

## SIZE-FRACTIONED PHOTOAUTOTROPHIC PRODUCTION IN A SHALLOW BAY\*

## PRODUCCION AUTOTROFICA FRACCIONADA POR TAMAÑOS EN UNA BAHIA SOMERA

Silvio Pantoja\*\* Humberto González\*\* y Patricio A. Bernal\*\*

### ABSTRACT

CO<sub>2</sub> assimilation measurements were carried out in Concepción Bay (36°40'S, 73°01'W), to evaluate size fractionated and total primary production in <sup>14</sup>C incubations.

The P-I curve shows an assimilation number of 3.82 mgC (mgChl-a)<sup>-1</sup> h<sup>-1</sup> and an α value of 0.0076 (mgC(mgChl-a)<sup>-1</sup> h<sup>-1</sup>)/uE m<sup>-2</sup> s<sup>-1</sup>, both parameters were used to estimate primary production in the water column using the Herman & Platt model (1983). Integrated production was calculated as 0.97 gC m<sup>-2</sup> d<sup>-1</sup>, four times higher than previous estimations reported off the Chilean coast.

At low irradiance (I = 109 uE m<sup>-2</sup> s<sup>-1</sup>), picoplankton and nanoplankton were responsible for 9.5% and 12.5% of the non-normalized production, whereas the net phytoplankton accounted for 64.6% of the photosynthetic assimilation. The chlorophyll normalized photosynthetic rate (productivity index) was higher for smaller than larger fractions, size dependant relationship with ecological significance at low light irradiances.

*Key words:* Primary production, Picoplankton, Chile.

### RESUMEN

Experimentos de asimilación de CO<sub>2</sub> se realizaron en la Bahía de Concepción (36°40'S, 73°01'W) con el fin de evaluar la producción primaria total y fraccionada a través de incubaciones con <sup>14</sup>C.

De la curva P-I resulta un número de asimilación de 3.82 mgC (mg Chl- a)<sup>-1</sup> h<sup>-1</sup> y un valor de α de 0.0076 (mgC(mgChl- a)<sup>-1</sup> h<sup>-1</sup>)/uE m<sup>-2</sup> s<sup>-1</sup>, los cuales fueron utilizados para estimar producción en la columna de agua usando el modelo de Herman & Platt (1983), cuya producción integrada se calculó en 0.97 gC m<sup>-2</sup> d<sup>-1</sup>, cuatro veces mayor que las informadas previamente para la costa de Chile.

A baja irradiancia (I = 109 uE m<sup>-2</sup> s<sup>-1</sup>), el picoplancton y el nanoplancton fueron responsables de un 9.5% y un 12.5% de la producción no normalizada, mientras que el fitoplancton de "red" (net-phytoplankton) contribuye con un 64.6% de la asimilación fotosintética. La tasa fotosintética normalizada por clorofila (índice de productividad) fue mayor en las fracciones pequeñas que en las fracciones de mayor tamaño, relación dependiente del tamaño y de importancia ecológica a bajas intensidades de luz.

*Palabras claves:* Producción primaria, Picoplancton, Chile.

### INTRODUCTION

Primary production estimates in Chilean waters are scarce and restricted to only a few works (Forsbergh & Joseph, 1963; Platt & Subba Rao, 1975). Observations from the Central Coast of Chile show the presence of sub-surface chlorophyll maximum layers (Bernal, 1986; González *et al.*, 1987) that supply suitable food for larval and juvenile fish survival, however this tropho-dynamic role has not been yet studied.

Recent developments in pelagic ecology have assigned a previously unrecognized role to marine prokaryotes by revealing that these organisms are more ubiquitous and abundant than was expected, and they are capable of extremely high rates of production (Hobbie *et al.*, 1977; Fuhrman & Azam, 1982; Azam *et al.*, 1983). The photo-autotrophic fraction of this prokaryote biomass, or picoplankton (< 2 μm), is widely distributed in the oceans (Waterbury *et al.*, 1979; Li & Platt, 1987) with the exception of

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\*\*Departamento de Oceanografía, Area de Biología y Tecnología del Mar (BIOTECMAR), Pontificia Universidad Católica de Chile, Sede Regional Talcahuano. Casilla 127, Talcahuano - Chile.

