	21.9	785.65	16.28	6.93
C-5 (20)	15˚10.7' S 75˚36.3' W	18	660	143.99	179.27	229.04	16.98	149.3	34.8	13.53
C-8 (19)	15˚17.0' S 75˚47.0' W	17	2175	101.56	219.98	381.36	22.57	334.95	23.89	10.09
C-10 (18)	15˚22.7' S 75˚58.5' W	16	4250	34.815	127.91	433.47	20.21	360.3	8.1	3.5
C-12 (35)	15˚29.0' S 76˚07.8' W	22	5100	123.55	-	158.08	20.25	97.95	32.82	11.27

In September 1976, values of NO_3^- ranging from 6.65 to 17.5 µM were observed throughout the euphotic zone from C-1 to C-12; they never fell below 6.65 µM. The maximum at the sea surface occurred at the upwelling center, C-3, ranging from 16.28 to the maximum of 17.5 µM. The section (Fig.8a) shows this high of 17.5 µM occurring below 20m inshore of the shelf-break (from C-1 to C-5). The lower values of NO_3^- at C-5 and C-12 reflect the result of biological uptake (Fig. 8a). The section of NO_2^- (Fig. 8b) had two subsurface maxima (1.1 µM) at 30m below the euphotic zone, the first near the coast, extended vertically into the water column at C-1; the second was a NO_2^- patch spreading horizontally between C-5 and C-8. Another maximum (1.6 µM) occurred at 20m at C-12; this one coincided with a NO_3^- minimum zone. Concentrations ranged from 0.51 to 1.6 µM, with a minimum in the water column at C-10 (Fig. 8b).

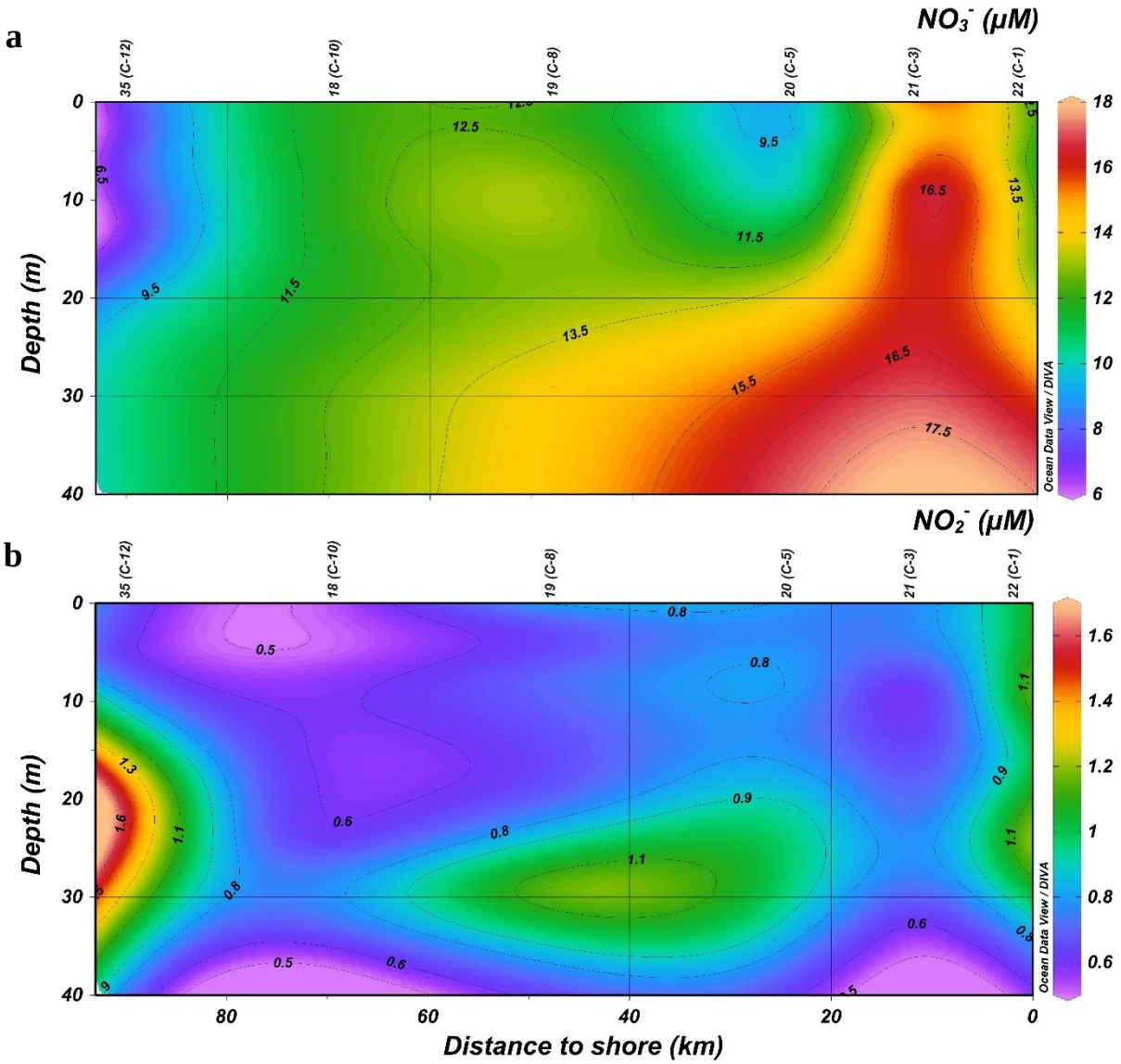


Fig. 8: (a) NO_3^- and (b) NO_2^- sections along the C-line from C-1 to C-12, respectively. Both sections represent the upwelling from 16th to 22th September 1976.

