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# DISTRIBUTION AND ANNUAL VARIATION OF TRICHODESMIUM THIEBAUTII GOMONT EX GOMONT (OSCILLATORIALES - CYANOPHYTA) IN TROPICAL WATERS OF NORTHEASTERN BRAZIL (WESTERN ATLANTIC)

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#### **ABSTRACT**

Studies about *Trichodesmium thiebautii* Gomont *ex* Gomont and the relations of its spatial and temporal distribution with the hydrological conditions were developed in the continental shelf of the coast of Pernambuco, Brazil. The samples were collected during the rainy (July 2005) and the dry (November 2006) seasons, on the surface and bottom, at six sampling points, distributed along two profiles perpendicular to the coast of Gaibú and Serrambi beaches. The collection points were approximately 5, 10 and 20 miles from the coast, corresponding to the isobaths of 10, 20 and 30 meters. *T. thiebautii* occurred in bothl periods, with less density in areas closer to the coast and higher further from the coast. The correlation of *T. thiebautii* was positive with pH and dissolved oxygen during the rainy season and with salinity and dissolved oxygen during the dry season. No correlation was observed with the nutrient salts.

**Key words**: hydrology, density, spatial variation

#### **RESUMO**

Estudos sobre *Trichodesmium thiebautii* Gomont ex Gomont e as relações de sua distribuição espacial e temporal com as condições hidrológicas, foram desenvolvidos na plataforma continental do litoral de Pernambuco, Brasil. As amostras foram coletadas durante as estações chuvosa (julho/2005) e seca (novembro/2006) na superfície e no fundo, em seis pontos de amostragem, distribuídos ao longo de dois perfis perpendiculares à costa das praias de Gaíbu e Serrambi. Os pontos de coleta localizaram-se aproximadamente a 5, 10 e 20 km da costa, correspondente às isobatas de 10, 20 e 30 metros. *T. thiebautii* ocorreu nos dois períodos estudados, com menor densidade em áreas mais próximas da costa e maior densidade em áreas mais afastadas da costa. A correlação de *T. thiebautii* foi positiva com pH e oxigênio dissolvido durante a estação chuvosa e com salinidade e oxigênio dissolvido durante a estação seca. Nenhuma correlação foi observada com os sais nutrientes.

Palavras-chave: hidrologia, densidade, variação espacial

## INTRODUCTION

The genus *Trichodesmium* Ehrenberg is considered an important component in the tropical Atlantic's planktonic flora, where it is sometimes found in dense growth in higher layers of the photic zone. It is recognized for its high productivity and capacity to assimilate atmospheric nitrogen, being responsible for the increase in planktonic biomass in tropical oligotrophic seas (Gallon et al. 1996; Tyrrell et al. 2003). Growth of some species may affect the physical and chemical properties of surface waters, and may sometimes produce toxins that have impacts on the ecology and on human health (Capone et al. 1997; Komárek & Anagnostides 2005).

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The first time the genus *Trichodesmium* was mentioned in Brazilian waters was by Darwin (1834) during his expedition along the coast of Bahia, when he described the growth of *T. erythraeum* Ehrenberg. Years later, the same species was cited along the coast of Pernambuco (northeastern Brazil), with the description of growth in areas near reef formations, characterized by high temperatures and elevated salinity and with the presence of *T. erythraeum* associated with the human disease called "Tamandaré Fever" (Sato et al. 1963).

Currently in Brazil, the genus is represented by the species *T. erythraeum*, *T. hildebrandtii* Gomont and *T. thiebautii* Gomont *ex* Gomont, of which *T. hildebrandtii* and *T. erythraeum* have been considered the most frequent and abundant (Sato et al. 1963; Sassi & Kutner 1982; Gianesella-Galvão et al. 1995; Medeiros et al. 1999; Sigueira et al. 2006).

The occurrence of *T. thiebautii* was reported along the southern coast of Brazil, where there are growths with toxic connotations as seen in bioassays with mollusks (Rörig et al. 1998) and, recently, in the northeastern waters of Brazil (Monteiro et al. 2010).

The objective of this paper is to quantify the density of *T. thiebautii* in the continental shelf of Pernambuco and correlate its spatial and temporal distribution with the area's hydrological conditions, as a means to contribute towards knowledge of the species' ecological characteristics in the northeastern tropical region of Brazil.