Polar waters. Li *et al.*, (1983), demonstrated experimentally that they are responsible for a substantial fraction (30 to 50%) of the total primary production in tropical oceanic and coastal pelagic ecosystems.

A primary production experiment was carried out in Concepción Bay (36°40'S, 73°01'W) in spring to evaluate the biomass and production of the size fractions at the depth of chlorophyll maxima. Fractionated photosynthetic rates and biomass were compared in order to establish the contribution of each size fraction. From the P vs I curve, the parameters  $\alpha$  and  $P_{\max}^B$  were obtained to model primary production in the water column using the vertical distribution of biomass (Herman & Platt, 1983).

## MATERIALS AND METHODS

On October 10, 1986, chlorophyll profiles were measured at a single station located at the mouth of Concepcion Bay (37°35'15"S, 73°01'30"W), with a continuous - flow fluorometer (Turner Designs Mod. 10-000R) in order to determine the depth of the chlorophyll maximum (6 m), and to collect water samples for  $^{14}\text{C}$  incubations.

On board, 19 light and 2 dark bottles were incubated with 5  $\mu\text{Ci}$  of  $\text{H}^{14}\text{CO}_3^-$  per 60 ml during 45 min in a irradiance gradient from 72.4 to 1144.0  $\text{uE m}^{-2} \text{s}^{-1}$ . Samples were homogenized only at the beginning of the experiment. Temperature was controlled by pumping sub-surface water ( $11 \pm 1^\circ\text{C}$ ) through a cooling chamber placed between a 250 Watt halogen light source and the incubation apparatus (modified from Lewis & Smith, 1983). This system, based on a modified overhead projector, allows the simultaneous incubation of 33 flasks (60 ml each) or alternatively of 45 scintillation vials (20 ml each), distributed over a Fresnel lens producing the light gradient (Figure 1). Scalar irradiance was measured using a Biospherical Instruments QSP 200-D underwater sensor fully submerged in an incubating flask filled with filtered seawater (0.45  $\mu\text{m}$ ) placed on the bottom plate glass of the cooling bath.

The size fractionation experiment was carried out at an average scalar irradiance of 109  $\text{uE m}^{-2} \text{s}^{-1}$ . Samples (2 light and 2 dark bottles) were gently filtered through Millipore membrane filters of the appropriate nominal pore-size under low-vacuum (15 cm Hg). The particulate fractions isolated directly by filtration were: 0.45-0.8  $\mu\text{m}$ , 0.8-40

$\mu\text{m}$ , 0.8-1.8  $\mu\text{m}$ , 0.8-210  $\mu\text{m}$ , and 0.45-335  $\mu\text{m}$  (i.e. whole range). The size-fractions between 0.45-1.8  $\mu\text{m}$  (picoplankton), 1.8 and 40  $\mu\text{m}$  (nannoplankton) and between 40 and 335  $\mu\text{m}$  (net phytoplankton) were inferred by subtraction. After filtration, the excess radioactive bicarbonate was removed with fuming HCl before addition of dioxane scintillation cocktail. Radioisotope samples of  $^{14}\text{C}$  and the corresponding sets of standards for "external standard channel ratio" were counted for two minutes (average error 7.3%) in a Beckmann Scintillation Counter LS 100C. Chlorophyll- *a* extracted with acetone, was determined for each fraction using the fluorometric method (Parsons *et al.*, 1984) with duplicate samples.

The use a log-scale abscissa in the presentation of the results of the size fractionation experiment is based on the formalization of the biomass spectra  $b(w)$  of Platt & Denman (1978). In the experiment reported here, the size intervals are not equal, and for each bar the value of biomass (ordinate) represents the integral for that size interval. Accordingly, the total biomass can be obtained by summing the chlorophyll values.

## RESULTS AND DISCUSSION

The results of the P-I experiment are shown as the P vs I curve in Figure 2, where the parameters  $\alpha$  and  $P_{\max}^B$  were estimated as 0.0076  $\text{mg C (mg Chl-a)}^{-1} \text{h}^{-1} / \text{uE m}^{-2} \text{s}^{-1}$  and 3.89  $\text{mg C (mg Chl-a)}^{-1} \text{h}^{-1}$ , respectively. The model of Herman & Platt (1983):