The chlorophyll α in the euphotic zone (Fig. 9) ranged from 0.67 to 8.34 mg/m^3, with a mean for all stations of 3.85 mg/m^3 or what is the same 3.85 µg/l. These values are lower than those obtained in March 1976 (18.4 µg/l) (Packard et al, 1978). The chlorophyll section revealed two maxima, the first one at C-5 and the second one at C-12 (Fig. 9). The first was displaced offshore by about 10 km from the silicate and nitrate maxima (Fig. 10 and 8a); the second coincided with a silicate minimum (Fig. 10) and extended down to 40m in the water column. As for the silicate, 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, ranging around 7.3 µM (from C-10 to C-12) (Fig. 10).

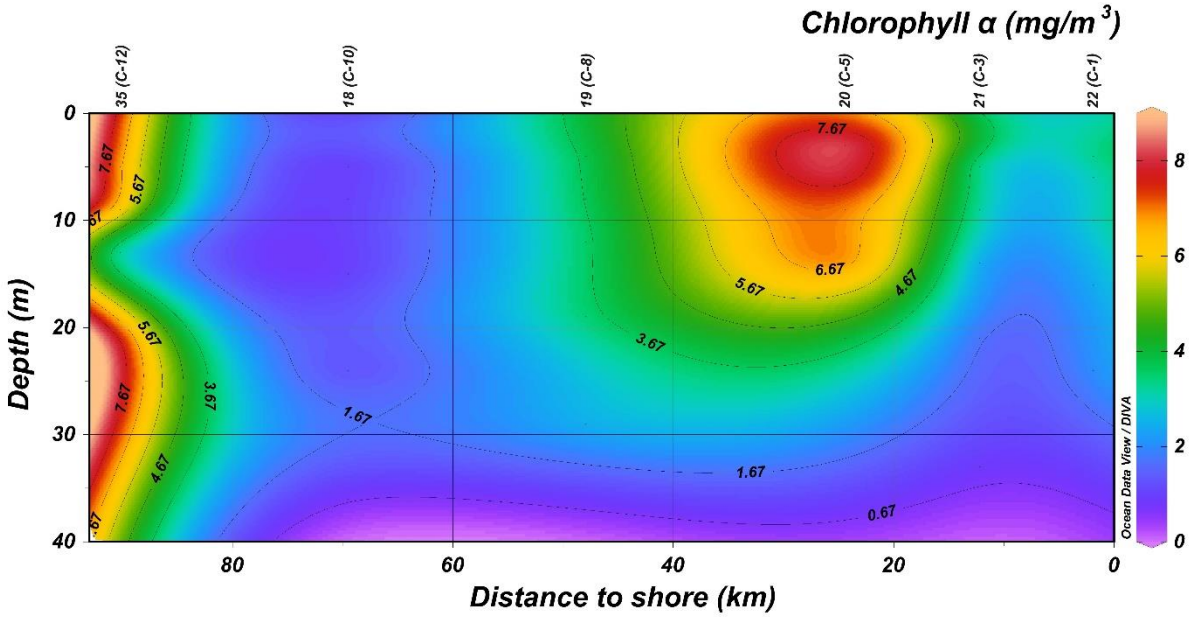


Fig. 9: Chlorophyll α distribution in the column water along the C-line from C-1 to C-12. The values represent the upwelling from 16th to 22th September 1976.

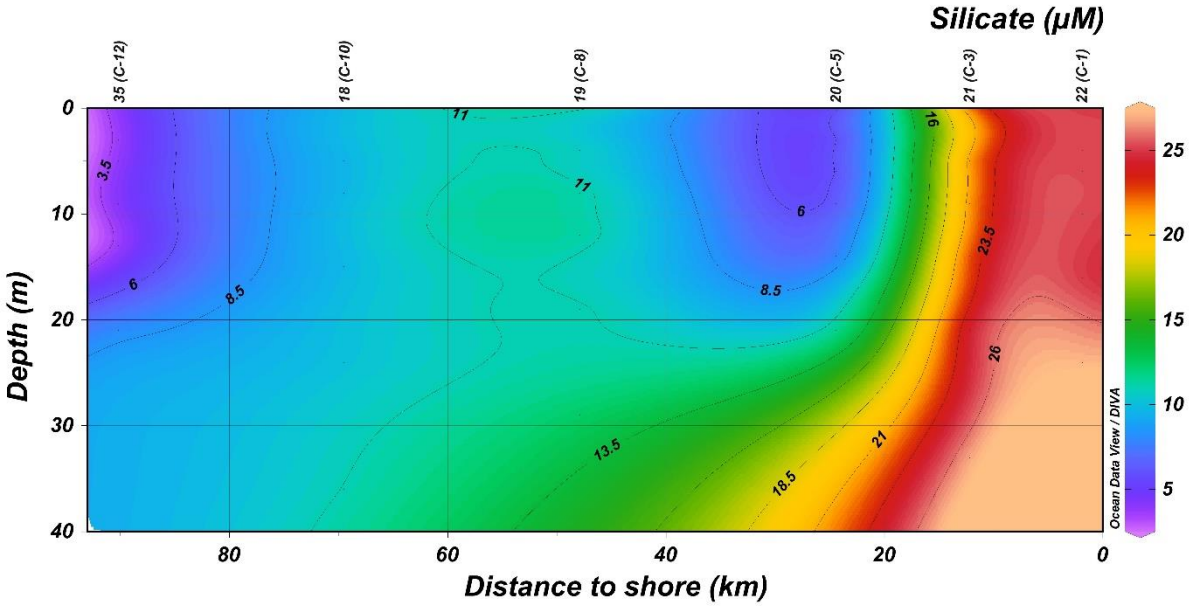


Fig. 10: Silicate distribution in the water column along the C-line from C-1 to C-12. The values represent the upwelling from 16th to 22th September 1976.