#### **MATERIAL AND METHODS**

The coast of Pernambuco extends between the coordinates  $7^{\circ}$  32' -  $8^{\circ}$  56' S and  $34^{\circ}$  49' -  $35^{\circ}$  11' W and it is located in a region with an As' climate (pseudo tropical) in the Köppen Classification. The annual average water temperature is  $27^{\circ}$ C and salinity ranges between 35 and 37 (Macedo et al. 2004). Rainfall is characterized in two annual periods: a rainy period, from March to August, with higher rainfall in May, June and July, and a dry period, extending from September to February, with October, November and December the driest months. Average rainfall is 2,106 mm per year (CPRH 2003).

The rivers that fertilize the shelf's waters are small in size, and the continental flow does not exceed 4 miles from the coast, being more efficient during the rainy season. Areas further away from food supplies depend on coastal currents (Koening & Macedo 1999).

Collections were taken from six points, distributed along two profiles perpendicular to the coast, located on the state's southern coast: one in front of the Gaibú beach (Point 1, 2 and 3) and another in front of the Serrambi beach (Point 4, 5 and 6). The points were approximately 5, 10 and 20 miles from the coast, corresponding to isobaths 10, 20 and 30 m in depth (Tab. 1).

**Table 1** — Sampling points in the continental shelf in the Pernambuco state (Northeastern Brazil).

POINTS	DEPTH (m)	GAIBÚ				
		Latitude	Longitude			
Pt 1	30	08°20'22,0"S	34°56'22,1" W			
Pt 2	20	08°21'15,0"S	34°53'35,5" W			
Pt 3	10	08°21'38,6"S 34°50'34,2" \				
		SERF	AMBI			
		SERF Latitude	AMBI Longitude			
Pt 4	30		Longitude			
Pt 4 Pt 5	30 20	Latitude				

The samples were collected from the surface and the bottom at each collection point in July 2005, for the rainy season, and in November 2006, for the dry season, using a Niskin Bottle. Part of each sample was kept in refrigeration and sent for abiotic parameter analyses. The values for salinity, dissolved oxygen, ammonia, nitrite, nitrate and phosphate were determined according to the methods described by Strickland and Parsons (1972). The method described by Grasshoff et al. (1983) was used for determining silicate content.

Another part was conserved with acetic lugol and sent for phytoplankton quantitative analyses, which were conducted in a Zeiss inverted optic microscope, model Axiovert 135M, and a zoom of 400X. For counting filaments and colonies, 10 ml sedimentation chambers were used and density (filament  $L^{-1}$ ) was estimated as per the method described by Villafañe & Reid (1995).

The values obtained for abiotic and biotic variables were submitted to correlation analysis (NTSYSpc - 2.10t) after linear correlations have been made.

#### **RESULTS**

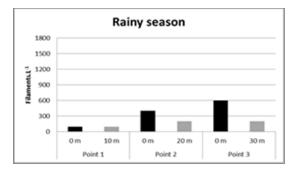
The density of *Trichodesmium thiebautii* filaments revealed spatial and temporal variations, with a minimum value of 100 filaments. $L^{-1}$ , observed at sites closer to the coast, and a maximum of 1,700 filaments. $L^{-1}$  at more distant sites.

At those sites located in the Gaibú profile, the species presented greater densities on the surface in the rainy and the dry seasons. The maximum number of filaments occurred on the surface at Point 2, with 1,000 filaments.L<sup>-1</sup>. In this profile, minimum values were always found at the deepest layers, with the minimum observed at Point 1 with 100 filaments.L<sup>-1</sup>. In general, higher values were always observed during the dry season, on the surface and at the most distant sites (Fig. 1).

At those sites located in the Serrambi profile, the maximum number of filaments also revealed variations between the surface and the bottom. Maximum density (1,700 filaments. $L^{-1}$ ) occurred on the surface at point 5, during the dry period, whereas the minimum (100 filaments. $L^{-1}$ ) was observed at points 4 and 6, during the rainy season, at the layer near the bottom, and also at points 4 and 6 in the dry season (Fig. 2).

Considering the two profiles analyzed, it is possible to observe that higher concentrations of filaments were always found on the surface and during the dry season.