$P^B = P_{\max}^B \tanh(\alpha I / P_{\max}^B)$ , was used to calculate production. The light attenuation coefficient ( $K_z$ ) was estimated as a function of chlorophyll- *a* concentration in the water column, according to the relationship described by Herman (1984) for the Peruvian coast:

$$K_z = 0.05 + 0.05 * \text{chlorophyll-a (mg m}^{-3}\text{)}$$

The profile of primary production is presented in Figure 3, where the production and the biomass profiles are plotted against depth. The resulting integrated production was 0.97  $\text{g C m}^{-2} \text{d}^{-1}$ , four times higher than the previous data cited as representative for the Chilean coast (Platt & Subba Rao, 1975). These data, originally reported by Forsbergh & Joseph (1963), correspond to coastal measurements off Antofagasta-Taltal (Expedition STEP-I, 1960) and to oceanic sta-

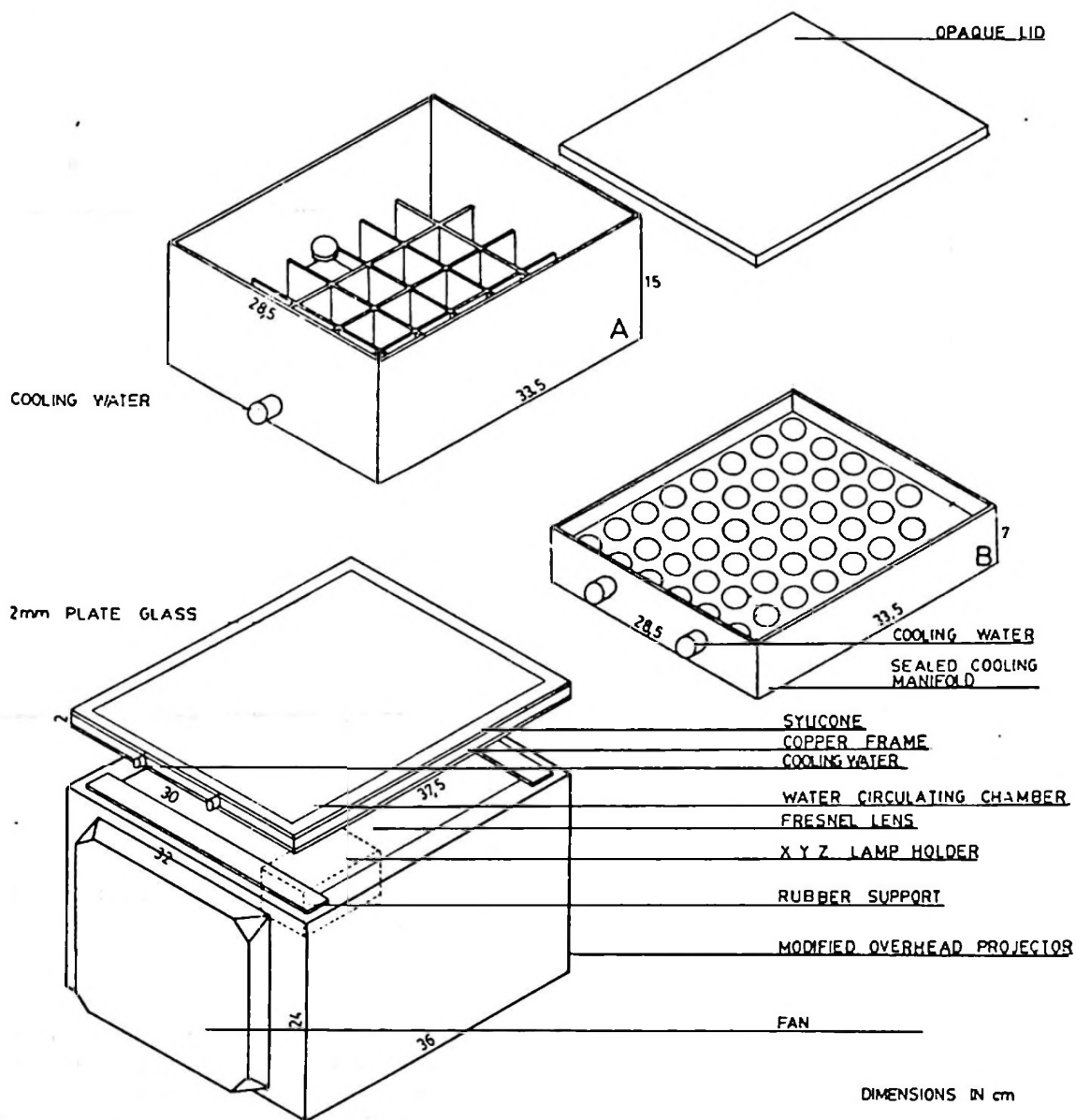


Figure 1. Schematic diagram of incubation system. A: bath for thirty three 60 ml flasks, B: sealed manifold for forty five 20 ml scintillation vials.