Nitrate reductase (NR) was measured in the euphotic zone throughout the water column at all stations. It ranged from 10 to 56 nM/h in September 1976 (Fig. 11a), higher than NR measured in March 1976 which ranged from 0 to 3.5 nM/h. Here, in contrast to the mesopelagic (subsurface) bacterial NR measurements made along the C-line in fall, 1977, special attention was paid to the phytoplankton in the light-rich surface waters where nitrate was plentiful and NR activity was extraordinary high. Results in the vertical distribution show high values in surface waters, where there is no limitation by light. Two maxima appear, the first and most intense one was at C-5; the second occurred at C-12 (Fig. 11a), coinciding with the maxima in the New Production (510 nM NO_3^- /h) calculated according Eq. 3 (Fig. 11b).

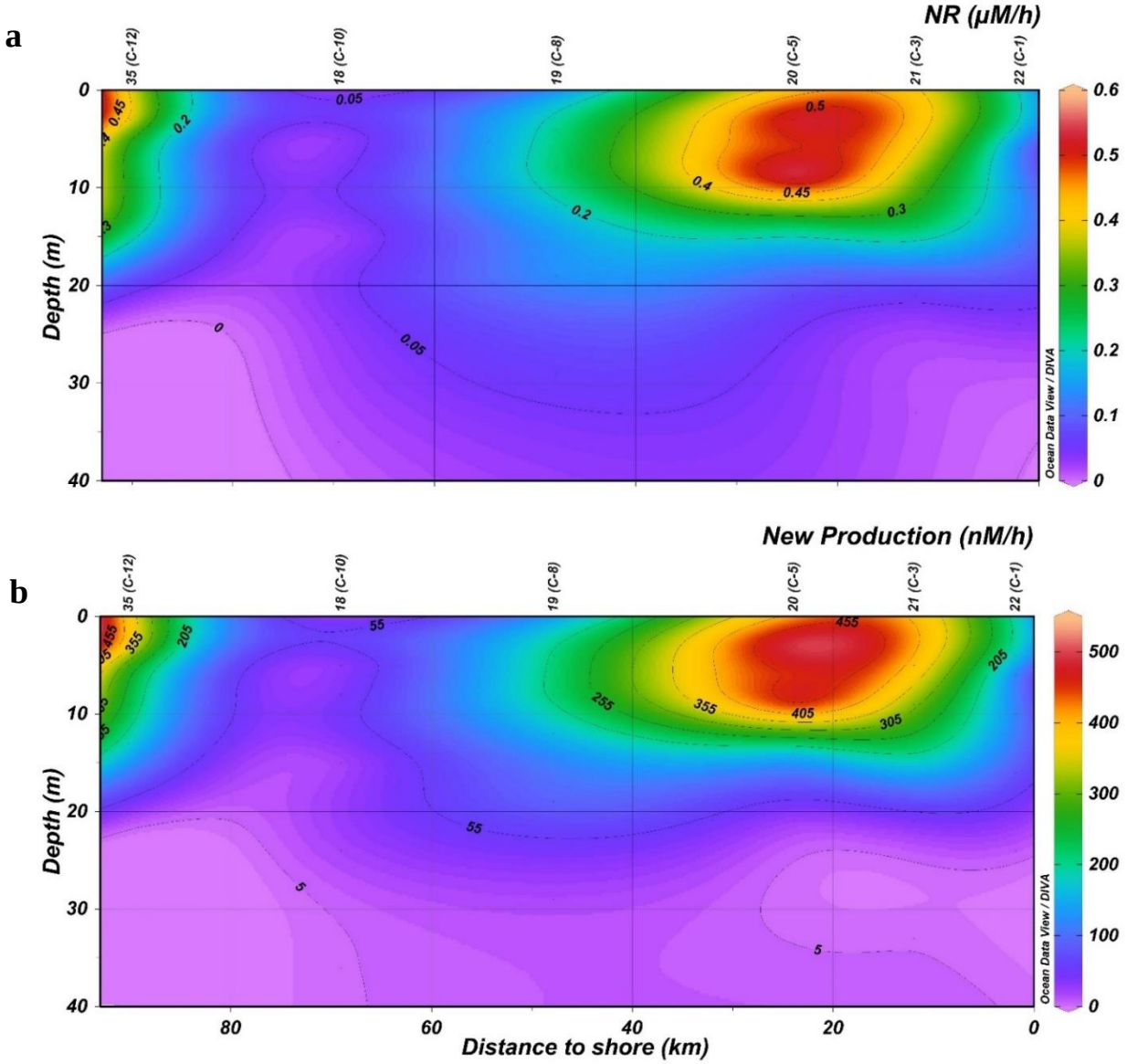


Fig. 11: (a) Distribution of the Nitrate Reductase activity expressed in nM. (b) New Production along the C-Line from C-1 to C-12 calculated from NR activity measured in phytoplankton, in the upwelling system of Peru coast, from 16th to 22th September 1976.