#### Gaibú



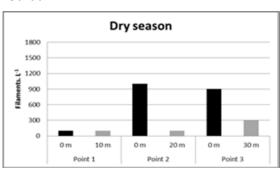
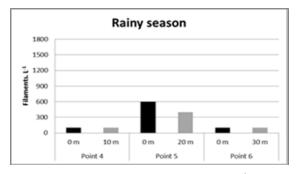
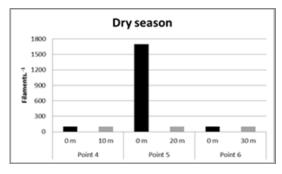


Figure 1 – Number of filaments.L<sup>-1</sup> of *Trichodesmium thiebautii* Gomont *ex* Gomont on the surface and at the bottom at the collection points in the Gaibú Profile (Northeastern Brazil), during the rainy season (July 2005) and the dry season (November 2006).

# Serrambi





**Figure 2** – Number of filaments.L<sup>-1</sup> of *Trichodesmium thiebautii* Gomont *ex* Gomont on the surface and at the bottom at the collection points in the Serrambi Profile (Northeastern Brazil), during the rainy season (July 2005) and the dry season (November 2006).

Average water temperature ranged between 26.10°C, at a depth of 10 m at Points 4, 5 and 6 (Serrambi) and 27.7°C, at a depth of 30 m at Point 3 (Gaibú) without any characterization of thermal stratification at the sampling points. Salinity revealed few annual variations with a minimum average of 33.19, on the surface at point 4 (Serrambi), and a maximum average of 36.38, at a depth of 30 m at Points 3 and 6 in the Gaibú and the Serrambi profiles. Dissolved oxygen and pH contents presented the same standard of variation as previous variables. In other words, there were small annual variations between the surface and bottom and between the six collection points (Fig.2). Low nutrient salts values were observed in both periods and at the six collection points, with small differences occurring between the surface and deepest layers (Tab. 2).

**Table 2** — Hydrological variables averages in the continental shelf in the Pernambuco state (Northeastern Brazil).

GAIBÚ											
	Depth	Temp.	Sal.	DO	pН	$NH_4$	NO <sub>2</sub>	NO <sub>3</sub>	PO <sub>4</sub>	SiO <sub>2</sub>	
	(m)	(°C)	(‰)	(mg/L)		(µg/L)	(µg/L)	(µg/L)	(µg/L)	(µg/L)	
Pt 1	0	27,4	33,73	4,88	8,55	0,012	0,140	0,180	0,046	12,316	
Pt 1	10	27,2	35,32	4,22	8,44	0,041	0,379	0,392	0,075	13,351	
Pt 2	0	27,2	35,58	4,65	8,55	0,001	0,555	0,453	0,006	8,731	
Pt 2	20	27,1	36,28	4,05	8,54	0,097	0,196	0,689	0,020	8,563	
Pt 3	0	27,4	35,58	4,66	8,60	0,001	0,076	0,162	0,001	14,862	
Pt 3	30	27,7	36,38	4,15	8,57	0,001	0,144	0,310	0,039	10,148	
SERRAMBI											
Pt 4	0	26,3	33,19	5,42	8,53	0,001	0,084	0,003	0,022	16,183	
Pt 4	10	26,1	34,52	4,31	8,25	0,001	0,149	0,920	0,035	25,767	
Pt 5	0	26,2	34,52	4,63	8,71	0,001	0,108	0,104	0,001	8,387	
Pt 5	20	26,1	35,85	4,16	8,55	0,001	0,174	0,318	0,009	13,573	
Pt 6	0	26,2	35,32	4,58	8,57	0,001	0,040	0,367	0,001	7,357	
Pt 6	30	26,1	36,38	4,49	8,64	0,001	0,077	0,353	0,021	5,172	

The correlation analysis between concentrations of *Trichodesmium thiebautii* and abiotic variables permitted a simultaneous interpretation of data, which revealed a positive correlation between filament density, pH and dissolved oxygen during the rainy season, and filament density, salinity and dissolved oxygen in the dry season. Both periods presented negative correlations between density, temperature and nutrients (Fig. 3).