tions about 280 km off Los Vilos (B/E Esmeralda Cruise, 1962). This discrepancy can be explained considering the different nature of the sampled environments (bay, shelf, open ocean) however, in interpreting it, allowance should also be made for the different methodologies employed, that according to common practice when published, involved radically different incubation times and counting techniques.

For the size fractionated experiment three types of presentation of the data are used: i) biomass (chlorophyll-*a*) as a function of

particle size (Figure 4-a), ii) non-normalized primary production (Figure 4-b), and iii) the productivity index  $P^B$  as a function of particle size, i.e. the normalized PP spectrum (Figure 4-c).

Picoplankton reached  $0.2 \text{ mg Chl-}a \text{ m}^{-3}$ , compared with  $7.21 \text{ mg Chl-}a \text{ m}^{-3}$  for the total biomass, (Figure 4-a) this corresponds to 2.8% to the photosynthetic biomass in the chlorophyll-*a* maximum layer at 6 m depth, while the nanoplankton contribute 16.9%. In an annual time series obtained in Concepcion Bay, the highest biomass of picoplank-

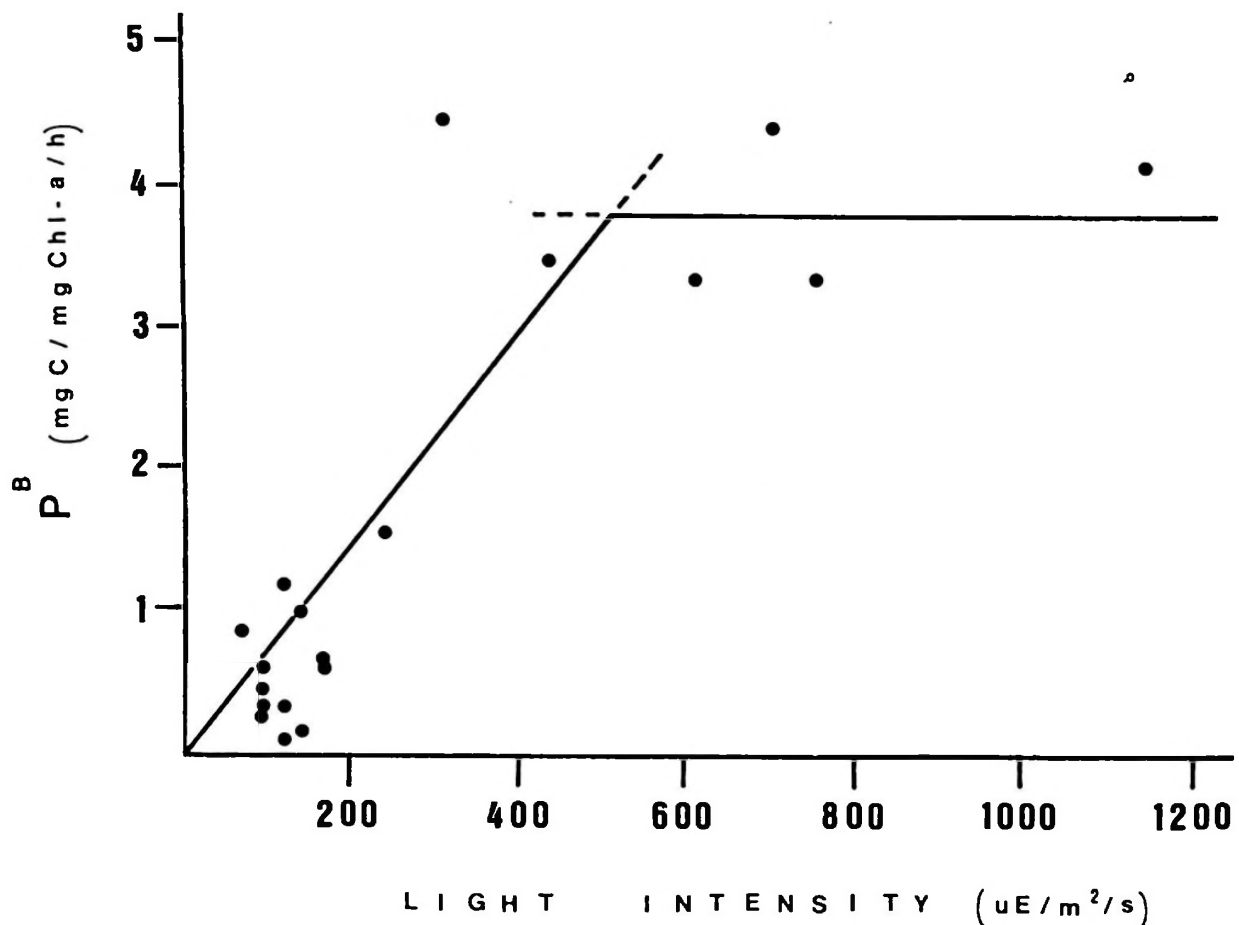


Figure 2. Photosynthesis ( $P^B$ ) versus incident irradiance ( $\mu\text{E m}^{-2} \text{s}^{-1}$ ) for samples obtained at the chlorophyll maximum depth at station 4BG.

ton has been found near the bottom at depth of 25 m, where it may represent up to 69% of the total biomass in winter (unpublished data). This is consistent with the hypothesis that autotrophic picoplankton might be particularly well adapted to grow at low light irradiances (Joint, 1987) and underlines the importance of studying the ecological role