The results obtained for the carbon productivity distribution are shown in Fig. 12. Measurements reveal high values in the surface water for both the 6 and 24 hours' incubation bottles. They ranged from 0 to 4.2 and 0 to 1.5 mmol/m^3/h of carbon per hour, respectively, which correlate with the NR values, arguing the close relationship between new production and carbon productivity.

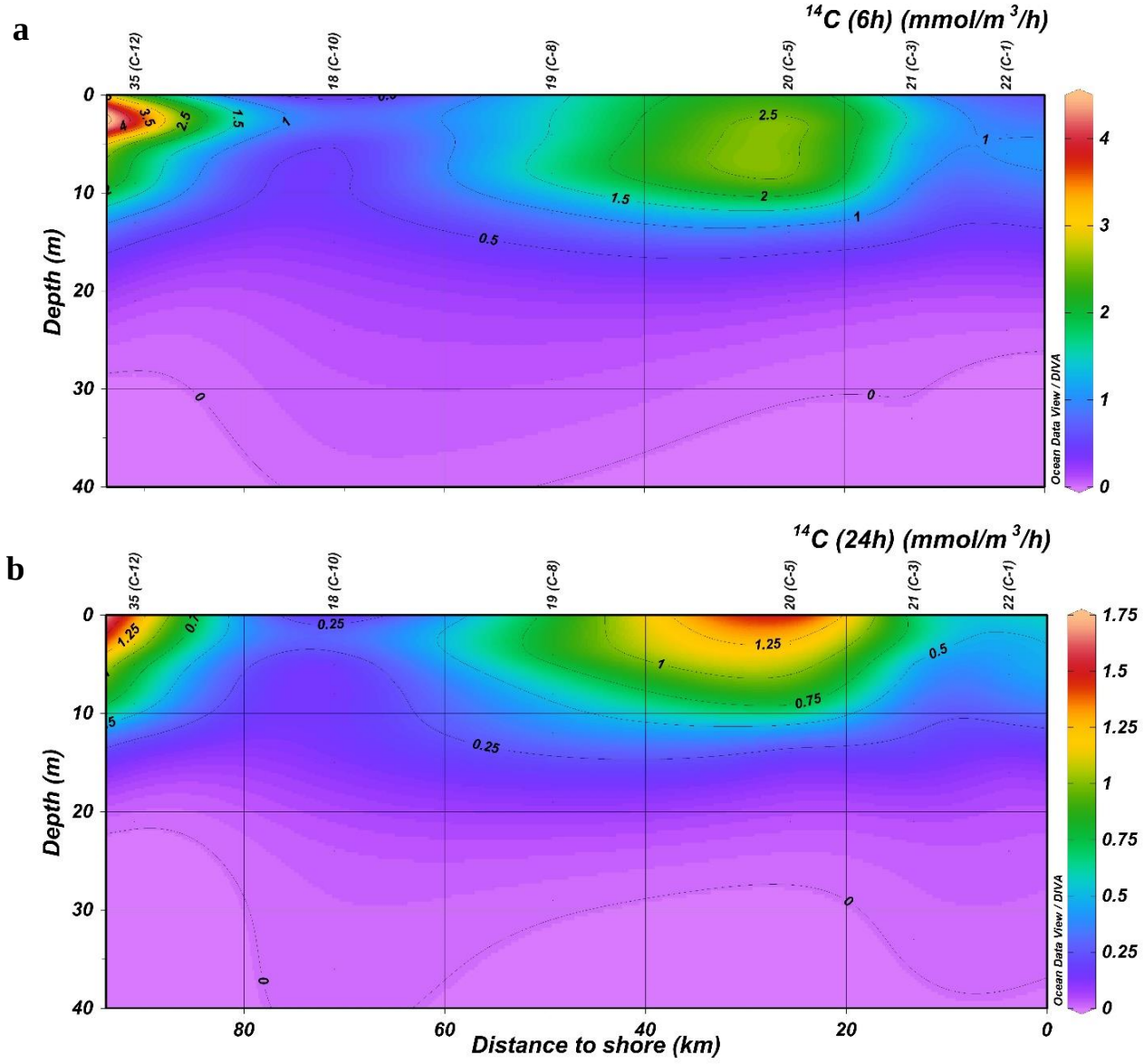


Fig. 12: ^{14}C-Productivity along the C-line from C-1 to C-12 as calculated from: (a) 6-hr and (b) 24-hr deck-incubated bottled phytoplankton. Both measurements correspond to the upwelling system of Peru coast from 16th to 22th September 1976.

RCO_2 was calculated from RO_2, data taken from a previous work from Packard et al. (2015), using a Redfield ratio (C/O_2) of 0.71 from Takahashi et al. (1985). In this case, the values ranged from 8.5 to 128 µmol O_2/l per hour. The profiles show a maximum at C-5, 20 km offshore, and another one at C-12, on the offshore side of the Peru Trench (Fig. 13). This distribution coincides with the values obtained for the potential respiration (ETS) measurements, which ranged from 2 to 15.6 µl O_2/l per hour (Fig. 14).