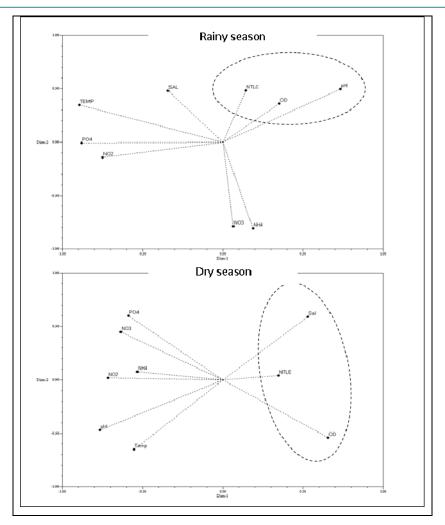


Figure 3 — Correlation between *Trichodesmium thiebautii* Gomont ex Gomont density and abiotic factors on the continental shelf of Pernambuco, in northeastern Brazil, during the rainy (July 2005) and the dry season (November 2006) (NTLC – number of trichomes per liter in the rainy season, NTLE – Number of trichomes per liter in the dry season, Temp – temperature, Sal – salinity, OD – dissolved oxygen, pH – hydrogenionic potential, NH<sub>4</sub> – ammonia, NO<sub>2</sub> – nitrite, NO<sub>3</sub> – nitrate, PO<sub>4</sub> – phosphate)

#### DISCUSSION

Pernambuco's continental shelf has oceanographic conditions typical of tropical seas and it is characterized by few annual variations in temperature (annual average of 27° C), elevated salinity values (annual average of 36.00 ups) and low nutrient content. Small annual and spatial variations also occur in dissolved oxygen and pH contents, conditioning an uniformity throughout the water column, with small thermal and saline stratifications.

These characteristics are the result not only of the location in a tropical climate region (Northeastern Brazil) but also of the continental shelf's geomorphological characteristics, which reveals a reduced width (ranging between 35 and 42 km), little depth (shelf break at around 50 to 60 m), soft declivity (around 1.5m/km). The rivers that fertilize the shelf's waters are small in size, and thus the extent of fertilization depends on the continental flow, which does not exceed 4 miles from the coast, being more efficient during the rainy season. Areas further away from food supplies depend on coastal currents (Koenig & Macedo 1999).

These environmental characteristics favor the establishment of *Trichodesmium thiebautii* throughout the year, with the number of filaments.L<sup>-1</sup> ranging between 100 filaments.L<sup>-1</sup>, in areas closer to the coast, and a maximum of 1,700 filaments.L<sup>-1</sup> at points further away and free from continental influence. The species is found at all collection point,

especially during the dry season, when hydrological conditions are characterized by clean and transparent waters and by low levels of nutrients, especially nitrite.

According to Carpenter (1983) and Carpenter et al. (1987), *Trichodesmium* growth can occur on the surface of the higher photic zone layers, and during periods of high intensity light, thus its representatives are frequently dominant in tropical and subtropical sea plankton. Some authors believe that other factors like calm seas with little wave action, salinity and high temperatures, weak winds and the presence of iron also contribute towards establishing *Trichodesmium* growth. High levels of light, orthophosphates and nitrogenated bases are also correlated with these growths (Gianesella-Galvão et al. 1995; Lugomela et al. 2002; Carpenter et al. 2004).

In the specific case of *T. thiebautii*, average density on the surface of the North Atlantic Ocean can reach 2,250 filaments.L<sup>-1</sup> (Carpenter et al. 2004), whereas east of the China Sea, densities were found ranging between 100 and 1000 filaments.L<sup>-1</sup> (Marumo & Asaoka, 1974).

On the Pernambuco shelf, quantitative values for *T. thiebautii* were very near those found by Marumo & Asaoka (1974), east of the China Sea, as well as data obtained from the Pacific Ocean, where densities ranged between 10 and 50 filaments.L<sup>-1</sup> at depths above 30 m, and 100 filaments.L<sup>-1</sup> at depths under 50 m. The values obtained in the Pernambuco shelf during the dry season, also proved to be near those observed by Carpenter et al. (2004) in a study conducted in the North Atlantic Ocean, where the authors observed an average density on the surface of 2,250 filaments.L<sup>-1</sup> between May and June 1994. This means the quantitative variations of *T. thiebautii* may occur in several parts of the world as a result of environmental conditions.