of the picoplankton close to the bottom in Concepcion Bay and other shallow bays. On the other hand, the net phytoplankton was predominant (78%) during the experiment, which is consistent with the report that large filamentous diatom species (i.e. *Skeletonema costatum* (Greville) Cleve, 1879; *Detonula pumila* (Castracane) Schuett, 1896), are numerically predominant in the phytoplankton assemblage during the upwelling season in spring-summer (Gonzalez et al., 1987).

The data for  $P^B$  (Figure 4-c) agree with previous observations that the highest photosynthetic rate is related to the smaller size

fractions of the particle-spectrum in the oceans (Li & Platt, 1987). The work by Morris & Glover (1981) with *Synechococcus* and the results of Platt et al. (1983) for  $P$  vs  $I$  parameters indicate that picoplankton should be better adapted to low light conditions than the larger phytoplankton. The photosynthetic rate of picoplankton at  $109 \mu\text{E m}^{-2} \text{s}^{-1}$  was measured at  $3.6 \text{ mg C}(\text{mg Chl-a})^{-1}\text{h}^{-1}$ , which is about twice that of the rest of the particle spectrum. However, despite its values for  $P^B$ , picoplankton primary production accounted for only 9.5% ( $0.72 \text{ mgC m}^{-3} \text{h}^{-1}$ ) of the total primary production (Figure 4-b), while the net phytoplankton contributes 64.6%. Although the pico- and nanoplankton present similar contributions in terms of photosynthetic carbon uptake (11% app), the picoplankton fraction shows the highest photosynthetic efficiency ( $3.6 \text{ mg C}(\text{mg Chl-a})^{-1}\text{h}^{-1}$ ), compared with  $0.78 \text{ mg C}(\text{mg Chl-a})^{-1}\text{h}^{-1}$  for