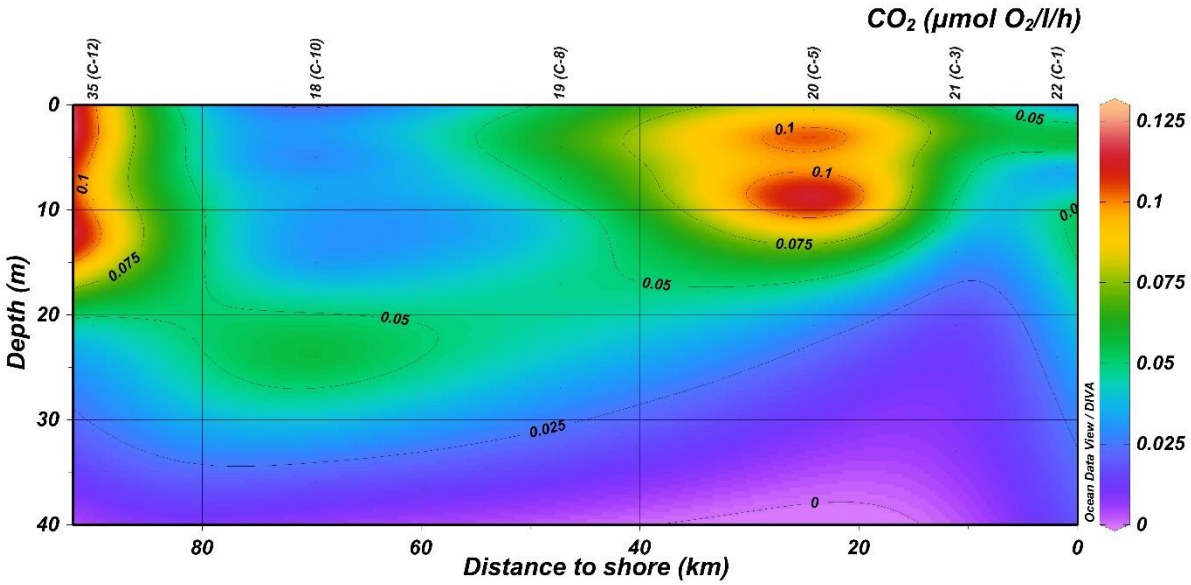


Fig. 13: CO_2 production along the C-line from respiratory O_2 consumption, calculated by Packard et al. (2015) data set, from 16th to 22th September 1976, in the Peruvian upwelling system.

In order to calculate the heterotrophic energy production (HEP), the formula (*HEP = 2 x 2.5 x 48 x RO_2*) from Packard et al. (2015), was applied. In this expression, the 2 is the number of electron pairs required to reduce O_2 to $2H_2O$, 2.5 is the ATP/2e^- ratio, 48 represents the Gibbs Free Energy (ΔG in J per mmol of ATP) and RO_2 is the respiratory oxygen consumption rate.

The calculations show low HEP values below 30 meters, where the minimum is 69.4 J/l per day. Higher HEP values occur in the surface layer, where a maximum between the first 10 meters depth occurs at C-5 (910 J l^{-1} d^{-1}) and another one at C-12 (1038 J l^{-1} d^{-1}) (Fig. 15), following the exact distribution as ETS and RCO_2 profiles (Fig. 14 and 13).

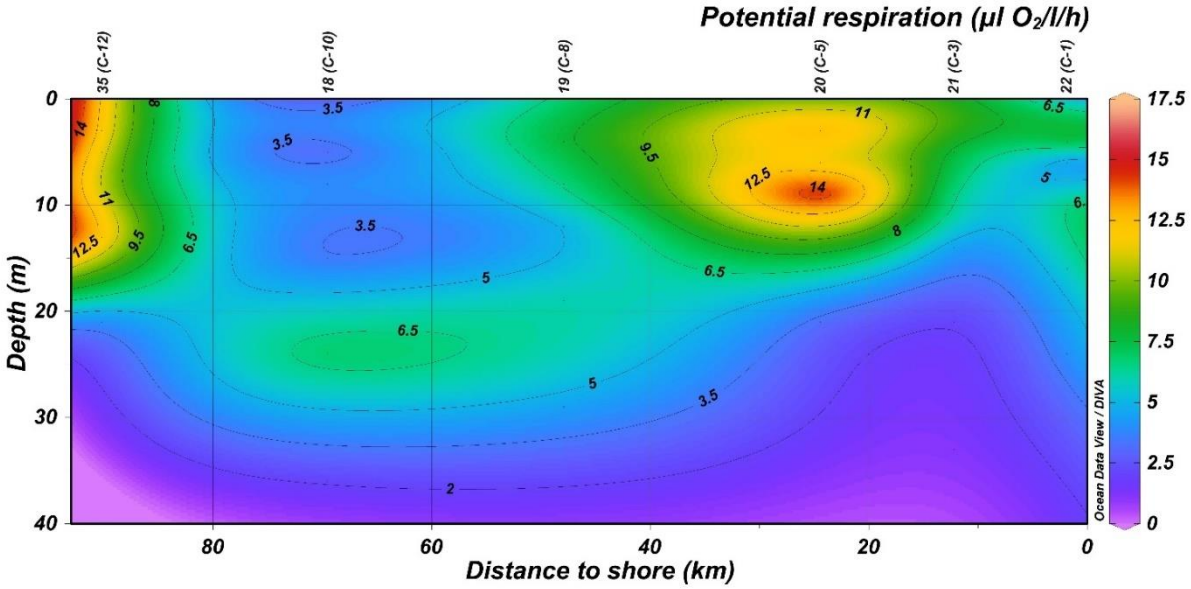


Fig. 14: Potential respiration (ETS) measurements along the C-line from C-1 to C-12, in the upwelling system of Peru coast, from 16th to 22th September 1976.