Higher concentrations of filaments found on the surface and in open seas show that in Pernambuco's continental shelf the species prefers surface ocean waters, a fact also observed by Post et al. (2002) in Aqaba Bay (Red Sea), where the species is found all year round, with higher concentrations on the surface. According to Lugomela et al. (2002), although the species occurs all year long, its presence in coastal areas of Tanzania, is mainly related to a higher concentration of nutrients, which occurred during the rainy season.

According to Monteiro et al. (2010), in Pernambuco's continental shelf, T. thiebautii is a frequent species that occurs in colonies in bunches, or rarely in bundles, with straight, cylindrical trichomes, ranging in length between 110 and 2,110  $\mu$ m, with square or slightly longer cells, measuring 5-10  $\mu$ m in width and 3-15  $\mu$ m in height. According to the authors, more robust colonies, with a greater number of trichomes, up to 100 trichomes/colony, are found during the dry season and in areas furthest from the coast, free of any influence from the continent. These data confirm that, although Pernambuco's continental shelf has very similar oceanographic conditions along its entire extension, the occurrence of T. thiebautii is related to the most oligotrophic and transparent areas, the evidence of which is proven by the colonies' morphometric characteristics and filament density.

The negative correlation observed during the rainy season and the dry season for *Trichodesmium thiebautii* with dissolved nutrients, confirms the association made by Marumo & Asaoka (1974), where the presence of the species was associated with a very small concentration of nutrient salts.

# CONCLUSIONS

The area presented clean and transparent waters and a very low concentrations of nutrient salts, characteristic of oligotrophic conditions;

*Trichodesmium thiebautii* occurred throughout the year, presenting maximum of filaments at points further away from the coast and free of continental influence;

*T. thiebautii* presented a positive correlation with pH and dissolved oxygen during the rainy season and with salinity and dissolved oxygen during the dry season.

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#### **REFERENCES**

- CAPONE, D. G.; ZEHR, J.; PAERL, H. W.; BERGMAN, B.; CARPENTER, E.J. *Trichodesmium,* a globally significant marine cyanobacterium. **Science**, v. 276, p. 1221-1229, 1997.
- CARPENTER, E. J. **Nitrogen in the marine environment**. In: CARPENTER, E.J.; CAPONE, D.G. (Eds.) New York, Academic Press. 1983.
- CARPENTER, E. J.; SCRANTON, M. I.; NOVELLI, P. C.; MICHAELS A. Validity of  $N_2$  fixation rate measurements in marine *Oscillatoria* (*Trichodesmium*). **Journal of Plankton Research**, v. 9, p. 1047-1056, 1987.
- CARPENTER, E. J.; SUBRAMANIAM, A.; CAPONE, D. G. Biomass and primary productivity of the cyanobacterium *Trichodesmium* spp. in the tropical N Atlantic ocean. **Deep-Sea Research**, v. 51, p. 173–203, 2004.
- CPRH Companhia Pernambucana do Meio Ambiente. **Diagnóstico sócio ambiental do litoral sul de Pernambuco**. CPRH, Recife, 2003.
- DARWIN, C. 1834. The Beagle diary. Cambridge University Press, Cambridge,.
- GALLON, J. R.; JONES D. A.; PAGE, T.S. *Trichodesmium*, the paradoxical diazotroph. **Algological Studies**, v. 83, p. 215–243. 1996.
- GIANESELLA-GALVÃO, S. M. F.; COSTA, M. P. F.; KUTNER, M. B. B. Bloom de *Oscillatoria* (*Trichodesmium*) *erythraeum* (Ehrenberg) Kützing in coastal waters of the Southweast Atlantic. **Publicação Especial Instituto do Oceanográfico**, v. 11, p. 133-140, 1995.
- GRASSHOFF, F. K.; EMRHARDT, M.; KREMLING, K. **Methods of seawater analysis**. 2. Ed. Verlag Chemie, New York, 1983. 419 p.
- KOENING, M. L.; MACEDO, S. J. Hydrology and phytoplankton community structure at Itamaracá Pernambuco (Northeast Brazil). **Brazilian Archives of Biology and Technology** v. 42, n. 4, p. 381-392, 1999.
- KOMÁREK, J.; ANAGNOSTIDES, K. Cyanoprokaryota: Oscillatoriales, 2. teil. Süsswasserflora von Mitteleuropa. Elsevier GmBH, München, 2005.
- LEITÃO; M. F. COSTA, (Org.). **Oceanografia: um cenário tropical**. Editora Bagaço, Recife, 2004, p. 255-286.
- LUGOMELA, C.; LYIMO T. J.; BRYCESON, I.; SEMESI, A. K.; BERGMAN, B. 2002. *Trichodesmium* in coastal waters of Tanzania: diversity, seasonality, nitrogen and carbon fixation. **Hydrobiologia**, v. 477, p. 1–13.
- MACEDO, S. J.; MUNIZ, K.; FLORES MONTES, M. J. Hidrologia da região costeira e plataforma continental do Estado de Pernambuco. In: E. ESKINAZI-LEÇA, S.; NEUMANN
- MARUMO, R.; ASAOKA, O. *Trichodesmium* in the east China Sea. **Journal of Oceanographical Society of Japan**, v. 30, p. 298-303, 1974.
- MEDEIROS, C.; MACEDO, S. J.; FEITOSA F. A. N.; KOENING, M. L. Hydrography and phytoplankton biomass and abundance of north-east brazilian waters. **Archive of Fishery and Marine Research**, v. 47 n. 2/3, p. 133-151, 1999.
- MONTEIRO, J. J. F.; ESKINAZI-LEÇA, E.; KOENING, M. L.; MACEDO, S. J. New record of *Trichodesmium thiebautii* Gomont ex Gomont (Oscillatoriales Cyanophyta) for the continental shelf of northeastern Brazil. **Acta Botanica Brasílica**, v. 24, p. 1104-1106, 2010.
- POST, A. F.; DEDEJ, Z.; GOTTLIEB, R.; LI, H.; THOMAS, D. N.; EL-ABSAWIβ, M; EL-NAGGAR, A.; EL- GHARABAWIβ, M; SOMMER, U. Spatial and temporal distribution of *Trichodesmium* spp. in the stratified Gulf of Aqaba, Red Sea. **Marine Ecology Progress Series**, v. 239, p. 241–250, 2002.
- RÖRIG, L. R. Studies on the ecology and toxicity of *Trichodesmium* spp. Blooms in southern Brazilian coast waters. In: Reguera, B. (ed.), **Harmful Algae**. Xunta de Galicia and IOC of UNESCO Publishers Santiago del Compostella, p. 22-25. 1998.