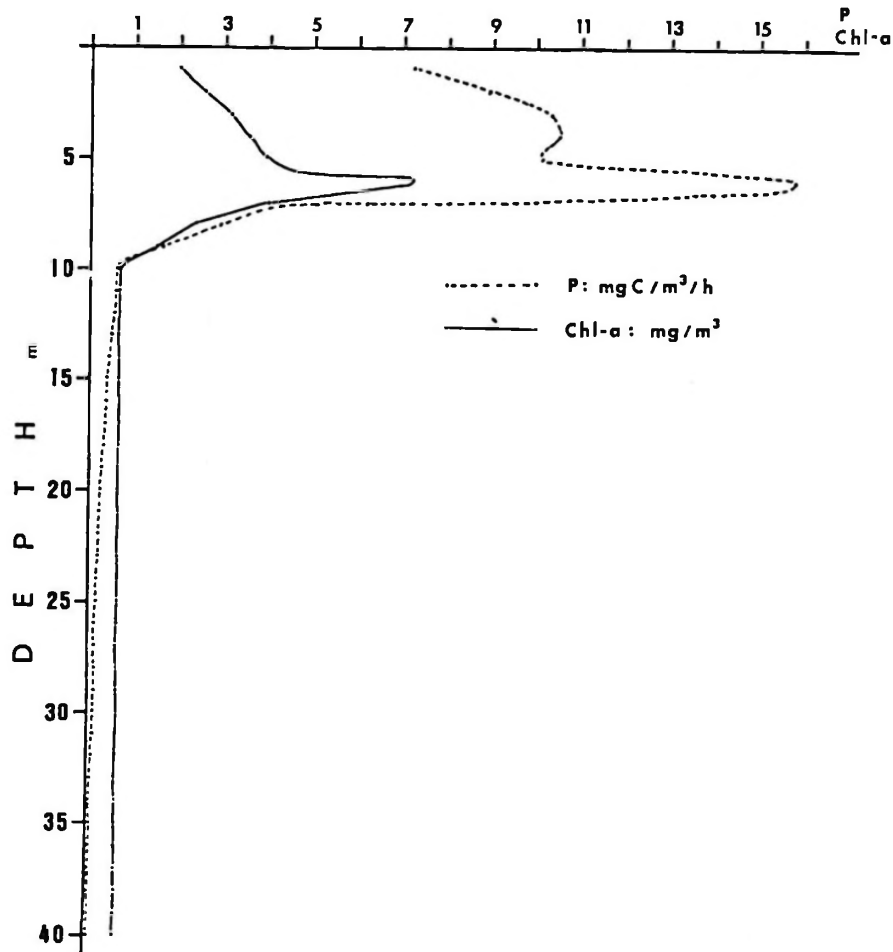


Figure 3. Chlorophyll- *a* ( $\text{mg m}^{-3}$ ) and estimated production ( $\text{mg C m}^{-3} \text{h}^{-1}$ ) profiles.

the nanoplankton. These data indicate that the picoplankton presents a photosynthetic efficiency five times that of the nanoplankton.

Considering the higher metabolic rates of picoplankton and the relatively low biomass of the larger fractions of the spectrum in winter, compared to the smaller particles, as it has been found in an unpublished study, it is reasonable to expect that the picoplankton is responsible for a larger fraction of the photosynthate produced during the winter than in the summer. This means that the still unquantified fluxes of energy involving this autotrophic fraction could be quite important during part of the annual cycle of production in the Bay, such as the role as prey for microzooplankton, proposed by Goldman & Caron (1985) for the autotrophic picoplankton in the bacterial loop model. According to Rassoulzadegan *et al.* (1988), the

ration of bacteria ingested by ciliates varied from 0.1 to 1.9  $\mu\text{gC l}^{-1} \text{d}^{-1}$  in a Mediterranean neritic area. The picoplankton production at Concepcion Bay was calculated as 8.6  $\mu\text{gC l}^{-1} \text{d}^{-1}$ , therefore, assuming similar ciliate bacterial ration, the ciliate populations could ingest between 1.2 to 22.1% of the picoplankton production in the bay. The evidence suggests that ciliates have also a considerable impact on nanoplanktonic production, consuming between 18% and 99% of the total nanoplankton production (Rassoulzadegan *et al.*, 1988). These authors report that ciliates smaller than 30  $\mu\text{m}$  take 72% picoplankton and 28% nanoplankton, those between 30-50  $\mu\text{m}$  take 30% pico- and 70% nanoplankton, while the larger ciliates (> 50  $\mu\text{m}$ ) take 95% nanoplankton and 5% picoplankton. In turn, changes in ciliate availability could also have an impact at higher trophic levels such as micro-zooplankton. It has