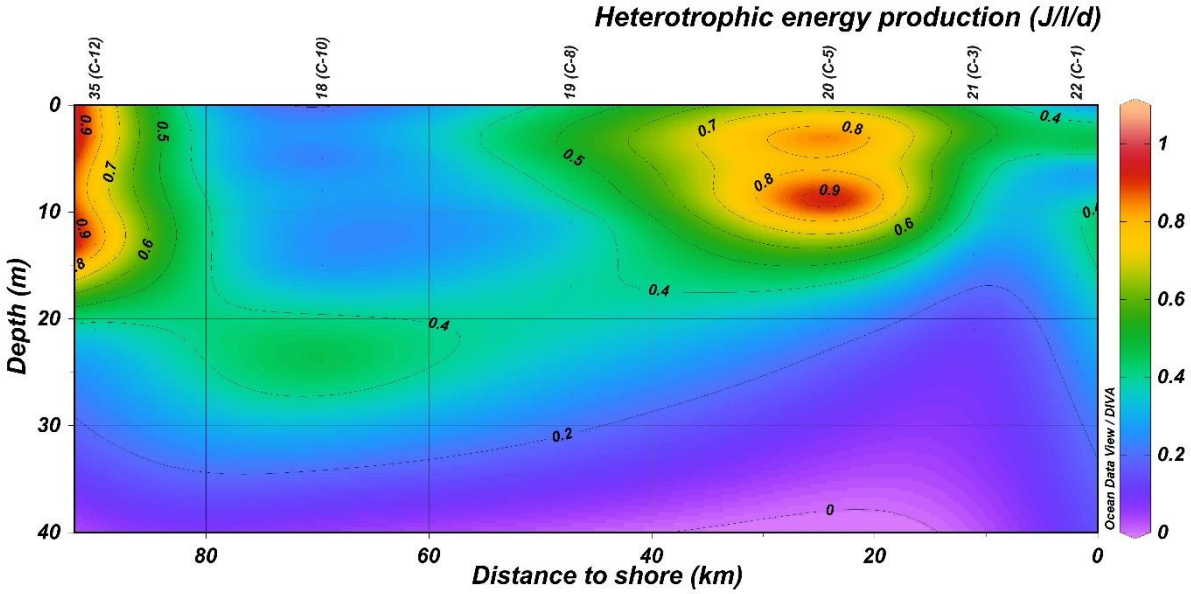


Fig. 15: HEP as ATP production along the C-line, from C-1 to C-12, in the euphotic zone, in the upwelling system of Peru coast, from 16th to 22th September 1976.

4. Discussion:

From our previous research (MacIsaac and Dugdale, 1969; Eppley et al, 1970; Packard et al, 1971; Dugdale, 1985; MacIssac et al, 1985; and Martinez, Packard & Blasco, 1987), we knew that NR activity was enhanced by light, followed a diel cycle, was stimulated by nitrate, and was inhibited by NH_4^+. We also knew that the production off Peru is driven by the vertical flux of inorganic nutrients, especially nitrate. Since 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 for potential new production. Packard et al (1971) hypothesized that nitrate uptake (R_{NO_3}-) would be a linear function of NR, related to NR by a constant (C) so that R_{NO_3}- would equal C [NR]. Blasco et al (1984) showed this to be true, but also showed that the linear relation was clouded by high variability. Here, we make an attempt to explore the use of Michaelis-Menten kinetics (Eq. 3) to use the variability to improve the prediction of R_{NO_3}-

Phytoplankton nitrogen metabolism is hypothetically responsible for NR activity in an euphotic zone seawater sample. Accordingly, it should be correlated with the Chlorophyll levels, but the literature is silent on this topic, so, from the field data in Packard and Jones (1978), we have tested this hypothesis in Fig. 16. We found a correlation between Chlorophyll and NR activity, but one with variability. This variability needs to be investigated experimentally, but we cannot do it with the data at hand. New investigations need to be done.