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SATÔ, S.; PARANAGUÁ, M. N.; ESKINAZI, E. On the mecanism of red tide of *Trichodesmium* in the Recife, northeastern Brazil, with some considerations of the relation to the human disease, "Tamandaré Fever", **Trabalhos do Instituto Oceanográfico da Universidade Federal de Pernambuco**, v. 5/6, p. 7- 49,1963.

SASSI, R.; KUTNER, M. B. B. Variação sazonal do fitoplâncton da região do Saco da Ribeira. **Boletim do Instituto Oceanográfico da Universidade de São Paulo**, v. 31, n. 2, p. 2942. 1982.

SIQUEIRA, A.; KOLM, E. H.; BRANDINI, F. P. Offshore distribution patterns of the cyanobacterium *Trichodesmium erythraeum* Ehrenberg and associated phyto and bacterioplankton in the southern Atlantic Coast (Paraná, Brazil), **Brazilian Archives of Biology and Technology**, v. 49, n. 2, p. 323-337, 2006.

STRICKLAND, J. D.; PARSONS, T. R. A pratical handbook of seawater analysis. **Bulettin Fisheries Research Board of Canada** v. 122, p. 1-172, 1972.

TYRRELL, T.; MARAÑÓN, E.; POUTON, A. J.; BOWIE, A. R.; HARBOUR, D.S. & WOOODW, E.M.S. -Scale latitudinal distribution of *Trichodesmium* spp. in the Atlantic Ocean. **Journal of Plankton Research**, v. 25, n. 4, p. 405-416, 2003.

VILLAFAÑE, V. E.; REID, M. H. F, Métodos de microscopia para la cuantificacion del fitoplâncton. In: K. ALVEAL; M. E. FERRARIO; E. C. OLIVEIRA & E. SAR (eds). **Manual métodos ficológicos**. Editora Anibal Pinto/Universidad de Concepción, Concepción, 1995, p. 169-186.