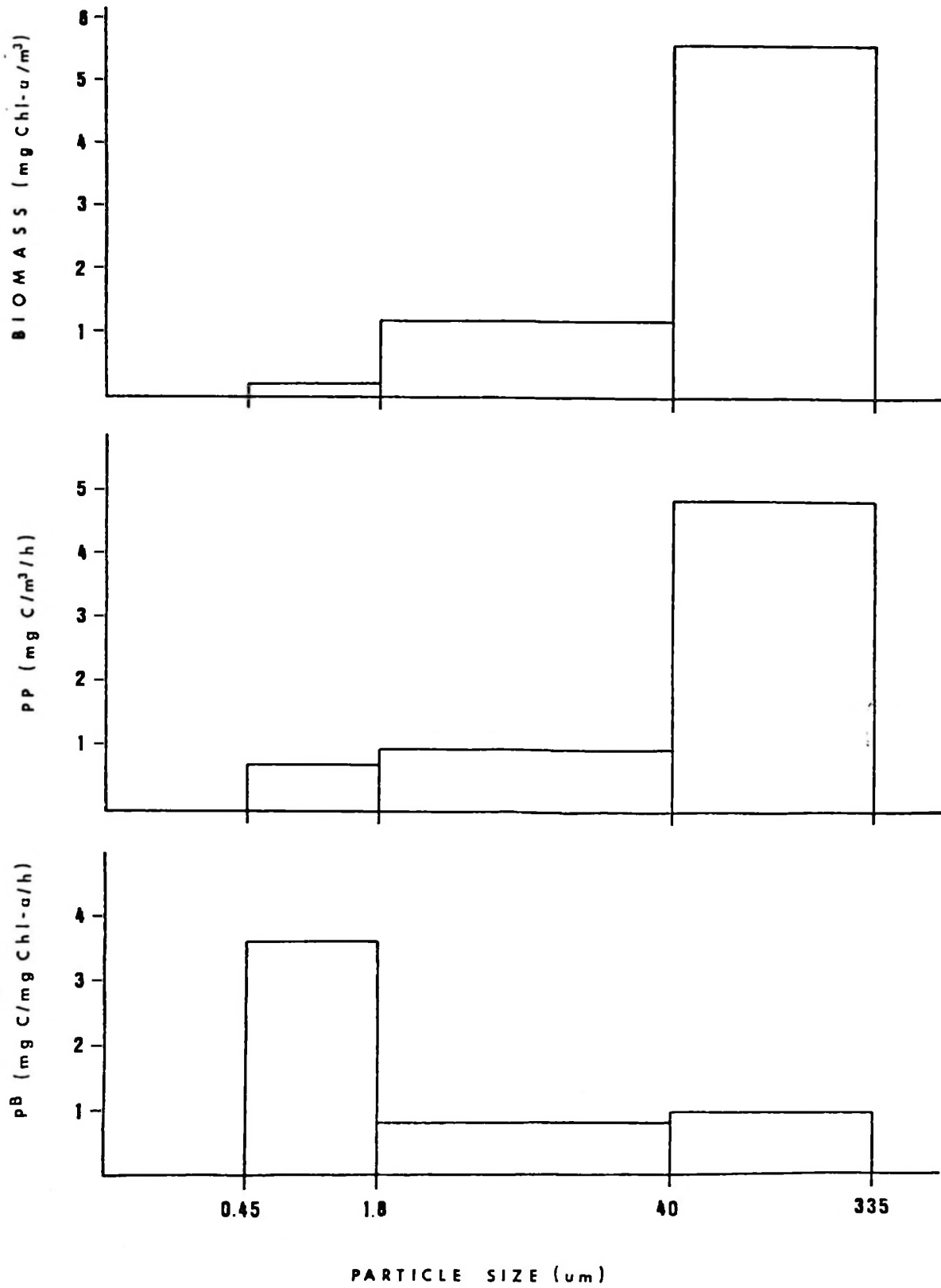


Figure 4. Size-fractionated Photo-autotrophic Biomass and Production: 4-a) Biomass ( $\text{mg Chl-a m}^{-3}$ ); 4-b) Primary Production ( $\text{mg C m}^{-3} \text{h}^{-1}$ ); 4-c) Normalized Primary Production ( $\text{mg C (mg Chl-a}^{-1}) \text{h}^{-1}$ ), particle size in  $\mu\text{m}$ .

been shown that small sized copepods such as *Acartia* prefer ciliates to phytoplankton in laboratory experiments (Stoecker and Sanders, 1985). This points to the lack of knowledge existing for Chilean waters with respect to micro-heterotrophs abundances and production and their role in coastal ecosystems.