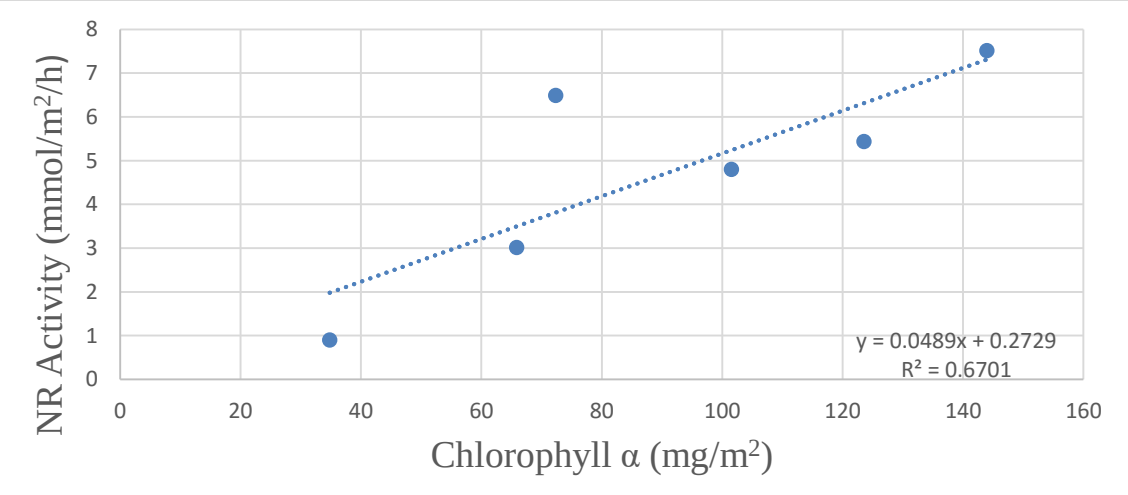
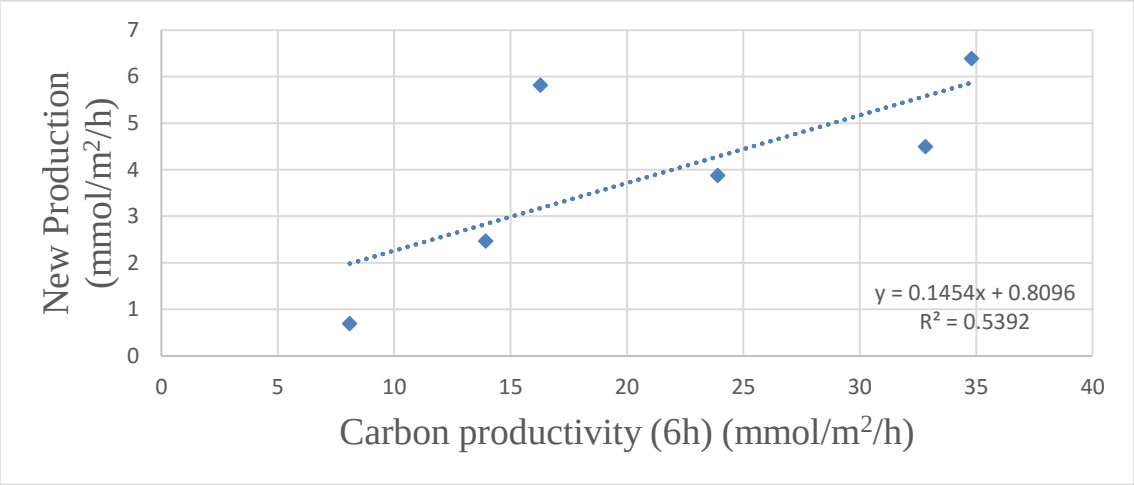


Fig. 16: Correlation between integrated NR activity and Chlorophyll.

Phytoplankton new production can be estimated from nitrate input to the system, an exercise in mass-balance, or from the rate of nitrate uptake by the phytoplankton community. Carbon fixation is based in both new and regenerated production (Dugdale and Groening, 1967; Dugdale, 1985). Furthermore, new production, by itself, is a good determinant of carbon flux in the ocean (Chavez & Barber, 1987). It is principally responsible for the organic matter exportation from the euphotic zone to the deep ocean, so, if the regenerated production is small then the new production should be directly correlated to the total primary production in the ocean (Eppley & Peterson, 2013). Figure 17 shows that this holds true for both our 6h-based and our 24h-based C-14 productivity data.

a



b

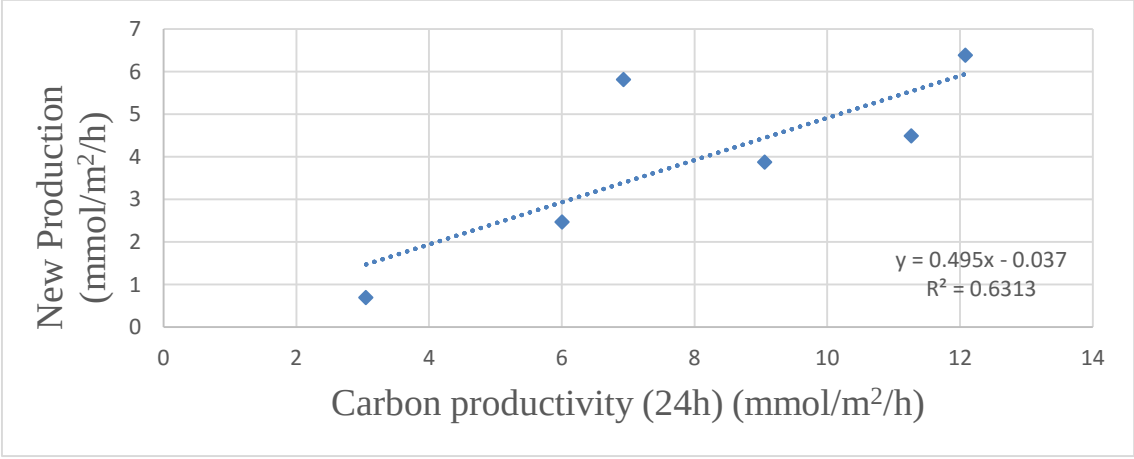


Fig. 17: Correlation between integrated New Production and Carbon productivity as calculated from: (a) 6-hr and (b) 24-hr deck-incubated bottled phytoplankton.

The two productivity data sets measure gross and net productivity, respectively. The difference is directly related to phytoplankton respiration. Since euphotic zone respiration was calculated from ETS activity in this data set in Packard et al., (2015) we can use these respiration calculations to compare with those from the difference in the productivity data. Distribution of potential respiration (ETS) and RCO_2 (Fig. 14 and 13) shows the same space variability, and coincide with the carbon productivity maximum and also with chlorophyll maximum at C-5 and C-12 (Fig. 12 and 9 respectively). This shows us the importance of the phytoplankton community respiration in the ocean, and suggest that these organisms are, indeed, responsible for the carbon exportation to the deep ocean. These results are in accordance with previous studies that consider the phytoplankton community as the key to understanding vertical carbon flux in marine ecosystems (Riley, 1951; Eppley and Peterson, 1979; Packard et al., 1988).

The HEP section (Fig. 15), represents the energy produced while ATP is being generated by respiratory O_2 consumption (RO_2), and since the principal purpose of respiration is to make ATP, this HEP should correlates with RCO_2 and potential respiration (ETS), as we can see in our results (Fig. 15, 13 and 14 respectively). This parameter is a new representation of ATP production in oceanographic analysis. The results obtained here show, as we have seen above, the similar spacial distribution of this variable with potential respiration (ETS) and RCO_2. For that reason, HEP can be an accurate indicator for the plankton community respiration and, hence, a possible key variable in calculating net community productivity, the importance of which has been discussed by Ducklow and Doney (2013).

5. Conclussions:

The model developed here calculates realistic levels of new production, but will need to be compared with new production measured by the ^{15}N technique. The main conclusions of this work are set forth below:

Nutrient and temperature sections across the Peru shelf at 15°S reveal strong upwelling in September 1976, making it an ideal research site to study New Production.

The maxima values of NR activity in surface waters show, as previous studies have shown (Packard et al., 1971), a light dependence of this enzyme, and provide measures of potential nitrate uptake upon which the model is based.

Modeled New Production, here, has the same distribution pattern as the primary production as measured by the C-15 method.

The distribution of the modeled New Production, here, reveals the highest values downstream of the upwelling center in a position over the continental slope where plankton respiration indicates a maximum of vertical carbon flux and high benthic respiration (Packard et al, 2015).

Acknowledgements

I would like to thank all the people who have made the development of this work possible. First of all I would like to thank May Gómez for let me be a part of the EOMAR group, guide me in the world of biological oceanography, offer me different alternatives for my study and supervise part of my TFT. My thanks also to the whole EOMAR group for their kindness and willingness to help. I would also like to thank my tutor and mentor Ted Packard, who introduced me in a complete new world which is the enzymology and the NR metabolism, thank him for the endless help, the innovative ideas and for be the main responsible for this work. Finally, I want to thank my family for their endless support, specially my parents, Verónica and Gregorio, my sister Miriam and last, but not least, Antonio Sanmartín for put up with my bad temper in the most difficult times of the process.

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TFT REPORT

1.- Detailed description of the activities performed for TFT

My work focused in develop a light-dependent, NO_3^--independent model of new production based on Nitrate Reductase activity (NR). To accomplish this aim, I started by searching in the literature the correct techniques to measure NR, how to convert it into New Production, the different ways the NO_3^- and the enzyme were related and all kind of information about the metabolic pathways and utilization of NO_3^- and derivated products in phytoplankton.

Once I had the necessary information I had to gather and organize all the data from different oceanographic researchs, and since this work focus in the measurements from the Coastal Upwelling Ecosystem Analysis (CUEA) JASON expedition from September 1976, all the data were collected from the corresponding technical reports provided by Ted Packard. With all the data I did oceanographic sections in order to show the distribution of the different nutrients in the water column. I also did regressions to observe the correlation between different parameters.

Finally, with all the figures and calculations done I had to discuss the results obtained comparing them with previous studies and different measurements from the data report.

2.- Received training

I learned the basics of enzymatic function in phytoplankton, specially nitrate reductase, and the different metabolic pathways of NO_3^-. I have also expanded my knowledge about "regenerated" and "new" production, thanks to my mentor Ted Packard, and I have learned to make a model to it. In order to build the oceanographic sections, I learned to use the software for exploration, analysis and visualization of oceanographic parameters, *Ocean Data View* (ODV).

3.- Integration and involvement within de department and relationship with the staff

I felt integrated within the investigation group from the beginning. At first Dra. May Gómez accepted me as a member of the group EOMAR and advised me on various studies that I could perform. Once I decided what I wanted to do for my TFT, Dr. Ted Packard, as my mentor, guided me through all the process and taught me all the necessary information to perform my study. Overall all the staff from the group where more than happy to help with anything I needed and there was a good atmosphere and a pleasant working environment.

As a member of EOMAR, all the other members made sure I felt integrated in the group. I also participated in FIMAR 2016 in Las Palmas de Gran Canaria and I will take part in the V international symposium of marine science in Alicante.

4.-Most significant negative and positive aspects of the TFT development

Most negative aspect of my TFT was the lack of time. This subject, as is New Production and Nitrate Reductase activity, is an interesting one for the oceanography research. There is still so much work to do to could understand all the processes that affect the productivity in the oceans, and I consider that the outcome of this study could have been much better if I had time to do it properly and investigate more about this complex process.

By other hand, most positive aspects of my TFT development was all the new knowledge I gained about the topic of Nitrate Reductase and NO_3^- metabolism from such an important scientist as is Ted Packard, who is also an expert in this subject; and getting involved in activities of the investigation group as is FIMAR and the future participation in a symposium.

5.- Personal assessment of the learning achievement throughout the TFT fulfillment

I have discovered a new side of the biological oceanography and I have learned about enzymology and metabolism, and although sometimes I've found it a little hard due to the complexity of the subject, I've found myself enjoying from learning new and different things who have also helped me to understand in a better way all the ocean and

its biochemical processes. I especially appreciate the opportunity to be part of an international congress and show the scientific community my work results and all the knowledge I have acquired.