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#### REFERENCES

- AZAM, F.; T. FENCHEL, J. G. FIELD, J.S. GRAY, L.A. MEYER-REIL & F. THINGSTAD. 1983. The ecological role of water-column microbes in the sea. *Marine Ecology Progress Series*, 10: 257-263.
- BERNAL, P.A. 1986. Flux and rate measurement of physical, chemical and biological variables in a highly productive environment. Informe Final Proyecto Fondecyt 1190/84. Pontificia Universidad Católica de Chile.
- FORSBERGH, E.D. & J. JOSEPH. 1963. Phytoplankton production in the South-eastern Pacific. *Nature*, 200 (4901): 87-88.
- FUHRMAN, J.A. & F. AZAM. 1982. Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. *Marine Biology*, 66: 109-120.
- GOLDMAN, J.C. & D.A. CARON. 1985. Experimental studies on an omnivorous microflagellate: implications for grazing and nutrient regeneration in the marine microbial food chain. *Deep - Sea Research*, 32: 899-915.
- GONZÁLEZ, H.; P. BERNAL & R. AHUMADA. 1987. Desarrollo de dominancia local en la taxocenosis de fitoplancton de Bahía Concepción, Chile, durante un evento de surgencia. *Revista Chilena de Historia Natural*, 60 (1): 19-35.
- HERMAN, A.W. & T. PLATT. 1983. Numerical modelling of diel carbon production and zooplankton grazing on the Scotian shelf based on observational data. *Ecological Modelling*, 18: 55-72.
- HERMAN, A. 1984. Vertical copepod aggregations and interactions with chlorophyll and production on the Peru shelf. *Continental Shelf Research*, 3 (2): 131-146.
- HOBBS, J.E.; R.J. DALEY & S. JASPER. 1977. Use of nucleopore filters for counting bacteria by fluorescence microscopy. *Applied and Environmental Microbiology*, 33 (5): 1225-1228.
- JOINT, I.R. 1987. Physiological ecology of picoplankton in various oceanographic provinces. In: *Photosynthetic Picoplankton*. Platt, T. & LI, W.K.W. (eds.), Canadian Bulletin of Fisheries and Aquatic Sciences, 214: 287-309, Ottawa, Canada.
- LEWIS, M.R. & J.C. SMITH. 1983. A small volume, short-incubation-time method for measurement of photosynthesis as a function of incident irradiance. *Marine Ecology Progress Series*, 13: 99-102.
- LI, W.K.W.; D.V. SUBBA RAO, W.G. HARRISON, J.C. SMITH, J.J. CULLEN, B. IRWIN & T. PLATT. 1983. Autotrophic picoplankton in the tropical ocean. *Science*, 219: 292-295.
- LI, W.K.W. & T. PLATT. 1987. Photosynthetic picoplankton in the ocean. *Science Progress, Oxford*, 71: 117-132.
- MORRIS, I. & H.E. GLOVER. 1981. Physiology of photosynthesis by marine coccoid cyanobacteria-some ecological implications. *Limnology and Oceanography*, 30: 957-961.
- PARSONS, T.R.; Y. MAITA & C.M. LALLI. 1984. *A Manual of Chemical and Biological Methods for Seawater Analysis*, Pergamon Press, Oxford, 173 p.
- PLATT, T. & D.V. SUBBA RAO. 1975. Primary production of marine microphytes. In: *Photosynthesis and Productivity in Different Environments*, International Biological Programme, Vol. 3., Cambridge University Press, Great Britain, pp. 249-280.
- PLATT, T. & K. DENMAN. 1978. The structure of pelagic marine ecosystems. *Rapports et Procès-Verbaux des Réunions du Conseil International pour l'Exploration de la Mer*, 173: 60-65.
- PLATT, T.; D.V. SUBBA RAO & B. IRWIN. 1983. Photosynthesis of picoplankton in the oligotrophic ocean. *Nature*, 300: 702-704.
- RASSOULZADEGAN, F.; M. LAVAL-PEUTO & R.W. SHELDON. 1988. Partitioning of the food ratio of marine ciliates between pico- and nanoplankton. *Hydrobiologia*, 159: 75-88.
- STOECKER, D.K. & N.K. SANDERS. 1985. Differential grazing by *Acartia tonsa* on a dinoflagellate and a tintinid. *Journal of Plankton Research*, 7: 85-100.
- WATERBURY, J.B.; S.W. WATSON, R.R.L. GUILLARD & L.E. BRAND. 1979. Widespread occurrence of a unicellular, marine, planktonic, cyanobacterium. *Nature*, 277: 293-294.

